ECOCYCLES
ISSN 2416-2140

OPEN ACCESS
Scientific journal of the
European Ecocycles Society

Ecocycles, Vol. 10, No. 1, pp. 1-17 (2024)
DOI: 10.19040/ecocycles.v10i1.391

RESEARCH ARTICLE

Feeding Mars: A pilot study growing vegetables using aquaponic effluent fertiliser in simulant and analogue Martian regoliths

Benz Kotzen[1] Marcos Paradelo[2] Lorenzo Fruscella[1]
[1]*School of Design, University of Greenwich, London, SE10 9LS, United Kingdom*
[2]*Natural Resources Institute, University of Greenwich, Chatham, Kent, United Kingdom*
Corresponding author: Benz Kotzen. email: b.kotzen@gre.ac.uk

Abstract – The Feeding Mars study was devised as a small, pilot proof of concept study to research the potential for using aquaponic effluents as an additive to regoliths which on Mars and the Moon are devoid of organic material and thus lacking microbes which assist in the delivery of water and nutrients to the plants via their roots. This research investigates aquaponics as a way to potentially produce fish and vegetal products in regoliths on Mars and the Moon as well as in extreme environments on Earth. In order to settle on Mars, settlers will have to grow their own food in systems that are self-perpetuating, with little or no inputs being brought from Earth once these systems have been established. This means that nutrients from the fish water can be used to grow plants in the hydroponic parts of the aquaponic system but also potentially in the Martian regoliths which are treated with effluents taken from aquaponic systems. Once production is established additional nutrients can be sourced from the arisings and waste, both from the fish (that are processed and eaten) and the plants, which can be used as compost to turn the regoliths into soil. In order to have fish in space, there is also the need for the systems to be self-sustaining in the production of fish feed.

The key outcomes of the project were that all the species grown (potatoes, tomatoes, dwarf beans, carrots, lettuce, spring onions, chives and basil) indicate the potential to be grown in regoliths with the addition of aquaponic effluents. A significant result was that on the whole the plants that were grown with the addition of aquaponic effluents were greener than those grown in the horticultural soil, indicating that the nutrient supply was adequate. However, a key lesson learned is that germination and thus development of the plants grown in the Mars simulant and analogue was slower than those grown in the horticultural soil. Thus, developing nutrients in the soil before planting is necessary as it is with agriculture and horticulture practices on Earth, where manuring/fertilization occurs before planting. The consequence of this research, and the envisaged research to follow is not only for extra-terrestrial environments. The Earth has its own hostile environments, characterised with regoliths and other unproductive soils, and aquaponic water and aquaponic wastes can readily be envisaged as providing solutions to growing nutritious food in areas where agriculture is not currently viable. The research was undertaken in an exhibition gallery setting at the University of Greenwich in order to encourage public interest and dialogue, which it did.

Keywords – Mars, Moon, settlement, food production on Mars, aquaponics, aquaponic effluents, regoliths, Mars simulants, Mars analogue.

Received: January 4, 2024 Accepted: January 21, 2024

1. INTRODUCTION

The Feeding Mars pilot study has its foundations in the research being carried out on soil based aquaponics at the University of Greenwich. The research investigates the potential benefits for including soil in aquaponic systems and also in using fish water and fish sludge as a manure in horticultural and agricultural scenarios as discussed in Palm et al. 2018 and Palm et al., 2023 under the heading of "aquaponics farming". Results of research growing onions using aquaponic derived effluents indicate the all-round better performance of onions grown in soil with these added effluents compared to onions grown with added manure (Fruscella et al., 2023).

To support Mars missions, NASA has developed several different types of Mars regolith simulant, some of which have been used for crop trials. JSC Mars-1 was developed in 1997 by the Johnson Space Center (JSC) based on the physical characteristics of the Martian surface derived from orbital/remote observations and those made at the two Viking lander sites (VL-1 and VL-2). Made from volcanic ash collected from the Pu'u Nene cinder cone in Hawaii, JSC Mars-1 sought to approximate the reflectance spectrum, mineralogy, chemical composition, grain size, density, porosity, and magnetic properties of the Martian regolith (Allen et al., 1998a; Allen et al., 1998b). The simulant has been used in trials growing Swiss chard (Gilrain et al., 1999) and sweet potato (Mortley et al., 2000). JSC Mars-1A was subsequently developed as a nearly identical simulant obtained from the same source, and has been used in trials to grow tomato, rye, garden cress, leek, quinoa, pea, radish, spinach, rocket, and chives (Wamelink et al., 2014; Wamelink et al., 2019).

Since the development of JSC Mars-1, three additional landing sites were examined which revealed a range of Martian surface characteristics. At the Mars Pathfinder site, the regolith is a combination of thin drifts of bright red, fine-grained material, soil-like deposits and rocks, whilst the Mars Exploration Rover (MER) Spirit found that the floor of Gusev Crater is dominated by basaltic sand grains and lithic fragments that have been disrupted by impact events and modified by eolian processes. At the same time MER Opportunity discovered that surface material throughout Meridiani Planum is dominated by sand-sized basaltic grains, sulfate-rich outcrop debris, and hematite-rich spherules and fragments. Mojave Mars Simulant (MMS) was developed using Saddleback Basalt from the Mojave Desert (Peters et al., 2008). Using material mined from the same area, The Martian Garden (https://www.themartiangarden.com) has developed two commercial simulants, MMS-1 and MMS-2. MMS-1 mainly consists of plagioclases in mixture with amorphous materials and zeolite, containing essential plant nutrients such as K, Ca, Mg and Fe, but lacking organic C, N, P and S (Caporale et al., 2020; Duri et al., 2020). It is an alkaline (pH 8.86) and coarse textured substrate, lacking adequate water holding capacity and occurrence of fine particles exerting colloidal properties. Therefore, to adequately sustain the plant growth, it needs to be amended with composted organic materials, enhancing the physicochemical properties, the water-holding capacity, and the availability of plant nutrients (Caporale et al., 2023a). An experiment growing green and red Salanova butterhead lettuce (Lactuca sativa L. var. capitata) in four different mixtures of MMS-1 and compost investigated the impact of compost rate on both crop performance and the nutritive value (Caporale et al., 2020; Duri et al., 2020). The addition to MMS-1 of different rates of manure from monogastric animals as a surrogate for human excreta was assessed in terms of the effect on fresh yield, organic acid, carotenoid content, antioxidant activity, and phenolic profile of lettuce (Duri et al., 2022a; Caporale et al., 2022; Caporale et al. 2023c). Caporale et al. (2023a) used MMS-1 amended with green compost to grow potatoes. Caporale et al. 2023b used MMS-1 mixed with different volumes of sphagnum peat to grow soybean. Berni et al., (2023) used MMS-1 to grow Italian ryegrass (Lolium multiflorum). Wamelink et al., (2022) grew rucola (Eruca sativa) in Martian Garden MMS mixed with harvested organic matter from a previous experiment and amended with pig slurry as a surrogate for human excreta in order to evaluate the effect on plant growth.

Unlike JSC Mars 1-A and MMS, Mars Global Simulant (MGS-1) was synthesized from individual components to match the minerology of Martian regolith, rather than starting with bulk material from a single location on Earth. MGS-1 is based on the Rocknest windblown soil in Gale Crater, which is thought to be representative of basaltic soils at various landing sites and remote sensing locations, and consequently it represents the average of a substantially larger surface area of Mars than prior simulants (Cannon et al., 2019). The simulant has been used in trials on lettuce grown in vermicomposted MGS-1 (Russell et al., 2022).

Trials by Eichler et al., (2021) to evaluate the potential of JSC Mars-1A, Martian Garden Mars Mojave Simulant (MMS-1), and Mars Global Simulant (MGS-1) for growing lettuce confirmed that none of these simulants are capable of supporting plant growth in the absence of nutrient supplementation, since they are deficient in copper, zinc and boron, all of which are critical micronutrients for plant growth.

In addition to crop production trials using regolith simulants, a number of experiments have been conducted using analogues designed to approximate Martian soils in terms of their mineralogical and chemical composition. Ramírez et al., (2019) tested the responses of various potato genotypes grown in soil from the La Joya desert in Southern Peru, whilst Peyrussen (2021) used analogues sampled in the Utah desert to grow radish and spearmint. Oze et al., (2021) created a regolith analogue representative of the Gusev Crater using New Zealand basalts and volcanic glass to grow amaranth and common bean), and Kasiviswanathan et al. (2022) first grew alfalfa in a ground basalt regolith analogue and subsequently used this as a soil simulant in the same analogue to grow turnip, radish, and lettuce.

The experiment reported here set out to test the efficacy of aquaculture effluent as a fertiliser in a Martian simulant and a Martian analogue. The viability of freshwater aquaculture effluent as fertiliser has been demonstrated for a wide variety of food crops grown in terrestrial soils, including wheat (Al-Jaloud et al.,, 1993), barley (Hussain and Al-Jaloud, 1998; Stevenson et al., 2010), maize (Abdul-Rahman et al., 2011; Osaigbovo et al., 2010), sorghum (Kolozsvári et al., 2022), soybean (Abdelraouf, 2017), amaranth (Ojobor and Tobih, 2015), potato (Abdelraouf, 2017), common bean (Meso et al., 2014), tomato (Castro et al., 2006; Pattillo et al., 2020), pepper (Omotade et al., 2019; Palada et al., 2019), chicory (Lenz et al., 2021a), cabbage (Elsbaay and Darwesh, 2022), lettuce (Lenz et al., 2021b), radish (Abdul-Rahman et al., 2011), cucumber (Ndubuisi, 2019), onion (Abdelraouf et al., 2016; Abdelraouf, 2017), basil (Omeir et al., 2020), marjoram (Kimera et al., 2021a) and oregano (Kimera et al., 2021b).

This paper discusses a small pilot/proof of concept study, to ascertain whether aquaponic fish effluent could be used in sterile regoliths to produce vegetables. The driver behind the study was thus not to collect large amounts of data on replicates of each vegetable used, but rather to see whether these vegetables, could survive and perhaps even be successful when comparing these with a small number grown in a control horticultural soil. Statistical analysis thus has not been seen to be key to this study. A remarkable aspect of this work was that it was designed to be publicly accessible. It was installed in an exhibition gallery at the University of Greenwich in order to maximise exposure of the experiment to the students and staff at the University as well as visitors to the University and the adjacent all access café (http://www.greenwichunigalleries.co.uk/feeding-mars-m-a-r-s-mars-aquaponic-research-study).

2. MATERIALS AND METHODS

2.1. Substrates

Two types of Martian regolith simulant were purchased from The Martian Garden (https://www.themartiangarden.com): MMS1-U-BR Unsorted Mojave Mars Simulant and MMS1-C-BR Coarse Grade (hereafter referred to as M1) (Figure 1). The two simulants were mixed at a ratio of 50:50 following recommendations by the manufacturer. This simulant is hereafter referred to as M1. As the simulant is costly to buy and import to the UK, in order to be able to increase the number of trials an analogue regolith (hereafter referred to as M2) was devised using the formula created by the Chicago Botanic Garden (Johnson 2017) and made using materials available in the United Kingdom: 2 parts crushed basalt, 2 parts basalt dust, 1 part horticultural sharp sand, and 0.2 parts feldspar potash. The control was a peat-free multi-purpose compost made from wood fibre, composted bark and coir, which had previously been used to grow vegetables successfully. All three substrates were analysed for pH, nitrate (N), phosphorus (P) and potassium (K) (Table 1). The substrates were not modified in any way prior to the start of the experiment.

Table 1. Indicative parameters of the three substrates

Substrate type	pH[1]	Nitrate (N)[2]	Phosphorus (P)[2]	Potassium (K)[2]
M1	8.7	Low	Very low	Low
M2	8.7	Low	Very low	Low
Control	6.2	Low	Medium-high	Medium

[1] Tested using a Hagen Nutrafin freshwater test kit. 50g of each substrate was mixed with 150ml of distilled water, stirred, left over night and stirred again before testing. [2] Tested using a Testwest soil test kit.

Figure 1. Feeding Mars experiment photo showing 3 different types of dry soil/regolith used: Left = normal horticultural soil , Right = Martian simulant regolith from the Martian Garden (USA), Middle = home manufactured Martian simulant regolith based on Chicago Botanical Garden formula.

2.2. Species selection and experimental design

The vegetable species chosen were designed to provide a nutritious and diverse diet for settlers on Mars: potatoes, tomatoes, dwarf French beans, carrots, lettuce, spring onion, chives and basil. HDPE plastic pots were used for all plants except for the potatoes, which were grown in fabric pots (Table 2). The pots were placed inside a Gorilla grow tent (4.725 m3) on trays to collect any excess runoff water (Figures 2 and 3).

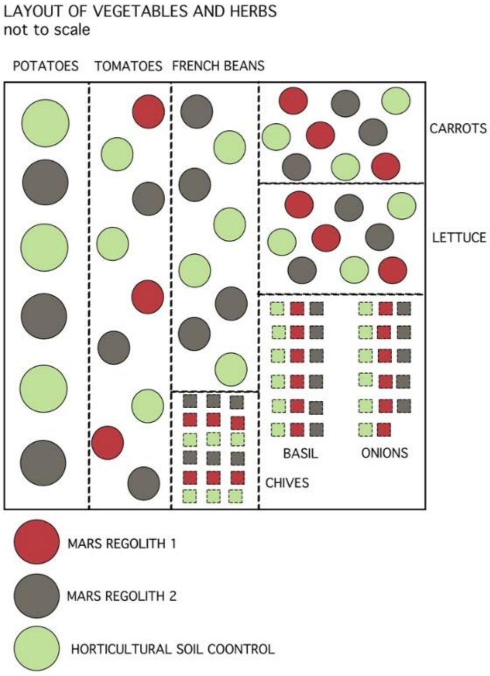

Figure 2. Illustration of the arrangement of species within the grow tent

Figure 3. Photograph of the arrangement of pots in the grow tent prior to planting

The temperature in the tent was very constant, ranging between 25.1 °C and 25.9 °C. Relative humidity varied between 27% and 40%, with air coming in through vents at the bottom and top of the sides of the tent, whilst air was extracted 24 hours a day using a Rhino Pro Fan and filter with air passing to the outside of the tent via sound insulated acoustic ducting. Lighting was provided by a Lumatek Zeus 600W LED (PPF 1620 µmol/s, photon efficacy 2.7 µmol/J) linear multi-light bar fixture hung from the frame of the tent. The lighting was attached to a timer which was programmed to be on for 14 hours per day. The experiment started on 17 March 2022 and was concluded on 28 May 2022.

2.3. Irrigation

The plants were irrigated approximately every 48 hours in the morning. Each pot was given 150 ml per day, or as much as was required to wet the whole pot. The vegetable species with smaller seeds were irrigated with a mister, so as not to disturb the soil prior to germination. The control plants were irrigated with potable tap water, whilst the plants in the M1 and M2 substrates were watered with a mixture of 50% fish water and 50% anaerobically digested fish sludge derived from the aquaponic system at the University of Greenwich (Table 3). The system was stocked with around three hundred adult Nile tilapia (*Oreochromis niloticus*), ranging 200–500g in weight. The fish were fed twice a day, once in the morning and once in the afternoon, with Aller Aqua Primo 6mm sinking pellet feed (37% Crude Protein,12% Crude Fat, 32.5% Nitrogen-free Extracts, 7% Ash, 3.5% Fibre, 1% Phosphorus, 19.6MJ Gross Energy, 16MJ Digestible Energy), for a total of 200g of feed per day. The fish were not directly used in the experiment. Fish water and fish sludge were taken from the system, which is done on a regular basis as part of management, without any detriment to the fish. The aquaponics system is registered by the Cefas Health Inspectorate under the Aquatic Animal Health (England and Wales) Regulations 2009.

Table 2. Vegetable species grown, plant pots, and substrates (see Figures 2 and 3)

	Latin name	**No. of pots / size cm / volume litres**	**No. pots M1**	**No. pots M2**	**No. pots Hort**	**No. plants per pot**
Potato	*Solanum tuberosum*	6 / 26 / 10	0	3	3	1
Tomato	*Solanum lycopersicum*	9 / 18 / 2.5	3	3	3	2
Dwarf French bean	*Phaseolus vulgaris 'Purple teepee'*	8 / 16 / 2	0	4	4	2
Carrot	*Daucus carota subsp. sativus*	9 / 16 / 1	3	3	3	Multiple
Lettuce	*Lactuca sativa*	9 / 16 / 1	3	3	3	Multiple
Spring onion	*Allium fistulosa*	12 / 8.5 / 0.35	4	4	4	1
Chives	*Allium schoenoprasum*	12 / 8.5 / 0.35	4	4	4	Multiple
Basil	*Ocimum basilicum*	12 / 8.5 / 0.35	4	4	4	Multiple

.

Table 3. Parameters of the fish water, fish sludge and tap water

Parameter	**Unit**	**Fish water[1]**	**Fish sludge[1]**	**Tap water[2]**
pH	-	6.0	5.6	7.4
Nitrate (NO_3)	mg/l	37.4	117.2	24.5
Phosphorus	mg/l	7.5	85.7	-
Potassium	mg/l	0.8	22.7	-
Calcium	mg/l	108.2	267.2	-
Magnesium	mg/l	13.53	29.36	5.2
Boron	mg/l	0.07	0.15	0.06
Sulphate (SO_4)	mg/l	149.1	212.0	69.8
Sodium	mg/l	38.6	48.2	28.9
Alkalinity (as HCO_3)	mg/l	83.0	53.0	275.7
Conductivity	uS/cm	628	1542	636
Chloride	mg/l	61.4	77.7	47.0

Parameter	Unit	Fish water[1]	Fish sludge[1]	Tap water[2]
Carbonate	mg/l	<10	<10	-
Total dissolved solids (TDS)	mg/l	579.6	1079.4	-

[1] Fish water and fish sludge analysed by NRM Labs on 18 May 2022 https://cawood.co.uk/nrm. [2] Tap water parameters retrieved from Thames Water https://waterquality.p.cloudapps.thameswater.co.uk/api/waterquality/Zone/SLE30

2.4. Measurements and Analyses

The dates of germination of the different species in each substrate were recorded, and observations were made on the conditions of the plants two weeks and five weeks after seeding (on 30 March and 22 April 2022). At the end of the experiment on 28 May 2022 the plants were harvested, yield parameters were measured, and observations were made on the condition of the plants, including the growth of the root systems.

To determine the effect of the aquaponic effluents on the water holding capacity of the simulant and analogue regoliths a WP4C dew point potentiometer was used to build water release curves before and after the experiment. At the end of the experiment samples of the two regolith types were collected from the tomato pots, air dried and divided into subsamples. A defined amount of water was added to each subsample and left to equilibrate overnight. After measuring the water potential, each sample was oven dried (at 105°C) for 24 hours and the dry weight was measured.

The produce was not tested for taste nor analysed for nutrient content. This will be undertaken at the next stages of the research.

3. RESULTS

3.1. Germination rate

Germination rate was quickest in the control substrate. The first plants to germinate were the French beans, four days after seeding, with plants of the other species appearing within one week. Germination in M1 and M2 was slower (Table 4). Two weeks after seeding all of the species had emerged in all of the substrates, but not in all of the pots.

Table 4. Germination rate one week after seeding (25 March 2022)

Species	M1	M2	Control
Potato	-	0	2
Tomato	0	1	2
French bean	-	1	4
Carrot	0	0	2
Lettuce	1	1	2
Spring onion	0	0	3
Basil	0	2	4
Chives	0	0	2
Total	1	5	21

3.2. Potatoes

Only the M2 and control substrates were used in this experiment, with one plant per pot and three plants per substrate. Two weeks after seeding all three plants in the control and two in M2 had emerged and needed to be topped up with additional substrate as is commonly done when growing potatoes. Five weeks after seeding the plants in the control were bushier than those in M2 and were starting to flower, but had become chlorotic with iron (Fe) and magnesium (Mg) deficiencies, whilst the plants in M2 were a much darker green (Figure 4). At the end of the experiment the plants in the control had larger and heavier tubers (Figure 5; Table 5).

Figure 4. Potato plants after 5 weeks the potatoes are almost all the same size, but the regolith (M2) grown potatoes are much greener (middle plant on the photo) where the plants on either side grown in horticultural soil show signs of yellowing, most likely iron and magnesium deficiencies.

Figure 5 Potato plants at the end of the experiment: control (left) and M2 (right)

Table 5: Physical parameters of the potatoes at the end of the experiment

Substrate type	M2-1	M2-2	M2-3	Control-1	Control-2	Control-3
Plant height (cm)	55	*	*	55	40	*
Plant diameter (cm)	25	*	*	35	25	*
No. of tubers	5	4	5	5	6	6
Total weight of tubers (g)	112	82	140	434	277	235
Largest single weight (g)	34	29	47	120	50	56
Largest tuber diameter (cm)	4.5	3.5	4.7	6.7	7.25	8

*spindly

3.3. Tomatoes

Two plants were seeded in each pot, with three pots per substrate. Two weeks after seeding the plants in the control substrate were growing well, whilst those in M1 and M2 were much smaller. Five weeks after seeding the plants in the control were still growing more strongly and had started to flower, but were showing signs of magnesium deficiency; the plants in M2 were faring better than those in M1. At the end of the experiment all the plants in M1 and M2 were spindly with poor root development, although those in M2 managed to produce flowers. The plants in the control performed best, being more vigorous with good root and plant development, and healthier than those grown in M1 and M2, although plants in all substrates suffered from magnesium deficiency, and those in M1 experienced leaf curl (Figure 6; Table 6).

Figure 6. Tomato plants from left to right: control, M1 (showing leaf curl), M2

Table 6. Physical parameters of the tomatoes at the end of the experiment

Substrate type	M1	M2	Control
Max plant height (cm)	*	48	30
Max plant diameter (cm)	*	*	30

3.3. Beans

Only the M2 and control substrates were used in this experiment, with two seeds in 4 pots of each substrate. Two weeks after seeding all plants were doing well, although those in M2 were slightly smaller than the control. Five weeks after seeding the plants in the control were still taller and had started to flower, but the leaves were light green with a purple tinge, whilst the plants in M2 were a darker green and also flowering. At the end of the experiment the plants in M2 were spindly with a poorly developed root system and green fruits. The plants in the control had a more developed root system, the plants were sturdier, and the fruits had ripened (Figure 7; Table 7).

Figure 7. Dwarf French bean plants in the control (left) and M2 (right)

Table 7. Physical parameters of the beans at the end of the experiment

Substrate type	M2	Control
Max. plant height (cm)	48	50
Max plant diameter (cm)	30	30

3.4. Spring onions

Four pots of each substrate were planted with one seed per pot. Two weeks after seeding plants were growing in all of the control pots, whilst only one plant had emerged in each of M1 and M2. Five weeks after seeding all plants had emerged, with the tallest being in the control. At the end of the experiment plants in all substrates had poor bulb development whilst leaf growth was similar, but overall growth parameters were better in the control (Table 8). The plants grown in the control also had the most comprehensive root structure, including fine roots penetrating to the bottom of the pots. The plants in M1 and M2 had a coarser root structure with fewer fine roots; those in M2 were denser than those in M1 (Figure 8, Table 8).

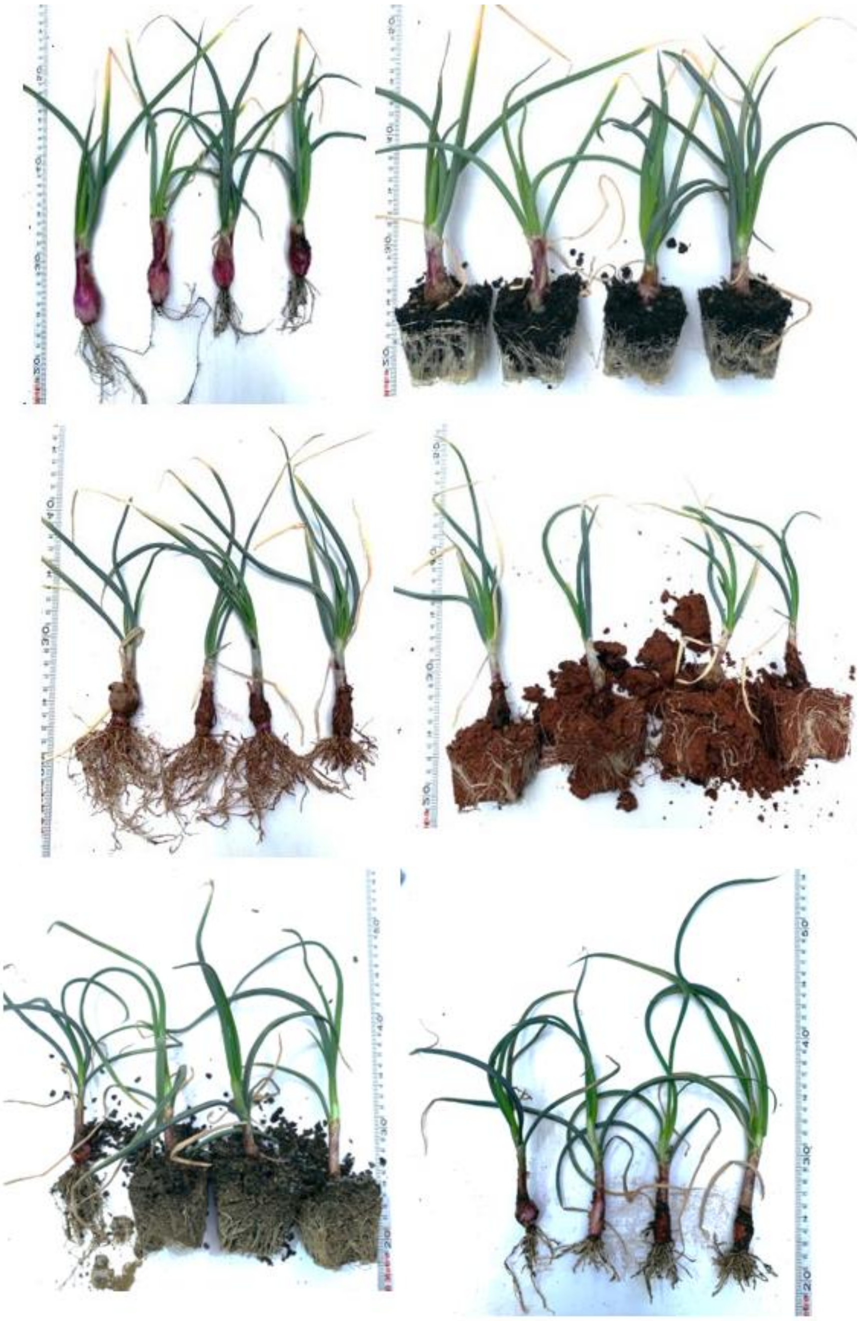

Figure 8. Spring onion plants at the end of the experiment: control (above), M1 (middle) and M2 (bottom)

Table 8. Physical parameters of the spring onions at the end of the experiment

Substrate type	**M1**	**M2**	**Control**
Max plant height from bulb (cm)	25	32	35
Max bulb diameter (cm)	2.2	1.6	2.9
Total weight (g)	37	55	106
Largest single bulb weight (g)	12	15	33

3.5. Chives

Multiple seeds were sown in four pots of each substrate. Two weeks after seeding plants were growing well in the control, whilst in M1 they were just starting to emerge, and had not yet germinated in M2. Germination in M1 and M2 was still poor five weeks after seeding, although one plant in M2 was growing very well. At the end of the experiment germination in M2 was still poor, but the plants that did grow were larger and more vigorous those in the control (Figures 9a and b). M1 had comparatively poor germination and poor growth (Table 9).

Figure 9a. Chive plants (control) at the end of the experiment

Figure 9b. Chive plants (M2) at the end of the experiment

Table 9. Physical parameters of the chives at the end of the experiment

Substrate type	**M1**	**M2**	**Control**
Max. plant height (cm)	7	24	17
Max stem diameter (cm)	1	4	2

3.6. Lettuce

Multiple seeds were planted in three pots of each substrate. Two weeks after seeding plants in all pots of the control were growing well, those in one pot of M1 were emerging well, and those in M2 were emerging more slowly. Five weeks after seeding the plants in the control were starting to be overcrowded and were becoming chlorotic. One pot of M1 and one pot of M2 had no germination, whilst the plants in the other two pots of each substrate were smaller than those in the control, with the exception of one plant in M2 which was well-developed and had a dark green colour (Figure 10).

Figure 10. Lettuce plants after 5 weeks in M1, control (Hort) and M2

At the end of the experiment the plants in the control substrate had good leaf growth and root development, and the chlorosis had reduced. The plants in M2 also showed good leaf growth and root development, and the size of the plants in both substrates was very similar, whilst the plants in M1 did not grow (Table 10, Figures 11a and b).

Table 10: Physical parameters of the lettuces at the end of the experiment

Substrate type	**M1**	**M2**	**Control**
Max. plant height (cm)	*	15	13

Figure 11a. Lettuce plants at the end of the experiment: Control in horticultural soil

Figure 11b. Lettuce plants at the end of the experiment: M2

3.7. Carrots

Multiple seeds were sown in three pots of each substrate. Two weeks after seeding the plants in the control had already developed their second pair of leaves, plants were emerging in two of the three pots of the M1 substrate, whilst germination had only occurred in one pot of M2. Five weeks after seeding the plants in the control were larger than those in M1 and M2. At the end of the experiment the plants in M1 and M2 were still more spindly than those in the control (Figure 12, Table 11). Best germination and growth therefore occurred in the control, followed by M2 and M1.

Table 11. Physical parameters of the carrots at the end of the experiment

Substrate type	M1	M2	Control
Max. plant height (cm)	15	21	20
Max stem diameter (cm)	3	4	6

Figure 12. Carrot plants at the end of the experiment: control (left), M1 (centre), M2 (right)

3.8. Basil

Multiple seeds were planted in four pots of each substrate. Two weeks after seeding the control plants were growing well, whilst those in M1 and M2 were emerging. Five weeks after seeding the plants in the control were larger than those in M1 and M2, but were starting to look chlorotic; the plants in M1 were smaller than those in M2. At the end of the experiment the plants in the M1 substrate were still smaller, with poorly developed roots, and some of the plants had failed to reach the flowering stage. However, the leaves were darker green than those in the control. Plant size in M2 and the control was comparable, and both reached the flowering stage (Figure 13, Table 12). Given the chlorosis in the control plants, the best growth was observed in the M2 simulant.

Table 12: Physical parameters of the basil at the end of the experiment

Substrate type	**M1**	**M2**	**Control**
Max. plant height (cm)	22	32	32

Figure 13. Basil plants at the end of the experiment: control (left), M1 (middle), M2 (right)

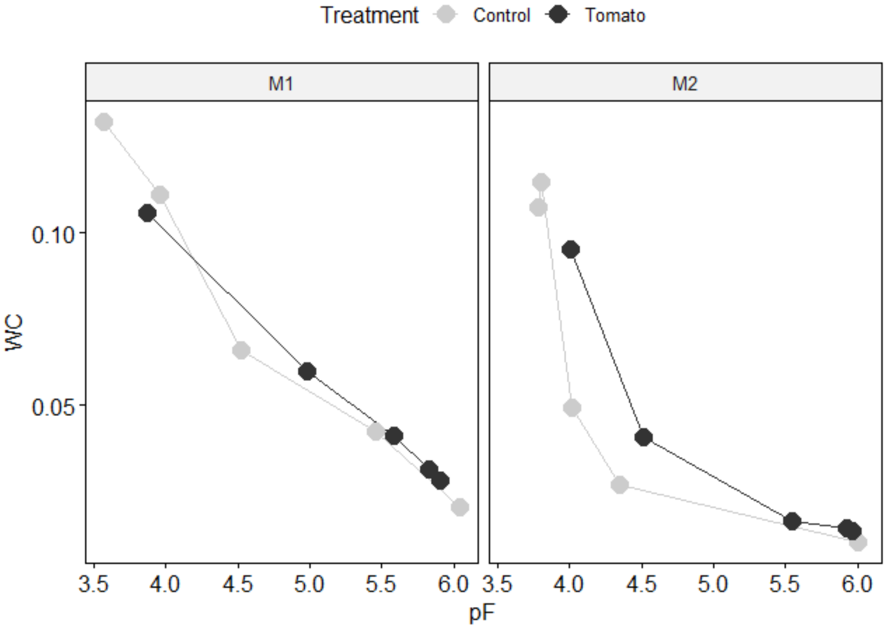

Figure 14. Water release curves for M1 and M2 before (Control) and after (Tomato) the experiment

3.9. Regolith water holding capacity

The water release curves before the experiments (Figure 14) show that the M1 simulant had the capacity to retain more water than the M2 analogue. At the end of the experiment the M1 simulant did not show any difference in the water release curve, whilst the M2 analogue showed an increase in water retention.

4. DISCUSSION

The germination rate of the plants in the control substrate was better than that in the M1 simulant and M2 analogue in all of the species trials, resulting in healthier and more robust looking plants after two weeks. The delay in germination in M1 and M2 was not envisaged, but in hindsight it could have been predicted. At the outset of the study it was considered that the seeds/tubers would provide their own source of nutrients during germination and that the plants would have germinated in the different substrates at approximately the same time. However, this was evidently not the case. It is considered that the reason for delayed germination in M1 and M2 was mainly because of the high pH values of 8.7, which inhibit seed germination (Perez-Fernandez et al., 2006).

Furthermore, there was no organic material in these substrates to hold water and thus keep the seeds damp, and the greater density of fines may have restricted air in the soil which seedling roots require. In retrospect, fertigation of the simulant and analogue substrates prior to seeding would have provided an instant supply of nutrients and an amelioration of the soil texture which would have improved germination.

Following germination and initial growth, greater variability was observed across the different species and substrates. After five weeks it became evident that some of the plants growing in the control substrate were affected by nutrient deficiencies, including nitrogen and potassium (French beans), and iron and magnesium (potatoes). This is probably due to fact that the multi-purpose compost used was formulated for general purposes, and not specifically for vegetables which have high nutrient demands. Despite the fact that the plants seeded in M1 and M2 were initially slower to germinate and grow, with the exception of the tomatoes they did not suffer from nutrient deficiencies. This can only be attributed to the greater availability of nutrients in these substrates which were irrigated with aquaponic effluents.

In all of the crop trials where it was used, the M1 simulant performed worse than the M2 analogue. It is most likely that this is due to the smaller sized particles and with the waterlogging of the regolith, limiting air to the roots and/or some toxicity and the ability for nutrient uptake because of the high pH.

With the exception of basil, lettuce and chives, the plants in the control substrate produced larger, healthier plants than those grown in the M2 analogue. This is because of a more conducive pH, microbial communities already in the soil and air and water availability at the roots.

5. CONCLUSIONS

This pilot study is the first to investigate the potential for aquaponics to be a key part of extra-terrestrial agricultural systems which may have the ability to provide Martian explorers and settlers with a consistent supply of nutritious food. The issue for growing vegetal produce on Mars and the Moon as well, is that the surface substrates are characterised by regoliths which comprise, basaltic bedrock that has been weathered into finer material over millennia. On the Moon the surface is 26% basaltic on the near side and 2% on the far side (Becca M., 2015). These regoliths which may provide suitable minerals for plant growth, however, do not have the nutrients, particularly (N, P and K) that are necessary for leafy growth, flowering and fruiting. The regoliths, in essence, lack any decomposed organic material to also hold water and provide interstices in the substrate to allow air to reach the plant roots. In order to grow plants, what is required is to transform the regoliths into soil. However, it can readily be argued that soil is not required to grow plants. All that is required is water, light, air and nutrients. In a Martian or lunar context, light and air would be provided in controlled indoor environments and it would be quite possible to grow plants using hydroponics, where the plants obtain their nutrients which are added to water which is directed to the plant roots. However, the issue is where do the hydroponic nutrients come from? They would need to be transported from Earth to the settlements which would most likely be costly and at least as far as Mars is concerned, which is approximately 8 months travel away, it would make food production and thus life for humans precarious because what would happen if supply of nutrients failed. The principal of self-reliance is thus crucial to long-term settlement on Mars and one of the ways of providing healthy nutritious produce which could be self-sustaining is using aquaponic systems to provide vegetables, herbs and indeed flowers, but also animal protein. Studies at the University of Greenwich (Fruscella et al., 2023) indicates that using fish effluents is an effective way of providing the required fertilizer for plants in soils and in addition to this the residues from the plants and the fish that have been eaten, can also be an important additive to the regoliths which will help to produce healthy soils which are capable of growing plants.

The literature on using Martian regolith simulants is quite extensive and there have been successes in growing various types of herbs in these less than fertile substrates. This research, which is the first to use aquaponic effluents (50% aquaponic water and 50% sludge water from an aerobic bioreactor), confirms that potatoes, tomatoes, beans, onions, chives, lettuce, carrots and basil can indeed be grown in Martian simulants. In this pilot the formula provide by the Chicago Botanic Garden (M2) proved to provide better results that the Martian simulant (M1) imported from ‘The Martian Garden’ in the USA. It was transparently evident that germination was much slower and poorer in the “Martian substrates” compared to the horticultural compost control and the lesson learned is that germination would most likely have improved if some fertilization of the regoliths would have been undertaken prior to seeding. This would have provided an instant supply of nutrients and an amelioration of the soil texture which would have improved germination. Another very noticeable finding of the pilot study was that, whilst the horticultural compost did not provide sufficient or the timely supply of nutrients to some plants, indicated by yellowing and purpling of leaves, on the whole the leaves of the plants provided with aquaponic effluents were much greener and healthier looking. Whilst the aquaponics water provides the required nitrogen (N), the addition of the aerobically provided sludge (provides the phosphorous and potassium that is needed. In this pilot, the delivery of nutrients to the plants was continuous but not added prior to planting. However, normal agricultural and horticultural practices would normally add the nutrients (organic or inorganic fertilizer into the soil before planting. Thus, further studies would need to investigate whether it is best to supply these fish based nutrients into the substrate before or during cultivation.

In conclusion it is also important to note that this research focusing on growing plants in extreme environments with regoliths is important for Earth based locations where agricultural and horticultural potentials are severely restricted. If the results indicate that plants can be grown using simulated Martian simulants, there is no reason to expect that the plants could be grown in Earth based regoliths, where nutrients would be supplied, as envisaged on the Moon and Mars by fish effluents as well as by adding the composted waste arisings from the plants themselves as well as fish waste, in the same way that horticultural composts are added to provide increased nutrients, water retention and improved soil structures which aid plant growth.

This proof of concept study indicates that some vegetables can be grown using fish effluent in a sterile regolith Martian simulant and analogue. But this does not make aquaponics and aquaponic farming (in soil) as described by Palm et al. 2018 and Palm et al., 2023 feasible on the Moon or Mars from an economic point of view. However, the cost of transporting food to the Moon and Mars is considered to be hugely expensive although these projected costs varies considerably according to different sources. Asakawa (2021) reports that the cost is as high as US$900,000 /kg for transport to the Moon and to Mars, Nield (2015) notes a sum of US$175,000. This 2nd sum is based on the cost of US$700 million to send NASA's Curiosity Rover to Mars (Nield, 2015). With such projected costs it is not surprising that numerous space agencies including The Japan Aerospace Exploration Agency note the need to produce food on site with '*the goal to create a food supply system that can sustainably secure practically all required nutrients by reusing waste, including organic waste, even in an airlocked environment of a moon base'* (Asakawa, 2021).

Aquaponics and aquaponically derived effluents would not be the only way that food could be produced on the Moon and Mars and other systems such as hydroponic and aeroponic systems could be effective as well. But initially at least dissolvable fertilizers would need to be transported by spaceship, before any 'home-grown' fertilizers could be brewed from the arisings from the system, for example through biodigesters and/or vermicomposting. Even then it is unlikely that the nutrient mix will provide every macro/micronutrient required for effective plant growth. As noted in the Introduction various plants have been tested in Martian simulated regoliths. Eichler et al., (2021) however, confirmed that nutrient supplementation would be required as the regoliths were deficient in nutrients. The advantage of aquaponics is that both the fish and plants and the fish and plant waste can be used to supply nutrients as part of a circular production system with or without soil (Figure 15) Commercial fish waste derived fertilizers are indeed available and are produced for plant and vegetable production. Bhuimbar and Dandge (2022) note that converting '*fish waste into organic biofertilizer could be an efficient, novel, eco-friendly approach to overcome environmental issues as well as adverse agricultural problems associated with the use of chemical fertilizers*' and thus fish waste could provide an effective fertilizer on settlements on the Moon and Mars.

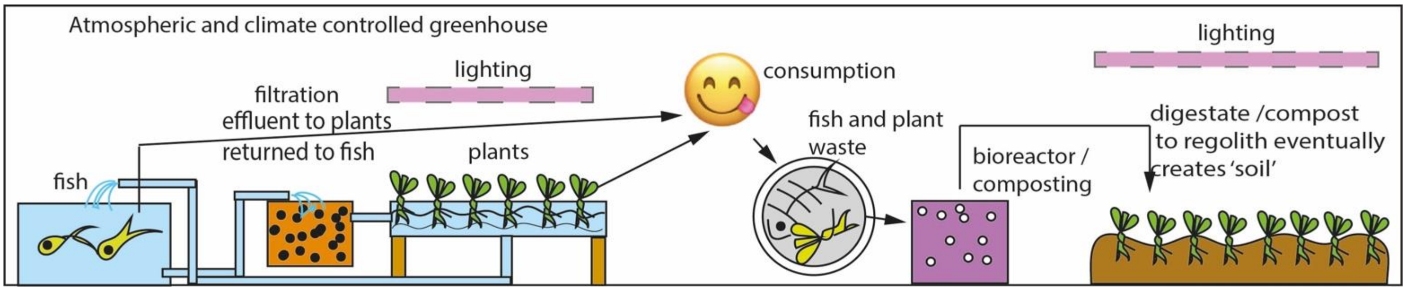

Figure 15. Diagram of fish and plant production within an atmosphere and climate controlled production environment

Devising aquaponic systems for the use on other planets also raises a number of issues:

- It is most likely that over time most people will become vegetarian. The Guardian newspaper notes that this could happen by 2050, due to water scarcity (The Guardian, 2012). If the future of human nutrition does not include eating flesh, then it appears counter to this trend to introduce the eating of flesh in space.

- A second issue arises. The question needs to be asked, whether it is ethical to take another animal species from Earth to an extra-terrestrial environment, even if it is safe for these animals to survive. It could be argued that taking fish to Mars and the Moon can be seen to be pollutive, if pollution is determined to comprise matter out of place as suggested by Kotzen et al., 2020. Taking this argument to its logical conclusion then the very presence of people and their spaceships and other paraphernalia may be considered to be pollutive.

- Many issues regarding food production in extra-terrestrial environments would need to be researched including the effects of less gravity and radiation. Tack et al.,(2021) note that whilst germination percentages were not affected by radiation, biomass and growth of cress and rye were significantly reduced by 32% and 48% respectively. The supply of water is a key issue and here it interesting to note that aquaponics itself is perceived by many authors to use much less water up to 90%, than normal agriculture and horticulture but the exact amounts would differ according to many variables. In any event, finding water, transporting it, storing it and maintaining it is likely to be an expensive business. Closing the circle and using the waste from the aquaponics system to fertilize the Martian or lunar substrates is considered here to be essential for people to live on Mars and within this scenario it will be necessary, if fish are involved to grow fish feed ingredients within and outside the aquaponic system. Numerous studies indicate successes with alternative protein and oil sources to fish meal which is usually derived from caught marine fish. These include, soy, pulses, algae and black soldier fly larvae. Raising black soldier flies and their larvae on Mars or the Moon further adds to the conundrum of bringing in other species into these pristine environments. There is no chance of ecological contamination or disruption as ecology does not exist, as far as we know, in these environments and any escapees from their protected growing environment would die immediately due to the extreme temperatures, radiation and winds.

Finally, this short study was always envisaged as a precursor to a much bigger and collaborative research project. A very positive aspect of the study was that it was located in the 'Project Space', part of the Stephen Lawrence Gallery, Stockwell Street Building, University of Greenwich, as an exhibition (http://www.greenwichunigalleries.co.uk/feeding-mars-m-a-r-s-mars-aquaponic-research-study/) in an area that was open both to students, staff and the general public who were visiting the University and/or the adjacent coffee shop. The space was designed as an immersive installation with video images of Mars and Mars dust and larger CNC cut boards simulating the surface topography of Mars which that could be touched by blind and partially sighted people to give an indication of Martian surface landforms. Explanations of the research and the installation were displayed in text and images on the walls (Figure 16). This "open access" arrangement meant that people could engage physically with the project and they were able to speak directly to those involved in carrying out the day-to-day tasks and monitoring of the research. The outcome of this was very positive and this approach should be considered for many types of research where there is particular public interest.

Figure 16. The Feeding Mars Exhibition 17th March-22nd May 2022. The scope of the experiment was extended to create and immersive experience as part of the communications strategy

ACKNOWLEDGEMENTS

This research and paper were partly funded by the Faculty of Liberal Arts and Sciences HEIF Fund. Additionally, the research was undertaken in the Feeding Mars installation held at the University of Greenwich and the authors would like to thank David Waterworth and the Stephen Lawrence Gallery. Thanks also to; Dr Sarah Milliken, Dr Marcos Paradelo Perez, Dr Lorenzo Fruscella, Dr Andrew Knight-Hill, Kam Rehal and Emmanouil Kanellos.

REFERENCES

Abdelraouf, R.E., Abou-Hussein, S.D., Badr, M.A., El-Tohamy, N.M. (2016) Safe and sustainable fertilization technology with using fish water effluent as a new bio-source for fertilizing. *Acta Horticulturae* 1142, 41–48. DOI: 10.17660/ActaHortic.2016.1142.7

Abdelraouf R.E. (2017) Reuse of fish farm drainage water in irrigation. In: Negm, A. (ed) *Unconventional Water*

Resources and Agriculture in Egypt, pp. 393–410. Springer, Cham.
DOI: doi.org/10.1007/698_2017_92

Abdul-Rahman, S., Saoud, I.P., Owaied, M.K., Holail, H., Farajalla, N., Haidar, M., Ghanawi, J. (2011) Improving water efficiency in semi-arid regions through integrated aquaculture/agriculture. *Journal of Applied Aquaculture* 23, 212–230.
DOI: 10.1080/10454438.2011.600629

Al-Jaloud, A.A., Hussain, G., Alsadon, A.A., Siddiqui, A.Q., Al Najada, A. (1993) Use of aquaculture effluent as a supplemental source of nitrogen fertilizer to wheat crop. Arid Soil Research and Rehabilitation 7 (3), 233–241.
DOI: 10.1080/15324989309381353

Allen, C.C., Morris, R.V., Jager, K.M., Golden, D.C., Lindstrom, D.J., Lindstrom, M.M., Lockwood, J.P. (1998a) Martian regolith simulant Mars-1. *Lunar and Planetary Science* XXIX, March 16–20, 1998, Houston, Texas.
https://www.lpi.usra.edu/meetings/LPSC98/pdf/1690.pdf

Allen, C.C., Jager, K.M., Morris, R.V., Lindstrom, D.J., Lindstrom, M.M., Lockwood, J.P. (1998b) Martian soil simulant available for scientific, educational study. *Eos* 79(34),405–409.
https://agupubs.onlinelibrary.wiley.com/doi/pdfdirect/10.1029/98EO00309

Asakawa T. (2021) Japan gov't sets sights on moon-base food production initiatives for future missions. The Mainchini, 24/09/2021,
https://mainichi.jp/english/articles/20210923/p2a/00m/0sc/014000c

Berni, R., Leclercq, C.C., Roux, P., Hausman, J-F., Renaut, J. (2023) A molecular study of Italian ryegrass grown on Martian regolith simulant. *Science of the Total Environment* 854, 158774.
DOI: 10.1016/j.scitotenv.2022.158774

Bhuimbar M.V. and Dandge P.B. (2023) Production of Organic Liquid Biofertilizer from Fish Waste and Study of its Plant Growth Promoting Effect. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 93, 235–243 (2023). Springer Link
DOI: 10.1007/s40011-022-01413-8

Cannon, K.M., Britt, D.T., Smith, T.M., Fritsche, R.F., Batcheldor, D. (2019) Mars global simulant MGS-1: a Rocknest-based open standard for basaltic Martian regolith simulants. *Icarus* 317, 470–478.
DOI: 10.1016/j.icarus.2018.08.019

Caporale, A.G., Vingiani, S., Palladino, M., El-Nakhel, C., Duri, L.G., Pannico, A., Rouphael, Y. De Pascale, S., Adamo P. (2020) Geo-mineralogical characterisation of Mars simulant MMS-1 and appraisal of substrate physico-chemical properties and crop performance obtained with variable green compost amendment rates. *Science of the Total Environment* 720,137543.
DOI: 10.1016/j.scitotenv.2020.137543

Caporale, A.G., Amato, M., Duri, L.G., Bochicchio, R., De Pascale, S., Simeone, G.D.R., Palladino, M., Pannico, A., Rao, M.A., Rouphael, Y., Adamo, P. (2022) Can lunar and Martian soils support food plant production? Effects of horse/swine monogastric manure fertilisation on regolith simulants enzymatic activity, nutrient bioavailability, and lettuce growth. *Plants* 11, 3345.
DOI: 10.3390/plants11233345

Caporale, A.G., Paradiso, R., Liuzzi, G., Arouna, N., De Pascale, S., Adamo, P. (2023a) Can peat amendment of Mars regolith simulant allow soybean cultivation in Mars bioregenerative life support systems? *Plants* 12, 64.
DOI: 10.3390/plants12010064

Caporale, A.G., Paradiso, R., Liuzzi, G., Palladino, M., Amitrano, C., Arena, C., Arouna, N., Verrillo, M., Cozzolino, V., De Pascale, S., Adamo, P. (2023b) Green compost amendment improves potato plant performance on Mars regolith stimulant as substrate for cultivation in space. *Plant Soil.*
DOI: 10.1007/s11104-022-05860-0

Caporale, A.G., Palladino, M., De Pascale, S., Duri, L.G., Rouphael, Y., Adamo, P. (2023c) How to make the Lunar and Martian soils suitable for food production – Assessing the changes after manure addition and implications for plant growth. Journal of Environmental Management325,116455.
DOI: 10.1016/j.envman.2022.116455

Castro, R.S., Borges Azevedo, C.M.S., Bezerra-Neto, F. (2006) Increasing cherry tomato yield using fish effluent as irrigation water in Northeast Brazil. *Scientia Horticulturae*110,44–50.
DOI: 10.1016/j.scienta.2006.06.006

Duri, L.G., El-Nakhel, C., Caporale, A.G., Ciriello, M., Graziani, G., Pannico, A., Palladino, M., Ritieni, A., De Pascale, S., Vingiani, S., Adamo, P., Rouphael, Y. (2020) Mars regolith simulant ameliorated by compost as in situ cultivation substrate improves lettuce growth and nutritional aspects. *Plants* 9, 628.
DOI: 10.3390/plants9050628

Duri, L.G., Pannico, A., Petropoulos, S.A., Caporale, A.G., Adamo, P., Graziani, G., Ritieni, A., De Pascale, S., Rouphael, Y. (2022a) Bioactive compounds and antioxidant activity of lettuce grown in different mixtures of monogastric-based manure with lunar and Martian soils. *Frontiers in Nutrition* 9, 890786.
DOI: 10.3389/fnut.2022.890786

Duri, L.G., Caporale, A.G., Rouphael, Y., Vingiani, S., Palladino, M., De Pascale, S., Adamo, P. (2022b) The potential for lunar and Martian regolith simulants to sustain

plant growth: a multidisciplinary overview. *Frontiers in Astronomy and Space Sciences* 8, 747821.
DOI: fspas.2021.747821

Eichler, A., Hadland, N., Pickett, D., Masaitis, D., Handy, D., Perez, A., Batcheldor, D., Wheeler, B., Palmer, A. (2021) Challenging the agricultural viability of martian regolith simulants. *Icarus* 354, 114022.
DOI: 10.1016/j.icarus.2020.114022

Elsbaay, A.M. and Darwesh, M.R. (2022) Effect of fish effluent on cabbage yield under organic mulching conditions. *Agricultural Engineering International* 24 (3), 1–11.

Fackrell, L.E., Schroeder, P.A., Thompson, A., Stockstill-Cahill, K., Hibbitts, C.A. (2021) Development of Martian regolith and bedrock simulants: potential and limitations of Martian regolith as an in-situ resource. *Icarus* 354, 114055.
DOI: 10.1016/j.icarus.2020.114055

Fruscella L., Kotzen B., Paradelo M., Milliken., (2023) Investigating the effects of fish effluents as organic fertilisers on onion (Allium cepa) yield, soil nutrients, and soil microbiome, Scientia Horticulturae, Volume 321, 2023, 112297
DOI: 10.1016/j.scienta.2023.112297.

Gilrain, M.R., Hogan, J.A., Cowan, R.M., Finstein, M.S., Logendra, L.S. (1999) *Preliminary Study of Greenhouse Grown Swiss Chard in Mixtures of Compost and Mars Regolith Simulant*. SAE Technical Paper 1999-01-2021.
DOI: 10.4271/1999-01-2021

Guinan, E.F. (2018) Mars gardens in the university - red thumbs: growing vegetables in Martian regolith simulant. *AAS* 231, 401.06.
https://ui.adsabs.harvard.edu/abs/2018AAS...23140106G/abstract

Harris, F., Dobbs, J., Atkins, D., Ippolito, J.A., Stewart, J.E. (2021) Soil fertility interactions with *Sinorhizobium*-legume symbiosis in a simulated Martian regolith; effects on nitrogen content and plant health. *PLoS ONE* 16(9), e0257053.
DOI: 10.1371/journal.pone.0257053

Hussain, G. and Al-Jaloud, A.A. (1998) Effect of irrigation and nitrogen on yield, yield components and water use efficiency of barley in Saudi Arabia. *Agricultural Water Management* 36 (1), 55–70.
DOI: 10.1016/S0378-3774(97)00045-0

Johnson, K. (2017) Growing plants in Martian soil. Chicago Botanic Garden
https://www.chicagobotanic.org/blog/how_to/growing_plants_martian_soil

Kasiviswanathan, P., Swanner, E.D., Halverson, L.J., Vijayapalani, P. (2022) Farming on Mars: treatment of basaltic regolith soil and briny water simulants sustains soil growth. *PLoS ONE* 17 (8), e0272209.
DOI: 10.1371/journal.pone.0272209

Kimera, F., Sewilam, H., Fouad, W.M., Suloma, A. (2021a) Efficient utilization of aquaculture effluents to maximize plant growth, yield, and essential oils composition of Origanum majorana cultivation. *Annals of Agricultural Sciences* 66 (1), 1–7.
DOI: 10.1016/j.aoas.2020.11.002

Kimera, F., Sewilam, H., Fouad, W.M., Suloma, A. (2021b) Sustainable production of Origanum syriacum L. using fish effluents improved plant growth, yield, and essential oil composition. *Heliyon* 7 (3), e06423.
DOI: 10.1016/j.heliyon.2021.e06423

Kolozsvári, I., Kun, Á., Janscó, M., Palágyi, A., Bozán, C., Gyuricza, C. (2022) Agronomic performance of grain sorghum (Sorghum bicolor (L.) Moench) cultivars under intensive fish farm effluent irrigation. *Agronomy* 12, 1185.
DOI: 10.3390/agronomy12051185

Kotzen. B., Branquinho C. and Prasse R. (2020) Does the exotic equal pollution? Landscape methods for solving the dilemma of using native versus non-native plant species in drylands, LDD – Land Degradation and Development, Volume31, Issue18, December 2020, Pages2925-2935. https://onlinelibrary.wiley.com/doi/full/10.1002/ldr.3650

Lenz, G.L., Loss, A., Lourenzi C.R., Lopes, D.L., Siebeneichler, L., Brunetto, G. (2021a) Common chicory production in aquaponics and in soil fertilized with aquaponics sludge. *Scientia Horticulturae* 281, 109946.
DOI: 10.1016/j.scienta.2021.109946

Lenz, G.L., Loss, A., Lourenzi, C.R., Luiz de Alcantara Lopes, D., Siebeneichler, L. de M., Brunetto, G. (2021b) Lettuce growth in aquaponic system and in soil fertilized with fish sludge. *Aquaculture Research* 52 (10), 5008–5021.
DOI: 10.1111/are.15372

Meso, M.B., Wood, C.W., Karanja, N.K., Veverica, K.L., Woomer, P.L., Kinyali, S.M. (2004) Effect of fish pond effluents irrigation on French beans in central Kenya. *Communications in Soil Science and Plant Analysis* 35 (7–8), 1021–1031.
DOI: 10.1081/CSS-120030578

Mortley, D.G., Aglan, H.A., Bonsi, C.K., Hill, W.A. (2000) *Growth of Sweetpotato in Lunar and Mars Simulants*. SAE Technical Paper 2000-01-2289.
DOI: 10.4271/2000-01-2289

Ndubuisi, N.L. (2019) Response of fish pond effluent on soil chemical properties and growth of cucumber (Cucumis sativus) in Igbariam, south eastern Nigeria. *International Journal of Current Microbiology and Applied Sciences* 8 (2), 2799–2807.
DOI: 10.2139/ssrn.3463863

Nield D. (2015) Here's The Official Cost of Sending a Letter to Mars, Science Alert, 09/12/2015, https://www.sciencealert.com/here-s-the-official-cost-of-sending-a-letter-to-mars

Ojobor, S.A. and Tobih, F.O. (2015) Effects of fish pond effluent and inorganic fertilizer on Amaranthus yield and soil chemical properties in Asaba, Delta State, Nigeria. *Journal of Agriculture and Environmental Sciences* 4 (1), 237–244. DOI: 10.15640/jaes.v4n1a29

Omeir, M.K., Jafari, A., Shirmardi, M., Roosta, H. (2020) Effects of irrigation with fish farm effluent on nutrient content of basil and purslane. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* 90 (4), 825–831.

Omotade, I.F., Alatise, M.O., Olanrewaju, O.O. (2019) Growth and yield performance of hot pepper using aquaculture wastewater. *Agricultural Engineering International* 21 (2), 18–25.

Osaigbovo, A.U., Orfue, E.R., Nwaoguala, C.N.C. (2010) Effect of fish pond effluent on some soil chemical properties and vegetative growth of maize (Zea mays L.). *Journal of Sustainable Agriculture and the Environment* 12, 123–131.

Palada, M.C., Cole, W.M., Crossman, S.M.A. (1999) Influence of effluents from intensive aquaculture and sludge on growth and yield of bell peppers. *Journal of Sustainable Agriculture* 14 (4), 85–103. DOI: 10.1300/J064v14n04_08

Palm H. W., Knaus U., Appelbaum S., Goddek S., Strauch S. M., Vermeulen T., Jijakli H. M., and Kotzen B., owards commercial aquaponics: a review of systems, designs, scales and nomenclature. Aquacult Int.26,813–842 (2018). DOI: 10.1007/s10499-018-0249-z

Palm HW, Knaus U, Kotzen B. Aquaponics nomenclature matters: It is about principles and technologies and not as much about coupling. *Rev Aquac*. 2024; 16(1): 473-490. DOI: 10.1111/raq.12847

Pattillo, D.A., Foshee, W.G., Blythe, E.K., Pickens, J., Wells, D., Monday, T.A., Hanson, T.R. (2020) Performance of aquaculture effluent for tomato production in outdoor raised beds. *HortTechnology* 30 (5),624–631. DOI: 10.21273/HORTTECH04655-20

Pérez-Fernández. M.A., Calvo-Magro, E., Montanero-Fernández, J., Oyola-Velasco, J.A. (2006) Seed germination in response to chemicals: effect of nitrogen and pH in the media. Journal of Environmental Biology 27 (1), 13–20.

Peters, G.H., Abbey, W., Bearmana, G.H., Mungasa, G.S., Smith, J.A., Anderson, R.C., Douglas, S., Beegle, L.W. (2008) Mojave Mars simulant – Characterization of a new geologic Mars analog. *Icarus* 197, 470–479. DOI: 10.1016/j.icarus.2008.05.004

Poulet, L., Engeling, K., Hatch, T., Stahl-Rommel, S., Justiniano, Y-A.V., Castro-Wallace, S., Bunchek, J., Monje, O., Hummerick, M., Khodadad, C.L.M., Spencer, L.E., Pechous, J., Johnson, C.M., Fritsche, R., Massa, G.D., Romeyn, M.W., O'Rourke, A.E., Wheeler, R.W. (2022) Large-scale crop production for the Moon and Mars: current gaps and future perspectives. *Frontiers in Astronomy and Space Sciences* 8, 733944 DOI. 10.3389/fspas.2021.733944

Peyrussen, F. (2021) Hydrogels improve plant growth in Mars analog conditions. *Frontiers in Astronomy and Space Sciences* 8, 729278. DOI: 10.3389/fspas.2021.729278

Rainwater, R. & Mukherjee, A. (2021) The legume-rhizobia symbiosis can be supported on Mars soil simulants. *PLoS ONE* 16 (2), e0259957. DOI: 10.1371/journal.pone.0259957

Ramírez, D.A., Kreuze, J., Amoros, W., Valdivia-Silva, J.E., Ranck, J., Garcia, S., Salas, E., Yactayo, W. (2019) Extreme salinity as a challenge to grow potatoes under Mars-like soil conditions: targeting promising genotypes. *International Journal of Astrobiology* 18, 18–24. DOI: 10.1017/S1473550417000453

Russell, J., De Leon, P., Stutte, G.W. (2022) Evaluation of candidate crop plant *Lactuca sativa* in biologically enhanced Martian regolith. *51st International Conference on Environmental Systems*, 10-14 July 2022, Saint Paul, MN. https://hdl.handle.net/2346/89608

Stevenson, K.T., Fitzsimmons, K.M., Clay, P.A., Alessa, L., Kliskey, A. (2010) Integration of aquaculture and arid lands agriculture for water reuse and reduced fertilizer dependency. *Experimental Agriculture* 46 (2), 173–190. DOI: 10.1017/S0014479709990871

Tack, N., Wamelink, G.W.W., Denkova, A.G., Schouwenburg, M., Hilhorst H., Wolterbeek, H.T. and Goedhart P.W., (2021) Influence of Martian Radiation-like Conditions on the Growth of Secale cereale and Lepidium sativum, Frontiers in Astronomy and Space Sciences, Volume 8-2021, DOI: 10.3389/fspas.2021.665649

Wamelink, G.W.W., Frissel, J.Y., Krijnen, W.H.J., Verwoert, M.R., Goedhart, P.W. (2014) Can plants grow on Mars and the moon: a growth experiment on Mars and moon soil simulants. *PLoS One* 9, e103138. DOI: 10.1371/journal.pone.0103138

Wamelink, G.W.W., Frissel, J.Y., Krijnen, W.H.J., Verwoert, M.R. (2019) Crop growth and viability of seeds on Mars and moon soil simulants. *Open Agriculture* 4, 509–516. DOI: https://doi.org/10.1515/opag-2019-0051

Wamelink, G.W.W., Schug, L., Frissel, J.Y., Lubbers, I. (2022) Growth of Rucola on Mars soil simulant under the influence of pig slurry and earthworms. *Open Agriculture* 7, 238–248.
DOI: 10.1515/opag-2022-0079