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The transfer of unsterilized material from Mars to Phobos: Laboratory tests, modelling and statistical evaluation


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Abstract
Sample return missions to Phobos are the subject of future exploration plans. Given the proximity of Phobos to Mars, Mars' potential to have supported life, and the possibility of material transfer from Mars to Phobos, careful consideration of planetary protection is required. If life exists, or ever existed, on Mars, there is a possibility that material carrying organisms could be present on Phobos and be collected by a sample return mission such as the Japanese Martian Moons eXplorer (MMX). Here we describe laboratory experiments, theoretical modelling and statistical analysis undertaken to quantify whether the likelihood of a sample from Phobos material containing unsterilized material transferred from Mars is less than 10⁻⁶, the threshold to transition between restricted and unrestricted sample return classification for planetary protection. We have created heat, impact and radiation sterilization models based on the Phobos environment, and through statistical analyses investigated the level of sterilization expected for martian material transferred to Phobos. These analyses indicate that radiation is the major sterilization factor, sterilizing the Phobos surface over timescales of millions of years. The specific events of most relevance in the Phobos sample return context are the 'young' cratering events on Mars that result in Zunil-sized craters, which can emplace a large mass of martian material on Phobos, in a short period of time, thus inhibiting the effects of radiation sterilization. Major unknowns that cannot yet be constrained accurately enough are found to drive the results – the most critical being the determination of exact crater ages to statistical certainty, and the initial biological loading on Mars prior to transfer. We find that, when taking a conservative perspective and assuming the best-case scenario for organism survival, for a 100 g sample of the Phobos regolith to be below the planetary protection requirement for unrestricted sample return, the initial biological loading on Mars must be <8.2 × 10³ cfu kg⁻¹. For the planned MMX mission, a ~10 g sample to be obtained from a 25–30 mm diameter core as planned would require an initial martian biological loading to be <1.6 × 10⁴ cfu kg⁻¹, in order to remain compliant with the planetary protection threshold.

1. Introduction
1.1. Study context
The presence of martian material in Phobos' regolith is a key factor to consider during the process of classifying the planetary protection (PP) status of Phobos samples returned to Earth. The Martian Moons eXploration (MMX) mission by the Japanese Space Agency (JAXA) plans to return approximately 100 g (>10 g minimum) of material from the surface of Phobos to Earth in 2029, the analysis of which will contribute to determining the origin of the martian moon, and to understanding the evolution of the martian system (Usui et al., 2018). However, sample return missions have a strict need for PP classification to prevent both forward contamination of 'special regions' where life may be present, and backward contamination of the terrestrial system. In isolation, Phobos is not considered a body that could harbour (or could have harboured) life and is therefore not considered a special region, but there is a likelihood that martian material, ejected by
impact processes on Mars, could reach the orbit of Phobos and be emplaced on (and in) the Phobos regolith. Since Mars contains many special regions that could have been an abode for life in the past (e.g., Rummel et al., 2014), it is critical that Phobos sample return PP classification considers the transfer and sterilization of material from Mars to Phobos.

Previous studies have estimated that a 200 g regolith sample from Phobos could contain on the order of 50 mg of Mars surface material; upper estimates have pushed this martian component to 250 mg in a 200 g regolith sample (Ramsley and Head 2013). This martian ejecta material is likely to be across most of the Phobos surface and, once there, will undergo thermal processes through impact and sustained exposure to the solar and galactic radiation environment. Biological material transferred from Mars to Phobos is likely to be sterilized by such processes, however the degree of sterilization expected by these processes needs to be understood, in order to make a sensible judgement on the PP status of the returned sample.

In 2015, the European Space Agency (ESA) defined the “Sterilization limits for sample return planetary protection measures” study (SterLim) to evaluate the probability of collecting unsterilized martian material from the surface of Phobos. This study combined laboratory simulations of impact and radiation sterilization processes to define appropriate sterilization models, with modelling of impact process events and the radiation environment experienced at the surface of Phobos (and as a function of depth). These experimental and theoretical findings were then evaluated in a statistical model, to evaluate whether the probability of finding an unsterilized particle in a Phobos regolith sample is below the required PP threshold for restricted sample return status.

The driving requirement from ESA for investigating the potential planetary protection status of a Phobos sample return activity is described by one of many requirements for the study, namely Requirement-10 (Req-10):

The probability that a single unsterilized particle from Mars ≥10 nm in diameter is in a sample returned from Phobos shall be ≤ 1 x 10⁻⁶.

This requirement sets the context for this study and the subsequent recommendations for planetary protection status, and the experimental and modelling experiments were constructed in order to best address this. In the context of the MMX mission, we also assume the following in respect to the sampling process likely to collect return samples:

• Mass of sample: ~ 10 g (sampling area not known)
• Sample material is a mixture of fine powder to large grains.
• The material will only be obtained from the top “dry” layer of Phobos

1.2. Key processes

How martian material is transferred to Phobos, and how it is sterilized, are closely connected. The basic process by which martian material is transferred to Phobos is described in Ramsley and Head (2013) and shown in Fig. 1. Material (containing biological material) is naturally ejected, from Mars, as a result of a hypervelocity impact on Mars. The ejection velocity from Mars (ignoring rotational effects of Mars for simplicity) required to reach Phobos and Deimos is dependent on the ejection angle, ranging from 4.01 to 4.31 km s⁻¹ and 4.65 – 4.70 km s⁻¹ for Phobos and Deimos respectively. The ejected martian material, upon reaching Phobos, undergoes a second impact event, ejecting material (including of martian origin) from Phobos (in the first impact on Mars the martian material is the target, and in the second on Phobos the martian material becomes the projectile). This material enters orbit about Mars, forming a debris cloud, and its orbit crosses that of Phobos. During the period in the orbit round Mars (often lasting centuries) the material experiences the thermal and radiation environment of a martian orbit. Smaller particle sizes are removed from the orbiting debris cloud and re-enter Mars’ atmosphere or escape Mars orbit, and after some period, the material re-collides which Phobos. This process continues for most material, but a small amount achieves martian escape velocity (5.03 km s⁻¹) and leaves the system. Most material is eventually ejected below Phobos’ escape velocity, and rapidly falls to the surface and joins the Phobos regolith where it is then experiences Phobos’ thermal and radiation environment. A small proportion of this material may undergo many hypervelocity impacts on the Phobos surface, resulting in fast and transient ‘flash’ heating.

Given these processes, martian material should be distributed fairly uniformly across Phobos and a mission returning (material) from Phobos has a possibility of returning it. However, key for planetary protection is how sterile is such material is having been subjected to hypervelocity impacts, radiation and temperature environments. This defines the motivation of this work to investigate these processes both through tests and simulation (modelling).

1.3. Methodological approach

Closely coupled modelling and experiment is required to investigate the transfer of unsterilized material from Mars to Phobos. In this study, three sterilization processes were experimentally tested: thermal, radiation and impact. The results of these experiments are used to produce two sterilization models: one for thermal sterilization (relating to the impact process), and another for radiation sterilization (i.e., exposure to galactic cosmic rays (GCRs) and solar energetic particles (SEPs)). It is assumed that the primary mechanism for sterilization during impacts is due to the heating experienced, so both thermal and impact tests are performed to provide the necessary inputs for impact sterilisation modelling. The thermal, impact and radiation sterilisation models will vary depending on the organism type, due to the inherent varying resilience between organisms.

The fitting procedure of data to modelling is common for all testing and the possible models of sterilization are derived based on theoretical expectations. The models depend on unknown parameters and are fitted to the experimentally measured sterilization by optimising the parameters. Errors on the fitted parameters are derived and from the parameter errors, the 3 standard deviation (sd), or 99% confidence level, is established. When the model for sterilization is evaluated, the parameters used are taken from the 3sd/99% confidence level using a conservative value (i.e., least sterilization). For the thermal sterilization during impact there is inherent chaotic variability and different parameters are employed to give lower, middle and upper limits of the results. These models are then used to assess the sterilization of material transferred between Mars and its moons.

Some theoretical assumptions are fundamental to the approach taken in this study. Firstly, the martian moons are airless and dry, and it is not considered possible that they could have indigenous life. Secondly, material transferred from Mars could contain life and, if in spore form, this could remain viable on the moons for extended periods but, since the rate of transfer of material from Mars to its moons, is limited, the potential to transfer life is low. This potential is modelled as part of this study. Thirdly, material impacts the moon at high velocity and is exposed to natural radiation contributing to sterilization.

Impact and radiation sterilization have been tested experimentally on terrestrial analogues, as will be described in this paper. The sterilization measured in these tests is naturally limited to the total number of viable organisms that can be loaded on a substrate, and more importantly, the detectable level of life after sterilization. This limits the range of sterilization that can be achieved in a laboratory simulation; the ‘real world’ scenarios occurring on Phobos far exceed the domain in which terrestrial tests can be performed. Hence the extension of the laboratory measurements is achieved by modelling, with the model fit taken as conservative by being at a 99% confidence level.
2. Materials and methods

2.1. Biological models

Biological model organisms were required for the study following ESA-defined requirements. Four groups were identified: bacterial spores, simple organisms, *Deinococcus radiodurans* and resistant small viruses. For each of these groups, with the exception of *Deinococcus radiodurans*, a selection process was undertaken to identify the most appropriate organism for use to demonstrate resistance to each of the sterilization processes. Practical criteria were considered, for example, where organisms needed to be grown to a high enough titre for the substrate loading, and to be grown to these titres within the project’s timeline. Further, biological models needed to survive the desiccation process during test preparation procedures. The biological models were also required to be in risk group 1 to reduce any potential for infection of staff.

The organisms chosen as biological models for use in the study were: *Bacillus atrophaeus* endospores (DSM 2277, bacterial spores, also referred to as *B. atro*); *Deinococcus radiodurans* (DSM 20,539, also referred to as *D. rad*); *Brevundimonas diminuta* (DSM 1635, simple organisms, also referred to as *B. dim*) and MS2 E. coli phage (DSM 13,767, resistant small viruses, referred to as MS2).

2.1.1. Preparation of working suspensions

Creation of stocks of each biological model, kept on parent plates was performed by resuscitating freeze-dried vials according to suppliers’ instructions. Parent stocks were stored at 4 °C and used to create fresh, high titre working stock solutions.

*B. diminuta* and *D. radiodurans* were cultured by adding a 10 µl loop of growth from working stock plates to 100 ml broth (Tryptone Soya Broth (TSB) at 30 °C, and Tryptone Glucose Yeast extract (TGY) broth at 32 °C, respectively) and growing for 20–24 h in a 120 rpm dry shaking incubator. They were harvested by centrifugation (2000 × g for 20 min) and washing of the bacterial pellet in water twice. This method produced working stocks of 10^{10} cfu ml^{-1} for both biological models. *B. atrophaeus* spores were produced from parent stocks by adding a 10 µl loop of growth into 50 ml TSB and growing overnight at 37 °C in static conditions. 5 ml of this culture was then added to SPOX agar (sporulation agar: 3 g tryptone, 3 g yeast extract, 4 g nutrient broth No.2, 3 g peptone, 4 mg MnCl2·4H2O, 4 mg CaCl2·2 H2O, heated in 500 ml deionized water and added to 20 g agar, dissolved by steaming in 500 ml deionized water. This was then autoclaved at 121 °C for 15 min and place 150 ml in Roux bottles and left to set horizontally. Following horizontal incubation at 37 °C for 24 h, spores were harvested from the agar by adding 50 ml of sterile distilled water and carefully scraping the surface to disturb the growth. The washings were heat shocked at 60 °C for 30 min, then washed by centrifuging at 3000 × g for 20 min and washing of the pellet in water three times, producing working stocks of 10^{9} spores ml^{-1}. MS2 requires resuscitation of both the E. coli host and phage itself, to give parent stock plates of the host and parent stock solution of the phage. This was performed as per suppliers’ instructions. Working stocks of MS2 solution were created by inoculating 100 ml of sterile TSB in a baffled flask with a 10 µl loop of parent E. coli growth (less than 24 h old). This was placed into a dry shaking incubator and aerated for 150 min at 37 °C at 120 rpm, and 10^{11} plaque forming units (pfu) of parent MS2 solution was added and aerated for a further 4 h. The suspension was then centrifuged for 20 min at 2000 × g, and the supernatant transferred to a clean flask, to give a 10^{12} pfu ml^{-1} solution.

Once working stocks were produced, they were used with 3% Bovine Serum Albumin (BSA), added as a protective agent against desiccation. Addition of BSA increased survival rates of the control biological models to match the time scales of the testing regimes.
2.1.2. Growth and recovery validation in ideal conditions

Parent and working stocks, test samples and control samples were all enumerated for numbers of viable organisms in the same manner. The bacteria- and spore-containing samples were assayed by serial dilution, 1 in 10 in PBST (Phosphate buffered saline in 0.01% Tween), and 100 µl spread onto appropriate agar, incubated at the appropriate temperatures then cfu enumerated by eye. B. diminuta produced 2 mm cream cfu on TSA at 30 °C after 48 h, B. atrophaeus spores produced 3–4 mm orange cfu on TSA after overnight incubation at 37 °C and D. radiodurans produced 2 mm pink cfu on PCA (Plate count agar) after 3–5 days at 32 °C.

MS2 was assayed by co-incubation with its host overnight at 37 °C by creating a fresh E. coli stock plate (incubated on TSA at 37 °C for 19–20 h). A 10 µl loop of growth was transferred from the plate to 10 ml sterile nutrient broth and incubated for 4 h 20 min. Soft phage agar was melted, and then cooled to circa 45 °C just before use. 100 µl of the MS2 suspension to be assayed (a serial 1 in 10 dilution) was added to the molten soft phage agar with 3 drops of the E. coli suspension, mixed gently and poured onto a TSA plate. Following agar solidification and inversion of plates, plaques (areas of clearing in the lawn of E. coli growth) were countable the next day.

2.1.3. Validation of application and recovery from test substrates

High titre solutions with BSA were used to optimise and validate recovery of each organism from each test substrate used. All substrates were sterilized, placed in petri dishes and loaded with organisms in a ventilating Class II Biosafety cabinet for the minimum drying time of 60 min, before the lids were replaced and dishes placed in darkness at room temperature for the remaining time of the exposure. The delays were reflective of the required times estimated to create, test, transport and process the substrates within the test regimes.

The metal and basalt substrates were sterilized by autoclaving (121 °C for 15 min) and drying (at circa 40 °C for at least 6 h). The regolith was sterilized by heating in a drying oven at 120 °C for 24 h, or by gamma irradiation when facilities were available. Sterilization was performed in this manner prior to all testing regimes.

2.1.4. Transportation of samples between each facility

To ensure correction for losses during transportation of samples pre- and post-test to and from their respective facilities, transportation was validated.

For samples for heat testing, platinum filaments were loaded with 5 µl of the biological model working suspensions using a pipette within a laminar flow cabinet at the Open University (OU) then left to dry for 60 min. The Pt filaments were immediately placed into PBST, then couriered at both ambient and 4 °C to Public Health England (PHE) for processing. Survival rates of the heat-treated organisms in PBST over time were also investigated at 4- and 8-days post-test, with storage at 4 °C in between.

Aluminium strips for irradiation were loaded with 5 µl of the biological model working suspension using a pipette within a Class II biological safety cabinet at PHE with organisms and their top surfaces kept protected from physical damage that could result from the strips being fixed in their experiment mountings before being transported to the radiation facilities. As gamma irradiation takes place at −80 °C in thick-walled test chambers, the samples remain cold for hours after exposure. Therefore, the test chambers were removed from the radiation facility once exposed and brought to PHE as soon as logistically possible. They were then brought up to room temperature before being opened, the strips removed from the target benches, and processed within the same day.

Pre-drilled basalt projectiles were loaded with 0.5 µl of biological models using a pipette, allowed to dry within a Class II safety cabinet at PHE and then a further 0.5 µl of the biological model suspension was added which, again, was then left to dry before use. As impact samples pre- and post-testing were dry regolith or basalt, and not likely to degrade as liquid samples might (as verified by control tests), they were transported at room temperature immediately post-impact from the OU and processed as soon as they arrived at PHE.

2.1.5. Sterility validation

Blank substrates were assessed throughout the test regimes, to ensure levels of background organisms did not interfere with results and interpretation. During radiation exposure, Al strips were added to the test mountings that had no organisms on them, and processed alongside the test and control samples. During impact testing, blank projectiles were fired in the test set up and the resulting impacted regolith analysed. Swabs of the internal surfaces of the impact test facility were also periodically taken before and after cleaning, to ensure the test set up remained clean. During heat testing, blank Pt filaments were subjected to heat conditions and also used as witness strips within the laminar flow hood during testing. These were processed alongside the test samples.

2.2. Radiation exposure

2.2.1. Radiation test setup

For this study, in order to maintain a conservative approach to the radiation sterilization, only gamma radiation was considered (as opposed to including proton and alpha radiation). Proton and alpha radiation tests were conducted, but results were inconclusive due to inconsistency in the derived results, namely higher sterilization observed for D. radiodurans with increasing dose compared to other work. The source of this inconsistency remains under investigation, with one possible explanation being due to potential induced reactivity of the oxide layer of the aluminium substrate used for the experiments due to the use of proton/alpha particles, enhancing the sterilizing potential of the dose received. Whilst this observation means that the proton/alpha information cannot be considered in this study, it does offer a potentially interesting planetary protection consideration (i.e., enhanced radiation sterilization due to substrate type). For the purposes of this study, however, by considering only gamma radiation we ensure a conservative interpretation and thus appropriate precautionary margins for planetary protection purposes.

The gamma irradiation was performed at the Synergy Health Gamma Plant (UK). Experiments were conducted at −80 °C, a temperature between the thermal extremes experiences on Phobos (−130 °C to −2 °C, e.g., Giuranna et al., 2011). Pragmatically, this is also a feasible temperature at which to keep the experiment cool over the course of several days while the experiments take place (via dry ice replenishment); performing experiments across the entire temperature range experienced on Phobos is unpractical.

The organisms were deposited onto autoclaved, dried Al strips (Fig. 2(a)), and subsequently fixed onto a target bench (Fig. 2(b)) for the gamma irradiation. The gamma irradiation setup consisted of a DN63CF full nipple and two end flanges (Fig. 2(c)). One flange incorporated a gas feedthrough to allow backfilling with nitrogen to ensure an inert atmosphere as per ESA requirements. The second flange housed the target bench and incorporated a temperature feedthrough to allow temperature verification to be performed prior to the irradiation. The numbers of biological models loaded onto the aluminium strips in 5 µl aliquots (as detailed previously) were as per Table 2-1. Each gamma test chamber contained all 4 biological models, arranged on the target bench as shown in Fig. 3. The locations of the alanine dosimeters (TLDs) are also shown.

After sealing, each tube was backfilled with N₂ to provide an inert atmosphere, over-pressured to 1.1 bar and immediately vented to just over 1 bar to ensure a positive pressure. The test chambers were then placed in polystyrene boxes packed with dry ice shortly after assembly in order to achieve the required experimental temperature prior to exposure (−80 °C achieved after 3 h).

The planned dose levels for the exposures are shown in Table 2-2.
These doses were chosen in order to cover both low-level dose increments for less radiation resistant organisms, as well as very high doses for more radiation tolerant organisms.

The test chambers were taken to the test facility, and returned for processing according to Table 2-2. To prevent condensation forming on the inner surface of the tubes, post-irradiation they were allowed to warm to laboratory temperature for approximately four hours after removal from dry ice. All tubes were disassembled and the Al strips assayed, as previously described, on the same day as arrival. Alanine dosimeters were analysed under the same conditions.

### Radiation exposure modelling

The radiation simulation work was undertaken using the GRAS (Geant4 Radiation Analysis for Space) application (Santin et al., 2005; GRAS, 2015). Geant4 v10.1 patch 2 was used, with the following physics models as default:

- Fritiof (FTF) for high energies for ions with \( Z \geq 2 \), and the quark-gluon-string model (QGS) for high-energy protons and neutrons.
- Liege intranuclear cascade (INCL++) for intermediate energies.
- ParticleHP for low energies (particularly coupled neutron-\( \gamma \)-ray physics).
- Standard EM physics with options 3 (which are appropriate for dose-versus-depth calculations of ions).

The surface of Phobos was simulated as planar, and the volume of uniform density and composition. The simulated regolith composition is based primarily on carbonaceous chondrite composition, which is compositionally analogous with the Phobos regolith, specifically, the Tagish Lake meteorite (Mittlefehldt, 2002). The oxygen composition is based on the analysis of the Murchison meteorite (Jarosewich, 1971), Ti, Co, Mn from the analysis of Brown et al. (2000), Cr from Mittlefehldt (2002) and N from Pearson et al. (2006). With the exception of hydrogen, the exact elemental composition is not critical in defining radiation dose (Truscott et al., 2009). Hydrogen can cause strong thermalisation of the neutron flux but is not believed to be a significant constituent of the Phobos surface.

### Table 2-1

<table>
<thead>
<tr>
<th>Biological model</th>
<th>Gamma tests loading</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. atrophaeus</em></td>
<td>( 4.1 \times 10^8 ) cfu</td>
<td>( 6.36 \times 10^7 )</td>
</tr>
<tr>
<td><em>B. diminuta</em></td>
<td>( 7.5 \times 10^8 ) cfu</td>
<td>( 1.06 \times 10^7 )</td>
</tr>
<tr>
<td>MS2 phage</td>
<td>( 6.53 \times 10^8 ) cfu</td>
<td>( 7.42 \times 10^7 )</td>
</tr>
<tr>
<td><em>D. radiodurans</em></td>
<td>( 9.4 \times 10^7 ) cfu</td>
<td>( 1.77 \times 10^6 )</td>
</tr>
</tbody>
</table>

Note INCL++ transfers nucleus-nucleus collisions for \( A > 18 \) to the Binary Cascade model.

- ParticleHP for low energies (particularly coupled neutron-\( \gamma \)-ray physics).
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### Table 2-2

<table>
<thead>
<tr>
<th>Samples</th>
<th>Days since inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 kGy + control</td>
<td>2</td>
</tr>
<tr>
<td>3 kGy + control</td>
<td>2</td>
</tr>
<tr>
<td>10 kGy + control</td>
<td>4</td>
</tr>
<tr>
<td>25 kGy + control</td>
<td>4</td>
</tr>
<tr>
<td>50 kGy + control</td>
<td>7</td>
</tr>
</tbody>
</table>

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### Fig. 2

(a) Sample deposition strip, 40 × 5 mm. (b) Mounted target bench with organism-loaded strips and alanine dosimeter. (c) Some assembled tubes, sealed.

### Fig. 3

Placement of biological models and alanine dosimeters (TLDs) on each of eight test benches. Right-hand side attaches to copper block.
The GCR spectra have been defined for ions from protons to nickel based on the ISO Standard 15,390 model (ISO, 2004). The model includes changes due to the heliospheric potential, \( \Phi \), which varies as a function of solar activity and suppresses the low-energy component of the spectrum below 1 GeV/nuc when the \( \Phi \) is high. In order to account for the long-term variation of \( \Phi \), information on cosmogenic \(^{14}\)C and \(^{10}\)Be from Steinhilber et al. (2012) are used to weight the GCR spectra and radiation transport simulation results. Using this approach, the GCR environment is averaged over 9400 years.

The mean SEP proton and \( \alpha \)-particle environments below 300 MeV/nuc have been determined using all event data from the ESA SEPEM (Solar Energetic Particle Environment Model) reference datasets (SEPEM). The data are derived from carefully cross-calibrated GOES spacecraft measurements covering the time period 1974–2015, and energy from 5 to 300 MeV/nuc. To account for energies greater than 300 MeV/nuc, IPRAm data (Heynderickx et al., 2014) were used.

A correction based on SOLPENCO2 simulations was applied to account for the average heliolarodial dependence of the SEP flux between 1 AU and Mars (Heynderickx et al., 2014). Note that SOLPENCO2 models only the helioradial propagation of protons, and therefore we assume that the helioradial dependence is approximately the same for protons and \( \alpha \)-particles with the same MeV/nuc.

The analysis of the radiation exposure as a function of depth was performed based on two quantities:

- Total ionising dose (TID) per incident ion flux within each layer (dose units in Gy). The dose is calculated for the Phobos regolith, since the radiation field experienced by organisms should be dictated by the local environment.
- The flux of particles per incident ion flux at boundaries as a function of LET (in MeV cm\(^{-1}\)).
- The intervals in the depth and maximum depth at which energy deposition was recorded by the simulation were dependent upon the source spectrum being modelled.

For GCRs, which have very hard spectra and show less variation in the source spectrum being modelled.

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- The flux of particles per incident ion flux at boundaries as a function of LET (in MeV cm\(^{-1}\)).
- The intervals in the depth and maximum depth at which energy deposition was recorded by the simulation were dependent upon the source spectrum being modelled.

A primary requirement for simulating the impact process of martian ejecta material impacting Phobos is the capability to fire projectiles at a target over a range of impact velocities from a few hundred ms\(^{-1}\) to a few km s\(^{-1}\). To achieve this, the All Axis Light Gas Gun (AALGG) at the OU was used (Fig. 4). The AALGG is a two-stage gun capable of firing projectiles up to 3 mm in diameter into targets up to 50 cm in diameter.

To attain lower velocities (500–1000 m s\(^{-1}\)), the AALGG was used as a single stage gas gun, using a pressurised gas reservoir and burst disc to fire the projectile. This also requires the characterisation of different gases, gas pressures and burst disc construction.

For shots at \( \sim 1 \) km s\(^{-1}\), the AALGG was used in two-stage mode. The first stage consists of a piston accelerated by an explosive charge, which subsequently compresses a volume of a light gas (nitrogen or hydrogen). Once a threshold pressure is reached, a burst disc ruptures and the pressure pulse propels the projectile (up to 3 mm diameter) forwards at velocities of up to \( \sim 4.5 \) km s\(^{-1}\).

A range of impact angles can be invoked because the AALGG has a tilting frame, allowing firing of the LGG from its horizontal position through any angle to fully vertical. This uniquely allows impact experiments into loose material.

The projectile material used was a fine-grained Icelandic basalt (from the OU's rock store), representing the major martian crustal unit type. The block of basalt (200 \( \times \) 120 \( \times \) 120 mm) was cut into slices approximately 3 mm thick and cored using a 3 mm diamond core drill. The slices were cleaned using sterile water. A 0.75 mm diameter hole was then drilled on the smooth top face along the axis of the projectile (2 mm depth) to create a cavity for the biological sample to be loaded into (Fig. 5).

The specification of the target material for use in Phase 2 (hereafter referred to as 'Phobos-1C') is based, predominantly, on our limited knowledge of the Phobos regolith inferred composition. In the absence of a sample of the regolith, the composition of the Tagish lake carbonaceous chondrite was chosen as a compositional proxy of the Phobos regolith, as for the radiation modelling. An alternative Phobos regolith target was also developed (‘Phobos-1P’), which more accurately represented our best current knowledge of the physical properties of the Phobos regolith (as opposed to the composition). However, given that Phobos-1P was produced from a cement-based material and resulted in a highly alkaline suspension when mixed with recovery media, this inactivated the organisms. Phobos-1P was therefore not used in this study.

To adequately mimic the composition of the Phobos regolith, the composition of the Tagish lake meteorite was approximated by its main mineralogical components: anhydrous mineralogy, phyllosilicates and an organic component. However, is likely that agglutinates (and to some extent glass particulates) will be present in the Phobos regolith, which are not present within chondritic meteorites. These components are known to be generated in the lunar regolith via micrometeorite bombardment and an analogous process may occur on the Phobos surface; the presence of agglutinates on Phobos has not yet been confirmed but is assumed that the abundance of agglutinates is roughly equivalent to that in the lunar regolith. Hence, the target mineralogical composition of a Phobos regolith was determined to be: 46% anhydrous mineralogy; 35% phyllosilicate; 4% organic component; 15% pseudo-agglutinate.

Procurement of the feedstock materials and production of the simulant was undertaken at the United States Geological Survey (USGS) facility in the Denver Federal Center, Colorado (see Patel et al., 2019).

To minimise processing of the final simulant, the feedstock components were initially and individually milled to grain sizes coarser than 15 mm diameter. The components were then mixed and sieved into three size fractions: fine fraction (<425 \( \mu \)m); medium fraction (1.2–3.3 mm) and coarse fraction (>5 mm but <15 mm). For the tests reported here, the fine fraction was used.

The target containers (Al cans, 63 mm diameter) were autoclaved prior to use. 100 g of material was decanted into the container and baked in an oven for 1 h at 100 °C in order to remove any trapped volatiles. The target container was then placed within a secondary larger container. Prior to installation of the container, the area around the target location was lined with sterile foil, in order to catch any ejecta that exited beyond the secondary container.

For angled impact shots, given the different orientation, a modified can was needed to avoid interference of the container edges with the ejecta plume (Fig. 6). The modified can was then placed in a secondary, larger container and secured using an o-ring.

Once mounted, the chamber was closed and the facility pumped down and maintained at a pressure <133 Pa, and the shot performed. After the shot, the material within the container was sieved using a 0.5 mm sieve, in order to capture any projectile fragments that may not be retrieved by the sub-sampling recovery approach (see Section 3.3.4).

A comparison of the ejecta dynamics between experiment and modelled impact was used to check the overall correlation of test and model results. This was achieved through the use of high-speed imaging (FASTCAM SA-Z Model 2100 K M1 camera) to observe the evolution of...
the impact and ejector cone as a function of time.

The velocities tested are shown in Table 2-3, and the approach was to begin by testing the conservative case. Thus, the low speed shot (500–750 m s\(^{-1}\), minimum expected sterilization) was performed first. In order to avoid total sterilization (which, although informative, does not help specify model parameters), the second velocity range was intermediate (1–1.99 km/s). The final velocity was then defined to be faster or slower based dependent on sterilization results, and was chosen to be slower (0.75–0.99 km s\(^{-1}\)).

The non-normal impact angle was chosen to be approximately 30–40°, being close to the angle of repose of the target material and thus allowing an easier firing setup with the facility in a horizontal configuration.

### 2.3.2. Impact modelling approach

In order to apply the information gained from laboratory experiments to the ‘real world’ Phobos context, a modelling approach was adopted to experimentally establish a heat sterilization model (see Section 3.3.2) and, subsequently, a means of predicting the extent of heating experienced by the projectile during impact. Combining these then provides a prediction of impact sterilization. The study of the heating during impact was achieved by using the EDEN hydrocode (Arber et al., 2001; Longbottom et al., 2012; Milne et al., 2017), which is well suited to modelling the very short time transient effects of a high speed impact, particularly during the very early phases of the impact process until the material temperatures stabilise.

Impacts generates shock waves that propagate through materials leading to transient high temperatures and pressures over timescales that are of order 1 µs. This is followed by considerable motion, which begins excavation of the crater. Part of this process involves inelastic compressions and rarefactions that results in heating of target materials that will diffuse on much longer timescales. It is assumed that this heat is the main cause of the inactivation of the organisms.

#### Material models

There are two key materials that require suitable models, the basalt projectile and the regolith simulant. For the basalt we have employed the Mie–Gruneisen equation of state (Melosh 1989). This model is convenient for implementation but is also a reasonable representation of the actual material. Basalt is a crystalline rock composed of constituent minerals. The weakest link, whether this be the strength of the crystals or the bonds between them, will determine the strength of the material as a whole. When the stress wave reaches this magnitude, the material will breakup and then behave quite differently. In the model, this strength is uniform across the whole material but in reality there will be spatial variation in the strength of the material due to variations in the structure and the presence of relatively weak and

<table>
<thead>
<tr>
<th>Impact angle</th>
<th>Velocity range /km s(^{-1})</th>
<th>Range 1</th>
<th>Range 2</th>
<th>Range 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 to 0.75 km s(^{-1})</td>
<td>14 shots</td>
<td>14 shots</td>
<td>14 shots</td>
</tr>
<tr>
<td>0° (vertical)</td>
<td>1 to 1.99 km s(^{-1})</td>
<td>14 shots</td>
<td>14 shots</td>
<td>14 shots</td>
</tr>
<tr>
<td>– 30–40° (horizontal)</td>
<td>0.75–0.99 km s(^{-1})</td>
<td>14 shots</td>
<td>14 shots</td>
<td>14 shots</td>
</tr>
</tbody>
</table>

Fig. 4. All Axis Light Gas Gun in (left) vertical and (right) horizontal mode.

Fig. 5. Completed projectile.

Fig. 6. Modified target can used for angled impact tests.
2.3.3. Heat test setup

In order to support interpretation of the hypervelocity impact experiments and modelling of impact sterilization from a thermal perspective, heat tests were conducted. These used a Pyrolyser 2000 Filament Pulse Pyrolyser (Pyrolab, Sweden) in a Bassair HEPA-filtered laminar flow hood, within a restricted-access ISO Class 7 clean room at the OU. Resistive heating of a Pt filament was used to deliver a controllable pulse of heat to the filament and, thus, any material deposited on the filament.

The Pyrolyser system consists of a pyrolysis chamber, a Pt filament (5 × 25 mm) and a control unit with PC interface (Fig. 7). Filaments were unique to each test, autoclaved before use and calibrated via the manufacturer’s recommended method to assure temperature accuracy. The accuracy of calibration was assessed by running two blanks before each test. Prior to dispensing, the vial containing the biological models were heated resistively within the laminar flow cabinet and left to dry under clean room conditions. Samples of each biological model working suspension were applied to the centre of a Pt filament within the laminar flow cabinet and left to dry under clean room conditions. Samples of each biological model working suspension were applied to the centre of a Pt filament within the laminar flow cabinet and left to dry under clean room conditions.

After drying, the Pt filament was heated resistively within the pyrolysis chamber by applying a 50 A burst of current to achieve a rapid temperature rise to the desired value (Tmax). Heating was conducted in a constant He flow (6 ml min\(^{-1}\)) and chamber temperature (Tc) (50 °C). An all-glass impinger was connected to the Pyrolyser to collect exhaust gases, which were also transported for analysis. Further details regarding the test set up and operation can be found within Pearson et al. (2019).

The samples were heated for 0.125, 0.25, 0.5 and 1 s to temperature of 200–1000 °C (100 °C intervals). The precise Tmax achieved was recorded by a built-in photodiode (above 550 °C) and resistivity monitoring, outputted as a time-temperature profile. Experiments were carried out in triplicate.

All sample preparation and tests were conducted within the laminar hood, which was cleaned and sterilized before testing commenced and between tests, and all apparatus was autoclaved before use. Sterility was confirmed as described above. Samples (and witness strips) were transferred between test and processing sites by courier, with temperatures maintained at 4 °C. This process was validated prior to the experimental campaign to assure no degradation of samples.

2.4. Statistical model

2.4.1. Mars–Phobos transfer

The process through which material is transferred between Mars and Phobos follows several stages, the modelling of which is discussed in more detail in Summers (2019), and is summarised here.

Impacts on the martian surface result from impactors typically from the asteroid belt. The distribution of impact velocities is taken from Minton and Malhotra (2010) and is modelled as:

\[
dP = \frac{(v - a)\exp(-v/b)}{(b\exp(-b/a))} \frac{dv}{v}
\]

where \(a = 6500 \text{ m s}^{-1}\) and \(b = 3588 \text{ m s}^{-1}\) gives the best fit to Minton and Malhotra (2010).

Each martian impact creates a crater, the distribution of which (with size and time) is known from Hartmann and Daubar (2017).

This is modelled as:

\[
\frac{d}{dt} \frac{dn}{dA} = \left(10^{-5}/(s/[m])^2 + 10^{-6}/(s/[m])^3\right) \frac{[\text{craters/km}^2/m/y]}{(25^{0.25} - 0.5^{0.25})}
\]

which provides the best fit to the martian isochron in Hartmann and Daubar (2017).

These equations are used to predict the rate of mass ejection from Mars, both in terms of mass transferred and velocity ejected.

The mass velocity relationship is translated into a density of mass.
flowing out through a solid angle, at the radial distance of the Phobos orbit. This is then multiplied by the solid angle projection of Phobos, projected onto this sphere with the velocity of the ejecta at the orbit of Phobos. This gives a prediction for the mass transferred from the impact to Phobos, as a function of the angle between the crater and Phobos. The phasing of Phobos in its orbit with respect to the exact time of impact is unknown, but the Phobos orbit is equatorial. The location of craters on Mars are known, and this enables a model for the angle between the crater and Phobos to be formed.

The velocity of mass ejection from Mars is mapped to the velocity of mass arrival at Phobos using standard gravitational dynamics. This is then vector summed with the orbital velocity of Phobos, to give the velocity of impact on Phobos, which is then used in the impact modelling to ultimately give the impact sterilization.

2.4.2. Monte Carlo model

By following mass transfer between Mars and Phobos, the transfer of theoretical biological material can be followed and its sterilization modelled. A Monte Carlo simulation which follows mass is used, specifically calculating the integral of the differential mass with respect to the theoretical biological material can be followed and its sterilization modelled. A Monte Carlo simulation which follows mass is used, specifically calculating the integral of the differential mass with respect to the variables simulated.

The loading of biological material is unknown for Mars, so some assumptions need to be made about potential biological loading. We chose to use a Mars-analogue terrain and field site, the Atacama desert, in order to establish ranges of biological loading. Navarro-Gonzalez et al. (2003) reported the following levels of biological loading in the Atacama desert between sample points:

- In the driest area of Yungay, one in five measurements confirmed the presence of life.
- Where no life was detected, the resolution of the technique was assessed to be \(10^5\) cfu g\(^{-1}\).
- Where life was detected it was at a level of \(10^4\) cfu g\(^{-1}\).
- Elsewhere in the Atacama measurements up to \(10^7\) cfu g\(^{-1}\) were seen.

Hence, in this study, three example loadings of life have been considered, as shown in Table 2-4.

The depth to which material is deposited on Phobos is not clear and will be strongly dependent on the nature of the regolith and the size of the impactor – and these are not well known. In the impact on Mars, high pressures will be experienced that will fragment material. Also, some martian craters have secondary craters (observed in the rays from the primary crater), the size of which indicate than at least some of the ejecta from the primary crater must be of a reasonable size. Hence, there is no confidence on the size distribution of material ejected during a martian impact, and thus little knowledge on the size of impactors on Phobos.

Given the lack of knowledge of depth of deposition for martian material on Phobos, coupled with the need to take a conservative approach in the interests of PP classification, we assume an emplacement depth of 50 cm. This is the depth the martian material is deposited at, and is the maximum expected sampling depth that will be achieved by future missions. Between depths of 1 cm and 1 m, the radiation exposure is reasonably constant, so the depth of deposition within this range is expected to have little effect. Thus, considering sterilization of a depth of 50 cm satisfies the conservative approach. The period over which the radiation is considered is determined by the range of estimates of the relevant crater age on Mars.

All these considerations are combined to give the mass transfer from Mars, which remains unsterilized at the current time, and thus the biological material transfer to Phobos.

3. Results

3.1. Phobos radiation environment

Fig. 8 (left) shows the predicted annual ionising dose as a function of depth within the Phobos surface due to several of the major cosmic ray ion species (H, He, C, N, O, Mg, Si and Fe), as well as for ions heavier than nickel and the total dose from all ion species. For depths greater than \(\approx 0.5\) m and \(<6\) m, the reduction in dose is approximately exponential with depth (evident from the nearly straight-lines in the plots), but at shallow depths the ionising dose is relatively flat. For the protons at shallow depths, there is a slight increase in dose as a function of depth from slowing primary protons and secondary particle production (including nuclear fragments).

The uncertainties in the GCR doses shown in Fig. 8 have only been included for the total from all species, and combine the uncertainties in the external GCR environment with the errors introduced from the Monte Carlo (Poisson) statistics.

The softer SEP spectra means both the penetration of the primary particles in the generation/influence of secondary protons and neutrons from nuclear interactions is much lower than for the GCR case. Fig. 8 (right) therefore shows much more rapid reduction in the dose-versus-depth profile both due to protons and \(\alpha\)-particles, with the latter contributing between one and two orders of magnitude lower dose than protons. The combined effect of SEP protons and \(\alpha\)-particles dominates over the average GCR environment from all ion species up to depths of \(\approx 2\) cm into the soil.

At greater depth within the soil, the radioactive decays of \(^{232}\)Th, \(^{238}\)U, and \(^{40}\)K as well as their radioactive progeny are expected to become the major contributor to the ionising dose. Based on the abundance of these radioisotopes in the Tagish Lake sample, the combined effects of these primordial radionuclides is predicted to provide an annual dose \((1.3 \pm 0.2) \times 10^{-4}\) Gy yr\(^{-1}\), and therefore will begin to dominate below \(\approx 5\) m.

3.2. Gamma radiation test results

3.2.1. Doses received

The results for the TLD dosimeters within each radiation test chamber are shown in Table 3-1, including the mean internal dose rate. The target dose was marginally exceeded in the first four test chambers, but in general was on target.

3.2.2. Biological recoveries

Initial testing of the survival of the biological models after being dried onto aluminium coupons and stored at room temperature is shown in Table 3-2. The results show that after 30 min of storage there is an initial log reduction in recoverable numbers for all organisms ranging from \(-0.1\) log for \(D.\ radiodurans\) to \(-0.59\) log for \(B.\ diminuta\). The recoveries then decreased further for all of the biological models with the exception of \(B.\ atrophaeus\) spores that exhibited similar log

<table>
<thead>
<tr>
<th>Categories of biological loading considered for this study, taken from Navarro-Gonzalez et al. (2003).</th>
<th>Biological loading (cfu kg(^{-1}))</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>(10^5)</td>
<td>Compatible with measurement resolution in Yungay</td>
</tr>
<tr>
<td>Medium</td>
<td>(10^7)</td>
<td>Compatible with highest level of life seen in Yungay</td>
</tr>
<tr>
<td>High</td>
<td>(10^{10})</td>
<td>Compatible with highest level of life seen anywhere in the Atacama</td>
</tr>
</tbody>
</table>
reductions over the three sample time points (30 min, 7 days and 14 days).

Given that the radiation exposure experiments were being performed at −80 °C, the unirradiated control experiments were therefore held at the same temperature, to ensure as accurate a representation as possible.

The gamma irradiation results for log reduction of the recoveries of the *D. radiodurans*, MS2 coliphage and *B. diminuta* biological models using the cold positive controls are shown in Table 3-3. The results for *D. radiodurans* and MS2 show an increasing log reduction with greater exposure to gamma rays. *D. radiodurans* is inactivated at the 48. kGy dose. MS2 shows increased resistance to gamma irradiation in comparison to the other biological models, with only a 4.82 log reduction from the unexposed cold positive controls at a dose of 48.5 kGy. Low level recovery of *B. diminuta* is seen at 1.4 kGy but higher dosages than this completely inactivate the biological model, consistent with the heat and impact test results (see Sections 3.3.1 and 3.3.4) which demonstrate it is not a robust organism. Positive and negative controls showed the appropriate growth and negative growth of the correct biological model respectively.

### 3.2.3. Radiation sterilization models

The γ-ray irradiation results shown in Table 3-3 have been used to produce regression fits assuming an exponential-decay dependence between the surviving fraction of the microorganism, \(N/N_0\), and the dose \(D\) (in kGy):

\[
\frac{N}{N_0} = \exp(\lambda D)
\]

Here \(\lambda\) is a constant <0. Alternatively, it is convenient to define the log₁₀ reduction, \(R_{10}\):

\[
R_{10}(D) = \frac{D}{D_{10}}
\]

where \(D_{10}\) is the dose required to produce one order of magnitude reduction in the surviving fraction, and is given by:

\[
D_{10} = \frac{-\ln(10)}{\lambda}
\]

The calculation of the fit parameters, shown in bold text in Table 3-4, is based on all non-zero results for the relevant microorganism in Table 3-3, with the TID for each data point contributing equally to the fit, i.e., no weighting was applied to the data. Estimation of the uncertainties in \(\lambda\) and \(D_{10}\) is based on the standard equations for linear regression.

Fig. 9 shows the fits compared with the γ-ray results for each of the micro-organisms. The graphs also show the influence of ± 1σ and ± 3σ uncertainty in the mean fit based on the values for the uncertainties given in Table 3-4.

The γ-ray data for the *D. radiodurans* irradiation generally show a consistent trend with dose. However, the measurements at 1 kGy are unexpected, with more microorganisms appearing to survive after irradiation compared with the unirradiated positive. The first row in
From the text, it is evident that the research pertains to the gamma irradiation biological results of *D. radiodurans*. The passage further elaborates on the differences observed in survival rates after irradiation, attributing the variations to the careful measures taken in the test chamber post-irradiation, which could have resulted in an anomaly. The text also discusses the results of the experiment, highlighting the significant effort made to treat all samples in the same manner, despite the presence of an anomaly.

### Table 3-3: Gamma irradiation biological results.

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Unirradiated (control) cfu</th>
<th>Irradiated (test) cfu</th>
<th>log_{10} change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>sd</td>
<td>n</td>
</tr>
<tr>
<td><strong>D. radiodurans</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>7.98E + 07</td>
<td>2.84E + 06</td>
<td>3</td>
</tr>
<tr>
<td>3.8</td>
<td>7.48E + 07</td>
<td>4.86E + 06</td>
<td>3</td>
</tr>
<tr>
<td>13.3</td>
<td>8.77E + 07</td>
<td>1.06E + 07</td>
<td>3</td>
</tr>
<tr>
<td>27.9</td>
<td>8.12E + 07</td>
<td>1.14E + 07</td>
<td>3</td>
</tr>
<tr>
<td>48.5</td>
<td>8.13E + 07</td>
<td>&lt;1.11</td>
<td>0.00E + 00</td>
</tr>
<tr>
<td><strong>MS2 phage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>1.38E + 08</td>
<td>2.40E + 07</td>
<td>3</td>
</tr>
<tr>
<td>3.8</td>
<td>2.15E + 07</td>
<td>1.69E + 08</td>
<td>3</td>
</tr>
<tr>
<td>13.3</td>
<td>9.60E + 07</td>
<td>1.48E + 07</td>
<td>3</td>
</tr>
<tr>
<td>27.9</td>
<td>1.21E + 08</td>
<td>1.64E + 07</td>
<td>3</td>
</tr>
<tr>
<td>48.5</td>
<td>2.34E + 08</td>
<td>9.71E + 07</td>
<td>3</td>
</tr>
<tr>
<td><strong>R. diminuta</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>2.71E + 06</td>
<td>1.10E + 06</td>
<td>3</td>
</tr>
<tr>
<td>3.8</td>
<td>9.27E + 06</td>
<td>2.03E + 06</td>
<td>3</td>
</tr>
<tr>
<td>13.3</td>
<td>4.20E + 06</td>
<td>1.69E + 06</td>
<td>3</td>
</tr>
<tr>
<td>27.9</td>
<td>2.73E + 06</td>
<td>1.76E + 06</td>
<td>3</td>
</tr>
<tr>
<td>48.5</td>
<td>2.50E + 06</td>
<td>7.27E + 05</td>
<td>3</td>
</tr>
<tr>
<td><strong>B. atrophaeus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>1.61E + 08</td>
<td>1.20E + 07</td>
<td>3</td>
</tr>
<tr>
<td>3.8</td>
<td>1.37E + 08</td>
<td>3.04E + 07</td>
<td>2</td>
</tr>
<tr>
<td>13.3</td>
<td>1.61E + 08</td>
<td>1.20E + 07</td>
<td>3</td>
</tr>
<tr>
<td>27.9</td>
<td>1.37E + 08</td>
<td>3.04E + 07</td>
<td>2</td>
</tr>
<tr>
<td>48.5</td>
<td>1.37E + 08</td>
<td>3.04E + 07</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 3-4: Exponential fit and log_{10} reduction parameters based on γ-ray irradiation results.

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>λ [kGy⁻¹] (log. reduction)</th>
<th>D_{10} [kGy]</th>
<th>Correlation coefficient</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D. radiodurans</strong></td>
<td>−0.438 ± 0.027</td>
<td>5.26 ± 0.33</td>
<td>0.959</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−0.440 ± 0.028</td>
<td>5.23 ± 0.33</td>
<td>0.969</td>
<td>Excluding three results at 1 kGy where N/N_0 &gt; 1.0</td>
</tr>
<tr>
<td><strong>MS2 coliphage</strong></td>
<td>−0.226 ± 0.006</td>
<td>10.2 ± 0.3</td>
<td>0.990</td>
<td></td>
</tr>
<tr>
<td><strong>B. atrophaeus</strong></td>
<td>−1.330 ± 0.026</td>
<td>1.731 ± 0.033</td>
<td>0.997</td>
<td></td>
</tr>
<tr>
<td><strong>B. diminuta</strong></td>
<td>−10.4</td>
<td>0.22</td>
<td>(N/A)</td>
<td>Recommended worst case (“worst of 3 samples”)</td>
</tr>
<tr>
<td></td>
<td>−9.56</td>
<td>0.24</td>
<td>(N/A)</td>
<td></td>
</tr>
</tbody>
</table>
The two other samples from the 1 kGy irradiation showed no detectable cfu, and therefore the mean surviving fraction is $6.8 \times 10^{-7}$ and the uncertainty in the mean is $6.8 \times 10^{-7}$. Whilst Table 3-4 provides values for $\lambda$ and $D_{10}$ based on this mean surviving fraction at 1 kGy, for our analysis we use the fit to the single non-zero data point, giving a value of $D_{10} = 0.24$ kGy.

The radiation sterilization models used for the micro-organism in the remainder of this paper are based on the parameters identified in bold text in Table 3-4. Apart from $B. \text{diminuta}$, all the non-zero measurements of microorganism concentration have been used to generate the sterilization models. The maximum potential concentrations for the cases where no microorganisms were detected post-irradiation (marked in red text in the last column of Table 3-3) are consistent with the models based on the fits to the non-zero data.

- The detection limit for $D. \text{radiodurans}$ at 48.5 kGy ($\log_{10}[N/N_i] = -7.86$) exceeds the model fit results +1-sigma uncertainty ($\log_{10}[N/N_i] = -8.65$);
- For $B. \text{atropaheus}$, the detection limits ($\log_{10}[N/N_i] = -8.14$ at doses of 27.9 and 48.5 kGy) very significantly exceed the model fit results +3-sigma uncertainty ($\log_{10}[N/N_i] = -15.2$ and -26.4, respectively);
- For $B. \text{diminuta}$, the detection limits ($\log_{10}[N/N_i] = -6.92, -6.58, -6.39$ and -6.35, respectively at doses of 3.8, 13.3, 27.9 and 48.5 kGy) also significantly exceed the worst-case model fit results which are all $< -15.8$.

These results were therefore not included in Fig. 9 for reasons of clarity and relevance.

3.3. Heat and impact test results

The heat tests were conducted with heat pulses applied for 0.125, 0.25, 0.5 and 1 s. The actual temperatures attained for each organism as recorded by the photodiode and resistivity measurements, and each test duration, are shown in the biological recovery plots (Fig. 10).

3.3.1. Biological recoveries for heat tests

Table 3-5 shows that the $B. \text{atropaheus}$ spores and $D. \text{radiodurans}$ could be dried onto the Pt filaments and recovered consistently close to the starting concentration over the 24-h test period. The $B. \text{diminuta}$ showed an initial loss of viability within 30 min, with a further decrease of 1.6 logs by the 24-h timepoint. MS2 coliphage shows recovery similar to that initially loaded onto the filament but this then reduces after 6 and 24 h of storage. Due to these losses, all heat tests were performed within 30 min of drying of organisms onto platinum filaments. Organism recovery results in Fig. 10 were calculated against positive controls that had been dried for a comparable length of time, to account for any losses in viability due to drying.

The results from the heat tests for all organisms are shown in Fig. 10; each test is indicated by a datapoint. For $B. \text{atropaheus}$, the results show that at temperatures below 200 °C there is a negligible decrease in the survival fraction of the spores. Above this temperature, the survival fraction decreases linearly. The survival fraction of $D. \text{radiodurans}$ does not decrease until approximately 160 °C, above which there is rapid decrease in the survival fraction with increasing temperature. Survival fractions at the highest temperatures show a greater increase in variation. This organism shows a more rapid inactivation with temperature than the $B. \text{atropaheus}$ spores. Positive and negative controls showed the appropriate growth and negative growth of the correct biological model respectively.

The survival fraction of $B. \text{diminuta}$ is consistent between 50 °C and
125 °C; at temperatures above 150 °C the survival fractions rapidly decrease up to 250 °C at which point the testing was stopped because recoveries were close to and below the detection limits of the assay. The recovery of MS2 above 50 °C was similar to the starting concentration inoculated onto the Pt filaments; at temperatures of 125 °C and above, the survival fraction decreases in a linear pattern.

### 3.3.2. Heat sterilization model

The mathematical model is discussed in detail elsewhere (Pearson et al., 2019) but is briefly reprised here for convenience. We assume that the number of organisms (N) in a sample being sterilized by heat is given by the exponential decay law with a rate coefficient, k:

\[
\frac{dN}{dt} = -kN
\]

Commonly in the literature (e.g., Gottiparthi et al., 2014) a first order kinetics model is used for \( k \), which assumes the variation of the rate with temperature is given by:

\[
k = k_0 \exp\left(-\frac{b}{T}\right)
\]

where \( k_0 \) and \( b \) are constants to be determined. Given \( T \) as a function of time \( t \), the general solution for the exponential decay law with a rate coefficient, \( k \) is as follows,

\[
\ln\left(\frac{N}{N_0}\right) = -\int_0^\tau k_0 \exp\left(-\frac{b}{T(t)}\right) dt
\]

where \( N_0 \) and \( N_e \) are the initial and final organism numbers respectively and \( \tau \) is the end time of the experiment.

Because of the nature of the model, there is always a theoretically non-zero tail in the distribution and hence a non-zero sterilization rate. In addition, most cells have repair mechanisms that work to fix any damage that occurs to DNA. The fact that heat sterilization is possible at all indicates that, at moderate temperatures, the repair mechanisms can cope with the rate of damage but cannot mitigate the rate of damage at higher temperatures. For these reasons, a cut-off has been imposed with the sterilization model. All the experiments were performed with an ambient temperature of 50 °C and at this temperature no sterilization was seen, within statistical spread. For mathematical convenience, the model is set to a sterilization rate of 0 for all temperatures below 51 °C.

Using this model, any given experiment can be simulated provided the parameters \( k_0 \) and \( b \) are available. We determined these parameters for each organism by fitting the model to the experimental data, namely the temperature history. Due to the nature of the experimental procedure only an effective starting temperature is available for each of the heating tests, so a method was constructed for estimating the…

---

**Table 3-5**

Log reduction of biological models from platinum filaments after drying and storage for 30 min, 6 and 24 h, standard deviations are shown in brackets.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Log reduction of biological models</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 mins</td>
</tr>
<tr>
<td>Bacillus atrophaeus</td>
<td>−0.21 (0.03)</td>
</tr>
<tr>
<td>Brevundimonas diminuta</td>
<td>−0.32 (0.05)</td>
</tr>
<tr>
<td>Deinococcus radiodurans</td>
<td>−0.08 (0.12)</td>
</tr>
<tr>
<td>MS-2 coliphage</td>
<td>0.03 (0.04)</td>
</tr>
</tbody>
</table>

Fig. 10. Flash heating log reduction results for B. atrophaeus, D. radiodurans, B. dimunita and MS2.
temperature during cooling. The temperature data from the heat tests has been used to estimate the cooling rates for each organism.

**Model fitting**: The models were fitted to the data for each organism by using the least squares method. The results of the model fitting are summarised in Fig. 11. The fits are qualitatively satisfactory but where there is significant scatter in the higher temperature regions the model has been shifted slightly away from the apparent trend line at lower temperatures. We have no reason to believe that the scatter is not real so we must retain these data points with the consequences that go with it.

The *B. atrophaeus* fit is cleanest but this reflects the quality of the data for this case. This organism is also the hardest, as expected, being a spore. *B. atrophaeus* reaches a log sterilization of −10 at 500 °C whereas for the other organisms this occurs at 250–275 °C. The MS2 curve is relatively shallow whereas *B. diminuta* and *D. radiodurans* show much faster increase in sterilization with temperature. This is reflected in the size of the \( b \) parameter. That the three biological models, *B. diminuta*, *D. radiodurans* and MS2 behave similarly but are significantly less robust than *B. atrophaeus* is appropriate, given the distinct nature of *B. atrophaeus*.

### 3.3.3. Impact test summary

The impact test campaign was implemented first with the ‘low speed’ range shots (500–750 m s\(^{-1}\)), followed by the ‘faster’ velocity range (1–1.99 km s\(^{-1}\)). Given the observed recoveries from the ‘faster’ shots, we chose to concentrate on the intermediate velocities, in order to best characterise the sterilization relationship with velocity. The final velocity range was chosen to be 1–1.2 km s\(^{-1}\). The velocities recorded for each shot are shown in the biological recovery plots (Figs. 15–18).

### 3.3.4. Biological recoveries for impact tests

Validation of the organism survival on, and subsequent recovery from, the basalt projectile is demonstrated in Table 3-6. *D. radiodurans* shows the most consistent recovery from the basalt projectiles over the three timepoints of the validation study. *B. atrophaeus* spores loaded into the projectile showed some variability in recovery over the test period ranging from a log reduction of −0.37 after 110 min to an increase of 0.13 logs after 7 days of storage. Both *B. diminuta* and MS2 coliphage showed increasing reductions in the recovery of the time-points of the study from 110 min to 14 days. Positive and negative controls showed the appropriate growth and negative growth of the correct biological model respectively.

The large amount (>200 g) of regolith used as target material during each hypervelocity test meant that direct processing of the entire target for each impact was not possible with the available time and resources. Therefore, a sub-sampling recovery approach was devised to

---

**Table 3-6**

Log reduction of biological models from basalt projectiles after drying and storage for 110 min, 7 and 14 days, standard deviations are shown in brackets.

<table>
<thead>
<tr>
<th>Organism</th>
<th>110 min</th>
<th>7 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus atrophaeus</em></td>
<td>−0.37 (0.27)</td>
<td>0.13 (0.10)</td>
<td>−0.27 (0.06)</td>
</tr>
<tr>
<td><em>Brevundimonas diminuta</em></td>
<td>−0.23 (0.11)</td>
<td>−1.07 (0.90)</td>
<td>−1.25 (0.33)</td>
</tr>
<tr>
<td><em>Deinococcus radiodurans</em></td>
<td>0.14 (0.10)</td>
<td>−0.12 (0.09)</td>
<td>0.02 (0.14)</td>
</tr>
<tr>
<td>MS-2 coliphage</td>
<td>−0.16 (0.12)</td>
<td>−0.64 (0.18)</td>
<td>−1.05 (0.17)</td>
</tr>
</tbody>
</table>

---

**Fig. 11.** Heat sterilization for each organism and the fitted models.
allow for fewer sub-samples of the regolith to be processed. The sub-sampling approach was fully validated prior to implementation for the impact test experiments.

Validation included comparing recovery results of sub-sampled and whole-sampled inoculated regolith. This consisted of six 100 g pots of regolith, which were each inoculated with a known number of *B. atrophaeus* spores and left to dry for 1 h. Three of these 100 g samples were decanted entirely into 10 × 10 g fractions (designated "Whole"), with all 10 fractions processed for organisms as described below. The remaining three pots had five ×10 g fractions decanted from them and processed (designated "Sub"). The results in Table 3-7 show there is consistency between each fraction of the sample that is processed. The sub-sampling process provides repeatable recovery from each of the fractions. For sub-sampling, 0.2% was recovered (SD ± 0.646), compared to 1.92% recovery (SD ± 0.21) when the whole sample was processed. There was no significant difference in the variation between the two groups of sub samples when compared using the Mann-Whitney Rank Sum test (*P = 0.539*). Z scores for individual fractions show they don't deviate from the average of that repeat by more than 2 standard deviations for 43 of 45 fractions. The whole and sub-sampling recoveries also did not differ significantly from each other (*P = 0.539*).

Processing of the post-test regolith involved thoroughly mixing the entire sample, then subdividing it into weighed 10 g sub-samples. To a 10 g subsample, 20 ml of PBS-T was added, the tubes vortexed for 10 min and then left to settle for 20 min to ensure particulate settling. The supernatant was carefully removed by pipetting and transferred to a sterile labelled universal, and its volume recorded. To the remaining regolith slurry, 10 ml of fresh PBS-T was added and vortex mixed for 10 min and then left to settle for 20 min to ensure particulate settling. The mixture was then centrifuged for 30 s at an acceleration of 1000xg. The resulting supernatant was transferred into a sterile labelled universal by pipetting and the volume recorded. A final wash step was undertaken using the same procedure with 10 ml PBS-T. This method produced a sample of supernatant from each of the 3 washes of the regolith, for each of the sub-sampled portions. Four out of 10 sub-sample portions for the low and medium speed impacts were processed in this manner, and 3 out of 10 for the high-speed impacts.

The supernatants were then processed either by directly plating (without centrifugation and colony counting), or by filtration of the supernatant and then placing this filter onto growth media, in this manner, and 3 out of 10 for the high-speed impacts.

### Table 3-7

Sub-sampling validation. Percentage recoveries compared to known number of added spores, in triplicate for each experiment type. Z-scores for each sample are given in brackets (z-score is how many standard deviations a data point is from the repeat mean).

<table>
<thead>
<tr>
<th>Sub-fraction number</th>
<th>Whole.1</th>
<th>Whole.2</th>
<th>Whole.3</th>
<th>Sub.1</th>
<th>Sub.2</th>
<th>Sub.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.49 [−0.41]</td>
<td>1.74 [−0.16]</td>
<td>1.75 [−0.63]</td>
<td>1.11 [−0.87]</td>
<td>2.04 [−0.34]</td>
<td>2.99 [1.09]</td>
</tr>
<tr>
<td>2</td>
<td>2.02 [0.34]</td>
<td>1.70 [−0.23]</td>
<td>3.37 [1.82]</td>
<td>1.47 [0.86]</td>
<td>1.93 [−0.52]</td>
<td>2.32 [−0.44]</td>
</tr>
<tr>
<td>3</td>
<td>1.02 [−1.07]</td>
<td>1.89 [0.13]</td>
<td>2.23 [0.10]</td>
<td>1.15 [−0.69]</td>
<td>2.29 [0.04]</td>
<td>2.38 [−0.29]</td>
</tr>
<tr>
<td>4</td>
<td>1.84 [0.09]</td>
<td>1.42 [−0.76]</td>
<td>3.06 [1.35]</td>
<td>1.17 [−0.60]</td>
<td>1.70 [−0.86]</td>
<td>2.93 [0.93]</td>
</tr>
<tr>
<td>5</td>
<td>1.58 [−0.28]</td>
<td>3.22 [2.69]</td>
<td>2.37 [0.31]</td>
<td>1.55 [1.29]</td>
<td>3.36 [1.69]</td>
<td>1.94 [−1.29]</td>
</tr>
<tr>
<td>6</td>
<td>1.78 [0.00]</td>
<td>1.52 [−0.57]</td>
<td>2.49 [0.49]</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>7</td>
<td>1.84 [0.08]</td>
<td>1.68 [−0.28]</td>
<td>1.45 [−1.09]</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>8</td>
<td>1.26 [−0.73]</td>
<td>1.69 [−0.26]</td>
<td>1.65 [−0.79]</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>9</td>
<td>1.36 [−0.59]</td>
<td>1.42 [−0.77]</td>
<td>1.52 [−0.98]</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>10</td>
<td>3.61 [2.57]</td>
<td>1.93 [0.21]</td>
<td>1.80 [−0.57]</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Ave. recovery per rep. (sd)</td>
<td>1.78 (0.71)</td>
<td>1.82 (0.52)</td>
<td>2.17 (0.66)</td>
<td>1.29 (0.21)</td>
<td>2.26 (0.65)</td>
<td>2.51 (0.44)</td>
</tr>
<tr>
<td>Ave. recovery per entire sample (sd)</td>
<td>1.9 (0.2)</td>
<td>2.0 (0.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
impact velocities as high as ∼1.5 km s\(^{-1}\). This strength is too high for observed persistence of relatively large fragments of projectile found between the two. It can be seen that the ejecta curtain propagation is well matched from the simulation and the experiments are taken at the same times. Apart from Fig. 12(a), the frames of the simulations now show a good match to the tests. In Fig. 12, the simulations can also show these effects, and by modifying the parameters of the regolith model the timings can be adjusted. Having done this, the simulations can produce a sterilization model that is relatively simple to implement. For application of a sterilization model in a Monte Carlo analysis, it is highly desirable to produce a sterilization model that is relatively simple to implement. The use of hydrocode simulations in a Monte Carlo analysis is not realistic, and it is necessary to extract a simpler model from the hydrocode simulations to ensure that the statistical calculations are tractable.

Having already constructed a heat inactivation model, the next step was to determine how much heating the organisms receive. To this end, a number of simulations were carried out to determine the kinetic energy transferred to the organisms during the impact tests. The physical properties of the basalt at the walls of the cylindrical hole in the projectile were monitored during the modelling runs. Fig. 13 shows the specific internal energy at four points around the wall of the projectile hole. In the early stages of the impact there were some large spikes of heating, but these do not last and a steady increase of heat is indicated by the asymptotic internal energy reached at later times. It is notable that the final heat value is similar for all points.

Looking at the data over a range of impact speeds, the fraction of speeds used in the tests. This may be a side effect of the use of a fluid model for the regolith but use of a two-phase mixture would have been prohibitively expensive given the resources available. All the models are constrained to fit the experiments and thus they retain validity in this respect for the results presented here.

Fitting the model to the experimental data: For application of a sterilization model in a Monte Carlo analysis, it is highly desirable to produce a sterilization model that is relatively simple to implement. The use of hydrocode simulations in a Monte Carlo analysis is not realistic, and it is necessary to extract a simpler model from the hydrocode simulations to ensure that the statistical calculations are tractable.

Having already constructed a heat inactivation model, the next step was to determine how much heating the organisms receive. To this end, a number of simulations were carried out to determine the kinetic energy transferred to the organisms during the impact tests. The physical properties of the basalt at the walls of the cylindrical hole in the projectile were monitored during the modelling runs. Fig. 13 shows the specific internal energy at four points around the wall of the projectile hole. In the early stages of the impact there were some large spikes of heating, but these do not last and a steady increase of heat is indicated by the asymptotic internal energy reached at later times. It is notable that the final heat value is similar for all points.

Looking at the data over a range of impact speeds, the fraction of

---

**Table 3-8**

Hypervelocity impact test biological results for D. radiodurans. Text in red denotes tests where result was zero; in this case the limit of detection of the assay was used to calculate the minimum log\(_{10}\) reduction achieved. A result of 0 cfu per 1 ml (entirety of wash volume of projectile fragment) is recorded as a limit of detection of <1 cfu recovered in total, a value of 1 is used to allow calculation of log change in recovery, and marked in red. *4 replicates performed in error.

<table>
<thead>
<tr>
<th>PHE test ID</th>
<th>Unimpacted projectile control</th>
<th>Impacted sample</th>
<th>log(_{10}) change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Speed (km s(^{-1}))</td>
<td>Orientation</td>
<td>ave cfu</td>
</tr>
<tr>
<td>Low (−0.5)</td>
<td>1.07E + 07</td>
<td>Vert.</td>
<td>1.07E + 07</td>
</tr>
<tr>
<td></td>
<td>1.22E + 07</td>
<td>Horiz.</td>
<td>2.83E + 06</td>
</tr>
<tr>
<td>medium (−1.4)</td>
<td>1.03E + 07</td>
<td>Vert.</td>
<td>2.12E + 06</td>
</tr>
<tr>
<td></td>
<td>1.14E + 07</td>
<td>Horiz.</td>
<td>NA</td>
</tr>
<tr>
<td>high (−1.8)</td>
<td>1.21E + 07</td>
<td>Vert.</td>
<td>2.93E + 06</td>
</tr>
<tr>
<td></td>
<td>9.53E + 06</td>
<td>Horiz.</td>
<td>2.83E + 06</td>
</tr>
</tbody>
</table>

**Table 3-9**

Hypervelocity impact test biological results for B. diminuta. Text in red denotes tests where result was zero; in this case the limit of detection of the assay was used to calculate the minimum log\(_{10}\) reduction achieved. The lowest expected result from regolith washes is 1 cfu from one sub-sample portion (i.e.: 1 cfu found in one wash volume, 0 cfu found across the other wash volumes), therefore the lowest expected result from one medium velocity shot fired is 1 cfu ÷3 × 10, to give the number of expected cfu from all 10 portions, <3 cfu cfu. A value of 3.3 cfu is used to allow for calculations of log change in recovery, marked in red. *Medium vertical, for one replicate, regolith mass was only 80 g.

<table>
<thead>
<tr>
<th>PHE test ID</th>
<th>Unimpacted projectile control</th>
<th>Impacted sample</th>
<th>log(_{10}) change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Speed (km/s)</td>
<td>Orientation</td>
<td>ave cfu</td>
</tr>
<tr>
<td>Low (−0.5)</td>
<td>1.26E + 07</td>
<td>Vert.</td>
<td>1.26E + 07</td>
</tr>
<tr>
<td></td>
<td>8.48E + 06</td>
<td>Horiz.</td>
<td>3.85E + 06</td>
</tr>
<tr>
<td>Medium (−1.2)</td>
<td>3.00E + 06</td>
<td>Vert.</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>2.35E + 06</td>
<td>Horiz.</td>
<td>N/A</td>
</tr>
<tr>
<td>High (−1.8)</td>
<td>3.45E + 07</td>
<td>Vert.</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>1.74E + 07</td>
<td>Horiz.</td>
<td>4.31E + 06</td>
</tr>
</tbody>
</table>

---

**Gottiparthi et al. (2014).**

Given the dominance of heat as the sterilization mechanism, constructing a model for impact effects on organisms is a way to predict the rate of kill for each species at each temperature. This model is obtained from the heat test results and covered in Section 3.3.2. To complete the model, it is necessary to determine the temperature history experienced by the organisms during the impact, through hydrodynamic simulations of the impact tests.

Hydrodynamic model of impact tests: To validate and calibrate the models employed, some comparisons have been made between the simulations and the tests. Using high-speed cameras to capture the dynamics of the impact process, the speed of propagation of the expanding crown can be seen as well as the form of the ejecta curtain. The simulations can also show these effects, and by modifying the parameters of the regolith model the timings can be adjusted. Having done this, the simulations now show a good match to the tests. In Fig. 12, the simulations and the tests are compared. Apart from Fig. 12(a), the frames from the simulation and the experiments are taken at the same times and it can be seen that the ejecta curtain propagation is well matched between the two.

The strength of the basalt projectile was set to 12 GPa based on the specific internal energy at four points around the wall of the cylindrical hole in the projectile were monitored during the modelling runs. Fig. 13 shows the energy transferred to the organisms during the impact tests. The physical properties of the basalt at the walls of the cylindrical hole in the projectile were monitored during the modelling runs. Fig. 13 shows the specific internal energy at four points around the wall of the projectile hole. In the early stages of the impact there were some large spikes of heating, but these do not last and a steady increase of heat is indicated by the asymptotic internal energy reached at later times. It is notable that the final heat value is similar for all points.
pre-impact kinetic energy density converted to heat (the light blue line in Fig. 14) averages at \(\sim 6 \pm 2\%\) at low speeds. At higher speeds this changes to 10 ± 5% and shows much greater variability. The change in behaviour corresponds to the impact speed regime where the projectile breaks up. When the projectile is more completely broken up it is able to follow the flow of the regolith. This results in a more chaotic form of motion and hence greater variability in the end result. For the purposes of constructing an easily computed model, the mean value is adopted with a linear transition from low-speed value to high-speed. This accounts for the fact that the model projectile will entirely fragment at one impact speed whereas the test basalt projectile becomes progressively more fragmented as the impact speed rises.

Following the injection of heat during an impact, the next step is to estimate the cooling rate, \(\beta\), for the organisms. The modelling establishes the starting temperature, but the rate of cooling may have an influence over the sterilization process. This rate of cooling is extremely complex to model, and is likely to have significant dependence on the thickness of the layer of cells, what they are attached to and the surrounding conditions etc. The cooling rates determined from the heat sterilization tests are not applicable to the impacts due to the difference in physical properties and scaling, so for the purposes of this work the cooling rate was treated as an unknown parameter that was varied to obtain the best fit to the data. This has been performed for each or- iginating condition etc. The cooling rates determined from the heat sterilization tests are not applicable to the impacts due to the difference in physical properties and scaling, so for the purposes of this work the cooling rate was treated as an unknown parameter that was varied to obtain the best fit to the data. This has been performed for each or-

The results of the models are shown in Figs. 15–18. In these plots, the resulting model is plotted as the centre line with the upper and lower lines being based on the ± 1 variance of the energy transfer fraction and the outer curves are for energy transfers that are ± 1 variation as per Fig. 14.

The level of scatter in the experimental results makes interpretation difficult. It is reassuring, however, to see that a definite trend is observable, and that the variability seems to be inherent in the nature of the chaotic impact process. Prediction of results from any single impact is meaningless though, so a statistical approach that considers all of the results was required (see Section 4).

It is noticeable that the test results show no discernible difference between the vertical and horizontal (\(\sim 40\%) shots. It is likely that this is only significant for shallower angles. Another factor that is apparent is that the tests where fragments were recovered are mainly those at the slowest speeds. However, this is by no means consistent and there are cases when fragmentation occurs at high speeds and high levels of sterilization.

The fitting of the model is done by first determining the offset due to bio-assay losses based on data points for the low velocities around 500 m s\(^{-1}\) when no sterilization is expected. Outliers are excluded since these almost certainly are the result of viable organic material that has not been accounted for. The cooling rate is then adjusted until the model fits the data in the sense that it passes through the data points evenly. Effectively this a least squares fit performed manually rather than by calculation, since in this case a numerical method was not found to provide significantly more accuracy given the large variability. The resulting model is plotted as the centre line with the upper and lower lines being based on the ± 1 variance of the energy transfer function.

### Table 3-11

<table>
<thead>
<tr>
<th>PHE test ID</th>
<th>Unimpacted projectile control</th>
<th>Impacted sample</th>
<th>log_{10} change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed (km/s)</td>
<td>Orientation ave cfu sd n</td>
<td>Result type ave cfu sd n</td>
<td></td>
</tr>
<tr>
<td>Low ((&lt;\sim 0.5))</td>
<td>Vert. 2.35E + 07 NA 1</td>
<td>Regolith washes 1.08E + 05 1.43E + 05 2</td>
<td>−2.34</td>
</tr>
<tr>
<td></td>
<td>Horiz. 1.72E + 07 NA 1</td>
<td>Regolith washes 2.82E + 07 3.59E + 07 2</td>
<td>0.08</td>
</tr>
<tr>
<td>Medium ((&lt;\sim 1.2))</td>
<td>Vert. 1.96E + 07 1.40E + 07 2</td>
<td>Regolith washes 1.14E + 05 1.59E + 05 2</td>
<td>−2.18</td>
</tr>
<tr>
<td></td>
<td>Horiz. 1.70E + 07 NA 1</td>
<td>Projectile fragment 1.44E + 07 8.47E + 06 2</td>
<td>−0.08</td>
</tr>
<tr>
<td>High ((&lt;\sim 1.6))</td>
<td>Vert. 1.54E + 07 1.27E + 06 2</td>
<td>Regolith washes 5.60E + 04 5.43E + 04 2</td>
<td>−2.54</td>
</tr>
<tr>
<td></td>
<td>Horiz. 2.35E + 07 4.95E + 06 2</td>
<td>Projectile fragment 2.85E + 05 3.11E + 05 2</td>
<td>−1.84</td>
</tr>
</tbody>
</table>

*No projectile fragments produced for vertical/high speed shots. *To assess fraction sub-sampling efficiency, all 100 g of impacted regolith was analysed.
Impact sterilization model and parameters: The model for the experiments has just one input parameter, the speed of the projectile, \( v \). This is converted to the kinetic energy transferred to internal energy per unit mass, \( E \), in the material that the organisms are exposed to:

\[
E = f_v \left( \frac{1}{2} v^2 \right)
\]

where \( f_v \) is a random variable, dependent on the impact speed, given by the model described above (Fig. 14). The corresponding change in temperature, \( \Delta T \), is then given by:

\[
\Delta T = \frac{E}{C_v}
\]

where \( C_v \) is the specific heat capacity of the projectile material. In this case the value 840 J/kg/K has been used for the basalt. The temperature that the organisms are raised to is then simply this change plus the ambient temperature, \( T_a \). The temperature history is then given by:

\[
T(t) = T_a + \Delta T e^{-\beta t}
\]

where \( \beta \) is the cooling rate used to fit the model to the data. The values for the various species are listed in Table 3-12.

The sterilization \( S = \ln(N_t/N_0) \) is computed from the integral

\[
S = \ln \left( \frac{N_t}{N_0} \right) = \int_0^{t_e} k_v e^{-b/T(t_e) t_e} dt
\]

where the \( k_v \) and \( b \) are as described above in the heat inactivation modelling and \( t_e \) is the time after which there is no further sterilization. As a side note, in the heat inactivation tests, \( t_e \) was the time at which the temperature dropped to 50 °C (the heat test ambient baseline).

3.3.6. Phobos impact model

A series of hydrodynamics simulations of impacts of basalt projectiles on Phobos regolith have been carried out to determine how the material is heated so that the starting temperature can be calculated for the sterilization calculation. The mean transfer function for each part of the projectile has been recorded and the result is shown in Fig. 19, which shows a cross-section of the projectile viewed from above.

The peak transfer function is observed to be at the front “shoulder” of the projectile which is surprising since the natural assumption would
be for the peak to occur at the front face. This is true of the initial transient peak temperature, but the steady temperature (that reached after the initial transient behaviour has subsided and equivalent to the transfer function) behaves differently. The effect of impact on the regolith at the front of the projectile is purely compressive whereas at the shoulder it is more a shearing action. Thus, it is reasonable that this region receives the most net heating. Equally notable is the large volume of projectile material that receives very little heating and hence little sterilization.

Putting all of the above work together results in a suitable, “engineering” model of the impact process, which can be used in the statistical analysis. The relative constancy of the heat transfer function with impact speed also allows the necessary computations to be
that is deflected from the regolith. This is illustrated in Fig. 20 where it is lost from the mesh it is possible to estimate the amount of material material escapes untouched. By recording the mass of the projectile that the point at which the impact is grazing incidence and virtually all as per a vertical impact. This effect continues with lower angles up to zero for grazing incidence. The rest of the material impacts the regolith escapes grows as the angle of incidence decreases where the angle is characteristic speed, the impact velocity, remains the same. Doubling the size of the projectile whilst keeping the same impact speed means the same things will happen but on a doubled timescale. Consequently, the characteristic speed, the impact velocity, remains the same. Doubling the size of the projectile whilst keeping the same impact speed means that the same things will happen but on a doubled timescale.

Scaling effects: Beyond the radius of the projectile this problem has no length scale, so it scales hydrodynamically. Doubling the length-scale of the problem also doubles the timescale. Consequently, the characteristic speed, the impact velocity, remains the same. Doubling the size of the projectile whilst keeping the same impact speed means that the same things will happen but on a doubled timescale.

Angle of incidence: Some 3D simulations have been carried out to examine the effects of angle of incidence from vertical through to 45° the projectile is fully impacted against target material so the temperature behaviour is the same as for vertical impacts. Looking at lower angles of incidence, the simulations show that some of the projectile material is deflected from the surface. The amount of material that escapes grows as the angle of incidence decreases where the angle is zero for grazing incidence. The rest of the material impacts the regolith as per a vertical impact. This effect continues with lower angles up to the point at which the impact is grazing incidence and virtually all material escapes untouched. By recording the mass of the projectile that is lost from the mesh it is possible to estimate the amount of material that is deflected from the regolith. This is illustrated in Fig. 20 where it is apparent that the relationship between deflected mass and angle of incidence is mostly linear. This is not perfect as there is a chaotic nature to the impact process and the measurement of the mass loss is not perfect. Hence some variability in the data is evident, but this can serve as a good basis for a model of angle dependence to be used in the statistical analysis.

Performed without the need for hydrodynamic simulations of every conceivable impact. Although the heat transfer rate varies over all impacts with the same speed, this can be factored in to the statistical calculations. The mean heat transfer ratio at any location in the projectile can be converted into a temperature increase by dividing by the specific heat capacity of the basalt. The initial temperature for the sterilization calculation is then given by this temperature increase added to whatever the temperature of the projectile was prior to impact. Finally, applying the heat sterilization algorithm discussed above provides the organism survival rate.

Table 3-12

<table>
<thead>
<tr>
<th>Species</th>
<th>Cooling rate (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. aro</td>
<td>6</td>
</tr>
<tr>
<td>D Rad</td>
<td>35</td>
</tr>
<tr>
<td>B Dim</td>
<td>18</td>
</tr>
<tr>
<td>MS2</td>
<td>32</td>
</tr>
</tbody>
</table>

Fig. 19. Mean heat transfer ratio. The diameter of the projectile is across the lower left axis (x) centred at 1.5. The radius normal to this diameter is along the right hand axis (y). The initial point of impact is the origin on this plot with the projectile travelling from right to left. The mean transfer function is plotted on the vertical axis.

Fig. 20. “Reflected” projectile mass as a function of angle of incidence.

4. Statistical analysis and discussion

First, we consider where the mass transferred from Mars to Phobos originates from. The details in this section are explored in greater detail in Summers (2019). Craters occur with a distribution given by the martian isochron (Hartmann and Daubar, 2017). The total mass transferred from Mars to Phobos, on average per year, is shown in Fig. 21.

Most mass is transferred from the largest craters, however the larger craters are also the rarest. What dominates is, as the craters increase in size, the mass ejected increases more rapidly than the fall in crater rate. Fig. 21 demonstrates that in almost all time periods, the largest crater created on Mars in that period will transfer the majority of the mass to Phobos. Focussing on the known recent large craters, the mass transfer to Phobos is given in Table 4-1.

This illustrates how most mass comes from the larger craters, with Mojave transferring more than all other craters combined. However, the age of the Mojave crater is estimated at between 3–8 million years (Hartmann et al., 2010); and where sterilization increases with time there is the possibility that the Zunil crater, with its age between 100,000 years and a million years (Werner et al., 2014), may transfer more unsterilized life (see later discussion).

A key point to note here is in regard to the accuracy of crater ages. At geological timescales, crater counts have aged the Zunil crater with great accuracy (less than a million years, Werner et al., 2014). However, from the perspective of radiation sterilization, this age accuracy of a factor of 10 corresponds to a change in sterilization of an order of magnitude, and is therefore a statistically significant effect in terms of planetary protection. The reality is that the only craters which have been aged with sufficient accuracy are those that have been observed to appear on Mars since detailed observations began with the Viking orbiters in the 1970s. This only applies to a few craters, and these are all quite small. This uncertainty in crater aging in general is an unavoidable problem when determining the likelihood of unsterilized martian material being present on Phobos or Deimos.

Finally, to estimate how potential biological material from Mars is sterilized on its journey to Phobos, all models are run in the Monte Carlo simulation. For the sake of completeness, we also calculate the values for transfer to Mars’ smaller moon, Deimos, as a comparison.

The first impact with Phobos results in an approximate 50% survival rate due to hypervelocity impact; this is largely independent of organism type. This is a low rate of sterilization and has been traced back to the nature of the hypervelocity impact; during this impact the material is typically fragmented down to individual grains. At this size it behaves as a fluid, and in the impact its motion is quite chaotic. The chaotic nature of the impactor gives rise to great variation in the internal temperature of the impactor and the fraction that is unheated
undergoes little sterilization. Deimos impacts experience less sterilization than Phobos impacts as the impactors are generally at lower velocity (most probable impact velocity for Deimos is 2 km s^{-1}, compared to 3.5 km s^{-1} for Phobos).

Given that most material ejected from Mars is likely to be low mass (in terms of size distribution), on impact with Phobos/Deimos the material is likely to be deposited near to the surface. At the surface of Phobos (and Deimos) the radiation environment is at its worst, with the radiation dose received by material deposited proportional to the time of exposure. This, in turn gives (for each of the organisms considered in this study) a generic timescale over which where the population is decreased by 1/e. This time scale is shown in Fig. 22 for each organism.

Organisms present at the surface are sterilized over a period up to centuries. This means that after a millennium, most organisms will be sterilized. When the material is buried to between 1 cm and 1 m depth, the subsurface environment extends the sterilization timescales by 3 orders of magnitude. Since any near-term sample return mission to Phobos is unlikely to sample from below 1 m, and the sterilization rate is approximately constant between 1 cm and 1 m, a conservative 50 cm depth was chosen, as discussed earlier.

Considering the major contributing craters to material transfer defined in Table 4-1, coupled with the range of biological loadings defined in Table 2-4, we arrive at the potential values of transferred biological material to Phobos per cratering event as shown in Table 4-2.

Taking into account the potential ages of the craters concerned, and coupling in the sterilization factors due to hypervelocity impact and radiation exposure after deposition, we can determine the level of unsterilized material present on Phobos, for each cratering event on Mars (Table 4-2).

Table 4-2 also demonstrates how the mass varies due to unknowns...
An assumption here is that the material is collected below 1 cm where the GCR contribution is the dominant component of radiation, and gives an approximately uniform radiation dose down to 1 m. So it can be seen for a typical mass transfer, and a 25 mm diameter corer, the level of loading of life on the original Mars ejecta material needs to be below $1.6 \times 10^9$ cfu/kg.

An additional complication arises because of the methods by which young craters on Mars have been identified. Werner et al. (2014) used measurements from THEMIS to find crater rays, but this was only achievable over 50% of the martian surface, specifically the equatorial region. Therefore, for 50% of Mars, crater ages are uncertain; these may be relevant for this study. The Mars isochron (Hartmann et al. 2017) predicts that there is probably one >10 km diameter crater younger than a million years; as with Zunil, a single 10 km crater has potential to transfer sufficient material to the martian moons to get close to the planetary protection limit.

However, consider 60 km diameter craters such as Mojave. From the isochron, a crater of that size should be generated on average every 30 million years. The Mojave crater is likely to be only 3 Ma; however in the half of Mars where the THEMIS aging technique of Werner et al. (2014) cannot be applied, such craters happen with an average period of below 100 million years. In any 100-year period, a 60 km diameter crater in the equatorial area of Mars and the probability of this being over the $10^{-6}$ planetary protection limit would transfer over $10^6$ kg of material to Phobos. This would be distributed across just one side of the moon, unknown because of the phasing of Phobos in its orbit. In that area there would be, on average, 778 mg/m$^2$ on Phobos or, expressed differently, a 10 cm$^2$ sample size would contain <1 mg of martian material. Material deposited within 100 years prior to sample collection will have undergone almost no radiation sterilization, independent of depth buried, and the collision with Phobos would provide only minimal sterilization. Given this, the level of loading of biological material in the 1 mg of martian material would need to be below $10^6$ cfu kg$^{-1}$ for the sample to remain unrestricted in terms of planetary protection. Not knowing the initial loading of biological material in the ejecta from Mars is therefore a critical knowledge gap to accurately assess this problem.

For the case of material ejected by Zunil, at 3sd confidence level, this would emulate a total mass of about 1 mg of martian material per m$^2$ on Phobos. For a 10 cm$^2$ sample, this corresponds to a total emplaced mass of about 1 µg martian material. To reduce the probability
Table 4-5

<table>
<thead>
<tr>
<th>Sample area</th>
<th>Sampled mass and depth</th>
<th>Mass of unsterilized martian material expected in sample after 100 kY</th>
<th>Mars biological loading in order to meet 10−6 requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 cm²</td>
<td>30 g @ 3 cm</td>
<td>61 ng</td>
<td>1.6&lt;sub&gt;4&lt;/sub&gt;&lt;sub&gt;6&lt;/sub&gt; cfu kg&lt;sup&gt;−1&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 cm²</td>
<td>120 g @ 6 cm</td>
<td>122 ng</td>
<td>8.2&lt;sub&gt;6&lt;/sub&gt;&lt;sub&gt;3&lt;/sub&gt; cfu kg&lt;sup&gt;−1&lt;/sup&gt;</td>
</tr>
<tr>
<td>20 cm²</td>
<td>400 g @ 10 cm</td>
<td>244 ng</td>
<td>4.1&lt;sub&gt;3&lt;/sub&gt;&lt;sub&gt;6&lt;/sub&gt; cfu kg&lt;sup&gt;−1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

of it containing organisms below 10<sup>−6</sup> would require an initial loading of biological matter in the Mars ejecta of <8.2 × 10<sup>3</sup> cfu kg<sup>−1</sup>. Table 4-5 shows, for three different sample areas/masses, what the subsequent expected mass of unsterilized martian material would be after a residence period of 100 kY. Table 4-5 also shows the ‘upper limit’ of original martian biological loading (in cfu kg<sup>−1</sup>) needed in order to meet the 10<sup>−6</sup> requirement for unrestricted sample return.

The nature of the problem studied here hinges on several input parameters that we only presently have limited information about. Thus, for the purposes of this study, the most meaningful interpretation of the results is to define the acceptable upper limit on the ‘Mars loading level of organisms’, arrived at by consideration of the sterilization and statistical models presented here. As the mass of the collected sample and the depth it is collected at increases, the upper limit of initial loading of organisms on Mars decreases. The major unknowns contributing to the uncertainty in this analysis are not presently tractable – namely the starting loading of organisms on Mars, the phasing of Phobos in its orbit at the time of Mars ejection, the size distribution of the ejected material and the exact age of large-scale craters on Mars to sufficient accuracy. The main conclusion of this work, therefore, in regard to Phobos sample return within an unrestricted sample return category, is to minimise the sample mass collected, and to collect it from as shallow a depth on Phobos as possible.

Declaration of Competing Interest

There are no conflicts of interest to declare for this submission.

Acknowledgments

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References

GRAS: http://space-env.esa.int/R_8d/9d/gras/.
M.L. Patel, et al.
M.R. Patel, S. Werner, regarding impact and crater discussions throughout the study, and G. Kminek in particular.

Table 4-5

| Mass of unsterilized material after 100 kY and acceptable biological loadings to meet the 10<sup>−6</sup> requirement, assuming a Znuni crater age of 100,000 years. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Sample area     | Sampled mass and depth | Mass of unsterilized martian material expected in sample after 100 kY | Mars biological loading in order to meet 10<sup>−6</sup> requirement |
| 5 cm²           | 30 g @ 3 cm      | 61 ng           | 1.6<sub>4</sub><sub>6</sub> cfu kg<sup>−1</sup> |
| 10 cm²          | 120 g @ 6 cm     | 122 ng          | 8.2<sub>6</sub><sub>3</sub> cfu kg<sup>−1</sup> |
| 20 cm²          | 400 g @ 10 cm    | 244 ng          | 4.1<sub>3</sub><sub>6</sub> cfu kg<sup>−1</sup> |

life sciences in space research (this issue).