Testing the Abilities of Native Illinois Plants to Effectively Remove Lead from Water

By:

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Abstract:

In today's world, there is an increasing number of contaminated environments that are in need of remediation efforts. While traditional remediation methods have been proven to be effective, they are not always the best option in terms of cost, appeal, and additional side effects that can occur. While more natural methods of remediation exist, little research has been conducted on them, causing them to not be readily known as possible options to undertake for remediation of a contaminated site. One such method is phytoremediation. This method involves using live plants to successfully remove heavy metals and other organic and non-organic pollutants from contaminated soils and waters. This experiment tested the ability of native plants in Illinois to remove lead from polluted waters. Five species of plants were tested and are as follows: The Water Hyacinth, Sawtooth Sunflower, Nodding-Bur Marigold, Common Buttonbush and Lizards Tail. The Water Hyacinths, Sawtooth Sunflowers, Common Buttonbush and Nodding-Bur Marigolds successfully grew in a hydroponic system. The first three were able to uptake an average of 24.59 ppm (0.02459 g/kg), 0.865 ppm (0.000865 g/kg), and 0.555 ppm (0.000555 g/kg) of lead respectively. Through the use of a T-Test, these plants statistically demonstrated the ability to be used as potential remediators for sites contaminated with lead. With this study and additional extensive research on these plants and the remediation technique, phytoremediation has the potential to grow into a more widely used and accepted method of remediation for contaminated areas.

Introduction:

Heavy metal contamination is becoming a more frequent and large-scale issue across the world. It is mainly seen in soils, but it can also be found in plants and water. This contamination can stem from several sources such as fertilizer and pesticide use, wastewater, mining and other industrial uses (Okieimen & Wuana, 2011). These metals can be found in all three phases which makes it difficult for the current state of soils to be fully assessed and furthermore makes it difficult to decide if and how to treat the affected areas. Traditional remediation techniques can be placed into several categories; Isolation, Immobilization, Toxicity/Mobility Reduction, Physical Separation and Extraction (Okieimen & Wuana, 2011). Each category can then go on and be broken down into several sub-categories (Okieimen & Wuana, 2011). Although there are numerous techniques to choose from when it comes to heavy metal remediation, costs, effectiveness, long-term solution abilities and overall feasibility play a huge role in deciding how an area will be treated. While many of these techniques can be effective the associated costs and risks make those forms of treatment not possible or unwanted, which leads to contaminated areas not being treated completely or ignored and not seen as a current threat to plants, animals, humans or the environment. One of the most reliable techniques, in terms of cost and feasibility, belongs to the toxicity and or mobility reduction category and it is known as phytoremediation.

Phytoremediation is a biotechnical tool used to remove or rehabilitate contaminated soil and water (Golubev, 2001). The process involves the use of living plants known as hyperaccumulators to uptake organic and inorganic contaminates and either store them in leaves and stalks or convert the contaminants into less harmful forms (Pilon-Smits, 2005). Studies on this method have said that "currently, phytoremediation is an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil and water. This technology is environmental friendly and potentially cost effective" (Tangahu V, et al., 2011, p. 1) As a cheaper and greener method of cleaning up a heavily polluted area, phytoremediation can return an environment to a safe and natural state. It also takes advantage of using plants that are already natural available or easy to obtain to perform the remediation while equipment or chemicals that would be needed for other methods are not a limiting factor. Since a wide variety of plants can be used, based off the site-specific needs, the remediation could practically be invisible to those who live locally as well as any who are unaware of treatment being carried out. Another turn-on to this technique is that it can be carried out on site. The soil or water that is contaminated does not need to be collected and transported to offsite labs or other facilities to receive treatment, which makes the area easier and faster to treat and prevents the unwanted spread of the pollutants.

While phytoremediation is not a new technology, it is one of the remediation techniques that has not been widely studied or tested. Few studies have been conducted on the ability of plants to absorb metals both in soil and water, and have demonstrated that phytoremediation is not just limited to treating contaminated soils. One study involved the use of three aquatic species of plants to treat municipal waste water (Kamal, Ghaly, Mahmoud, & Cote, 2004). "All the three plants were able to remove Fe, Zn, Cu, and Hg from the contaminated water. The average removal efficiency for the three-plant species was 99.8%, 76.7%, 41.62%, and 33.9% of Hg, Fe, Cu, and Zn, respectively" (Kamal, Ghaly, Mahmoud, & Cote, 2004). The high efficiency rate for the three plants in this study alone provide great support for implementing phytoremediation techniques for water treatment. While this waste water study and other supporting research have demonstrated the ability of plants to accumulate many different metals, one metal stands out from the rest when it comes to water and that is lead.

Lead is one of many heavy metals that has been known to cause serious health issues for humans, plants and animals. It exists naturally in many things in low concentrations that do not pose serious threats, but once levels become elevated, major health issues can arise. As a heavy metal, lead is toxic and exposure to it should be very limited as it can cause severe health issues as well as lead to death. The Center for Disease Control (CDC) states that lead can affect nearly every system in the human body and exposure is nearly impossible to see. Exposure to lead on a short and long-term case can cause anemia, kidney and brain damage, high-blood pressure and infertility (CDC, 2017). When exposure to lead is prolonged or in a high concentration, lead poisoning can occur. In a 1994 article on lead poisoning, it states that in the United States lead poisoning was "the most common disease of environmental origin" (Landrigan & Todd, 1994). While this disease can be treated, it can leave behind lasting health effects, like permanent neurological or kidney damage.

Children are affected by lead exposure and poisoning more than adults as they cannot tolerate as much lead in their bodies. Serious neurological issues have been linked to lead exposure or poisoning in children (CDC, 2017). When in the womb, overexposure to lead can cause premature births and stunted growth (Landrigan & Todd, 1994). Young children exposed to lead can have delayed growth and impaired neurological development (Landrigan & Todd, 1994). Since there is no set "safe" level of lead that can be in childrens' bodies, it is extremely important to reduce and prevent any possible route of exposure to lead they could come by. Lead exposure has the same effects on animals as it does humans. When it comes to plants, phytotoxicity can occur which can stunt the growth of plants, prevent germination and lead to deformities. If the amount of lead or other heavy metal is too high for the plants to handle, it will kill them. When areas are found to have high levels of lead, it is of major concern for the overall health of the area that measures are taken to reduce the levels of lead.

In recent years, lead has been in the headlines more frequently for water contamination. One of the largest well-known instances occurred in Flint, Michigan. While the crisis began in

2014, it did not come to public attention until 2016 (CNN Library , 2017). The local drinking water source was changed and when it began to flow through the outdated water delivery system, it caused the water to become contaminated with lead (CNN Library , 2017). A state of emergency was announced in early 2016 that left the residents of Flint using bottled water for drinking, bathing, cooking, and cleaning as the lead levels were dangerously high (CNN Library , 2017). Although this documented lead contamination and many others were in part caused by old lead pipes and connectors, other water supplies such as lakes, ponds, rivers and streams can become contaminated with lead too. This type of contamination is caused from industrial or agricultural practices and include untreated wastewaters, fertilizers and pesticide use, soil leaching and more. Lead or any other heavy metal can be extremely detrimental to an ecosystem in large untreated amounts since they can harm every organism that encounters the water source. Since these heavy metals have little to no natural benefit to water sources, it is imperative for the metals to be removed as quickly as possible before serious damage occurs.

Literature Review:

In Orissa, India a study was conducted on the wastewater of the Sukinda chromite mines. The wastewater from the mining activities of Sukinda was found to contain high levels of hexavalent chromium, which can cause serious health issues if inhaled or ingested (Saha, Sarkar, & Shinde, 2017). Since India is still a developing area in most parts, there is little money to provide proper treatment for such wastewaters. As a low-cost alternative to traditional costly chemical-based remediation, phytoremediation using Water Hyacinth was tested to see if the technique could be used to treat this wastewater (Saha, Sarkar, & Shinde, 2017).

The Water Hyacinth was chosen for this study because of the following characteristics. The Water Hyacinth plant is a known hyperaccumulator, produces a large amount of biomass, has a very high tolerance to growing in polluted waters and as an aquatic plant, it was an easy choice to use for a water remediation experiment (Saha, Sarkar, & Shinde, 2017). It is for these same reasons why Water Hyacinths were chosen to be the positive control in my experiment. For this experiment performed in India, Water Hyacinths were collected from Jamshedpur, India and placed in tanks with previously tested wastewater from the Sukinda chromate mines (Saha, Sarkar, & Shinde, 2017). A series of plastic tanks containing 5 and 100 liters of wastewater were set up, plants placed in them and the tests were run for 0,5,10 and 15 days (Saha, Sarkar, & Shinde, 2017). The results of this study showed that the Water Hyacinth was in fact effective in reducing the concentration of the hexavalent chromium.

This study also contained a standardized test of the Water Hyacinth, in water that was tainted with known concentrations of hexavalent chromium (Saha, Sarkar, & Shinde, 2017). The procedure was the same as above and those results also showed the Water Hyacinth being efficient in removing the hexavalent chromium from the water. At the low concentration level of 0.25-2 ppm, the plant was able to remove around 90% of the hexavalent chromium from the water (Saha, Sarkar, & Shinde, 2017). As the concentration increased, the plants were able to still remove similar amounts of the hexavalent chromium, but it took several more days for them to do so (Saha, Sarkar, & Shinde, 2017). At a 0.5 ppm concentration it only took 8 days for 99% of the hexavalent chromium to be removed, while at 0.75-2 mg/L it took between 10-15 days (Saha, Sarkar, & Shinde, 2017).

While the plants in this study were previously growing in another area before being taken to the lab setting, in the proposed experiment the plants were grown from seed. This is so the plants were completely acclimated to growing in aquatic conditions. It was also being done so there was more control over what the plants were up-taking during the growing process and have a better understanding of what the plants contained when it came time to analyze the biomass contents. Although this experiment used hexavalent chromium as the contaminate, the results for using lead as the contaminate for the water hyacinth are projected to be similar as this study as well as several others note that water hyacinth is very effective in removing heavy metals from contaminated waters.

In another similar study, the ability of the Chinese Cabbage to accumulate lead from contaminated soil was tested (Corley & Mutiti, 2017). This study was born from the concern over lead exposure through food (Corley & Mutiti, 2017). Since numerous plants can uptake heavy metals and other contaminates through their roots while still growing and maintaining a heathy stature, it can be difficult to see that those plants have lead in them. This leads to the plants being harvested and sold for consumption to unknowing consumers who will be exposed to whatever was absorbed during the growing period (Corley & Mutiti, 2017). When there is an exposure to heavy metals, it can have serious health effects and even lead to death if the level of exposure is high enough.

Three different lead compounds, lead nitrate, lead carbonate and lead sulfide were used in varying concentrations to contaminate soil that the Chinese Cabbage was then grown in (Corley & Mutiti, 2017). The cabbages were allowed to grow in the contaminated soils for ten weeks, with samples of the cabbages being taken after four, eight and ten weeks of growth (Corley & Mutiti, 2017). The soil concentrations were broken up into three levels, 400-mg kg^{-1}, 550-mg kg^{-1} and 600-mg kg^{-1} and the actual concentration of lead in each soil treatment fell within a range between each level (Corley & Mutiti, 2017). It was found that the highest lead accumulation by the cabbage came from the 600-mg kg^{-1} range after four weeks of growth (Corley & Mutiti, 2017). The overall average for the three levels of concentrations for four and eight weeks of growth was 20-mg kg^{-1} (Corley & Mutiti, 2017). While the highest accumulation occurred in the 600-mg kg^{-1} range, the cabbages grown in the lower concentrations were still able to accumulate significant amounts of lead but not until after eight weeks of growth (Corley & Mutiti, 2017).

While this study was conducted using soil as the contaminated growing median, the results are still comparable to using water as the contaminated growing median as plants can grow just as well in water as they can in soil. The lead nitrate treatment did not have the highest overall uptake by the cabbages, but it still showed the ability to be taken up by plants which is why, with this study and the chemical properties of lead nitrate, it is the contaminate of interest for this phytoremediation experiment. Since the plants for my experiment should grow similar to how they would in soil, it will be noted how there could be a possible lead uptake cap that the plants could reach like the cabbages in this study did. While the plants that were chosen are different than the cabbages used in the experiment, based off the overall physical uptake abilities of plants, the experimental plants should reach a point to where lead uptake is at a maximum, but the plants continue to produce more biomass.

As previously mentioned, lead pollution and exposure can cause serious health effects. In a case study conducted in 2001, a 66-year-old man from Australia suffered from lead poisoning for 2 years (Mangas, Visvanathan, & van, 2011). The man had been in and out of numerous hospitals due to abdominal pain where he received CT scans, ultrasounds, endoscopies, colonoscopies, barium enemas and constipation treatments with no results or pain relief occurring (Mangas, Visvanathan, & van, 2011). Other health issues such as dramatic weight loss, personality change, memory impairment, drowsiness, difficult food retainment and more, none of which could be explained by the conducted tests and treatments (Mangas, Visvanathan, &

van, 2011). The blood examination showed that the man was anemic and had basophilic stippling, which is a usual sign of lead poisoning (Mangas, Visvanathan, & van, 2011). Upon being tested for lead poisoning, the man had a blood lead level of 98 mu g/dl, ten times the national goal set for lead levels in Australians (Mangas, Visvanathan, & van, 2011).

The extremely elevated level lead to a thorough investigation of the man's lifestyle. He had worked at a tool factory before being hospitalized for the lead poisoning (Mangas, Visvanathan, & van, 2011). An investigation there reveled no lead sources from the machinery or tools used by the man, so the investigation then switched to his home where the water supply, household items, food, and homemade items were tested for abnormal lead levels (Mangas, Visvanathan, & van, 2011). Upon testing some homemade wine, it was found that the two batches had significantly high levels of lead (Mangas, Visvanathan, & van, 2011). In Australia, the lead level limit in wine is 0.2 mg/L. In samples taken from one batch of wine, the lead levels were 14 mg/L and 55 mg/L and in the other batch 4.5mg/L (Mangas, Visvanathan, & van, 2011). All the samples greatly exceed the set standard and were determined to be the cause of the man's lead poisoning as he had consumed a large amount of the homemade wine (Mangas, Visvanathan, & van, 2011). While most wines contain small amounts of lead, the levels of the homemade wine were exceedingly high. To figure out the source of the lead the investigators tested all the equipment and materials used by the man (Mangas, Visvanathan, & van, 2011). They found out that the tub used to hold crushed grapes for 7 days to pre-ferment was corroded and lead was leaching into the grapes (Mangas, Visvanathan, & van, 2011).

Although this is a less frequent route for lead exposure and poisoning, this case study and many others demonstrate the ability of lead to find its way into the everyday life of people. Whether it is in food that was grown in contaminated soil, water that flows through an outdated lead pipe system, inhaled dust particles from factory settings, or a bathtub used to make wine, lead is an ever-present threat and can cause serious issues. While the man was able to receive treatment, and get rid of many of his symptoms, that is not always the case with some people. Lead effects everyone differently and can at first be invisible to medical professionals, since lead poisoning is a less common disease to encounter. It is for this reason as well as numerous others, that lead needs to be under better management practices as well as have faster and stronger clean-up methods for places or objects that contain high levels of lead. Lead can be seen as a silent killer and without proper measures being taken to control its levels of exposure, it can greatly damage an entire area or population in a short amount of time.

Experimental Inspiration

When heavy metal contamination in an area shows signs of posing serious threats to human health, the Environmental Protection Agency can declare the area a superfund site and have it places on the National Priority List (EPA, 2017). Once declared, these areas are put under management of the EPA to be treated. My hometown of DePue, IL can be seen as one of the EPA's listed superfund sites in the U.S (EPA, 2017). The town was home to the New Jersey Zinc/Mobil Chemical zinc smelting facility and phosphate fertilizer plant. During their time of operation, untreated waste was discharged into the nearby lake, Lake DePue. Once the companies shut their doors, a huge slag pile was left on the property, full of numerous heavy metals that have leached into nearby homeowners' yards as well into a small wetland that connects to the lake. This contamination has been going on since the late 1960s and it is still happening to this date as the slag pile still lies, in its entirety, at its final resting location.

Few cleanup options have been proposed for the soil, such as the offer to residents to have their yards excavated and replaced with new soil and grass. While that is a notable clean-up proposal, it is expensive, unsightly, and leaves room for contaminates to be missed or other damages to the area to occur. The excavation method may seem like an easy simple fix for the soil, but when it comes to the lake the water cannot just be removed and replaced and since there has not been a cleanup proposal, phytoremediation offers a solution to removing the heavy metals in the lake.

As a low cost and more eco-friendly option, phytoremediation has proven to be a reasonable and successful cleanup technique as well as a more aesthetically-pleasing one (Dietz & Schnoor, 2001). By finding ways to remediate the area, interested partisans or other organizations can use similar techniques for contaminated areas and help rehabilitate those ecosystems. Figures 1 and 2 show a breakdown of the superfund and NPL sites just in the US that are still in need of remediation. The costs and benefits for phytoremediation alone show that there would be little loss or risk with performing this method of cleanup as plants are relatively cheap and easy to care for and allow for the treatment to be hidden from plain sight. The heavy metals have already affected the lake as it is advised to not the eat the fish caught from the lake or swim in the lake. Thirty years ago, the lake was healthy enough for all recreational and industrial activities and now it is primarily used for boating by few people. While the lake is not a determining factor in the success of the town like it used to, it is still important to preserve the natural freshwater source and its surrounding environment.

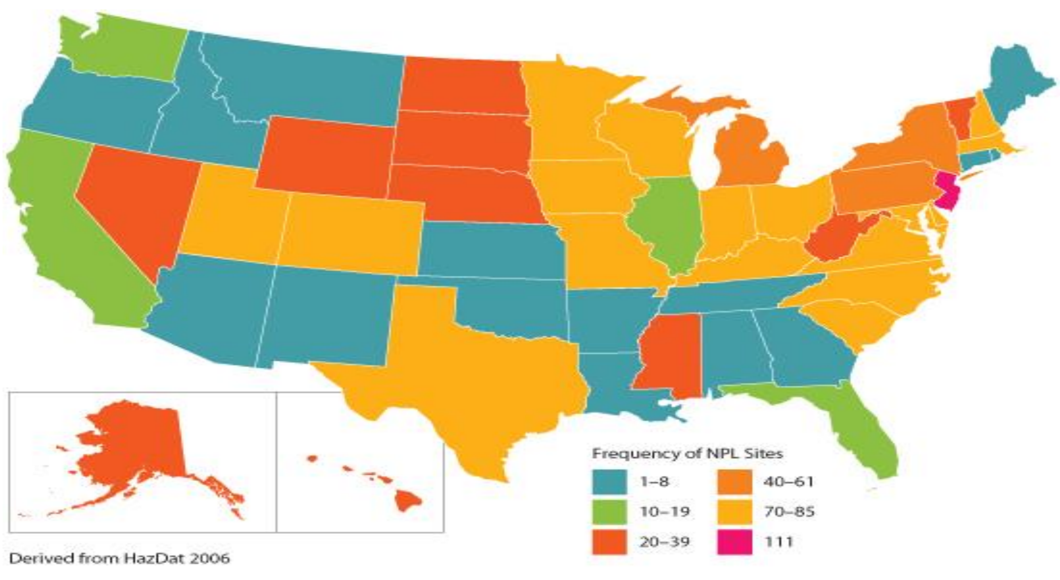

Figure 1: Frequency of NPL sites across the US, with blue being under ten and pink being over 110 sites per state (EPA, 2007)

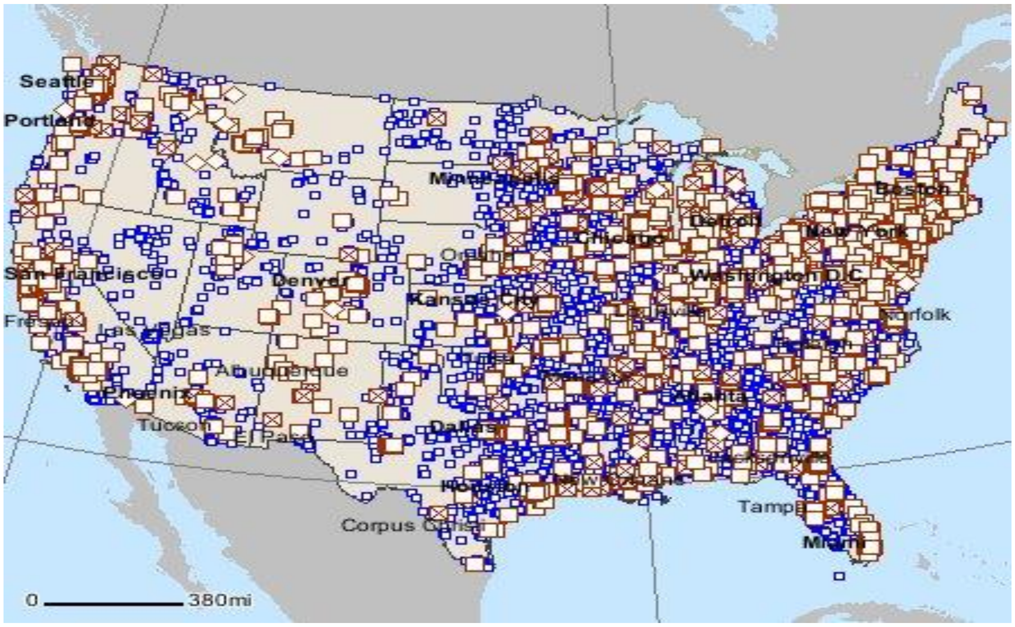

Figure 2: The red boxes display the declared superfund sites in the US. Retrieved from: https://toxmap-classic.nlm.nih.gov/toxmap/combo/mapControls.do

Experimental Overview

As previously stated, phytoremediation has only been tested on small scales for water treatment. As a technique that is beginning to gain interest and popularity, it is important for more controlled studies to be conducted on plants that can accumulate lead. This leads to the technique gaining more support and credibility for its implementation on sites that require remediation efforts to be taken. With that idea in mind and the inspiration from my hometown, my proposed experiment was to look at the ability of native plants to Illinois to remove lead from water. To test this idea and mimic water treatment, hydroponic systems were used and can be seen below.

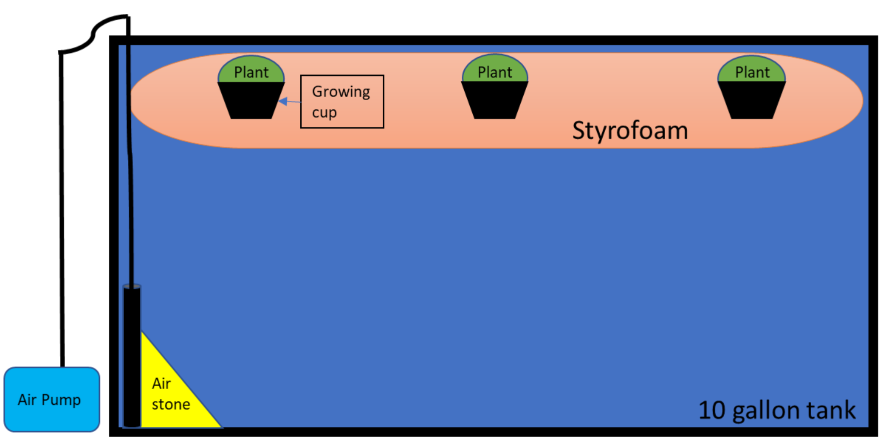

Figure 3: A typical hydroponics system involves a tank, air source and plants. For this experiment, ten-gallon tanks were used along with basic air stones and Styrofoam to hold the growing cups with the plants above the water. While many systems have a nutrient delivery system, nutrients will be directly added when needed.

Hydroponics is a growing technique that involves directly growing plants in water instead of soil. There are several types of hydroponic systems ranging in difficulty, space and time requirements, and costs. For this experiment, a basic hydroponic system was used. Hydroponics, when properly setup, allows for the fast and easy growth of plants as they are in constant contact with water, light, and nutrients. This system can provide plants with everything they need to successfully grow. In traditional soil methods, plants can be limited or have to compete with other factors to obtain the water, nutrients and light they require. Due to the interest of phytoremediation on an aquatic level, a hydroponic system allowed for the greatest growing and treatment median to mimic the conditions plants would face if used on a lake or pond cleanup. While the systems are controlled for the experiment and artificially contaminated water is used, water found in nature that is already contaminated could potentially be obtained and used in these hydroponic systems to test treatments possibilities.

With many of the well-known hyperaccumulators being invasive species, the question of whether this technique, if used in the field, can be properly performed without allowing the invasive plans to spread arises. Invasive species are any organism that is not native to an area, and that if introduced, can have severe effects on an ecosystem. Many invasive plant species out-compete native species for resources and can immediately take over an area. During remediation efforts, these plants would be grown in the soil or water. If not properly regulated, they could spread and colonize the area, which would have devastating effects. By looking to use native species, the high risk of using invasive species is gone. If the native species were able to spread outside of the remediation site, there would not be any serious effects or changes to the local ecosystem.

Many of the known hyperaccumulating plants can handle more than one heavy metal and other contaminates. While the lake in my hometown is contaminated with more than one heavy metal, lead is one that can be related to more on a public sense. Lead-tainted drinking water has made national headlines numerous times, especially in recent years, and the effect of lead on human health has been a widely studied topic for hundreds of years, making it a very well-known metal. For that reason, lead, in the form of lead nitrate, was the only metal tested in this experiment.

The overall goal of this study is to test the possibility of plants native to Illinois to be used in aquatic phytoremediation efforts. Furthermore, I looked to answer my original study question of: Can phytoremediation be used on large scales, such as on lakes? I hypothesized that all of the selected plants would be able to grow in these hydroponic systems and for those that grew, they would all be able to uptake lead.

Methods:

Five different species of plants were used for this study. One of the five species used was an invasive, the Common Water Hyacinth (*Eichhornia crassipes*) and due to its known remediation abilities, it was the positive control. The other four species of plants are native to Illinois and are as follows: Sawtooth Sunflower (*Helianthus grosseserratus*), Nodding Bur-Marigold (*Bidens cernua*), Common Buttonbush (*Cephalanthus occidentalis*), and Lizard’s Tail (*Saururus cernuus*). The first two listed are more traditional terrestrial plants that can grow in wet conditions while the other two are aquatic plants. Each plant, fully grown, can be seen below.

The Common Water Hyacinth
Eichhornia crassipes
Photo obtained from:
http://www.bbc.co.uk/blogs/gardening/2011/11/a-drop-of-exotica-water-hyacin.shtml

Sawtooth Sunflower
Helianthus grosseserratus
Photo obtained from:
http://www.illinoiswildflowers.info/prairie/plantx/swt_sunflowerx.htm

Nodding Bur-Marigold
Bidens cernua
Photo obtained from:
https://www.prairiemoon.com/bidens-cernua-nodding-bur-marigold-prairie-moon-nursery.html

Common Buttonbush
Cephalanthus occidentalis
Photo obtained from:
https://gobotany.newenglandwild.org/species/cephalanthus/occidentalis/

Lizard's Tail
Saururus cernuus
Photo obtained from:
http://www.ibiblio.org/carrborocitizen/flora/2009/06/step-softly-among-the-water-dragons/

Lead nitrate was obtained, and fourteen 0.5000-0.5005 g samples were measured out using weigh boats and an analytical balance. Each sample was dissolved in deionized water (DI) while in the weigh boat and then quantitively transferred to a 125 mL Erlenmeyer flask where the solution was diluted with 100.00 ml of DI water. The original lead nitrate concentration for each tank was ~14.7 ppm. Next, twelve ten-gallon fish tanks were obtained, placed in the school's greenhouse and filled with around nine gallons of DI water. Each tank was given its own air stone to provide a constant flow of oxygen into the water. A visual representation of the greenhouse set-up is in Figure 4.

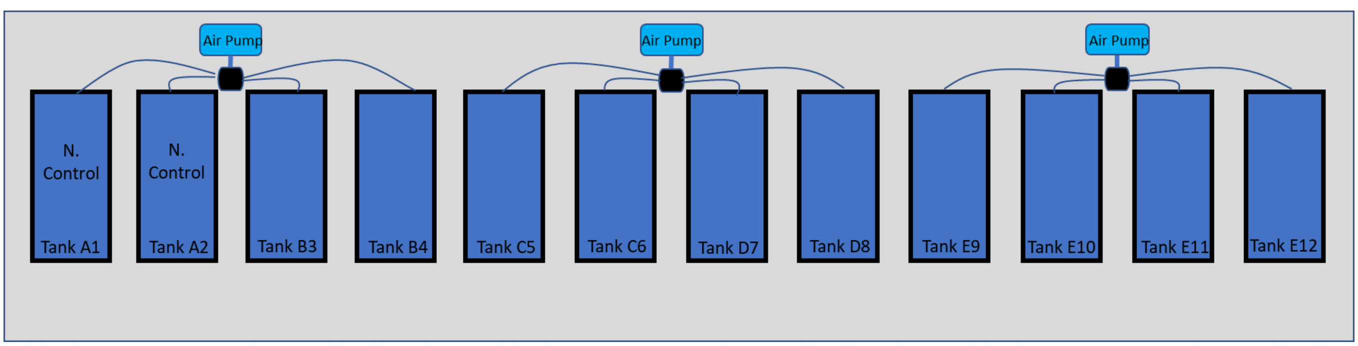

Figure 4: This diagram shows the greenhouse setup with twelve ten-gallon tanks lined along the table. The letter and number label combination are used to show the replicates for the negative control and the four native plants. There are four E tanks for the time-lapse experiment. The labels also show how much lead was added to each tank.

After the tanks were set up, 3 seeds of each plant were placed in growing sponges inside plastic growing cups. The growing sponge acts as a medium for the plant roots to attach to like they would if they were growing in soil. The plastic cups were 2 inches deep and can be seen in Figure 5. The cups were then placed in a Styrofoam sheet that assists in holding the plant up on top of the water. Ten cups for each species of plants were added to their own tank and labeled accordingly. One tank for the experimental set-up in the greenhouse can be seen in Figure 6.

Figure 5: A growing cup with a growing median cube placed inside

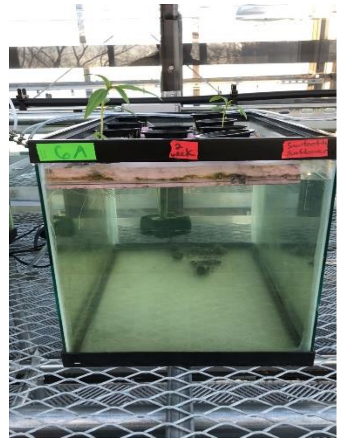

Figure 6: A complete set-up of one of the tanks. Included is the air stone, Styrofoam and growing cups containing seeds.

The Water Hyacinth could not be purchased from seed and therefore could not be placed in the greenhouse. Two ten-gallon tanks were placed in a climate controlled classroom, filled with nine gallons of DI water and given their own air stones. This set-up is depicted in Figure 7. Due to their biological adaptation for water, the Water Hyacinths were able to float on their own. Since the plants had to be transported live, they did not all arrive in similar health with half of the plants having lost their root systems. In order to have one complete set-up for the control, the ten plants that still had their root systems were placed in the same tank while the ten plants that lost their roots were put in the same tank. This was done in hopes of having at least ten samples to test after six weeks.

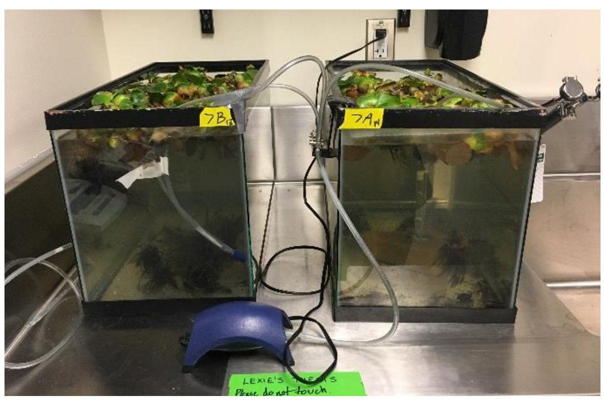

Figure 7: The Water Hyacinth tank setups

Once all of the plants were placed in the water, the lead nitrate solutions were quantitatively added to each tank and the total volume level in the tank was marked. Next, six pellets of the fertilizer in Figure 7 were added to each tank which supplied nutrients for the plants for the duration of the experiment. Every two days the tanks were checked, and additional DI water was added when the level dipped below the initial marked volume line.

Figure 6: The brand of fertilizer used to supply nutrients to the plants.

The plants grew undisturbed for six weeks. After six weeks of growth, the plants were removed from the water. For the plants that grew, they were removed from the growing cups and each species was placed in its own jar and dried in the oven. The method used for lead analysis was EPA Method 200.7, Revision 4.4. Due to the short growing period for the plants, there was not enough biomass to fulfill the sample size called for by the EPA. To have testable samples, every plant grown in a tank was ground up together with a mortar and pestle to increase overall sample size. The EPA method called for a 1.0 g sample from each plant. The only plants with enough biomass for a 1.0 g sample were the Sawtooth Sunflowers and Water Hyacinths. The Nodding-Bur Marigold and Common Buttonbush did not grow enough to produce a combined sample biomass over 1.0 g. All of the biomass that could be recovered from these plants were used.

To prepare the ground-up plants for analysis, they were placed in individual beakers where 4 mL of (1+1) nitric acid[1] and 10 mL (1+4) hydrochloric acid[2] were added to each beaker to breakdown and liquify the plants. These solutions were then boiled for 30 minutes. After boiling, the solutions were quantitatively transferred into 100 mL volumetric flasks and diluted to the mark with ultra-pure DI water. A sample from each solution was then taken and ran through the Microwave Plasma Atomic Emission Spectrometer (MPAES) to detect and measure any lead present.

Five water samples from each of the negative control tanks were taken. To prepare for analysis, each sample had to be acidified to a pH of ~2 with (1+1) nitric acid. Next, 50 mL of water was taken from each sample and transferred to separate beakers. To each beaker, 1 mL of

[1] (1+1) Nitric Acid solution consists of 50 mL Nitric Acid and 50 mL ultra-pure DI water.

[2] (1+4) Hydrochloric Acid solution consists of 20 mL Hydrochloric Acid and 80 mL ultra-pure DI water.

(1+1) nitric acid and 0.5 mL of hydrochloric acid was added and after, the samples were heated and reduced in volume to 20 mL. Once at 20 mL, the samples were transferred to 25 mL volumetric flasks and diluted to the mark with ultra-pure DI water. These samples were then run through the MPAES to measure the lead concentrations. As the negative control, the data from the water samples was not used in the results as theoretically, the concentrations should have stayed around 14.7 ppm. I also decided to leave the water samples out of my results as I was more focused on the plant samples.

To convert the data from the MPAES into concentrations, two standard curves were made. A stock solution of nitric acid, hydrochloric acid and ultra-pure water was made, where a $1/20^{th}$ dilution was performed to make 8 standard solutions of known concentrations. Serial dilutions were used to make the standard solutions. The 8 standards were run twice through the MPAES, once in the beginning and once at the halfway point between my experimental samples to increase the quality of the standard curves when using the best fit line to solve for unknown concentrations. The 8 standards had lower and upper detection limits for measured intensities of ~400 and ~55,000, respectively. Once the plant samples were analyzed, the Nodding-Bur Marigold had measured intensities that were under 100. I decided to not include this data in the results as the best fit line equation from the standard curve would not accurately represent the true value of lead in the plant samples since they are significantly lower than the lower detection limit. The Common Buttonbush samples were slightly under the 400-intensity level, but since the numbers were within 100 of the detection limit, I decided to use these numbers in the results to have a greater comparison aspect for my experiment.

Once the numbers were converted from intensity into ppm, they were transferred into SPSS, averaged for each tank, and then ran in a One-Way T-Test. The results from the replicates were combined with the initial results for a total of ten samples for each species. The measured concentrations of lead for each species were graphed in excel.

An additional two tanks of the sawtooth sunflower were used for a time-lapse experiment for a total of four tanks with these species growing in them. The original plan was to test plant samples from a tank at 2, 4, and 6 weeks of growth to better assess the plants performance when on a timed schedule. However, the 2 and 4-week tanks had little to no growth over those times periods, therefore there was no biomass to test for lead. The 2 and 4-week tanks were allowed to grow for the remaining weeks, but no samples were taken from them at the end of 6 weeks. This was done to make the number of samples for each plant species even.

Results

After analyzing the plant samples, the amount of lead found in each sample per species was averaged. The average lead uptake was graphed in Excel to compare the performance of the plants to one another and can be seen in Figure 7. The Nodding-Bur Marigold average was excluded from this figure as well as from the following T-Test. As stated earlier, the water samples were also left out of the T-Test as I solely wanted to statistically compare the plants. Figure 8 shows the results of the one sample T-Test with p-values above 0.05, showing success in the experiment and leading to the acceptance of the proposed hypothesis that if the plant grows, then it will uptake lead.

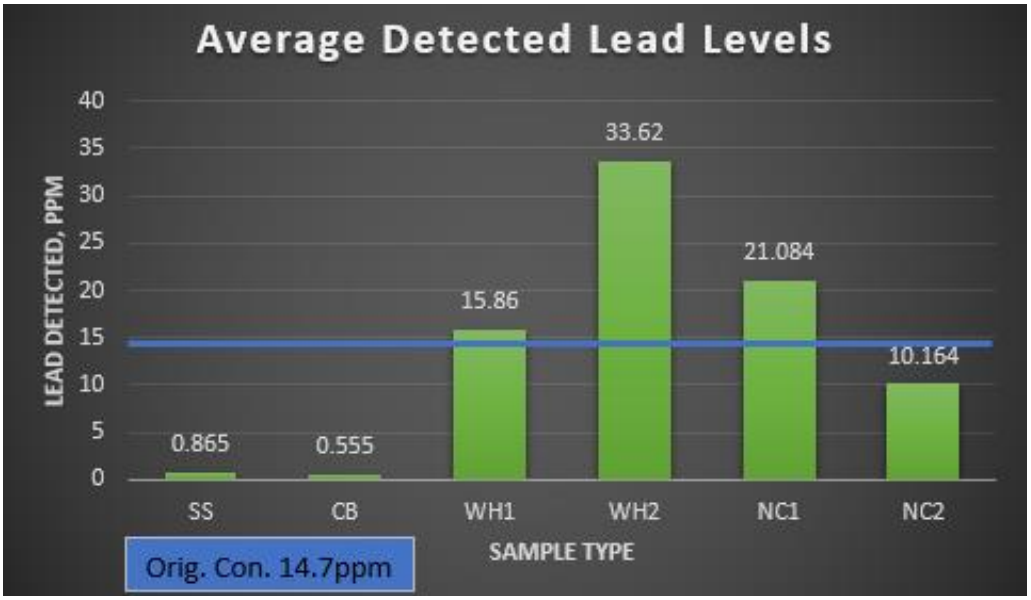

Figure 7: The average lead uptake of the plants that grew and both controls. SS: Sawtooth Sunflower, CB: Common Buttonbush, WH1-2: Water Hyacinths, NC1-2: Negative Control

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
SS	1.559	1	.363	.86500	-6.1869	7.9169
CB	5.286	1	.119	.55500	-.7792	1.8892
WH1	1.042	1	.487	15.86000	-177.5284	209.2484
WH2	3.133	1	.197	33.62000	-102.7176	169.9576

Figure 8: The results of the One-Sample T-Test, demonstrating that three species were successful in up taking lead as the p-values were greater than 0. SS: Sawtooth Sunflower, CB: Common Buttonbush, WH1-2: Water Hyacinths

Discussion

Over the course of six weeks, three out of the four plants were able to grow in the hydroponic systems. The only plant that was unable to grow was the Lizards Tail, which lead to the rejection of the first proposed hypothesis that all of the plants would be able to grow in a hydroponic system. Since the Sawtooth Sunflower, Nodding-Bur Marigold, and Common Buttonbush samples were all found to have detectable levels of lead, the second proposed hypothesis of if the plants grow, then they will be able uptake lead was accepted. A misconception that could be applied to the lead that was taken up is that since the plants were constantly in contact with the water which was also the main route for nutrients to be delivered that the dissolved lead would be taken up regardless of the plant species. While many plants can be grown in hydroponic systems, not every plant can grow in the presence of heavy metals. The plants that would either not grow in the first place or would not survive long after sprouting due to the metal poisoning the plant. To be shown as a reliable hyperaccumulator, the plant must grow and survive in these polluted environments. Due to the success of the three plants that grew, it can be said that they are capable of being hyperaccumulators, but more testing and on larger scales would have to be performed to confirm their abilities. Since the Lizards Tail did not grow, it can be hypothesized that this species cannot survive in a total aquatic system or cannot grow in the presence of lead. Other factors could have also affected the growth of this species and more testing would need to be performed if the Lizards Tail would be of interest for further studies on growing in an aquatic system and accumulating heavy metals.

Throughout the six-week experiment period, the Water Hyacinths slowly started to die, but they were still able to uptake an average of 24.59 ppm 0r 0.02459g/kg of lead. This was the highest average lead uptake among all the plants. This came as no surprise as the Water Hyacinths are well-known for their heavy metal uptake abilities, which is why they were used as the positive control in order to compare the native plants performance to them. The higher uptake average could also be attributed to the fact that the Water Hyacinths were full grown when the experiment started and could have already had lead in them or other metals that would cause the MPAES to detect it as lead. With their well-developed root systems and large size, it is probable they were able to uptake more lead since the remaining plants had to grow from seed and did not have enough time to develop as much as the Water Hyacinths. They also grew in a separate environment from the other plants, which could have affected the lead uptake as well. To have a true comparison among the different plant species, the Water Hyacinths would need to be grown from seed alongside the other plants in the same environment.

When assessing the performance of the native plants that grew, the Sawtooth Sunflower had the highest uptake average of 0.865 ppm or 0.000865 g/kg. This means that the Sunflowers were able to uptake 5.88% of the original lead concentration in the tank. This came as no surprise as members of the sunflower family are known hyperaccumulators. The Common Buttonbush had the second highest uptake of the natives at 0.555 ppm or 0.000555 g/kg. This comes out to 3.78% of the original concentration of lead. While these percentages are small, they show that the experiment was successful in finding lead in the plant sample. To further confirm the abilities and find the full potential of these plants, repeated studies would need to be done.

While the Sawtooth Sunflowers took in the most lead, the Nodding-Bur Marigolds had the most growth in terms of plants that sprouted. Out of the twenty total growing cups with seeds, each cup successfully sprouted. Some of the plants did not completely break through to the surface from the growing sponge, but the seeds inside did sprout and if given more time to grow, would have broken through to the surface. The Sawtooth Sunflowers had a total of nine plants grow and four of them were over three inches tall with well-developed root systems. With the size of the root systems, it is possible that there was too much competition for nutrients among the plants and only a few of the plants could successfully grow. A larger tank or less plants per tank could be used to see if this is a possible explanation as to why few plants grew. The Common Buttonbush only had four plants sprout which can attribute to the low trace of lead found in the samples. The plants that did grow were very small, so a competition for nutrients may not be the reason as to why few grew. To test this theory of competition, tanks of varying sizes and amount of plants per tank could be used in a future study. When looking to perform this method of remediation, the amount of plants that will grow becomes an important factor to review, especially when it comes to the size of the site being remediated.

The combined water samples from both negative control tanks had a lead average of 15.624 ppm. When looking at each individual tank, tank 1 had an average of 21.084 ppm while tank 2 had an average of 10.164 ppm. This is 143.43% and 69.14% of the original concentrations, respectively. When starting the experiment, I believed that the concentrations would remain the same or be at least slightly smaller. These percentages, however, show that the concentrations in both tanks drastically changed over the course of 6 weeks. The unexpected amounts of lead can be attributed to several causes. Where the tanks were in the greenhouse, a large fan constantly blew on the two tanks and lead to increased evaporation. This could have led to some of the lead being lost from the tank through evaporation. It is also possible that with the constant changing water level the lead could have solidified again and stuck to the side of tank which would have caused that amount to not show up in the samples that were taken. It is also possible that some of the lead was absorbed into the sponge that held the air hose under the water. The sponge was washed with DI water and wrung three times to get as much of the original lead solution out, but some could have remained trapped in it. Lastly, when refilling the tanks to make up for the evaporated water, the original water could have overflown from the tank onto the floor when new water was dumped in. To explain this loss of lead, tanks not in front of a fan could be tested in the same manner as well as different air hose holders that don't absorb water. More care can also go into pouring in water to the tanks to prevent spills. Tank 1 had over 100% of the original concentration of lead. The best possible explanations could be that the original concentration of this tank was higher than 14.7 ppm or there was some form of cross contamination between the tanks.

The T-Test was run to statistically show if the experiment was successful in the sense that there were detectable levels of lead in the plant samples. A test value of zero in this experiment shows that any p-value over zero was significant. Figure 8 shows that every p-value was above zero and therefore significant and successful in proving the hypothesis that if a plant grows then it will absorb lead. The Sawtooth Sunflowers had a p-value of 0.363, Common Buttonbush at 0.119, and the two Water Hyacinth samples had p-values of 0.487 and 0.197. When this experiment was initially formed, the idea was to individually compare the lead up-take performance of each plant against each other with an ANOVA. This was to see what plant(s) performed the best and should be further tested to find its true phytoremediation potential. Since plant growth is sporadic, it was not possible to plan for the potential plant growth. Due to the uneven plant growth, the original plan had to be replaced by the use of the T-Test. To use the original plan, more tanks and plants would need to be used in order to have more plant material to test.

The proposed time lapse experiment was unsuccessful. After two weeks of growth, there was one small plant growing in the two-week tank. After four weeks of growth, there was only 3 small plants growing in the four-week tank. It was decided that there was not enough biomass to test for any traces of lead, so these tanks were put aside, and no samples were taken to be tested from them. For the time lapse to work, a longer total experimental time frame would be necessary. The Sawtooth Sunflowers that did grow did not really show significant growth until four and a half weeks in.

Overall, the experiment was successful as the results showed that plants native to Illinois are capable of removing lead from water. While three of the four plants grew, the ones that did grow were all found to contain traces of lead. The most successful species, in terms of lead accumulation, was the Sawtooth Sunflower and the most successful species to grow in the hydroponic system was the Nodding-Bur Marigold. Based off these initial results, it can be concluded that the Sawtooth Sunflower and Nodding-Bur Marigold have the potential to be used as hyperaccumulators to remove lead from contaminated water. Longer and larger scaled experiments with other variables such as more heavy metals, additional pollutants and nondeionized water would have to be conducted on these two plants to further demonstrate their potential as well as find their limitations before being recommended as plants for site remediation. It is possible that these plants could be used to remove lead and possibly other heavy metals from a lake or pond like the one from my hometown, but as already stated further tests would need to be conducted before recommending these plants for this type of remediation.

Future Directions and Improvements

While this experiment was statistically successful, there are several steps that could be taken to improve upon the experimental setup and overall study. If this experiment was run again one of the main changes would be a longer time frame. While six weeks was the longest possible time frame that could be achieved for this experiment, the initial ideal time frame was ten weeks since for most plants, that is a significant amount of time for growth. A longer growing period would have allowed for the ANOVA to be run and for a comparison amongst the plants to be conducted. This comparison would help show which plant was the most successful, statistically, and should be pursued for further phytoremediation research on using native plants to remediate contaminated sites. The longer growing period would also allow for more biomass to be obtained to more accurately follow the EPA Method. The Sawtooth Sunflowers and Water Hyacinths had enough biomass for a 1.0 g sample, but the other plants fell short. With the amount of acids and water added used to form the sample solutions being scaled for a 1.0 g plant sample, the solutions were not accurately made for the plants that fell below a 1.0 g sample weight. It was decided that scaling down the acids added to the plant samples would be too difficult and time consuming. If this experiment was conducted again, I would make sure there is enough plant matter to accurately make the sample solutions, as well as have more samples for each plant.

As previously mentioned, to have the true comparison for the Water Hyacinths, growing them from seeds at the same time as the other plants could be done. As a heavily regulated invasive species, it is difficult to obtain Water Hyacinth seeds, so a different well-known hyperaccumulator with seeds that can be purchased could be used as the positive control instead. Also, to prevent outside factors from affecting the success of the plants, all the hydroponic systems would need to be contained in the same area. Larger tanks with varying amounts of plants in them could also be tested to find the ideal amount of plants that can grow and be sustained in a certain size tank.

Several other factors could be taken to improve upon this study. One factor would be to reduce the amount of plants per tank or use a larger tank to reduce the possibility of nutrient competition. Another would be to reduce the potential of cross contamination by better spacing out the tanks so water from one tank could not enter the other and changing the original metal concentration. I would also use less plant species, if ran again, to make it easier when making the acid solutions, analyzing them with MPAES and comparing the final data sets.

Since my hometown lake is the inspiration behind this entire experiment, I would like to be able to use water from the lake in the hydroponic systems. I would like to test if these same plants can grow in the water with several major heavy metal pollutants as well as remove some or all the pollutants from it. Ideally, I would want this experiment to be conducted on a larger scale to mimic a real lake treatment and see if this technique is a viable method of remediation. While my original plants would likely perform differently due to what pollutants are within the water, I would still want to fully test these native plants as they are a safer, cheaper and more manageable option than other remediation plants and techniques. If my original plants were unsuccessful, another group of plants would need to be tested to find a mix of plants that could remove a significant amount of pollutants and possibly restore the lake to its pre-contamination state, over time. With a lake as large as this, it is most likely not possible that plants alone could help remediate the lake, but it could be a successful method to reduce the overall pollution and serve as a step in the right direction towards completely restoring the lake.

Phytoremediation is an up and coming remediation method and I believe that if more extensive research is put into this technique for larger scale cleanups, it can be a low-cost and effective method to recommend for the cleanup of polluted aquatic environments. Having more affordable and low maintenance methods of remediation could lead to action being taken. With so many NPL and superfund sites in the US alone, we are in desperate need of an effective remediation method that is also cost-effective.

Conclusion

The results of this experiment show that native plants to Illinois have the potential to be used in aquatic phytoremediation cleanups. The Sawtooth Sunflower and Common Buttonbush demonstrated significant abilities to remove lead from water and with further research could be used to remediate larger scale environments that are not controlled, such as the lake in my hometown. With the low-cost, visual appeal and ease of phytoremediation, this technique should continue to be studied and implemented in remediation efforts as the need for treatment of contaminated ecosystems, such as superfund sites and NPL sites, grows in the US and worldwide.

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