Survival of lichens and bacteria exposed to outer space conditions – Results of the Lithopanspermia experiments

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ABSTRACT

In the space experiments Lithopanspermia, experimental support was provided to the likelihood of the lithopanspermia concept that considers a viable transport of microorganisms between the terrestrial planets by means of meteorites. The rock colonising lichens Rhizocarpon geographicum and Xanthoria elegans, the vagrant lichen Aspicilia fruticulosa, and endolithic and endoaridic communities of cyanobacteria and bacteria with their natural rock substrate were exposed to space for 10 days onboard the Biopan facility of the European Space Agency (ESA). Biopan was closed during launch and re-entry. In addition, in the Stone facility, one sample of R. geographicum on its natural granite substrate was attached at the outer surface of the re-entry capsule close to the stagnation point, only protected by a thin cover of glass textolite. Post-flight analysis, which included determination of the photosynthetic activity, LIVE/DEAD staining, and germination capacity of the ascospores, demonstrated that all three lichen were quite resistant to outer space conditions, which include the full spectrum of solar extraterrestrial electromagnetic radiation or selected wavelength ranges. This high resistance of the lichens to space appears to be due to their symbiotic nature and protection by their upper pigmented layer, the cortex. In contrast, the rock- or halite-inhabiting bacteria were severely damaged by the same exposure. After atmospheric re-entry, the granite of the Stone sample was transformed into a glassy, nearly homogenous material, with several friction striae. None of the lichen cells survived this re-entry process. The data suggest that lichens are suitable candidates for testing the concept of lithopanspermia, because they are extremely resistant to the harsh environment of outer space. The more critical event is the atmospheric re-entry after being captured by a planet. Experiments simulating the re-entry process of a microbe-carrying meteoroid did not show any survivors.

1. Introduction

The concept of Panspermia, i.e. the propagation of microscopic forms of life between planets, originally formulated by Lord Kelvin (Thompson, 1871) and then further developed by Arrhenius (1903), was revived when meteorites were detected that originated from Mars (Becker and Pepin, 1984; Warren, 1994; Gladman, 1997). They provided evidence that rock fragments can travel from Mars to Earth in the solid state, and some of them experienced moderate shock pressures and temperatures (Melosh, 1984; Vickery and Melosh, 1987; Weiss et al., 2000; Head et al., 2002; Artemieva and Ivanov, 2004; Fritz et al., 2005). On Earth, a number of microbial communities have been found to inhabit subsurface environments (Pedersen, 2000). The rocks can act as a protective layer which protects the organism from environmental extremes, such as...
Among the organisms tested, spores of *Bacillus subtilis* (Horneck et al., 1994). However, direct exposure to the solar extraterrestrial UV radiation, reduced their survival by orders of magnitude. So far, lichens are the only known organisms that can survive the intense solar UV radiation that is present in the outer atmosphere (Horneck et al., 2008). In this scenario, microorganisms have to cope with three major phases of stress: the escape from the home environment, the exposure to space, and the landing on a foreign planet. This concept, that life can be transferred from one planet to another by a meteorite, is known as "panspermia" (Nicholson et al., 2000; Benardini et al., 2003; Cockell, 2008).

Experimental evidence for a potential impact explosion of viable microorganisms from Mars and Mars-like planets has been provided in systematic shock recovery experiments simulating shock pressures between 5 and 50 GPa, a range that has been determined for martian meteorites (Artemieva and Ivanov, 2004; Fritz et al., 2005). This work demonstrated a well-defined shock window for the transfer of rock-inhabiting microorganisms from Mars to Earth by impact explosion, which ranged between 5 and 45 GPa for spores of *Bacillus subtilis* and the lichen Xanthoria elegans, but was restricted to 5–10 GPa for the rock-inhabiting cyanobacterium *Chroococcidiopsis* (Stöffler et al., 2007; Horneck et al., 2008).

In space, microorganisms are confronted with an extremely hostile environment, characterised by a high vacuum (10^-7 – 10^-4 Pa), an intense field of ionising radiation of solar and galactic origin, unfiltred solar UV radiation and extreme temperatures (-120 to +120 °C). Space technology has provided the facilities to study the survival of different microorganisms in the harsh environment of space (Demets et al., 2005; Baglioni et al., 2007). Among the organisms tested, spores of *B. subtilis* (Horneck et al., 1994; 2001; Horbeck, 1993; Retberg et al., 2002), the lichens *Rhizocarpon geographicum* and *X. elegans* (Sancho et al., 2007) and adults and eggs of the tardigrades *Richtersius coronifer* and *Monti­sum tardigradum* (Jönsson et al., 2008) turned out to be the most resistant ones. *B. subtilis* (70%) spores survived 2107 days in space, on board of the NASA Long Duration Exposure Facility (LDEF), when shielded against solar UV (Horneck et al., 1994). However, direct exposure to the solar extraterrestrial UV radiation, reduced their survival by orders of magnitude. So far, lichens are the only known organisms that can survive exposure to the complex matrix of all parameters of space including solar extraterrestrial UV radiation, as tested during the two-week flight of Biopan-5 (Sancho et al., 2007; de los Rios et al., 2010). It has been suggested that this symbiotic system, composed of fungal and photosynthetic cells (cyanobacteria or algae), which is covered by a thick and pigmented cortex (Gauslaa and Solhaug, 2004), provides efficient shielding against the hostile parameters of outer space (Sancho et al., 2009).

If sufficiently shielded by meteorite-like material, endolithic microorganisms may survive the journey through space and finally be captured, with their host rock, by another planet. If the planet has an atmosphere, which might make it habitable (Lammer et al., 2009), the outer shell of the rocks is subjected to very high temperatures during atmospheric entry. So far, microorganisms that were embedded in 2 cm thick rocks fitted at the outer surface of a re-entry capsule (Stone facilities of Fotón missions), thereby simulating the entry of a meteorite, did not survive this entry process (Brandstätter et al., 2008; Westall and de la Torre-Noetzli, 2008).

The aim of this work is to obtain further information on the mechanisms of the resistance of rock-colonising microbial communities and lichens to outer space conditions, including space vacuum and different spectral ranges of solar extraterrestrial electromagnetic radiation. In the Lithopanspermia/Biopan experiment, a variety of microbial and symbiotic communities together with their natural habitat (rock or halite) were exposed to defined space conditions during the Biopan-6 flight of ESA on board of a Russian Fotón satellite. Besides viability and ultra-structural integrity of the whole systems, we investigated the resistance of ascospores to space and the role of the lichen cortex and pigments in protecting the underlying cells.

Complementary to Lithopanspermia/Biopan the aim of the Lithopanspermia/Stone experiment was to identify the physical, chemical and biological modifications caused by atmospheric entry in meteorites and to their possibly embedded microorganisms. We used the lichen *R. geographicum* as biological rock-inhabiting model system to test its ability to survive during entry into the Earth atmosphere.

With these studies we provided additional experimental evidence that microorganisms, residing in the interior of rocks, may be suitable candidates for an interplanetary transfer of microorganisms, as required by the lithopanspermia scenario.

2. Materials and methods

2.1. Spaceflight experiments

The experiment Lithopanspermia/Biopan was part of the payload of the Biopan-6 space mission of ESA mounted on the Earth-orbiting FOTON satellite. Biopan is a pan-shaped facility with the Lithopanspermia/Biopan hardware mounted within its lid (for further details see Appendix A). The biological samples were accommodated inside the Lithopanspermia hardware, which allowed access to space vacuum and selected wavelength ranges of solar extraterrestrial electromagnetic radiation: λ > 110 nm, λ > 200 nm, λ > 290 nm, λ > 400 nm, when in Earth orbit. Biopan was hermetically closed during launch and re-entry, and opened by telecommand after reaching its orbital altitude, thereby exposing the biological samples to the selected space parameters. Total flight duration was 10 days. After landing the samples were recovered and analysed.

The Lithopanspermia/Stone experiment was attached to the outer shell of the Fotón re-entry capsule as part of the Stone facility. A rock sample with the lichen *R. geographicum* was directly mounted onto the outer shell of Fotón with the biological layer facing the satellite. It was covered by a thin layer of a glass textile, but otherwise it was open to space during launch, spaceflight and landing (see Appendix A for further details).

2.2. Biological samples

2.2.1. Lichens

Epilithic lichens were collected together with their rock substrate. The crustose lichen *R. geographicum* was collected at the Plataforma de Gredos (Sierra de Gredos, Avila, 2020 m a.s.l.,
40°17'N, 5°14'19"W), a region with a predominant lithology of granite with phenocrysts, which is predominantly colonised by *R. geographicum* (Sancho et al., 2001). The area is characterised by a continental climate, where conditions (temperature variations, humidity, and solar UV and VIS radiation) are extremely harsh and where *R. geographicum* grows in “map-shapes”, contributing to rock-weathering. Rocks with flat surfaces and homogeneous rich thallus colonisation (50 mm and more in diameter) were selected. In order to assess the protecting effect of the cortex, samples with intact cortex as well as those without cortex, which was mechanically removed by aid of a scalpel as described in de la Torre et al. (2007a) were used for the flight experiment. Although the latter method seems to be quite aggressive, field studies have shown that the photosynthetic activity of the lichen was not impaired by this treatment (de la Torre et al., 2007a).

Rocks with the epilithic placoid crustose lichen *X. elegans* (Link.) Th. Fr. were collected at Peñones de San Francisco (Sierra Nevada, Granada, Spain, 2400 m a.s.l., 37°06'N, 3°23'W). Samples with homogeneous covering of lichen thalli were selected. In order to assess the shielding effect of the pigments, samples with intact thallus as well as those without pigments, which were extracted by use of pure acetone 100%, according to Gauslaa and Ustvedt (2003), were used for the flight experiment.

Samples of *R. geographicum* and *X. elegans* with fruiting bodies (containing ascospores) were collected near the Sanetsch glacier and the Gornergrat glacier, Zermatt (Wallis, Switzerland, between 2000 m and 3300 m, GPS coordinates Sanetsch (2000 m): 46°21.799'N, 007°17.844'E, Zermatt/Gornergrat (3100 m): 45°59'06.68"N, 7°47'38.27"E, Zermatt/Hohthálli (3270 m): 45°59'21.02"N, 7°48'09.86"E), in early summertime (mid of June) when the majority of the ascospores of *X. elegans* are fully developed in the mentioned habitat. For *R. geographicum*, the high degree of melanin incrustation and the anatomical and morphological structure of the ascospores in divided compartments were taken as indication of the final stage of spore development.

For the *Lithopanspermia/Biopan* experiment of each lichen/rock type, 12 cylindrical samples (9.5 mm in height, 6.9 mm in diameter, less than 1 g in weight) were cut by use of a diamond point saw machine (Fig. 1A). They were divided in three parallel sets of four samples serving as flight samples, flight spare samples and ground control samples, respectively. For *Lithopanspermia/Stone* a disc-shaped sample, with 45 mm diameter and 4 mm thickness, was cut out of a granite rock colonised with a homogeneous thallus of *R. geographicum* (Fig. A3A).

The vagrant lichen *Aspicilia fruticulosa* was collected on clayey soils in continental high basins of Central Spain (Guadalajara, Zarzates, 40°45'14"N, 02°11'51"W, 1260 m a.s.l.). Vagrant or erratic lichens living unattached to the substrate are well known from the continental deserts and arid areas of Middle Asia, Eurasia, North America and Northern Africa. *A. fruticulosa* typically develops a globular fruticose and compact thallus up to 2.5 cm diameter, made up of numerous dichotomous or sympodial branching (Fig. 1B). At the top branches show more or less circular pseudocyphellae that appear as white regions which lack of cortical layer and expose the medulla to the atmosphere (Sancho et al., 2000).

### 2.2.2. Endoevaporitic microbial communities

Samples of halite (NaCl) crust with visible signs of colonisation in their cavities by endoevaporitic communities represented mainly by photoautotrophic cyanobacteria accompanied by heterotrophic bacteria as described previously by Wierzchos et al. (2006) were obtained from Salar Grande in the Atacama Desert.

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**Fig. 1.** Flight samples (A) of epilithic lichens on their natural rock substrate: *Xanthoria elegans* (FX) and *Rhizocarpon geographicum* (FR) and (B) of *Aspicilia fruticulosa*.**
2.4.1. Lichens

2.4. Pre- and post-flight analysis of biological samples

2.3. Rock samples

2.2.3. Endolithic cyanobacterial communities

Rocks naturally colonised by epilithic and boring (enueolithic) communities of cyanobacteria were sampled from coastal limestone/sandstone cliffs in Beer, Devon, UK. The rocks are colonised by a diverse cyanobacteria assemblage including species of Leptolyngbya, Neurocapsa and Phormidium as determined from a 16S rDNA clone library and isolation studies on the communities (Olsson-Francis et al., 2010). The cliffs at Beer, are dominated by Cretaceous nodular chalk limestone, and the samples were collected from the upper greensand zone. The rock substrate itself is not as important as the microbial colonists which could potentially colonise any rock surface. However, sedimentary rocks such as the ones we examined have been shown to survive shock pressures associated with impact ejection and atmospheric entry (Brack et al., 2002; Horneck et al., 2008; Moeller et al., 2008). Samples of rock were cut into blocks with a surface of approximately 1 cm² for the Lithopanspermia/Biopan experiment.

To test the ability of resting states of cyanobacteria with known desciontion resistance to survive space conditions, the rocks were also seeded with akinetes (resting cells) induced from Anabaena cylindrica cultures (Yamanoto, 1975). Anabaena was obtained from the Pasteur Culture Collection (PCC 6309). The organism was grown in BG-11 medium (Rippka et al., 1979) at 25 °C, under natural sunlight and day/night cycle. Akinetes were induced by transferring log phase cells into an iron limited BG-11 medium, followed by three washes in the same medium. After five weeks of growth, the akinetes were harvested by allowing them to settle to the bottom of the flask. They were then washed in medium and transferred to dd H₂O. Akinetes were stored in a refrigerator at 4 °C until required. Akinetes (100 µl) were added to the surface of each of the rocks, and then dried. Samples were either used for the space experiment or as ground controls.

2.3. Rock samples

For the Lithopanspermia/Stone experiment a granite rock colonised with R. geographicum was collected at the Plataforma de Gredos (Sierra de Gredos, Ávila, 2020 m a.s.l., 40°17'N, 5°14'19"W). Textural-, mineralogical- and geochemical analyses, as described in Section 2.5, were performed with two pieces of fresh granite previous to the space flight. The composition of the samples was: 50% feldspar (white to pink large subhedral prismatic crystals), 30% quartz (semi-transparent to white anhedral-subhedral crystals) and 20% mica (mainly elongated and fibrous-radial biotite and chlorite crystals). Quartz low (SiO₂), anorthoclase (Na, K)Al₂Si₂O₆, albite, ordered (NaAlSi₃O₈), biotite (K(Mg, Fe₂)₃Si₃O₁₀(F, OH)₂) and ferroan clinohlorite (Mg, Fe₂)(Si, Al₂)₂O₄(OH)₆) were specifically detected by XRD. Feldspar grains showed incipient to advanced sericitic alteration and small fractures filled with quartz and micas, irregular inclusions of ore minerals (mainly pyrite) occurred dispersed in the silicate groundmass (Fig. 2). Geochemically, the granite sample displayed significant amounts of Pb (21 ppm), Cr (25 ppm), Zn (81 ppm), V (85 ppm) and Zr (206 ppm).

2.4. Pre- and post-flight analysis of biological samples

2.4.1. Lichens

To determine the activity of the photosystem II (PSII) of the photobiont of the lichens, the samples of R. geographicum, X. elegans and A. fruticulosa were reactivated in a climatic chamber under controlled conditions as follows: constant temperature of 10 °C, 12 h light and 12 h dark cycles for 72 h. Irradiation with photosynthetic active light was performed by use of a mercury lamp with a 100 µmol m⁻² s⁻¹ photosynthetic photon flux density (PPFD). For rehydration, the samples were sprayed twice a day with deionised water. Then the activity of the photosystem II (PSII) of the photobiont was measured by use of a Mini-PAM fluorometer (Heinz Walz GmbH), as described previously (Sancho et al., 2007; de la Torre Noetzel et al., 2007b). The lichens were rewatered immediately before each measurement. The optimum quantum yield of chlorophyll a was determined by fluorescence measurements after 10 min of dark adaptation according to Schreiber et al. (1994) from the equation:

\[ \frac{F_v}{F_m} = \frac{F_m - F_o}{F_m} \]

with \( F_v \) = variable fluorescence yield, \( F_m \) = maximal fluorescence yield, and \( F_o \) = minimal fluorescence yield. This optimum quantum yield of photosystem II was taken as an indication of the PSII activity of the photobiont of the lichen after the exposure to the space parameters. The relative PSII activity was determined from the ratio of the \( F_v/F_m \) of the flight sample to the pre-flight data of the same sample.

In addition, the viability of the lichens R. geographicum and X. elegans - of the entire lichen thallus, and separately of the mycobiont hyphae and photobiont cells embedded in the lichen symbiotic structure - was determined by Confocal Laser Scanning Microscopy (CLSM) (Leica TCS-NT/Confo Systems Software) analysis of samples stained with LIVE/DEAD-dye, FUN 1 (INVITROGEN, Molecular Probes) (de Vera, 2005; de Vera et al., 2003, 2004a,b, 2008; Horneck et al., 2008; Onofri et al., 2008). For this LIVE/DEAD analysis a short reactivation time of 15 h was applied. Channel-imaging was correlated with contour images and overlay images to improve recognition of visual damage. Channel-imaging emission filters in three different fluorescence ranges were applied. Band pass filters at 548 nm, 559 nm, and 506 nm were used for green, red, and blue fluorescence, respectively. From the quantitative data on the viability of the exposed samples that were stained by LIVE/DEAD-dye, FUN 1 mean values were determined, which indicated the deviation of the ratio of vital tissue \( t_v \) to the whole lichen thallus \( t_t \). Viable and non-viable cells of both symbionts, the photobiont and mycobiont, in the lichen tissue were quantified by the cell counting program of Image-J and by CLSM fluorescence.
2.4.2. Endoevaporitic microorganisms

The frozen specimens were cryo-fractured in the preparation unit. After the washing procedure the samples were glued by use of neutral plasticine dots on the lids of Petri-dishes; the lids were then placed over the dishes, which were filled with a Malt Yeast/ampicillin (MY/amp) extract. A consecutive drying procedure caused the ascospores to leave the fruiting bodies and to spread on the MY/amp medium. Light microscope observations and photographic documentation were performed during the following weeks to determine the germination and growth capacity of space-exposed samples in relation to the ground controls. From the images, the rate of germination and growth (in percent) of space exposed ascospores was determined by use of the cell counting program Image-Tool.

Lichen thalli of *A. fruticulosa* were examined using the Low Temperature Scanning Electron Microscopy (LTSEM) technique following de los Ríos et al. (2005). Small lichen fragments were fixed onto the specimen holder of the cryo-transfer system (Oxford CT1500), plunged into sub-cooled liquid nitrogen, and then transferred to the scanning electron microscope (SEM) via an air-lock transfer device. The frozen specimens were cryo-fractured in the preparation unit and transferred directly via a second air lock to the microscope cold stage where they were etched for 2 min at −90 °C. After ice sublimation, the etched surfaces were gold-sputter coated in the preparation unit. Samples were subsequently transferred onto the cold stage of the SEM chamber. Fractured and etched surfaces were observed under a DSM960 Zeiss SEM microscope at −135 °C by bacteria or other fungal spores that may have occurred after opening of the *Biopan* hardware. After the washing procedure the samples were glued by use of neutral plasticine dots on the lids of Petri-dishes; the lids were then placed over the dishes, which were filled with a Malt Yeast/ampicillin (MY/amp) extract. A consecutive drying procedure caused the ascospores to leave the fruiting bodies and to spread on the MY/amp medium. Light microscope observations and photographic documentation were performed during the following weeks to determine the germination and growth capacity of space-exposed samples in relation to the ground controls. From the images, the rate of germination and growth (in percent) of space exposed ascospores was determined by use of the cell counting program Image-Tool.

2.4.3. Endolithic cyanobacteria

After space flight, the viability of endoevaporitic halite microbial communities, comprising photosynthetic and heterotrophic microbial communities, was tested using the nucleic acid double-staining (NADS) procedure, which combines a general nucleic acid dye – Sybr Green 1 (SB1) with a membrane integrity probe propidium iodide (PI) (Falcioni et al., 2008). Both dyes stain RNA and DNA (Haugland, 2002) yet differ in their spectral characteristics, the former staining bacteria with intact cell membranes stain fluorescent green, while bacteria with damaged membranes appear fluorescent red. This bacterial viability kit is a sensitive, single-step assay for discriminating between live and dead bacterial cells.

To test for viable endolithic cyanobacteria after space flight, the rocks with endolithic microbial communities were halved and incubated in 5 ml of BG-11 medium and filtered sea water. The samples were exposed to sunlight and natural day/night cycles at 25 °C, for two months. The surfaces of the rocks were scraped with a blade and inoculated into BG-11 or seawater media and spread onto plates of the same composition. Cyanobacteria were identified by morphological and molecular techniques as follows: (i) examination at 1000 times magnification on a Leica DMRL microscope equipped with a epifluorescence microscope, which allowed to determine whether the cyanobacteria were in resting or vegetative state; (ii) identification of cyanobacteria by amplification of the 16S rDNA gene by PCR and specific primers (Nubel et al., 1997). The reaction mixture contained: 5 µl of culture; 200 µM dNTP; 1 µM primers; 5 U of Taq DNA polymerase; 1 × PCR Buffer (20 mM Tris-HCl (pH 8.4), 50 mM KCl) and 2.5 mM MgCl2 (Invitrogen, Paisley, UK). Amplification consisted of incubation at 94 °C for 15 min: this was followed by 35 cycles of 1 min at 94 °C, 1 min at 60 °C and a 1 min extension at 72 °C, with a final extension of 5 min at 72 °C. Sequences were phylogenetically classified and their nearest 16S rDNA sequences identified in the GenBank database.

2.5. Pre- and post-flight analysis of rock samples of Lithopanspermia/Stone

Analyses of the textural, mineralogical and geochemical characteristics of the granitic rocks of Lithopanspermia/Stone experiment were performed using the following set of analysing techniques: a combination of transmitted and reflected light microscopy (Nikon E600 POL polarising microscope), X-ray Diffraction (Seifert XRD 3003 T-T), Fourier transform infrared spectroscopy (Nexus Nicolet FTIR) with a microscope attached (Spectra-Tech IR-Plan Microscope) and inductively coupled plasma mass spectroscopy (ELAN 9000 ICP-MS).

3. Results

3.1. Lithopanspermia/Biopan

Within the Lithopanspermia/Biopan experiment the biological samples were exposed to the different parameters of space according to the sample-test-parameter plan (Table 1). After retrieval, the samples were distributed to the different laboratories for analysis of the effects of the space parameters applied to them.

3.1.1. Lichens after space exposure

The PSII activity of the flight samples of the lichens *R. geographicum*, *X. elegans* and *A. fruticulosa* was determined after 72 h of hydration and pre-adaptation of the desiccated specimens (Table 1). Intact thalli of all three lichen species that were exposed to space environment except solar electromagnetic radiation (dark flight controls) reached 100% PSII activity within experimental error compared to the pre-flight data of the same samples. These data show that the photosynthetic activity of the samples had rapidly recovered after the flight. A similar high relative PSII activity was measured in the ground laboratory controls (data not shown). Irradiation with solar extraterrestrial radiation during the space mission did not significantly reduce the PSII activity of the lichens, irrespective of the wavelength range applied (Table 1). Only in *X. elegans*, exposed to >400 nm sunlight, the PSII activity was reduced by 20%. This extremely high resistance of the photosynthetic systems of the lichens to outer space conditions, including solar extraterrestrial electromagnetic radiation, confirms earlier observations.
The reason for this variation of "cortex-depleted" lichens may be explained by a possible incomplete removal of the cortex in some samples, that show high PSII activity, e.g. those exposed to \( \lambda > 200 \text{ nm} \) and \( \lambda > 400 \text{ nm} \). Surprisingly, flight samples exposed to solar radiation of \( \lambda > 400 \text{ nm} \) showed the highest reduction in PSII activity. Unfortunately, the limited space in the Lithopansperma flight hardware did not allow accommodating more than one sample per test parameter, so that a statistical analysis of the data is not possible. When comparing the data of the Sun exposed to the flight dark samples, they support the suggestion that the cortex with its pigments acts as a protective endogenous shield against solar electromagnetic radiation. Sun-screening pigments are well known from different lichen species (Solhaug et al., 2003), especially in those living in Arctic habitats (Nybakken et al., 2004) and in high mountain areas, where the cortex protected R. geographicum from the harsh environment governing that milieu (de la Torre et al., 2007a). A Sun-screening effect of the pigments was also observed in flight samples of X. elegans, where a parallel set of samples had been depigmented before flight (Table 1). Their relative PSII activity was reduced, at least in samples exposed to solar extraterrestrial radiation in the ranges of \( \lambda > 200 \text{ nm} \) and \( \lambda > 400 \text{ nm} \). However, because the PSII activity of the depigmented dark flight samples was \( 76.6 \pm 19.6\% \) lower than that of the intact dark flight samples \( (100.3 \pm 4.0\%) \), it is more likely that depigmentation per se made the lichens more sensitive to the complex matrix of all parameters of space applied to them. Again, more studies in space with more samples are required in order to assess the significance of those observations.

Viability of the lichens R. geographicum and X. elegans after space exposure was determined as active staining index, i.e., the rate of viable to non-viable cells of the lichen tissue measured by the CLSM technique. In this case, the viability index of the flown dark controls of both species (ranging from about 79\% to 82\%) was slightly reduced compared to the ground control (94\%) (Table 1). The Sun-exposed flight samples showed a higher loss in viability than the dark controls. R. geographicum was especially damaged by the full spectrum of solar extraterrestrial radiation \( (\lambda > 110 \text{ nm}) \), when the vi­ability dropped to 52.3\% (compared to 94\% viability of the ground controls). Viability of Sun-exposed flight samples of X. elegans was reduced to values from 67\% to 75\% (compared to ground control of 95\%), however a dependence of viability on the spectral range of solar UV was not observed (Table 1). The CLSM technique allows also differentiating between damaged photobiont and mycobiont cells. Whereas in X. elegans photobiont cells were more affected (less stained by FUN I and no physiologic activity), in R. geographicum a higher degree of damage occurred in the mycobiont cells. Because in both investigated species, 2/3 of the lichen thallus is composed of mycobiont cells, the higher sensitivity of the mycobiont cells in R. geographicum is the reason for its higher loss of viability compared to X. elegans (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Biological system*</th>
<th>Specification</th>
<th>Space parameter</th>
<th>Relative PSII activity (%)</th>
<th>Viability (LIVE/DEAD analysis) (%)</th>
<th>Germination capacity (%)</th>
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<td><strong>Rhizocarpon geographicum</strong></td>
<td>Intact thalli</td>
<td>Cosmic radiation (mGy)</td>
<td>UV/VIS (nm)</td>
<td>Relative PSII activity (%)</td>
<td>Viability (LIVE/DEAD analysis) (%)</td>
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<tr>
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<tr>
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<td>Intact thalli</td>
<td>Cosmic radiation (mGy)</td>
<td>UV/VIS (nm)</td>
<td>Relative PSII activity (%)</td>
<td>Viability (LIVE/DEAD analysis) (%)</td>
</tr>
<tr>
<td>4 ± 0.5</td>
<td>Dark</td>
<td>&gt;400</td>
<td>95.9 ± 13.4</td>
<td>ND</td>
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</tr>
<tr>
<td>100 ± 20</td>
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<tr>
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<tr>
<td>100 ± 20</td>
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<tr>
<td>100 ± 20</td>
<td>&gt;400</td>
<td>34.1</td>
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<td><strong>Xanthoria elegans</strong></td>
<td>Intact thalli</td>
<td>Cosmic radiation (mGy)</td>
<td>UV/VIS (nm)</td>
<td>Relative PSII activity (%)</td>
<td>Viability (LIVE/DEAD analysis) (%)</td>
</tr>
<tr>
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<td>100.3 ± 14.0</td>
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<tr>
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<td>82.8 ± 11.7</td>
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<tr>
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<td>72.9 ± 12.6</td>
<td>82.5 ± 16.7</td>
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</tr>
<tr>
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<td>67.2 ± 9.2</td>
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<td>UV/VIS (nm)</td>
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<td>Viability (LIVE/DEAD analysis) (%)</td>
</tr>
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<tr>
<td><strong>Apthelia fruticulosa</strong></td>
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<td>Cosmic radiation (mGy)</td>
<td>UV/VIS (nm)</td>
<td>Relative PSII activity (%)</td>
<td>Viability (LIVE/DEAD analysis) (%)</td>
</tr>
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<tr>
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<tr>
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<td>ND</td>
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</tr>
</tbody>
</table>

NA = not applicable.
ND = not determined.
* In addition, samples of endoevaporites and endolithic cyanobacteria were exposed to the same conditions.
Ultrastructural analysis by LTSEM technique revealed the integrity of both the algal (black arrow) and fungal (white arrow) cell walls in thalli of *A. fruticulosa* exposed to the full spectrum of solar extraterrestrial radiation of $\lambda > 110$ nm, (Fig. 3).

### 3.1.2. Germination and growth capacity of ascospores after space exposure

After space exposure, ascospores were isolated from the thalli of *R. geographicum* and *X. elegans* and their germination and growth were studied. For all flight samples, the ascospores of *X. elegans* showed a germination index between 75% and 90% (Table 1) (compared to 97% of the ground control). The highest loss in germination was found in ascospores from samples exposed to the full extraterrestrial solar UV ($\lambda > 100$ nm). An even higher germination index (81–100%) was found for *R. geographicum* flight samples.

After 1–3 days of incubation, the ascospores of *X. elegans* flight samples started germination by forming the initiation hypha. This was 1–2 days earlier than observed for the ground controls (starting point 2–4 days). The following steps were identical with the controls: first branching after 10 days and the formation of network mycelia after 22 days.

In contrast to the flight samples, ascospores of the ground controls of *R. geographicum* did not show any germination under the applied laboratory conditions (0% germination index). It seems that the space conditions have triggered the germination process in the ascospores of *R. geographicum*, so that they started germination, when brought in contact with the nutrient medium in the laboratory. In their natural habitats in high mountain regions (2500–3200 m), desiccation is essential for breaking up the protective ascosporic membranes and cell walls to allow germination and growth of the lichen mycobiont of *R. geographicum*. Probably, desiccation by space vacuum exerted a similar triggering of germination in the ascospores of the flight samples of *R. geographicum* as it occurs in nature.

### 3.1.3. Endolithic halite microbial communities after space exposure

The applied NADS technology enables differentiation between bacteria with intact and damaged cytoplasmic membranes (Berney et al., 2007), which to a certain extent allows also differentiating between active and dead cells. In Fig. 4 the live bacterial cells show green fluorescence and dead bacteria emit a red fluorescence signal. Fig. 4a gives an example of live cyanobacteria and live heterotrophic bacteria of the ground control. Note the intense red autofluorescence of the photoautotrophic cells (open arrow in Fig. 4a). Nucleic acids stained with SB1, appeared as yellow spots (green SB1 signal on a background of red autofluorescence gives a net yellow signal) within the cyanobacterial cytoplasm, indicating cell membrane integrity and thus their viability. Live heterotrophic bacterial cells were also distinctly stained with SB1 (arrow in Fig. 4a). Fig. 4b shows microorganisms of a flight dark sample. This image reveals a high proportion of live cyanobacterial and...
heterotrophic bacterial cells (green SB1 signals). In the flight sample that was exposed to the full spectrum of solar extraterrestrial electromagnetic radiation (>110 nm), cell integrity was less preserved (Fig. 4c). Most of the cyanobacteria and heterotrophic bacteria cells in this sample showed red PI fluorescence indicating dead cells with damaged membranes. Only around a tenth (visual estimate and counting) of the heterotrophic and photoautotrophic cells emitted green fluorescence indicating they were intact and alive. In addition, one has to consider, that whereas the membrane-compromised bacterial cells can be clearly considered dead — those cells that appear “intact” may also be damaged to a certain extent. This has especially been observed for UV-irradiated bacteria (Villarino et al., 2000). Comparable images were obtained for flight samples that were exposed to solar extraterrestrial radiation at $\lambda > 200$ nm (Fig. 4d). The percentage of live cyanobacteria cells as well as heterotrophic bacteria gradually increased from flight samples exposed to solar extraterrestrial radiation at $\lambda > 290$ nm (Fig. 4e) to those, exposed solely to VIS ($\lambda > 400$ nm). In the latter case, the quantity of live heterotrophic bacterial cells was comparable to that observed in dark flight samples.

3.1.4. Cryptoendolithic microbial communities from the Beer rock after space exposure

Addition of BG-11 and seawater to the rocks from the ground control experiment resulted in green mats forming on the surface of the rocks after eight weeks. The green mats were cultured, and after one month, growth was visible on the plates (Fig. 5A). Examination of the colonies under the microscope identified both vegetative and resting state cyanobacteria. The predominant vegetative morphology was identified by 16S rDNA analysis as *Leptolyngbya* species. *Leptolyngbya* are prevalent in the 16S rDNA library prepared from the natural rocks (Olsson-Francis et al., 2010).

For rocks exposed to Low Earth Orbit (LEO) conditions in the Lithopanspermia experiment, the green epilithic growth on the surface of the rock turned brown after two days (this was not observed with the ground control rocks). After eight weeks, no green mats were distinguishable; however, the surface was spread onto plates to test for growth. After a month, green colonies appeared on the rocks that were not exposed to the Sun, i.e. dark control flight samples. The green filamentous colonies grew from small fragments of rock on the plate (Fig. 5B and C). Microscopic studies revealed that colonies from the space-exposed samples were the same as those of the ground controls. The organisms grew as long chains of spherical cells morphologically identical to *Anabaena* (Fig. 5D). 16S rDNA PCR using cyanobacteria specific primers confirmed them to be *Anabaena*.

3.2. Results of Lithopanspermia/Stone

3.2.1. Mineralogical, textural and geochemical changes of the granite during the atmospheric re-entry process

The *Foton* capsule with the *Stone* facility entered Earth’s atmosphere with a velocity of 7.7 km/s, a velocity, which was below the 12–20 km/s of medium sized meteoroids. The atmospheric re-entry process of the granite sample in the Lithopanspermia/Stone experiment caused a general mineralogical and geochemical transformation into a glassy, nearly homogeneous material (Fig. 6). At micrometric scale, it occasionally displayed imprints of atmospheric flight that resembled those found on meteorite fusion crust (e.g. friction striae), as well as some particular features (principally semi-transparent to black glass droplets and rough and clean areas), which are similar to those found in some meteorites and pseudometeories (Genge and Grady, 1998; Martinez-Frias et al., 1999). SEM-EDX analysis of the glass indicated chemical variations (wt.%) of Na (0.77–1.96), Mg (0.40–2.89), Al (2.51–7.94), Ca (0.87–4.49), K (0.27–2.70), Si (14.45–24.25) and O (61.94–69.95). Some microdomains were made of pure silica.

3.2.2. Survival of the lichen *R. geographicum* after the atmospheric re-entry process

The lichen *R. geographicum* on its natural granite habitat was one of the four Stone-samples facility on *Foton* M3 facing to the backside. During the entry process the samples experienced temperatures that were high enough to melt silica and basalt. None of the biological Stone samples survived this atmospheric entry (Westall and de la Torre-Noetzel, 2008). It has been argued that either the 2 cm rock coverage was not thick enough to protect the microorganisms, or that hot gases released during ablation.
might be the main reason for the measured reduction in the lichen thallus. However, the detailed study of some centimetre-size fragments showed that some textural microdomains (e.g., smooth and rough surfaces, blebs, friction-trace-like features) can be distinguished.

pervaded the space between the sample and the sample holder leading to intense local heating. This assumption was confirmed by surface melting observed at the non-exposed surface of the rock samples (Brandstätter et al., 2008).

4. Discussion

The data from the Lithopanspermia/Biopan experiment clearly demonstrates the extraordinary survival capacity of lichens in outer space. Besides the lichens R. geographicum and X. elegans, which were already studied in the experiment LICENS on board of Biopan-5 (Sancho et al., 2007, 2009; de los Ríos et al., 2010), the vagrant lichen A. fruticulosa showed a similar high resistance to all space parameters with regard to their photosynthetic activity: cosmic radiation, space vacuum as well as the full spectrum of solar extraterrestrial electromagnetic radiation (Table 1). Further studies of the gas exchange rate of flown and space-exposed A. fruticulosa thalli revealed normal values of respiration and photosynthesis (Sancho et al., 2009).

The viability of the dark flight samples determined by LIVE/DEAD staining and CLSM analysis was slightly reduced, by about 20% (Table 1), and even more in the Sun-exposed samples (up to about 40%). We hypothesise that the mycobionts are especially vulnerable to space and that the high fraction of mycobionts in R. geographicum might be the main reason for the measured reduction in viability. It seems that the photobionts in their natural symbiotic system were extremely well protected against the harsh environment of space. Protection was provided by the differentiated cortex with Sun-screening pigments as well as by live or dead cells of the surrounding fungus.

From the point of view of Lithopanspermia, the high resistance of ascospores is of particular importance, as they are protected by the fruiting bodies and by secondary lichen metabolites, such as melanin and usnic acid deposits in R. geographicum (BeGora and Fahselt, 2000). An additional protection is exerted by the presence of extrapolymeric substances (Kappen, 1988; Honegger, 1993; de los Ríos et al., 2002, 2003; Flemming and Wingender, 2001). In addition, desiccation caused by space vacuum, facilitated the germination capacity of the ascospores of R. geographicum. Ascospores in the fruiting bodies and photobiont cells from deep layers of the lichen thallus are the best protected cells and candidates for surviving natural transfer between planets (Mileikowsky et al., 2000). Together they comprise all prerequisites for generating a new lichen thallus on the new planet, if conditions are favourable for life.

In contrast to the high resistance of lichens, which are composed of cyanobacteria as photobiont and a fungus as mycobiont, cyanobacterial communities within their natural habitat, such as halite or limestone/sandstone, were less resistant to prolonged periods in outer space. It seems that vegetative cells of many naturally occurring cyanobacteria would lose viability once extracted from their natural environment. The limestone/sandstone rocks were predominantly colonised by Leptolyngbya species; however, 16S rDNA analysis and isolation experiments with freshly isolated rocks, demonstrated that the cyanobacterial community of the rocks were highly diverse (Olsson-Francis et al., 2010). In addition, in the vegetative state, cyanobacteria that survive isolation from their natural environment would be killed by the extreme conditions of space. However, some extremophilic cyanobacteria, such as an unidentified species of Synechococcus isolated from gypsum–halite crystals were reported to largely survive the harsh conditions of outer space experienced on a two-week flight on Biopan-1 (Mancinelli et al., 1998).

The experiments reported here suggest that many widespread cyanobacterial communities are not capable of surviving prolonged periods in outer space. However, resting state cyanobacteria are likely to confer greater resistance to space conditions. Akinetes are resting state cyanobacteria and are known to survive decades of desiccating conditions (Olsson-Francis et al., 2009). Furthermore, resting state cyanobacteria do not require sunlight and are therefore not limited to the upper surface of the rock. Thus, akinetes, or similar resting state phototrophic organisms, might provide a mechanism for escaping the adverse conditions experienced at the surface of the rock during atmospheric entry (Cockell et al., 2007; Cockell, 2008). These characteristics make resting cells of cyanobacteria one ideal model system for understanding the interplanetary transfer of microorganisms.

The space experiments reported here have shown that lichens can survive short-term, i.e. 10 days, exposure to outer space. From laboratory experiments mimicking impact ejection at shock pressures up to 50 GPa it is known, that photobiont cells and particularly ascospores of X. elegans are able to survive impact ejection (Stöffler et al., 2007; Horneck et al., 2008). The crucial stage in lithopanspermia seems to be the process of capturing by another planet and entry and landing on that planet. So far, in the Lithopanspermia/Stone experiment, all cells were killed during the entry process. In order to perform a more realistic simulation of the entry of meteorites carrying endolithic microorganisms, rock of different composition, e.g. basalts, should be tested and the technical concept of Stone needs to be modified so that larger, and in particular thicker rock samples can be accommodated. However, samples thicker than 2 cm can for safety reasons not be installed on a Fotan capsule. The very thick and resistant heat shield of the Fotan spacecraft will safely do its job even if equipped with recesses to accommodate Stone as it is now, but a further deepening of the recesses in the heat shield to make room for thicker Stone samples has justifiably been rejected by the Fotan engineers and safety people.

Natural activities, such as rearrangements and relocations of soil material by glaciers or liquid water may lead to situations, where lichens, lichen fragments or their symbionts are better protected by soil material, and thereby better protected for all three steps of lithopanspermia. Therefore, on one hand, we cannot completely exclude the likelihood of a successful transfer of lichen material between two planets in the Solar System, on the other...
hand, we are also not sure about the opposite (complete disintegration of biological material and no survival during interplanetary transfer of lichens). Further experiments in space and in simulation facilities on ground with different types of rock-colonising biological communities and rock material are envisaged to amend knowledge for assessing the likelihood of lithopanspermia.

Acknowledgments

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Appendix A

A.1. Lithopanspermia flight hardware

A.1.1. Lithopanspermia on Biopan

One unit of the Lithopanspermia experiments was part of the payload of the Biopan-6 space mission of ESA. Biopan is a cylindrical exposure container of 38 cm in diameter for biological samples, installed on the outside of the re-entry capsule of a Russian Foton satellite (Fig. A1) (Demets et al., 2005; Baglioni et al., 2007). After reaching an orbital altitude of approximately 300 km, the lid of Biopan folds open thereby exposing the experimental samples to the outer space environment. Biopan is equipped with in-built sensors of temperature, pressure, and UV and VIS for monitoring space environmental parameters relevant for the experimental objectives (Horneck et al., 2001).

The hardware of Lithopanspermia/Biopan was constructed of aluminum-silica alloy-ISO Al Mg Si (A16082 T6) and consisted of a top (level-1) and a bottom (level-2) plate, each dimensioned 146 mm x 129 mm x 23 mm, and each accommodating 36 cylindrical sample cells of 13 mm in diameter and 9 mm in height (Fig. A2). The cells of the top plate were covered by optical long-pass filters with the following characteristics: (i) MgF₂, which is transparent for the complete spectrum of extraterrestrial solar electromagnetic radiation of \( \lambda > 110 \) nm; (ii) SQO synthetic quartz transmitting solar electromagnetic radiation of \( \lambda > 200 \) nm, thereby simulating the UV and VIS radiation climate on the surface of Mars; (iii) long-pass filter for \( \lambda > 290 \) nm to simulate the terrestrial UV and VIS radiation climate (as a control) and (iv) for \( \lambda > 400 \) nm thereby cutting off all solar UV radiation. Reference samples in the bottom plate were kept in the dark during the whole mission. For allowing access of space vacuum to the samples a channel system...
was drilled in- and between the cells. A tubular membrane (polyethersulfone PES (371WPET12, Berghof Filtrations- und Anlagetechnik GmbH&Co KG, Eningen, Germany) with a pore size of 100 kD and a diameter of 12 mm was inserted in each cell to prevent possible contamination between adjacent cells through the channels.

The samples inside the cells, and the optical filters on top of the cells, were fixed by a mixture of silicone and primer (RTV-576 with primer SS41555).

The hardware (except the optical filters) was covered by a white coated (paint SG121FD) thin plate (146 mm x 129 mm x 2 mm) to reduce temperature excursions and to limit the temperature gradient in relation with the Biopan structure. Mechanical function of this white plate was to clamp the optical filters and the experimental package of Lithopanspermia to Biopan’s mounting plate. Steel bolts (14 M5 bolts) were used to secure Lithopanspermia onto the lid plate of Biopan and to hide the bolts, which fixed the top and bottom plates (6 M5 bolts). Total mass of the experimental package was 636.7 g.

In addition to the Biopan-provided sensors, a thermoluminescence dosimeter (TLD) assembly Litho-Dose (Fig. A2D) was integrated adjacent to the sample cells, to register the depth dose distribution of cosmic radiation (Reitz et al., 2002; Olko et al., 2006). On the opposite side, one of the AD590 temperature sensors from Biopan was located. The time profile of the radiation dose during the mission was recorded by another experiment on Biopan-6, called R3D (Dachev, 2008).

A.1.2. Lithopanspermia as part of Stone

The Stone experiments of ESA consisted of four disc-shaped test samples in the outer surface of the heat shield of the Fotón M3 re-entry capsule around the point where the spacecraft is subjected to the highest stress upon atmospheric entry (stagnation point). One of the test samples of Stone was the Lithopanspermia unit. It was composed of three parts: (i) the lichen *R. geographicum* on its natural granitic habitat (a disc of 45 mm diameter and 4 mm thickness); (ii) a spacer (a 2 mm thick ring made out of optical fibre G10FR4 with 70 mm outer and 40 mm inner diameter), which separated the sample from the holder at the surface of the capsule (Fig. A3A); and (iii) a cover of hat-like shape (with a base of 70 mm outer and 45 mm inner diameter and a 10 mm high tube with 45 mm inner diameter that was overarched by a dome of 6 mm height at its highest point), which protected the sample against the extremely high friction and temperature conditions reached during re-entry (Fig. A3B and C). This cover was designed to simulate the external layer of a meteorite. It was fabricated from the same material that was used for the ablative heat shield of the Fotón capsule, i.e., glass textile (a glass fibre reinforced phenolic resin material). (Fig. A3B and C). The lichen-on-granite sample was accommodated between the spacer and the cover with the biological layer oriented inwards, towards the capsule. The three parts – spacer, lichen-granite sample and cover – were glued together as one block, using silicone RTV-566 with primer SS41555, and inserted in an annular Stone holder which was bolted into a recess in the skin of the Fotón capsule, with the 6-mm high dome (Fig. A3B) protruding from the smooth Fotón surface.

A.2. Flight protocol

The Lithopanspermia/Stone sample (Fig. A3) was delivered to ESA’s technical Center ESTEC in Noordwijk, The Netherlands, by the end of January 2007. It was then sent to Russia in February 2007 for integration in the skin of the Fotón M3 capsule. For the Lithopanspermia/Biopan experiment, on the 12th of August 2007, at the principal investigators laboratory INTA, the biological samples were integrated in the different sample cells of the Lithopanspermia hardware according to the sample-test parameter plan. The experiment was then transported to ESTEC, where on 6th of September 2007 it was fixed in the lid of Biopan-6 (Fig. A1). The fully integrated Biopan-6 facility was then transported to the launch site Baikonur in Kazakhstan for accommodation on the Fotón M3 satellite. Fotón M3 with Biopan-6 and Stone was launched on 14th of September 2007. It reached a near-circular orbit with a maximum apogee of 302 km and minimum perigee of 263 km. The orbital inclination was 63°. The orbital period of Fotón M3 was 89.9 min, alternating Sun-illuminated periods with darkness periods.
Fig. A4. Temperature profile of the Biopan-6 mission, measured in the bottom part (upper curve) and the lid (lower curve) of the facility.

Fig. A5. Solar irradiance data of Lithopanspermia measured with solar sensors of different spectral sensitivity (courtesy of Kayser-Threde, München).
corresponding to the spacecraft crossing the night side of the Earth. Having completed 190 orbital loops in 12 days with the lid of Biopan-6 open for 10 days, the spacecraft landed in the vicinity of Kostanay, Kazakhstan on 26 September 2007. Biopan-6 and Stone were dismounted from the capsule and transported to ESTEC for de-integration of the samples and further analysis in the investigators’ laboratory.

A3. Lithopanspermia flight data

During each orbital loop, the Fotón M3 satellite was about 35 min in the Earth’s shadow and 55 min in the Sun, resulting in temperature fluctuations of about 10 °C per orbit. Temperature was further influenced by orbital parameters resulting in temperature fluctuations at the position of the Lithopanspermia hardware between -23 °C and +16 °C (Fig. A4). Because Fotón is a non-stabilized free-flying satellite, which slowly rotates during its orbital journey, the samples were arbitrarily insulated for short intervals (minutes) depending on the orientation of the satellite (Fig. A5). The depth dose profile measured in the Litho-Dose experiment steeply declined with shielding mass from 100 ± 20 mGy at level-1 (top layer, Sun exposed) of the experiment down to 4 ± 0.5 mGy at level-2 (bottom layer, dark control samples) (Fig. A6). These data are comparable with those of previous Biopan missions (Table A1).

Table A1

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<th>Cosmic radiation (mGy)</th>
<th>Temperature (°C)</th>
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<tr>
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<td>11 501</td>
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<tr>
<td>5</td>
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<td>240</td>
<td>11 800</td>
<td>4 ± 0.5</td>
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Table A1 Environmental data from Biopan-1 through Biopan-6 missions (data for Biopan-1–3 from Hornbeck et al. (2001), for Biopan-5 from Sancho et al. (2007)).

References


