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Research article

Turning earthworms into moonworms: Earthworms colonization of lunar regolith as a bioengineering approach supporting future crop growth in space

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ABSTRACT

The earthworms beneficial effects on soils may be promising to improve lunar soil fertility, enabling the use of local substrates for space farming. Herein, we investigated the effects of the lunar regolith simulant (LHS-1) at different concentrations in cow manure mixtures on the survival and fitness of *Eisenia fetida*. During 14 and 60-day experiments, although *E. fetida* showed an increased mortality with LHS-1 alone, most of the population survived. More numerous tunnels were observed when exposed to the higher concentrations of LHS-1 (poor in nutrients for earthworms). This may be related to an increased mobility for food search. The cocoons production was not affected by different substrate treatments, except for the highest concentration of LHS-1. No effects of different LHS-1 concentrations on the amount of ingested substrate were recorded. This study shows that *E. fetida* can potentially colonize lunar regolith representing a future valuable biological tool for supporting crops growth on the Moon.

1. Introduction

Terraforming and ecopoiesis (processes by which extra-terrestrial environments are modified and colonized by life, making space and/or other planets habitable) [1,2] are fascinating concepts that are suddenly shifting into the focus of planetary science, aerospace technology, bioengineering, and life science due to the recent renewed interest in returning to the Moon (e.g. NASA's Artemis program), and sending humans to Mars by the 2030s [3–6]. A major challenge of long-term missions in space for humans is represented by the limited stowage of life-support resources, and waste management [7,8]. Currently, food is provided from terrestrial sources, and the production of potable water and oxygen relies on physicochemical processes [9,10]. Anderson et al. [11] estimated that an individual member of a crew would consume 1.83 kg of food and 2.50 kg of water per day, thus the overall life-support payload (e.g. food and water) needed for a 3-year mission to mars would be of several tons per person.

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Bioregenerative life support systems (BLSS) represent a recent and promising space technology based on the culture of biological life forms (mainly vegetables) *in situ* to satisfy the metabolic needs of a crew by providing food, producing oxygen, fixing carbon dioxide and purifying water [12]. However, also in this case, a crucial starting amount of resources from Earth (water, fertilizers, culture substrates) is needed, hampering the implementation feasibility of BLSS and space growing of crops.

Cultivation in controlled environments directly on the lunar soil would give numerous advantages in terms of reduction of logistics costs, and transportation of resources from Earth. Extensive studies have been conducted on several plant species to investigate their responses to the lunar material (e.g. lunar regolith and its simulants) [13–18]. However, it has been reported that although plants can potentially extract few nutrients from lunar regolith [19,20], this is not enough to ensure a proper and complete crop cycle [21]. Moreover, very recently it has been demonstrated that lunar regolith is a stressful substrate for plants. In fact, *Arabidopsis thaliana* plants grown on lunar regolith show the expression of a set of genes associated with stress [22]. The lunar regolith is very different from terrestrial soils, not only in terms of mineral composition. A terrestrial soil contains minerals, air, water, but most of all organic matter and living biota [23]. So, plants and soil organisms are tightly linked, as the latter improve the soil structure, aerate it, and favour water infiltration, as well as are responsible for nutrient availability, waste decomposition, and more [24–26].

Among Earth's telluric organisms, earthworms play a key role in the plant-soil system providing highly beneficial effects to the soil fertility [27,28]. Earthworms are well recognized to contribute to enhance plant production through improving soil structure, cycling of nutrients, and potentially harbouring gut microbiota with plant growth promoting activity [29–36].

The potential integration of such organisms in BLSS and space farming strategies would significantly leverage crop cultivation on off-Earth agricultural systems. In a recent experiment, two earthworm genera (*Caligonella* and *Dendrobaena*) were added to plants cultivated in Mars soil simulant to investigate how they affect the growth of several crops, but no significant effects were found [37].

With particular reference to agriculture on Moon, earthworms may contribute to provide a natural soil fertility approach, promoting processes making lunar regolith closer to an earth-like soil, thus suitable to sustain crop growth. However, lunar soil may represent a harsh substrate for earthworms, and information on how they behave on regolith has not yet been explored.

In this study we carried out a pilot study to test the effects of the lunar regolith simulant (e.g. LHS-1 Lunar Highlands Simulant) on the survival and fitness of *Eisenia fetida* Savigny (Oligochaeta: Lumbricidae), an earthworm species commonly used for vermicomposting tolerating several adverse environmental conditions and habitat perturbations, thus also representing an elective model as bioindicator [38]. Interestingly, lunar simulant synthesis is based on real Moon soil samplings from Apollo missions [39], compared to simulants of other planets (e.g. Mars) [40], thus this would make experimental results closer to those obtainable with native lunar soil. Herein, we determined the mortality, reproduction, tunnel formation, and rate of ingestion at various concentrations of lunar regolith simulant mixed with cow manure to investigate the potential of *E. fetida* in colonizing the Moon soil.

2. Materials and methods

2.1. Ethic statement

The present study adheres to the legal requirements of Italian legislation (D.M. 116,192), and EU regulation [41]. No specific permits are needed in using Oligochaeta in the country where the experiments were conducted.

2.2. Animal maintenance

Eisenia fetida individuals from a commercially mass-reared strain were provided along their feeding substratum (cow manure) by Centro Lombricoltura Toscano (San Giuliano Terme, Pisa, Italy). *E. fetida* redworms were maintained under laboratory controlled environment at 20 ± 2 °C, following the OECD guidelines [41]. For the tests, adult earthworms presenting a well-developed clitellum and weighing between 300 and 600 mg were used [41–43].

2.3. Lunar regolith simulant

Lunar regolith simulants reproduce the chemical compositions, mineralogy, particle size distributions, as well as engineering properties of lunar soils [39].

Herein, we used the LHS-1 Lunar Highlands Simulant (CLASS Exolith Lab, Orlando, Florida, USA), that reproduces with high-fidelity a generic highlands location on the Moon, by accurately combining both mineral and rock fragments with the particle size distribution to match that of typical Apollo soils.

2.4. Experiment description

LHS-1 was mixed with cow manure (normally used as *E. fetida* substrate) dried at 60 °C, to have LHS-1 concentrations of a) 0, b) 25, c) 50, d) 75, and e) 100%. These substrate treatments (e.g. pure LHS-1 and cow manure, and their different mixtures) were put in separate experimental arenas (200 × 200 × 15 mm) filling them by 5 mm, and ensuring a constant moisture content of 60% [41] using a mobile soil-moisture sensor. Five adult individuals, previously starved for 2 days to empty their guts, were transferred in each experimental arena containing different substrate treatments. After 14 days [44] the percentage of dead individuals, as well as the number of tunnels produced by the earthworms per experimental arena were recorded. Tunnels are well visible on the surface of the substrate as excreted clumps by earthworms along their dug paths.

E. fetida reproduction was evaluated by determining the cocoon production of the surviving earthworms over a 60 day experiment [44] in the same conditions described earlier. In the 60 day exposure experiment, mortality was again recorded, while tunnels production could not be reliably assessed after this period.

The ingestion of the different substrate treatments by *E. fetida* was determined by locating starved individual earthworms in Petri dishes (12 cm diameter, 2.5 cm height) containing different substrates for 4 days. Subsequently, *E. fetida* individuals were transferred in Petri dishes with no substrates for 2 days to empty their guts. The casts ejected were dried, and weighed.

For the mortality, reproduction, tunnel formation, and ingestion tests 15 replicates were carried out for each substrate treatment. Fig. 1 shows an *E. fetida* adult individual (Fig. 1A), several cocoon produced by matures *E. fetida* (Fig. 1B), a handful of the LHS-1 Lunar Highlands Simulant (Fig. 1C).

2.5. Statistical analysis

Data about the impact of different substrate treatments on the mortality, tunnel formation, and cocoon production of *E. fetida* individuals, as well as on their ingestion were neither normally distributed (Shapiro–Wilk test, goodness of fit $p < 0.05$) nor homoscedastic (Levene's test, $p < 0.05$), thus they were analyzed relying on non-parametric statistics. In particular, the Kruskal–Wallis test, followed by Dunn's multiple comparison test with Bonferroni correction, was used. All data were analyzed by using R software v3.6.1 [45].

3. Results

Our study showed how substrate treatments with various concentrations of LHS-1 had a different influence on the mortality, tunnel formation, and reproduction performance of *E. fetida*.

In the 14-day experiment, mortality of *E. fetida* was significantly affected by different concentrations of LHS-1 ($\chi^2 = 16.96$, $d.f. = 4$, $P = 0.0020$). The percentage of dead individuals was higher for earthworms exposed to the concentration e) compared to those exposed to the concentrations a) ($Z = 3.34$; $P = 0.0084$), b) ($Z = 3.34$; $P = 0.0084$), d) ($Z = 3.34$; $P = 0.0084$) (Fig. 2A).

In the 60-day experiment, *E. fetida* showed a significant difference in mortality when exposed to different concentrations of LHS-1 ($\chi2 = 41.40$, $d.f. = 4$, $P = 0.0020$). The dead individuals percentage was higher for earthworms exposed to the concentration e) compared to those exposed to the concentrations a) ($Z = 5.17$; $P < 0.0001$), b) ($Z = 5.55$; $P < 0.0001$), c) ($Z = 4.79$; $P < 0.0001$), d) ($Z = 4.41$; $P = 0.0001$) (Fig. 2B).

The overall number of tunnel formation was not significantly different in experimental arenas containing substrate treatments with different concentrations of LHS-1 ($\chi2 = 9.41$, $d.f. = 4$, $P = 0.0516$) (Fig. 3A). However, the number of tunnel formation per Earthworm (n. of tunnels/n. of individuals for each experimental arena) was significantly different in experimental arenas with substrate treatments containing different concentrations of LHS-1 ($\chi2 = 19.52$, $d.f. = 4$, $P = 0.0006$). The number of tunnels was higher in the experimental arena containing the substrate treatment with concentration e) compared to those containing the concentrations a) ($Z = 3.55$; $P = 0.0038$), b) ($Z = 3.65$; $P = 0.0026$), c ($Z = 3.23$; $P = 0.0120$) (Fig. 3B).

The cocoon production was importantly influenced by the different LHS-1 concentrations of the substrate treatments ($\chi2 = 46.15$,

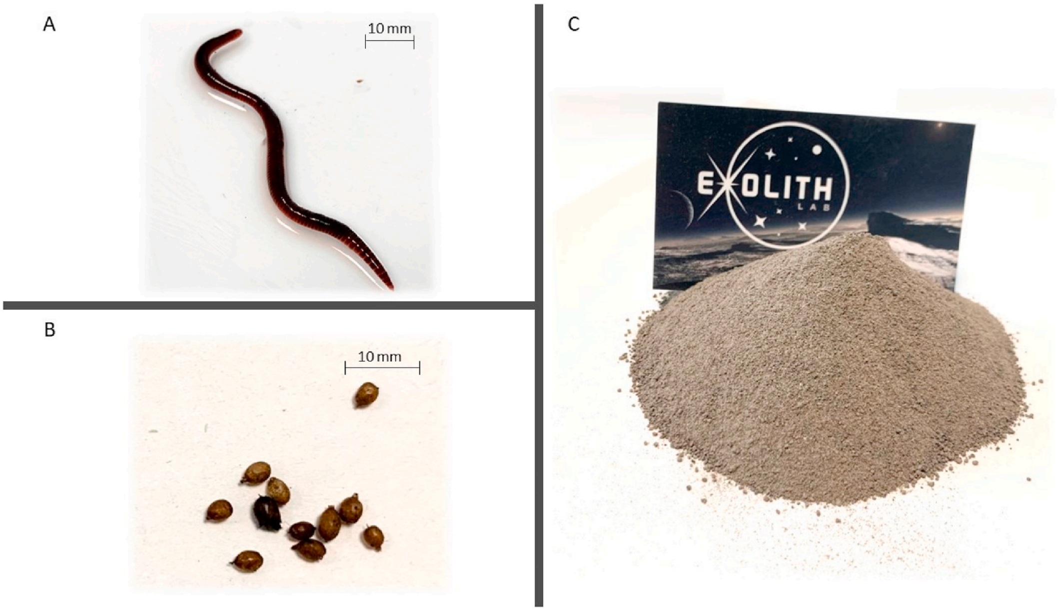

Fig. 1. (A) *Eisenia fetida* individual. (B) Cocoons produced by *E. fetida*. (C) A handful of the LHS-1 Lunar Highlands Simulant.

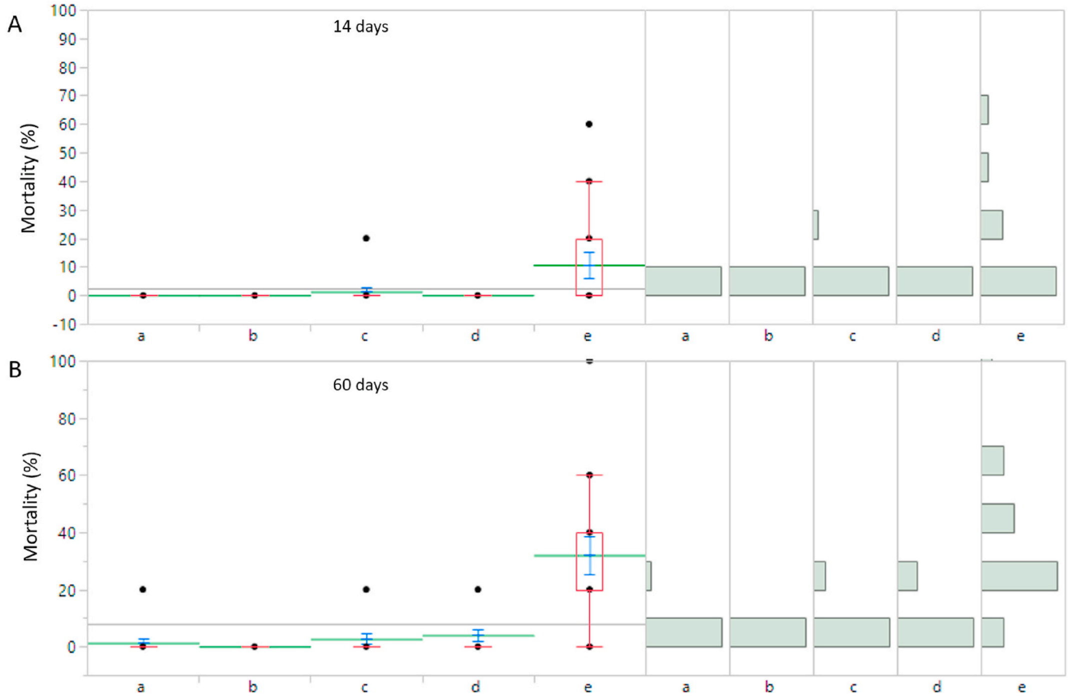

Fig. 2. Mortality percentage of *Eisenia fetida* individuals post-exposure for 14 days (A) and for 60 days (B) to different concentrations of LHS-1 Lunar Highlands Simulant. LHS-1 concentrations: a) 0%, b) 25%, c) 50%, d) 75%, and e) 100%. In each box plot the median (red line) and its range of dispersion (lower and upper quartiles, as well as outliers) are indicated. The mean (green line), and the standard error value (blue T-bars) are also included. Each box plot reports on its right histograms describing data distribution. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

d.f. = 4, $P < 0.0001$). The number of cocoons was lower in the experimental arena containing the concentration e) compared to those containing the concentrations a ($Z = -6.13$; $P < 0.0001$), b ($Z = -5.44$; $P < 0.0001$), c ($Z = -4.11$; $P = 0.0004$), d ($Z = -3.21$; $P = 0.0133$). The number of cocoons was lower in the experimental arena containing the concentration d) compared to that containing the concentrations a ($Z = -2.91$; $P = 0.0356$) (Fig. 4A).

Different LHS-1 concentrations of the substrate treatments also affected the cocoon production per individual (n. of cocoons/n. of individuals for each experimental arena) ($\chi 2 = 15.02$, *d.f.* = 4, $P = 0.0046$). The number of cocoons per individual was lower in the experimental arena containing the concentration e) compared to that containing the concentrations a ($Z = -3.76$; $P = 0.0017$) (Fig. 4B).

E. fetida showed no significant differences in the ingestion of the different substrate treatments ($\chi 2 = 9.41$, *d.f.* = 4, $P = 0.0516$) (Fig. 5).

4. Discussion

Epigeic earthworm species have been reported to tolerate diverse harsh environments while remaining stable during habitat perturbations [38]. So, we selected the epigeic redworm *E. fetida* as an organisms potentially able to survive in a substrate unsuitable for life, such as lunar soil. This preliminary study shows the earthworm *E. fetida* can potentially colonize lunar regolith in controlled environments (our experiments were conducted on Earth, thus excluding microgravity, high radiation levels, and other conditions encountered on space), contributing to facilitate current BLLSs and space farming methods by exploiting their ability to increase soil fertility as a bioengineered approach to support crops growth also on the Moon.

E. fetida showed to highly tolerate substrate treatments with different LHS-1 concentrations. Although a significant increasing in mortality was observed in the substrate treatment containing just LHS-1 (concentration e), most of the population survived in both the 14 and 60-day experiments. Unlike anecic earthworm species that can extract nutrients also from mineral soil, *E. fetida* is an epigeic species, mainly feeding on organic residues [46], thus the increased mortality in the substrate treatment e), especially in the 60-day experiment, can be explained by the lack of organic food. Also the higher number of tunnels in the substrate treatments with higher concentrations of LHS-1 (poor in nutrients for earthworms) may be related to an increased locomotion activity to search for food [27]. However, epigeic species such as *E. fetida* are excellent soil ecosystem engineers, being able to inhabit contaminated harsh habitats and remediate polluted wastes turning them into valuable vermicompost [47,48], as well as they exhibit high reproduction rate [49], all factors that are essential to fast colonize new environments. *E. fetida* is considered a r-selected species [50], thus its high reproductive rates balance the high mortality may occur in harsh environmental conditions [27]. Our results showed that the overall number of

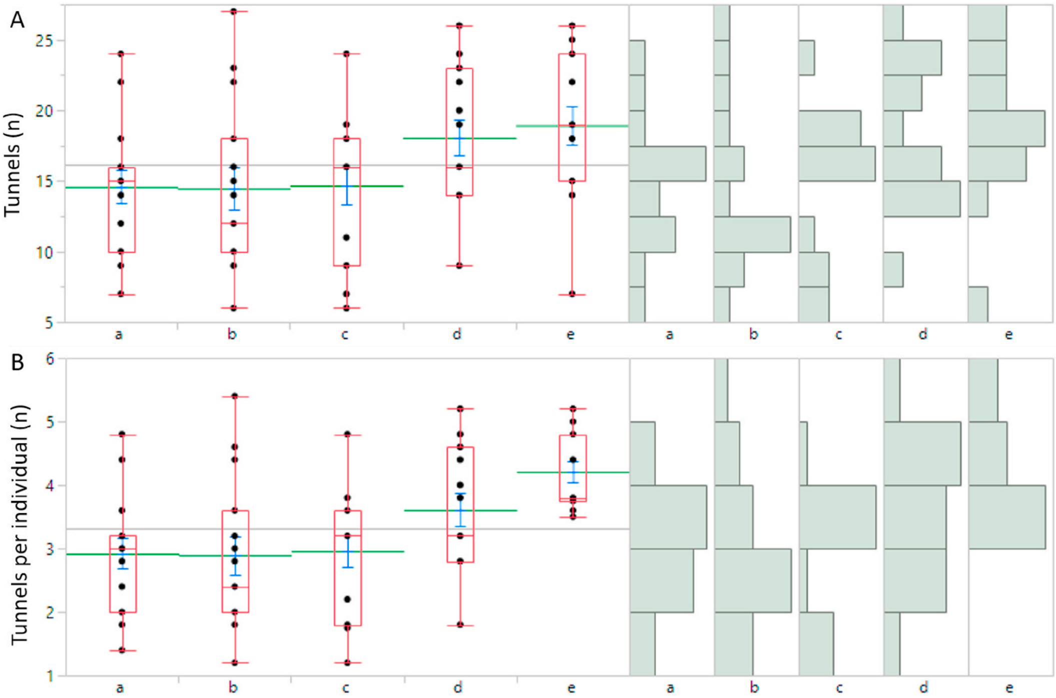

Fig. 3. Overall number of tunnel formation (A), and number of tunnel formation per *Eisenia fetida* individual (B) during the 14-day exposure to different concentrations of LHS-1 Lunar Highlands Simulant. LHS-1 concentrations: a) 0%, b) 25%, c) 50%, d) 75%, and e) 100%. In each box plot the median (red line) and its range of dispersion (lower and upper quartiles, as well as outliers) are indicated. The mean (green line), and the standard error value (blue T-bars) are also included. Each box plot reports on its right histograms describing data distribution. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

cocoons decreased at the highest concentrations of LHS-1 in the substrate treatment. However, these observations were due to the lower number of surviving individuals in those experimental arenas. So, considering the production of cocoons per individual, the number of cocoons was not significantly different among different substrate treatments, except for the concentration e) in which the cocoons were produced, but in lower numbers.

Food quality and quantity play a crucial role in earthworm ingestion rates [51]. It has been reported that ingestion is higher if food quality is low [44]. However, no effects of different LHS-1 concentrations on the amount of substrate treatments ingested were observed. Earthworms produce an intestinal mucus rich in enzymes and microorganisms that may protect them against direct effects of particles [52]. This mucus is produced mostly when earthworms ingest material poor in organic matter inducing a priming effect for microorganisms enhancing the uptake of nutrients. Higher LHS-1 concentrations can stimulate the production of more mucus, thus helping earthworms in uptalking nourishing substances.

This research provides a first step towards the establishment of a sustainable agroecosystem for space farming on possible Moon colonies. Farming in space is thought to take place in controlled condition chambers artificially supplying light, temperature, relative humidity, and gases (e.g. oxygen, carbon dioxide), as well as making use of local resources (e.g. regolith) [53], although lunar regolith is really nutrient poor to ensure adequate crops growth [21]. Earthworms may help in compensating the quality of lunar soil. In the first phase of colonization, these organisms can be used to possibly enrich the lunar regolith with microbiota having plant growth promoting activity [32,33]. Furthermore, crop waste and/or human faeces may feed earthworms which in turn would produce, and mix with the regolith, fertile compost for plants [54,55].

In future studies, we will also investigated the response to lunar regolith of earthworm species belonging to different categories, (e.g., epigeic, anecic, and endogeic) according to their type of feeding strategy and ecological nature [56]. In this framework, it will be useful to explore possible terrestrial soils that have the most similar mineralogy and physical properties to the lunar regolith simulant used by referring to the international soil classification system of the World Reference Base [57]. Understanding the telluric biocenosis of these soils will help the selection of earthworms species, and other organisms that can possibly be used to colonize the lunar soil.

Overall, our results show how *E. fetida* may adapt and colonize the Moon soil providing a valuable biological tool to promote extra-terrestrial soil creation processes [58], with a consequent possible increase in soil fertility and primary production [27,59,60].

5. Conclusions

This research reports the earthworm *E. fetida* can survive postexposure to lunar regolith, potentially colonizing and supporting crops growth on the Moon. The experiments were carried out in controlled conditions on Earth, thus other environmental factors

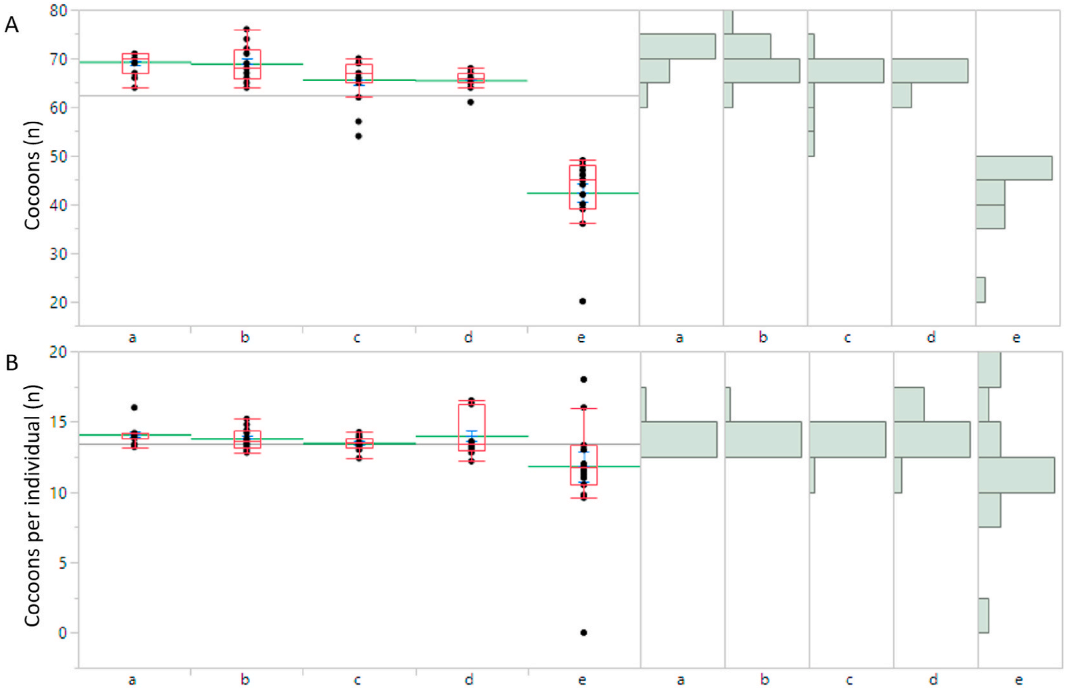

Fig. 4. Overall cocoon production (A), and cocoon production per *Eisenia fetida* individuals (B) during the 60-day exposure to different concentrations of LHS-1 Lunar Highlands Simulant. LHS-1 concentrations: a) 0%, b) 25%, c) 50%, d) 75%, and e) 100%. In each box plot the median (red line) and its range of dispersion (lower and upper quartiles, as well as outliers) are indicated. The mean (green line), and the standard error value (blue T-bars) are also included. Each box plot reports on its right histograms describing data distribution. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

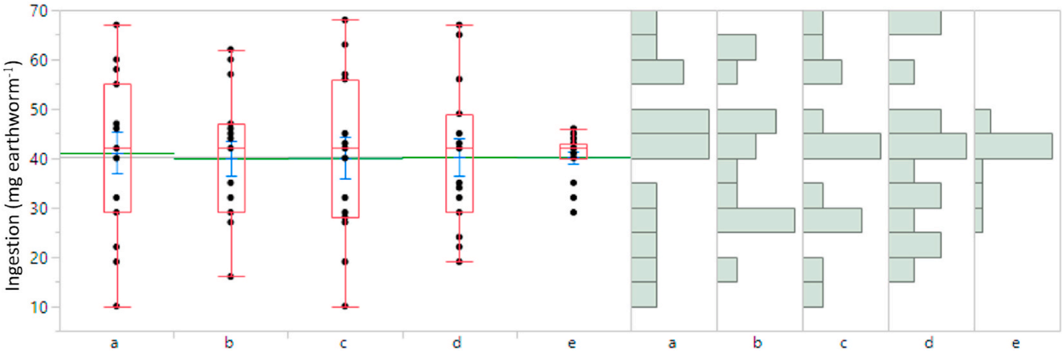

Fig. 5. Ingestion per *Eisenia fetida* individual of substrate treatments with different LHS-1 concentrations. LHS-1 concentrations: a) 0%, b) 25%, c) 50%, d) 75%, and e) 100%. In each box plot the median (red line) and its range of dispersion (lower and upper quartiles, as well as outliers) are indicated. The mean (green line), and the standard error value (blue T-bars) are also included. Each box plot reports on its right histograms describing data distribution. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

present in space, such as microgravity and cosmic radiations, should be also evaluated in future low Earth orbit (LEO) studies to better reproduce the environment encountered on the Moon. This study reports on the ability of earthworms to survive and reproduce on LHS-1. However, the effect these organisms have on the LHS-1 agronomic properties is still unknown. Also, more information on chemical and physical features of the lunar simulant potentially affecting the plant growth should be assessed in future works. Overall, results from this study encourages further research on how earthworms may contribute to provide a natural approach promoting lunar regolith to sustain crop growth.

Author contribution statement

Donato Romano: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Adriano Di Giovanni; Cesare Stefanini: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Chiara Pucciariello: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] M.J. Fogg, Terraforming: a review for environmentalists, Environmentalist 13 (1) (1993) 7–17.
[2] P. Todd, M.A. Kurk, E. Boland, D. Thomas, C. Scherzer, Mars ecopoiesis test bed: on earth and on the red planet, in: 41st COSPAR Scientific Assembly, vol. 41, 2016. F3-2.
[3] M. Smith, D. Craig, N. Herrmann, E. Mahoney, J. Krezel, N. McIntyre, K. Goodliff, The Artemis program: an overview of nasa's Activities to return humans to the moon, in: 2020 IEEE Aerospace Conference, IEEE, 2020, March, pp. 1–10.
[4] B.M. Jakosky, C.S. Edwards, Inventory of CO 2 available for terraforming Mars, Nat. Astron. 2 (8) (2018) 634–639.
[5] K. Szocik, Ethical, political and legal challenges relating to colonizing and terraforming Mars, in: Terraforming Mars, 2021, pp. 123–134.
[6] D. Miranda, 2020 NASA Technology Taxonomy (No. HQ-E-DAA-TN76545), 2020.
[7] H. Yokota, Space agriculture on Mars using hyper-thermophilic aerobic bacteria, Habitation 10 (2006) 191.
[8] T. Verbeelen, N. Leys, R. Ganigué, F. Mastroleo, Development of nitrogen recycling strategies for bioregenerative life support systems in space, Front. Microbiol. 12 (2021), 700810.
[9] R.M. Bagdigian, J. Dake, G. Gentry, M. Gault, International space station environmental control and life support system mass and crewtime utilization in comparison to a long duration human space exploration mission, in: 45th International Conference on Environmental Systems, 2015. http://hdl.handle.net/2346/64374.
[10] F. Volpin, U. Badeti, C. Wang, J. Jiang, J. Vogel, S. Freguia, et al., Urine treatment on the international space station: current practice and novel approaches, Membranes 10 (11) (2020) 327.
[11] M.S. Anderson, M.K. Ewert, J.F. Keener, Life Support Baseline Values and Assumptions Document (No. NASA/TP-2015-218570/REV1), 2018.
[12] D.P. Häder, M. Braun, R. Hemmersbach, Bioregenerative Life Support Systems in Space research, in: Gravitational Biology I, Springer, Cham, 2018, pp. 113–122.
[13] P.S. Baur, C.H. Walkinshaw, R.S. Halliwell, V.E. Scholes, Morphology of Nicotiana tabacum cells grown in contact with lunar material, Can. J. Bot. 51 (1) (1973) 151–156.
[14] C.S. Brown, T.W. Tibbitts, J.G. Croxdale, R.M. Wheeler, Potato tuber formation in the spaceflight environment, Life Support Biosph. Sci. 4 (1–2) (1997) 71–76.
[15] A. Kuang, M.E. Musgrave, S.W. Matthews, Modification of reproductive development in *Arabidopsis thaliana* under spaceflight conditions, Planta 198 (4) (1996) 588–594.
[16] A.M. Visscher, A.L. Paul, M. Kirst, A.K. Alling, S. Silverstone, G. Nechitailo, et al., Effects of a spaceflight environment on heritable changes in wheat gene expression, Astrobiology 9 (4) (2009) 359–367.
[17] Y. Rouphael, S.A. Petropoulos, C. El-Nakhel, A. Pannico, M.C. Kyriacou, M. Giordano, et al., Reducing energy requirements in future Bioregenerative life support systems (BLSSs): performance and bioactive composition of diverse lettuce genotypes grown under optimal and suboptimal light conditions, Front. Plant Sci. 10 (2019) 1305.
[18] G.W.W. Wamelink, J.Y. Frissel, W.H.J. Krijnen, M.R. Verwoert, Crop growth and viability of seeds on Mars and Moon soil simulants, in: Terraforming Mars, 2021, pp. 313–329.
[19] C.H. Walkinshaw, H.C. Sweet, S. Venketeswaran, W.H. Horne, Results of Apollo 11 and 12 quarantine studies on plants, Bioscience 20 (24) (1970) 1297–1302.
[20] R.J. Ferl, A.L. Paul, Lunar plant biology—a review of the Apollo era, Astrobiology 10 (3) (2010) 261–274.
[21] G.W. Wamelink, J.Y. Frissel, W.H. Krijnen, M.R. Verwoert, P.W. Goedhart, Can plants grow on Mars and the moon: a growth experiment on Mars and moon soil simulants, PLoS One 9 (8) (2014), e103138.

[22] A.L. Paul, S.M. Elardo, R. Ferl, Plants grown in Apollo lunar regolith present stress-associated transcriptomes that inform prospects for lunar exploration, Commun. Biol. 5 (1) (2022) 1–9.

[23] Z.A. Sylvain, D.H. Wall, Linking soil biodiversity and vegetation: implications for a changing planet, Am. J. Bot. 98 (3) (2011) 517–527.

[24] A. Sofo, A.N. Mininni, P. Ricciuti, Soil macrofauna: a key factor for increasing soil fertility and promoting sustainable soil use in fruit orchard agrosystems, Agronomy 10 (4) (2020) 456.

[25] P.F. Hendrix, D.A. Crossley, J.M. Blair, D.C. Coleman, Soil biota as components of sustainable agroecosystems, in: Sustainable Agricultural Systems, CRC press, 2020, pp. 637–654.

[26] C. Pucciariello, A. Boscari, A. Tagliani, R. Brouquisse, P. Perata, Exploring legume-rhizobia symbiotic models for waterlogging tolerance, Front. Plant Sci. 10 (2019) 578.

[27] P. Lavelle, Earthworm activities and the soil system, Biol. Fertil. Soils 6 (3) (1988) 237–251.

[28] N.Q. Arancon, C.A. Edwards, P. Bierman, C. Welch, J.D. Metzger, Influences of vermicomposts on field strawberries: 1. Effects on growth and yields, Bioresour. Technol. 93 (2) (2004) 145–153.

[29] J.L. Johnson-Maynard, K.J. Umiker, S.O. Guy, Earthworm dynamics and soil physical properties in the first three years of no-till management, Soil Tillage Res. 94 (2) (2007) 338–345.

[30] C. Gilot, Effects of a tropical geophageous earthworm, *M. anomala* (Megascolecidae), on soil characteristics and production of a yam crop in Ivory Coast, Soil Biol. Biochem. 29 (3–4) (1997) 353–359.

[31] U. Tomati, E. Galli, Earthworms, soil fertility and plant productivity, Acta Zool. Fennica 196 (11) (1995).

[32] Y. Chen, Y. Zhang, X. Shi, L. Xu, L. Zhang, L. Zhang, The succession of GH6 cellulase-producing microbial communities and temporal profile of GH6 gene abundance during vermicomposting of maize stover and cow dung, Bioresour. Technol. 344 (2022), 126242.

[33] S. Suthar, Evidence of plant hormone like substances in vermiwash: an ecologically safe option of synthetic chemicals for sustainable farming, Ecol. Eng. 36 (8) (2010) 1089–1092.

[34] A. Banerjee, J.K. Biswas, D. Pant, B. Sarkar, P. Chaudhuri, M. Rai, E. Meers, Enteric bacteria from the earthworm (*Metaphire posthuma*) promote plant growth and remediate toxic trace elements, J. Environ. Manag. 250 (2019), 109530.

[35] S. Al-Maliki, D.K. Al-Taey, H.Z. Al-Mammori, Earthworms and eco-consequences: considerations to soil biological indicators and plant function: a review, Acta Ecol. Sin. 41 (2021) 512–523.

[36] S. Houida, L. Yakkou, L. Kaya, S. Bilen, M. Fadil, M. Raouane, A. El Harti, S. Amghar, Biopriming of maize seeds with plant growth-promoting bacteria isolated from the earthworm *Aporrectodea molleri*: effect on seed germination and seedling growth, Lett. Appl. Microbiol. (2022), https://doi.org/10.1111/lam.13693.

[37] G.W.W. Wamelink, L. Schug, J.Y. Frissel, I. Lubbers, Growth of Rucola on Mars soil simulant under the influence of pig slurry and earthworms, Open Agric. 7 (1) (2022) 238–248.

[38] J. Nahmani, M.E. Hodson, S. Black, A review of studies performed to assess metal uptake by earthworms, Environ. Pollut. 145 (2) (2007) 402–424.

[39] V.S. Engelschiøn, S.R. Eriksson, A. Cowley, M. Fateri, A. Meurisse, U. Kueppers, M. Sperl, EAC-1A: a novel large-volume lunar regolith simulant, Sci. Rep. 10 (1) (2020) 1–9.

[40] A.N. Scott, C. Oze, Y. Tang, A. O'Loughlin, Development of a Martian regolith simulant for in-situ resource utilization testing, Acta Astronaut. 131 (2017) 45–49.

[41] European Commission, Commission Recommendations of 18 June 2007 on Guidelines for the Accommodation and Care of Animals Used for Experimental and Other Scientific Purposes, 2007. Annex II to European Council Directive 86/609. See 2007/526/EC. Retrieved from, http://eurex.europa.eu/LexUriServ/LexUriServ.do?uri1/4OJ:L. 2007.197:0001:0089:EN:PDF.

[42] OECD, Earthworm Reproduction Test. Guideline for Testing Chemicals No.222, OECD, Paris, France, 2004.

[43] C. Baeza, C. Cifuentes, P. González, A. Araneda, R. Barra, Experimental exposure of *Lumbricus terrestris* to microplastics, Water, Air, Soil Pollut. 231 (6) (2020) 1–10.

[44] ISO 11268-1, Soil Quality - Effects of Pollutants on Earthworms – Part 1: Determination of Acute Toxicity to *Eisenia fetida/Eisenia Andrei*, 2012.

[45] E. Huerta Lwanga, H. Gertsen, H. Gooren, P. Peters, T. Salánki, Van Der Ploeg, et al., Microplastics in the terrestrial ecosystem: implications for *Lumbricus terrestris* (Oligochaeta, Lumbricidae), Environ. Sci. Technol. 50 (5) (2016) 2685–2691.

[46] R Development Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2019.

[47] S. Gajalakshmi, E.V. Ramasamy, S.A. Abbasi, Potential of two epigeic and two anecic earthworm species in vermicomposting of water hyacinth, Bioresour. Technol. 76 (3) (2001) 177–181.

[48] E. Olchawa, M. Bzowska, S.R. Stürzenbaum, A.J. Morgan, B. Plytycz, Heavy metals affect the coelomocyte-bacteria balance in earthworms: environmental interactions between abiotic and biotic stressors, Environ. Pollut. 142 (2) (2006) 373–381.

[49] S. Pattnaik, M.V. Reddy, Heavy metals remediation from urban wastes using three species of earthworm (*Eudrilus eugeniae, Eisenia fetida* and *Perionyx excavatus*), J. Environ. Chem. Ecotoxicol. 3 (14) (2011) 345–356.

[50] A. Rorat, H. Suleiman, A. Grobelak, A. Grosser, M. Kacprzak, B. Płytycz, F. Vandenbulcke, Interactions between sewage sludge-amended soil and earthworms—comparison between *Eisenia fetida* and *Eisenia andrei* composting species, Environ. Sci. Pollut. Control Ser. 23 (4) (2016) 3026–3035.

[51] J.E. Satchell, r worms and K Worms: A basis for classifying lumbricid earthworm strategies, in: Soil Biology as Related to Land Use Practices: Proceedings of the VII International Soil Zoology Colloquium, EPA, 1980, pp. 848–863.

[52] J.P. Curry, O. Schmidt, The feeding ecology of earthworms–a review, Pedobiologia 50 (6) (2007) 463–477.

[53] D. Trigo, I. Barois, M.H. Garvin, E. Huerta, S. Irisson, P. Lavelle, Mutualism between earthworms and soil microflora, Pedobiologia 43 (6) (1999) 866–873.

[54] O. Monje, G.W. Stutte, G.D. Goins, D.M. Porterfield, G.E. Bingham, Farming in space: environmental and biophysical concerns, Adv. Space Res. 31 (1) (2003) 151–167.

[55] A. Ahmad, Z. Aslam, K. Bellitürk, N. Iqbal, S. Naeem, M. Idrees, et al., Vermicomposting methods from different wastes: an environment friendly, economically viable and socially acceptable approach for crop nutrition: a review, Int. J. Food Sci. Agric. 5 (1) (2021) 58–68.

[56] C. Schröder, F. Häfner, O.C. Larsen, A. Krause, Urban organic waste for urban farming: growing lettuce using vermicompost and thermophilic compost, Agronomy 11 (6) (2021) 1175.

[57] T. Sizmur, E.L. Tilston, J. Charnock, B. Palumbo-Roe, M.J. Watts, M.E. Hodson, Impacts of epigeic, anecic and endogeic earthworms on metal and metalloid mobility and availability, J. Environ. Monit. 13 (2) (2011) 266–273.

[58] IUSS Working Group WRB, World Reference Base for Soil Resources. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps, fourth ed., International Union of Soil Sciences (IUSS), Vienna, Austria, 2022.

[59] G.H. Baker, G. Brown, K. Butt, J.P. Curry, J. Scullion, Introduced earthworms in agricultural and reclaimed land: their ecology and influences on soil properties, plant production and other soil biota, Biol. Invasions 8 (6) (2006) 1301–1316.

[60] J.W. Van Groenigen, I.M. Lubbers, H.M. Vos, G.G. Brown, G.B. De Deyn, K.J. Van Groenigen, Earthworms increase plant production: a meta-analysis, Sci. Rep. 4 (1) (2014) 1–7.