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Iron Bioaccumulation in *Lemna minor* (Duckweed) and *Pleurotus ostreatus* (Oyster Mushrooms)

Mine tailings have a major impact on the environment and ecology of a specific location. The Gold King Mine Spill unleashed millions of gallons of contaminated water into the Animas and San Juan Watersheds. One of the elements contained in the spill included iron. Iron itself usually is not in high enough concentrations to be considered a contaminant. It is of interest, however, due to its ability to form complexes with other, more dangerous, elements. Iron can also be used as an analogue for other transition metal behavior, such as that of manganese, copper, and chromium. The primary objective of this project is to determine the ability in which duckweed is able to remediate iron in an aquatic system and what processes can be done with the duckweed after remediation has occurred. A secondary objective is to investigate the plant-microbe associations to determine what role microbes play in bioaccumulation.

Preliminary results were collected, followed by a repeated study which yielded more results. The preliminary study and experimental set-up is explained in the following few sentences. Duckweed (*L. minor*) was grown in 12 water-filled vats measuring 34.6 cm in length, 21 cm in width, and 12.4 cm in height and placed under a fluorescent light table. 9 of the 12 vats were used as the experimental group and the remaining 3 were used as the control group. There were varying levels of chelated iron product added to each vat in the experimental group and a 20-20-20 fertilizer added to all 12 vats. All vats contained 0.10 kg of duckweed, except the controls, and 2.9 kg of water. Each vat in the control group received a different treatment. One vat contained duckweed with 20-20-20 fertilizer, one contained duckweed and tap water with no fertilizer, and the third vat contained 20-20-20 fertilizer with the addition of chelated iron, but no duckweed. The fluorescent lights were placed on a timer to allow for a 12 hour photoperiod and a 12 hour dark period, to keep the light and dark variables constant. The duckweed was grown for a month's period of time and samples were collected during various time points throughout the entirety of the experiment. Each sample set was then oven-dried at 65^{o}C, weighed to 0.5g, digested using EPA method 3052, and analyzed for total iron content by way of Inductively Coupled Plasma-Optical Emission Spectrometry (hereafter referred to as ICP-OES). The tap water was also analyzed for total iron content to avoid skewing the data collected for total iron content of the duckweed.

Once the duckweed was grown after a one month period of time, collected, digested, and results were obtained, 10 to 15 individual plant samples were separated from each vat containing duckweed and kept in a 4^{o}C freezer to inhibit any external microbial activity not already present in the sample. A total of 20 individual duckweed plants were then plated onto two different sugar substrates, with 2 replicates of each. The two substrates used were Potato-Dextrose AGAR and Acidified Potato-Dextrose AGAR. The Acidified Potato-Dextrose AGAR was used to culture the fungus, as acidification makes it difficult for bacteria to grow, and the Potato-

Dextrose AGAR was used for bacterial colonization. All samples were placed in a lit room to encourage growth. Once fungi and bacteria started growing, they were then isolated onto separate AGAR plates to obtain a pure culture. Some of the bacterial plates were contaminated with mites, however. To remedy this, the plates were placed in a -20^{o}C freezer to slow the movement of the mites, viewed under a microscope to detect the location of the mites, and a clean sample was then taken and placed onto Water AGAR to retain the bacterial growth but get rid of any mites or unwanted sample contaminants.

Polymerase Chain Reaction (PCR) was then conducted on the fungal samples and sent to a lab for genetic analysis to determine the exact species that were grown. The results indicated that the three different species of fungus grown were *Alternaria alternata, Plectosphaerella cucumerina,* and *Cladosporium tenuissimum*. Literature review was then conducted to try and identify the species possibly responsible for the uptake of iron in the duckweed. Findings from the literature review suggest that *Alternaria alternata* is most likely responsible for the plant's iron uptake ability, as it can mycosynthesize iron ion particles.

After obtaining the preliminary results, the experiment was set up again, but on a larger scale. Setting up the experiment a second time required more space than a single light table was able to provide, therefore it was set up in a greenhouse as opposed to a single light table. The vat sizes remained the same (34.6 cm (L) x 21 cm (W) x 12.4 cm (H)) as did the water weight (2.9 kg) and the weight of the duckweed added (0.10 kg). The iron concentration levels did not vary from the preliminary data either (0.5g product, 5g product, and 50g product, respectively) and 1g of 20-20-20 fertilizer continued to be used as well.

Experimental blank vats were set up consisting of 2 different treatments replicated 2 times per treatment. Water was added to each vat with 5g of added iron product and 1g of fertilizer in one treatment and no added fertilizer in the second treatment. Water samples were taken every day over a 5 day period and analyzed for total iron content via ICP-OES. The idea behind the experimental blanks was to determine how iron acts in water without the addition of duckweed. The treatment that received the fertilizer was to determine if the iron was binding to any nutrient in the fertilizer in any way. Results obtained showed that it was not. The purpose of the treatment that did not receive the fertilizer was to determine how much iron was precipitating out of solution and adhering to the walls of the plastic vats. These results can then be deducted from overall iron content found in the duckweed and left in solution to eliminate any variables that may be contributing to skewed results. Experimental blank results will help explain and solidify other data obtained.

Control vats were then set up to include aerated and non-aerated vats with duckweed, water, and fertilizer, with no added iron product. There were 3 replicates of each control totaling 6 vats overall. Fertilizer was added to maintain plant growth and health. Samples of water and duckweed were taken every day for a 5 day period and analyzed for total iron content via microwave digestion (for the duckweed only) and ICP-OES (for both duckweed and water). These results will later be compared to results obtained from the experimental units.

Experimental unit vats were then set up to, again, include aerated and non-aerated vats to determine if aeration makes a significant difference in bioaccumulation ability or not. The experimental units consisted of water, duckweed, fertilizer, and low to high concentrations of added iron product. There were 3 treatments (0.5g iron product, 5g iron product, and 50g iron product) x 3 replicates per treatment x 2 (aerated and non-aerated vats) for a total of 18 experimental unit vats overall. Water and duckweed samples were taken every day for a 5 day time period and analyzed for total iron content using the analytical tools described above. Results show that duckweed is removing iron from solution but it is unclear whether it is absorbing it into the plant or the iron particles are just adhering onto the roots. The objective of this study is not concerned over the mechanism of uptake, however, just in overall uptake by the plant. The highest amount of iron found in the duckweed was about 20,000 parts per million (ppm).

After the 5 day time period, all of the duckweed was collected, weighed, and placed into plastic Folgers Coffee bins with aeration and drainage holes on the top, bottom, and sides of each container for composting. Literature shows that duckweed by itself takes a long time to compost and has not fully composted to this day. Mycelial growth was found in the control compost bin and strands were removed and placed into the bins showing no signs of degradation in hopes to colonize those bins and accelerate the decomposition. No results have yet been obtained.

A third experimental trial was set up with 2 sets of experimental blanks (identical to previous trials) and controls, low amounts of added iron supplement, and high amounts of iron supplement; all non-aerated. Each treatment was replicated 5 times. The vats were aerated over the course of one week to help dissolve the iron before any duckweed was added. Water and duckweed samples were taken initially and every 3 days over the course of 2 weeks to be analyzed using ICP-OES. Water samples were acidified using nitric acid (HNO_3) to inhibit microbial activity and bring the iron into solution. Acidification also preserves the samples for later analysis. Results are pending.

Future directions of this project include setting up the experiment again, but on a larger scale, recognizing problems associated with the preliminary finding and correcting those problems, composting the duckweed once the experimental trial is over, growing *Pleurotus ostreatus* (oyster mushrooms) using the duckweed compost, and analyzing the oyster mushrooms to see if the fruiting bodies have absorbed any of the iron. If they do absorb the iron, we can determine how much was absorbed, how much iron was bound in the compost, and how much may have precipitated out of solution. Progress will continue as the research moves forward.

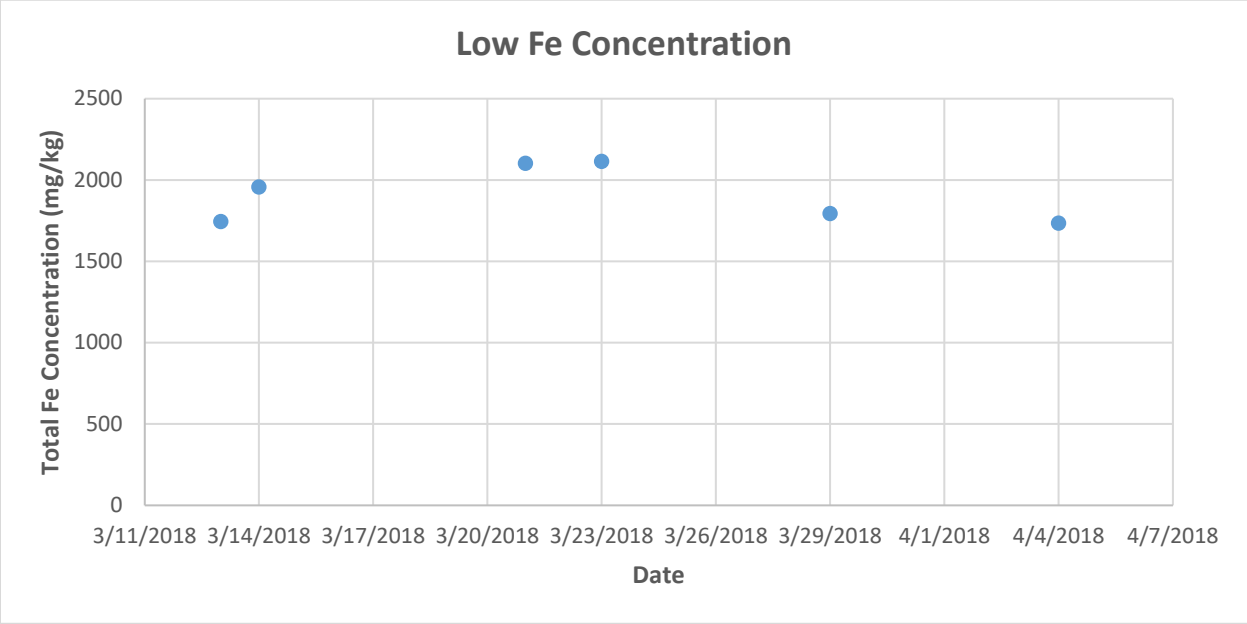
This research has the potential to benefit many individuals and agencies. Concluded results can be beneficial to water research agencies, mining companies, environmental agencies, mycologists, and possibly humans with anemia. Water research agencies can benefit from this research because it approaches the problem of how to decontaminate the contaminated waterways. The decontamination of the waterways is being approached in an environmentally friendly way by using plants as opposed to conventional remediation methods, therefore environmental agencies can also utilize the results by taking a non-traditional approach to contaminant removal. Mining agencies can benefit as well by observing the results, seeing the

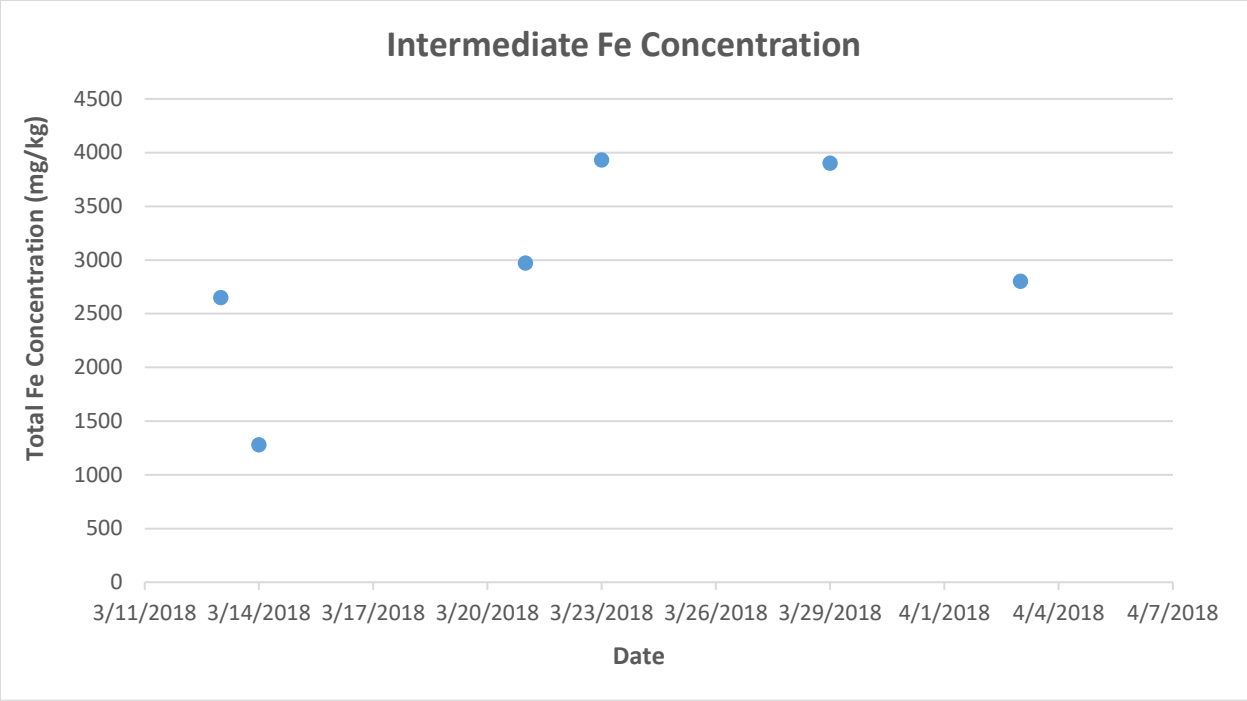
effects mine tailings have on the environment and certain ecosystems, and, from that, create a more environmentally friendly way of mining. Mycologists, or fungal scientists, can also benefit from these results, as I will be working with oyster mushrooms to study their iron accumulating abilities for mycoremediation purposes. If the results show that the mushrooms uptake the iron in significant amounts, this information can be used biomedically to assist anyone suffering from an iron deficient diet.

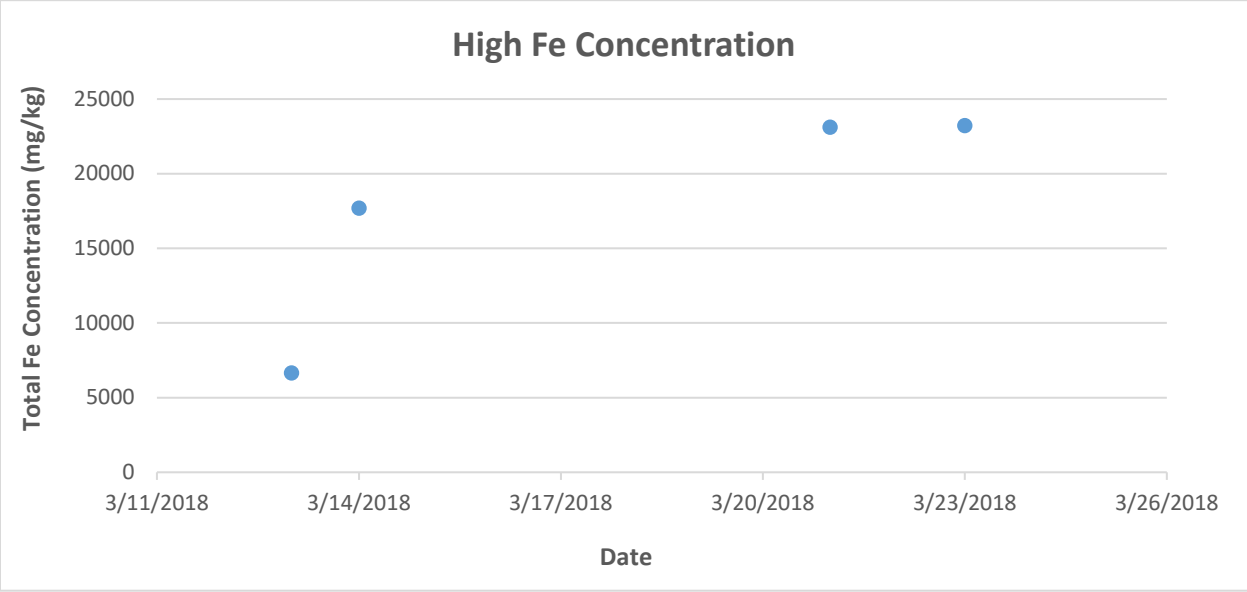
I owe a tremendous amount of gratitude to Dr. April Ulery and Dr. Soum Sanogo for their assistance in this project and its components. I also owe a tremendous amount of gratitude to one of my lab team members, Hunter Windsor, for assistance in the experimental set-up and for ordering necessary supplies. I would like to acknowledge and thank Barbara Hunter as well, for completing the plant and water digests and analyzing my samples. I also would not have been able to do this without the grant money awarded to me by NMWRRI that helped order supplies and the duckweed. All funds allocated towards this research have been spent to include purchases of duckweed, aerators, tubing, air stones, sample analyses, etc.

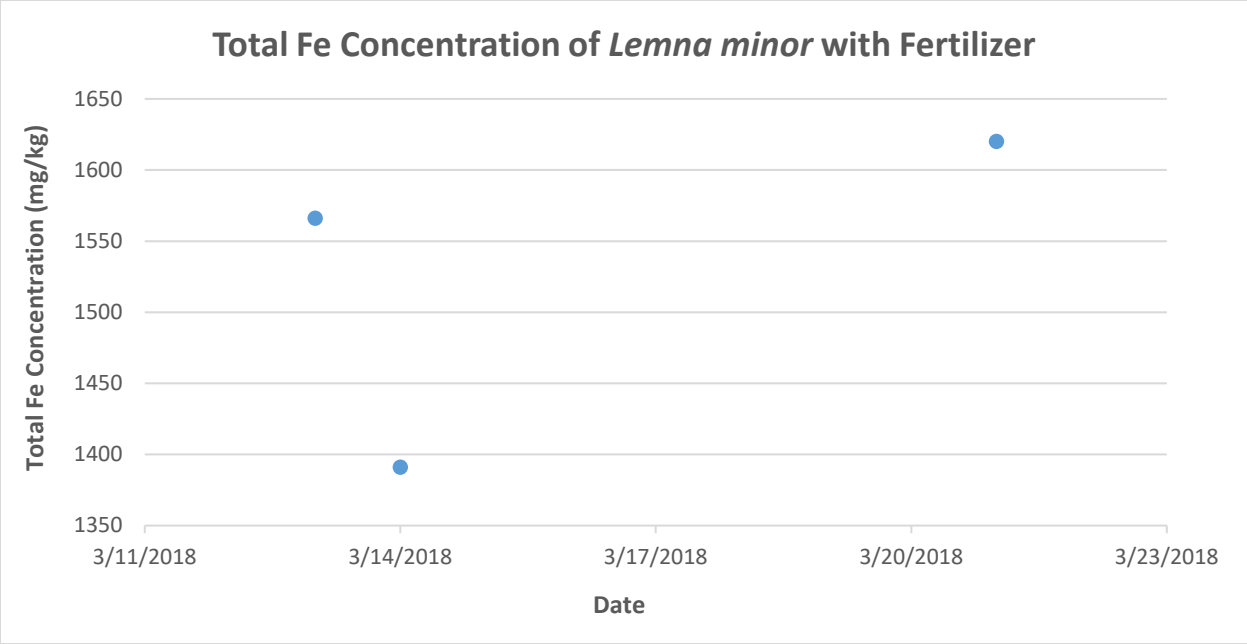
My future career plans are inclusive of obtaining my master's degree, followed by my PhD and Post-Doc, so I can stay in academia and run my own research lab where I plan to continue studying phytoremediation and environmental contaminants. I would like to expand my research, once running my own lab, to study the effects of contaminants, not only in groundwater, but in soils as well. Along with that, I would also like to become a University Professor, which will allow me to essentially train the next generation of scientists.

Below are scatter plots representing my preliminary results.




Intermediate Fe Concentration
Total Fe Concentration (mg/kg)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
3/11/2018
3/14/2018
3/17/2018
3/20/2018
3/23/2018
3/26/2018
3/29/2018
4/1/2018
4/4/2018
4/7/2018
Date


High Fe Concentration
Total Fe Concentration (mg/kg)
25000
20000
15000
10000
5000
0
3/11/2018
3/14/2018
3/17/2018
3/20/2018
3/23/2018
3/26/2018
Date


Total Fe Concentration of *Lemna minor* with Fertilizer
Total Fe Concentration (mg/kg)
1650
1600
1550
1500
1450
1400
1350
3/11/2018
3/14/2018
3/17/2018
3/20/2018
3/23/2018
Date