

Planetary Bioresources and Astroecology

1. Planetary Microcosm Bioassays of Martian and Carbonaceous Chondrite Materials: Nutrients, Electrolyte Solutions, and Algal and Plant Responses

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The biological fertilities of planetary materials can be assessed using microcosms based on meteorites. This study applies microcosm tests to martian meteorites and analogues and to carbonaceous chondrites. The biological fertilities of these materials are rated based on the soluble electrolyte nutrients, the growth of mesophile and cold-tolerant algae, and plant tissue cultures. The results show that the meteorites, in particular the Murchison CM2 carbonaceous chondrite and DaG 476 martian shergottite, contain high levels of water-extractable Ca, Mg, and $\text{SO}_4\text{-S}$. The martian meteorites DaG 476 and EETA 79001 also contain higher levels of extractable essential nutrients $\text{NO}_3\text{-N}$ ($0.013\text{--}0.017\text{ g kg}^{-1}$) and $\text{PO}_4\text{-P}$ ($0.019\text{--}0.046\text{ g kg}^{-1}$) than the terrestrial analogues. The yields of most of the water-extractable electrolytes vary only by factors of 2–3 under a wide range of planetary conditions. However, the long-term extractable phosphate increases significantly under a CO_2 atmosphere. The biological yields of algae and plant tissue cultures correlate with extractable $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$, identifying these as the limiting nutrients. Mesophilic algae and *Asparagus officinalis* cultures are identified as useful bioassay agents. A fertility rating system based on microcosm tests is proposed. The results rate the fertilities in the order martian basalts > terrestrial basalt, agricultural soil > carbonaceous chondrites, lava ash > cumulate igneous rock. The results demonstrate the application of planetary microcosms in experimental astroecology to rate planetary materials as targets for astrobiology exploration and as potential space bioresources. For example, the extractable materials in Murchison suggest that concentrated internal solutions in carbonaceous asteroids (3.8 mol L^{-1} electrolytes and 10 g L^{-1} organics) can support and disperse microorganisms introduced by natural or directed panspermia in early solar systems. The results also suggest that carbonaceous asteroids and martian basalts can serve as potential future resources for substantial biological populations in the Solar System.

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INTRODUCTION

Rocks and soils in early aqueous planetary environments may have provided resources for the origins of life and nutrients for early microorganisms. The objects of interest include carbonaceous asteroids during aqueous alteration and local igneous rocks or comets and meteorites that land within aqueous planetary environments. Similar materials are also of interest as potential soils for space-based agriculture (Ming and Henninger 1989). The present work demonstrates the use of planetary microcosms based on meteorites and terrestrial analogues to assay the biological potentials of these materials.

Meteorites can play several roles in which biological fertility is significant. For example, carbonaceous chondrites and similar dust or cometary materials import organics to planets (Chyba and Sagan 1992, Greenberg and Li 1998). Carbonaceous chondrite asteroids during aqueous alteration and carbonaceous meteorites on aqueous planets contain water in their pores and may form highly concentrated solutions that facilitate biogenesis (Mautner *et al.* 1995, Mautner 1997a, Bodnar and Zolensky 2000, Cohen and Coker 2000). Meteorites may also actively transport indigenous microorganisms or those introduced by natural or directed panspermia among asteroids and planets (Arrhenius 1908, Crick and Orgel 1973, Chyba and McDonald 1995, Mautner 1997b, Mileikowsky *et al.* 2000). After impact on aqueous planets, the meteorites will constitute the first nutrient environments for the embedded microorganisms (Mautner 1997a, Mautner *et al.* 1997).

There are indications that many space materials can indeed support life. First, their mineral and organic constituents are similar to terrestrial rocks that support diverse geomicrobiology. Algae growing on meteorite dust in Greenland were observed as early as 1870 [Leslie 1879 (p. 65 refers to observations of algae by the botanist Berggren on cryoconite, later identified

as micrometeorite dust by Maurette *et al.* 1986]. The nutrient values of organic planetary materials were demonstrated on a synthetic terrestrial analogue, tholin (Stoker *et al.* 1990). The Murchison CM2 meteorite was observed to have soil fertility parameters comparable to productive terrestrial soils (Mautner 1997b, Mautner 1999). Murchison extracts were observed to support various soil microorganisms such as the oligotrophs *Flavobacterium oryzihabitans* and *Nocardia asteroides*. By exploratory application of genetically modified organisms to planetary materials, experiments with *Pseudomonas fluorescence* with *Vibrio fischeri lux* genes showed that meteorite organics can serve as the sole carbon source (Mautner *et al.* 1997). Indications were also found that the Murchison materials support the anaerobic thermophile eubacterium *Thermotoga maritima* and the aerobic thermophile *Thermus aquaticus* (H. W. Morgan, quoted in Mautner *et al.* 1997). In contrast, the Allende meteorite was observed to inhibit biological growth. Martian minerals may have also supported microorganisms in the past (McKay *et al.* 1996). Various carbonaceous and martian meteorites were found recently to contain microorganisms from terrestrial contamination (Steele *et al.* 2000).

These observations suggest that diverse planetary materials can support biological activity. Their biological properties, as represented by meteorites or future return samples, should be assayed systematically in a way similar to agricultural soils (McLaren and Cameron 1996, Beare *et al.* 1997). Previous and more recent microbial experiments with meteorites constituted limited planetary microcosms for such purposes (Mautner *et al.* 1997, Mautner 1997a) and have been extended recently to more complex microbial populations (Mautner 2002).

The objectives of the present study are: (1) to introduce planetary microcosms as tools for the bioassay of planetary materials; (2) to measure the contents of water-extractable nutrient electrolytes in meteorites and analogues; (3) to use the extracts in microcosms for testing biological responses; (4) to identify useful bioassay agents; (5) to check the consistency of various nutrient and biological assays, and to develop a rating method based on the assays; (6) to apply microcosm rating to the biological fertilities of some actual and simulated planetary materials.

EXPERIMENTAL

1. Materials. The rock samples used were martian and carbonaceous chondrite meteorites and terrestrial analogues. The Dar al Gani 476 (DaG 476) meteorite and Elephant Moraine 79001 (EETA 79001) lithology A are both basaltic shergottites (McSween and Jarosewitz 1983). DaG 476 contains a fine-grained pyroxene and feldspathic-glass ground mass also containing sulfides and phosphates. Both meteorites contain olivine, orthopyroxene, and chromite. The DaG 476 meteorite was subject to extensive terrestrial weathering, leading to the formation of carbonates. Phosphate minerals in the shergottites include merrillite and chlorapatite. The comparative mineralogy

of DaG 476 and EETA 79001 was discussed recently (Zipfel *et al.* 2000).

The Nakhla meteorite is a cumulate igneous rock. Its main component is augite, with some olivine and minor other minerals (McSween and Treiman 1998). As only small amounts of Nakhla were available, the Theo's Flow lava formation in Canada, which was described as closely similar in mineralogy, was used as a terrestrial analogue (Friedman 1998). A further basalt sample containing 65% labradorite feldspar, 25% clinopyroxene, and 10% magnetite from Timaru, New Zealand was also used.

Further terrestrial analogues included NASA simulants of lunar and martian soils. The Mars soil simulant JSC Mars-1 is a sample of lava ash from the Pu'u Nene volcano in Hawaii (Allen *et al.* 1998). It contains Ca-feldspar and minor magnetite, olivine, augite pyroxene, and glass, including a highly weathered glassy matrix. It also contains nanophase ferric oxide similar to that inferred for martian soil. The lunar simulant JSC-1 is a glass-rich volcanic ash from the San Francisco volcanic field near Flagstaff, Arizona (McKay *et al.* 1993). Its elemental composition is similar to that of Apollo 14 soil sample 14163, and it contains plagioclase, clinopyroxene, orthopyroxene, olivine, magnetite, ilmenite, and apatite. In addition, a representative terrestrial agricultural soil, from the Templeton area in New Zealand, Udis Ustochrept, which is a fine loamy mixed, mesic soil, was also used for comparison.

Two carbonaceous chondrites, Allende and Murchison, were also used. The mineralogies of both are well known (Fuchs *et al.* 1973, Bunch and Chang 1980, Komacki and Wood 1984) and were reviewed recently (Brierly and Jones 1998). The main component of Murchison is a phyllosilicate formed by aqueous alteration in the parent body.

2. Extraction and analysis. Samples of meteorites and terrestrial analogues were ground in agate mortar to yield particle size distributions similar to those of terrestrial soils (Mautner and Sinaj 2002). Extractions were carried out in polythene tubes washed in 10% acetic acid for 24 h and rinsed four times with deionized water to remove electrolyte impurities.

The extractions were performed using deionized and Millipore filtered water with resistivity >18 Mohm-cm. The water was degassed by bubbling with N₂, denoted as "H₂O/N₂" in the following. In some extractions, the effects of early planetary CO₂ atmospheres were simulated by extracting the samples in deionized water saturated with CO₂ at pH 3.9, with the extraction vials sealed under CO₂ and placed in sealed jars filled with CO₂ as a further barrier against exchange with the atmosphere. These conditions are denoted as "H₂O/CO₂" in the following.

The powders were extracted at solid/solution ratios and extraction times as described in Table I. Extractions at 20°C were carried out with shaking on an orbital shaker. Hydrothermal extracts were obtained by placing the sealed extraction tubes in an autoclave and extracting under standard sterilizing conditions at 121°C for 15 min. For analysis, the powders were separated from the solution by centrifuging.

TABLE I
Concentrations of Water-Extractable Electrolytes (g kg⁻¹) in Carbonaceous Chondrites, Martian Meteorites, Martian and Lunar Soil Analogues, and Terrestrial Soil Solutions

Materials	Extraction conditions ^a	Ca	Mg	Na	K	Cl	NO ₃ -N	SO ₄ -S	PO ₄ -P
Carbonaceous chondrites									
Allende	HT ^b	0.097 (0.012)	0.20 (0.01)	0.060 (0.014)	0.034 (0.006)	0.10 (0.01)	0.004 (0.002)	0.36 (0.01)	0.0075 ^c
	H ₂ O/CO ₂ ^d	0.20 (0.03)	0.30 (0.05)	0.30 (0.04)	0.034 (0.004)	0.06 (0.01)	0.003 (0.002)	0.38 (0.01)	0.005
Murchison	HT ^b	3.0 (0.2)	4.0 (0.1)	1.4 (0.4)	0.34 (0.06)	0.44 (0.08)	0.008 (0.003)	7.6 (0.2)	0.0050 ^c
	H ₂ O/CO ₂ ^d	2.8 (0.5)	1.7 (0.1)	2.4 (0.1)	0.18 (0.01)	0.28 (0.01)	0.008 (0.002)	6.8 (0.4)	0.0048
	0.01 kg L ^{-1 e}	4.2	3.5	2.6	0.21	0.20	0.004	4.4	
	0.1 kg L ^{-1 e}	2.0	2.4	2.0	0.13	0.26		5.2	
	>1 kg L ^{-1 e}	1.4	1.7	1.9	0.11	0.18	0.012	5.7	
Mars meteorites									
Dar al Gani 476	HT ^b	1.0 (0.3)	0.38 (0.02)	0.067 (0.047)	0.064 (0.013)	0.074 (0.021)	0.017	0.92 (0.24)	0.019
	H ₂ O/CO ₂ ^d	1.1 (0.9)	0.58 (0.24)	0.040	0.032	0.06	0.015 (0.007)	0.88 (0.31)	0.024 ^f
EETA 79001	HT ^b	0.18	0.084	0.076	0.016	0.037	0.013	0.048	0.046
	H ₂ O/CO ₂ ^d	0.42	0.17	0.043	0.006	0.24	0.013	0.031	0.037 ^g
Mars and lunar analogues									
Basalt	HT ^b	0.09 (0.01)	0.037 (0.039)	0.088 (0.011)	0.032 (0.002)	0.056 (0.014)	0.002 (0.001)	0.008 (0.001)	0.013
	Theo's Flow	HT ^b	0.24 (0.02)	0.077 (0.051)	0.034 (0.011)	0.007 (0.002)	0.044 (0.014)	0.002 (0.001)	0.008 (0.001)
JSC Mars-1 simulant	H ₂ O/CO ₂ ^d	0.81 (0.01)	0.14 (0.08)	0.026 (0.007)	0.012 (0.003)	0.03 (0.01)	<0.001	0.004 (0.001)	0.0002
	HT ^b	0.15 (0.06)	0.014 (0.002)	0.011 (0.007)	0.11 (0.02)	0.048 (0.010)	0.004 (0.001)	0.012 (0.004)	0.003
Lunar simulant	H ₂ O/CO ₂ ^d	0.36 (0.06)	0.08 (0.02)	0.027 (0.008)	0.13 (0.01)	0.02 (0.01)	0.002 (0.001)	<0.001	0.0006
	HT ^b	0.16 (0.02)	0.004 (0.002)	0.10 (0.01)	0.027 (0.007)	0.048 (0.019)	0.002 (0.001)	0.018 (0.001)	0.017
Terrestrial soils									
Templeton soil	HT ^b	0.04 (0.02)	0.004 (0.002)	0.04 (0.01)	0.03 (0.007)	0.018 (0.010)	<0.001 (0.001)	0.007 (0.001)	0.001 ^h
Terrestrial soil solution (range) ⁱ		0.001–0.060	0.0007–0.1	0.009–0.03	0.001–0.011	0.007–0.050	0.002–0.80	<0.003–5	0.000001–0.03

^a All extractions at solid/solution ratios of 0.1 g mL⁻¹ unless noted otherwise.

^b Hydrothermal extractions at 121°C for 15 min. Average results of four replicates with standard deviations as shown in parentheses.

^c Average of present measurements (Allende 0.0051 g kg⁻¹, Murchison 0.0045 g kg⁻¹) and literature results (Allende 0.0098 g kg⁻¹, Murchison 0.0055 g kg⁻¹) (Mautner 1997a).

^d Extractions in water saturated with carbon dioxide (H₂O/CO₂) at a solid/solution ratio of 0.1 kg L⁻¹ at 20°C for 24 h.

^e Extraction in H₂O/N₂ at 20°C for 4 days. Solid/solution ratio as noted; >1 kg L⁻¹ represents the average of approximately constant extractable amounts at solid/solution ratios of 1, 2, 4, and 10 kg L⁻¹ (Mautner 2002).

^f Extraction in H₂O/CO₂ at a solid/solution ratio of 0.01 kg L⁻¹ at 20°C for 8 days.

^g Extraction in H₂O/CO₂ at a solid/solution ratio of 0.1 kg L⁻¹ at 20°C for 1 day.

^h Extraction in H₂O/N₂ at a solid/solution ratio of 0.1 kg L⁻¹ at 20°C for 4 days.

ⁱ Soil solution values units of g L⁻¹ (Bowen 1966).

Trace metals were extracted from Allende and Murchison at $r_{\text{solid/solution}} = 0.027 \text{ kg L}^{-1}$ by 1 M NH₄OAc solution, a standard soil extractant (Blakemore *et al.* 1987), and analyzed by ICP-MS at Hill Laboratories, Hamilton, New Zealand.

Anions in the extracts were analyzed by ion exchange chromatography using a Waters Ion Exchange Chromatograph and Waters Baseline 810 software. The method used was Waters Ion Chromatography Method A-102 "Anion Exchange Analysis

Using IC-Pak A HC Column Borate/Gluconate Eluent," with the samples filtered through a 0.1- μm filter paper prior to analysis. Cations were analyzed by a Shimadzu AA-6200 Atomic Absorption Flame Emission Spectrophotometer.

Analysis of the extracted phosphate was performed by developing the solutions with malachite green reagent at 4:1 solution/reagent ratios for 1 h and measuring the absorbance at 630 nm (van Veldhoven and Mannaerts 1987). In addition, total phosphorus content was determined by extraction of 10–100 mg samples with 10 ml of 70% HNO_3 solution overnight, adding 4 ml perchloric acid and heating to 210°C, and cooling and diluting appropriately for analysis by the malachite green reagent. Isotope exchange kinetics (IEK) assays of bioavailable phosphate were performed on a suspension of 100 mg solid in 10 ml deionized water that was allowed to equilibrate with the solution for 24 h. A small amount of $^{33}\text{PO}_4^{3-}$ was then added and solution samples were withdrawn 1, 10, 30, and 60 min after introducing the labeled tracer and the radioactivity in the solution was measured in a scintillation counter (Frossard and Sinaj 1997).

The meteorite sample sizes of 10–40 mg that are available per analysis are much smaller than the 1- to 10-g samples used in standard soil analysis. The small samples required soil microanalysis methods that were developed for meteorites previously (Mautner 1997a). The microanalysis methods for one of the analytical procedures, extractions with 1 M ammonium acetate, could be compared with conventional analysis by 10–30 laboratories worldwide, compiled by the International Soil Analytical Exchange Program (SAEP; Wageningen University, Department of Environmental Sciences, The Netherlands, WEPAL@MAIL. BenP.wagur.NL). The microanalysis data fell in the reported range. Comparison with the SAEP results leads to an estimated coefficient of variation ($cv = \text{std}/\text{mean}$) of 0.3 in our microanalysis measurements. This uncertainty estimate is consistent with the average cv of the results in Table I obtained from four replicate samples in each extraction. In addition to the usual factors in the SAEP interlaboratory scatter, the uncertainties in our data are due to the small available sample sizes. This leads to low concentrations of the analyzed elements in the extracts, often close to the detection limits of 10^{-4} – 10^{-3} g L^{-1} for anions and cations and 10^{-5} g L^{-1} for phosphate.

3. *Plant and algal cultures.* For the plant tissue culture experiments, the meteorites and analogues were extracted and sterilized at 121°C for 15 min at the ratio of 100 mg solid powder in 0.5 ml deionized water, except EETA 79001 where 10 mg of solid was extracted in 0.2 ml of water. For each plant culture medium, 45 μL of the extracts and 5 mg of the extracted solid powder were transferred to a 2-ml polythene microfuge tube. For blanks, deionized water was used instead of the extracts. *Arabidopsis* seeds were germinated and grown in these extracts directly. For asparagus and potato tissue cultures, to each 45 μL of extracts or water, a further 45 μL of 5 mM NH_4NO_3 solution +3% sucrose was added as nitrogen and carbon sources, leading to 2.5 mM NH_4NO_3 and 1.5% sucrose in the culture solutions.

Algal culture media were prepared by extracting 20 mg of the solids in 0.2 ml deionized water and mixing with equal volumes of 10 mM NH_4NO_3 or with deionized water. Two culture methods were used. In the first method, for testing benthic growth, both the extract and the extracted solid powder were placed in a cavity microscope slide partially covered with a slide cover and kept in a closed dessicator at 100% humidity to prevent evaporation. This somewhat reduced the light flux and these cultures were grown at $<80 \mu\text{E m}^{-2} \text{ s}^{-1}$. In the second method, used for population counting experiments, the aqueous extracts and only traces of the extracted powder were used under full illumination at $80 \mu\text{E m}^{-2} \text{ s}^{-1}$ in sealed vials, which were opened periodically for air exchange.

The inoculant algal cultures were grown in standard nutrient medium and washed by four cycles of centrifuging and washing with deionized water. This procedure dilutes the nutrient medium remaining in the algal sample by factors of 10^6 – 10^8 to negligible levels. The final washed algal pellets were dispersed in deionized water and diluted to give inoculations that used 20 μL of the mixed and washed algal cultures to yield starting populations of 10^4 CFU mL^{-1} of each algae in the microcosms. Algal populations in 20- μL samples were counted by direct microscopic count in a haemocytometer chamber.

Plant tissue cultures were established from *in vitro Asparagus officinalis*, cultivar "Limbras 10" genotype ASC 69. Apical meristem shoot tips about 1 mm long were dissected from 4–8 cm plants grown on agar. The parent plants developed to full size in agar in one month but were kept in agar for an additional three months to assure that all the nutrients in the medium were exhausted. This procedure produces plants that are depleted of nutrients, making them more responsive to nutrients in the mineral extracts. Well-formed globular apical shoot tips could be removed uniformly from each plant. Potato plants *Solanum tuberosum* cultivar Iwa were grown using a similar procedure. Plants of *Arabidopsis thaliana* strain "Landsberg Erecta" were germinated from seed on filter paper wetted with deionized water and grown to about 10 mm full size, which is achievable on the seed nutrients alone, before introduction to the culture media.

All plant cultures were grown for 20 days in closed microfuge tubes in standard tissue culture growth chambers under illumination by cool white fluorescent lights with an incident light flux on the samples of $80 \mu\text{E m}^{-2} \text{ s}^{-1}$ using 16-h light–8-h dark photocycles. Algal cultures were grown in similar growth chambers in vials with a punctured cap that allowed air exchange. The algal cultures were contained in glass jars saturated with water vapor to allow full light exposure but prevent evaporation.

RESULTS

1. *Extractable nutrient electrolytes in Mars meteorites and analogues.* The concentrations of extractable materials in the powdered solids are listed in Table I. The majority of the reported values are the average of four replicate measurements with the standard deviations as listed. The average of the cv

values (std/mean) for these measurements is 0.28, consistent with our general experience in similar extraction measurements. This may be assumed as the estimated uncertainty also for those results in Table I where less replicates were performed due to the scarcity of materials.

The water-extractable solutes may be calculated in terms of the concentration of extractable element in the solid, c_{solid} (g kg^{-1}), and the concentration established in the extract solution, c_{aq} (g L^{-1}). For a given extraction, the two quantities are related by

$$\begin{aligned} c_{\text{aq}} (\text{g L}^{-1}) &= c_{\text{solid}} (\text{g kg}^{-1}) w_{\text{solid}} (\text{kg}) / V_{\text{aq}} (\text{L}) \\ &= c_{\text{solid}} (\text{g kg}^{-1}) r_{\text{solid/solution}} (\text{kg L}^{-1}), \end{aligned} \quad (1)$$

where w_{solid} is the weight of solid subject to extraction, V_{aq} is the volume of the extract, and $r_{\text{solid/solution}}$ is the solid/solution ratio.

2. *Anions and cations.* The amounts of extracted elements depend on several variables such as the solid/solution ratio, pH, and temperature. Most of the data in Table I relate to extraction under mild hydrothermal conditions (121°C , 15 min, solid/solutions ratio of 0.1 kg L^{-1}). Extractions under more moderate conditions at 20°C in $\text{H}_2\text{O}/\text{N}_2$ generally yielded comparable amounts usually lower up to a factor of 2, as can be observed by comparing in Table I the Murchison results at 0.1 kg L^{-1} for hydrothermal vs $\text{H}_2\text{O}/\text{N}_2$ extractions. Using higher solid/solution ratios generally decreases the extracted amounts per unit weight of the solid, as also observed in Table I for Murchison, but the effect is small over the wide range of solid/solution ratios shown. Varying the extraction time for the materials in Table I from 2 to 8 days and in some cases up to 30 days changed the extracted amounts only within a factor of 2 in most cases.

Table I also shows the effects of extraction under a 1 atm CO_2 atmosphere, which models the atmospheres of early Earth and Mars (Kasting and Mishna 2000). The resulting high levels of dissolved carbonic acid and low pH can affect the bioavailabilities of ions. Table I shows that the amounts extracted hydrothermally in $\text{H}_2\text{O}/\text{N}_2$ or at 20°C by $\text{H}_2\text{O}/\text{CO}_2$ are generally similar, differing the extracted amounts by less than a factor of 2 in most cases.

Altogether, the extractions in Table I cover a wide range of planetary and asteroid conditions, from 20 to 121°C and, for Murchison, from an excess of water to an excess of solid and also acidic solutions (pH 3.9) under CO_2 -dominated atmospheres. The results show that the amounts of extractable electrolytes vary only moderately, generally less than a factor of 2, under a wide range of plausible planetary conditions.

The nutrients extracted from the meteorites may be compared in Table I with those extracted from terrestrial analogues. The highest levels of extractable Ca, Mg, Na, K, Cl, and SO_4 were found in Murchison, as was also observed in other extractions (Mautner 1997a, 2002), and these were up to an order of magnitude higher than in most of the other materials.

Although quite different in origin and mineralogy, the martian DaG 476 also yielded higher Ca, Mg, and $\text{SO}_4\text{-S}$ than the other materials, and K and Cl were also in the high range. On the other hand, the other martian sample, EETA 79001, yielded soluble electrolytes comparable with the terrestrial samples. Notable in the martian meteorites are the relatively high levels of the limiting nutrients $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ compared with that found in the other materials.

The 7.6 mg g^{-1} sulfate extractable from Murchison was about two orders of magnitude higher than in the terrestrial materials. The high levels of Ca and $\text{SO}_4\text{-S}$ may be derived from gypsum crystals observed on the Murchison surfaces (Fuchs *et al.* 1973) and may also be related to the high total S content of 3% in Murchison (Jarosewich 1971, Lovering *et al.* 1971). Note that the extractable sulfate is significantly higher in all the meteorites than in the terrestrial materials examined. The lowest amounts of most extractable electrolytes were found in the sample terrestrial soil, which, unlike the meteorites and rocks, have been subject to extensive weathering and leaching, although even with these amounts of nutrients, it is a productive agricultural soil.

In most of the electrolytes, the Allende CV3 meteorite yielded less soluble amounts by factors of 5–40 lower than in Murchison, as was also found in other extractions at various conditions (Mautner 1997a, 2002). The concentrations in Allende are comparable to those in the terrestrial rocks, except for relatively high Mg and $\text{SO}_4\text{-S}$. A few extractions, however, yielded several times less Ca, Mg, and $\text{SO}_4\text{-S}$ than the results in Table I, suggesting that Allende may be inhomogeneous in these components on the scale of 20- to 100-mg samples.

The concentrations in the extracts (c_{aq} , g L^{-1}) can be obtained according to Eq. (1) by multiplying the c_{solid} values in Table I by the solid/solution ratio, which is 0.1 g kg^{-1} in most samples. The resulting concentrations can be compared with those in terrestrial soil solutions shown in Table I. These solutions, diluted by a factor of 2, were applied in the algal and plant cultures described in the following. Except for the large concentrations obtained from Murchison, the other meteorites and simulants yielded solution concentrations within the range of terrestrial soil solutions. However, the limiting nutrient $\text{NO}_3\text{-N}$ in most of the extracts was lower by about an order of magnitude than the lower limit of soil solutions, even for the martian meteorites, which were the richest in extractable nitrate.

The other limiting nutrient is extractable phosphate. Because of its significance, it is desirable to characterize available phosphate in more depth. A quantitative assessment of long-term availability can be obtained by the isotope exchange kinetic method (Frossard and Sinaj 1997). The method, as described in the Experimental section, is suitable for meteorite studies as it can use small, 100-mg samples.

This method can assess, for example, phosphate available through extraction by a root system during three months. Applied to the present materials, isotope exchange measurements showed that the phosphate available in three months is higher by factors of 17–200 for the materials used than the short-term

TABLE II
Yields and Statistical Analysis of *Asparagus officinalis* Tissue Cultures Grown on Extracts of Meteorites and Simulants^a

Material	Fresh weight (mg) (mean and std dev.)	Sample vs blank <i>p</i> values ^b	Sample vs DaG 476 <i>p</i> values ^b	Sample vs Murchison <i>p</i> values ^b	Number of samples
Blank water	0.23 (0.03)	—	0.000	0.118	18
Allende set A	0.08 (0.07)	0.086	0.000	0.009	4
Murchison	0.32 (0.04)	0.064	0.050	—	14
Theo's Flow Nakhla simulant	0.35 (0.04)	0.075	0.136	0.609	15
Hawaii lava Mars simulant	0.38 (0.04)	0.008	0.370	0.283	14
Basalt	0.43 (0.06)	0.008	0.936	0.149	6
DaG 476	0.44 (0.04)	0.000	—	0.050	14
Allende set B	0.55 (0.05)	0.000	0.111	0.001	8
EETA 79001	0.58 (0.20)	0.000	0.020	0.000	11
MS nutrient medium	5.79 (0.18)	0.000	0.000	0.000	14
Reduced light					
Allende set B	0.30 (0.06)	0.004 ^c	1.000 ^d	0.231 ^d	6
Murchison	0.40 (0.05)	0.237 ^c	0.231 ^d	—	9
DaG 476	0.30 (0.06)	0.075 ^c	—	0.231 ^d	6

^a Yields (and standard errors) (mg) of *Asparagus officinalis* tissue cultures grown on extracts of meteorites or terrestrial analogues.

^b Mann–Whitney nonparametric analysis *p* values.

^c Mann–Whitney *p* values comparing the yields in the extract indicated by the row headings in full and reduced light.

^d Mann–Whitney *p* values comparing the yields in the extracts indicated by the row and column headings, both grown in reduced light.

soluble amount. An exception was DaG 476 where this ratio was only 1.6, possibly because the amount of soluble P is already high. The amount of (PO₄-P) (3 months) was higher by about an order of magnitude in the carbonaceous chondrites than in the other materials. Altogether, 13.0 and 43.9% of the total elemental P content was long-term bioavailable in Allende and Murchison while only 1–6% of the P present was bioavailable in the igneous materials.

The effects of extraction by H₂O/CO₂ for 21 days on the long-term available PO₄-P was substantial. This protracted exposure to H₂O/CO₂ increased the available P from 13.0 to 51.6% of the total P content in Allende, from 43.9 to 90.4% in Murchison, from 1.6 to 11.6% in DaG 476, from 6.4 to 97.6% in Theo's Flow, and from 2.5 to 40.5% in Hawaii lava. A more extensive study of extractable and of long-term bioavailable phosphate in planetary materials will be presented elsewhere (Mautner and Sinaj 2002).

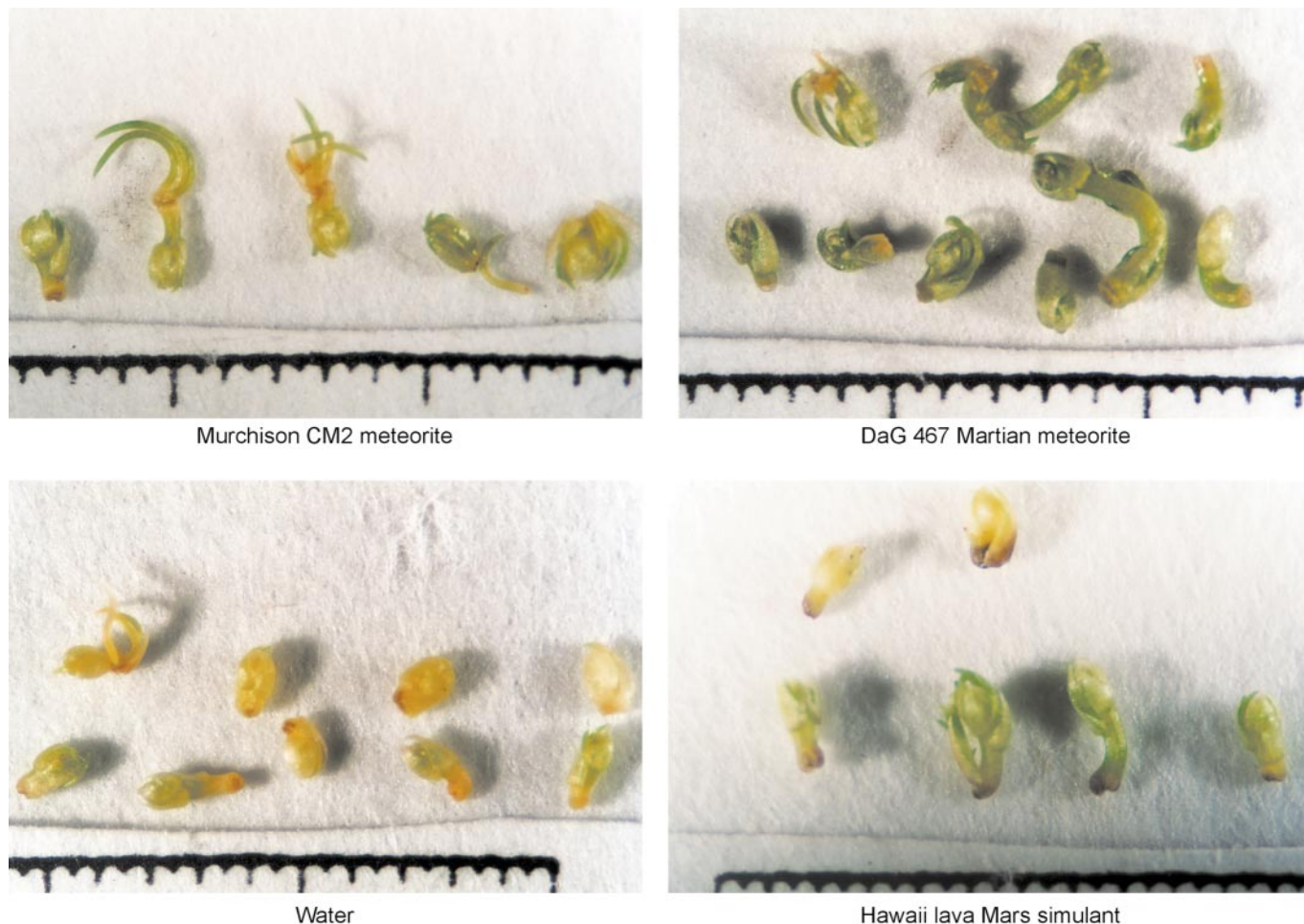
3. *Plant bioassays.* In previous studies, asparagus and potato tissue cultures showed that Murchison had a nutrient effect, with optimal growth from extractions at solid/solution ratios of 0.05–0.1 kg L⁻¹. In contrast, the extracts of Allende indicated an inhibitory, possibly toxic effect (Mautner 1997a, Mautner *et al.* 1997).

The present work examined the effects of extracts of additional planetary materials. The concentrations of the nutrients in the various extracts may be calculated from Table I as discussed previously. Two sets of each culture were grown with

the combined number of replicate plants as shown in Table II. Figure 1 illustrates the products obtained in one set of experiments. Significant differences were observed among the various media. The products in the control water blanks, supplemented only by nitrate and sucrose, showed the least growth, possibly only cell enlargement supported by stored nutrients in the starting meristem tips. In comparison, the meteorite and lava ash products showed more coloration and development. The plants in the extracts of the Hawaii lava Mars simulant and some of the DaG 476 products showed more stem development than the Murchison products. Some of the Murchison products showed a partial reddening that may indicate phosphorus deficiency. As noted in Fig. 1, the DaG 476 products showed the most differentiated development and the deepest green coloration, possibly due to the high phosphate content.

Cultures in one set of the Allende extracts showed brown coloration, decreased size, and lower fresh weights even than the water blank, as was also observed previously (Mautner 1997a). These effects suggest low nutrient concentrations or the presence of toxic elements. However, extracts from a different fragment of Allende showed more green coloration and development and had one of the highest yields. The differences between the two sets may reflect the inhomogeneity noted previously in the concentrations of extractable elements from different Allende fragments. The inhibitory fragment is denoted in Table II as Allende A and the nutrient fragment is denoted as Allende B.

Table II shows the yields determined as fresh weights, since the dry masses of <0.1 mg were too small to measure.



Murchison CM2 meteorite

DaG 467 Martian meteorite

Water

Hawaii lava Mars simulant

FIG. 1. Plant tissue cultures of *Asparagus officinalis* in meteorite and soil extracts, all supplemented with $5 \text{ mM L}^{-1} \text{ NH}_4\text{NO}_3$ and 3% sucrose. Small ticks are 0.5 mm apart.

Statistical analysis was performed to calculate the Mann–Whitney p values comparing the weights of the product plants in each pair of media. The p value obtained is inversely related to the statistical difference, and a value of $p < 0.050$ indicates a statistically significant difference between the two sets. A more strict but less sensitive indicator may be obtained using a protected mean separation test.

The statistical analysis may be applied to answer these questions: (1) Is any given treatment statistically different from blank water? (2) Are any two treatments significantly different from each other? (3) How does any given treatment compare with a full nutrient medium (Murashige and Skoog 1962)?

As to the last question, all of the yields in Table II are much lower than in the full medium and are, correspondingly, statistically distinct from the other media ($p = 0.000$). The lower yields in the extracts may reflect the absence, or unbalanced amounts, of nutrients.

In comparing the extracts with blank water, Table II shows increased yields with low p values, indicating that the extracts provided useable nutrients. The yields in the extracts of the two

martian meteorites were among the highest. Tables II, columns 2 and 4 show that the DaG 476 extracts were most similar in yield and gave the highest p values when compared with the terrestrial basalt and lava ash, which are of similar mineralogical origin. The Murchison products showed the closest, possibly coincidental, similarity in yield and highest p value compared with the Theo's Flow cumulative igneous rock.

Tissue cultures of *Solanum tuberosum* (potato) were also observed. The Hawaii lava extracts produced strong green coloration, while the Murchison and DaG 476 extracts produced a brown coloration. The average fresh weights of these products were higher than the asparagus yields, in the order Hawaii Lava, 2.2; DaG 476, 1.8; Murchison, 1.7; Allende and Theo's Flow, 1.6 mg. However, none of the extract sets were statistically different from each other, with the minimum p value of 0.451 between any two sets. These observations are similar to previous results with variously supplemented Murchison extracts, where the potato cultures also gave higher weights but smaller statistical differences among the various media than asparagus (Mautner *et al.* 1997).

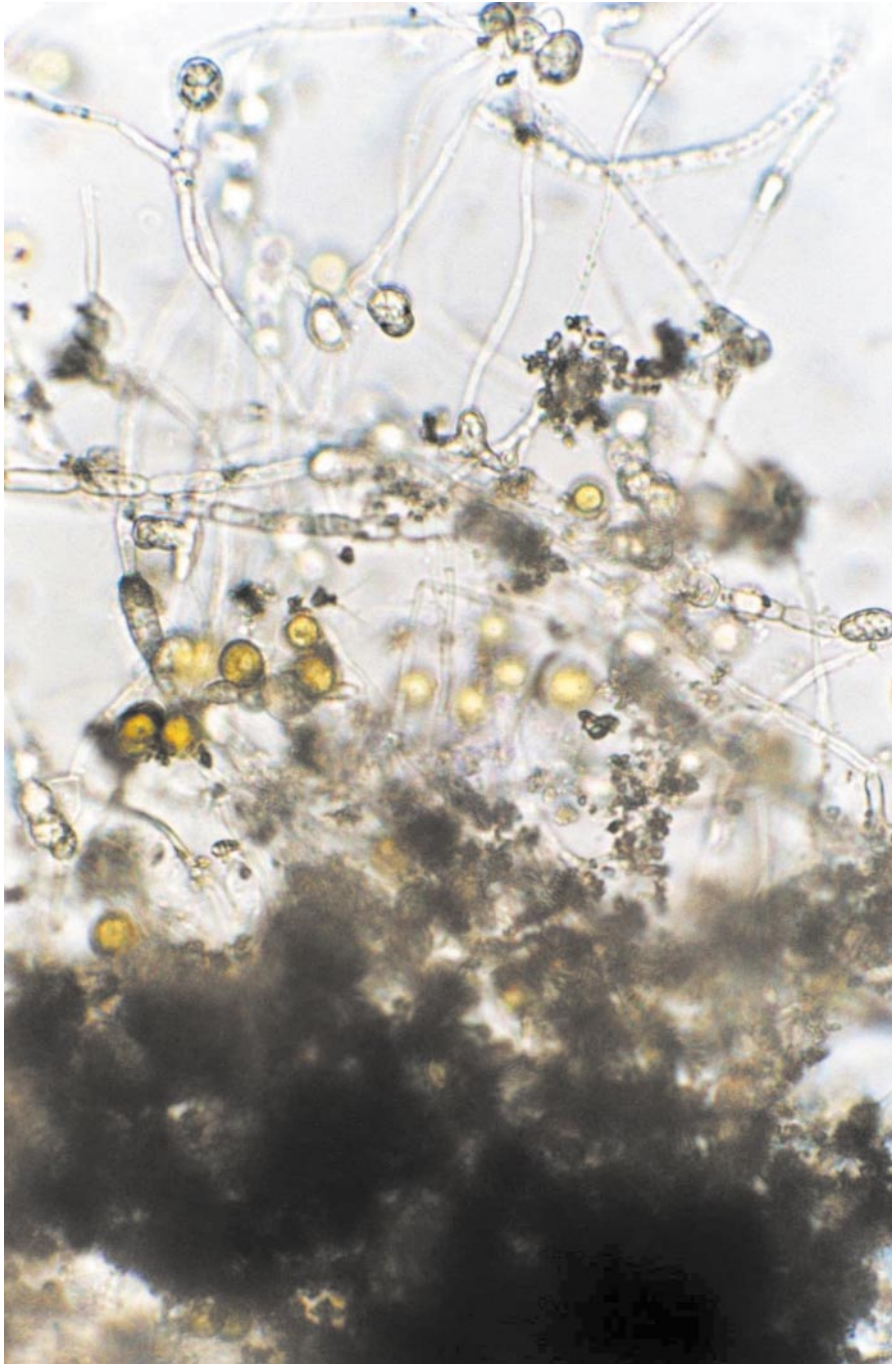


FIG. 2. Benthic growth of algae and fungi on powder of Dar al Gani 476 Mars meteorite. Yellow/green circles—*Chlorella*; white filament—fungal filaments; white spores—fungal spores. For scale, compare *Chlorella* in Fig. 3.

Alternative bioassay agents may be plants grown from seeds. In the present work *Arabidopsis thaliana* was grown in the meteorite and rock extracts to plants of about 10 mm. In the first 2 weeks the plants grown in all the media were similar as they used the seed nutrients. After 4–6 weeks, the relative effects of the various extracts were qualitatively similar to those observed in the asparagus tissue cultures, except for most of the plants in the extracts of Allende and Murchison that yellowed

and degraded, suggesting toxic effects. Plants grown from seed merit further work as they may produce more uniform results than tissue cultures where it is hard to achieve uniform starting meristem tips.

Light intensity may affect the sensitivity of tissue cultures to the various media, and it will also be an important variable in planetary biology at varying heliocentric distances. The present cultures were developed at typical tissue culture growth chamber

light fluxes of $80 \mu\text{E m}^{-2} \text{s}^{-1}$, about 10% of the solar constant on Earth, as more intense light damages the cultures. This light flux is near the O_2 production/utilization compensation point. In relation to astrobiology, this light-flux corresponds to the solar constant at about 3 AU (asteroid belt). To test light-flux effects, cultures of Murchison, Allende B, and DaG 476 were grown in a partially shaded area of the growth chamber at a reduced flux of $12 \mu\text{E m}^{-2} \text{s}^{-1}$, equivalent to the solar constant at about 9 AU (Saturn).

A comparison of the yields of each extract in full vs reduced light shows that the yields of the Allende B and DaG 476 extracts were smaller in reduced light than in full light, and the Mann–Whitney analysis showed small p values in these comparisons; i.e., this is a statistically meaningful effect of light reduction in these media (Table II, columns 2 and 3, footnote c, bottom rows). In Murchison extracts the light effects were statistically less significant.

It is also of interest if the light affects the differences between the yields in the various media. To examine this, the p values were calculated to compare pairwise the yields in Allende set B, Murchison, and DaG 476 extracts, all in reduced light (Table II, columns 2, 4, and 5, footnote d, bottom rows). These p values in reduced light were larger and showed less statistical distinctness than the yields in the same pairs of extracts in full light.

These observations suggest that light intensity is a significant variable in astrobiology. Nevertheless, the products observed in reduced light suggest that solar irradiation may support plant growth, although reduced, at the distances of the asteroid belt, Jupiter, and Saturn.

4. Algal bioassays. Algae are the first colonizers in many terrestrial environments and therefore are also candidates for planetary terraforming. Algae are therefore relevant to planetary bioassays. They are also convenient because their sizes allow microscopic analysis.

In the present experiments we used mesophilic algae isolated from soil in Canterbury, New Zealand. Preliminary experiments were also performed with cold-tolerant algae from sub-Antarctic islands. Mixed algal populations were used to allow a degree of natural selection, testing whether a dominant species will emerge or a diverse stable ecosystem is established in the microcosms.

The algae were identified microscopically to genus level as *Leptoyngbya sp.* (filamentous blue-green), *Klebsormidium sp.* and *Stichococcus sp.* (filamentous green), *Chlorella sp.* (green unicellular), *Chlorosarcinopsis sp.* (green unicellular in aggregates), and *Navicula sp.* (diatom). The inoculant cultures also contained flagellate bacteria of about $1 \mu\text{m}$ in diameter.

In the cultures grown on cavity slides as described previously, the meteorite extracts and solids served as minaturized planetary microcosms simulating small ponds. The growth of the algae was examined microscopically at weekly intervals. Most of the growth of the filamentous blue-green algae and *Chlorella* occurred as benthic growth.

Green cells and filaments were observed to survive for at least four weeks in most extracts. The best growth, illustrated in Fig. 2, was observed with the DaG 476 extract and solids. In contrast, in the Allende A materials growth ceased after 6–10 days and only shells of algae were observed later, consistent with the inhibitory effects of this material already noted. Figure 2 also shows a dense growth of fungal filaments and spores. Since the DaG 476 material is not known to contain organics, the fungal growth appears to be supported by algal detritus and enhanced by the high extractable phosphate content of this meteorite.

The benthic growth did not allow the microscopic counting of cell populations because of the interference of the solids. For quantitative measurements cultures were grown in extracts in vials, without the solids present, and counted in a haemocytometer chamber. Unicellular *Chlorella* as single cells and some *Chlorosarcinopsis* in aggregates of 2–20 cells were dominant in all the extract cultures. A few filaments, 8–40 μm in length and containing 2–10 cells were observed, as well as occasional short *Klebsormidium* filaments and *Stichococcus* fragments, with cell populations smaller by an order of magnitude than the unicellular algae. Figure 3 shows examples of the algal populations obtained after 32 days. The unicellular chlorophytes dominated in all the cultures, but the diversity of organisms varied in the different extracts. Fungal spores were also observed especially in the Murchison extracts, where they may be supported by the organic content of the meteorite. This is similar to the efficient growth of heterotrophic bacteria and fungi observed in other cultures of Murchison extracts (Mautner 1997a, 2002). Although the starting inoculants contained diatoms, no diatom populations were observed after 1–4 weeks.

Growth curves for cultures without added nitrate are shown in Fig. 4. The relative cell populations after 34 days followed a similar order as in the plant tissue cultures. Here also the yields of DaG 476 exceeded Hawaii lava and Murchison, while the Allende A extracts gave the lowest populations.

The population levels were higher in the cultures containing added nitrate, but the relative populations in the various extracts followed a similar order to that without nitrate. The populations grew for about 8 days and remained approximately constant for a further 8–20 days. After 8 days the product populations were DaG 476, 1.1×10^6 ; Murchison, 0.30×10^6 ; Hawaii lava, 0.27×10^6 ; Theo's Flow, 0.23×10^6 ; and Allende A, 0.12×10^6 cells mL^{-1} . These relative yields were consistent with the trends in the extracts without added nitrate and in the plant tissue cultures with added nitrate. The fact that added nitrate had little effect on the relative yields suggests that phosphate rather than nitrate was the limiting nutrient. Cultures of the same algae were also grown in more complex microcosms of Allende and Murchison that also included bacteria and fungi inoculant from a wetland (Mautner 2002).

Cold tolerance is required for potential growth on the asteroids and outer planets. For this reason, preliminary experiments were performed with cold-tolerant *Chlorella sp.*, *Stichococcus sp.*, and filamentous blue-green *Oscillatoriacie sp.* collected

from sub-Antarctic islands. The cultures were grown in meteorite extracts at 12°C inoculated with 10^4 CFU mL^{-1} of each alga. After 60 days, the combined populations of *Chlorella* and *Stichococcus* were: Nutrient solution, 25.9; Basalt, 6.1; Allende B,

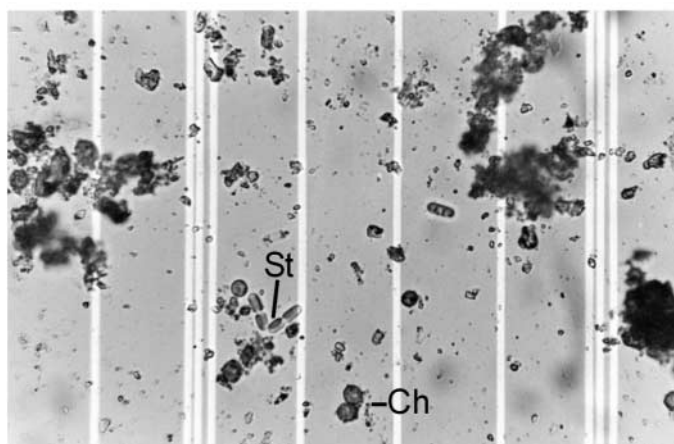
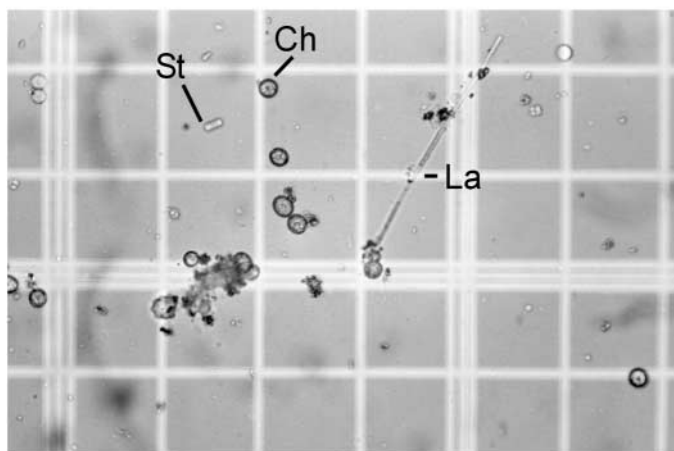
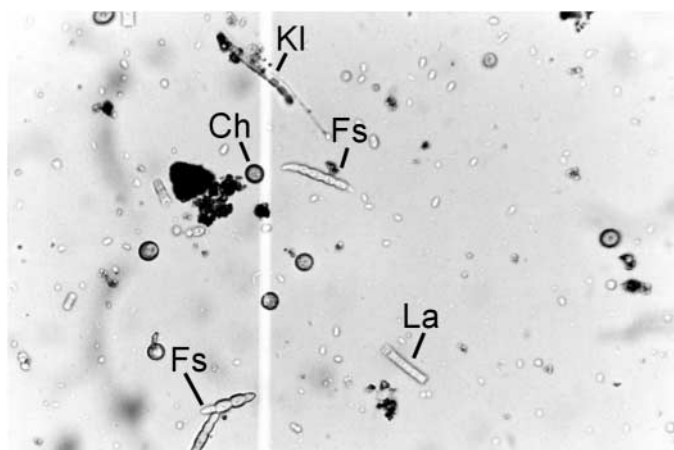


FIG. 3. Algal populations observed in meteorite and soil extracts supplemented by 0.5 mM NH_4NO_3 after 32 days of growth. (Top) Murchison. (Middle) DaG 476. (Bottom) Hawaii lava Mars simulant. Ch—*Chlorella*; Kl—*Klebsormidium*; La—*Leptolyngya*; St—*Stichococcus*; Fs—fungal spores. The spacing between the counting chamber lines is 0.2 mm.

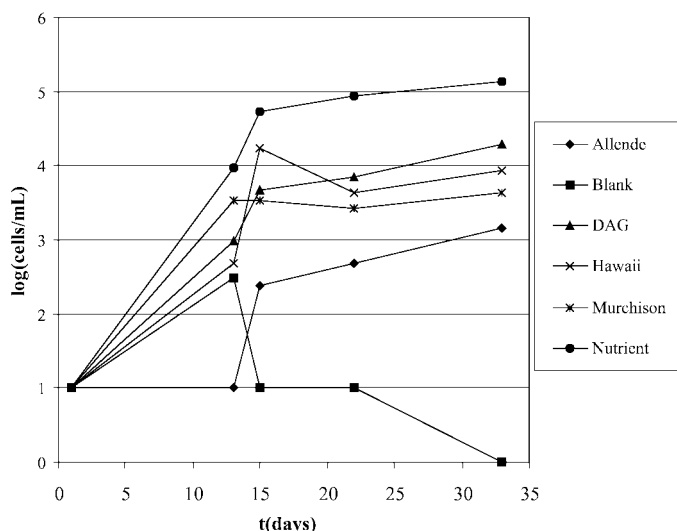


FIG. 4. Algal population growth curves in meteorite and simulant extracts and BG-11 nutrient medium. Note the order of yields: BG-11 nutrient medium > DaG 476 martian meteorite > Hawaii lava Mars simulant > Murchison > Allende A > deionized water.

2.5; Hawaii lava, 1.5; Lunar Simulant, 1.3; DaG 476, 1.1; Theo's Flow, 1.0; Murchison, 0.9; Templeton soil, 0.6; blank water control, 0.2×10^6 CFU mL^{-1} . In these cultures also, the filamentous blue-green algae, here *Oscillatoriacie*, did not grow well and disappeared after 20 days. Further increased viable populations, especially of *Stichococcus*, were observed for at least eight months in these cultures.

In addition to the algal growth, all of the cultures contained motile microorganisms of about 1 μm in diameter. The bacteria attained observable populations after 2–4 weeks, and at later times they become numerically the largest population, at the ratio of 2–4 microorganisms per algal cell. The populations were highest in the extracts Templeton Soil, Murchison, and Hawaii lava, in correlation with the organic contents of the solids. When cultured on nutrient or potato agar in the dark, the microorganisms yielded bright orange colonies, but not when the agar was supplemented with chlortetracyclin (auremycin) antibacterial agent. The microorganisms were motile heterotrophic bacteria supported by organics in the extracts and by the algal metabolites and decay products.

DISCUSSION

1. *Design considerations for planetary microcosms.* The design of planetary microcosms depends on the objectives of the simulation, such as the testing of planetary materials in relation to early life or as future ecosystems. Early planetary conditions may have been reducing or dominated by carbon dioxide. In contrast, terraforming will aim to create habitable oxygen-rich environments. Our present tests address the latter environment, but anaerobic tests are also under way.

To assess a complex planetary ecosystem, it is necessary to examine the mutual effects of atmospheric, aqueous, and geological processes. Such studies may be limited, however, by the available amounts of meteorites. For example, the analysis of extracted nutrient cations commonly involves atomic absorption spectroscopy or ICP-MS, and for anions, ion exchange chromatography. These techniques require about 1 mL solutions containing 10^{-6} g of solute, usually from solids that contain them in soluble forms mostly in concentrations of 10^{-3} – 1 g kg^{-1} (see Table I); i.e., they require 0.001–1 g of mineral samples. Phosphate can be quantified reliably using colorimetry in 1-mL samples at concentrations of 10^{-5} g L^{-1} and is usually contained at extractable levels of 10^{-4} – 10^{-2} g kg^{-1} (see Table I), requiring 0.001–0.1 g of mineral samples.

For biological tests, the microcosm must contain enough nutrients to support the population. In plant bioassays, the sample plants from tissue cultures yield typically fresh weights of 10^{-3} g containing 1 g kg^{-1} or a net 10^{-6} g of macronutrients such as Ca or K. This requires 0.001–1 g of mineral sample per plant and usually 4–10 plants per experiment to allow statistical comparisons (see Table III).

For microbial or algal microcosms, population levels of 10^7 cells mL^{-1} in 1-mL cultures need to be supported. With an average algal mass of 10^{-10} g cell^{-1} again a macronutrient content 10^{-6} g extracted from 0.001–1 g of solid may be required. For tests using bacteria, populations of 10^7 cells mL^{-1} of microorganisms of mass 10^{-13} – 10^{-12} g cell^{-1} , the requirements are smaller, 10^{-9} – 10^{-8} g of nutrient extracted from 10^{-6} – 10^{-2} g of solid.

The material requirements for chemical analysis and plant and algal microcosm cultures are therefore usually 0.001–1 g per test. A comprehensive ecological study of a microcosm may require 10 or more chemical and biological analyses and 0.01–10 g of material. These considerations illustrate that microcosm simulations are possible with small amounts of meteorite materials, but the experiments must be designed judiciously.

2. Simulated solutions in asteroid, cometary, and meteorite interiors. Extracts obtained under planetary conditions can simulate interior solutions in meteorites and their parent bodies during aqueous alteration in surface layers of cometary nuclei during perihelion passes, and in meteorites after in fall or after infall to aqueous early planets. In fact, the high soluble salt contents in Murchison may have been deposited during the alteration process, after the water evaporated or was absorbed by serpentine formation (Tomeoka and Buseck 1985, Bodnar and Zolensky 2000, Cohen and Coker 2000). Similarly, high levels of soluble salts in the Nakhla martian meteorite were considered to be evaporates from a salty ocean (Sawyer *et al.* 2000), and this may also apply to DaG 476.

To simulate these interior solutions, the solids should be extracted at the high solid/solution ratios that apply in the internal pores. In Murchison, which has a porosity of 23%, this requires a solid/solution ratio of about 10 kg L^{-1} . Recent studies of extractions in the range of 1–10 kg L^{-1} showed that the

amounts extracted per gram solid remain constant in this range of solid/solution ratios, at the values listed Table I (Mautner 2002). For extractions at $r_{\text{solid/solution}} = 10$ kg L^{-1} the resulting solution concentrations in moles per liter can be obtained by multiplying the values in Table I by 10/MW (molecular weight of the solute). The results are 0.4, 0.7, 0.8, and 0.03 mol L^{-1} for the cations Ca^{2+} , Mg^{2+} , Na^+ , and K^+ and 0.05, 0.009, 1.8, and 0.002 mol L^{-1} for Cl^- , NO_3^- , SO_4^{2-} and PO_4^{3-} , respectively. The total measured ion concentration is 3.8 mol L^{-1} with an ionic charge of 6.7 equivalents L^{-1} . Similarly, from the amount of soluble organics one can estimate a concentration of organics (using average MW = 100) as 0.1 mol L^{-1} (Mautner *et al.* 1995).

From a biological point of view, trace elements are significant as micronutrients. Extractions of Murchison in water at $r_{\text{solid/solution}} = 0.021$ kg L^{-1} yielded only Mn at 0.005 and Ni at 0.1 g kg^{-1} , while more aggressive extraction in 1 M ammonium acetate yielded B, 0.0005; Fe, 0.009; Mn, 0.011; Al, 0.00003; Cd, 0.0038; Cr, 0.0002; Cu, 0.0005; and Ni, 0.1 g kg^{-1} . Converted to solution concentrations at the solid/solution ratios of 10 kg L^{-1} applicable in asteroid pores, all the concentrations are in the range found in soil solutions and can be sufficient as micronutrients. However, the high concentration of Ni may be toxic.

The calculated concentrations show that the pores of carbonaceous chondrite parent asteroids and CM2 meteorites may contain highly concentrated electrolytes and organics under aqueous conditions. Can microorganisms grow in these solutions? As a test, samples of Murchison that were wetted at solid/solution ratios of 10 kg L^{-1} were inoculated with microbial isolates from a wetland (Mautner 2002). The cultures produced microbial communities of several algal species; bacteria assigned tentatively as *Clavibacter michiganense*, *Microbacterium imperiale*, *Eurobacterium saperdae*, *Pseudomonas putida*, and *Corynebacterium sp.*; and a filamentous fungus. These microbial populations grew in these concentrated meteorite–asteroid interior solutions for over a year.

3. An assessment of the biological test agents. It is of interest to correlate the responses of the various test organisms to each other and to correlate these biological responses with the nutrient contents of the various media.

The algal populations produce yields in units of CFU per milliliter and the plant cultures in units of plant mass in milligrams. To compare these responses on a common basis, the yield of each culture in medium X was normalized using Eq. (2) to the yield in the respective optimized medium:

$$\text{yield X (\%)} = 100[\text{yield}(X) - \text{yield}(\text{blank water})] / [\text{yield}(\text{in optimized medium})]. \quad (2)$$

The algal populations were normalized to those obtained in BG-11 medium (Rippka 1979) and the plant fresh weights to those obtained in an MS medium (Murashige and Skoog 1962). The yields in water and in optimized media used for the normalization were: mesophile algae, 0 and 1.4×10^5 CFU ml^{-1} ;

TABLE III
Relative Algal and Plant Culture Yields,^a Limiting Nutrients, and a Bioassay Ranking of Meteorites and Analogous Rocks

	Mesophile algae ^{a,b}	Antarctic algae ^{a,c}	Asparagus ^a	Potato ^a	Mean relative yield ^d	Rating by algal yield ^e	Rating by average yield ^e	Rating by NO ₃ -N ^e	Rating by PO ₄ -P ^e	Mean Z score and fertility rating ^f
Allende B	—	8.4	5.5	7.6	7.2	+	++	+	+	-0.22 M
Murchison	3.1	2.4	1.6	8.1	3.8	+	+	++	+	-0.57 M
DaG 476	14.1	3.1	3.6	9.0	7.4	++	++	+++	++	0.58 H
EETA 79001	14.4	—	6.0	—	10.1	+++	++	+++	+++	1.32 VH
Basalt	14.2	22.4	3.6	—	13.3	+++	+++	+	++	0.65 H
Hawaii lava	4.7	4.7	2.6	11.1	5.8	+	+	+	O	-0.56 M
Lunar simulant	0.0	3.7	—	6.0	3.2	O	O	O	++	-0.36 M
Theo's Flow	0.7	2.7	2.1	7.7	3.3	O	O	+	O	-1.01 L
Templeton soil	18.6	1.2	—	—	9.9	++	++	O	+	0.21 H
<i>cv</i> ^g	0.84	0.62	0.48	0.21	0.49					

^a Yields of algal populations and tissue culture products, normalized to yields in optimized media (see Eq. (2) and following text).

^b Normalized algal populations after 34 days.

^c Normalized algal populations after 60 days.

^d Mean over columns $j = 2-5$ of the normalized yields x_{ij} for material i .

^e Rated using the standard normal variate (Z score = $(x_{ij} - \mu_j)/\sigma_j$) (see Eq. (3) and following text).

^f Average of the Z_{ij} values for each material, and rating based on this value (see text).

^g Coefficient of variation $cv = \text{std}/\text{mean}$ of the values in each column.

Antarctic algae, 2.9×10^5 and 2.6×10^7 CFU ml⁻¹; asparagus, 0.23 and 5.79 mg; and potato, 0.2 and 18.0 mg. Although the normalized yields as shown in Table III are low, they do show differences among the various extracts. The overall biological response to each extract is reflected in Table III, column 6 by the mean of the normalized yields.

It is of interest whether the relative responses to the various media were correlated among the various test organisms. Pearson correlation analysis of the yields showed the highest correlation coefficient between the yields of mesophile algae and asparagus cultures (cor. coeff. = 0.716) and the smallest correlation coefficient between the yields of the Antarctic algae and potato cultures (cor. coeff. = 0.005).

A discriminating biological assay agent should give distinct responses to different media. In this respect, a large scatter of the responses of a given organism to various media indicates the ability of that organism to discriminate among the media. A measure of this scatter is the coefficient of variation ($cv = \text{std}/\text{mean}$) of the set of responses of each assay agent j (columns 2-5, Table III) to the set of media in rows i , with the resulting cv values for each test agent j shown in the last row of Table III. By this measure the organisms applied are discriminating in the order of cv values: Mesophilic algae (0.84) > Antarctic algae (0.62) > asparagus (0.48) > potato (0.21).

4. *Relation between nutrients and biological responses.* The responses of the various test organisms should correlate with the nutrients in the mineral extracts. To test this a Pearson correlation analysis was carried out between the normalized yields of each algal and plant test organism in Table III and the nutrient concentrations were derived from the data in Table I. The biological yields showed negative correlations with most of the

electrolytes in the extracts, i.e., Ca, Mg, Na, K, Cl, and SO₄-S, with correlation coefficients mostly between -0.2 and -0.5, but positive correlations with the NO₃-N and PO₄-P concentrations. The largest positive correlation coefficients were obtained with the yields of mesophilic algae, which showed correlation coefficients of +0.28 with NO₃-N and +0.32 with PO₄-P. The asparagus yields also gave positive correlation coefficients of +0.31 with NO₃-N and +0.74 with PO₄-P. The results indicate that PO₄-P and to a lesser degree NO₃-N are the limiting nutrients.

The overall biological response is measured by the combined average normalized yields of the algae and plants in Table III, column 6. An analysis of these yields vs the concentrations of nutrients gave the correlation coefficients -0.38 for Ca, -0.36 for Mg, -0.34 for Na, -0.36 for K, 0.37 for Cl, +0.08 for NO₃-N, -0.36 for SO₄-S, and +0.35 for PO₄-P. These results again suggest that nitrate and especially phosphate are the limiting nutrients in these materials.

An alternative way to identify the limiting nutrients is to compare the relative amounts of the nutrients in each medium with the relative amounts of these nutrients in biomass. For example, the concentration of nutrient X_i in extract i may be normalized to the limiting nutrient (NO₃-N) in the same extract. The normalized concentrations ($[X]/[\text{NO}_3\text{-N}]_i$) in solution can be compared with the normalized concentration of the same nutrient ($[X]/[\text{NO}_3\text{-N}]_{\text{biomass}}$) in algal biomass (Bowen 1966). The ratios of the normalized concentrations $[X/(\text{NO}_3\text{-N})]_i/[X/(\text{NO}_3\text{-N})]_{\text{biomass}}$ show the excess of the element in extract i over the amount needed to construct algal biomass when all the NO₃-N in the extract is converted to biomass. The comparison shows, for example, that if all the NO₃-N extracted from EETA 79001 is converted to plant biomass, Ca in this extract is in excess by

a factor of 23; Mg, 59; Na, 145; K, 2.6; Cl, 42; SO₄-S, 34; and PO₄-P, 252, over the amount required to complement NO₃-N. Similarly, if the available NO₃-N in Murchison is converted to algal biomass, Ca is in excess by a factor of 493; Mg, 1428; Na, 80; K, 12; Cl, 177; SO₄-S, 1188; and PO₄-P, 3.5 to form algal biomass. This analysis further suggests that NO₃-N is the limiting nutrient in the martian meteorites while in Murchison both NO₃-N and PO₄-P are the limiting nutrients. Of course, this analysis does not include C, which is available on Mars from the atmosphere and in Murchison from the 2% organic fraction.

The high fertility of the martian meteorites in the final ranking in Table III may be due primarily to the relative high extractable nitrate and phosphate. Conversely, Murchison ranks only medium despite its high content of extractable electrolytes, probably because of the relatively low levels of nitrate and phosphate.

5. *A bioassay of martian and meteorite materials and analogues.* The results of the biological tests and nutrient analysis can be combined for an overall rating of the biological potentials of the tested materials. The rating is based on the average algal yields, plants yields, combined average biological yield, and concentrations of the limiting nutrients NO₃-N and PO₄-P. These different criteria must be accounted for in a form that allows comparisons among tests measured in different units. In this work, a standard variate *Z* score was applied. This relates the result of each test on material *i* to the average and standard deviation of the same test over all the materials, using

$$Z_{ij} = (x_{ij} - \mu_j) / \sigma_j. \quad (3)$$

Here x_{ij} is the value of result *j* (biological yield, columns 2–6 in Table III, or nutrient concentration, columns 8 and 10 in Table I) for a given material *i* (rows 1–11 in Table III), and μ_j and σ_j are the mean and standard deviation of results of test *j* averaged over all the materials. The resulting *Z* scores were then used to rate material *i* according to result *j*, as follows: $Z_{ij} > 1$ is rated +++; $0 < Z_{ij} < 1$ is rated ++; $-1 < Z_{ij} < 0$ is rated +; and $Z_{ij} < -1$ is rated O. For example, the average of the mean normalized biological yields in Table III, column 6 is $\mu_j = 7.11\%$, with $\sigma_j = \pm 3.49\%$. Using Eq. (3), these values combined with the normalized biological yield of 10.1% of EETA 79001 gave a *Z* score of 0.86, which was rated as ++ (Table I, column 8).

The criteria rated in this manner in Table III are the mean algal yield (average *Z* score for mesophilic and Antarctic algae, Table III, columns 2 and 3), the overall mean biological (algal and plant) yield (Table III, column 6), and the extractable NO₃-N and PO₄-P concentrations from Table I. Finally, an overall rating is assigned to each material *i* based on the mean of the *Z* scores for material *i* over the criteria *j* applied, as shown in Table III, column 11. The ratings VH, H, M, or L were assigned to the fertility of the material on the same basis as the + + +, etc., values assigned in the preceding paragraph. For example, the

average of the *Z* scores for EETA79001 in the various tests was 1.32, which was rated as VH.

The various fertility criteria obtained by this procedure in Table III show internal consistency. The basaltic martian meteorites and the terrestrial basalt sample rate ++ or +++ in most individual tests and VH or H in the overall evaluation. The two carbonaceous chondrites Allende and Murchison rate + or ++ in the tests and M overall. The two lava ash NASA simulants, JSC Mars-1 and the JSC-1 lunar simulant, and the Theo's Flow Nakhla analogue rate O or + in the tests and M in the overall rating. The tests therefore assign comparable ratings to related materials. The martian materials rate higher than their proposed mineralogical or physical analogues, suggesting that biological simulants should be selected by different criteria than mineralogical or physical simulants. The result that the fertility tests group similar materials together suggests that the tests are internally consistent.

CONCLUSIONS

1. *Microcosm bioassays.* The present work tests the application of planetary microcosms to assay the biological fertilities of extraterrestrial materials, using small samples of 10–100 mg suitable for meteorite studies. The procedure in this work consists of:

- (1) aqueous extraction, and analysis, of nutrient electrolytes at solid/solution ratios of 0.01–10 kg L⁻¹, using 0.1 kg L⁻¹ in most cases;
- (2) biological tests using mixed algal populations that allow a degree of natural selection by the tested material and tests using plant tissue cultures;
- (3) correlation analysis among the biological responses to various meteorites, to test the consistency of the bioassays;
- (4) using the bioassay to identify the most discriminating and sensitive test organisms;
- (5) correlation analysis between the overall biological responses and extracted nutrients to identify the critical limiting nutrients; and
- (6) based on the extractable nutrients and biological responses, assigning a fertility ranking to various materials.

The various tests applied were generally consistent. Mesophile green algae and asparagus tissue cultures were found to be sensitive and discriminating bioassay agents. Testing the correlation between nutrients and biological yields suggests that NO₃-N and PO₄-P are the limiting nutrients in the meteorites. The fertility ranking groups materials in the order martian basalts > terrestrial basalts, agricultural soil > carbonaceous chondrites, lava ash > cumulate igneous rock. The tests rate materials of similar mineralogy together in terms of fertility. The relation between the relative biological yields and the extractable nutrients is also reasonable. These observations suggest that the bioassays are internally consistent.

2. *Implications for astrobiology and astroecology.* Astrobiology concerns the past evolution and potential future of life in the Universe (Morrison 2001). The microcosm results are relevant to these areas, although the present small microcosm models can provide only a few indications of the biological potentials.

Based on the present results, carbonaceous asteroids during aqueous alteration and meteorites after infall to aqueous planets can form concentrated internal solutions of nutrient electrolytes (3.8 mol L^{-1} electrolytes) and soluble organics (10 g L^{-1}) as discussed elsewhere (Mautner 2002). These trapped solutions can allow the multistep synthesis of complex organics, contributing to biogenesis. The observed microbial and algal growth suggest that these solutions can also support early microorganisms. These observations may support tentative observations of Dinoflagellate and Chysomonad algal fossils in carbonaceous chondrites meteorites (Claus and Nagy 1961, Urey 1962). If nutrient solutions and microorganisms are indeed present in early aqueous carbonaceous asteroids, frequent collisions among these objects may facilitate the growth and dispersion of the microorganisms in solar nebulae, making them favorable environments for natural panspermia and useful targets for directed panspermia (Mautner 1997b, 2002).

The results show that light intensity is a significant variable in astrobiology at various heliocentric distances. The observed yields in reduced light suggest that solar irradiation can support plant growth, although at reduced yields, even at large heliocentric distances corresponding to the asteroid belt and Jupiter, and Saturn and their moons.

The results on nutrients allow estimating the biomass sustainably by planetary materials, for example, carbonaceous chondrite asteroids that were proposed as space resources (O'Neill 1974, O'Neill 1977, O'Leary 1977, Lewis 1993, Sagan 1994, Dyson 2000). Based on Table I, the 10^{22} kg total carbonaceous chondrite material in the asteroid belt (Lewis 1997) contains 8×10^{16} kg extractable $\text{NO}_3\text{-N}$ and 5×10^{16} kg extractable $\text{PO}_4\text{-P}$. If distributed as synthetic soils, these materials can sustain up to 10^{18} kg microbial or plant biomass and correspondingly larger human populations than the estimated terrestrial biomass of 10^{15} kg (Bowen 1966). Similar carbonaceous chondrite materials in Phobos and Deimos may also be useful soil resources. The high extractable $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ contents of the martian meteorites suggest that igneous martian rocks can be processed into useful soils for terraforming. The large sustainable biomass by these various materials suggests that planetary resources can accommodate substantial biological and human activity.

In summary, planetary microcosms based on meteorites are useful in experimental astrobiology and astroecology. Larger samples from return missions or in situ studies will allow more bioassay agents and the simultaneous monitoring of nutrients and biota. Presently, studies with small meteorite samples can provide consistent bioassays and a fertility ranking for targeting searches for past life and for identifying future bioresources. The

results suggest that the fertilities of planetary materials are fundamentally similar to or exceed terrestrial soils. Consequently, lifeforms viable on Earth may be sustained by asteroid and planetary materials in this and other solar systems.

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