Learning Microbial DNA Extraction Techniques in Mexico and Building New Partnerships to Improve Zinc Nutrition in Maize

Zinc deficiency in staple foods affects nearly two billion people worldwide, impacting human health and nutrition as well as soil fertility. In maize-based systems, particularly in the U.S. Midwest and Mexico, conservation agriculture (CA) practices such as cover cropping, manure application, and no-tillage offer a promising yet underexplored strategy to improve soil zinc availability and crop uptake. These practices could mitigate unfavorable soil conditions by creating an environment that alleviates crop zinc deficiency, primarily by providing optimal growth conditions for microorganisms that enhance plant zinc availability.

As part of my PhD dissertation, I am collaborating with researchers from the Institute of Geology at the National Autonomous University of Mexico (UNAM). We have established controlled experimental trials in Illinois to investigate the effects of cover cropping, manure application, and no-tillage on zinc content in maize. Through our partnership with UNAM, we have initiated trials to collaborate with smallholder farmers in Mexico who already practice conservation agriculture.

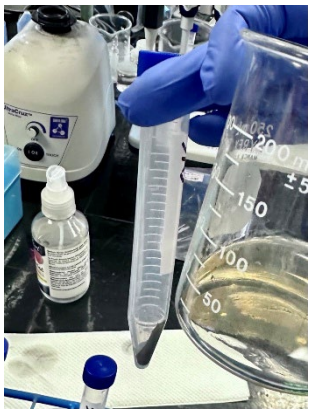
1. Learning how to extract microbial DNA from rhizosphere and bulk soil

Funding from the ACES International Graduate Grant supported this project's focus on the microbial community. My collaborator, Dr. Rocio Alcantara, is an expert in microbiology and utilizes molecular DNA and RNA tools to detect key microorganisms involved in biogeochemical processes within soil and water systems. In November and December, I traveled to Mexico to learn how to extract microbial DNA in bulk and rhizosphere soil samples collected from smallholder farms in Tlaxcala, Nayarit, and Hidalgo. Dr. Alcantara was my primary advisor while I was there, and her PhD student guided me through the process. I then completed the experiments independently, allowing me to gain the skills needed to conduct them on our research plots in Illinois.

For the rhizosphere samples, I took smaller clippings from the maize root sample (Figure 1A.) and carefully removed excess bulk soil. I then separated the soil from the root sample through a cleaning process that involved using a vortex and centrifuge to wash the root with sterilized water (Figure 1B). The soil was then separated from the root and the water (Figure 1C).

(A)

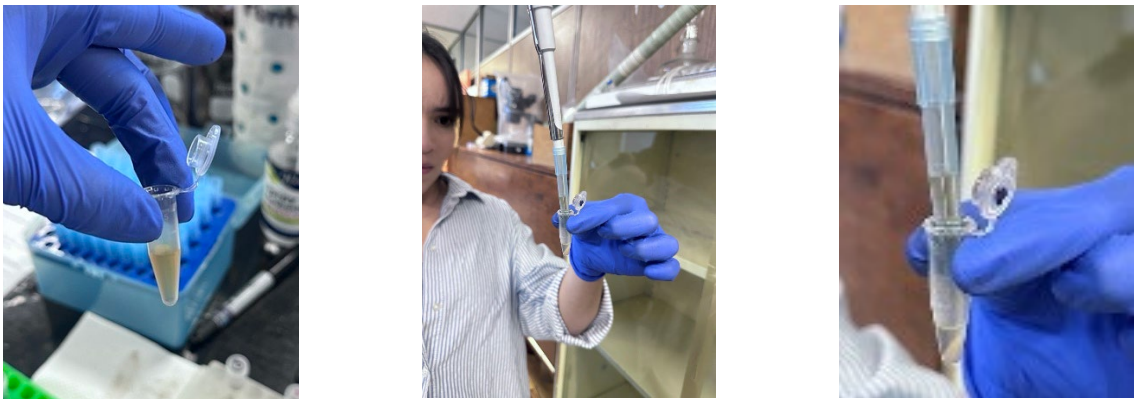
(B)



(C)

Figure 1. Rhizosphere soil removal from root.

After containing only the soil sample, the soil will be mixed with beads and a lysis buffer solution. This disperses the soil, dissolves the humic acids, breaks down the cells, and protects the DNA. The soil will then be separated from the new solution containing the cell contents (Figure 2A). I then remove the remaining soil and use an inhibitor to separate the sample into DNA and a white pellet of substances such as humic acids and cell debris (Figure 2B/C).



(A) **(B)** **(C)**

Figure 2. The process of removing the soil and separating the DNA from other humic and cell debris.

After the DNA is separated as much as possible, I began a multi-step process of creating a more concentrated DNA solution and removing any remaining contaminants, such as proteins. I then quantified the DNA concentration in this sample. Each location showed a difference in the amount of DNA extracted, which can be related to the soil environment (Figure 3). These concentrations have now been sent off for complete DNA sequencing.

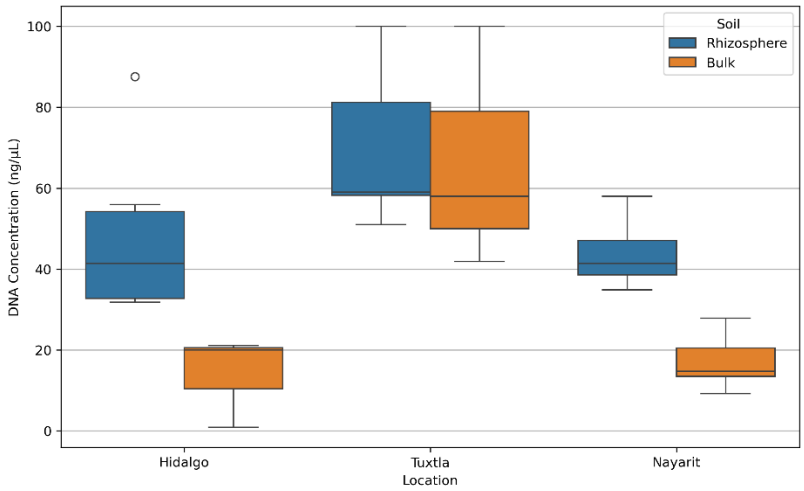


Figure 3. Soil rhizosphere and bulk DNA extractions for Hidalgo, Tuxtla, and Nayarit.

2. Visiting the International Maize and Wheat Improvement Center (CIMMYT)

Prior to leaving for Mexico, my UNAM collaborators and I met with researchers from CIMMYT. We learned that they are also working to understand the effects of conservation agriculture on food nutrition. We then scheduled a visit to CIMMYT. Dr. Alcantara and I visited CIMMYT in Texcoco at the end of my trip to Mexico. We learned about the various research platforms managed by CIMMYT and walked through the experimental sites in Texcoco. During our visit, we discussed what our partnership would entail. As a

result of our visit and new collaboration, I traveled to CIMMYT this past March and April to help physically and chemically characterize over 800 maize samples collected from more than 30 of CIMMYT's research platforms. I am now analyzing the zinc content in this data and identifying a region for a more in-depth study. I will return in the fall to collect soil, root, and grain samples for this study.

Figure 4. Visiting CIMMYT & its research platforms in Texcoco, Mexico.

Acknowledgements

I would like to thank ACES for providing international travel funding for graduate students like me. Traveling to Mexico was a fantastic experience both academically and personally. This experience has allowed me to learn new skills and form exciting new collaborations that will continue to shape my academic career. One of the reasons I chose to come to the University of Illinois is because of the strong emphasis on global opportunities, so I am incredibly grateful ACES has programs in place to make these experiences financially accessible.

I would also like to thank my PhD advisor, Dr. Prasanta Kalita, for his guidance and for continually encouraging me to seek opportunities like these. I would also like to thank my UNAM advisors, Dr. Rocio Alcantara and Dr. Milton Soto-Barajas, for guiding me throughout my time in Mexico.