The Microbiology of Crops Grown in eXposed Root On-Orbit Test System (XROOTS) on ISS.

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Introduction

To test the eXposed Root On-Orbit Test System (XROOTS) on orbit, a series of four XROOTS demo grow-outs were performed on ISS. Samples were collected at three harvests from the 2nd grow-out (Harvest 2: radish and mizuna) and the 3rd grow-out (Harvest 3: dwarf wheat, lettuce, and Harvest 4: tomato, pea).

Harvest of radish (cv. Cherry Belle) and mizuna plants from second grow – Astronaut Samantha Cristoforetti.

Third and fourth grow–out: lettuce, wheat, tomato, pea.

Samples were returned with the Crew 4 on October 10th, 2022, and SpX-26 on January 11, 2023.

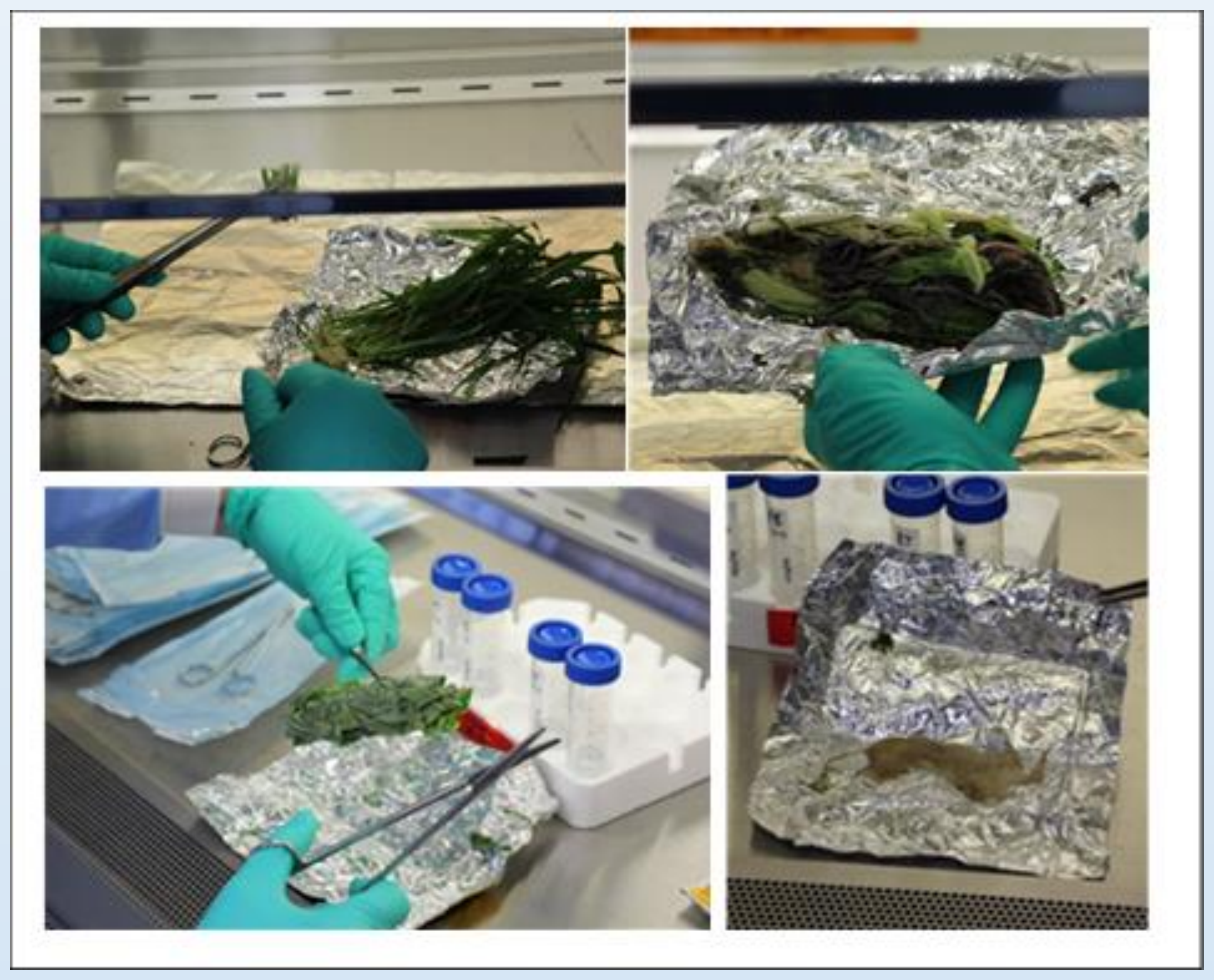
Objectives

The objectives for the analysis are listed below. This poster addresses results from the microbiological analysis.

- Identify microbial communities on plant samples, plant growth hardware surfaces, and in water samples.
- Screen for potential foodborne pathogens.

Methods

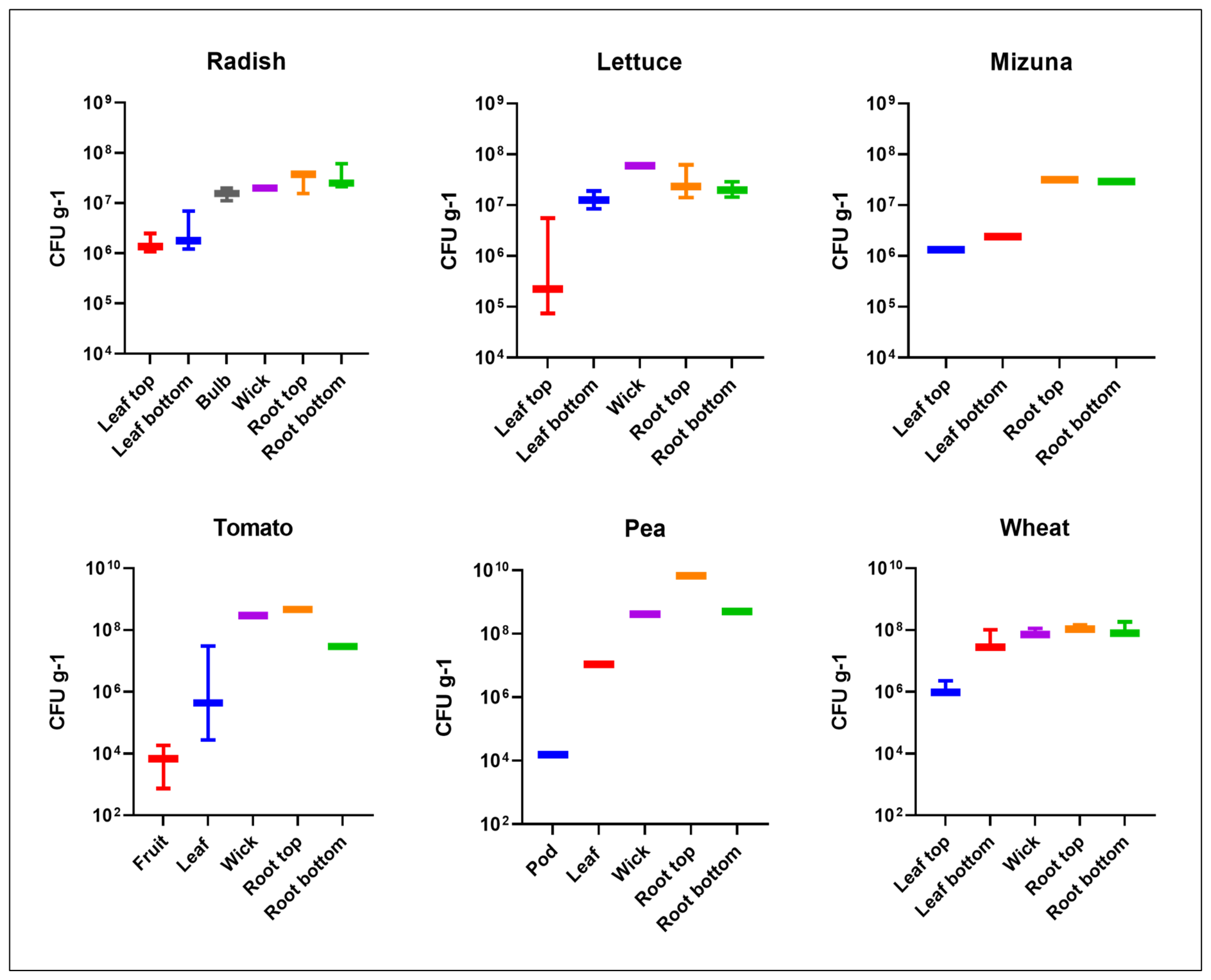
Frozen leaf and root samples were divided into proximal and distal sections to determine spatial differences in microbial load. Tomato fruit, pea, radish storage root and wick material were all processed in phosphate buffered saline before plating. All sample buffers from plant tissue, wicks, and swabs as well as water samples were diluted and plated onto trypticase soy agar (TSA) for bacteria and inhibitory mold agar (IMA) for fungi. Individual bacterial and fungal phenotypes were identified using Biolog Micro ID system or MicroSeq 16S rDNA and the MicroSeq D2 LSA rDNA using the ABI 3500 Genetic Analyzer Sequencing System. Petrifilms and *Salmonella* selective media were used to identify and enumerate *Escherichia coli*/coliform, *Staphylococcus aureus* and *Salmonella sp* for microbial food safety screening.



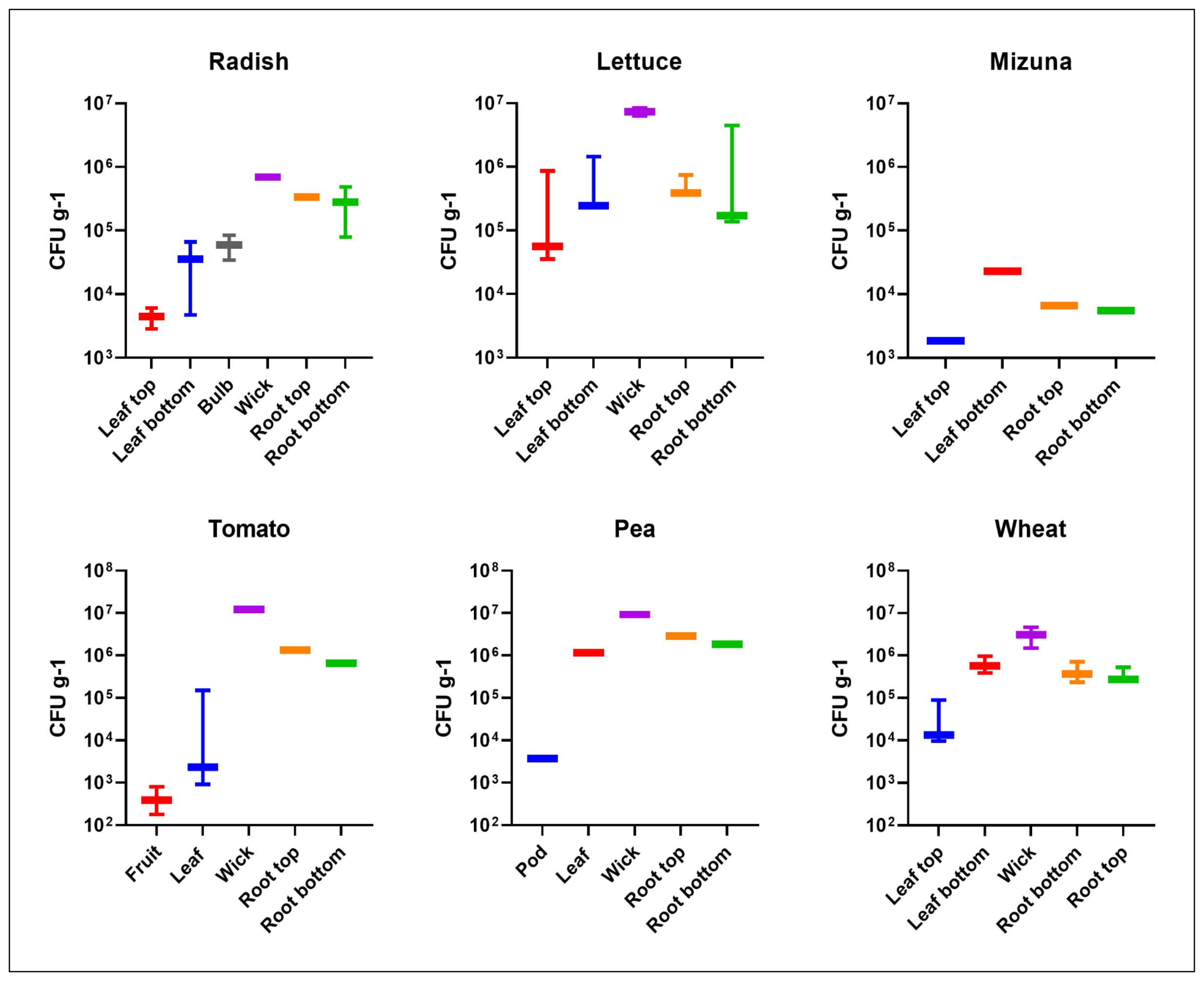
Leaf and root samples were divided into proximal and distal sections to determine spatial differences in microbial load.

Results

Bacterial counts (APC CFU/gram fresh weight)



Fungal counts (APC CFU/gram fresh weight)



Bacterial and fungal counts (CFU/g) on plant materials and wicking. Vertical bars indicate max and min values where more than one sample was available for each sample type.

Results

- The bacterial counts on the distal leaf sections were lower than the roots, leaf proximal section, and wicks in all samples. The tomato fruit and the pea pod had the lowest average counts.
- Microbial counts from the leaves harvested from XROOTS were not out of the range of counts found on the leaves from previous Veggie grown leafy greens (Khodadad et al, 2020, Hummerick et al, 2021).
- In addition, the wick samples analyzed from XROOTS showed similar trends as those from previous Veggie samples.
- Surface sample microbial counts from the root module (RM) were reduced 2.1 to 7.5-fold after cleaning with ProSan sanitizing wipes except for the RM 4 in Harvest 4.
- Fungal counts were also reduced 1.6 to 8.9-fold with the exception of RM 2 in Harvest 3 and RM 4 in Harvest 4, in which the counts remained similar after cleaning.
- Bacterial counts in irrigation water ranged from 65 CFU/mL to 3,800 CFU/mL, the highest from the third harvest.
- All screening tests for potential food borne pathogenic bacteria were negative. (Corroborated by community sequencing).
- Many of the isolates were found in all XROOTS sample sets, for example, the fungus *Fusarium.*
- Additionally, *Chryseobacterium*, *Pseudomonas*, *Rhodotorula*, *Sporothrix*, *Exophalia and Trichoderma* were found in most sample sets.
- *Fusarium solani* was identified in plants, water and swabs from harvests 3 and 4. *F. solani* can cause root rot in plants but is a common soil inhabitant.
- There may be some plant species dependent patterns. For example, for tomato and pea plant samples for the 4th harvest, the types of microbes are different especially for the leaf samples and the root bottom samples.
- From the swab samples collected from the 4th harvest, the microbial species located on the Veggie bellows tended to differ from those on the root module surfaces.

Conclusion

Microbial surveys of ISS grown plants and the associated hardware provide a fuller picture of the ISS microbiome that can be applied to future ISS missions and enable successful deep space crop production and plant research.

References

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