UNIVERSITY OF BATH

Nuffield Foundation

LUNAR MISSION ONE

The possible sources and risk of lunar contamination

By Samuel Thomsen

Abstract

In this article, the different types of biological contamination, biomixy, sapromixy, phagomixy and ecomixy, are defined. assembly Detection methods are explored, as are methods of sterilisation. The conditions present on the moon are looked at and assessed in relation to their threat and possible support to life. The probability that life could survive is explored, with research into the exposure of microorganisms to outer space being discussed. Different sources of contamination and the impact of contaminants are compared to the likelihood of contamination occurring. The effect of all these factors are considered and used to provide advice on what action lunar mission 1 should consider taking.

Aim

To identify the risk and sources of contamination and identify microorganisms which might be capable of surviving on the moon

Rationale/context

Findings from the investigation will be used to advise the Lunar mission 1 investigation on sources of contamination, risks of contamination, which microbes could possibly survive and whether it’s necessary to decontaminate any objects being sent to the moon.

Introduction

Lunar Mission 1 is a non-profit organisation, set up as a joint public and private sector endeavour which plans to launch an unmanned mission to the moon in 2024 and drill between 20 and 100m at the Shackleton crater (see figure 1). Once the borehole has been drilled, a time capsule will be placed in it which is expected to be left there for 1 billion years. The time capsule will contain an Archive of life, hair samples and media sent in from the general public. They hope to get people from every country in the world involved. It's plans are part of the space exploration road map.

The possible risks of contamination of the moon and what they would constitute will be assessed. The possible spread of microbes will also be researched and it may be attempted to find suitable model for it.

An attempt to identity the sources of contamination will be made. To do this, past findings involving microbes on the moon, experiments preformed in space on the survival of microbes and at microbes which could survive the conditions found on the moon (especially around the Shackleton crater), such as extremophiles, will be looked at.

How certain microbes could possibly survive on the lunar surface will be researched. This will be done by observing the adaptions they have to survive in extreme conditions, such as microbes found on and around geothermal vents at the bottom of the ocean which experience extreme cold and heat, similar to that of the moon.

In This investigation, for each stage, several secondary sources will be used to draw clear lines of thought, these lines of thought will be analysed and compared, then used to reach a conclusion on the best course of action regarding the possible sources and risk of lunar contamination in relation to lunar mission 1.

Additionally current procedures for sterilisation and the reasoning behind them, observing their advantages and limitations, will be researched. Possible improvements to existing techniques, looking at a range of other scenarios where sterilisation to avoid contamination is applied may then be suggested.

Ultimately, this investigation will try to outline and assess what the best possible procedure for Lunar Mission 1 to sterilise equipment and avoid contamination will be.

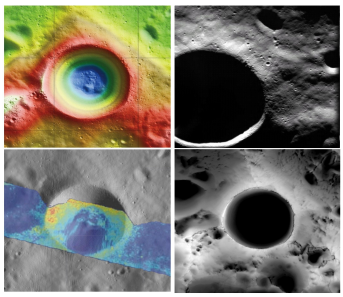

Figure 1: The shakleton crater, the proposed site of drilling

Methodology

The report will be structured as a literature review. Secondary sources will be searched for by using the internet, through online searches and directories like google scholar and researchgate, and the university library for physical copies. Secondary sources, for example articles and papers, will be collected from relevant sources, such as specific websites, ran by qualified organisations and individuals, and peer-reviewed, widely respected journals with high impact ratings. A clear line of argument will be identified in each data source and used to reach a conclusion, where possible.

Results and discussion

4 different types of contamination, each with specific risks where identified identified.[1] Sagan names and defines each as the following:

- Biomixy: The Moon may contain no indigenous living organisms, and may be incapable of supporting terrestrial organisms. Nevertheless, there may be relics of primitive indigenous organisms and deposited cosmobiota on or beneath the surface.

- Sapromixy: The Moon may contain no indigenous living organisms, and may be incapable of supporting terrestrial organisms. But subsurface prebiological organic matter may exist which would be indistinguishable from deposited terrestrial organic matter, either biological or abiological in origin.

- Phagomixy: The Moon may contain no indigenous living organisms, but may be capable of supporting some terrestrial organisms. This would require subsurface organic matter, moisture, and heat sources. The possibility then exists that a deposited terrestrial microorganism, in the absence of biological competitors or predators, will multiply at a geometric rate limited only by the availability of water and metabolites. Such a biological explosion might in a short time destroy large quantities of organic matter produced in the early history of the Moon.

- Ecomixy: The Moon may contain indigenous living organisms. There is then the possibility that deposited terrestrial microorganisms, by competition with or parasitism upon even one species of lunar organism, will disrupt the autochthonous ecology completely.

  Assembly and pre-launch contamination can be determined by the swab-rinse method[2]. This method, in addition to fallout strips and air samplers, was used during the construction of Apollo 6 to assess contamination in intramural environments used for the assembly and testing of the spacecraft.

The swab-rinse method was used to determine the surface contamination on Apollo command and lunar modules. Preliminary results indicated that microbial contamination accumulates on exposed stainless steel surfaces, there is airborne contamination in high bay areas and that unmanned spacecraft's have lower levels of contamination[2].

The swab-rinse method was performed by having 60 sterile steal strips exposed to air in the environment. Every week 6 strips where assayed by placing each bottle in 50ml peptone water (soluble protein solution). These bottles where then placed in a tank and insonated (exposed to ultrasonic energy). After insonation, 4 5ml portions from each bottle where collected and placed in agar. 2 agar plates where incubated aerobically and 2 anaerobically in Brewer anaerobic jars. The Interior sampling locations where areas most likely to be contaminated. In these locations, 20 swabs where taken each sampling period from the quadrants. NASA also currently uses black lights (see figure 2) to look for organics on object surfaces[3].

Figure 2: Black light contamination inspection for the Lunar Reconnaissance Orbiter (LRO) [3].

A sterilisation procedure that could be used is detailed by Nicks et al. and was used for the ranger missions[4]. The procedures adopted required most pieces of equipment to be sealed and heated to 125°C for 24 hours prior to being incorporated into the spacecraft. For elements that can't be heated, other techniques where used.

For example, aseptic assembly, use of special sterilants and environmental control the number of microbes. Once the spacecraft was assembled, it was soaked in an atmosphere of sterilising gas, 12% ethylene oxide and 88% feon (weight), for a minimum of 11 hours for a final surface sterilisation. However some of the components where wavered for sterilisation.

From their findings, Nicks et al. emphasise that the early design should select components which are compatible with the planned sterilisation methods to be used. The outside of the craft doesn't require sterilisation because the solar radiation will kill microbes on the spacecraft's shell[5].

The decontamination procedures for the ranger missions where made more stringent than necessary to develop the technology of decontaminating spacecrafts for later missions[4]. The reason for sterilisation, given by Nicks et al., is that it reduces sample contamination, since it localises any contamination to a small area on the moon and means the chance of microbe proliferation is very low.

The 1958 committee on contamination by extra-terrestrial exploration states sterilisation should be carried out only for practice in regards to the moon[5]. This is because Living cells can't survive on lunar surface due to a lack of water at a high vacuum. Though they argue that the level of macromolecule contamination should be limited. They propose this could be done by restricting landing sites, so future research isn't affected. This supports that sapromixy may be a possibility.

The validity of the committee's findings vary because they where published in a highly respected and ranking publication, the journal Nature, but the findings and conclusions where made before any extra-terrestrial exploration occurred. Overall the findings seem valid since they are corroborated by other sources.

The idea that there is a low probability of biological contamination on the moon is further supported by Nicks et al.[4] and Sagan[1]. Though it is believed that contamination has been the cause for several mission failures, such as the 2014 space X CRS-3[3].

Sagan states the 3 major hazards for survival of terrestrial life on the moon as being temperature, particulate (corpuscular) radiation and solar electromagnetic radiation[1]. In addition; the lack of oxygen, water, energy sources and nutrients prevents surface microbe reproduction.

The temperature is an issue due to the surface temperature ranging, during a lunar day and night, between 100 to -180°C. However at a depth of 0.5M, the temperature range is less extreme, between 0 to -70°C. In addition, the thermostability of microbes is improved in dry vacuum state. This means temperature may be less of an issue than other factors.

From the Equation: t = 214ap(D/l) [1 -e- (/P)pa P-' logio (No/N), which gives the time (s) which the population (No) of organisms receive a lethal dose of radiation, the survival time of exposed microbes on the surface can be calculated[1]. This indicates all microorganisms will be killed within a few hours by UV radiation on the surface.

However, underground microorganisms could survive. In addition the surface dust may shield a few microorganisms. This may be further enhanced by the depths proposed for lunar mission 1's bore hole, being 20 to 100m into the crust.

The moon surface is covered in a layer of regolith (See figure 3). Regolith is the equivalent of terrestrial soil on worlds without signs of surface solvents (i.e. water) and is the outermost rocky material. The moon has a thick layer, from several to over 10m in depth in the lunar Maria and highlands[6].

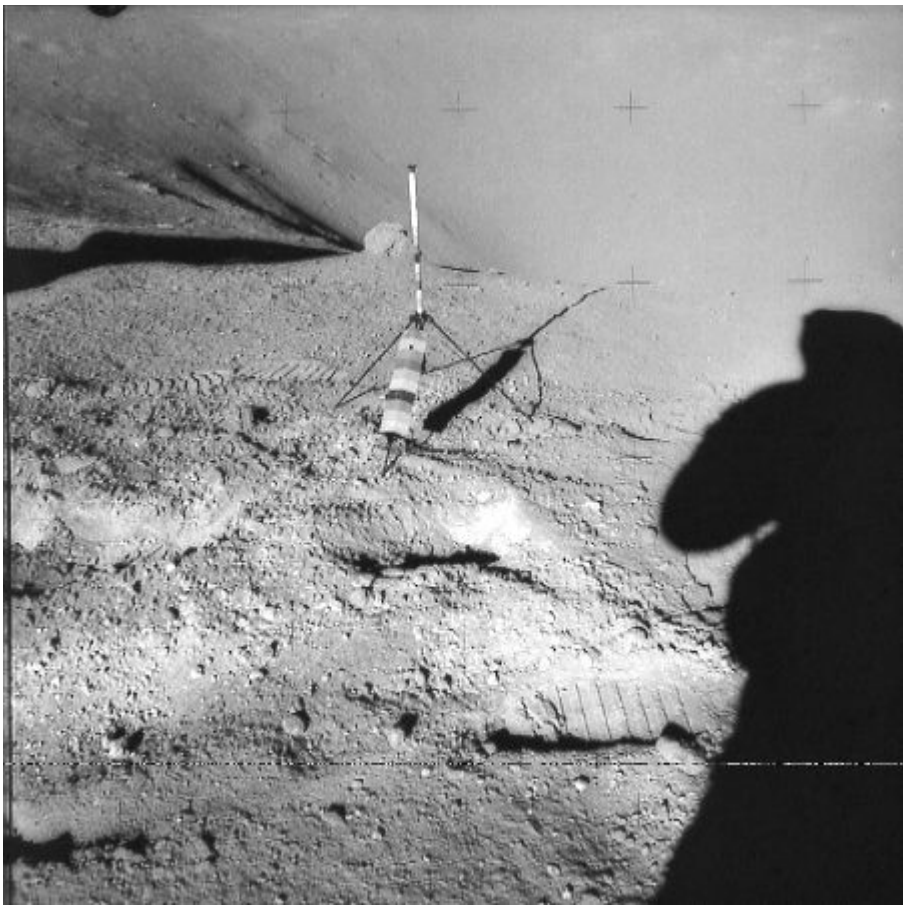

Figure 3: Apollo 16 astronaut footprint in regolith.

Non-living organic cell compounds, for example catalase, cytochromes, sulfhydryl compounds and photoreactivation mechanisms, could, due to their high levels of resistance to radiation, exist after the cell has died[1]. This could cause several processes to continue to occur. However the exact time that they would remain intact is unclear.

Within the regolith, amino acids have been detected in low concentrations, along with hydrocarbons and urea. The source of these has been under a lot of debate. From recent analysis, a large amount of the amino acids present seem to be from biological contamination. Though some appear to be indigenous to the moon. This appears to be direct evidence of sapromixy occurring, since the terrestrial amino samples seem to overwhelm the lunar native amino acids.

Looking at the thickness of the moons atmosphere and the strength of it's magnetic field, Sagan comes to the conclusion that the exposure levels are equivalent to that of free space for unprotected microorganisms[1].

Figure 4: A tardigrade, also known as a water bear.

Looking at life forms that could survive these conditions, the bacterium *Streptococcus* survived, inside the foam of a camera, lunar surface exposure[6]. Tardigrades (see figure 4), small animals approximately 1mm in size, have been exposed to open space, for example in the TARDIS experiment (see figure 5)[8], survived and even reproduced, resulting in eggs producing viable young, while exposed[9].

Tardigrades can survive for decades in a dessicated form, survive extreme temperatures, ranging from near -274°C to over 150°C and have a extreme resistance to radiation[8]. Tardigrades are also quite abundant and widespread. This makes it possible that they could easily come into contact with any objects being sent to the moon.

Figure 5: The foton-M3 capsule which orbited the tardigrades in open space for 12 days in the TARDIS experiment.

Many other microorganisms, fungi and plants have been exposed to open space, for example in the EXPOSE-R experiment, where biological specimens where exposed for over 22 months (see figure 6)[10]. Microbes shielded from direct UV exposure where capable of reproducing again when placed in favourable conditions. However the EXPOSE-R experiment found UV exposure adversely affects microbial activity. This was partly indicated by their lower resistance to antibiotics, making reducing the risk of them becoming pathogenic. Also no microbes, even in spores, survived complete exposure to UV radiation in space.

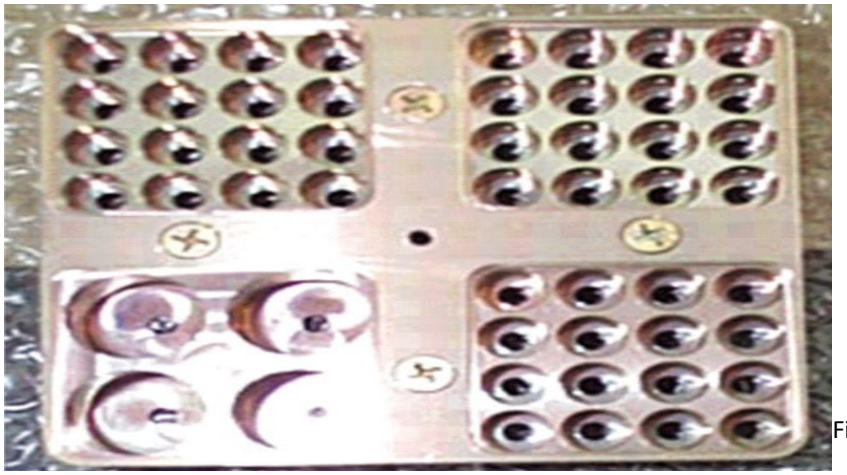

Figure 6: A sample tray from the EXPOSE-R experiment which was mounted to the international space station.

The EXPOSE-E experiment, which looks at the survival rate of *Bacillus* bacteria, further supports that full UV kills most microbes (see figure 7 and 8)[11]. Some of the samples where taken from an airlock in the spacecraft assembly facility, since they would be likely contaminants. Though, they did have some bacteria survive. They attested this to chance of the bacteria being in cracks in the spacecraft, which shielded them.

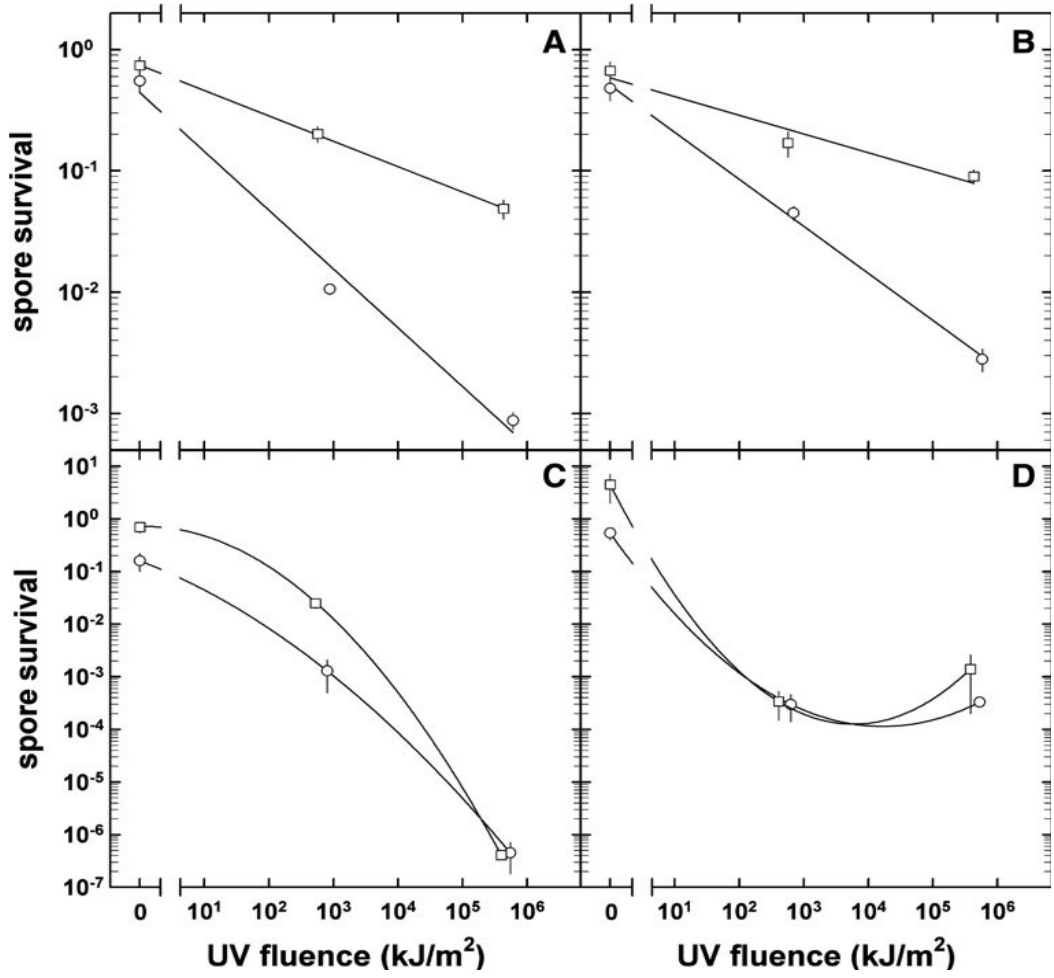

Figure 7: Graph showing the survival of bacteria spores at different UV levels in space. Circles generally had no protection while squares where partly shielded.

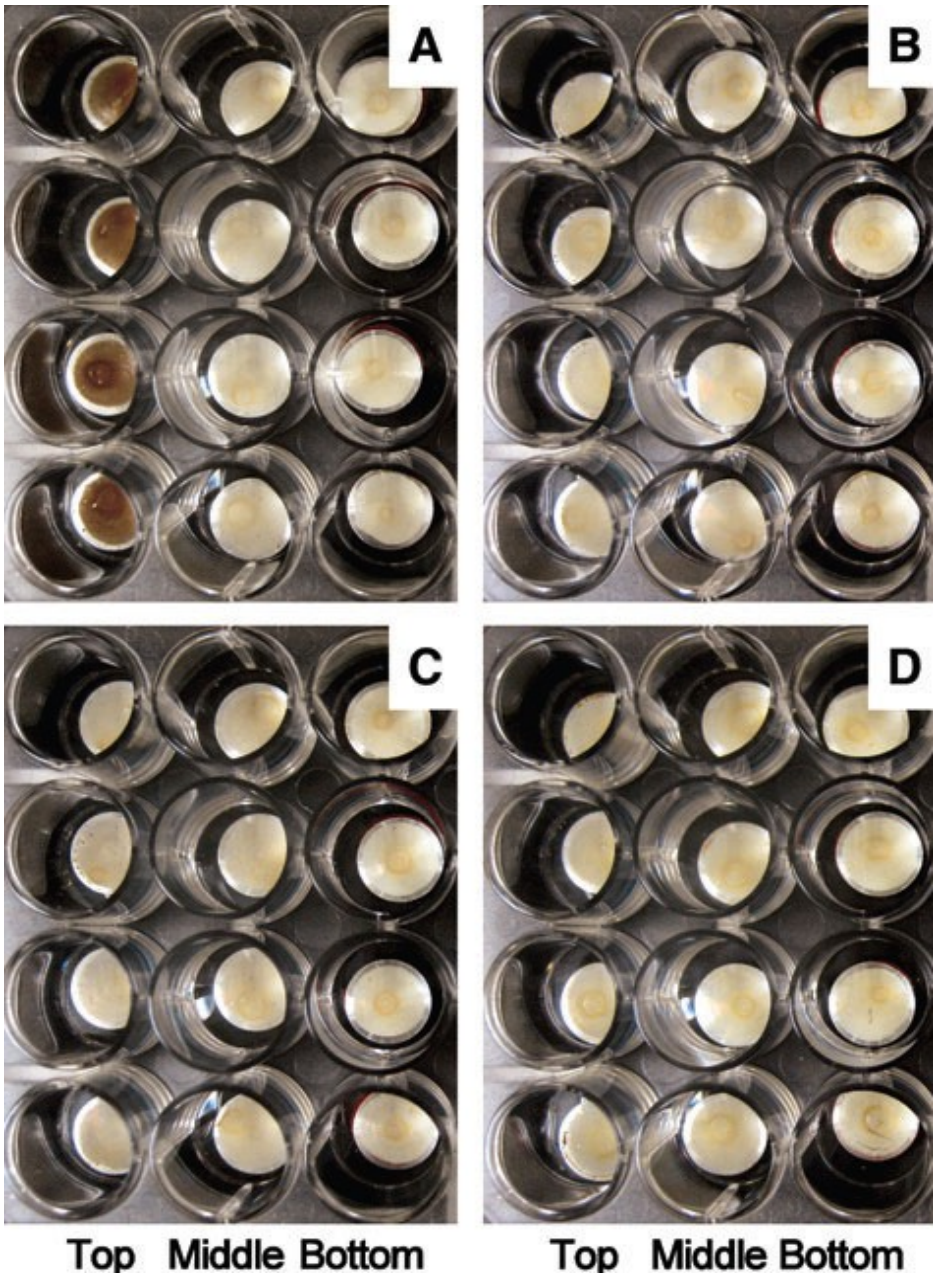

Figure 8: Photo of the multilayer of B. subtilis 168 spores on the aluminum coupon after spaceflight, taken during the deintegration of EXPOSE-E. (A) ‘‘Trip toMars’’ spores, full (100%) Sun exposure (k ‡ 110 nm); (B) ‘‘Trip to Mars’’ spores, attenuated (0.1%) solar radiation (k ‡ 110 nm); (C) ‘‘Stay on Mars’’ spores, full (100%) Sun exposure (k ‡ 200 nm); (D) ‘‘Stay on Mars’’ spores, attenuated (0.1%) solar radiation (k ‡ 200 nm).

A community of Bacteria in a subterranean Antarctic lake have been found, in high salinity and low temperature conditions[12]. These organisms appear to survive by exploiting the chemical reactions occurring between the brine solution and the sediment. This seems to supports that phagomixy may be possible, since it demonstrates microorganisms surviving in harsh conditions using the geology to grow. Though the lack of liquid water on the moon seems to mean this is improbable.

In Further support of phagomixy being a possibility, several species of mineral inhabiting microbes, such as halophilic bacteria, *Bacillus*, fungi, lichens and cyanobacteria, have survived in space, with *Bacillus subtilis* having survived for nearly 6 years in space[13]. Though these organisms are generally vulnerable to UV radiation, if unshielded, which further supporting Sagan's findings[1].

Sagan, in his summery, states the probability of 'explosive reproduction of terrestrial microorganisms in indigenous lunar organic matter' as 'remote'[1].

The validity of Sagan's paper varies across the content because, although it is a reliable source, being published in a peer-reviewed journal, and Sagan being a renowned scientist in the field, its age reduces its validity. This is because, at the time of writing, very little research had been done on the subject, with no manned moon mission having been completed, the first (Apollo 11) occurring in 1969. As a result several developments have been made in the field, making parts of the article, irrelevant or inaccurate. Sagan himself highlights that more research needs to be done on the microstructure of the Moon's surface needs to be done to corroborate part of his findings conclusion, stating it as being of 'great importance'. Considering this, while any findings proven wrong by later research should be disregarded, other findings, still found to be relevant should be considered valid.

Evaluation

The search method used was reliable since all the data used was collected from reliable sources, which where critiqued. This means a conclusion can be made form the findings. Being a literature review, no primary data was collected, making it difficult to investigate certain factors and that the direction of research was dependent on previous research available. A large number of the sources where not modern but from the mid 20th century, some before extra-terrestrial exploration had even began.

The Reason for large number of dated sources is due to little interest in The moon's biological potential since it's been largely established that it's incapable of supporting life. In addition, the sheer cost of sending missions to the moon, meaning few have occurred on the moon since the Apollo missions, has likely greatly restricted research in the area. Also, initially, there was only access to publicly available papers. This was because a profile and email for a academic institution wasn't available, meaning databases, such as research gate, couldn't be used to access most papers. Outside of the academic institution, even less was available due to not having an academic IP address which allows access to a greater range of literature at some websites.

Though, later, greater access to papers became available, due to having access to an academic IP address, in and out of the university, and being shown how to access databases from the university's library web page using provided logins and the university's IP address.

However this was still limited to what was available from the databases used like the web of science and what the university had licences and subscriptions to.

The effect of this was that not as many and a wide array of sources where used for the research than possibly could have been used. In future, the research could be improved by having direct communication with individuals working in the area. Since they could provide insight into recent findings and research.

Conclusion

While any introduced microbes will likely perish from UV radiation exposure, there is a remote chance, if no steps to decontaminate any objects going to the lunar surface, a suitably resilient microbe, capable at metabolising at low temperatures, could survive within the borehole. Though it is improbable it would be capable of reproducing. This could have future implications regarding the development of a lunar base near the area, due to the possibility of a Pathogenic microbe surviving in the area. Of greater probability, there could also be implications for research into the moon's lithology, regarding organic molecules, due to contamination of samples from the area. While the risk of contamination is low, Lunar Mission 1 is advised to take care to avoid the introduction of microbes onto any objects destined for the lunar surface.

Thus, it is formally recommended that decontamination should be considered, and carried out where possible, from the beginning, and at every stage, of the construction of the lander module.

Reference:

Figure 1: The shakleton crater, the proposed site of drilling

Source: lunar mission 1

Figure 2: Figure 2: Black light contamination inspection for the Lunar Reconnaissance Orbiter (LRO)

Source: 3 Skow, M. CONTAMINATION CONTROL AND POSSIBLE USE OF NDE TECHNIQUES. (2005)

Figure 3: Apollo 16 astronaut footprint in regolith

Source: Apollo image archive http://apollo.sese.asu.edu/LIW/20080826.html

Figure 4: A tardigrade, also known as a water bear

source: 9 BBC. Tardigrades return from the dead.

Figure 5: The foton-M3 capsule which orbited the tardigrades in open space for 12 days in the TARDIS experiment

source: 8 Tiny animals survive exposure to space.

Figure 6: A sample tray from the EXPOSE-R experiment which was mounted to the international space station

source: 10 Novikova, N. *et al.* Study of the effects of the outer space environment on dormant forms of microorganisms, fungi and plants in the 'expose-r' experiment.

Figure 7: Graph showing the survival of bacteria spores at different UV levels in space. Circles generally had no protection while squares where partly shielded

source: 11 Horneck, G. *et al.* Resistance of bacterial Endospores to outer space for planetary protection Purposes—Experiment PROTECT of the EXPOSE-E mission.

Figure 8:

source: 11 Horneck, G. *et al.* Resistance of bacterial Endospores to outer space for planetary protection Purposes—Experiment PROTECT of the EXPOSE-E mission.

1 Sagan, C. BIOLOGICAL CONTAMINATION OF THE MOON. Astronomy **46**, 396–402 (1960).

available from: http://europepmc.org/backend/ptpmcrender.fcgi?accid=PMC222850&blobtype=pdf

[date accessed: 1/8/16]

2 Puleo, J. R., Fields, N. D., Moore, B. & Graves, R. C. Microbial contamination associated with the Apollo 6 spacecraft during final assembly and testing. Space Life Sciences **2**, 48–56 (1970).

Available from:

http://download.springer.com/static/pdf/180/art%253A10.1007%252FBF00928955.pdf?originUrl=http%3A%2F%2Flink.springer.com%2Farticle%2F10.1007%2FBF00928955&token2=exp=1470062987~acl=%2Fstatic%2Fpdf%2F180%2Fart%25253A10.1007%25252FBF00928955.pdf%3ForiginUrl%3Dhttp%253A

%252F%252Flink.springer.com%252Farticle%252F10.1007%252FBF00928955*~hmac=83d780c79c7c1e9e133e69693eb3614a74f461b8585f2dfb3145ea45d792f999

[date accessed: 1/8/16]

3 Skow, M. CONTAMINATION CONTROL AND POSSIBLE USE OF NDE TECHNIQUES. (2005)

Available from:

http://www.nasa.gov/sites/default/files/files/M_Skow-Contamination_Control_and_Use_of_NDE_Techniques.pdf

[date accessed: 1/8/16]

notes: NASA use black lights to look for organics on surfaces.

4 Nicks, O. W. & Reynolds, O. E. Decontamination and sterilization of lunar and planetary spacecraft. *Letters* **142**, 539–540, 10.1126/science.142.3592.539, http://science.sciencemag.org/content/142/3592/539 (1963)

[date accessed: 1/8/16]

5 Contamination by extra-terrestrial exploration. *Nature* **183**, 925–928, 10.1038/183925a0 (1959)
available from: http://www.nature.com/nature/journal/v183/n4666/pdf/183925a0.pdf
[date accessed: 1/8/16]

6 Scharf, C. A. *Extrasolar planets and Astrobiology*. (University Science Books,U.S.: United States, 2008)

7 Elsila, J. E. *et al.* The origin of amino acids in lunar regolith samples. *Geochimica et Cosmochimica Acta***172**, 357–369, 10.1016/j.gca.2015.10.008, http://www.sciencedirect.com.ezproxy1.bath.ac.uk/science/article/pii/S0016703715005906 (2016)

[date accessed: 7/9/16]

8 Tiny animals survive exposure to space. http://www.esa.int/Our_Activities/Human_Spaceflight/Research/Tiny_animals_survive_exposure_to_space. (2008)

[date accessed: 1/8/16]

9 BBC. Tardigrades return from the dead. http://www.bbc.co.uk/earth/story/20150313-the-toughest-animals-on-earth. (2015)

[date accessed: 1/8/16]

10 Novikova, N. *et al.* Study of the effects of the outer space environment on dormant forms of microorganisms, fungi and plants in the 'expose-r' experiment. *International Journal of Astrobiology***14**, 137–142, http//dx..org/10.1017/S1473550414000731, https://www.researchgate.net/publication/271207691_Study_of_the_effects_of_the_outer_space_environment_on_dormant_forms_of_microorganisms_fungi_and_plants_in_the_%27Expose-R%27_experiment?enrichId=rgreq-11ce7090cf602152ea8b8c8684ee2436-XXX&enrichSource=Y292ZXJQYWdlOzI3MTIwNzY5MTtBUzoxODkzMjIxODM1ODU3OTRAMTQyMjE0OTMzNDk4Mw%3D%3D&el=1_x_3 (2015)

[date accessed: 7/9/16]

11 Horneck, G. *et al.* Resistance of bacterial Endospores to outer space for planetary protection Purposes—Experiment PROTECT of the EXPOSE-E mission. *Astrobiology* **12**, 445–456, 10.1089/ast.2011.0737 (2012)

available from: https://www.researchgate.net/publication/225288543_Resistance_of_bacterial_endospores_to_outer_space_for_planetary_protection_purposes-experiment_PROTECT_of_the_EXPOSE-E_Mission

[date accessed: 1/8/16]

12 NASA researchers discover ancient microbes in Antarctic lake. NASA (2012). at <http://www.nasa.gov/home/hqnews/2012/nov/HQ_12-408_Lake_Vida_Microbes.html>

available from:

http://www.nasa.gov/home/hqnews/2012/nov/HQ_12-408_Lake_Vida_Microbes.html

[date accessed: 1/8/16]

13 Cockell, C. S. Geomicrobiology beyond earth: Microbe–mineral interactions in space exploration and settlement. *Trends in Microbiology* **18**, 308–314, 10.1016/j.tim.2010.03.005,

http://www.sciencedirect.com.ezproxy1.bath.ac.uk/science/article/pii/S0966842X10000430 (2010)

[date accessed: 7/9/16]

Acknowledgements

I would like to thank the Nuffield research program, The University of Bath, particularly Sue, Gillian and Andrew and Lunar mission 1 particularly Paul Bennet.