

ESRM - Undergraduate Research Experience

Introduction

Organophosphates are an organic compound made up of phosphate ester groups. Specifically, the pesticide nature of these compounds will be focused on for this project. In general, a pesticide is a substance used for destroying, or killing, insects and other organisms that may be harmful to plants or animals. However, the overuse of such pesticides can lead to many problems. They contaminate soil, water, and vegetation that can have a severe effect on the environment, as well as contaminating water and food.

The organophosphates focused on for this study were Paraoxon ethyl (Px) and Methyl Parathion (MP). Paraoxon ethyl is the active ingredient of the insecticide parathion, and it works to inhibit the acetylcholine, a neurotransmitter that is responsible for the contraction of muscles. This substance has been proven to be dangerous to humans and animals. On the other hand, Methyl Parathion is another insecticide, but it is most commonly used on cotton. The EPA, or the Environmental Protection Agency, has put many limitations on the use of it due to the potential risks.

Bioremediation is the use of microorganisms to consume or degrade environmental pollutants in order to clean up a polluted site. Yeast, bacteria, algae, and fungi have been studied as ways to clean up aquaculture waste, inorganic pollutants, heavy metals contamination, and antibiotic contamination. For this study, organophosphate-degrading bacteria would be the focus. Specifically, soil samples were collected from various places, and they were tested for any kind of bacteria that can degrade the organophosphates, Paraoxon ethyl and Methyl Parathion.

Methodology

Week 0

After collecting soil samples and recording the exact location using the GPS coordinates, 1 gram of soil was inoculated in LB, or Luria-Bertani, broth. This would serve to cultivate the bacteria found in the sample that will be used with the organophosphates. The soil inoculations were incubated for one week at thirty degrees celsius.

Week 1

Four tubes of pesticides were prepared and added to a carbon selection media, CSM, or sulfur selection media, SSM. One milliliter of the LB bacterial culture was transferred to four milliliters of the CSM and SSM solutions with Px, and later, due to the late shipment of the MP, 1 milliliter of the LB bacterial culture was added to the CSM and SSM solutions. The Px tubes were incubated at thirty degrees celsius for one week, and the MP tubes were incubated at thirty-seven degrees celsius for one day.

Weeks 2-5

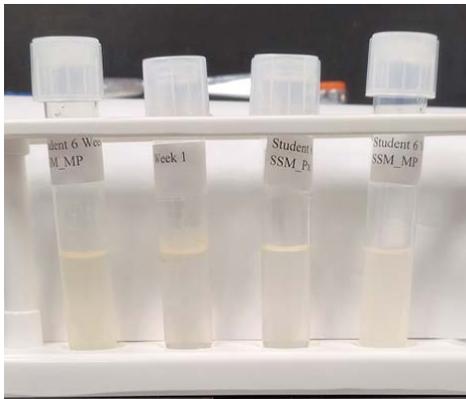
The same procedure was followed. 200 microliters of the previous week's culture was transferred to the corresponding current week's tube. The four tubes, CSM_Px, SSM_Px, CSM_MP, and SSM_MP were all inoculated and incubated accordingly. Each week, pictures were taken of the last tubes. It is important to note that a yellow color or hue to the originally clear solutions would indicate the ability to use the pesticides by the bacteria.

Results

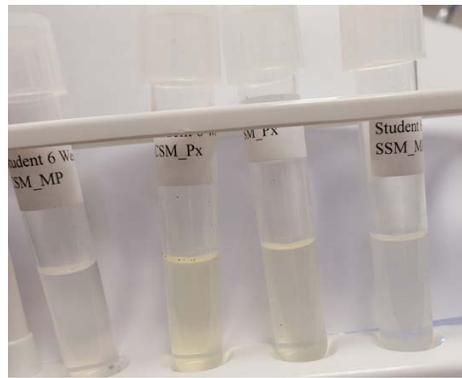
At the beginning of each week, a picture was taken to monitor any of the solutions turning yellow. Week One tubes showed very little change. The clear solutions of CSM_Px, SSM_Px, and SSM_MP turned foggy, but the CSM_MP got the slightest hue of yellow.

However, it was not strong enough to declare that organophosphate-degrading bacteria was present. Week Two had two tubes, CSM_Px and SSM_Px, start to turn yellow. Week Three had the strongest results with CSM_Px and SSM_Px being bright yellow, but the MP solutions remained clear. Week Four and Five had the same yellow results on the Px tubes. Below are the pictures that show the yellow solutions that have bacteria capable of degrading organophosphates.

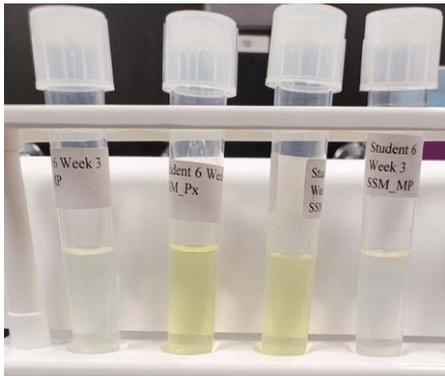
Pictures : Order Left to Right - CSM_MP, CSM_Px, SSM_Px, SSM_MP



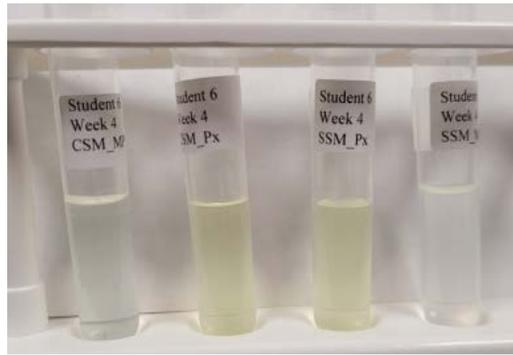
Week 1



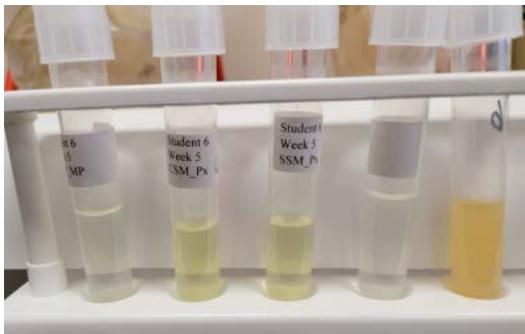
Week 2



Week 3

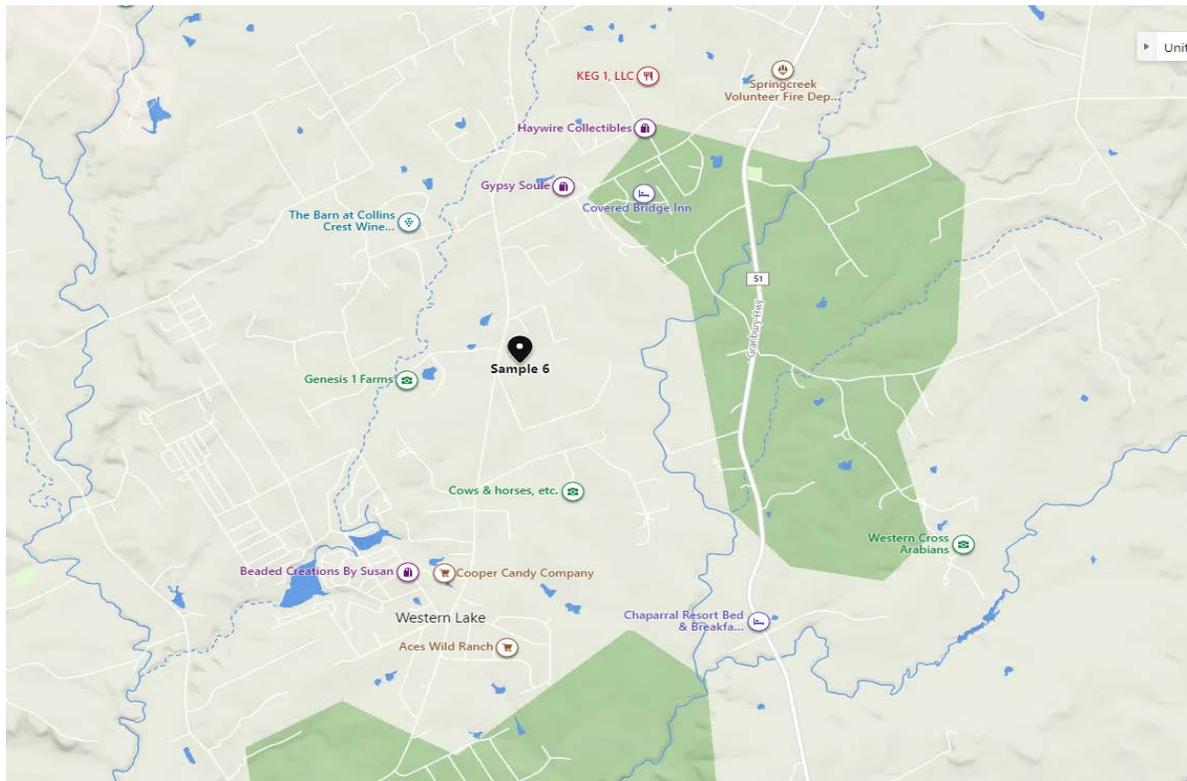


Week 4



*Week 5 *Far Right : LB Broth Growth**

In the last image above, “Week 5”, the far right tube was used to grow the bacteria from the CSM_Px solution. The bacteria that did grow would later be used to plate colonies to further understand the amount and possibly start identifying the species of bacteria present. The soil sample six from GPS coordinates, 32.637, -97.796, used for the CSM_Px solution was plated and showed $3.40E+08$ colony-forming units. A map is shown below of where this sample was collected. It is important to note that overall, out of seventeen different soil samples, only the Px solutions showed any sign of bacteria that could degrade the organophosphate. Of these solutions, only fourteen were moved forward to the LB Broth. The CSM and SSM solutions both had bacteria grow in the LB Broth, and most of them that were plated came back with a good plate count. After running a t-test on the plate counts, there was no statistical difference detected.



Soil Sample 6 GPS Map : Exact Location 32.637, -97.796

Discussion

In conclusion, most of the soil samples collected contained some kind of bacteria that was capable of degrading organophosphates. This shows potential for future advancements to clean the soil of the pesticide pollutants. Prior to the experiment, I hypothesized that there would be very little bacteria that would be able to break down the pesticides Px and MP in either the CSM or SSM solutions. However, after comparing the results from the class as a whole, I was proven wrong. Personally, I thought this was impressive. It is hard to comprehend that there is bacteria in most of the soil samples that could degrade and metabolize the pesticides.

For my sample, Student 6, I had two tubes, SSM_Px and CSM_Px, show bright enough yellow to move on to inoculation in the LB Broth. However, only the CSM_Px sample grew. This is shown in the figure for week 5, the far right tube. The next few weeks would be spent plating, and it was fascinating to see the growth.

I am beyond thankful for the opportunity to participate in the research project, and I am excited to see the project continue to grow. An important takeaway is to be more aware of the various forms of pollutants, and their everlasting effect on the soil and other environmental factors. I look forward to advancements in using bacteria to rid the soil of the pesticide contaminants, or organophosphates, using bioremediation