
# Microbiological profile of multi-species leafy greens grown simultaneously in the Veggie vegetable production systems on ISS

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The Veggie facility on ISS  was used to demonstrate the suitability of the leafy greens,  red romaine ‘Outredgeous’ lettuce, Waldman’s green lettuce, and mizuna in a mixed crop configuration (Figure 1).


*Figure 1. VEG 03 D, E, and F planting arrangement. W.G. = Waldman’s green lettuce, O.L= Outredgeous red romaine lettuce. Red font indicates pillows that were frozen and sent back for analysis.*

# Sample processing

*Table 1. Dates including plant, pillow and swab sample processing. Numbers in parentheses indicates which Veggie facility was used.*

| | Plant initiation | Harvest | Return | Plant analysis | Pillow analysis | Swab analysis |
|---|---|---|---|---|---|---|
| **Veg 03D (2)** | 9/27/17 | 11/23/17 | 5/18 and 10/18 | 5/14/18 | 7/10/18 | 6/29/18 |
| **Veg 03E (1)** | 2/6/18 | 4/6/18 | 10/18 | 10/4/18 | 11/27/18 | 11/19/18 |
| **Veg 03F (2)** | 2/9/18 | 4/9/18 | 10/18 | 10/4/18 | 11/27/18 | 11/19/18 |

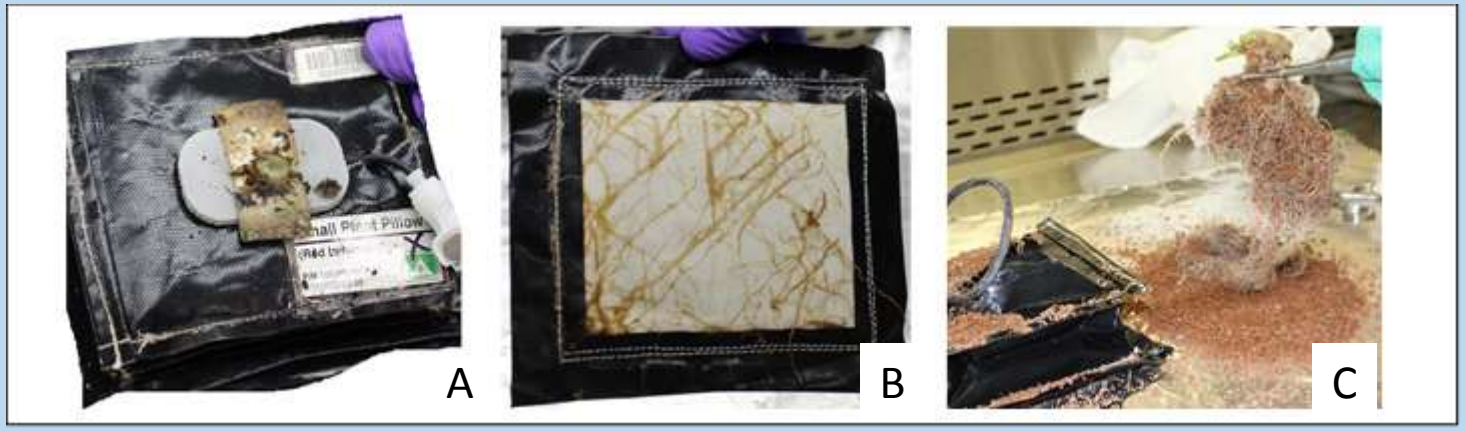




*Figure 2. Rooting pillow components and sample collection. A. Pillow used to grow red romaine lettuce, B. Nomex pillow bottom with roots. C. Removal of root sample. D. Plant tissue.*

# Microbiology Methods

- Post harvest:
- Samples taken from both ground and flight
  - Plant surfaces, Root zone
  - Wicks and Substrate

- Heterotrophic bacterial & fungal plate counts

- Media specific screening
  - *E. coli*
  - *Salmonella sp*
  - *Staphylococcus aureus*

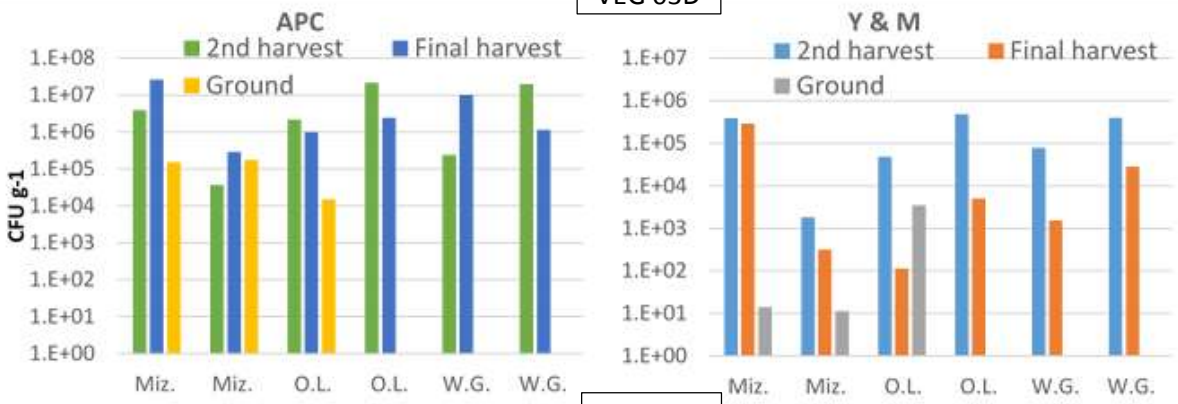
- Microbe Identification


Plant Leaves

Substrate & Root Zone

# Microbial counts: Leaves

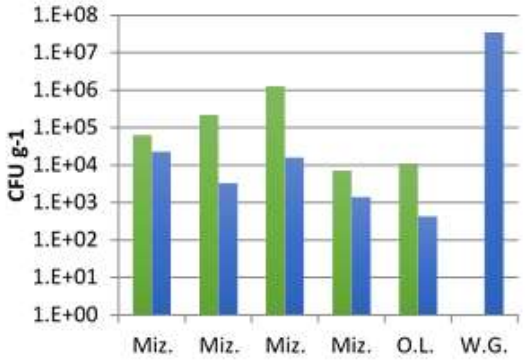
VEG 03D

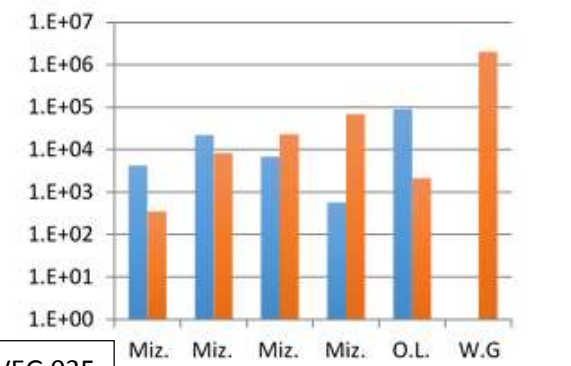




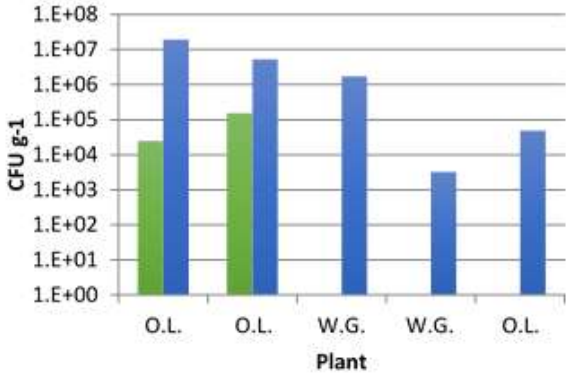


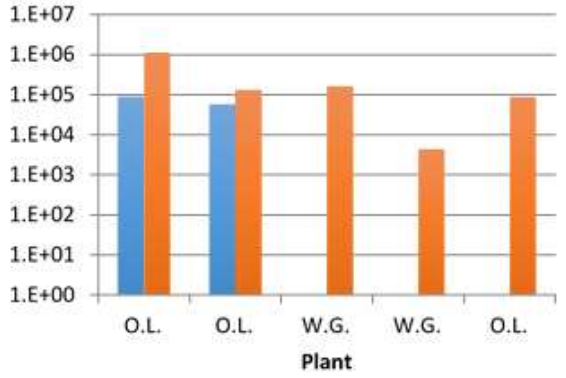
VEG 03E



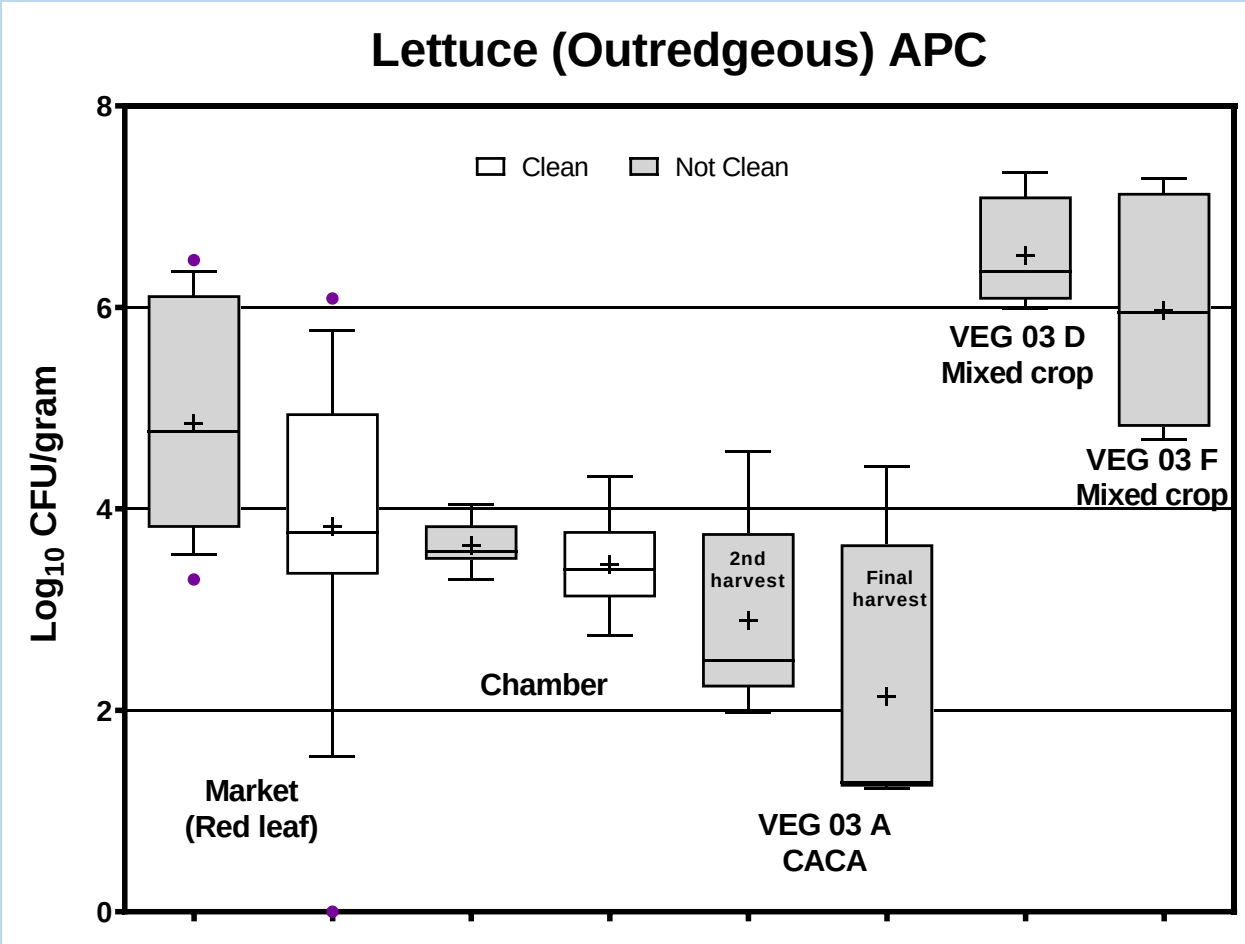

VEG 03F

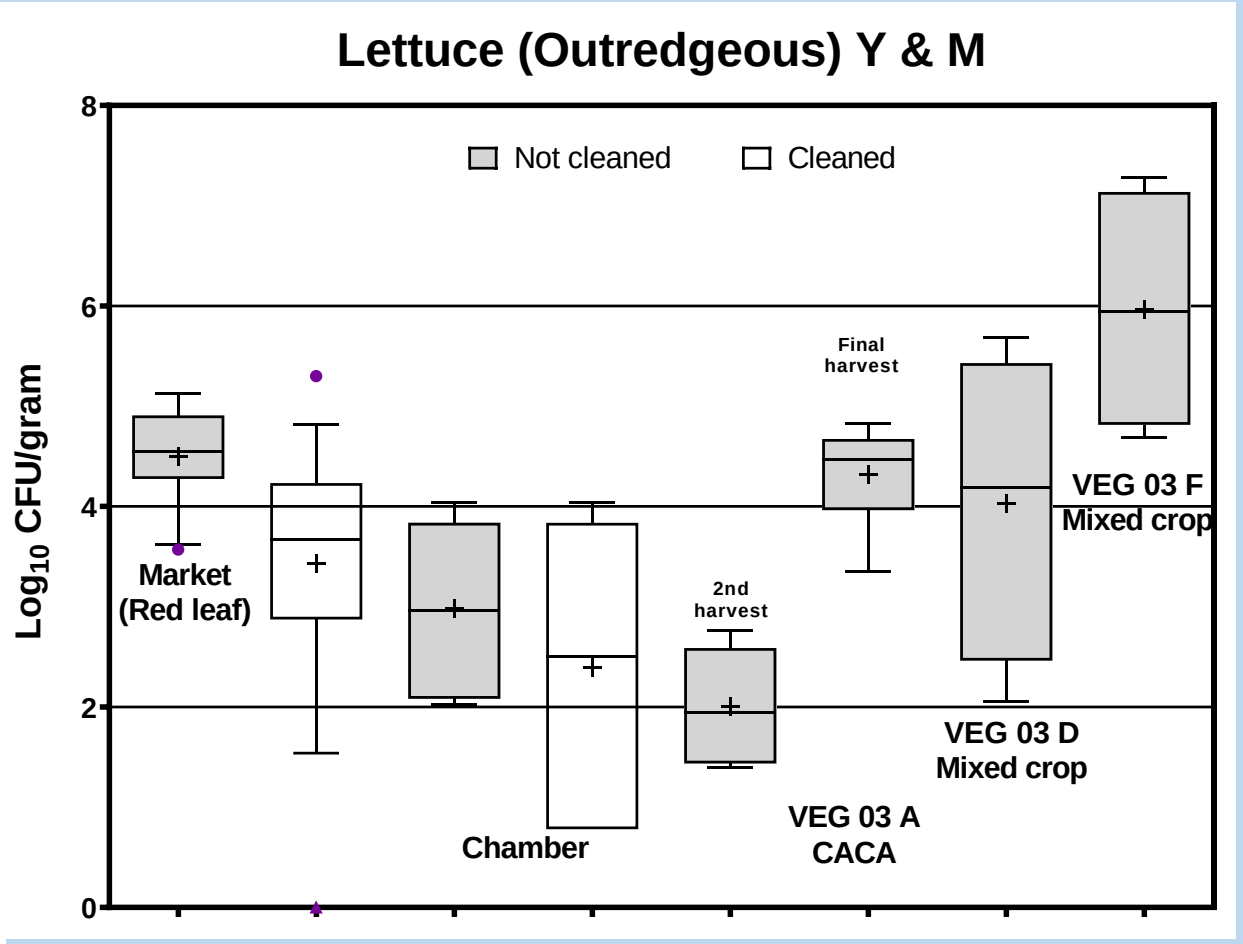



- Bacterial and fungal counts from VEG 03D exceeded current NASA food quality standards for APC (10,000 in any 2 samples) and yeast and mold (1,000 in any 2 samples) in 100% and 83% of the samples respectively.
- Samples from VEG 03 E and F also exceeded the standards, 50% exceeded APC from VEG 03E and 86% from VEG 03F. 82% of VEG 03E samples and 100% of VEG 03F samples exceeded the allowable number for yeast and mold.
- None of the pathogens screened for (*E. coli, S. aureus, Salmonella sp.* and *Aspergillus flavus)* were recovered from selective media.

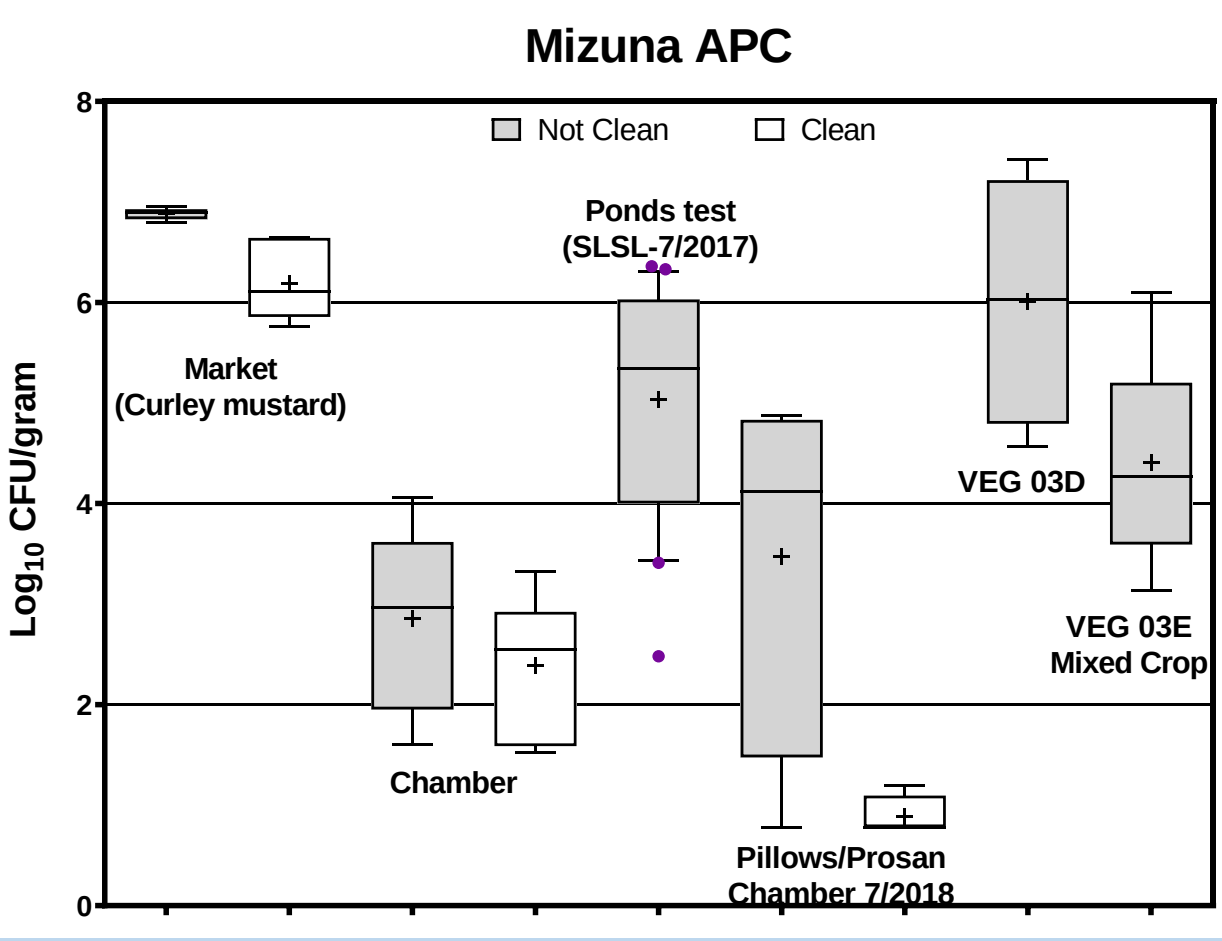
# Compared to……?

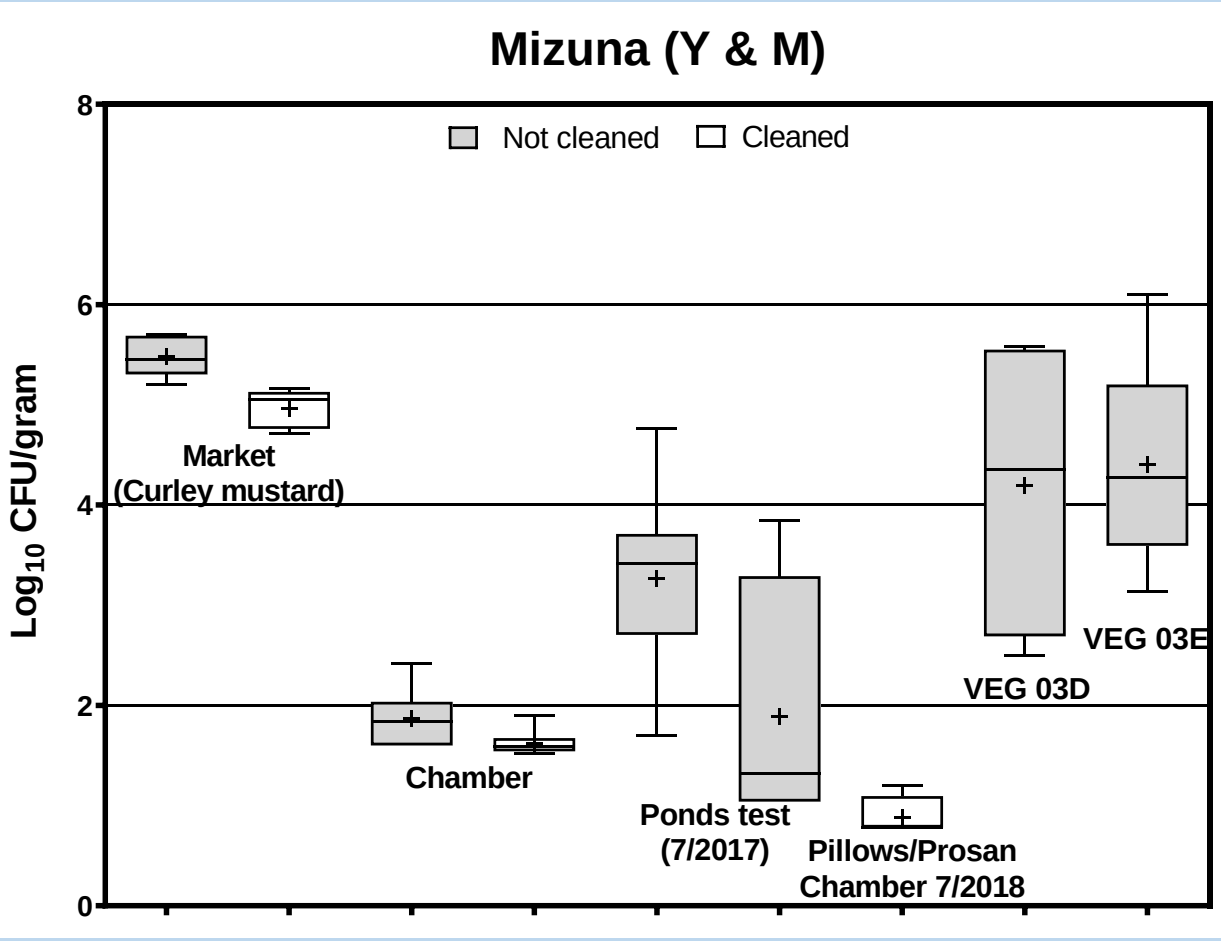



Box and whiskers (10-90 percentile) plots for aerobic plate counts and yeast and mold counts on outredgeous lettuce grown under a variety of conditions. Purple circles indicate outliers. Chamber=growth medium with timed release fertilizer/sub irrigation, ISS conditions. VEG 03 A,D,E,F=in veggie pillows on ISS. Veg 04 D, E and F were mixed crop.

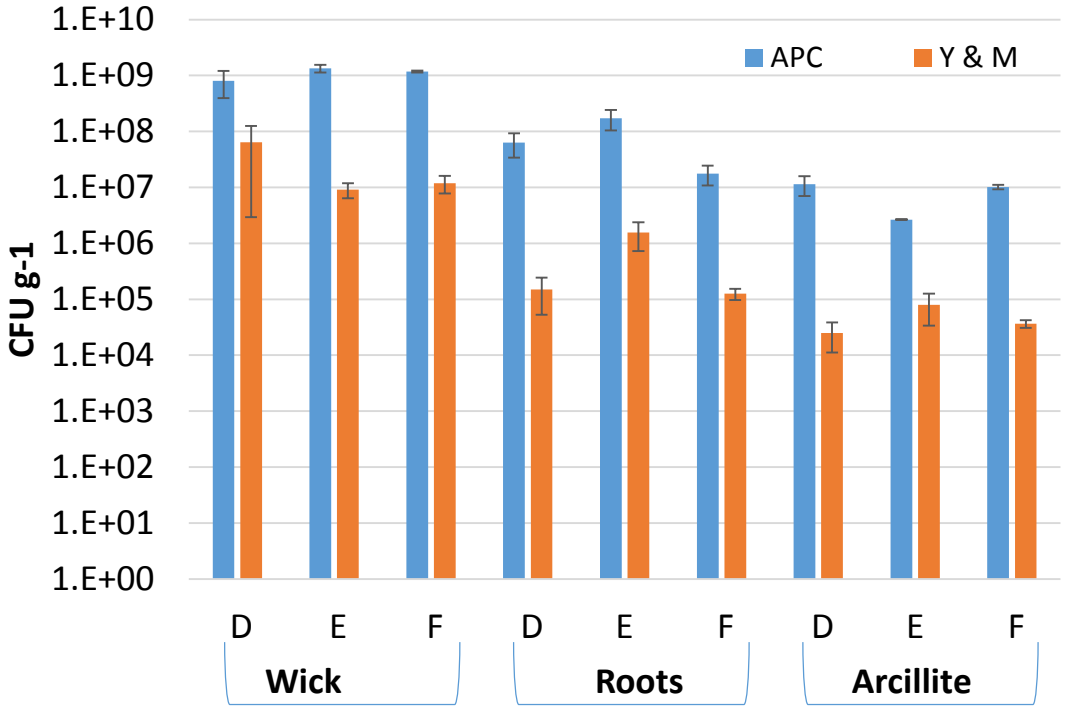
# Compared to……?



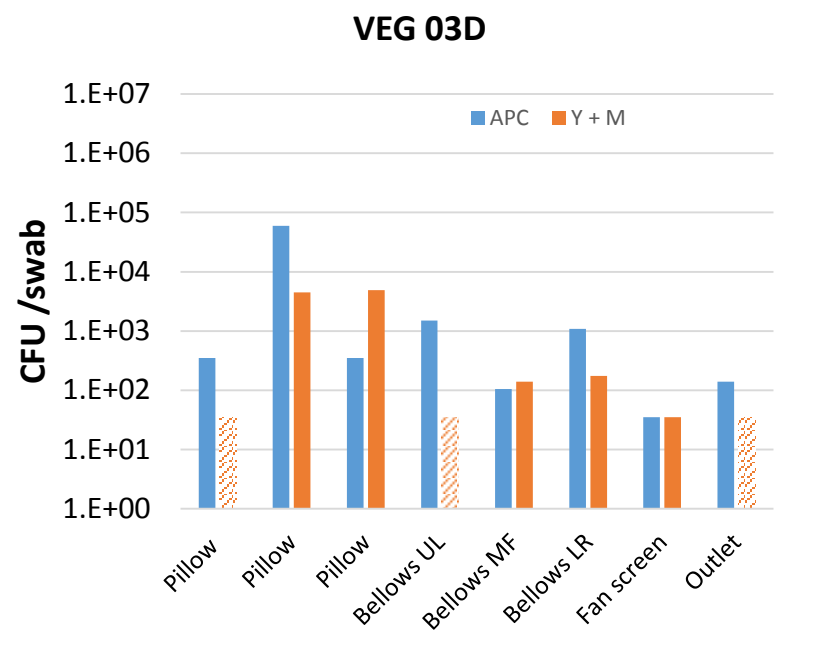

Box and whiskers (10-90 percentile) plots for aerobic plate counts and yeast and mold counts on mizuna grown under a variety of conditions. Purple circles indicate outliers. Chamber=growth medium with timed release fertilizer/sub irrigation, ISS conditions. VEG 03 D,E=in veggie pillows on ISS. Veg 04 D, E were mixed crop.

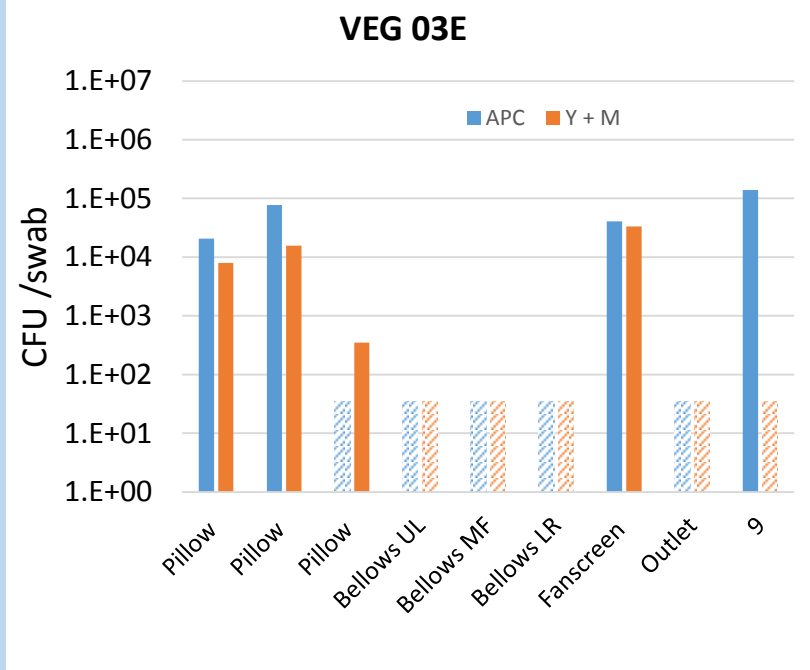
# Microbial counts: Pillows

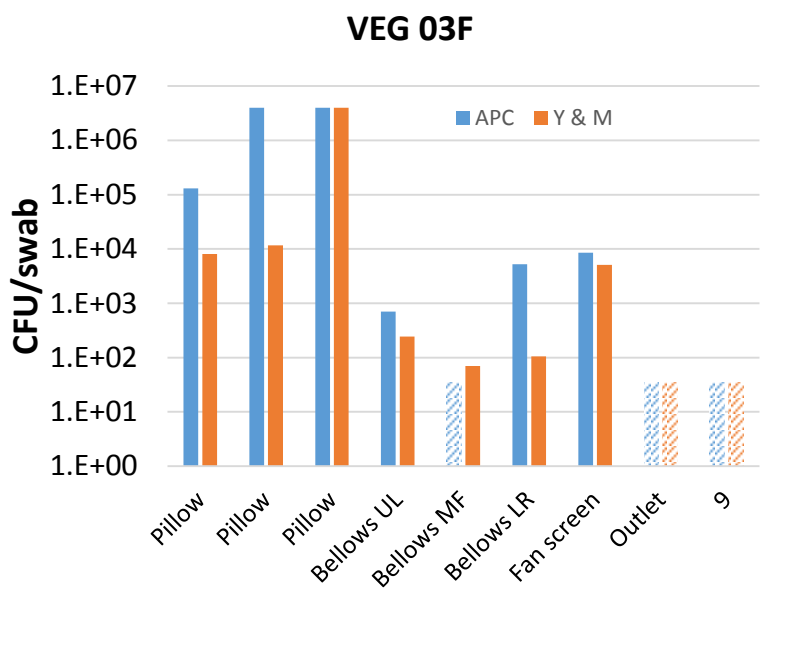



- Samples from pillows including wicking material, roots and arcillite showed lower bacterial counts in the arcillite from VEG 03E ($p$=0.02).
- Fungal counts were higher in the roots from the same pillows from Veg 03E.
- There is no difference in the counts on the wick.

# Microbial counts: Swabs




*Bacterial (APC) and fungal (Y & M) counts on surface samples from Veggie surfaces. Striped bars indicate detection limit and samples that are below detection limit.*

- Bellows samples were relatively low in counts from approximately 1,000 to below detection limit for fungi.
- Counts from the surface of the fan screen were highest from VEG 03E and pillow surfaces from VEG 03F had the highest counts of both bacteria and fungi.
- These surfaces are sampled after harvest but before the Veggie facility is wiped down with disinfectant wipes.

# Identified bacterial isolates.

| Bacteria | swabs |
|---|---|
| Bacillus pumilus | D |
| Bacillus altitudinis, | D |
| Bacillus pumilus strain PD4 | D |
| Bacillus subtilis | D |
| Chryseobacterium gregarium | E,F |
| Enterobacter hormaechei, | D |
| Fictibacillus arsenicus | F |
| Gardinella vaginalis | D |
| Microbacterium marytipicum | E,F |
| Nocardioidaceae | F |
| Novosphingobium capsulatum | E,F |
| Paenibacillus spp. | F |
| Paenibacillus harenae | E,F |
| Pantoea agglomerans | D |
| Pantoea vagans | D |
| Pseudomonas fulva | D,E,F |
| Pseudomonas tundra | F |
| Rhodococcus | E |
| Staphylococcus pseudintermedius | E,F |
| Staphylococcus saprophyticus | D,E,F |
| Staphylococcus sciuri | D |
| Staphylococcus sp | D |

| Bacteria | wick |
|---|---|
| Acidovorax avenae | F |
| Bacillus spp. | D |
| Brevundimonas vesiculans | F |
| Burkholderia sp | D,E,F |
| Burkholderia pyrocinnia | D,F |
| Burkholderia cepacia | D |
| Curtobacterium citreum | F |
| Flavomonas oqyzihabitans | D |
| Klebsiella pneumoniae ozaenae | F |
| Klebsiella pneumoniae pneumoniae | D,F |
| Leifsonia aquatica, | D,E,F |
| Methylobacterium rhodinum | D |
| Microbacterium sp | D,E,F |
| Paenibacillus pabuli | E,F |
| Pantoea sp. | F |
| Paracoccus yeei | F |
| Pseudomonas fulva | D |
| Rhizobium sp. | D,E |
| Staphylococcus saprophyticus | F |
| Staphylococcus sp | D |

| Bacteria | Arcillite |
|---|---|
| Amphibacillus tropicus | D |
| Bacillus spp. | D |
| Brevundimonas vesiculans | F |
| Burkholderia sp | D,F |
| Burkholderia pyrocinnia, | D,F |
| Burkholderia cepacia, | D |
| Clavibacter sp | D |
| Cupriavidis panculus | F |
| Flavomonas oqyzihabitans | D |
| Gordonia terrae | D |
| Leifsonia aquatica | D,E,F |
| Leifsonia shinshuensis | F |
| Methylobacterium spp. | D |
| Methylobacterium rhodinum | D |
| Microbacterium sp | D,E,F |
| Micrococcus sp | D |

| Bacteria | Roots |
|---|---|
| Burkholderia pyrocinnia, | D,F |
| Burkholderia cepacia, | D |
| Flavomonas oqyzihabitans | D |
| Leifsonia aquatica, | D |
| Methylobacterium rhodinum | D |
| Microbacterium sp | D,E,F |
| Rhizobium sp. | D,E |

| Bacteria | leaf |
|---|---|
| Achromobacter piechaudi | E,F |
| Amphibacillus tropicus | D |
| Burkholderia pyrocinnia | D |
| Chryseobacterium gleum | D |
| Curtobacterium flaccumfaciens | E,F |
| Klebsiella pneumoniae ozaenae | D |
| Klebsiella pneumoniae pneumoniae | D |
| Kocura palustris | D |
| Microbacterium marytipicum | D,E |
| Microbacterium sp | E |
| Microbacterium testaceum | D |
| Micrococcus sp | E |
| Paenibacillus xylanilyticus | E,F |
| Pantoea agglomerans | D |
| Pectobacterium cypripedii | D |
| Pseudomonas asplenii | E,F |
| Pseudomonas fulva | D,E |
| Staphylococcus aureus ss aureus | D,E,F |
| Staphylococcus epidermidus | D |
| Staphylococcus capitus | D |
| Staphylococcus pseudintermedius | E |
| Staphylococcus sp | D |

# Identified fungal isolates.

| Fungi | swabs | wick | roots | arcillite | plants |
|---|---|---|---|---|---|
| Aspergillus spp | D,E,F | D,E,F | D,E,F | D,E,F | D,E,F |
| Fusarium oxysporum | D,E,F | D,E,F | D,E.F | D,E,F | D,E,F |
| Penicillium spp | F | D,F | | D,E | D,E,F |
| Rhodotorula spp | D,F | D,E,F | D,E,F | D,E,F | D,E,F |
| Exophiala spp | | | | E | |
| Purpureocillium lilacinum | E,F | | | | |

# In Summary

- There was no correlation between repeated harvests and increased microbial counts on the leaves of leafy greens.
- Leaves from Veg 03 D,E and F exceed NASA APC limits for non-thermo-stabilized food.
- Screening for some human pathogens yielded negative results.
- A higher bacterial load was seen on Veg 03 D, E and F mizuna and lettuce leaves when compared to chamber grown and previous Veggie grown crops.
- This research provided valuable information for future food production-related research on the ISS, which will help to enable deep space food production capabilities for supporting crew health.

VEGGIE
Vegetable Production System

This work is funded by NASA space biology.