Introduction

Pesticides are one of the many organic pollutants that contaminate our environment. These chemicals, if ingested or exposed to, can cause "serious public health problems and considerable damage to agricultural and natural ecosystems" (2). They are so widely used that they have been proven to cause health problems such as cancer, ADHD, Alzheimer's Disease and even certain variations of birth defects (1). This is known for making it very critical to find a way to decrease the amount of pesticides in use. Getting rid of these organic pollutants, particularly pesticides, is the goal of this experiment. The process of bioremediation uses microbes such as bacteria because they have lots of metabolic pathways, which makes them excellent metabolizers compared to other plants and animals. Organic compounds can be more easily metabolized by bacteria compared to inorganic compounds. One of the two media's we used is carbon select media (CSM), which is deficient in carbon. This being said, the only way bacteria can get the carbon they need is to break down the paraoxon or methyl parathion pesticides. Sulfur select media (SSM), is the second media used which does not contain sulfur. The bacteria would have to break down the methyl parathion pesticide in order to obtain the sulfur that they need. In the experiment performed, a soil sample was inoculated and the supernatant was used to test the bacteria from the soil against the pesticides: paraoxon (Px) in Carbon Selective Medium (CSM), paraoxon (Px) in Sulfur Selective Medium (SSM), methyl parathion (MP) in CSM and finally, SSM. Our experimental results will allow us to gain a greater knowledge on what kinds of bacteria can break down pesticides through bioremediation.

Methodology

Week 0 (first week) a soil sample was collected into a light protected 15mL conical tube. The sample was then kept at room temperature until ready to be submerged in Luria broth (LB).

1.108g of the soil sample was measured out and then was ready to be inoculated in LB. After being submerged in LB the tube was capped loosely and incubated at 30°C at 200 rpm for 1 week. Week 1 two tubes filled with 4mL media each were given. The pesticides: CSM_Px, SSM_Px, CSM_MP, and SSM_MP were used every week to detect any growth or decay of pesticides against the bacteria. 0.2 mL of the soil broth was added to each of the tubes filled with 5 mL of the media (1:25 dilutions) and capped immediately, and ready for incubation for the next week. For Weeks 2 through 5 the process of adding 1 mL of each pesticide from the prior week was added to the next new set of fresh pesticides. Then incubated throughout the next week and results were recorded. However during Week 4 there was a mix up in the pesticide inoculation, so the SSM_Px tube was discarded and no longer a part of the experiment.

After week 5 anything that was bright yellow was revitalized. By adding 1 mL of the week 5 media into 4 mL of the revitalized media (1:5 dilution). The samples were then plated and the colonies were counted.

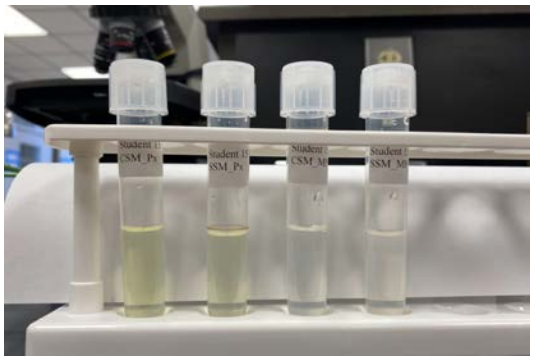
Results

Overall, the results for sample #15 observed gradual or no color change throughout the weeks. Indicating metabolization of pesticides in the media or no color where there is indication of no growth/metabolization.

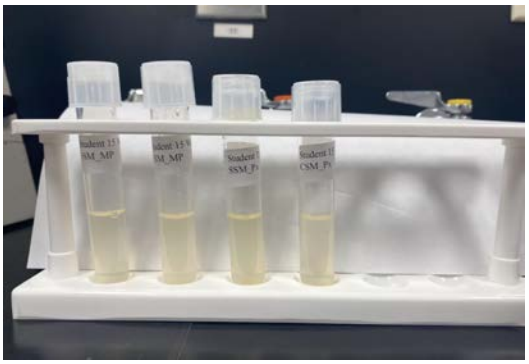
Student #15	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
Pesticide:						
CSM_Px	×	Very light yellow	Slightly darker yellow	Even darker yellow	No color	Yellow
SSM_Px	×	Very light yellow	Very light yellow	Slightly darker yellow	×	×

CSM_MP	×	Very light yellow	No color	Very light yellow	Very light yellow	Very light yellow
SSM_MP	×	Very light yellow	No color	No color	No color	No color

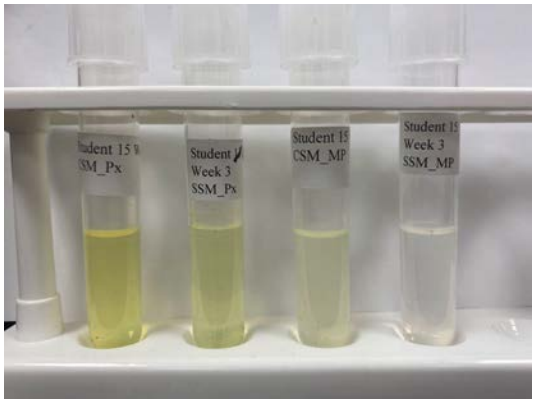
Week 1:



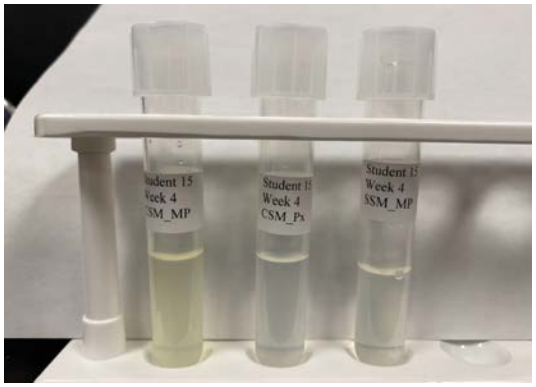
Week 2:



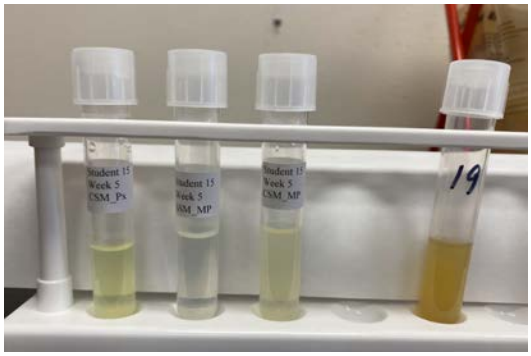
Week 3:



Week 4:



Week 5:



Week five samples were plated and the colonies were counted by each student involved in the experiment. In the table below, we concluded that we had no methyl parathion, only parxonon. Almost all samples did grow in LB, including #15, and obtained a plate count.

Plate Count #15: 1.22E+08 colony forming units (cfu)

ESRM Spring 2022 Growth Results:

Sample #	Student #	MP or PX	CSM or SSM	Growth in LB	cfu count	BIMS v. Other
1	1	Px	CSM	YES	6.20E+07	BIMS
2	1	Px	SSM	YES	4.00E+04	BIMS
3	2	Px	SSM	YES		
4	3	Px	CSM	YES	3.50E+07	Other
5	5	Px	CSM	YES	1.22E+08	Other
6	6	Px	CSM	YES	3.40E+08	BIMS
7	6	Px	SSM	NO		
8	8	Px	CSM	YES	2.86E+06	BIMS
9	8	Px	SSM	YES		
10	9	Px	CSM	YES	2.25E+04	BIMS
11	10	Px	CSM	YES	7.20E+07	BIMS
12	10	Px	SSM	NO		
13	11	Px	SSM	YES	2.80E+02	Other
14	11	Px	CSM	YES	3.20E+08	Other
15	12	Px	CSM	YES	1.22E+08	Other
16	13	Px	CSM	YES	6.00E+06	BIMS
17	13	Px	SSM	NO		

18	14	Px	CSM	YES		
19	15	Px	CSM	YES	1.76E+08	Other
20	16	Px	CSM	YES	3.00E+06	Other

T Test Results:

P value = 0.519233712

Discussion/Conclusion

A t-test was performed on the plate count results to see if the p-value was significant or insignificant. If the results proved to be significant ($p < 0.05$) it further supports the hypothesis that microbes can be used to break down pesticides in the environment. If the results proved to be insignificant then it can be concluded that something went wrong in the experiment, or that something should be improved. The t-test performed concluded that the results were insignificant because the p-value was greater than 0.05. The samples indicated breakdown in CSM and SSM of the paraoxon pesticides tested proven by the bright yellow indicator.

The possible reason for the insignificant results and no methyl parathion growth could have been because of a vendor issue or a sample collection issue. Other studies were done specifically from the Houston area where there tends to be a lot of pesticide pollution therefore making Houston a better location than Stephenville. So, it is possible that Stephenville and wherever the samples were collected hindered our results. The vendor specific issue could be due to the fact that some of the products, like the methyl parathion pesticide, that we used were no longer good or it was just a bad batch.

Works Cited

1. Jakuboski, Samantha. “The Dangers of Pesticides .” *Nature News*, Nature Publishing Group, 25 July 2011, https://www.nature.com/scitable/blog/green-science/the_dangers_of_pesticides/#:~:text=After%20countless%20studies%2C%20pesticides%20have,system%2C%20and%20the%20endocrine%20system.
2. Pimentel, David, and Michael Burgess. “Environmental and Economic Benefits of Reducing Pesticide Use.” *SpringerLink*, Springer Netherlands, 1 Jan. 1970, https://link.springer.com/chapter/10.1007/978-94-007-7796-5_5.