Application of the Enviro-Mist Method to Cannabis Flower: Nondestructive Sample Remediation for Microbial Contamination

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Abstract

The purpose of this study was to determine the efficacy of Enviro-Mist method directly applied to cannabis products. Cannabis Flower was inoculated with *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Escherichila Coli* Shigella Spp, *Salmonella*, *Staphylococcus aureus,* yeasts and molds and subsequently treated using Ionized Hydrogen Peroxide (iHP) to the dried material. Dielectric Barrier Discharge technology was used to enhance decontamination and increase aeration. Measurements were taken on moisture, residual solvents, potency and microbial load. A non-Inoculated sample was used as base control for potency, whereas an inoculated sample was used to show base line for microbial testing. Visual and organoleptic characteristics were not adulterated. Potency results of the cannabis plant were not affected. No additional residual solvents were found. The process was successful in complete remediation of all microbial contaminants.

1. Introduction

Remediation or decontamination can be found in Title 310 Oklahoma State Department of Health Chapter 681.[1] A Harvest Batch that fails a microbial testing must be remediated, decontaminated, or disposed of in accordance with state law. [1] Harvest batches are currently limited to 10 lbs. [1] The ability to remediate or decontaminate microbial contamination is of paramount interest to all licensed grows in the State of Oklahoma, of which there are 7,364[2].

2. Materials and Methods

2.1 Potency Control Sample

200 mg of homogenized flower was used to determine the baseline potency of the flower via HPLC.

2.2 Strain Choice

Microbial stains were chosen based on the current requirement for microbial contamination set forth by Title 310 Oklahoma State Department of Health Chapter 681. Medical Marijuana Regulations. [1] *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Escherichila Coli* Shigella Spp, *Salmonella*, *Staphylococcus aureus,* yeasts and molds

2.3 Sample Preparation and Inoculation

5 grams of flower was inoculated with aerosol positive control media containing *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Escherichila Coli* Shigella Spp, *Salmonella*, yeasts and molds.

A second sample of 5 grams was homogenized and treated without microbial inoculation. This sampling was used for potency.

2.4 Procedure for Treatment

iHP was applied to dried material. Dose was calculated by the room's cubic footage, by amount of dried cannabis product, and volume of area where the product sat. Dielectric Barrier Discharge technology aeration time was calculated based on the room dose.

2.5 Microbiological Testing

Testing was performed in accordance with SOP.T.40.046 (Plating) covered under the ISO/IEC 17025:20177 accreditation of Analytical Edge Discoveries, LLC DBA Kaycha Labs Oklahoma.

2.5.1 Table 1: OMMA Acceptable Limits

Microbial Testing	
Substance	Acceptance Limits
Shiga-Toxin producing *E. coli* (STEC)-Bacteria	<1 Colony forming Unit (CFU) per gram
Salmonella species-Bacteria	<1 Colony forming Unit (CFU) per gram
Aspergillus niger	<1 Colony forming Unit (CFU) per gram
Aspergillus fumigatus	<1 Colony forming Unit (CFU) per gram
Aspergillus terreus	<1 Colony forming Unit (CFU) per gram
Aspergillus flavus	<1 Colony forming Unit (CFU) per gram
Total Yeast and Mold	<10^4 Colony forming Unit (CFU) per gram

2.6 Potency Testing

Testing was performed in accordance with SOP.T.30.050, SOP.T 40.020 (HPLC) covered under the ISO/IEC 17025:20177 accreditation of Analytical Edge Discoveries, LLC DBA Kaycha Labs Oklahoma. Acceptance Criteria was set + or – 15% based on the most stringent repeatability for chemical analysis of CCV's in Title 310 Oklahoma State Department of Health Chapter 681. Medical Marijuana Regulations. [1]

Sample homogeneity was achieved by bead milling.

2.7 Effects on Quality

The appearance and organoleptic qualities of the plant were observed before and after treatment by the Enviro-Mist Method. Moisture

content was also measured using MOC63u Moisture Balance. Moisture tests are used as correction factors for cannabinoid testing

2.8 Moisture Content

Moisture Content Testing was performed in accordance with SOP.T.40.011, SOP.T 40.020 (Moisture Analyzer) covered under the ISO/IEC 17025:20177 accreditation of Analytical Edge Discoveries, LLC DBA Kaycha Labs Oklahoma. Moisture content was measured before and after treatment by the Enviro-Mist Method.

2.9 Residual Solvents

Testing was performed in accordance with SOP.T.40.050, SOP.T 30.052 (GC-MS) covered under the ISO/IEC 17025:20177 accreditation of Analytical Edge Discoveries, LLC DBA Kaycha Labs Oklahoma. Samples were measured before and after to verify no adulteration of the flower.

3. Results and Discussion

3.1 Potency Results

Based on the following data, there was not a systematic decrease in cannabinoid potency in the major, minor, acidic and neutral cannabinoids in the flower samples provided. No consistent concentration decreases were observed and in some cases the concentration increased. This is due to the inhomogeneous nature of the plant. CBN and d8-THC are the degradation products of d9-THC, and no increase is observed to make the claim degradation is occurring.

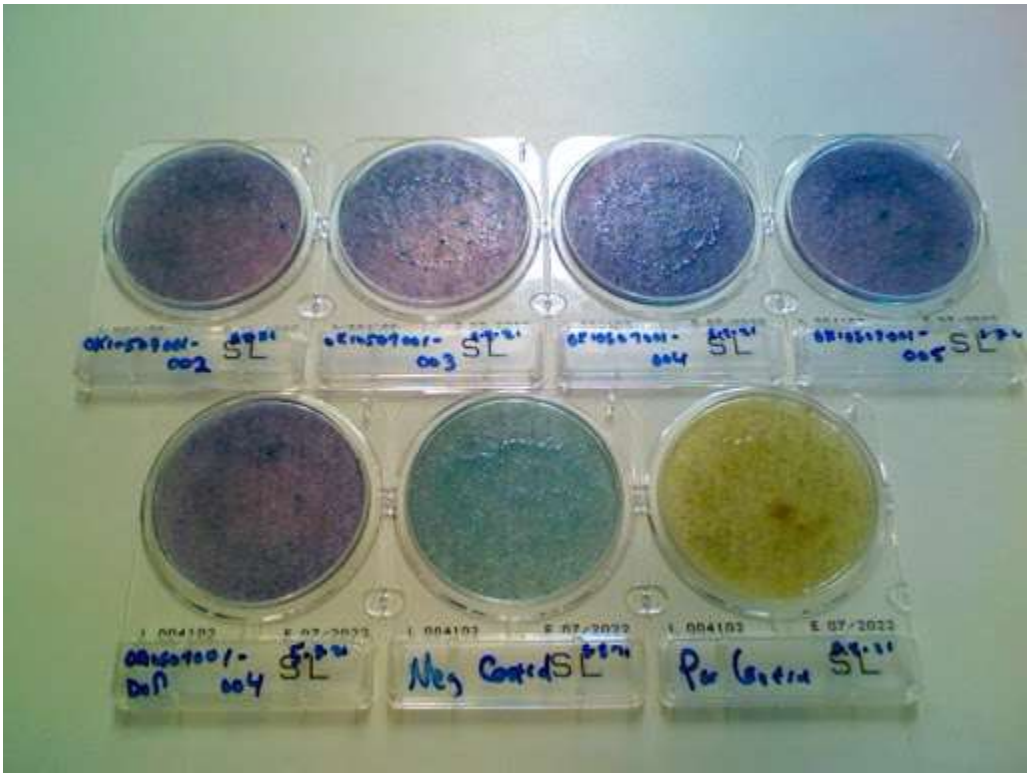
3.1.1 Table 2: Potency results for pre and post treatment

Sample ID	**OK10507001-001POT**	**OK10507001-003POT**	**OK10507001-004POT**	**OK10507001-005POT**
Sample Name	Potency Pre Spike and Treat	Micro Spike Treated 1	Micro Spike Treated 2	Micro Spike Treated 3
CBC	0.064	0.089	0.060	0.069
CBD	0.005	0.008	0.006	0.003
CBDA	0.030	0.034	0.029	0.030
CBDV	0	0	0	0
CBG	0.099	0.106	0.109	0.076
CBGA	0.526	0.507	0.607	0.462
CBN	0.064	0.107	0.055	0.064
D8-THC	0.019	0.025	0.025	0.020
D9-THC	2.270	3.161	2.247	2.300
THCA	16.210	16.026	16.371	13.810
THCV	0.014	0.021	0.014	0.012

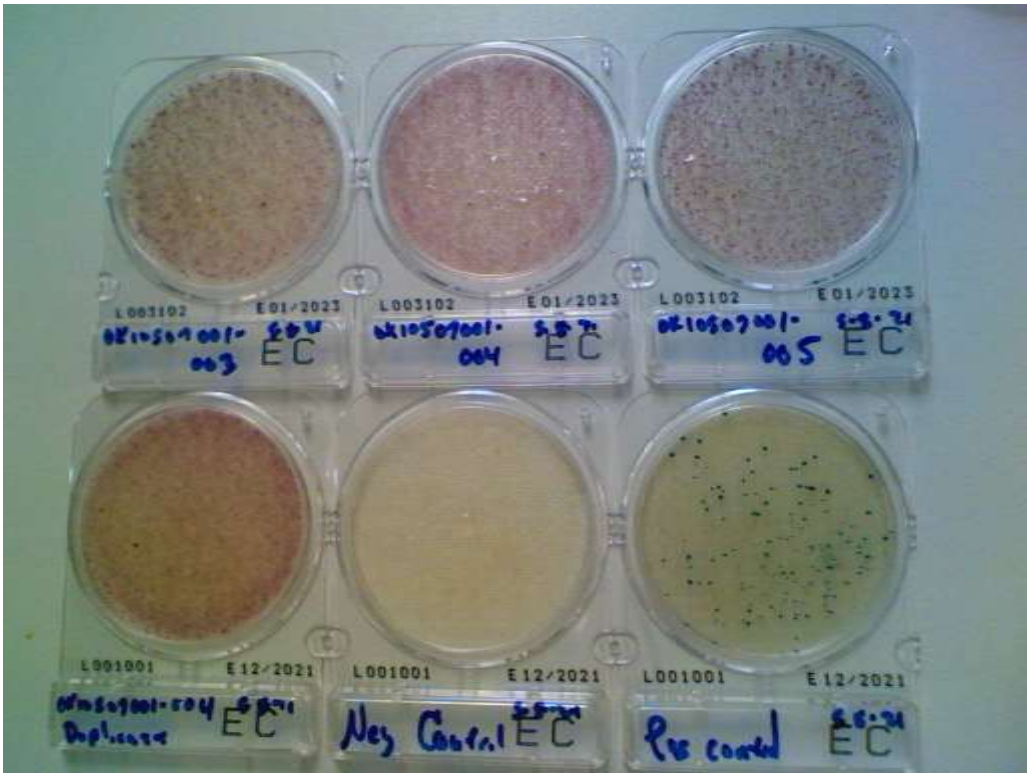
3.2 Microbial Results

For the Bacterial testing the results were as follows. Plate Image 1 shows *Salmonella* growth in the contaminated sample. It shows no growth of *Salmonella* in the treated samples.
Plate Image 2 shows the *Escherichila Coli* Shigella Spp plate. No growth of *Escherichila Coli* Shigella Spp in the color change media. Plate Image 3 shows *Staphylococcus aureus.* No growth on any plates. Only growth on positive control. Plate Image 4 and Plate Image 5 show *Aspergillus.* Plate Image 6 shows Total Yeast and Mold.

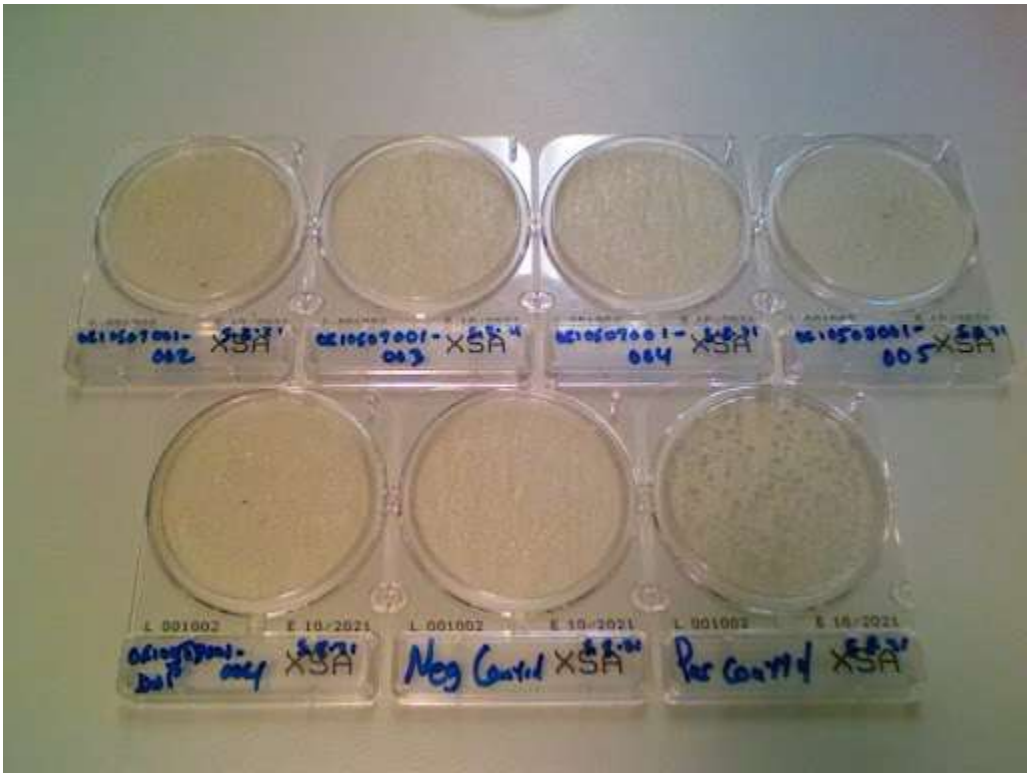
3.2.1 Plate Image 1: Salmonella



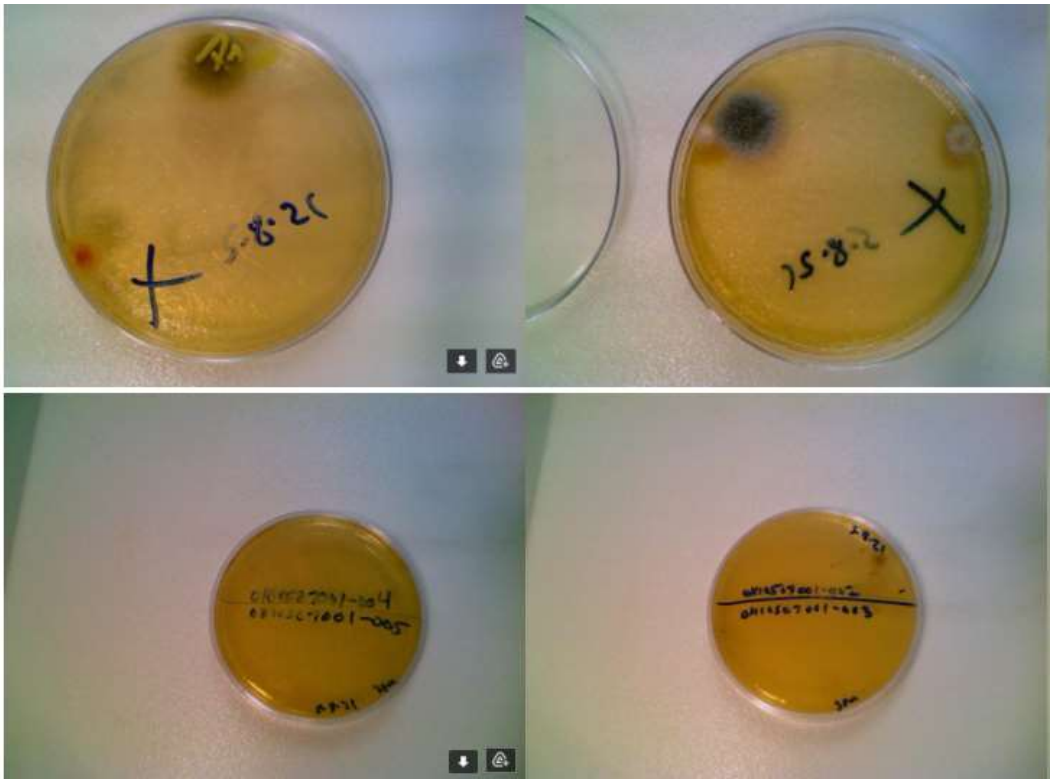
3.2.2 Plate Image 2 : E Coli



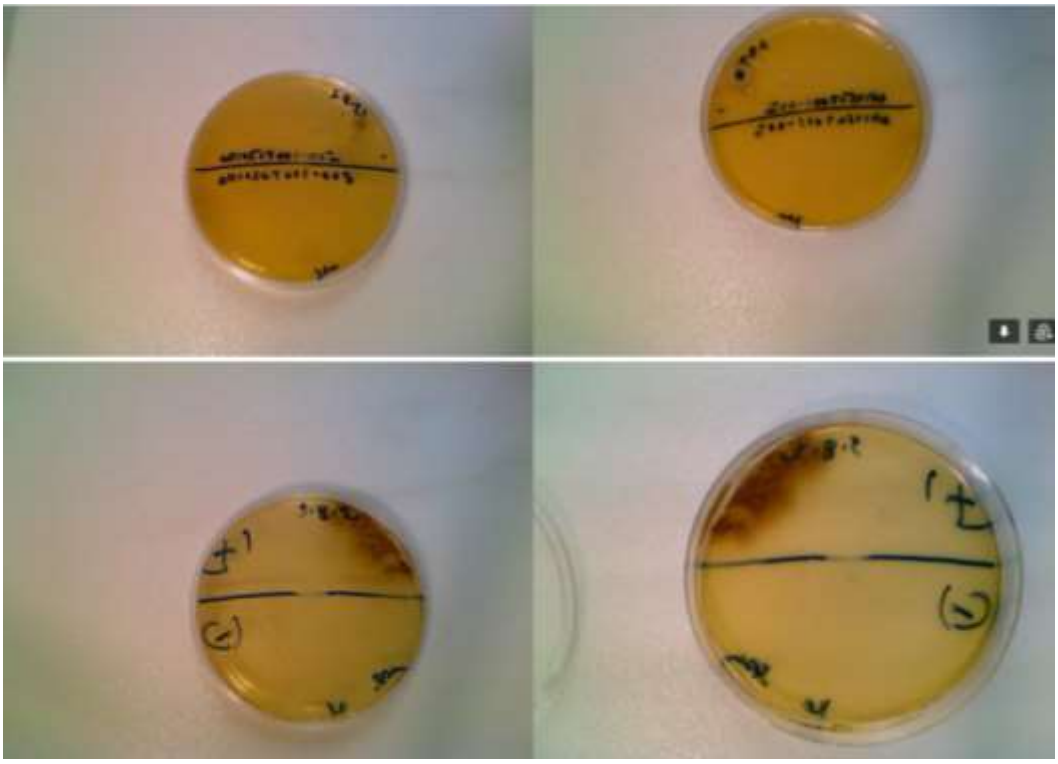
3.2.3 Plate Image 3: Staph



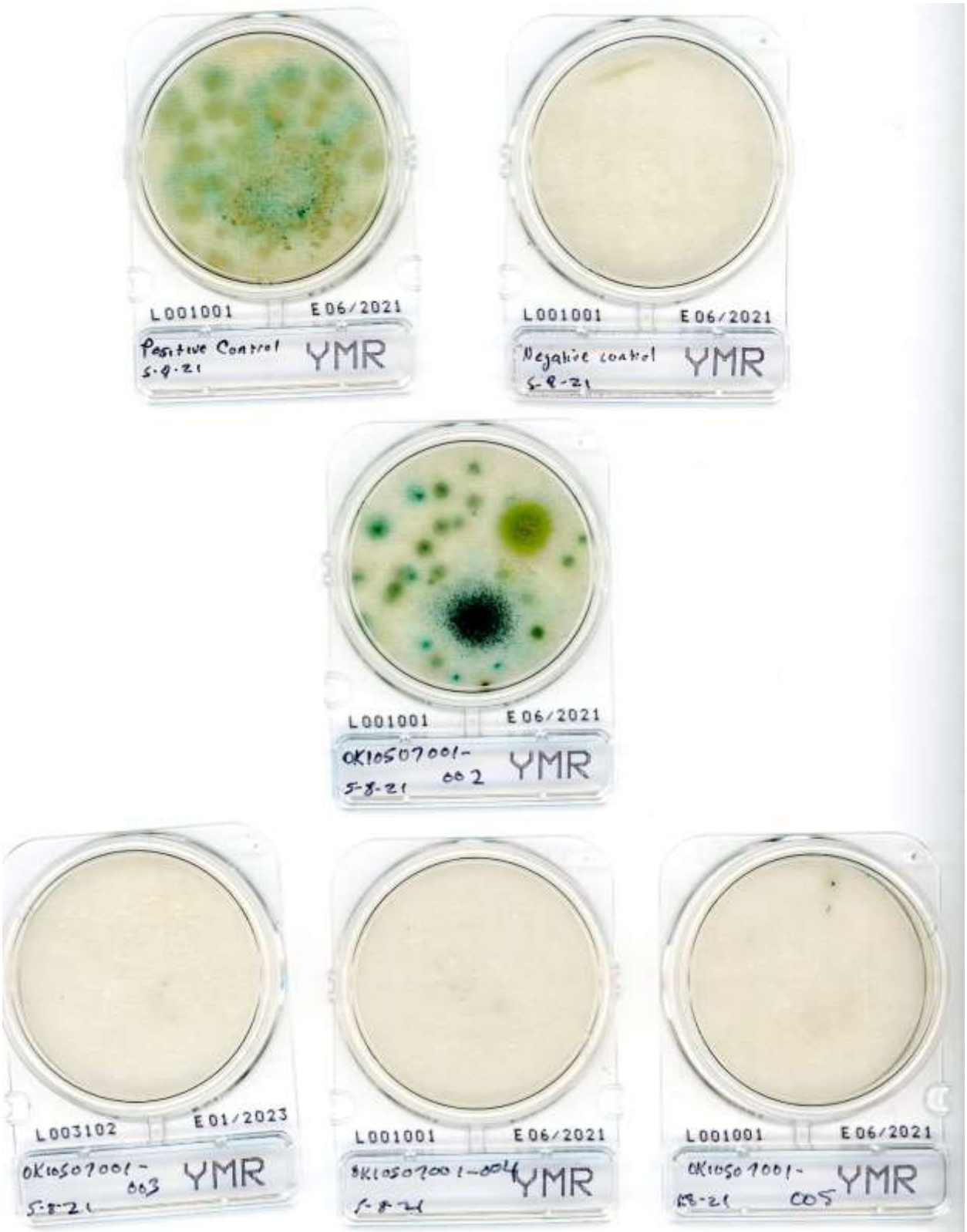
3.2.4 Plate Image 4: Aspergillus



3.2.5 Plate Image 5: Aspergillus



3.2.6 Plate Image 6: Total Yeast and Mold



3.3 Effects on Cannabis Flower Quality

No visual or organoleptic adulterations were observed. The flower is unaltered to the naked eye.

3.4 Moisture Content

Moisture content of the pre-treated flower was 11.94%. Moisture content of the post-treated flower was 15.8%, 17.19%, 16.87%. An average increase of 4.68%

3.5 Residual Solvents

All samples pre and post treatment received passing results for residual solvents.

4. Conclusion

The microbial load used in this experiment was sufficient to fail a product by OMMA standards for all analytes. The limits used by the State of Oklahoma to fail can be found in Table 1 The Enviro-Mist Method was able to remediate *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Escherichila Coli Shigella Spp, Salmonella, Staphylococcus aureus,* yeasts and molds. An increase in moisture content post treatment was observed at 4.68%. Additional time and ventilation will be needed after treatment for moisture content to return to pretreatment levels. Potency was not adulterated. Visual and organoleptic characteristics were not adulterated. No new residual solvents were found in the remediated samples. Overall, the results demonstrated the Enviro-Mist Method's ability to improve microbial safety without undue product adulteration.

5. Acknowledgements

The authors thank Enviro-Mist Inc. for graciously providing the remediation procedures, technician and specialized equipment in order to perform this study.

6. References

(1) TITLE 310. OKLAHOMA STATE DEPARTMENT of HEALTH CHAPTER 681. MEDICAL MARIJUANA REGULATIONS "Unofficial Publication"; https://oklahoma.gov/content/dam/ok/en/omma/docs/osdh_current_rules.pdf

(2) *OKLAHOMA MEDICAL MARIJUANA AUTHORITY. LIST of LICENSED GROWERS*; https://oklahoma.gov/content/dam/ok/en/omma/docs/business-lists/omma_growers_list.pdf