Antarctic regolith as prospective substrate for cultivation of plants in space analog habitat greenhouses: Seed germination and early growth study of broccoli in aqueous and acidic dilutions

Štěpán Krejčí[1*], Hector-Andreas Stavrakakis[2], Miloš Barták[1], Jiří Sekerák[1], Dimitra Argyrou[2]

[1]*Masaryk Universit, Faculty of Science, Department of Experimental Biology, Laboratory of Photosynthetic processes, Kamenice 5, 625 00 Brno, Czech Republic*
[2]*National Technical University of Athens, School of Mining and Metallurgical Engineering, Department of Geological Sciences, Athens, Greece*

Abstract
Food prouction for the needs of space mission crews has posed one of the leading concerns of recent space research. One of the arguably best terrestrial analogs of extraterrestrial habitats are the polar research stations, such as those found in Antarctica. Plants cultivation, offering a valulable source of fresh food, have been a prominent research topic not only for their significance for space analog experiments, but also for the needs of the scientists working at these stations. One of the approaches is the *In Situ* Resource Utilization (ISRU), which in this case can be adopted by cultivating crops directly in the local soil. Our study aims to evaluate early growth phase and photosynthetic performance of *Brassica oleracea* var. *botrytis italica* in Antarctic regolith collected at foothill of the Berry Hill mesa, James Ross Island, Antarctica. Fine grained regolith consisting primarily of hyaloclastic breccias was collected and transported to the laboratories in the Czech Republic. For germination and growth of the experimental plants, leachates were prepared from the regolith using deionized water and 0.11 M acetic acid. Individuals of *B. oleracea* were cultivated from seeds in a Murashige-Skoog (MS) liquid solution under controlled conditions (T = 21°C, PAR = 120 µmol m^{-2} s^{-1}) either without addition of regolith leachates (control) or with addition of leachates done by using demineralized water and weak acetic acid. Growth rate, and photosynthetic activity of the experimental plants were measured by chlorophyll fluorescence in 1 day intervals for 21 days. We measured (1) F_V/F_M (potential yield of photochemical photosynthetic processes), and (2) Φ_{PSII} (effective quantum yield of photosystem II). It was showed that acidic leachate either fully inhibited germination or had a strong inhibitory effect on *B. oleracea*. Water leachates added to the MS medium had moderately strong inhibitory effects on F_V/F_M and Φ_{PSII}. The experimental plants showed decreased but still satisfactory growth rate. The results are promising for follow-up studies aimed to understand and expand the experimental plant growth in Antarctic regolith and its potential association with ISRU purposes.

DOI: 10.5817/CPR2024-2-20

Received November 29, 2024, *accepted* January 15, 2025.
[*]Corresponding author: Š. Krejčí <17stpnkrjc@gmail.com>
Acknowledgements: The authors are grateful for support provided by the Czech Antarctic Research Programme 2024 (CARP 2024) and laboratory facilities (EEL MU Brno, ECOPOLARIS project).

Key words: photosynthesis, chlorophyll fluorescence, *Brassica oleracea*, space greenhouse analogue, space missions, agriculture, ISRU

Introduction

The interest in space biology and exploration into various aspects of plant cultivation in space has been increasing in last decades (for review *see* Lorenz et al. 2024). The studies addressing plants in space reasearch might be divided into two principal subgroups: (1) those focused on microautotrophs like algae and cyanobacteria, and (2) those exploring vascular plants (*e.g.* Eichler et al. 2021). The latter ones have used several approches ranging from preflight test done in Earth laboratories, orbital experiments done onboard of International Space Station (ISS) using growth chamber facilities (*e.g.* Massa et al. 2021), and the experiments done in space analogues, typically plant biomass producing chambers located in remote locations such as deserts and polar regions. In the last decades, several projects attempted to cultivate vascular plants in the Earth facilities for the application in future space missions (Gitelson et al. 2003, Nitta 2005, Fu et al. 2016, Wheeler 2017, Dreschel et al. 2018, Wang et al. 2019).

Recently, the EDEN ISS project is the newest major space greenhouse analog project located in Antarctica (EDEN ISS facility near the German Neumayer III) focused on technical, technological, operational and scientific aspects of plant cultivation (for overview *see* Zabel et al. 2020). The study reports the results of 9 month-long experiment, cultivation of a wide range of plants including crops and vegetables (more than 268 kg of fresh food harvested from the 12.5 m^2 cultivation area). The study describes a closed, controlled environment using an aeroponic system, LED illumination, the irrigation water's pH control system, nutrient solution preparation unit and high-pressure pumps that spray a fine nutrient mist inside the root compartment. Plant health status at the EDEN ISS is remotely monitored based on photos and spectreal reflectance parameters, effectively reducing the required crew time (Zeidler et al. 2019). Microbial monitoring in the EDEN ISS cultivation untit is performed as well (Fahrion et al. 2020). Based on the experience gained during last years of running EDEN ISS, a recommendation for future directions has been formulated, *i.e.* to design and subsequently operate a ground test demonstrator for a Lunar greenhouse (Maiwald 2023). Another study (Poulet et al. 2021) evaluated crew time needed for plant cultivation from 13 experiments conducted between July 2014 and December 2019 at the EDEN ISS.

Since the early days of Antarctica exploration, numerous facilities and systems were utilized for growing plants, utilizing various set-ups, including imported non-native soils and local substrates. Later, however, use of hydroponics has become a standard method in antarctic greenhouses and plant cultivation units, such as *e.g.* automatic hydroponic system for plant cultivation in artificial environment (Campiotti et al. 2008). Other systems exploiting hydroponic-based plant cultivation in Antarctica are reported in the review of Bamsey et al. (2015). Recent technologies tested in Antarctica use aeroponic cultivation, *e.g.* EDEN ISS infrastructure (Zeidler et al. 2021, Schubert et al. 2018, Zabel et al. 2015) and irrigated soil-based systems[2].

The use of substrates with nutrient solution addition is another approach in plant growth test performed at Earth facilities. The Vegetable Production System (Veggie) used for plant production within the Hawai'i Space Exploration Analog and Simulation (HI-SEAS), which was later operated in space conditions within the ISS, exploited *Arcillite* (calcinated clay, a substrate composed of kaolin clay fired at a high temperature) supplemented with nutrients (Hatch et al. 2022).

Alternative method of plant cultivation in space conditions, that is being considered, particularly for the surface of celestial bodies of the most imminent human interest, like the Moon or Mars, is planting the crops directly into the native surficial rock substrate – regolith. This approach is understood as an adaptation of the general concept of ISRU. In some studies, Lunar regolith and its analogues were used to cultivate plants (tomatoes, yellow bell peppers, green okra and sweet basil, Houston Museum of Natural Science – Luke Jerram experiments[1]). Similarly, Martian regolith analogues (basaltic regolith simulant) have been used to cultivate turnip (*Brassica rapa*), radish (*Raphanus sativus*), lettuce (*Lactuca sativa*) or alfalfa (*Medicago sativa*) as shown by Kasiviswanathan et al. (2022) for germination, early growth and biomass production. Plant growth and physiological processes are typically slower in Martian regolith simulants than in natural Earth soils. However, additon of organic matter to the Martian simulant improves chemical and microbiological parameters of the Martian regolith simulant, even though the development of plants is improved only to a small extent as shown for vermicompost (an organic fertilizser made from compost that has passed through the digestive tract of earthworms) and wheat cultivation by Przemieniecki et al. (2024). Other tested experimental treatments of extraterrestrial regolith simulants aimed at improving their agricultural properties include for example manure addition (Caporale et al. 2022) or addition of organic solid waste consisting of plant residues and feces (Yao et al. 2022).

In our study, we focused on Antarctic volcanic regolith that has been shown to have good affinity with Lunar regolith (Coufalík 2024) as a proposed substrate for plant cultivation in space analog habitat greenhouses. The study aimed to germinate and grow broccoli (*Brassica oleracea* var. *botrytis italica*) in Murashige-Skoog (MS) liquid medium with additition of aqueous and/or acidic leachate. We hypothesized that experimental plant growth and primary photosynthetic processes will be negatively affected by the leaches, which proved to be true. However, a degree of leachate dillution done before the addition to the MS medium may alleviate negative effects and stimulate plant growth and development in comparison to control (pure MS medium).

Material and Methods

Description of regolith collection site

Regolith samples were collected from the northern deglaciated part of the James Ross Island called the Ulu peninsula. The area represents the largest continuous deglaciated area in the region of NE Antarctic peninsula (*sensu* Terauds and Lee 2016). The deglaciated part represents 1.2% of the total ice-free area in Antarctica (Kavan et al. 2017). Cryosols are formed on the deglaciated area. Recent study (Daher et al. 2022) showed that cryosols formed on volcanic rocks at the James Ross island had higher contents of Al_2O_3, Fe_2O_3, CaO, MgO, and some trace elements oxides (TiO_2, SO_3, MnO, P_2O_5, Cr_2O_3, SrO, and ZrO_2) than sedimentary soils from the same region.

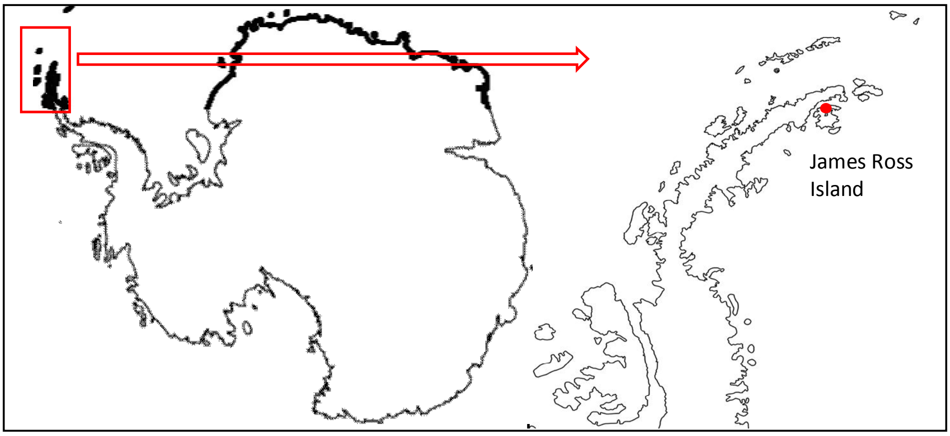

Fig. 1. Location of sampling site on the northern part of James Ross Island (JRI), Antarctica.

Climatic parameters of deglaciated area reported for the northern part of JRI (J. G. Mendel station site, 63° 48′ 02″ S, 57° 52′ 56″ W) are as follows. The mean annual air temperature of the site is −7.0°C (Hrbáček et al. 2016). Mean daily temperatures, however, fluctuate according the season. They achieve the values above 5°C and below −30°C in the austral summer and winter seasons, respectively (Láska et al. 2011). The mean annual precipitation ranges from 300 to 500 mm (van Lipzig et al. 2004). Collection site was located about 2.5 km SE from the J. G. Mendel station (*see* Fig. 1). Fine grained regolith consisting primarily of hyaloclastic breccias was collected (about 900 g) at foothill of the Berry Hill mesa (63° 48´ 36´´ S, 57° 50´ 45´´ W) and dried out under natural conditions. After drying, the regolith was transported to the Czech Republic where it was used for the plant germination and growth rate experiments, preparation of leachates in particular.

Fig. 2. View on regolith collected at James Ross Island showing natural grain size fractions.

Regolith leachates preparation

For germination and plant growth experiments, leachates were prepared from the regolith using deionized water and 0.11 M acetic acids. First, the finer components of Antarctic regolith sample were crushed and homogenized into fine particles in order to attain a bulk mean composition. The extraction of a leachate was done on a shaker (GFL 3018, Germany) in 0.11 mol/L CH_3COOH (Suprapur, 96%, Merck, Germany) in a ratio of 0.5 g of sample per 20 mL of solution at laboratory temperature for 16 h (for extraction details *see* Coufalík et al. 2024). Extraction in deionized water was carried out under the same conditions as described above. Then, the extracts were centrifuged (Centrifuge 5804R, Eppendorf AG, Germany) at 9000 g for 10 min., and collected into HDPE scintillation vials (Kartell, Italy).

Seed sterilization, sowing and plant cultivation

Individuals of broccoli (*Brassica oleracea* var. *botrytis italica*) were cultivated from seeds in a MS liquid growth solution in a special cultivation miniboxes (*see* Fig. 3) in sterile environment under controlled conditions of a growth chamber (T = 21°C, PAR = 120 µmol m^{-2} s^{-1}). Seeds were placed into a MS medium in individual holes of microbiological plate with and without addition of the above-described regolith leachates and their dilutes, respectively (*see* below).

Before placing them in the leachates and enclosing them into the cultivtion miniboxes, the seeds were surficially sterilized following this procedure:

1. 5 min. washing with 50% ethanol

2. 10 min. washing with 20% solution of bleach

3. 3 x 5 min. washing with sterile distilled water

In the first experiment, seed were sown into the original, undiluted leachates. This treatment resulted in complete suppression of seed germination. Following this outcome, the leachates were diluted in two dilution ratios. For the subsequent growth experiment, the following treatments were used: (1) control (no leachate addition), (2) 10 times diluted, and (3) 100 times diluted leachate. In the following text, the treatments are abbreviated:

C - control

W10D - addition of 10 times diluted water leachate

W100D - addition of 100 times diluted water leachate

A10D - addition of 10 times diluted acidic leachate

A100D - addition of 100 times diluted acidic leachate

Statistical analysis

The effects of treatments (C, W10D, W100D, A10D, and A100D) on growth and physiological parameters were evaluated by a ONE-WAY ANOVA (Statistica), if not stated differently.

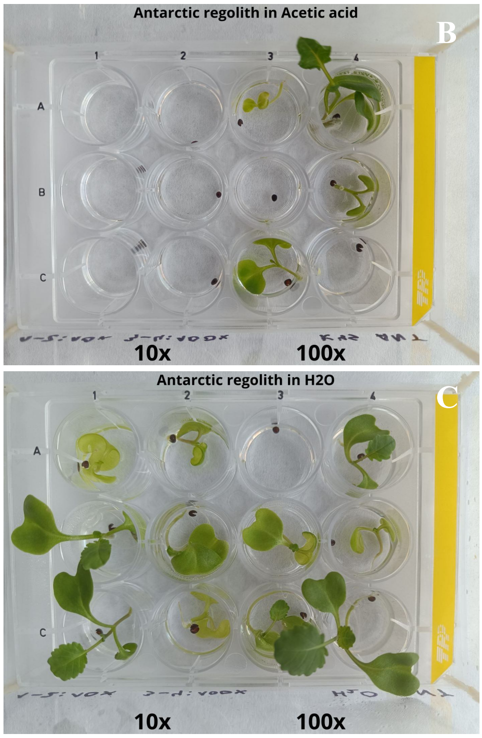

Fig. 3. Cultivation units consisting of a microbiological plates (hole diameter 22 mm) and a plactic cover (left - A). Top view on *B. oleracea* plants germinating in MS cultivation medium with addtion of acetic leachete A10D and A100D (upper right - B), and water leachate W10D, W100D (lower right - C). The photo was taken on day 10 of cultivation.

Monitoring of photosynthetic activity

The effects of acidic and water regolith leachates on the early phases of *B. oleracea* growth and photosynthetic performance was assessed by chlorophyll fluorescence imaging system (Open FluorCam fluorometer, Photon Systems Instruments, Drásov, Czech Republic) using the method described earlier (Barták et al. 2021). Chlorophyll fluorescence parameters were measured in 1 d interval for 15 days. Plants were dark-adapted for 5 min. before starting the measurements of slow Kautsky kinetics supplemented with saturation pulses. A single measurement started with a low light applied to dark-adapted germinating plants in order to induce the basal chlorophyll fluorescence F_0. Then, a 2s-lasting saturation pulse was applied to induce the maximum chlorophyll fluorescence level (F_M), followed by a 30 s dark period. After that, the germinating plants were exposed to a 300s-lasting actinic light (AL) period in order to induce slow Kautsky kinetics, *i.e.* a rapid increase reaching maximum (F_P) followed by a slow polyphasic decline in chlorophyll to a steady-state chlorophyll fluorescence (F_S) found at the end of actinic light period. Another saturation pulse was then applied to induce $F_M{'}$ (maximum chlorophyll fluorescence in the light-adapted state). After switching off the actinic light, the background ChlF ($F_0{'}$) was recorded). A final saturation pulse was then given to induce $F_M{''}$ level after a 40 s dark period. The following chlorophyll fluorescence parameters (for definitions *see* Roháček 2002) were finally calculated by FluorCam software: F_V/F_M (maximum (potential) yield of photosynthetic processes in PSII), Φ_{PSII} (effective quantum yield of photosynthetic processes of PSII), qP (fraction of PS II centres in open states), and NPQ (non-photochemical quenching).

Results

Both the water and the acidic leachates prepared from the antarctic regolith caused 100% suppression of germination in the sowed broccoli seeds. Following this result, the two dilutions (10D and 100D) were prepared for next experiment.

In this subsequent step, addition of leachates into a MS medium led to an inhibition of experimental plants growth (Fig. 5). While W10D and W100D water leachates caused significant, but similar (10D *vs* 100D) decrease in growth, acidic leachate A10D caused full inhibition of germination and, therefore, no apparent growth. The A100D acidic treatment caused decreased germination (66.6%) and the most pronounced growth inhibition which was apparent especially in the first part of growth period (*see* days 0-9, Fig. 4). In majority of cases, the growth was biphasic. It showed two different growth rates recorded for the periods (1) 0-10 d (slower), and (2) 10-15 d (faster). While the slower growth rate is attributed to the development of cotyledons, the faster one is related to the development of primary leaves.

Physiological processes in *Brassica oleracea* var. *botrytis italica* related to primary photosynthesis were monitored by several chlorophyll fluorescence parameters. Negative effect of leachate addition into growth medium on the capacity of photosynthetic processes in PSII (F_V/F_M) was apparent especially at the begining of growth period (Fig. 5). The grates difference in F_V/F_M was found on day 1 between control and acidic A10D treatment. Then, F_V/F_M in the leachate treatments tended to reach untreated control values in a course of cultivation time. In all treatments, however, absolute F_V/F_M value decreased with cultivation time.

Effective quantum yield of photochemical photosynthetic processes in PSII (Φ_{PSII}) is considered an indicator of photosynthetic linear electron transport in between PSII and PSI as well as overal photosynthesis expressing an equilibrium between primary photochemical and secondary biochemical photosynthetic processes. At the begining of cultivation period (day 1-2), Φ_{PSII} in control was slighly, but insignificantly higher than in the leachate treatments. Negative effect of leachate addition into growth medium on Φ_{PSII} increased slightly in a course of cultivation time. The largest negative effect was apparent for W10D water leachate at the end of cultivation period (*see* Fig. 5).

During the early germination, the experimental plants activated protective mechanisms, *i.e.* non-photochemical quenching. The difference in NPQ between untreated control and the leachate treatments was largest at the begining of cultivation time (day 0-1) indicating that the addition of leachates into the growth medium led to a stress to PSII and consequent increase in NPQ. The difference in NPQ between untreated control and the leachate treatments, however, diminished with cultivation time resulting in almost zero difference found for NPQ at the end of cultivation period. Throughout the cultivation time, however, NPQ values were found highest in the A100D treatments, while the difference between control, W10D, and the W100D treatments was much smaller.

Photochemical quenching (qP) of chlorophyll fluorescence is considered a measure of the fraction of open PSII reaction centres, or the redox state of the plastoquinone pool. In our experiment, the highest difference in qP was found between control and the A100D treatment at the begining of growth period. In a course of cultivation time the difference between control and the leachate treatments (W10D, W100D, and A100D) got smaller. The qP value, however was still found higher in control than in the leachate treatments at the end of cultivation period.

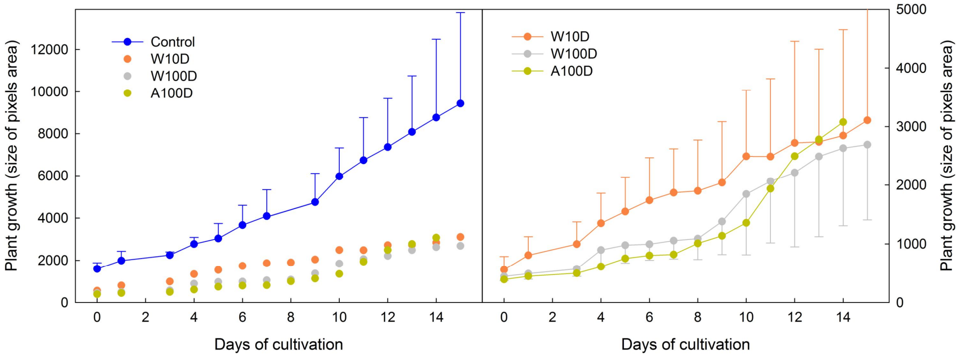

Fig. 4. Growth of *Brassica oleracea* var. *botrytis italica* as expresses as plant projection area derived from chlorophyll fluorencence imaging (number of pixels fitting the image of germinating plants). Left: untreated control and all leachate treatments (water leachate 10D, 100D, and acidic leachate 10D). Right: detail of plant growth in the treatmntes with leachate addition. Means ± standard deviations are presented.

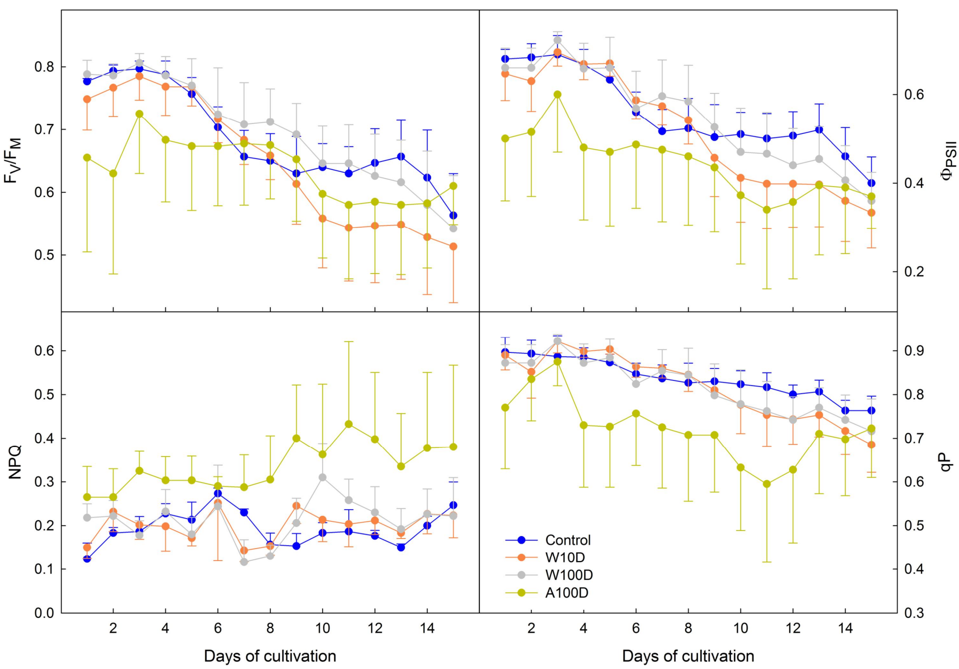

Fig. 5. Chlorophyll fluorescence parameters related to the activity of photosystem II of *Brassica oleracea* var. *botrytis italica* recorded for a two week growth period (days 1 to 15). Capacity of photosynthetic processes in PSII (F_V/F_M), effective quantum yield of PSII (Φ_{PSII}), non-photochemical quenching (NPQ), and photochemical quenching (qP) are presented. Data point represent means of 3-5 replicates. Standard deviations are presented only for A100D treatment. The treatments are Control (pure Murashige–Skoog growth medium), water leachate diluted ten times (MS medium with addition of W10D), water leachate diluted 100 times (MS medium with addition of W100D), and acidic leachate diluted 100 times (MS medium plus A100D). Means ± standard deviations are presented.

Discussion

Leachates from Antarctic regolith added to the liquid growth solution led to a decrease in primary photosynthetic processes related to photosystem II, especially in the early germination and growth period. The negative effect can be attributed to the co-action of ions relased from the regolith during a leachate preparation. Acidic leaching apparently led to an increased content of the elements (compared to aqueous leaching) since there was significant decrease in growth when a hundred times diluted acidic leachate was used (A100D), while full inhibition of germination was apparent in A10D). Earlier study showed a high content of mobile fraction heavy metals including mercury in regolith samples from James Ross Island (Zvěřina et al. 2014). Therefore, we may attribute the decrease in PSII functioning to co-action of heavy metals leached from regolith grains during leachate preparation, their negative effects in chloroplasts and heavy metals-induced alterations in nutrient uptake from growth solutions. However, The content of Cd, Cr, Cu, Ni and Pb in the deionised water and acetic acid solution used was below the detection limit for ETAAS. The zinc content in water was 0.5 μg/L and the zinc content in acetic acid was 1.1 μg/L. In recent studies, heavy metals-induced inhibition of PSII functioning is reported for a variety of plants (Qin et al. 2023).

Our study indicated that original (undiluted) leachate treatments led to absolute suppression of germination and that their dilutions caused inhibition of early growth rate of *Brassica oleracea* var. *botrytis italica*. Negative effects on primary photosynthetic processes, however, were apparent only in the early growth, as the difference between regolith-untreated control and the treatments affected by water and/or acidic leachates decreased with the time of cultivation. This suggests that cultivation of plants in Antarctic regolith of similar nature, *e.g.* similar chemical composition and mineralogical characteristics as the one used in our study (for details regarding the chemical composition and mineralogy see Coufalík et al. 2024) can be considered fit for use in follow up studies. Additionally, the system exploiting cultivation of experimental plants in the regolith from James Ross Island (Antarctica), and the watering process with different amount and proportion of fertilizing compounds will be tested to provide guidelines for the biotechnologies exploiting bioregenerative life-support systems (BLSS) for space applications and potential future ISRU applications. The improvement of Lunar and Martial regolith as well as their simulants for plant cultivation has recently garnered growing interest (Eichler et al. 2021, Fackrel et al. 2021). This effort faces several chalenges, such as *e.g.* a high content of metal ions, salt precipitation and high pH caused by addition of organic matter alone, interaction with microbiota (for review *see* Ding et al. 2024). However, such trend is quite promising since several recent studies focused on biofertilisation of Lunar and/or Martian regolith (Duri et al. 2022) or their simulants (Zhao et al. 2024) with an organic matter in order to improve the agricultural potential of these substrates as plant growth media in future space farming (Caporale et al. 2023). Morover, the number of plant species tested in such growth systems has been increasing last decade (Eichler et al. 2021, Fackrel et al. 2021, Rickard et al. 2021, Gonçalves et al. 2024).

References

BAMSEY, M., ZABEL, P., ZEIDLER, C., GYIMESI, D., SCHUBERT, D., KOHLBERG, E., MENGEDOHT, D., RAE, J. and GRAHAM, T. (2015): Review of Antarctic greenhouses and plant production facilities: A historical account of food plants on the ice. 45th International Conference on Environmental Systems, 12-16 July 2015, Bellevue, Washington, 36 p.

BARTÁK, M., HÁJEK, J., OREKHOVA, A., VILLAGRA, J., MARÍN, C., PALFNER, G. and CASANOVA-KATNY, A. (2021): Inhibition of primary photosynthesis in desiccating Antarctic lichens differing in their photobionts, thallus morphology, and spectral properties. *Microorganisms*, 9: 818. doi: 10.3390/microorganisms9040818

CAMPIOTTI, C.A., BALDUCCHI, R., DONDI, F., ALONZO, G., CATANESE, V., INCROCCI, L. and BIBBIANI, C. (2008): The PULSA (Plant-Based Unit For Life Support in Antarctica): A sustainable plant food production technology for remote and isolated environments. ISHS Acta Horticulturae 801: International Symposium on High Technology for Greenhouse System Management: Greensys2007. *Acta Horticulturae*, 801: 417-424. doi: 10.17660/ActaHortic. 2008.801.44

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. and 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(23): 3345. doi: 10.3390/plants11233345

CAPORALE, A. G., PALLADINO, M., DE PASCALE, S., DURI, L. G., ROUPHAEL, Y. and ADAMO, P. (2023): 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 Management*, 325(Pt A): 116455. doi: 10.1016/j.jenvman.2022.116455

COUFALÍK, P., STAVRAKAKIS, H-A. and ARGYROU, D. (2024): Chemical analysis of Antarctic regolith and lunar regolith simulant as prospective substrates for experiments with plants. *Czech Polar Reports*, 14(2): 241-254. doi: 10.5817/CPR2024-2-15

DAHER, M., FERNANDES-FILHO, E., FRANCELINO, M., COSTA, L. and SCHAEFER, C. (2022): Geochemistry of semi-arid Cryosols on volcanic and sedimentary materials from James Ross Island, Antarctica. *Geoderma Regional*, 28: e00490. doi: 10.1016/j.geodrs.2022.e00490

DING, J., XU, Y., TAN, J., ZHANG, H., XIONG, X., MEI, C., LI, M. and XIE, G. (2024): How to make lunar soil suitable for cultivation? - A review. *The Science of the Total Environment*, 948: 174603. https://doi.org/10.1016/j.scitotenv.2024.174603

DRESCHEL, T. W., KNOTT, W. M., PRINCE, R. P., SAGER, J. C. and WHEELER, R. M. (2018): From project mercury to the breadboard project. *Proceedings of the 48th International Conference on Environmental Systems*, Boston, MA.

DURI, L., CAPORALE, A., ROUPHAEL, Y., VINGIANI, S., PALLADINO, M., DE PASCALE, S. and ADAMO, P. (2022): The potential for lunar and martian regolith simulants to sustain plant growth: A multidisciplinary overview. *Frontiers in Astronomy and Space Sciences*, 8: 747821. doi: 10.3389/fspas.2021.747821

EICHLER, A., HADLAND, N., PICKETT, D. E., MASAITIS, D., HANDY, D. C., PEREZ, A., BATCHELDOR, D., WHEELER, B. A. and PALMER, A. G. (2021): Challenging the agricultural viability of martian regolith simulants. *Icarus*, 354: 114022.

FACKRELL, L. E., SCHROEDER, P. A., THOMPSON, A., STOCKSTILL-CAHILL, K. and 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.

FAHRION, J., FINK, C., ZABEL, P., SCHUBERT, D., MYSARA, M., VAN HOUDT, R., EIKMANNS, B., BEBLO-VRANESEVIC, K. and RETTBERG, P. (2020): Microbial monitoring in the EDEN ISS greenhouse, a mobile test facility in Antarctica. *Frontiers in Microbiology*, 11: 525. doi: 10.3389/fmicb.2020.00525

FU, Y., LI, L., XIE, B., DONG, C., WANG, M., JIA, B., SHAO, L., DONG, Y., DENG, S., LIU, H., LIU, G., LIU, B., HU, D. and LIU, H. (2016): How to establish a bioregenerative life support system for long-term crewed missions to the Moon or Mars. *Astrobiology*, 16(12): 925-936. doi: 10.1089/ast.2016.1477

GITELSON, I. I., LISOVSKY, G. M. and MACELROY, R. D. (2003): Manmade closed ecological systems. Earth space institute book series. New York, NY: Taylor & Francis, 416 p.

GONÇALVES, R., WAMELINK, G. W. W., VAN DER PUTTEN, P. and EVERS, J. B. (2024): Intercropping on Mars: A promising system to optimise fresh food production in future martian colonies. *PloS One*, 19(5): e0302149. doi: 10.1371/journal.pone.0302149

HATCH, T. R., MCQUILLEN, J. B., WASSERMAN, M. and WEISLOGEL, M. M. (2022): Plant water management in microgravity. *Proceedings*, 51st International Conference on Environmental Systems, 10-14 July 2022, St. Paul, Minnesota, USA, 12 p.

HRBÁČEK, F., LÁSKA, K., NÝVLT, D., ENGEL, Z. and OLIVA, M. (2016): Active layer thickness variability on James Ross island, eastern Antarctic peninsula. *In*: F. Gunther, A. Morgenstern (Eds.): International Conference on Permafrost, Bibliothek Wissenschaftspark Albert Einstein, Potsdam.

KASIVISWANATHAN, P., SWANNER, E. D., HALVERSON, L. J. and VIJAYAPALANI, P. (2022): Farming on Mars: Treatment of basaltic regolith soil and briny water simulants sustains plant growth. *PLoS One*, 17(8): e0272209. doi: 10.1371/journal.pone.0272209

KAVAN, J., ONDRUCH, J., NÝVLT, D., HRBÁČEK, F., CARRIVICK, J. L. and LÁSKA, K. (2017): Seasonal hydrological and suspended sediment transport dynamics in proglacial streams, James Ross Island, Antarctica. *Geografiska Annnaler Series A – Physical Geography*, 99(1): 38-55. doi: 10.1080/04353676.2016.1257914

LÁSKA, K., BARTÁK, M., HÁJEK, J., PROŠEK, P. and BOHUSLAVOVÁ, O. (2011): Climatic and ecological characteristics of deglaciated area of James Ross island, Antarctica, with a special respect to vegetation cover. *Czech Polar Reports*, 1: 49-62. doi: 10.5817/CPR2011-1-5

LICHTENTHALER, H. K., BUSCHMANN, C. and KNAPP, M. (2005): How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio R Fd of leaves with the PAM fluorometer. *Photosynthetica*, 43(3).

LORENZ, C., MELLE, B.H., ARENA, C. (2024): Where is the plant-space research going? An overview on the last two decades through bibliometric network multi-analysis. *Acta Astronautica*, 221: 26-33.

MAIWALD, V. A., KYUNGHWAN, K., VRAKKING, V. and ZEIDLER, C. (2023): From Antarctic prototype to ground test demonstrator for a lunar greenhouse. *Acta Astronautica*, 212: 246-260. doi: 10.1016/j.actaastro.2023.08.012

MASSA, G. D., WHEELER, R. M., MORROW, R. C. and LEVINE, H. G. (2016): Growth chambers on the international space station for large plants. *Acta Horticulturae*, 1134: 215-222. doi: 10.17660/ActaHortic.2016.1134.29

NITTA, K. (2005): The Mini-Earth facility and present status of habitation experiment program. *Advances in Space Research* (the official journal of the Committee on Space Research-COSPAR), 35(9): 1531-1538. doi: 10.1016/j.asr.2005.03.100

PANOVA, G. G., TEPLYAKOV, A. V., NOVAK, A. B., LEVINSKIKH, M. A., UDALOVA, O. R., MIRSKAYA, G. V., KHOMYAKOV, Y. V., SHVED, D. M., ILYIN, E. A., KULESHOVA, T. E., KANASH, E. V. and CHESNOKOV, Y. V. (2023): Growth and Development of leaf vegetable crops under conditions of the phytotechnical complex in Antarctica. *Agronomy* (*Basel*), 13(12): 3038. doi: 10.3390/agronomy13123038

POULET, L., ZEIDLER, C., BUNCHEK, J., ZABEL, P., VRAKKING, V., SCHUBERT, D., MASSA, G. and WHEELER, R. (2021): Crew time in a space greenhouse using data from analog missions and Veggie. *Life Sciences in Space Research*, 31: 101-112. doi: 10.1016/j.lssr.2021.08.002

PRZEMIENIECKI, S. W., KALISZ, B., KATZER, J., WAMELINK, G. W. W., KOSEWSKA, O., KOSEWSKA, A., SOWIŃSKI, P. and MASTALERZ, J. (2024): Effect of vermicompost on rhizobiome and the growth of wheat on Martian regolith simulant. *The Science of the Total Environment*, 935: 173299. doi: 10.1016/j.scitotenv.2024.173299

QIN, J., JIANG, X., QIN, J., ZHAO, H., DAI, M., LIU, H. and CHEN, X. (2023): Effects of lead pollution on photosynthetic characteristics and chlorophyll fluorescence parameters of different populations of *Miscanthus floridulus*. *Processes*, 11: 1562. doi: 10.3390/pr11051562

RICKARD, CH. P., BODE, R. F. (2021): *Sporobolus airoides* as a Pioneer Plant for Lunar Regolith. 17th Earth and Space Conference, *Proceedings*, pp. 211–221.

ROHÁČEK, K. (2002): Chlorophyll fluorescence parameters: The definitions, photosynthetic meaning, nad mutual relationships. *Photosynthetica*, 40: 13-29.

SCHUBERT, D., BAMSEY, M., ZABEL, P., ZEIDLER, C. and VRAKKING, V. (2018): Status of the EDEN ISS Greenhouse after on-site installation in Antarctica. 48th International Conference on Environmental Systems 8-12 July 2018, Albuquerque, New Mexico. https://elib.dlr.de/121867/1/ICES-2018-140.pdf

TERAUDS, A., LEE, J. (2016): Antarctic biogeography revisited: Updating the Antarctic conservation biogeographic regions. *Diversity and Distributions*, 22: 836-840.

VAN LIPZIG, N. P. M., KING, J. C. and LACHLAN-COPE, T. A. (2004): Precipitation, sublimation, and snow drift in the Antarctic peninsula region from a regional atmospheric model. *Journal of Geophysical Research: Atmospheres*, 109: D24106. doi: 10.1029/2004JD004701

WANG, M., CHEN, D. and WANLIN, G. (2019): Evaluation of the growth, photosynthetic characteristics, antioxidant capacity, biomass yield and quality of tomato using aeroponics, hydroponics and porous tube-vermiculite systems in bio-regenerative life support systems. *Life Sciences in Space Research*, 22: 68-75. doi: 10.1016/j.lssr.2019.07.008

WHEELER, R. M. (2017): Agriculture for space: People and places paving the way. *Open Agric*, 2: 1401. doi: 10.1515/opag-2017-0002

YAO, Z.Y., FENG, J. and LIU, H. (2021): Bioweathering improvement of lunar soil simulant improves the cultivated wheat's seedling length. *Acta Astronautica*, 193: 1-8. doi: 10.1016/j.actaastro.2021.12.055.

ZABEL, P., ZEIDLER, C., VRAKKING, V., DORN, M. and SCHUBERT, D. (2020): Biomass production of the EDEN ISS Space Greenhouse in Antarctica during the 2018 experiment phase. *Frontiers in Plant Science*, 11: 656. doi: 10.3389/fpls.2020.00656

ZABEL, P., BAMSEY, M., ZEIDLER, C., VRAKKING, V., JOHANNES, B., RETTBERG, P., SCHUBERT, D., ROMBERG, O., IMHOF, B., DAVENPORT, R., HOHENEDER, W., WACLAVICEK, R., GILBERT, C., HOGLE, M., BATTISTELLI, A., STEFANONI, W., MOSCATELLO, S., PROIETTI, S., SANTI, G., NAZZARO, F., FRATIANNI, F., COPPOLA, R., DIXON, M.A., STASIAK, M., KOHLBERG, E., MENGEDOHT, D., BUCCHIERI, L., MAZZOLENI, E., FETTER, V., HUMMEL, T., BOSCHERI, G., MASSOBRIO, F., LAMANTEA, M., LOBASCIO, C., PETRINI, A., ADAMI, M., BONZANO, G., FIORE, L., DUECK, T.A., STANGHELLINI, C., BOCHENEK, G., GILLEY, A., MCKEON-BENNETT, M.M., STUTTE, G.W., LARKIN, T., MOANE, S., MURRAY, P., DOWNEY, P.J., FORTEZZA, R. and CERIELLO, A. (2015): Introducing EDEN ISS - A European project on advancing plant cultivation technologies and operations. doi: 10.5281/ZENODO.34391

ZEIDLER, C., ZABEL, P., VRAKKING, V., DORN, M., BAMSEY, M., SCHUBERT, D., CERIELLO, A., FORTEZZA, R., DE SIMONE, D., STANGHELLINI, C., KEMPKES, F., MEINEN, E., MENCARELLI, A., SWINKELS, G. J., PAUL, A. L. and FERL, R. J. (2019): The plant health monitoring system of the EDEN ISS space greenhouse in Antarctica during the 2018 experiment phase. *Frontiers in Plant Science*, 10: 1457. doi: 10.3389/fpls.2019.01457

ZEIDLER, C., WOECKNER, G., SCHÖNING, J., VRAKKING, V., ZABEL, P., DORN, M., SCHUBERT, D., STECKELBERG, B. and STAKEMANN, J. (2021): Crew time and workload in the EDEN ISS greenhouse in Antarctica. *Life Sciences in Space Research*, 31: 131-149. doi: 10.1016/j.lssr.2021.06.003

ZHAO, Y., LUO, R., ZHANG, H., YUAN, L., FANG, X., TONG, X., QIAN, Y., ZHOU, Z., YANG, Y., ZENG, X., LI, J., XU, X., XIE, Q., GONG, B. and GUO, J. (2024): The effects of simulated Martian regolith on Arabidopsis growth, circadian rhythms and rhizosphere microbiota. *Plant and Soil*, doi: 10.1007/s11104-024-06970-7

ZVĚŘINA, O., LÁSKA, K., CERVENKA, R., KUTA, J., COUFALÍK, P. and KOMÁREK, J. (2014): Analysis of mercury and other heavy metals accumulated in lichen *Usnea antarctica* from James Ross Island, Antarctica. *Environmental Monitoring and Assessment*, 186(12): 9089-9100. doi: 10.1007/s10661-014-4068-z

Web sources / Other sources

[1] Luke Jerram experiments
https://blog.hmns.org/2020/07/science-in-action-Martian-farming/

[2] EVONIK
https://corporate.evonik.cn/en/growing-vegetables-in-permanent-ice-568.html