BIOLOGICAL STERILIZATION OF RETURNED MARS SAMPLES. C. C. Allen[1], F. G. Albert[2], J. Combie[2], R. J. Bodnar[3], V. E. Hamilton[4], B. L. Jolliff[5], K. Kuebler[5], A. Wang[5], D. J. Lindstrom[6], P. A. Morris[7], R. V. Morris[6], R. W. Murray[8], L. E. Nyquist[6], P. D. Simpson[9], A. Steele[10], S. J. Symes[6]
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Introduction: Martian rock and soil, collected by robotic spacecraft, will be returned to terrestrial laboratories early in the next century. Current plans call for the samples to be immediately placed into biological containment and tested for signs of present or past life and biological hazards. It is recommended that "Controlled distribution of unsterilized materials from Mars should occur only if rigorous analyses determine that the materials do not constitute a biological hazard. If any portion of the sample is removed from containment prior to completion of these analyses it should first be sterilized." [1]. While sterilization of Mars samples may not be required, an acceptable method must be available before the samples are returned to Earth. The sterilization method should be capable of destroying a wide range of organisms with minimal effects on the geologic samples.

A variety of biological sterilization techniques and materials are currently in use, including dry heat, high pressure steam, gases, plasmas and ionizing radiation. Gamma radiation is routinely used to inactivate viruses and destroy bacteria in medical research. Many commercial sterilizers use ^{60}Co, which emits gamma photons of 1.17 and 1.33 MeV. Absorbed doses of approximately 1 Mrad (10^8 ergs/g) destroy most bacteria [2].

This study investigates the effects of lethal doses of ^{60}Co gamma radiation on materials similar to those anticipated to be returned from Mars. The goals are to determine the gamma dose required to kill microorganisms in rock and soil samples and to determine the effects of gamma sterilization on the samples' isotopic, chemical and physical properties.

Samples and Methods: Twelve types of rocks and minerals were tested to assess the effects of gamma radiation: basalt, halite, carbonaceous chert, Mars soil simulant (weathered volcanic ash), carbonaceous chondrite meteorite, plagioclase, olivine, pyroxene, aragonite, montmorillonite clay, quartz and gypsum. Chips and powder samples were prepared in a tungsten carbide shatterbox and placed in ~1 cm^3 plastic vials.

Samples of two bacteria species, *Clostridium sporogenes* and *Bacillus subtilis*, were inserted into 8 x 17 mm cores of the fine-grained basalt. The cores were irradiated and the surviving bacteria were cultured. Naturally-occurring fungi and bacteria were also cultured from irradiated 1 cm^3 splits of Mars soil simulant. Rock cores and soil samples of these dimensions will be collected by the Athena instrument package on Mars for return to Earth [3].

The samples were irradiated with ^{60}Co gamma photons in a commercial sterilizer. Sets of samples were exposed to total doses of 0.3, 3 and 30 Mrad at ambient temperatures (< 49°C). Splits of all samples, prepared identically but not irradiated, were kept as controls.

Results: *Microorganisms in rock and soil*. The viabilities of *C. sporogenes* and *B. subtilis*, partially shielded within basalt cores, were significantly reduced at an absorbed gamma dose of 0.3 Mrad. Viability fell to near zero following a dose of 3 Mrad. Natural populations of fungi and bacteria in Mars soil simulant were destroyed or greatly reduced at a radiation dose of 0.3 Mrad, and entirely eliminated when the dose was increased to 3 Mrad. The highest experimental dose, 30 Mrad, was conservatively lethal with respect to all of these microorganisms.

Induced radioactivity. Each sample set was monitored for induced radioactivity by measuring gamma radiation over the energy range 0.05 to 2.0 MeV. The samples exposed to 0.3 Mrad produced 36 gamma ray peaks, all attributable to natural K, Th or U, or to ^{137}Cs produced by atmospheric nuclear tests. The net count rate attributable to these peaks across the entire energy range was 0.521 ± 0.003 counts/sec. The sample set exposed to 30 Mrad produced the same 36 gamma peaks and an identical net count rate. Thus, the combined sample sets showed no detectable evidence of induced radioactivity.

Isotopes and age dating. The ratios of ^{87}Sr/^{86}Sr, ^{149}Sm/^{152}Sm and ^{150}Sm/^{152}Sm were measured for unirradiated and irradiated (30 Mrad) samples of basalt using standard procedures for radiometric age dating. The ratios were indistinguishable within analytical error. Thus, radiometric ages for basalt, determined by the Sm-Nd or Rb-Sr methods, should not be affected by gamma doses as high as 30 Mrad.

Elemental composition. Concentrations of 28 major, minor and trace elements in powdered basalt were measured by INAA. A set of 17 elemental concentrations was determined by ICP-ES. Most major and minor element concentrations were indistinguishable, within analytical error, among sample sets. Concentration differences in tungsten of ~10 ppm were due to contamination from the shatterbox. The variations among splits of several trace elements exceeded analytical error but did not change systematically with radiation dose, and were attributed to natural inhomogeneities. In summary, no compositional changes due to irradiation were detected.

Crystal structure. The XRD patterns of control and irradiated (30 Mrad) powders were essentially identical for all samples. For the dominant peaks, all interplanar spacings were unchanged to a precision of < 0.5%. The peak widths of control and irradiated samples were indistinguishable. The basal spacing of montmorillonite, which is extremely sensitive to temperature and degree of hydration, was not affected by irradiation. No peaks were observed in spectra from the irradiated samples which were not present in spectra from the controls, and vice versa, indicating that no crystalline phases were formed nor destroyed. In particular, there was no evidence for the dehydration of gypsum to anhydrite.

Thermal IR (5-25 μm) spectra of these geologic materials exhibited absorption features due to the interaction of light with vibrational motions of atoms in the crystal lattices. Spectra of pyroxene, aragonite, gypsum and Mars soil simulant showed no changes in band positions resulting from radiation doses as high as 30 Mrad. Spectra of halite exhibited minor changes, attributable to variations in trace elements among the splits.

Raman spectroscopy provided data on the mineralogy of chip samples, including basalt, plagioclase, olivine, aragonite, quartz and gypsum. No changes in either the peak intensities nor peak positions of spectra from the irradiated (30 Mrad) splits relative to the controls were observed.

These three techniques provided complementary data on the samples' crystal structures. In no case were changes attributable to irradiation detected.

Fluid inclusions. Changes in the range of equilibration temperatures in quartz crystal fluid inclusions were measured but could not be ascribed unequivocally to irradiation effects.

Optical properties. Crystals of quartz, initially colorless and transparent, were colored deep brown and darkened by irradiation. The effect increased with dose, most apparently between 0.3 and 3 Mrad. Darkening was concentrated in bands parallel to the crystal faces. The dark bands correlated with Al_2O_3 concentrations of approximately 250 ppm, versus 100 ppm in the intervening light bands. Halite darkened and turned deep blue with increasing dose. Color and brightness changes were not apparent in other samples.

All samples were analyzed by diffuse reflectance spectroscopy at 20°C over the range 350 to 2100 nm (visible to near IR). Only quartz, halite, aragonite and carbonaceous chert showed changes in spectral properties clearly related to irradiation. Changes in quartz and halite spectra were consistent with the darkening and coloration seen visually. Spectral changes were confined to wavelengths below ~1000 nm for quartz and ~1400 nm for halite. Aragonite and carbonaceous chert became uniformly brighter over the entire spectral range, following radiation doses of 0.3 Mrad and higher.

Thermoluminescence. Irradiation altered the thermoluminescence (TL) properties of powdered quartz and plagioclase. The characteristic TL glow curves of these minerals were completely masked, particularly by a dose of 30 Mrad. The natural TL signals of basalt, Mars soil simulant and carbonaceous chondrite samples were too low to resolve irradiation effects. Studies of the natural TL properties of some returned Mars samples could be compromised by gamma sterilization.

Conclusions: Gamma photons from ^{60}Co, in absorbed doses on the order of 3 Mrad, destroyed two species of bacteria placed in basalt cores, as well as a variety of fungi and bacteria in simulated Mars soil. Gamma doses as high as 30 Mrad produced no measurable changes in the radiometric isotope ratios nor the elemental composition of fine-grained basalt. This dose level also had no measurable effects on the crystal structure of any of twelve geologic materials. The only detectable effects of irradiation were color and/or spectral changes in quartz, halite, aragonite and carbonaceous chert, increases in the TL of quartz and plagioclase and possible alteration of some fluid inclusions. If samples returned from Mars require sterilization, gamma irradiation is an attractive option.

References: [1] Space Studies Board, *Mars Sample Return Issues and Recommendations*, National Research Council, Wash., DC, 1997. [2] Battista, J. R., *Ann. Rev. Microbiol., 51*, 203-224, 1997. [3] Squyres, S. et al., *LPS XXIX*, 1998.