Evaluation of Low-Pressure Cold Plasma for Disinfection of ISS Grown Produce and Metallic Instrumentation

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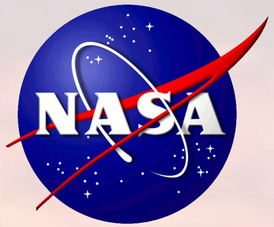
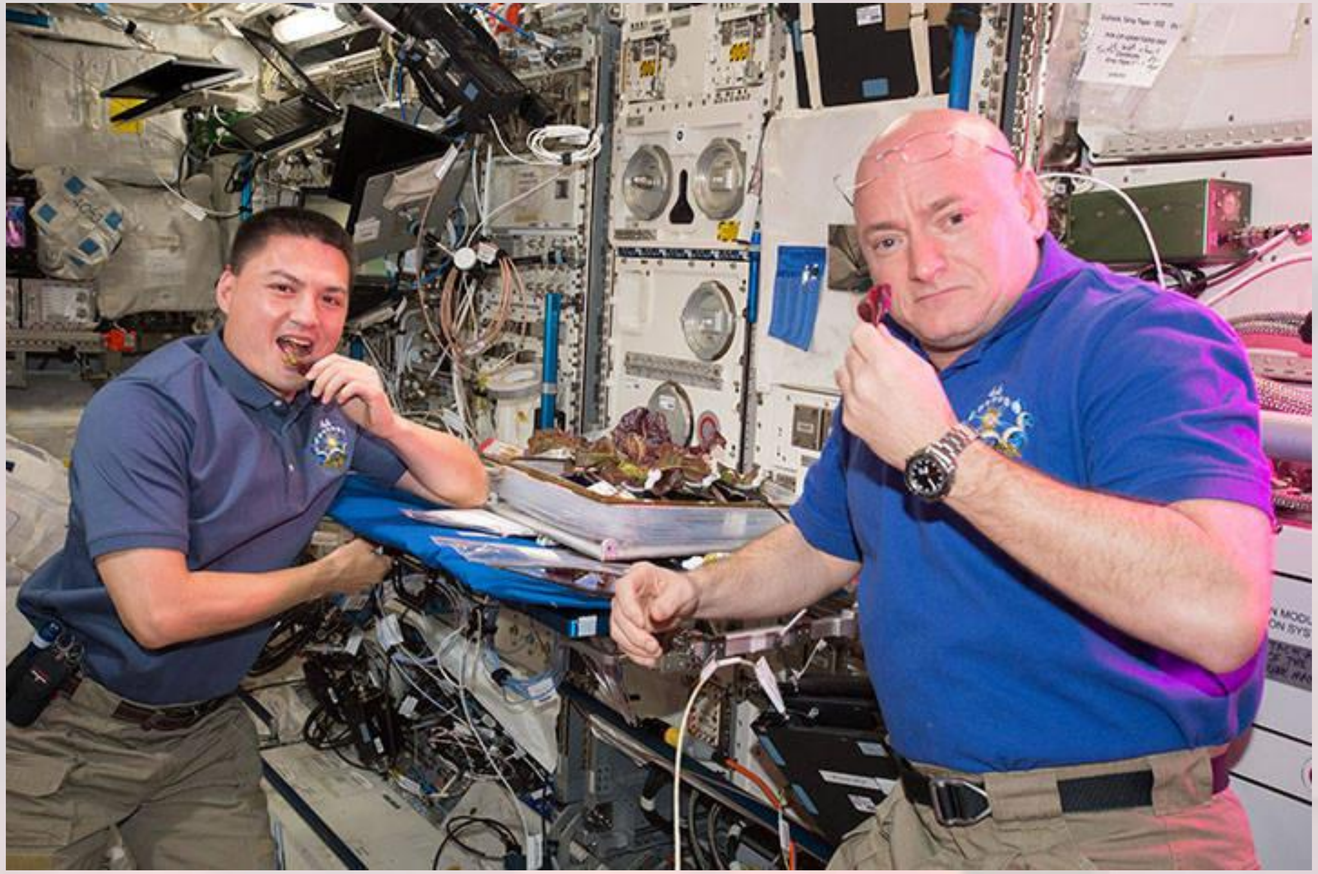


Microbes on ISS



NASA astronauts Scott Kelly and Kjell Lindgren take a bite of red romaine lettuce grown in Veggie. Photo credit NASA

- Food
- Instruments
- Potable water system
- Fluid delivery systems
- Surfaces

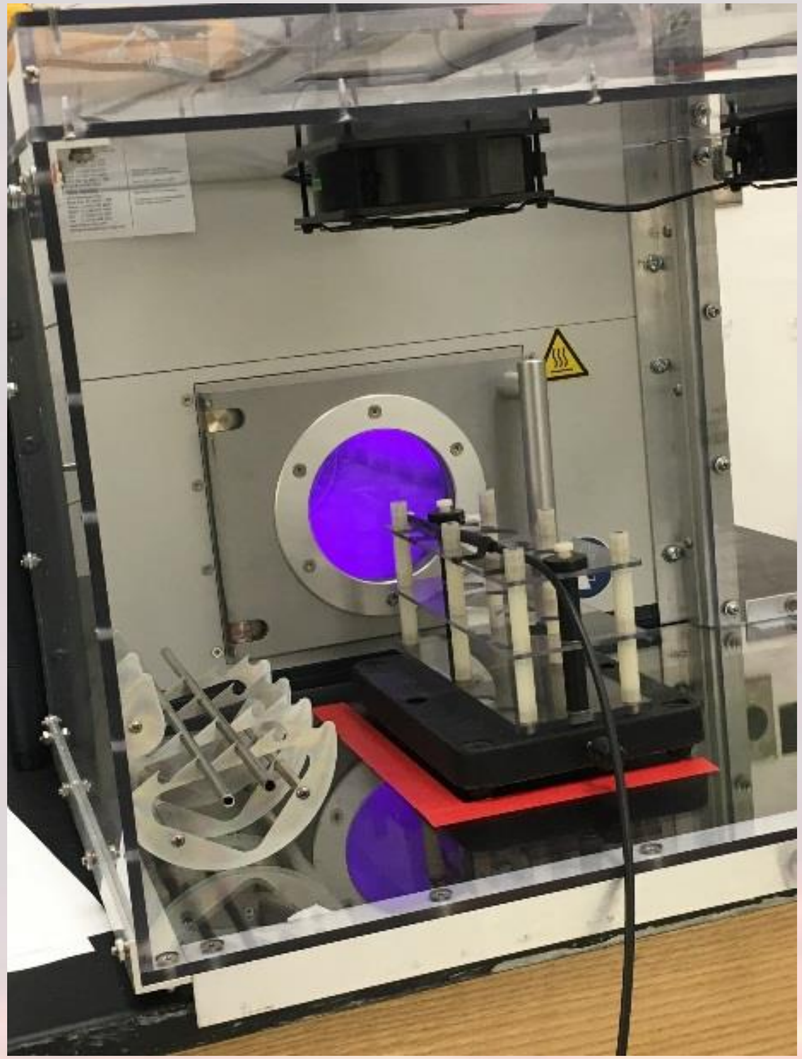
Disinfection using plasma

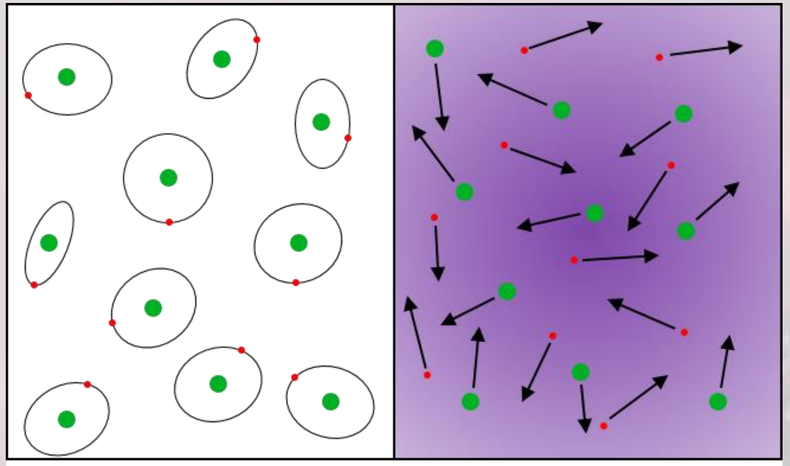
- Shown to be effective at precision cleaning aerospace hardware at Kennedy Space Center.
- Relatively new technology being investigated for disinfecting agricultural commodities and medical instruments.
- Plasma cleaning is a dry, non-thermal process, which can provide broad-spectrum antimicrobial activity.
- Microgravity compatible since cold plasma uses no liquids and is able to penetrate even the smallest cracks and crevices.

Objectives

- Determine plasma conditions, i.e. vacuum pressure and duration of plasma treatment and any effect on plant tissues.
- Evaluate the efficacy of plasma treatment for produce disinfection.
- Evaluate the efficacy of plasma treatment for disinfection/sterilization of solid items such as utensils and medical supplies.

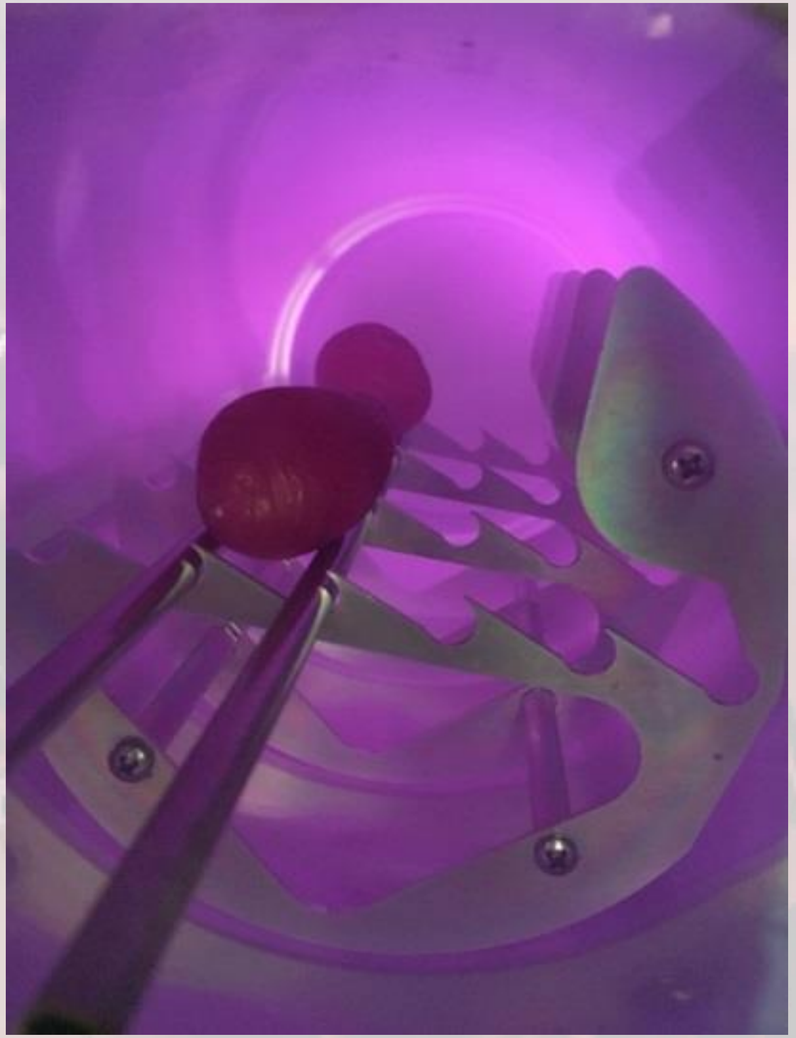
Background





Gas is excited by energy supplied in a vacuum.
Reactive species and UV are generated dependent on source gas.

- Oxidation of biomolecules
- Damage to DNA
- “Sandblasting” effect



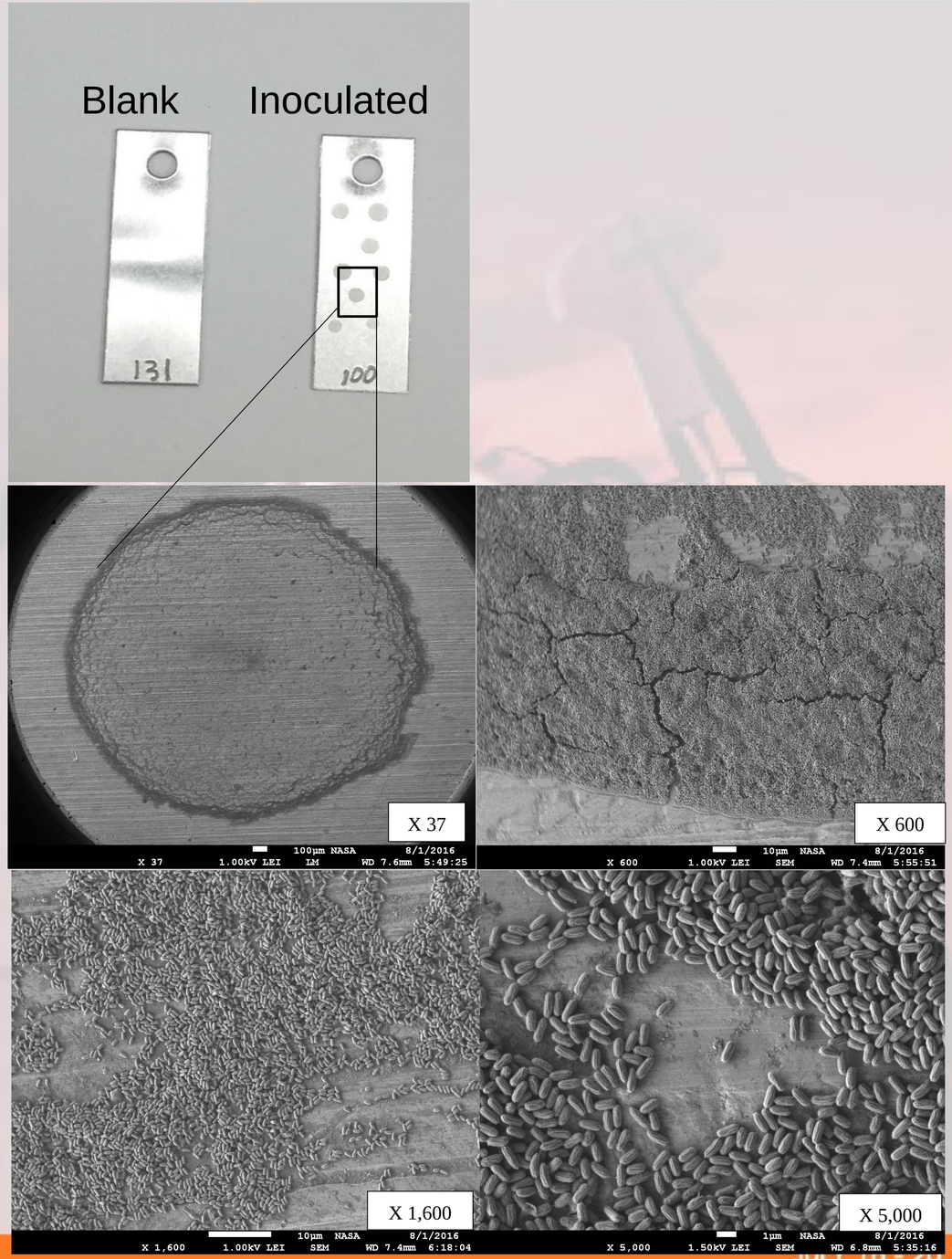
Optimization

- Conditions for metallic coupons were based on previous precision cleaning techniques.
- Moisture present in the produce required adjustments to the low pressure settings to be able to maintain plasma and the integrity of the item for the duration of testing

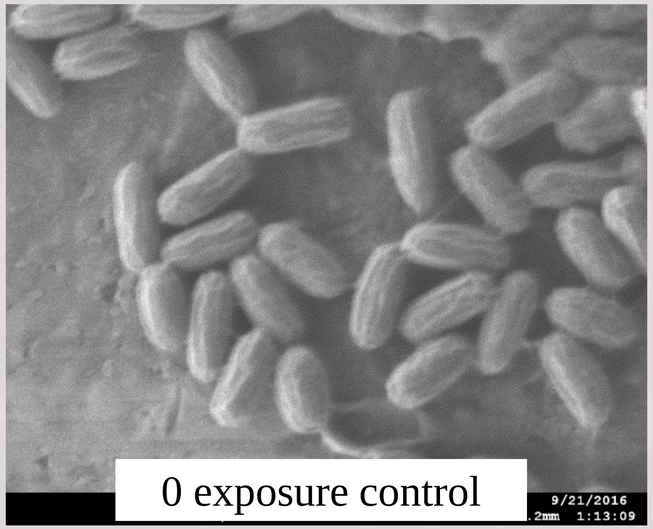
Item	Pressure (mbar)	Quantity/Run	Exposure Time (min)
Metallic Coupons	0.10	Up to 10	5, 10, 15, 30, 60
Cherry Tomatoes	0.60 and 0.80	5	5, 10, 15
Radishes	0.80	3	5, 10, 15
Peppers	0.80	2	5, 10, 15
Cabbage	Could not be determined	2	None

Metal Surfaces

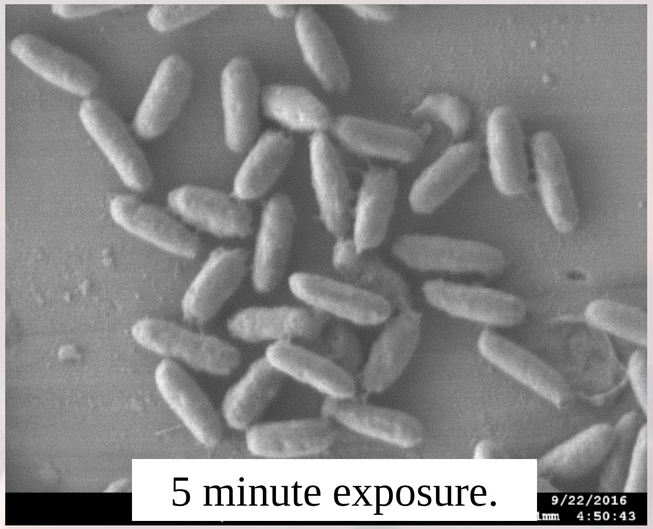
- Coupons were inoculated with ~10^7 *Bacillus pumilus* spores or *E. coli* cells per coupon.
- Coupons exposed to plasma at different exposure times, up to 60 min. Controls were subjected to low pressure only.
- Analysis done by:
  - ✓ Scanning electron microscopy (SEM) imaging (*B. pumilus*)
  - ✓ Calculation of log reduction using Most Probable Number technique (*B. pumilus*)
  - ✓ Plate counts (*E. coli*)



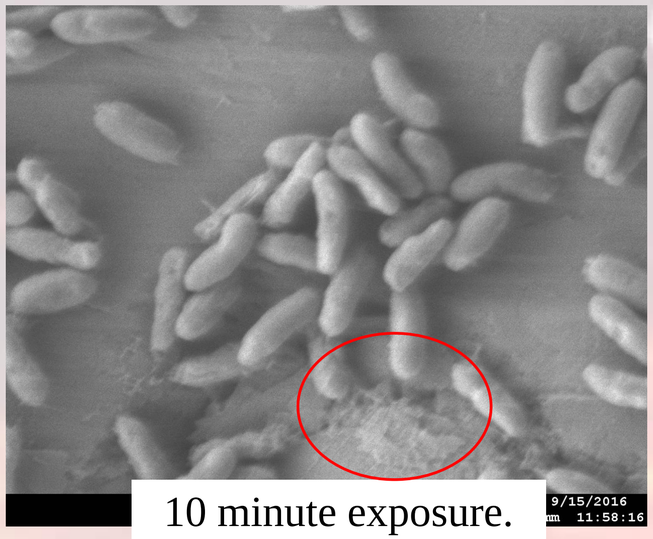
Results (SEM) Plasma treatments



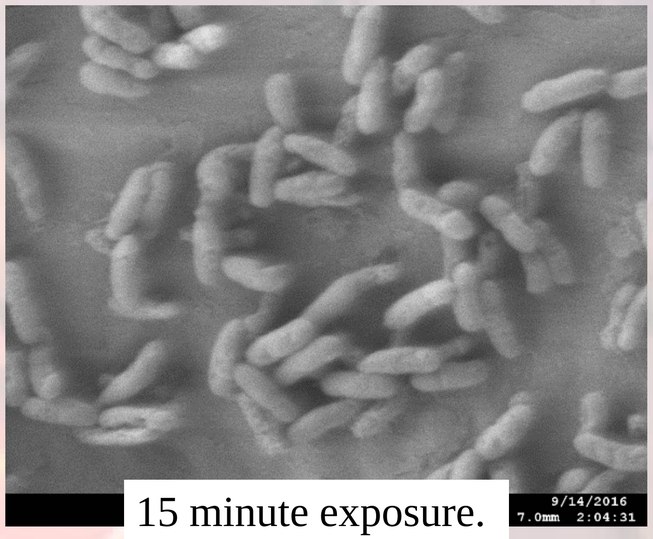
0 exposure control



5 minute exposure.



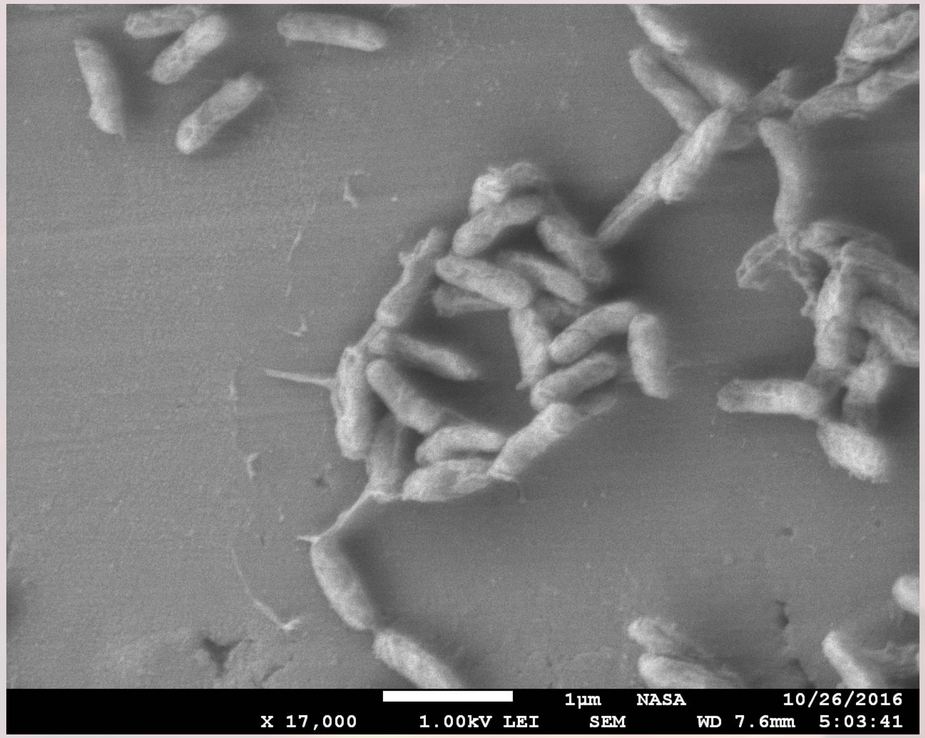
10 minute exposure.

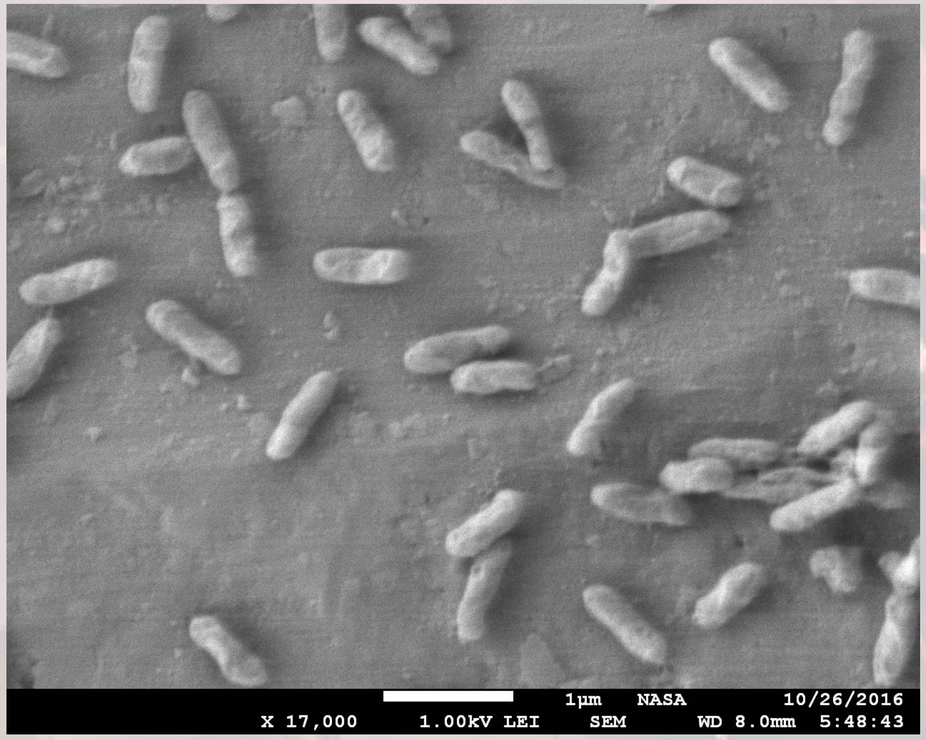


15 minute exposure.

- Smaller in size
- Surfaces are smooth and pitted. Spore coat proteins affected?
- Extracellular material evident after 10 and 15 min treatment.

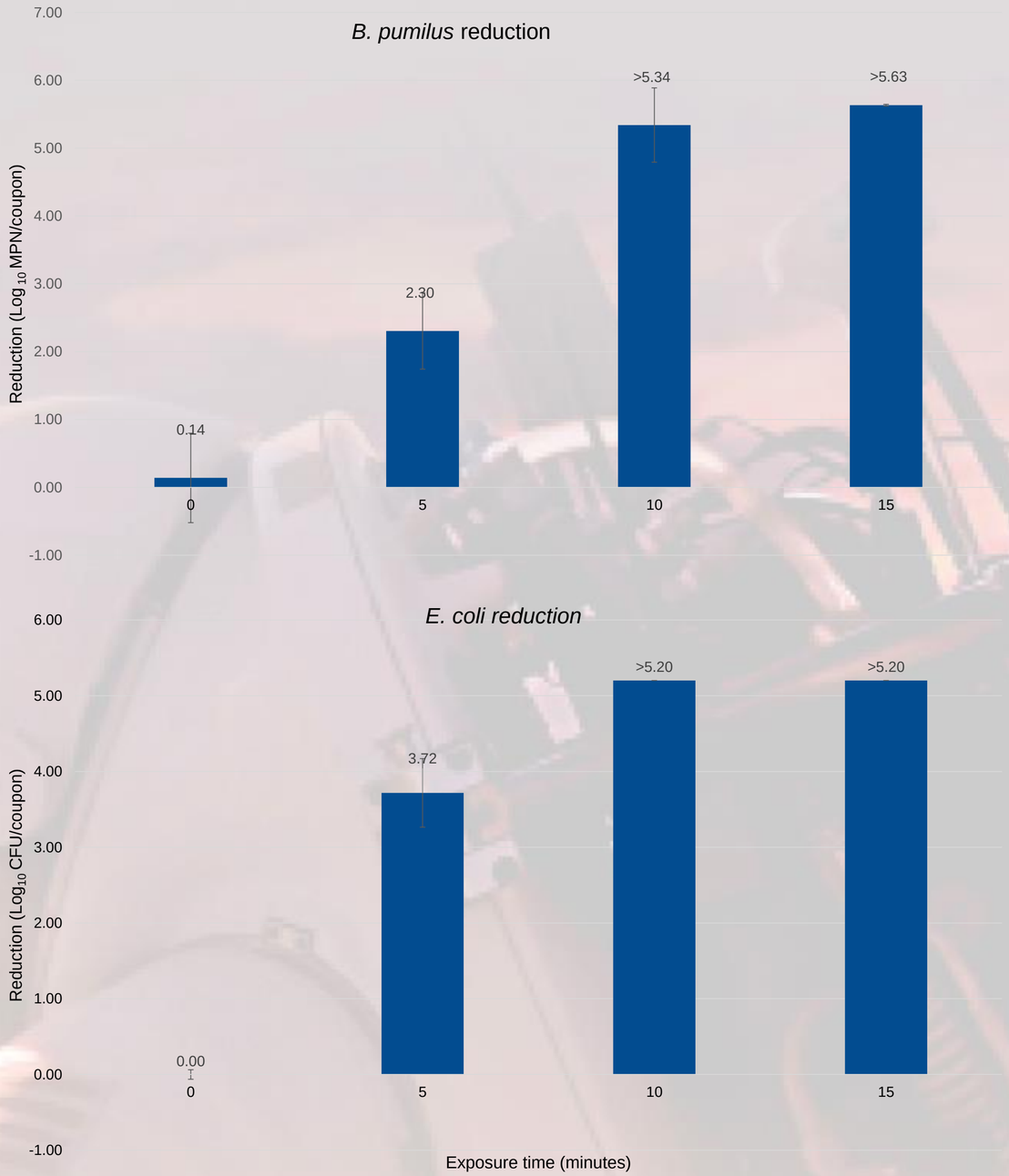
30 and 60 minute treatment.





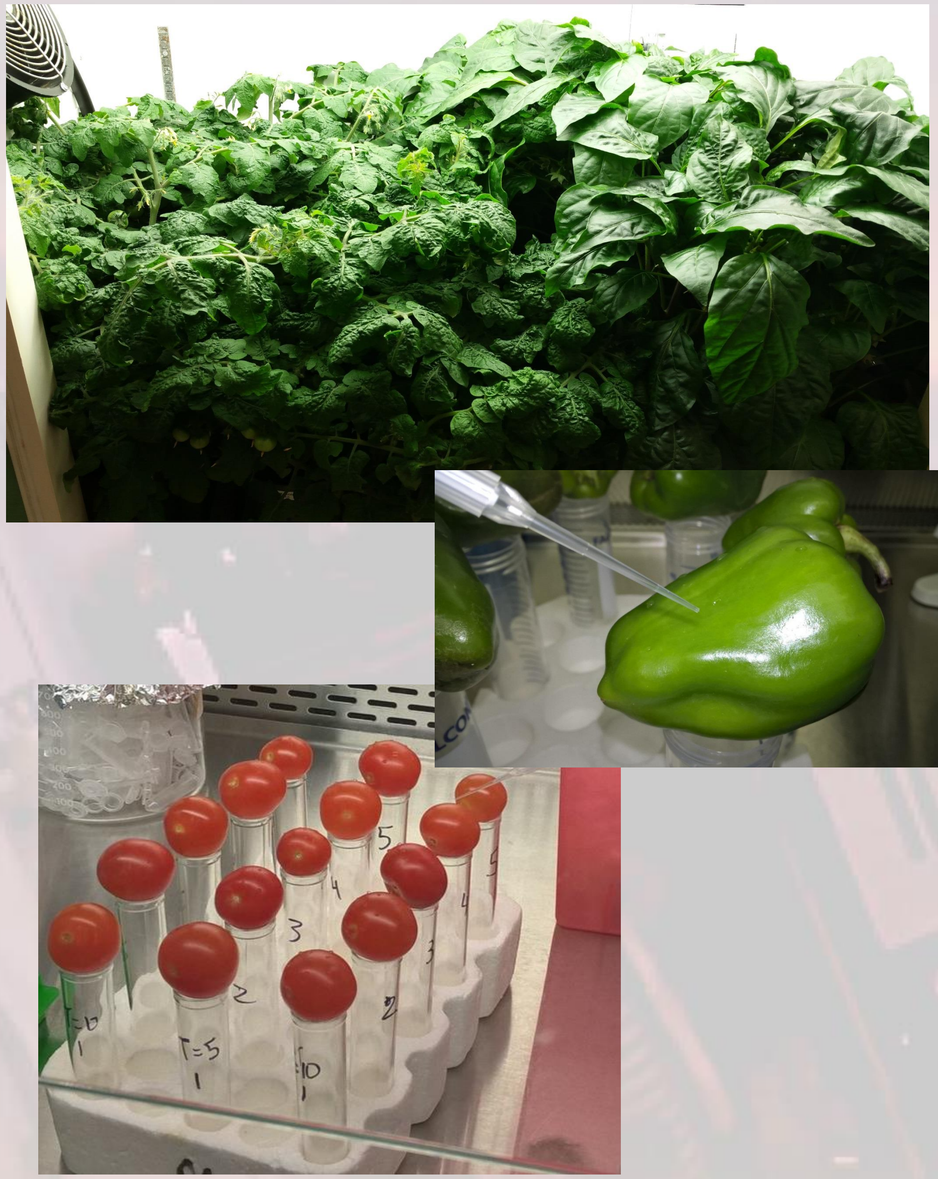
Results. Viability

- Maximum log reduction is achieved with 10 minute treatment for *E. coli and B. pumilus*
  - Decimal reduction value=1.9 minutes for B. pumilus. Treatment time for a single Log_{10} reduction calculated.
- Theoretically 15 min should reduce~ 7.5 log_{10} Bacillus spores



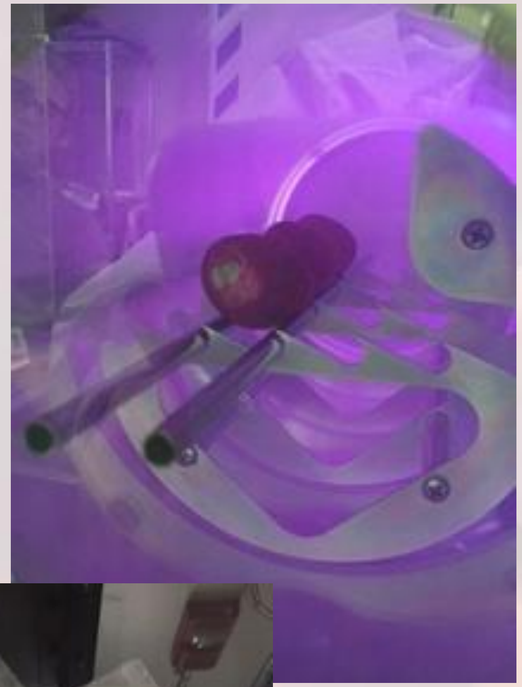
Produce

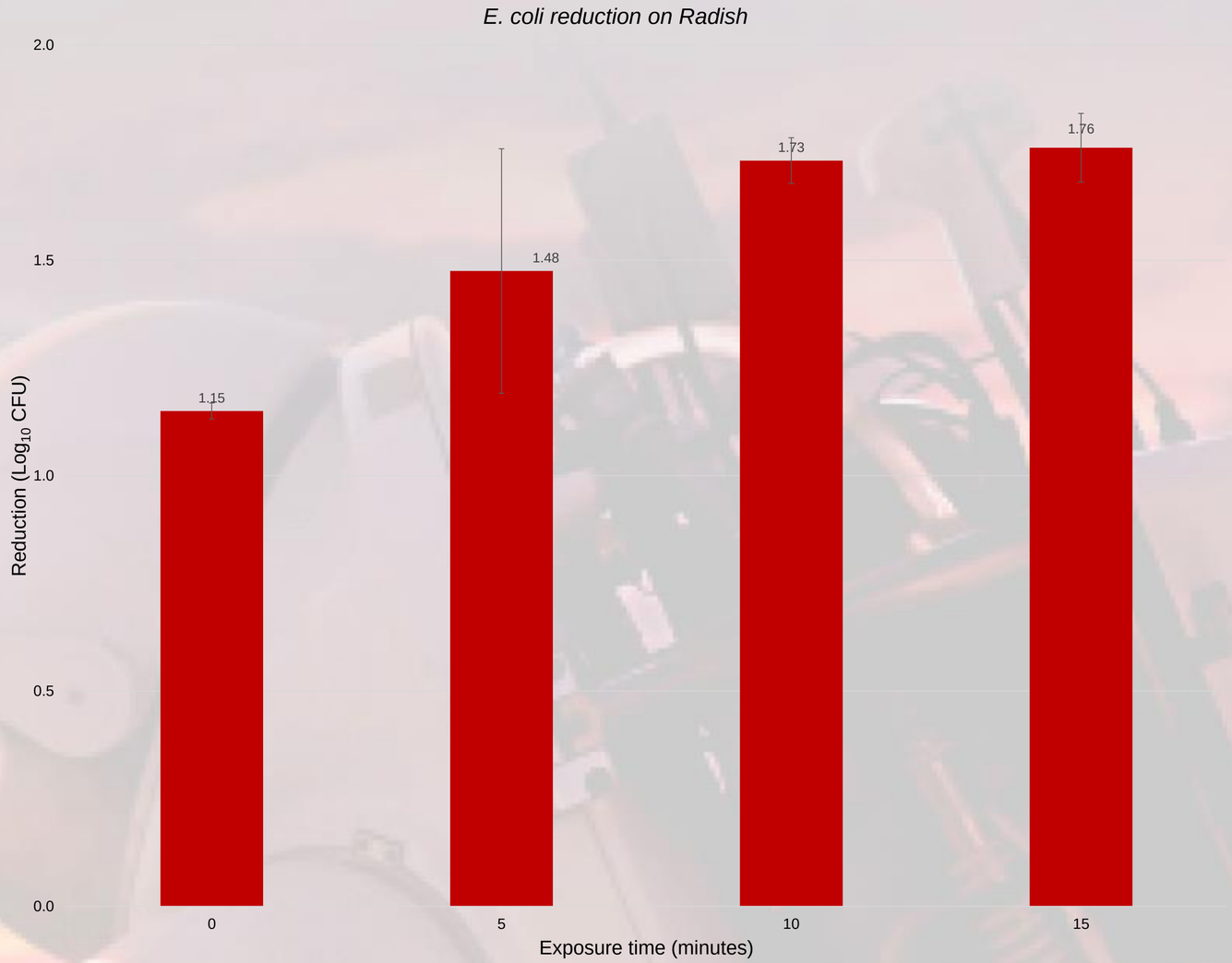
- Produce grown in controlled environment chambers at KSC.
- Selected candidate crops for Veggie VPU.
- Inoculated with ~ 10^7 *E. coli* cells/piece.
- Exposure times tested were 0 (low pressure for 15 min),5, 10 and 15 minutes.
- Analysis done by:
  - ✓ calculation of bacteria log reduction using plate counts
- No changes in temperature were observed when moisture was not present.
- Freezing or tissue damage was detected when water was present.



RADISHES

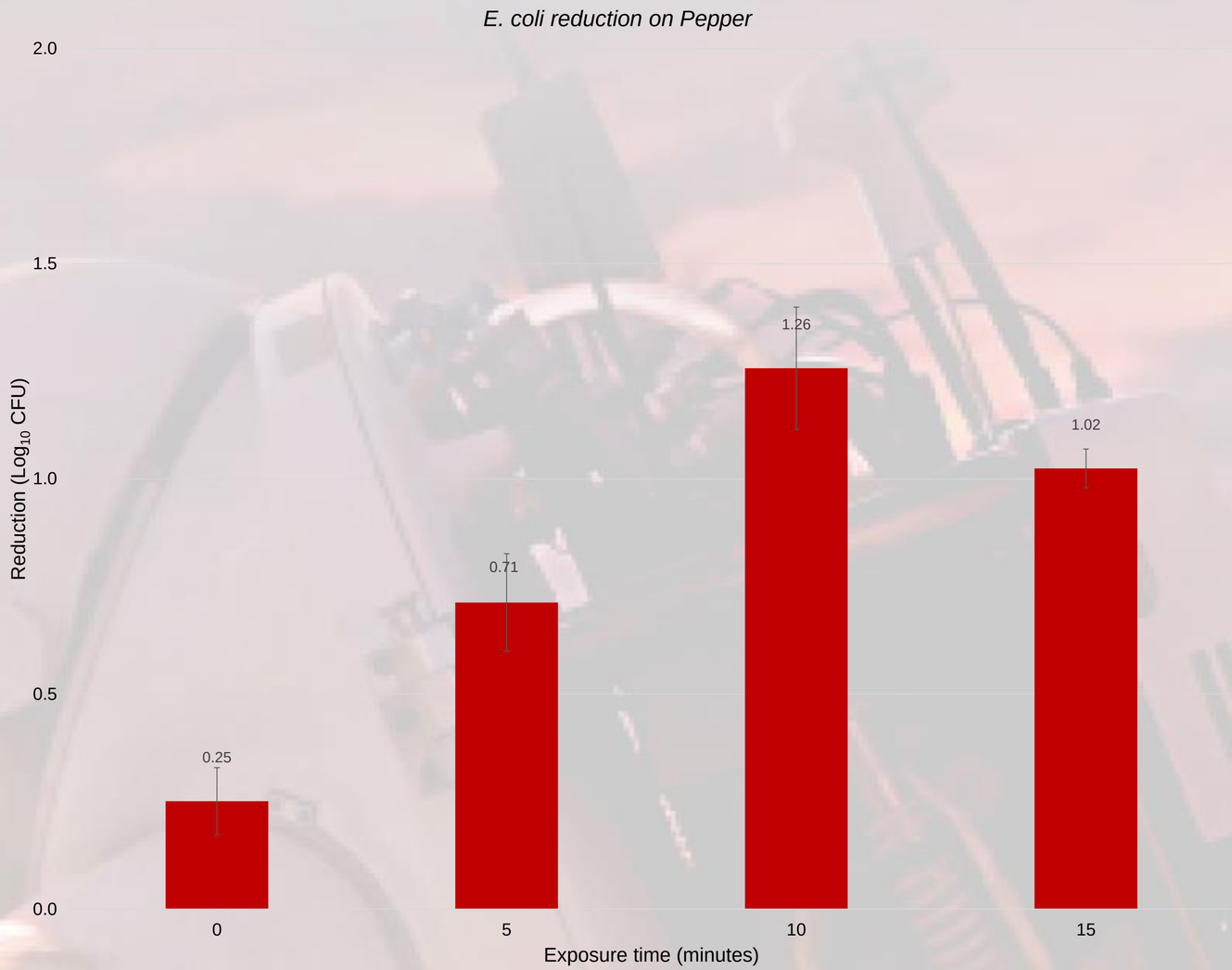
- ✓ Did not present any damage that could lead to test failing





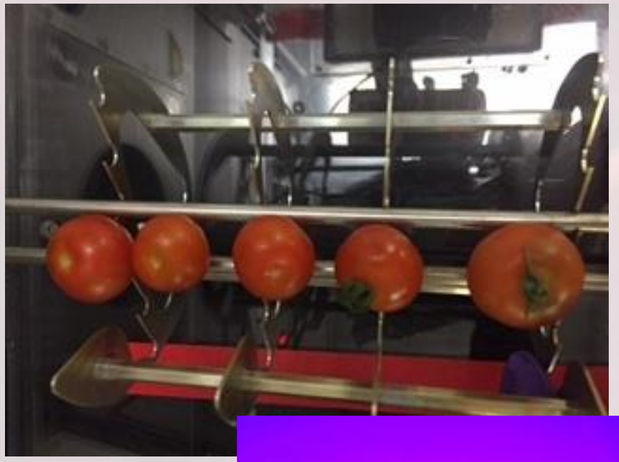
PEPPERS

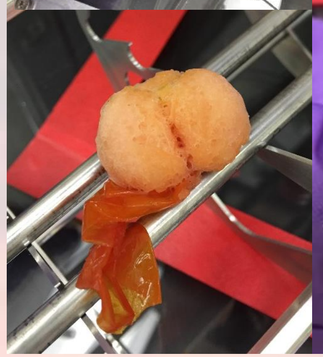
- ✓ Open peppers could lead to test failure and skin damage



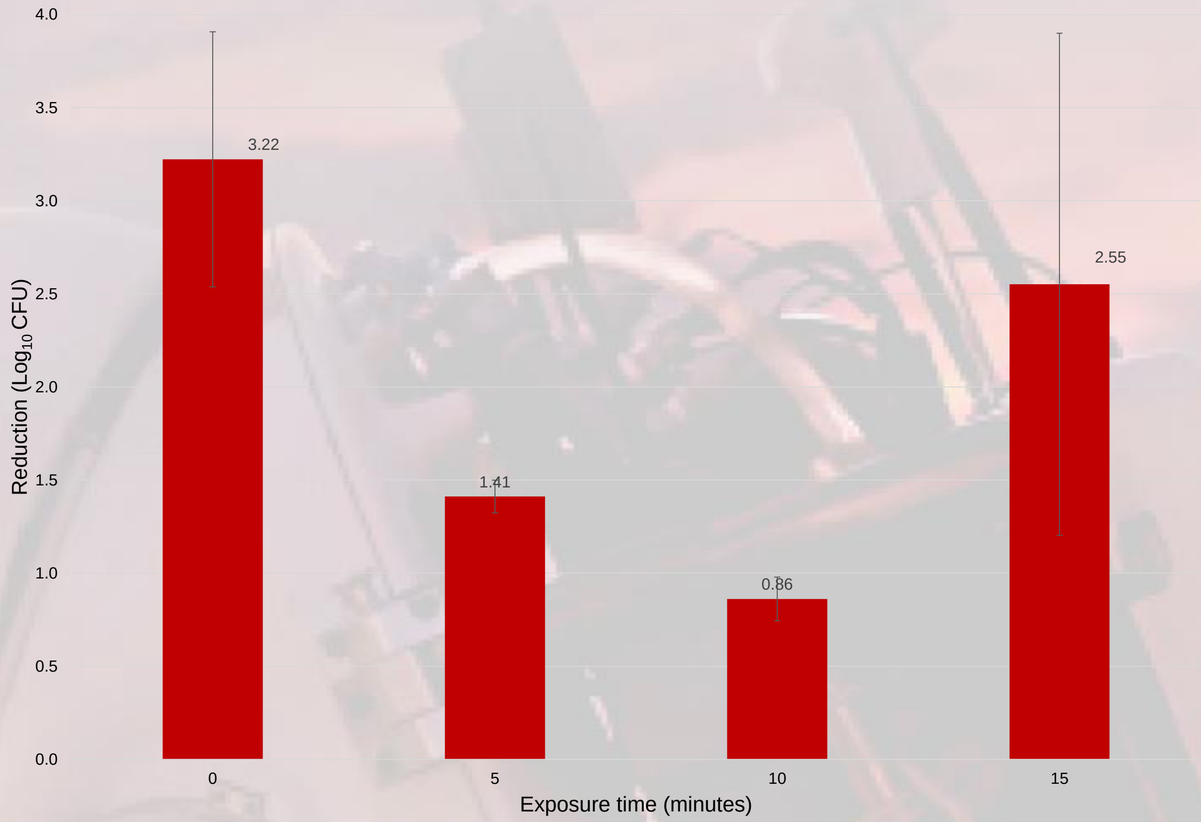
TOMATOES

- ✓ If tomato was very ripe and water detected through removed stalk considerable damage was observed



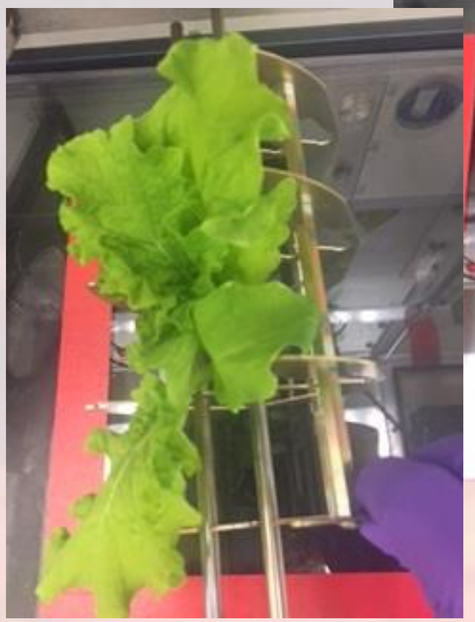


E. coli reduction on Tomato



CHINESE CABBAGE

- ✓ Failed due to water content
- ✓ Significant tissue damage



Potable Water Dispenser (PWD) Needle

- Successfully disinfected a piece of spaceflight hardware, the Potable Water Dispenser (PWD) needle that is used on the International Space Station (ISS). The needle is used by astronauts to rehydrate food packaging.
- The PWD needle was inoculated with a challenge organism inside the body of the needle and disinfected with cold plasma.

Conclusions

- Plasma is effective in killing spores of *B. pumilus and E. coli cells* on solid surfaces (>5.5 log reduction) within 10 minutes of exposure time.
- Treatment was less effective on reducing *E. coli* on produce.
- Low pressure cold plasma can effect plant tissues i.e. quality.

Future work

- Test efficacy of process on solid items inoculated with the fungus *Aspergillus niger.*
- Test sterilization of a variety of solid items inoculated with both test organisms.
  - ISS Potable water dispenser needle
  - Medical tools (ex. Hemostat, scalpel)
  - Plastic 3-D printed items

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Questions?