In Situ Biological Contamination studies of the Moon: Implications for Future Planetary Protection and Life Detection Missions

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Abstract: NASA and ESA have outlined visions for solar system exploration that will include a series of lunar robotic precursor missions to prepare for, and support a human return to the Moon, and future human exploration of Mars and other destinations. One of the guiding principles for exploration is to pursue compelling scientific questions about the origin and evolution of life. The search for life on objects such as Mars will require that all spacecraft and instrumentation be sufficiently cleaned and sterilized prior to launch to ensure that the scientific integrity of extraterrestrial samples is not jeopardized by terrestrial organic contamination. Under the Committee on Space Research's (COSPAR's) current planetary protection policy for the Moon, no sterilization procedures are required for outbound lunar spacecraft, nor is there yet a planetary protection category for human missions. Future *in situ* investigations of a variety of locations on the Moon by highly sensitive instruments designed to search for biologically derived organic compounds would help assess the contamination of the Moon by lunar spacecraft. These studies could also provide valuable "ground truth" data for Mars sample return missions and help define planetary protection requirements for future Mars bound spacecraft carrying life detection experiments. In addition, studies of the impact of terrestrial contamination of the lunar surface by the *Apollo* astronauts could provide valuable data to help refine future Mars surface exploration plans for a human mission to Mars.

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The Committee on Space Research (COSPAR) of the International Council for Science (ICSU) was established in 1958 to promote international level scientific research in space. One of the continuing tasks of COSPAR has been to address planetary protection issues related to the Moon, Mars, and other planetary bodies. The current COSPAR planetary protection policy states that space exploration should be conducted so as to avoid forward biological contamination of planetary bodies by outbound spacecraft that could jeopardize the search for extraterrestrial life (DeVincenzi and Stabekis, 1983; Rummel *et al.*, 2002). The current planetary protection policy for the Moon related to forward contamination is not at all stringent (Category I) since the probability that terrestrial life can grow in the harsh environment on the lunar surface is very low. Even survival on the lunar surface is difficult to imagine with the Moon's nearly nonexistent atmosphere, intense ultraviolet (UV), galactic and solar cosmic radiation, lack of liquid water, and large temperature extremes. However, experiments carried out on NASA's Long Duration Exposure Facility (LDEF) have shown that even after 6 years in space, a large fraction of spore forming bacteria will survive if they are not directly exposed to solar UV radiation (Horneck *et al.*, 1994). These results certainly suggest that bacteria can be delivered to the surface of the Moon by robotic spacecraft. Although bacterial growth on the Moon remains unlikely, survival of terrestrial bacteria on non-UV exposed regions, such as the interiors of lunar spacecraft, the permanently shadowed south polar region of the Moon, or below the surface cannot be ruled out. Analysis of selected components returned from the unmanned *Surveyor III* probe, including the television camera that spent over two years on the lunar surface found viable *Streptococcus mitis* bacteria from a sample of foam collected inside the camera housing (Mitchell and Ellis, 1972). However, all of the other camera components did not contain bacteria (Knittel et al. 1971), and it has been suggested that contamination of the foam occurred during analysis in the Lunar Receiving Laboratory (Rummel, 2004). Future microbiological investigations of the *Apollo* site materials that have been exposed to the lunar environment for over 30 years could help resolve the *Surveyor III* issue.

It also should be emphasized that even if bacteria delivered by lunar spacecraft are inactivated or sterilized on the Moon, due to the harsh surface conditions, organic compounds from dead cells will remain and could leave a terrestrial fingerprint in lunar samples returned to Earth. A typical terrestrial microorganism such as an *E. coli* cell has a dry weight of 10^{-13} grams and is comprised of protein amino acids (57%), nucleic acids (24%), lipids (9%) and other

material (Neidhardt *et al.* 1990). Therefore, in addition to dry heat sterilization needed to kill most bacterial cells on spacecraft surfaces, cleaning with a variety of organic solvents and degassing is required to minimize the organic load of the spacecraft and sample collection hardward. Most *Apollo* spacecraft hardware surfaces were cleaned to organic contamination levels of 10-100 ng/cm^2, and the lunar soil sampling equipment and storage boxes were precision cleaned at the White Sands Test Facility in New Mexico to a level of 1 ng/cm^2 for polished planar surfaces (Johnston et al. 1975). Estimates of the total organic contamination to lunar samples from the *Apollo 11* and *12* missions based on spacecraft cleanliness was in the 0.1 to 100 part per billion (ppb) range (Flory and Simoneit, 1972). Based on the *Apollo* spacecraft bioburden and the survival of terrestrial microorganisms on the lunar surface, it was estimated that only 10^{-4} to 10^{-5} viable microorganisms per square meter of lunar surface were present at the time the Apollo samples were collected (Dillon *et al.* 1973). *Apollo* soil samples returned to the Earth were immediately analyzed for bacterial and organic contaminants in the Lunar Receiving Laboratory. Although no viable organisms were detected in the *Apollo 11* and *12* samples (Oyama 1970; Holland and Simmons, 1973), varying levels of organic contamination in the returned samples were reported. Burlingame (1970) reported an organic contamination level of 5 ppb for some *Apollo 11* samples, while others reported no organic contamination above the 1 ppb level (Mitchell *et al.* 1971;). Porphyrine-like pigments were also found in some *Apollo* samples at the trace ng to pg level by Hodgson and coworkers (1971). Terrestrial amino acid contaminants were also observed at concentrations of up to 70 ppb (Hare *et al.*, 1970; Gehrke, 1975; Harada *et al.*, 1971; Brinton and Bada, 1996). However, since these lunar samples were not analyzed for traces of organic compounds on the surface of the Moon, it remains unclear how much if any of the amino acid contamination in the lunar soils occurred during collection.

In addition to concerns about surface organic contamination of the lunar collection tools and regolith samples themselves both during collection and after return to Earth, a variety of other potential sources of contamination during the *Apollo* missions were noted by Simoneit and Flory (1970) including, (1) dimethyl hydrazine and nitrogen tetroxide exhaust products from the lunar descent engine and reaction control system engines; (2) lunar module outgassing; (3) astronaut spacesuit leakage and venting of life support back pack; (4) particulate material from spacesuit or other sources during EVA; and (5) venting of lunar module fule and oxidizer tanks, cabin, and waste systems. Measurements of hydrogen and oxygen isotopes of water extracted

from lunar soils revealed that the water was primarily of terrestrial origin, probably from the Apollo spacecraft and astronauts (Epstein and Taylor, 1972). During Apollo 17 *in situ* measurements on the lunar surface by the Lunar Atmospheric Composition Experiment (LACE) provided evidence for traces of methane, ammonia, and carbon dioxide in the lunar atmosphere (Hoffman and Hodges, 1975). Although these volatiles may be indigenous to the Moon resulting from chemical reactions between solar implanted ions or exchange with the lunar polar cold traps, contamination by the Apollo spacecraft or the astronauts themselves cannot be ruled out as a possible source. At present it is not known whether or not past human or spacecraft contamination of the Moon is detectable in localized regions, or limited to the *Apollo* landing sites, themselves. Future *in situ* evolved gas measurements of the lunar regolith (ten Kate *et al.* 2010) at previous *Apollo* landing sites as well as "pristine" polar sites are needed to help constrain the origin of lunar volatiles and to understand the extent and persistence of volatile contamination during *Apollo.*

Although the lunar surface environment may represent a worst-case scenario for the survival of microorganisms and even terrestrial organic matter, lunar exploration provides a unique opportunity to use the Moon as a test-bed for future Mars exploration, where the search for evidence of life has become a primary objective. NASA is planning to a series of robotic orbiters and landers to the Moon, Mars, and small bodies such as asteroids to prepare for future manned missions to these destinations. ESA, as part of its Aurora exploration program, is also planning a similar set of robotic precursor missions in a similar timeframe. For these missions, *in situ* measurements that target key organic biomarkers and other volatiles in lunar soil samples as well as on spacecraft surfaces could be carried out using highly sensitive instruments on landers and rovers. These "ground truth" experiments on the Moon also would be particularly useful for assessing the degree of organic contamination in lunar soil samples prior to their return to Earth, as well as the stability of organic compounds in sun-exposed and shadowed regions on the surface of the Moon. Furthermore, *in situ* experiments carried out at previous lunar landing sites such as *Apollo* could provide important information regarding the extent that previous extravehicular activities by the *Apollo* astronauts contaminated the Moon during lunar surface operations.

The use of sensitive robotic experiments to detect contamination that may still be present ~~nearly~~ over 40 years after humans first explored the surface of the Moon may be critical to help

establish a contamination baseline, but there are broader contamination challenges regarding a more sustained human presence on both the Moon and Mars. Such considerations should be kept in mind as we prepare for sustained human exploration (McKay and Davis, 1989; Lupisella, 1999). Human exploration could, in fact, confound the search for life on Mars, since the presence of humans will dramatically increase the amount of terrestrial organic material, potentially making the detection of indigenous organic matter exceedingly difficult, if not impossible. Future robotic and human missions to the Moon could provide a unique opportunity to carry out ground-truth experiments using *in situ* life detection instruments to help understand the extent of forward contamination by robotic spacecraft and human presence over a limited range of conditions and time. Ultimately, these experiments will help guide future planetary protection requirements and implementation procedures for robotic and human missions to Mars.

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