NASA should protect Earth in 2030s as if Mars samples contain mirror life – a review for attention of space agencies

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This preprint is currently in process of development and not yet peer reviewed. For the questions for NASA please jump to:

- Questions for NASA - and why NASA's main argument is invalid

This review focuses on worst case scenarios for the same reason we need to consider house fires for smoke detectors.

It is around 120,00 words, not including references. The last comprehensive Mars sample return study is from 2009. This review needs to cover at least some of the new research since then, and sometimes completely new topics in a preliminary way.

However this review is designed to be easy to navigate. If you start with the Questions for NASA you can drill down to increasing detail for each of the points. For a first overview of the paper just read section titles in the all sections: page at the end of the paper. The section titles are written like mini-abstracts and summarize the sections. I plan a shorter paper based on it.

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Abstract

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Figure 1 : quote from (Sagan, 1973):

"The likelihood that such pathogens exist is probably small, but we cannot take even a small risk with a billion lives."

Background image shows Sagan with a model of the Viking lander (NASA, 2017)

In the late 2020s to 2030s, China, and NASA / ESA and Japan plan to return samples from Mars. We need to keep Earth's biosphere safe from any Martian microbes. Japan's agency JAXA has the simplest mission, to return samples from the top few centimeters of Mars's innermost moon Phobos.

JAXA can safely return unsterilized samples without any precautions, because any microbes already withstood ejection from Mars, most recently, 700,000 years ago. Then on Phobos they were sterilized similarly to martian meteorites arriving at Earth today from that ancient impact.

JAXA warned this meteorite argument is not valid for samples from the Martian surface. NASA's draft EIS incorrectly says any life from Jezero crater can get here faster and better protected in a meteorite than in a sample tube. Surface dirt and dust can't get here at all.

NASA's EIS also proposes to return its samples to a Biosafety Level 4 facility. However, the European Space Foundation study in 2012 set size limits well beyond capabilities of a BSL-4 and indeed beyond any current air filter capabilities.

We can avoid all these issues and keep Earth 100% safe by sterilizing samples before they get here, with the equivalent of a few hundred million years of Mars surface ionizing radiation. This has virtually no effect on geology, while terrestrial contamination in Perseverance's samples makes most astrobiology impossible.

We can greatly increase science value with contamination free samples in a sterile container returned to a martian gravity centrifuge in an unmanned satellite above GEO, to start Sagan's ***"vigorous program of unmanned exobiology".***

This is a review of central results in planetary protection literature, with new worst case scenarios such as mirror life, to encourage space agencies to ensure Earth's biosphere is adequately protected when they return samples from Mars.

Review of central results in the recent planetary protection literature for Mars Sample Return missions for attention of space agencies

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This review focuses on NASA's draft EIS only because it is the first environmental impact statement for a Mars sample return ever published.

NASA paid so much attention to planetary protection in the past, yet as described in the abstract and in more detail in this paper even they have major lapses in their environmental impact statement. This suggests other space agencies can easily do the same. For a list of the main issues found in the draft EIS see:

- Questions for NASA - and why NASA's main argument is invalid
- Reasons for these questions: mistakes in NASA's draft EIS and the report of the sterilization working group

For the recommendations see:

- **Recommendations for space agencies generally** – the simplest way to keep Earth safe is to sterilize any samples returned from Mars before they reach Earth – this can be done with ionizing radiation – sterilization would have virtually no effect on geology and most likely no effect on astrobiology for preliminary samples – priority to return samples free from forward contamination by terrestrial life

- **Recommendations for NASA** – need to restart the process with a scientifically credible Environmental Impact Statement – simplest approach is to sterilize samples before they are returned to Earth - this retains virtually all geology and most likely has no impact on astrobiology – a valid environmental impact statement should at least look at pre-sterilized samples as a reasonable alternative that keeps Earth 100% safe

It would be a major omission to omit all of the many new findings since the last comprehensive Mars sample return study in 2009 (SSB, 2009).

- This paper frequently covers recent research findings – because if it didn't it would be 13 years out of date – however it is not itself a comprehensive review and shouldn't be used as such

This paper is written to be maximally accessible. See:

- Note on use of language – this paper is designed to be maximally accessible – by careful use of vocabulary and grammatical structures, but never with loss of precision in the meaning of the text

No, life on Mars can't get to Earth faster and better protected in meteorites than in a sample tube - the 2009 Mars sample return study warns against this argument as does the 2019 Phobos sample return study - indeed martian surface brines, ice, salts, dirt and dust can't get to Earth at all

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Let's start with the meteorite argument. NASA's Environmental Impact Statement (EIS) argues that (NASA, 2022 : 3-3):

> *The natural delivery of Mars materials can provide better protection and faster transit than the current MSR mission concept.*

However, the NRC Mars Sample Return study in 2009 said, in the section ***"Martian meteorites, Large-Scale effects and Planetary Protection"*** (SSB, 2009: 47)

> *The potential hazards posed for Earth by viable organisms surviving in samples [are] significantly greater with a Mars sample return than if the same organisms were brought to Earth via impact-mediated ejection from Mars*

The NRC goes on to say (SSB, 2009: 48):

> *... Thus it is not appropriate to argue that the existence of martian meteorites on Earth negate the need to treat as potentially hazardous any samples returned from Mars by robotic spacecraft.*

So, how did NASA's EIS come to such a different conclusion?

They argue that potential Mars microbes would be expected to survive ejection from Mars (NASA, 2022: 3-3):

> ***First, potential Mars microbes would be expected to survive ejection forces and pressure (National Academies of Sciences, …, 2019), …***

Their cite there is the planetary protection study for a sample return from Phobos, the innermost moon of Mars. But their cite explicitly says on page 4 that its recommendations do not apply to future sample return missions from Mars itself. (SSB, 2019 : 4):

The main differences between MSR and Phobos/Deimos sample return missions are as follows:

- ***Many surface materials couldn't mechanically withstand ejection from Mars.***
- *The samples would also be selected on the surface for scientific interest which makes them different from martian meteorites which weren't selected for science interest before ejection.*
- *The samples wouldn't be sterilized by ejection, impact on Phobos or by exposure to ionizing radiation on the surface of Phobos for hundreds of thousands of years before collection.*

Therefore, the committee finds that the content of this report and, specifically, the recommendations presented in it do not apply to future sample return missions from Mars itself.

NASA's source explain this in more detail in Chapter 3 (SSB, 2019 : 43). This is what they say about the third of those three points, they say that samples on the Martian surface may well come from environments that mechanically cannot become a Mars meteorite.

The reasoning regarding natural flux does not apply directly to samples returned from the Mars surface.

- *The material will be gently sampled and returned directly to Earth.*
- ***The sample may well come from an environment that mechanically cannot become a Mars meteorite.***
- *The microbes may not be able to survive impact ejection and transport through space.*
- *Samples with current liquid water and recent ice seem especially fragile to natural transport to Earth.*

Finding: The committee finds that the content of this report and, specifically, the recommendations in it do not apply to future sample return missions from Mars itself.

*S*o, in short, NASA's draft EIS gets to its conclusion of ***"better protection and faster transit"*** in martian meteorites through incomplete citing. They summarized the main ideas of the Phobos sample return study but missed the warnings on pages 4 and 43 of its main cite, the Phobos sample return report which says clearly that their reasoning and their recommendations should NOT be used for samples returned from Mars.(SSB, 2019 : 4) (SSB, 2019 : 43).

Any life in Mars surface dust, salts, and dirt would NOT be better protected in a meteorite as they couldn't mechanically survive ejection at all, as they would burn up in the atmosphere before reaching escape velocity. Also a microbe adapted to live on the surface of Mars might well be unable to survive ejection and the desiccating effect of vacuum while a sample tube is like a miniature space-craft for a microbe complete with a small amount of the Martian atmosphere.

The draft EIS also misses the warning on page 48 of the 2009 NRC Mars Sample Return report that it is inappropriate to use the meteorite argument for samples returned from Mars (SSB, 2009: 48)

Any life in Martian meteorites DOES get here faster and better protected than samples returned from PHOBOS because Phobos samples survived ejection from Mars and spent hundreds of thousands of years getting sterilized on the surface of Phobos – so it is safe for Japan to return samples unsterilized without special precautions – and why this reasoning DOES NOT apply to samples from MARS

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So where does the “faster and better protected” idea come from? It is because the Phobos sample and the Martian meteorites have had a similar history since they were on Mars (SSB, 2019 : 38 ff).

The most recent opportunity for any life to get from Mars to Phobos OR to Earth was after the impact that formed the Zunil crater on Mars around a million years ago (direct crater count suggests 700,000 years ago) (Hartmann et al, 2010).

So any meteorites from Mars arriving at Earth today from the Zunil crater ejecta spent at least the last several hundreds of thousands of years in space, while any samples we return from Phobos take longer than the meteorites arriving today on Earth, because they left Mars at the same time and haven’t yet got here.

Then as for “better protected”, our martian meteorites last left Mars at least 700,000 years ago (ejection ages between 0.7 and 18.5 million years ago (Udry et al, 2020:table S4)). So, they compared two chains of events, a Phobos sample return and transfer on a meteorite.

We don’t know how long it took exactly, but our best estimate is 700,000 years old for the Zunil crater, so let’s use that figure to help illustrate how they are an exact parallel of each other:

> **Phobos sample return:** Ejection from Mars → Shock of impact on Phobos → Remains in top 10 cm of the Phobos surface for 700,000 years → returned to Earth in the Phobos sample return
>
> **Meteorites from Mars arriving today:** Ejection from Mars → spends 700,000 years in space traveling from Mars to Earth → fireball of reentry into Earth’s atmosphere and delivered to Earth.

They found that the amount of sterilization is similar for ejection in both cases. Martian meteorites need a higher ejection velocity to reach Earth than to reach Phobos but a small percentage of the ejecta is only weakly shocked so the difference between the two cases is modest. (SSB, 2019: 59).

The amount of sterilization between ejection from Mars and arrival on Earth is also similar in both cases, as the samples get similar ionizing radiation, whether resting on the surface of Phobos or traveling to Earth in a meteorite.

There are two differences

- Phobos sample: Shock of impact on Phobos
- Mars meteorite: Fireball of re-entry into Earth's atmosphere

They found that any microbes from Mars would be far more sterilized by the shock of an impact into Phobos than a reentry fireball to Earth because only the surface of the rock is heated (SSB, 2019: 40).

For this part of the calculation, the committee assumed a 10% survival of microbes (underestimating a likely 80 to 100% survival) (SSB, 2019 : 40).

The Phobos sample return study estimates that about 100 kilograms of Martian meteorites arrives every year and that about 100,000 tons of material have been delivered to Earth from the Zunil impact in the last million years.

Compared to these figures the amount of material returned from the surface of Phobos will be small.

This is the backward contamination version of Greenberg's "Natural contamination standard" (Greenberg et al, 2001).

> *"As long as the probability of people infecting other planets with terrestrial microbes is substantially smaller than the probability that such contamination happens naturally, exploration activities would, in our view, be doing no harm. We call this concept the natural contamination standard."*

The reasoning is that if Earth frequently encounters Martian life anyway, we have no need to protect Earth with special precautions,

The Phobos sample return analysis may have a slight omission here for photosynthetic life which lives near the surfaces of rocks and so is more vulnerable to the fireball of re-entry than most life. But if so, it seems a minor issue because the meteorites we have from Mars also come from at least 3 meters below the surface in the very cold dry southern uplands. See the supplementary data:

- New: extending the JAXA analysis to photosynthetic life on or near the surface of any Martian meteorites

In this way the Phobos sample return study argues that any microbes on Phobos are safe to return to Earth because they **ALREADY** survived ejection from Mars similarly to any microbes in Martian meteorites.

They do **NOT** argue that all species of microbes on Mars can get to Phobos or to Earth, never mind get here faster or better protected.

The only previous use of this argument I can find in the planetary protection literature is by Zubrin in a non peer reviewed op ed (Zubrin, 2000). Here is how he puts it:

> *In the first place, if there are or ever were organisms on or near the Martian surface, then the Earth has already been, and continues to be, exposed to them.*
>
> *Over the past billions of years, millions of tons of Martian surface material have been blasted off the surface of the Red Planet by meteorite strikes, and a considerable amount of this material has traveled through space to land on Earth.*
>
> *Although each SNC meteorite must wander through space for millions of years before arrival at Earth, it is the opinion of experts in the area that neither this extended period traveling through hard vacuum nor the traumas associated with ejection from Mars and arrival at Earth would be sufficient to sterilize these objects, if they originally contained bacterial spores.*
>
> *Furthermore, on the basis of the amount of SNC meteorites we have found, it has been estimated that these Martian rocks continue to rain down upon the Earth at a rate of about 500 kilograms (more than 1,000 pounds) per year.*

As we've seen, his reasoning would not survive peer review. Although some hardy microbes could survive deep within a rock, any life in the kilograms of rocks from Mars had to survive ejection from Mars, and any life arriving today has also spent hundreds of thousands of years traveling through space and many terrestrial microbes couldn't survive even a short time in space conditions or ejection into space. We don't know the capabilities of Martian life, but it is rather remarkable that any terrestrial life has the potential to survive transits between planets.

There is no reason to suppose Zubrin influenced the authors of NASA's draft EIS. However, there may be a common background to explain the many striking similarities between his arguments and the arguments in the EIS. I go into that below:

- There are many parallels between the arguments in the draft EIS and Zubrin's op ed – no reason to believe there was any direct influence – but there may be a common background

Terrestrial analogy of invasive starlings in the USA and the invasive diatom Didymo in New Zealand – it's life that CAN'T get to Earth by itself that matters for backwards contamination – while for panspermia the focus is on life that CAN get to Earth

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NASA's draft EIS also supports their argument with research on panspermia, transfer of life between planets (NASA, 2022: 3-3):.

> *Thus, if potentially harmful microbes were abundant on the Martian surface it is likely they already would have been transferred to Earth by this natural process (Fajardo-Cavazos et al. 2005, Horneck et al. 2008, Howard et al. 2013).*

The first of those cites is to an experiment that did show the very hardy microbe bacillus subtilis survived re-entry in a rock attached to a sub-orbital sounding rocket with re-entry velocity of 1.2 km / sec. That is within an order of magnitude of re-entry speeds for a Mars meteorite (Fajardo-Cavazos et al, 2005).

B. subtilis is indeed a candidate for a microbe that might get from Mars to Earth in a meteorite, if it is found on Mars. Other evidence showed b. subtilis could withstand the shock of ejection from Mars and some microbes could survive 300,000 years transit from Mars to Earth with just 10 cm of shielding from ionizing radiation within the meteorite (Cockell, 2008).

However, panspermia has a different focus from planetary protection:

- **Panspermia:** the search is for ***any species that could have got here*** from Mars (if life ever evolved there). We only need it to happen for ***one microbe once*** in the last several billion years to establish panspermia.
- **Planetary protection:** To establish Earth is safe from invasive species from Mars, we need to ask if there is ***any scenario*** with present day Martian species that ***can't get here***, or can only get here with great difficulty.

For a valid backwards contamination argument we need ***all species we could return from Mars, in all potential scenarios,*** to have equal or better opportunities to get here on meteorites than in a sample tube.

It may help to use an analogy with terrestrial species of birds crossing the Atlantic. European Barn swallows were in the Americas already. However, European starlings lack the swallow's ability to fly across the Atlantic, which is how they could become an invasive species in the USA (US DOA, 2017).

Figure 2: Starling photo from: ([Johnstone, 2017](#))

Barn swallow photo from ([Batbander, 2020](#))

Didymosphenia geminata from (Spaulding,n.d.)

Many freshwater microbes can't cross oceans either. Non native microbes sometimes cause issues even on Earth. In the Great Lakes, non native S. binderanus blooms caused taste and odor problems in drinking water in the 1950s through to 1970 and clogged water-treatment-plant filters in the lower lakes. Some of the invasive diatoms may have made native diatoms locally extinct and one of them removed so much dissolved silica, it had an ecosystem level effect, creating conditions for large blooms of cyanobacteria along the coasts, which out competed other species (Spaulding et al, 2010:556-7)

The clearest example is the freshwater diatom "Didymo" (Didymosphenia geminatum) (Schmidt, n.d.) which causes many problems in New Zealand (Spaulding et al, 2010:558). This is an example sign warning sailors about the risk of carrying didymo to another lake.

.

Figure 3: Text on sign: Your boat may now be carrying didymo. Please clean using approved methods. Protect our waters …

Image from: (Thorney¿?. 2006)

Didymo can't even get from one freshwater lake to another on the same island without human help (Spaulding et al, 2010). It could never get from Mars to Earth.

It is far harder to cross the vacuum of space than to cross oceans. Although, as we saw, remarkably some hardy microbes on Earth such as b. subtilis may be able to do this (Cockell, 2008), many could never survive months in vacuum conditions or the other challenges involved in crossing from one planet to another.

Three scenarios for Martian life in Perseverance's samples – blown in the dust storms, microhabitats, and native life with novel capabilities

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We will find that there are three main scenarios which could lead to Martian life in Perseverance's samples

1. Spores, biofilm fragments and hardy propagules blown there from elsewhere on Mars (Billi et al, 2019a), (Billi et al, 2019b).
2. Microhabitats which we can't detect from orbit or even using Perseverance, like the microhabitats in terrestrial deserts, such as water that forms through spontaneous condensation of water vapour in micropores in salt or gypsum deposits (Vítek et al, 2010) (Wierzchos, 2012) (Conley, 2016) (Davies, 2014)
3. Native Martian life adapted over billions of years with capabilities terrestrial life doesn't have.

Martian species evolved to live in the very cold salty brines found by Curiosity may be unable to get here in a meteorite

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On that third of those three scenarios, Native Martian life adapted over billions of years with capabilities terrestrial life doesn't have, Curiosity found salty brines that last for a few hours in the late evening / early morning in Gale crater (Martin-Torres et al, 2015). These brines are far too cold for terrestrial life at -73°C, but otherwise habitable. As the day progresses, the surface brines get warm enough for life but too salty, then dry out completely.

So, could Martian life be adapted to inhabit such cold brines?

Terrestrial life often uses biofilms to inhabit Mars analogue deserts and the 2015 MEPAG review suggested life on Mars may do the same (SSB, 2015 :11). Nilton Renno suggests microbes might use biofilms (Pires, 2015). These would likely be a mix of many species working together for extra resilience like the grit crust in the Atacama desert (Jung et al, 2020).

Curiosity detected these brines in Gale Crater even on the surface (0 cm) through to late spring, see Martin-Torres et al, 2015:fig 3b) and they were still present on the surface through to 6 am on the last day it detected surface brines, in the southern hemisphere spring. Later on that same day, surface temperatures reached 288 °K = 15 °C (Martin-Torres et al, 2015:fig 2a).

A Martian biofilm might include analogues of desert mosses. These absorb water vapour and water quickly and then retain it for a long time. Grimmia sessitana is a candidate moss, collected in the alps, that might be able to live in suitable conditions on Mars (Huwe et al., 2019). Some desert mosses have adaptations to retain water in dry conditions, and a martian moss analogue, adapted over millions of years, might have extra adaptations similarly to cactuses which have stomata, pores that close in daylight and open at night, to trap water (the opposite way around from how stomata work on most plants).

We will look at these and some other ways life could make the brines more habitable below, based on strategies used by terrestrial life, or methods possibly unique to Martian life such as a novel biochemistry:

- Martian life could be more capable of coping with Martian conditions than terrestrial life – e.g. survive better in dust storms or cope better with cold temperatures and temperature changes – and ways a martian biofilm could retain water in ultracold night time brines through to the midday warmth – fine hairs that swell when hydrated, pores that close in daytime like cactuses – chemicals that speed up metabolism, slow generation times and novel biochemistry

Such a biofilm would face many challenges. It's not likely terrestrial microbes could inhabit such cold brines in the forward direction (unless already made much more habitable by Martian life). But these midday temperatures in the Martian spring at Gale crater are potentially habitable for any organism or biofilm able to absorb the cold salty brines at night (perhaps passively while dormant) and able to retain water through to the daytime.

Perseverance can't detect these brines without Curiosity's DAN instrument, but calculations suggest they would also be found in Jezero crater; and last longer before drying out than they do in Gale crater (Chevrier et al, 2020: figure 7). As measured by Perseverance the ground temperature in Jezero crater often varies from well below -70 °C to well above 15 °C in a single day (Afri et al, 2022: Figure 3). Because Perseverance can't detect the brines, there is no way to know if Jezero crater also has surface brines followed by high midday temperatures, as for Gale crater.

See:

- Microbes from near the surface in Jezero crater would withstand temperatures varying from below -70 °C to above 15 °C in a single day – and major changes in humidity and pressure – this is likely to favour polyextremophiles – and martian life would likely be able to resist higher levels of stresses like UV, low humidity, vacuum, desiccation, and ionizing radiation – and may be able to fix nitrogen at low concentrations – which seems likely to make it easier not harder for them to survive on Earth

These brines couldn't get to Earth, and there is no particular reason why life adapted to live in surface brines would also be able to live deep within Martian rocks and get into a meteorite to Earth, or if they could, survive ejection from Mars. Species in the biofilms might only survive desiccation within propagules or biofilm fragments on the surface and not in the vacuum of space conditions..

Martian life that spreads in dust storms as biofilm fragments may include species that can't get here in a meteorite

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This is the first scenario, life in the dust, from our:

- Three scenarios for Martian life in Perseverance's samples – blown in the dust storms, microhabitats, and native life with novel capabilities

Billi et al found biofilm fragments mixed with regolith 0.015 to 0.03 mm thick could resist the UV equivalent to 8 hours of full martian sunlight. That gives fragments enough time to travel over 100 km in light martian winds of 5 meters per second (Billi et al, 2019a), (Billi et al, 2019b). We will find that they could also travel at night and travel much further before sterilization in dust storms. These fragments could help start up a new biofilm faster. It's even possible that Mars continues to have biofilms spread via fragments at times when the conditions are no longer favourable to grow a biofilm as a colony developing from single microbes or spores (Mosca et al, 2019).

Also solitary microbial spores might spread as viable spores in the dust, perhaps attached to a dust grain and shielded from UV in dust storms (Bak et al., 2019).

Martian life might spread slowly like this even with only one spore or biofilm fragment succeeding every few millennia. This possibility is discussed below in:

- 2019: A thin (0.03 microns thick) fragment of desiccated biofilm of chroococcidiopsis would be still viable after blowing 100 km in moderate winds (5 meters per sec) in full Martian sunlight (and following sections)

The biofilms, as for the example of terrestrial diatoms, could be perfectly adapted to survive and propagate on Mars, and yet have no evolutionary pressure to withstand extreme shock, vacuum, life below the surface of a rock, and so on. Some or all of species that make up biofilms might depend on conditions in the biofilm, and have no way to get to Earth independently on a meteorite. For a biofilm fragment, a sealed sample tube is like a miniature spaceship complete with a small amount of martian atmosphere.

Native life that colonizes micropores or micrometer thick layers of melting frost over millions of years may be unable to get here in a meteorite

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This is the second scenario, life in microhabitats, from our:

- Three scenarios for Martian life in Perseverance's samples – blown in the dust storms, microhabitats, and native life with novel capabilities

Life that depends on micropores in salt which it slowly colonises over millennia or millions of years might well have no capability to get to Earth in a meteorite. There's also no reason why life that depends on melting frost could get here in a meteorite.

We'll look at various suggested scenarios for life that could be possible at Jezero crater, for instance, Jezero crater can also have life in micropores in salt or gypsum (Conley, 2016) (Davies, 2014),

- 2015: the MEPAG2 review draws attention to potential for local microenvironments to provide habitats for life that can't be detected in large scale surveys – with example of micropores in salt or gypsum

Or it could use melting frost.

- 2021: Potential for melting frost to form a "dew" of microns thick layers of fresh liquid water trapped by a temperature inversion - it could persist for a few hours even in Jezero crater – as an example to show the potential for future surprise microhabitats

We could find novel microhabitats and life with novel adaptations as surprising as the martian geysers (Kieffer et al., 2006) were when discovered. We might not be able to predict these from terrestrial analogues. See:

- Mars has had many geological surprises like the CO_2 geysers – once we start to look in earnest we may find many astrobiological surprises too

There is no particular reason any of these habitats would have native life able to get to Earth from Mars on a meteorite.

If we find familiar terrestrial life and discover it got to Earth in a meteorite before – this is like finding swallows in the Americas – we may be missing the starlings – so it does NOT show all species in the samples are safe

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If we do find a familiar terrestrial species in the sample, that's like the swallow in the Americas, this will only show that that one species crossed between the planets. To drop planetary protection, we need to continue to look for the starlings in this analogy. We need to show **all species** we might return from Mars are either harmless for some other reason or already get here on meteorites.

Even for microbes that can survive ejection from Mars, they may not be able to survive re-entry to Earth. In a similar experiment to b. subtilis, using an aeroshell re-entering at 7.5 km / sec, Cockell tested whether photosynthetic life would survive the fireball of re-entry, since it typically grows near the surface of rocks. He tested the very hardy blue-green algae Chroococcidiopsis and found that not only the algae but all associated organics were destroyed at a typical growing depth (Cockell, 2008).

So even if we find exceptionally hardy life on Mars, it might not always have the adaptations to let it get to Earth. Also a microbe can survive ejection yet be unable to withstand desiccation from the vacuum of space conditions.

We might find b. subtilis on Mars that got here in meteorites and in the very same sample we might find a chroococcidiopsis analogue that is as hardy as chroococcidiopsis but never got here.

Actually we wouldn't immediately know that b. subtilis on Mars is safe either. There could be strains adapted to Mars over billions of years with novel capabilities in the very different conditions on Mars that never got here.

2015 (overturning results from 2014): Jezero crater seems uninhabited from orbit – but so do Mars analogue deserts on Earth – the 2015 MEPAG review overturned all the conclusions NASA rely on from 2014 – saying life might be transported in dust storms, or live locally in microhabitats and biofilms that can make deserts locally more habitable

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[question]

The draft EIS says the Martian surface is too inhospitable for life to survive in Jezero crater, where Perseverance is collecting samples (NASA, 2022: 1-6):

> *Consensus opinion within the astrobiology scientific community supports a conclusion that the Martian surface is too inhospitable for life to survive there today, particularly at the location and shallow depth (6.4 centimeters [2.5 inches]) being sampled by the Perseverance rover in Jezero Crater, which was chosen as the sampling area because it could have had the right conditions to support life in the ancient past, billions of years ago (Rummel et al. 2014, Grant et al. 2018).*

To support this, NASA's draft EIS refers to SR-SAG2 (Rummel et al , 2014).

However this misses out the review commissioned by NASA and ESA in 2015, the MEPAG review (SSB, 2015) which modified all the main conclusions that NASA relies on for this statement about Jezero crater. NASA don't' cite this review even though they commissioned it.

First, let's go through a brief summary of the main points in the MEPAG review.

The MEPAG review warns that maps such as the ones NASA relied on to select Jezero crater as a landing site represent an incomplete state of knowledge (SSB, 2015 :28):

> *Maps that illustrate the distribution of specific relevant landforms or other surface features can only represent the current (and incomplete) state of knowledge for a specific time—knowledge that will certainly be subject to change or be updated as new information is obtained*

This means that we can't decide in advance from orbit which areas are safe from forward contamination. More on this below in this section.

The MEPAG review also says SR-SAG2 didn't discuss the potential for life to be transported in the dust in the atmosphere (e.g. dust storms) (SSB, 2015 : 12).

> *"The SR-SAG2 report does not adequately discuss the transport of material in the martian atmosphere. The issue is especially worthy of consideration because if survival is possible during atmospheric transport, the designation of Special Regions becomes more difficult, or even irrelevant."*

Here, "special regions" (SSB, 2015 :6) are regions where terrestrial organisms are likely to propagate. The second half of the definition isn't used much given that we don't yet know capabilities of any putative Martian life:

> "within which terrestrial organisms are likely to propagate, or a region which is interpreted to have a high potential for the existence of extant martian life forms."

If terrestrial life can be spread from anywhere to anywhere on Mars it becomes much harder or impossible to map out safe regions for forward contamination, depending how easily it can spread.

[See below: 2015 MEPAG review: potential for viable life transported through the atmosphere (for instance in dust storms)]

The MEPAG review says SR-SAG2 only briefly considered the implications of our lack of knowledge of microenvironments on Mars (SSB, 2015 :12).

> *Physical and chemical conditions in microenvironments can be substantially different from those of larger scales. Although the SR-SAG2 report considered the microenvironment (Finding 3-10), the implications of the lack of knowledge about microscale conditions was only briefly considered.*

[See below 2015: the MEPAG2 review draws attention to the potential for local microenvironments to provide habitats for life that can't be detected in large scale surveys – and illustrative examples of micropores in salts or gypsum, and Curiosity's salty brines]

The MEPAG review draws special attention to biofilms. These aren't discussed in SR-SAG2 (it has only one mention of the word). (SSB, 2015 :11)

> *Given the wide distribution and advantages that communities of organisms have when they live as biofilms enmeshed in copious amounts of EPS [substances that microbes can produce around them to help make a "home" in a hostile environment], it is likely that any microbial stowaways that could survive the trip to Mars would need to develop biofilms to be able to establish themselves in clement microenvironments in Special Regions so that they could grow and replicate.*

Biofilms are of especial importance in the other direction from Mars to Earth, for backwards contamination as in a scenario with native martian life, it has had millions of years to evolve communities of microbes adapted to the Martian surface conditions. Also if there are microhabitats for martian life in Jezero crater, Martian biofilms have had millions of years to find them.

[See below 2015 MEPAG review: microbes can use biofilms to create conditions favourable to them in otherwise uninhabitable microniches – this need to build up a biofilm first reduces the risk for forward contamination for spacecraft with low bioloads – however such niches could be inhabited by Martian life that already lives in biofilms adapted for millions of years]

SR-SAG2 relies on maps like this. This map shows that the equatorial region of Mars including Perseverance's landing site in Jezero crater has no shallow ice observed or suspected

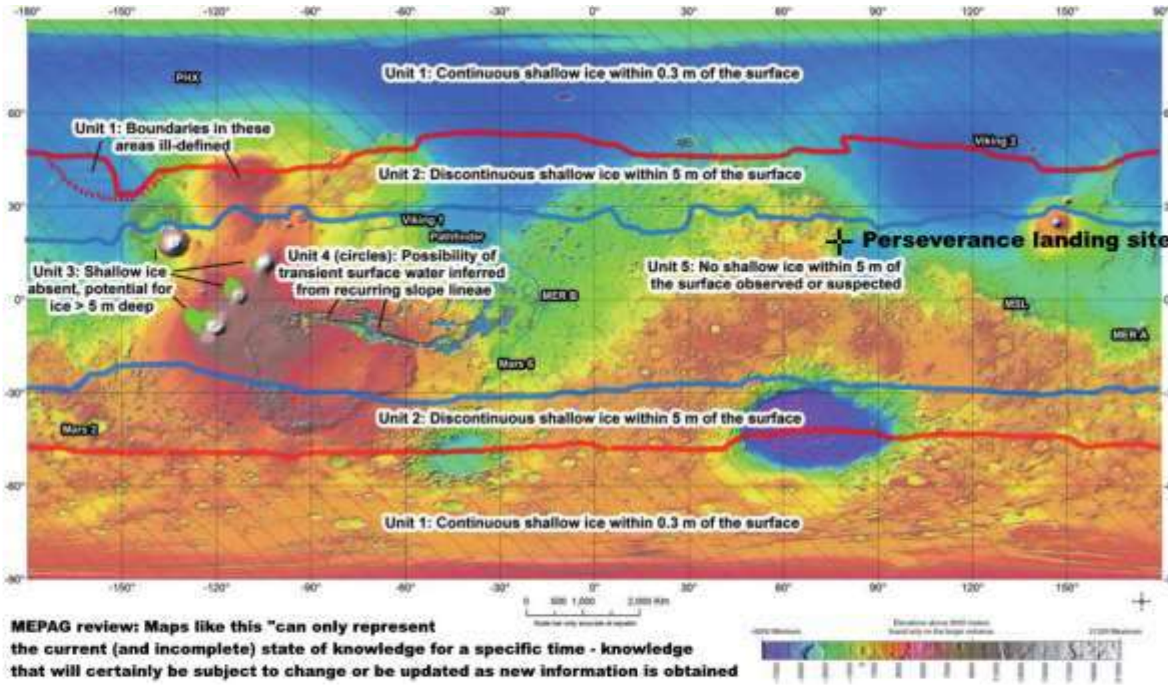

Figure 4: Source: (Rummel et al , 2014 : Fig. 45) Colour coding shows elevation. Perseverance landing site shown at 18.44°N 77.45°E (NASA, 2022)

SR-SAG2 then maps out the Recurrent Slope Linea (RSLs) (Rummel et al , 2014 : Fig. 47). These grow down slopes in spring, broaden through the summer and fade away in the autumn on sun facing slopes on Mars (McEwen, 2011). At the time the favoured explanation was that they were the indirect result of subsurface brine seeps which could be potentially habitable (McEwen, 2011).

More recent research strongly suggests these RSLs are caused by dust flows though this is not yet proven. The way the RSLs grow in spring may be because of seasonal variations in wind speed, direction, and turbulence (Stillman et al., 2021). They may be supplied by bouncing sand grains and movement of dust down the slopes triggered by dust devils [like miniature tornadoes on Mars]. However none of this can be verified as many of the dust devils won't leave tracks detectable from orbit (McEwen et al, 2021). We probably can't resolve this from orbit and need in situ observations (Stillman et al., 2021).

This debate illustrates the issues with use of maps to detect habitability on the ground. The habitability of the RSLs remains unknown in the papers reviewed here, though it is much less favoured than with the science of 2011.

These RSLs can be detected from orbit and SR-SAG2 recognizes the need for buffer zones around them.

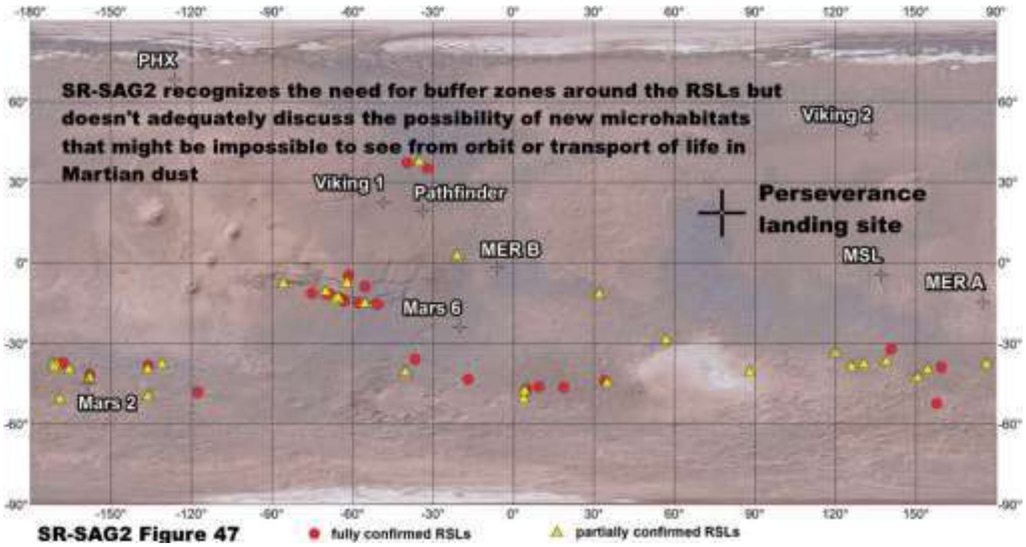

Figure 5: Source (Rummel et al , 2014 : Fig. 47)

The 2014 report uses these maps to map out "Special regions" which are defined as regions (SSB, 2015 :6).

> "within which terrestrial organisms are likely to propagate, or a region which is interpreted to have a high potential for the existence of extant martian life forms."

In the future we may visit the RSLs to search for extant martian life and to find out what causes them. However, with our present level of knowledge about them, our rovers such as Perseverance aren't sufficiently sterilized to approach these regions, so we avoid them to protect against forward contamination.

The 2015 MEPAG Review of this 2014 report says such maps can only represent the current (and incomplete) state of knowledge for a specific time (SSB, 2015 :28).

> *In general, the review committee contends that the use of maps to delineate regions with a lower or higher probability to host Special Regions is most useful if the maps are accompanied by cautionary remarks on their limitations. Maps that illustrate the distribution of specific relevant landforms or other surface features can only represent the current (and incomplete) state of knowledge for a specific time—knowledge that will certainly be subject to change or be updated as new information is obtained.*
>
> …
>
> *Identification of a Special Region needs a multiscale approach ... and thus, as far as missions to Mars are concerned, conservatism demands that each landing ellipse be scrutinized on a case-by-case basis.*

Jezero crater does seem uninhabited from orbit, but there are many ways it could have habitats not easy to detect from so far away as the RSLs.

Many potential microhabitats would be undetectable from orbit, as we see from Mars analogue deserts on Earth. It would be impossible to detect micropores in the gypsum or salt pillars in the Atacama desert from orbit. Mars may have micropores like those (see below):

- 2015: the MEPAG2 review draws attention to potential for local microenvironments to provide habitats for life that can't be detected in large scale surveys – with example of micropores in salt or gypsum

It is feasible to get very high resolution from orbit around Mars in principle because of the thin atmosphere but it needs larger mirrors. Our highest resolution images come from HiRISE on the Mars Reconnaissance Orbiter, with a resolution of 30 to 60 cms per pixel (NASA, n.d.).

We could detect potential past microhabitats with an orbital visible imager with a resolution of 1 cm per pixel. We could identify bulk features consistent with a past habitat at a resolution of 10 to 15 cms, but not reliably (not diagnostic) (Cabrol, 2018). But even 1 cm per pixel wouldn't detect micropores optically from orbit or the brines Curiosity detected. Our rovers can't even see these potential microhabitats on the ground.

This paper needs to look at highlights of the most recent research into the two possibilities looked at by the MEPAG review, or it would be 8 years out of date:

- dust transport
- that the Martian surface might not be as uninhabitable as it seems because of microhabitats (like the micropores), and because of the way microbes can use biofilms to inhabit regions that are otherwise uninhabitable.

Also since this paper is about planetary protection for Earth, it needs to look at possibilities available to Martian life which are important for backwards contamination, but which don't apply to the few microbes, short timescale and known biology of the terrestrial life on our rovers.

The potential for Martian life in regions like Jezero crater, or to spread in the dust, hasn't been looked at in a comprehensive review since 2009. This paper would be 13 years out of date if it didn't cover these developments. Examples include:

- Life on Mars may be adapted to conditions beyond the range of terrestrial life.
- Martian life has had time to colonize habitats that may take thousands of years to colonize. The SR-SAG2 had a limited time frame and said it didn't need to consider forward contamination that takes more than 500 years to get established (Rummel et al , 2014:894).
- Martian life could use fragments of biofilms blown in the winds to create its own microhabitat in a region that is otherwise inhospitable to life (Billi et al, 2019b). Mosca et al suggest it could do that even if local conditions don't permit it to establish a biofilm by slowly growing from a few microbes today, so long as some time in the past biofilms

were able to form, propagating ever since then using these broken off fragments (Mosca et al, 2019). The small numbers of microbes on spacecraft sent to Mars so far can't build up biofilms and the MEPAG Review says whether there are enough terrestrial microbes on a spacecraft to build up a biofilm is a central question in forward contamination (SSB, 2015 :11).

Then there is another possibility for both forward contamination by terrestrial and backward contamination by martian life that's been researched since the MEPAG review

- Microbes can be protected inside larger dust grains up to half a millimeter in size which can travel hundreds to thousands of kilometers by bouncing across the sand dunes a few meters at a time in the dust storms (Kok, 2010:4). (Bak et al., 2019).

All these factors mean even the MEPAG review is out of date.

As for the 2009 study, so much has changed since then. For instance the 2009 study doesn't even consider caves (the word "cave" doesn't' occur in it). Penny Bolton's first paper predates the 2009 study (Boston et al, 2006) but didn't get much attention. Caves are now considered to be one of the top four candidates for indigenous Martian life by many astrobiologists along with ice, salts and the deep subsurface (Carrier et al, 2020:Abstract). SR-SAG2 and the MEPAG review consider them briefly but only in the context of forwards contamination.

This paper turned up research into numerous new scenarios which suggest some potential for returning extant life in the samples. We will look at this in detail in the following sections:

- **2015: MEPAG2 review draws attention to potential for viable life transported through the atmosphere (for instance in dust storms)**

    - 2019: A thin (0.03 microns thick) fragment of desiccated biofilm of chroococcidiopsis would be still viable after blowing 100 km in moderate winds (5 meters per sec) in full Martian sunlight
    - 2019: Curiosity found UV radiation fell by 97% at the start of the 2018 dust storm, which could increase Billi et al's 100 km to 1000s of kilometers in Martian dust storms
    - 2017: individual microbes can travel in dust storms imbedded in a dust grain for extra protection from UV
    - 2019: Microbes can be protected by bouncing sand grains up to half a millimeter in diameter traveling meters in each bounce, and some (less than 1 in 1000) b. subtilis spores remain viable after hundreds to thousands of kilometers of travel in simulation experiments
    - New: Martian life could have spores with extra layers to protect against UV in dust storms - or fruiting bodies or other propagules detached by strong winds protected by outer layers of altruistic social bacteria - and martian life could use strong

biomaterials similar to chitin (found in hard parts of insects but also in fungi and lichens) to protect from impact bounces

- **2015: MEPAG2 review draws attention to the potential for local microenvironments to provide habitats for life that can't be detected in large scale surveys – and illustrative examples of micropores in salts or gypsum, and Curiosity's salty brines**

  - 2021: Potential for melting frost to form a "dew" of microns thick layers of fresh liquid water even in Jezero crater – as an example to show the potential for future surprise microhabitats

- **2015: MEPAG review also draws attention to biofilms that microbes may need to use to create conditions favourable to them in otherwise uninhabitable microniches – this reduces the risk for forward contamination for spacecraft with low bioloads – however this argument doesn't work for backwards contamination – such niches could be inhabited by Martian life that already lives in biofilms**

  - NEW: Life adjusted to Mars has had millions of years to set up biofilms – and slowly colonize microhabitats we may not yet know exist
  - A Martian biofilm might consist of many species that evolved together to inhabit local conditions over millions of years, similarly to the Atacama Desert grit crust [2020]
  - Martian life could be more capable of coping with Martian conditions than terrestrial life – e.g. survive better in dust storms or cope better with cold temperatures and temperature changes – and ways a martian biofilm could retain water in ultracold night time brines through to the midday warmth – fine hairs that swell when hydrated, pores that close in daytime like cactuses – chemicals that speed up metabolism, slow generation times and novel biochemistry
  - Many ways native martian life could make brines more habitable

- **2010: Martian life could inhabit caves that vent to the surface – many types of cave can only be detected by in situ observation unlike the easier to detect lava tube skylights**
-
  - 2016: NASA discovered potential for current habitats for terrestrial life in Gale crater AFTER Curiosity's landing

The general picture is that research since 2015 suggests some potential for life in Jezero crater. In some scenarios life can live in Jezero crater itself in biofilms and / or microhabitats. In other scenarios, Perseverance's sample tubes could return life brought to Jezero crater from distant regions of Mars in dust storms.

If you wish to skip the rest of this section on topics such as dust transport, microhabitats, biofilms and potential for Martian life to be adapted to Martian conditions, and go to the next main section it is:

- NASA's draft EIS argues that existing credible evidence suggests Mars has not been habitable to Earth life for millions of years — yet their cite for this sentence is about a search for current localized habitable regions on Mars – another conclusion reached through a citing error

In more detail on the potential for returning Martian life from Jezero crater:

2015 MEPAG review: potential for viable life transported through the atmosphere (for instance in dust storms)

Next section – all sections – previous section
[question]

The MEPAG review says the SR-SAG2 report doesn't adequately discuss transport of material in the atmosphere (e.g. dust storms). (SSB, 2015 : 12).

> *"The SR-SAG2 report does not adequately discuss the transport of material in the martian atmosphere. The issue is especially worthy of consideration because if survival is possible during atmospheric transport, the designation of Special Regions becomes more difficult, or even irrelevant."*

Here, "special regions" (SSB, 2015 :6) are regions where terrestrial organisms are likely to propagate. The second half of the definition isn't used much given that we don't yet know capabilities of any putative Martian life:

> "within which terrestrial organisms are likely to propagate, or a region which is interpreted to have a high potential for the existence of extant martian life forms."

But if life can be transported from almost anywhere on Mars to almost anywhere it would be impossible to distinguish any particular regions as safe for forward contamination. In this case even if landing sites aren't special regions, they could still potentially seed the Martian dust with life that could propagate in special regions elsewhere on Mars, which is why they say that in the worst case if terrestrial life can propagate easily in the dust it becomes almost irrelevant to single out regions as "special" that need to be avoided.

SR-SAG2 highlights the potential for dust to attenuate the UV and for microbes to be protected growing as aggregates (SSB, 2015 : 12)

> *Atmospheric transport can move microbial cells and spores over long distances, as is known from investigations of foreign microbes delivered to North America from Africa via Saharan dust … and Asia …*

...

In addition to dilution effects, the flux of ultraviolet radiation within the martian atmosphere would be deleterious to most airborne microbes and spores.

However, dust could attenuate this radiation and enhance microbial viability. In addition, for microbes growing not as single cells but as tetrades or larger cell chains, clusters, or aggregates, the inner cells are protected against ultraviolet radiation. Examples are methanogenic archaea like Methanosarcina, halophilic archaea like Halococcus, or cyanobacteria like Gloeocapsa. This is certainly something that could be studied and confirmed or rejected in terrestrial Mars simulation chambers where such transport processes for microbes (e.g., by dust storms) are investigated. The SR-SAG2 report does not adequately discuss the transport of material in the martian atmosphere.

Both reports are about terrestrial life, for forward contamination.

Neither of these reviews examine the possibility of indigenous martian microbes better adapted to the Martian conditions than terrestrial life, because that was not the remit. Also, most of the research into dust transport since then focuses on the potential for forward contamination by terrestrial life.

The evidence so far suggests transport of terrestrial microbes in the dust might be possible, but not easy, and more feasible in biofilm fragments. Our rovers have undoubtedly brought viable microbial spores to Mars, but not enough to introduce biofilms. Most microbes that survived are likely in cracks on the rovers, and though some may drop off into the dirt, many wouldn't be able to take advantage of the local conditions on Mars.

We have taken some care to reduce contamination of our rovers in the forwards direction. Even taking account of the new research into dust transport, we hopefully haven't yet contaminated Mars in the forwards direction. But native evolved life might well be able to spread more easily in the dust storms than terrestrial life, and the samples will include dust and dirt from the Martian surface.

2019: A thin (0.03 microns thick) fragment of desiccated biofilm of chroococcidiopsis would be still viable after blowing 100 km in moderate winds (5 meters per sec) in full Martian sunlight

Next section – all sections – previous section

In 2019, Billi et al found that a fragment of dried biofilm of the blue-green algae chroococcidiopsis mixed with regolith only 0.015 to 0.03 millimeters thick could survive 469 days of Mars surface UV in conditions of partial shade on Mars simulated using a 0.1% filter (Billi et al, 2019b). They calculate that this dose is equivalent to 8 hours of full sunlight on Mars, and that eight hours is enough time to transport the biofilm more than 100 km at typical wind speeds of 5 m/s, though they add that they only tested for the effects of UV and not the effects of perchlorates (Billi et al, 2019a).

Perchlorates in the dust may make this more challenging for terrestrial life because UV has been shown to reduce the survival times, at least for b. subtilis, perhaps by converting them to the more reactive chlorates and chlorites. However, this is not tested for polyextremophiles that may be more resilient (Wadsworth et al, 2017). Also, dust storms would reduce UV reducing this effect and increase the survival times for individual microbes. The biofilm mixed with regolith would also shelter microbes within it from UV. Also, Martian microbes would have had the opportunity to evolve to become more resilient to the martian conditions.

Billi et al conclude Mars (Billi et al, 2019b).

> *… Our findings support the hypothesis that opportunistic colonization of protected niches on Mars, such as in fissures, cracks, and microcaves in rocks or soil, could have enabled life to remain viable while being transported to a new habitat*

In this way martian biofilms could hop from one microhabitat to another a few tens of kilometers at a time, similarly to the way desert nomads use oases to cross deserts.

2019: Curiosity found UV radiation fell by 97% at the start of the 2018 dust storm, which could increase Billi et al’s 100 km to 1000s of kilometers in Martian dust storms – and Mosca et al’s suggestion that biofilm fragments established in the past could continue to propagate even if Mars doesn’t have conditions to start a new biofilm today

Next section – all sections – previous section

Occasional global dust storms cover much of Mars (Shirley, 2015). They typically start in the south, in the southern spring or summer, encircle the planet in southern latitudes then extend north across the equator. Curiosity was able to give direct observations of surface UV during the 2018 global dust storm, and found that it fell by 97% at the start of this storm, and remained at similar low levels for about three weeks (solar longitude 195 to 205) (Smith, 2019).

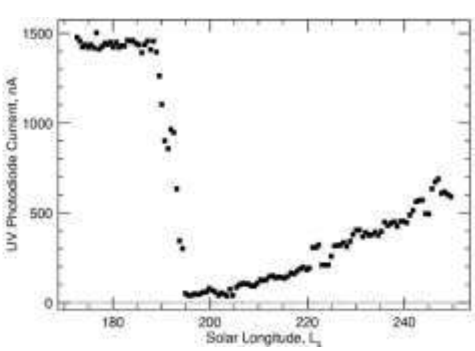

Figure 6: UV measurements by upward pointing photodiodes on the REMS instrument suite on Curiosity. The UV fell by 97% at the onset of the dust storm (Figure 5 of Smith, 2019)

Scaling up from Billi et al's 100 km in 8 hours of full sunlight (Billi et al, 2019a), this suggests a single dust storm could transport biofilm fragments protected from UV for thousands of kilometers, similarly to the dust transport from the Gobi desert to Japan (Maki et al, 2019).

[New] Small biofilm fragments like these can continue moving at night too, when there is no UV radiation. Even during the dust storms, the wind speeds continue at night at 3-4 meters per second increasing to 10 meters per second or more in the day time – at least as measured from the Insight lander for the dust storm in 2019. Before the dust storms, the night wind speeds were above 5 meters per second for most of the night, increasing to a maximum of around 10 meters per second just before dawn at around 4 am. So there seems significant potential for transport of biofilms for large distances at night.

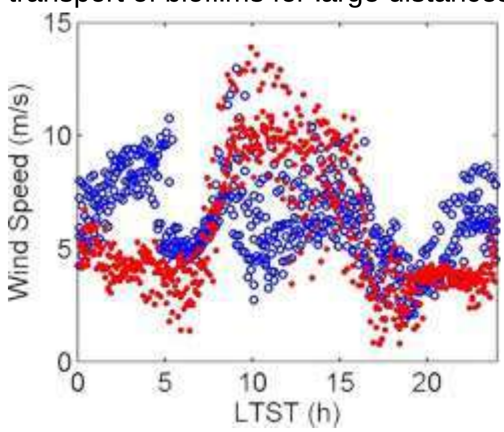

Figure 7: Blue dots show the wind speeds 5 days before the 2019 dust storm as measured from the Insight lander site, about 5 meters per second at night, increasing to 10 meters per second in the middle of the day.

Red dots show the wind speeds 5 days after onset which range from around 3 meters per second most of the night to above 10 meters per second in the middle of the day.

(Viúdez-Moreiras et al., 2020: figure 5).

Mars doesn't need to have conditions to grow biofilms from single cells today. Mosca et al suggest if such a biofilm ever occurred in Martian history, it could continue to be transported from niches that become unfavourable to more favourable niches on Mars through to the present. (Mosca et al, 2019). So, Mars could continue to have life propagated using biofilm fragments from the same ancestral biofilm, potentially even millions of years after the ancestral biofilm first formed and long after the occurrence of any conditions favorable for it to form again from single cells.

This gives a way native evolved Martian life might be able to survive on Mars in conditions a single microbe or spore, Martian or terrestrial, could never colonize. This gives another way for Martian life to colonize apparently uninhabitable regions in Jezero crater.

2017: individual microbes can travel in dust storms imbedded in a dust grain for extra protection from UV

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Individual microbes might also be able to get transported in the dust including in storms. Microbes can get attached to dust grains in tests (van Heereveld et al, 2017) (Osman et al 2008). Sagan suggested a viable microorganism could be imbedded in a dust grain and be protected from the UV by the iron oxides in the dust (Sagan et al, 1967).

The protection from UV by the dust grain could also help with protection from the harmful effects of UV activated perchlorates along with the other factors discussed above in:

- 2019: A thin (0.03 microns thick) fragment of desiccated biofilm of chroococcidiopsis would be still viable after blowing 100 km in moderate winds (5 meters per sec) in full Martian sunlight

2019: Individual microbes can travel in sand grains up to half a millimeter in diameter that bounce repeatedly over the sand dunes, traveling meters in each bounce – a few b. subtilis spores remain viable after bouncing continuously for up to thousands of kilometers in simulation experiments (< 1 in 1000 remain viable)

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The Martian sand dunes have typical grain sizes of half a millimeter, or 500 microns. The Martian winds, far too weak in the thin atmosphere to suspend these grains, can still pick up these half millimeter diameter grains in a bouncing motion, called saltation.

Once the grains start bouncing, Mars's low gravity and lower vertical drag lets them travel higher and further with each bounce than on Earth. A strong wind can lift the grains a few meters horizontally with each bounce and lift them to a height of 10s of cms, (Kok, 2010:fig.3b).

There are two important wind speeds for this process. The wind needs to go above the fluid threshold to start grains bouncing, and after that it has to stay above the lower impact threshold to keep the bounces going after each impact. The impact threshold on Mars is approximately a tenth or less of the fluid threshold (Kok, 2010:fig.1). That's a much bigger difference than for Earth where the wind speeds can get fast enough to set the grains bouncing at only a little over the impact threshold, with a ratio of 0.82 for loose sand.

This means that on Mars, if a gust of wind just over the fluid threshold detaches a particle from the surface, it will continue to bounce across the dunes until the wind speed drops to below the much lower impact threshold (Kok, 2010). The upshot is that even though the winds are much weaker on Mars, with occasional gusts to get them started, dust grains can then move continuously in the winds on Mars much as they can on Earth (Kok, 2010).

> *The small ratio of the impact and fluid thresholds allows Martian saltation to occur for much lower wind speeds than previously thought possible. Indeed, once saltation is initiated by a localized wind gust, it will continue downwind until the wind speed falls by approximately an order of magnitude to a value below the impact threshold*

As an example, Proctor crater has typical wind speeds at only a third of the fluid threshold, however the instantaneous wind speed will occasionally exceed the fluid threshold and then the dust grains keep going until wind speeds falls to below the impact threshold which is far less than typical wind speeds in this crater (Kok, 2010:4).

So, once a dust grain on Mars starts bouncing it typically continues bouncing over and over, for a long time.

The bounces themselves can destroy spores, through mechanical stress. Bak et al. tested this process with spores of b. subtilis, in grains of Icelandic basalt to simulate Martian basalt, half of the spores were killed in a minute. They simulated Martian atmospheric pressure but not the other conditions like UV exposure, perchlorates or cold. These spores were completely destroyed. Nearly all the remaining spores were destroyed within a day (Bak et al., 2019).

However, Bak et al found some spores viable after days of bouncing. They speculated that possibly these spores were protected inside cavities in the dust grains (Bak et al., 2019). The number of viable particles is reduced to 0.5% of the original after a day of tumbling time (Bak et al., 2019:4) (Minns et al., 2022). Bak et al found the number of viable spores is reduced more than 1000 fold after 5 days of bounces, but after that the remaining spores were reduced only 50% after another 5 days of bounces for a total of 10 days (Bak et al., 2019:6). (Bak et al., 2019:Fig 2).

Bak's particles bounced in a tumbler at a rate of 60 bounces per minute, or 86,400 bounces a day (Bak et al., 2019:3). This means that 5 days corresponds to 432,000 bounces.

Kok found a horizontal particle speed of 3 to 10 meters per second for a particle of 500 microns in diameter (red dashed line in (Kok, 2010:fig.3a)) and a bounce distance of 1 to 4.5 meters (red dashed line in (Kok, 2010:fig.3b)). Kok et al.'s 500 micron particle is a little larger than Kok et al's 100 micron particles which would have longer bounces, and so, travel further for the same amount of damage.

432,000 bounces for a 500 micron particle corresponds to:

- 432 kilometers at 1 meter per hop and it would travel that distance in about 40 hours at 3 meters per second (86.4 km in 10 hours for 0.5% viability)

- 1944 kilometers at 4.5 meters per hop, and it would travel that distance in about 54 hours at 10 meters per second (388.8 km in 10.8 hours for 0.5% viability)

(Minns et al., use larger hop size using a different calculation method, and get higher figures (Minns et al., 2022))

Minns et al. said sand grains on Mars are rounded after billions of years of bouncing, and suggest it could increase the survival fraction (Minns et al., 2022). Bak et al. agreed this needs attention and also raise the issue of the particles building up static electricity and discharging it during the bounces which might reduce the survival rate at low atmospheric pressures, though they didn't notice a large effect of lower pressure (Bak et al., 2022).

Bak et al. found the survival curve fitted a power law, approximating a straight line on a log log plot. Doubling the number of bounces approximately halves the number of viable spores left (Bak et al., 2019:6). (Bak et al., 2019:Fig 2). If it continues like this, and there are enough spores for some to be viable after a million-fold reduction, there could still be a few viable spores after several years of bouncing and hundreds of thousands of kilometers of travel. This seems quite promising for a native martian organism if there is anywhere on Mars, habitable enough to produce millions of spores that can get to the surface, or indeed over long periods of time. Even if only hundreds of spores a year got into the bouncing grains from a habitat, over hundreds of thousands of years, some would potentially survive travel for hundreds of thousands of kilometers.

Bak et al plan more experiments. The data so far suggests that though most b. subtilis spores would be killed in the bouncing martian sand grains, a small proportion of spores could perhaps be transported for thousands of kilometers, or more, and remain viable.

The species they tested, b. subtilis, is a reasonable organism to choose for a forwards contamination experiment as it is highly resistant to radiation and oxidizing chemicals. However they only tested one organism, other terrestrial microbes might be more hardy. Spores of native martian life would have evolved to resist these bounces and a higher percentage may survive for longer in Martian conditions. As for the dust grains, some of the microbes could be protected from UV by cracks in the grains, and they may have evolved adaptions to dust transport.

New: Martian life could have spores with extra layers to protect against UV in dust storms – or fruiting bodies or other propagules detached by strong winds protected by outer layers of altruistic social bacteria - and martian life could use strong biomaterials similar to chitin (found in hard parts of insects but also in fungi and lichens) to protect from impact bounces

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This is a speculative section about possible adaptations of native Martian life to transport in dust storms. From the previous sections, especially Billi et al's experiments (Billi et al, 2019a), (Billi et al, 2019b), it seems likely Martian life could propagate in the dust as fragments of biofilms even if it only has similar capabilities to terrestrial life. However, after billions of years of evolution, martian life might well be better adapted than terrestrial life.

Spores are already protected from reactive chemicals by multiple coat and crust layers (Cortesão et al, 2019). This makes them far more resistant to oxidizing agents, bactericidal agents, chlorites, hypochlorites etc than vegetative cells (Sella et al, 2014). Spores are also more UV resistant than vegetative cells. Some terrestrial spores can withstand many hours of UV radiation on Mars, including one strain still viable after 28 hours of simulated direct UV radiation in Mars simulation surface conditions (Galletta et al, 2010). This helps protect them from high levels of UV which they encounter high in Earth's atmosphere and in mountainous and polar regions.

However, after billions of years of adaptation in the harsh conditions on Mars, any Martian spores could have more layers of protection than terrestrial spores and resist UV for even longer than those 28 hours.

Martian life might also develop colonial ways of surviving in dust storms in the larger clusters or aggregates of microbes like the terrestrial analogues described by the MEPAG review (SSB, 2015 :12, section Translocation of terrestrial contamination). Perhaps Martian microbial life might also evolve larger bacterial fruiting bodies similarly to those of the myxobacteria ("slime bacteria"). These have some bacteria that altruistically develop into non reproductive cells to protect the spores inside (Muñoz-Dorado et al, 2016: Fig 1).

Simple multicellular martian life could reproduce by just breaking apart in dust storms (fragmentation). Fungi are able to reproduce from fragments of their filaments (hyphae). Red algae (rhodophyta) also often propagate in the same way. Their propagules are fragments of the parent plant which break off from the main body (thallus) and give rise to a new individual (Cecere et al, 2011). The evolution could begin with fragments broken off in winds due to the impact splashes of the half millmeter sand grains bouncing on the dunes of the previous section and the natural movements of the dunes. Bacteria might create propagules extended above the surface that get torn off by stronger winds in dust storms or dust devils.

Then by the results of the last section, a sufficiently hardy propagule half a mm in diameter could potentially be transported great distances in dust storms by the saltation bounces. It's one way that any native life might adapt to spread more easily in the martian conditions.

A half millimeter diameter propagule, or 500 microns in diameter, typical in size for saltation on Mars (Kok, 2010:fig.3b) can contain many spores. At a maximal packing density of $\frac{\boldsymbol{\pi}}{3\sqrt{2}} \approx 0.74048$, which is the densest packing density possible for congruent spheres (Hales, 1998) (Hales et al, 2017), it can contain $\frac{\boldsymbol{\pi}}{3\sqrt{2}} \times \frac{4}{3}\boldsymbol{\pi} \times 250^3 = \frac{\boldsymbol{\pi}^2 \times \sqrt{2} \times 250^3}{9} =$ 24. 2 million spherical spores or microbes in dormant state at 1 micron diameter.

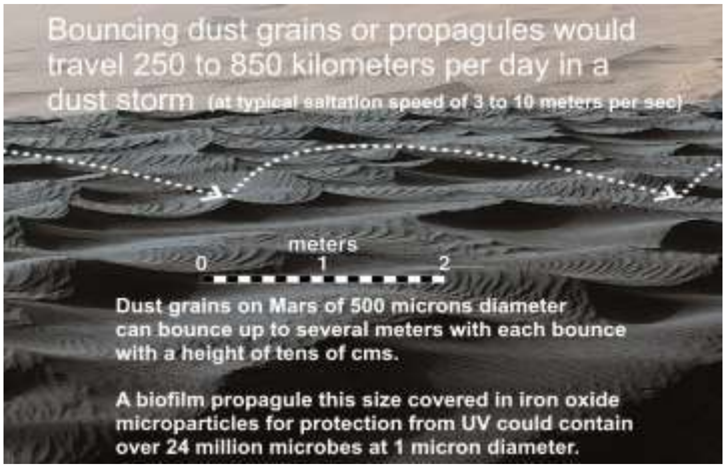

Figure : Bouncing dust grains or propagules would travel 250 to 850 kilometers per day in a dust storm (at typical saltation speed of 3 to 10 meters per sec).

Dust grains on Mars of 500 microns diameter can bounce up to several meters with each bounce with a height of tens of cms. A biofilm propagule this size covered in iron oxide microparticles for protection from UV could contain over 24 million microbes at 1 micron diameter.

Artist's impression of a typical bounce based on figure 2b from (Almeida et al, 2020) superimposed on photograph of the top of a large sand dune taken by Curiosity on December 23, 2015 (NASA, 2016)

As with the biofilm fragments, larger propagules could help martian life to get off to a head start in an environment where a single microbe might not be able to survive.

The fruiting bodies might evolve extra protection against UV, perhaps an agglutinated external cyst of iron oxides to protect themselves from UV held together by secreted organics similarly to the external cysts of foraminifera (Heinz et al, 2005).

Speculating further, Martian life might also perhaps evolve specialized biological materials with much stronger protective layers than iron oxide to protect themselves from both UV and from damage from impacts during the saltation bounces. To take one very speculative example [new]: chitin is an essential component of the cell walls of fungi such as Aspergillus (Brauer et al., 2023) and the fungal component of lichens (Lenardon et al, 2010). It's the same material insects use for their exoskeletons and jaws. Chitin has a Mohs hardness of 7 – 7.5 (Zhang et al, 2020) similar to quartz (King, n.d.). Chitin nanofibers have a Young's modulus of elasticity of more than 150 GPa (Vincent et al, 2004), higher than copper or titanium alloy and not far below wrought iron or steel (Engineering ToolBox, 2003). There might be a strong evolutionary pressure on Martian propagules to evolve chitin materials for protection (or some similarly hard native polymer) perhaps as nanofibers in the biofilm. These might help protect the propagules as they bounce over the Martian surface in the winds.

2015: MEPAG2 review draws attention to the potential for local microenvironments to provide habitats for life that can't be detected in large scale surveys – and illustrative examples of micropores in salts or gypsum, and Curiosity's salty brines

Next section – all sections – previous section
[question]

The 2014 report does consider microenvironments, but the 2015 MEPAG review says it only briefly considered the implications of our lack of knowledge of them. First this is what the 2014 report said (Rummel et al , 2014:904).

> ***Finding 3-10:*** *Determining the continuity/heterogeneity of microscale conditions over time and space is a major challenge to interpreting when and where Special Regions occur on Mars.*

It then gives a list of seven naturally occurring microenvironments on Mars:

- **Vapor-phase water available** Vapor or aerosols in planet's atmosphere; within soil cavities, porous rocks, etc.; within or beneath spacecraft or spacecraft debris
- **Ice-related** Liquid or vapor-phase water coming off frost, solid ice, regolith or subsurface ice crystals, glaciers
- **Brine-related** Liquid water in deliquescing salts, in channels within ice, on the surface of ice, within salt crystals within halite or other types of ''rock salt''
- **Aqueous films on rock or soil grains** Liquid water on regolith particles of their components such as clay minerals, on surface of ice, on and within rocks, on surfaces of spacecraft
- **Groundwater and thermal springs** (macroenvironments) Liquid water
- **Places receiving periodic condensation or dew** Liquid water on regolith particles of their components such as clay minerals, on surface of ice, on and within rocks, on surfaces of spacecraft
- **Water in minerals** Liquid water bound to minerals

The 2015 MEPAG review says that though SR-SAG2 considered these microenvironments it only briefly considered the implications of our lack of knowledge of them (SSB, 2015 :11 - 12).

> *Physical and chemical conditions in microenvironments can be substantially different from those of larger scales. Although the SR-SAG2 report considered the microenvironment (Finding 3-10), the implications of the lack of knowledge about microscale conditions was only briefly considered.*
> *…*
> *Craters, and even microenvironments underneath and on the underside of rocks, could potentially provide favorable conditions for the establishment of life on Mars, potentially*

leading to the recognition of Special Regions where landscape-scale temperature and humidity conditions would not enable it.

…

The review committee agrees with Finding 3-10 of the SR-SAG2 report but stresses the significance of the microenvironment and the role it might play on the definition of a Special Region in areas that (macroscopically speaking) would not be considered as such.

Also (SSB, 2015 :28).

Identification of a Special Region needs a multiscale approach ... and thus, as far as missions to Mars are concerned, conservatism demands that each landing ellipse be scrutinized on a case-by-case basis. Maps, which come necessarily at a fixed scale, can only provide information at that scale and are, therefore, generalizations

Several of the seven microenvironments mentioned by SR-SAG2 are relevant to Jezero crater. We have already looked at an illustrative example of one of them.

> **"Brine-related** Liquid water in deliquescing salts, in channels within ice, on the surface of ice, within salt crystals within halite or other types of 'rock salt'" (Rummel et al , 2014:904).

The Curiosity brines are an example here as we discussed above (Martin-Torres et al, 2015), which could be made more habitable using biofilms biofilms (Pires, 2015).

> **"Vapor-phase water available** Vapor or aerosols in planet's atmosphere; within soil cavities, porous rocks, etc.; within or beneath spacecraft or spacecraft debris" (Rummel et al , 2014:904).

Microbes can inhabit micropores in salt deposits in deserts when the air is otherwise too dry through spontaneous condensation of water vapour in the micropores (Vítek et al, 2010) (Wierzchos, 2012). Cassie Conley (Conley, 2016) and, separately, Paul Davies (Davies, 2014) have suggested these as potential habitats on present day Mars. Microbes can use micropores in gypsum too, at an external humidity of only 60%, imbibing water at higher humidity, gradually becoming more dessicated below 60% humidity. (Wierzchos et al, 2011: figure 1).

Jezero crater doesn't have the large bright salt deposits of Mount Sharp (Lerner, 2019). The orbital measurements suggest that it has salts almost everywhere, along with other minerals that form through interaction with water, but only at levels of at most a few percent, suggesting water flowed there only briefly in the past (Wiens et al., 2022).

However these salts are likely to be patchy rather than uniformly mixed. Perseverance has found micropatches of gypsum (calcium sulfate) already along with perchlorates. However unlike the perchlorates found in Gale crater, the white features here fill gaps in the rock, likely as the result of processes involving water (Scheller et al., 2022:2).

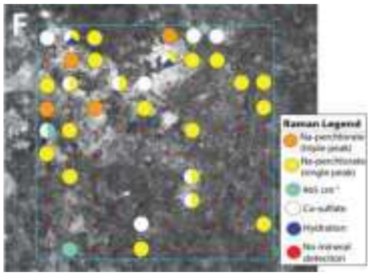

Figure 8: (Scheller et al., 2022: Fig. 1)

This is the region sampled, the Raman target is the square shown in white, less than 1 cm across, so these deposits are millimeter scale.

Figure 9: (Scheller et al., 2022: Fig. 1)

Even small deposits of gypsum or other salts could have micropores which could be a potential microhabitat for martian microbes.

2021: Potential for melting frost to form a “dew” of microns thick layers of fresh liquid water trapped by a temperature inversion - it could persist for a few hours even in Jezero crater – as an example to show the potential for future surprise microhabitats

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This idea fits another of the examples of potential local microhabitats mentioned in S-SRAG2, liquid water from melting frost (Rummel et al , 2014:904):

> **“Ice-related** Liquid or vapor-phase water coming off frost, solid ice, regolith or subsurface ice crystals, glaciers”

Fresh water is stable against freezing and boiling over 29% of the surface of Mars, but it is not stable against evaporation because the partial pressure of water vapour in the Martian atmosphere is two orders of magnitude too low (Martinez et al., 2013:2). However fresh water could form temporarily in special conditions, if there is some buffering of the water vapour. This could happen after rapid melting of ice, if it melts faster than the evaporation rate. This may be possible, on: (Martinez et al., 2013:2.1).

"slopes facing the sun, under clear sky and calm wind conditions, at locations with low surface albedo and low soil conductivity"

However there is another way that liquid water could form as the frost melts. Mars gets a temperature inversion of warm air over cold air, which prevents convection and can trap the water vapor close to the surface as the frost melts.

The Viking 2 lander (NASA, 1997) and Phoenix lander (NASA, 2008) both imaged daytime frosts on the surface. The other rovers haven't photographed them but there are estimates that a few tens of microns of frost could have formed in Gale crater at night (Martínez et al., 2013). That's enough to be useful water for a microbe as it melts.

There is possible direct detection of frosts in Gale crater a few microns thick (Gough et al., 2020). Perseverance is currently attempting to detect frost in Jezero crater using Laser Induced Breakdown Spectroscopy (LIBS) and Raman spectroscopy of nearby rocks which they have named Snowy Mountain and Red Mountain, as well as by using the microphone to detect the sound of the LIBS laser "zap" (NASA, n.d.).

If frost exists on the Snowy Mountain target, we should detect hydrogen in the LIBS spectra and O-H bonds in the Raman spectra in greater quantities than in the Red Mountain spectra. We're also listening for a soft acoustic signal in the first LIBS shot of Snowy Mountain which could indicate a frost layer as thin as ~10 microns.

This idea goes back to Gilbert Levin and his son Ron Levin, who suggested a cool humid layer could be trapped near the surface as dawn approaches, in a temperature inversion, overlain by a layer of warm air. This might lead to thin films of water that form briefly in the early morning then evaporate. Chris McKay, agrees that this process could form a layer of liquid though it may not last long (Abe, 2001).

The frost would form at night, and melt in daytime, but the temperature inversion would trap the moisture long enough for it to survive evaporation for up to several hours.

Experimental work suggests this is a plausible scenario. This experiment simulated conditions in Gale crater, with small droplets of water forming and then evaporating. (Ramachandran et al, 2021)

Our experiments show how a pool of water is formed and remains stable for about 3.5 to 4.5 h while evaporating and releasing water to the dry atmosphere.

These experiments simulate evaporation under wind-free conditions. This scenario is not unrealistic as, according to REMS observations, the night-time to sunrise winds may be very mild with speeds under 2 m/s

There is another way Mars could surprise us, a scenario with present day fresh liquid water at 0 °C, this time as a distant source for microbes transported in the dust storms from polar regions. Most of the meltwater in Antarctica actually forms due to the solid state greenhouse effect. Ice and snow is optically transparent and traps heat, so it tends to melt a layer of liquid water about half a meter below the surface where the balance of the amount of light received and the thermal insulation is optimal.

The same process on Mars would melt liquid water at a depth of about 5 cms even with surface temperatures on Mars as low as 180 °K (-93 °C). If Mars has snow or ice with similar optical and thermal properties to the Antarctic snow and ice, and there is no particular reason to suppose it wouldn't be, we should find melt water 5 cms below the surface of the ice, on sun facing slopes in polar regions in summer. See below:

- 2009, 2014: Possible future surprise discovery of large quantities of fresh water on Mars: ice lets light through and traps heat, which melts ice half a meter below the surface in Antarctica -– if Martian ice is similar, its polar regions should have meltwater in summer, ~5 cms below the surface, even with surface temperatures below -90 °C – Mars may also have miniature melt ponds around sun warmed dust grains

2015 MEPAG review: microbes can use biofilms to create conditions favourable to them in otherwise uninhabitable microniches – this need to build up a biofilm first reduces the risk for forward contamination for spacecraft with low bioloads – however such niches could be inhabited by Martian life that already lives in biofilms adapted for millions of years

Next section – all sections – previous section
[question]

The 2015 MEPAG review also discusses how microbes in biofilms modify microhabitats by surrounding themselves with "extrapolymeric substances" – proteins, polysaccharides, lipids, DNA and other molecules. These can make microenvironments far more habitable for microbes and help them cope with environmental stressors (SSB, 2015 :11).

SR-SAG2 doesn't discuss biofilms. It has only one mention of the word (Rummel et al , 2014 : 944):

> *The synergy of multiple factors that enable enhanced microbial survival and growth (i.e., storage mechanisms,* ***biofilms****, and the structure of microbial communities), and mechanisms that may allow for temporal separation in microbial resource use.*

The 2015 MEPAG review starts the biofilm section saying: (SSB, 2015 :11).

The SR-SAG2 report identified the ability of microorganisms to withstand multiple stressors as an important area of research..

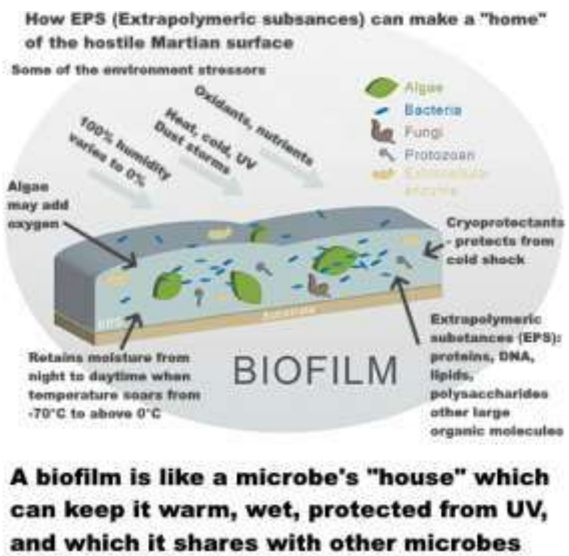

Figure 10: Graphic adapted from figure 2 of (Sabater et al., 2016)

Microbes in biofilms can use those extrapolymetric substances (EPS) to inhabit ecological niches that would otherwise be uninhabitable (SSB, 2015 :11)

The majority of known microbial communities on Earth are able to produce EPS, and the protection provided by this matrix enlarges their physical and chemical limits for metabolic processes and replication. EPS also enhances their tolerance to simultaneously occurring multiple stressors and enables the occupation of otherwise uninhabitable ecological niches in the microscale and macroscale.

This helps with planetary protection in the forward direction, as a spacecraft from Earth may need to carry enough terrestrial life with them to Mars to establish a biofilm (SSB, 2015 :11)

Given the wide distribution and advantages that communities of organisms have when they live as biofilms enmeshed in copious amounts of EPS, it is likely that any microbial stowaways that could survive the trip to Mars would need to develop biofilms to be able to establish themselves in clement microenvironments in Special Regions so that they could grow and replicate.

So when asking if spacecraft could be sources of forward contamination on Mars a central question is whether the spacecraft has enough terrestrial life on it to be able to establish a biofilm on Mars. It's not about the species only but about how many microbes there are to establish a "beachhead" on the martian surface for terrestrial life to start growing there.

However this is a study of forwards contamination and any native Martian life may have already established this beachhead millions of years ago.

NEW: Life adjusted to Mars has had millions of years to set up biofilms – and slowly colonize microhabitats we may not yet know exist

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In such very cold conditions, the Martian surface life may colonize very slowly. There is evidence species of lichens and mosses are still in the process of recolonizing Antarctica since the last ice age, with species diversity dependent on distance from the nearest geothermally active sites that provided refuges during the ice ages (Fraser et al, 2014).

We need to consider microhabitats that might take many millennia to slowly colonize in the very cold martian conditions as well as that idea of present day biofilms spread via fragments that originated from biofilms that first formed in slightly more habitable conditions a few million years ago (Mosca et al, 2019).

SR-SAG2 had a 500 year time-frame for forward contamination. They didn't consider processes that could lead to colonization over millennia. (Rummel et al , 2014:894) *(Sun et al, 1999)*..

> *The actual low temperature limits of terrestrial organisms are currently unknown, primarily due to technological constraints of detecting extremely low rates of metabolism and cell division. But even if the actual low temperature limits of terrestrial organisms are lower than the currently known empirically deetermined limits, the actual limits may not be relevant to defining Special Regions for the given 500-year time frame because cell division and metabolism would be so slow.*
>
> *For example, cryptoendolithic microbial communities of the Antarctic Dry Valleys (where temperatures rarely exceed 0°C) successfully invade and colonize sandstones over 1000 to 10,000 years (Sun and Friedmann, 1999). Therefore, we examined the currently known empirically determined limits of cell division and metabolism at low temperatures and did not consider theoretical limits or extrapolations based on current knowledge [edited 10^3 to 10^4 to 1000 to 10,000]*

Martian life has had millions of years to colonize any potential microhabitats and billions of years of evolution to develop species and biofilms adapted to the Martian conditions.

We can't know that Jezero crater is uninhabitable everywhere for martian life without detailed study looking for:

- Biofilms that might modify conditions in Jezero crater to make it habitable to martian life, such as Nilton Renno's biofilms (Pires, 2015)
- Fragments of biofilms or native propagules in the dust

- Life in the micropores in salt deposits suggested by (Conley, 2016) and, separately, Paul Davies (Davies, 2014)
- Life in other potential local habitats and microhabitats,
- Searching with an open mind with versatile instruments so that we can find microhabitats in situ that could be new to science, and potentially inhabited by a novel exobiology.

Mars may have many surprises for us still, as we saw with the melting frost example.

- 2021: Potential for melting frost to form a “dew” of microns thick layers of fresh liquid water even in Jezero crater – as an example to show the potential for future surprise microhabitats

Whether or not we find that particular microhabitat, we could get many future surprises once we send instruments to Mars better able to detect microhabitats like this, and search them for life.

A Martian biofilm might consist of many species that evolved together to inhabit local conditions over millions of years, similarly to the Atacama Desert grit crust [2020]

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[2020] The Atacama grit crust got its name because it grows around and within small grit sized pebbles about 6 mm in diameter, turning them black over hundreds of square kilometers of the desert (Jung et al, 2022). Biocrusts are common in deserts but this particular biocrust has interesting special adaptations relevant to Mars. It is tolerant to high UV, and adapted to rapid changes of temperature and humidity. It can photosynthesize with the lowest amount of water known for any such community worldwide (Jung et al, 2022). Also, though frequent wetting and drying is normally lethal for similar communities, it causes no problem for the grit crust which can respond rapidly to fogs that blow in over the desert (Jung et al, 2020).

It is made up of a mix of blue-green algae (similar to chroococcidiopsis), green algae such as chlorella, black rock inhabiting fungi, lichens such as Pleopsidium chlorophanum and other microbes. The climate in the Atacama desert has been stable for at least 150 million years giving time for the Atacama gritcrust to evolve to adapt to it. (Jung et al, 2022).

The Atacama grit crust has been considered as a possible pioneer biofilm for preparing extraterrestrial soils for human colonization (Jung et al, 2022). Perhaps the likely evolution of the gritcrust over 150 million years may also be a analogue for a native Martian biofilm if such exists.

An analogous biofilm on Mars would have had billions of years to adapt to the current Martian conditions as the planet slowly became less habitable, with the species that are able to work together for greater resilience forming the most successful biofilm colonizers.

That would be especially so for Mosca's idea of a biofilm that is no longer able to propagate as individual microbes colonizing one by one, but can only establish a foothold as biofilm fragments (Mosca et al, 2019). The most successful biofilm fragments would include organisms that cooperate well with each other for mutual support.

The lichen Pleopsidium chlorophanum, one of the lichens found in the grit crust, is one of the top candidates for a terrestrial lichen that might be able to survive on Mars. See:

- 2014: Example of an alpine lichen Pleopsidium chlorophanum found in places like California and the Alps that also grows in Mars analogue conditions in Antarctica and can survive and even grow in Mars simulation conditions – this shows even higher life from Mars could be adapted to live on Earth

In addition to the lichen Pleopsidium Chloraphpanum, there are many black rock inhabiting fungi that may be able to survive on Mars, and some blue-green algae, especially chroococcidiopsis, so a Martian biofilm potentially might also have close analogues to the components of the Atacama gritcrust.

- Several candidate terrestrial microbes and even higher organisms such as lichens may be able to survive on Mars, with promising results in Mars simulation chambers, suggesting a possibility that their Mars analogues may be able to live on Earth

Martian life could be more capable of coping with Martian conditions than terrestrial life – e.g. survive better in dust storms or cope better with cold temperatures and temperature changes – and ways a martian biofilm could retain water in ultracold night time brines through to the midday warmth – fine hairs that swell when hydrated, pores that close in daytime like cactuses – chemicals that speed up metabolism, slow generation times and novel biochemistry

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The MEPAG and MEPAG review studied forwards contamination, so didn't look at potentially more capable martian life. Any life on Mars has had billions of years to evolve to survive transfer better in dust storms or to adapt to colder temperatures or the many sudden day / night changes in temperature and humidity.

Martian life might also use novel biochemistry (Schulze-Makuch et al, 2010a) (Houtkooper et al, 2006), or use the abundant martian "chaotropic agents" such as the perchlorates, which speed up a cell's chemical processes at low temperatures and can reduce the lowest temperatures for cell division for many microbial species (Rummel et al , 2014:897).

The biofilms could generate hydrogel-like EPS's to retain water but this only works to prevent slow desiccation (Dabravolski et al., 2022).

Experiments in 2019 (Huwe et al., 2019) suggested that Mars could also have microscopic moss like plants, which are interesting because of their ability to absorb water rapidly, in seconds, and retain it for a long time (Tao et al., 2012). Huwe et al. tested a moss Grimmia sessitana collected in the alps in a terrestrial Mars simulation chamber (Huwe et al., 2019) and also tested in BIOMEX, the Mars simulation experiment that was attached to the outside of the ISS (De Vera et al., 2019) though I can't find any paper on the results for Grimmia from the BIOMEX experiment.

In the terrestrial Mars simulation experiment (Huwe et al., 2019), its vitality wasn't affected by extreme temperature or vacuum though direct exposure to simulated Mars surface sunlight did reduce its viability by 6% each day for 31 days (Huwe et al., 2019),. They tested rapid day night cycles from -25°C to 60°C which had no effect. They didn't need to cycle it all the way down to below -70°C as it has already been shown to be able to survive immersion in liquid helium at only 0.65°C unharmed (Becquerel, 1951) (Lenne et al, 2010). They found that vacuum and the Mars like atmosphere had no effect on it. It can remain viable when desiccated for a long time. The samples they used had been stored desiccated for 3 years and an Antarctic species was revived after 400 years in ice. (Huwe et al., 2019:228).

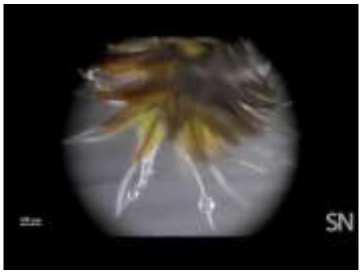
Huwe et al. suggest another moss for future study, Ceratodon purpureus, as it might have higher UV resistance. It's also called "common roof moss" or "fire moss", a widely distributed terrestrial moss with strains adapted to habitats as diverse as cold dry deserts from the Antarctic peninsula, and hot dry deserts in Australia. It produces red anthocyanin pigments that screen it from UV and act as an antioxidant, and it has mechanisms that let it dissipate overdosed light for photosynthesis as harmless heat energy by a process called nonphotochemical quenching (Huwe et al., 2019:228). It has diversified into many clades with one of the clades in Antarctica isolated for millions of years (Biersma et al, 2020).

Desert mosses can absorb water very quickly. In a study of the desert moss Syntrichia caninervis it could hydrate in seconds (Tao et al., 2012). It retained water using specialized structures including leaf hair points which are long thin hairs at the end of the leaves of the mosses which take up water and expand, which likely reduces evaporation (Tao et al., 2012).

The leaf hairs

1. absorb water through capilliary action which would otherwise evaporate, which slows down evaporation
2. make the mosses more reflective, reflecting away sunlight
3. expand as they hydrate and so form a 3D mesh which blocks evaporation

This reduces evaporation from the hydrated moss for as long as it has high water content. Then martian organisms might have evolved to take up humidity directly from the air. Some lichens do this. They might even be able to make their own micropores similar to the gypsum and salt micropores. The desert moss Syntrichia caninervis which is found in desert biocrusts throughout the world does something like this, with an "upside-down" water collection system that collects water droplets which condense onto microgrooves within its leaf hair points and it rapidly funnels those down to the plant below (Pan et al., 2016).



Video: Demystifying desert moss hydration | Science News

These seem likely capabilities for native Martian life to explore in billions of years of evolution. Perhaps also a native evolved higher lifeform on Mars could evolve structures that mimic the gypsum micropores, collecting liquid water in the pores, which it then absorbs. Adaptations like this to make the brines habitable for moss-like plants at a microscale would make the biofilm more habitable to other life too. In the hyperarid Martain conditions any moss-like plants would be likely to be microscopic and to hide from direct sunlight beneath dirt, so would be hard to spot without dedicated searches looking for biosignatures in the dirt.

[NEW] This is speculative. Could a biofilm also use pores (stomata) to let in humidity at night and retain it in daytime, like a cactus? Terrestrial lichens don't have these pores, but terrestrial mosses do. Some mosses can open and close their pores like plants do (Chater et al, 2011). Each pore is surrounded by two "guard cells" which open or close in response to various signals (Brodribb et al., 2020).

Also some mosses can open and close pores (stomata) like plants (Chater et al, 2011). . I couldn't find a paper about terrestrial biocrust doing this, but could a biofilm be covered by a martian organism that evolved pores that close in daylight like the stomata of cactuses to hold in the water?

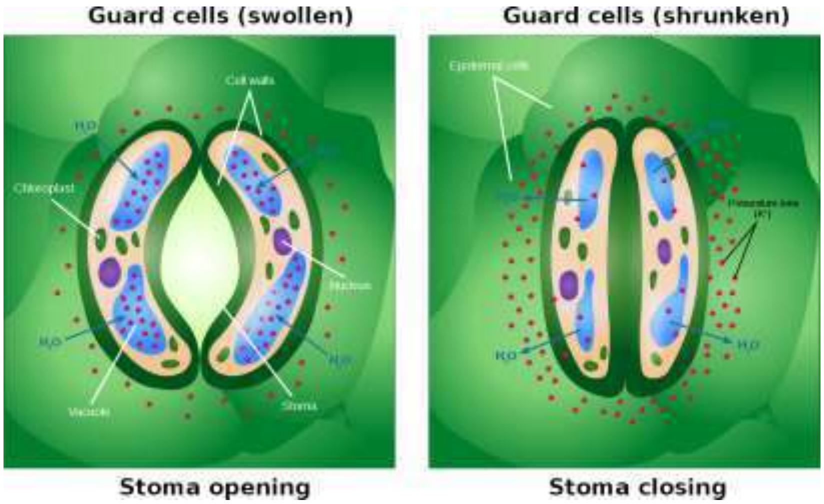


Figure 11: (Zifa, 2016)

Although terrestrial desert mosses don't seem to use their stomata to retain water, perhaps a moss-like martian organism could cover the biofilm, with pores that close to retain the water as the biofilm warms and starts to dry out. Given the very low temperatures, perhaps the pores could even start to close passively in response to desiccation at temperatures too low for metabolism. Or open at night, close in daytime.

Then we need to allow for the unexpected. We've had many surprises in extraterrestrial geophysics such as the Martian geysers (Kieffer et al., 2006) and the Phoenix leg droplets (Renno et al, 2009), which have no equivalents on Earth. We could have astrobiological surprises too, adaptations life evolved in martian conditions so unique that life on Earth has never explored them.

Many ways native martian life could make brines more habitable

Next section – all sections – previous section

The previous sections give many ways biofilms could make brines more habitable including the ones Curiosity found

- hydrogel-like substances (EPS) (SSB, 2015 :11), to reduce dehydration, though these are slow acting in terrestrial biofilms (Dabravolski et al., 2022).
- hydrate rapidly like mosses and then retain the water and release it slowly in daytime.
- leaf hair points that expand rapidly when hydrated and reduce evaporation, and perhaps stomata like pores that close to retain water through to daytime (previous section)
- perchlorates and similar self generated chemicals to speed up their metabolism at lower temperatures (chaotropic agents) (Rummel et al , 2014:897),
- very long generation times with a doubling time of millennia or more (Rummel et al , 2014:897). As seems to happen with some terrestrial cold loving microbes.

- novel biochemistry, perhaps using hydrogen peroxide or perchlorates (Schulze-Makuch et al, 2010a).

2010: Martian life could inhabit caves that vent to the surface – many types of cave can only be detected by in situ observation unlike the easier to detect lava tube skylights

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Caves are important for forward contamination, but even more so for backwards contamination because there is potential for contamination that's almost all one way, outwards from the cave. A cave with a small entrance might be at low risk for contamination by microbes spread in the dust from a rover on Mars, but if the cave environment is productive for life, and there are ways for life in the cave to reach the surface, it might be a major source of microbes that are spread in the dust storms. For instance Boston suggested the methane plumes could come from subsurface caves (Boston et al, 2006). If so, perhaps subsurface life could also be lofted onto the Martian surface from the caves.

Although Penelope Boston suggested searching for life in caves on Mars over a decade ago, there's been greatly increased interest in Martian caves over the last decade. There is no occurrence of the search term in a search of the NRC report in 2009. (SSB, 2009: cave). By the 2020 conference "Mars extant life: what's next?" caves were one of the four top priorities for a search for extant (i.e. current) life on Mars kix.wcfa2l3gtnu (Carrier et al, 2020:Abstract):

> *A powerful theme that permeated the conference is that the key to the search for martian extant life lies in identifying and exploring refugia ("oases"), where conditions are either permanently or episodically significantly more hospitable than average.*
>
> *Based on our existing knowledge of Mars, conference participants highlighted four potential martian refugium (not listed in priority order): Caves, Deep Subsurface, Ices, and Salts.*

A new review on back contamination risks for a Mars sample return updating (SSB, 2009) would surely have an extensive section discussing the potential for back contamination from caves similarly to the sections for forward contamination in SR-SAG2 which has over 900 words on it (Rummel et al , 2014:920-21) and the MEPAG review which has over 700 words on it (SSB, 2015 : 24).

SR-SAG2 and the MEPAG review both discuss caves and agree that there is potential for forward contamination of them. They are treated as Uncertain Regions and treated as Special Regions until proven otherwise (SSB, 2015 : 24)

> SR-SAG2 Finding 4-11: On Earth, special geomorphic regions such as caves can provide radically different environments from the immediately overlying surface

> environments providing enhanced levels of environmental protection for potential contaminating organisms. The extent of such geomorphic regions on Mars and their enhancement (if any) of habitability are currently unknown.

MEPAG Review looks at the potential for life to survive in such caves writing (SSB, 2009 : 24):

> Although their number and sizes are largely unknown, caves and other subsurface cavities on Mars would represent environments with ambient conditions (e.g., temperature, humidity, exposure to radiation) that are very different from those at the surface, and most probably, those conditions are likely to be favorable for microbial colonization. Consideration of caves and subsurface cavities is paramount for two reasons. First, they provide a protected environment (e.g., from extremely low temperatures and radiation). Second, they can provide a means by which terrestrial contamination can access martian subsurface environments.
>
> …
>
> In conclusion, there could be a number of possible primary sources of the necessary ingredients for life inside caves and subsurface cavities on Mars, and therefore, they are best classified as Uncertain Regions and treated as Special Regions until proven otherwise.

SR-SAG2 lists the possibilities as:

- Drained lava tubes
- Caves formed by tension fractures
- Caves in the salts left after evaporation of salty water – e.g. in gypsum

The MEPAG review adds:

- Caves formed by water running underground (like many terrestrial caves)
- Caves formed by mud volcanoes or material expelled by hydrothermal processes

Penelope Boston adds glacier caves, wind scoured caves, and caves formed from water by moving fine particles instead of dissolving the rock, and sublimational caves because of ice and dry ice subliming directly into the atmosphere. Many of these could be connected to the surface. These are the main types of caves she looks at (Boston, 2010):

1. **Solutional caves** (e.g. on Earth, caves in limestone and other materials that can be dissolved, either through acid, or water)
2. **Melt caves** (e.g. lava tubes and glacier caves)
3. **Fracture caves** (e.g. due to faulting)
4. **Erosional cave**s (e.g. wind scoured caves, and coastal caves eroded by the sea)
5. **Suffosional caves** - a rare type of cave on the Earth, where fine particles are moved by water, leaving the larger particles behind - so the rock does not dissolve, just the fine particles are removed.
6. **Mars could have sublimational caves** caused by dry ice and ordinary ice subliming directly into the atmosphere.

She points out a few processes that may be unique to Mars, including that last category of sublimational caves. Amongst many other ideas she suggests:

1. **For the solutional caves**, the abundance of sulfur on Mars may make sulfuric acid caves more common than they are on Mars. There's also the possibility of liquid CO_2 (which forms under pressure, at depth, e.g. in a cliff wall) forming caves.
2. **For the melt caves**, the lava tubes on Mars are far larger than lava tubes we find on Earth.

She talks in more depth about the possibility of sulfuric acid caves on Mars and suggests the methane plumes found by Curiosity could come from subsurface caves in (Boston et al, 2006)

The solutional cave and rock fracture caves are particularly challenging to detect from orbit. (Boston et al, 2006). For backwards contamination what matters most is the potential for nearby undetected caves that might be able to spread Martian microbes to the samples in dust storms or dust devils.

On Earth many caves are not easy to find as with the example of the Lascaux cave paintings. Ravidat's dog Robot got entangled in a toppled Juniper tree and he discovered the hole that lead to the cave as a result of going to his dog's rescue. The toppled Juniper revealed the hole (Eshleman et al, 2008).

A concealed cave on Mars might be revealed as a result of a slump of sand. The cave entrances also would be hard to detect remotely. Many caves on Earth can only be seen if you walk right up to them.

This shows a sand slump detected by Curiosity from the changes between the image on the left and the image on the right:

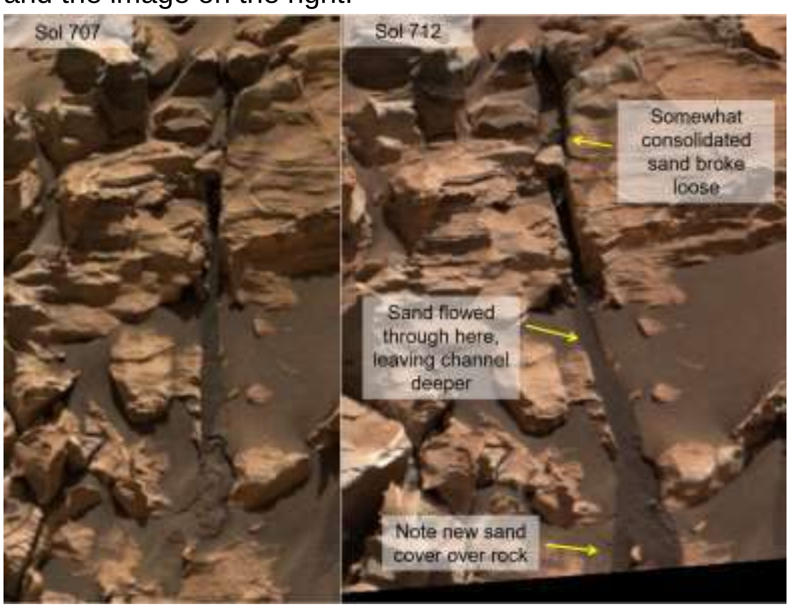

Figure 12: Slide 15 from (Vasaveda, 2015)

A slump like that could reveal an entrance to a previously unknown cave. In addition, a large subsurface cave with only a small entrance to the surface could exist in complex terrain such as shown in that photo and often found in Jezero crater or Gale crater. It could be undetected not only from orbit but also undetected by the rover or even marscopters.

2016: NASA discovered potential for current habitats for terrestrial life in Gale crater AFTER Curiosity's landing

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[question]

We have an example already of how our knowledge of a landing site can change after a rover lands. Like Perseverance, Curiosity is not sufficiently sterilized to visit regions where terrestrial life could spread. NASA thought Gale Crater had no risk for forward contamination. But then they discovered potential habitats for terrestrial life in Gale Crater after Curiosity's landing (JPL, 2016).

These were possible RSLs, those features that grow down slopes in spring, widen in summer and fade in the autumn (McEwen, 2011). As we mentioned the latest research continues to favour the dry dominated mechanism, i.e. that they aren't habitable.

- Mars analogue wet streaks in the McMurdo dry valleys fade over multiple years (Toner et al, 2022).
- The RSLs on Mars became more active after the 2018 global dust storm and though this is also consistent with a wet mechanism supplemented by dust, the observations seem more consistent with a dust mechanism (McEwen et al, 2021).
- The apparent connection with hydrated salts now seems an artefact of the data processing (McEwen et al, 2021)..
- The RSLs are found in locations not consistent with groundwater discharge such as the peaks of mountains (McEwen et al, 2021)..
- Frosts and deliquescing salts can't supply enough water to be the main mechanism (McEwen et al, 2021)..
- They may be caused by dust and bouncing sand grains and triggered by the dust devils, most of which don't produce tracks detectable from orbit (McEwen et al, 2021).
- A close study of RSLs in three representative craters can't confirm the dry mechanism but the authors favour it, because they are not correlated with the times of year for brines for the wet mechanism and two of the sites may not have enough humidity to form brines (Stillman et al., 2021).

However, we don't have the surface resolution yet to be able to investigate them in detail and we can't directly test for humidity near the surface, as the effects on the atmosphere would be too small to detect with current capabilities (Kurokawa et al., 2022).

Although the features close to the rover were ambiguous and not definitely RSLs, mission planners were concerned that Curiosity was not sufficiently sterilized to approach them because of the risk of forward contamination by terrestrial life, in case terrestrial life might be able to inhabit them. After discussion they made a tentative decision that it could approach within a couple of kilometers to image them but not study close up (Witze, 2016).

Of numerous candidates, only two were considered to resemble RSLs sufficiently for concern, the sites 12 and 13 circled in orange in this slide (Dundas et al., 2015) (Vasaveda, 2015).

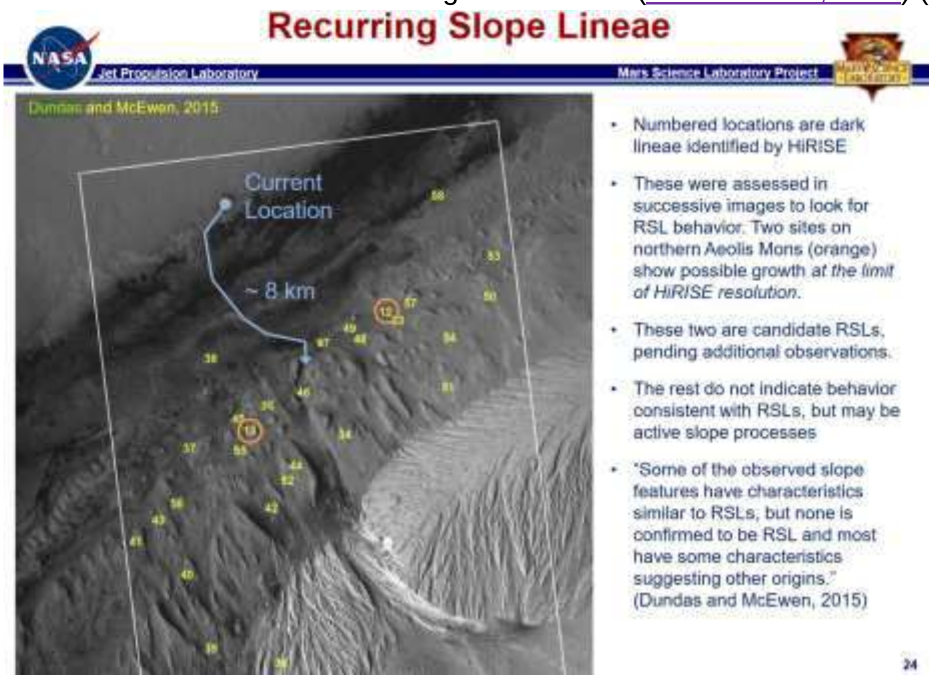

Figure 13: Slide 24 from (Vasaveda, 2015)

Curiosity is currently exploring the region of the possible RSLs but hasn't approached either of those candidates (NASA, n.d.).

Draft EIS says "Existing credible evidence suggests that conditions on Mars have not been amenable to supporting life as we know it for millions of years" – their main cite says "exploration of Mars … will establish whether localised habitable regions currently exist" – another conclusion based on a citing error

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[question]

Another central argument in NASA's draft EIS is that Mars is lifeless anyway. The draft EIS says (NASA, 2022: 1-6):

> Existing credible evidence suggests that conditions on Mars have not been amenable to supporting life as we know it for millions of years (… National Research Council 2022).

Yet their most recent source for this sentence is about searches for currently habitable environments on Mars! (Smith et al, 2022: 393)

> *Section title: "Are There Chemical, Morphological and / or Physiologic / Metabolic or Other Biosignatures in* ***Currently Habitable Environments*** *in the Solar System*
>
> *The exploration of … Mars (Curiosity, Perseverance) will help establish whether localised habitable regions* ***currently exist*** *within these seemingly uninhabitable worlds.*
>
> *[Emphasis on "currently" mine]*
>
> *(cited by NASA's EIS as National Research Council 2022)*

Once more, as for the meteorite argument, NASA got to this conclusion through a citing error.

It's a surprising error given NASA itself was involved in extensive discussions about whether to divert Curiosity away from potential current habitats for terrestrial life in Gale crater (JPL, 2016) as we saw in the previous section.

Although, as we saw, the RSLs are looking increasingly unlikely to be candidates for habitats themselves, we don't have the surface resolution yet to investigate them in detail. We can't directly test for humidity near the surface, as the effects on the atmosphere would be too small to detect with current capabilities (Kurokawa et al., 2022).

We don't yet know for sure that the RSLs are unhabitable to terrestrial life. If they are not habitable, there are many other potential habitats on Mars for terrestrial life and even more so for possibly better adapted martian life.

The only previous use of this argument I can find in the planetary protection literature is by Zubrin in a non peer reviewed op ed (Zubrin, 2000).

> *The fact of the matter is that life almost certainly does not exist on the Martian surface. There is no liquid water on the surface-the average surface temperature and atmospheric pressure will not allow it. Moreover, the planet is covered with oxidizing dust and bathed in ultraviolet radiation. Both of these features-peroxides and ultraviolet light-are commonly used on Earth as methods of sterilization. If there is life on Mars now, it almost surely must be ensconced in exceptional environments, such as heated hydrothermal reservoirs underground.*

His reasoning here is invalid and would not survive peer review. Even in the period from the 1970s through to the discovery of the Phoenix leg droplets in 2009 all previous planetary protection studies said we have to assume there could be viable life in samples returned from Mars, even when the surface of Mars seemed completely arid.

On Zubrin's two points here about UV and perchlorates:

- UV is a form of light and is blocked by a mm or so of dust, and attenuated in shadows or by translucent materials like ice, gypsum and salt. Some terrestrial microbes are adapted to high levels of UV, for instance because they are adapted to cloudless conditions in terrestrial deserts and especially in Antarctica, where there is less protection from UV by ozone, at high altitudes, in Antarctica and high altitudes in the Andes where there is less protection from UV by the atmosphere. A monitor in the high Andes setting a UV record in 2014 with a UV index of 40 more similar to typical Martian than Terrestrial levels (Gronstal, 2014) (Cabrol et al., 2014)
- Perchlorates are much less corrosive at lower temperatures and are useful on Mars as an oxidiser for martian life, there are terrestrial microbes also that metabolize perchlorates (Rummel et al , 2014). Martian life could use perchlorates to speed up their metabolism at lower temperatures (chaotropic agents) (Rummel et al , 2014:897), and it may even use perchlorates internally in a novel biochemistry (Schulze-Makuch et al, 2010a)

There is no reason to suppose Zubrin influenced the authors of the EIS, but there may be a common background to explain the many striking similarities between his arguments and the arguments in the EIS. I go into that below:

- There are many parallels between the arguments in the draft EIS and Zubrin's op ed – no reason to believe there was any direct influence – but there may be a common background

Potential for more habitable distant regions as sources for viable martian life in the dust in Jezero crater

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Mars may have more habitable brines than the ones Curiosity found, ones that retain more water through to warmer conditions naturally, and even fresh liquid water.

We covered potential habitats formed by evaporating frosts, micropores in salt deposits, and caves in Jezero crater. If we don't find them in Jezero crater, they may be found in more distant regions of Mars and be a source for viable life in the dust in Jezero crater.

- 2015: the MEPAG2 review draws attention to potential for local microenvironments to provide habitats for life that can't be detected in large scale surveys – with example of micropores in salt or gypsum
- 2021: Potential for melting frost to form a "dew" of microns thick layers of fresh liquid water trapped by a temperature inversion - it could persist for a few hours even in Jezero crater – as an example to show the potential for future surprise microhabitats
- 2010: Martian life could inhabit caves that vent to the surface – many types of cave can only be detected by in situ observation unlike the easier to detect lava tube skylights

But there may be many new possibilities for distant habitats, which could also be relevant to Jezero crater as sources of life for spores in the dust. We cover some of them below:

- 2009, 2014: Possible future surprise discovery of large quantities of fresh water on Mars: ice lets light through and traps heat, which melts ice half a meter below the surface in Antarctica -– if Martian ice is similar, its polar regions should have meltwater in summer, ~5 cms below the surface, even with surface temperatures below -90 °C – Mars may also have miniature melt ponds around sun warmed dust grains
- The paradox of abundant spores of heat adapted geobacillus spores in cold places - and potential that present day Mars has similarly abundant heat adapted spores from hydrothermal systems, perhaps produced by the rootless cones, fumaroles, or ice fumaroles – some might have been active in the last few million years – some might even be active today

Also Renno's salt on ice which we look at in:

- Astrobiologists have a range of views on whether current habitats for terrestrial life exist on Mars – sometimes revising their assessments after discoveries suggesting new microhabitats on Mars or new ways that life can grow in extreme conditions – example of brines formed when salt overlays ice and lichens that can grow using humidity alone

Also, any Martian brines will be far more stable in low lying areas with higher atmospheric pressure and high humidity, like the deep Hellas impact basin which is close to equatorial areas. Chevrier et all suggests these may be interesting targets for present day habitability looking at brines just below the surface deep enough for the dirt above them to suppress evaporation but close enough to the surface to be warmed by the sun (Chevrier et al, 2020). The evaporation rate can be less than 1 mm per hour for fresh liquid water in some regions. So brines or liquid fresh water produced by some process in Hellas crater could last for significantly longer (Temel et al., 2021)

Then there's another idea which doesn't seem to have been explored. This is based on the discovery of a newly formed crater which excavated ice boulders from the subsurface in an equatorial region. Perhaps ice boulders thrown up by impact gardening in equatorial regions could sustain life in temporary habitats that last as long as the ice remains on the surface before it evaporates away completely.

- Value of targeting a newly formed crater on Mars as an alternative to drilling meters below the surface – with example of a crater that excavated ice boulders from the Amazonis planitia in the equatorial regions in 2022 – also value of developing a 100% sterile marscopter, rover or complete lander

Astrobiologists have a range of views on whether current habitats for terrestrial life exist on Mars – sometimes revising their

assessments after discoveries suggesting new microhabitats on Mars or new ways that life can grow in extreme conditions – example of brines formed when salt overlays ice and lichens that can grow using humidity alone

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Astrobiologists have a wide range of views, but most of the published statements are on the lines that current habitats suitable for terrestrial life likely do exist on Mars. Many say habitats are more likely underground, in the deep subsurface or in caves, but others say there could be microhabitats for life over much of the surface of Mars, especially associated with salts.

It's a separate question whether any habitats if they exist could be inhabited already by native martian life. There again we have a range of views, all the way to a few who think we may have detected the effects of life already with the Viking labelled release experiment in the 1970s. A significant number of astrobiologists think there is a realistic possibility that Mars has native microbial life today (Carrier et al, 2020:804).

Astrobiologists often revise their assessments about the possibility of habitats on Mars after they make discoveries for new potential habitats or ways that life could survive on Mars.

Rummel and Conley, both former planetary protection officers for NASA, put it like this: (Rummel et al , 2014)

> *"Claims that reducing planetary protection requirements wouldn't be harmful, because Earth life can't grow on Mars, may be reassuring as opinion, but the facts are that we keep discovering life growing in extreme conditions on Earth that resemble conditions on Mars. We also keep discovering conditions on Mars that are more similar—though perhaps only at microbial scales—to inhabited environments on Earth, which is where the concept of Special Regions initially came from."*

[2014] Renno and his team used the Michigan Mars Environmental Chamber (Fischer et al., 2013) to simulate droplets similar to the ones on the Phoenix lander' legs (Renno et al, 2009) and found they formed within a few tens of minutes at -50 °C when salt overlaid ice (Fischer et al., 2014). After they achieved this, Renno said (Renno, 2014):

> *This is a small amount of liquid water. But for a bacteria, that would be a huge swimming pool – a little droplet of water is a huge amount of water for a bacteria. So, a small amount of water is enough for you to be able to create conditions for Mars to be habitable today. And we believe this is possible in the shallow subsurface, and even the surface of the Mars polar region for a few hours per day during the spring.*

These are the droplets they simulated

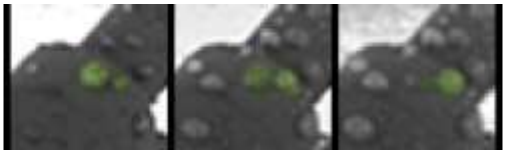

Figure 14: Possible droplets on the legs of the Phoenix lander – they appeared to merge and sometimes fall off. In this sequence of frames, the rightmost of the two droplets – highlighted in green on this black and white image – grows and seems to do so by taking up the water from its companion to the left, which shrinks (Gronstall, 2014)

Renno doesn't go into details but though the temperature of the droplets in their experiment is very cold at -50 °C (Fischer et al., 2014), it is not as cold as the Curiosity brines, and as with the Curiosity brines it's possible life could exploit them with biofilms, and also grow at lower temperatures using the chaotropic agents which speed up some biochemical reactions, etc (Rummel et al , 2014:897).

[2014] De Vera et al. showed that an Antarctic lichen not only survived but grew in Mars simulation conditions similar to Antarctica with no liquid water just water vapor, and after that they wrote: (de Vera et al, 2014):

> *This work strongly supports the interconnected notions*
> *(i) that terrestrial life most likely can adapt physiologically to live on Mars (hence justifying stringent measures to prevent human activities from contaminating/infecting Mars with terrestrial organisms);*
> *(ii) that in searching for extant life on Mars we should focus on "protected putative habitats"; and*
> *(iii) that early-originating(Noachian Period) indigenous Martian life might still survive in such micro-niches despite Mars' cooling and drying during the last 4 billion years*

For the background see below

- 2014: Example of an alpine lichen Pleopsidium chlorophanum found in places like California and the Alps that also grows in Mars analogue conditions in Antarctica and can survive and even grow in Mars simulation conditions – this shows even higher life from Mars could be adapted to live on Earth

[2018] Stamenković with his modelling found new possibilities for brines on Mars to take up substantial amounts of oxygen in cold conditions (Stamenković et al, 2018) and after that he said (Wall, 2018) :

> *There is still so much about the Martian habitability that we do not understand, and it's long overdue to send another mission that tackles the question of subsurface water and potential extant life on Mars, and looks for these signals*

[2020] In the 2020 conference "Mars extant life: what's next?" kix.wcfa2l3gtnu a significant fraction of the participants thought that there is a possibility Mars has extant life (Carrier et al, 2020:Abstract):.

Primary conclusions are as follows: A significant subset of conference attendees concluded that there is a realistic possibility that Mars hosts indigenous microbial life. A powerful theme that permeated the conference is that the key to the search for martian extant life lies in identifying and exploring refugia ("oases"), where conditions are either permanently or episodically significantly more hospitable than average.

Based on our existing knowledge of Mars, conference participants highlighted four potential martian refugium (not listed in priority order): Caves, Deep Subsurface, Ices, and Salts.

Also later in the report (Carrier et al, 2020:804):

A significant subset of the actively publishing Mars science community who are experts in various disciplines of relevance to interpreting habitability and astrobiology concluded that there exists a realistic possibility that Mars hosts indigenous microbial life and that there are testable hypotheses for seeking it.

The report from the conference looks into those four categories. It singles out surface and near surface salts as one of the priority targets for future rover missions because of the potential of finding extant life, including life that uses the sunlight for energy, phototrophs (Carrier et al, 2020:797)

One major advantage of salts as a potentially habitable microenvironment is that they may provide a protected environment for extant life on Mars very close to the surface and may harbor phototrophs …. The salts themselves may serve as a UV shield, while allowing the limited sunlight to be accessible to the microbes.

They single out salts as favorable for an astrobiological sample return (Carrier et al, 2020: *797*).

Near-surface exploration accessibility makes salt deposits a favorable target for exploring for extant life on Mars.

They agree that we'd be able to detect life so long as the chemistry is similar to Earth life and so long as the samples can be free of forward contamination (Carrier et al, 2020: *801*)

Conference attendees agreed that we would be able to detect extant life in a sample return mission using modern biological techniques with the suite of instruments at our disposal on Earth—if the life-forms present were based on Earth biogeochemistry
…
This is based on the assumption that the collected sample has been returned without getting contaminated during collection or transportation.

Cockell: There is a high chance of habitable environments on Mars – if we look at many planets and don't find life we will have to try to find out what happened that was unusual on Earth

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Cockell, who has written extensively about the possibility of uninhabited habitats on Mars (Cockell, 2014) says that there is a high chance that there are habitable environments. But we don't know what the origin of life requires, so it's not possible to say if there is life there, if we look for at many planets and many environments, and don't find life, it will mean life is very rare (Deighton, 2016)

> *Most microbes can grow in different types of extremes and the extremes that we are looking at, things like radiation, perchlorate salts and also sulphate salts (found on Mars), they will grow in that. It's just a question of trying to determine what the limits are and that's the work we're doing at the moment. Anywhere where we've gone to the deep subsurface (on earth) today, where there is liquid water, there is a high chance that environments are habitable,*
>
> *Simply because Mars is a planet of volcanic rock, and when volcanic rock weathers that provides an environment for microbes to grow and reproduce, I think we can already say there is a high chance there are habitable environments.*
>
> 'At the moment we just don't know what the origin of life requires, going from simple chemicals to self-replicating microbe,'
>
> 'If we looked at many planets, many environments and didn't find life, then that would tell us that life is extremely rare and that early spark was an unusual event.
>
> 'And then we'd have to try and find out exactly why it was, and what happened in those early stages of life that was unusual on the earth.'

[2008] [2013] Stabilised swansong biosphere: a way for Mars to stay habitable but only barely habitable for billions of years over a wide range of volcanic emissions scenarios – whenever it gets warm enough for liquid water it rapidly loses much of the CO_2 from its volcanoes into carbonates

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This section combines results from research by O'Malley-Jones into swansong biospheres (O'Malley-James et al, 2013) with Nolan's idea that the Mars atmosphere could stabilize at around the triple point of water, because the water enhances carbonate formation and as Mars gets drier, the amount of carbon dioxide it can remove from the atmosphere falls greatly (Nolan, 2008 : 137). There are similar earlier ideas by Haberly and Kahn based on abiotic photosynthesis (Kahn 1985) (Haberle et al, 2001).

The CO_2 from the original Martian atmosphere would have disappeared long ago if it weren't for emissions from volcanoes. Right now they are geologically inactive, or are active at such a low level we haven't yet detected it. However, they were active in the very recent geological past, with some evidence of explosive volcanism as recently as 53 to 210 thousand years ago (Horvath et al, 2021).

Mars also gets CO_2 from infall of organics from meteorites (Frantseva et al, 2018) (Goetz et al, 2016:247). These organics will react with superoxygenated surface layers, and ionizing radiation will also split the surface organics back to gases such as methane and carbon dioxide and water vapour. Mars gets more CO_2 from comets, directly or as a result of methane and organics in the comets reacting with the surface organics.

However none of these are continuous supplies. Sometimes Mars will get more and sometimes less CO_2. Its habitability will also vary depending on other factors like the tilt of its axis and how circular or elliptical its orbit is.

If that was the complete story, it would seem Mars must surely go through times with less CO_2 than today unless we see it by remarkable coincidence at its least habitable. For instance, starting from the present, if Mars continues to remain volcanically inactive for tens or hundreds of millions of years the CO_2 will gradually be removed until the pressure falls below the triple point of water. Eventually, even in low lying levels like the Hellas basin the Martian surface will be completely inhospitable to life.

This simple argument, that we are unlikely to see Mars at its least habitable, suggests that though we have clear evidence that water flows and forms lakes on Mars sometimes, at other times the Martian surface is likely to become completely inhospitable to life. This mean any surface life there today had to get to the surface from below, or evolve anew over the last few million years.

O'Malley-Jones coined the term "Swansong biosphere" to refer to the phase of a planet's history when it is still habitable but is gradually becoming less habitable. The swansong phase for Mars started billions of years ago as it cooled down (O'Malley-James et al, 2013) (O'Malley-James et al, 2014)..

It would be quite a coincidence for Mars to continue as a barely habitable swansong biosphere at over 3 billion years from when it had a thick enough atmosphere for oceans.

However, there is another way of looking at this. The Martian atmospheric pressure at 0°C is remarkably close to 6.1 millibars, the triple point of water, the balance of temperature and pressure where water can exist as ice, water vapour or liquid water. Low lying regions have pressures high enough for water to be stable briefly for a few hours at some times in the year and the pressures in the mountains are so low that liquid water in any form, even the brines, is likely impossible. In Hellas basin the atmospheric pressure by one model it is 12.4 millibars and water would boil at 10°C (Schulze-Makuch et al, 2010b).

Most of the CO_2 in the martian atmosphere comes from volcanoes. It seems a remarkable coincidence to spot the Martian atmosphere at the exact point in time when it is so close to the triple point, desert like in appearance, yet, with substantial chemical interaction of water with the atmosphere. This may be as remarkable a coincidence as to spot it at its least habitable.

Suppose it is more than a coincidence? That's lead to some authors proposing that there is some process removing CO_2 from the atmosphere. If this process involves liquid water, it could explain how the atmosphere has stayed so close to the triple point of water.

There are two main non biological ways Mars can lose its CO_2, to interplanetary space through carbon dioxide sputtering in the upper atmosphere, and into water by forming carbonates (Hu et al, 2015). Of those two processes, only the rate of loss through carbonate formation could be sensitive to the presence of liquid water. If the atmosphere increases above the triple point for water as a result of volcanic outgassing, this leads to liquid water forming on the surface of Mars, either as open springs, rivers and lakes, or as transitory pockets of water or moisture. This increases the rate of carbonate formation through carbon dioxide dissolving in the water. Then as CO_2 is removed, the atmosphere thins, and with hardly any water left, the rate of carbonate formation drops almost to zero and the atmosphere stabilizes, as long as there is enough CO_2 still emitted to keep the atmosphere from vanishing altogether.

Nolan suggested this process of carbonate formation could bring the Mars atmosphere back to the triple point of water whenever the pressure goes above this point (Nolan, 2008 : 137). Kahn and Haberle et al. suggested abiotic photosynthesis as another process that could have the same effect of keeping the atmosphere close to the triple point of water (Kahn 1985) (Haberle et al, 2001).

Mars does have significant levels of carbonates in the Martian dust. By thermal infrared spectra, the dust contains 2-5% of carbonates by weight, enough to sequester several bars of CO_2 atmosphere (Bandfield et al., 2003) (Niles et al., 2013: section 3.1). This is just accounting for carbonates in the dust. There are more carbonates in surface rocks (Niles et al., 2013: sections 3.2, 4), and yet more detected in the subsurface through study of Martian meteorites (Niles et al., 2013: section 2).

We could examine the chemical composition of carbonates in the dust to help reveal when they were formed. Bandfield et al say we can expect to find a mix of magnesium and calcium carbonates and oxides if they formed in a dry and thin atmosphere like the present day climate on Mars, while we can expect to find calcium carbonates associated with clays (smectite clays) if the weathering occurred in wetter conditions with a thicker CO_2 atmosphere (Bandfield et al., 2003).

We do have evidence carbonates are continuing to form on Mars from the meteorites, with evidence of carbonate formation at 3.9 billion years ago (ALH84001), ~600 million years ago (Nakhla) and < 200 million years ago (EETA 79001) (Niles et al, 2010). This is for rocks from at least three meters below the surface (Head et al, 2002). There has to be a continuing input of CO_2 into the system to form these carbonates.

This proposal is that over a wide range of emissions scenarios for Martian volcanoes, and for most of the geological history of a Mars-like planet, we expect to see its atmosphere stabilized close to the triple point of water. This abiotic feedback, process which slows down carbonate formation when the atmosphere thins and the water dries up, could extend the swansong phase of a Mars-like planet for billions of years and perhaps almost indefinitely.

A smaller planet like Mars with no plate tectonics would be likely to reach its swansong phase early on, as it wouldn't have enough volcanism to maintain a thick atmosphere especially with the losses to carbonates. However, by this hypothesis even such a small planet with no plate tectonics might have enough volcanism to extend its swansong phase for billions of years, because of the reduced levels of sequestration of CO_2 in such dry conditions.

We could detect this in our study of exoplanets once we can detect the atmospheric composition of Mars-like planets in the habitable zone but too small for active plate tectonics. If this hypothesis is correct we should find that such planets frequently have atmospheres close to the triple point of water.

NEW: Swansong Gaia: photosynthetic life could sequester CO_2 into organics to stabilize a swansong biosphere for billions of years over an even wider range of volcanic CO_2 emission scenarios - a thin atmosphere close to the triple point of water might even be a weak biosignature for a Mars-like planet

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In the original preprint this review is based on I suggested photosynthetic life could remove CO_2 from the atmosphere, and help to stabilize it close to the triple point of water over a much wider range of emissions scenarios for the volcanoes. This might even be a weak biosignature, to see a planet like Mars with the atmosphere so close to the triple point of water. This is similar to what Kleidon calls "Anti-Gaia" – a Gaia that makes its planet less habitable (Kleidon, 2002). But it has an extra wrinkle to it, that it has a feedback to stop the process going all the way to an uninhabitable planet. It's a stabilized low biomass end state. That's the reason for coining a new name "Swansong Gaia" instead of "Anti-Gaia".

We might have seen the signature of life based photosynthesis already on Mars, though there are many competing abiotic explanations.

Curiosity observed an excess of oxygen in summer and a deficit of oxygen in winter. The oxygen levels rose in spring and summer to levels 30% above those explainable by chemistry alone and dropped back to normal levels in the fall. Methane similarly rises by more than 60% of expected levels in summer, and also spikes randomly and unpredictably (Shekhtman, 2019). The authors write (Trainer et al, 2019:3021):

Though Mars has the potential to generate significant O_2 release due to abundances of oxidants in/at its surface, the mechanisms by which O_2 could be quickly generated and then quickly destroyed are completely unknown. As with all surprising results, we hope that continued in situ, experimental, and theoretical results may shed light on this intriguing observation.

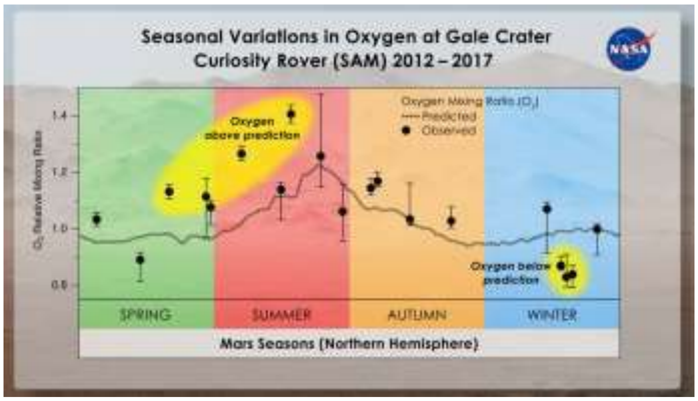

Figure : the regions highlighted in yellow show the excess of oxygen in spring to summer and deficit in winter over the expected seasonal variation as measured by Curiosity.

Credits: Melissa Trainer/Dan Gallagher/NASA Goddard (Shekhtman, 2019)

There is a weak correlation suggesting less oxygen is generated when there is more dust in the air. This is something one would expect from photosynthesis.

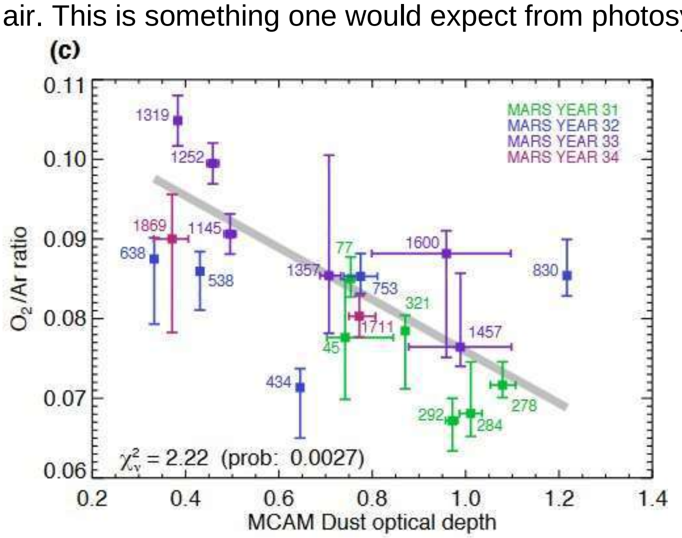

Figure : less oxygen is produced when the atmosphere lets less light through (greater optical depth means less light gets through because the optical depth is the natural logarithm of the ratio of the light that reaches the top of the atmosphere to the amount that gets through to the surface)
From figure 59 of Supporting information for (Trainer et al, 2019)

Optical depth of 0.3 means 74% of the light is let through. Optical depth of 1.1 means 33% of the light is let through.

If photosynthesic life produced the oxygen, it increased it by around 400 ppm or about 10^{20} molecules / cm^2 if the atmospheric oxygen came from the surface directly below the atmosphere (page 3017 of Trainer et al, 2019) which works out at about 0.006 moles of oxygen per square cm or about 26.6 grams of oxygen per square meter (calculated as molecular weight of oxygen * area of meter in square cms * 10 ^ 20 / Avogadro's number).

Our desert crusts on Earth can be productive enough for this. Garcia-Pichel et al recorded 950-2640 g O_2/m^2/year in a desert crust in Utah, using photosynthesis in a surface layer only a few mm thick. Other results for desert crusts are similar (Garcia-Pichel et al, 1996, summarized in Cockel et al, 2009 table 1).

It's more of a challenge to achieve such a fast metabolism in the very cold Martian brine layers.

The figures may be more plausible if a biofilm can retain the water through to the much warmer daytime temperatures, and if life is abundant on Mars.

There's another way such high levels of oxygen production could be more plausible, and this is, if martian life is better at photosynthesis than terrestrial life. In the challenging Martian conditions, it could have evolved a faster form of photosynthesis than the Calvin cycle. Then if it had a variable antenna size to cope with dust storm conditions, this would let it use light more efficiently at all light levels. It could also improve on most terrestrial photosynthesis by capturing nearly the full spectrum of sunlight (like seaweeds). Combining all these possibilities, Martian life could potentially achieve roughly a ten-fold increase in efficiency compared to terrestrial photosynthesis. We discuss these possibilities below.

- NEW: Martian life could be better at photosynthesis than terrestrial life since terrestrial photosynthesis works at well below its theoretical peak efficiency and the lower light levels on Mars might favour evolution of more efficient photosynthesis

Martian life could also be adapted to the colder conditions on Mars with a faster metabolism, using chaotropic agents such as the perchlorates which can speed up reactions at low temperatures.

Trainer who is the lead author of the paper on the Curiosity oxygen results, interviewed by Scientific American, put it like this: (Andrew, 2019):

> *On Earth, photosynthesis and respiration by living things cause tiny fluctuations in our planet's otherwise steady oxygen concentration. We shouldn't expect this on Mars, though. "That's far out," Telling says: Mars appears too inhospitable for a critical mass of life capable of sustaining either process. "It's almost certainly going to be a nonbiological chemical reaction."*

Trainer herself does not rule out a biological explanation, but nevertheless underscores its unlikeliness. “People in the community like to say that it will be the explanation of last resort, because that would be so monumental,” she says. There are abiotic mechanisms aplenty, both known and unknown, to rule out first before leaping to any more sensational claims.

The biotic explanation would require Martian life to be very abundant or very productive of oxygen compared to terrestrial life or both. It would also need to have developed photosynthesis. As for the amount that needs to be sequestered to keep the Swansong Gaia in equilibrium, it would depend on the current input of CO_2 from volcanoes which we don’t know.

However, if Mars did ever evolve photosynthetic life, it would speed up the natural processes of carbon sequestration, and help maintain Mars close to the triple point of water for higher levels of CO_2 emissions. In this proposal, life sets up feedback cycles that limit its own growth.

It would work similarly to abiotic photosynthesis, but would act faster to restore the atmosphere to its barely habitable state after a pulse of volcanic activity or impact of a large comet. It could also keep the surface barely habitable over a wider range of CO_2 supply levels to the atmosphere.

As with the terrestrial Gaia there may be not just one cycle, but several interlocking and reinforcing cycles. There are many biological pathways that bacteria can use to form calcite (cement) for instance, with some of them used in self healing concrete (Rummel et al, 2017) (Dhami et al, 2013) One method may be of special interest to Mars. A consortium of methane oxidising and sulfate reducing bacteria can convert underground aquifers to calcite through anaerobic oxidation of methane (Rummel et al, 2017) (Drake et al, 2015). Perhaps these conditions may occur in the Martian subsurface, for instance at the sources of the methane plumes if these originate in geothermally heated underground aquifers.

This might explain the trace levels of methane too (Yung et al, 2018) (Klusman et al, 2022), with another parallel methane cycle.

The methanotrophs might grow in a layer above the methanogens to catch the methane. Since they form calcite, this could block it off and prevent it reaching the surface. From time to time some of it would break through and this could explain the methane spikes.

In this case the methanotrophs render their subsurface habitat less habitable by converting some of the water to calcite and blocking off their own supply of methane. If this happens near the surface, the warmer conditions may act as a feedback to block off the methane more and so reduce the warming effect on the atmosphere. By forming calcite the methanotrophs also take CO_2 from the atmosphere.

Another cycle might involve siderite (iron carbonate), which is produced in prodigious amounts by some Fe(III)-reducing bacteria (Onstott et al, 2019), which is a proposed metabolism for subsurface life on Mars (Parnell et al, 2016) (Onstott et al, 2019), these could also form carbonates with other metals such as copper (Onstott et al, 2019). As the methanogens warm the planet, more water would be available in subsurface layers suitable for Fe (III) reduction and the formation of siderite would help to cool it down again.

Another cycle could involve forams. On Earth forams evolved calcareous tests (shells) less than half a billion years ago during the Cambrian explosion (Boudaugher-Fadel, 2018:46, Fig 2.1). However the Martian water was oxygen rich long before we have evidence of abundant oxygen on Earth.

So, it's possible Mars evolved microorganisms with carbonate shells at an earlier stage of evolution. Planktic foraminifera produce as much as half the terrestrial particular carbonate flux to the ocean floor at a rate of around 2.9 gigatons per year (Jacob et al, 2017). So this process might cause a similar increase in the carbonate flux on Mars and be a significant increase over the abiotic processes. Forams incorporate oxygen from the water to make their shells, rather than from the atmosphere. However, Martian lakes and seas could have oxygen rich surface layers, similarly to Gale crater lake (Hurowitz, 2017) (Doyle, 2017) (Lanza et al, 2014) (NASA, 2017).

Terrestrial forams can be single cell secondary consumers, or kleptoplasts, ingesting chloroplasts from green algae to photosynthesize (Serôdio et al, 2014). The Martian analogues of forams could be either of those, or they might be themselves photosynthetic. They would need a reservoir to survive in between the warmer spells, perhaps in the Antarctic meltwater?

There's one possible weak biosignature in the meteorites that might suggest some of the Martian carbonates come from life. Life preferentially uses the lighter stable isotope of carbon, carbon 12 because it leads to lower energy costs than the heavier stable isotope carbon 13 (USGS, n.d.) so life processes tend to separate out the two isotopes. The carbon 13 ratios ($\delta^{13}C$ ratios) in the meteorites are highly variable, from -20% to +42%. Ratios on Earth fall into a similar wide range from -25% to +30%. Jull et al suggest fractionation by life as one of hypothetical explanation (Jull et al, 1995 : 1667).

It's a weak biosignature as they mention another possible explanation, that the atmosphere is the source for the high carbon 13 ratio and the low carbon ratio could be due to magma that is low in carbon 13 (Jull et al, 1995 : 1667). This is a plausible explanation as the current ratio is 46%±4% as measured by Curiosity (Webster et al, 2013). The early Martian atmosphere may have had a composition of 10% to 20% (Shaheen et al., 2015).

Then Mars is likely to need nitrogen for life in a Swansong Gaia. Earth's atmosphere is maintained at its high levels by denitrification. Capone et al. say that on a planet with oceans and continents, since nitrates are so readily soluble in water, without denitrification, nitrogen on the land would be substantially depleted, nitrogen would end up in the ocean and terrestrial life would be impossible (Capone et al., 2006).

If life on Mars is as on Earth and returns nitrogen to the atmosphere through denitrification, during a warming spell after a pulse of CO_2, life would use denitrification of nitrate deposits to produce the nitrogen for photosynthetic life to spread through the planet and to continue to remove the CO_2 from the atmosphere for as long as it is produced.

There may be just enough nitrogen for nitrogen fixation.

- Microbes from near the surface in Jezero crater would withstand temperatures varying from below -70 °C to above 15 °C in a single day – and major changes in humidity and pressure – this is likely to favour polyextremophiles – and martian life would likely be able to resist higher levels of stresses like UV, low humidity, vacuum, desiccation, and ionizing radiation – and may be able to fix nitrogen at low concentrations – which seems likely to make it easier not harder for them to survive on Earth

if Mars life behaves like life in Mars analogue deserts a complex picture of denitrification / nitrogen fixation arises (Shen et al, 2021) which reinforces the Swansong Gaia effect.

In the driest conditions the research of Shen et al suggests there is no biotic nitrogen fixation and no denitrification, just nitrate assimilation with the nitrates fixed abiotically from the atmosphere. They deduce this based on the isotope ratios for nitrogen and oxygen.

In wetter sites they detected more complex pathways and denitrification dominates. They found that some denitrification does occur in some hyperarid sites (Shen et al, 2021).

They suggest (Shen et al, 2021).

> *"These results suggest that N cycling on the more recent dry Mars might be dominated by nitrate assimilation that cycles atmospheric nitrate and exchanges water O during intermittent wetting, resulting stable isotope biosignatures could shift away from martian atmospheric nitrate endmember.*
>
> *"Early wetter Mars could nurture putative life that metabolized nitrate with traceable paleoenvironmental isotopic markers similar to microbial denitrification and nitrification stored in deep subsurface."*

This suggests a scenario where as Mars becomes more habitable after a warming pulse, life through denitrification makes the biosphere more habitable for nitrogen fixing photosynthetic life, which would strengthen the Swansong Gaia effect, with the CO_2 removed more rapidly the thicker the atmosphere.

As the CO_2 is removed and the planet becomes drier, the balance between denitrification and nitrification shifts in the other direction. Over much of Mars, where nitrates are less available, life is limited by the nitrogen fixation before it reaches the point where it is limited by the availability of liquid water. Once CO_2 levels get low enough so denitrification stops, nitrogen levels in the atmosphere fall, and photosynthetic life is reduced. With less photosynthesis to remove it, CO_2 from volcanoes builds up again until denitrification produces enough nitrogen for photosynthetic life to flourish enough to take the CO_2 out of the atmosphere.

In this scenario we can expect levels of nitrogen in the atmosphere to be close to the lowest limit for nitrogen fixation. Some nitrogen fixation is still possible but at less than peak efficiency.

This is an extra feedback to keep the planet at a warming level high enough to keep nitrogen fixation and denitrification in balance, and at a level where some nitrogen fixating photosynthetic life is possible throughout the planet. In this scenario, Mars has enough water for much of it to be more like the wetter parts of terrestrial Mars analogue hyper arid deserts, where denitrification begins to dominate over nitrification. This might brines if they retain enough water for denitrification, and meltwater below the surface of the ice in the polar regions. Antarctica has a rich diversity of cold adapted denitrifying bacteria (Cabezas et al., 2022).
See:

- 2009, 2014: Possible future surprise discovery of large quantities of fresh water on Mars: ice lets light through and traps heat, which melts ice half a meter below the surface in Antarctica -– if Martian ice is similar, its polar regions should have meltwater in summer, ~5 cms below the surface, even with surface temperatures below -90 °C – Mars may also have miniature melt ponds around sun warmed dust grains

Some or all of the nitrogen for photosynthetic life in a Swansong Gaia may also come from comets which may be enough to balance the nitrogen lost through nitrogen fixation (Poch et al, 2020).

Figure 15: Swansong Gaia hypothesis. Modern Mars looks sterile, but photosynthetic life might take CO_2 out of the atmosphere when it gets warm enough for liquid water, keeping Mars barely habitable. This would work with a wide range of CO_2 emission scenarios

Image credits: NASA's Goddard Space Flight Center from: (Steigerwald, 2019)

When the Martian atmosphere briefly thickens, life would colonize the lakes and rivers, and draw down a lot of CO_2 until it brings the pressure down low enough to get back to the triple point of water. The original paper goes into this in detail, and may be of interest to explore in the future.

On Earth, photosynthetic life stops it from getting too hot, keeping it in a very habitable state by converting most of the CO_2 to oxygen, reducing the greenhouse warming (Richardson, 2019).

However even on Earth our Gaia is not necessarily optimally habitable. Schulze-Makuch et al reason that our highest levels of biomass are in tropical rainforests, so a warmer world would be likely to have more biomass. Also, by analogy with the early carboniferous era which produced our coal and shale oil deposits, it’s possible a 5°C warmer Earth could sustain more biomass than at present, depending on which organisms inhabit it (Schulze-Makuch et al., 2020:1397). Also Earth was significantly less habitable during the ice ages. Also on theoretical grounds, if the continents were all covered in forests it would increase biomass, and the amount of carbon sequestered in the biosphere (Kleidon, 2002).

So life doesn’t necessarily make a planet optimally habitable and on an already cold Mars-like planet with a thin atmosphere, with no tectonic drift to return CO_2 to the atmosphere via volcanoes, any process that reduces global warming makes it less rather than more habitable.

The suggestion here is that when photosynthetic life sequesters CO_2 on a Mars-like planet, it makes the planet significantly less habitable for itself and then maintains it at that barely habitable state indefinitely.

We could verify or refute either of these ideas of a stabilizes Swansong biosphere, or a Swansong Gaia, once we can do detailed in situ measurements on Mars, looking for life based photosynthesis or abiotic processes.

We could also get indirect evidence from study of exoplanets, if we find a statistical anomaly where Mars-like planets have either thick atmospheres or thin atmospheres close to the triple point but with a gap in between with very few planets with intermediate thickness atmospheres.

If life based Swansong Gaias are common, we might be able to detect biotic photosynthesis on the Mars-like exoplanets with atmospheres that are briefly thicker and more habitable. If we find the same statistical anomaly, but see no signatures of life in Mars-like planets with briefly thicker atmospheres, this might suggest abiotic photosynthesis is more common.

In the other direction, though Earth may seem close to optimal for terrestrial life, it may be very far from optimal if we consider some hypothetical alternative biochemistry that is less dependent on phosphorous and has more efficient photosynthesis and less energy intensive nitrogen fixation and so on. See:

- NEW: Enhanced Gaia – ways that introduced Martian life could be beneficial to humans, ecosystems and Earth’s biosphere

The Viking landers in the 1970s remain our only attempt to search for life on Mars – a few astrobiologists think its labelled release may have already detected life in the 1970s – while others say the data can be explained by complex chemistry – we haven't sent the follow up experiments needed to finally resolve this debate and we can't deduce anything about whether Perseverance might return life even if the Viking experiment did find complex chemistry

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We have only sent two spacecraft to Mars to search for life in situ, the two Viking landers in the 1970s. The results were ambiguous, confused by the reactive chemistry on Mars.

The most sensitive experiment, the Viking labelled release, seemed to detect life. This experiment added dirt to organics labelled with carbon 14 and tested for radioactive evolved gases (such as carbon dioxide or methane).

The Viking labelled release experiment was so sensitive, that in tests before the mission it found life in a half gram sample from Victoria valley in Antarctica with only 50 cultivable cells in it (colony forming units), or 100 cultivable cells per gram (Levin et al, 1976).

A second life detection experiment didn't detect life, but was less sensitive than the labelled release.

However, the Viking chemical analysis experiment (TV-GC-MS) didn't detect any likely looking organics. It heated the samples to 200°, 350°, and 500°C to evaporate small organic molecules and break up large ones which it then analysed by separating them chemically and then by mass. The only organics it found were:

- Dichloromethane CH2Cl2 (Viking Lander 1).
- Chloromethane CH3Cl (Viking Lander 2)

The experimenters dismissed these as likely due to terrestrial contamination, even though they weren't detected in blank runs on Mars, because the chlorine 37 / 35 isotope ratios were similar to Earth isotope ratios.

However we now know that Mars has perchlorates, first discovered by Phoenix in 2009 (Hecht et al., 2009). These chlorohydrocarbons are exactly what you'd get from 0.1% organics reacting with the perchlorates when heated in the Viking ovens for analysis (Navarro-González et al, 2010).

Perchlorates also figure in the most developed non biological theory for the apparent detection of life. Quinn et al in 2013 suggested that the perchlorates in the soil were decomposed through gamma radiation to hypochlorite (ClO^-), trapped oxygen, and chlorine dioxide. Then the hypochlorite reacted with the ^{14}C-labelled alanine to produce chloroalanine which then decomposed to produce the ^{14}C-labelled CO_2. (Quinn et al, 2013). This didn't explain everything and a follow up paper by Georgiou et al filled out the picture some more but is still not a complete explanation (Georgiou et al, 2017).

However there are points in favour of the hypothesis of life too. Levin and Straat in a paper published in 2016 review some of the issues they have found with this and other abiotic proposals (Levin et al, 2016).

1. Two of the labelled release experiments got inactivated after storage in darkness for several months
2. Activity of the soil is significantly reduced if heated first to 50 °C.

Miller's reanalysis of the old Viking data in 2002 found an offset of the evolved gases from the diurnal maximum temperature by two hours. This is especially hard to explain by abiotic processes, as the evolved gases would take only 20 minutes to reach the detector. As an expert on circadian rhythms, Miller said they look like circadian rhythms (Levin et al, 2016) (Miller et al, 2002). He suggested this may be a biosignature in the data. A later complexity analysis seemed to support this interpretation (Bianciardi et al, 2012)

So, it can't be regarded as settled yet either way. Probably most astrobiologists would say it's very complex chemistry.

However these new insights lead some astrobiologists to say they think there is a strong chance the Viking landers detected life on Mars already.

Miller et al (Miller et al, 2002).

> *"Did Viking Lander biology experiments detect life on Mars? ... Recent observations of circadian rhythmicity in microorganisms and entrainment of terrestrial circadian rhythms by low amplitude temperature cycles argue that a Martian circadian rhythm in the LR experiment may constitute a biosignature."*

Bianciardi et al (Bianciardi et al, 2012)

> *"These analyses support the interpretation that the Viking LR experiment did detect extant microbial life on Mars"*

Levin et al (Levin et al, 2016)

> *"It is concluded that extant life is a strong possibility, that abiotic interpretations of the LR data are not conclusive, and that, even setting our conclusion aside, biology should still be considered as an explanation for the LR experiment."*

Davila et al. wrote: (Davila et al, 2010).

> *"… the immediate strategy for Mars exploration cannot focus only on past life based on the result of the Viking missions, particularly given that recent analyses call for a re-evaluation of some of these results. It also cannot be based on the assumption that the surface of Mars is uniformly prohibitive for extant life, since research contributed in the past 30 years in extreme environments on Earth has shown that life is possible under extremes of cold and dryness."*

What the Viking landers found was either life or very complex chemistry. Some day we'll know the answer. We need follow up experiments to help resolve this question definitively, but we haven't sent them yet.

If the Viking landers did find life it must be widespread on Mars, either in microhabitats or dispersed in the dust, since they got similar results in two sites thousands of miles apart.

If they didn't find life, this doesn't mean there is no life on Mars, as they were stationary landers with no capability to explore and go looking for life. They were limited to whatever they might reach using a scoop extended from the lander.

We can't deduce anything from this either way about whether or not Perseverance could return life bearing samples. Even if somehow we resolve this debate, and show the Viking landers only found complex chemistry in the 1970s, there's the problem of "microbial dark matter". Any native life might not have met the conditions it needed to revive in the experiment, a likely scenario since on Earth most life can't yet be cultivated in the laboratory (Dance, 2020). Also life in Mars analogue deserts is often patchy and if neither lander found life, this doesn't rule out a a patch of life even just a couple of meters from the lander.

Also if we were able to prove that what Viking found was chemistry, it wouldn't rule out the possibility of a viable spore or propagule in the Perseverance sample return, blown in the dust from elsewhere on Mars, or even local indigenous life in Jezero crater in a micrometers thick biofilm microhabitat in the dirt which Perseverance sampled by chance and neither Viking lander did.

There is an asymmetry built into our life searches on Mars. If we find indigenous life we may be able to confirm it quite quickly. If we don't find life we might not be able to deduce much without many extensive follow up searches.

2012: The European Space Foundation study reduced the size of particle to contain from 0.2 microns to 0.01 microns at the one in a million threshold, and added that it is not acceptable to release a particle of 0.05 microns or larger under any circumstance – this is well beyond the capabilities of NASA's proposed BSL-4

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[question]

The draft EIS says they would use many of the basic principles of a Biosafety level 4 facility (BSL-4): (NASA, 2022: S-4):

> ***The material would remain contained until examined and confirmed safe or sterilized for distribution to terrestrial science laboratories. NASA and its partners would use many of the basic principles that Biosafety Level 4 (BSL-4) laboratories use today to contain, handle, and study materials that are known or suspected to be hazardous.***

The draft EIS doesn't mention that the European Space Foundation study in 2012 reduced the size of the smallest particle we need to contain at the 1 in a million level from 0.25 ± 0.05 microns to 0.01 microns (Ammann et al, 2012:19). All larger particles would also have to be contained at the 1 in a million level. Also they made it clear that this means a 1 in a million chance of release of a single particle in the entire lifetime of the facility. This is well beyond the capabilities of a BSL-4.

The ESF also said a particle of 0.05 microns or larger shouldn't be released under any circumstances because of the discovery that ultramicrobacteria remain viable after passing through 0.1 micron nanopores (Ammann et al, 2012:21). This is how they summarize it graphically:

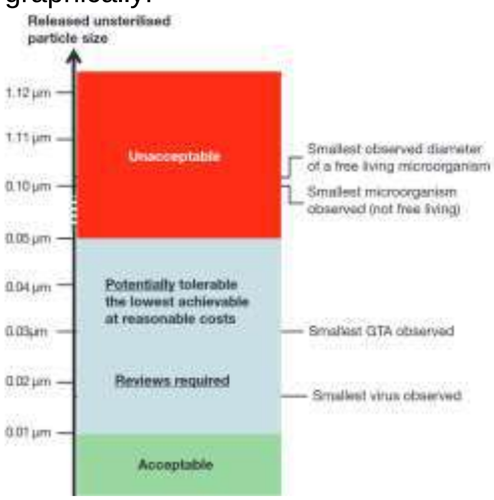

Figure 16: from (Ammann et al, 2012:21).

They summarize the decision on page 48 as "Recommendation 7" (Ammann et al, 2012:48):

> ***RECOMMENDATION 7:***
> ***The probability that a single unsterilised particle of 0.01 µm diameter or greater is released into the Earth's environment shall be less than 10^{-6} .***
> ***…***
> ***The release of a single unsterilized particle larger than 0.05 µm is not acceptable under any circumstances***

This is how the 2012 ESF report explained its decision at the time study (Ammann et al, 2012:3):

> ***The value for the maximum particle size was derived from the NRC-SSB 1999 report 'Size Limits of Very Small Microorganisms: Proceedings of a Workshop', which declared that 0.25 ± 0.05 μm was the lower size limit for life as we know it (NRC, 1999). However, the past decade has shown enormous advances in microbiology, and microbes in the 0.10–0.15 μm range have been discovered in various environments. Therefore, the value for the maximum particle size that could be released into the Earth's biosphere is revisited and re-evaluated in this report. Also, the current level of assurance of preventing the release of a Mars particle is reconsidered.***

They made this change after a discovery of fast horizontal gene transfer to distantly related archaea in sea water via Gene Transfer Agents (GTA) (Ammann et al, 2012:19):

> **Surprisingly, it is now estimated that GTA transduction rates are more than a million times higher than previously reported for viral transduction rates in marine environments. Clearly, GTAs are a major source of genetic diversity in marine bacteria.**

The ESF base this on research that showed that archaea can readily transfer novel capabilities to other distantly related species of archaea overnight in sea water (Maxmen, 2010) (McDaniel, 2010).

The EIS doesn't cite the ESF study. NASA's Mars sample return biological safety report does cite it (Craven et al., 2021:4) but doesn't mention the update on the limit of size and doesn't mention the gene transfer agents. Instead they have an extensive discussion of prions. Prions are not listed as a risk by the ESF study (Ammann et al, 2012) or the NRC study (SSB, 2009).

This is a visual comparison of the change in the size limit. The 0.01 microns bar is shown as the potential theoretical size limit a future review might decide on to contain early life ribocells. This is also the size limit for the GTAs for the one in a million threshold.

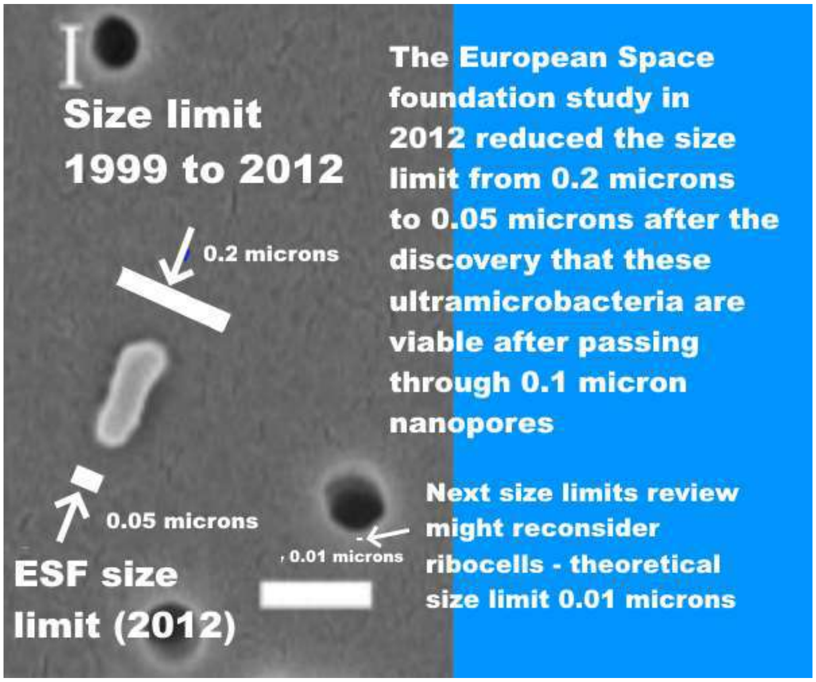

Figure 17: SEM of a bacterium that passed through a 100 nm filter (0.1 microns), larger white bar is 200 nm in length (Liu et al, 2019).

In support of the 0.05 micron size limit, the ESF (Ammann et al, 2012:15): cited two studies that found ultramicrobacteria were still viable after passing through 0.1 micron nanopores in freshwater from Greenland (Miteva et al, 2005), and eight different sites in Switzerland (Wang et al, 2007).

The ESF study also approached this theoretically and found that a minimal size free living cell based on terrestrial biology has a diameter of 0.15 to 0.2 microns if it's spherical but can have a width of less than 0.1 microns and a variable length greater than 0.2 microns. They also say that it's possible smaller cells exist which have an obligatory requirement to co-exist with other organisms as the source of the required genes or gene products (Ammann et al, 2012:15). The ultramicrobacteria that pass through 0.1 micron nanopores for instance in the images by Liu et al are indeed elongated (Liu et al, 2019). Less than 0.1 microns in diameter, but 0.2 microns in length.

NASA's sample return biological safety report doesn't consider ultramicrobacteria or the size limit for small organisms (Craven et al., 2021:4).

The ESF requirement is beyond the range for testing HEPA filters – only tested down to 0.1 to 0.2 microns

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The requirements for a BSL-4 facility depend on standards for HEPA filters. Typically it will use biosafety class III cabinets (which can be used for all biosafety levels). A biosafety class III cabinet has to be exhausted to the outside air through two HEPA filters or a HEPA filter and an air incinerator (Richmond et al, 2000:37). We will look at the alternative of an air incinerator in the next section.

These HEPA filters are required to trap 99.97% of particles of 0.3 microns in diameter and 99.99% of particles of greater or smaller size (WHO, 2003:35). These standards don't set any size limit for 100% containment.

In the US, HEPA filters are tested down to 0.1- 0.2 microns (depending on the class of filter, some are tested only at 0.3 microns). In Europe they are tested at the most penetrating particle size which may vary depending on the filter. In both cases, the filters are tested according to probabilities (Zhou et al, 2007) (EMW n.d.).

There is a higher standard than HEPA available. ULPA level 17 filters are rated to filter out 99.999995 percent of particles (BS, 2009:8) in the range 0.12 microns to 0.25 microns (BS, 2009:4), according to BS EN 1822-1:2009, the British implementation of the European standard (BS, 2009).

However even ULPA filters don't comply with the ESF standard of no release of a 0.05 micron particle in any circumstances. They aren't even tested in this size range.

Alternative of an air incinerator for the second HEPA filter – not tested for ultramicrobacteria imbedded in a dust grain – or the scenario of Martian spores that evolved extra layers to make them more resilient than terrestr ial test spores – or for 100% containment

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The standards for a biosafety class III cabinet for a BSL-4 laboratory do include an option to use an air incinerator instead of the second HEPA filter (Richmond et al, 2000:37), which might potentially be a way to contain very small cells.

The NIH guidelines for research involving recombinant or synthetic nucleic acid molecules specifies that these air incinerators need to be tested against a challenge aerosol of hardy spores, either b. subtilis var niger spores, or b. stearothermophilus spores (Meyer et al., 2019).

However, for a Mars sample return, it also has to contain potential Martian life potentially more resilient than b. subtilis var niger or b. stearothermophilus after millions of years of evolution in the extreme conditions on Mars. It may have:
:

- evolved to resist perchlorates, hydrogen peroxide, UV, ionizing radiation, low pressure and low humidity,
- spores may have evolved additional protective layers that make it more resilient to incineration.

see above:

- New: Martian life could have spores with extra layers to protect against UV in dust storms - or fruiting bodies or other propagules detached by strong winds protected by outer layers of altruistic social bacteria - and martian life could use strong biomaterials similar to chitin (found in hard parts of insects but also in fungi and lichens) to protect from impact bounces

By the ESF requirement, it has to contain 100% of spores at all sizes above 0.05 microns in diameter, not just small spores.

- It also has to contain ultramicrobacteria at 0.05 microns or GTAs at 0.01 microns – these incinerators aren't tested for ultramicrobacteria.

- It may have to contain life based on a different biology which may be more heat resistant than terrestrial life. As an example, PNA, has been proposed as the backbone in place of DNA and RNA for replicating biomolecules in a PNA-world "soup" before terrestrial life (Nelson et al., 2000). This is significantly more heat resistant than RNA (Jasiński et al., 2019).

  In one experimental test of PNA heated for 150 to 200 ms (Jasiński et al., 2019 : Fig 10).
    - No RNA – RNA base pairs are stable beyond 350 C
    - All PNA – PNA base pairs are stable up to 420 C

  When heated for 200 ms, half the double strands melt, i.e. separate, at (Jasiński et al., 2019 : Table 1)
    - 314.6 C for RNA – RNA
    - 347 C, for PNA – PNA

  In a test of melting for 200 ms, (Jasiński et al., 2019 : Fig 4).
    - Nearly all RNA – RNA strands separate at 340 C
    - Almost none of the PNA – PNA strands separate at 340 C

  this suggests the possibility that a second genesis of life could use biomolecules that are significantly more stable than terrestrial biomolecule

- The standards require more than a 100 billion fold reduction in spores – this doesn't quite reach the 100% assurance that it won't release a single particle of 0.05 microns in the facility lifespan.

There are other issues to look at. For instance, any martian life in the samples may be already imbedded in Martian dust grains, see

- 2017: individual microbes can travel in dust storms imbedded in a dust grain for extra protection from UV

Even small dust grains could give extra protection from air incineration to microbes imbedded in a crack in the grain.

- The ultramicrobacteria could be imbedded in small dust grains from Mars for extra protection

It is also important to consider maintenance of the incinerator, testing and replacement. This also

- must not permit release of a single unsterilized particle of 0.05 microns.
- All the maintenance of all the filters over the lifetime of the facility should be included in the calculation of the 1 in a million chance of release of a single particle at 0.01 microns

Also those size limit requirements need to be updated based on review of the level of assurance and size limit which hasn't happened.

In short, an air incinerator can't be added in an ad hoc way to "fix" the draft EIS.

The EIS would need to restart with a new technology review based on examining whether such technology could be used to contain an alien biology to the required level of assurance. The size limit and level of assurance needs to be updated first before doing this review. These are some of the things such a review would need to consider.

Then the public have to be given the opportunity to comment on a scientifically credible EIS that also evaluates reasonable alternatives like sterilizing the samples before they reach Earth.

NEW: If the ESF requirement is met using air filters it seems to need new breakthrough technology rather than incremental improvements

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Current air filters can't achieve 100% containment at any small particle size.

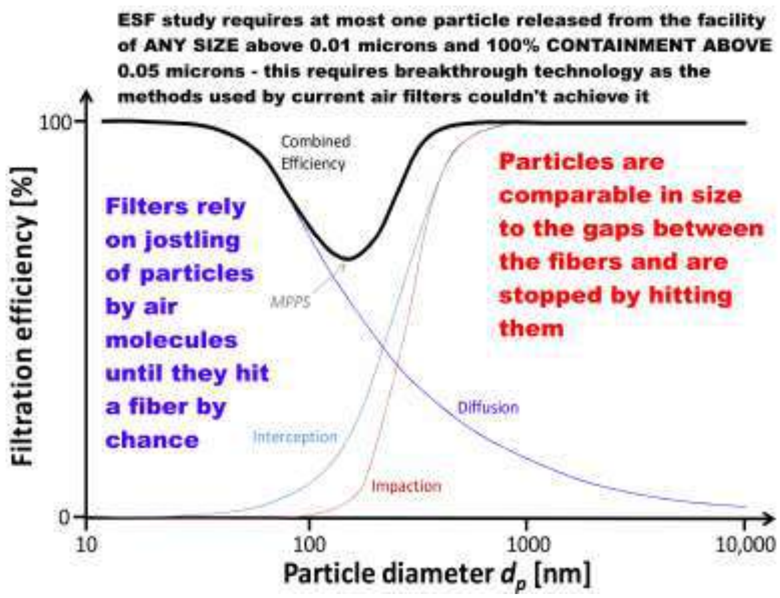

Figure 18: from (Todea et al, 2020: fig 1)

Filters generally have a maximum penetrating particle size at around 0.1 microns. Below this size the nanoparticles are far smaller than the gaps between the fibers, so they rely on Brownian motion – the random jostling of the particles by air molecules until some by chance hit the fibers. Above this size they rely more on the fibers directly stopping the particles. See section 4.3 and figure 5 from (Borojeni et al, 2022:7) and (Todea et al, 2020: fig 1)

Recent air filter technology reviews don't mention any attempts to achieve 100% containment above any size. Also they don't mention anything approaching 1 in a million chance of releasing a single particle in the lifetime of a facility at all sizes above 0.01 microns (Borojeni et al, 2022:7). The 100% requirement would seem to need some new breakthrough technique rather than incremental changes such as more layers of filters or varying the spacing as those couldn't get it all the way to 100% containment of such small particles.

It may be possible to achieve 100% containment of 0.05 micron particles in water under high pressure. A 2020 review of the literature found several studies that achieve a million fold reduction or more of small viruses in water. (Singh et al, 2020:6.3). That doesn't quite meet the target but Singh et al found one study using carbon nanotubes loaded with silver that achieved 100% removal of very small viruses such as the polio, noro and Coxsackie viruses (Kim et al, 2016), (Singh et al, 2020:6.3).. The poliovirus is only 0.03 microns in diameter (Hogle, 2002).

However these filters for smaller nanoparticles for water treatment are easily damaged, through chemical and biological deterioration by aging, scratches by particle like substances, or fouling of the membrane (Singh et al, 2020:8). If we achieve filtration at this standard in the air, the air filters may also have similar maintenance issues.

Also there is an issue with testing filters over this very small size range. The filters are tested with challenge aerosols such as dioctylphthal (DOP) generated on the intake side of the filter, and measured with a photometer on the discharge side (Richmond et al, 2000:33). These photometers have limited sensitivity to nanoaerosols below the 100 nm limit.

In a study of a DOP aerosol using TSI model 8130 Automated Filter Tester in 2008 (table III of Eninger et al, 2008), particles below 100 nm (0.1 microns) constituted 10% of the count of particles in the test aerosol, and 0.3% of the mass. However they provided almost none of the light scatter in the testing photometer (less than 0.01%).

Any new filter technology would need to specify how they will be checked and replaced. Biosafety level III cabinets need to be checked annually (Richmond et al, 2000:33) and equipment will sometimes need to be repaired. HEPA filters often fail these annual tests and need replacement. When these filters are changed, the Biological Safety Cabinets (BSCs) must be decontaminated (WHO, 2003:35). The method for decontamination would also need to be devised as well as the method for keeping the Mars samples undamaged during decontamination.

This technology may not be impossible. But by analogy with the situation for HEPA filters, it would seem to require a significant research program that hasn't yet been started to:

- develop filters to achieve the ESF requirement,
- design ways to test the filters,
- design methods to maintain them and replace them while preserving containment of the samples,
- set the necessary standards that the filters must meet, and
- confirm that the required standards have been achieved

ESF study said values for required level of assurance and the size limit need to be revisited periodically based on changes in scientific knowledge and risk perception

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[question]

The ESF study said that future reductions in the size limit are indeed possible. They expected later reductions to happen at a slower pace, but say the size limit will need to be reviewed in the future, adding (Ammann et al, 2012:21):

> *Based on our current knowledge and techniques (especially genomics), one can assume that if the expected minimum size for viruses, GTAs or free-living microorganisms decreases in the future, and this is indeed possible, it will be at a slower pace than over the past 15 years*
>
> *However, no one can disregard the possibility that future discoveries of new agents, entities and mechanisms may shatter our current understanding on minimum size for biological entities. As a consequence, **it is recommended that the size requirement***

as presented above is reviewed and reconsidered on a regular basis.
[bolding as in original cited text]
…
RECOMMENDATION 8: Considering that (i) scientific knowledge as well as risk perception can evolve at a rapid pace over the time, and (ii) from design to curation, an MSR mission will last more than a decade, the ESF-ESSC Study Group recommends that values on level of assurance and maximum size of released particle are re-evaluated on a regular basis

By 2022, a decade later, another review is certainly required.

The next review may examine new research into extremely small early life cells such as ribocells with enzymes made from fragments of RNA instead of proteins (Kun, 2021). Steven Benner and Paul Davies say the small 0.01 micron diameter structures in the martian meteorite ALH84001 are consistent with RNA world cells (Benner et al, 2010: 37). Panel 4 for the 1999 workshop estimated a minimum size of 12 nm in diameter and 120 nanometers in length for early life RNA world cells, if there is an efficient mechanism for packing its RNA (SSB, 1999: 117).

As we learn more about the mystery of the first cell, these researches may lead to a review of the size limit to accommodate new ideas (Kun, 2021).

We are a long way from solving the mystery of the first cell, but more and more of the puzzle- pieces are known. The problems, both dynamical and structural, have been identified, and for some, solutions proposed.

A team of researches lead by professor Joyce are using directed evolution to produce an RNA enzyme, or ribozyme which is able to catalyze its own replication. It is now able to replicate its own smaller ancestor but can't yet replicate itself. But a longer strand may be able to replicate itself. If they achieve this they will show an RNA world cell can in theory replicate its own RNA without using proteins (Portillo et al., 2021).

RNA world life may be the most developed idea of an early life cell, but there are many other possibilities. This field has expanded greatly. PNA and TNA (NASA, 2001) are the best known of numerous ways now known to construct the backbone of an informational biopolymer These can then be combined in a couple of dozen different paring systems to form a system of two informational biopolymers, just as in terrestrial life, DNA is paired with RNA, and some of these might have been available to early life (Anosova et al, 2015).

Can our size limits apply to all these possibilities and others we haven't through of yet, independent of the molecular basis of the biology? That's another question the review may need to revisit.

This review needs to be done first before developing the filter and / or air incinerator technology and relevant testing requirements, as requirements could change as a result.

Draft EIS does mention a 0.05 micron limit – but not for the BSL-4, only for the return capsule – and without mentioning the ESF study

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My comment of May 15th alerted NASA to this issue. I said in its first two paragraphs (Walker, 2022a)

> ***Are you aware of the ESF Mars Sample Return*** (Ammann et al, 2012:14ff)? ***It said "The release of a single unsterilized particle larger than 0.05 μm is not acceptable under any circumstances". This is to contain starvation limited ultramicrobacteria which pass through 0.1 micron filters*** (Miteva et al, 2005). ***Any Martian microbes may be starvation limited.***
>
> ***This 100% containment at 0.05 microns is well beyond capabilities of BSL4 facilities. Even ULPA level 17 filters only contain 99.999995 percent of particles tested only to 0.12 microns*** (BS, 2009:4)***.***

NASA didn't respond to this comment in the section of the draft EIS where they respond to public comments. They did mention a 0.05 micron limit but not in the context of the ESF Study or a BSL-4 so it wasn't a response to my comment.

They mention a 0.05 micron limit in their response to this question from members of the general public: (NASA, 2022: 4-7):

> What is the smallest Mars particle that is forbidden to be on the capsule carried to Earth? Dust level, bacteria level, virus level, prion level?

They respond that the minimum size is 50 nm – for the capsule on the journey back to Earth (NASA, 2022: 4-7):

> A number of studies (National Research Council 1999, Heim et al. 2017) have estimated the minimum sizes for life forms from fundamental inputs such as the genetic material required to permit a cell to perform basic functions [e.g., (Glass et al. 2006)], observations in extreme environments [e.g., (Comoli et al. 2009)] or theoretical constraints that would apply to astrobiology investigations (Lingam 2021).
>
> Values from such studies have been used to inform findings on best practices for sample return missions and MSR has considered those findings in selecting 50 nm for engineering requirements.

Their first cite is to the National Research Council study in 1999. Panel 1 gave a minimum size of 250 nm ± 50 nm for spherical cells(SSB, 1999: 2), which remained the minimum size for containment for a Mars sample return through to the 2012 ESF study (Ammann et al, 2012:3):.

So that can't be their source for a 50 nm requirement. Panel 3 of the same study, which looked into hypothetical early life RNA world cells finds the minimal gene size could fit into spherical cells of 50 nm diameter (SSB, 1999: 117). This is the only occurrence of a 50 nm figure that I found in NASA's cites for this sentence. However panel 3 also said if RNA world cells are elongated, they could be as narrow as 12 nm in diameter (SSB, 1999: 117) as we saw in the previous section:

- ESF study said values for required level of assurance and the size limit need to be revisited periodically based on changes in scientific knowledge and risk perception

NASA's cite Heim et al gives 250 nm as the minimum diameter for a prokaryote, and is not a primary source as it just uses the 1999 NRC cite as its source (Heim et al, 2017).

Lingam gives 0.2 micron as a limit in diameter for spherical microbes able to sense chemical gradients and move in response to them (Lingam, 2021:17), down to around 0.1 microns if the gradients are steep (Lingam, 2021:17). It assumes cells are spherical, and says elongated cells could be smaller in minimum diameter (Lingam, 2021:3).

Lingam may not be relevant to the smallest cell size for Mars, as many microbes are not able to move by themselves, including most fungi, and many blue-green algae. For instance chroococcidiopsis, a top candidate for a terrestrial microbe that may be able to survive on Mars can't move by itself. Only the youngest daughter cells (baeocytes) of a similar cyanobacteria Myxosarcina are able to move, gliding across surfaces for a short while after they first form (Sanders et al, 2021).

NASA's other two cites don't try to estimate minimal sizes for cells. Glass et al is about a search for a minimal genome but all it says about the size of their cells is that they passed them through 0.22 micron nanofilters to break up clumps into single cells (Glass et al, 2006). Comoli et al is a paper about a successful attempt to image one particular sub-micron microbe with its inner membrane fitted to an ellipsoid of 402 nm by 442 nm by 312 nm in diameter. It is shown in association with much smaller particles that they concluded are probably viruses (Comolli et al, 2009).

It's not clear from these cites why NASA selected 0.05 microns for its engineering requirements for particle release from the return capsule. However since they don't cite the ESF study it is likely to be nothing to do with the ESF study. They also make no connection with HEPA filters or the recommendation to use a BSL-4.

The National Reaserch Council study from 2009 warns the potential for even LARGE SCALE harm to human health and the environment isn't demonstrably zero – NASA's draft EIS conclusion that there is no significant risk of even SMALL SCALE

environmental effects seems a minority view amongst microbiologists – they don't alert the reader to the existence of any other view on the topic

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[question]

Another major change made by NASA's EIS compared to previous published work on planetary protection is a finding of no significant risk of environmental effects for life returned from Mars. This is what the draft EIS says (NASA, 2022:3-16):

> The relatively low probability of an inadvertent reentry combined with the assessment that samples are unlikely to pose a risk of significant ecological impact or other significant harmful effects support the judgement **that the potential environmental impacts would not be significant.**

We can compare this with the National Research Council's 2009 study, which said the potential for [even] large-scale negative effects appears to be low but is not demonstrably zero (SSB, 2009 : 48).

> The committee found that **the potential for large-scale negative effects on Earth's inhabitants or environments by a returned martian life form appears to be low, but is not demonstrably zero**

The NRC study also said that it is not possible to assess future negative impacts caused by delivery of putative extraterrestrial life, based on current evidence (SSB, 2009 : 48).

> … **it is not possible to assess past or future negative impacts caused by the delivery of putative extraterrestrial life**, based on current evidence.

When comparing the two statements, bear in mind that the NRC is looking at the potential for large scale harm to human health or the environment. While NASA's EIS comes to a conclusion of no significant effects – i.e. no effects of any significance, even minor effects.

Previous planetary protection studies have stressed the need to involve other agencies, and many legal processes to keep Earth safe. We look at this legislation in detail in:

- If NASA or another space agency accepts the NRC study's assessment that the risk of large scale effects on human health or the environment is not demonstrably zero – this has major legal ramifications domestically, with agencies such as the DoA, CDC, NOAA etc involved and also internationally and through international treaties with the FAO, WHO etc involved as well as potentially domestic laws of other countries

However, NASA seems to be of the opinion this mission is not covered by these legal requirements to look closely at environmental effects. At least they are not mentioned. So the difference in language here has major legal implications. It seems that by using this language "**that the potential environmental impacts would not be significant**" they feel they are no longer required to look into it any further.

The EIS doesn't alert the reader to the conclusions of the National Research Council study in 2009, or, as we'll see, many other cites that came to the same conclusion as the NRC that we need to consider the potential for even large scale harm to the environment or to human health.

That passage also gives the impression the only risk is from an inadvertent reentry.

However the Mars sample return planetary protection studies only briefly cover re-entry and focus in detail on issues with breach of containment after return to Earth, especially during sample handling. See:

- 2012: The European Space Foundation study reduced the size of particle to contain from 0.2 microns to 0.01 microns at the one in a million threshold, and added that it is not acceptable to release a particle of 0.05 microns or larger under any circumstance – this is well beyond the capabilities of NASA's proposed BSL-4

This is important because by presenting it as a mission where the main risk is a mishap during re-entry, they don't need to examine issues of containment, quarantine, lab safety etc with a novel organism of unknown biology when they discuss sample handling later in the EIS (NASA, 2022:S-4).

The discrepancy this time is a result of NASA using the conclusions of NASA's biological safety report for a Mars sample return by its sterilization working group, which concludes that (Craven et al., 2021):

> "*the presence of a direct pathogen on Mars is likely to have a near-zero probability"*

It also concludes that (Craven et al., 2021:6-7):

> "*Thus, it is plausible that any Martian microbe, after it arrives on Earth, would not be viable on Earth due to a lack of its required Martian nutritional and environmental conditions."*

I haven't been able to find a previous study in recent times that came to the same conclusions as them. Also, these two statements seem to represent a minority view amongst microbiologists (MacGregor et al, 2001) .

The EIS doesn't alert the reader to the discrepancy between previous studies and the conclusions of the sterilizing working group.

NASA's biological safety report does quote the NRC study but singles out a different passage, from the section of the NRC study discussing the potential for large scale negative pathogenic effects on humans (Craven et al., 2021:4).

> *'...the potential for large-scale pathogenic effects arising from the release of small quantities of pristine Mars samples is still regarded as being very low.'*
>
> *...'extreme environments on Earth have not yet yielded any examples of life forms that are pathogenic to humans'*

They omit the following "However" which leads to the section ending more cautiously than it began. The NRC study goes on to say that they ***have*** yielded microbes from hydrothermal vents with **close evolutionary connections** with human pathogens and concludes that (Craven et al., 2021:4).

> *since the potential risks of pathogenesis cannot be reduced to zero, a conservative approach to planetary protection will be essential, with rigorous requirements for sample containment and testing protocols of life forms that are pathogenic to humans'*

NASA's biological safety report doesn't mention this passage. We will discuss that section below in:

- Sample return biological safety report gives an example of an e. coli strain it says became toxic by coexisting with humans – it doesn't cite the NRC's counterexample of a human pathogen which shares many virulence genes with species adapted to hydrothermal vents – meanwhile even its e. coli example might have developed Shiga's toxin (poison) to prevent itself from being eaten by protozoa in biofilms – the origin of its virulence remains an open question

The passage from the NRC concludes that since the potential risk for life returned from Mars can't be reduced to zero a conservative approach to planetary protection is essential (SSB, 2009: 46)

> *"… It follows that, since the potential risks of pathogenesis cannot be reduced to zero, a conservative approach to planetary protection will be essential, with rigorous requirements for sample containment and testing protocols of life forms that are pathogenic to humans'*

NASA's biological safety report for the samples argues that martian life has a near zero chance to harm us because it didn't co-evolve with us and that plausibly it would be unable to survive on Earth because it's used to extreme conditions on Mars – these arguments were previously presented in an op. ed. by Zubrin in 2000 – planetary protection experts at the time found many errors in this reasoning and said it was like a recommendation to build a house without smoke detectors

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NASA's biological safety report for the samples uses two main arguments to reach its conclusions, which we can summarize as:

- that Martian life didn't co-evolve with humans so it can't harm us

That's their main argument for the conclusion (Craven et al., 2021):

> "*the presence of a direct pathogen on Mars is likely to have a near-zero probability"*

- that Martian life would be extremophile, only able to survive in the extreme conditions on Mars

That's their main argument for the conclusion that (Craven et al., 2021:6-7):

> "*Thus, it is plausible that any Martian microbe, after it arrives on Earth, would not be viable on Earth due to a lack of its required Martian nutritional and environmental conditions.*

The next few sections discuss how they came to use such arguments, and a possible common background with space colonization enthusiasts. If you want to skip to the arguments in the sterilization working group report, go to:

- Argument that martian pathogens wouldn't be adapted to humans or other Earth hosts misses a disease of biofilms that opportunistically infects human lungs - legionnaires' disease

I have been unable to find any previous planetary protection studies that argue this way, even when Mars was considered to be almost as arid as the Moon. The sterilization working group also give no cites to any previous sources that use either of these arguments.

I did find identical arguments in one earlier source, which they don't cite. It is a ***non peer reviewed op. ed.*** by the space engineer and Mars colonization proponent Robert Zubrin (Zubrin, 2000).

Since they don't cite this op ed., there is no reason to suppose any direct influence. But there are striking parallels which we will look at in the next section which may be due to a common shared background.

- There are many parallels between the arguments in the draft EIS and Zubrin's op ed – no reason to believe there was any direct influence – but there may be a common background

Many in the space exploration / colonization community have been convinced by Zubrin's arguments, and would likely find the conclusions of the sterilization working group convincing because they resemble Zubrin's ideas. So, let's start by looking at Zubrin's arguments.

First is Zubrin's argument that pathogens have to evolve in humans to harm us (Zubrin, 2000):

> *But couldn't such life, if somehow unearthed by astronauts, be harmful? Absolutely not. Why? Because disease organisms are keyed to their hosts. Like all other organisms, they are specially adapted to life in a particular environment. In the case of human disease organisms, this environment is the interior of the human body or of a closely related species, such as another mammal. For almost 4 billion years, the pathogens that afflict humans today have waged a continuous biological arms race with the defenses developed by our ancestors. An organism that has not evolved to breach our defenses and survive in the microcosmic free-fire zone that constitutes our interiors will have no chance of successfully attacking us. This is why humans do not catch Dutch elm disease and trees do not catch colds. Any indigenous Martian host organism would be far more distantly related to humans than are elm trees.*
>
> *There is no evidence for the existence of (and every reason to believe the impossibility of) macroscopic Martian fauna and flora. Without indigenous hosts, the existence of Martian pathogens is impossible. And if there were hosts, the huge differences between them and terrestrial species would make the idea of common diseases an absurdity.*

This may seem very convincing, that a microbe would need an indigenous multicellular host to develop into a pathogen, that is, until you start to look for counterexamples. Studies on planetary protection found several specific examples of ways Martian life can be pathogenic to humans without ever encountering any multicellular life. They include analogies with (Warmflash, 2007).

- Legionnaires' disease, tetanus, botulism, ergot disease, and others, none of them adapted to humans.

See:

- Argument that martian pathogens wouldn't be adapted to humans or other Earth hosts misses a disease of biofilms that opportunistically infects human lungs - Legionnaires' disease
  [and following sections]

Then comes Zubrin's argument that Martian life can't compete with terrestrial life

> *Equally absurd is the idea of independent Martian microbes coming to Earth and competing with terrestrial microorganisms in the open environment. Microorganisms are adapted to specific environments. The notion of Martian organisms out-competing terrestrial species on their home ground (or terrestrial species overwhelming Martian microbes on Mars) is as silly as the idea that sharks transported to the plains of Africa would replace lions as the local ecosystem's leading predator.*

This again may seem convincing as Mars seems such an alien world. However we keep finding extremophiles on Earth, and not only that, extremophiles that are also able to live in ordinary non extreme conditions.

- NASA's biological safety report agrees on the potential for an invasive Martian species to harm or displace terrestrial photosynthetic bacteria – but says it's plausible life adapted to Martian conditions such as the temperatures and pressures plausibly wouldn't be viable on Earth – their own cite mentions Planococcus Halocryophilus, a microbe which lives in Arctic permafrost soils and likely grows in sub zero brine veins down to at least -15 °C, with an optimal growth temperature of 25°C and growth up to 37 °C (human blood temperature)
  [and following sections]

We also have many candidate terrestrial microbes now that do well in Mars simulation chambers and may be able to live on Mars in favourable conditions there.

- Many candidate microbes such as the blue green algae chroococcidiopsis and even higher life like lichens have been proposed as Mars analogue organisms, some tested with promising results in Mars simulation chambers, so it's biologically credible a species can have adaptations to live on both planets

John Rummel, who was NASA's planetary protection officer at the time, gave several counter examples to Zubrin's arguments, and wrote: (Rummel et al., 2000).

> *NASA ' s current policy, as recommended by the US National Research Council, is not extreme. Rather, it is based on the sound principle that a sample from Mars should be contained until scientists find it does not contain a biohazard …*
>
> *Still, he insists that Mars life unrelated to Earth organisms couldn't possibly cause harm. How does he know, when we have precisely zero experience with life unrelated to Earth life? … How ought others judge the cost-benefit ratio of Mars exploration if we don't take simple precautions to avoid potentially harmful consequences? Harshly, I suspect.*

Margaret Race in her response said we do need to take care to protect Earth and that his proposal to drop planetary protection is like building a house without smoke detectors (Rummel et al., 2000).

"He's confident in our impressive technological prowess; he's raring to go and doesn't want anything to slow down or stop our exploration of Mars - especially not burdensome regulations based on very small risks and scientific uncertainty.
...
If he were an architect, would he suggest designing buildings without smoke detectors or fire extinguishers?

Figure 19: *Background graphics:Smoke detector (Rockmelder, 2007) House on fire (LAFD, 2018)*

NASA's draft EIS and the report of the sterilization working group don't cite Zubrin, so there is nothing to suggest a direct influence. However, there is a striking parallel.

There are two other striking parallels between Zubrin's op ed and NASA's Environmental Impact Statement.

Zubrin's op ed is the only previous use of the meteorite argument for a Mars sample return that I could find.

- Any life in Martian meteorites DOES get here faster and better protected than samples returned from PHOBOS because Phobos samples survived ejection from Mars and spent hundreds of thousands of years getting sterilized on the surface of Phobos – so it is safe for Japan to return samples unsterilized without special precautions – and why this reasoning DOES NOT apply to samples from MARS

As we saw, the planetary protection literature for Mars warns against its use for Mars.

Also Zubrin's op ed is the only previous article on planetary protection I could find that argues that there is evidence suggesting that there is no life on Mars.

- Draft EIS says "Existing credible evidence suggests that conditions on Mars have not been amenable to supporting life as we know it for millions of years" – their main cite says "exploration of Mars … will establish whether localised habitable regions currently exist" – another conclusion based on a citing error

There are many parallels between the arguments in the draft EIS and Zubrin's op ed – no reason to believe there was any direct influence – but there may be a common background

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There is no reason to believe there was any direct influence of Robert Zubrin on the sterilization Working Group. However, there may be a common background leading to the striking similarities we found with their arguments and also elsewhere in the draft EIS, which we noticed in the previous section.

Both space colonization enthusiasts and the NASA engineers and scientists are likely to have a strong interest in science fiction, and in spacecraft and exploration of the solar system, and are likely to prioritize Mars missions far higher than the general public. This may lead to them paying less attention to safety issues than the general public, because of their enthusiasm for the mission. There are other factors too that could lead to them having a different perception of the risks for a sample return.

Common factors include (links to the sections where I discuss these factors):

- 1. Engineering focus – NASA engineers have been tasked with returning samples from Mars to Earth
- 2. The new fast track NEPA process may encourage the view that they don't need to spend much time looking into the details, as their EIS won't get the close scrutiny by regulators it had before when the process took many years
- 3. The example of Apollo – few are aware the Apollo procedures had no scientific peer review and were not considered adequate even with the science of the 1960s
- 4. Inspiration of science fiction
- 5. Space colonization enthusiasts who see parallels between themselves and the settlers of the American west

Then I look at how in the larger picture it might actually be a big positive for human space exploration to find a second genesis on Mars that can never be returned to Earth even though it would mean humans can never land on Mars safely.

- The larger picture: how a scenario of mirror life microbes on the Mars surface could actually invigorate space exploration, as a forever unattainable human frontier - still studied and exploited by avatar robotic explorers controlled from orbit – with many other places for humans to explore in person, on the Moon, moons of Mars, asteroids, independently orbiting space settlements, aerostats above Venus clouds, Jupiter's moon Callisto, Saturn's moon Titan and beyond

I explore this all in:

- Factors for space agencies to look out for that may lead to them assigning planetary protection of Earth much less significance and attention than the general public

If you want to skip to the arguments in the sterilization working group report, to to:

- Argument that martian pathogens wouldn't be adapted to humans or other Earth hosts misses a disease of biofilms that opportunistically infects human lungs - legionnaires' disease

First though, I wish to include a short section to help the general public who may read this paper.

Analogy of a smoke detector – Perseverance will only find life if it is very easy to find on Mars – it is not visiting the most likely locations for present day life on Mars – not searching for life – no sure way to identify life in situ – not sterilized sufficiently to approach a potential habitat – and martian microbes may well be harmless – but we need to take precautions for worst cases as for a house fire – and as a precedent for future potentially more risky missions

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For anyone who might read this paper and panic and expect the worst, Margaret Race's smoke detector analogy may help. We need to look at the worst case scenarios, even though most people never get a house fire.

This particular mission is not designed to search for present day life or microhabitats. It will only return life if it is very common on Mars, as spores, biofilms etc.

Also if Mars does have life, there is no particular reason to expect it to be harmful. There are many other possibilities. It could be a harmless "drop in" replacement for terrestrial life, or it could be beneficial. Mars could also have no life, early life, or life precursors so primitive they are unable to compete with modern life.

However, just as it is wise to install smoke detectors in all houses rather than just the most at risk houses, it is wise to set a precedent to keep Earth safe for this sample return (likely low risk) and not just for future sample returns that may have more potential to return life. We don't yet know what scenario we have on Mars, so we have to take precautions. We also need to set a precedent for future samples returned from other areas of Mars more likely to have life, as well as samples returned from other locations like Enceladus, Europa or Ceres, that a space agency needs to take its responsibilities seriously and do a proper and thorough planetary protection study.

[This section of the paper is important for two reasons, to help people who panic easily as well as to help forestall any future infodemic about risks of a sample return.]

Argument in NASA's sample return biological safety report that martian pathogens wouldn't be adapted to humans or other Earth hosts misses a disease of biofilms that opportunistically infects human lungs - legionnaires' disease

Next section – all sections – previous section
[question]

The author would like to thank the sterilization working group for the effort formulating their position in a scientifically precise way with example diseases. The counterexamples were often subtle and in some cases they led this review to consider topics that seem new to the planetary protection literature. These will surely be expanded on in future sample return backwards contamination studies by experts.

First, arguing from many examples of pathogens adapted to humans, NASA's sample return biological safety report says the risk of a direct pathogen of humans is near-zero (Craven et al., 2021:6)

> *Since any putative Martian microorganism would not have experienced long-term evolutionary contact with humans (or other Earth host),* ***the presence of a direct pathogen on Mars is likely to have a near-zero probability.***

The NRC study by contrast concludes its section on the potential for large scale negative pathogenic effects on humans by saying that the potential risks of large scale negative effects from a disease of humans cannot be reduced to zero (SSB, 2009 : 45-6)

> *"TITLE: Types of large scale effects*
>
> *SUBTITLE: Large scale negative pathogenic effects on humans*
> *…*
> *… It follows that,* ***since the potential risks of pathogenesis [disease causing infection of humans] cannot be reduced to zero****, a conservative approach to planetary protection will be essential, with rigorous requirements for sample containment and testing protocols of life forms that are pathogenic to humans.*

NASA's sample return biosafety report does cite part of this section earlier (Craven et al., 2021:4) but not in this paragraph where they discuss the potential of martian microbes that are human pathogens

They proceed by enumerating a list of human pathogens and generalizing from that list. Their list includes:

- two diseases humans catch as a result of handling diseased animals (Ebola (CDC, n.d.) and HIV (CDC, n.d.),
- two diseases that infect humans via mosquitoes, malaria (CDC, n.d.) and yellow fever (CDC, n.d.) where malaria is spread human to human and yellow fever from monkeys.
- They also mention Kaposi sarcoma caused by a virus of humans, (Mayo clinic, n.d.) and Schistosomiasis also known as bilharzia, caused by a parasitic worm transmitted from freshwater snails in tropical conditions (CDC, n.d.).

We can agree on this, none of these are credible analogues for a pathogen from Mars.

They give two other examples which are less convincing and open up interesting questions.

For Escherichia coli strain 0157:H see below:

- The sterilization working group's report gives an example of an e. coli strain that they say became toxic by coexisting with humans – however the NRC report gave an example of human pathogens with close evolutionary connections with microbes in hydrothermal vents – meanwhile Łoś et al suggested their example, e. coli, strain 0157:H7, might have evolved Shiga's toxin (poison) to deter protozoan grazing in biofilms and only uses it opportunistically in humans
[and following sections]

For Candidiasis yeast infections, see below:

- NEW: sample return biological safety report mentions an opportunistic fungal pathogen, Candidiasis adapted to humans – but misses the counter-example of Aspergillus, not adapted to us – an estimated 200,000 life-threatening cases of invasive aspergillosis a year – mortality 30% to 95% - invasive because of capabilities martian life may share such as its ability to respond quickly to rapid changes in humidity and temperature, very efficient at taking up nutrients and storing them, and able to tolerate low oxygen levels in the lungs
[and following sections]

This section doesn't cite any of the planetary protection literature on the potential for pathogens of humans and potential terrestrial anlogues. They give no cites for the diseases they mention either (I added cites to their CDC pages for this paper).

Their only cite for this entire paragraph about human pathogens is to a paper about the last common bilaterian ancestor. They use this to support their statement that mosquitoes (which transmit malaria and yellow fever) and snails (which transmit schistosomiasis) had a last common ancestor 600 to 1,200 million years ago (Erwin et al., 2002).

Their list of human pathogens misses out the important exception of Legionnaires' disease , an example from (Warmflash, 2007). This a disease of biofilms and protozoa that also infects human lungs and sometimes can kill us, yet it's evolved to live in biofilms, not to attack humans.

Researchers into Legionella say that to the microbe Legionnella pneumophila, human lungs must seem like biofilms, and the macrophages in our lungs must seem like large protozoa *(Alberts et al 2002)*.

Legionnella pneumophila isn't an exact analogue for a microbe we could return from Mars in these sample tubes, as it needs an oxygen rich aquatic environment to survive, can't survive drying and can't form spores. But it is a useful example to show that a martian microbe could be preadapted to live in human lungs without ever encountering anything except biofilms. It can infect our lungs because of a close resemblance in relevant details between the conditions L. pneumophila encounters in lungs, and in biofilms.

Warmflash used Legionnaires' disease to challenge whether there is a need for human pathogens to co-evolve with us (Warmflash, 2007):

> *In essence, all that a potentially infectious human pathogen needs to emerge and persist is to grow and live naturally under conditions that are similar to those that it might later encounter in a human host. On Mars, these conditions might be met in a particular niche within the extracellular environment of a biofilm, or within the intracellular environment of another single-celled Martian organism.*
>
> *...*
>
> *To be sure, the genetic similarity between humans and protozoa is much greater than could be expected between humans and the Martian host of a Martian microbe.*
>
> *Even in the context of a planetary biosphere that is limited to single-celled life, and even where there is unlikely to have been a co-evolution between agent and host organism, the possibility of infectious agents, even an invasive type, cannot be ruled out.*

The report argues later that it's plausible martian life would be unable to survive on Earth. If that was true this section on human pathogens would be redundant. If martian life can never survive terrestrial conditions, how can it have any potential human pathogens?

However we'll find out that there is no real barrier to martian life spreading to terrestrial conditions. See below:

- NASA's biological safety report agrees on the potential for an invasive Martian species to harm or displace terrestrial photosynthetic bacteria – but says it's plausible life adapted to Martian conditions such as the temperatures and pressures plausibly wouldn't be viable on Earth – their own cite mentions Planococcus Halocryophilus, a microbe which lives in Arctic permafrost soils and likely grows in sub zero brine veins down to at least -15 °C, with an optimal growth temperature of 25°C and growth up to 37 °C (human blood temperature)

First we'll focus on human pathogens as for the report.

Sample return biological safety report gives an example of an e. coli strain it says became toxic by coexisting with humans – it doesn't cite the NRC's counterexample of a human pathogen which shares many virulence genes with species adapted to hydrothermal vents – meanwhile even its e. coli example might have developed Shiga's toxin (poison) to prevent itself from being eaten by protozoa in biofilms – the origin of its virulence remains an open question

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[question]

A toxin here means a poison produced by any living organism, including microbes and a hydrothermal vent is like a geyser but on the sea floor, bringing hot water and volcanic gases into the sea. NASA's biological safety report mentions that microbes which co-exist with humans can evolve the ability to make a toxin that harms humans (Craven et al., 2021:6).

> *Existing microorganisms that coexist with humans over long periods of time can also cause new diseases when the organism takes on new pathogenicity, such as the Escherichia coli strain 0157:H7 that acquired a gene for Shiga toxin, …*

This is part of the sterilization working group's reasoning towards the conclusion of a near zero probability of harm to human health.

However they don't consider the possibility that organisms can become toxic WITHOUT co-evolving with humans.

Also they don't consider the possibility that a new microbe from Mars could co-evolve with us after it gets to Earth. Any new microbe is a change to Earth's biosphere for all time. We need to consider effects of evolution on Earth in the near future, and on future generations. A microbe can become pathogenic which wasn't before, or evolve to become more pathogenic as with their example of Shiga's toxin.

The 2009 NRC review which NASA's EIS refers to elsewhere adds a counter example of hydrothermal vent organisms which are evolutionarily close to human pathogens (SSB, 2009: 46):

> *"**However**, it is worth noting in this context that interesting evolutionary connections between alpha proteobacteria and human pathogens have recently been demonstrated for natural hydrothermal environments on Earth*
>
> *… it follows that, since the potential risks of pathogenesis cannot be reduced to zero, a conservative approach to planetary protection will be essential, with rigorous requirements for sample containment and testing protocols of life forms that are pathogenic to humans'*

The NRC cite is to two species of microbes that live in the hot hydrothermal vents on the sea floor. These are strains of the class epsilon-Proteobacteria, (Nakagawa et al, 2007) now reclassified as Epsilonbacteraeota (Waite et al, 2017).

These organisms don't harm us, but their close relatives can. Helicobacter can cause stomach ulcers and Campylobacter can cause acute gastrointestinal disease in humans (Cornelius et al, 2012). These pathogens harm us through virulence genes they share with the hydrothermal vent organisms. The same adaptations that help them survive in their ecological niches in hydrothermal vents also help them survive in humans (Waite et al, 2017):

> *Although they are nonpathogenic, both deep-sea vent epsilon-Proteobacteria share many virulence genes with pathogenic epsilon-Proteobacteria, [they give a list of virulence genes, and other capabilities that enhance virulence]*
> *… these provide ecological advantages for hydrothermal vent epsilon-Proteobacteria who thrive in their deep-sea habitat and are essential for both the efficient colonization and persistent infections of their pathogenic relatives.*

[2013] Also, it turns out that we haven't even proven that e. coli strain 0157:H7 became toxic in humans through co-evolution with humans. In 2013, Łoś et al put forward an alternative hypothesis that e. coli strain 0157:H evolved Shiga's toxin in biofilms, to protect itself from being eaten by protozoa. They reasoned that (Łoś et al, 2013):

- e. coli strain 0157:H rarely spreads human to human. It only does this during rare outbreaks
- e.coli kills itself in humans when it produces Shiga's toxin – so it only benefits other e. coli altruistically by destroying the white blood cells (phagocytes) that attack them.
- e. coli stays alive in biofilms when it produces Shiga's toxin – the toxin only kills the attacking protozoa.

In short, their reasoning is that Shiga's toxin seems to be more beneficial to e. coli in biofilms than in humans, and that e.coli seems unlikely to have had the opportunity to evolve Shiga's toxin in humans either since the outbreaks don't spread enough for much evolution.

[2018] Experiments since then led to conflicting results. Some studies find Shiga's toxin has a survival advantage in biofilms, helping e. coli to resist the protozoa *T. pyriformis* by surviving in the protozoa's food vacuoles. It also helps e. coli to kill the protozoa *A. castellanii and T. thermophila.* Other studies found Shiga's toxin is not able to protect it from the protozoa A. castellanii and can even decrease its ability to resist predation by A. castellanii *(Sun et al, 2018)*

In short, it's not clear how E. coli strain 0157:H7 evolved its virulence, but it remains possible that it evolved it in biofilms to resist protozoan grazing rather than in humans *(Sun et al, 2018):*

In conclusion, evolution of mechanisms that allow for survival within protozoa may have selected for traits that also allow bacteria to escape that harmful effects of phagocytes [in humans].

If E. coli strain 0157:H7 did evolve this virulence in biofilms, it is a similar example to Legionnaires' disease in the last section, a pathogen that evolved its ability to harm humans in a biofilm rather than through coexisting with humans.

Also, whether this particular microbe E. coli strain 0157:H7 developed Shiga's toxin in biofilms or by interacting with humans, Łoś et al's proposed mechanism suggests a plausible scenario for pathogenic martian life. A martian microbe might already be able to make a toxin which it evolved to resist grazing by larger single cell predators on Mars. Once brought to Earth, it might use this same toxin opportunistically to destroy white blood cells in the human immune response when they try to attack it.

NASA's biological safety report doesn't mention clear examples of microbes which produce accidental poisons without any co-evolution with humans or higher life, such as tetanus which kills thousands of unvaccinated newborns every year

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[question]

The sterilization working group's report has another major omission in its discussion of Shiga's toxin (Craven et al., 2021:6):

Existing microorganisms that coexist with humans over long periods of time can also cause new diseases when the organism takes on new pathogenicity, such as the Escherichia coli strain 0157:H7 that acquired a gene for Shiga toxin, …

The origin of shiga's toxin is already unclear as we saw in the previous section. So that's an example of incomplete citing already.

However they also missed several much clearer examples in the planetary protection literature of microbes that secrete accidental toxins that harm us, and were not the result of co-evolution with humans or any other higher lifeforms. Warmflash et al give the examples of tetanus, which is locally infectious and accidentally toxic, and botulism which harms humans by contaminating food without infecting us, (Warmflash, 2007).

Locally infectious organisms, which do not multiply systemically within a host but which produce a toxin which the host can absorb, perhaps through an infected wound, may also be possible on a planet that harbors single-celled life. Clostridia is an example of an anaerobic genus that often lives as spores in soils and some of its species are important human pathogens, including C. tetani and C. perfringens, which are locally infectious in

wounds, where they release toxins that can be life-threatening through systemic effects (C. tetani) or local effects (C. perfringens)
…
There are terrestrial examples of organisms that are pathogenic to humans without being infectious, meaning that the organisms do not need to live or replicate on nor in humans in order to intoxicate them. For example another Clostridia species, C. botulinum, produces spores that can contaminate food that is stored under anaerobic conditions, allowing the spores to germinate. The bacteria release an exotoxin into the food which, if ingested, blocks the release of acetylcholine from presynaptic nerve endings at the neuromuscular junction …. This leads to flaccid paralysis, which can be fatal.

We can now protect babies with tetanus vaccines, and they are widely available, yet tetanus still kills thousands of newborns every year in weaker economies as cases of neonatal tetanus [neonatal means in the first 28 days of a baby's life] (WHO, n.d.) . Tetanus is a common anaerobic (non oxygen using) soil bacteria. The tetanus toxin is made by a plasmid (pE88), a small self-contained circular DNA molecule, which is independently evolved from the main genome of the microbe. The origins of this plasmid are unclear as it seems to be unique to Cloristidium tetani (Brüggemann et al., 2003).

There are many other clear examples of toxins produced by fungi, some of which didn't co-evolve with higher life, and which spoil crops and poison humans that eat them. We'll cover this below in:

- Aspergillus molds also spoil crops and so harm humans indirectly and eating the toxin aflatoxin can lead to the sometimes life-threatening condition of aflatoxicosis

NEW: An unrelated exobiology may produce many novel bioactive compounds which could be of great benefit, but the difference in biochemistry could also lead to more accidental toxins than terrestrial life, and in some scenarios, the internal chemistry of an unfamiliar exobiology could be accidentally toxic

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We could expect an unrelated exobiology to produce novel bioactive compounds, since that is what life need to do to survive, grow, and reproduce. These could harm us or help us. Let's look briefly first at some of the ways they can help us.

Biochemicals from unrelated or distantly related martian life may be of great value to us. Many modern medicines are based on bioactive compounds from microbes (Abdel-Razek et al., 2020). Indeed botulism toxin itself, properly used, has many medical benefits (Jankovic, 2004).

If we do find martian life, it may bring new medicines, or benefit us in many ways. For instance aspergillus niger, a bacteria whose natural habitat is soil and decaying vegetation, is used for industrial production of citric acid for beverages, food, detergents, cosmetics and pharmaceuticals (Behera, 2020).

Extremophile fungi may be a source of bioactive compounds for medically useful drugs (Chávez et al., 2015). However bioactive compounds for medicine have to be screened for toxicity (Madariaga et al., 2019).

There are many other ways a novel biology could benefit humans and our biosphere. See:

- NEW: Enhanced Gaia – ways that introduced Martian life could be beneficial to humans, ecosystems and Earth's biosphere

However for the topic of back contamination and what we need to do to protect Earth, what matters is whether it can also harm us.

Some of the ways it can do this include:

- Allergens, e.g. various Aspergillus species can trigger asthma (Latgé, 1999) and are not adapted to humans (McCormick et al, 2010). We cover this in detail below:
    - NEW: Possibility of an allergic response to harmless alien life – or indeed a new genus of familiar life - if it is recognized by the immune system but not by the inflammation dampening Treg cells - allergic bronchopulmonary aspergillosis affects around 4.8 million people globally and chronic pulmonary aspergillosis, affects 400,000 globally – these figures could be higher for an allergic response to extraterrestrial life - if a normally functioning human immune system doesn't recognize the need to dampen its response

- Secondary metabolites, e.g. which inhibit the growth of other microbes, Wallemia which is adapted to low water activity in salt or sugary solutions spoils food with secondary metabolites, the most toxic is wallimidione (Desroches et al, 2014). As another example, Aspergillus species produce numerous toxic secondary metabolites (Pfliegler et al., 2020)
- protoxins, which when metabolized break down into toxic products., such as methanol which is converted into toxins when digested (Mégarbane, 2005), or hypoglycin A, which is broken down into the highly toxic MCPA-CoA on digestion and can lead to the fatal Jamaican vomiting sickness after eating the unripe fruit of the Ackee tree, a national foodstuff in Jamaica (Holson, 2015).
- The chemistry of alien cells may itself be toxic to Earth life. Martian life might use hydrogen peroxide and perchlorates in its intracellular fluids in place of the chlorides used by Earth life, similarly to the composition of the brines it inhabits (Schulze-Makuch et al, 2010a).

- As well as the internal chemistry, the waste products and metabolic intermediaries could also be accidentally toxic or allergenic.

So, even if we find unrelated Martian organisms, it would be no great surprise to find they produce bioactive compounds which affect humans in various ways, sometimes beneficial, sometimes harmful.

Whether we want to introduce martian life itself to Earth would depend on what the effects would be on our biosphere and on humans and the animals within it.

One scenario is that novel martian life has potential for mixed effects on Earth's biosphere, with some positive effects, and some negative. If so, we might prefer to leave it on Mars and exploit it on Mars and export only the products of martian life to Earth, even if the life itself could also lead to some benefits on Earth.

In a situation where we believe martian life will have mixed effects, we will have a difficult decision about whether or not to return the life itself to Earth. That's especially so if it affects some ecosystems or some human communities positively and some negatively. Probably most would agree it is not ethical to return something that has a risk of harm to a significant fraction of the world population, even if overall it's a benefit.

NEW: NASA's sample return biological safety report mentions an opportunistic fungal pathogen, Candidiasis adapted to humans – but misses the counter-example of Aspergillus, not adapted to us – an estimated 200,000 life-threatening cases of invasive aspergillosis a year – mortality 30% to 95% - invasive because of capabilities martian life may share such as its ability to respond quickly to rapid changes in humidity and temperature, very efficient at taking up nutrients and storing them, and able to tolerate low oxygen levels in the lungs

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[question]

The sterilization working group report said fungal infections such as candidiasis yeast opportunistically infected people with compromised immune systems as a result of coexisting with humans for a long time (Craven et al., 2021:6):

> *Existing microorganisms that coexist with humans over long periods of time can also …*
>
> *opportunistically infect a host with a weakened or compromised immune system such as candidiasis yeast infections*

As they say there,

- candidiasis yeast adapted to humans (Alves et al., 2010).

The second main genus of opportunistic fungal infections that kill humans is adapted to mammals:

- cryptococcus adapted to mammalian hosts (Kronstad et al, 2012).

However that's not true of the third main genus of opportunistic fungal infections that kill humans

- aspergillus fumigatus [the main aspergillus pathogen of humans] is not adapted to a pathogenic lifestyle [in any other organism] (McCormick et al, 2010).

  According to our current knowledge A. fumigatus lacks sophisticated virulence factors that are solely dedicated to permit a pathogenic lifestyle.

So once more they found an example consistent with their conclusion, but missed an example that disproves it.

As for the second part of their statement, (Craven et al., 2021:6):

> *opportunistically infect a host with a weakened or compromised immune system such as candidiasis yeast infections*

There are many people who have a weakened or compromised immune system. There are estimated to be more than 200,000 life threatening Aspergillus infections a year with mortality rates varying from 30 to 95%.

Disease (most common species)	Location	Estimated life-threatening infections/ year at that location*	Mortality rates (% in infected populations)*
Opportunistic invasive mycoses			
Aspergillosis (*Aspergillus fumigatus*)	Worldwide	>200,000	30–95
Candidiasis (*Candida albicans*)	Worldwide	>400,000	46–75
Cryptococcosis (*Cryptococcus neoformans*)	Worldwide	>1,000,000	20–70
Mucormycosis (*Rhizopus oryzae*)	Worldwide	>10,000	30–90
Pneumocystis (*Pneumocystis jirovecii*)	Worldwide	>400,000	20–80

Figure 20: (Brown et al, 2012:table 1)

Invasive aspergillosis has an overall 50% fatality rate but a near 100% fatality rate if the diagnosis is missed or delayed (Brown et al, 2012:3).

Those most at risk of the life threatening condition invasive aspergillosis include those with autoimmune disorders, cancer, inflammatory diseases, previous septic conditions, those treated with high-dose corticosteroids for cytokine storm syndrome and patients in ICU for severe influenza or COVID (Thammahong, 2021).

A fungus from Mars would be likely to grow best at colder conditions. However, there are strains of chroococcidiopsis in the nasopharyngeal microbiota (Ventero et al, 2022), in human milk from Gambia (Lackey et al, 2019), and in Sri Lankan reservoirs (Magana-Arachchi et al, 2013).

Also the rock inhabiting black fungus Exophiala jeanselmei MA 2853 which responded well to exposure to Mars simulation conditions with no signs of stress (Zakharova et al, 2014) has close relatives that live in the human microbiome which are occasionally pathogenic (Zakharova et al, 2014) and is occasionally an opportunistic pathogen itself (Wu et al., 2022) and close relatives are sometimes fatal (Zeng et al., 2007). So we can't rule out the possibility of a black fungus, say, from Mars that may be able to adapt relatively quickly to warmer conditions in humans. For more about this see below:

- NEW: Many terrestrial fungi do well in Mars simulation chambers – a fungal disease from Mars would be likely to be hard to distinguish from tuburculosis through testing or medical imaging - and with likely no effective antifungals available initially or for some time

COVID associated pulmonary aspergillosis (CAPA) is especially difficult to diagnose because symptoms resemble severe COVID and it can be hard to detect in blood tests. It affects 10% of patients in ICU (originally 20%) typically about 8 days after admission and typically more than half of those affected die, it can invade the blood cells a few days later with mortality of 80% at that point even with antifungal treatment (Hoenigl et al., 2022).

People who are not immunocompromised can also be affected by an allergic reaction. Allergic bronchopulmonary aspergillosis affects 2.5% of patients with asthma, and an estimated 4.8 million people globally. Chronic pulmonary aspergillosis (CPA) affects around 400,000 globally and only occurs in people who are not immunocompromised with symptoms of “weight loss, profound fatigue, productive cough, significant shortness of breath, and life-threatening hemoptysis [spitting out of blood from the lungs]” (Denning et al., 2013).

The most common microbes that cause invasive Aspergilliosis in humans are A. fumigatus, A. flavus, A. niger, A. terreus, and A. nidulans with A. fumigatus causing 90% of all human cases (Denning, 1998). A. fumigatus and *A. nidulans*, have no adaptations to a pathogenic lifestyle. *A. Flavus* has generalist adaptations to infect wounds in plant, animal and insect hosts though not adapted to any specific species (St. Leger et al., 2000).

Researchers find the top three Aspergillus species that infect humans A. Fumigatus, A. flavus and A. terreus. are not closely related to each other, and all have close relatives that rarely infect humans (Gibbons et al., 2013). A. fumigatus may be the most common cause of serious infections in humans just because its spores are very common in the environment, they are buoyant, it can grow well at blood temperature, and its spores are coated with a water repellent coat (hydrophobin) which makes them inert as far as the immune system is concerned (Gibbons et al., 2013).

Aspergillus is pathogenic in humans because of factors that make the fungi very resilient in extreme conditions, stress resistant, able to respond rapidly to dehydration and rehydration, able to form biofilms and penetrate tissues mechanically with filaments, which also break off and can spread through the body, able to withstand low oxygen in damaged lung tissue and so on.

These are not adaptations to humans and many of them are also likely to be shared by Martian fungi.

Paulussen et al. put it like this (Paulussen et al, 2017):

> *Collectively, the aspergilli are remarkable fungi. … there are numerous aspects of* Aspergillus *cell biology and ecology (including their metabolic dexterity when adapting to nutritional and biophysical challenges) which contribute to their status as, arguably, the most potent opportunistic fungal pathogens of mammalian hosts.*
>
> *Aspergillus species are able to utilize a wide range of substrates, highly efficient at acquiring such resources, and can store considerable quantities of nutrients within the cell; all traits which contribute to their energy-generating capacity and competitive ability*
>
> *…*
>
> *Species of Aspergillus are also among the most stress-tolerant microbes thus far characterized in relation to, for example, low water activity, osmotic stress, resistance to extreme temperatures, longevity, chaotropicity, hydrophobicity and oxidative stress*

Microbes returned from Mars could be even more stress tolerant than Aspergillus species, to all those conditions.

It may help to give a summary of some of the key points.

From the section: "Biophysical capabilities and ecophysiology of pathogenic *Aspergillus* species" (Paulussen et al, 2017) aspergillus species are able to be pathogenic in humans because of many adaptations in extreme environments, as a result of which they:

- Can recover and rehydrate rapidly from desiccation – this lets inhaled desiccated spores recover rapidly to colonize the respiratory tract
- Have large amounts of melanin which can protect the cell membranes from breakdown (lysis) by the immune system
- Produce enzymes that break down proteins (proteases)
- Produce branching filaments (hyphae) which mechanically penetrate tissues and can also break up into fragments that can spread rapidly through immunocomromised patients
- All these activities require a lot of energy and Aspergillus species are able to colonize a wide range of media, highly efficient at taking up nutrients and converting it to energy
- Amongst the most stress tolerant of species, able to resist extreme high temperatures, highly tolerant of low temperatures, also oxidative stress (including hydrogen peroxide), UV, carbon and nitrogen starvation, and ionizing radiation (Paulussen et al, 2017: table 2),
- Able to protect themselves from chaotropic agents like urea and ethanol, by stabilizing their proteins, producing more proteins, more energy, and modifying their membrane

composition and increase production of enzymes that remove reactive oxygen species. [there are many chaotropic agents on Mars such as the perchlorates]

- Able to tolerate low oxygen levels in the lungs (as low as 1% partial pressure in inflamed tissue) and some strains can function without oxygen (Paulussen et al, 2017: table 2)
- Many specific adaptations to stress including ability to produce EPS for biofilms, accumulate large amounts of melanin in the cell walls, and protein stabilization mechanisms, and they can synthesize solutes like glycerol for protection from the environment around them. The glycerol can protect against freeze thawing (Paulussen et al, 2017: table 2)
- Naturally resistant to antifungals generally - some species of aspergillus can pump antifungals out of the cell (using efflux pump transport proteins) and also secrete plastic like substances (polymeric substances) to reduce contact with the antifungals, the melanin in the cell walls can bind to antifungals and they can use heat shock responses to reduce the entry of antifungals (Paulussen et al, 2017: table 1)

These are all capabilities that could be shared by a new genus of fungi from Mars. Indeed, aspergillus niger, one of the occasional opportunistic pathogens of humans, also happens to have high resistance to UV for a terrestrial microbe, higher than some microbes from Mars analogue environments. It was tested in a Mars analogue experiment in a high altitude stratospheric balloon because of potential for forward contamination of a human mission to Mars (it's found on the outside of spacecraft and is one of the species of microbe in the ISS) and was still viable after 5 hours of UV at levels similar to full daylight on the Martian surface (Cortesão et al, 2021:table 3) and ionizing radiation between a third and a quarter of Mars surface conditions (Cortesão et al, 2021)

Aspergillus is capable of micro-evolution as it spreads through the body after it infects a host (Ballard et al, 2018). The same could be true of any putative Martian fungus after it infects a human host.

This suggests a scenario where, just like Aspergillus fumigatus, a martian fungus could be pre-adapted for spreading rapidly through a human host because of adaptations to extreme stress conditions on Mars including low oxygen, UV, ionizing radiation, rapid changes in temperature and humidity, and likely evolutionary adaptations for rapid recovery and rehydration after desiccation.

The other two main genera of fungi pathogenic for humans bring out a larger philosophical point. Species from the candida and cryptococcus genera are fungal parasites of protozoa, not just higher life (Gonçalves et al., 2019). We might perhaps return fungal parasites of similar microbes on Mars.

A fungal parasite of a martian microbial host might evolve on Earth over time to adapt to higher life and humans. Shouldn't we look at the possibility for a new genus of fungi from Mars to gradually adapt to human hosts. even if they are not immediately able to be more than a minor nuisance?

We don't know how quickly martian fungi could adapt to higher life, especially given that terrestrial microbes might have no adaptations to protect against it.

So there are two possibilities here, a new genus of Martian fungi already able to infect humans similarly to Aspergillus, but evading our immune system which never learnt to recognize it, and t a new genus of Martian fungi which can already infect other microbes, which would have no adaptations to stop it initially, and later evolves to infect humans at some point in the future.

They could of course also be harmful to us indirectly through effects on our ecosystems even if they only infect terrestrial microbes, depending on the effects of the infections. For more on the potential for fungal pathogens of martian microbes to infect terrestrial microbes see below:

- NEW: Microbes from Mars could have pathogens that can infect terrestrial microbes – example of fungal pathogens of phytoplankton and cyanobacteria – cyanobacteria have specific antifungal adaptations to the phylum that attacks them, the chytrids and may have no adaptations to a novel phylum from Mars

Aspergillus molds also spoil crops and so harm humans indirectly and eating the toxin aflatoxin can lead to the sometimes life-threatening condition of aflatoxicosis

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Warmflash et al. also gave the example of ergot disease, a disease of some crops that accidentally harms humans (Warmflash, 2007). This isn't a very close analogue for the scenario of a maritan fungus, as ergot toxins are produced by fungi specialized to infect ovaries of grasses (Miedaner et al, 2015) which of course don't occur on Mars.

However, there are many other species of molds that produce toxins (mycotoxins). Some of these toxins can accidentally harm humans and they also damage crops, causing millions of dollars of economic loss per year (Hussein et al., 2001).

The molds from the Aspergillus genus may be a better analogue for the scenario of a fungus returned in a Mars sample return mission. These molds are either not adapted to higher life at all or only have generalist adaptations. They produce alfatoxins, which are amongst the most poisonous mycotoxins in food, along with several other toxins that spoil food (WHO, n.d.).

The natural habitats for Aspergillus species include soil and decaying vegetation. They are often opportunistic pathogens in plants and animals and spoil hay, grain, and crops (Dagenais et al. 2009). They produce numerous mycotoxins including aflatoxin, gliotoxin and ochratoxins (Pfliegler et ak, 2020). Large doses of aflatoxin cause the acute poisoning, aflatoxicosis which can be life threatening, and there is some evidence this toxin also causes liver cancer in humans (WHO, n.d.). The aspergillus species which most often damages crops is *A. Flavus* which has generalist adaptations to infect wounds in plant, animal and insect hosts though not adapted to any specific species (St. Leger et al., 2000).

Some of the Aspergillus toxins protect against insects, but some of the gene clusters that produce these toxins are activated only when interacting with other microbes. The toxins protect against other microbes and also provide a competitive advantage with other microbes. Aspergillus also protects itself against its own toxins

For more evidence that the toxins are used to compete with other microbes, when humans domesticated A. oryzae to make saki, the A., oryzae can only do part of the process. It converts starches to sugars and needed to work with brewer's yeast to convert sugar to alcohol. As it evoved to match the needs of humans it needed to work with brewer's yeast, and to do this it significantly downregulated its production of the mycotoxins aflatoxin and cyclopiazonic acid. This is seen as more evidence that these toxins are used for competition with other microbes (Gibbons et al., 2013).

So one scenario here is that molds on Mars might have toxins they use in competition with other microbes on Mars. Those toxins might then damage our crops or harm higher life including humans when we eat the crops.

NEW: by analogy with terrestrial fungal diseases – a fungal disease from Mars would be likely to be hard to distinguish from tuburculosis through testing or medical imaging – a new genus would likely have no effective antifungals available initially or for some time because fungi are evolutionarily close to humans making it hard to develop effective antifungals – and we need to consider this possibility as many terrestrial fungi do well in Mars simulation chambers including a strain of a black fungus sometimes pathogenic in humans

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[2015] We have many terrestrial fungi which do well in Mars simulation chambers suggesting in the other direction that it's a realistic possibility that Mars has fungi that would be able to grow on Earth. Many of our candidates for fungi that might live on Mars are rock inhabiting black fungi. These are able to adapt to extreme environments, hot and cold and other extremes such as high salinity, acidity, and dessication, and many have been able to colonize rocks in Antarctica (Selbmann et al, 2015).

One of these black fungi, Cryomyces antarcti was tested in the BIOMEX experiment simulating a Martian atmosphere, exterior to the ISS. At the end of the experiment it was not only still viable but showed only slight damage too fine to see with optical microscopy (Pacelli et al, 2017). These types of fungi have been given many names in the literature including "black yeasts" and "micro-colonial fungi".

[2014] One of these black fungi is closely related to human pathogens. That's *Exophiala jeanselmei MA 2853,* a rock inhabiting black fungus in moderate climates, which turned out to have the potential to survive and grow in the Mars simulation chamber of the German aerospace center with daily temperature changes from below -40°C to above 15°C and also simulating the day to night humidity cycle (Zakharova et al, 2014).

E. jeanselmei is sometimes a pathogen itself and its close relatives are sometimes fatal. In a review of 84 case reports since 1980, 2 out of 29 patients with normal functioning immune system died and 7 out of 55 immunocompromised cases died (Wu et al., 2022 : table 2). These were all classified at the time as E. jeanselmei, but many early cases may be other cryptic species of Exophilia that are clinically identical and can only be distinguished with gene sequencing such as such as *E. heteromorpha*, *E. lecanii-corni*, *E. oligosperma*, and *E. xenobiotica* (Zeng et al., 2007). E. jeanselmei itself is also sometimes an opportunistic pathogen of humans (Zeng et al., 2007). This for example is a case from 2010 confirmed by gene sequencing (Badali et al., 2010) as was the case studied by Wu et al. (Wu et al., 2022).

Fungal diseases are hard to diagnose. Let's look at aspergillus, our closest analogue to a fungal disease not adapted to humans. The test for fungal galactomannan (a component of the fungal cell wall) is 80% sensitive which means 20% of infections wouldn't be detected even if aspergillus is suspected (Brown et al, 2012:6).

However often aspergillus isn't the first guess of the doctor. Chronic pulmonary aspergillosis (CPA) has high mortality within 5 years, and is often confused with tuberculosis. It looks similar to tuburcuosis in medical images of the lungs, and also clinically. It can be distinguished from tuburculosis by testing for antibodies to Aspergillus. As of 2012 there was no standardised test for antibodies (Brown et al, 2012:6). There are many marketed tests now, but they are still not 100% reliable. The ELISA IgG antibody tests for CPA vary in accuracy but on average they are 93% reliable (sensitivity) but with 3% false positives (97% specificity) (as of 2020). One of the issues here is distinguishing between harmless colonization and the disease (Volpe Chaves et al., 2020).

Fungi are evolutionarily closer to humans than most microbes, which makes it harder to develop antifungals. The introduction of echinocandins and third-generation triazoles improved the options for antifungal therapy but they have had modest success in preventing death from fungi (Brown et al, 2012:6).

A fungal disease from Mars might be initially similarly hard to diagnose and confused with other diseases like tuberculosis. It might be hard to distinguish between harmless colonization by the fungus from Mars, and the invasive disease. It might take some time to develop effective tests for it. Also, it might be similarly hard to develop antifungals to protect against it.

NEW: Our immune system responses are highly specific to each of the three genera of opportunistic human fungal pathogens – without the necessary pathogen associated molecular patterns (PAMPS) we might all be immunocompromised to a new genus of fungi from Mars

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In the next section, we'll look at warnings by Sagan, Lederberg and others that there is a possibility that our immune system can't detect alien pathogens generally. First, let's look at a the opportunistic fungal pathogens.

Although Aspergillus isn't adapted to us, our immune systems are adapted to defend against it, and we'd likely have far more cases of severe Aspergillus without those adaptations.

Would our immune system be able to detect a fungal infection by an alien fungus from Mars, and if so could it stop it?

Our immune system probably stops many fungal infections by recognizing particular patterns, the pathogen-associated molecular patterns (PAMPs). It likely does this using pattern recognition receptors (PRRs) which then trigger the immune response.

These are targeted to the molecular patterns from the most common fungi that attack humans, species from three genera: Candida, Aspergillus, and Cryptococcus with different molecular patterns specific to each genus (Kumar et al, 2018 : table 1)

Looking at the two fungal genera that infect via the lungs, I have shown in bold the patterns and the receptors shared in common between the two genera:

Aspergillus fumigatus

> **PAMPs*:*** β-1,3-glucan, chitin, galactomannan, DHN-melanin
> **PRRs: TLR2**, CLRs (dectin-1, **−2**, mincle, DC-SIGN), NLRs (NOD1, **NLRP3**), CR3, PTX3 MelLec

Cryptococcus neoformans

> **PAMPs:** Mannose,capsular polysaccharide, glucuronoxylomannan
> **PRRs:** TLRs (**−2**,-4), CLRs (**dectin-2**, MR), NLRs (**NLRP3**)

Suppose hypothetically that our human immune system only ever encountered Cryptococcus, and never encountered Candida or Aspergillus. It would have three pattern recognition receptors it could potentially use with Aspergillus,

> **PRRs:** TLR2, Dectin-2 and NLRP3.

However, none of its acquired pathogen-associated molecular patterns would work with Aspergillus. It still wouldn't see it.

> **PAMPs:** None

Similarly, our immune system might not have genus specific PAMPs for a martian fungus in a novel genus with a shared terrestrial biology. It's not at all likely to have PAMPs for a fungus with a totally alien biochemistry.

So it seems indeed, that there is some potential that we might all be immunocompromised against a fourth opportunistically pathogenic genus of fungi from Mars. We are likely even more immunocompromised if challenged by fungi with a totally alien biochemistry.

It could also go the other way, that our immune system is overactive when we encounter alien life and we might get an allergic reaction, which can potentially even be life threatening.

- NEW: Possibility of an allergic response to harmless alien life – or indeed a new genus of familiar life - if it is recognized by the immune system but not by the inflammation dampening Treg cells - allergic bronchopulmonary aspergillosis affects around 4.8 million people globally and chronic pulmonary aspergillosis, affects 400,000 globally – these figures could be higher if a normally functioning human immune system doesn't recognize the need to dampen its response

But first lets look at the potential that our immune system doesn't recognize alien life at all.

Warnings by some astrobiologists such as Sagan and Lederberg that in worst case we could be in effect immunocompromised to an entire exobiology from Mars

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[question]

Some astrobiologists say that there is a possibility that more generally, in a worst case scenario, we might all be in effect immunocompromised to an entire exobiology from Mars. Joshua Lederberg, a key figure in early work on planetary protection (Scharf, 2016) put it like this (Lederberg, 1999b):

> ***Joshua Lederberg:*** *Whether a microorganism from Mars exists and could attack us is more conjectural. If so, it might be a zoonosis [infectious disease that jumps to humans] to beat all others*

In that paper he is looking at the dilemma of a parasite that if it proliferates too fast it risks killing its host and few parasites benefit from the death of the host, but if it proliferates too slowly it risks being overwhelmed by its host's immune system within a week to 10 days unless it can develop stealth tactics to continue to evade it after that.

So pathogens find a balance between the two. But a microorganism from Mars hasn't been through this process.

Lederberg goes on to argue our immune system and defenses are keyed to various chemicals produced by Earth life such as peptides and carbohydrates [A peptide is made up of amino acids like a protein but with a short chain, 2 to 50. A chain of 10 or more is sometimes called a polypeptide (University of Queensland, 2017)]. Mars life might use different chemicals.

> *On the one hand, how could microbes from Mars be pathogenic for hosts on Earth when so many subtle adaptations are needed for any new organisms to come into a host and cause disease? Dozens if not hundreds of bacterial genes need to work in concert to enable a microorganism to be a pathogen.* ***On the other hand, microorganisms make little besides proteins and carbohydrates, and the human or other mammalian immune systems typically respond to peptides or carbohydrates produced by invading pathogens.***

He concludes:

> ***Joshua Lederberg:*** *Thus, although the hypothetical parasite from Mars is not adapted to live in a host from Earth, our immune systems are not equipped to cope with totally alien parasites: a conceptual impasse."*

In another paper, he considers two possibilities, that martian life is mystified by us, or in the worst case, our immune system doesn't recognize the attackers as life, and does nothing to stop them, saying it likely takes as fine tuning for a microbe to moderate itself as to take on the defensive barriers of a strange host (Lederberg, 1999a).

> ***Joshua Lederberg:*** *Many serious emerging infections are zoonotic transfers, including HIV, hantavirus, plague, and tickborne rickettsioses. In many of these cases human infection is incidental to the natural history of the microbe. Probably most inter-species transfers are totally innocuous, hence invisible. Many others will be neutral. We pay close attention to those where the microbe-host balance is disrupted by the change in genomic environment, has not yet reached new equilibrium, and manifests a rule-breaker.*
>
> *It likely takes as delicate fine-tuning for a microbe to moderate itself as it does to take on the defensive barriers of a new and strange host.*
>
> *New zoonoses are not alien encounters, as the microbe involved usually has a history of successful parasitosis in another species- even if that experience is as distant as transovarian propagation in a tick.*
>
> *These earthly encounters raise questions for those concerned about interplanetary travel and ensuing exposure to microbes that might be found on other celestial bodies. If*

Martian microorganisms ever make it here, will they be totally mystified and defeated by terrestrial metabolism, perhaps even before they challenge immune defenses? Or will they have a field day in light of our own total naivete in dealing with their "aggressins"?

Technical note: from the context, "aggressins" doesn't seem to be used in its technical sense of a substance a microorganism produces to inhibit or destroy the host's ability to defend itself (Casadevall et al., 2001:338) since "our own total naivety" implies no immune response to inhibit.

Carl Sagan, discussing the potential effect of Martian life on humans, put it like this (Sagan, 1973)

> ***Carl Sagan:*** *On the one hand, we can argue that Martian organisms cannot cause any serious problems to terrestrial organisms, because there has been no biological contact for 4.5 billion years between Martian and terrestrial organisms. On the other hand, we can argue equally well that terrestrial organisms have evolved no defenses against potential Martian pathogens, precisely because there has been no such contact for 4.5 billion years.*

Perhaps microplastics would be a useful analog here. At 10 microns or less in diameter, they can potentially cross into the blood stream, for instance through the submicron barrier in the lungs, and access all organs and at 0.1 microns or less they can penetrate the skin through to the blood stream (Campanale et al, 2020). Our bodies are to some extent permeable to small particles that our immune system ignores.

So far, these ideas seem to have had no attention in the planetary protection literature since Lederberg's two papers. The paper (Lederberg, 1999a) has sixteen cites in Google Scholar, and the paper (Lederberg, 1999b) has seven cites in Google Scholar. None of these cites are to planetary protection discussions. The 2009 NRC Mars Sample Return study (SSB, 2009) doesn't cite or mention Lederberg's 2009 papers, see search results.

All this needs to be looked at by experts. This paper suggests that there is enough here to merit attention in a future Mars sample return study, as part of our assessment of what needs to be done to protect Earth.

The Mars sample return studies so far proceed almost entirely by analogy with specific examples of terrestrial pathogens, with little or no discussion of potential effects on terrestrial organisms of a totally different exobiology.

It's natural for out studies to focus on terrestrial examples as we have no actual examples of extraterrestrial life. However, Lederberg's papers show that it is possible to discuss possible effects of exobiology without any example lifeforms.

I have found no further discussion of these ideas in the planetary protection literature. I hope at some point this will be covered.

Meanwhile, to try to expand on his brief comments a little, it might help to look at some ways that our immune system would likely fail to recognize an alien exobiology as harmful. I made a start on this below:

- NEW: How our body's first line of defence could miss alien life –antimicrobial peptides might not work with an alien exobiology – and its second line of defence might also fail if dentritic cells fail to recognize the need to split alien life into antigens to present to T-cells

Then there are a couple of other possibilities that don't seem to have been considered in the planetary protection literature so far.

Even if terrestrial and martian life are mutually mystified, if we have lots of extraterrestrial spores they might cause problems to terrestrial biology similarly to nanoplastics and microplastics when those are in large quantities. I explore this below:

- NEW: Scenario of an alien biology that produces large numbers of spores that our immune system can't see and in turn do nothing to our bodies and are completely inert like microplastics and nanoplastics – even this could be harmful to terrestrial life

Then our immune system might overreact to an alien biology with an allergic response, or overreact to minor damage with inflammation.

- NEW: Possibility of an allergic response to harmless alien life – or indeed a new genus of familiar life - if it is recognized by the immune system but not by the inflammation dampening Treg cells - allergic bronchopulmonary aspergillosis affects around 4.8 million people globally and chronic pulmonary aspergillosis, affects 400,000 globally – these figures could be higher if a normally functioning human immune system doesn't recognize the need to dampen its response

I have been unable to find any expert treatment of these topics, so this is preliminary. There is no way that this review could cover those topics adequately. The main intention here is to suggest that these are topics that can be studied and need to be looked at.

Then just as our body's defences might not work with alien microbes, our antifungal and antibiotic medicines might not work with them either and it might take some time to develop an antibiotic that targets an alien biology.

- NEW: unrelated Martian life might not have the cell processes targeted by our antifungals and antibiotics – while related Martian life could be accidentally resistant like the accidental resistance to the new synthetic antibiotic quinolones in Shewanella algae and might transfer that resistance to terrestrial life

This is a more techy question about whether a martian pathogen could infect white blood cells like the microbe that causes Legionnella disease.

- NEW: Could the host of a martian pathogen on Mars be similar enough to protozoa to infect the white blood cells in our immune system as for Legionnaires' disease? This seems to be an open question

Then this is another topic that I can't find any previous discussion for – the potential for alien fungal pathogens of microbes.

- NEW: Microbes from Mars could have pathogens that can infect terrestrial microbes – example of fungal pathogens of phytoplankton and cyanobacteria – cyanobacteria depend on specific antifungal adaptations to protect against fungi in the chytrid phylum, so may have no adaptations to a novel fungal phylum from Mars

This is about whether the same issue of naivety of our immune system to alien pathogens could apply to all life including terrestrial microbial life.

- NEW: Claudius Gros's worst case scenario for forward contamination – if this scenario can be applied in reverse, nearly all higher life eventually goes extinct outside habitats, though it takes a long period of time

If you wish to skip the next few sections on how the immune systems of terrestrial life might fail to recognize an alien exobiology, or overreact to it, the next main section is:

- NASA's biological safety report agrees on the potential for an invasive Martian species to harm or displace terrestrial photosynthetic bacteria – but says life adapted to Martian conditions such as the temperatures and pressures plausibly wouldn't be able to survive on Earth – their own cite mentions Planococcus Halocryophilus, a microbe which lives in Arctic permafrost soils and likely grows in sub zero brine veins down to at least -15 °C, with an optimal growth temperature of 25°C and growth up to 37 °C (human blood temperature)

NEW: How our body's first line of defence could miss alien life – antimicrobial peptides might not work with an alien exobiology – and its second line of defence might also fail if dentritic cells fail to recognize the need to split alien life into antigens to present to T-cells

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If we find a second genesis of life based on a different alien biology, we might not be able to detect it easily with our instruments. (Carrier et al, 2020: *801*)

Meanwhile, it is important to note that if the life-form were based on another biochemistry, modern techniques might be too specific.

RNA world life might use fragments of RNA instead of proteins. It might not use amino acids. Or a second genesis might use a different backbone from RNA or DNA such as TNA or PNA. We look briefly at some of the research here:

- ESF study said values for required level of assurance and the size limit need to be revisited periodically based on changes in scientific knowledge and risk perception

Just as we might not be able to detect completely alien life with our instruments, our immune systems might not spot it either. This doesn't seem to be discussed in any detail in the planetary protection literature. We just have those two paragraphs by Lederberg which have never been followed up, at least not in the literature that cites the papers. It is a very technical subject. I will try to make a first start at it here, but it needs expert attention.

Our skin's first line of defence consists of sixteen broad spectrum antimicrobial peptides (Abdo et al, 2020 : table 1). A peptide is shorter version of a protein, made up of amino acids like a protein but with a short chain, 2 to 50. A chain of 10 or more is sometimes called a polypeptide (University of Queensland, 2017). Some of these very broad spectrum like Dermacidin which disrupts the cell walls of bacteria and others are more specific to particular types of microbe.

An alien microbe based on a different biology might break up the toxic peptides, or extrude the toxins, or might bind to them and so make them harmless. Terrestrial pathogens do all these things to defend themselves. An unfamiliar exobiology may do these things just because it is different from terrestrial life and functions in a different way (Peschel et al., 2006).

Some peptides are specific to particular genera of terrestrial life (Abdo et al, 2020 : table 1) so those would likely have no effect on an alien microbe.

The broadest spectrum peptides in the table still have to interfere with specific cell processes of particular types of terrestrial microbes while avoiding harming the host cells. This discrimination seems to be based on the composition of cell membranes. Microbes tend to have negatively charged outer cell walls while eukaryote cells (the cells with a nucleus, of multicellular life) are covered with various chemicals that make them neutrally charged (Hancock et al., 2000) (Lei et al., 2019).

In more detail these broad spectrum antimicrobials rely on negatively charged outermost acid groups in cell walls (acid groups donate protons to water to become negatively charged) to attract a positively charged cation. They then use molecules that are water attracting at one end and water repelling at the other end to bridge into the center of the cell wall and then across it similarly to the lipid bilayer that makes up the cell wall, and so are able to construct a breach in the cell wall (Hancock et al., 2006). There are various other ideas for how this works including the barrel staves or toroidal model which makes pores in the cell wall, or the carpet model where it disrupts the cell surface more generally (Lei et al: Fig 3). Dermadectin, one of the most common and potent broad spectrum antimicrobials in the human skin imbeds itself in the membranes of microbes to form an ion channel which greatly increases the permeability of the cell wall for water and ions and can dissipate the transmembrane electrical potential in as little as a ten thousandth of a second (Song et al, 2013). The cell then dies as it needs to maintain that potential to survive.

A peptide that constructs a breach in the cell wall like that could be very damaging to an alien microbe, if its biology is similar enough to terrestrial pathogens. However these natural antimicrobials are limited in potency because of the risk of harming the host and of harming beneficial microbes as well.

An alien biology might happen to have cell membranes that are neutrally charged on the outside, like eukaryote cell walls, making them immune to many of the antimicrobials.

More generally, alien microbes might escape harm because the cell walls of the alien microbe more closely resemble the cells of the host or beneficial microbes than typical terrestrial pathogens. Or it might be for the opposite reason that alien cells are just too different to be affected by the antimicrobials.

Lei, J., Sun, L., Huang, S., Zhu, C., Li, P., He, J., Mackey, V., Coy, D.H. and He, Q., 2019. The antimicrobial peptides and their potential clinical applications. American journal of translational research, 11(7), p.3919.

> *AMPs can exert antimicrobial effects without harming normal cells likely due to the positive charge(s) on the α-helix surface of AMPs can interact with negatively charged membranes of microbes, while the membranes of eukaryotic cells are composed of uncharged neutral phospholipids, sphingomyelins and cholesterol*

Some of the broad spectrum antimicrobials can also target fungi, which are eukaryotes, so have cell walls resembling the host. They bind to specific receptors in fungal cell walls to get inside, and then once inside they damage the myochondria that provide energy to the fungal cell (Lei et al., 2019 : mitochondrial attack). This might not work with an alien microbe which might not have those receptors.

There are many other types of antibiotic. Penicillin for instance binds to transpeptidase which is essential for cross linking in the final stage of cell wall synthesis to make rigid cell walls (Yocum et al, 1980). Microbes develop resistance to penicillin by using different enzymes for this cross-linking (Gordon et al, 2000). Similarly other antibiotics target specific enzymes and processes within living cells based on Earth's biochemistry (Kapoor et al, 2017).

An alien biochemistry might not have those enzymes or processes.

As a second line of defense, the adaptive immune system has to recognize a specific species of alien microbe as a potential intruder. To do this, its T-cells have to create antibodies to pre-processed pieces of the intruder, the antigens, on antigen presenting cells. These then tag the pathogen and are the signal for the neutrophils to dispose of them (a special type of white blood cell). But first, our body has to break up the alien intruder into antigens or the T-cells won't make any antibodies to them.

Before any of this can happen, our dendritic cells have to recognize the alien microbe as a potential pathogen using pattern recognition receptors. Once they recognize a pathogen, they capture it into a small container, a vesicle, where it is broken up into smaller particles in a process called "Receptor mediated phagocytosis" (recognition and ingestion of large particles). They then process these smaller fragments into antigens to present to the T-cells (Liu, 2016).

As we saw with fungi earlier, our immune system might not recognize the patterns of even a new genus of fungi from Mars.

- NEW: Our immune system responses are highly specific to each of the three genera of opportunistic human fungal pathogens – without the necessary pathogen associated molecular patterns (PAMPS) we might all be immunocompromised to a new genus of fungi from Mars

In this scenario, our dendritic cells don't have the receptors or patterns to recognize the alien organism. So they never process the invader into antigens to show to the T-cells, and the T cells never "see" the invader and mount no response.

So, in short, at the first line of defence, the alien organism might not be affected by the antimicrobials. It would be likely not to have the particular cell processes targeted by the more specific antimicrobials like penicillin. For the broadest spectrum antimicrobials, it might be either too different from terrestrial life or too similar to multicellular life in the way its cell wall works and it might also bind to the antimicrobials themselves or break them up or extrude them.

At the second line of defence, if the alien life doesn't match any of the standard patterns, as we saw could happen with a new genera of fungi, the dendritic cells might never notice the alien organism, and so never process it into antigens, and so our body never mounts an immune response.

If our immune system doesn't notice the alien microbes, then as for microplastics, at 10 microns or less in diameter, they can potentially cross into the blood stream, and access all organs through our lungs. At 0.1 microns or less even our skin is permeable to them (Campanale et al, 2020). Once the alien life is inside us it does whatever it does, e.g. hydrates fast and grows filaments seeking for nutrients it can use, in the case of an alien fungus resembling Aspergillus.

NEW: Worst case scenario - If a martian microbe can grow in the sea, soil, and fresh water like chroococcidiopsis, is adapted to spread in the wind in Martian dust storms, and outcompetes terrestrial biology, e.g. better at photosynthesis or nitrogen fixation, it could be found globally after introduction to Earth in weeks to months, and be one of the most common microbes in our soils and oceans in years to decades or sooner, far more common than nanoplastics or microplastics

As with the analogy of smoke detectors and house fires, we need to look at potential worst case scenarios here for proper contingency planning.

If martian microbes do escape and spread through Earth's biosphere, to start with, every microbiome will have many more of the terrestrial microbes. However, the alien biology might have some advantages over terrestrial life, such as

- more efficient photosynthesis, see below:
    - NEW: Martian life could be better at photosynthesis than terrestrial life since terrestrial photosynthesis works at well below its theoretical peak efficiency and the lower light levels on Mars might favour evolution of more efficient photosynthesis
- better at nitrogen fixation – nitrogen fixation is an energy demanding process and it's a challenge for cyanobacteria especially as they produce oxygen which reacts with the nitrogenase needing strategies to keep it separate (such as nitrogen fixation at night) (Bueno Batista et al., 2019)

  I haven't found papers looking at ways nitrogen could be adapted to be more energy efficient like the ones on improving on the efficiency of natural photosynthesis, but it seems likely that there is room for more efficient, less energy demanding nitrogen fixation
- not limited by some elements that terrestrial life requires, example, it might be able to use phosphorus in the absence of sulfur (Davies et al, 2009)
- a biology that can adapt to a wider range of temperature conditions and grow faster than terrestrial life, perhaps especially in colder conditions,

This could just be beneficial and lead to an enhanced Gaia. See:

- NEW: Enhanced Gaia – ways that introduced Martian life could be beneficial to humans, ecosystems and Earth's biosphere

But here we are looking at the possibility that it could be harmful.

Then
- its organics might be indigestible to terrestrial life, as for mirror life. Martian mirror life could be pre-adapted to digest normal organics because most of the organics on Mars are produced abiotically and so contain equal amounts of normal and mirror organics.

    - NEW: Example worst case scenario of a mirror life chroococcidiopsis analogue from Mars which gradually converts organics in ecosystems into indigestible mirror organics
- Another way that an alien biology could be indigestible to terrestrial life is if its proteins are based on different amino acids. There are numerous biologically feasible amino acids both naturally occurring or ones that an extraterrestrial biology might synthesize. See below:
    - NEW: Chroococcidiopsis indica produces an accidental neurotoxin, BMAA, which resembles serine and by replacing it, can cause protein misfolding – leading to the possibility that novel amino acids from a novel exobiology could also cause protein misfolding

In another scenario, Mars has a non terrestrial shadow biosphere which can't get from Mars to Earth, co-existing with a shared biosphere of terrestrial life. The shadow biosphere, though adapted to Martian conditions, never developed the ability to withstand the vacuum of space, pressures of ejection into space etc.

This would give the Martian shadow biosphere a tremendous advantage, even ir it has a completely novel molecular basis like TNA, or PNA, and perhaps using a different vocabulary of amino acids, or it doesn't use amino acids or proteins at all. In this scenario the martian life has co-evolved with terrestrial life on Mars for billions of years, and learnt to use terrestrial organics (if it is feasible to do so with its biology).

In this scenario:

- Martian life has adapted to digest terrestrial organics for billions of years giving it an initial advantage over terrestrial life which hasn't learnt to digest alien organics,
- Martian life might have evolved the ability to infect terrestrial microbes as for the chytrid fungi of terrestrial microbes. See below:
    - NEW: Microbes from Mars could have pathogens that can infect terrestrial microbes – example of fungal pathogens of phytoplankton and cyanobacteria – cyanobacteria depend on specific antifungal adaptations to protect against fungi in the chytrid phylum, so may have no adaptations to a novel fungal phylum from Mars

Then Mars might have a biosphere, or a shadow biosphere of minute nanobe sized cells with a more efficient, simpler biology, which lets it produce tiny cells beyond the lower limits of terrestrial life - like ribocells, or the nanobes in the Shadow Biosphere hypothesis which was considered some time back for terrestrial life.

Nanobes have several advantages, similarly to ultramicrobacteria but more so, whether it's a shadow biosphere or if this is the only form of life on Mars.

- alien cells might be numerically more numerous than terrestrial cells for the same mass
- very small cells could escape grazing by larger grazing amoebas which don't notice it
- very small cells could be able to use nutrients better in nutrient poor conditions like ultramicrobacteria

See:

- NEW: Closely related worst case scenario of a shadow biosphere of small mirror life nanobes that produce indigestible mirror life biofilms on Earth with small cells advantages that they take up nutrients faster and avoid protozoan grazing

The Martian life would be used to colder temperatures than much of Earth. That might slow it down initially.

However we'll see that some martian life might be pre-adapted to warm temperatures on Earth and rapidly adapt and spread through species sorting which could lead to rapid growth of species that can live at higher temperatures and even already have optimal growth at higher temperatures than currently found in Jezero crater (or whichever environment life is returned from):

- NASA's biological safety report agrees on the potential for an invasive Martian species to harm or displace terrestrial photosynthetic bacteria – but says it's plausible life adapted to Martian conditions such as the temperatures and pressures plausibly wouldn't be viable on Earth – their own cite mentions Planococcus Halocryophilus, a microbe which lives in Arctic permafrost soils and likely grows in sub zero brine veins down to at least -15 °C, with an optimal growth temperature of 25°C and growth up to 37 °C (human blood temperature)

And following sections including

- Mars surface temperatures can reach 35°C in the shade in summer – some species of Martian surface life may be pre-adapted to hotter, even hydrothermal conditions in geologically recent Mars – and emerge through species sorting – persist in small numbers in surface biofilms and spread and adapt rapidly when they encounter far warmer conditions

So, this seems a potential scenario we need to look at. In this scenario we return a microbe which can replicate in a terrestrial environment right away, and is also pre-adapted to be able to compete with terrestrial life.

In the forwards direction from Earth to Mars, Carl Sagan once calculated a terrestrial microbe with a slow generation time of two months could, in the absence of other ecological limitations reproduce to the point where there is as much of it on Mars as in all the terrestrial soils, within a decade (Sagan et al, 1968). Of course that will never happen on Mars because it isn't habitable enough to reach such levels but it could happen to Martian life returned to Earth, this time limited mainly by competition with terrestrial microbes. Terrestrial microbes often have generation times of hours rather than months.

A microbe similar to the blue-green algae chroococcidiopsis and adapted to Mars might
- Be able to live in the oceans, deserts, and soil
- Spread easily in the wind (because it would have adapted to spread in Martian winds)
- Within weeks to months, if it spreads easily in the wind, there might be spores everywhere on Earth.

- If the microbe finds the sea, or soils, or fresh water or any habitat to its liking, it might not be many years or decades before it is one of the most common species in that habitat and is found in large numbers almost everywhere on Earth.

Eventually the numbers of alien microbes might build up, potentially rapidly with exponential growth. It might become one of the most common microbes in our soils, oceans and lakes in a few years to decades

Even if there are far fewer than for terrestrial microbes there may be many more than for nanoplastics or microplastics in our environment. So that leads to our next scenario, where terrestrial and alien life ignore each other but the vast numbers of alien spores still can cause problems for us similarly to the inert materials of nanoplastics and microplastics.

NEW: Scenario of an alien biology that produces large numbers of spores that our immune system can't see – in this scenario the alien spores also do nothing to our bodies and are completely inert like microplastics and nanoplastics – even this could be harmful to terrestrial life

Next section – all sections – previous section

Even if the alien biology of martian microbes is as mystified by terrestrial biology as our immune system is mystified by it, it could still harm us, especially if we have large quantities of cells of alien biology in our biosphere.
They could enter the blood stream via our lungs, or smaller particles could enter via our skin, and then access all organs (Campanale et al, 2020).

We'll look here at the simplest problem, from the analogy with nanoplastics and microplastics, that inert alien spores get covered in sticky plasma, and clump together to cause blood clots.

Polystyrene nanoplastics can form Polystyrene-protein coronas enclosing them, through interaction with blood. This new biological entity hides them from the immune system and lets them translocate to all organs. (Gopinath et al, 2019)

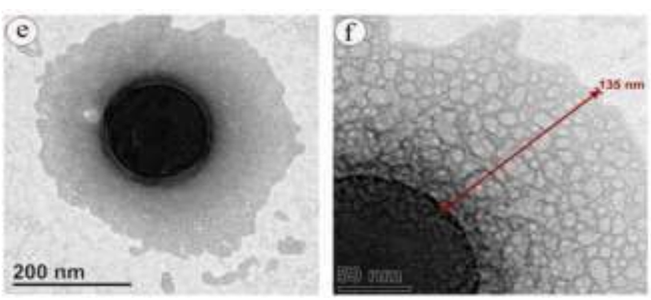

Figure 21: Coronated polystyrene nanoplastics. An alien biology ignored by the immune system might perhaps interact with the blood plasma in the same way on entering the blood and form alien chemicals /

protein coronas that would hide it from the immune system and make it more likely for our cells to ingest them

[Detail from figure 2 of (Gopinath et al, 2019)]

These can merge to make larger accumulations of cells since they stick to each other.

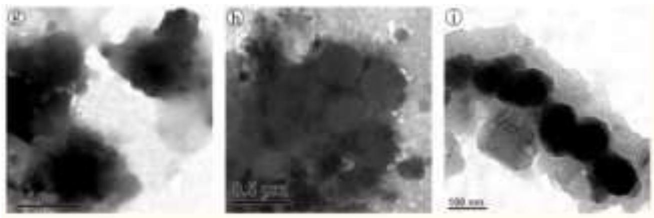

Figure 22: Coalescence of protein coronas of nanoplastics and microplastics in human blood plasma. The levels of nanoplastics in our bodies are low enough for this to not be a serious issue. However if coronated alien cells do this then it could lead to circulation issues and heart attacks.

[Detail from figure 2 of (Gopinath et al, 2019)]

We don't get noticeable problems from micro and nanoplastics, because they are few in number and only some produce these polystyrene - protein coronas. But if at some time in the future our environment is filled with trillions of alien spores, with our body essentially permeable to them, and if these also get covered in similar protein coronas in our blood stream, this seems a possible scenario to consider, whether clumps of alien spores stuck together by protein coronas in our arteries may cause problems such as heart attacks.

There are other more complex issues with nanoplastics and microplastics covered in the next section such as chronic inflammation that again we don't get as they are too few in number.

NEW: Possibility of an allergic response to harmless alien life – or indeed a new genus of familiar life - if it is recognized by the immune system but not by the inflammation dampening Treg cells - allergic bronchopulmonary aspergillosis affects around 4.8 million people globally and chronic pulmonary aspergillosis, affects 400,000 globally – these figures could be higher for an allergic response to extraterrestrial life - if a normally functioning human immune system doesn't recognize the need to dampen its response

Next section – all sections – previous section

We have been looking at the case where alien life isn't noticed by our immune system. Now we'll look at the opposite problem. Suppose, instead, the alien organism is detected, and processed into antigens by the dendritic cells, which are then taken up by T-cells.

The challenge for the immune system is to get the right balance between not responding to the alien exobiology at all and over responding in an allergic reaction.

So far, I have found no discussion of the potential for allergic reactions to an alien biology in the planetary protection literature. It seems to deserve attention in future backward contamination studies. Putative martian life won't be harmless if it can cause severe allergic reactions. In the worst cases these can even be fatal.

Our immune system has to make sure that its T cells don't attack the body's own cells, and don't harm beneficial microbes either. One of many ways it does this is to use Treg cells that have an anti-inflammatory effect (Clark, 2010)

> *The immune system is faced with the difficult problem of mounting immune responses to dangerous pathogens while maintaining tolerance to the body's own tissues and to harmless or commensal organisms. Regulatory T cells (Tregs) are one of many mechanisms developed by the immune system to enforce tolerance to harmless and self antigens.*

These dampen down allergic responses to harmless microbes, for instance in the lungs the Treg cells prevent allergic responses to dust mites, Aspergillus fumigatus and plant pollen. Similarly Treg cells in the gut and other barrier tissues help to dampen down responses to the many different species of microbes we are exposed to (Attias et al., 2019).

Our immune system also has to clear the aspergillus microbes from our lungs, but at the same time it has to avoid over-reacting in a harmful inflammatory response.

This response is modulated by T-helper cells and almost all classes of T-helper cells are involved in this response and need to be finely regulated in a healthy individual. The most important ones for our adaptive immune response to aspergillus are the Th1, Th17, Th22, Th2, Th9, Treg and Tr1 cells (Dewi et al., 2017).

Our Treg cells might

- misrecognise the alien life as familiar and dampen down the response when our body really needs protection,
- for harmless life, dampen the response down so much it can't clear the microbes from our lungs
- fail to dampen down an allergic reaction to a harmless alien microbe.

So there is a delicate balancing act here. It's not clear how our immune system learns to respond appropriately to harmless microbes with the allergen specific Treg cells. But there's evidence children exposed to allergens early on in dairy farms are less likely to develop asthma, especially if they have early life exposure to hay, unprocessed cows milk, manure and contact with cows and straw (Deckers et al., 2021). If this can be generalized to life with an alien biology, then since we all have no previous exposure to alien life, all except the youngest children might be prone to allergic reactions to it. That's assuming that our immune system is able to develop Treg responses to it to moderate the allergic response.

Allergic bronchopulmonary aspergillosis affects 2.5% of patients with asthma, and an estimated 4.8 million people globally. Chronic pulmonary aspergillosis (CPA) affects around 400,000 globally and only occurs in people who are not immunocompromised with symptoms of "weight loss, profound fatigue, productive cough, significant shortness of breath, and life-threatening hemoptysis [spitting out of blood from the lungs]" (Denning et al., 2013).

So far we've looked at the response to the alien invader itself.

Our immune system can also respond to the damage even if it can't see the invader, with DAMPs (Damage Associated Molecular Patterns) a bit like the PAMPs mentioned above, but they respond to cells that get damaged, rather than the agents that damage them.

DAMPs help trigger inflammation, which can turn the area of your body red. That's because of disease fighting cells leaking out of the blood stream into the surrounding tissue. However sometimes the inflammation can cause more damage leading to DAMPs responding to the damage caused by the inflammation they themselves triggered in a positive feedback loop leading to chronic inflammation (Cunha et al., 2012). DAMPs are involved in many chronic inflammation disorders (Roh et al, 2018).

DAMPS are also involved in sterile inflammation, inflammation caused by over reaction to non living particles, such as the reaction to silicon particles in silicosis. Detailed imaging shows the white blood cells called macrophages try to destroy the silica particles, and fail, which damages the white blood cells. That leads to the inflammation response and feedbacks leading to the chronic diseaise (Kazazian, 2014:4). We also get sterile inflammation to other non living particles such as urea crystals (in gout) and microplastics.

So this seems another possible scenario, that the alien biology completely ignores our biology but the alien microbes either contain material our white blood cells can't destroy, or perhaps a hard coating to protect themselves from Martian dust-storms. They could lead to an inflammation response without any direct harm. In this scenario our immune system harms itself in its attempts to attack them.

Perhaps sterile inflammation from microplastics is our closest analogy to sterile inflammation from a mutually mystified alien biology (Yang et al, 2022). It's broadly similar to other forms of sterile inflammation. This figure shows one proposal for what may be happening in detail with microplastics. The reactive oxygen species, DAMP, inflammation and cell death are all detected but the other details need to be clarified.

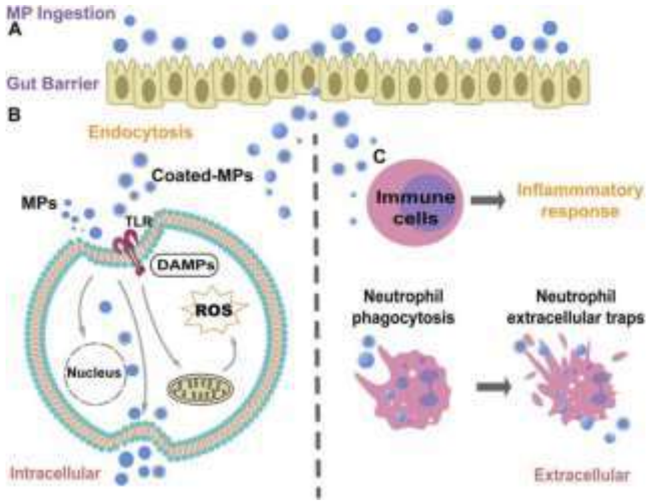

Figure 23: figure 4 from (Yang et al, 2022)

First, coated microplastics (MP) shown in blue get taken up by the white blood cells (macrophages). The microplastics interfere with the mitochondria, the energy powerhouses of the cell that turn oxygen into energy. This leads to a build up of very reactive highly oxygenated chemicals like peroxides, perchlorates etc which leads to oxidative stress (Yang et al, 2022).

Next in response to the oxidative stress, the white blood cell may self destruct (apoptosis or programmed cell death) (Yang et al, 2022).

The DAMPs then may be activated by the damage to the white blood cell, which triggers an inflammation response. When the white blood cell breaks open, it may release the microplastics to start the process again (Yang et al, 2022).

Another thing that can happen is that the immune system makes antibodies to pathogens that got stuck in the protein coronas that got stuck to the microplastics. When those antibodies attach to the pathogens, that attracts another type of white blood cell, the neutrophils that look for antibody tagged objects to destroy. The neutrophils also try to deal with the microplastics with no success. Like the macrophages, they try to deal with the microplastics, with no success and may self destruct. But this time the neutrophils form tendrils extended from the cells, called “extracellular traps” which trap the microplastics (Yang et al, 2022).

The protein coronas can also take up external pollutants and chemicals which increases the toxic effects of the coated microplastics (Yang et al, 2022).

In another scenario chemicals that make up the alien microbes are toxic for our body or they get degraded to toxins by our immune system or converted to toxins by interacting with our biology. For details see above

- NEW: An unrelated exobiology may produce many novel bioactive compounds which could be of great benefit, but the difference in biochemistry could also lead to more accidental toxins than terrestrial life, and in some scenarios, the internal chemistry of an unfamiliar exobiology could be accidentally toxic

In short, if our immune system does detect totally unfamiliar alien life, we found it has a delicate balance between under and over-reaction. In the previous sections we found that if the immune system misrecognizes an alien pathogen as harmless, the pathogen can enter the blood stream and access all organs unopposed, as for microplastics.

- NEW: How our body's first line of defence could miss alien life –antimicrobial peptides might not work with an alien exobiology – and its second line of defence might also fail if dentritic cells fail to recognize the need to split alien life into antigens to present to T-cells

Even if it's harmless, large amounts of harmless material entering the bloodstream could cause problems such as blood clots.

- NEW: Scenario of an alien biology that produces large numbers of spores that our immune system can't see and in turn do nothing to our bodies and are completely inert like microplastics and nanoplastics – even this could be harmful to terrestrial life

Then in this section we found that if our immune system misrecognizes benign alien life as harmful, it could lead to an allergic response to a harmless microbe.

We also found another possible kind of over-reaction, that our immune system could overreact to minor damage with inflammation, or it could respond to damage caused to white blood cells in the immune system which harm themselves trying to remove particles produced by alien life in the lungs, which could build up into chronic inflammation similar to gout or silicosis. Also if the alien microbes or spores behave similarly to some microplastics, relatively inert but able to bind to proteins, this could form a protein corona around the microplastic, then our white blood cells might try to dispose of the spores and destroy themselves in the process. Our adaptive immune system could also make antibodies to pathogens that get stuck in the protein coronas and try to eliminate those and again destroy themselves. If this is possible, the neutrophils would also destroy themselves trying to clear the antibody tagged spores, which would contribute to the chronic inflammation in an over-reaction to otherwise relatively harmless microbes or spores.

NEW: unrelated Martian life might not have the cell processes targeted by our antifungals and antibiotics – while related Martian life could be accidentally resistant like the accidental resistance to the new synthetic antibiotic quinolones in Shewanella algae and might transfer that resistance to terrestrial life

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If a pathogen from Mars did evade our immune system we'd turn to our antibiotics and antifungals to treat it. But Martian life might be naturally resistant to all our antibiotic and antifungals without ever encountering terrestrial life.

Let's take penicillin as an example. It binds to transpeptidase which is essential for cross linking in the final stage of cell wall synthesis to make rigid cell walls (Yocum et al, 1980). One way microbes develop resistance to penicillin is by using different enzymes for this cross-linking (Gordon et al, 2000). It is similar for our other antibotics. They target specific enzymes and processes within living cells based on Earth's biochemistry (Kapoor et al, 2017). An alien biochemistry might not have those enzymes or processes.

We have only a few effective antifungal medicines, making antifungal resistant microbes a problem (Cowen et al, 2015). Alien life might be naturally antifungal resistant, if they don't have the biochemistry targeted by antifungal medicines.

Eventually the greater difference between terrestrial and alien life might be a weakness for the martian pathogens, as we develop medicines that target an alien biochemistry or even a new genus of terrestrial fungi. There is some work on broad spectrum antifungals, similarly to our natural broad spectrum antimicrobial peptides. Perhaps something similar would work for alien biology. The challenge, as for their use against terrestrial microbes, is to provide them in a way that avoids harming the host (Hancock et al., 2006).

Whatever the mode of action of a new antibiotic targeting alien life, it takes much expense and a great deal of research to develop a new antibiotic or antifungal. It is easy to find substances that kill bacteria. The challenge is to find substances that kill bacteria, and also don't harm humans. For novel classes of antibiotic 1 in 30 completes the research process. The process typically takes ten to 15 years and costs about $1 billion for each new antibiotic or antifungal (Welcome Foundation, n.d.). It would be a top priority, and that could speed up the process with larger trials etc. But there could be a period of time after first encountering the alien life when we don't have any effective antibiotics to treat it.

Also the search for a new antibiotic for humans usually starts from naturally occurring antibiotics in other organisms. With pathogens based on an alien biochemistry there might not be any naturally occurring antibiotic candidates to use as a basis for this research.

Another possible issue is that closely related Martian life might have antibiotic resistant genes it can transfer to terrestrial life. When human pathogens develop antibiotic resistance, this often comes from other microbes by horizontal gene transfer, as they arise too quickly for the microbes to evolve it themselves. These resistance genes are found for every type of antimicrobial (Martínez, 2012). They didn't evolve in response to our antifungals as they are found even in microbes that have been isolated in a cave for 4 million years (Bhullar et al., 2012).

Many of our naturally occurring antibiotic resistance genes probably originate in microbes that make those antibiotics themselves and need the resistance gene to protect themselves from their own antibiotics. But the gene that gives antibiotic resistance to quinolones, a new non naturally occurring synthetic antibiotic, seems to have originated in a Shewanella algae which doesn't produce antibiotics itself. It seems likely to have a different role in it (Martínez, 2012). So related martian microbes might be accidentally antibiotic resistant in the same way.

Shewanella algae is an example to show even related Martian microbes such as a new genus of fungi could have antibiotic resistance through genes evolved for other purposes in the novel multiply extreme conditions on Mars. This might accidentally lead to their internal processes changing in ways that make even our synthetic antibiotics no longer effective. Then as with Shewanella, they might transfer this resistance to terrestrial life (Martínez, 2012).

NEW: Could a martian pathogen on Mars have a host similar enough to protozoa so that it can infect white blood cells in our immune system as for Legionnaires' disease? This seems to be an open question

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Here we return to Warmflash's comment based on the analogy of Legionnaires' disease which uses the same method to infect white blood cells (phagocytes) of our immune system as it uses to infect protozoa in biofilms: (Warmflash, 2007):

> *In essence, all that a potentially infectious human pathogen needs to emerge and persist is to grow and live naturally under conditions that are similar to those that it might later encounter in a human host. On Mars, these conditions might be met in a particular niche within the extracellular environment of a biofilm, or within the intracellular environment of another single-celled Martian organism.*
>
> *It is important to note the numerous biofilms observed aboard the Mir space station, which were found on surfaces and within water plumbing. These films were often multi-species and included bacteria, fungi, and protozoa.*
>
> *To be sure, the genetic similarity between humans and protozoa is much greater than could be expected between humans and the Martian host of a Martian microbe.*
>
> *Even in the context of a planetary biosphere that is limited to single-celled life, and even where there is unlikely to have been a co-evolution between agent and host organism, the possibility of infectious agents, even an invasive type, cannot be ruled out.*

Could there be protozoa on Mars, or more generally, larger bacterial grazers that play a similar role in biofilms?

Many protozoa can resist extreme conditions including dessication and are common in terrestrial soils (Stout, n.d.). Anaerobic protozoa can reach up to a quarter of the growth efficiency of aerobic protozoa (Priya et al, 2008). Also, Stamenković showed cold martian brines can in principle take up oxygen to a surprising degree so there may be aerobes in them too (Stamenković et al, 2018)

So it seems plausible a martian ecology could include an analogue of protozoa.

If so they might well have pathogens. That leads to the next question – could Martian hosts of these pathogens be related to terrestrial protozoa? I haven't been able to find any panspermia studies for protozoa. They can be very resilient, but it's a big ask for a large protozoan host to survive transfer from Mars to Earth on a meteorite.

If Martian hosts are unrelated, Warmflash suggests it is an open question for now. How well would the defences of a protozoan respond to a parasite of a protozoan analogue from Mars?

If we include the possibility of a different exobiology altogether, we have a similar question to the one tackled by Sagan and Lederberg, extended to diseases like Legionnaire's disease that infect protozoa and may be able to infect the white blood cells in our lungs (phagocytes).

So far we've been looking at humans as an example of multicellular life. But would even a single cell microbe be able to defend itself from an alien exobiology? In the next section we look at this using the analogy of fungi again.

NEW: Microbes from Mars could have pathogens that can infect terrestrial microbes – example of fungal pathogens of phytoplankton and cyanobacteria – cyanobacteria depend on specific antifungal adaptations to protect against fungi in the chytrid phylum, so may have no adaptations to a novel fungal phylum from Mars

Next section – all sections – previous section

This is another topic that seems to be new to the planetary protection literature. We saw that fungi like aspergillus could affect humans without being adapted to us,

- NEW: NASA's sample return biological safety report mentions an opportunistic fungal pathogen, Candidiasis adapted to humans – but misses the counter-example of Aspergillus, not adapted to us – an estimated 200,000 life-threatening cases of invasive aspergillosis a year – mortality 30% to 95% - invasive because of capabilities martian life may share such as its ability to respond quickly to rapid changes in humidity and temperature, very efficient at taking up nutrients and storing them, and able to tolerate low oxygen levels in the lungs

However, fungi infect microbial hosts too, including protozoa (Gonçalves et al., 2019), biofilms, phytoplankton and cyanobacteria. Most fungal pathogens in freshwater and sea water are chytrids, the informal name for the Chytridiomycetes class (the only class) in the Chytridiomycota phylum (Comeau et al, 2016). Class is a couple of levels up from Genus in the Linnean classification (Domain, Kingdom, Phylum, Class, Order, Family, Genus, Species) (Boundless, 2022).

The chytrid class is best known for the amphibian fungal disease chytridiomycosis which is caused by the fungus (Batrachochytrium dendrobatidis), which had severe effects on many amphibian species around the world (Australian government, n.d.). However it is also the main class of fungi that attack microbes.

Chytrids have a fossil record that goes back half a billion years and are the simplest of the fungi (Roehl, 2016). They are the only fungi with zoospores ("baby" fungi) that can swim to find a new host to infect using hair-like flagella to propel themselves. Some chytrid species live on dead organics, others are parasites of algae, microscopic worms, plants and amphibians. Some are useful in ecosystems because of the way they can break up cellulose, chitin and keratin (Roehl, 2016).

Some chytrid fungi don't need oxygen (anaerobic). Like other fungi (and higher life), they do have cells with a nucleus, called eukaryotes, and other smaller organelles (miniature organ-like structures) within the cells that generate energy for the cell. However, where most eukaryotes use mitochondria as their energy source, which use oxygen, eukaryotes that grow without oxygen use hydrogenosomes (van der Giezen, 2002). These anaerobic chytrid fungi break up cellulose in the stomachs of ruminants like sheep and cows, so chytrids are often useful. However, they can also be pathogens and harm their hosts.

As an example of a chytrid pathogen, Rhizophydium megarrhizum infects blue-green alge (cyanobacteria). It begins its life cycle as a free swimming zoospore which actively looks for cyanobacteria and phytoplankton to infect. It lets its host capture it (encystment) then penetrates the host and extracts nutrients, killing it. It is a generalist which adapts quickly to a new host species in just 200 days (Agha et al, 2018).

Cyanobacteria (blue-green algae) defend themselves from chytrid fungi similarly to the way humans defend themselves from fungi, using peptides specific to this genus of fungi. The antifungals they use are microcystins, microviridins, or anabaenopeptins.

In one study, researchers used genetic engineering to knock out the capability of a strain of the cyanobacteria Planktothrix to make these antifungals. When they did this, the cyanobacteria lost its resilience to the fungi. In one example the wild type cyanobacteria were completely immune to one of the chytrid strains they studied while it could infect all the cyanobacteria mutants even with just one of these classes of antifungals removed (Rohrlack et al., 2013). So the cyanobacteria seem to need all three types of antifungal for protection against chytrids.

So, in this scenario, terrestrial phytoplankton and blue-green algae encounter a novel fungal phylum from Mars already adapted to infect microbes on Mars, also perhaps able to infect Martian biofilms, lichens, or protozoa analogues just as the chytrids do on Earth, but they aren't chytrids, they are a new genus or class. Terrestrial microbes wouldn't have any antifungals adapted to this phylum, and so, might have no resistance to it.

After spreading through the terrestrial microbes, a novel fungal phylum from Mars could also evolve to attack higher life, as for the disease of amphibians. But we have already seen that fungal diseases from Mars might already be able to infect higher life without any need for adaptations. See above:

- NEW: NASA's sample return biological safety report mentions an opportunistic fungal pathogen, Candidiasis adapted to humans – but misses the counter-example of Aspergillus, not adapted to us – an estimated 200,000 life-threatening cases of invasive aspergillosis a year – mortality 30% to 95% - invasive because of capabilities martian life may share such as its ability to respond quickly to rapid changes in humidity and temperature, very efficient at taking up nutrients and storing them, and able to tolerate low oxygen levels in the lungs
  [and following sections]

So the main interest here is that fungi from Mars could also give an example of a disease of microbes. This might even extend Sagan and Lederberg's scenario to microbes. We look at this further below:

- NEW: Claudius Gros's worst case scenario for forward contamination – if this scenario can be applied in reverse, nearly all higher life eventually goes extinct outside habitats, though it takes a long period of time

This is not a human extinction scenario but it is of course a scenario to avoid

- Humans could survive even Lederberg's scenario and even Gros's scenario (in reverse) by covering Earth with large enclosed habitats using modern technology – and we could preserve nearly all our biodiversity – over millions of years the result may have a more diverse biochemistry with interesting new lifeforms – but if these are possible scenarios they are ones to avoid

NASA’s biological safety report agrees on the potential for an invasive Martian species to harm or displace terrestrial photosynthetic bacteria – but says it’s plausible life adapted to Martian conditions such as the temperatures and pressures plausibly wouldn’t be viable on Earth – their own cite mentions Planococcus Halocryophilus, a microbe which lives in Arctic permafrost soils and likely grows in sub zero brine veins down to at least -15 °C, with an optimal growth temperature of 25°C and growth up to 37 °C (human blood temperature)

Next section – all sections – previous section
[question]

NASA’s biological safety report agrees that planetary protection must consider not just human health but the entire biota of Earth. It agrees that if an invasive Martian species is possible, it could have serious effects, for instance it could potentially harm or even displace photosynthetic bacteria (Craven et al., 2021:6-7):

> *Photosynthetic bacteria such as Prochlorococcus are among the most abundant organisms on Earth and intensely important for the health of oxygen-respiring organisms, such as humans and animals. … Planetary protection must consider not just human health directly, but the entire biota of Earth.*

They then give a list of ways that ecosystems can be damaged,

- Direct cellular infections (which they consider to be unlikely – but as we saw in the previous sections, it needs to be considered as a possibility even for humans as well as microbes)
- Competition for resources
- Production of biotoxic metabolites
- Displacement of organisms.

They conclude:

> *Planetary protection must consider not just human health directly, but the entire biota of Earth.*

They then argue that Martian microbes wouldn’t be able to survive on Earth. This is a little puzzling. If they are so confident Martian life can’t survive on Earth, why was there a need for a section on human pathogens? They don’t give any cite to previous use of this argument in the literature and as I said the only previous occurrence I can find is Zubrin’s op. ed. See above:

- NASA’s biological safety report for the samples argues that martian life has a near zero chance to harm us because it didn’t co-evolve with us and that plausibly it would be unable to survive on Earth because it's used to extreme conditions on Mars – these arguments were previously presented in an op. ed. by Zubrin in 2000 – planetary protection experts at the time found many errors in this reasoning and said it was like a recommendation to build a house without smoke detectors

However it is clear they find this argument convincing, as did the authors who used this reasoning to support the draft EIS. Others are likely to find it convincing too. So we do need to look at it carefully.

Since this argument has never been suggested in the mainstream planetary protection literature as a possibility, there isn't any previous discussion to rely on. So we need to go into a fair bit of detail to discuss it properly.

For this argument they use examples of extremophiles that can't live in our normal habitat to argue it's plausible any martian microbe would not be viable on Earth, and so, that martian life couldn't cause any environmental issues (Craven et al., 2021:6-7)

> *There are many described extremophiles that may survive in environments that are extreme to human or animal life* ***(e.g. extremes of temperature or pressure)*** *but do not survive under conditions in our normal habitat* (Merino et al. 2019) *… Thus, it is plausible that any Martian microbe, after it arrives on Earth,* ***would not be viable on Earth due to a lack of its required Martian nutritional and environmental conditions****.*
>
> *[bolding added for the two examples in brackets]*

This has a major omission, polyextremophiles that live in a wide range of extreme environments and can often also live in normal environments.

Their own cite (Merino et al, 2019) includes one remarkable polyextremophile, amongst many extremophiles that can only tolerate a narrow range of conditions. The widest range of all in Merino et al's table is Planococcus Halocryophilus with a salinity range 0 to 19% and temperature range -15 °C to 37 °C

Strain	Domain	Extremophile Type	Isolation ecosystem	Temperature (°C)
Picrophilus oshimae KAW 2/2	*Archaea*	Hypercidophile	Hot springs, Solfataras	47–65 (60)[a]
Serpentinomonas sp. B1	*Bacteria*	Alkaliphile	Serpentinizing system (water)	18–37 (30)
Methanopyrus kandleri 116	*Archaea*	Hyperthermophile	Deep-sea hydrothermal vent	90–**122** (105)
Planococcus halocryophilus Or1	*Bacteria*	Halopsychrophile	Sea ice core	**−15**–37 (25)
Halarsenatibacter silvermanii SLAS-1	*Bacteria*	Haloalkaliphile	Soda lake	28–55 (44)
Thermococcus piezophilus CDGS	*Archaea*	Piezothermophile	Deep-sea hydrothermal vent	60–95 (75)
Haloarchaeal strains GN-2 and GN-5	*Archaea*	Xerophile	Solar salterns (brine)	nr

[a]*Data presented as range (optimum) for each parameter. nr, not reported in the original publication.*

Figure 24: (Merino et al, 2019: table 3)

This is p. Halocryophilus Or1 isolated from Canadian permafrost (Mykytczuk, 2012), likely grows in sub-zero brine veins around soil particles at an ambient temperature of around -16°C. The researchers found it has an optimal growth temperature of 25°C and can continue to grow right up to 37 °C (human blood temperature) tested (Mykytczuk et al., 2013).

The -15 °C in that table isn't likely to be the lowest limit for growth as p. Halocryophilus Or1 shows metabolic activity down to at least -25 °C which is the lowest temperature tested (Mykytczuk et al., 2013). It's hard to study growth at low temperatures, as it takes 1,000 to 10,000 years for microbes to successfully colonize granite in the McMurdo dry valleys. (Rummel et al , 2014:894) *(Sun et al, 1999)* So it's certainly possible that p. Halocryophilus can grow colonies extremely slowly at −25 °C. It might be able to grow at even lower temperatures as that's the lowest tested for metabolic activity.

On the lower limit of the temperature range for life, Merino et al. say *(Merino et al, 2019):*

> ***Around -26°C to -10°C, microbial cells will likely become vitrified (without intracellular freezing), enabling cells to survive low temperatures***
>
> ***…Thermodynamic considerations suggest that life might be impossible below -40°C, thus the current theoretical boundaries for life are -40°C to 150°C. It is still possible, however, that the boundary conditions of life might extend past these limits, and the surpassing of previous historical theoretical limits suggest that future studies might unveil unexpected adaptation strategies.***

This seems another example of incomplete citing as the Sterilizing Working Group don't mention this organism in their own cite, and don't give any plausibility argument to explain how adaptations to lower temperatures on Mars would mean an organism like this couldn't survive on Earth.

Pressure is the only other example extreme condition they specifically mention(Craven et al., 2021:6-7)

> **“*(e.g. extremes of temperature or pressure)”***.

But they don’t give any reasoning to say how adaptation to low pressure could make it hard for a Martian extremophile microbe to live on Earth. Merino et al. only mentions extremophiles that require high pressure (Merino et al, 2019).

> “Several hyperthermophiles (growth at >80°C) must grow at high pressure conditions because high pressure allows water to remain liquid at higher temperatures, with an upper theoretical limit of 407°C at 29.8 MPa pressure”

That’s not relevant to the low pressure of the Martian surface in Jezero crater.

On low pressure, Merino et al. say several terrestrial organisms can survive exposure to space conditions for months to years and say low pressure is not likely to affect microbial survival (Merino et al, 2019).:

> *In contrast to high pressure environments, the low pressure found at high altitude in mountain formations (0.0033 MPa at the summit of Mount Everest) is unlikely to affect microbial survival per se, and the lowest pressure is found in space vacuum or low Earth orbit (10^{-13} to 10^{-10} MPa). … Despite this, several prokaryotes, fungi, and lichen can survive exposure for several months to years under space conditions*

There isn’t anything here to suggest microbes adjusted to the lower atmospheric pressures on Mars would have problems in air at terrestrial pressures. Indeed it shows some microbes at least can tolerate both low pressure and terrestrial pressures.

NASA’s biological safety report says a martian microbe might be unable to find its required nutrients on Earth – many microbes find almost all the nutrients they need except water, and sunlight, from basalt which is abundant on both Mars and Earth

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[question]

The samples biological safety report also mentions nutritional requirements (Craven et al., 2021:6-7)

> *Thus, it is plausible that any Martian microbe, after it arrives on Earth,* ***would not be viable on Earth due to a lack of its required Martian nutritional and environmental***

conditions*.*

This isn't elaborated on either.

Many microbes that live in and on basalt as prime producers and get nearly all their nutrients from basalt. They would find the same nutrients on Earth as on Mars.

There are some nutrients that are common on Mars and rare on Earth such as the perchlorates (which are found in some terrestrial deserts). But it doesn't seem plausible that ALL martian microbes would be limited by a need for nutrients found ONLY on Mars.

As an example, the blue green algae Chroococcidiopsis is one of our main candidates for a Mars analogue organism, and is a prime producer, which means, it doesn't depend on any other life. It only needs

- sunlight, with other metabolic pathways it can use such as hydrogen
- trace elements which it can get from the basalt or other rocks on Mars,
- water,
- a source of CO_2 (for carbon fixation) and nitrogen (for nitrogen fixation) or any other suitable sources for carbon and nitrogen.

Chroococcidiopsis has been found growing 750 meters below the Atlantic sea bed (Li et al, 2020), surviving on just gabbro, water, and hydrogen. Gabbro is a rock chemically equivalent to basalt (King, H., n.d.). Terrestrial basalt is one of the most common terrestrial rocks (Washington University, n.d), and it is the most common rock on Mars making up most of its surface (Payré et al, 2022). It has all the trace elements life needs, and it even has enough carbon and nitrogen to support millions of cells per gram without nitrogen or carbon fixation (Fisk et al., 1999:11806, section 3.1).

Mars could plausibly have life similar to the terrestrial blue-green algae with similar nutritional requirements. If so, it could find almost anywhere on Earth with access to rock (in the form of basalt at least), water and sunlight or some other form of chemical energy it can access. Terrestrial basalts are good analogues for Martian basalt (Cockell et al., 2019).

If martian life is able to fix nitrogen and photosynthesize it doesn't need carbon or nitrogen either but can get those from the atmosphere. But it can also find abundant carbon and nitrogen in many terrestrial ecosystems, and as we saw, it will find some of those elements even in basalt (Fisk et al., 1999:11806, section 3.1). In short there seems to be plenty of potential for Mars to have microbes with nutritional requirements they can satisfy as easily on Earth as on Mars.

Microbes with high levels of resistance to ionizing radiation like radiodurans and chroococcidiopsis do grow a little slower and have a longer reproduction time – but do still co-exist in the same habitats as less resistant life

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Chroococcidiopsis is a top candidate for a Mars analogue organism, partly because some strains have very high levels of ionizing radiation resistance (Li et al, 2022). Chroococcidiopsis is able to survive and remain viable in the temperature and pressure conditions of Mars analogue chambers and in theory could grow on Mars if it had a source of water and nutrients (Billi et al., 2011).

However this is not such an unusual feature. We know of many other very radioresistant microbes. The first microbe with the capabilities to resist high levels of ionizing radiation was deinococcus radiodurans, first discovered in radiation sterilized cans of ham in 1956, which means it was living in the ham before sterilization (Seckbach et al., 2015) (Anderson, 1956) (Krisko et al, 2013).

John Rummel, NASA's planetary protection officer at the time, used radiodurans in his response to Zubrin's op. ed, referring to radiodurans's ability to grow in nuclear plant environments as an analogy to show that similarly a microbe adapted to Mars may already have capabilities to adapt to conditions on Earth it never encountered before (Rummel et al., 2000),

Radiodurans can repair 100 double strand breaks per chromosome without any loss of viability or mutation of its genome (Minton, 1994). Radiodurans has this capability without ever encountering radiation sterilization in its evolutionary history.

There are several theories to explain why organisms like chroococcidiopsis and radiodurans are so radioresistant. According to the review of the literature by Shurvak et al, (Shuryak et al, 2019):

1. radioresistance probably is a byproduct of resistance to oxidative stress, and damage to the DNA from desiccation, UV, heavy metals, and other agents. Many microbes isolated from exposed areas and deserts have high levels of radioresistance
2. however they don't always go together, some very desiccation resistant microbes are not strongly resistant to ionizing radiation and vice versa.
3. Microbes can evolve radioresistance quickly, and even microbes with already high levels of radioresistance can increase it through multiple rounds of exposure to high doses of ionizing radiation and then rapidly growing the survivors
4. These increases in radioresistance are associated with subtle changes in DNA repair and metabolic functions

This suggests that plausibly many or most organisms have the capability to develop radioresistance.

So why don't all microbes have this capability already? Probably because it has downsides, again summarizing, (Shuryak et al, 2019).

- Many radioresistant prokaryotes (cells without a nucleus) and fungi grow more slowly than their radiosensitive relatives.
- Radioresistant microbe spend more resources on stress resistance
- Radioresistant microbes also stop and repair their DNA before reproducing which means they take more time to reproduce

So plausibly there are downsides to radioresistance but Shurak et al also say that microbes can evolve radioresistance rapidly.

One experiment to test how fast cells can evolve to exceptionally high levels of radioresistance, started with a radioresistant e. coli strain which could withstand 2,000 Gy (Harris et al, 2009). It was exposed to gradually increasing doses of ionizing radiation. Each time it was only exposed for long enough to kill more than 99% of the population. After 20 cycles of this process, it could resist 10,000 Gy, close to the radioresistance of radiodurans.

The newly evolved radioresistant strain kept that radioresistance to 10,000 Gy for 100 generations of growth in normal conditions. This most radioresistant strain of e. coli was also 4,500 times better at surviving at 3,000 Gy than the original strain, see (Harris et al, 2009:fig 1) and the text following figure 1. Then, the founder strain couldn't survive at all at 5,000 Gy, which destroyed all DNA in the founder strain. In the radioresistant e. coli, repair at 5,000 Gy happened too fast for the recovery to be due to normal replication (i.e. with the damaged daughter cells dying). This shows that e. coli used active DNA repair for its increased radiation resistance.

Since multiple species of terrestrial biology can achieve high levels of radioresistance already after short periods of accelerated evolution, life evolved on Mars for billions of years is likely to be very radioresistant, possibly far more so than terrestrial life.

This extra resilience to ionizing radiation in turn might mean marian life devotes more of its resources to radioresistance. As with radioresistant terrestrial life, it might grow more slowly, and it might also check over its DNA while reproducing and so, take longer to reproduce than its terrestrial analogue.

However radiodurans is found widely on Earth, including in cans of ham where radiodurans was first discovered. Other radioresistant microbes are similar, such as some strains of chroococcidiopsis. Their radioresistance doesn't stop them from growing in more normal situations.

So, though radioresistance seems to have a penalty associated with it, it's not likely that ionizing radiation resistance will make it impossible for martian life to survive here.

Also, though I didn't find any studies on how long it takes microbes to drop those extra precautions, or on whether radiodurans can evolve to lose its resistance, it seems plausible that over time martian life could gradually evolve to remove those unnecessary extra protections against ionizing radiation, and spread more rapidly as it spreads on a planet with less ionizing radiation.

Microbes from near the surface in Jezero crater would withstand temperatures varying from below -70 °C to above 15 °C in a single day – and major changes in humidity and pressure – this is likely to favour polyextremophiles – and martian life would likely be able to resist higher levels of stresses like UV, low humidity, vacuum, desiccation, and ionizing radiation – and may be able to fix nitrogen at low concentrations – which seems likely to make it easier not harder for them to survive on Earth

Next section – all sections – previous section
[question]

Martian conditions seem likely to favor polyextremophiles able to survive multiple extremes, for instance life that can survive both warm and extremely cold conditions, and dry and very humid conditions. This also suggests that not only is it possible but highly likely that life adapted to Mars could survive somewhere on Earth, especially if it is adapted to the surface conditions.

As for other factors, any Martian life might well be better able than its terrestrial analogue to survive desiccation, UV, ionizing radiation, and low atmospheric pressure as a result of evolving in those conditions for billions of years. However there seems no reason why it would ***depend*** on any of those things.

To guarantee no backwards contamination issues we need ALL possibilities for life in the samples to find it impossible to live on Earth and impossible to adapt to commonly found terrestrial conditions after it escapes containment. It just takes one exception and we have a possibility for a martian organism that could be invasive if it gets here, and then spread through our biosphere.

Let's look at this in more detail.

The sterilization working group report doesn't go into details. It only mentions temperature and pressure as environmental factors that extremophiles can be adjusted to that can make it impossible for them to live in less extreme conditions (Craven et al., 2021:6-7):

There are many described extremophiles that may survive in environments that are extreme to human or animal life ***(e.g. extremes of temperature or pressure)*** *but do not survive under conditions in our normal habitat*

… Thus, it is plausible that any Martian microbe, after it arrives on Earth, would not be viable on Earth due to a lack of its required Martian nutritional and environmental conditions.

[bolding added]

They don't say that those two examples apply to Mars particularly but it's all we have to go on. We saw in the last section that low pressures on Mars can cause problems for some terrestrial life in the forward direction, but it doesn't seem likely that microbial life would ***depend*** on low pressure. Their cite doesn't suggest that there would be extremophiles that depend on Mars like low pressure, and they don't give any examples themselves. We would need all putative Martian life to depend on low pressure.

That leaves temperature of the two things they specifically mention. As we saw in the last section Planococcus Halocryophilus Or1 was isolated from permafrost at a temperature of -16°C, though its optimal growth temperature is 25°C and it can continue to grow right up to human blood temperature (Mykytczuk et al., 2013).

But we may need to expand on that a little. Why would any Martian species be likely to be polyextremophile like that, adapted to both cold and warm conditions? Might it be a special feature of Earth's biosphere, that because parts of Earth are warm, we have microbes adapted to warm places flourishing in cold places?

Well no. First Mars can get a little warmer than most realize. Perseverance's two regolith samples are from surface dirt (NASA, 2022).

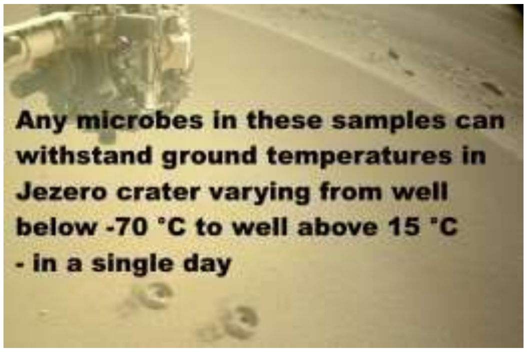

Figure 25: Location photo of Perseverance's two regolith samples (NASA, 2022).

As measured by Perseverance the ground temperature in Jezero crater can vary from well below -70 °C to well above 15 °C in a single day (Afri et al, 2022: Figure 3).

Perseverance's landing site is at 18.44°N 77.45°E (NASA, 2022). The ground temperatures in Jezero Crater reach their maximum at around the northern hemisphere fall equinox (solar longitude Ls180) rather than the summer solstice (solar longitude Ls90). This is as expected for regions close to the equator, and happens because Mars is furthest from the sun close to the northern summer solstice (Newman et al., 2021: 6.2).

This shows the annual cycle for eight models. They vary in predicted peak temperatures by around 10 °C, with GEM Mars predicting a maximum of around 13 °C at the northern hemisphere fall equinox.

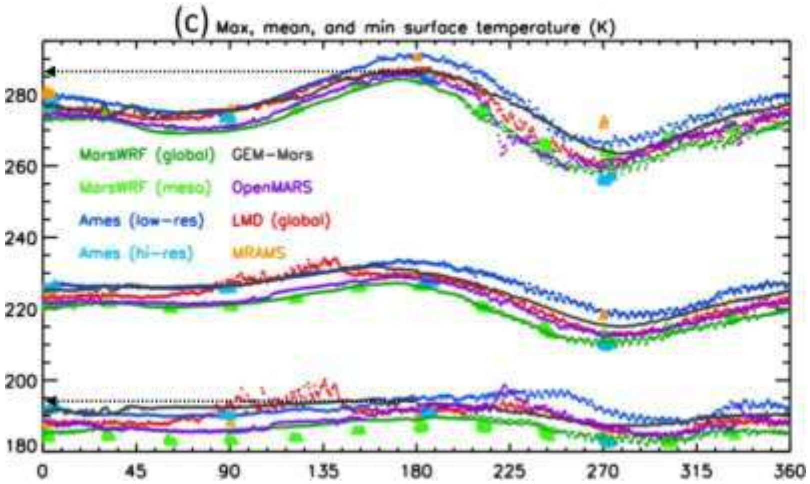

Figure 26: from: (Newman et al., 2021:Fig 5)

There can be significant variation around these predicted temperatures, as Mars has large scale coherent weather systems such as warm fronts (Young et al., 2022: Fig. 1).

The northern hemisphere autumn equinox, LS 180, was on Feb 24 2022 from the Mars Calendar (Planetary Society, n.d.). This is counted as mission sol 361 for Perseverance, as seen in the caption for an image as acquired on Feb. 24, 2022 (Sol 361) (NASA, 2022)

Perseverance often measures surface temperatures that vary from below -70 °C to above 15°C in a single day. Examples

- sol 361 varied -74.33 °C to 16.96 °C (198.82 °K to to 290.11 °K).

Later in the martian autumn it found even warmer temperatures:

- sol 380 ranged from -65.05 °C to 18.84 °C (208.1°K to 291.99 °K).

This is based on the calibrated data, details see: (Rodriguez-Manfredi et al, 2021). For the data files used

- Perseverance ground temperature in this paper's Supplementary Information

These measurements match well to the predicted diurnal variation at Ls180 from models. This shows the prediction for two of the models, in an earlier publication from 2020. 0 °C is a little over 273 °K.

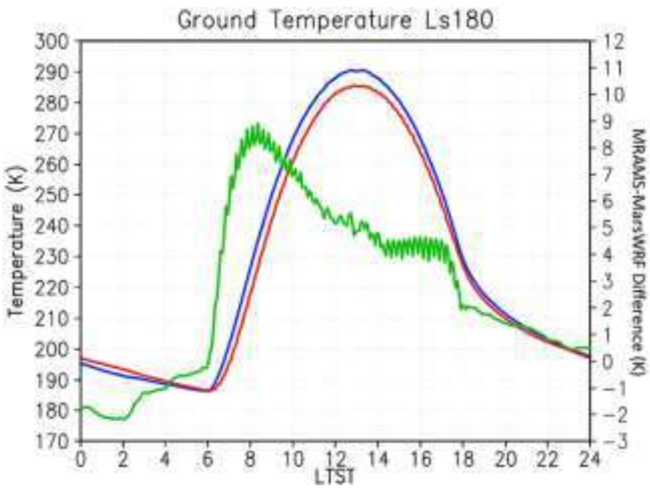

Figure 27: Predicted ground temperature for Jezero Crater for the autumn equinox at Perseverance's site (LS 180) in blue forthe Mars Regional Atmospheric Modeling System (red line shows the Mars Weather Research and Forecasting model and green line shows the differences between the two). From (Pla-García, 2020: Fig. 3)

So, any martian life in the top few millimeters of the Martian surface in Perseverance crater needs to withstand extreme changes of temperature from below -70 °C to above 15 °C in a single day. It could certainly survive warmer conditions. Then there's the question if it could grow at above 15 °C. It's possible to stare at the data and convince yourself either way, but it seems not impossible, for instance if it can absorb moisture rapidly like mosses and then retain water through to the daytime. We covered that above in the discussion of Curiosity brines. See:

- Many ways native martian life could make brines more habitable (and previous sections)

If Martian life can grow up to 0 °C that brings a large part of Earth's surface within range, anywhere that gets cold enough for frosts on occasion. Even if it needs temperatures below -15 °C, that is still a large part of the terrestrial surface that experiences those temperatures at least some of the time in winter.

The very low night time temperatures aren't necessarily a problem for survival for Martian life evolved to live in those conditions. Terrestrial life can survive temperatures below -70 °C. Indeed some mosses and algae can survive immersion in liquid nitrogen at -193 C and they can even survive immersion in liquid helium at only 0.05 degrees above absolute zero (Lenne et al, 2010),

Any martian life is also likely to be UV resistant, ionizing resistant, radioresistant and able to tolerate low atmospheric pressures and low relative humidity in daytime. We have terrestrial life that does all that. It's likely to live in partial shade and since UV is light, it is easily blocked by a millimeter or so of dirt. None of those capabilities are likely to make it impossible to survive on Earth though it may make it slower growing as we saw in the previous section.

- Microbes with high levels of resistance to ionizing radiation like radiodurans and chroococcidiopsis do grow a little slower and have a longer reproduction time – but do still co-exist in the same habitats as less resistant life

For completeness let's look at the other factors. The gravity, atmospheric pressure and atmospheric composition for Mars is different, and it has temperatures that at times go below any temperatures recording on Earth. Almost everything else is duplicated somewhere on Earth in Mars analogue sites (Preston et al, 2013). For instance the high concentrations of perchlorates are duplicated in the Atacama desert, almost as high as the levels found by Phoenix (Fairén et al., 2010:836)

The lower gravity on Mars isn't likely to cause problems for terrestrial life. Microbes grow in the ISS in zero gravity, so there isn't any major obstacle to microbes adjusted to low gravity surviving on Earth.

The humidity on Mars is very variable and depending on location can reach 100% at night in winter and varies to close to 0% in spring to summer in daytime, and the pressure also varies greatly from day to night. This seems likely to encourage polyextremophiles that can tolerate any humidity level, rather than extremophiles adjusted to extreme low humidity.

The oxygen in Earth's atmosphere seems unlikely to cause problems. Mars may have photosynthetic life that produces oxygen when it converts CO_2 to organics. The Martian atmosphere also has 0.13 - 0.19% oxygen (Trainer et al) in the atmosphere and oxygen is also possibly present in the cold brines at levels high enough for oxygen using life (Stamenković et al, 2018).

Terrestrial life uses antioxidants to tame the oxygen in its environment, similar to the terrestrial antioxidant enzymes such as superoxide dismutase to convert superoxide radicals into hydrogen peroxide, and catalase to convert hydrogen peroxide into water and oxygen gas (Goodsell, 2004).

Curiosity discovered seasonal oxygen in Gale crater, 30% higher than expected in spring to summer. It could be produced abiotically or by low levels of present day life. (Trainer et al, 2019:3021) (Shekhtman, 2019)

Indeed, Martian surface conditions are superoxygenated. Martian salts include chlorides and sulfides as on Earth but also their oxygenated and superoxygenated forms, chlorates, sulfates and perchlorates, and we also find hydrogen peroxide on Mars. All this might make Martian life if anything better adjusted to oxygen stress than terrestrial life.

So, Mars has native Martian life, it is also likely to have antioxidants to tame the Martian superoxidants, similarly to terrestrial life (Goodsell, 2004).

This doesn't mean it has to be dependent on perchlorates or hydrogen peroxide. Mars life might use perchlorates as oxidants, as a source of energy (Rummel et al , 2014). But again, at least if martian life is like terrestrial life, it's not likely that ***all martian life depends*** on perchlorates as a source of energy. Even if it did, there are those perchlorates in Mars analogue sites such as the hyperarid core of the Atacama desert (Fairén et al., 2010:836).

It's possible to hypothesize a martian lifeform that depends on perchlorates and hydrogen peroxide for a faster metabolism in very cold conditions (Schulze-Makuch et al, 2010a). Their hypothetical organism couldn't survive for long above 10 °C with high humidity. (Houtkooper et al, 2006). Its biochemistry would limit its range on Earth to cold dry Mars analogue conditions. Even then it might find a niche here.

Martian life that depends on perchlorates represent a possible best case scenario for backwards contamination. But it doesn't mean that this is what we will find on Mars, it's just a scenario. For planetary protection we need to look at worst case rather than best case scenarios until we know what we have on Mars. Also, if it can't survive on Earth it can't survive in warm wet conditions on Mars and especially not in hydrothermal systems, which Mars had in the geologically recent past (Scanlon et al, 2014), and may possibly have below the surface even today (Horvath et al, 2021). This would suggest that even if we find martian life that can't survive warm conditions this could co-exist with other life that can. We look at this some more below in:

- Mars surface temperatures can reach 35°C in the shade in summer – some species of Martian surface life may be pre-adapted to hotter, even hydrothermal conditions in geologically recent Mars – and emerge through species sorting – persist in small numbers in surface biofilms and spread and adapt rapidly when they encounter far warmer conditions

Then there's nitrogen, but it's hard to see why the extra nitrogen in Earth's atmosphere would cause problems. Microbes from Mars might not be able to fix nitrogen, but then many terrestrial microbes can't either. Also it's not impossible that there are nitrogen fixers on Mars. It's just on the border of possible. Experiments so far tested some cold tolerant microbes from Antarctica in air at normal atmospheric pressure but with nitrogen reduced to only 0.2 mbars similarly to Mars (Mancinelli, 1993) following (Klingler et al, 1989). These microbes could still fix nitrogen after simulating the temperature and UV flux of Mars (Sakon et al, 2005) (Sakon et al, 2006). More experiments are needed in Mars simulation chambers for 0.2 mbar nitrogen at a total pressure of 6 mbars similar to Mars (Sakon et al, 2006).

In a scenario where Mars has nitrogen fixers, they may be better able to fix nitrogen than terrestrial life. However, the abundant nitrogen on Earth would not be likely to be a problem for them.

In summary, there don't seem to be any major issues that would prevent life adapted to Mars surface conditions from living on Earth.

Indeed there may be scenarios where Martian life is better at living on Earth than terrestrial life. We covered this above in:

- NEW: Worst case scenario - If a martian microbe can grow in the sea, soil, and fresh water like chroococcidiopsis, is adapted to spread in the wind in Martian dust storms, and outcompetes terrestrial biology, e.g. better at photosynthesis or nitrogen fixation, it could be found globally after introduction to Earth in weeks to months, and be one of the most common microbes in our soils and oceans in years to decades or sooner, far more common than nanoplastics or microplastics

Mars surface temperatures can reach 35°C in the shade in summer – some species of Martian surface life may be pre-adapted to hotter, even hydrothermal conditions in geologically recent Mars – and emerge through species sorting – persist in small numbers in surface biofilms and spread and adapt rapidly when they encounter far warmer conditions

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We saw that Planococcus Halocryophilus Or1 which grows in permafrost soils is also able to grow right up to human blood temperature (Mykytczuk et al., 2013). Could Martian life grow at such high temperatures?

The issue of optimal temperatures for growth is more acute for human pathogens. Any life that can live in or on the human body needs to be able to survive at or near body temperatures, although a pathogen adjusted to cooler conditions can harm us if it contaminates food or is an allergen or toxin in the air.

By definition, psychrophiles (cold loving microbes) have optimal growth at 15°C or below and psychrotrophs (cold tolerant microbes also known as psychrotolerant) are able to grow below 15°C but prefer to grow at higher temperatures, doing well at around 20°C (typical room temperature in the US) (Moyer et al., 2007).

In a likely scenario, most Martian life in Jezero crater has optimal growth temperatures at 15°C or below, psychrophiles. However this doesn't rule out the possibility of life on Mars that has optimal growth temperatures much higher.

If martian life does have biofilms, which can retain water through to day time, there could be evolutionary pressure for species in these biofilms to acclimatize to grow rapidly for the short time it experiences very warm near surface conditions in summer, which as we saw could reach 15 °C (Martin-Torres et al, 2015:fig 2a). on the same day that surface brines have enough humidity for life at below -70 C in the early morning through to 6 am (Martin-Torres et al, 2015:fig 3b)

The Spirit rover measured temperatures of up to about 35°C in summer (though it had no way to detect if there were any surface brines) and down to about -90°C in winter (NASA, 2007). That is the temperature in the shade, so may be similar to the temperature experienced by any native life in the dust.

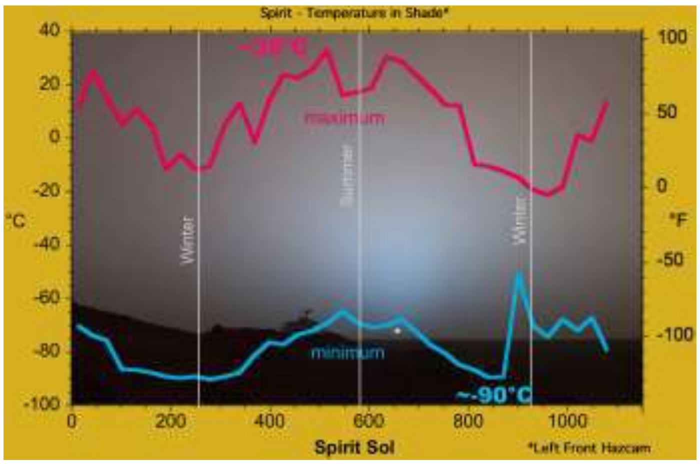

Figure 28: graphic from (NASA, 2007), added the text for the maximum and minimum temperature as described in the source.

Whether or not martian life is adapted to grow at the temperatures of human blood, it may well be able to survive such temperatures.

In another scenario, Mars has rare species of Martian surface life that inherited capabilities to adapt to warmer conditions than it encounters on the surface in Jezero crater today, for instance from species adapted to hydrothermal conditions. This commonly happens on Earth.

Smith et al. list three ways microbes can adapt to warmer conditions (Smith et al., 2022).

- **acclimatize** by up or down regulating genes, and altering the fatty acid composition of cell walls, this can happen in minutes to days
- **adapt** by selection of the genetic variation a population has naturally, some combination of traits that works well at higher temperatures, or by mutation, or by recombination.
    - With archaea, adaptation can also involve taking up genetic traits from other archaea through gene transfer agents, or in the other direction if they can transferring some of their capabilities to terrestrial life, which can happen overnight in seawater as we saw in the discussion of the ESF study

- **Respond with species sorting –** supposing we return a fragment of biofilm from Mars, it would likely include a diverse species mix and typically a terrestrial microbiome has a some rare species in it with capabilities the other microbes don't have.

Smith et al. tested species sorting with six samples taken from a long term field experiment site, Nash's field, in Berkshire, UK in 2016. They kept each soil sample hydrated at a constant temperature in the laboratory at 4, 10, 21, 30, 40, and 50°C for 4 weeks to simulate species sorting though they didn't supplement them with any additional nutrients (Smith et al., 2022).

The highest temperature they tested, 50°C, is likely well above the most extreme temperatures Berkshire soils experienced in thousands of years. The UK reached 40°C for the first time in recorded history in summer 2020. According to one model, before global warming the UK likely encountered this temperature only every 2,000 to 10,000 years (Christidis et al, 2020: figure 6d).

Smith et al. then took microbes from each soil sample and incubated them in agar plates until colonies formed. They found that if they incubated the sample at the same temperature it was sorted in the constant temperature phase, the new community had microbes that were much better adapted to the conditions. This was especially striking for the tests at 50°C.

Without sorting, the dominant species when incubated at 50 °C had optimal temperatures ranging from below 20 to above 35°C. After sorting by keeping the soil hydrated at 50°C for 4 weeks, the dominant species incubated at 50°C had optimal temperatures ranging from below 50°C to well above 55°C (Smith et al., 2016) (reading from the diagram).

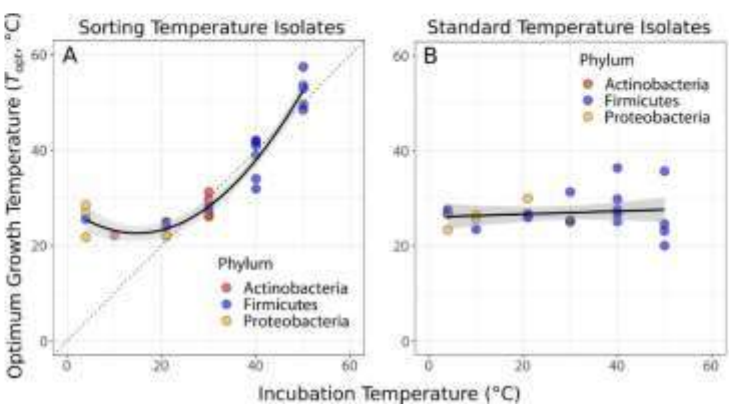

Figure 29: from (Smith et al., 2016: Fig 2)

From these experiments it seems that species sorting could give a community of Martian microbes a far faster way to adapt to terrestrial conditions than evolution or gene expression, provided it has even rare species able to live at higher temperatures in the surface communities.

Mars had warmer conditions in the geologically relatively recent past. As recently as 210 million years ago, a volcanic eruption on the flanks of Arsia Mons melted enough ice for two lakes of 40 cubic kilometers each and a third one of 20 cubic kilometers of subsurface melt, which would have stayed melted for centuries to millenia insulated by surface ice (Scanlon et al, 2014).

These would likely have subsurface high temperature habitable regions from volcano / ice interactions such as hydrothermal pools, and tuyas, a flat topped volcano beneath ice with liquid water forming around it (Glenister et al, 2021).

The paradox of abundant spores of heat adapted geobacillus spores in cold places - and potential that present day Mars has similarly abundant heat adapted spores from hydrothermal systems, perhaps produced by the rootless cones, fumaroles, or ice fumaroles – some might have been active in the last few million years – some might even be active today

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Mars has been geologically active in the very recent past, only the last few million years. The rootless cones (volcanic cones without a magma chamber below them) show evidence of steam explosions and hydrothermal systems that may have remained above 0 C for up to 1,300 years (Hamilton et al, 2010), with some of them possibly active as recently as less than 20 million years ago (Stacey, 2019), and there is evidence of explosive volcanism 53 to 210 thousand years ago by crater counting, which suggests some potential for present day subsurface hydrothermal activity (Horvath et al, 2021).

Mars may even have undetected hydrothermal pools in caves even today which vent to the surface from time to time, or did so in the recent past. If so, there may be rare species in surface microbial communities grow best in very warm conditions as with the microbes found by Smith et al. in their species sorting experiment. In this scenario, these thermophiles likely don'tt do well in the present Martian surface conditions. They are in low numbers, outcompeted by other microbes, but they persist in small numbers, or may only persist as numerous viable spores, ready to grow when they meet the right conditions.

This exact scenario happens on Earth with the genus geobacillus. It's been called the geobacillus paradox. These microbes do best in hydrothermal vents but they are found in surprisingly large numbers almost everywhere researchers look, including in cool soils and cool ocean floors (Zeigler, 2013). We can add more cold climate examples to Zeigler's review, as since then, geobacillus spores have also been collected from the air over the McMurdo dry valleys in Antarctica (Bottos et al., 2014) and the soil of raised beaches in G King George Island, the largest of the South Shetland islands 120 km off the south coast of Antarctica (Zhang et al., 2018)

Geobacillus spores are probably so common because their spores are very durable. This would let them accumulate to such large numbers over long periods of time. Zeigler argues that they seem to have lifespans of at least millennia, possibly more. They are highly resistant to ionizing radiation and UV, perhaps as much so as radiodurans. They are also just the right size to get transported in the atmosphere and dust storms. It's possible that they get released in large numbers on rare favourable conditions (Zeigler, 2013).

This seems a possible scenario for present day Mars if it has hydrothermal systems active recently, perhaps in caves or even as active fumaroles (openings that vent steam and volcanic gases), that vent to the surface, with some of the spores spreading in the dust. These vents may release large numbers of spores from time to time, but only rarely, during times of volcanic activity when the hydrothermal systems become more productive or activate. Then the spores from those occasional periods of activity could spread in the dust storms, protected from UV and ionizing radiation and accumulate in the dirt. In this scenario these spores from hydrothermal systems or fumaroles on mars could still be viable, even if they aren't able to replicate in the current martian surface conditions.

Could there be active fumaroles even today? Fumaroles might be hard to spot on Mars as the humid air might form ice towers in colder regions of Mars, like the ice fumaroles on mount Erebus in Antarctica. These ice towers would hide most of the thermal signature. In their terrestrial analogues, life lives in dark humid caves inside the tower, with a humidity of 80% to 100%, and most microbes use chemical redox gradients for energy. Hoffmann suggested looking for circular hot spots a few degrees warmer than the surroundings and up to 100 meters in diameter. The towers would be up to 30 meters high in the low Martian gravity. The terrestrial towers often collapse and reform over a timescale of decades (Cousins et al, 2011), (Hoffmann et al, 2003).

Figure 30: Fumarole ice chimney on mount Erberus (Turner, 2012). Hoffmann et al. suggest its Mars analogue could be up to 30 meters high and up to 100 meters in diameter in Martian gravity and may be hard to spot because the ice would mask its thermal signature from orbit (Hoffmann et al, 2003).

Mars might have undetected fumaroles today or it may have ones that were active in the geologically recent past that might spread spores in the dust storms, similarly to the geobacillus spores on Earth. This gives a possible way for Perseverance's samples to contain spores adapted to high temperatures in hydrothermal conditions. They might also have been produced by rootless cones (Hamilton et al, 2010) in the geologically recent past less than 20 million years ago (Stacey, 2019) or perhaps even at the times of explosive volcanism 53,000 to 210,000 years ago (Horvath et al, 2021).

In this scenario, if we return a fragment of a biofilm, say, it may have a diverse species mix just from the life best adapted to current conditions in Jezero crater, perhaps some with optimal growth temperatures well above 0°C if they can retain water through to daytime in Jezero crater.

But then, as for the field in Berkshire, there may be some that are able to do well at significantly higher temperatures than any they encounter in Jezero crater today. Those then may be like the NRC study's example (SSB, 2009: 46) of Epsilonbacteraeota (Waite et al, 2017), found in natural hydrothermal vents, which may not be many steps away from pathogenic strains such as Helicobacter (Cornelius et al, 2012) as we saw above in

- Sample return biological safety report gives an example of an e. coli strain it says became toxic by coexisting with humans – it doesn't cite the NRC's counterexample of a human pathogen which shares many virulence genes with species adapted to hydrothermal vents – meanwhile even its e. coli example might have developed Shiga's toxin (poison) to prevent itself from being eaten by protozoa in biofilms – the origin of its virulence remains an open question

All this suggests a scenario where a martian sample might already have heat adapted strains able to grow on or in our bodies at up to human body temperature, not that different from Planococcus Halocryophilus Or1 (Mykytczuk et al., 2013), or the strains of chroococcidiopsis found in the nasopharyngeal microbiota (Ventero et al, 2022), and in human milk from Gambia (Lackey et al, 2019), also black fungi such as the pathogenic close relatives of the black fungus Exophiala jeanselmei MA 2853 which responded well to exposure to Mars simulation conditions (Zakharova et al, 2014)

See above:

- NASA's biological safety report agrees on the potential for an invasive Martian species to harm or displace terrestrial photosynthetic bacteria – but says it's plausible life adapted to Martian conditions such as the temperatures and pressures plausibly wouldn't be viable on Earth – their own cite mentions Planococcus Halocryophilus, a microbe which lives in Arctic permafrost soils and likely grows in sub zero brine veins down to at least -15 °C, with an optimal growth temperature of 25°C and growth up to 37 °C (human blood temperature)
- NEW: by analogy with terrestrial fungal diseases – a fungal disease from Mars would be likely to be hard to distinguish from tuburculosis through testing or medical imaging – a new genus would likely have no effective antifungals available initially or for some time because fungi are evolutionarily close to humans making it hard to develop effective antifungals – and we need to consider this possibility as many terrestrial fungi do well in Mars simulation chambers including a strain of a black fungus sometimes pathogenic in humans

In this scenario, it's also possible, as for the geobacillus paradox, that Mars may even have microbes able to survive at far higher temperatures than human body temperature.

Martian microbes can evolve small adaptations to terrestrial conditions such as higher growth rates, more efficient use of food and increased upper temperature limit for growth over weeks to years

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Let's also look at adaptation. Microbes have such a short replication time that they can adapt rapidly allowing researchers to study evolution in action in the laboratory (McDonald, 2019). A martian microbe would only need to establish a foothold on Earth to start the process of adapting to more terrestrial conditions. If the temperatures it encounters on Earth are within the range of temperatures where it can already grow on Mars, it may be able to adapt quickly to higher growth rates at those temperatures, and higher yields [how efficiently it uses the food it finds]. It may also be able to increase the upper temperature limit of growth by a degree or more, quite quickly.

In one study relevant to Mars, experimenters tested a cold adapted Antarctic bacterium with a growth range of −2.5°C to 29°C which shows signs of heat stress above 20 C. First, it was pre-adapted to 15 C. After a further 900 generations of evolution at gradually increasing temperatures it was able to grow at 30°C, one degree beyond the original temperature limit for growth. The ancestral strain could survive up to 30°C but not grow. The evolved strain could survive up to 31°C (Toll-Riera et al., 2022)

Another experiment looked at the yield [the mass of bacteria produced per mass of substrate consumed] of an e. coli clone. They started with a strain adapted to the same medium for 2000 generations at 37°C. Its yield near doubled at 42.2°C (1.94 fold increase) after 2000 generations in parallel in 115 separate cultures. This lead to 1331 mutations in total at 600 sites (Tenaillon et al., 2012).

Some microbes can also rapidly increase their critical high temperature (CHT), the temperature limit for growth by several degrees. In one experiment *Z. mobilis* TISTR 548's temperature limit for growth increased by 3°C from 38°C to 41°C. (Kosaka et al., 2019: table 1)

This suggests that a microbe from Mars that gradually spreads through the terrestrial biosphere might be able to change its temperature limits and its optimal temperature for growth relatively rapidly in numerous sub strains adapted to the different conditions it finds. It could also evolve to grow faster in the conditions it encounters relatively quickly. These are relatively short term experiments with small numbers of microbes compared to the conditions a new microbe would find spreading for the first time through Earth's biosphere.

These experiments don't test the potential for gene transfer from terrestrial to related martian life, which is another way that a species could adapt genetically, which probably needs to be considered, with archea able to transfer capabilities overnight to other microbes in seawater (Maxmen, 2010) (McDaniel, 2010). Martian life might have similar capabilities to transfer capabilities with each other.

With gene transfer, sufficiently closely related martian life could also transfer novel capabilities to terrestrial life and so spread its genetic capabilities to habitats it can't inhabit itself. Alternatively, closely related terrestrial life might transfer capabilities via gene transfer to the new martian species which would make it easier for martian life to survive in a terrestrial habitat.

Many candidate microbes such as the blue green algae chroococcidiopsis and even higher life like lichens have been proposed as Mars analogue organisms, some tested with promising results in Mars simulation chambers, so it's biologically credible a species can have adaptations to live on both planets

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[question]

We now have a wide range of candidate terrestrial microbes and even higher organisms such as lichens that could potentially survive and even grow in various candidate microhabitats on Mars. This shows that it's biologically possible for a species of microbe or even higher life to be adapted to both planets.

This is not so surprising as we saw almost all the most challenging Martian conditions are duplicated somewhere on Earth in Mars analogue sites (Preston et al, 2013) (Fairén et al., 2010:836). Only its ionizing radiation levels and the vacuum of space, the extreme cold, and the near vacuum of the Martian atmosphere are not duplicated here, of the ones that are severe stressors,. But many terrestrial microbes turn to be pre-adapted to ionizing radiation and vacuum, probably partly because of desiccation resistance, and many go into a state of vitrification at low temperatures. Some mosses and algae can survive immersion in liquid nitrogen at -193 C and even liquid helium at only 0.05 degrees above absolute zero (Lenne et al, 2010), so would be able to survive the very low temperatures on Mars.

See above:

- Microbes from near the surface in Jezero crater would withstand temperatures varying from below -70 °C to above 15 °C in a single day – and major changes in humidity and pressure – this is likely to favour polyextremophiles – while microbes able to resist stresses like UV, low humidity, vacuum, and ionizing radiation do not require a non-terrestrial biology and there is no reason for them to be dependent on these conditions to survive

Some of these candidates have been tested in terrestrial Mars simulation chambers. Others have been tested in the BIOMEC experiment on the exterior of the ISS for their ability to survive in Mars simulation conditions complete with ionizing radiation. I will quote just the summary for each category from (Sielaff et al., 2019):

- **“Methanogens:** The results from laboratory studies show it might be possible for some methanogens to inhabit the subsurface of Mars due to their tolerance to low pressure, desiccation, and perchlorate salts.”
- **“Bacteria:** The described studies show that the organisms with the highest potential for survival of Martian conditions are likely to be spore-forming bacteria which show resistance to multiple extreme physicochemical factors. It is important to determine if vegetative cells of spore-formers and nonspore-formers could withstand long-term simulated Martian conditions. The microorganisms isolated from various Earth environments show this potential, but more research is needed on studying the limits of life for bacteria in the context of Mars habitability.”
- **“Fungi:** Based on the study results, the fungi studies survived exposure to simulated Martian conditions in various capacities. Their resistance to radiation might be an important advantage over other microbial forms with regard to survival under Martian conditions.”
- **“Lichens:** Although Mars would present a harsh environment for sustaining life on its surface due in part to the high amount of radiation, … an environment protective against high levels of radiation could be present on the Martian surface, which may allow for the survival and proliferation of photosynthetic organisms [such as iron, salts, snows and crystalline rocks] These environments may allow for photosynthetic growth by lichens and other photosynthetic organisms on the surface, while allowing for a protective niche from the harsh environment.”
- **“Mosses:** These results showed that bryophytes have a high potential for survival in Martian conditions, although more research is needed. Even though UV exposure did not inhibit photosynthesis completely, it would be necessary to determine if bryophytes can conduct photosynthetic activity for extended periods under these conditions.”

Some individual species or genera of special interest:

- Chroococcidiopsis – UV and radioresistant can form a single species ecosystem, and only requires CO_2, sunlight and trace elements to survive (Billi et al., 2011)
    - tested in BIOMEX (De Vera et al., 2019)
    - sometimes found in human microbiome in the upper throat behind the nose (Ventero et al, 2022) and human breast milk (Lackey et al, 2019).
    - one strain produces a potential neurotoxin BMAA which can cause Lou Gerig syndrome, the disease Steven Hawking had (Cox et al., 2005:fig 2), See: NEW: Chroococcidiopsis indica produces an accidental neurotoxin, BMAA, which resembles serine and by replacing it, can cause protein misfolding – leading to the possibility that novel amino acids from a novel exobiology could also cause protein misfolding

- Methanogenic archaea such as *Methanosarcina soligelidi (*Maus et al, 2020) (Serrano et al., 2019)

- Alkalilimnicola ehrlichii MLHE-1 (Euryarchaeota)

- able to use CO in Mars simulation conditions, in salty brines in conditions similar to those of the Recurrent Slope Linea for the water potential and temperature range, and could grow in oxygen free conditions if nitrates are present, and unaffected by magnesium perchlorate and low atmospheric pressure (10 mbar) (King, 2015)

- Halorubrum str. BV (Proteobacteria)
  - also did well in similar conditions to Alkalilimnicola ehrlichii, simulating the RSLs (King, 2015).

- rock inhabiting black fungi, Cryomyces antarcticus (an extremophile fungi, one of several from Antarctic dry deserts) and Knufia perforans,
  - adapted and recovered metabolic activity during exposure to a simulated Mars environment for 7 days using only night time humidity of the air; no chemical signs of stress (Pacelli et al, 2017)
  - Cryomyces antarcticus was tested in BIOMEX (De Vera et al., 2019)

- Rock inhabiting black fungus Exophiala jeanselmei MA 2853
  - also adapted and recovered metabolic activity during exposure to a simulated Mars environment for 7 days using only night time humidity of the air; no chemical signs of stress (Zakharova et al, 2014)
  - close relatives found in the human microbiome which are occasionally pathogenic (Zakharova et al, 2014)
  - occasionally an opportunistic pathogen itself (Wu et al., 2022) and close relatives are sometimes fatal (Zeng et al., 2007).
    See: NEW: by analogy with terrestrial fungal diseases – a fungal disease from Mars would be likely to be hard to distinguish from tuburculosis through testing or medical imaging – a new genus would likely have no effective antifungals available initially or for some time because fungi are evolutionarily close to humans making it hard to develop effective antifungals – and we need to consider this possibility as many terrestrial fungi do well in Mars simulation chambers including a strain of a black fungus sometimes pathogenic in humans

- Lichens such as Xanthoria elegans, Pleopsidium chlorophanum (de Vera et al, 2014) and Rhizocarpon geographicum
  - Xanthoria elegans and Rhizocarpon geographicum tested in BIOMEX (De Vera et al., 2019)
  - for more on Pleopsidium chlorophanum, see next section
    2014: Example of an alpine lichen Pleopsidium chlorophanum found in places like California and the Alps that also grows in Mars analogue conditions in Antarctica and can survive and even grow in Mars simulation conditions – this shows even higher life from Mars could be adapted to live on Earth

- Mosses Grimmia sessitana collected in the alps (Huwe et al., 2019)
  - tested in BIOMEX (De Vera et al., 2019)

2014: Example of an alpine lichen Pleopsidium chlorophanum found in places like California and the Alps that also grows in Mars analogue conditions in Antarctica and can survive and even grow in Mars simulation conditions – this shows even potentially some multicellular life from Mars could be able to live on both planets

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Not just microbes, even higher life from Mars could be adapted to live on Earth. One of our best candidates for a lichen to survive on Mars is Pleopsidium chlorophanum (gold cobblestone lichen), an alpine lichen that also grows in Antarctica in Mars analogue conditions and also grows in warmer alpine locations in places such as Europe and California.

Figure 31: Pleopsidium chlorophanum showing its different growing habits.

The photograph to the left shows its semi-endolithic growth in Antarctic conditions. You can see that it has fragmented the granite, and that pieces of the granite are partly covering it, possibly helping to protect from UV light. Photograph credit DLR taken at an altitude of 1492 m above sea level at "Black Ridge" in North Victoria Land, Antarctica (de Vera et al, 2014:figure 1).

The image to the right shows its more usual growing habit in California, above Lake Isabella, in the Kern River area (Sharnov, 1989)

Summarizing details about it from (de Vera et al, 2014), this lichen is able to cope with high UV, low temperatures and dryness in cracks, probably adaptive behaviour to protect it from UV light and desiccation. It remains metabolically active in temperatures down to -20 °C, and can absorb small amounts of liquid water from the atmosphere in an environment where it is only surrounded by ice and snow. The relative humidity in the lichen's niche microhabitat varies from 57% to 79% as the temperature varies from -6 °C to -8 °C and externally it varies from 23% to 46% as the external temperature varies from 8 °C to -8 °C.

In their 34 day Mars simulation chamber experiment the temperature varied between -50 °C and +21 °C, and the relative humidity varied between 0.1% and 75% (because warmer air has lower humidity for the same water content).

The atmosphere approximates conditions encountered in the equatorial and lower lattitude regions of Mars.

When exposed to full UV levels the fungus component of the lichen Pleopsidium chlorophanum died, and it wasn't clear if the algae component was still photosynthesizing,

However, when partially shaded from the UV light, as it is in its natural habitats in Antarctica, both fungus and algae survived, and the algae remained photosynthetically active throughout. Also new growth of the lichen was observed. Photosynthetic activity continued to increase for the duration of the experiment, showing that the lichen adapted to the Mars conditions.

This is remarkable as the fungus is an aerobe, growing in an atmosphere with no appreciable amount of oxygen and 95% CO_2. It seems that the algae provides it with enough oxygen to survive.

The lichen was grown in Sulfatic Mars Regolith Simulant, without ice. Photosynthetic activity was strongly correlated with the beginning and the end of the simulated Martian day, when atmospheric water vapour could condense on the soil and be absorbed by it, and could probably also form cold brines with the salts in the simulated Martian regolith.

The pressure used for the experiment was 700 – 800 Pa, above the triple point of pure water at 600 Pa and consistent with the conditions measured by Curiosity in Gale crater.

The experimenters concluded some lichens and cyanobacteria can probably adapt to Mars conditions, taking advantage of the night time humidity, and that it is possible that life from early Mars could have adapted to these conditions and still survive today in microniches on the surface (de Vera et al, 2014).

If lichens like Pleopsidium chlorophanum on Earth can grow in Mars simulation conditions, then it's biologically possible the other way around that any lichens on Mars may be able to grow on Earth.

So, not only microbial life, also higher life from Mars could potentially be able to colonize Earth.

2009, 2014: Possible future surprise discovery of large quantities of fresh water on Mars: ice lets light through and traps heat, which melts ice half a meter below the surface in Antarctica -– if Martian ice is similar, its polar regions should have meltwater in summer, ~5 cms below the surface, even with surface temperatures below -90 °C – Mars may also have miniature melt ponds around sun warmed dust grains

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If Mars has sources for fresh liquid water, that greatly expands the range of terrestrial species that can live there and would give many new possibilities for indigenous martian life.

We already mentioned the potential for fresh liquid water in Jezero crater from melting frost.

- 2021: Potential for melting frost to form a “dew” of microns thick layers of fresh liquid water even in Jezero crater – as an example to show the potential for future surprise microhabitats

That’s not the only way Mars could have fresh liquid water. Another possible future surprise discovery might be thin layers of liquid fresh water over large areas of the polar regions of Mars not far below the surface of the ice.

When researchers dig a half meter or so below the surface of the Antarctic snow and blue ice, they often find layers of fresh liquid water. They find this water in Antarctica even when surface temperatures are far too cold for ice to melt (Liston et al, 2005: p 1470 and fig 1).

This happens because the snow and ice is thermally insulating but optically transparent. Here optically transparent doesn’t mean optically clear. It can be like frosted glass, you can’t see through it, but it lets light through.

The covering insulating layer of snow or ice is thick enough at half a meter below the surface to hold in the heat, and enough sunlight gets through to warm up the snow or ice until it melts. This continues until the melt layer gets to a thickness of centimeters and even tens of centimeters. Actually, far more ice melts in the subsurface of Antarctica than on the surface. Researchers estimated that all except 46 out of 362.5 cubic kilometers of snow melted per year was subsurface melt in 1991–2000, while all except 2 out of 59.4 cubic kilometers of blue ice melted per year in that same time period was subsurface melt. (Liston et al, 2005).

On a summer day on Mars in the polar regions it would take just one day to melt a layer 1mm thick at a depth of about 5 cm below the surface, even with surface temperatures on Mars as low as 180 °K (-93 °C). This thin layer should remain liquid even at night, through to the next day and gradually increase in depth to centimeters and tens of centimeters (Möhlmann et al, 2009) (Martinez et al., 2013:2.2.2) (Martinez et al., 2013:3.1.2).

This process should happen anywhere on Mars with sun facing optically transparent snow or ice, but it would not be easy to spot from orbit because of the ice cover and the thinness of the layers of water. It’s essentially a cryptic habitat. All that’s necessary is for the snow and ice to be optically transparent and thermally insulating, as it is on Earth and there is no particular reason why it wouldn’t be. A layer of fresh water tens of centimeters thick is like an ocean for a microbe and there would be large areas of this water and it would give access to sunlight with the UV filtered out, as well as to trace elements in any salts, dust and dirt mixed in the ice.

There is another way to get fresh water inside the ice in Antarctica, as melt ponds around dust and rock fragments in the ice which would likely get covered in ice. This should also work on Mars (Möhlmann, 2010).

The way this works in Antarctica and indeed anywhere with ice sheets or glaciers is that dust, microbes and small rock particles, blown by the wind settle on the surface. Because they absorb heat from the sunlight, they warm up in the sunlight. When they settle on ice like this, they are called “cryoconites”. This then melts the ice around them and they sink into the ice forming striking cylindrical holes in the ice. In most ice sheets and glaciers, the holes remain open to the surface. In the McMurdo dry valleys, closest Antarctic analogue to Mars, the cryoconite holes freeze over forming an ice lid a few centimeters thick over the liquid water with air in between like a miniature greenhouse. It works similarly to the meltwater layer for the ice but with the melting enhanced by the warming of the rocks (Zamora, 2018).. Fountain et al. found with rough calculations that the cryoconite holes contribute at least 13% of the meltwater for Canada glacier in the McMurdo dry valleys (Fountain et al., 2004)

They provide a refuge for life in ice sheets, rich in microbes such as mats of various types of cyanobacteria, bacteria, algae, and higher life such as rotifers and tardigrades (Vincent et al., 2000). Vincent et al., suggest they may have provided a refuge for life during snowball Earth when Earth were covered or almost all covered in ice sheets and sea ice a billion years ago. The algal mats in the cryoconite holes are as productive as algal blooms in the polar seas (Vincent et al., 2000: 141). This shows how it works:

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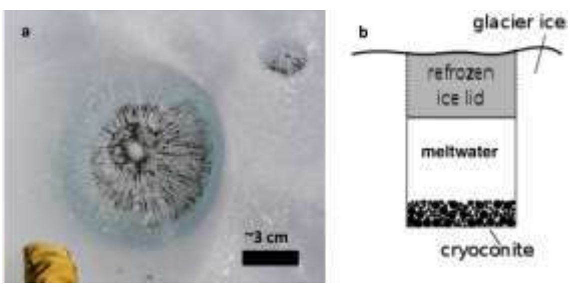

Figure 32: Two ice covered cryoconite holes on the left and sketch of how they work on the right (Zamora, 2018:2)

These cryoconite holes have been proposed as a way that life could survive and propagate in the polar ice caps on Mars, as well as possibly comets and Europa (Hoover et al., 2004) (Corenblit et al., 2019:14). On Mars as in Antarctica if there is life in the dust and sand grains that heat up to form the hole initially, these then inoculate them with life.

Zawierucha et al. suggest that microbial communities from cryoconite holes in Antarctica would be good candidates for model organisms in astrobiology including study of the possibility of higher life on other planets because they can withstand very cold conditions, and abrupt changes of conditions, can go into hybernation, many have dark pigments and are protected from UV, and they propagate easily from one glacier to another amongst other similarities and rotifers are highly resistant to ionizing radiation (Zawierucha et al., 2017) [rotifers depend on oxygen, so they aren’t an exact analogue unless the water on Mars is oxygen rich (Stamenković et al, 2018)]

Scientists have found possible indirect evidence on Mars for both these processes. The subsurface ice melt is one of the two possible explanations for the flow-like features in Richardson crater. These form in the debris of the Martian CO_2 geysers in early spring, extend down slopes in summer, and fade away in autumn, in a seasonal cycle not unlike the RSLs. (Martinez et al., 2013:3.1.2).

Figure 33: Since the images are taken centered at different points on the Mars surface, it is impossible to line up all the features exactly between the images. I've aligned the flow like features in the vertical center of the image by preference. Walker, 2015

These sometimes get confused with the similar looking Northern hemisphere flow-like features which are probably best explained by a dry formation mechanism involving dust and dry ice (Martinez et al., 2013:3.1.2).

The Richardson crater features seem to be formed by liquid water. Both current models explain these features as flowing salty water (brines). In one of them these flows are fed by fresh water from a subsurface ice melt similarly to the Antarctica subsurface melt. In the other model they are fed by undercooled interfacial water – a thin layer of liquid water below the normal freezing point for ice that can form covering the surface of rocks in ice because of interactions between water and the rock with molecules attaching themselves to the rock (Martinez et al., 2013:3.1.2). The two processes are described in (Martinez et al., 2013:2.2.1 and 2.2.2).

As for water melting about dust grains, in Antarctica a similar process around meteorites forms gypsum which could be a clue to the same process on Mars. We have discovered large surface deposits of gypsum around the polar ice caps. This is puzzling because gypsum is soft and easily eroded with no obvious source to replenish it in this region (Fishbaugh, 2007) and this is one possible explanation for these deposits (Losiak et al., 2014). Martian rocks or dust on the surface could lead to surface melts that survive up to a few hours on warm windless days (Losiak et al., 2014), or they could be trapped in the snow with the ice freezing and keeping the water from evaporating similarly to the solid state greenhouse effect (Möhlmann, 2010), a process that should be effective between a few centimeters depth down to ten meters below the surface. That could easily explain the otherwise puzzling gypsum deposits.

If Mars does have freshwater habitats in the polar regions, this is a potential habitat for a wide range of microbial species to exploit. Similar Antarctic habitats have a diverse ecosystem of mcrobes, both in the fresh water and also in salty brines that form as the fresh water mixes with local salts (Doytchinov et al, 2022).

At the moment there is no direct evidence of fresh water on Mars. However, these proposed microhabitats would be undetectable by the instruments and spacecraft we have sent to Mars so far. Mars has surprised us many times and it's not impossible that it surprises us again with fresh water. These proposals seem to have a lot going for them, since there seems no particular reason why these processes, which occur widely on Earth, wouldn't happen similarly on Mars.

Then there's another possibility that I can't find in the literature of Mars yet. So it's a speculative suggestion in this paper, but it's just combining those ideas with the discovery of ice boulders thrown up by meteorite collisions in regions close to the equator where there is ice near the surface.

If the ice boulders are optically translucent, as seems likely, they would have subsurface melt water too on sunny days in summer. Also, the dust from the dust storms and the bouncing sand grains would be sure to settle on their surfaces and might form cryoconite holes and inoculate them with any microbes that may find their way into the dust, which would be another possible habitat. We couldn't see this from orbit. The resolution isn't good enough to see typical McMurdo dry valley cryoconite holes at up to multicentimeter scales.

See:

- Value of targeting a newly formed crater on Mars as an alternative to drilling meters below the surface – with example of a crater that excavated ice boulders from the Amazonis planitia in the equatorial regions in 2022 – also value of developing a 100% sterile marscopter, rover or complete lander

The remarkable polyextremophile genus, the blue green algae chroococcidiopsis, one of our top candidate Mars analogue organisms, has strains in many terrestrial habitats, and sometimes in the human microbiome

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The remarkable ability of Radiodurans to repair multiple double strand breaks of DNA is also shared by one of our top candidates for a terrestrial microbe to survive on Mars, dessication resistant desert strains of the blue green algae chroococcidiopsis. The BIOMEX experiment on the exterior of the ISS tested Chroococcidiopsis sp. ASB-02, a species isolated from the Urad Middle Banner desert in inner Mongolia, and it remained viable after exposure to cosmic radiation in Mars simulation conditions (Li et al, 2022).

A microbe from Mars only needs to find a niche somewhere on Earth that it can survive in, then it can evolve and adapt and proliferate to other habitats. Species from the genus chroococcidiopsis flourish from Antarctic cliffs to the Atacama desert (Bahl et al, 2011) or from Sri Lankan reservoirs (Magana-Arachchi et al, 2013) to the Chinese sea (Xu et al, 2016:111). As a prime producer chroococcidiopsis survives on just rock, water, and light, fixing CO_2 and nitrogen from the atmosphere.

Chroococcidiopsis is an ancient polyextremophile with numerous alternative metabolic pathways it can use, including nitrogen fixation, methanotrophy, sulfate reduction, nitrate reduction etc (KEGG, n.d.), with strains of chroococcidiopsis even able to grow in complete darkness with viable populations 750 meters below the Atlantic sea bed (Li et al, 2020). In this habitat chroococcidiopsis strains can get energy by oxidising hydrogen produced in the rocks by various abiotic processes (Puente-Sánchez et al., 2018).

Chroococcidiopsis like many bacteria reproduces asexually through cell division, making the distinction between a species and a strain rather fluid as they can't interbreed, though they can share genes via horizontal gene transfer with other bacteria.

Species of chroococcidiopsis are also sometimes found in the human microbiome, for instance in the upper throat behind the nose (Ventero et al, 2022) and human breast milk (Lackey et al, 2019).

NEW: Chroococcidiopsis indica produces an accidental neurotoxin, BMAA, which resembles the amino acid serine and by replacing it, can cause protein misfolding and may be a contributing cause to Lou Gheric disease, the disease which Steven Hawking had – leading to the possibility that novel amino acids from a novel exobiology could also cause protein misfolding

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One strain of Chroococcidiopsis, chroococcidiopsis indica produces BMAA (Cox et al., 2005:fig 2), which may be a contributing cause to neurodegenerative diseases such as ALS which Steven Hawking suffered from, as it can bind to serine transfer RNA and so get misincorporated into proteins in place of serine. This leads to protein misfolding and these misfolded proteins have been found in nerve cells of people with ALS (Holtcamp, 2012).

This leads to interesting questions about what the effects might be of an extraterrestrial biology not based on terrestrial amino acids.

An extraterrestrial biology could have proteins built up of many more amino acids than the 20 encoded in RNA and used to build proteins in terrestrial biology. There are 140 that occur elsewhere in terrestrial biology, but not in proteins (Ambrogelly et al., 2007). 52 amino acids have been identified in the Murchison meteorite (Cronin, 1983). A computer search turned up nearly 4,000 biologically reasonable amino acids (Meringer, 2013) (Doyle, 2014). Many of those won't occur in nature, but terrestrial biology also includes non natural amino acids. Meanwhile also many of the natural amino acids don't occur in terrestrial biology and might potentially be used in extraterrestrial biology.

The suggestion here is that some extraterrestrial amino acids could get misincorporated for terrestrial amino acids similarly to the way BMAA is misincorporated for serine. That then may lead to the proteins folding into different shapes, as for BMAA. Proteobacteria in our gut may provide some protection against BMAA by removing it (Baugh et al, 2017). However there might be no helpful microbes to protect us by removing similarly close analogs of our amino acids from an alien biochemistry.

NEW: Martian life could be better at photosynthesis than terrestrial life since terrestrial photosynthesis works at well below its theoretical peak efficiency and the lower light levels on Mars might favour evolution of more efficient photosynthesis

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Photosynthetic life on Earth operates at well below its theoretical peak efficiency for photosynthesis. Bains et al suggest this may be a many pathways event. They suggest that perhaps oxygenic photosynthesis could evolve in many ways, but with very low probability of achieving all the necessary steps. So terrestrial life only happened to evolve it once. As a perhaps more plausible alternative, they suggest that it could be a "pulling up the ladder" event where once the niche was filled, with a photosynthesizer not limited by the need for an electron donor such as sulfide, Fe(II) or hydrogen it was hard for a new photosynthesizer to evolve again (Bains et al, 2016).

Terrestrial photosynthesis rejects 50% of the incoming sunlight, mainly in the red part of the spectrum, leading to the distinctive "red edge". The purple bacteria and lichens don't have this "red edge" and Martian life would be likely to use red light like the purple bacteria, because of the high absorption of blue light by dust (Kiang, 2007).

Oxygenic photosynthesis goes through two photosystems, 1 and 2, and both use the same frequencies of light. The efficiency could be doubled by using red light for one of the two systems (Blankenship et al, 2011:808).

Martian life might also be able to use the full range of the spectrum. Terrestrial seaweeds are dark brown in colour because they use accessory pigments like fucoxanthin to gather the blue-green component of light rejected by chlorophyll. These then transfer the energy to the chlorophyll and so to the photosynthetic reaction centers. They do this so that they can use sunlight at only 1% of surface levels so it helps to use the blue-green light that passes through seawater (Caron et al, 2001).

A hypothetical Martian microbe with faster photosynthesis might find it useful to capture the full spectrum, especially in the low light levels on Mars. This would double its theoretical efficiency compared to terrestrial life.

According to Mellis, it would be possible to increase the typical 3% efficiency of green algae three fold, close to the theoretical maximum of 8 to 10% by truncating the light-harvesting chlorophyll antenna size (Mellis, 2009). Experiments back this up, though with smaller improvements (instead of tripling, they achieve modest increases of 55% to 60%) (Kirst, 2014). Terrestrial life likely uses a larger antenna than is needed, either to block out light from competitors, or because it allows it to capture more light at lower light levels with lower cell densities (Ort et al, 2015:8530) (Negi et al, 2020:15).

Although terrestrial life uses a fixed antenna size, cells have been designed using bioengineering that adjust the antenna size depending on the light intensity so that they achieve high efficiency both at low and high light levels compared to wild-type strains, doubling and even tripling the yields of the wild-type strains (Negi et al, 2020:15). A Martian photosynthetic organism would experience large changes in light levels with a need to capture light during dust storms if possible, and also to capture as much as possible during conditions of bright sunlight. So it might already have an adjustable antenna size, combining the advantages of both small and large antennas.

A Martian photoautotroph would only need a small improvement in efficiency compared to terrestrial life to be competitive with our photoautotrophs in the oceans, and there seem to be possibilities for major increases in efficiency. This Martian photoautotroph then might replace the natural species in our oceans.

In the best case, this could just be a drop-in replacement for terrestrial life. In this best case it has minimal effect on the diversity of the terrestrial marine microbiota which survives but in smaller populations, and increases the productivity of the oceans. It might also have fewer or no exotoxins and might not form algal blooms. In the other direction though, in the worst case, it's inedible, or produces many accidental toxins, is so competitive that the new marine biota is almost a monoculture, or its growing habit produces thick algal mats that block out light below the surface, or any or all of those.

Mars has had many geological surprises like the CO_2 geysers – once we start to look in earnest we may find many astrobiological surprises too

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Mars has had many geological surprises for us. CO_2 geysers (Kieffer et al., 2006), increasing evidence of a shallow northern sea in the ancient past (Berad, 2022) (Cardenas et al., 2022), deltas like the one in Jezero crater, the lake in Gale crater, or perhaps smaller lakes (Michalski, 2021) (Liu et al., 2021), the perchlorates (Hand, 2008), the droplets on the Phoenix lander (Renno et al, 2009). It may have astrobiological surprises too.

Figure 34: Artist's impression by Ron Miller of the martial CO_2 geysers that form in spring in the polar regions (Miller, 2006) (JPL, 2006).

This leads to our new worst case scenario: mirror life. This could compete with terrestrial photoautotrophs even if it is no better or even less efficient at photosynthesis, and could be combined with better photosynthesis for a worst-worst case scenario.

NEW: Example worst case scenario of a mirror life chroococcidiopsis analogue from Mars which gradually converts organics in ecosystems into indigestible mirror organics

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See:

- Questions for NASA – and why NASA's main argument is invalid

The planetary protection literature doesn't cover many scenarios in depth. In a search for new scenarios, I found many ways life from Mars can harm humans, our crops or ecosystems – as well as many ways it can be harmless or beneficial. This is a paper I had been working on for some years but not yet published when NASA published its draft environmental impact statement (Walker, 2022b).

It may help to briefly mention one detailed worst case scenario, which as far as I know is new to the topic, to encourage space agencies to treat planetary protection more rigorously.

This worst case but scientifically very interesting scenario is independently evolved mirror life. It could include an algae like chroococcidiopsis able to survive on just rock, water, CO_2, nitrogen and sunlight, but with the DNA flipped the other way, everything flipped as in a mirror.

Nearly all terrestrial DNA spirals the same way. Most of the organics that make up terrestrial life are asymmetrical and so can have two mirror forms, like your left and right hand – but they nearly all are only found in one form in terrestrial life. That's because the molecules fit together a bit like an intricate mechanism, and the enzymes, and translation machinery and ribosomes which construct proteins and so on wouldn't work as intended if some of the pieces were flipped as in a mirror.

When a molecule can occur in two mirror forms, like your hands, it's called chiral - the word chiral is derived from the Greek word χειρ (*kheir*) for hand. Terrestrial life is homochiral, which means that nearly all of its asymmetrical (chiral) molecules occur in only one of its two mirror forms. Also terrestrial life for the most part can't use any mirror organics it finds and just ignores them.

Figure 35: Background image from (NOAA, n.d.), DNA spiral from (Pusey, 2012)

If we could flip a cake in 3D, like reflecting it in a mirror, all the way down to its molecules, we might be able to eat it, like artificial sweeteners, but our metabolism couldn't do anything with the flipped starches or proteins, and many fats would also be inaccessible (Dinan et al, 2007)

We don't know how terrestrial life became homochiral, with many proposed mechanisms (Blackmond, 2019). Some experts say it is *"luck of the draw"* (Brazil, 2015).

The theory of punctuated chirality suggests that early on as life was just starting to evolve, there were patches of chemicals that worked together with each other in chiral networks which expand converting a non chiral substrate into chiral organics and where two chiral networks of opposite chirality meet there are ways for them to slowly convert each other to the opposite chirality.. For instance one network might consist of the chemicals used by terrestrial life, and another might consist of all the same chemicals but flipped as in a mirror and these chemicals can include enzymes that turn the substrate into chiral molecules (Gleiser et al., 2008a) (Gleiser et al, 2008b). Both chiral networks also have to maintain themselves against the pressure of racemization – unlike our hands, these small molecules can spontaneously flip to the opposite sense. They do it faster at higher temperatures and also UV and ionization radiation can cause them to flip (Cataldo et al., 2005).

However there would be many such patches, some of one chirality some the opposite, and they would expand and flip each other back and forth in chirality on an environmental scale, with these flips perhaps frequent in Early Earth (Gleiser et al., 2008a), until one of them got established as the basis for the evolution of life. If so, depending on how the flips went on Mars, life could easily have evolved from chemicals with the opposite chiral bias to Earth life (Gleiser et al, 2008b).

Our analysis predicts that other planetary platforms in this solar system and elsewhere could have developed an opposite chiral bias.

They predict that in the universe as a whole if an organic is found in a large sample of independently evolved forms of life it should occur in both forms (Gleiser et al, 2008b).

As a consequence, a statistically large sampling of extraterrestrial stereochemistry would be necessarily racemic on average

This means that if you have the same chemical in many different independently evolved forms of life, then there will be roughly equal amounts of both symmetries of that chemical in the universe. E.g, if many independently evolved forms of life use glucose, there should be roughly the same amount of D and L glucose in the universe.

Summarizing this as a graphic:

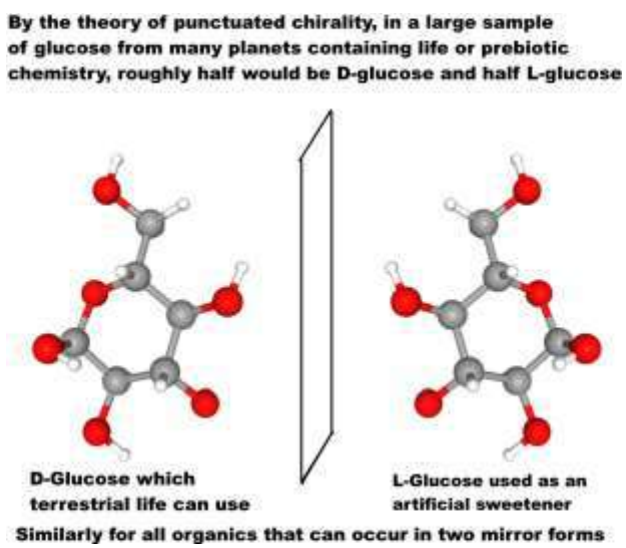

Figure 36: Graphic for L-glucose from (NCBI, n.d.) and D-glucose by reflecting the graphic horizontally. Grey: Carbon, Red: oxygen, white: Hydrogen.

Synthetic biologists plan to gradually flip ordinary to mirror life over a period of a decade or so – and will make sure synthetic mirror life is engineered to depend on chemicals only available in the laboratory. They warn escape of mirror life could cause major transformations of the terrestrial biosphere by locking up organics in unusable mirror forms (Bohannon, 2010).

The biggest risk here is if mirror life gets enzymes (isomerases) that transform ordinary organic molecules into their mirror form. A few rare terrestrial microbes already use this in reverse to eat mirror organics (Pikuta et al, 2016). In the worst case scenario, mirror life has the enzymes to let it consume ordinary organics, but terrestrial life can't make anything of the mirror organics (Bohannon, 2010). ***Kasting*** *"It would quickly consume all*

the available nutrients. This would leave fewer or perhaps no nutrients for normal organisms."
The *CO_2 in the ocean would get taken up by inedible mirror cells and so draw down CO_2 from the atmosphere. He calculated that in around 300 years half of Earth's CO_2 would be gone. At that point most land plants couldn't photosynthesize, including all agricultural crops except corn and cane sugar (which use C-4 photosynthesis which can work with almost no CO_2).*

"All agricultural crops other than corn and sugar cane would die,"

… "People might be able to subsist for a few hundred years, but things would be getting pretty grim much more quickly than that."

At 600 years they envision a new ice age with almost no *CO_2 left.*

The article continues (Bohannon, 2010):

—both Kasting and Church think mirror predators would evolve, but whatever life existed on Earth by that point wouldn't include us.

Martian life likely already has the isomerases to metabolize organics of opposite sense, whether it is mirror or normal life - because nearly all organics are either made abiotically locally, or are infall from comets, asteroids and interplanetary dust, with organics of both senses.

Eventually terrestrial microbes likely develop isomerases to metabolize mirror life, but higher life couldn't evolve so quickly. The outcome is a mix of normal and mirror organics. In Kasting and Church's worst case scenario mirror life would need to retain the edge over normal life in this evolutionary race.

I think we would survive however with modern technology.

Humans wouldn't go extinct even if we returned mirror-life and it made Earth uninhabitable for higher terrestrial life – over periods of decades to centuries we'd cover Earth with habitats for ourselves and the rest of our biosphere similarly to the habitats for space settlements and proposals for paraterraforming – but it is potentially a severely diminished world to leave to future generations

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We have already designed almost self-sustaining space habitats like the early Russian BIOS-3 based on plants grown for food, and oxygen. The plants in turn take up carbon dioxide and water from humans, and our metabolisms use precisely the amount of oxygen that is generated by the plants we eat as they grow, making for a self sustaining closed system. This should work in space, a more challenging situation than an enclosed habitat in a world dominated by mirror life (Salisbury et al, 1997) (Johansson, 2006) .

We could enclose large areas of Earth with its tropical jungles, coral reefs etc, in habitats like this, similarly to Biosphere 2 (UA, n.d.).

It would happen slowly, maybe decades to generations, by the estimates of the synthetic biologists (Bohannon, 2010). We would have enough time to recognize what is happening and build habitats to survive in. Also, we would be able to preserve much of the Earth's biodiversity including all the plants with preservable seeds (which is most of them).

In these worst case mirror life scenarios, there may be many ways to reduce or at least slow down the impacts on Earth's biosphere, perhaps with engineered normal life predators of mirror life. Or if Mars has both forms of life, we may be able to import normal life predators of mirror life from Mars.

We could develop new crops that are able to metabolize the mirror organics and be grown outside the habitats. We might be able to engineer predators of the mirror life photobionts to return the CO2 to the atmosphere, or maybe return them from Mars.

However, these are scenarios to avoid, with consequences hard to predict and likely to be difficult to direct to a desired outcome. Such a paraterraformed Earth could severely diminish life prospects for several generations.

NEW: Closely related worst case scenario of a shadow biosphere of small mirror life nanobes that produce indigestible mirror life biofilms on Earth with small cells advantages that they take up nutrients faster and avoid protozoan grazing

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For a closely related scenario, Earth and Mars exchange normal life, but Mars has a shadow biosphere with a different biochemistry that never got here like the hypothesis of a terrestrial shadow biosphere of nanobes (Cleland, 2019, pp 213 - 214) which could co-exist with modern life. Earth doesn't seem to have one (yet) but small cells have an advantage in an environment with low nutrient concentrations, as they have a larger surface to volume ratio, and so take up nutrients more efficiently. They would also avoid protozoan grazing (Ghuneim et al, 2018).

In this second mirror life scenario, Martian mirror life cells have a less sophisticated biology, but compete in a shadow biosphere on Earth because of their small size, with the extra advantage that they form mirror organics biofilms. These shadow biosphere biofilms are inedible to most terrestrial life and expand.

The mirror nanobes could have evolved in regions separated by physical barriers, for instance after a volcanic eruption such as volcanic eruption on the flanks of Arsia Mons 210 million years ago, which likely lead to 100 cubic kilometers of subsurface melt in three lakes, which would have stayed melted for centuries to millenia insulated by surface ice (Scanlon et al, 2014).

With the punctuated chirality model, Gleiser et al. see it as an unlikely possibility that the very early stage of prebiotic chemistry they are looking at could start in hydrothermal vents, but if it could, indeed different vents would vary in chirality. However, there is too much mixing from the circulation of the oceans for them to stay as separate networks indefinitely, as they gradually evolve to life (Gleiser et al, 2008b:6). But on early Mars and indeed later on, through to the present there were many possibilities for life to start again a second time possibly not inoculated by life that already existed elsewhere on the planet.

We could devise other examples such as ordinary life co-existing with mirror life with similar capabilities to normal life so it has a mix of normal and mirror life analogues of terrestrial blue green algae. This would allow martian life to take most advantage of the infall of achiral organics. Also, more speculatively, it cold have chirality indifferent life using enzymes such as Joyce's RNA enzyme which can replicate RNA of opposite chirality including its own mirror version (Joyce, 2007) (Sczepanski, 2014) (Singer, 2014)

NEW: Claudius Gros's worst case scenario for forward contamination – if this scenario can be applied in reverse, nearly all higher life eventually goes extinct outside habitats, though it takes a long period of time

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For completeness perhaps I should mention this scenario too. It is an extension of the ideas of Sagan and Lederberg about the potential that we might have no protection against an exobiology from Mars in the worst case.

- Warnings by some astrobiologists such as Sagan and Lederberg that in worst case we could be in effect immunocompromised to an entire exobiology from Mars

This is not a human extinction scenario but if it is possible, it is of course a scenario to avoid, see next section:

- Humans could survive even Lederberg's scenario and even Gros's scenario (in reverse) by covering Earth with large enclosed habitats using modern technology – and we could preserve nearly all our biodiversity – over millions of years the result may have a more diverse biochemistry with interesting new lifeforms – but if these are possible scenarios they are ones to avoid

If Sagan and Lederberg's alternative scenario was true of terrestrial life mystified by an alien pathogen, it wouldn't just apply to humans but potentially to all terrestrial life. So, what would the outcome be if we returned life that none of Earth's multicellular organisms has immunity to or has natural defences against?

It seems we should look at this possibility, if it is a possibility.

As with Sagan and Lederberg's warnings, it is impossible to go to the expert reviews for comment on this because it isn't covered in the backward contamination studies for a Mars sample return. We have some very respected experts saying it is something we need to consider as a possibility in the published scientific literature on the topic, but that is the end of the dialog.

In the forward direction, the physicist Claudius Gros looks at a clash of interpenetrating biospheres in his paper on a "Genesis project" to develop ecospheres on transiently habitable planets. Gros reasons that the key to functioning of the immune system of multicellular organisms, plants or animals, is recognition of "non-self". He presumes that biological defense mechanisms evolve only when the threat is actually present and they don't evolve to respond to a never encountered theoretical possibility (Gros, 2016).

> *"How likely is it then, that 'non-self' recognition will work also for alien microbes?"*
>
> *"Here we presume, that general evolutionary principles hold. Namely, that biological defense mechanisms evolve only when the threat is actually present and not just a theoretical possibility. Under this assumption the outlook for two clashing complex biospheres becomes quite dire."*
>
> *"In the best case scenario the microbes of one of the biospheres will eat at first through the higher multicellular organisms of the other biosphere. Primitive multicellular organisms may however survive the onslaught through a strategy involving rapid reproduction and adaption. The overall extinction rates could then be kept, together with the respective recovery times, 1–10 Ma [million years], to levels comparable to that of terrestrial mass extinction events."*
>
> *"In the worst case scenario more or less all multicellular organism of the planet targeted for human settlement would be eradicated. The host planet would then be reduced to a microbial slush in a pre-cambrian state, with considerably prolonged recovery times. The leftovers of the terrestrial and the indigenous biospheres may coexist in the end in terms of 'shadow biospheres' "*

Is this argument valid, and can we apply this argument in reverse for backwards contamination?

If it can be applied in reverse, then in the worst case scenario,. terrestrial life is naive and offers no resistance when eaten by Martian life. The worst case would be that almost all multicellular organisms on Earth could be eradicated. All that would be left would be some small rapidly evolving organisms.

It might be a possible scenario that microbial life also wouldn't be immune, for instance from bacterial or fungal pathogens of an alien exobiology. See

- NEW: Microbes from Mars could have pathogens that can infect terrestrial microbes – example of fungal pathogens of phytoplankton and cyanobacteria – cyanobacteria have specific antifungal adaptations to the phylum that attacks them, the chytrids and may have no adaptations to a novel phylum from Mars

If it is possible to generalize from this idea of a fungal pathogen of microbes of a related but novel phylum to an unrelated biochemistry, it may be a possible scenario that all terrestrial life, both higher life forms and microbial life has no defences against pathogens from a completely alien exobiology.

The main difference here between microbes and higher life is that terrestrial microbes with their much shorter generation time and their much larger populations would be able to adapt far faster than higher life to resist a fungus or similar pathogen from a novel exobiology.

If Claudius Gros is right, and his ideas can be used in reverse and also extended to the microbial level, new microbes from a novel exobiology could also be vulnerable to the Chytrid fungi too, which might protect us from them to some extent.

Perhaps such a scenario is impossible. But it might also be that we were lucky and that many civilizations at the same early level of technology, at the time of Apollo, return alien life from a nearby biosphere and make themselves extinct. If so it is not correct to assume that our luck will continue to hold if we continue to explore in the same way as before.

As far as I know, none of the planetary protection studies have looked at such a scenario, or at the scenarios of Lederbeg and Sagan mentioned above:

- Warnings by some astrobiologists such as Sagan and Lederberg that in worst case we could be in effect immunocompromised to an entire exobiology from Mars

So this is to draw the attention of experts to their scenarios.

I suggest future planetary protection studies of the effects of a Mars sample return use Lederberg's papers as a starting point, and also consider Gros's ideas. Based on this we need to look at the potential impacts on human health and more generally on Earth's biosphere of a totally alien unrelated exobiology such as mirror life or life not based on DNA.

The backward contamination studies so far proceed almost entirely by analogy with known effects of terrestrial life. They only briefly mention the potential impact of returning samples of life based on a different biochemistry or don't discuss it at all.

I haven't been able to find any specific scenarios of a second genesis such as mirror life in the planetary protection literature to date. That is why it seemed important to introduce a specific example in this paper. Also it seemed important to draw attention to Lederberg's papers and

Gros's suggestion, and to start the process of examining whether those scenarios are also possible.

Humans could survive even Lederberg's scenario and even Gros's scenario (in reverse) by covering Earth with large enclosed habitats using modern technology – and we could preserve nearly all our biodiversity – over millions of years the result may have a more diverse biochemistry with interesting new lifeforms – but if these are possible scenarios they are ones to avoid

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We could survive even this scenario without going extinct, by paraterraforming Earth as described above in:

- Humans wouldn't go extinct even if we returned mirror-life and it made Earth uninhabitable for higher terrestrial life – over periods of decades to centuries we'd cover Earth with habitats for ourselves and the rest of our biosphere similarly to the habitats for space settlements and proposals for paraterraforming – but it would be a severely diminished world to leave to future generations

We would have to be more careful, because in this scenario, microbial life outside the habitats could be pathogenic for humans. But it would likely still be easier than living in a space habitat on another planet or the Moon.

Eventually life outside the habitats would reach an equilibrium, with small microscopic single cell and multicellular terrestrial lifeforms able to evolve fast enough to take advantage of the new microbial environments. Over millions of years, perhaps faster with assistance from humans, there would be higher life forms again able to survive in an environment with both kinds of biology. Perhaps humans also could artificially adapt our progeny to survive outside habitats or find ways to supplement their own immune systems so that they are protected from extraterrestrial microbes that our naive immune systems don't recognize as life.

The end result might well be a far more biodiverse world, maybe even with new forms of higher life similar to lichens, the result of cooperation between a fungus and an algae, but this time based on mutual beneficial interactions between terrestrial life and an unrelated exobiology from Mars. But essentially this process would turn Earth into an alien planet for macroscopic terrestrial biology in its current (original) form.

We have the technology to survive this scenario today, but it would have been much harder with the early technology of the 1960s. The first "bubble boy" David Vetter who lived his life in an isolation room was born in 1971 (Gannon, 2012). Without experience of such technology, humans in the 1960s might have had great difficulty adapting to survive back contamination of

Earth's biosphere if our biology is not able to protect itself naturally against the alien life. This would have depended how fast it spread. It might have spread slowly enough so that we would have develop the technology in time.

NEW: Enhanced Gaia – ways that introduced Martian life could be beneficial to humans, ecosystems and Earth's biosphere

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So far we've focused on situations where biosphere collisions are harmful. We need to focus on scenarios where there is indeed a need to protect Earth for the same reason that a designer of a smoke detector has to focus on house fires.

However, we should also recognize that the introduction of extraterrestrial life to our biosphere could be harmless and also be beneficial, as Rummel mentioned in his foreword to "When Biospheres Collide" (Meltzer, 2012)

> *"Likewise, we don't know what would happen if alien organisms were introduced into Earth's biosphere. Would a close relationship (and a benign one) be obvious to all, or will Martian life be so alien as to be unnoticed by both Earth organisms and human defenses? We really have no data to address these questions, and considerate scientists fear conducting these experiments without proper safeguards. After all, this is the only biosphere we currently know – and we do love it!"*

We have examples from multicellular life to show that invasive species aren't always harmful. Schlaepfer et al did a survey of invasive species and in their table 1 they find many non native species that are actually beneficial. Some were deliberately introduced for their value for conservation, but many of the best examples were introduced unintentionally (Schlaepfer et al, 2011).

Schlaepfer doesn't list any microbial examples. What could benign interactions with terrestrial life look like for Martian microbes? We can get some ideas from looking at the ways that beneficial terrestrial microbes help higher life, and from capabilities that Martian life might have that might be of value in potential habitats where terrestrial life has limited growth. Let's for the moment look at just the positives without looking for possible downsides of any of the interactions.

Here are a few suggestions:

- More efficient photosynthetic life from Mars could increase the rate of sequestration of CO_2 in the sea and on land, improve soil organic content, and perhaps help with reduction of CO_2 levels in the atmosphere
- More efficient photosynthesis could increase the productivity of oceans
- Better at nitrogen fixation, which is an energy intensive process (Bueno Batista et al., 2019) and may have room for optimization similarly to photosynthesis.

- Adapt to a wider range of temperature conditions and grow faster than terrestrial life, perhaps especially in colder conditions
- Better at phosphorus and iron mobilization, and so improve our soils
- Help with crop yields as endophytes– the plant equivalent of the human microbiome – these are fungi, bacteria and other microbes that live between the cells of plants (endophytes) without harming them and often beneficially (Gouda et al., 2016) (Afzal et al, 2019) that co-exist with plants
- – they help by acquiring nutrients for the plant, generating hormones that help the plant, protect it from pathogens, insects and herbivores, produce antibiotics and protect it against stresses from the environment such as high and low temperatures, drought, salinity and toxic metals (Baron et al., 2022) and help with crops in the same way (White et al., 2019)
- Endophytes produce unusual and valuable organic substances that solve not only plant health problems but human health problems too (Montana State University, n.d.) (Gouda et al., 2016), for instance Taxol, the world's first billion dollar anti-cancer agent, currently sourced from the bark of yew trees but there is research underway to produce it from a strain of Aspergillus fumigatus that is able to coexist with certain yew trees as an endophyte (Kumar et al., 2019) (El-Sayed et all, 2020).
- Martian life might aid digestion, like terrestrial life does, or enter into other beneficial forms of symbiosis with humans
- Martian life might be better at metabolizing cellulose and aid the digestion of ruminants
- Martian life could produce beneficial bioactive molecules as part of the human microbiome. These could include molecules that are antiviral, antibacterial, antifungal, insecticides, molecules that kill cancer cells, immunosuppressants, and antioxidants – we get all of those from beneficial microbes that are already in our microbiome. (Borges et al, 2009).
- Extremophile fungi may be a source of bioactive compounds for medically useful drugs (Chávez et al., 2015) after screening for toxicity (Madariaga et al., 2019).
- Martian life could increase species richness by gene transfer to Earth microbes, leading to more biodiverse microbial populations.
- Martian extremophiles could be able to cope with drier conditions than terrestrial life through adaptations to retain humidity on Mars, and colonize microhabitats in deserts and eroded landscapes barely habitable to terrestrial life, helping with reversal of desertification
- Most of the surface layers of our oceans are almost uninhabitable to life, except near to the coasts, because of the limitation of nitrogen, phosphorus, iron and silica (needed for diatom shells) (Bristow et al, 2017). If extraterrestrial life has different nutrient requirements, it may be able to inhabit these "ocean deserts" and form the basis of an expanded food web in the ocean which may be accessible to terrestrial ocean life.
- Not limited by some elements that terrestrial life requires, for instance it could use arsenic in place of phosphorous, which would help in deep hydrothermal vents and desert varnish (Davies et al, 2009:245) – or it might use phosphorous in place of sulfur, RNA world life enzymes use phosphorus instead of proteins which need sulfur (Davies et al, 2009) – which might find a home in acid sulphate soils (Queensland Government, n.d.)
- Martian microbes with more efficient photosynthesis might be useful to generate biofuels from sunlight and water (Schenk et al, 2008)

- Martian life might be accidentally toxic and control harmful microbes or insects

We saw before that introduced life that's better than terrestrial life could be harmful. But that's with our focus on worst case scenarios:

- NEW: Worst case scenario - If a martian microbe can grow in the sea, soil, and fresh water like chroococcidiopsis, is adapted to spread in the wind in Martian dust storms, and outcompetes terrestrial biology, e.g. better at photosynthesis or nitrogen fixation, it could be found globally after introduction to Earth in weeks to months, and be one of the most common microbes in our soils and oceans in years to decades or sooner, far more common than nanoplastics or microplastics

Here we are looking at the flip side of that. An introduced biology that is better than terrestrial life could be beneficial by making our biosphere more productive and in many other ways.

Martian life might also have many commercial applications. See below:

- Why we might want to protect species on other planets as we protect species on this planet – intrinsic value like a work of art – perhaps an ethical right to exist as a species – commercial value like the billion dollar industry for enzymes from extremophiles – health benefits for medicine and bioactive compounds – and comparison with the now extinct Australian gastric brooding frog

Introduced martian life could also have mixed effects, beneficial for some organisms and in some ecosytems and harmful in other contexts.

It can also be harmless. We could return a "drop in replacement" for terrestrial life. Just return another slightly different strain of chroococcidiopsis say not much different from returning life from another terrestrial desert. Or it could be life that has no chance of competing on Earth, an example might be Woese's early life transformable cells which don't compete at the cellular level but evolve through massive parallel evolution and Darwinian evolution only of the components of cells with "all the cellular componentry altered and/or displaced through *HGT [Horizontal Gene Transfer]" (*Woese, 2002). Such cells would likely be extremely vulnerable to modern life.

But here, let's continue to focus on the best-best case scenario of enhanced Gaia.

Our planet is not necessarily optimal for global biomass (Kleidon, 2002), and it may have had significantly higher biomass during the early carbonaceous period when most of its land area was covered in the first tropical jungles Schulze-Makuch et al., 2020:1397). More on this towards the end of the earlier section on swansong Gaia:

- NEW: Swansong Gaia: photosynthetic life could sequester CO_2 into organics to stabilize a swansong biosphere for billions of years over an even wider range of volcanic CO_2

emission scenarios - a thin atmosphere close to the triple point of water might even be a weak biosignature for a Mars-like planet

Perhaps extraterrestrial life with additional capabilities could enhance the productivity of the terrestrial Gaia.

Return of Martian life might

- create a new enhanced Gaia system that has significantly more surface biomass and biodiversity than the one we have today, maybe even more than during the early carbonaceous period.
- add a new domain of life with almost entirely beneficial interactions similarly to the Archaea
- add to biodiversity with new forms of multicellular life based on a different biochemistry, or multicellular life in a different domain of life from the eukaryotes, with a more ancient common ancestor which only enhances the diversity of our biosphere.

NEW: Amongst a million extra-terrestrial civilizations that return unsterilized unstudied life – how many would find they harmed the biosphere of their home world? We don't know

Next section – all sections – previous section

Amongst a million extra-terrestrial civilizations that return an unsterilized sample of life from a nearby biosphere without studying it first, and limited technological capabilities to contain it, we don't know how many would find they have harmed the biosphere of their home world.

It could be anywhere in the range from no effect or beneficial to frequently harmful.

- it is never seriously harmful, it usually leads to an enhanced Gaia, or has no effect, and is almost always a beneficial process or harmless,
- [many other possibilities], all the way to
- most civilization's biospheres are seriously degraded after they return unstudied unsterilized life.

We have nothing by way of previous experience to guide us here.

If NASA or another space agency accepts the NRC study's assessment that the risk of large scale effects on human health or the environment is not demonstrably zero – this has major legal ramifications domestically, with agencies such as the DoA, CDC, NOAA etc involved and also internationally and through international treaties with the FAO, WHO etc involved as well as potentially domestic laws of other countries

Next section – all sections – previous section

There are numerous legal ramifications if a space agency such as NASA takes on board the assessment of the National Research Council's study in 2009, that the risk from martian life of minor or major global harm to humans or the environment can't be assessed, and though likely low is not demonstrably zero (SSB, 2009: 48).

In the US, NASA itself, as a federal agency, is mandated to consider such matters as (NASA, 2012):

- impact on the environment,
- impact on the oceans,
- impact on the great lakes,
- escape of invasive species,
- lab biosecurity against theft

Uhran et al mention many other agencies likely to declare an interest including (Uhran et al, 2019) (Meltzer, 2012:454) (Race, 1996).

- CDC (for potential impact on human health),
- Department of Agriculture (for potential impact on livestock and crops),
- NOAA (for potential impact on oceans and fisheries after a splashdown in the sea)
- Occupational Safety and Health Administration, to consider questions of quarantine if a scientist or technician gets contaminated by a sample
- Department of the Interior which is the steward for public land and wild animals which could be affected by release of Martian microbes
- Fish and Wildlife Service for the DoI who maintain an invasive species containment program and may see back contamination as a possible source of invasive species

Although the sources I used don't mention this, it seems likely that European countries such as the UK and the EU would get involved at some point since it is a joint ESA / NASA mission. The Directive 2001/42/EC might apply (EU, 2001), and the Espoo convention (UNECE, n.d.) if the mission is seen as having potential for transboundary effects.

It seems unlikely that worst case scenarios would be ignored as the legal proceedings continue. If the legal discussions expand to focus on these scenarios, this could involve many other organizations.

No matter which country is involved in planning a Mars sample return mission, at some stage, international agencies like the Food and Agriculture Organization may get involved, because of potential impact on agriculture and fisheries and global food supplies, and the World Health Organization because of effects on human health globally if a new organism is returned that can be spread to other countries.

International treaties would be triggered and domestic laws of other countries are also likely to be triggered. Race and Urhan et al summarize some of these potential legal ramification see: (Uhran et al, 2019) (Race, 1996).

In the USA, the Environmental Protection Agency partners with the United Nations Environment Program (UNEP), and Arctic Council, so they'd likely get involved (EPA, n.d.).

Indeed, there would be few aspects of human life that would not be relevant in some way in discussions of the very worst case scenarios. As the legal process continues, surely there would be open public debate about these scenarios, and if the discussion expands in this way, potentially it might lead to much wider involvement in the international community. It would be necessary to convince the public, and interested experts in all these agencies that this is a safe mission and that all their concerns have been answered.

In short, great care is taken to make sure that Earth is kept safe.

NASA's draft EIS if approved will bypass all legal precautions – not just for the USA – another country could use the same arguments, and the EIS as precedent – to claim there is no need to contain Mars samples at all from anywhere on Mars – even places that are believed to have high potential for present day life – However NASA's EIS is surely going to be challenged at some point or the presidential directive will over turn it or at some point the mission will be stopped in its current form and have to do a proper assessment – the sooner the better

Next section – all sections – previous section
[question]

NASA's draft EIS, if approved, and never challenged, will bypass all legal issues mentioned in the previous section, by claiming that there is no significant risk to the environment for released samples.

This would become a precedent for other countries, including small countries that haven't paid much attention to the planetary protection literature. They could use NASA's EIS as a model for their own planetary protection measures. But there is nothing in the reasoning in the EIS to prevent them simplifying it like this:

1. Existing credible evidence shows Mars has been uninhabitable for millions of years – [argument]
2. If there is present day life anywhere on Mars, it can get here faster and better protected in a meteorite than in a sample tube – [argument]
3. If life that hasn't got here already escapes from a BSL-4 there is effectively no chance of even small scale harm to humans or to the environment – [argument]

From this they could in good faith conclude:

4. So we don't need a BSL-4
5. Also we can return a sample from anywhere on Mars without any precautions including samples of ice, dirt and salts from the polar regions or regions that some scientists think may include possible habitats for present day martian life.

In this way they could return samples with no containment, from anywhere on Mars, bypassing both their own internal legislation and international law. They could in good faith say to their people and their politicians that NASA's EIS as precedent means they don't need to consult any other countries.

We will see however this is unlikely to happen. At some point NASA's plan will be challenged.

If NASA's EIS isn't challenged, their mission plan may be challenged as part of the presidential directive to consider potential for allegations of large scale effects.

If it gets past that, the worst case for NASA is public outcry leads to them having to divert the samples on the return journey from Mars. At that point it's pretty certain the EIS would be examined and shown to not be scientifically credible.

This paper is looking at this at a far earlier stage than if these issues are ignored until later.

For details, see:

- This doesn't look like the broad acceptance which Rummel et al said is essential for success of this mission – if NASA continues with this action, it is vulnerable to being stopped in the future

NASA’s draft EIS fails NEPA requirement for a valid Environmental Impact Statement to ensure scientific integrity – with missing cites and cites that overturn the sentences they are cited to

Next section – all sections – previous section
[question]

NASA’s draft fails several of NEPA’s central requirements for a valid EIS.

> *Agencies shall* ensure *the professional integrity, including scientific integrity, of the discussions and analyses in environmental impact statements*
> § 1502.23 [Links directly to the legal text]

The EIS has major issues, mainly

- **Currency:** uses out of date research, with major omissions of later studies that overturn results it relies on.
- **Accuracy:** sentences in the EIS are contradicted by the cites attached to those sentences, and the reader isn’t alerted to this discrepancy
- **Accuracy:** doesn’t mention views opposed to their conclusions in their own sources or other sources with views that contradict the agency’s conclusions in the EIS.

A credible scientific report needs to be reviewed carefully to eliminate or minimize such errors (Blakeslee, 2004) (Tripp, n.d.) (Nausman, n.d.). For a list of the main issues found in the draft EIS see:

- Questions for NASA - and why NASA’s main argument is invalid
- Reasons for these questions: controversial or mistaken statements in NASA's draft EIS and the report of the sterilization working group

On the last point of omissions of opposing views (Feldman et al., n.d.)

> *An agency must address in an EIS “responsible opposing view[s].” Courts have interpreted this regulation as requiring agencies to address opposing scientific viewpoints. In recent years, courts have given an agency’s response to opposing scientific viewpoints deferential treatment, so long as the agency addressed the opposing statements and differing opinions in a meaningful way during the decision-making process.*

So, for instance on the topic of environmental effects, it seems the courts would be able to pass it as a valid Environmental Impact Statement under NEPA based on NASA’s own statement that in their view there is no significant risk of environmental effects, so long as NASA alert the reader to the opposing views in sources such as the

- the NRC Mars sample return study in 2009
- the ESF Mars sample return study in 2012

and so long as NASA address these differences of view in a meaningful way in the EIS.

Presumably NASA would also need to discuss the reasons the ESF and the NRC gave for their views, and explain why they came to a different view.

However, the views in the ESF and NRC studies on environmental effects are not mentioned. So, it would seem to fail this requirement for a valid EIS.

It wouldn't matter here if they are simply unaware of the opposing views. Part of the process of preparing a scientifically credible study is to do a sufficiently thorough literature survey to find opposing views. It is not as if they are hard to find in the literature. They cite both the 2009 and the 2012 studies themselves. They just needed to read their own cites carefully.

For a discussion of the views they omitted see:

- The National Reaserch Council study from 2009 warns the potential for even LARGE SCALE harm to human health and the environment isn't demonstrably zero – NASA's draft EIS conclusion that there is no significant risk of even SMALL SCALE environmental effects seems a minority view amongst microbiologists – they don't alert the reader to the existence of any other view on the topic
  [And following sections]

For a short summary of some of the main views omitted, see the section

- Large scale effects in Questions for NASA - and why NASA's main argument is invalid
- For a list of the main issues found in the draft EIS see:
- Questions for NASA - and why NASA's main argument is invalid
- Reasons for these questions: controversial or mistaken statements in NASA's draft EIS and the report of the sterilization working group

NASA's draft EIS fails the NEPA's requirement to consider reasonable alternatives in detail so that reviewers may evaluate their comparative merits – it doesn't examine the reasonable alternatives to sterilize samples in space first – or to delay the mission until it can be done safely

Next section – all sections – previous section
[question]

Another of the NEPA's central requirements for a valid EIS.

> *(a) Evaluate reasonable alternatives to the proposed action, and, for alternatives that the agency eliminated from detailed study, briefly discuss the reasons for their elimination.*

(b) Discuss each alternative considered in detail, including the proposed action, so that reviewers may evaluate their comparative merits.
§ 1502.14 [links directly to legal text]

NASA's EIS doesn't have rigorous analysis of ANY alternative except "no action". Reasonable alternatives include sterilizing samples in space before they approach humans or our biosphere.

See:

- We can forestall all these issues and make the mission 100% safe by sterilizing samples before they reach Earth – NEW

Or delaying the mission until it can be done safely. See:

- Other commentators raised significant issues – including one of the principle authors of NASA's probabilistic risk assessment guide who said a better statement of options should include the possibility of delaying the return until the risks are better understood

Although the option to sterilize first isn't discussed in the draft EIS, NASA should have been aware of this as a suggested alternative. I suggested the reasonable alternative of sterilization first in the first round of comments in the public comment they received on May 15, 2022 (Walker, 2022a):

I propose two possible solutions in my article.

1. sterilize samples during the return journey, perhaps with nanoscale X-ray emitters. Present day life in the sample would be recognizable after sterilization OR

2. return unsterilized samples to a safe orbit where astrobiologists study them remotely using miniature instruments designed for life detection on Mars. Return sterilized sub-samples to Earth immediately;

As a safe orbit, this paper recommends the Laplace plane above GEO where ring particles would orbit if we had a ring system.

7 other commentators in the first round of comments from April through to May also suggested sterilization first in public comments. See:

- Public comments on the draft EIS: 50 members of the public out of 63 commenting said test first, sterilize first, or stop mission, and likely have similar views to Carl Sagan – who said that this is a qualitatively different situation from a human pathogen in a BSL-4 and NASA shouldn't take even a low level of risk with Earth's biosphere – 9 specifically mentioned unprecedented harm

Past litigation has sometimes completely halted agency actions for failing the NEPA requirement to look at reasonable alternatives – just because the EIS didn't look at them – not based on any assessment of whether the alternatives are better or worse than the proposed actions – by a 7th circuit decision in 1997

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[question]

By the U.S. Court of Appeals for the Seventh Circuit in *Simmons v. U.S. Army Corps of Engineers*, (1997) 120 F.3d 664 (7th Cir.) ("7th circuit decision"), it is contrary to NEPA for agencies to

> ***"contrive a purpose so slender as to define competing `reasonable alternatives' out of consideration (and even out of existence)."***

This is the judgement:

> **One obvious way for an agency to slip past the strictures of NEPA is to contrive a purpose so slender as to define competing "reasonable alternatives" out of consideration (and even out of existence). The federal courts cannot condone an agency's frustration of Congressional will.** If the agency constricts the definition of the project's purpose and thereby excludes what truly are reasonable alternatives, the EIS cannot fulfill its role. Nor can the agency satisfy the Act. 42 U.S.C. § 4332(2)(E)

In that test case the Justice vacated the action, i.e. ruled that it can't go ahead. The U.S. Army Corps of Engineers wanted to build a reservoir but wrote the Purpose and Need section so narrowly as to exclude the possibility of considering two smaller reservoirs in place of one larger reservoir.

That was enough for them to lose their case and they weren't permitted to build any reservoir. The justice's decision was not based on two smaller reservoirs being better. The agency was prevented from building any reservoir just because they improperly excluding two smaller reservoirs from consideration.

The Council on Environmental Quality clarified that the requirement to consider reasonable alternatives persists even after its narrowing of scope in its 2021 revision of NEPA (CEQ, 2022).

> The revision clarifies that agencies have discretion to consider a variety of factors when assessing an application for an authorization, removing the requirement that an agency base the purpose and need on the goals of an applicant and the agency's statutory authority.
>
> *See also, e.g., Nat'l Parks & Conservation Ass'n* v. *Bureau of Land Mgmt.*, 606 F.3d 1058, 1070 (9th Cir. 2010) ("Agencies enjoy `considerable discretion' to define the purpose and need of a project.

> However, `an agency cannot define its objectives in unreasonably narrow terms.'

It would take an expert in US environmental law to confirm, but previous cases seem to suggest considerable legal jeopardy for NASA to define its purpose and need so narrowly.

It's not enough to mention reasonable alternatives, they also need adequate consideration. So NASA also needs to take great care in a new EIS to present the needs of the public adequately in the needs and purpose section.

The CEQ mention *National Parks v. Bureau of Land Mgmt*, 606 F.3d 1058 (9th Cir. 2009), In this case the EIS did mention many reasonable alternatives but dismissed them because of narrowly drawn up project objectives. The justices ruled:

> Agencies enjoy "considerable discretion" to define the purpose and need of a project. *Friends of Southeast's Future v. Morrison*, 153 F.3d 1059, 1066 (9th Cir. 1998).
>
> However, "an agency cannot define its objectives in unreasonably narrow terms." *City of Carmel-By-The-Sea v. United States Dep't. of Transp.*, 123 F.3d 1142, 1155 (9th Cir. 1997).
>
> As the *Friends* court stated, "An agency may not define the objectives of its action in terms so unreasonably narrow that only one alternative from among the environmentally benign ones in the agency's power would accomplish the goals of the agency's action, and the EIS would become a foreordained formality."
>
> …
>
> The BLM proposed several alternatives that would have been responsive to the need to meet long-term landfill demand, such as a landfill on other Kaiser property, waste diversion, offsite landfill locations, landfill mining, alternative Townsite locations, and alternative Townsite uses. The BLM did not, however, consider these options in any detail because each of these alternatives failed to meet the narrowly drawn project objectives, which required that Kaiser's private needs be met.
>
> …
>
> Our holdings in *Friends* and *Carmel-By-The-Sea* forbid the BLM to define its objectives in unreasonably narrow terms. The BLM may not circumvent this proscription by adopting private interests to draft a narrow purpose and need statement that excludes alternatives that fail to meet specific private objectives, yet that was the result of the process here.

The case was taken to the Supreme Court in 2010 but they declined to hear the case. Kaiser Ventures abandoned their plans to develop the Eagle Mountain Landfill site (Flaccus, 2013) and filed for bankruptcy, it was passed on to the local council which finally scrapped the plans in

2023 as it was no longer needed because of recycling and reduced volume of waste (Press Enterprise, 2013)

Similarly, NASA's needs and purpose section in the draft EIS is narrowly focused on the needs of NASA for the mission which includes an artificial requirement to return unsterilized samples to Earth for "safety testing" which is not needed for a pre-sterilized sample return and seems to serve no purpose.

It doesn't mention the published views of Carl Sagan (Sagan, 1973), and the views of many in the general public including in the first round of public comments that we need to protect Earth's biosphere as highest priority, and that this need overrides any mission purposes. See:

- Carl Sagan and others warning we can't take even a small risk with a billion lives – this could be formalized into law as a requirement to use the prohibitory precautionary principle whenever there is any appreciable risk for harm unprecedented in human history

It seems that in a new EIS, it's not enough for NASA to discuss reasonable alternatives such as sterilizing the samples before they reach earth. NASA also needs to give proper consideration to the views and the needs of the general public. For instance it could state in the Needs and Purpose section that some in the public consider that there is an overriding need to protect Earth's biosphere and keep it safe from any life in the samples.

Again this would need the attention of an expert in NEPA environmental law to evaluate properly.

NASA's draft EIS fails the NEPA's requirement to use an interdisciplinary approach including the social sciences, by failing to involve the public early on, not just in the USA but through fora open to representatives from all countries globally, as recommended in sample return studies – so the public weren't given the opportunity to comment on a scientifically valid draft EIS

Next section – all sections – previous section
[question]

Another of the NEPA's central requirements for a valid EIS.

> *Agencies shall prepare environmental impact statements using an interdisciplinary approach that will ensure the integrated use of the natural and social sciences and the environmental design arts … The disciplines of the preparers shall be appropriate to the scope and issues identified in the scoping process*
> § 1507.2 [links directly to legal text]

Mars sample return studies emphasize the need to involve the public early on, not just in the USA, but through fora open to representatives from all countries globally because negative impacts could affect countries beyond the ones involved directly in the mission (Ammann et al, 2012:59)

> *RECOMMENDATION 3*
>
> *Potential risks from an MSR are characterised by their complexity, uncertainty and ambiguity, as defined by the International Risk Governance Committee's risk governance framework. As a consequence, civil society, the key stakeholders, the scientific community and relevant agencies' staff should be involved in the process of risk governance as soon as possible.*
>
> *In this context, transparent communication covering the accountability, the benefits, the risks and the uncertainties related to an MSR is crucial throughout the whole process. Tools to effectively interact with individual groups should be developed (e.g. a risk map).*
>
> *RECOMMENDATION 4*
>
> *Potential negative consequences resulting from an unintended release could be borne by a larger set of countries than those involved in the programme. It is recommended that mechanisms and fora dedicated to ethical and social issues of the risks and benefits raised by an MSR are set up at the international level and are open to representatives of all countries*

The public weren't involved early on in that way. Not only that, those in the public who did discover NASA's request for public comment weren't given the opportunity to comment on a scientifically valid EIS.

I hope NASA and other space agencies can ensure a mishap like this can never happen again

Other commentators raised significant issues – including one of the principle authors of NASA's probabilistic risk assessment guide who said a better statement of options should include the possibility of delaying the return until the risks are better understood

Next section – all sections – previous section

Several other commentators raised significant issues including some of the ones already mentioned as well as new ones (Dehel, 2022) (DiGregorio, 2022) (Everline, 2022) .

Everline, a JPL employee and a principal author of NASA's probabilistic risk assessment guide (Stamatelatos, 2011), made a detailed public comment which said (Everline, 2022)

> ***Chester Everline:*** *A better statement of options should include the possibility of delaying the return of Mars samples until the risks associated with their return are better understood*

The Council of Environmental Quality says the first step is to contact the agency to resolve issues, however NASA has not yet responded to attempts to contact them on this topic

Next section – all sections – previous section

[question]

The Council of Environmental Quality say the first step is to contact the agency to resolve issues (COEQ, 2007:28):

> *Your first line of recourse should be with the individual that the agency has identified as being in charge of this particular process.*

The natural point of contact is NASA's planetary protection office. They haven't responded to my email about the issues I raised after the draft EIS was published.

The comments section of the draft EIS didn't include responses to substantial issues I raised in the previous round of comments in May (Walker, 2022a)

NEPA posted a letter to the public comments page on the last day of the public comments period, December 7th which doesn't mention the many significant issues I or anyone else raised with the draft EIS (EPA, 2022).

There seems no way forward by way of dialog with NASA at this point in time. It's also not appropriate to try to work with other employees of NASA to resolve this issue when NASA's planetary protection office aren't responding.

I encourage NASA to respond. I encourage any reviewers for this paper to ask NASA the same questions.

Sagan and Lederberg would have written a paper like this – sadly most major authors on planetary protection for a Mars sample return either died or are employees of NASA or ESA or retired from those organizations, and so can't say much – so it seems to be up to me to get the ball rolling

Next section – all sections – previous section

My background is I have been working on a paper specifically on NASA's Mars sample return mission for many years now (Walker, 2022b). I haven't yet submitted it for publication anywhere.

There are many other authors you would expect to write a paper on this topic of the need for NASA to take more care over planetary protection. Sagan and Lederberg sadly died. Gill Levin also passed away just before the process of NASA's draft EIS started. However there are many still alive today who have written extensively on a Mars sample return.

But sadly many of those are former NASA or ESA planetary protection officers or employees. They are authors, co-authors or contributors for most of the recent substantial research on a Mars sample return. There doesn't seem to be much awareness more widely at present.

The issue here is you can't expect an employee or ex employee of NASA to publicly challenge their agency's environmental impact statement. For example, John Rummel is author or co-author or contributor of much of the literature on the topic (Rummel, n.d). But as a former NASA planetary protection officer it's no surprise that he just deferred to the planetary protection office when I tried to contact him about it.

So far I have had no response from the Planetary protection office. I will keep trying. I don't know who will reply if I get through. NASA'S current planetary protection officer J. Nick Benardini has done a fair bit of work on planetary protection measures to prevent forward contamination for a Mars mission but doesn't seem to have any papers on the topic of back contamination from a Mars sample return (JPL, n.d.)

According to recommendations in the planetary protection literature, NASA should have taken account of the ESF size limit review in 2012, and commissioned a new review of the size limit and level of assurance taking account of the views of the general public (Ammann et al, 2012:21). They should have set up fora to engage with the general public internationally so that we work together on the way forward (Ammann et al, 2012:59). Also the science and our understanding of Mars has progressed so much NASA should have done a new Mars sample return study before doing the EIS because the one in 2009 (SSB, 2009) is way out of date.

They didn't do any of those things.

That is why this paper is 120,000 words long. There is no way this is a substitute for a new Mars sample return review, but it was important to cover some of the main topics such a review would cover in a preliminary way, since there hasn't been a major review since 2009.

For an overview of this paper go to all sections. The titles are like mini abstracts and the most important sections in bold.

Questions for NASA – and why NASA's main argument is invalid

Next section – all sections – previous section

NASA are normally so credible and they have been world leading on planetary protection ever since the Viking landers. NASA, please return to the care you took before and pay attention to Carl Sagan's call to treat protection of Earth as of the highest priority. If you haven't read it do check the Abstract. Please also see the previous section:

- Sagan and Lederberg would have written a paper like this – sadly most major authors on planetary protection for a Mars sample return either died or are employees of NASA or ESA or retired from those organizations, and so can't say much – so it seems to be up to me to get the ball rolling

We have to prepare for worst case scenarios and not just optimistic best case scenarios as for house fires and smoke detectors.

Astrobiologists agree we can't know indigenous Martian life would be based on the same biology as terrestrial life. One respected theory of the origins of life is punctuated chirality. Almost all terrestrial life would find mirror life starches and proteins indigestible, while mirror life from Mars would be likely to have adaptations to metabolize ordinary organics based on infall from space from comets asteroids and interplanetary dust or possibly co-existing with terrestrial life on Mars. Please ensure your precautions would protect Earth even from mirror life. For details about why it is so important not to return mirror life, see:

- NEW: Example worst case scenario of a mirror life chroococcidiopsis analogue from Mars which gradually converts organics in ecosystems into indigestible mirror organics

This is NASA's argument.

1. **Existing credible evidence shows Mars has been uninhabitable for millions of years**
   – CONTRADICTED BY NASA'S OWN CITE AND WORK PROTECTING MARS – [question] – [details]
2. **If there is present day life anywhere else on Mars it can't get to Jezero crater**
   - NASA COMMISSIONED A STUDY THAT OVERTURNS THE STUDY THEY RELY ON – [question] – [details]
3. **If there is present day life anywhere on Mars, including in Jezero crater, it can get to Earth faster and better protected in a meteorite than in a sample tube**
   – THEIR MAIN CITE SAYS THE METEORITE ARGUMENT CAN'T BE USED FOR MARS – [question] – [details]
4. **However despite all that, out of an abundance of caution NASA will contain the samples in a BSL-4 (Biosafety level 4 facility) which they say is adequate to contain any hazard**)
   - DON'T MENTION 2012 ESF STUDY WITH REQUIREMENTS BEYOND A BSL-4 – [question] – [details]
5. **If Martian life escapes from the BSL-4, potential environmental impacts would not be significant**
   – CONTRADICTS ALL PREVIOUS PLANETARY PROTECTION LITERATURE – [question] – [details]
6. **However we must do safety testing of any samples before they are released to ordinary labs from the BSL-4**
   – TOO MUCH TERRESTRIAL CONTAMINATION FOR SAFETY TESTING – [question] – [details]
7. **Because safety testing requires samples to be returned unsterilized to a BSL-4 on Earth, they can't consider any other alternative such as the reasonable alternative to sterilize samples before they reach Earth**
   – NO NEED TO TEST SAMPLES STERILIZED BEFORE THEY REACH EARTH – [question] – [details]

1 and 3 are invalid by their own cites. 2, 4, and 5 are invalid because of missing cites. 6 and 7 are invalid on closer examination.

This leads to the following questions for NASA:

2012 ESF Mars Sample Return size limit review:

- Are you aware that the European Space Foundation (ESF) Mars Sample Return study in 2012 reduced the size limit from 0.2 microns to 0.01 microns for the 1 in a million threshold and required 100% containment at 0.05 microns and that this is well beyond the capabilities of a BSL-4?

  If so, why doesn't the EIS mention this change and why isn't the reader alerted to this discrepancy?
  [details] – [section] – [argument]
- Are you aware that the ESF recommended that the size limit and level of assurance is reviewed regularly? If so, why isn't this recommendation considered?
  [details] – [section]

Meteorite argument for samples returned from the Mars surface

- When you say life can get from Mars to Earth faster and better protected in a meteorite – are you aware that your own cite, for a Phobos sample return specifically says not to use their meteorite argument for samples returned from the Mars surface – and are you aware that the NRC Mars sample return study also warns against this argument? If so, why isn't the reader alerted to this discrepancy?
  [details] – [section] – [argument]

2015 MEPAG review:

- Are you aware of the 2015 MEPAG review that overturned all the findings you rely on to say that life couldn't get to Jezero crater from elsewhere on Mars? If so, why doesn't the EIS cite it?
  [details] – [section] – [section] – [section] – [section] – [argument]
  Are you aware your most recent "credible evidence" for "**conditions on Mars have not been amenable to supporting life as we know it for millions of years**" says "exploration of … Mars … will help establish whether localised habitable regions **currently exist** within these seemingly uninhabitable worlds"? If so, why isn't the reader alerted to this discrepancy?
  [details] – [section] – [section] – [argument]

Large scale effects

- Are you aware the NRC sample return study in 2009 said "the potential for large-scale negative effects on Earth's inhabitants or environments … appears to be low, but is not

demonstrably zero"? If so, why isn't the reader alerted to this discrepancy when the EIS says "the potential environmental impacts would not be significant" ?
[details] – [section] – [argument]

- Are you aware of warnings about the potential that we have no defences against alien life by Joshua Lederberg and others? If so, why doesn't the EIS discuss them?
[details] – [section]

Mars microbes as pathogens of humans, these are questions for your sterilization working group about its report:

First when you said (Craven et al., 2021:6):

"*the presence of a direct pathogen on Mars is likely to have a near-zero probability"*

- Are you aware that Legionella pneumophila is a disease of biofilms that also opportunistically infects humans as Legionnaires' disease, which is sometimes lethal, and is not adapted to multicellular life? If so why isn't this disease mentioned in the discussion of whether pathogens have to coexist with humans to harm us?
[details] – [section]
- Are you aware that the fungus Aspergillus fumigatus is not adapted to any multicellular host and causes an estimated 200,000 life threatening cases of invasive aspergillosis a year, mainly in immunocompromised people, with a 30% to 95% mortality rate? If so, why isn't this fungus mentioned in the discussion of Candidas yeast's adaptations to humans?
[details] – [section]
- Are you aware of the example from the NRC sample return report of an independently evolved hydrothermal vent organism that shares many virulence genes with a human pathogen? If so why isn't this included in the discussion of Shiga's toxin?
[details] – [section]
- Are you aware that the toxin produced by Clostridium tetani is not a result of adaptation to humans and neonatal tetanus kills thousands of unvaccinated newborns every year? If so, why isn't this mentioned in the discussion of Shiga's toxin?
[details] – [section]

Potential for martian microbes to survive on Earth, more questions for your sterilization working group about its report:

Also when you said (Craven et al., 2021:6-7):

"*Thus, it is plausible that any Martian microbe, after it arrives on Earth, would not be viable on Earth due to a lack of its required Martian nutritional and environmental conditions."*

- Are you aware that the extremophile paper you cited lists Planococcus Halocryophilus, a microbe isolated from permafrost at an ambient temperature of about -16 °C, which has an optimal growth temperature of 25 °C and can grow at temperatures up to 37 °C

(temperature of human blood) and salinity 0% to 19%? If so why isn't this microbe discussed in your suggestion that it's plausible that life adjusted to Martian conditions such as temperatures and pressures would not be viable on Earth?
[details] – [section]

- Did you have any examples of extreme conditions microbes face on Mars that could prevent them surviving on Earth? If you didn't have specific examples, why doesn't your report mention this limitation in your analysis?
[details] – [section]
- Are you aware that there are many candidates for terrestrial life that may be able to survive on Mars and one of our top candidates, the blue-green algae chroococcidiopsis, has as nutrient requirements only basalt, sunlight and water, and basalt a rock commonly found on Mars and on Earth? If so, why isn't the reader informed of this?
[details] – [section] – [section]

Scoping and requirement for "safety testing"

- With your requirement of "Safety testing", are you aware that the expected level of forward contamination of 0.7 nanograms per gram per biosignature means all samples will test positive and go to hold and critical review, which will make the safety testing pointless?
[details] – [section] – [section] – [section] – [argument]
- Why wasn't the option considered to sterilize samples before they reach Earth's biosphere which wouldn't need safety testing? Why wasn't the public given the opportunity to comment on this option, which would keep Earth 100% safe?
[details] – [section] – [argument]

Procedure:

- As you surely know, NEPA requires agencies to ensure scientific integrity in an Environmental Impact Statement, so, do you know how the EIS come to have so many citing errors of central importance to your arguments, and can NASA ensure this won't happen again in any future EIS and also ensure you will consider reasonable alternatives and pursue an interdisciplinary approach as required by NEPA?
[details] – [section] – [section] – [section] – [section]
- The Council for Environmental Quality says the first step is to contact the agency to resolve issues, so, can you respond to these questions?
[details] – [section]

The simplest answer is that it is all a big mistake, and they weren't aware of any of those things. If so fine, we all make mistakes! But that means we need to start again with a scientifically credible EIS starting with a new size limit review etc.

At some point NASA are going to have to look at these questions and others like them. The public response to the EIS so far shows many will want answers. If these are indeed valid questions, the sooner NASA look at them the easier and less costly the solutions, and the fewer the complications.

Need to set a good precedent for other countries

This leads to another question:

- Other countries will use NASA's Environmental Impact Statement as a precedent and template for their own impact statements.
  How important is it to set a good precedent for other countries?

Here I hope the answer is "Very".

If the EIS is not challenged, other countries, relying on NASA's EIS, may in good faith:

- return samples from anywhere on Mars without any precautions
- leave out the apparently unnecessary BSL-4 and "abundance of caution".

See:

- NASA's draft EIS if approved will bypass all legal precautions – not just for the USA – another country could use the same arguments, and the EIS as precedent – to claim there is no need to contain Mars samples at all from anywhere on Mars – even places that are believed to have high potential for present day life – However NASA's EIS is surely going to be challenged at some point or the presidential directive will over turn it or at some point the mission will be stopped in its current form and have to do a proper assessment – the sooner the better

The solution of sterilizing samples is likely to

- cost less
- keep Earth 100% safe,
- preserve virtually all the same science value, and
- give a good precedent other space agencies can follow easily.

See:

- Recommendations – not enough to "fix" with ad hoc addition of an air incinerator which has many issues to be examined – NASA need to provide a scientifically credible EIS first – simplest and lowest cost solution is to sterilize all samples before return to Earth with virtually the same science return – meanwhile a bonus pre-sterilized sample container sent to Mars on the ESF sample fetch rover could greatly increase the mission's astrobiological interest while keeping Earth 100% safe

For an overview of this paper go to all sections. The titles are like mini abstracts and the most important sections in bold.

Reasons for these questions: mistakes in NASA's draft EIS and the report of the sterilization working group

Next section – all sections – previous section

Here is a list of the mistakes in the EIS or the report of the sterilization working group which that list of questions is based on, with links to the sections of this paper that discuss them:

2012 ESF Mars Sample Return size limit review:

Are you aware that the European Space Foundation (ESF) Mars Sample Return study in 2012 reduced the size limit from 0.2 microns to 0.01 microns for the 1 in a million threshold and required 100% containment at 0.05 microns and that this is well beyond the capabilities of a BSL-4?

If so, why doesn't the EIS mention this change and why isn't the reader alerted to this discrepancy?
[summary] – [argument]

Draft EIS (NASA, 2022: S-4):

> *"The material would remain contained until examined and confirmed safe or sterilized for distribution to terrestrial science laboratories. NASA and its partners would use many of the basic principles that Biosafety Level 4 (BSL-4) laboratories use today to contain, handle, and study materials that are known or suspected to be hazardous."*

2012 ESF Mars sample return study: (Ammann et al, 2012:48):

> *RECOMMENDATION 7:*
> *The probability that a single unsterilised particle of 0.01 μm diameter or greater is released into the Earth's environment shall be less than 10^{-6}.*
> *…*
> *The release of a single unsterilized particle larger than 0.05 μm is not acceptable under any circumstances*

This is well beyond the capability of a BSL-4. See:

- 2012: The European Space Foundation study reduced the size of particle to contain from 0.2 microns to 0.01 microns at the one in a million threshold, and added that it is not acceptable to release a particle of 0.05 microns or larger under any circumstance – this is well beyond the capabilities of NASA's proposed BSL-4
  [and following sections]

Are you aware that the ESF recommended that the size limit and level of assurance is reviewed regularly? If so, why isn't this recommendation considered?
[summary]

2012 ESF Mars Sample Return Study (Ammann et al, 2012:21):

> *RECOMMENDATION 8: Considering that (i) scientific knowledge as well as risk perception can evolve at a rapid pace over the time, and (ii) from design to curation, an MSR mission will last more than a decade, the ESF-ESSC Study Group recommends that values on level of assurance and maximum size of released particle are re-evaluated on a regular basis*

See:

- ESF study said values for required level of assurance and the size limit need to be revisited periodically based on changes in scientific knowledge and risk perception

Meteorite argument for samples returned from the Mars surface

When you say life can get from Mars to Earth faster and better protected in a meteorite – are you aware that your own cite, for a Phobos sample return specifically says not to use their meteorite argument for samples returned from the Mars surface – and are you aware that the NRC Mars sample return study also warns against this argument? If so, why isn't the reader alerted to this discrepancy?
[summary] – [argument]

Draft EIS (NASA, 2022: 3-3):

> *"The natural delivery of Mars materials can provide better protection and faster transit than the current MSR mission concept."*

2009 NRC Mars Sample Return Study (SSB, 2009: 47)

> *Thus, the potential hazards posed for Earth by viable organisms surviving in samples ~~is~~ [are] significantly greater with a Mars sample return than if the same organisms were brought to Earth via impact-mediated ejection from Mars*

The NRC goes on to say (SSB, 2009: 48):

> *… Thus it is not appropriate to argue that the existence of martian meteorites on Earth negate the need to treat as potentially hazardous any samples returned from Mars by robotic spacecraft.*

The 2019 study of planetary protection requirements for Japan's Phobos sample return says (SSB, 2019 : 43) (split the sentences into bullet points):

There are several reasons why Mars sample return (MSR) missions differ from those for collecting samples from Phobos and Deimos, including the following:

…

- *The material will be gently sampled and returned directly to Earth.*
- *The sample may well come from an environment that mechanically cannot become a Mars meteorite.*
- *The microbes may not be able to survive impact ejection and transport through space.*
- *Samples with current liquid water and recent ice seem especially fragile to natural transport to Earth.*

Finding: The committee finds that the content of this report and, specifically, the recommendations in it do not apply to future sample return missions from Mars itself.

See:

- No, life on Mars can't get to Earth faster and better protected in meteorites than in a sample tube - the 2009 Mars sample return study warns against this argument as does the 2019 Phobos sample return study - indeed martian surface brines, ice, salts, dirt and dust can't get to Earth at all
  [and following sections]

2015 MEPAG review:

Are you aware of the 2015 MEPAG review that overturned all the findings you rely on to say that life couldn't get to Jezero crater from elsewhere on Mars? If so, why doesn't the EIS cite it?
[summary] – [argument]

Draft EIS (NASA, 2022: 1-6):

"Consensus opinion within the astrobiology scientific community supports a conclusion that the Martian surface is too inhospitable for life to survive there today, particularly at the location and shallow depth (6.4 centimeters [2.5 inches]) being sampled by the Perseverance rover in Jezero Crater, which was chosen as the sampling area because it could have had the right conditions to support life in the ancient past, billions of years ago."

The MEPAG review warns that maps such as the ones NASA relied on to select Jezero crater as a landing site represent an incomplete state of knowledge (SSB, 2015 :28):

Maps that illustrate the distribution of specific relevant landforms or other surface features can only represent the current (and incomplete) state of knowledge for a *specific time—knowledge that will certainly be subject to change or be updated as new information is obtained*

See:

- 2015 (overturning results from 2014): Jezero crater seems uninhabited from orbit – but so do Mars analogue deserts on Earth – the 2015 MEPAG review overturned all the conclusions NASA rely on from 2014 – saying life might be transported in dust storms, or live locally in microhabitats and biofilms that can make deserts locally more habitable
  [And following sections]

The MEPAG review says SR-SAG2 didn't discuss transport of material in the atmosphere (e.g. dust storms) (SSB, 2015 : 12).

> *"The SR-SAG2 report does not adequately discuss the transport of material in the martian atmosphere. The issue is especially worthy of consideration because if survival is possible during atmospheric transport, the designation of Special Regions becomes more difficult, or even irrelevant."*

See:

- 2015 MEPAG review: potential for viable life transported through the atmosphere (for instance in dust storms)
  [And following sections]

The MEPAG review says that SR-SAG2 only briefly considered the implications of our lack of knowledge of microenvironments on Mars (SSB, 2015 :https://nap.nationalacademies.org/read/21816/chapter/4?term=dust - 12 11).

> *Physical and chemical conditions in microenvironments can be substantially different from those of larger scales. Although the SR-SAG2 report considered the microenvironment (Finding 3-10), the implications of the lack of knowledge about microscale conditions was only briefly considered.*

See:

- 2015: the MEPAG2 review draws attention to the potential for local microenvironments to provide habitats for life that can't be detected in large scale surveys – and illustrative examples of micropores in salts or gypsum, and Curiosity's salty brines
  [And following sections]

The MEPAG review draws attention to biofilms which aren't discussed in SR-SAG2 (it has only one mention of the word). (SSB, 2015 :11)

> *Given the wide distribution and advantages that communities of organisms have when they live as biofilms enmeshed in copious amounts of EPS [substances that microbes can produce around them to help make a "home" in a hostile environment], it is likely that any microbial stowaways that could survive the trip to Mars would need to develop*

biofilms to be able to establish themselves in clement microenvironments in Special Regions so that they could grow and replicate.

Biofilms are of especial importance for backwards contamination as putative martian life would have had millions of years to evolve communities of microbes adapted to the Martian surface conditions and to establish them in Jezero crater if they are possible there.

- 2015 MEPAG review: microbes can use biofilms to create conditions favourable to them in otherwise uninhabitable microniches – this need to build up a biofilm first reduces the risk for forward contamination for spacecraft with low bioloads – however such niches could be inhabited by Martian life that already lives in biofilms adapted for millions of years
  [And following sections]

Are you aware your most recent "credible evidence" for "conditions on Mars have not been amenable to supporting life as we know it for millions of years" is a source that says "exploration of … Mars … will help establish whether localised habitable regions currently exist within these seemingly uninhabitable worlds"? If so, why isn't the reader alerted to this discrepancy?
[summary] – [argument]

Draft EIS (NASA, 2022:1-6):

> Existing credible evidence suggests that conditions on Mars have not been amenable to supporting life as we know it for millions of years (… National Research Council 2022).

Your most recent source for this sentence is about searches for currently habitable environments on Mars! (Smith et al, 2022: 393)

> *Section title: "Are There Chemical, Morphological and / or Physiologic / Metabolic or Other Biosignatures in* ***Currently Habitable Environments*** *in the Solar System*
>
> *The exploration of … Mars (Curiosity, Perseverance) will help establish whether localised habitable regions* ***currently exist*** *within these seemingly uninhabitable worlds.*
>
> [Emphasis on "currently" mine]

See:
- NASA's draft EIS argues that existing credible evidence suggests Mars has not been habitable to Earth life for millions of years — yet their cite for this sentence is about a search for current localized habitable regions on Mars – another conclusion reached through a citing error

See also:
- 2016: NASA discovered potential for current habitats for terrestrial life in Gale crater AFTER Curiosity's landing

Large scale effects:

Are you aware the NRC sample return study in 2009 said "the potential for large-scale negative effects on Earth's inhabitants or environments … appears to be low, but is not demonstrably zero"? If so, why isn't the reader alerted to this discrepancy when the EIS says "the potential environmental impacts would not be significant"?
[summary] – [argument]

Draft EIS (NASA, 2022:3-3):

> *"The relatively low probability of an inadvertent reentry combined with the assessment that samples are unlikely to pose a risk of significant ecological impact or other significant harmful effects support the judgement* ***that the potential environmental impacts would not be significant."***

2009 NRC Mars Sample Return Study (SSB, 2009 : 48)

> The committee found that **the potential for large-scale negative effects on Earth's inhabitants or environments by a returned martian life form appears to be low, but is not demonstrably zero**
>
> … **it is not possible to assess past or future negative impacts caused by the delivery of putative extraterrestrial life**, based on current evidence.
> …
> … It follows that, since the potential risks of pathogenesis cannot be reduced to zero, a conservative approach to planetary protection will be essential, **with rigorous requirements for sample containment and testing protocols of life forms that are pathogenic to humans**

See:

- The National Reaserch Council study from 2009 warns the potential for even LARGE SCALE harm to human health and the environment isn't demonstrably zero – NASA's draft EIS conclusion that there is no significant risk of even SMALL SCALE environmental effects seems a minority view amongst microbiologists – they don't alert the reader to the existence of any other view on the topic

Are you aware of the warnings about the potential that we have no defences against alien life by Joshua Lederberg and others? If so, why doesn't the EIS discuss them?
[summary]
Draft EIS Sterilization Working Group report (Craven et al., 2021:6)

> *"Since any putative Martian microorganism would not have experienced long-term evolutionary contact with humans (or other Earth host),* ***the presence of a direct pathogen on Mars is likely to have a near-zero probability."***

Joshua Lederberg (Lederberg, 1999b):

> ***Joshua Lederberg:*** *Whether a microorganism from Mars exists and could attack us is more conjectural. If so, it might be a zoonosis [infectious disease that jumps to humans] to beat all others*

See:

- Warnings by some astrobiologists such as Sagan and Lederberg that in worst case we could be in effect immunocompromised to an entire exobiology from Mars
  [And previous sections]

Mars microbes as pathogens of humans, these are questions for your sterilization working group about its report:

Are you aware that Legionella pneumophila is a disease of biofilms that also opportunistically infects humans as Legionnaires' disease, which is sometimes lethal, and is not adapted to multicellular life? If so why isn't this disease mentioned in the discussion of whether pathogens have to coexist with humans to harm us?
[summary]
Draft EIS Sterilization Working Group report (Craven et al., 2021:6)

> *Since any putative Martian microorganism would not have experienced long-term evolutionary contact with humans (or other Earth host),* ***the presence of a direct pathogen on Mars is likely to have a near-zero probability.***

Warmflash used Legionnaires' disease to challenge whether there is a need for human pathogens to co-evolve with us (Warmflash, 2007):

> *In essence, all that a potentially infectious human pathogen needs to emerge and persist is to grow and live naturally under conditions that are similar to those that it might later encounter in a human host. On Mars, these conditions might be met in a particular niche within the extracellular environment of a biofilm, or within the intracellular environment of another single-celled Martian organism.*
>
> *…*
>
> *To be sure, the genetic similarity between humans and protozoa is much greater than could be expected between humans and the Martian host of a Martian microbe.*
>
> *Even in the context of a planetary biosphere that is limited to single-celled life, and even where there is unlikely to have been a co-evolution between agent and host organism, the possibility of infectious agents, even an invasive type, cannot be ruled out.*

See:

- Argument that martian pathogens wouldn't be adapted to humans or other Earth hosts misses a disease of biofilms that opportunistically infects human lungs - legionnaires' disease

Are you aware that the fungus Aspergillus fumigatus is not adapted to any multicellular host and causes an estimated 200,000 life threatening cases of invasive aspergillosis a year, mainly in immunocompromised people, with a 30% to 95% mortality rate? If so, why isn't this fungus mentioned in the discussion of Candidas yeast's adaptations to humans?
[summary]
Draft EIS Sterilization Working Group report (Craven et al., 2021:6):

> *Existing microorganisms that coexist with humans over long periods of time can also …*
>
> *opportunistically infect a host with a weakened or compromised immune system such as candidiasis yeast infections*

From this list of the most common opportunistic invasive fungal diseases, Aspergillus is at the top alongside Candidiasis

Disease (most common species)	Location	Estimated life-threatening infections/ year at that location*	Mortality rates (% in infected populations)*
Opportunistic invasive mycoses			
Aspergillosis (*Aspergillus fumigatus*)	Worldwide	>200,000	30–95
Candidiasis (*Candida albicans*)	Worldwide	>400,000	46–75
Cryptococcosis (*Cryptococcus neoformans*)	Worldwide	>1,000,000	20–70
Mucormycosis (*Rhizopus oryzae*)	Worldwide	>10,000	30–90
Pneumocystis (*Pneumocystis jirovecii*)	Worldwide	>400,000	20–80

Figure 37: (Brown et al, 2012:table 1).

It's not adapted to humans or indeed as a pathogen of any higher life (McCormick et al, 2010).

> *According to our current knowledge A. fumigatus lacks sophisticated virulence factors that are solely dedicated to permit a pathogenic lifestyle.*

See:

- NEW: NASA's sample return biological safety report mentions an opportunistic fungal pathogen, Candidiasis adapted to humans – but misses the counter-example of Aspergillus, not adapted to us – an estimated 200,000 life-threatening cases of invasive aspergillosis a year – mortality 30% to 95% - invasive because of capabilities martian life may share such as its ability to respond quickly to rapid changes in humidity and temperature, very efficient at taking up nutrients and storing them, and able to tolerate low oxygen levels in the lungs

Are you aware of the example from the NRC sample return report of an independently evolved hydrothermal vent organism that shares many virulence genes with a human pathogen? If so why isn't this included in the discussion of Shiga's toxin?
[summary]

Draft EIS Sterilization Working Group report (Craven et al., 2021:6).

> *Existing microorganisms that coexist with humans over long periods of time can also cause new diseases when the organism takes on new pathogenicity, such as the Escherichia coli strain 0157:H7 that acquired a gene for Shiga toxin, …*

2009 NRC Mars Sample Return Study (SSB, 2009: 46):

> *"**However**, it is worth noting in this context that interesting evolutionary connections between alpha proteobacteria and human pathogens have recently been demonstrated for natural hydrothermal environments on Earth … it follows that, since the potential risks of pathogenesis cannot be reduced to zero, a conservative approach to planetary protection will be essential, with rigorous requirements for sample containment and testing protocols of life forms that are pathogenic to humans'*

See:

- The sterilization working group's report mentions a strain of e. coli that they hypothesize became toxic by coexisting with humans – however the NRC sample return report gave an example of an independently evolved hydrothermal vent organism that shares many virulence genes with a human pathogen – martian microbes would continue to evolve on Earth – and this omits the suggestion by Łoś et al that e. coli developed Shiga's toxin to deter protozoan grazing in biofilms and only uses it opportunistically in humans

Are you aware that the toxin produced by Clostridium tetani is not a result of adaptation to humans and neonatal tetanus kills thousands of unvaccinated newborns every year? If so, why isn't this mentioned in the discussion of Shiga's toxin?
[summary]

Draft EIS Sterilization Working Group report (Craven et al., 2021:6).

> *Existing microorganisms that coexist with humans over long periods of time can also cause new diseases when the organism takes on new pathogenicity, such as the Escherichia coli strain 0157:H7 that acquired a gene for Shiga toxin, …*

Warmflash et al give examples such as tetanus, locally infectious (Warmflash, 2007).

Locally infectious organisms, which do not multiply systemically within a host but which produce a toxin which the host can absorb, perhaps through an infected wound, may also be possible on a planet that harbors single-celled life. Clostridia is an example of an anaerobic genus that often lives as spores in soils and some of its species are important human pathogens, including C. tetani and C. perfringens, which are locally infectious in wounds, where they release toxins that can be life-threatening through systemic effects (C. tetani) or local effects (C. perfringens)

We can now protect babies with widely available tetanus vaccines, yet tetanus still kills thousands of newborns every year in weaker economies (WHO, n.d.) .

See:

- NASA's biological safety report doesn't mention clear examples of microbes which produce accidental poisons without any co-evolution with humans or higher life, such as tetanus which kills thousands of unvaccinated newborns every year

Are you aware that the extremophile paper you cited lists Planococcus Halocryophilus, a microbe isolated from permafrost at an ambient temperature of about -16 °C, which shows activity down to the lowest temperature tested of -25 °C, and verified growth in the lab from -15 °C to 37 °C (temperature of human blood) and salinity 0% to 19%? If so why isn't this microbe discussed in your report?
[summary]

Draft EIS Sterilization Working Group report (Craven et al., 2021:6-7):

> *There are many described extremophiles that may survive in environments that are extreme to human or animal life* ***(e.g. extremes of temperature or pressure)*** *but do not survive under conditions in our normal habitat* (Merino et al. 2019).
> *… Thus,* it is plausible that any Martian microbe, after it arrives on Earth, would not be viable on Earth due to a lack of its required Martian nutritional and environmental conditions.

One of the extremophiles listed in their cite (Merino et al, 2019: table 3) is Planococcus Halocryophilus with a temperature range -15 °C to 37 °C and optimal growth 25 °C which was actually isolated from permafrost soil, where it like inhabits cold brines in the soil (Mykytczuk et al., 2013) (Mykytczuk, 2012).

Strain	Domain	Extremophile Type	Isolation ecosystem	Temperature (°C)
Picrophilus oshimae KAW 2/2	*Archaea*	Hypercidophile	Hot springs, Solfataras	47–65 (60)[a]
Serpentinomonas sp. B1	*Bacteria*	Alkaliphile	Serpentinizing system (water)	18–37 (30)
Methanopyrus kandleri 116	*Archaea*	Hyperthermophile	Deep-sea hydrothermal vent	90–**122** (105)
Planococcus halocryophilus Or1	*Bacteria*	Halopsychrophile	Sea ice core	**−15**–37 (25)
Halarsenatibacter silvermanii SLAS-1	*Bacteria*	Haloalkaliphile	Soda lake	28–55 (44)
Thermococcus piezophilus CDGS	*Archaea*	Piezothermophile	Deep-sea hydrothermal vent	60–95 (75)
Haloarchaeal strains GN-2 and GN-5	*Archaea*	Xerophile	Solar salterns (brine)	nr

[a]*Data presented as range (optimum) for each parameter. nr, not reported in the original publication.*

Figure 38: (Merino et al, 2019: table 3)

See:

- NASA's biological safety report agrees on the potential for an invasive Martian species to harm or displace terrestrial photosynthetic bacteria – but says life adapted to Martian conditions such as the temperatures and pressures plausibly wouldn't be able to survive on Earth – their own cite mentions Planococcus Halocryophilus, a microbe which lives in Arctic permafrost soils and likely grows in sub zero brine veins down to at least -15 °C with an optimal growth temperature of 25°C and growth up to 37 °C (human blood temperature)

Did you have any examples of extreme conditions microbes face on Mars that could prevent them surviving on Earth? If you didn't have specific examples, why doesn't your report mention this limitation in your analysis?
[summary]

Draft EIS Sterilization Working Group report (Craven et al., 2021:6-7)

> *"There are many described extremophiles that may survive in environments that are extreme to human or animal life (e.g. extremes of temperature or pressure) but do not survive under conditions in our normal habitat … Thus, it is plausible that any Martian microbe, after it arrives on Earth, would not be viable on Earth due to a lack of its required Martian nutritional and environmental conditions."*

See:

- Microbes from near the surface in Jezero crater would withstand temperatures varying from below -70 °C to above 15 °C in a single day – and major changes in humidity and pressure – this is likely to favour polyextremophiles – while microbes able to resist stresses like UV, low humidity, vacuum, and ionizing radiation do not require a non-terrestrial biology and there is no reason for them to be dependent on these conditions to survive
  [And following sections]

Are you aware that there are many candidates for terrestrial life that may be able to survive on Mars and one of our top candidates, the blue-green algae chroococcidiopsis, has as nutrient requirements only basalt, sunlight and water, and basalt a rock commonly found on Mars and on Earth? If so, why isn't the reader informed of this?
[summary]

Examples of many candidate organisms to survive on both Earth and Mars here:

- Many candidate microbes such as the blue green algae chroococcidiopsis and even higher life like lichens have been proposed as Mars analogue organisms, some tested with promising results in Mars simulation chambers, so it's biologically credible a species can have adaptations to live on both planets

On the nutritional requirements (Craven et al., 2021:6-7)

> *Thus, it is plausible that any Martian microbe, after it arrives on Earth,* ***would not be viable on Earth due to a lack of its required Martian nutritional and environmental conditions****.*

Many microbes only need basalt, sunlight and water. The blue-green algae chrooccocidiopsis is an example and is one of our top candidates for a microbe that may be able to survive on Mars

NASA's biological safety report says a martian microbe might be unable to find its required nutrients on Earth – many microbes find almost all the nutrients they need except water, and sunlight, from basalt which is abundant on both Mars and Earth **Error! Bookmark not defined.**

Scoping and requirement for "safety testing"

With your requirement of "Safety testing", are you aware that the expected level of forward contamination of 0.7 nanograms per gram per biosignature means all samples will test positive and go to hold and critical review, which will make the safety testing pointless?
[summary] – [argument]

Draft EIS (NASA, 2022:3-3)

> *These same principles regarding the importance of using terrestrial laboratories to enable the best scientific return also apply to the care and attention to detail that would*

be required to conduct a proper and comprehensive sample safety assessment in a proposed SRF.

See:

- NEW: Sadly Perseverance's permitted levels of 0.7 nanograms per gram for their most abundant biosignatures would overwhelm any faint signature of biosignatures from past life and it would also mask even as many as thousands of cells per gram of present day ultramicrobacteria, though it could spot present day life if there are many spores per gram in the dust
- So sterilization preserves virtually all geological interest with minimal impact on astrobiological impact – but NASA's EIS doesn't permit it due to a requirement for "safety testing"
- NEW: "safety testing" can never prove it is safe to release unsterilized samples – level of forward contamination guarantees all samples test positive for life – no guaranteed biosignature to distinguish terrestrial from potential martian life – most or all tests will find sequences new to science as nearly all terrestrial microbes are unsequenced – we don't know in advance how to detect extraterrestrial biochemistry – we can't reliably cultivate even most species of terrestrial life – and it is impossible in practice to predict effects of introducing unknown extraterrestrial life to Earth's biosphere – so the required "safety testing" serves no useful purpose

  [And previous and following sections]

Why wasn't the option considered to sterilize samples before they return? Why wasn't the public given the opportunity to comment on this option, which would keep Earth 100% safe?

[summary] – [argument]

The sterilization working group said *"… it is impossible to remove all risk without ceasing space exploration".* (Craven et al., 2021:4).

> *"While **it is impossible to remove all risk without ceasing space exploration**, … There is always some level of risk associated with exploration into the unknown, and it was the goal of the SWG to help manage the risks of possible adverse effects to the Earth's biosphere while maintaining the science integrity of the returned samples."*

Yes, we can't eliminate risks to robotic spacecraft and astronauts that venture into space. However it ***is*** possible to eliminate all risk to Earth's biosphere from life from other planets, by the simple method of

- not returning samples at all, or
- sterilizing them first,

If that is what we decide as a civilization, it IS possible to prioritize the safety of Earth's biosphere over everything else in our explorations and explore space in a way that is 100% safe for Earth's biosphere.

See:

- NEW: We can forestall all these issues and make the mission 100% safe by sterilizing samples before they reach Earth – it is impossible to eliminate all risks to spacecraft and astronauts from space exploration into the unknown – but it IS possible to eliminate all risks to Earth's biosphere

Procedure:

As you surely know, NEPA requires agencies to ensure scientific integrity in an Environmental Impact Statement, so, do you know how the EIS come to have so many citing errors of central importance to your arguments, and can NASA ensure this won't happen again in any future EIS and also ensure you will consider reasonable alternatives and pursue an interdisciplinary approach as required by NEPA?
[summary]

> *Agencies shall* ensure *the professional integrity, including scientific integrity, of the discussions and analyses in environmental impact statements*
> § 1502.23

- NASA's draft EIS fails NEPA requirement for a valid Environmental Impact Statement to ensure scientific integrity – with missing cites and cites that overturn the sentences they are cited to

See also:

- NASA's draft EIS fails the NEPA's requirement to consider reasonable alternatives in detail so that reviewers may evaluate their comparative merits – it doesn't examine the reasonable alternatives to sterilize samples in space first – or to delay the mission until it can be done safely
- Past litigation has sometimes completely halted agency actions for failing the NEPA requirement to look at reasonable alternatives – just because the EIS didn't look at them – not based on any assessment of whether the alternatives are better or worse than the proposed actions – by a 7th circuit decision in 1997
- NASA's draft EIS fails the NEPA's requirement to use an interdisciplinary approach including the social sciences, by failing to involve the public early on, not just in the USA but through fora open to representatives from all countries globally, as recommended in sample return studies – so the public weren't given the opportunity to comment on a scientifically valid draft EIS

The Council for Environmental Quality says the first step is to contact the agency to resolve issues, so, can you respond to these questions?
[summary]

Council of Environmental Quality (COEQ, 2007:28):

Your first line of recourse should be with the individual that the agency has identified as being in charge of this particular process.

- The Council of Environmental Quality says the first step is to contact the agency to resolve issues, however NASA has not yet responded to attempts to contact them on this topic

Recommendations – scientific credibility can't be "fixed" e.g. with ad hoc addition of an air incinerator – but there is a simple and low cost solution – to sterilize all samples before return to Earth with virtually the same science return – and a bonus pre-sterilized sample container sent to Mars on the ESF sample fetch rover could greatly increase the mission's astrobiological interest – while keeping Earth 100% safe

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For a summary of these recommendations, and links to the relevant sections, see:

- **Recommendations for space agencies generally –** the simplest way to keep Earth safe is to sterilize any samples returned from Mars before they reach Earth – this can be done with ionizing radiation – sterilization would have virtually no effect on geology and most likely no effect on astrobiology for preliminary samples – priority to return samples free from forward contamination by terrestrial life

- **Recommendations for NASA –** need to restart the process with a scientifically credible Environmental Impact Statement – simplest approach is to sterilize samples before they are returned to Earth - this retains virtually all geology and most likely has no impact on astrobiology – a valid environmental impact statement should at least look at pre-sterilized samples as a reasonable alternative that keeps Earth 100% safe

Chris McKay has suggested adding an air incinerator in place of the second HEPA filter to try to bring a BSL-4 standard up to the requirements of the ESF study (private communication).

This could be an option in a FUTURE EIS, though it's not clear it would be suitable as there are many issues to look at, see:

- Alternative of an air incinerator for the second HEPA filter – not tested for ultramicrobacteria imbedded in a dust grain – or the scenario of Martian spores that evolved extra layers to make them more resilient than terrestr ial test spores – or for 100% containment

But for this EIS, the public were never given the opportunity to comment on a scientifically credible EIS that also evaluates reasonable alternatives like sterilizing the samples before they reach Earth.

The NEPA guidelines are clear that an environmental impact statement shouldn't go ahead if it doesn't fulfil those requirements to

- maintain scientific credibility and to
- look at reasonable alternatives to the proposed action.

Agency actions have been stopped through litigation that failed this requirement. See (above):

- Past litigation has sometimes completely halted agency actions for failing the NEPA requirement to look at reasonable alternatives – just because the EIS didn't look at them – not based on any assessment of whether the alternatives are better or worse than the proposed actions – by a 7th circuit decision in 1997

If NASA do persist with their current draft EIS, it seems vulnerable to litigation. If it survives that, it is vulnerable to the president's directive on large scale effects, and the worst case would be increasing awareness in the early 2030s and public concern leading to the sample return diverted in the 2030s.

Many in the general public value Earth's biosphere highly. The issues of scientific credibility of the EIS described in this paper will be discovered as soon as there is widespread scrutiny of it by other scientists, the general public, other agencies or countries, or in any legal case.

See:

- This doesn't look like the broad acceptance which Rummel et al said is essential for success of this mission – if NASA continues with this action, it is vulnerable to being stopped in the future

Recommendations:

We do have at least one possible solution that preserves virtually all the science while keeping Earth 100% safe.

NASA say that

1. We can do virtually the same geological science with a sterilized sample as with an unsterilized sample.

It's actually the same situation for astrobiology, because we can't expect to do much astrobiology because of forward contamination:

2. Permitted level of forward contamination of the samples, at nanograms per gram of sample - is so high for astrobiology it gives no chance of detecting past life
3. ionizing radiation reduces any past life organics from grams to attograms (billionths of a billionth of a gram)

This suggests

4. reasonable alternative of sterilizing a sample before it is returned to Earth
   - suggested in first round of public comments
   - no need for safety testing as only sterilized samples get returned
   - virtually same science return

   Likely costs the same or less:
   - no aeroshell to take to Mars and back again
   - increased cost for a sterilizing satellite in a safe orbit above GEO
   - but eliminates cost for a receiving laboratory with technology that doesn't yet exist.

   much less risk of opposition from the general public

5. as a bonus ESA can send a pre-sterilized container to return contamination free samples of dirt, dust, and a pebble from the Mars surface

   - Only sterilized subsamples returned to Earth
   - Safety testing even of contamination free samples still can't achieve the high levels of assurance of safety likely required by the public
   - Humans can't safely handle unsterilized samples in space because human quarantine can't protect Earth from mirror life, crop pathogens or human pathogens with symptomless carriers

6. Can safely return contamination free samples to a centrifuge spinning to simulate Mars gravity in a satellite in a safe high orbit above GEO, which can be the same satellite used to pre-sterilize samples to return to Earth

   - Should present as just a significant first step in Sagan's "vigorous program of unmanned exobiology"
   - not expected to settle central questions in astrobiology
   - Makes it possible to look for trace levels of organics in the dust and dirt and do a first search for spores or other viable life in the dust
   - let's us study chemistry of the martian dirt to see if this can explain the Viking experiments
   - Detects present day life only if very abundant on Mars
   - Unlikely to detect past life without in situ searches first

7. A marscopter with pre-sterilized sample handling capabilities could return a pebble from a recently formed small crater excavated to a depth of at least 2 meters

    - Should present as just our first test for organics that might be preserved from early Mars
    - not expected to find past life either without in situ searches, as organics would almost certainly be altered by fluids flowing through the rocks and mixed with organic infall from space and indigenously produced organics
    - a significant step forward to prepare for future searches in situ

We will look into these ideas in detail later in this paper and they are summarized with links to those sections in

- **Recommendations for NASA –** need to prepare a scientifically credible EIS and restart the process – simplest approach is to sterilize samples before they are returned to Earth which retains virtually all geology and most likely has no impact on astrobiology – a valid environmental impact statement should at least consider sterilized samples as a reasonable alternative

As the NRC Mars sample return study in 2008 observed, we can't actually assess the level of risk until we know more about Mars – it could be zero or it could be far higher than expected

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This mission raises many novel ethical and legislative questions. First, as the NRC observed, we can't actually assess the current level of risk (SSB, 2009: 48):

> … **it is not possible to assess past or future negative impacts caused by the delivery of putative extraterrestrial life**, based on current evidence.

Let's link that statement to a couple of possible future scenarios.

- If later we find only prebiotic synthesis on Mars, or slowly and imperfectly reproducing life with a biochemistry compatible with terrestrial predators, our risk from an unsterilized sample return is zero. Our main risk is in the forward direction that we might lose the chance to discover and investigate early life or prebiotic synthesis on Mars.

However,

- if later we discover a mirror life analogue of chroococcidiopsis on Mars, our risk from an unsterilized sample return of even large scale harm is far higher than we currently assess it to be.

Worst case scenarios introduce novel ethical and legal questions – is a 1 in a million level of risk acceptable for a scenario that could adversely affect the biosphere of Earth in the very worst case?

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The very worst case scenarios for martian life such as mirror life also introduce novel ethical and legal questions about levels of risk we are prepared to take for outcomes that could be exceptionally severe in the worst case.

Kelly has traced the 1 in a million figure back to a 1 in 100 million figure in a 1961 article, introduced by Mantel et al for the purpose of discussion (Mantel et al, 1961). When asked why he chose this figure he replied ***"We just pulled it out of a hat"*** (Kelly, 1991). The FDA adopted this in 1973 but it became 1 in a million when the final rule was issued. Graham (Graham, 1993) says in practice, EPA's air office tries to reduce the risk to as many people as possible to 1 in a million and the maximally exposed individual to 1 in 10,000. In other situations, EPA recommends a range of risk levels from 1 in 100,000 to 1 in 10 million, and sometimes approves at a level of 1 in 10,000.

This is an ad hoc ethical decision by regulators about levels of acceptable risk, which got accepted more widely by legislators and the general public.

It also doesn't take account of human error. There are many examples, such as a SARS outbreak in 2003 in Taiwan which happened because a technician skipped the standard procedure after a spill, because it would make him late for a conference (Demaneuf, 2020).

Other escapes could happen from equipment failure. During the Apollo sample returns, two technicians had to go into isolation after a leak was found in a sample handling glove for Apollo 11 (Meltzer, 2012:485), and 11 technicians in a similar incident for Apollo 12 (Meltzer, 2012:241).

All these issues such as the level of assurance and how to take account of human error needs especially close scrutiny once we assess a potential for novel and unprecedented large scale harm. We might also want to consider other issues such as accidents, a fire at the facility, even a plane crash into the facility, or criminal actions.

Synthetic biologists suggest a safety mechanism for synthetic life should be many orders of magnitude safer than a BSL-4

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Synthetic biology already permits the creation of inheritable synthetic life such as life with hachimoji DNA, inheritable DNA with four extra bases for a total of 8 instead of the 4 used by terrestrial life (Hoshika et al, 2019). They make sure this is safe by designing the four extra nucleotides so that they depend on chemicals only available in the laboratory.

Some synthetic biologists have suggested that a safety mechanism to contain synthetic life should be many orders of magnitude safer than any contemporary biosafety device. Schmidt puts it like this (Schmidt, 2010)

> *The ultimate goal would be a safety device with a probability to fail below 10^{-40}, which equals approximately the number of cells that ever lived on earth (and never produced a non-DNA non-RNA life form). Of course, 10^{-40} sounds utterly dystopic (and we could never test it in a life time), maybe 10^{-20} is more than enough. The probability also needs to reflect the potential impact, in our case the establishment of an XNA ecosystem in the environment, and how threatening we believe this is.*
>
> *The most important aspect, however, is that the new safety mechanism should be several orders of magnitude safer than any contemporary biosafety mechanism.*

We can't rely on the same risk-benefit calculus for release of SARS and for release of mirror life, without legislative / executive / public involvement to decide if this is what we should do.

NEW: Society places very high value on the environment and given the potential for large scale effects, we might require Earth is kept 100% safe for this mission – i.e. use the prohibitory precautionary principle

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This mission also leads to novel questions about variations on the precautionary principle – principles to do with how we need to handle situations where the level of risk can't currently be assessed because the science is incomplete.

The ESF study considered variations on the precautionary principle (Ammann et al, 2012:25) based an analysis of the principle by Stewart (Stewart, 2002), including:

- **Best Available Technology Precautionary Principle**: Activities that present an uncertain potential for significant harm should be subject to best technology available requirements to minimise the risk of harm unless the proponent of the activity shows that they present no appreciable risk of harm.
- **Prohibitory Precautionary Principle**: Activities that present an uncertain potential for significant harm should be prohibited unless the proponent of the activity shows that they present no appreciable risk of harm

The ESF ruled out the Prohibitory Precautionary Principle on the basis that it would simply lead to cancellation of the mission (Ammann et al, 2012:25):

> *It is not possible to demonstrate that the return of a Mars sample presents no appreciable risk of harm. Therefore, if applied, the Prohibitory Precautionary Principle approach would simply lead to the cancellation of the MSR mission.*

They did this as experts mandated to find the safest way to conduct the mission.

However Stewart, elsewhere in that same paper, suggests there may be situations where prohibition may be needed, since society places very high value on the environment and its protection (Stewart, 2002:15).

Carl Sagan and others warning we can't take even a small risk with a billion lives – this could be formalized into law as a requirement to use the prohibitory precautionary principle whenever there is any appreciable risk for harm unprecedented in human history

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Chester Everline in his comment said (Everline, 2022):

> *A possible consequence of unsuccessful containment is an ecological catastrophe. Although such an occurrence is unlikely, NASA should at least be clear regarding what level of risk it is willing to assume (for the biosphere of the entire planet)*

This of course isn't NASA's decision alone. We are all stakeholders here. Carl Sagan said we can't take even a small risk with a billion lives (Sagan, 1973):

> ***Carl Sagan:*** *Because of the danger of back-contamination of Earth, I firmly believe that manned landings on Mars should be postponed until the beginning of the next century, after a vigorous program of unmanned Martian exobiology and terrestrial epidemiology.*
>
> *…. I, myself, would love to be involved in the first manned expedition to Mars. But an exhaustive program of unmanned biological exploration of Mars is necessary first**. The likelihood that such pathogens exist is probably small, but we cannot take even a small risk with a billion lives**.*

In the terminology of the precautionary principle, Sagan was saying that we use the prohibitory version discussed in the previous section, that we shouldn't take even a small risk.

Gill Levin, who died shortly before the EIS, said the same, as recorded on video by Dehel and mentioned in his public comment (Dehel, 2022).

> ***Gill Levin:*** *I believe people will realize, especially after the Covid-19 catastrophe, that even if there's only a small chance that something could be contagious and pathogenic, coming from a foreign planet, I don't think it's worth taking that chance….you don't take unnecessary chances where the risk-to-benefit ratio is almost infinite."*

DiGreggorio in his public comment quotes from an interview he did with Dr Carl Woese who also expressed a similar sentiment (DiGregorio, 2022)

> ***Carl Woese:*** *Unless you can rule out the chance that it might do harm, you should not embark on such a course*

So this is a question we need to decide as a civilization. Is a sample return mission one where we should consider the prohibitory version of the principle discussed in the previous section? See above:

- NEW Society places very high value on the environment and given the potential for large scale effects, we might require Earth is kept 100% safe for this mission – i.e. use the prohibitory precautionary principle

It's clear where Woese, Levin and Sagan would stand on that point. One possible outcome of public debate on this topic is to formalize Woese, Levin and Sagan's ethical views on this topic into legislation. The general public, and legislators, could decide that if an action has potential for unprecedented levels of harm to human health or the environment, the prohibitory version of the principle should always be used.

Perhaps it might be formulated something like this (for illustrative purposes only not a proposal):

> *If it is impossible to show that there is no appreciable risk of unprecedented levels of harm to public health or the environment, the Prohibitory version of the Precautionary Principle must always be used*

Unprecedented here means unprecedented in human history (e.g. mass extinction level events).

The decision about acceptable levels of risk for large scale harm is an ethical decision and can't be decided on the basis of science or engineering

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This decision is something that needs global public debate.

NASA are likely to set a higher priority to completing the mission assigned to them than the general public. However they aren't the only stakeholders. We are all potentially affected in the worst case. It needs to be opened out to larger debate.

This is something we can't decide on the basis of science or engineering. It is an ethical and legislative choice. As Randolph put it (Randolph, 2009 : 292)

> *The risk of back contamination is not zero. There is always some risk. In this case, the problem of risk – even extremely low risk – is exacerbated because the consequences of back contamination could be quite severe.* ***Without being overly dramatic, the***

consequences might well include the extinction of species and the destruction of whole ecosystems*. Humans could also be threatened with death or a significant decrease in life prospects*

In this situation, what is an ethically acceptable level of risk, even if it is quite low? This is not a technical question for scientists and engineers. Rather it is a moral question concerning accepting risk

Public comments on the draft EIS: 50 members of the public out of 63 commenting said test first, sterilize first, or stop mission, and likely have similar views to Carl Sagan – who said that this is a qualitatively different situation from a human pathogen in a BSL-4 and NASA shouldn't take even a low level of risk with Earth's biosphere – 9 specifically mentioned unprecedented harm

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The public comments aren't a poll, but they do show that many members of the public have similar views to Carl Sagan, Gill Levin, Carl Woese and others, that this is a qualitatively different situation from a known pathogen in a BSL-4 lab and that we shouldn't take even a low level of risk.

- Carl Sagan and others warning we can't take even a small risk with a billion lives – this could be formalized into law as a requirement to use the prohibitory precautionary principle whenever there is any appreciable risk for harm unprecedented in human history

Out of 63 comments from the general public, 9 specifically mention the potential for unprecedented harm in one way or another and 50 said some combination of test first, sterilize first or stop mission.
I think it is also reasonable to assume that all or nearly all the ones that said, test first, sterilize first or stop mission would support Carl Sagan's quote (Sagan, 1973):

"… we cannot take even a small risk with a billion lives."

Here are the comments summarized, and I've shown in bold the ones that said test first, sterilize first, or stop mission or specifically mention unprecedented harm and likely support Carl Sagan's statement that we can't take even a small risk with a billion lives.

So, as a rough estimate, 50 supporting some variation on Carl Sagan's view out of a total of 63 separate people commenting (selected one only for duplicate entries).

There could be a slight overcount here. 12 of those 50 were anonymous. With the public interface, it's not possible to know for sure if some of those were duplicate.

At any rate, several dozen distinct members of the public expressed views that suggest they would be in support of Sagan's quote, on a not very widely publicised EIS. Nine commentators specifically mention unprecedented harm.

This figure of 50 out of 63 shouldn't be read as a percentage of the public as it is not a poll. But it does show that at least several dozen of the members of the public who were reached in the not very well publicized second round of comments had concerns similar to Sagan.

- **stop mission, unprecedented harm – test first – study in separate module attached to ISS – test first – stop mission**
- **stop mission – test first – test first, unprecedented harm – Study in space or not at all, keep Earth 100% safe, our efforts to contain the samples may seem lax a generation from now – test first**

- **stop mission** – need clarity about security measures – off topic – alternative design - **keep Earth 100% safe**
- **unprecedented harm – stop mission, unprecedented harm** – alternative design – **test first – test first**

- **test first – unprecedented harm – test first – Test first – Don't return unless 100% safe – or sterilize first**
- **Don't return – don't return until 100% safe – test first – test first – test first**

- **ISS first – test first – test first – unknown risk, test first – sterilize first**
- extra precautions for EES reentry - **sterilize first – sterilize first – sterilize in space station first – sterilize first**

- **do not return – do not return – do not return** – send to Russia first **–** issues with disinfection of earth entry site
- **test first** – support EIS **– study in situ or space lab or sterilize first** – fully support, suggests more samples – – off topic (future missions need to be designed for reeuse)

- multiple cautious measures – support EIS – support EIS **– test or sterilize first – sterilize first**
- **test in situ or don't return – do not return – unprecedented harm, test first – unprecedented harm, return to space station**

- - and the four comments already mentioned by name **(Walker, 2022a) (Dehel, 2022) (DiGregorio, 2022) (Everline, 2022)**

Also notice that 12 said sterilize first, even though it's not listed as an alternative action in the EIS.

- **Don’t return unless 100% safe – or sterilize first – sterilize first – sterilize first – sterilize first – sterilize in space station first**
- **sterilize first – test or sterilize first – sterilize first – study in situ or space lab or sterilize first**
- Plus (Walker, 2022a) (Dehel, 2022) (DiGregorio, 2022)

For sterilization first it’s also relevant to look at the first round of comments as this shows it was proposed as a reasonable alternative before NASA drafted the EIS.

There were 8 comments asking NASA to sterilize the samples first as one option or the only option, in the first round of comments, in May:

- sterilize first – sterilize or test first – sterilize first – sterilize first – sterilize first
- sterilize first, test method of sterilization on Mars life in situ
- Plus (Walker, 2022a) and (Dehel, 2022) (suggestion in his attached file)

Nick Bostrom’s suggestion for a mathematical way to work with probabilistic risk assessment for low likelihood probabilities of unprecedented harm – to multiply the likelihood level by the number affected in the worst case

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Nick Bostrom derived an interesting way of working with this mathematically, which may help others to understand the perspective of those who think a one in a million chance of a severe impact like this is unacceptable.

Bostrom’s approach is to multiply the probability by the population to get the expected number impacted (Bostrom, 2002) This doesn’t mean that the intuition of Sagan and others is based on calculations like this. But this calculation may help with mutual understanding between those with and without that point of view.

Let’s apply his approach to an artificial example scenario. Suppose there is a 1 in a million chance that half the world’s population, 3.35 billion, is severely impacted.

Then he would multiply out 7.7 billion * (1/2) * 1 / million. This becomes an expected 3,350 people impacted by the sample return.

In this artificial scenario, back contamination, never actually harms 3,350 people. It either harms nobody or 3.35 billion. But multiplying out like that is a way to think about rare risks that harm lots of people.

If it is something that has long term future effects on our ecosystem, leaving Earth significantly less habitable to humans for all future time - the numbers become far greater. For instance, if you look forward 100,000 years, or 3,000 generations, and suppose that the 1 in a million chance affects half the population for 100,000 years, those 3,250 people become 9.75 million.

Nick Bostrom suggests that this can give a way to think about these existential risks, that take us out of our instinctual responses.

His paper also looks at a way of calculating the impact for potential for human extinction. This part involves a calculation of the numbers of humans that would otherwise exist if we don't go extinct.

However, arguably there is no risk of human extinction with modern technology, even in the worst case back contamination scenario. Many. maybe all of us could survive using space technology by paraterraforming Earth – covering it with large enclosed habitats. However that would be a severely diminished world so the numbers severely affected are huge.

See:

- Humans could survive even Lederberg's scenario and even Gros's scenario (in reverse) by covering Earth with large enclosed habitats using modern technology – and we could preserve nearly all our biodiversity – over millions of years the result may have a more diverse biochemistry with interesting new lifeforms – but if these are possible scenarios they are ones to avoid

EPA's letter posted on the last day of public discussion says they didn't identify significant environmental concerns in their review of the EIS – with no mention of all the public comments raising concerns similar to Carl Sagan's

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EPA posted on the last day of public comments. Their letter says it didn't identify significant environmental concerns in its review of the EIS. It doesn't say anything about a need for NASA to respond to new issues raised in the comments by the general public mentioned in the previous section (EPA, 2022):

> *We appreciate NASA addressing EPA's concerns regarding water resources, unplanned releases and cultural/biological resources identified in the letter.*
>
> *Based on the review of the draft PEIS, EPA did not identify significant environmental concerns to be addressed in the Final EIS.*

From the previous sections, if Carl Sagan was still alive today he would surely have commented on the EIS, and raised the same concerns as many of the general public made, see above.

- Carl Sagan and others warning we can't take even a small risk with a billion lives – this could be formalized into law as a requirement to use the prohibitory precautionary principle whenever there is any appreciable risk for harm unprecedented in human history

This doesn't look like the broad acceptance which Rummel et al said is essential for success of this mission – if NASA continues with this action, it is vulnerable to being stopped in the future

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Rummel at al wrote (Rummel et al, 2002:96) :

> *"Broad acceptance at both lay public and scientific levels is essential to the overall success of this research effort."*

This doesn't look like broad acceptance of NASA's proposed action. It may be stopped at various points.

First NASA could withdraw the EIS, do the size limit review, do a scientifically rigorous EIS.

This seems far the best outcome for NASA. Not forced to do anything by a court decision. Not responding to public panic. They can decide in their own time how to proceed. For instance they can do a 100% safe mission using sterilize first, or they can work on other ideas, but it's all done in coordination with the general public, legal experts, ethicists, social scientists etc.

Even a last-minute conversion to a 100% safe mission could cause problems if NASA do it in response to panic from a distrustful public. Far better to get the public involved from the outset.

Assuming NASA continue with the EIS, it could be stopped by other agencies. As it is currently, the draft EIS says there would be no significant environmental effects, so they'd have no reason to look at it closely (NASA, 2022: 3-16):

> … support the judgement **that the potential environmental impacts would not be significant.**

But if any of them do look at it more closely they'd see these issues with the citing and sources and may stop it.

The next point it can be stopped is in a court case after the EIS is finished and published. There is no provision for legal challenges within NEPA, so it is done through judicial review, usually on the basis that: (Congressional Research Service, 2021).

- the agency failed to consider some of the impacts
- the agency failed to properly consider the weight of the impacts under review

They can only be taken to the courts by someone with “standing”. For this, they need to take part in the public comments or debate in the NEPA process, and need to be directly affected by the proposed action.

There you have to show that you are particularly affected by it, which is normally understood to mean more so than by others. If the petitioner claims NASA overlooked a worst case risk of global effects NASA could try to block it on the basis that in their hypothetical scenario they wouldn’t be affected more than anyone else in the world and so don’t have standing.

In the past, environmental cases have gone either way based on subtle legal arguments about whether environmental effects give the petitioner “standing” for the case (Birnbach, 1997).

If it does get as far as the courts, the case is usually (Congressional Research Service, 2021:Remedies in NEPA Litigation)

- referred back to the agency (such as NASA) for further proceedings

If that is all the court does, the agency can continue with the project while it does those proceedings.

However at this point the court can also order “equitable relief”

- the court can order the agency to stop the project going ahead
- order some other action (in this case perhaps order to sterilize the samples first?).

So if a case is taken out and it’s successful, that could lead to a justice asking NASA to either stop the mission or to use some other remedy such as to sterilize the samples first before they are returned to Earth.

If nobody takes them to court or NASA successfully block the case so it never reaches the court, the next step is the presidential directive NSC-25, which requires a review of large scale effects that could be reasonably expected to result in allegations of major or protracted effects. It has to be done even if the agency feels confident such allegations are false (Whitehouse, 1977):. This happens after the NEPA process is completed (Race, 1996).

If it gets past all those hurdles with little public awareness, it could be stopped at the last minute with samples already on their way back to Earth. At that point, if not before, experts would look at the published EIS and see it wasn’t scientifically credible.

Mounting global public concern could lead to Congress and the president acting to tell NASA to divert the mission away from Earth. A worst case here might be an infodemic about Mars life similar to the COVID infodemic, junk science, problems for NASA’s credibility, and issues with eventual return of even 100% safe sterilized samples.

NEW: We can forestall all these issues and make the mission 100% safe by sterilizing samples before they reach Earth – it is impossible to eliminate all risks to spacecraft and astronauts from space exploration into the unknown but it IS possible to eliminate all risks to Earth's biosphere

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[question]

There is a way to make the mission 100% safe from the outset. That is to sterilize the samples before they reach Earth. What's more we'll see that sterilization done carefully has virtually no impact on either the geological or the astrobiological interest of the mission.

The sterilization working group said "… it is impossible to remove all risk without ceasing space exploration". (Craven et al., 2021:4).

> *"While it is impossible to remove all risk without ceasing space exploration, … There is always some level of risk associated with exploration into the unknown, and it was the goal of the SWG to help manage the risks of possible adverse effects to the Earth's biosphere while maintaining the science integrity of the returned samples."*

However, there are different types of risk in space exploration.

It is true that there is no way to remove all risk of robotic spacecraft crashing or malfunctioning, or accidents for humans in space during space exploration.

However, we can ALWAYS **completely eliminate all risks of possible adverse effects to the Earth's biosphere or to the health of humans on Earth**

We can keep Earth safe, by simply not doing or delaying any activities that carry such risks, and prioritizing safety of Earth's biosphere over everything else in our explorations.

There is no risk to Earth's biosphere from in situ robotic exploration of Mars, or human exploration by telepresence from orbit around Mars. There is no risk if we sterilize samples before we return them to Earth. We will see that we can choose a level of sterilization that would eliminate any risk even from life of an unknown biology.

The safest approach here is to eliminate all risk to Earth's biosphere and human health while retaining the science interest of the returned samples as far as possible.

First for the geological interest. One way to sterilize the samples with minimal impact on geological studies is to duplicate the Martian surface ionizing radiation. Even the equivalent of 500 million years of surface radiation would have virtually no impact on geological interest, as rock samples from the ancient delta have had the same type of sterilizing radiation for 3 billion years, and Perseverance can't drill to ancient layers that were protected from surface ionizing radiation all that time.

We will see that this also has virtually no impact on the astrobiological interest, because of the high levels of forwards contamination (for astrobiology) which would overwhelm the faint signature of past life, and because it is targeting a region of Mars that is not likely to return abundant and easily recognizable present day life.

This solution can make the whole process far simpler, with none of the legal complexities of an unsterilized return. However if we do this, it is still important to keep the public fully involved, to coordinate and respond to questions and concerns, and liaise with the help of ethicists, legal experts, representatives of other countries and so on. It is not enough to ensure that the samples are 100% safe. We also need to make sure everyone agrees and understands they are 100% safe.

NEW: Sterilization with 3 million years equivalent of surface ionizing radiation will have virtually no effect on geological studies

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Allen et al tested the effect of sterilization of simulated Mars samples (Allen et al, 1999) with a gamma ray dose of 30 megarads, equivalent to 0.3 megagrays (one Mrad is 10,000 Gy). There was no effect on radiometric dating, rock composition, crystal structure, no dehydration of gypsum, no changes in the spectra of the components of the Mars soil simulant.

There was no effect on the basal spacing of montmorillonite, which is extremely sensitive to temperature and degree of hydration. The only change they found was a change in the colour of quartz (clear to deep brown) and halite crystals (to blue) and a change in their thermoluminescence properties.

Curiosity measured 76 milligrays a year on the Martian surface (Hassler, 2014). Rounding that up to 100 milligrays, then 0.3 megagrays corresponds to about 3 million years of surface radiation (likely an underestimate of the number of years).

0.3 megagrays is enough for a trillion fold reduction of radiodurans based on a million fold reduction at 0.14 megagrays (Horne et al, 2022).

Given that many of the rocks have already had 3 billion years of ionizing radiation, these changes have likely already happened to Mars surface deposits long ago.

Sterilization must be effective for any conceivable exobiology – 500 million years equivalent of ionizing radiation would reduce a gram of amino acids to a milligram and would likely be more than enough to sterilize the samples for any conceivable life with virtually no effect on the geology return, a lower dose like 50 million years equivalent to halve the amino acids or even less may also be more than enough but this needs attention of experts

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By (Kminek et al, 2006:4) 500 million years at 200 milligrays per year, i.e. 100 megagrays, reduces many amino acids to a millionth of the original concentration.

If we take a figure of 100 milligrays a year instead, based on Curiosity's 76 milligrays a year e (Hassler, 2014), 100 megagrays corresponds to a billion years to reduce many amino acids a million fold. Then 500 million years, or 50 megagrays would reduce many amino acids 1000 fold (this is because it's cumulative, if we applied 50 grays twice, the first dose reduces them 1000 fold, then the second dose reduces what's left 1000 fold leading to a million fold reduction).

More generally, based on those figures, the dose x in megagrays for an n-fold reduction is

$$x = 50 * \log(n) / \log(1000) = 50 * \log(n) / 3.$$

For example, a 4-fold reduction in amino acids needs around 10 megagrays or around 100 million years of surface radiation.

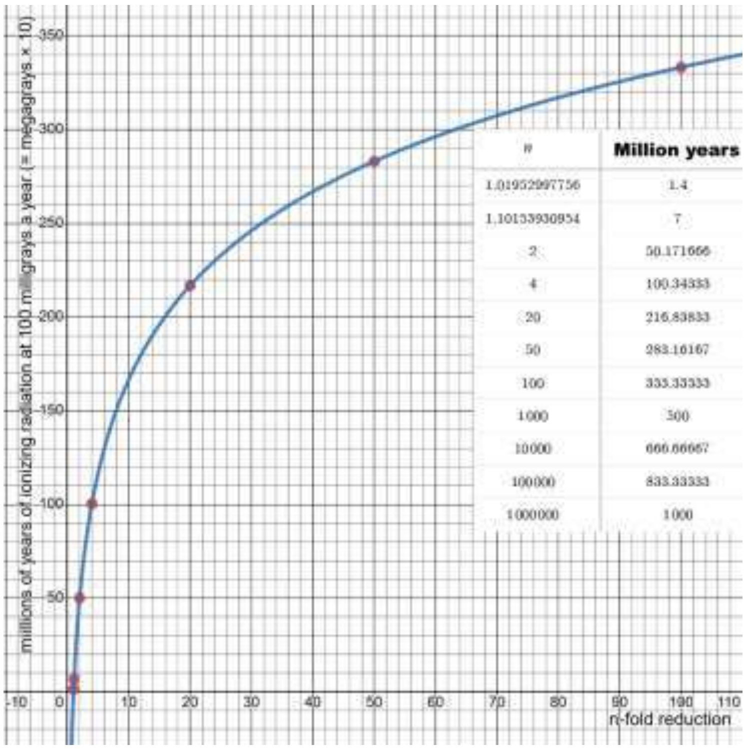

Figure 39: Dots show:

reduction: timescale
1.02: 1.4 million years (radiodurans)
1.1: 7 million years (5 times more hardy)
2: 50 million years
4: 100 million years
20: 217 million years
50: 283 million years
100: 333 million years
Graph and data for the dots available online from Desmos here

To destroy half the amino acids needs only 5 megagrays.

That might be more than enough since the number of viable Radiodurans microbes is reduced a million fold at 0.14 megagrays when it is at its most resilient, desiccated and frozen (Horne et al, 2022). This works out as an approximately 1.02 fold reduction in amino acids, destroying around 2% of the amino acids.

Mileikowsky et al suggested considering a hypothetical five times more resistant microbe on Mars for their modelling (Mileikowsky, 2000: p 401).

Using a simpler approach than they used, just to show how it works, a five times more resistant microbe would need 0.7 megagrays to reduce it a millionfold. That works out as a 1.1 fold reduction destroying about 10% of amino acids.

It would be for experts to consider what level of dose is needed to sterilize even unknown exobiology and there doesn't seem to be a thorough study of this in the literature.

For the purposes of this paper we'll use a value of 5 megagrays, or 50 million years worth of ionizing radiation, enough to halve the amounts of amino acids, but that's not intended as a recommendation. It's just for purposes of illustration. It is more than ten times the 0.3 megagrays value used by (Allen et al, 1999) above:

- NEW: Sterilization with 30 million years equivalent of surface ionizing radiation will have virtually no effect on geological studies

It's more than 5 timers the dose for a hypothetically 5 times more radioresistant microbe than radiodurans.

5 megagrays would be enough to reduce viability of the hypothetical microbe 5 times more radioresistant than radiodurans effectively to zero. Theoretically 4.9 megagrays would reduce a population of microbes five times more radioresistant than radiodurans 10^{42} fold. But maybe no microbe could be viable after losing half its amino acids. There might be a cut-off point that makes it impossible.

If experts think extraterrestrial life could be exceptionally hardy and survive even half of its amino acids destroyed we can use 50 megagrays to reduce them 1000-fold, equivalent to half a billion years of Mars surface radiation. Or achieve a million-fold reduction with 100 megagrays equivalent to a billion years of surface ionizing radiation. Even these high doses would likely have virtually no effect on the geological studies since the rocks have had much higher levels of ionizing radiation already.

Using high levels of sterilization for the samples would reduce the amounts of past organics left in the sample, but they will be undetectable anyway. There is likely so little left of past organics after 3 billion years of surface ionizing radiation that even a small amount of forward contamination will overwhelm it.

Meanwhile, if we are serious about starting a search for present day life in Jezero crater, we need to return samples without the forward contamination. We also need to return them for remote study in a high orbit far from Earth's biosphere. See:

On the remote chance we return present day life from Mars in the Perseverance samples it's not going to be easy to recognize or study it.

NOTE: We can also calculate the n-fold reduction from the dose as

n = 10^(3*x/50)

and the % destroyed for the dose x in megagrays as

100 – 100/ n.

This also gives a way to calculate the figure for the sterilization dose for JAXA samples. Any that got to Phobos over 18.5 million years ago had a dose of over 1.85 megagrays, so at least 22.5% of many of their amino acids have been destroyed since then. This calculation is referred to in:

- New: extending the JAXA analysis to photosynthetic life on or near the surface of any Martian meteorites

The 2009 study makes an argument that it is not necessary to use ionizing radiation doses more than life would experience on a meteorite traveling from Mars to Earth (SSB, 2009 : 46).

> *The preceding section in this chapter argued that if there are Mars organisms sufficiently robust to survive a realistic sterilization treatment in the quarantine facility, then some of these resistant organisms also would have survived transit to Earth in meteorites, and our planet already has been infected by them. Thus sample certification as "effectively sterilized" is appropriately based on verifying that the treatment used kills the most resistant known terrestrial organisms, and that the treatment is at least as harsh as that experienced by recent meteorites in Mars to Earth transit. Being substantially harsher than this will not be necessary.*

However this is not a valid argument as we saw in the discussion of the JAXA mission.

- Example scenario of martian life adapted to live in surface dust or dirt but unable to get to Earth on a meteorite - with terrestrial analogy of invasive starlings in the USA and the invasive diatom Didymo in New Zealand – it's life that CAN'T get to Earth by itself that matters for backwards contamination

A microbe on Mars might be able to resist sterilization during the journey back. But that doesn't prove that it can also withstand the

- desiccation of complete vacuum
- shock of ejection

Then if it can withstand both of those, there's also the issue that the materials it lives in may never get to Earth.

If a microbe typically lives in surface dust, dirt, salts or ice and depends on photosynthesis, then it is possible that it

- never gets into a rock sufficiently far below the surface to get to Earth, for instance if it relies on sunlight it may never or rarely get into the subsurface.

If it gets below the crust of a rock that is ejected from Mars it may be

- sterilized by the fireball of exit from the Martian atmosphere or the fireball of re-entry into Earth's atmosphere

So we need to ensure that any sterilization will destroy all viable life. It is not enough to supply sterilization levels equivalent to the journey from Mars to Earth in a meteorite.

Amino acids exposed to 3 billion years of surface radiation have been reduced from grams to attograms, a billionth of a billionth of a gram – meanwhile infall from space adds about 60 micrograms per gram but is constantly destroyed by surface processes

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Using the same result that the amino acids are reduced a thousand fold every 500 million years, amino acids that have been on the surface for 3 billion years got reduced by 10^{18} leaving only one attogram for every original gram of amino acids (a billionth of a nanogram, which in turn is a billionth of a gram).

These minute traces of past organics may also be mixed with infall from space (Frantseva et al, 2018). Many processes degrade the surface organics, but without them, Mars would have around 60 ppm or 60 micrograms per gram of organics infall, averaged over its entire surface to a depth of a hundred meters (Goetz et al, 2016:247) as well as indigenous abiotic synthesis.

Even if Mars had abundant life in the past, those attograms that remain will be completely swamped by infall from meteorites, comets, interplanetary dust and in situ abiotic processes .(Mulkidjanian, 2015) (Westall et al, 2015) (Franz et al, 2020).

This is why astrobiologists devised ultra sensitive instruments such as astrobionibbler able to detect just a single amino acid in a gram (Schirber, 2013) (Noell et al, 2016).

As for present day life, one microgram per gram is enough for ten million ultramicrobacteria at a tenth of a picogram each (see next section). Even if there are thousands of ultramicrobacteria they might be easily overwhelmed by organic infall in searches for biosignatures. But the situation is far worse because of the issue of forward contamination in the Perseverance samples.

NEW: Sadly Perseverance's permitted levels of 0.7 nanograms per gram for their most abundant biosignatures would overwhelm any faint signature of biosignatures from past life and it would also mask even as many as thousands of cells per gram of present day ultramicrobacteria, though it could spot present day life if there are many spores per gram in the dust

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[question]

Perseverance's engineers believe they achieved contamination levels for returned rock samples of (Boeder et al, 2020: table 6):

- 0.7 parts per billion or 0.7 nanograms per gram for their most abundant biosignatures.
- 8.1 parts per billion, or 8.1 nanograms per gram total organics

This means each gram of returned rock sample could have 8.1 nanograms of organics and up to 0.7 nanograms of contamination from DNA, or Glycine, or some other organic biosignature. The paper doesn't give a detailed breakdown of the estimated level achieved for each organic.

From the previous section a gram of early amino acids would be reduced to attograms (billionths of a billionth of a gram) by 3 billion years of surface ionizing radiation.

- Amino acids exposed to 3 billion years of surface radiation have been reduced from grams to attograms, a billionth of a billionth of a gram – meanwhile infall from space adds about 60 micrograms per gram but is constantly destroyed by surface processes

So it seems that sadly, Perseverance's permitted levels of forward contamination are too high for the samples to be likely to be of much astrobiological interest for past life, even if they returned a sample which when deposited consisted entirely of past life organics.

As for present day life, attendees to the 2020 conference "Mars extant life: what's next?" (Carrier et al, 2020:801) agreed that we would be able to detect extant life resembling terrestrial life if it has been returned without getting contaminated.

It's not so clear that we'd be able to detect unfamiliar life even without contamination. But if returned to Earth we can use a much wider range of instruments to search for it than we can send to Mars in situ.

So there is some value to astrobiology of returning samples to Earth if we think there is a chance they contain extant life, but have been unable to detect it in situ on Mars – however we need to ensure the samples aren't contaminated.

So how does the level of contamination compare with the organics we might find from extant life on Mars? The nutrient poor conditions there may favour ultramicrobacteria, as small cells take up nutrients more efficiently.

By definition an ultramicrobacteria has a volume of at most 0.1 cubic microns. A micron is a millionth of a meter or a 10,000th of a centimeter. So a cubic micron has a volume of a trillionth of a cubic centimeter, or a mass of a trillionth of a gram, or a thousandth of a nanogram (a picogram). So, at a volume of a tenth of a cubic micron each, 0.7 nanograms is equivalent to 7,000 ultramicrobacteria.

Figure 40: Sample tube photo from (NASA, n.d.pst)

There would need to be large numbers of ultramicrobacteria, tens of thousands to millions per sample tube, to have an easily detected biosignature for extant life. Those aren't large numbers for inhabited dirt but that's only likely if Martian life is abundant almost everywhere in the Martian dirt.

The Martian conditions might favour spore forming microbes and we might find spores in the Martian dust. The b. subtilis spore, one of the smallest spore forming microbes, is typically 1.2 μm long and 0.8 μm wide so its volume is about a cubic micron, increasing to 1.8 μm long and 1.2 μm wide on hydration (Chada et al., 2003). At one nanogram per spore, if there are a few dozen or a few hundred spores per tube we might detect them as significantly higher levels of biosignatures than the forward contamination.

Suppose we found a single viable organism in the sample. The sterilization requirement for living cells was no more than a 0.1% chance of a single viable terrestrial organism. This is per tube rather than per gram of sample. They estimate that they achieved a much more stringent 0.00048% (Boeder et al, 2020: table 6). If their estimate is accurate, this makes it no more than a 0.02% chance of finding a single viable terrestrial organism in at least one of the 38 tubes. So, discovery of a viable martian organism wouldn't be enough for a discovery of Martian life, but it would be suggestive.

From these figures, Perseverance seems unlikely to detect martian life, past or present in its sample tubes, even if by chance it returns it. It might detect it if it sampled a biofilm, or if there are many spores per gram in the regolith samples. If it found a single viable microbe, it would be suggestive but not definitive.

Small chance of returning recognizable recent or present day life if Perseverance samples a biofilm, or local concentration of life, or there are many spores per gram in the dust or lucky discovery of a microbe entombed in a crystal of salts or gypsum – but Perseverance isn't actively searching for this scenario

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We saw there is effectively no chance of returning recognizable past life with forward contamination at billionth of a gram levels and traces of past life amino acids reduced to a billionth of a billionth of a gram for every gram of past life amino acids. See:

- NEW: Sadly Perseverance's permitted levels of 0.7 nanograms per gram for their most abundant biosignatures would overwhelm any faint signature of biosignatures from past life or even as many as thousands of cells per gram of present day life, even if viable

There may be a small chance for detecting recent or present day life if Perseverance samples a biofilm or some other concentration of present day life, or if there are many spores per gram of present day life. Another way we could detect a clear signal of extant or recent past life on Mars would be the lucky discovery of a microbe from the present day or recent past entombed in a fluid inclusion in a crystal of salts such as halite (common salt), or gypsum (Carrier et al, 2020 : 797 and figure 4).

This photo shows microbes entombed in an 830 million year old salt crystal. If we found something like this in the samples we'd know it is native life, but the chance of such a discovery is very low, without any capability to detect life in situ.

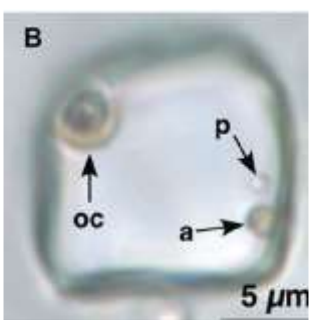

Figure 41: (Schreder-Gomes et al., 2022: figure 3) Caption: Clear prokaryotic cocci (p), orange algal cell (a), and organic compound halo (oc) around air bubble; plane transmitted light.

Benison et al. found many microbes entombed in gypsum in Mars analogue shallow acid salty lakes in the high Andes, where crystals form of various salts such as halite and gypsum, and write (Benison et al., 2014):

> *Could microfossils and/or viable microorganisms be trapped in gypsum on Mars as they are in gypsum on Earth? It is likely that abundant sulfate sand grains on Mars contain fluid inclusions similar to those in the acid-precipitated bottom-growth and reworked gypsum we discuss here.*
>
> *We suggest that gypsum on Mars would have entrapped, as solid inclusions and within fluid inclusions, any microorganisms and/or organic compounds that were present in its parent waters. Therefore, fluid inclusions and solid inclusions hosted by salt minerals may be the best place to continue the search for life on Mars*

It does seem a possible scenario, to find life entombed in crystals of martian salts, which we could recognize as native because there is no possibility of forward contamination. However, it will likely need a new mission that actively searches for these inclusions in salt deposits on Mars. Such, a new mission could also be designed to prevent forward contamination.

Why NASA permitted forward contamination – they could have put tubes and tools for sample collection in a bag to keep them sterile but their engineers worried that this would jeopardize sample collection if they couldn’t open the bag on Mars

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You might wonder,

> *“Why did they permit contamination at all?”*

We do have the technology to make 100% sterile sample tubes free of any organics and to do the same for the sample collection tools. There are various ways to do this including baking the tubes and tools in an oven.

However, engineers need a way to protect the tubes and tools from recontamination until after launch. The easiest way to do that is to put them in a bag to keep them sterile, but engineers worried this risks jeopardizing the mission, if Perseverance got to Mars and then they found that the bag couldn’t be opened (Redd, 2015).

The sample collection tubes, can be baked in a hot oven. As temperatures reach 500°Celsius (930°Fahrenheit), any organic materials are baked away and/or oxidized into carbon dioxide. As long as the materials can withstand those temperatures, it comes out of the oven completely clean. Contamination from air—specifically, air on Earth—is harder to avoid.

...

"Air is full or organic compounds—particles such as dust, volatiles in the form of gas," Sessions said. "At the level of parts per billion, they're everywhere."

…

On the surface, the answer appears simple—put the sample collection tools inside an air-tight bag and transport it to Mars, keeping the material from ever coming in contact with Earth's atmosphere. But such a solution comes with its own problems.

"The engineers were very worried about this," Sessions said. "Imagine getting to Mars, and you can't get the bags open."

So, instead, after baking and sterilization, the sample tubes were exposed to the atmosphere in a clean room. They also had to be handled by technicians when they were placed in the rover. This decision made 100% sterilization impossible. So NASA went for less strict requirements.

It would have been a major challenge to keep the rock samples contamination free given the complexity of Perseverance. It wouldn't be enough to have sterile sample tubes. They would need also to keep the sample collection tools 100% sterile and free of organics.

That includes the drill bit and the complex mechanical equipment to deposit the sample in the tube and seal the tube. They would need to cover it in a container or barrier, and need to a way to remove the protective barrier after launch.

This would be mission critical for sample collection. If the barrier or container couldn't be opened, Perseverance could still do its observations on Mars but would not be able to acquire any samples. Engineers always try to reduce the number of mission critical steps as far as possible, so it is natural for them to design the mission without a containing barrier for the sample acquisition equipment.

However, sadly, the result is a mission is of far less interest for astrobiology, with almost no chance of detecting organics from past life, and only a very small chance of detecting it from recent or present day life.

So sterilization preserves virtually all geological interest, and because of the forward contamination would most likely have minimal impact on astrobiological interest – but NASA's EIS doesn't permit it due to a requirement for "safety testing"

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[question]

NASA's draft EIS doesn't permit sterilization as an alternative because it says unsterilized samples need to be returned to Earth for "safety testing" in its Purpose and Need (NASA, 2022: 3-3)

> *These same principles regarding the importance of using terrestrial laboratories to enable the best scientific return also apply to the care and attention to detail that would be required to conduct a proper and comprehensive sample safety assessment in a proposed SRF.*

However, there is no need for safety testing for samples that are sterilized before they reach Earth as they are made safe by sterilization.

Also this is a reasonable alternative since virtually all the geological interest is preserved by sterilization and the forward contamination is so high for astrobiology that sterilization would have minimal impact on the astrobiology interest too.

So, this requirement seems to improperly exclude a reasonable alternative. By the 7th circuit decision mentioned above, it is contrary to NEPA for agencies to

> ***"contrive a purpose so slender as to define competing `reasonable alternatives' out of consideration (and even out of existence)."***

See (above):

- Past litigation has sometimes completely halted agency actions for failing the NEPA requirement to look at reasonable alternatives – just because the EIS didn't look at them – not based on any assessment of whether the alternatives are better or worse than the proposed actions – by a 7th circuit decision in 1997

NEW: “safety testing” can never prove it is safe to release unsterilized samples – level of forward contamination guarantees all samples test positive for life – no guaranteed biosignature to distinguish terrestrial from potential martian life – most or all tests will find sequences new to science as nearly all terrestrial microbes are unsequenced – we don’t know in advance how to detect extraterrestrial biochemistry – we can’t reliably cultivate even most species of terrestrial life – and it is impossible in practice to predict effects of introducing unknown extraterrestrial life to Earth’s biosphere – so the required safety testing serves no useful purpose

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[question]

Meanwhile if samples are returned unsterilized, NASA’s “safety testing” seems to serve no useful purpose. By their own cite (Kminek et al., 2022) it is practically impossible to assess the environmental impact if life is found, so the only testing they can do is for presence of life or not.

> *During the Working Group’s deliberations, it became clear that a comprehensive assessment to predict the effects of introducing life in new environments or ecologies is difficult and practically impossible, even for terrestrial life and certainly more so for unknown extraterrestrial life.*

This cite goes on to discuss how to test for life by checking for biosignatures. However, by Perseverance’s permitted levels of forward contamination, they are guaranteed to generate false positives for all the samples tested. The next stage is that the samples all go to “hold and critical review”.

This cite doesn’t say what would happen next, but we currently have no way to reliably distinguish terrestrial from potential martian biosignatures.

Sequencing won’t work. We could recognize familiar life like chroococcidiopsis which we already cultivate and have already sequenced. However, the vast majority of microbial species haven’t been characterized or sequenced or cultivated in the laboratory; the problem of “microbial dark matter” (Dance, 2020). We don’t even have any chance of a genetic catalogue of all the microbes that were sent to Mars on Perseverance. Every swab had a different mix of microbes and several of them had microbes not only not found on any of the other swabs but not closely related to any species identified before on Earth. This is not unusual, indeed this is expected and normal.

- Swabs of Perseverance’s clean room found many microbes only detected through their 16S RNA ribosome subunit, with four not closely resembling any known terrestrial life (Hendrickson et al., 2021). If we detect other novel sequences like this after taking them to Mars and back, it will be impossible to prove they aren’t martian.

They could be sure that they didn't closely resemble an known life because the 16S RNA ribosome subunit is very stable, and is the basis for the modern classification method for microbes and other organisms due to Carl Woese (Sapp et al., 2013). It is a short section of RNA mixed with proteins to make up the structure of the ribosome used to translate RNA into proteins.

Also we can't reliably test for viable martian life by attempting to cultivate it.

- Even terrestrial life is often impossible to cultivate in laboratory conditions. Microbes may need a nutrient poor medium, have generation times of 6 months or more, or depend on other microbes in biofilms for amino acids or even require other microbes for their nucleotides, yet be found in widespread (often harsher) habitats to their liking outside the laboratory (Solden et al, 2015).

Another caveat here. Even if we find life as familiar as chroococcidiopsis, the question still arises – is it terrestrial or is it evidence of panspermia? We'd need to study it closely to see if it is sufficiently identical to any terrestrial strain.

In this case we know we will find sequences from many species of terrestrial life. So, how can we expect to prove that amongst all that terrestrial contamination, there is no unfamiliar or unrelated life? Even no mirror life? Never mind closely related life with new capabilities? Surely this is impossible.

NEW: Too early for any form of safety testing at the level of assurance needed for potential large scale harm – even for samples returned in sterile containers with no forward contamination – after destructively testing 10,000 grains of dust the 10,001th grain could have an undetected viable microbe imbedded in it

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We saw in the previous section that we can't do safety testing for the Perseverance samples because of the high level of forward contamination (for astrobiology). However we could add bonus samples from Mars with no contamination, as we'll see below. So let's look at that situation.

The 2009 study already drew attention to the issue of safety testing, raising issues that are not adequately discussed in the draft EIS (SSB, 2009 :52).

Their main points are:

- BSL-4 facilities don't routinely test soils, rocks, and other materials for pathogens unless they are implicated in a disease

- At the highest containment level testing is increasingly limited to viral agents of somewhat known molecular basis

They assume that before the samples are returned, a battery of tests is developed to detect life and do biohazards detection.

Then they draw attention to “The problem of sample heterogeneity”, the uneven distribution of life.

- Life tends to live in gaps in rocks or associated with particular phases of minerals. Their examples include gaps between granules, holes weathered out by liquids, and networks of microfractures.
- Life is also often found in small quantities of water or gas trapped in the rock

They recommend a process of

1. Examining the outside of the sample and any evolved gases in the sample return facility
2. Removing samples to external laboratories safely enclosed in containers, to study with scanning x-ray imaging, micro-CT scanning and other methods to map out the distribution of minerals and biological elements on the microscale
3. Returning to the laboratory to take microsamples from those microscopic areas within the samples for biohazard testing.

However, this will only work if life in the sample is relatively abundant. In this section, they didn’t consider the issue of detecting individual viable spores or other propagules that may have got carried to the samples in dust storms.

From simulated wind blown Martian dust, microbes can indeed get attached to a dust particle and blown in the winds as we saw above (van Heereveld et al, 2017) (Osman et al 2008). A viable microorganism could be imbedded in a dust grain (Sagan et al, 1968). See above:

- 2017: individual microbes can travel in dust storms imbedded in a dust grain for extra protection from UV

The iron oxides shield the microbe from UV but also hide a microbe from non destructive tests, such as Raman spectroscopy or autofluorescence which in any case are also less reliable.

Even if we have a test guaranteed to identify Martian life, which we don’t have yet because of the issue of a potentially unfamiliar biochemistry, we could destructively test 10,000 grains of uncontaminated dust individually – and perhaps the 10,001th grain has a viable microbe which we can’t detect without destroying it. We could destructively test 10 grams for biosignatures, and the next milligram contains a viable microbe.

Also this process of testing microsamples wouldn't even 'be guaranteed to detect a colony of life growing in the sample. As we'll see below, the life on Mars could be very uneven, as Cockell put it: (Cockell et al, 2019c)

> *Every sample Is a 'microbial island'*

See below:

- The complexity of searching for past life on Mars – why preservation of organics of past life is so hard on Mars and we likely need in situ searches to find that "sweet spot" where past life deposits were not significantly degraded by the fluids it inhabited, or by the harsh conditions since then – without in situ searches the samples are more of a technology demo for astrobiology

Even if we examine 10,000 microscale features, all similar, e.g. 10,000 voids or 10,000 fluid inclusions, the next unexamined feature could be the one with the colony of Martian life in it.

Then there's the issue that we don't know for sure what to look for by way of biochemistry. We can't construct a test that is guaranteed to detect Martian life until we have a better idea what we are looking for as we saw in the previous section.

- NEW: "safety testing" can never prove it is safe to release unsterilized samples – level of forward contamination guarantees all samples test positive for life – no guaranteed biosignature to distinguish terrestrial from potential martian life – most or all tests will find sequences new to science as nearly all terrestrial microbes are unsequenced – we don't know in advance how to detect extraterrestrial biochemistry – we can't reliably cultivate even most species of terrestrial life – and it is impossible in practice to predict effects of introducing unknown extraterrestrial life to Earth's biosphere – so the required safety testing serves no useful purpose

So, it's actually too early to do ***any*** form of safety testing, even with samples returned in sterile containers, at least, not at the level of assurance needed when there is potential for large scale harm. We conclude that any such samples need to be sterilized before they can be declared safe, until we have a much better understanding of the scenario we face on Mars than we do at present.

NEW: Conclusion: "Safety testing" is not feasible at present, and sterilization keeps Earth 100% safe with likely virtually no difference to the science return

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In short, this “safety testing” for unsterilized samples can’t be used to keep Earth safe at our current level of knowledge. It may be possible later once we know more about Mars, how to identify the life and what harmful capabilities it has if any.

Meanwhile sterilization keeps Earth 100% safe with virtually no difference to the geological or astrobiological science return.

That leaves the practical question, how can we sterilize samples before they reach Earth, while still maintaining effectively zero risk of harm? We have to sterilize the samples in a way that has no risk of the unsterilized samples entering into a chain of contact with Earth’s biosphere.

NEW: Samples can be safely sterilized in a satellite similar to geostationary satellites, but positioned in a safe orbit tens of thousands of kilometers above GEO

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The simplest solution might be to use nanoscale x-ray emitters on the return journey. In one experiment the tube operates at 50 kV with an emission beam current of 140 μA, or a power output of 7 watts which would suggest power consumption of 10s of watts, far less than a conventional X-ray. The dose at 3 cm is 8.19 Gy per minute (Kim et al, 2016).

Depending on the dose, for our illustrative example of 5 megagrays, to halve the amino acids, this would require 1.15 years of continuous operation, so, several of them would be needed per sample for a six month journey back, which might then run into issues of available power. Lower doses would be easier. Also, they were blocked by just 3 mm of a copper collimeter and may be blocked by the walls of the tungsten sample tubes. So it seems a challenge to find enough power to sterilize the entire sample using X-rays in six months with the amount of power likely available from solar panels.

If we don’t have sufficient solar power available for that, we can return it to a larger satellite similar to a geostationary satellite for sterilization.

Cobalt 60 sources can be very heavy, with much of the mass for the shielding. X-rays might be better as they can be switched on and off and adjusted. A satellite above GEO can potentially have a significant power supply for generating X-rays. The Inmarsat 5 F1 has a power supply of 15 kilowatts on launch (Inmarsat, 2013).

Whatever method we use to bring the spacecraft back to above GEO, we can target the Laplace plane inclined at approximately 7.2° from the equatorial plane. This is a proposed “graveyard orbit” for GEO satellites at end of lifetime as even large light fragments of cladding from the satellites stay trapped well away from GEO, through the balance of the light pressure from the sun and gravity (Rosengren et al, 2013). It’s where ring particles would orbit if Earth had a ring system.

The sterilizing sample could be placed, say, 50,000 km or 100,000 km above this proposed GEO disposal orbit. This is very safe as the delta V is over 1 km / second to both Earth and the Moon and it would also be safe for GEO and far from the proposed Laplace plane GEO graveyard orbit.

The “Earth Entry Vehicle” can be converted into an “Above GEO Insertion Vehicle” by replacing the aeroshell with extra fuel – and returned to this orbit without concerns about aerobraking or even flybys of the Earth or Moon – we can use the EEV’s ion thruster for low energy ballistic transfer

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We need to avoid aerobraking and we can do that using “ballistic capture”, also known as “weak stability boundary transfers” (Topputo et al, 2015), the low delta v, fuel efficient, three or four body transfer orbits first used for the Japanese Hiten mission in 1990 (Belbruno, 2018). The ESA Earth Return Orbiter will use continuous low thrust transfer (Huesing et al, 2019), ideal for ballistic capture.

One way is to return the sample via a lunar retrograde orbit (actually a prograde orbit around Earth but retrograde around the Moon). Lock et al. proposed returning a sample from Mars using a close flyby of Earth followed by a flyby of the Moon which then can get captured in a lunar retrograde orbit with a delta v of only 100 meters per second (Lock et al, 2014). First they need to reduce the speed of the spacecraft relative to Earth on the flight back, to make this capture easier. This increases the fuel mass by about 30% for their hypothetical mission. Assuming a 1000 kg dry mass and fuel mass of just under 370 kg for the direct flight with aerocapture, this increases to a fuel mass of just under 480 kg for the low energy transfer (Lock et al, 2014).

From the lunar retrograde orbit, it is easy to transfer to Lunar L2 (LL2) (Ming, 2009), the gravitational point of balance beyond the far side of the Moon as seen from Earth. From there it can do a low energy transfer to a Lunar L1 halo between Earth and the near side, and then it can get to above GEO, using lunar flybys in fuel efficient ballistic transfer trajectories again, to reduce the total delta v requirements.

This does need a little more fuel than the direct flight and aerocapture for the same amount of dry mass. However, the dry mass to return is reduced by the mass of the aeroshell, which can be replaced by fuel, and a lower dry mass requires less fuel for the transfer. So, though more fuel is needed, the total mass including the dry mass might not be much different.

There is another especially promising low energy trajectory that may be worth mentioning as it avoids even flybys of Earth or the Moon. This is the reverse of the trajectory in (Kakoi et al, 2014:fig 13). If a spacecraft is placed just beyond the Lunar L2 position on the far side of the Moon from Earth, and it doesn't use rocket motors to stay in place, it will spiral outwards away from the Moon in wider and wider spirals along the "halo orbit manifold". There's a similar outwards spiral for the Sun Earth L2.

This sample return uses those halo orbit manifolds in reverse. It first spirals from ballistic capture to a halo orbit around Sun Earth L2, the unstable gravitational point of balance between Earth and sun, which is on the far side of Earth from the sun as well outside the orbit of the Moon. It gets there at just the right moment to dovetail to a similar spiral down to Lunar L2. For details see (Kakoi et al, 2014:fig 10) which needs to be used in reverse. Then as before it dovetails to a low energy transfer to a Lunar L1 halo between Earth and the near side, and then the spacecraft slowly reduces the size of its orbit around Earth and circularize it in an orbit well above GEO as before

NEW: This keeps Earth 100% safe with virtually no loss to science and little change in NASA's budget – adds the cost of a Sample Sterilizing Satellite but saves on the mass of the aeroshell and the cost of a Sample Receiving Facility – estimated at $471 million in 2015 US dollars for the 1999 technology specifications and would likely cost more today if the technology can be developed

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Launch costs to an orbit above GEO wouldn't be prohibitive for NASA. For an example, the Falcon 9 can deliver 8.3 tons to Geostationary Transfer Orbit (GTO) at a cost of $67 million and the Falcon Heavy can already deliver 26.7 tons to GTO at a cost of $97 million for the reusable rocket (SpaceX, n.d.) As we approach the 2030s, launch costs are sure to go down further. SpaceX might be flying the super-heavy by the 2030s.

There is very little by way of extra delta v to transfer to a higher orbit at say 100,000 km. I've done an online calculator to help (Walker, n.d.). Here is an example:

> Missions start in LEO at altitude 250 km and velocity 7.755 km / sec.
>
> Transfer to GEO at altitude 35786.154 km and velocity 3.075 km / sec:
> **Delta V** = 3.912 km / sec = 2.44 km / sec (leave LEO) + 1.472 km / sec (insertion to GEO).
>
> Transfer from LEO to orbit at altitude 100000 km and velocity 1.936 km / sec:
> **Delta V** = 4.158km / sec = 2.886 km / sec (leave LEO) + 1.273 km / sec (insertion to orbit at 100000 km).

Transfer from LEO to the orbit at altitude 100000 km needs:
Extra delta V = 0.246 km / sec
- compared to the transfer to GEO.

You can enter any altitude for the orbit above GEO and the calculator will show the amount of the extra delta v (Walker, n.d.). It is a simple calculator that doesn't take account of any change in inclination. Also it doesn't take account of the gravity of the sun or moon. Especially for higher orbits, missions sending payloads to the satellite from Earth might use the gravity of the Moon to assist the orbital transfer, as with the rescue mission for Asia-Sat-3 / HGS-1 in 1998 (Ocampo, 2005) (Siddiqi, 2018: 203)

With this option, NASA has the extra cost of the sterilizing satellite, but save on several other costs including the cost of a sample receiving facility on Earth, estimated at $471 million in 2015 dollars (Mattingly, 2010:20) based on the 1999 size limit.

There are no designs available or costs for a facility to comply with the 2012 ESF size limit review but it would likely cost more than that estimate, if it is feasible at all.

This option also saves on the mass of the aeroshell, and the fuel budget to take the aeroshell to Mars and back again for the Earth Entry Vehicle. .

For the cost saved for the sample receiving facility, it would be possible to send a large satellite to geostationary orbit. Also universities might well be interested to join in on the cost. The sample receiving satellite could also be a special item for a budget in Congress if there is enough public interest.

In this way we keep Earth 100% safe, with virtually no loss to science and little change in overall budget.

We can't expect samples returned from Mars at this stage to answer central questions in astrobiology even with bonus samples except with extraordinary luck – astrobiologists emphasize that we need to search in situ first – our aim instead is to find a way to turn this into a far more interesting first step for astrobiology

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We will find that with more ambition, with bonus samples collected in a sterile container, and not much change in the budget, we can transform this into a much more interesting mission for astrobiology. However, we need to be clear first, not to raise expectations to levels that are likely to be impossible to fulfil.

A white paper submitted to the decadal review by astrobiologists emphasized the need to be able to detect life in situ before we can intelligently decide which samples to return demonstration (Bada et al, 2009:7):

> ***We feel that organic detection efforts over the next two decades via investment into advanced in situ robotic instrumentation are fundamental in support of a future intelligent MSR mission.***
>
> *Currently, MSR is regarded by much of the scientific community as largely weighted towards a technology demonstration as the rationale for good astrobiology will not be apparent until we discover more about our neighboring planet.*

Other studies came to the same conclusion (Paige, 2000) (Davila et al, 2010). Most recently in 2020 (Carrier et al, 2020: 802)

> *Future missions would therefore benefit from the development of instruments capable of direct and unambiguous detection of extant life in situ, and improvements are needed in capabilities for sample preparation to optimize biosignature detection. Spacecraft resources should support a sufficient number of sample analyses to support replicate analyses, positive and negative controls.*
>
> *Contamination control should be coupled with contamination knowledge so that Earth-sourced material can be eliminated as a possible source of any biological material discovered in Martian samples.*

Perseverance's geology focus dates back to an oversight present from the mission's inception a decade ago. The decadal review in its summing up said (Space Studies Board, 2012:17).

> ***Mars science has reached a level of sophistication such that fundamental advances in addressing the important questions above will come only from analysis of returned samples.***

They relied on a 2002 paper, Safe on Mars from a time with a much simpler understanding of Mars and less capable instruments for in situ studies (Space Studies Board, 2002a, chapter 5:38) and even then it said that:

> ***"If such capabilities were to become available, one advantage is that the experiment would not be limited by the small amount of material that a Mars sample return mission would provide. What is more, with the use of rovers, an in situ experiment could be conducted over a wide range of locations."***

The instruments were already far smaller and more capable just 7 years later at the time of the paper by Bada et al. Since then astrobiological instruments continued to get smaller and more capable, while our understanding of past and present day habitability of Mars gets more complex. The now overwhelming case for in situ study for astrobiology continues to get stronger.

It is unrealistic to expect at this stage to answer central questions in astrobiology such as whether Mars ever had life in the past or whether it has life currently, though it is reasonable to hope for it.

We have the example of ALH84001, the Mars meteorite which at one time was thought to perhaps have clear evidence of life, but then turned out to be potentially abiotic, and still remains controversial to the present. This controversy made it clear to astrobiologists how difficult it is likely to be to prove that an ancient structure resembling microbes is life.

As Harry McSween put it in 1997 (McSween, 1997)

> *"this controversy continues to help define strategies and sharpen tools that will be required for a Mars exploration program focused on the search for life."*

Bada et al used this example to highlight how challenging it is to try to resolve central questions of astrobiology with a sample return (Bada et al, 2009:1)

> *In the worst scenario, we would mortgage the exploration program to return an arbitrary sample that proves to be as ambiguous with respect to the search for life as ALH84001.*

To answer either of those questions with Perseverance's samples and a few contamination free bonus samples would require a large measure of luck.

We will see in the next section that there is probably almost no potential for finding clear evidence of past life until we can search for it in situ, even with a few contamination free bonus samples.

However, we can make major first steps with bonus samples. We can find a lot about abiotic organics on Mars in the past and present, and conditions there that might confuse life searches, which can let us "hit the floor running" for future in situ searches on Mars.

We will find that there may be some potential to find evidence of present day life if it is reasonably abundant, especially in the salts, possibly also in the dirt and dust.

The complexity of searching for past life on Mars – why preservation of organics of past life is so hard on Mars and we likely need in situ searches to find that "sweet spot" where past life deposits were not significantly degraded by the fluids it inhabited, or by the harsh conditions since then – without in situ searches the samples are more of a technology demo for astrobiology

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First, in the present day, microbes may inhabit millimeter scale features in the rock. Cockell used terrestrial obsidian to gain insights into colonization of Martian rocks. Although obsidian hasn't been directly detected on Mars, the processes are similar. He found that many cubic centimeter samples are lifeless. He recommended a minimum sample size of ten cubic centimeters from a Martian rock, for a reasonable chance to detect life in it.

As he put it: (Cockell et al, 2019c)

> *Every sample Is a 'microbial island'*

What about the past though? Might Mars have the equivalent of our coal beds or shale deposits, vast thick layers of life?

Well, it's true Mars was far more habitable for terrestrial life at the time when the deposits in Jezero crater formed than today. However, Earth's coal and shale deposits are relatively young. It is harder to find traces of life from the distant past, and we don't know in advance where to look in the geological record for past life on Mars (Beaty, 2019) (Cockell et al, 2019c).

Even on Earth, we have little by way of organics from the most ancient stromatolites, and finding those was a huge challenge (Allwood, 2009). It is especially hard to find terrestrial organics that predate photosynthesis. The problem is most past organics are destroyed by geological processes and ionizing radiation from radioactive elements in the rocks.

On Mars also, most organics would be destroyed over billions of years, though some of the processes are different. The main processes include ionizing radiation, radioactive decay of elements in the rock, and reactive chemistry of the hydrogen peroxide and perchlorates (Grotzinger, 2013) (McMahon et al, 2018)..

This is especially so for surface or near surface organics. Grams would be reduced to attograms by ionizing radiation levels far higher than for Earth, as we saw above.

- Amino acids exposed to 3 billion years of surface radiation have been reduced from grams to attograms, a billionth of a billionth of a gram – meanwhile infall from space adds about 60 micrograms per gram but is constantly destroyed by surface processes

Also Earth's great deposits of shales and coal depended on photosynthesis, as did the stromatolites. Martian life might never have developed photosynthesis, or if it did, it might not have done so until after the Jezero crater deposits formed 3 billion years ago (Summons et al, 2011:21) Westall et al suggest evolution of photosynthesis would have been more challenging on Mars than on Earth as the water might have been covered in ice most of the time because of the lower levels of sunlight. (Westall et al, 2013:894). In the scenario without photosynthesis (Hays et al, 2017), its past or present day distribution is likely to be even more heterogeneous (patchy) than in Earth's deserts.

Perseverance and Curiosity both found organics indistinguishable from organics from meteorites (JPL, 2021). Indeed, most Martian organics are expected to come from meteorites, interplanetary dust, and comets, or from indigenous organics from abiotic processes including abiotic photosynthesis (Mulkidjanian, 2015) and electrochemical reduction of mantle materials (Westall et al, 2015) (Franz et al, 2020).

So how do we find life amongst all the abiotic organics? Several authors suggested variations on a "follow the nitrogen" strategy to look for life. Capone et al. say that in a planet with both land and oceans, the presence of nitrogen in any form on the land is hard to explain without life supplied chemistry, because nitrates tend to dissolve in the presence of small amounts of water, and nitrogen has few geological reservoirs, apart from a few clays with ammonium substituted for potassium. Except for nitrates in very dry places, most would end up in the oceans (Capone et al., 2006). Shannon put's it like this (Shannon, 2006)

> ***An oasis of liquid water on Mars would still be presumed sterile if no nitrogen—and therefore no nitrogen containing organic compounds—were detected. In this way, nitrogen might be a better target than the water itself.***

Bada et al suggested to look for biosignatures such as chirality by first looking for specific nitrogenous compounds used by life such as amino acids which may be preserved as components of stable fatty acids (such as lipids), sugars, and peptidoglycans (mureins, constituents of cell walls) (Bada et al, 2009).

All life on Earth requires nitrogen. Also, there are theoretical reasons for expecting alien organic life to use nitrogen, as the weaker nitrogen based amide bonds are essential for the processes by which DNA is replicated. Mars, compared with Earth, has little nitrogen, either in the air or in the soil. Jeff Bada explained it like this, interviewed for an article for NASA's Astrobiology Magazine (Schirber, 2013).

> ***"No other atom than nitrogen can form the diverse set of hydrogen-bonded compounds found to be fundamental in the biologically central processes of replication and catalysis"***

So, we may need to look for past sources of nitrogen as well as organics. Even then we may also need to look for a source of energy in the past which might not necessarily be sunlight.

Then there's the issue of preservation. We don't yet know which Martian processes are most conducive to preserving fine structure and organics from past life. The preservation of biosignatures of past life depends on the history after deposition (Grotzinger, 2013) (McMahon et al, 2018). First, the organics can be degraded, altered or removed.

- Organics can be degraded by cosmic radiation if they remain close to the surface for hundreds of millions of years before they are buried, or are temporarily exposed to surface conditions at some later stage in their history.
- Later flooding can wash out organics.
- Warm conditions can flip organics to the mirror form of the molecule until there is a mix of both forms losing the chiral signature it may have had originally.
- Then the organics can be modified by chemical reactions, for instance with perchlorates, hydrogen peroxide and oxygen.

Preservation of the biosignatures also depends on the conditions in which the organics are deposited (Hays et al, 2017).

- The very conditions that make a habitat suitable for life, such as the flowing water that creates chemical redox gradients in a hydrothermal vent which life exploits for energy, can also degrade the biosignatures that help us to recognize that life.
- Similarly, the water that deposits the clays and other minerals that help preserve the biosignatures can also in turn destroy those biosignatures.

So we may need to find a sweet spot, where deposits from past life were also not degraded too much by the fluids that provided the habitat for life (Hays et al, 2017), for it to be preserved well enough to be recognized

Cockell et al say (Cockell et al, 2019b) that the processes that could preserve life on Mars are different from Earth because amongst other things, Mars

- Has no tectonic subsidence or burial to preserve life
- High concentrations of oxidizing agents such as perchlorates
- Tendency for acidic chemistries in habitats (which leads to less preservation of organics) and lower temperatures (which leads to less biomass)

Because of the acid conditions and the oxidising chemistry, amongst other reasons, a thriving past ecosystem could leave no preserved biomass on Mars. However, Cockell et al consider that it is unlikely that nothing has been preserved of past ecosystems anywhere on Mars if it was inhabited on a planetary scale. If many lifeless sample are returned from many contexts, it would suggest that Mars never had life.

Cockell et al say that a more likely scenario resulting from these challenges is that potential past life signatures are found but are ambiguous or close to detection limits, leading to similar debates as for ALH84001, where some think life was found and others think it wasn't (Cockell et al, 2019b).

Grotzinger, Project Scientist for the Curiosity Mars Science Laboratory mission, suggests we may need to find a "magic mineral" on Mars that preserves ancient life. He says that for Earth this key mineral was chert, a discovery from 1954. There may be similar key discoveries that open up a window into past life on Mars (Grotzinger, 2013) (Grotzinger, 2014).

The situation isn't hopeless. Indeed these astrobiologists are reasonably optimistic that if there was life on Mars in the past, we will find it eventually. However, the general impression from the literature is that they expect it to be a major challenge to find past life even if it was abundant on Mars. That is why so many of the astrobiologists say we should search for past life in situ first. Our first objective is to find it at all.

With this background we should be careful not to raise hopes with the public that we will find out whether or not Mars had life in the past with a few samples from a mission like Perseverance.

However, if we can drill, or find a way to access samples excavated from a few meters below the surface, it is more plausible that we can return past organics, and make a start on finding out how past organics have been processed and changed over the billions of years since they were deposited. In this way we can make a start on learning what we need to look out for in future in situ searches.

Also, even though we have no prospect of in situ searches for present day life at present there are some samples we could return of great interest to astrobiology, the salts, dirt, dust and atmosphere.

NEW: We can transform this into a much more interesting first step for astrobiology with little change in the overall budget by adding bonus samples collected in a STERILE container sent on the ESF fetch rover – the aim is to return dust, dirt, ideally salts, compressed gas from the atmosphere – and some pebbles for a technology demo of a contamination free rock sample

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One thing astrobiologists are sure to do once they can send life detection instruments to Mars is to study the dirt, dust and salts in situ on Mars.

- The dust is like a collection of tiny rocks from the rest of Mars. If we are lucky, a sample of dust might snag some viable life, or the remains of life, along with the dust.

- The dirt can help us understand conditions on the surface of Mars. We already were surprised by the perchlorates in the dirt, and can't know what other surprises we might find that may lead to new research directions (David, 2015).
- The salts may be inhabited by microbes or may preserve evidence of life processes or trapped microbes from the past.

The studies of the dust, dirt and dust could also resolve the puzzle of the Viking labelled release. Did it find life or complex chemistry? See:

- The Viking landers in the 1970s remain our only attempt to search for life on Mars – a few astrobiologists think its labelled release may have already detected life in the 1970s – while others say the data can be explained by complex chemistry – we haven't sent the follow up experiments needed to finally resolve this debate and we can't deduce anything about whether Perseverance might return life even if the Viking experiment did find complex chemistry

If Viking found life, these samples have a high chance to return viable or dead propagules in the dust or dirt, and if instead we find products of complex chemistry, we can use that to refine the chemical explanations of the Viking results. This would make Mars surface simulation experiments and studies more accurate – and perhaps be of interest for prebiotic synthesis?

Astrobiologists are especially interested to study the salts. In the summary of the 2020 conference "Mars extant life: what's next?" salts are singled out as of interest for a sample return, indeed it is the only suggestion they make for a near future sample return. Salts are of great interest because (Carrier et al, 2020:797)

- We might find viable or at least identifiable microbes in fluid inclusions in the salts
- Spectroscopic analysis could uncover biochemicals synthesized by life or resulting from breakdown of life organics
- Salts and brines could contain dissolved solutions and pockets of gas, such as perchlorates, nitrates, sulfates, organics and methane that life could exploit
- The salts could also give access to sunlight for life that can make use of it.

They say salts (including gypsum as well as the halite salts) are of interest because (Carrier et al, 2020:797)

- There is a lot of life in hypersaline environments on Earth
- Microbes are often preserved intact along with easily detectable carotenoid pigment biosignatures
- Salts attenuate UV which would help protect life on Mars
- Salts deliquesce protecting life from long term deliquescence
- Deliquescence of salts can provide potential liquid resources for life.
- Salts could also preserve ongoing evolutionary processes
  [That could include cases where life has gone extinct but then evolves anew in

uninhabited habitats Mars (Cockell, 2014) – perhaps it could evolve anew even when there is extant life elsewhere on Mars]

- Salts extend the temperature range of liquid brines

They don't discuss NASA's sample return mission specifically. But a bonus sample of salts would be sure to get the interest and attention of astrobiologists interested in the possibility of extant life on Mars, if collected in sterile containers.

Another suggestion, we might also find evidence in uncontaminated samples of subtle abiotic processes such as abiotic photosynthesis, or abiotic nitrogen fixation. Mars could have an abiotic nitrogen cycle with photochemically produced HNO_3 fixed in thin (0.2 to 5 nm) pure water metastable interfacial films, potentially supporting up to one kilogram of fixed nitrogen per square meter (Boxe et al, 2012)

To make a start on these questions, we could add a STERILE container to send to Mars on the ESF fetch rover to return bonus samples of dirt, dust, and if possible salts, and atmosphere without forward contamination.

We have already sent an atmospheric compressor to Mars on Perseverance but it is used for Moxie, an independent experiment to test options for creating fuel from the atmosphere, not connected with the sampling experiments. Jakosky et al propose sending a similar experiment to Mars in a small sample tube sized container or alternatively a larger container to return 100 cc of atmosphere. This is how it works.

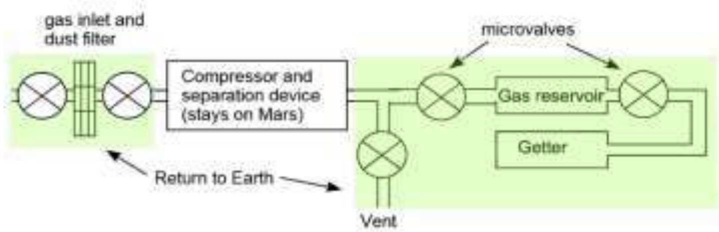

Figure 42: (Jakosky et al, 2021: Fig 6)

First it uses the getter to remove evolved gases from the container wall. Then it closes one microvalve and opens another to get an atmospheric sample. Finally it closes both microvalves to the gas container and opens the vent to run more atmosphere through the compressor to collect dust in the filter (Jakosky et al, 2021)

Assuming a volume of, say, 50 cc of dust, and a dust density of 0.5 grams per cc, it could return up to 25 grams of dust.

This is enough to detect life at around one cell per gram or less. This is also a useful first upper bound of the amount of life in the dust if none is returned.

For astrobiology, it might be useful to do the air collection at night or in the early morning to detect the composition of the air at times of high humidity. Ideally two air collectors, one for daytime sampling and one for night / early morning.

We can then add a scoop of dirt and return all these samples of dust, dirt and atmosphere in a separate small sealed sterile container which goes to Mars on the ESF fetch rover.

The active agent in the Viking experiment was de-activated after storage of the dirt in darkness for several months (Levin et al, 2016). So it's possible that the active agent, whatever it was, chemistry, prebiotic synthesis, or life, could be de-activated during the return journey.

If practical in terms of engineering, it may help to add a window to the container with a neutral filter, similarly to the Mars simulation chamber in the BIOMEX experiment. This would let sunlight in to illuminate part of the sample, duplicating surface conditions on Mars (ideally with day / night changes) which might possibly preserve the active agent whatever it was. The rest of the sample would be protected from UV in darkness, perhaps just kept dark by the shading of the dirt or dust itself.

We could also make Perseverance into a far better sample return technology demo for astrobiology for rocks too, by returning CLEAN rock samples. The ESA fetch rover could use a sterilized scoop and pick up a sample of dirt along with a few small pebbles / rocks. This would demonstrate the capability to return rock samples without forwards contamination. Also, by returning a clean rock, could help with fine details of surface chemical / abiotic processes which could help with the life searches in situ later on.

NEW: It is impossible to use quarantine to protect Earth's biosphere if humans handle the samples in orbit – the Apollo quarantine procedures never had peer review and missed the issue of a symptomless superspreader – and this can't keep out mirror life, or molds like the one that killed two plants on the ISS – keeping humans well away from the samples also avoids forward contamination for very sensitive measurements

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Some authors have proposed using humans to study the samples in space. The idea is that technicians who are prepared to risk their lives in interest of science would study the samples in orbit, as for the Anteus report (Devincenzi, 1981) or on the Moon (Schrunk et al., 2007 : 146), and then we return the samples to Earth once they know they are safe.

Several members of the public also suggested this idea of returning the samples to a space station where they would be handled by human volunteers in public comments to NASA's draft EIS. Their comments are listed amongst the others in the section above:

- Public comments on the draft EIS: 50 members of the public out of 63 commenting said test first, sterilize first, or stop mission, and likely have similar views to Carl Sagan – who said that this is a qualitatively different situation from a human pathogen in a BSL-4 and NASA shouldn't take even a low level of risk with Earth's biosphere – 9 specifically mentioned unprecedented harm

This may seem a plausible solution since the Apollo astronauts had 3 weeks quarantine to protect Earth's biosphere. The same approach was used to protect technicians who handled the Apollo samples in the laboratory. Technicians had to go into quarantine at least twice after a breach of sample containment during sample handling (Meltzer, 2012:485) (Meltzer, 2012:241)

However the Apollo procedures were decided internally and only released on the day of launch, and this was never subject to legal review or public scrutiny (Meltzer, 2012:452).

Carl Sagan gave the example of leprosy for the "vexing question of the latency period" (Sagan, 1973:130)

> *There is also the vexing question of the latency period. If we expose terrestrial organisms to* Martian *pathogens, how long must we wait before we can be convinced that the pathogen-host relationship is understood? For example, the latency period for leprosy is more than a decade.*

We now know that leprosy can take 20 years or more to show symptoms (WHO, 2019).

The Apollo 3 week period was a compromise. NASA commissioned Baylor university to design a protocol for them to use. Baylor university recommended a quarantine period of at least 30 days with 60 days preferrable. (NASA, 1967 : 22).

NASA decided on three weeks as a compromise between no quarantine and the recommended 30 to 60 days. Their reasoning was that if astronauts and technicians can get through three weeks without adverse effects, any pathogen in the lunar samples is not fast acting. They reasoned that this gives time to prepare a remedial action, if a slower acting agent spreads outside the laboratory.

Richard Bryan Erb was the manager of the Lunar Receiving laboratory from 1969 to 1970 (Carroll, 2019). This is what he said about the quarantine protocols as interviewed in 1999 (Butler, 1999)

> **Erb:** The lab worked. It worked well. We had some problems with the breaches in the biological barrier, and people would get contaminated in the labs, and then we'd dump them into quarantine as well. We had projected that this sort of thing could happen and that we would have lab technicians exposed and have to add them to the crew that was being quarantined. I believe it was a reasonably rigorous quarantine and an effective demonstration that there were no effects of the lunar material that showed up quickly.

> You never know whether something might show up in thirty years. There are viruses and things that will show up long after the fact, but the theory was that if you can go through a quarantine for three weeks, which was the time set, without adverse effect, then you're obviously not dealing with something that is rapidly reacting and dangerous, so you would have time to prepare a remedial action. It was a good trade, I think, between a hazard, which was not very likely, but a risk of perhaps life on Earth, which was immense. So it was one of those fundamentally indeterminate things, and you just had to make a judgment call.

The length of quarantine period is just one of many issues. Once experts on infectious disease get involved they will find many other issues.

Once the issue is raised of possible pathogens of humans, the Occupational Safety and Health Administration in the USA is sure to declare an interest for questions of quarantine. The WHO is likely to declare an interest at an international level (Uhran et al, 2019).

These experts on infectious diseases are sure to raise the issue of a lifelong symptomless carrier / superspeader of an unknown pathogen. The best known example, which any infectious disease expert will know about, is Typhoid Mary, who had to be isolated through to her death because she was a spreader of typhoid, but never showed any symptoms of typhoid herself (Korr, 2020). Quarantine periods are based on the body clearing the pathogen. But how do we know our bodies would clear a Martian pathogen ever?

No quarantine period can be long enough for a lifelong symptomless carrier, or indeed a life long or long term carrier with symptoms as with immunocompromised COVID patients (Abbasi, 2021), or HIV patients. This may be a possibility for a novel pathogen based on a novel biochemistry, that our immune system doesn't recognize as life, and so, can't clear from the body.

Also, in a similar issue, quarantine can't protect Earth from mirror life or indeed fungal diseases, or other pathogens which are harmful to other plants or Earth's biosphere but don't harm humans. Two zinnia plants on the ISS died of a fungal disease fusarium oxysporum (NASA, 2016) probably brought there on an astronaut's microbiome (Urbaniak et al, 2018).

Figure 43: Mold growing on a Zinnia plant in the ISS. The mold fusarium oxysporum likely got to the ISS in the microbiome of an astronaut (Urbaniak et al, 2018). Two of the four infected plants died (NASA, 2016).

Human quarantine wouldn't be a reliable method to keep a pathogen of terrestrial plants out of the terrestrial biosphere, at least until we know if there is life on Mars and what its capabilities are.

This fungal disease disease fusarium oxysporum is also an occasional opportunistic pathogen of humans (Urbaniak al, 2019). but didn't harm the healthy astronaut who brought it to the ISS. In the same way a fungal pathogen from Mars could be harmless to young healthy technicians but be devastating to people with various health conditions or immunocompromised.

The ISS has a preflight "Health stabilization program" which uses vaccination and a 14 day quarantine to help prevent upper respiratory infection and gastroenteritis (NASA, n.d.). But this of course is not able to keep out crop pathogens or human pathogens that are rarely harmful to healthy people.

As another example, Aspergillus Flavus and Aspergillus niger are amongst the most common fungal spores in the HEPA filters on the ISS and found at relatively high concentrations compared to US homes (Vesper et al, 2008). Human quarantine couldn't keep these out either as they can lead to harmless colonization of healthy humans but harmful pathogens of immunocompromised. See (above):

- NEW: NASA's sample return biological safety report mentions an opportunistic fungal pathogen, Candidiasis adapted to humans – but misses the counter-example of Aspergillus, not adapted to us – an estimated 200,000 life-threatening cases of invasive aspergillosis a year – mortality 30% to 95% - invasive because of capabilities martian life may share such as its ability to respond quickly to rapid changes in humidity and temperature, very efficient at taking up nutrients and storing them, and able to tolerate low oxygen levels in the lungs

As we mentioned, the blue green algae chroococcidiopsis are sometimes found in the human microbiome including in the nasopharynx (upper part of the throat behind the nose) (Ventero et al, 2022), and in human milk from Gambia (Lackey et al, 2019) and martian life might be pre-adapted to warmer temperatures. See

- Mars surface temperatures can reach 35°C in the shade in summer – and possibility that some species of Martian surface life are pre-adapted to hydrothermal conditions in geologically recent Mars – and may be present in small numbers in surface biofilms

So it's unlikely a mirror life chroococcidiopsis analogue could be reliably kept out of Earth's biosphere by human quarantine of technicians or astronauts, as needed for the scenario of mirror life on Mars.

- NEW: Example worst case scenario of a mirror life chroococcidiopsis analogue from Mars which gradually converts organics in ecosystems into indigestible mirror organics

Then there are the fungal pathogens of microbes. If a fungus will only harm photobionts, and microbial life, there is likely to be no way to keep it out with quarantine. See above

- NEW: Microbes from Mars could have pathogens that can infect terrestrial microbes – example of fungal pathogens of phytoplankton and cyanobacteria – cyanobacteria depend on specific antifungal adaptations to protect against fungi in the chytrid phylum, so may have no adaptations to a novel fungal phylum from Mars

Then, there is the vexing issue of serious medical incidents. If one of the Apollo astronauts became seriously ill and needed urgent treatment that wasn't available within the quarantine facility, NASA's stated plan was to immediately take them out of quarantine and to a hospital, as an authorized breach of quarantine (Meltzer, 2012:229).

> *If a serious medical emergency had occurred that was beyond the capabilities of CRA [Crew Reception Area] equipment, NASA would have rushed the afflicted person from LRL to a hospital, regardless of quarantine requirements.*
>
> *Although such a situation did not occur, this was another example of NASA's policy to prioritize the lives of its people above back contamination requirements.*

Suppose a technician in quarantine had a sudden medical condition such as a heart attack, unrelated to sample handling, that required urgent expert attention in a hospital. Suppose even that a technicians life-threatening condition is suspected to be caused by the sample. It would be hard to legally or ethically justify keeping the technician isolated, unless there was clear proof that removing them from quarantine constituted an overriding significant danger to others.

Carl Sagan and Cyrus Levinthal raised the issue that "in emergencies the safety of the crew transcends the quarantine requirement." in a meeting of the Planetary Biology Subcommittee in 1967 (Mangus, 2004:34).

Even if something turned up that was pathogenic, deadly, and contagious, and we knew it was transmitting rapidly between technicians in quarantine, it would still be hard to know what to do, as Erb said interviewed in 1999 (Butler, 1999)

> ***Erb:** You know, you fantasize about some of these scenarios, too. I thought supposing we do find something really deadly. What is the action? And it went through our minds that, well, you might, in fact, have to sacrifice everybody in the laboratory and bulldoze it under 100 feet of dirt.*

Which of course is not ethically acceptable. But what do you do?

The ethical conundrum here is that an unknown and probably low probability of severe risk to Earth's environment or to other humans or organisms is difficult to balance against the immediate certainty that without treatment an individual may die. The legal issue is one of human rights; could a technician legally be kept in quarantine in this situation, even if it is transmitting person to person in the laboratory, when it is known that removing them could save their life?

So any quarantine measures would be likely to adopt a similar policy to the one for the Apollo astronauts, that the quarantine would be breached if a technician's life was at stake, while taking precautions to try to limit further spread. It would be ethically difficult to argue for any other policy. However if this is the policy, it significantly reduces the capability of the quarantine procedures to protect Earth.

So, quarantine can't be used to protect Earth from putative martian organisms with unknown capabilities.

The planetary protection literature rarely mentions these issues with quarantine. As we saw, Sagan raised the issue with using a quarantine period to protect Earth's biosphere from an unknown biohazard as early as 1973 (Sagan, 1973:130). It isn't discussed in either the 2009 (SSB, 2009) or the 2012 (Ammann et al, 2012) sample return studies. It's had little or no attention in the planetary protection literature since Sagan's remark in 1973. All I have found so far is that interview with Erb where he briefly mentions that these questions will need to be considered again (Butler, 1999)

> ***Erb:*** *It'll be interesting to go through this again as we tackle Mars samples return, because in three or four years we'll be coming back with samples from Mars, and we'll have to think through all the same decisions, but now with, I think, a much greater likelihood of life forms from Mars*.

Quarantine **may** be useful in the future for **some scenarios**. Even then it couldn't be used in all scenarios, as the example of mirror life shows. It would also be either challenging or impossible to use quarantine to keep out fungal diseases of humans, higher life or microbes, depending on the disease.

So, at least until we know the scenario we face on Mars, we have to return bonus samples too, to an unmanned satellite. This is also a far lower cost solution than a space station staffed by human technicians.

An unmanned satellite also lets us study martian life without the forward contamination in a human occupied space station, as ultramicrobacteria can get through HEPA filters both ways.

NEW: These clean samples will be studied above geostationary orbit in Mars simulation conditions with a Martian gravity centrifuge – they are not intended for safety testing - and humans never go near the satellite

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These clean samples could be studied above geostationary orbit, in Mars simulation conditions with a centrifuge for artificial martian gravity – which would make it unique as a facility, as we can't simulate martian gravity accurately on Earth.

These samples would be returned to a small robotic satellite and NOT a human occupied space station like the ISS.

This orbital lab is still not for "safety testing". Suppose we successfully cultivate life from the sample, and detect familiar life, a novel strain of a familiar microbe such as chroococcidiopsis. Even then, this could bring new capabilities to Earth acquired from billions of years of evolution in Martian conditions. For instance it is reasonably likely to be better adapted to cold, to rapid fluctuations in temperature, to ionizing radiation, to desiccation and amongst its many metabolic pathways, it may have the ability to metabolize mirror organics because much of the organics it encounters on Mars is achiral. These changes may be harmless but amongst them all there may be some adaptation that causes problems when returned to Earth.

Also if we find familiar life, it would be hard to prove that there is no unfamiliar life in the sample as we saw in the example of a mirror-life nanoboes. As with terrestrial life much of it could be uncultivable in laboratory conditions yet do fine in nutrient poor and more challenging situations outside the orbital lab. Also if there was a mix of some life with similar biology to terrestrial life and some with unfamiliar biology we might detect the familiar biology first.

Also as we saw, even if we test 10,000 grains of dust we don't know that the 10,001th grain is safe to return. See above:

- NEW: Too early for any form of safety testing at the level of assurance needed for potential large scale harm – even for samples returned in sterile containers with no forward contamination – after destructively testing 10,000 grains of dust the 10,001th grain could have a viable microbe in it

These dust and dirt samples are just the first step in Sagan's "exhaustive program of unmanned biological exploration of Mars"– and a first try out for supersensitive instruments astrobiologists developed to find life in situ – next step is to send some of the same instruments to Mars so we know what to return

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The dust and dirt samples are just a start. There is likely no shortcut alternative to Sagan's (Sagan, 1973):

> "*exhaustive program of unmanned biological exploration of Mars".*

This orbiting astrobiology lab is the equivalent of one geostationary satellite far above GEO. Humans can study the dust, dirt and atmosphere as they would on Mars using exquisitely sensitive in situ instruments designed for end to end sample preparation to analysis – these already exist such as the Life Marker Chip LDChip300 (antibodies) almost sent on Exomars but descoped (Parro et al, 2011) target mass of less than 1 kg (ESA, n.d.), the gene sequencer SETG (Mojarro et al, 2016)., astrobionibbler able to detect a single amino acid in a gram

(Schirber, 2013) (Noell et al, 2016), a chiral version of the Viking labelled release experiment (Anbar et al, 2012), and many others.

Figure 44: Graphic shows: GEOS17 (Clark,, 2018) just to have an image of a geostationary satellite, not that it would be a $2.5 billion dollar satellite.SETG from (Mojarro et al, 2016) Astrobionibbler from (Elleman, 2014) ISS centrifugal motor for plant experiments, dialable to any level from microgravity to 2 g (NASA, n.d.)

Most of the studies astrobiologists want to do with their samples can be done using instruments in situ on Mars and so could also be done in the orbiting satellite. Only the ones in bold boxes in this graphic currently need to be done on Earth (Carrier et al, 2020:8022)

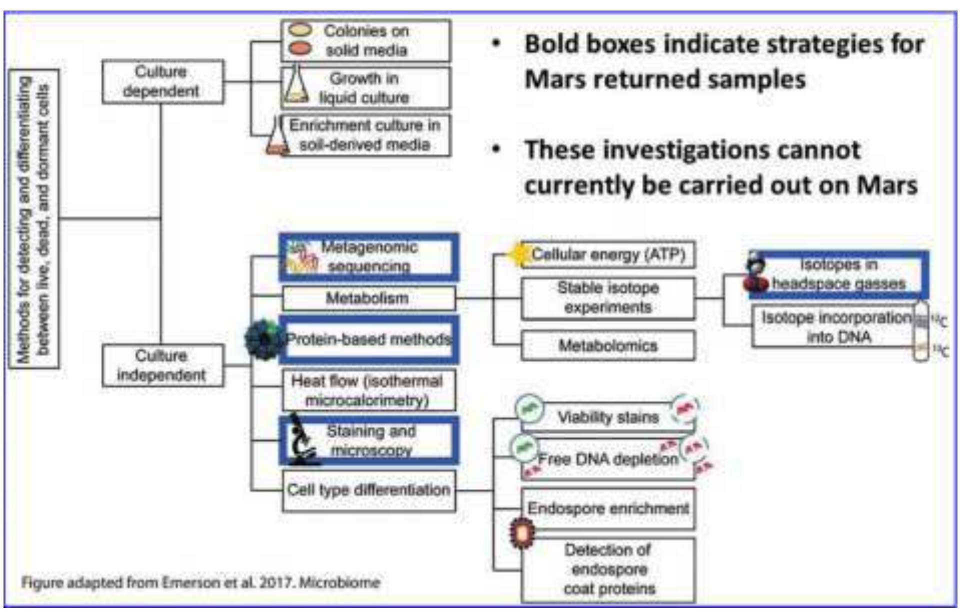

Figure 45: from (Carrier et al, 2020: fig 10) adapted by Mackelprang from (Emerson et al, 2017: fig 1)

Returning a sample from Earth gives the advantage that we can use many more instruments to study the sample, but we get a similar advantage if we return samples to a satellite above GEO. We can still send many more instruments to above GEO than we could send to Mars, and we also have a faster turn around, we can build new instruments and send them to the satellite above GEO based on discoveries made previously.

We need a wide range of instruments in order to have the best chance of detecting life as often multiple biosignatures are required simultaneously to detect life with confidence (Westall et al, 2015).

Take the example of the chiral labelled release. Although this is an excellent experiment when it is combined with others, on its own it can generate a false positive or a false negative.

- False positive – the chiral labelled release could detect complex prebiotic chemistry, for instance if the active agent consists of chiral organics on Mars, in a prebiotic chiral network as for the punctuated chirality hypothesis. This would of course be very interesting, but not life (Gleiser et al, 2008b).

- False negative – the active agent could be chirality indifferent if it is either
    1. A mix of life of both chiralities, see NEW: Closely related worst case scenario of a shadow biosphere of mirror life nanobes that produce indigestible mirror life biofilms on Earth

       OR
    2. Chirality indifferent life ("ambidextrous"), for instance Joyce's RNA enzyme in its D or right hand form can make copies of L RNA and its L or left handed version can copy D RNA. (Joyce, 2007) (Sczepanski, 2014) (Singer, 2014)

Astrobiologists have already designed many other instruments to send to Mars and such a satellite would stimulate work to develop many more light weight in situ life detectors. Here are some of the others already at various stages of development, some not so well known as the ones already listed. Many of these are from a study for a Europa lander mission which I use as a source here (Hand et al, 2017)

- A method to check for redox reactions directly by measuring the electrons and protons they liberate. This is sensitive to small numbers of microbes and has the advantage that it could detect life even if not based on carbon or any form of conventional chemistry we know of (Abrevaya et al, 2010).
- Tests for autofluoescence. Aromatic amino acids (incorporating a ring of six carbons) fluoresce when stimulated with deep UV at wavelengths less than 250 nm. Chlorophyll and some other biological organics also autofluoresce (Hand et al, 2017).

- We could also use fluorescent dyes that bond to specific macromolecules such as lipids, proteins and nucleic acids (Hand et al, 2017) ,
- We can also use this autofluorescence to directly search for the activity of swimming microbes (Hand et al, 2017).
- An off-axis holographic microscope to let the focus be adjusted after the image is taken making it easier to image individual microbes in a liquid medium (Lindensmith et al, 2016),
- Raman microspectroscopy synchronized with visible light can do a chemical analysis of the microbes directly (Hand et al, 2017),
- Superresolution optical microscopy, which can go beyond the usual optical resolution limit of 200 nm to observe nanobacteria (Hand et al, 2017)
- A miniature variable pressure electron microscope that combines imaging with in situ chemical analysis (Gaskin et al, 2012)

These are all very small instruments, miniaturized to be light enough to send to Mars, at up to a few kilograms each. However for the suggestion of a receiving satellite above GEO, we can also consider heavier instruments, with over 25 tons per payload. Probably more by the 2030s. We could consider sending much heavier instruments up there. Also, maybe some of the ones that can't yet be done in situ on Mars could be sent to above GEO by the 2030s and later may be miniaturized enough for Mars or suitable once we have a larger payload to Mars.

This approach has similar advantages for a sample to returnnto Earth without the risk of forward contamination of the samples and without the risk of backwards contamination of Earth.

This is especially useful if we find unfamiliar life. For instance if we find mirror life, and perhaps not even based on DNA we may need to use a wide range of instruments to study it. (Carrier et al, 2020:801)

> *Meanwhile, it is important to note that if the life-form were based on another biochemistry, modern techniques might be too specific. We determined that using a well-designed suite of multiple advanced detection techniques, which provide complementary information for life detection, would be especially important. The advantage of a return mission would thus be in the ability to access a multitude of more sensitive instruments and wide-ranging laboratory techniques than is possible for in situ missions. We agreed that life-detection instrument development programs should be a priority and that more research should be conducted to understand what signals of life may be universal and how to best detect those.*

We might be able to develop new techniques too. For instance, maybe we can adapt SETG to sequence an alien gene sequence. It wouldn't be feasible to send all possible gene sequencers for all possible forms of exobiology to Mars but we might with work be able to figure out how to sequence DNA with extra bases. Maybe even with mirror life?

Anything like this will be far easier with a returned sample above GEO than for a rover on Mars, but without the risk to our biosphere of returning samples to Earth's surface.

In the other direction, if we find familiar life on Mars, we shouldn't jump to the conclusion that all life on Mars is familiar without more study. It could co-exist with unfamiliar life as with the idea of the mirror nanobes shadow biosphere.

Why we can't return samples to the Moon – at least for now – because under COSPAR guidelines we need to keep the Moon free from contamination too and because above GEO is far more accessible

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The Moon may seem a better place to return the samples if we have a continuous human presence in a base on the Moon by the 2030s. Humans close by could reduce latency for teleoperation and might make it easier to add or remove equipment and supplies.

However, latency for telerobotics from Earth would remain reasonably low above GEO, and we can send multiple ton missions up there at low cost (SpaceX, n.d.).

We can't return the sample to the Moon anyway for now. COSPAR guidelines for category 5 (sample return) missions currently say that (COSPAR, 2011) (Debus, 2004)

> *"(The Moon must be protected from back contamination to retain freedom from planetary protection requirements on Earth-Moon travel)".*

So, at least for now, we need to protect the Moon as for Earth.

However the Moon may be useful for a sample receiving facility in the future, especially once we have a permanent presence there, and once we have a better understanding of what is in the samples. For instance if we have to deal with mirror life on Mars, we might return it to a telerobotic facility on the Moon which might be easier to service than a satellite above GEO, with humans nearby, and even set up habitats on the lunar surface inhabited by mirror life to study it and maybe exploit it for exports to Earth – but it would be far easier to keep 100% safe than a facility on Earth (Schrunk et al., 2007 : 146).

Figure 46: A lunar facility might be useful in the future. Hazardous Biology Facility on the Moon, telerobotically attended, surrounded by vacuum - Artist's impression, illustration by Madhu Thangavelu and Paul DiMare © (Schrunk et al., 2007 : 146).

In the future, a lunar sample return facility like this could be useful for any hazardous biology generally. It could function as an extra biohazard level above biohazard 4.

As another example, a lunar facility would be a good place to experiment with synthetic biology using XNA in place of DNA. We could use a facility on the Moon, to minimize any risk of it affecting Earth, and do the experiments using telerbotics and using robotic avatars.

Even if life did escape from the facility, e.g. after a meteorite strike, there are no dust storms, and it can only be transported through electrostatically levitating lunar dust. It would be thoroughly sterilized by UV radiation before long.

For extra safety, we could turn the region around the facility into glass by sintering the dust, and remove any dust that strays onto that glass regularly. Also there are many lunar caves. A lunar Mars Receiving Facility in an isolated lunar cave dedicated to it would be even more protected, even from quite large meteorite strikes. This could be part of our future long term. See below:

- The larger picture: how a scenario of mirror life microbes on the Mars surface could actually invigorate space exploration, as a forever unattainable human frontier - still studied and exploited by avatar robotic explorers controlled from orbit – with many other places for humans to explore in person, on the Moon, moons of Mars, asteroids, independently orbiting space settlements, aerostats above Venus clouds, Jupiter's moon Callisto, Saturn's moon Titan and beyond

But for now, a facility above GEO is easier to access and service and keeps the Moon free from contamination.

NEW: With yet more ambition we can search for past organics using a Marscopter to return pebbles excavated by a recent small impact crater to a depth of 2 meters or more – this could be both a technology demo, and a first look at organics from 3 billion years ago - though unlikely to return a clear sample of past life until we have the ability to search for it in situ on Mars

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With yet more ambition, we could search for past organics. It might be enough to sterilize a separate sample collector which is lifted by the Marscopter and used to acquire a rock sample.

We could search for a crater recently excavated to 2 meters and the marscopter could search for an exposed pebble which by the geological context was exposed from 2 meters depth, and with minimally degraded organics. This is not likely to return recognizable past life unless the marscopter has in situ multiple biosignature detection, but this could be a start towards investigating organics from 3 billion years ago in Jezero crater, and how it's been chemically altered since then, to use to help plan future in situ studies and later sample returns.

A typical small crater of 16 to 32 meters in diameter can excavate the surface of Mars to more than 2 meters. An observational study by Daubar et al found that this size of crater excavated the surface to depths of between 2 and 9 meters (based on seven newly formed craters at this size in Figure 4 of Daubar et al, 2014).

This is deep enough to find organics not significantly damaged by cosmic radiation even after three billion years. It may also be able to penetrate below the average depth of impact gardening by meteorites. The fines dominated regolith in Jezero crater is estimated as 2 to 5 meters thick (Schuyler et al, 2020).

In the size range 16 to 32 meters the crater rate is about 2.57 craters per square kilometer every ten million years (1.9 + 0.67 for first two rows in table 1 of Hartmann et al, 2017).

Perseverance can travel 200 meters per day (NASA, 2020). In 90 days, it can travel 18 km and access 2616 craters in this size range and less than 10 million years old. In actuality it averages a little over a tenth of that speed, with all the science stops, and has travelled 18.64 km in 764 sols (though not on a straight line).

We can calculate the probability that there is at least one crater of this size younger than a given age x from the number younger than 10 million years old as

$$100 - 100\left(\frac{10\,million - x}{10\,million}\right)^n$$

where n is the number of craters accessible to Perseverance, in this case n=2616.

This yields a near certainty of a crater at most 30,000 years old within any 18 km radius. The probability here is calculated in the range 0 to 100 as a percentage rather than the range 0 to 1 as mathematicians and scientists more usually do it, for accessibility.

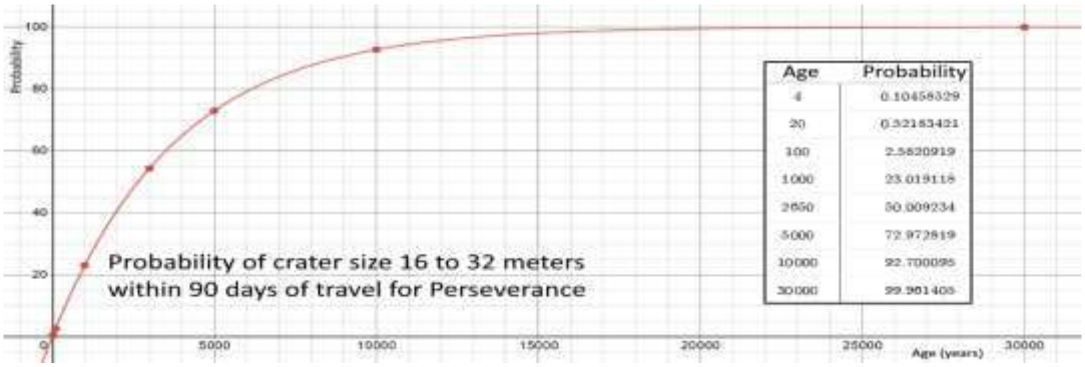

Figure 47: Probability of crater size 16 to 32 meters within 90 days of travel for Perseverance - graph available online from Desmos here

We find that in this size range there is a

- 50% chance of a crater less than 2,650 years old
- 2.6% chance of a crater less than 100 years old
- half percent chance of a crater less than 20 years old
- one chance in a thousand of a new crater forming in 4 years.

Going to older craters, there is a

- 92.7% chance of a crater less than 10,000 years old.
- 99.96% chance of a crater less than 30,000 years old.
- 99.9998% chance of a crater less than 50,000 years old
- 99.9999999996% chance of a crater less than 100,000 years old

So it would seem a near certainty that Perseverance can find a young crater in this size range less than 100,000 years old, and a decent chance of one younger than 10,000 years old which it could reach. Then it could work out an itinerary to visit it in the next 2-3 years.

We could use orbital images combined perhaps with observations from above using the Marscopter to estimate the ages of the young craters.

At this size, we can only find a newly formed crater with before and after images if we are exceptionally lucky but the chance goes up or smaller craters and longer time intervals.

Using a similar calculation and for that same 18 km maximum travel distance I found a

- 97% chance of a new crater at least 2 meters wide in the next decade
- a roughly 50 50 chance of a new crater at least 4-8 meters within a decade - which can excavate to half a meter.

For more details for this and some of the other suggestions here see my draft for a future paper (Walker, 2022b).

As we saw above, it's unlikely that we find past life without the ability to search for it in situ.

- We can't expect samples returned from Mars at this stage to answer central questions in astrobiology even with bonus samples except with extraordinary luck – astrobiologists emphasize that we need to search in situ first – our aim instead is to find a way to turn this into a far more interesting first step for astrobiology

We'd be very lucky to find a newly formed crater that excavated a sample of recognizable life with all the alteration processes of the last 3 billion years. However it would be easier to return a sample of past organics likely chemically altered since then which would give a much clearer picture of the conditions in Jezero crater and the processes that have acted on the deposits since then and help guide future in situ searches and prepare for them.

For future missions we could target newly formed craters using before and after images as part of the site selection process. This could be useful even for a mission that can drill as it gives easy access to the subsurface without drilling.

NEW: With even more ambition we can use new technology developed for oil wells, ovens, electric cars and Venus landers to make a 100% sterile marscopter, by specifying components able to resist heating at 300 °C for several hours – it can then be flown to sensitive locations with no risk of forward contamination and can retrieve samples with no risk of backwards contamination – the same technology could be used to develop a 100% sterile complete rover for in situ life searches on Mars

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We don't sterilize our spacecraft as much as for the Viking lander, relying on the harsh conditions there to take the place of the hours the Viking landers spent in ovens. This saved about $100 million per mission (Chang, 2015).

That made sense in the late 20th century, but since then the conditions aren't as harsh as were thought. Still, our rovers likely shed only a few occasional spores and viable microbes which would then find it hard to survive on Mars given the harsh conditions there. Hopefully the chance per mission still very low. But the more missions we have the more the risk of forward contamination.

The Viking rovers weren't 100% sterile. They had an estimated 30 cultivable spores per spacecraft (Barengoltz, 2005:3). There would be between hundreds and thousands of viable organism per spore. One modern estimate for the clean rooms used for Perseverance using various techniques to test directly for viable microbes found a ratio between hundreds for some methods of estimation, up to 12,091, which they say is probably an overestimate. That would make it between thousands and tens of thousands of viable microbes per spacecraft, though that would be reduced further by the journey and the conditions on Mars (Hendrickson et al., 2017).

We will see that our technology has advanced so much since Viking it's now feasible to have a 100% sterile lander or rover on Mars.

We could start with a much easier project, a 100% sterile marscopter. We can do that by specifying components that are not affected by preheating to a few hours at 300 °C, and heat it during the flight to Mars, or else sterilize it on Earth and enclose it in a bag that is opened on Mars.

The sterilized copter could be flown to nearby RSLs or other sensitive locations with no risk of forward contamination, and it could be used to return contamination free rock samples such as pebbles or rock fragments from crater rims or crater floors. Our technology has advanced since the Viking landers which were baked for 112 °C for 30 hours, enough for a million-fold reduction of the originally low population (Beauchamp, 2012).

We now have high temperature microprocessors and memory devices for oil wells, aviation and electric cars. Their heat resilience means they don't have to be cooled, and they can be placed closer to heat sources such as engines. This helps with cost, weight and most important, reliability (Watson et al, 2012).

- At 250 °C the half life of the RNA bases under hydrolysis is between 1 and 35 minutes, and at 350 °C the half-lives are between 2 and 15 seconds (Levy et al, 1998). Eight of the 20 amino acids have been proven to not just evaporate or liquify but to decompose at temperatures between 185°C for Q (Glutamine) to 280°C for H (Histamine) (Weiss et al, 2018) There might be other more recalcitrant organics remaining but it seems that this should be sufficient to eliminate both forwards and backwards contamination.

The Venus lander teams sketched out a design for a largely mechanical rover with minimal onboard electronics capable of functioning at Venus surface temperatures of around 500 °C, as part of Venus Rover studies. The researchers proposed that the same approach could be useful for planetary protection (Sauder et al, 2017, section 6.2).

Since then, temperature technology continued to improve. NASA's HOTTECH program has developed sensors, imagers, solar arrays, batteries, electric motors, actuators, and other technologies that work even at 500 C for at least 60 days for a Venus surface lander (NASA, n.d.). The Long-lived in-Situ solar system explorer (LLISSE) is a design for a complete in situ Venus surface probe that can meet this specification with no active cooling (Kremic et al, 2021).

By using some of that technology plus commercial off the shelf components we could achieve specifications for a Marscopter that is essentially the same machine, except that it can be heated to 300 °C for a few hours, finally perhaps cleaned with carbon dioxide snow – and shipped to Mars in a container only opened on the surface

The marscopter might be a good starting point for a 100% sterile probe, as it is small, doesn't have so many components as a rover, and could potentially travel far enough to reach sensitive sites near a rover which can't go up to them itself. For instance if it had a 100% sterile Marscopter, Curiosity could us it to inspect the potential RSLs close up. It wouldn't take much heat at 300 °C to achieve a 100% sterile marscopter. It could then be used to collect contamination free samples for return for analysis in the automated lab above GEO.

This could be the first of many 100% sterile rovers we could use in the future to explore sensitive areas of Mars. The 100% sterile Marscopter would incur one off cost for the research effort, but after that the extra costs of using those components, or sterilizing it or packing it in a suitable container to remain sterile to Mars is not likely to be a significant % of the total mission cost per copter for future missions, especially in proportion to the science value of contamination free exploration of special regions and vulnerable microhabitats. As we develop experience we can use similar methods for more and more complex 100% sterile rovers, landers, aerobots etc on Mars.

NEW: The satellite above GEO could include a Mars simulation chamber, similar to BIOMEX but much greater fidelity, simulating Mars gravity, variation of temperature, pressure and humidity between day and night, seasons, ionizing radiation, UV levels for dust storms etc

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First, to prevent forward contamination with terrestrial life to eliminate the risk of false positives, the satellite would need to be presterilized of all terrestrial life, perhaps by heating to 300 C and CO_2 snow, similarly to the Marscopter. Then as for Viking it would be covered in a shroud until it reaches space. This is necessary for the best science anyway.

Reagents could be sterilized using ionizing radiation or whatever method is appropriate to the reagent and then kept sterile in a separate container. The result would be a clean facility at levels hard to achieve on Earth.

Then the satellite could become a basis for a far more sophisticated Mars simulation chamber than BIOMEX. It could have adjustable filters and blinds simulating day night cycles on Mars, and even seasonal cycles with UV levels as for Mars by simply filtering down the sunlight to half terrestrial intensity and then filtering out more of it to simulate the effect of the Martina atmosphere. Then more filters could simulate the shading and UV shading in dust storms.

The simulation chamber could be shielded from cosmic radiation and solar storms to a level needed to approximate the ionizing radiation levels on the surface of Mars. Water vapour could be added / removed to help approximate the seasonal cycles on Mars and the day night pressure and humidity cycles would happen to an extent automatically by the warming and cooling. Perhaps even the Martian frosts could be simulated and the pressure / temperature gradients over the near Martian surface?

In this way it could become a facility also for studies of terrestrial analogues similarly to BIOMEX but dedicated to Mars simulation chamber studies 24/7. The simulated Mars gravity, the natural cosmic and solar ionizing radiation and the natural sunlight would make it far more straightforward to simulate Mars surface conditions to far greater fidelity than we can ever do on Earth.

In this way we can study the returned samples including any martian salts, dirt and dust in close to the natural conditions on Mars. We can also use those to construct more accurate regolith analogues and test terrestrial life in the regolith analogues in separate chambers again in very close to Mars surface conditions.

NEW: The satellite above GEO could later expand to a receiving station for samples throughout the solar system including Ceres, and eventually Europa and Enceladus

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The new satellite would also be an investment for the future as it could be expanded to a receiving station for samples from everywhere in the solar system including the largest asteroid in the asteroid belt, Ceres, and Saturn's moon Enceladus, both of which have potential for life, Enceladus could have life in its subsurface ocean, with plumes of water ejected into space (Neveu et al, 2020). Ceres could have life in a subsurface mud ocean because of evidence that it may still have internal heating and cryovolcanism (like a volcano but with liquid water instead of lava) and because of evidence of hydrated salt, hydrohalite ($NaCl \cdot 2H_2O$) which must have been exposed recently (Castillo-Rogez, 2022)..

NASA may return samples from Ceres in the late 2030s with the mission already recommended in the Decadal review. This would return samples from the ice deposits in Occator crater which may also be one of the places where salty water from the subsurface muddy ocean is exposed to space (Castillo-Rogez, 2022). (Carter, 2022) (Castillo-Rogez, 2022).

Then perhaps further in the future, there are proposals to return samples from Enceladus captured by flying through the plumes. The return to Earth risks the samples getting further altered through reentry heating, landing shock and heating, and forward contamination from terrestrial life (Neveu et al, 2020).

Figure 48: (Neveu et al, 2020: Figure 4).

Neveu et al comment (Neveu et al, 2020):

> *Organisms in Enceladus' ocean would be highly unlikely to survive ejection to space, sample capture, exposure to radiation during the back cruise, Earth reentry, and/or any exposure to the relatively oxidizing conditions of Earth's surface*

Return to a sample above GEO eliminates two of those four challenges, the Earth reentry, and the oxidizing conditions of Earth’s surface.

For returned samples they give four advantages over study in situ in orbit around Enceladus.

1. More modern instruments (spacecraft can only have instruments available at the time of launch and usually selected long before launch)
   - Return to above GEO also fulfils this

2. Instruments that cannot be miniaturized

    - Return to above GEO partially fulfils this – much larger instruments can be sent to above GEO

3. Complex wet chemistry protocols or sample preparation steps
    - May be some options for return to above GEO with artificial gravity and low latency telepresence
4. A much more diverse suite of techniques than could be accommodated on any spacecraft.
    - Return to above GEO also fulfils this.

The satellite above GEO meets two of those four challenges and partially fulfils the remaining two.

We can then apply the remaining techniques with pre-sterilized samples returned to Earth.

A Europa sample return might not need planetary protection since any surface ice samples would be sterilized already by the high levels of ionizing radiation.

However, with Europa, there is some evidence suggests the chaos terrain in Thera Macula may be in the process of forming over a rising layer of liquid water heated from below (rather similar to the way that magma plumes form on Earth) (Schmidt et al, 2011). This liquid water would be shielded from thermal imaging by an insulating layer a few centimeters thick (Abramov et al, 2013).

Europa may also have water plumes as for Enceladus (Lesage et al, 2022) and if so, these may bring water from the near surface to space, and may have viable life in them as for Enceladus. NASA's Europa Clipper will help resolve this question when it gets there in 2030 (NASA, 2022).

If Europa does have near surface liquid water, it could potentially have indigenous near surface life, as it would be partially shielded by the ice from the very high levels of surface ionizing radiation (NASA, 2011).

If so, we may also need to protect Earth from samples from Europa's near subsurface in the near future. They could be handled in similar ways to Ceres and Enceladus samples.

It would be easy to add simulation chambers that can be adjusted to any gravity and any temperature and light intensity. These could then handle samples returned from anywhere in the solar system.

NEW: NASA have an opportunity to set a precedent for other space agencies and future NASA missions to keep Earth 100% safe – and if we find life on Mars that can never be returned safely it may stimulate rather than discourage space exploration and settlement

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NASA have an opportunity to set a precedent for other space agencies and themselves to keep Earth safe in the future. Other countries are likely to follow its example, or indeed, collaborate in a multi-national astrobiology sample handling and pre-processing lab above GEO. This could be done in a similar spirit to the ISS but far lower cost, with nations adding extra modules, and equipment to examine the samples that can be used collaboratively.

If we do find life on Mars that can never be returned safely, this may stimulate rather than discourage vigorous space exploration and settlement. The first astronauts to Mars might study the surface remotely in a spectacular orbit, a sun synchronous Molniya orbit as proposed by the Mars HERRO study. This orbit is tilted at 117 degrees and it is easy to get to as it needs less delta v than a landing on Earth's moon, and is similar to the minimal delta v Mars capture orbit (Oleson et al, 2013) (Valinia, 2012)

In this orbit astronauts fly near to both poles twice a day and skim in close over the equatorial regions, over different parts of Mars on the opposite sides of the planet twice a day. The maximum latency is 147 ms or less than a sixth of a second which means they can control rovers anywhere on Mars in close to real time throughout the orbit, and then they can operate experiments or do anything that needs fine control when close to Mars in each orbit.

This is a video I did to simulate the orbit for the HERRO study. It uses a futuristic spacecraft just because that was an easy way to make the video in the simulator I used. It's speeded up 100 times.

Video: One Orbit Flyby, Time 100x: Mars Molniya Orbit Telerobotic Exploration in HERRO Mission

Early astronaut explorers would likely use two spacecraft joined via tethers for artificial gravity to stay healthy, simulating mars gravity perhaps, and then operate surface marscopters, rovers and other surface assets, similarly to avatars in a computer game.

This is what it might look like from inside the spacecraft

Composite of photo from the Cupola of the ISS (Coleman, C, 2011) and Hubble photo of Mars (Hubble, 2003)

It would be similar to exploring the Venus surface, or the Jupiter cloud decks or other parts of the solar system where humans can’t go safely.

Main image: NASA, 2012 “*Safely tucked inside orbiting habitat, space explorers use telepresence to operate machinery on Mars, even lobbing a sample of the Red Planet to the outpost for detailed study."*

Inset image of a tele-operated Centaur as an insert. Carter Emmart / NASA Ames research center (Mann, 2012)

In a scenario where Mars has mirror life or other life that can never be returned to Earth, settlers in orbital settlements or on the Martian moons would first use pre-sterilized rovers but as the settlement develops, they would later make rovers in surface factories controlled as in the game of civilization. These would be free of any terrestrial life already since they were made on Mars.

The Venus surface rover technology now gives us as a civilization the option to continue exploration in the forwards direction with 100% protection of life on other planets from terrestrial contamination

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In the forwards direction, originally planetary protection was based on the idea that life on Mars has to survive long enough to satisfy human curiosity. The aim was to conduct a reasonable number of biological experiments before it becomes impossible to study a contamination free Mars. As Sagan et al put it, (Sagan et al, 1967).

> ***"The desirability of performing a large number of biological experiments on the Martian surface before there is a sizeable probability of contamination"***.

In their discussion of issues with forwards contamination of Europa, Greenberg and Tuft looked at the opposite extreme, what they called the "Prime directive" by analogy with Star Trek, that we should make sure there is no risk of forward contamination, and wrote (Greenberg et al, 2001).

> *"The problem with this principle is that, if rigorously applied, it would likely bring exploration of some of the most interesting moons and planets to a halt."*

However since then as we saw, we've developed the technical capability to make 100% sterile robotic explorers. This means we now have the option to explore moons and planets of the solar system with no risk of forward contamination as we saw above

- NEW: With even more ambition we can make a 100% sterile marscopter by specifying components able to resist heating at 300 °C for several hours – it can then be flown to sensitive locations with no risk of forward contamination and retrieve samples with no risk of backwards contamination

The marscopter might be a good starting point as it is small, doesn't have so many components as a rover, and is ideal for targeting sensitive sites near a rover. It wouldn't take much heat at 300 °C to achieve a 100% sterile marscopter. We can go on to develop 100% sterile cave bots, borrowing moles, balloons, miniature planes, probes, and build on those to achieve 100% sterile complete rovers.

This is now a decision for us as a civilization. It is no longer a case of weighing up whether to do the science at all or to protect the solar system 100% in the forwards direction. With the Venus surface rover technology it has now become a matter of public choices, priorities, budget and planning whether we

- protect the solar system 100% from both forward and backwards contamination with virtually no impact on science, but with a little more upfront cost, or
- continue with the current policy of reducing contamination but not eliminating it, with the aim to do a significant amount of science while Mars remains reasonably contamination free.

Why we might want to protect microbial species on other planets as we protect species on this planet – intrinsic value like a work of art – perhaps an ethical right to exist as a species – commercial value like the billion dollar industry for enzymes from extremophiles – health benefits for medicine and bioactive compounds – and comparison with the now extinct Australian gastric brooding frog

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So, why might we decide to be more careful to protect microbes on Mars than we have so far, using 100% sterile marscopters, and eventually drones and rovers? If we find a second genesis on Mars it will be independently evolved from the ground up. It is highly unlikely to use exactly the same chemical structures in exactly the same ways to do the same things.

The interior of a microbe is immensely complex. These molecular visualizations by WEHI give an idea of the complexity of our genetic machinery and how it operates. The idea of including these videos is just to give an overview of how intricate it is.

Video: DNA animation (2002-2014) by Drew Berry and Etsuko Uno

In more detail, how DNA is transcribed to RNA and then made into a protein

Video: From DNA to Protein

And this is the cytoskeleton which is what gives the cell its support for movement and also is used as a kind of trackway for internal processes.

Video: [Cell Organelles 2 Cytoskeleton]()

Those, and many other intricate processes are going on right now in every cell of every terrestrial organism, and the processes are almost identical in them all using the same or almost identical chemical structures.

Even if martian life is based on DNA and RNA, it surely won't be identical in all these intricate details unless it is related.

Indeed, it may be very different in how it copies DNA, how it translates it to RNA, how it translates RNA to proteins (if it uses proteins), how activates it or inactivates DNA, how it corrects for errors, and so on. The cell cytoskeleton may be different, how it moves, how it maintains its structure. There may be many other differences.

To take one example, we mentioned before one study found nearly 4,000 biologically plausible amino acids Earth life could have used. See:

- NEW: Chroococcidiopsis indica produces an accidental neurotoxin, BMAA, which resembles serine and by replacing it, can cause protein misfolding – leading to the possibility that novel amino acids from a novel exobiology could also cause protein misfolding

Does Martian life use the same amino acids as terrestrial life? Does it use the same translation table to map nucleotide triples to amino acids?

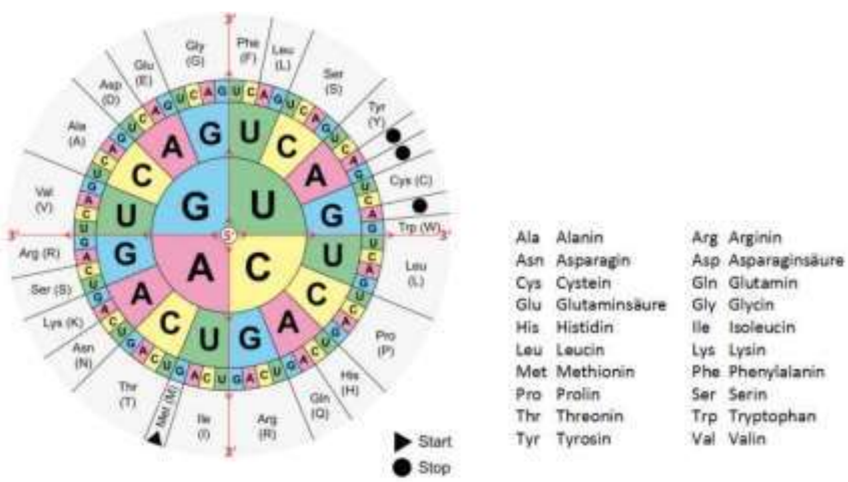

Figure 49: codon table shows how RNA triplets are translated into amino acids. You read it from the centre outwards, e.g. GUG, GYA, GUC and GUU all are translated into Valin (Mouagip, 2021).

This has a lot of redundancy and could in principle code up to 62 different amino acids together with a start and stop codon.

Is this particular list of twenty amino acids in some way optimal or is it a historical accident, and other life does it differently?

Might life based on a different biochemistry which originated independently from Earth life have a different codon table for the same bases and amino acids? Might it use more, or fewer amino acids? Or not use amino acids at all? Might it use different bases and amino acids?

The interior of a living cell is so complex with so many components interacting together. Suppose we discover a second genesis of life on Mars that works on a different basis, independently evolved life on Mars, even if only microbial, even if just one species that survives in one small habitat on Mars. This novel microbe may be as complex as terrestrial life, but so different that at the atomic level it is as different from terrestrial life as a coral reef is from a tropical jungle at the scale of animals, plants, corals etc. It may have been living on Mars for billions of years.

Let's look at a terrestrial example to see why we might want to protect martian life. The two Australian species of gastric-brooding frogs had a unique adaptation, the female, frog incubated its young in its stomach. Normally when we eat food this triggers production of peptins and acid to digest the food and then it triggers the stomach to empty its contents into the small intestine. But the tadpoles secreted enzymes that switched off those processes so that they could live in their mother's stomach.

At the time, scientists started studies to find out how they did this. This might have led to new treatments for human peptic ulcers which afflict 25 million people in the USA every year, but they had to stop because the frogs went extinct (Chivian et al, 2008). The frogs probably went extinct because of the imported chytrid fungus which grew on their skins and made many amphibian species extinct. There was some hope that the northern species might still exist in the wild, but an expedition in 2021 didn't find any traces of it, though it did find other rare frogs (Groves, 2021).

Video: Meet the Gastric Brooding Frog (Rothschild, 2017)

This is quite a close analogy for forward contamination. We might introduce fungi to Mars, and martian life might be defenseless against it. The chytrid phylum is very diverse and includes fungi that are parasitic on cyanobacteria and other microbes as we saw above.

- NEW: Microbes from Mars could have pathogens that can infect terrestrial microbes – example of fungal pathogens of phytoplankton and cyanobacteria – cyanobacteria have specific antifungal adaptations to the phylum that attacks them, the chytrids and may have no adaptations to a novel phylum from Mars

If martian organisms never encountered chytrids, and some compatible fungal pathogen of cyanobacteria from this phylum gets to Mars and survives there, it could make martian microbial species extinct.

We can get some idea of the potential value of extraterrestrial life from the study of extremophiles which led to enzymes that are widely used (Sarmiento et al, 2015).

- Cold adapted enzymes for detergents. These work at lower temperatures which removes the need for heating and saves energy. The global market for these enzymes is valued at $1 billion
- Enzymes and microbes that work well at lower temperatures are used in the food industry, including proving the dough at lower temperatures for bread making, to clarify fruit juices, for lactose free foods, and syrups.
- In the textile, cement, cosmetic industries (antioxidants active down to 20 C)
- Heat adapted extremophiles are used for wood pulp and paper processing, and production of glucose from starch (e.g. from maize).
- in various research techniques for experts studying DNA and RNA

They are used to reduce costs, make the processes more eco friendly, reduce CO_2 emissions, enable more efficient faster processing, etc.

The cold adapted enzymes are more active, so less of the enzyme is needed, and they can be used at lower temperatures, saving energy.

The heat adapted enzymes are active and efficient at high temperatures, extreme pH values, high concentrations of the substrate, and high pressures, resist organic solvants, easier to separate during purification and catalyze faster reactions.

Discoveries from study of a novel biochemistry could be far more radical than these enzymes, with implications for pharmaceuticals, understanding processes in medicine, agriculture, nanotechnology, and many other fields. They may form materials with novel properties too (many materials we use in everyday life are the results of biology).

These lifeforms if they exist, and any discoveries that would flow from them, are as much part of our natural heritage as human beings living in our solar system as the Gastric brooding frog and any of the other myriad species that we share our planet with. The questions that arise are similar.

Given that the public highly value species such as the gastric brooding frog it’s possible they may also place high value on conservation of microbes on Mars too.

Chris McKay has discussed an approach he calls planetary ecosynthesis. If we do find a second genesis of life on Mars, biologically different from terrestrial life – it could be that the life still lingers in isolated refugia, but is headed towards future extinction.

In this scenario, we might decide not just to preserve it but to enhance conditions for growth and make Mars more habitable for Martian life, to whatever extent we can. He suggests this could greatly benefit humans, and more than outweigh any utilitarian value from attempts to make Mars itself more habitable for terrestrial life (McKay, 2009).

> *Perhaps the most interesting and challenging case is that in which Mars has, or had, life and this life represents a distinct and second genesis.*
>
> *I would argue that if there is a second genesis of life on Mars, its enormous potential for practical benefit to humans in terms of knowledge should motivate us to preserve it and to enhance conditions for its growth. Observations of Mars show that currently there is no global biosphere on that planet and if life is present it is in isolated refugia or dormant. It is possible that life present on Mars today is at risk of extinction if we do not alter the Martian environment so as to enhance its global habitability.*
>
> *An appreciation for the potential utility and value of the restoration of a Martian biota does not depend on the assignment of intrinsic value to alternative lifeforms. The creation of a second biosphere using a second genesis of life could be of great utilitarian value for humans in terms of the knowledge derived ranging from basic biology to global ecology. And a case can be made that its' value exceeds the opportunity cost of not establishing human settlements on Mars.*

Additional reason to protect Mars from forward contamination - even one species with a different origin would make our understanding of biology multi-dimensional and greatly enhance synthetic biology – and if we lose life from Mars, even if it is only microbial – we are not likely to be able to reverse extinction even if we find perfectly preserved but dead cells

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Suppose we find microbes on Mars but make them extinct, by mistake. In this scenario, as for the gastric brooding frog, we may try to de-extinct them.

Researchers were able to make an embryo of the gastric brooding frog. The cells duplicated a number of times but then stopped. There is no way to know when or if we'll make the necessary breakthrough to grow a gastric brooding frog embryo into a tadpole and then reproduce the conditions it needed to grow to maturity in the stomach of a mother frog (Groves, 2021).

In the same way if we make native martian life extinct, there may be no way to de-extinct it, even if we have a fair bit of information and even genetic material from it. Even if we have complete but dead cell, perhaps cryopreserved in a salt crystal, most likely we can't de-extinct it. Even with terrestrial life, we can revive viable dormant cells but not dead cells.

To see how hard this is, let's look a the example of mirror life. It may seem simple. Don't we "just" need to put together all the chemicals that make up a terrestrial cell but in their mirror form?

First, it's an enormous challenge even to synthesize mirror images of proteins or enzymes or other biomolecules from scratch. The first step is to synthesize a mirror image of the ribosome, the protein factory that converts messenger RNA into proteins. Then we need to synthesize the mirror images of the proteins that add amino acids to the chains. We can then build up mirror messenger RNA somehow, and then use mirror messenger RNA together with that mirror ribosome to make mirror proteins. But it's a difficult job (Service, 2022).

Then, it's not enough to have the biochemicals. Suppose we now have the mirror RNA, mirror ribosome and mirror proteins. If we tried to make a living mirror-life cell by mixing biochemicals in a solution and filling a membrane with them, they would immediately start to react with each other as we try to build the cell. We don't know how to make a living cell from a dead cell.

The plan instead is take a new living cell and slowly transform it one step at a time, all the way from normal life to finally, hopefully, mirror life. For this to work, we need intermediate functioning cells that consist partly of mirror and partly of normal biochemicals. Remarkably the synthetic biologists think that this is possible, though it is a major challenge and may take a decade or more to complete (Bohannon, 2010) (Peplow, 2016).

We can't just mix together its component chemicals, because so much depends on everything else. To make ribosomes we need mRNA which needs DNA and the translation machinery which needs enzymes made by ribosomes. Then, the ribosomes themselves are enzymes that need to be made using mRNA and other ribosomes.

In short the ribosomes need ribosomes to make themselves. The mRNA needs mRNA and ribosomes to make the enzymes to translate the DNA into mRNA, and so on. As soon as you start to build your cell, before it is complete, everything will start reacting and you are left with a mess.

Terrestrial life must have evolved originally from chemistry. However, this likely happened through a long chain of simpler living cells as ancestors that gradually got more and more complicated. We can't do that (though some researchers are trying to duplicate it). The only way we know to make a living cell is to start with another living cell.

So, in our scenario of newly extinct Martian life, maybe even extinct a year before, we won't be able to de-extinct it from non viable cells.

The most we could do is modify terrestrial life step by step until we get to something that resembles martian life, just as we may do for synthetic mirror life. But depending on what martian life is like, that might not be possible, there might not be intermediate steps from terrestrial life to martian life which are all viable living cells. If it is possible it would be a huge challenge and if we succeeded the result would be unlikely to be an exact copy of Martian life in all respects.

Even we find a new form of life with, say, 10 amino acids out of 20 amino acids different from terrestrial biology and a new codon table would greatly expand the vocabulary of life. We could make new life forms by changing / introducing any of those amino acids, and it becomes a multi-dimensional discipline, with numerous other extrapolated exobiologies that we could postulate for other planets – and perhaps attempt to synthesize in the laboratory.

Suppose we find another scenario, independently evolved life which uses exactly the same vocabulary of amino acids, and all the structures and the codon table of independently evolved life are identical to terrestrial life. That would be extraordinary and we would want to find out why. We would be eager to find out what is different if anything.

Most likely there would be many differences to study.

If the life is extinct, we could still attempt this but we could only do it by studying dead life. With no examples of living cells of the exobiology it would be far harder to know how it worked when alive, its life cycle, metabolism, how it moved, how it ate food etc. It would be very similar to trying to understand the gastric brooding frog from museum specimens. De-extincting it would be like the attempt to de-extinct the gastric brooding frog but likely to be far harder.

In another scenario, we find martian life, and don't make it extinct immediately but it can no longer compete with terrestrial life on Mars. In this case, it might still be hard to get it to grow, because of the issues with cultivating microbes in the laboratory even for terrestrial life. Depending on how widespread native Martian life is on Mars and how rapidly terrestrial life is overcoming it, we might find ourselves in a race against time to try to rescue species of Martian microbial life before they go extinct.

The general public need to know how major the difference is between a discovery of present day life on Mars and a discovery of newly extinct microbial life on Mars that we ourselves made extinct. The public need this information to make properly informed policy decisions, fully aware of the potential consequences of our decisions.

New version of the precautionary principle for potential super positive assets to help clarify decision making

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The precautionary principle was developed to help deal with new unprecedented challenges faced by humans. We looked at some variants for backwards contamination. See above:

- NEW: Society places very high value on the environment and given the potential for large scale effects, we might require Earth is kept 100% safe for this mission – i.e. use the prohibitory precautionary principle

If we contaminate Mars, Europa or Enceladus with Earth life, there is no risk of harm to human health or the environment of Earth. It's more like missing out on an unexpected gift of treasure. We risk losing a potential future benefit of great value, something we never knew we had, such as discovery of living microbes from a second genesis of life in our solar system.

The general case is as a civilization we may risk losing something of tremendous value by some course of action, but it's something we don't have available to us yet and we can't prove this treasure exists.

> **Proposed definition:** a "super positive asset" is an asset which if it exists is expected to have vast positive transformative effects on us, our children and all future generations and civilizations.
>
> For example, some alternative form of life or early life on Mars could revolutionize biology, could potentially benefit medicine, agriculture, and indeed anything that we do that uses products of life, also nanotechnology. It could potentially, in the best case scenario be a hugely positive transformative discovery.

There might be future discoveries waiting on Mars such as XNA life (life based on a different informational polymer from DNA) or insights into the early stages of prebiotic synthesis between life and non life. They might be discoveries future generations will only be able to make by travelling interstellar distances (and maybe not even then if there is something special about our solar system for evolution of life).

Let's use the text of the Wingspread consensus statement on the precautionary principle, which seems a good starting point for developing a positive variant of it (Raffensperger, 1998)

Let's replace "threats of harm to human health or the environment" by "super positive asset" in the precautionary principle

> When an activity ~~raises threats of harm to human health or the environment~~,

To:

> When an activity [**impacts on a potential super positive asset that may be of overwhelming positive and transformative value for humanity**]

We can rephrase the rest of it accordingly in a positive way. We get something like this:

> "When an activity **impacts on a potential super positive asset that may be of overwhelming positive and transformative value for humanity**, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically.
>
> "In this context the proponent of an activity, rather than the public, should bear the burden of proof.
>
> "The process of applying the precautionary principle for potential **superpositive assets** must be open, informed and democratic and must include potentially affected parties. It must also involve an examination of the full range of alternatives, including no action."

As with the precautionary principle, the burden of proof is on the proponents of the activity to show that we don't need to take precautionary measures, that we don't risk losing a superpositive asset.

Until we can do a biological survey of Mars, there is no way to prove Earth microbes could make Mars life extinct, or to prove they will cause no harm. Also we have no way to decide how much of a benefit such a discovery can make to scientific knowledge, commerce, human health etc.

This would be a motive to do such a survey to decide one way or another if we do risk losing a superpositive asset on Mars.

As with the precautionary principle there can be variants, such as best technology, prohibitory and so on, depending on how much importance we assign as a society to these superpositive assets.

The main point is not so much the detailed wording of the principle. It is a recognition that we need public involvement here. We have a decision to make as a civilization about how much we value potential but not yet discovered superpositive assets, and about what level of precautions we consider are necessary to protect them.

This isn't a decision to be made by scientists. Nor is it a decision to be made by enthusiasts for a particular activity. Rather, it is a decision that concerns all of society. It also is a decision that impacts on the legacy we leave for future generations.

NEW: With care we might later be able to return even mirror life to Earth even with the prohibitory version of the precautionary principle – sketch for a potentially 100% safe lab protected by an oil sump and in a large oven for end of lifetime sterilization

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Even if we use the prohibitory version of the precautionary principle and find mirror life on Mars, it might be possible to return it to a future sample receiving laboratory on Earth with appropriate precautions. I sketch out an idea for a way this could be done in my preprint (Walker, 2022b) using a titanium sphere surrounded by a Whipple shield for containment during re-entry, and black box flight recorder technology for protection during transport.

Then the final analysis is in a telerobotic facility accessed via a sump filled with vacuum stable light oil sterilized with ionizing radiation and heated at high temperatures, and the whole thing inside a large externally maintained oven in a nuclear fallout shelter for end of life sterilization, because we can't assume that what we return will ever be proven safe for Earth's biosphere.

Figure 50: shows the LAS fully robotic floor plan for a Mars sample receiving facility placed inside an oven for end of laboratory lifetime sterilization of the facility and accessed via two airlocks and a sump for 100% containment of even mirror life nanobes.
Sketch of telerobotic facility Credit NASA / LAS (Hsu, 2009)

Photo of Cultybraggan nuclear bunker (Clark, 2009)

This is just a sketch of basic scientific ideas not an engineering proposal. There might be simpler ways to do it but this sketch may be enough to establish the possibility to return life to Earth even with the high standard of “no appreciable risk” for the prohibitory version of the precautionary principle.

However, if we have a scientific outpost on the Moon or indeed Phobos by the time we want to return samples to a larger laboratory, it may be preferrable to return it to the Moon or indeed Phobos, for telerobotic study rather than to a telerobotic facility on Earth protected by an oil sump or by whatever other method we might devise. For instance if one considers the remote possibility of a meteorite strike on the facility to be relevant, there is no risk to Earth’s biosphere if it is on the Moon but there is a risk to Earth’s biosphere (exceedingly small) if it is on Earth. See above:

- Why we can’t return samples to the Moon – at least for now – because under COSPAR guidelines we need to keep the Moon free from contamination too and because above GEO is far more accessible

NEW: We can explore and exploit Mars without humans on the surface, as part of a vigorous program of exploration and perhaps settlement throughout the solar system

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We can explore and exploit Mars without humans on the surface, settling the Martian moons and orbital space habitats, as part of vigorous exploration and perhaps settlement throughout the solar system. Humans and robots work together each doing what it does best. Torrence Johnson, Galileo Chief Scientist, put it like this in the foreword to Meltzer's "Mission to Jupiter" (Meltzer, 2007)

> ***Torrence Johnson:*** *What we call robotic exploration is in fact human exploration. The crews sitting in the control room at Jet Propulsion Laboratory as well as everyone out there who can log on to the Internet can take a look at what's going on. So, in effect, we are all standing on the bridge of Starship Enterprise*

My aim with this review is to do everything I can to help make sure voices and concerns of the public are heard. My wish is that this will encourage space agencies to do a rigorous scientific review with full public involvement. I am sure somehow, the public will get their say, though I don't yet see clearly how exactly it will happen.

Recommendations for space agencies generally – the simplest way to keep Earth safe is to sterilize any samples returned from Mars before they reach Earth – this can be done with ionizing radiation – sterilization would have virtually no effect on geology and most likely no effect on astrobiology for preliminary samples – priority to return samples free from forward contamination by terrestrial life

Next section – all sections – previous section

It's understandable that space agencies compete to be first to return a sample from Mars. The conclusion of this paper is that we don't currently have the technology to contain unsterilized samples that fulfil the size limit requirement of the ESF study in 2012. The cost of such a facility will likely be high if it can be developed. The simplest solution, which also keeps Earth 100% safe, is to sterilize samples before they reach Earth.

Space agencies shouldn't present a mission to return samples from Mars as a way to decide central questions in astrobiology. We are unlikely to find present day or past life without in situ searches. The only real possibility of finding clear evidence of life early on is if the Viking missions did detect life already in the 1970s, or if life is very common in the dirt or dust on Mars.

Any such mission should be presented as an early step in Carl Sagan’s vigorous program in unmanned exobiology. It should be presented as part of a longer term plan to search for present day and past life in situ on Mars itself. See:

- We can’t expect samples returned from Mars at this stage to answer central questions in astrobiology even with bonus samples except with extraordinary luck – astrobiologists emphasize that we need to search in situ first – our aim instead is to find a way to turn this into a far more interesting first step for astrobiology

Any space agency can return interesting microsamples from Mars early on in a relatively easy way. They can follow the example of “SCIM”, a proposed mission to use aerogel collectors to skim the Martian atmosphere and return micron sized dust particles (Leshing, 2002). The proposal is to dip into the Mars atmosphere during its dusty season, and pick up a sample of dusty air, to return to Earth.

This mission plan uses a "free return" trajectory. As soon as it leaves Earth's vicinity, it's on a trajectory to skim the Mars atmosphere and return to Earth with only minor course corrections after that (Leshin, 2002) (Savage, 2002). This is a feasible sample return option for India, Japan, the UAE, or any space agency that develops the ability to send a mission to Mars orbit or to fly past Mars.

Laurie Leshin, interviewed by Space.com, describes it like this (Tillman, 2014)

> *"Think of it as a microscopic average rock collection from Mars"*

Video: SCIM Mission to Mars narrated

A small sample like that can be sterilized during the journey back using nanoscale X-ray emitters or similar – with little effect on its science value as it is not likely to contain viable life.

The next step up is Chris McKay’s mission concept. Design the simplest lowest cost way to return a sample from Mars. No rover, just a lander like the Viking lander but with a Mars ascent stage. Just grab it and return (David, 2015).

> *"**The first thing is getting a mission that scoops up a bunch of loose dirt, puts it in a box and brings it back to Earth**."*

He motivates it by hazards for astronauts in the dirt such as the perchlorates. However the dirt is also of significant astrobiological interest as we saw.

If an agency returns a dirt sample like this, ideally it should be collected in a sterile container to prevent forward contamination, or it loses most of its astrobiological interest. It may be of interest to add a sub compartment with a window so that part of the sample is kept in conditions resembling the Martian surface, ideally simulating day / night cycles, because the active ingredient in the Viking labelled release mission was inactivated by storage in darkness.

If there are concerns that opening the sterile container would be mission critical, the mission could include a separate container with permitted forward contamination. This would return samples of geological interest but likely of little astrobiological interest:

This mission can be greatly enhanced in interest if it can also collect a compressed sample of atmosphere and of dust, and if possible a sample of salt from the Martian surface. It can also be enhanced by a few pebbles for a technical demo to return our first contamination free rocks from Mars.

See above:

- NEW: We can transform this into a much more interesting first step for astrobiology with little change in the overall budget by adding bonus samples collected in a STERILE container sent on the ESF fetch rover – the aim is to return dust, dirt, ideally salts, compressed gas from the atmosphere – and some pebbles for a technology demo of a contamination free rock sample

It is not possible to do safety testing of samples that we think may have potential for martian life, even if they are clean samples collected without forward contamination. See:

- NEW: Too early for any form of safety testing at the level of assurance needed for potential large scale harm – even for samples returned in sterile containers with no forward contamination – after destructively testing 10,000 grains of dust the 10,001th grain could have an undetected viable microbe imbedded in it

Samples that we think may have a potential for current life from Mars can be sterilized and returned directly to Earth and if sterilized carefully, for instance, using X-rays would still be of astrobiological interest, the life could still be recognized as such. We can sterilize a small sample using nanoscale X-ray emitters during the journey back. We might need to return a larger sample to a sterilizing satellite. See:

- NEW: Samples can be safely sterilized in a satellite similar to geostationary satellites, but positioned in a safe orbit tens of thousands of kilometers above GEO

We could also return samples unsterilized to a Mars simulation chamber remotely operated in a satellite above GEO as described above in:

- NEW: These clean samples will be studied above geostationary orbit in Mars simulation conditions with a Martian gravity centrifuge – they are not intended for safety testing - and humans never go near the satellite

Perhaps this sample receiving satellite above GEO could be operated internationally, similarly to the ISS but as a much smaller robotic facility rather than one staffed by humans. As it grows in complexity, it could use the same approach of docked modules, like the ISS in miniature. The modules could be operated by different countries with the use of telerobotics to move samples and other equipment from one module to another.

Value of targeting a newly formed crater on Mars as an alternative to drilling meters below the surface – with example of a crater that excavated ice boulders from the Amazonis planitia in the equatorial regions in 2022 – also value of developing a 100% sterile marscopter, rover or complete lander to search for present day life

Next section – all sections – previous section

Surface ionizing radiation would reduce past organics from three billion years ago from grams to attograms. See:

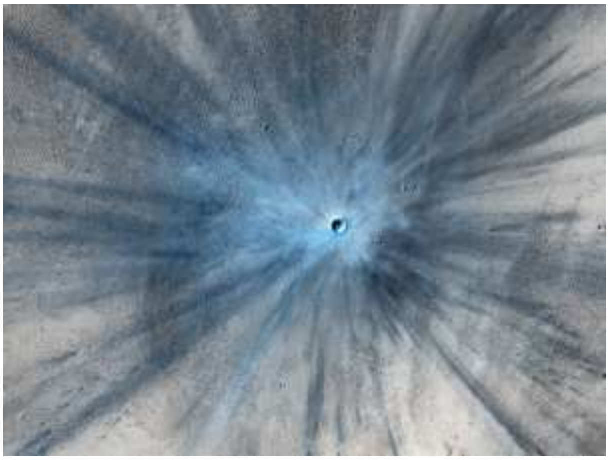
- Amino acids exposed to 3 billion years of surface radiation have been reduced from grams to attograms, a billionth of a billionth of a gram – meanwhile infall from space adds about 60 micrograms per gram but is constantly destroyed by surface processes

If we can return a sample that was buried quickly, and remained buried until just a few decades ago, there's a possibility that it's had almost no exposure to cosmic radiation or solar storms since it was deposited.

That suggests a space agency can greatly increase the science value of demo rock samples returned from Mars by targeting pebbles from recently excavated craters on Mars. A new mission has the advantage that it could target one of the hundreds of craters which formed on Mars since we started photographing it from orbit. It's still not likely to return past life except with extraordinary luck because past organics would have been altered so much since then and it might not even have deposited life there originally. It is likely to be hard to find past life and it's best to look for it in situ on Mars first. But it could be a very interesting first step to return organics from billions of years ago with little by way of ioinizing radiation so we can look at what else happened to it.

- We can't expect samples returned from Mars at this stage to answer central questions in astrobiology even with bonus samples except with extraordinary luck – astrobiologists emphasize that we need to search in situ first – our aim instead is to find a way to turn this into a far more interesting first step for astrobiology

NASA estimate that every year Mars gets over 200 new craters at 3.9 meters in diameter or larger (NASA, 2014). We can detect some of these using high resolution before and after images. This is a particularly striking example from 2014, 30 meters in diameter, surrounded by rays of debris thrown out from it (NASA, 2014). In this enhanced false colour image the blue is the result of excavating the reddish dust.



With so many new craters a year, some have likely formed in the last few decades within regions of astrobiological interest such as deltas, lake beds and the large northern hemisphere salt flats explored by the Phoenix rover.

Even a 4 meter diameter crater can excavate to a depth of half a meter or more and may be of interest especially if it impacts on a deposit of interest for the search for past life. At 8 to 16 meters the crater depth is often a meter or more and a typical crater of 16 to 32 meters will excavate the surface to more than 2 meters (Daubar et al, 2014: Fig. 4),.

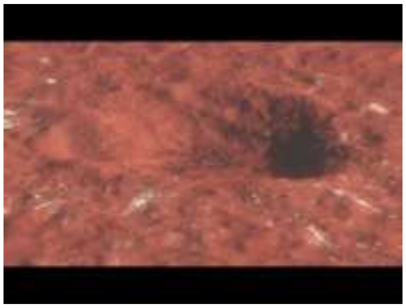
Dauber et al. measured 32 newly excavated craters on Mars with a depth of from 1.9 to 5.22 meters. See supplementary data table 1:

- Dauber et al's table of newly excavated craters on Mars has 32 craters with a measured depth of at least 1.9 meters, ranging up to 5.22 meters

Amongst the new craters found so far, there's one especially interesting target in the search for present day life on Mars. However, it likely needs a sterilized rover to prevent forwards contamination by terrestrial life.

In October 2022, the Insight Lander detected a magnitude 4 earthquake from an impact that made a crater 21 meters deep and 150 meters across and sent debris as far as 37 kilometers from the impact point.

This excavated ice boulders in the equatorial regions of Mars in Amazonis Planitia near the flanks of Olympus Mons (JPL, 2022).



Video: Flyover of Mars Impact Using HiRISE Data (Animation)

This would be of special interest for in situ study and also for a sample return as it would give an opportunity to return ice from Mars buried for millions of years and excavated recently from the subsurface. This crater threw up boulders from the subsurface of the Amazonis Planitia region which is especially interesting for recent or even maybe present day life because lava flowed there less than 24 million years ago.

(JPL, 2022)

Fuller et al highlight Amazonis Planitia as a potentially astrobiologically interesting region on the flanks of Olympus Mons. If there was life there at the time, perhaps living below the surface in subsurface groundwater at the time of the lava flows, they suggest that it's highly likely it erupted to the surface, where standing water formed briefly. Any standing water would freeze over rapidly and the ice evaporate into the thin atmosphere leaving traces of any life on the surface where we may be able to detect it still today (Fuller et al.,. 2002)

Suggestion: similarly to geobacillus on Earth, might it have also scattered resilient spores throughout the surface of Mars back then, 24 million years ago? See:

- The paradox of abundant spores of heat adapted geobacillus spores in cold places - and potential that present day Mars has similarly abundant heat adapted spores from hydrothermal systems, perhaps produced by the rootless cones, fumaroles, or ice fumaroles – some might have been active in the last few million years – some might even be active today

This is also a region with rootless cones (volcanic cones without a magma chamber below them) active even more recently, some as recently as 10 million years ago (Lanagan et al., 2001). Rootless cones may have had hydrothermal systems above 0 C for up to 1,300 years (Hamilton et al, 2010)

But it might be interesting for present day life not just past life. The ice in the ice boulders thrown up by the impact might itself be habitable for life today, especially if the ice lets light through (is optically translucent). The optically translucent but thermally insulating ice should lead to an ice melt layer 5 cms below the surface of the boulder just as we saw may happen in the polar regions of Mars. See:

- 2009, 2014: Possible future surprise discovery of large quantities of fresh water on Mars: ice lets light through and traps heat, which melts ice half a meter below the surface in Antarctica -– if Martian ice is similar, its polar regions should have meltwater in summer, ~5 cms below the surface, even with surface temperatures below -90 °C – Mars may also have miniature melt ponds around sun warmed dust grains

Life could get there from below or it might reach the region as viable spores or propagules in the dust, dirt or bouncing dust grains.

Some of the bouncing sand grains would fall on the ice boulders, and they would then be warmed by the sun. If they form miniature melt ponds they could melt their way down into the ice (called cryoconite holes), maybe to the point where the melt pond freezes over to trap liquid water similarly to the way the cryoconite holes freeze over in the McMurdo dry valleys in Antarctica, again like the suggestion for the polar regions of Mars. The sand grains might bring life with them.

For details, again see:

- 2009, 2014: Possible future surprise discovery of large quantities of fresh water on Mars: ice lets light through and traps heat, which melts ice half a meter below the surface in Antarctica -– if Martian ice is similar, its polar regions should have meltwater in summer, ~5 cms below the surface, even with surface temperatures below -90 °C – Mars may also have miniature melt ponds around sun warmed dust grains

Another possibility is that this impact might have thrown up salt that lies on top of the ice on the boulders or elsewhere, a similar scenario to the experimental simulation by Nilton Renno's team of the droplets that formed on the leg of the Phoenix lander, which we cover briefly in

- Astrobiologists have a range of views on whether current habitats for terrestrial life exist on Mars – sometimes revising their assessments after discoveries suggesting new microhabitats on Mars or new ways that life can grow in extreme conditions

If these microhabitats do exist, ice boulders thrown up by impact gardening in equatorial regions could sustain life in temporary habitats that last as long as the ice remains on the surface, before it evaporates away completely. Then the dust storms would pick up spores from the surface after the ice evaporates to start the process again.

Through to 2021 HiRISE observed 48 small impact craters or clusters in mid to high latitudes. The ice thrown up remains bright for months to years (Dundas et al., 2021: 12). The ice disappears more rapidly at lower latitudes. These impacts may also throw up pore filling ice which is impossible to detect from orbit as it darkens to the colour of the regolith in days (Dundas et al., 2021: 8).

Mars also has several exposed pole facing ice cliffs at low latitudes near equatorial regions, which also show that there is ice not far from the surface. This suggests that there is ice over much of the region that can be exposed in this way (Dundas et al., 2018) (Dundas et al., 2021: 4).

There might be enough ice exposed to sustain life that grows for as long as the ice persists, for months to years, then spreads to another crater and ice boulders in the dust with hardy long lived spores.

For the time being the only way to study these features is from orbit.

To study regions like this properly in situ, we need far more sterile rovers than the ones we have now, because of issues of forwards contamination. We could duplicate the approach used for Viking. But even that did have a small risk of forwards contamination as the two Viking spacecraft weren't sterilized 100%. They permitted 30 cultivable spores per spacecraft (Barengoltz, 2005:3) and likely several thousand or more viable non spore forming microbes (Hendrickson et al., 2017). Our technology at the time couldn't achieve 100% sterilization easily.

With present day technology we can go further and for perhaps not that different expense from the 1970s Viking missions, we now know how to make 100% sterile landers and rovers as discussed in:

- NEW: With even more ambition we can use new technology developed for oil wells, ovens, electric cars and Venus landers to make a 100% sterile marscopter, by specifying components able to resist heating at 300 °C for several hours – it can then be flown to sensitive locations with no risk of forward contamination and can retrieve samples with no risk of backwards contamination – the same technology could be used to develop a 100% sterile complete rover for in situ life searches on Mars

Once we have the design, future 100% sterile rovers would cost little more than our current contaminated rovers, as many of the components will be available already "off the shelf" as commercial components for high temperature applications. They would need to be enclosed after sterilization until after the launch as for Viking, but this needn't involve adding significant weight and the methods for enclosing them and releasing them from the enclosure after launch would be well developed.

Recommendations for NASA – need to prepare a scientifically credible EIS and restart the process – simplest approach is to sterilize samples before they are returned to Earth which retains virtually all geology and most likely has no impact on astrobiology – a valid environmental impact statement should at least consider sterilized samples as a reasonable alternative

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First, the Environmental Impact Statement needs to be scientifically credible:

- NASA's draft EIS fails NEPA requirement for a valid Environmental Impact Statement to ensure scientific integrity – with missing cites and cites that overturn the sentences they are cited to

At a minimum an independent reviewer needs to check cites are correctly summarized in the sentences they are attached to. Many of the errors found in the EIS would be spotted with this basic level of peer review.

Other errors were missed due to a limited literature review. This missed the 2015 MEPAG review of the 2014 SR-SAG2, the 2012 size limit revision in the ESF cite, and many counter examples in the planetary protection literature to the examples in the sterilizing working group report. See:

- Questions for NASA - and why NASA's main argument is invalid

One way to avoid issues of a limited literature review is to rely on authors already familiar with the planetary protection literature, and who have written extensively on the topic. The former NASA planetary protection officers John Rummel and Cassie Conley wouldn't be capable of such mistakes. John Rummel in particular is author, co-author or contributor to a significant fraction of the planetary protection literature on a Mars sample return. He is a co-author of the

2012 ESF Mars sample return study (Ammann et al, 2012:19) and is one of the individuals who gave advice or comments to the 2009 NRC study (SSB, 2009 : viii). He is principal author of the SR-SAG2 forward contamination study (Rummel et al , 2014), and one of the individuals who gave advice or comments to the MEPAG review (SSB, 2015 :xii).

A new EIS also needs to consider reasonable alternatives such as sterilizing the samples before they are returned to Earth

- NASA's draft EIS fails the NEPA's requirement to consider reasonable alternatives in detail so that reviewers may evaluate their comparative merits – as it doesn't examine the reasonable alternatives to sterilize samples in space first or to delay the mission until it can be done safely

Indeed, the simplest solution is to sterilize all samples before they are returned to Earth.

In this case all that's needed is

- A review of methods for sterilization adequate to sterilize even unfamiliar life that may be hardier even than radiodurans while preserving astrobiological interest, including using ionizing radiation or x-rays

The rock samples could simply be sterilized before they reach Earth during the return journey, or they can be returned to a satellite for sterilization in a safe orbit above GEO.

Then even with the Earth kept 100% safe through sterilization, it's important to engage with the public and get widespread agreement that the chosen method is effective and would keep Earth safe. The ESF study in 2012 recommended fora open to representatives from all countries globally because negative impacts could affect countries beyond the ones involved directly in the mission (Ammann et al, 2012:59)

This is just as important for a sterilized sample return as the public and other countries need to be in agreement that the sterilization method selected is effective.

- set up fora and other ways to engage with the public and interested experts in countries around the world to make sure that all are in agreement that the method of sterilization will keep Earth 100% safe.

We need to avoid the situation where dozens of members of the public comment on a not well publicised draft EIS saying that the mission needs to be stopped.

See:

- NASA's draft EIS fails the NEPA's requirement to use an interdisciplinary approach including the social sciences, by failing to involve the public early on, not just in the USA but through fora open to representatives from all countries globally, as recommended in sample return studies – so the public weren't given the opportunity to comment on a scientifically valid draft EIS

As a bonus NASA could add on the capabilities suggested in the previous section for space agencies to make it a far more interesting mission for astrobiology. Even a pre-sterilized sample of dirt, gas and dust collected in a clean sample container would greatly add to the interest of the mission, and especially so if the unsterilized samples can be studied as suggested in a safe orbit remotely by telerobotics like studying samples on Mars but without the latency.

If NASA wish to continue with the proposed action in the draft EIS, much more is needed.

- The general public must be given the opportunity to comment on a scientifically credible environmental impact statement which must also examine reasonable alternatives such as to keep Earth 100% safe by sterilizing the samples before the return to Earth.

The current EIS needs to be cancelled, even if the intention is to continue with the proposed action, as the general public didn't get the opportunity to comment on a valid EIS, which should make it invalid under NEPA.

Some of the main points. We need to:

- Review the level of assurance and size limit.

- Allow for end of mission sterilization of any equipment or materials that could be contaminated in case the sample contains mirror life or some other form of life that can never be released to the terrestrial environment.

- We can't rely on quarantine of technicians in case of a breach of containment, see:

  NEW: It is impossible to use quarantine to protect Earth's biosphere if humans handle the samples in orbit – the Apollo quarantine procedures never had peer review and missed the issue of a symptomless superspreader – and this can't keep out mirror life, or molds like the one that killed two plants on the ISS – keeping humans well away from the samples also avoids forward contamination for very sensitive measurements

  The facility most likely would need to use telerobotics.

- If the new proposal includes an air incinerator, we need to study the potential that Martian spores would be hardier than Aspergillus niger because of adaptations to the extreme Martian conditions or because of a different biological basis, e.g. PNA is more thermally stable than RNA

  Alternative of an air incinerator for the second HEPA filter – would need to be evaluated for containment of putative Martian life likely more resilient than standard test terrestrial spores – and for 100% containment

Also, any new valid EIS needs a proper comparison with reasonable alternatives including the ones outlined in this paper.

For a summary of the recommendations in bullet points see the end of the section above:

- Recommendations – scientific credibility can't be "fixed" e.g. with ad hoc addition of an air incinerator – but there is a simple and low cost solution – to sterilize all samples before return to Earth with virtually the same science return – and a bonus pre-sterilized sample container sent to Mars on the ESF sample fetch rover could greatly increase the mission's astrobiological interest – while keeping Earth 100% safe

Also, before a new EIS:

- We need to follow the ESF study's recommendation, to review the size limit of particle to be contained, and the level of assurance

- We also need a new Mars sample return planetary protection report to take account of the many advances in our understanding of Mars, of potential habitats on Mars, of Mars analogue terrestrial extremophiles, and of synthetic biology and the potential pathways for a second genesis of life.

- It also needs to broaden its remit and take account of some topics not previously considered such as fungal pathogens of blue-green algae, mirror life, Lederberg's two papers and the potential that we could all be immunocompromised to an extraterrstrial pathogen, the potential that we might have no defences against a new genus of fungi, the potential that our immune system might over-react to extraterrestrial biology with an allergic response or with sterile inflammation, and various other considerations that seem to be new to this review.

  It would help if the next Mars sample return study uses an interdisciplinary approach and gets help from other agencies including synthetic biologists, epidemiologists and experts from the WHO, experts on fungal pathogens of microbes, on allergic reactions and immune responses, and other areas of expertise.

The next section looks at some of the topics that a new Mars sample return planetary protection study would need to look at.

Topics that need to be covered in a future Mars sample return backwards contamination study (not likely to be a complete list)

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Based on the new material found in this review a new sample return study should consider many topics not previously considered in planetary protection studies. This is not likely to be a complete list of all the topics they need to consider. It is just a list of the main ones that turned up so far in this review.

First, based on the 2012 ESF recommendation to review the size limit and level of assurance, it needs to review:

- The size limit, including reviewing new research on the potential for non terrestrial biology such as ribocells. See:
  - ESF study said values for required level of assurance and the size limit need to be revisited periodically based on changes in scientific knowledge and risk perception

- level of assurance, with a consideration of Carl Sagan's view that we shouldn't take even a small risk with a billion lives

On that last point, it needs to:

- examine whether or not to adopt the prohibitory version of the precautionary principle, based on wishes of the public rather than priorities of space agencies
  - Carl Sagan and others warning we can't take even a small risk with a billion lives – this could be formalized into law as a requirement to use the prohibitory precautionary principle whenever there is any appreciable risk for harm unprecedented in human history

The review of the potential for extraterrestrial pathogens of humans should consider examples such as:

- Aspergillus
  - NEW: NASA's sample return biological safety report mentions an opportunistic fungal pathogen, Candidiasis adapted to humans – but misses the counter-example of Aspergillus, not adapted to us – an estimated 200,000 life-threatening cases of invasive aspergillosis a year – mortality 30% to 95% - invasive because of capabilities martian life may share such as its ability to respond quickly to rapid changes in humidity and temperature, very efficient at taking up nutrients and storing them, and able to tolerate low oxygen levels in the lungs

- Tetanus
  - Sterilizing subcommittee's report doesn't mention clear examples of microbes which express accidental toxins without coevolution with humans or higher life, such as tetanus which kills thousands of unvaccinated newborns every year

- Our immune response to a new genus of fungi as invasive as Aspergillus but with no pattern recognition capabilities to recognize it

- NEW: Our immune system responses are highly specific to each of the three genera of opportunistic human fungal pathogens – without the necessary pathogen associated molecular patterns (PAMPS) we might all be immunocompromised to a new genus of fungi from Mars

- Allergic reactions e.g. to fungi from Mars
  - NEW: Possibility of an allergic response to harmless alien life – or indeed a new genus of familiar life - if it is recognized by the immune system but not by the inflammation dampening Treg cells - allergic bronchopulmonary aspergillosis affects around 4.8 million people globally and chronic pulmonary aspergillosis, affects 400,000 globally – these figures could be higher for an allergic response to extraterrestrial life - if a normally functioning human immune system doesn't recognize the need to dampen its response

The review of whether life from Mars could affect terrestrial ecosystems and the Earth's biosphere should look at:

- Fungal parasites of microbes including parasites of photobionts
  - NEW: Microbes from Mars could have pathogens that can infect terrestrial microbes – example of fungal pathogens of phytoplankton and cyanobacteria – cyanobacteria depend on specific antifungal adaptations to protect against fungi in the chytrid phylum, so may have no adaptations to a novel fungal phylum from Mars

- Example of permafrost microbe with optimal growth temperature 25°C and capable of growth at human blood temperature
  - NASA's biological safety report agrees on the potential for an invasive Martian species to harm or displace terrestrial photosynthetic bacteria – but says life adapted to Martian conditions such as the temperatures and pressures plausibly wouldn't be able to survive on Earth – their own cite mentions Planococcus Halocryophilus, a microbe which lives in Arctic permafrost soils and likely grows in sub zero brine veins down to at least -15 °C, with an optimal growth temperature of 25°C and growth up to 37 °C (human blood temperature)
- Species sorting which could lead to species adapted to higher temperatures than currently found in the environment life is returned from
  - Mars surface temperatures can reach 35°C in the shade in summer – some species of Martian surface life may be pre-adapted to hotter, even hydrothermal conditions in geologically recent Mars – and emerge through species sorting – persist in small numbers in surface biofilms and spread and adapt rapidly when they encounter far warmer conditions
- Geobacillus paradox - the possibility that volcanic vents on Mars produce large numbers of hardy spores similarly to the geobacillus spores that then spread widely in the dust and may be found almost everywhere
  - The paradox of abundant spores of heat adapted geobacillus spores in cold places - and potential that present day Mars has similarly abundant heat adapted

spores from hydrothermal systems, perhaps produced by the rootless cones, fumaroles, or ice fumaroles – some might have been active in the last few million years – some might even be active today

A new sample return study should look at possibilities with no terrestrial analogue such as:

- Joshua Lederberg's two papers looking at the possibility that terrestrial immune systems have no defences against alien biology - and Claudius Gros's similar suggestion
    - Warnings by some astrobiologists such as Sagan and Lederberg that in worst case we could be in effect immunocompromised to an entire exobiology from Mars
- and the possibility that this extends to even microbial terrestrial life with no defences against alien microbes
    - NEW: Claudius Gros's worst case scenario for forward contamination – if this scenario can be applied in reverse, nearly all higher life eventually goes extinct outside habitats, though it takes a long period of time
- Effects of returning mirror life or other life with a radically different internal biology
    - NEW: Example worst case scenario of a mirror life chroococcidiopsis analogue from Mars which gradually converts organics in ecosystems into indigestible mirror organics
- Possibility that Martian life could have much smaller cells that take up nutrients faster and avoid protozoan grazing
    - NEW: Closely related worst case scenario of a shadow biosphere of small mirror life nanobes that produce indigestible mirror life biofilms on Earth with small cells advantages that they take up nutrients faster and avoid protozoan grazing
- Life that might be better at photosynthesis than terrestrial life
    - NEW: Martian life could be better at photosynthesis than terrestrial life since terrestrial photosynthesis works at well below its theoretical peak efficiency and the lower light levels on Mars might favour evolution of more efficient photosynthesis
- Life that might be better adapted than terrestrial life and spread rapidly
    - NEW: Worst case scenario - If a martian microbe can grow in the sea, soil, and fresh water like chroococcidiopsis, is adapted to spread in the wind in Martian dust storms, and outcompetes terrestrial biology, e.g. better at photosynthesis or nitrogen fixation, it could be found globally after introduction to Earth in weeks to months, and be one of the most common microbes in our soils and oceans in years to decades or sooner, far more common than nanoplastics or microplastics

- Life that could cause problems similarly to nanoplastics and microplastics even if terrestrial and martian life are mutually mystified and don't attack each other or defend against each other
    - NEW: Scenario of an alien biology that produces large numbers of spores that our immune system can't see and in turn do nothing to our bodies and are completely inert like microplastics and nanoplastics – even this could be harmful to terrestrial life

-

When considering whether samples could contain life it should look at:

- Potential for Martian life to make the Curiosity brines habitable through adaptations such as biofilms, perhaps covered with surface mosses that use hair structures that swell when hydrated to block escape of water vapour and maybe even micropores that close at times of lower humidity or in response to daylight
    - Martian life could be more capable of coping with Martian conditions than terrestrial life – e.g. survive better in dust storms or cope better with cold temperatures and temperature changes – and ways a martian biofilm could retain water in ultracold night time brines through to the midday warmth – fine hairs, pores that close in daytime like cactuses – chemicals that speed up metabolism, slow generation times and novel biochemistry
- New studies on transport of biofilms in the dust
    - 2019: A thin (0.03 microns thick) fragment of desiccated biofilm of chroococcidiopsis would be still viable after blowing 100 km in moderate winds (5 meters per sec) in full Martian sunlight
- suggestion that even if microbial life can't get started today, life on Mars could propagate via biofilm fragments
    - 2019: Curiosity found UV radiation fell by 97% at the start of the 2018 dust storm, which could increase Billi et al's 100 km to 1000s of kilometers in Martian dust storms – and Mosca et al's suggestion that biofilm fragments established in the past could continue to propagate even if Mars doesn't have conditions to start a new biofilm today
- Potential for individual microbes to survive in cracks in dust spores
    - 2017: individual microbes can travel in dust storms imbedded in a dust grain for extra protection from UV
- Potential for microbes to survive in cracks in bouncing grains of dust up to half a millimeter in size
    - 2019: Microbes can be protected by bouncing sand grains up to half a millimeter in diameter traveling meters in each bounce, and some (less than 1 in 1000) b. subtilis spores remain viable after hundreds to thousands of kilometers of travel in simulation experiments
- Potential for Martian life to evolve spores or other propagules with hardened outer shells to survive bouncing which can be propagated similarly to bouncing grains
    - New: Martian life could have spores with extra layers to protect against UV in dust storms - or fruiting bodies or other propagules detached by strong winds protected by outer layers of altruistic social bacteria - and martian life could use strong biomaterials similar to chitin (found in hard parts of insects but also in fungi and lichens) to protect from impact bounces

- The suggestion that Mars has fresh liquid water seasonally in the polar regions similarly to the subglacial melt in Antarctica which should happen at surface temperatures of -90 C because of the way ice lets light through but insulates the melt water and protects from evaporation in a vacuum which leads to ice melting at half a meter depth in Antarctica - in the different conditions on Mars it should melt at a depth of 5 cms

- 2009, 2014: Possible future surprise discovery of large quantities of fresh water on Mars: ice lets light through and traps heat, which melts ice half a meter below the surface in Antarctica -– if Martian ice is similar, its polar regions should have meltwater in summer, ~5 cms below the surface, even with surface temperatures below -90 °C – Mars may also have miniature melt ponds around sun warmed dust grains

For the idea of testing samples before release it should consider:

- Testing can't protect Earth from life in a sample - that after 10,000 dust grains tested destructively you can't deduce that the 10,001th grain is safe
    - NEW: Too early for any form of safety testing at the level of assurance needed for potential large scale harm – even for samples returned in sterile containers with no forward contamination – after destructively testing 10,000 grains of dust the 10,001th grain could have an undetected viable microbe imbedded in it

For human quarantine:

- Human quarantine can't work to protect Earth or even humans - issue of life-long symptomless superspreader like Typhoid Mary and that technicians might happen to be immune to a new fungal pathogen which most of the population have no immunity to –
- if a technician got ill with a potentially life threatening disease, ethically you can't leave them to die in quarantine – they would be removed but that's the very time when they need to be kept in to protect Earth
  Impossibility of keeping out a pathogen of crops or microbes with human quarantine - it can become part of the human microbiome as with the example of the crop pathogen brought to the ISS
- Impossibility of keeping out mirror life with human quarantine

These are all covered in

- NEW: It is impossible to use quarantine to protect Earth's biosphere if humans handle the samples in orbit – the Apollo quarantine procedures never had peer review and missed the issue of a symptomless superspreader – and this can't keep out mirror life, or molds like the one that killed two plants on the ISS – keeping humans well away from the samples also avoids forward contamination for very sensitive measurements

A Mars sample return study

- shouldn't be set up with a remit to find a way to return a sample safely

- should be tasked to evaluate whether or not current technology is not able to contain the samples to the required level of assurance, with "no" as a permissible answer

A Mars sample return back contamination study should be empowered to look at reasonable alternatives such as

- sterilizing all samples before they are returned to Earth,
    - NEW: We can forestall all these issues and make the mission 100% safe by sterilizing samples before they reach Earth

- returning some samples of especial astrobiological interest to a satellite above GEO for study remotely, and sterilizing anything returned from that satellite to Earth. See:
    - NEW: We can transform this into a much more interesting first step for astrobiology with little change in the overall budget by adding bonus samples collected in a STERILE container sent on the ESF fetch rover – the aim is to return dust, dirt, ideally salts, compressed gas from the atmosphere – and some pebbles for a technology demo of a contamination free rock sample
    - NEW: These clean samples will be studied above geostationary orbit in Mars simulation conditions with a Martian gravity centrifuge – they are not intended for safety testing - and humans never go near the satellite

This paper has identified a need for an interdisciplinary approach for planetary protection for a Mars sample return. For a thorough investigation of the topic, astrobiologists need to reach out to those with other areas of expertise including:

- synthetic biologists
- researchers into early life
- epidemiologists and experts in infectious diseases – the WHO and the CDC should be involved in any reasonably comprehensive Mars sample return study, and they wouldn't have missed the issue of symptomless carriers
- Experts on fungal pathogens of microbes,
- Experts on allergic reactions and immune responses
- Experts on the potential for improving on terrestrial photosynthesis and nitrogen fixation to consider whether extraterrestrial life could do the same.
- Experts on air filter technology
- Many other disciplines.

Then they need to follow up leads these experts suggest and suggestions from the general public as it is not easy to do a comprehensive study of the potential risks for extraterrestrial life from another planet.

We know of many possibilities today in the 2020s that wouldn't even be considered in the 1960s. So we also need to give some attention to the possibility of future developments in science and our understanding of Mars to bring up even more possibilities in the future that we are not yet aware of.

This review is NOT comprehensive and just suggests some of many areas that would need to be looked at.

The simplest approach is to sterilize the samples before the return to Earth. This doesn't need a comprehensive backwards contamination study – but it still needs care and attention, to look into the level of ionizing radiation needed to sterilize samples sufficiently to protect Earth.

Method and limitations

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This paper is written as a review for the general public to use, legislators, ethicists, decision makers and scientists of other disciplines, so is designed to be maximally accessible.

This paper has to consider research published after 2009 or it would be 13 years out of date. The final draft of the NRC study was finalized in late February 2009 and approved in March 2029 (SSB, 2009: viii), just before the discovery of the droplets on the legs of the Phoenix lander which was first announced on 17th March 2009 (Renno et al, 2009) and a lot has happened since then.

A new comprehensive review is needed but this is not that review.

Instead the cites here are selected as illustrative examples to answer the main mistakes in NASA's draft EIS which are treated as representative of mistakes other space agencies are likely to make.

This paper should NOT be used in lieu of a comprehensive review. I hope that NASA and ESA consider commissioning a new review as an urgent first priority if they still intend to return unsterilized samples to Earth.

This paper includes new worst case scenarios. These shouldn't be seen as likely. We need to look at those is for the same reason that we look at the scenario of a house fire when we design or install a smoke detector.

This paper covers some views from the planetary protection literature in depth such as Carl Sagan's view that we shouldn't take even a small risk with a billion lives, and Lederberg's two papers arguing that our immune systems might not recognize an alien pathogen. That's because NASA's draft EIS doesn't mention them. However this paper is not the place to advocate for or against any of those views. That is for public debate and for legislators and decision makers to look into.

This paper comes to the conclusion that it is possible to keep Earth 100% safe with minimal or no impact on science and even a major increase in science return with minimal effect on the budget with the bonus samples. This paper argues that this thesis needs to be looked at carefully, and if it is indeed true, this also is something the public need to know when making their decision.

However, readers should be aware that any paper like this is part of a dialog. We need to see what others say in response to that reasoning.

In more detail on these points:

Note on use of language – this paper is designed to be maximally accessible – by careful use of vocabulary and grammatical structures, but never with loss of precision in the meaning of the text

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I have written this paper to be maximally accessible to everyone – theologians, philosophers, lawyers, politicians, decision makers, the general public and autistic people.

I wish this choice to survive through to the final version of this paper if possible. Examples:

- I use the most widely accessible vocabulary available to convey the desired meaning
- I replace technical by non-technical terms when it can be done with no loss of precision
- I use non-scientific terms, non-technical terms generally, and non-mathematical language whenever if it is available with the same precision.

Examples of using non-technical terms when there is no loss of precision:

- Million instead of 10^6
- “Didymo” instead of Didymosphenia geminate

Where there is no ordinary language equivalent, I explain the term in ordinary language as far as is possible when it is first introduced. I may later use a shorter definition of the same term as a reminder.

As for the choice to make this paper maximally accessible to autistic people – as part of my voluntary work helping scared people over the internet, I am used to working with scared people, many of them autistic. I have learnt how to use simple and self contained sentence structures that even quite severely autistic people can understand quickly when in the middle of a panic attack.

This is a “win win” situation as I find this approach usually makes the sentences shorter, with fewer words. It also seems to make the text easier for everyone to parse quickly. You may not notice much difference. The most obvious change may be that sentences and clauses within sentences tend to be a little shorter than for most academic papers.

I did a blog post on the difference between how autistic and non autistic people preferentially parse sentences which may help the reader understand the choices I make in sentence structure, see (Walker, n.d.)

This paper frequently covers recent research findings – because if it didn't it would be 13 years out of date – however it is not itself a comprehensive review and shouldn't be used as such

Next section – all sections – previous section

[This section has internal to the relevant sections of the paper to make it easy to jump to them]

It would be a major omission to write this paper and not mention the brines first discovered by Curiosity in Gale crater, but they were discovered 6 years after the major National Research Council sample return study in 2009 (SSB, 2009), and three years after the European Space Foundation major revision in 2012 which also focused mainly on the size limit (Ammann et al, 2012). There have been numerous other major advances in our understanding of many topics relevant to this mission since those two studies, as we'll see.

Similarly it would be a major omission not to mention the work on transport of biofilms in dust storms or the transport of viable b. subtilis spores in saltation bounces. It would also be a major omission not to include the material on the potential for subsurface ice melt and melt ponds around dust grains in the polar regions which gives potential for a present day fresh water habitat on Mars.

This paper needs to refer to some of that newer research or it would be 13 years out of date. However it's not in any way comprehensive. It just draws attention to some of the most major findings of the last decade or so that NASA's draft EIS omits.

This review turned up many new topics such as mirror life, fungal diseases of blue green algae and other microbes, aspergillus as an analogue of a Martian pathogen not adapted to humans, the possibility mutually mystified alien microbes as inert as microplastics could harm us, possibility of a severe allergic reaction from a novel genus of fungi from Mars or a completely novel biology, the potential our immune system can't recognize a new fungal genus from another planet, possibilities for novel amino acids to cause protein misfolding, and many other topics.

It also found that human quarantine can't keep out a fungal pathogen of crops, mirror life, or even a human pathogen with symptomless carriers.

This review also turned up new research into potential microhabitats on Mars and potential for transport in the dust. It makes a start at adaptations Martian life might use to inhabit martian microhabitats and for more resilient spores, and many other topics.

I hope that this can encourage NASA and ESA to commission a new sample return study to look into these and many other many major new developments of the last decade thoroughly.

To help distinguish what is new and what is from the older studies, paragraphs are prefixed with the year of the research, if the research is from after the last major review for a Mars sample return in 2009. For forward contamination they are labelled with any date after the 2014 study including the 2015 MEPAG review because NASA's Environmental Impact Statement omitted it. Some other material may be new to the planetary protection literature, such as the new mirror life scenarios. These are labelled NEW. See:

- Topics that need to be covered in a future Mars sample return backwards contamination study (not likely to be a complete list)

Scope of this review – material likely to be of especial interest to space agencies, based on mistakes in NASA's draft EIS – rather than any attempt at a comprehensive review

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It is clearly impossible to attempt a comprehensive review to survey all the most important research since 2009, as it would need book length treatment and the participation of many experts. So, the cites here are selected in response to mistakes made in NASA's draft EIS. These are treated as likely to be representative of mistakes that other space agencies might make in similar Mars Sample Return environmental impact statements.

- Questions for NASA - and why NASA's main argument is invalid
- Reasons for these questions: controversial or mistaken statements in NASA's draft EIS and the report of the sterilization working group

This review doesn't attempt to be comprehensive in its responses to those questions either. Instead, the aim is simply to draw attention to some of the more important results of the last decade omitted from NASA's draft Environmental Impact Statement that decision makers need to be aware of. The studies mentioned should be seen only as a few illustrative examples drawn from a much large literature which a comprehensive review would need to look at.

This review also focuses on Jezero crater, however this can be thought of as a representative illustration, as the planetary protection concerns are relevant to just about any site likely to be selected for a near future Mars sample return study.

In the near future at least, samples are likely to be returned from equatorial regions since our spacecraft are not sterilized sufficiently for the polar regions, and likely to be returned from low altitude sites since the need for aerobraking makes a high altitude landing challenging.

Pretty much the entire surface of Mars is within reach of the global dust storms. Frosts are likely over much of the equatorial regions and high night-time humidity and the Curiosity brines are also likely to be widespread.

This paper includes new worst case scenarios – they shouldn't be considered likely – they are considered in detail for the same reason you consider the worst case scenario of a house fire when installing or designing a smoke alarm

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This paper includes several new worst-case scenarios which it covers in some detail. These are included to encourage space agencies to treat planetary protection more rigorously. There are many other scenarios where no life is returned or the life returned is easily contained or managed or is beneficial or harmless. Beneficial scenarios are covered only briefly in one section.

- NEW: Enhanced Gaia – ways that introduced Martian life could be beneficial to humans, ecosystems and Earth's biosphere

This doesn't mean that the worst case scenarios are more likely. It is just that they are ones that we need to consider carefully for planetary protection issues. Margaret Race's analogy of a smoke detector may help (Rummel et al., 2000).

When you install a smoke detector in a house, you need to consider the worst case scenario of a house fire and install it correctly so that it will detect a fire. This doesn't mean that you consider the house fire likely.

This paper covers several options and views not mentioned in NASA's draft EIS such as the option to sterilize samples before they reach Earth, and Carl Sagan's view that "we cannot take even a small risk with a billion lives" – public and legislators need this background to make properly informed decisions – but this paper shouldn't be taken as advocating for or against these options or views

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This paper covers the option of sterilizing the samples before they are returned to Earth in considerable detail. The aim is to help ensure that this option is considered carefully and thoroughly and the public and legislators have this information available when they make their decisions.

It's similar for the discussion of the ethics of when and whether we should require Earth to be 100% safe, based on a discussion of the prohibitory version of the precautionary principle in a discussion of Carl Sagan's quote (Sagan, 1973):

> ***"The likelihood that such pathogens exist is probably small, but we cannot take even a small risk with a billion lives."***

It's important that the public and legislators know about this view and the reasoning that Carl Sagan and others give for it.

This paper shouldn't be seen as advocating for or against these or any of the views or options discussed here. It's written carefully to abstract away from any views the author may have.

It's the same for the analysis at the end. This paper reasons that:

- we can keep Earth 100% safe by sterilizing samples before they are returned to Earth,
- this can be done with virtually no impact on geological or astrobiological science return, and
- the astrobiological sample return can be greatly boosted with minimal impact on cost with bonus samples of salt, dirt, atmosphere and dust.

This is part of a dialog. We need to see what conclusions others come to with their reasonings.

All this then can become input to the discussions by public and legislators which then leads to the final decision.

The main thing is that, as NEPA requires, we need to make these decisions based on a scientifically credible, clear and open process, where the full range of views can be expressed. It is also important that the public are part of this process, and not ignored or excluded.

Factors for space agencies to look out for that may lead to them assigning planetary protection of Earth much less significance and attention than the general public

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It seems worth drawing attention of space agencies to some factors that could lead to them weighting planetary protection differently from the general public. This might also help the general public to understand the point of view of NASA engineers and counteract any infodemic in advance. It is hard to see how NASA could make so many mistakes except by inattention.

Many of the mistakes are easy to spot. For instance their cite for the meteorite argument says on page 5 that it shouldn't be used to support the argument that it is used as a cite for. One wonders how they could have missed that.

- The meteorite argument can't be used for potential life in surface dust, salts and dirt as these materials can't mechanically survive ejection from Mars

For the other mistakes:

- Questions for NASA - and why NASA’s main argument is invalidh_questions_for_NASA
- Reasons for these questions: controversial or mistaken statements in NASA's draft EIS and the report of the sterilization working group

There is no way to know at this point why NASA’s EIS has so many mistakes. We can’t know if any of the factors considered here apply to this draft EIS.

However, whether or not they actually were factors in the mistakes NASA made, these are factors that could lead to mistakes like this and a review of them may help space agencies ensure they weigh planetary protection higher in their environmental impact statements.

This can help them to match the expectations of the general public.

1. Engineering focus – NASA engineers have been tasked with returning samples from Mars to Earth

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Margaret Race made a relevant point here. She says scientists are likely to focus on (Race, 1996)

1. technical details
2. mission requirements
3. engineering details
4. costs of the space operations and hardware

General public are likely to focus on

- risks and accidents
- whether NASA and other institutions can be trusted to do the mission
- worst case scenarios
- whether the methods of handling the sample, quarantine and containment of any Martian life are adequate

We see the results of this different focus in the report. It is just not something that greatly occupies the minds of the engineers and scientists who work on space projects, yet it is the main thing on the minds of members of the public.

For the engineers, and scientists directly involved in the mission it is "above their pay grade" to change it to a mission that sterilizes the samples first or to delay the sample return. Their focus is on completing the mission as specified for them, within budget and on time.

2. The new fast track NEPA process may encourage the view that they don't need to spend much time looking into the details, as their EIS won't get the close scrutiny by regulators it had before when the process took many years

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With the new fast track NEPA process, they are likely to feel there is less need to spend much time on checking the statement, especially since they have feedback from EPA that their draft EIS is already adequate.

See above:

- EPA's letter on last day of public discussion says they didn't identify significant environmental concerns in their review of the EIS – with no mention of the public comments raising concerns similar to Carl Sagan

3. The example of Apollo – few are aware the Apollo procedures had no scientific peer review and were not considered adequate even with the science of the 1960s

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Their plan closely parallels what the Apollo missions did. There are two natural questions one might have here. The first one:

3 (a) If it is good enough for Apollo – why wouldn't it be good enough today, updated a little to take account of modern science? – because the Apollo plans never had public scrutiny and failed internal peer review

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Amongst other issues with the Apollo plans, they never had public scrutiny in 1969. Also, they did have private scrutiny from other agencies within the USA and representatives of two of these agencies, the National Academy of Sciences and the Public Health Services, both told NASA that their plans were inadequate.

The NASA planetary protection plans for Apollo predate the National Environmental Policy Act (NEPA), which was published the year after the first Apollo landings. NASA's plans were made available to the public for the first time on the day of launch of Apollo 11. This gave no time for the public to scrutinize or comment on them (one of the things fixed by NEPA).

NASA did make an effort to protect Earth from the possibility of life in the lunar samples in 1969. However this attempt was largely symbolic.

Amongst many lapses, the astronauts opened the door of the Apollo 11 capsule after splashdown in the open sea, letting out air previously exposed to lunar dust from the landing module. There was dust in the astronauts' clothes. The astronauts donned biological isolation garments and exited into a life-raft bobbing in a heavy sea, and quickly swabbed the isolation garments with a bleach solution. They then weighted those swabs and dropped them into the sea. Finally they disinfected the raft with an iodine solution (Meltzer, 2012:404) and sank the raft (Meltzer, 2012:205).

These procedures wouldn't be enough to protect the sea from microbial spores even with the scientific understanding of the 1960s. The view of Vishniac of the National Academy of Sciences is summarized by Meltzer as : (Meltzer, 2012:203).

> *Opening and venting the spacecraft to Earth's atmosphere after splashdown would, in his view, make the rest of Apollo's elaborate quarantine program pointless.*

The chairman of the Interagency Committee, David Sencer, from Public Health Service said these plans violated the concept of biological containment (Meltzer, 2012:203).

However, NASA set up the internal Interagency Committee with a consensus requirement. All parties had to agree on any changes to NASA's plans, including NASA itself (Meltzer, 2012:129). This gave NASA the authority to block objections. It used this power in 1969 to block requests for more stringent precautions on the basis that there wasn't enough time left before the launch of Apollo 11 to add the precautions required by interagency experts.

It is impossible to know what scientists and the general public would have decided at the time if NEPA had predated Apollo 11. However there were ways that we could have made the Apollo missions completely safe, for instance using robotic sample returns similarly to the Soviet missions. It wouldn't have impacted on the science return much to sterilize the first robotic samples. The chance of life in the lunar samples or dust were already considered to be extremely low. NASA could have done a sterilized robotic sample return, or maybe several, and done enough studies to confirm that the Moon was as uninhabitable as it seemed from other observations. Then once NASA had a high level of confidence that the surface of the Moon was sterile they could have dropped all planetary protection protocols and sent humans.

So, even then NASA had the possibility to keep Earth 100% safe. But they didn't consider it. There was much less public awareness of any need to protect ecosystems and Earth's biosphere in the 1960s than there is today.

The second natural question one might have is, why would samples from Mars be harmful given that there was no harm from the samples returned from the Moon?

3 (b) There was no harm to Earth from the Apollo samples, so why would there be any harm to Earth from Mars samples? – Mars has a very different

– the difference is that the potential for life on Mars as we understand it today is far higher than it was for the Moon even as it was understood in the 1960s – and we might have been lucky with the Moon anyway.

Indeed if we had done as much robotic exploration of the Moon by 1969 as we have done for Mars today - we would already know by 1969 that the Moon was lifeless.

However, there are differences that make Mars a better candidate for surface native life today than the Moon seemed to be with the understanding of the 1960s.

In summary:

- **We have clear evidence today, that early Mars had conditions favourable for evolution of life, with lakes and even seas .** Though it's possible early Mars was ice covered much of the time (Vago et al, 2017) even an ice covered ocean would have habitable hydrothermal vents. Then there's evidence of at least two tsunamis, likely the result of impacts (Rodriguez et al, 2019) which suggest at least a temporary largely liquid ocean (Turbet et al, 2019), as recently as 3.4 billion years ago (Rodriguez et al, 2019)**.**
    - In the 1960s we had no clear evidence for a past habitable Moon. There was weak evidence suggesting the Mares were ancient sea beds (Gilvarry, 1964a) (Gilvarry, 1964b), but this evidence was not persuasive (and of course soon turned out to be false)
- **Curiosity has detected ultra cold salty brines on and near the surface of sand dunes just before dawn / after dusk and below the surface just after sun rises and just before the sun sets** (Martin-Torres et al, 2015).
    - There was no detection of liquid water on the Moon, just a hypothetical layer that could exist at a depth of tens of meters enriched with organics, far from the surface.

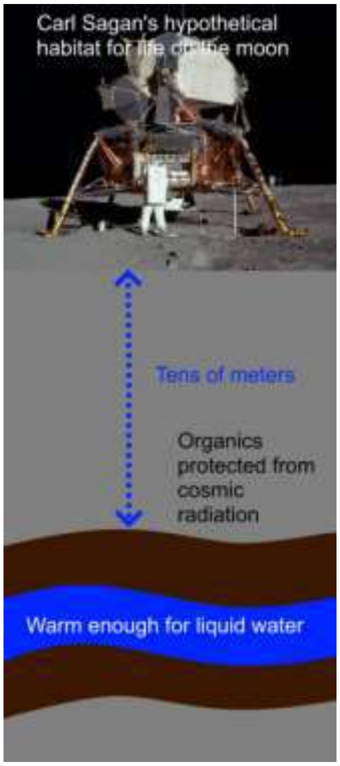

Figure 51: Sagan's hypothetical liquid water layer on the Moon (Sagan, 1961), photograph is of Buzz Aldrin and the Eagle lunar landing module, 1969 (NASA, 1995).

- **Mars has a sparse atmosphere humid enough for thin layers of frost to form at night in many regions.**
  See: 2021: Potential for melting frost to form a "dew" of microns thick layers of fresh liquid water even in Jezero crater – as an example to show the potential for future surprise microhabitats
  Also, some terrestrial blue green algae and lichen have been able to grow in Mars simulation conditions using just the night time humidity in partial shade.
  See: 2014: Example of an alpine lichen Pleopsidium chlorophanum found in places like California and the Alps that also grows in Mars analogue conditions in Antarctica and can survive and even grow in Mars simulation conditions – this shows even potentially some multicellular life from Mars could be able to live on both planets

  - The Moon has no atmosphere, only an exosphere. Frost can't form there at night, and by 1969 it was already clear no life could grow on the surface of the Moon. (Sagan, 1961:25) (Stern, 1999),.

- **The Martian dust storms may be able to transport spores from distant regions of Mars**. See: 2015: MEPAG2 review draws attention to potential for viable life transported through the atmosphere (for instance in dust storms) and following sections.

  - There is no way for life to be transported from distant parts of the Moon.

- **A small minority of scientists believe that the Viking landers may have detected life in the 1970s and the data from the Viking landers is not yet fully explained by either a biological or a chemical hypothesis.**

  See: The Viking landers in the 1970s remain our only attempt to search for life on Mars – a few astrobiologists think its labelled release may have already detected life in the 1970s – while others say the data can be explained by complex chemistry – we haven't sent the follow up experiments needed to finally resolve this debate and we can't deduce anything about whether Perseverance might return life even if the Viking experiment did find complex chemistry

  - There were no puzzling observations from lunar experiments that anyone interpreted as possibly due to life.

So, Mars has greater potential for life with our current understanding of Mars than the Moon ever did even with our understanding of the 1960s before Apollo.

Also, we may have been lucky with the Moon. Sagan's deep subsurface layer was not totally implausible, at the time. Then for a similar Moon but with that deep subsurface layer, there could be various processes that could move life from such a layer to the surface and for it to still be viable.

Another civilization in another star system might return life from a moon closely resembling ours superficially as we understood it at the time of Apollo, perhaps with a different accretion history.

As an example, Ceres is superficially somewhat similar to the Moon as we understood it in 1969. However the patches of ice in regions of permanent shade suggest it may have a subsurface liquid water or mud ocean, not unlike Sagan's hypothesis for a deep subsurface habitable layer for the Moon. We don't know if there is life on Ceres. We may return samples from Ceres in the late 2030s. We will need to take precautions to protect Earth in case there is life in those samples (Carter, 2022) (Castillo-Rogez, 2022).

4. Inspiration of science fiction

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Many who work for NASA have a deep interest in space exploration and space technology, so they would be likely to be inspired by science fiction stories about astronaut and space explorers.

Just about all the science fiction greats set stories on Mars. Examples include Arthur C. Clarke, Isaac Asimov, C.S. Lewis, Edgar Rice Burroughs, Robert Heinlein, H.G. Wells, Gregory Benford, Kurt Vennegut, Frederick Pohl, Ben Bova, Greg Bear, Ray Bradbury, Phillip K. Dick, Kim Stanley Robinson, Andy Weir, and Larry Niven amongst others.

None of these stories feature Martian life that harms Earth's biosphere. Indeed, as a science fiction enthusiast myself, I can't think of even a short story that features native life returned from Mars that causes large scale harm to Earth's biosphere or human health. If anyone reading this knows of any, do say.

That's understandable because it isn't a plot twist likely to appeal to a human reader and it wouldn't advance their story line in any way.

In many stories Mars is lifeless. In others, especially from the early 20th century, when Mars was thought to be more habitable, Mars has life but it's compatible with terrestrial life. Examples include the Edgar Rice Burroughs and C.S. Lewis stories. In these stories, Mars is seen as like another terrestrial continent like Australia perhaps, or Africa or the Americas with unusual creatures influenced by the low gravity.

Amongst these early famous Martian science fiction stories, one of the more notable fictional martians is Stanley G. Weinbaum's "Tweel", a cute intelligent creature in-between a plant and an animal, the last relic of an ancient Martian civilization (Weinbaum, 1945). Harmless to Earth.

Figure 52: Jarvis meeting Tweel. Painting in the Smithsonian's Mars gallery (Rowland, 2010)

The only major story involving Mars with a plot twist of large scale harm to living organisms, is H.G. Wells's "War of the World". But there it is resolved in human favour and the creatures harmed are the Martians.

Figure 53: M. Dudouyt's vision of the Martians from the 1917 edition of War of the Worlds (*Dickson, M. n.d.)*

In H.G. Wells' story, Martians had never encountered bacteria on their planet (Wells, 1898 : book 2 section VIII).

> *And scattered about it, some in their overturned war-machines, some in the now rigid handling-machines, and a dozen of them stark and silent and laid in a row, were the Martians—dead!—slain by the putrefactive and disease bacteria against which their systems were unprepared; slain as the red weed was being slain; slain, after all man's devices had failed, by the humblest things that God, in his wisdom, has put upon this earth.*
>
> *For so it had come about, as indeed I and many men might have foreseen had not terror and disaster blinded our minds. These germs of disease have taken toll of humanity since the beginning of things—taken toll of our prehuman ancestors since life began here. But by virtue of this natural selection of our kind we have developed resisting power; to no germs do we succumb without a struggle, and to many—those that cause putrefaction in dead matter, for instance—our living frames are altogether immune. But there are no bacteria in Mars, and directly these invaders arrived, directly they drank and fed, our microscopic allies began to work their overthrow. Already when I watched them they were irrevocably doomed, dying and rotting even as they went to and fro. It was inevitable. By the toll of a billion deaths man has bought his birthright of the earth, and it is his against all comers; it would still be his were the Martians ten times as mighty as they are. For neither do men live nor die in vain.*

This was the only example Carl Sagan had available to him when he referred to the backwards contamination risk as the plot twist of War of the Worlds, but in reverse:

Carl Sagan put it like this (Sagan, 1973:162):

> *"Precisely because Mars is an environment of great potential biological interest, it is possible that on Mars there are pathogens, organisms which, if transported to the terrestrial environment, might do enormous biological damage - a Martian plague, the twist in the plot of H. G. Wells' War of the Worlds, but in reverse. … The chance of such an infection may be very small, but the hazards, if it occurs, are certainly very high.*

The difference here is humans don't get to write the story line for a real-life return of microbes from Mars.

Perhaps it is time for a science fiction writer to write a ground-breaking work of science fiction where there is a risk of harm to Earth? But Carl Sagan's plot twist of the War of the Worlds in reverse wouldn't work as an inspiring science fiction work for humans. It likely needs to be some other plot.

5. Space colonization enthusiasts who see parallels between themselves and the settlers of the American west

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The inspiration of the American West is likely to be a factor indirectly for many Americans though we can't say that it is a factor for the EIS. The only previous article I found that resembles the report of the sterilization working group is Robert Zubrin's op. ed. for the Planetary report. They don't refer to Zubrin, and didn't go as far as he did in their conclusions, and if there is any connection it is likely to be indirect.

There is a striking similarity, the sterilization working group report is a more scientific version of what he says. It doesn't include any of the examples from the planetary protection literature that count against his arguments and it doesn't mention the major Mars sample return studies that ALL came to the opposite conclusion to his arguments. But this doesn't mean the motivation is the same. See above:

- The sterilization working group report argues martian life didn't co-evolve with us so can't harm us and that martian life would be extremophile not able to survive on Earth – these arguments were previously presented in an op. ed. by Zubrin in 2000 – and planetary protection experts at the time found many errors in them and said it was like a recommendation to build a house without smoke detectors

Robert Zubrin's motive at least is clear as he describes it eloquently himself. He sees Mars as the frontier that the US no longer has in the American West, now that the entire continent has been explored by Europeans (Zubrin, 1996).

> *Without a frontier from which to breathe life, the spirit that gave rise to the progressive humanistic culture that America has offered to the world for the past several centuries is fading. The issue is not just one of national loss — human progress needs a vanguard, and no replacement is in sight.*

He wants humans to colonize Mars and sees Mars colonists as essential for the vitality of Earth's civilizations. He is focused on the engineering and practicalities of this. He has arguments that seem plausible to him that there is no risk of harm to Earth. His views are very influential amongst space colonization enthusiasts. This in turn influences science fiction writers and may reinforce the tendency to envision Martian life as harmless to Earth in their fiction.

The sterilization working group use somewhat similar language but all they say by way of motivation is (Craven et al., 2021:4).

> *"While it is impossible to remove all risk without ceasing space exploration, …*
> *There is always some level of risk associated with exploration into the unknown …"*

We discuss this in:

- NEW: We can forestall all these issues and make the mission 100% safe by sterilizing samples before they reach Earth – it is impossible to eliminate all risks to spacecraft and astronauts from space exploration into the unknown – but it IS possible to eliminate all risks to Earth's biosphere

However whether it's relevant to the EIS or not, Zubrin's motivation is a possible factor for space agencies. Many may be inspired to join a space agency because of this idea of a new frontier in space and a belief that it will breathe vitality into our civilization.

.

This isn't the place to try to decide whether our civilization does need a physical frontier for vitality. Or to try to settle the long running debate in the US about Turner's "Frontier thesis". Or to ask if it really was a frontier given that the Americas were already colonized (Aron, 2016). Perhaps it's best looked at on the level of individual inspiration?

There are many in our civilization who find inspiration in frontiers in art, music, literature, in science and technology, in pushing the physical limits of their bodies in sports challenges, and so on. Others are inspired by the need to learn to live sustainably, or to protect species and stop and reverse biodiversity loss, looking for undiscovered species, or in horticulture, saving old varieties of apples (Brown, n.d.), or striving to breed a blue rose (Nanjaraj et al., 2018) or C-4 rice for higher yields with global warming, which they describe as one of the scientific "Grand Challenges" of the 21st century (C4 Rice, n.d.). Others want to push terrestrial physical limits of colonization such as living sustainably in deserts with saltwater greenhouses such as the Sahara forest project (SFP, 2020), or in floating cities (UNHabitat, 2022) and Buckminster Fuller and Osker worked on engineering details for a future with permanent dwellings in the air (Lippincott, n.d.). For others, advances in medicine or human rights may be what they see as important to the vitality of a civilization.

But there are those like Zubrin who do see physical exploration in space and settling too, as vital to our civilization.

For those of us who see things like that, it may help to look at a larger picture, Space can still be a vibrant frontier without humans colonizing the surface of Mars.

The larger picture: how a scenario of mirror life microbes on the Mars surface could actually invigorate space exploration, as a vibrant forever unattainable human frontier - still studied and exploited by avatar robotic explorers controlled from orbit – with many other places for humans to explore in person, on the Moon, moons of Mars, asteroids, independently orbiting space settlements, aerostats above Venus clouds, Jupiter's moon Callisto, Saturn's moon Titan and beyond

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The example of mirror life shows that it's not a foregone conclusion that Martian life is eventually proven safe for Earth. If we do find alien life on Mars which can never be returned safely to Earth, initially perhaps this is disappointing for aspiring space settlers, but it could invigorate space exploration and settlement. The public interest and the scientific and practical benefits to humans could be enormous from a completely alien biology such as mirror life.

A Mars with mirror life, perhaps, becomes a forever unattainable frontier, a world you can never actually land on, but can still explore with high fidelity telepresence. We have never had a frontier like that.

We have places on Earth we can't settle currently such as the deep ocean, even the shallow ocean is hard for us to inhabit, for now the atmosphere is hard to inhabit, we have only made a start on sea steading, and some places are well beyond current technology. We can't explore down to the core of our planet as Jules Verne envisioned in his "Journey to the center of the Earth" (Verne, 1897). But we have never had a frontier due to biology.

Might that "untravell'd world" (Tennyson, 1842) also lead to stimulus and challenges and invigorate our culture?

> *Yet all experience is an arch wherethro'*
> *Gleams that untravell'd world whose margin fades*
> *For ever and forever when I move.*
> *…*
> *And this gray spirit yearning in desire*
> *To follow knowledge like a sinking star,*
> *Beyond the utmost bound of human thought.*

Mars isn't such a prize as a planet for humans to inhabit as it seems to be visually from the attractive reddish looking terrain. There is no soil there, of course, it's just desert. And Mars is not habitable to humans in any ordinary sense of the word. The atmospheric pressure of 0.6 to 0.7% is well below the Armstrong limit of 6.3% where water and body fluids boil at body temperature (Murray et al, 2013). The atmospheric pressure is too low for our lungs to function, so we couldn't breathe even with bottled oxygen. We would go unconscious in seconds, and require a full body pressure suit to breathe.

Suppose we had a plateau on Earth, at a height of 45 kilometers (NASA, n.d.), five times higher than Mount Everest at 8,848.86 km (Dwyer, 2020). The pressure would be typical for Mars, but it would be far more habitable and it would be far easier to colonize such a high plateau than Mars. Yet if such a plateau occurred on Earth, we would likely have few living there permanently except to extract resources or for scientific study. We don't colonize most deserts, or the shallow continental shelves, which are far more habitable.

Lunar dust isn't laced with perchlorates like the Martian dust, but it was still a major issue for lunar astronauts. They all reported difficulties with the dust (Stubbs et al, 2007). Astronaut Eugene Cernan, the last man to walk on the Moon to date (NASA, 2017), described the lunar dust as one of the greatest inhibitors to a nominal operation on the Moon (Levine, 2020).

Mars has worse dust problems than the Moon. For instance, the Moon doesn't have dust storms. Several times a decade dust storms on Mars block out the sunlight for weeks making surface conditions as dark as night. On the Moon we can clear the dust from around a settlement, or melt it into paving slabs, and on any landing sites for our rockets, and eliminate the issue at least locally as well as along roads and tracks (Taylor et al., 2005). That would be only a temporary solution on Mars as the paved surface would get covered by dust again with the next dust storm and the dust would still be suspended in the air.

Lunar dust falls back in ballistic arcs, in an atmosphere so thin it is classified as an exosphere rather than an atmosphere.

However, on Mars, some of the finest dust kicked up by astronauts or rovers would linger in the air for some time even in calm weather without winds.

Inhaling a few milligrams of Martian dust could exceed the recommended maximum daily dose for perchlorates (Reference dose or RfD) (Davila et al, 2013). When the perchlorates are activated by ionizing radiation they may change to the more deadly chlorates and chlorites with some potential for more serious and immediate effects such as respiratory difficulties, headaches, skin burns, loss of consciousness and vomiting (Davila et al, 2013).

There are methods for dealing with this, used for dust suppression when mining uranium, lead or other heavy metal contaminated areas. But it adds to the complexity of Mars colonization (Davila et al, 2013).

The challenges to keep the dust out impact on most aspects of a mission to the surface (Rucker, 2017). It can be done. The suitport may be a solution for the problem of dust inside habitats (Boyle et al, 2013).

Figure 54: Suitport illustration from (Gernhard et al, 2008) reduces loss of air when exiting or entering the rover and greatly reduces the dust problem.

None of this makes Mars settlement impossible, but Mars does not seem to be an optimal place to colonize for its own sake. There has to be another reason to take the tremendous efforts needed to colonize a place like this. If we find instead that we have to explore Mars from orbit, actually it would be a safer and easier way to explore Mars.

There are many other places pioneering humans can explore and perhaps settle. Including the moons of Mars. Once we have the capability, humans in orbit around Mars can direct the rovers on the surface of Mars via telepresence from orbit, with binocular vision and haptic feedback as for the HERRO study (Oleson et al, 2013) (Valinia, 2012) and the first part of the Lockheed Martin "Stepping Stones" to Mars (Hopkins et al, 2011) (Kwong et al 2011) and Mars Base Camp (Cichan et al, 2017) studies as far as the human base camp on the moons of Mars exploring the surface via telepresence.

Several authors have suggested we could bootstrap space exploration with seed factories that make more equipment, and even copies of themselves (Freitas et al, 1981) (Kalil, 2014) (Metzger et al, 2013). So long as the original seed factory is sterile, any equipment made on Mars made by the small seed factory of 100% sterile manufacturing robots and 3D printers would itself also be sterile.

Then there's the Moon itself. There are resources on the Moon that may be valuable if the cost of returning them is low enough.

Hoyt's cislunar tether system is an ingenious way of using the gravitational potential difference between the Moon and Earth much like the way a syphon can lift water over the lip of a container to a lower place. It uses two spinning tethers, one lifts materials out of the lunar gravitational well, the other receives them in orbit around Earth. The system is powered by the flow of materials from the Moon to Earth which in turn lets astronauts and their provisions and tourists travel from Earth to the Moon again with no extra fuel except whatever is needed to fly to the lower tip of the tether in Earth's orbit in a hypersonic space plane (Hoyt et al., 2000).

This is a brief outline to show it works, first, it captures a payload launched to a low holding orbit and boosts it into a lunar transfer orbit to the Moon.

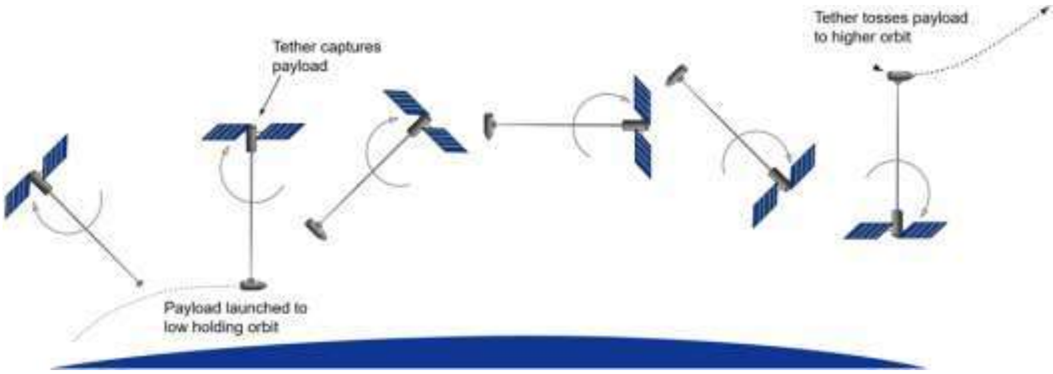

Figure 55: from ([Hoyt et al., 2000: Fig 1](#)).

The complete cislunar transport system looks like this:

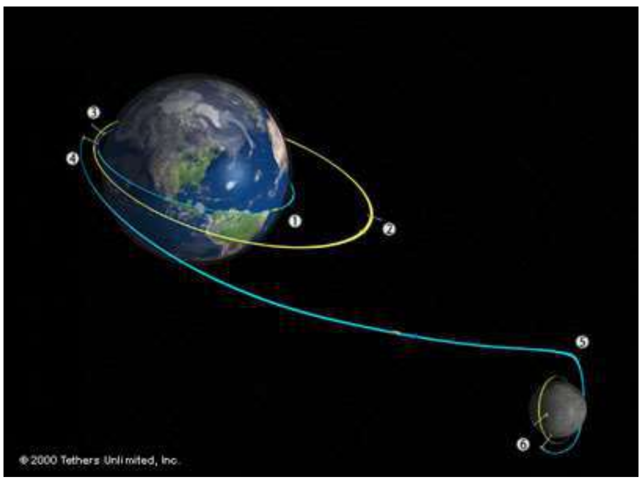

Figure 56: from ([Hoyt et al., 2000: Fig 2](#)). **"***The Cislunar Tether Transport System. (1) A payload is launched into a LEO holding orbit; (2) A Tether Boost Facility in elliptical, equatorial Earth orbit picks up the payload (3) and tosses it (4) into a lunar transfer trajectory. When it nears the Moon, (5), a Lunavator Tether (6) captures it and delivers it to the lunar surface."*

At the Moon end of the transport system, the tether can spin at just the right speed to be stationary relative to the Moon's surface when it is closest to the Moon make it easier to transfer passengers and materials to / from the Moon. The material flowing from Earth to the Moon is used to keep the lunavator tether spinning and to boost it.

Then in the other direction, material flowing from the Moon to Earth is used to boost the tether in Earth orbit to prevent it de-orbiting and to keep it turning. In this way both tethers are continually boosted and kept turning, and it turns out that the gravitational potential difference between the Moon and the Earth can be exploited to keep the system going with almost no extra fuel so long as more mass is transferred from the Moon to Earth than in the other direction. ([Hoyt et al., 2000](#)).

> *"By balancing the flow of mass to and from the Moon, the orbital momentum and energy of the system can be conserved, eliminating the need to expend large quantities of propellant to move the payloads back and forth"*

Both can be made with materials we have already. The Earth-orbit tether boost facility needs a mass 10.5 times the payloads it can handle and the lunar tether facility needs a mass less than 17 times the payload it can handle. So, once we have frequent payloads back and forth between the Earth and the Moon it will pay for itself in terms of payload mass quickly.

There are many details to the idea – that's just the outline of how it works (Hoyt et al., 2000).

The Moon is also a natural destination for space tourism; for our first science outposts. Once we have an industrial base on the Moon, it is a natural place to build spacecraft and rockets, to send to explore further afield. We will be able to launch spacecraft, mirrors, and space habitats of any size and shape from the surface. There is no need for a faring to make the payload aerodynamic and the low gravity makes it easy to launch heavy payloads (Metzger et al, 2013). The vacuum conditions also make it a natural place for chip manufacturing plants, for making some types of solar panels and so on.

On the Moon, we can even convert the surface into solar panels with an autonomous solar panel paving rover taking advantage of the vacuum conditions to make solar panels in situ, something we couldn't do on Mars (CAM, n.d.).

The moon is also far more suitable for human settlement than was thought at the time of Apollo. New results include the evidence of vast lunar lava tube caves useful for shelter from surface ionizing radiation. The largest signal from the Grail gravity data suggests a cave 3.5 km wide and 550 meters high, and these lunar caves can be tens of kilometers long (Chappaz et al., 2017). That's far larger than a Stanford torus space habitat. E.g. a ten kilometer long cave 3.5 km wide has a living area of 35 square kilometers or 35 million square meters. The Stanford torus has various sizes, the simplest has a projected living area of 680,000 square meters (Johnson et al, 1977:table 4.1, page 44)

Some of the lunar caves probably have an internal steady temperature of around -20 °C, potentially useful as a constant heat sink for a settlement (Daga et al., 2009). The challenge of providing energy during the lunar night is a similar challenge to providing energy during Martian dust storms. Then there are the peaks of almost eternal light at the poles with solar power 24/7 nearly year round (Foing, 2005), the polar ice and so on (NASA, 2019). The Moon is a place where we can make our first steps in sustainable living in space, within easy access of Earth for repairs, supplies, and emergency medvac back to Earth in only two days.

Further afield, as another frontier, it's likely humans explore Venus from the clouds, teleoperating assets on the surface. First they would use airships as with the HAVOC design (NASA, n.d.) and then more permanent aerostats. We need to be sure first that there is no life already in the Venusian clouds, or that if there is, that the sulfuric acid in the clouds makes it so alien there is no risk of forward or backward contamination.

Some enthusiasts think that humans could eventually settle in aerostats living above the clouds of Venus at a height where the temperature and pressure is similar to Earth normal and the skies are clear (Landis, 2003). The idea of colonizing the Venusian skies was first explored by the Soviets in the 1970s (Moskalenko, 1981). Venusian cloud settlers could mine a lot of their resources from the atmosphere and perhaps get other resources from the surface using grapples. What is harder to explain is a reason for living there apart from scientific study of the Venus surface. However, sky living does seem to be possible at least in terms of engineering. Buckminster Fuller's kilometer diameter "Cloud Nine" would float in Earth's atmosphere like a hot air balloon using the waste heat from the settlement and using his lightweight robust dome construction to make a habitat that is safe from even the strongest hurricanes. This was later elaborated in engineering detail to Osker's proposal for a one mile diameter Solar Thermal Atmospheric Research Station (STARS) (Lippincott, n.d.)

A Venusian space settlement, if it is possible, would be similar, but in the Venusian atmosphere using oxygen and nitrogen for buoyancy. A balloon filled with these gases will float in the CO_2 atmosphere much in the same way that a helium balloon floats in ours. Another idea is to suspend the habitat from a balloon filled with a mix of water and ammonia which would evaporate or condense depending on the external temperature, keeping the balloon stable at a particular temperature, and so, height, in the atmosphere (Moskalenko, 1981).

Whether we settle the Venus clouds or not, our exploratory outposts in the Venusian clouds would require far less mass launched from Earth for the same habitable volume than any other space habitats, except perhaps habitats in lunar caves. It would also require less technological sophistication for protection against acid than to contain the atmosphere in a vacuum and protect against cosmic radiation.

As our spacecraft get more capable (Adams et al, 2003) , humans can also explore and even colonize Callisto, outermost of the Galilean moons of Jupiter (McGuire et al, 2003) .

In the Jupiter system, Europa is very unsuitable for humans. It is well within Jupiter's very hazardous radiation belt where humans would die quickly even protected by a spacesuit. They couldn't survive long on or near the surface without very thick shielding. Then there's the forward and backward contamination risk if there is any native life there, especially if it has rising plumes of liquid water in places like Thera Macula (Schmidt et al, 2011).

However, Jupiter's Callisto has lower radiation levels than Mars and the same planetary protection classification as the Moon (Kerwick 2012). It is outside of Jupiter's ionizing radiation belt and also shielded by Jupiter's magnetic field from solar storms (Kerwick 2012) . Callisto also has rock and water ice in abundance. It may have a deep subsurface ocean but the surface is unaltered for likely billions of years so there is no forward or backward planetary protection issue. Also humans would have a scientific motive to live there, to study Jupiter's moons and the planet itself from close up and as a base for remote exploration of Europa particularly, with sterilized robotic explorers. As we explore the solar system Callisto is a natural place for a human base and eventually settlement. (Kerwick 2012)

NASA's Human Outer Planet Exploration (HOPE) program in 2003 selected Callisto as the optimal destination for humans beyond the orbit of Mars suitable for human surface exploration and with features of scientific interest (Callisto has a deep subsurface ocean). This summarizes their reasons for selecting Callisto (Adams et al, 2003:4)

- *Europa—Jupiter's smallest Galilean satellite and the second closest to the planet. Scientific interest is largely prompted by the likely presence of a submerged ocean with tidal heating, which could offer conditions conducive to the development of life. Europa's location within the Jovian radiation belts poses significant design problems, particularly when contemplating human surface exploration.*
- *Callisto—the second-largest Galilean satellite and the most distant from Jupiter. Scientific interest is prompted by the possibility of subsurface water. Callisto's distance from Jupiter places it in a significantly less hazardous radiation environment than Europa, potentially permitting human surface operations.*

…

The Jovian moon Callisto was selected because of the balance that it offers concerning scientific interest, design challenge severity, and the level of hazard to human operations posed by the local environment

.

Figure 57: Elon Musk's artist's impression of his spacecraft for a crew of 100, the Interplanetary Transport System. He said his spacecraft would use Europa as a refueling stop in the outer solar system. Callisto is a far better refueling stop because of the lethal ionizing radiation around Europa which is within Jupiter's radiation belts. The artist's impression actually more closely resembles Callisto as the surface of Europa is probably broken up and rough on the meter scale, at least with current understanding (SpaceX, 2016).

Inset shows artist's impression of an exploration base on Callisto (NASA, 2004:22)

Then there's Titan in Saturn's system, which like the Venusian clouds, has an atmospheric pressure similar to Earth, indeed greater. It is far easier to protect against cold than against vacuum or acid. Explorers only need a thick insulating high tech version of a diver's dry suit with insulation only 7.5 cm thick, with batteries to heat a visor and gloves, and they could explore Titan's surface using a closed circuit oxygen or air rebreather system (without bubbles) much like military and deep sea divers use (Nott 2009). The winds just a few kilometers above the surface can be a source of energy for a settlement, while surface winds are so light as to cause no issues (Hendrix et al, 2017). It also has organics for making plastics, a stable environment and complete protection from ionizing radiation and the smaller meteorites (as for Earth) . (Wohlforth et al, 2016a) (Wohlforth et al, 2016b). Unless there is cryovolcanism, "volcanoes" of liquid water instead of lava, there is no risk of forward contamination. As for backwards contamination, we'd need to find out what is there but it's plausible that Titan life if it exists would not be able to survive terrestrial temperatures

Whether any of these are easy places to live long term may depend also on the gravity requirements for human health which are not yet known. However it is not yet known if the gravity on the Moon or Mars is suitable for human health long term either. It's possible that they all need to be supplemented with the use of slow centrifuges spinning for artificial gravity during sleep, exercise etc.

If our aim is space settlement, planets may not necessarily be the obvious choice they seem to be. This is an observation that goes back to O'Neil in 1969 when he was teaching freshman physics, (Heppenheimer, 1977:chapter 2)

> ***The first answers they came up with indicated there was more than a thousand times the land area of Earth as the potential room for expansion. They concluded that the surface of a planet was not the best place for a technical civilization. The best places looked like new, artificial bodies in space, or inside-out planets.***
>
> ***The classical science-fiction idea, of course, is to settle on the surface of the moon or Mars, changing the conditions there as desired. It turned out that there were several things wrong with this, however. First, the solar system doesn't really provide all that much area on the planets—a few times the surface area of Earth, at most. And in almost all cases the conditions on these planets are very hard to work with.***

The asteroid belt has resources sufficient to make habitats with a thousand times the land area of Earth, slowly spinning for artificial gravity. Settlers can choose the climate and even atmospheric pressure and composition, and gravity level for the habitats.

Finally over the centuries, and millennia, with space habitats slowly spinning for artificial gravity and large thin film mirrors to focus sunlight, we could explore and settle the entire solar system to Pluto and beyond (Johnson et al, 1977: *175*)

"At all distances out to the orbit of Pluto and beyond, it is possible to obtain Earth-normal solar intensity with a concentrating mirror whose mass is small compared to that of the habitat.”

Once we have fusion power we have the entire Oort cloud to explore / settle / colonize. Once we can live in any ice dwarf in the Oort cloud, humans can live almost anywhere around any star in the galaxy as nearly all stars have clouds of comets and ice dwarfs surrounding them.

Robert Zubrin has an argument which he presented in the "Making of" episode 0 of season 1 of the National Geographic series Mars (Zubrin, 2016). 45:15

"I would say that we have not only the right, but the obligation, to go and establish ourselves on Mars. We are the creatures with all of our flaws that the Earth's biosphere has evolved to allow itself to reach out and establish itself on additional worlds. And we will take this nearly dead world and we will create a fully living world there. And so there'll be new species of birds and fish and plants. And it will be magnificent. No-one will be able to look on it and not feel prouder to be human.”

If Earth evolved us with the capability to reach out and establish itself on other worlds, it also evolved us with the intelligence, foresight, and deep scientific and ethical understanding to guide that exploration. We are the Earth's biosphere's guiding intelligences in space, and that may be one of our main roles.

For instance one of the main reasons for going into space may be to protect Earth from hazards (such as asteroids), or to find resources for use on Earth, or to increase our understanding of ourselves, and of science, biology and the universe, or indeed, as a place for adventure and recreation. We have found many benefits for Earth’s biosphere, already, through our satellites in Earth orbit.

It's not automatic that what anything humans find inspiring and want to do is going to be harmless to ourselves or to other creatures in our biosphere. For instance deliberate and accidental introduction of species have led to many problems, such as the results of the deliberate introduction of the European wild rabbit into Australia for sport (Fenner, 2010).

We have seen that there are many other places humans can settle – and introduce terrestrial life to, other than Mars. The enormous lunar caves, getting on for the size of an O’Neil cylinder, are already vast enough to introduce many birds and animals to, as Zubrin wishes, but it’s much easier to make them habitable by finding a way to provide light, including through the lunar night, and filling with air and setting up a functioning self enclosed ecosystem. That would e a major task but nothing like the enormous effort needed to try to transform the atmosphere of a planet to make it more habitable for terrestrial life.

The Mars Society itself, based on the work of Chris McKay, who has done a lot of work on terraforming, estimates that using four supergreenhouse gases CF_4, C_2F_6, C_3F_8, SF_6, Mars could be warmed up to the point where astronauts no longer need to wear pressure suits in a century or two and water may start to flow and rain fall (Kunzig, 2010).

This is a 2021 presentation where Chris McKay talks to the Mars Society about how Mars can be terraformed. At the end he also talks about the bioethics of terraforming.

Video: Dr. Chris McKay - Terraforming Mars - 2021 Mars Society Virtual Convention

In a 2001 paper, McKay et al. estimate that it can be done with one Pa of super greenhouse gases in the atmosphere (1/101,325th of Earth's atmospheric pressure). To make this would need 245 power stations rated at half a gigawatt each running 24/7 for a century manufacturing super greenhouse gases from fluoride ores they would need to mine on Mars (McKay et al., 2001:104).

In a 2004 paper they found an optimal mix of four fluoride based greenhouse gases that reduced the amount needed to 0.2 Pa (Marinova et al., 2005). That suggests 50 power stations running continuously would be enough. They estimate that this needs 25,700 years of Earth's yearly production of these greenhouse gases for the first century. The greenhouse gases need to be sustained to keep Mars warm, which needs about 3 times Earth's yearly production from then on (Marinova et al., 2005).

This is based on the assumption that Mars still retains enough CO_2 for an atmosphere, and that it retains enough water too, all frozen in the regolith. Possible issues here are that much of the CO_2 likely got sequestered in carbonates, as has happened to nearly all the CO_2 on Earth. Terrestrial CO_2 is cycled through volcanoes but Mars has little by way of volcanic activity. The other issue is that the water could have got split by ionizing radiation and the hydrogen lost to space. We don't yet have good estimates of the inventories on Mars of these volatiles but McKay et al. argue that plausible modelling suggests that Mars has sufficient reserves available still in volatile form (McKay et al., 2001:97-8). Jakosky et al. caution that it's possible much of the early atmosphere was lost to space, and that the CO_2 adsorbed in the regolith couldn't be released easily under warming climate scenarios (Jakosky et al., 2017).

> *The CO_2 available for emplacement into the atmosphere is exceedingly small. Only the polar CO 2 ice is readily accessible. Even if a large non-polar subsurface reservoir of carbonates or adsorbed gas were available, it could only be released by*

heating the materials; global heating would require raising the surface temperature and allowing heat to conduct into the subsurface, an inherently long-term process. Adsorbed gas could be released, but would be limited by the establishment of a new equilibrium between the pore/atmospheric gas and adsorbed gas.

If their assumptions are correct, even after 1000 years there would only be 5% oxygen. Redwoods might be able to grow by then, but it would take many millennia to reach oxygen levels suitable for humans to breathe (Kunzig, 2010).

Meanwhile, says botanist James Graham of the University of Wisconsin, human colonists could seed the red rock with a succession of ecosystems—first bacteria and lichens, which survive in Antarctica, later mosses, and after a millennium or so, redwoods. Coaxing breathable oxygen levels out of those forests, though, could take many millennia.

These are grand scale projects that might be possible as we develop as a space faring civilization. However, for far less than the technology and investment needed for 50 large power stations running continuously on Mars and the mining industry on Mars for all the fluorine and other materials needed to make these super greenhouse gases, we could have a self sustaining atmosphere in a lunar cave, or a Stanford Torus. Such a project could also be achieved far faster, within a few years and lead on to many more such settlements. Also we can learn valuable lessons about issues that may arise when establishing novel self sustaining ecosystems from our attempts to set up smaller scale habitats. If something goes wrong in the balance of species in a space habitat, in the worst case, the atmosphere can be vented, the soil sterilized and the project started again. These lessons may be valuable if we ever do terraform Mars.

If our aim is to extend Earth’s biosphere into space, it can be done faster and more efficiently using space habitats, which eventually can lead to habitats with a total of a thousand times the living area of Earth, just from the materials in the asteroid belt (Heppenheimer, 1977:chapter 2) and with the vast Kuiper belt and Oort cloud available for future expansion.

We don’t need to be in a rush to colonize Mars or to decide in advance that this is what we are going to do. We have time to work through the issues on the Moon and elsewhere, in space habitats, to be prepared, if that is the eventual decision.

By being careful now to protect Earth and other planets in our solar system we prepare for a future where we learn to live sustainably in the galaxy just as we currently need to live sustainably on Earth. By establishing a precedent to live sustainably in the galaxy, and to respect and protect other forms of life in the forward direction where we find it, we also protect other life and civilizations from our descendants throughout the galaxy, and we also protect our own way of life in our solar system from them too, as they explore in turn and return to Earth. As humans diversify and evolve, and perhaps also modify themselves with genetic engineering, we could be so different as to be almost like an alien civilization for them, for our distant descendants a million years later. By setting a precedent to explore in a way that protects other alien civilizations, we also protect ourselves in the future. I explore that in my "Pale Blue Dot" preprint (Walker, n.d.).

If Mars turns out to have native life we can never return to Earth, we have seen that there is no shortage of frontiers in the solar system, and new places humans can explore. Perhaps in the future we settle in some of those places and perhaps introduce other terrestrial life in habitats we make self sustaining.

However, wherever we go, Earth will remain the planet we evolved on and the one we are adapted to. Jeff Bezos put it like this (Boyle, 2016):

> *We have looked at this solar system. We have sent probes to every planet in this solar system. And believe me, this is the best planet. There is no doubt this is the one that you want to protect. This is the jewel. We evolved here, we're kind of made for this planet. It's gorgeous, and we can use space to protect it.*

As we explore in space, many would say that we do need to protect Earth as our top priority, and indeed use space to protect it. As Carl Sagan put it (Sagan,1997)

> ***...There is nowhere else, at least in the near future, to which our species could migrate. Visit, yes. Settle, not yet. Like it or not, for the moment, the Earth is where we make our stand.***

All sections – for an outline of this paper

Titles of sections are like mini-abstracts and summarize the details of each section. For a first overview of this paper read the section titles.

The most important sections are the top level headers, in bold.

Contents

Supplementary Information

Start – all sections – end

Perseverance ground temperature

This is supplementary information for the section:

- Microbes from near the surface in Jezero crater would withstand temperatures varying from below -70 °C to above 15 °C in a single day – and major changes in humidity and pressure – this is likely to favour polyextremophiles – and martian life would likely be able to resist higher levels of stresses like UV, low humidity, vacuum, dessication, and ionizing radiation – which seems likely to make it easier not harder for them to survive on Earth

The ground temperature data for Perseverance mission sols 361 and 380 uses the data page; Welcome to the Mars 2020 Perseverance archive which you can browse online by clicking on the Calibrated Data Link or the Derived Data where appropriate, for ground temperature you use the calibrated data.

- ground temperature from the TIRS data for sol 361 range 198.82 °K to to 290.11 °K.
- ground temperature from the TIRS data for sol 380 range 208.1°K to 291.99 °K

Data files used:
Perseverance TIRS data for sol 361.
Hyperlink:
https://atmos.nmsu.edu/PDS/data/PDS4/Mars2020/mars2020_meda/data_calibrated_env/sol_0300_0419/sol_0361/WE__0361___________CAL_TIRS_______________P02.CSV
All the calibrated data for sol 361
Hyperlink:
https://atmos.nmsu.edu/PDS/data/PDS4/Mars2020/mars2020_meda/data_calibrated_env/sol_0300_0419/sol_0380/

Perseverance TIRS data for sol 380.
Hyperlink:
https://atmos.nmsu.edu/PDS/data/PDS4/Mars2020/mars2020_meda/data_calibrated_env/sol_0300_0419/sol_0361/WE__0361___________CAL_TIRS_______________P02.CSV
All the data for sol 380
Hyperlink:
https://atmos.nmsu.edu/PDS/data/PDS4/Mars2020/mars2020_meda/data_calibrated_env/sol_0300_0419/sol_0380/

Dauber et al's table of newly excavated craters on Mars has 32 craters with a measured depth of at least 1.9 meters, ranging up to 5.22 meters

This is supplementary information for the section:
This is from table S1 in the supporting information for (Daubar et al, 2014). They used the shadow measurement technique, and out of 209 craters measured, 32 are at least 1.9 meters in depth. These are the depths ordered with the deepest first.

5.22±0.21
5.07±0.16
4.96±0.06
4.25±0.02
4.08±0.03
3.89±0.02
3.84±0.04
3.78±0.1
3.64±0.15
3.63±0.14
3.61±0.1
3.45±0.1
3.3±0.03
3.17±0.13
3.12±0.11
2.88±0.03
2.87±0.15
2.76±0.05
2.73±0.19
2.48±0.17
2.31±0.04
2.29±0.05
2.28±0.08
2.26±0.04
2.23±0.21
2.17±0.1
2.13±0.13
2.09±0.06
2.04±0.15
1.99±0.01
1.98±0.09
1.95±0.03

New: extending the JAXA analysis to photosynthetic life on or near the surface of any Martian meteorites

This is supplementary information for the section:

- Any life in Martian meteorites DOES get here faster and better protected than samples returned from PHOBOS because Phobos samples survived ejection from Mars and spent hundreds of thousands of years getting sterilized on the surface of Phobos – so it is safe for Japan to return samples unsterilized without special precautions – and why this reasoning DOES NOT apply to samples from MARS

There may be a slight omission in the Phobos sample return discussion of the fireball of re-entry as their 10% figure is based on life that inhabits the interior rather than just the surface of rocks. (SSB, 2019 : 40). The astrobiologist Charles Cockell tested the blue-green algae chroococcidiopsis. When he attached it at a typical growing depth on a re-entry aeroshell, he found that not only the algae, but all its associated organics were destroyed. He concluded (Cockell, 2008)

> *... Thus, the planetary exchange of photosynthesis might not be impossible, but quite specific physical situations and/or evolutionary innovations are required to create conditions where a photosynthetic organism happens to be buried deep within a rock during ejection to survive atmospheric transit.*

The question here is, could there be viable photosynthetic life on the surface of Phobos, which can't get to Earth in a meteorite.

I.e. life that is

- on Phobos in a surface layer of a rock
- living in a rock layer that would be destroyed during the re-entry fireball if it got to Earth on a meteorite

First, it helps that some photosynthetic life near the surface of the ejected rocks may be sterilized or destroyed already by the fireball of exit from Mars on its way to Phobos.

However the details here are uncertain because the total mass ejected by the Zunil impact was about 30% of the mass of the atmosphere above it, which could be enough to shield some of the ejecta from aerodynamic heating (SSB, 2019 : 27). This would seem to leave a possibility that some photosynthetic life could survive on the surface of a rock on Phobos.

However, second, all our martian meteorites come from locations where photosynthesis is unlikely.

They were

- thrown into space by glancing collisions into the high altitude southern uplands (Tornabene et al, 2006), where the thin atmosphere makes ejection to Earth easier.
- come from at least 3 meters below the surface (Head et al, 2002:1355),

- anywhere below 12 centimetres has a uniform temperature of around -73°C (Möhlmann, 2005:figure 2).

Third, there is yet another twist here to look at if we want to be as thorough as possible.

- The remarkable blue-green algae chroococcidiopsis can use alternative metabolic pathways to grow underground without any light (Li et al, 2020) (Puente-Sánchez et al., 2018).
- So, martian life capable of photosynthesis is not impossible deep underground, but even then it would be using other metabolic pathways with no reason to live near the surface of a rock, in complete darkness.

Fourth, there is another possible exception, life can use thermal radiation from a deep sea hydrothermal vent for photosynthesis (Beatty et al, 2005). The most recent possibility for a hydrothermal system in the southern uplands on Mars might be the rootless cones (volcanic cones without a magma chamber below them) which may have had hydrothermal systems above 0 C for up to 1,300 years (Hamilton et al, 2010) possibly active as recently as less than 20 million years ago (Stacey, 2019).

However, these are very different conditions from a deep sea hydrothermal vent. For this exception we need:

- photosynthetic life using thermal radiation for photosynthesis could be found in the rootless cones in the last 20 million years
- be ejected to Earth,
- with the photosynthetic life only found near the surface of the ejected rocks,

That is what is needed for it to be sterilized in Earth’s atmosphere but potentially survive impact on Phobos. This may need expert review but on the face of it, it seems an unlikely scenario to the point of not realistic.

Then we don’t need to go further back than 20 million years, because any life from earlier impacts that might get into the surface samples from Phobos has had over 22.5% of many of its amino acids destroyed – this is calculated in the discussion of sterilization dose in:

- 500 million years of ionizing radiation would reduce a gram of amino acids to a milligram

So, the Phobos sample return analysis seems correct with this minor tweak to account for photosynthetic life.

The more eyes that look at these studies the better given how important it is to protect Earth’s biosphere.

If these arguments are correct, it may also be safe to send astronauts to Phobos so long as they sterilize any materials before contact from deep below the surface.

The issue for astronauts with deep subsurface samples is that there may be potential for viable life on Phobos buried deep after ancient larger impacts on Mars, which have been shielded ever since and are still viable, but can't get to Earth currently, and perhaps potentially could have ejected photosynthetic life from the surface of Mars which got to Phobos but couldn't survive the fireball of re-entry to Earth's atmosphere.

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> *On Earth, photosynthesis and respiration by living things cause tiny fluctuations in our planet's otherwise steady oxygen concentration. We shouldn't expect this on Mars, though. "That's far out," Telling says: Mars appears too inhospitable for a critical mass of life capable of sustaining either process. "It's almost certainly going to be a nonbiological chemical reaction."*
>
> *Trainer herself does not rule out a biological explanation, but nevertheless underscores its unlikeliness. "People in the community like to say that it will be the explanation of last resort, because that would be so monumental," she says. There are abiotic mechanisms aplenty, both known and unknown, to rule out first before leaping to any more sensational claims.*

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>
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In this experiment, survival of the Chroococcidiopsis strain occurred only with those cells that were mixed with martian regolith simulant and plated as thin layers (about 15–30 μm, corresponding to 4–5 cell layers).

… Our finding suggests that a putative microbial life-form at least as resistant to desiccation and radiation as the investigated desert cyanobacterium could withstand some exposure to UV on the martian surface.

… Our findings support the hypothesis that opportunistic colonization of protected niches on Mars, such as in fissures, cracks, and microcaves in rocks or soil, could have enabled life to remain viable while being transported to a new habitat

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The survival of dried cells of strain CCMEE 123 when exposed to 21 days of simulated martian conditions further supports the employment of hot desert strains of Chroococcidiopsis in future approaches to mimic endolithic martian exposure. It was previously reported that the survival of dried cells of Chroococcidiopsis sp. CCMEE 029 (Negev Desert) under simulant martian soil or gneiss was not affected by a 4 h exposure to unattenuated martian UV flux. This shows that in theory such organisms could survive

or even grow on Mars or Mars-like planets if they had a source of water and nutrients; thus they could be active in such environments (Cockell et al., 2005)

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>
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>
> *The fungus Aspergillus niger and the bacterium Staphylococcus capitis subsp. capitis were included in this study because they are human-associated and opportunistic pathogens, and have both been previously detected on the International Space Station (ISS). Thus, they are likely to travel to Mars in crewed space missions …. Moreover, spores from A. niger might resist space travel on the outside of a spacecraft; therefore, understanding their survival potential in a Mars-like environment is of interest to planetary protection.*

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Even in this dessicated place, she found life: photosynthetic bacteria that had made a home in tiny chambers within halite salt crystals. There's a small amount of water retained inside the halite and, at night, it cools down and condenses both on the walls of the chambers and on the surface of the organisms that are sitting there.

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> *In 1998 EN 1822 came into effect. This was the first standard, which established a filter classification system for HEPA filters based on filtration process theory. EN 1822 also introduced the evaluation criterion MPPS (Most Penetrating Particle Size). MPPS is the particle size at which the air filter has its lowest arrestance. Not just a whim of nature, MPPS relates directly to physical mechanisms in the filtration process.*
>
> *The U.S. takes a different approach for filter classification of HEPA filters. The mother of all test procedures for these filters in the U.S. is MIL-STD-282, which was introduced in 1956. Other test procedures include e.g. IEST-RP-CC001 and IEST-RP-CC007. Each test procedure specifies certain particle sizes at which efficiency is evaluated. Depending on the filter class evaluated, this is done at 0.3 µm, 0.1 - 0.2 µm or 0.2 - 0.3 µm.*

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into what is now known as the Rotunda. With Marsal and two other boys he explored the cave and discovered the paintings.

For many years a story was told in which it was said that Lascaux was discovered by Ravidat's dog, Robot. There is some basis for this, as during the first trip Ravidat had been drawn to the toppled pine hole by the barking of Robot who had become entangled in its brambly overgrowth. However, it appears that Robot was not around when the boys went down through the hole.

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> *Similarly, the top three most common human pathogens,* A. fumigatus, A. flavus *and* A. terreus*, do not group together in the* Aspergillus *family tree and all possess relatives that rarely, if ever, infect humans. This lack of association between lifestyle and evolutionary affinity is probably because many of the traits render fungi into potent pathogens, agricultural pests, or cell factories are likely features that are generally associated with the saprophytic lifestyle and selected for survival in conditions independent of their current roles in pathogenesis, pestilence, or biotechnology.*
>
> *The advent of* Aspergillus *genomes has also augmented studies on understanding the regulation of SM gene clusters. One of the most interesting recent developments is the discovery that the velvet family of proteins, together with the global regulator* laeA *[73], form a complex that links and coordinates SM production with morphological differentiation [75, 76], which is in turn activated when a highly conserved signal transduction module receives the appropriate external environmental signals (Figure 3) [77]. This coupling of SM with development presumably evolved because the protection offered by the deposition of SMs into the spores is vital to propagation [76]. In line with this hypothesis,* A. nidulans *mutants deficient in the production of SMs are less toxic to their insect predators than the wild-type [78]. However, SMs are not only important in predator avoidance; evidence that certain* Aspergillus *SM gene clusters are activated only when physically interacting with other microbes [79], that SMs provide a competitive advantage [80], as well as the discovery of self-protection genes nested within others [81], suggest that SMs are also likely to be critical in interactions between* Aspergillus *and other microbes.*
>
> *Additional support for the hypothesis that SMs are critical components of fungal–microbial interactions comes from a recent study aimed at identifying the molecular signature of domestication in* A. oryzae *[7], one of the two fungi used in the making of*

sake in the last few millennia in the Far East. During sake making, A. oryzae *is responsible for breaking down rice starch into simpler sugars, a process that occurs, to a large degree, in parallel with the conversion of sugars to alcohol by the brewer's yeast* Saccharomyces cerevisiae*. In contrast to its wild relative* A. flavus*, the entire SM profile of* A. oryzae *is dramatically downregulated when grown on rice, including the gene clusters responsible for the synthesis of the mycotoxins aflatoxin and cyclopiazonic acid [7]. Because aflatoxin, and presumably other SMs as well, is genotoxic to* S. cerevisiae *[82] and its presence during fermentation would affect yeast survival and, consequently, sake making, the domestication process may have converted* A. oryzae *into a microbe that is 'friendly' to its other microbial co-inhabitants.*

…

This dominance of A. fumigatus *[as a human pathogen] is likely due to ecological traits, such as the high prevalence and buoyancy of its spores in the environment [85], as well as genetic ones, such as the ability to grow well at 37°C and the coating of its spores with a hydrophobin that renders them immunologically inert [86]*

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and CB2000 were repaired, with the normal NotI banding pattern clearly visible in the pulsed-field gel after 2 hours in both cases. Visible genome restoration appeared to peak after 3 to 4 h. The increase in genomic DNA was not due to growth of undamaged survivors. No increase in bacterial cell mass was evident in the cultures until 8 h and 5 h for CB1000 and CB2000, respectively. These results indicate that the genomic DNA was repaired well before the initiation of normal genome replication and cell division.

QUOTE One single colony isolate was taken from each of these populations, generating purified strains designated CB2000, CB3000, and CB4000. The founder, like other E. coli K-12 strains, is quite sensitive to IR; exposure to 3,000 Gy gamma radiation (60Co; 19 Gy/min) reduced the viability 4 orders of magnitude compared to that of the unirradiated culture (Fig. (Fig.2).2). The D37 value [37% survival] for CB1000 was 1,100 Gy, whereas the D37 value for CB2000 and CB3000 was 2,000 Gy—approximately threefold less than the D37 value measured for actively growing cultures of Deinococcus radiodurans R1 (41). The D37 value for the founder was 730 Gy. Higher doses of IR revealed a major improvement in resistance. CB1000, CB2000, and CB3000 exhibited 1,500- to 4,500-fold increases in survival relative to the founder after exposure to 3,000 Gy (Fig. (Fig.2).2). CB4000 was approximately 10-fold less radioresistant than the other isolates.

QUOTE 20 iterative cycles of irradiation and outgrowth. The length of each exposure was adjusted to kill >99% of the population, with this dose increasing from 2,000 Gy for the first cycle to 10,000 Gy on the last cycle

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Improved instrumentation on rovers that might detect and identify a diversity of potential in situ biosignatures, including ancient organic molecular biosignatures, designed with the ability to differentiate biotic and abiotic signals in micro- or macrostructures. Instrumentation could also be better attuned to the unique complications of biosignature preservation on Mars (e.g., deeper drilling to access potentially better preserved

organics)

The fluorescence spectrometers on SHERLOC can detect condensed carbon and aromatic organics by deep UV-induced fluorescence, and SHERLOC's Raman spectrometer will allow classification of aromatic and aliphatic organics. Raman spectrometry can also be used to detect minerals relevant to aqueous chemistry. While these measurements would allow us to identify reduced carbon compounds, there may not be sufficient structural information to distinguish between a biological signal and extraterrestrial organic input.

A major knowledge gap that will directly impact our ability to choose an appropriate landing site is what terrestrial analog environments might look like—what the biosignature signals might be—if photosynthetic microorganisms had not evolved and instead the environments were only inhabited by chemosynthetic microorganisms

4.4. Strategies and priorities

In many of the environments discussed, there is a dichotomy between habitability and preservation—many of the conditions that make an environment more habitable are destructive to one or more of the biosignatures of interest. For example, fluid flow in the subsurface of hydrothermal environments helps create the redox gradients that support communities that inhabit the outflow channel. Fluids are also essential for lithification and the associated decrease in permeability essential for long-term preservation. Preservation is enhanced by rapid burial and mineral precipitation that encases and lithifies biological materials in less permeable matrices—in these cases, silica from hydrothermal environments, or silica-enriched aqueous environments, is an important material for preservation. However, these same fluids can degrade biosignatures such as mineralogy, chemistry, and micro- and macrostructures. One strategy for astrobiological exploration has to be to seek out a "sweet spot" where these two balance each other so that long-term preservation is possible. This sweet spot may occur as conditions change through time.

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>
> … *However, once CAPA becomes angioinvasive and produces positive serum GM, mortality is more than 80%, even if systemic antifungal therapy is provided*
>
> … *Using these variable criteria, a median incidence of 20.1% (range 1.6–38%) was reported in patients with COVID-19 acute respiratory failure requiring invasive ventilation … bringing the prevalence of CAPA down to about 10% among invasively ventilated patients with COVID-19. However, incidence continues to vary widely between ICUs, due to non-uniform approaches to COVID-19 treatments, different burdens of Aspergillus exposure and differing diagnostic algorithms as well as genetic predisposing risk factors*

… The combination of dexamethasone and tocilizumab, invasive ventilation and older age, have been reported as risk factors for developing CAPA

… Unlike influenza-associated pulmonary aspergillosis, CAPA develops later and is diagnosed a median of 8 days after ICU admission

… CAPA has been consistently associated with COVID-19 mortality rates of more than 50%

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…
Dike-induced melting of ground ice and hydrothermal circulation could generate favorable conditions for recent or even extant habitable environments in the subsurface. These environments would be analogous to locations on Earth where volcanic activity

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After 7 years of air-drying, Chroococcidiopsis not only avoided genome degradation but preserved at least a sub-set of mRNAs and 16S ribosomal RNA.

... In the present work, the occurrence of survivors in dried biofilms and dried-UV-irradiated biofilms was proved by growth after transfer into liquid BG-11 medium (not shown) and by INT reduction after 72 h of rewetting.

Reshaping the boundaries of Chroococcidiopsis desiccation and UV tolerance has implications in the search for extra-terrestrial life since it contributes to defining the habitability of Mars and planets orbiting other stars. In fact, the UV dose used here corresponds to that of a few hours at Mars's equator (Cockell et al., 2000). Hence, considering that survivors occurred in the bottom layers of the biofilms (Baqué et al., 2013), it might be hypothesized that if a biofilm life form ever appeared during Mars's climatic history, it might have been transported in a dried state under UV radiation, from niches that had become unfavorable to niches that were inhabitable (Westall et al., 2013). The reported survival also suggests that intense UV radiation fluxes would not prevent the presence of phototrophic biofilms or their colonizing of the landmass of other planets.

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47 mm of mercury in bars = 6.3%

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> *A critical nutrient to the expansion of both subsurface and surface life on any planet is the availability of nitrogen as an aqueous species. On Earth, microorganisms evolved the ability to fix N2 into ammonia with the development of nitrogenase to overcome this constraint. Nitrogenases, Nif proteins, are complex enzymes, utilizing iron, molybdenum, and/or vanadium, that exist in both bacterial and archaeal domains. Phylogenetic comparison of genes that comprise nitrogenases and a complement of proteins required*

for their regulation indicate that nitrogenases emerged in anoxic sulfidic environments on Earth within obligate anaerobic thermophilic methanogens and were transferred to obligate anaerobic clostridia (Boyd et al., 2015), both common subsurface microorganisms. As Nif proteins were adopted first by the aerobic diazotrophic lineage Actinobacteria and then by the more recently evolved aerobic Proteobacterial and Cyanobacterial lineages, the Nif protein suite became more complex to protect the core MoFe-bearing proteins from O2 (Boyd et al., 2015). Although it is not clear whether the emergence of the more complex protein occurred prior to or after the Great Oxidation Event, it is certain that the ancestral protein emerged in an anoxic environment when the demands for aqueous nitrogen species exceeded the abiotic supply. The implications for martian ecosystems are that nitrogenase would have also likely emerged within an anaerobic subsurface environment, not in the oxic surface environment.

Experiments on the effects of low pN2 on diazotrophic nitrogen-fixing soil bacteria have shown that they could grow in N2 partial pressures of 5 mbar but not 1 mbar (Klingler et al., 1989). This result suggests that further experiments on wild-type species are required to determine whether the evolution of pN2 in the martian atmosphere was a significant deterrent to the expansion of early life, especially after Mars lost most of its atmosphere. Analyses of the nitrogen budget and of nitrogen cycling from deep subsurface environments in South Africa indicate that the pN2 is higher at depth than on the surface, that most of this N2 originates from the rock formations through nitrogen cycling, and that N2 is being actively fixed in the subsurface by microbial communities (Silver et al., 2012; Lau et al., 2016b). Given the presence of a cryosphere barrier to diffusion on Mars, the nitrogen availability and perhaps even the pN2 of subsurface brines are likely to be higher there than on the martian surface.

…

Meter-sized Fe(II)-rich carbonate/iron oxide concretions (Fig. 4) are found in Jurassic sandstone deposits of southwest Colorado that were formed at hundreds of meters' depth between 2 and 0.5 Ma as the Colorado River Basin was uplifted (McBride et al., 2003; Loope et al., 2010). Similar-sized ferroan calcite and siderite concretions occur in Late Paleocene/Early Eocene Wasatch Group sandstones, and siderite nodule-bearing cores from the formation (Lorenz et al., 1996) yielded thermophilic Fe(III)-reducing bacteria that were capable of producing prodigious quantities of siderite (Roh et al., 2002). In subaqueous systems unconstrained by rock matrix, authigenic carbonate mounds at CH4 and hydrocarbon seeps, formed from carbon mobilized by methane- and alkane-oxidizing microorganisms (Greinert et al., 2001; Formolo et al., 2004; Ussler and Paull, 2008), can be hundreds of meters tall and more than a kilometer wide (Klaucke et al., 2008).

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…
"A single terrestrial microorganism reproducing as slowly as once a month on Mars would, in the absence of other ecological limitations, result in less than a decade in a microbial population of the Martian soil comparable to that of the Earth's. This is an example of heuristic interest only, but it does indicate that the errors in problems of planetary contamination may be extremely serious."

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> *…*
> *Overall, neither frost nor deliquesced brines correlate with RSL activity except for the absence of frost when RSL at Palikir crater are active. Note the RH [Relative Humidity] in the near surface is also poorly constrained as additional in-situ measurements are needed to better calibrate the mesoscale models.*
>
> *We hypothesize that the rough correspondence of RSL activity with warmer parts of the year at most sites may actually be due to seasonal variations of wind speed, direction, and turbulence. Our MRAMS modeling does not show a clear correlation between upslope potential sediment transport and RSL seasonality. However, it is possible that more detailed modeling (higher resolution, varying surface roughness, better PDF evaluation for wind gusts) could find a better correlation.*
> *…*
>
> *We also conclude that if the dry RSL mechanism is correct then locations with favorable RSL geomorphology that lack RSL may lack a combination of strong winds and sand grains that can easily saltate on Mars (~100 μm in diameter).*
> *…*
> *We found that at two of the three RSL sites deliquescence never occurs, and at the third site (Rauna crater) the stability of brines does not correlate with RSL activity. The existence of small amounts of surface brines that could possibly trigger RSL grain flows via efflorescence and associated changes in cohesion appears to be unlikely. Likewise, the absence of frost is only correlated with RSL activity at Palikir crater, thus frost is likely not affecting RSL triggering.*

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> *…*
> *Applied to Mars, wet streaks are inconsistent with the surface expression and dynamics of RSL. Wet streaks propagate and fade over multiple years, drain onto low angled slopes, and have a characteristic pattern of dark downhill and lateral edges. In contrast, RSL are seasonal features, terminate on angle-of-repose slopes, and typically appear monochromatic. These inconsistencies provide evidence against brine flow hypotheses of RSL formation.*

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>
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The cause, Legionella pneumophila, is a facultative, Gram-negative rod that is one of several human pathogens now known to be carried in the intracellular environments of protozoan hosts. L. pneumophila can also persist, even outside of any host, as part of biofilms.

In essence, all that a potentially infectious human pathogen needs to emerge and persist is to grow and live naturally under conditions that are similar to those that it might later encounter in a human host. On Mars, these conditions might be met in a particular niche within the extracellular environment of a biofilm, or within the intracellular environment of another single-celled Martian organism. It is important to note the numerous biofilms observed aboard the Mir space station, which were found on surfaces and within water plumbing. These films were often multi-species and included bacteria, fungi, and protozoa.

To be sure, the genetic similarity between humans and protozoa is much greater than could be expected between humans and the Martian host of a Martian microbe.

However, the L. pneumophila example does bring into question the rationale of the need for host-pathogen coevolution. Even in the context of a planetary bio-sphere that is limited to single-celled life, and even where there is unlikely to have been a co-evolution between agent and host organism, the possibility of infectious agents, even an invasive type, cannot be ruled out.

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