Ionic Silver affects Lettuce Growth, Nutrient Uptake, Microbiome and Metabolome in a Hydroponics System

*LaShelle Spencer [1], Blake Costine [2], Tesia Irwin [3], Anirudha Dixit [2], Cory Spern [1], Angie Diaz [4], Trent Smith [5], and Aubrie O'Rourke [5],**

[1] Noetic Strategies, Inc., LASSO II, Kennedy Space Center, FL 32899, USA; lashelle.e.spencer@nasa.gov; cory.j.spern@nasa.gov; christina.l.khodadad-1@nasa.gov, [2] Aetos Systems Inc., LASSO II, Kennedy Space Center, FL 32899, USA; blake.m.costine@nasa.gov; anirudha.dixit@nasa.gov, [3] Astrion, LASSO II, Kennedy Space Center, FL 32899, USA; tesia.d.irwin@nasa.gov; wenyan.li-1@nasa.gov, [4] Bennett Aerospace, LASSO II, Kennedy Space Center, FL 32899, USA; angie.m.diaz@nasa.gov, [5] NASA Exploration Research and Technology, Kennedy Space Center, FL 32899, USA; trent.m.smith@nasa.gov;
* Correspondence: aubrie.e.orourke@nasa.gov

Background

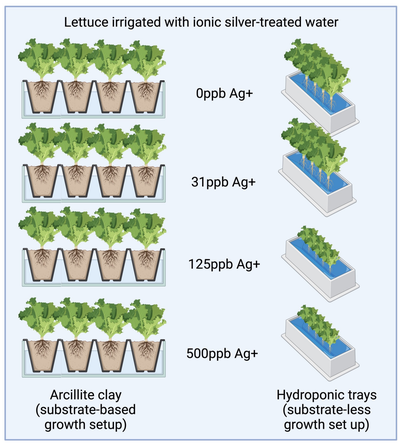
Spacecraft may utilize controlled-release silver technology in potable water systems. Ionic silver (Ag^+) is being investigated as a residual biocide for use in NASA spacecraft potable water systems on future crewed missions. Currently, plant chambers such as the Advanced Plant Habitat on the International Space Station recycle their own transpired water but require potable water to prime their water tanks and plumbing, and for water volume replenishment. This water will also be used to irrigate future spaceflight crop production systems; however, imparted silver ions are not planned to be removed from the potable water effluent. We recently published on the evaluation of ionic silver levels on the growth, plant nutrient content, and root zone microbiome of the space crop, *Lactuca sativa* cv. 'Outredgeous' lettuce. Prior to that work, there were no established guidelines for its use in the space crop production setting.

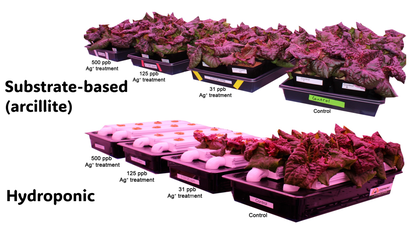


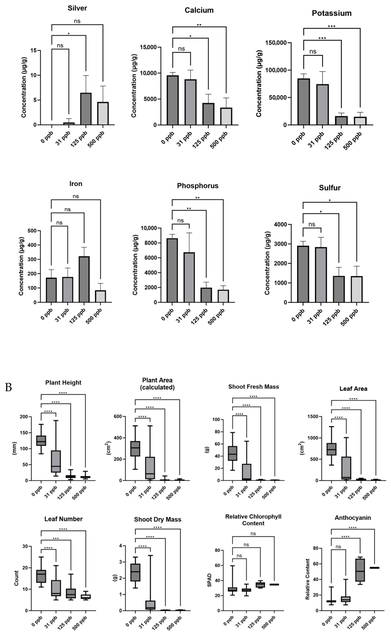


Introduction

Our work in *Lactuca sativa* cv. 'Outredgeous' lettuce determined that in a substrate-based system such as arcillite, lettuce plant growth, nutrient content, microbial diversity, and abundance are not affected by irrigation water with as high as 500 ppb ionic silver (Spencer *et al.* 2024). However, in a hydroponics system, lettuce plants exposed to silver-treated water showed reduced growth for shoots at 31 ppb silver and severely stunted growth at 125 and 500 ppb. Lettuce leaves from the hydroponic system showed the accumulation of silver and a reduced uptake of other essential elements as the silver concentration increased. It was also observed that the number of microbes associated with roots and water from the growth trays of the hydroponic system increased as the concentration of silver increased. In our present study, we investigate the metabolome of the shoots and the roots of the lettuce plants grown under the three silver concentrations to understand what metabolites are altered in the plant and what metabolites may be guiding this plant-microbial interaction.





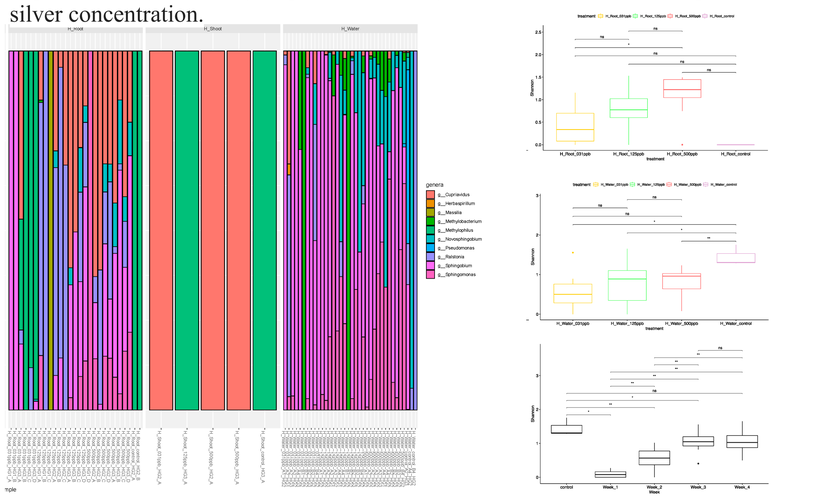


Results

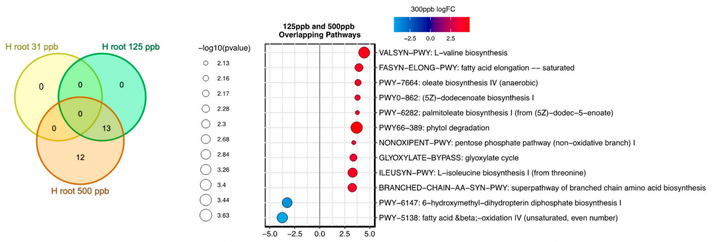
- Using a hydroponics system, lettuce plants exposed to silver-treated water showed reduced growth for shoots at 31 ppb silver and severely stunted growth at 125 and 500 ppb.

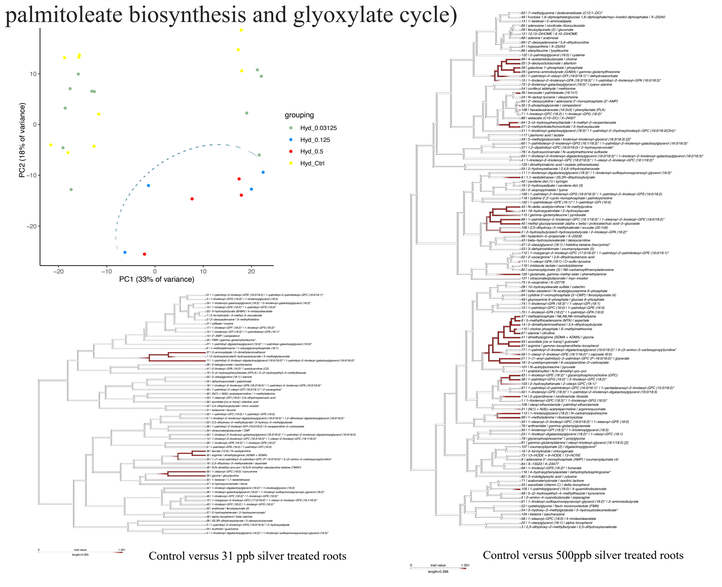


- The 125 ppb and 500 ppb ionic silver treatments displayed representatives of *Shingomonas*, *Sphingobium*, *Ralstonia*, *Novoshingobium*, and *Cupriavidus* in the roots and water of the hydroponic water tray but in different relative abundances.
- Shannon diversity for roots is not significantly different among treated and control but is significant for hydroponic water samples.
- Alpha diversity of the hydroponic water tray increases over time irrespective of silver concentration.



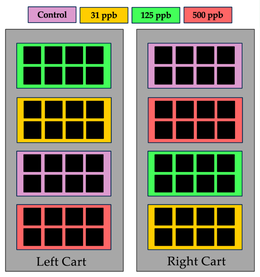
- **Metagenomics:** Thirteen differentially enriched pathways for the 125ppb silver-treated hydroponic root samples overlap with the differentially enriched pathways of the 500 ppb silver-treated hydroponic root samples when compared to the control hydroponic root samples.
- **Metabolomics:** PCA of total plant/microbe metabolites cluster according to control/31 ppb and 125/500 ppb silver water exposure. Upregulated metabolites illustrate the expression of enriched gene pathways found in metagenomics analysis. (e.g. palmitoleate biosynthesis and glyoxylate cycle)





Methods: Horticulture

Lettuce (*Lactuca sativa* cv. 'Outredgeous') was grown in environmental growth chambers (Percival PGW-48, Perry, IA, USA) located in the Space Station Processing Facility at NASA Kennedy Space Center, Florida (located at sea level with an atmospheric pressure of ~101 kPa). The chamber air temperature, relative humidity (RH), and CO_2 levels were maintained at 23 °C, 50%, and 3000 ppm, respectively, for all tests. These set points were selected to simulate an average environment of the ISS. Lighting was provided by overhead light-emitting diode (LED) grow lights (Percival, Perry, IA, USA). A dilution series of ionic silver was selected to encompass: (1) the highest possible dose (500 ppb) to dispense from the potable water system; (2) a dose (~125 ppb) close to the acceptable drinking water dose limits of silver, which is 100 ppb; and (3) the lowest dose (31 ppb) to represent a dose thought to have next to no adverse effects in a substrate-based system as reported in the literature.



Treatments included one control and three dilutions of Ag^+ residual biocide water (0 ppb/control, 31 ppb, 125 ppb, and 500 ppb). Each treatment was applied to two Nutrient Film Technique (NFT) hydroponic systems (each containing 8 plants) providing n = 16 technical replicates. Trays/systems were randomly positioned to minimize chamber effects. Each experiment was repeated with three grow outs to obtain a biological replication of three.

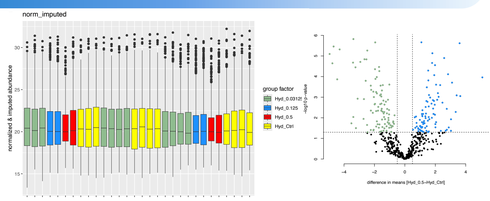
Arcillite was autoclaved and amended with 7.5 g/L T70 16-6-8 Nutricote controlled-release fertilizer. Pots were manually irrigated and checked daily. The hydroponic system water contained 0.5 × Hoagland's/Arnon solution. Each system had pH and electrical conductivity (EC) checked and adjusted manually daily. Nutrient solution changeouts were completed weekly. Plant samples were harvest after 28 days of growth.

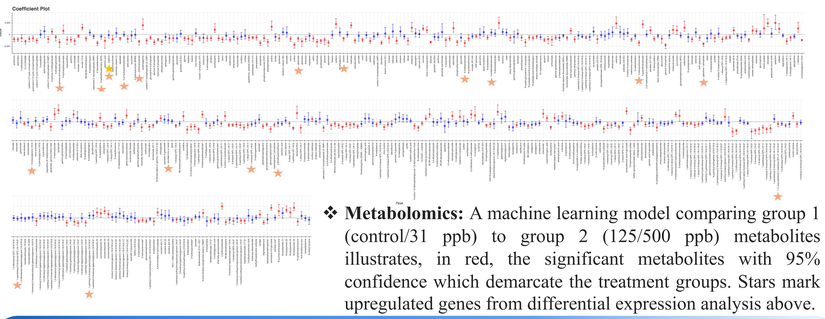
Methods: Molecular

Following DNA isolation, 1 µg of each sample was processed using NEBNext® Microbiome DNA Enrichment Kit (New England Biolabs, Ipswich, MA, USA) following the manufacturer's recommendations. Microbial enriched fractions were quantified using a Qubit dsDNA HS kit (Invitrogen, Carlsbad, CA, USA). Shotgun metagenomic sequencing libraries were prepared using an Illumina DNA Prep library preparation kit with unique dual indexes via IDT for Illumina DNA/RNA UD Indexes (Illumina Inc., San Diego, CA, USA). Libraries were sequenced as paired-end 150 bp sequences on a NextSeq 1000 instrument (V1.5.0.42699) at Kennedy Space Center. MetaPhlAn3 was used to provide the relative abundances of microbial genera within the samples by mapping the metagenomic forward and reverse reads to a set of clade-specific marker sequences representing bacterial and archaeal phylogenies. The MetaPhlAn3 output also provided a breakdown of the genomic pathways associated with 126 metagenomic samples with associated pathway abundances.

Methods: Chemical

ICP-OES instrument (Thermo Fisher Scientific iCAP 7000, Waltham, MA, USA) was used for elemental analysis of lettuce leaves. Metabolomic analysis was carried out by Metabolon for lettuce roots and output was visualized using MetaboDiff: an R package for differential metabolomic analysis and 'MetabWorkflow' from CIMCB.





- **Metabolomics:** A machine learning model comparing group 1 (control/31 ppb) to group 2 (125/500 ppb) metabolites illustrates, in red, the significant metabolites with 95% confidence which demarcate the treatment groups. Stars mark upregulated genes from differential expression analysis above.

Discussion

The metagenomes of bacteria which colonized the roots of lettuce treated with 125 or 500 ppb ionic silver in a hydroponic growth bed exhibited an enrichment of gene pathways associated with palmitoleate biosynthesis and the glyoxylate cycle as compared to lettuce roots under the control treatment without ionic silver. The glyoxylate cycle is an anaplerotic pathway - meaning the cycle carries out chemical reactions to provide intermediates necessary for metabolism - of the tricarboxylic acid cycle (Ramírez-Trujillo *et al.* 2016). This results in the ability for bacteria to utilize acetate, fatty acids, or poly-β-hydroxybutyrate (PHB) as precursors to drive metabolism and generate carbohydrates. Non-targeted metabolomics of the same samples from the metagenomics study showed a corresponding upregulation of glyoxylate precursor metabolites. Among these are the intermediates of palmitoleate biosynthesis. Palmitoleate is the conjugate base- the form when an acid donates a proton- of palmitoleic acid. In the context of this study palmitoleate-related fatty acids may be protective or signaling for microbes that associate with lettuce roots under silver stress. Arginine also appears to have a role in this relationship either as a nitrogen or carbon source.

Acknowledgments

Thank you to NASA Science Technology Mission Directorate (STMD) for Center Innovation Funding (CIF) in fiscal year 2022 to carry out this work.

References

Spencer, L. *et al.* Substrate Matters: Ionic Silver Alters Lettuce Growth, Nutrient Uptake, and Root Microbiome in a Hydroponics System. *Microorganisms* **12**, 515 (2024).

Ramírez-Trujillo, J. A., Dunn, M. F., Suárez-Rodríguez, R. & Hernández-Lucas, I. The Sinorhizobium meliloti glyoxylate cycle enzyme isocitrate lyase (AceA) is required for the utilization of poly-β-hydroxybutyrate during carbon starvation. *Ann. Microbiol.* **66**, 921–924 (2016).