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PHYTOREMEDIATION OF COPPER CONTAMINATED WASTE WATER USING LEMNA MINOR

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PHYTOREMEDIATION OF COPPER CONTAMINATED WASTE WATER USING LEMNA MINOR

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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ABSTRACT

The use of natural remediation methods to remove contaminants from waste water is becoming more popular. Plants have been used for several decades, yet their use for municipal waste water contaminated by heavy metals is limited to a few studies which focus on the Mercury and Chromium (Bennicelli, et.al, 2004). This study specifically attempted to determine the viability for using Lemna minor to remediate municipally generated wastewater contaminated with copper.

The study used 100 ml samples of wastewater, artificially spiked with 8 mg/L of copper sulfate and seeded with approximately 100 Lemna minor fronds. Each treatment was repeated 15 times and distilled water was added daily to maintain 100 ml samples.

The addition of Lemna minor statistically lowered the copper concentration of the treatment groups (55% reduction in total *Cu* concentration). No significant decrease was seen in the control groups. While Lemna minor has metals accumulation potential, its wide spread use is limited by the toxic effect of copper on Lemna minor at relatively low levels.

INTRODUCTION

In recent years, there has been a growing struggle to provide efficient, inexpensive, and environmentally friendly options for the remediation of heavy metals in municipal waste water. Currently, most facilities use chemical precipitation methods that can be costly and produce hazardous waste concerns of their own (Eisazadeh, 2008). This thesis attempted to determine whether Phytoremediation is a viable option for the removal of the heavy metal copper (*Cu*) from a municipally produced waste stream. “Phytoremediation is an innovative treatment technology that uses plants and trees to clean up contaminated soil and water. Plants can break down, or degrade, organic pollutants or stabilize metal contaminants by acting as filters or traps.” (EPA definition)

Specifically, this study employed the use of Lemna minor (Duckweed) in the reduction of the quantity of copper (a typical contaminant) found in the waste water stream in Henrico County, Virginia. In developing this study, waste water samples were collected directly from the waste stream and analyzed for copper content. Lemna minor was added to samples and a mass balance measurement was used to determine the reduction in the amount of copper. The study was conducted in a self constructed growth chamber in order to control as many variables as possible (see Methods for more detail).

Anthropogenic sources of copper entering the waste stream are generally from industrial generators such as tanneries, electronics manufacturers, and plating facilities, as well as residential copper piping. Large amounts of copper may enter directly into freshwater systems from mining and refining facilities as well. In low concentrations (<4.0 mg/L), copper appears to pose minimal risk to humans and is actually an essential element in the formation of hemoglobin and several metabolic functions (Agency for Toxic Substances and Disease Registry (ATSDR), 2010). Currently there are many homeopathic researchers and doctors who actually tout the health benefits of regular copper supplements. Copper is hailed as a treatment for everything from chronic yeast infections, to neurological disorders. High concentrations of copper, however, can be toxic when ingested at the gram level. Symptoms are usually limited to nausea and mild vomiting and recovery requires little action. In extremely high concentrations, or to those with liver and/or kidney concerns, patients may develop similar symptoms as seen with other heavy metals, including: anemia, anuria, and liver cirrhosis. Fatality is rare when treated by chelation with penicillamine or dimercaprol (Merck, 2010).

The primary concerning issue for copper contamination is an environmental one. Copper concentrations as low as 0.39 mg/L may be fatal to pond invertebrates and other organisms according to the Hazardous Substances Databank (National Institute of Health, 2010). Copper is known to bio-concentrate, or be absorbed at a greater rate than that at which it is lost, like all heavy metals, in many different organs in fish and mollusks (Owen, 1981). Due to copper’s potent effects, it is commonly used as an algaecide in ponds and lakes (usually in sulfate form). The free copper ions inhibit photosynthetic growth, pigment production and may disrupt potassium regulation. Sensitive algae species may suffer fatality from concentration as low as the part per billion (ppb) level. Copper has received little attention, in both the common media and academic research, when compared to other heavy metals such as lead, mercury, and arsenic, mainly due to this discrepancy between human and environmental impacts. When compared to the other heavy metals, little is known about the long term environmental impact or remediation techniques for copper and copper containing compounds. Regulation is also far less stringent for copper relative to other metals such as lead and mercury.

Numerous papers and studies have been completed in recent years in order to determine the impact of heavy metals (in general) on the environment and human health. The United States Environmental Protection Agency (EPA) regulations as well as state and local ordinances are used in the attempt to reduce the quantity of these heavy metals from entering the environment. The EPA regulates copper discharge under the National Pollutant Discharge Elimination System (NPDES) using the Biotic Ligand Model (BLM). Discharge limits vary by locality but typically range between 1.5 mg/L and 2.5 mg/L with action levels as low as 1.0 mg/L. The drinking water Maximum Contaminant Level Goal (MCLG), the point at which there is no known or expected risk to health, is set at 1.3 mg/L. The action level for copper is similarly set at 1.3 mg/L and controlled by Treatment Technique (TT) that reduces the corrosiveness of drinking water (EPA, 2010). If more than 10% of samples exceed this level (1.3 mg/L), drinking water facilities must take additional steps toward reduction.

While reduction at the source has been shown to be the most effective method in the reduction of environmental contamination, trace amounts of heavy metals are still regularly found in waste streams and waste water effluents with ultimate discharge to the environment (e.g. rivers, streams, and ponds). Many costly attempts have been made to remove these metals. Cunningham and Ow estimated, in their report "Promises and prospects of phytoremediation" (Cunningham, 1996) that it can cost a minimum of $250,000 per acre to clean up heavy metal contaminated sites. The most common method for remediation of heavy metals, including copper, from waste water or other contaminated water is through a coagulation/filtration method. This method involves removing metals by chemically or physically treating the contaminant so that the particles in solution agglomerate into larger particles which then settle as precipitates (Huang et al., 2004). The precipitates are collected by gravity separation or forced through filters to trap the particles. Once the particles are separated from the water they can be disposed of by land-filling or incineration. Concerns with this approach revolve primarily around high cost, addition of chemicals for precipitation and the safe transport/disposal of the sludge-like material that is the end product. In summary, "use of these methods for cleanup/disposal of contaminated water is very expensive and disruptive to the habitats that surround the water" (Tu and Ma, 2002).

An increasingly used option for the cleanup of contaminated water and soil is the use of plants. This method, termed "phytoremediation" or "phytoextraction", applies the natural tendency of some plants to bio-accumulate heavy metals and other toxic materials. In bio-accumulation, plants sequester a substance from the surrounding environment in higher concentrations than it is found. Under ideal conditions, certain plants may even metabolically alter the contaminant so that it no longer poses a health or environmental risk. Typically the plant absorbs the contaminant (heavy metal) through its root system. Previous research suggests that the absorption of heavy metals in free floating aquatic plants, such as Lemna minor, occur via the roots (Marschner, 1995). “Absorption of heavy metals is by passive movement through the cuticle, where the negative charges of the pectin and cutin polymers of the thin cuticle and the polygalacturonic acids of the cell walls create a suck inwards. Due to the increase in the charge density inwards, transport of positive metal ions takes place. (Prasad, 2006)

Lemna minor was chosen as the aquatic plant species for removal of copper in this study for several reasons. The most essential reason for its use in this study is that Lemna minor is well documented by other researchers in its ability to act as a bioaccumulator of heavy metals (Prasad, 2006). In fact, it has been suggested that Lemna minor has been nearly “exhaustively studied” in the field of bioremediation (Tripathi, 1991). Research with Lemna minor and heavy metals (*sp. Cu, Cd, Hg, Pb*) dates back to the early 1970’s at the advent of phytoremediation (Antonovics, 1971). Additionally, a study was published by Antonovics and Wu in the journal *New Phytologist* (1975) that concluded there was no significant impact on Lemna minor’s uptake potential of copper when other heavy metals were present. Since this study used wastewater, the presence of other heavy metals (e.g. zinc, lead, arsenic, etc.) is well within the realm of possibility. Antonovics’ research helped remove any concerns about the synergistic or nullifying effects of multiple metals. Additionally, Lemna minor has been shown to act as a hyperaccumlator for the heavy metal Nickel (in Nickel Chloride solution specifically) and thus is likely suitable for the use in accumulating copper or other heavy metals (Kara, 2003).

Another reason for using Lemna minor is its tolerance to a large range of habitats. Lemna minor can be found on almost every continent and in nearly all non-polar regions (Germplasm Resources Information Network (GRIN)). Since native species of Lemna minor can be found throughout the world, in-situ installations will not pose an invasive threat. Lemna minor is highly suitable for toxicity testing and remediation due to its small size, rapid growth, simple internal structure, and wide range of tolerances to environmental conditions such as pH and temperature (OECD, 2006). Lemna minor can be found under some of the most extreme toxic conditions such as sludge ponds and mining waste ponds, thus showing its potential for use in remediating these environments (Cyle, 2007).

MATERIALS AND METHODS

Through laboratory experimentation, samples were collected and analyzed to attempt to determine if phytoremediation using Lemna minor could produce a significant reduction in the copper content of the studied waste water. These waste water samples were collected directly from the influent side of the Henrico County Water Reclamation Facility (WRF). The WRF is a typical waste water treatment plant where water is treated using a combination of settling and biological treatment. Raw waste water enters the plants Primary phase where large sediments are screened and settled out. Water then flows to aeration tanks in the Secondary phase where water-borne micro-organisms are allowed to digest organic material. Waste water is again settled and filtered to remove remaining material (including the expired micro-organisms) prior to the Tertiary phase (disinfection).

Water samples for this study were obtained from the activated sludge tank (aeration tank) in the Secondary treatment phase. Sample water was then filtered using #200 mesh filter fabric to remove the majority of the sludge, leaving a nutrient rich (total nitrogen and phosphate) solution. After screening, the sample water was measured to quantify the total nitrogen and phosphate concentration. The sample was tested and found to have a negligible amount of copper at this point. Henrico waste water contains extremely low concentrations of copper due primarily to the limited number of commercial and industrial producers. Additionally, many would-be residential copper producers have plastic polyvinyl chloride (PVC) plumbing as opposed to traditional copper plumbing due to the relatively newer construction (post 1980's) when PVC became the standard for water lines.

Once the samples were processed and nutrient content ascertained, a calculated amount of chelated copper sulfate ($CuSO_4$) was added in order to achieve the desired copper content of 8 mg/L. Chelated copper sulfate was chosen to avoid possible misrepresentation from copper settling. Since copper does not readily dissolve in water, citric acid is added (by the manufacturer) to allow the copper to more easily dissolve in the solution and maintain consistent levels without natural settling. The 8 mg/L copper sulfate concentration was determined by exposing Lemna minor to incremental concentrations of copper sulfate to determine a lethal dosage. The maximum dosage of copper sulfate that Lemna minor is known to tolerate was previously determined to be approximately 15 mg/L $CuSO_4$. (Khellaf et.al.)

To determine the desired concentration, sample jars containing 100 ml of distilled water, were dosed with 4 mg/L, 6 mg/L, 8 mg/L, 10 mg/L, 12 mg/L and 14 mg/L of copper sulfate. After 9 days the Lemna minor was examined to determine the viability at the corresponding concentration. In the jars containing the copper sulfate solution of 10 mg/L and above, nearly all the Lemna minor fronds (leaf-like structures) were significantly yellowed or dead. The 4 and 6 mg/L jars showed no ill effects. The 8 mg/L copper sulfate jar had a few fronds (approximately 10%) that were beginning to yellow, but otherwise appeared healthy. Thus (8 mg/L) was chosen as the maximum concentration of copper sulfate which Lemna minor could tolerate.

The waste water samples were divided into treatment and control groups in order to obtain comparison data for analysis. Each sample contained 100 milliliters (ml) of filtered (to remove the micro-organisms and sediments created during the waste water treatment process), contaminated waste water with the desired concentration of copper sulfate (8 mg/L) which was verified using a readily available chemical test kit (Aquarium Pharmaceutical, ASIN: B0006JDWH8) containing the reagents Sodium Diethyliditiocarbamate Trihydrate and Tetrasodium EDTA. Each sample from the treatment group was seeded with 5 ml of Lemna minor (approximately 100 fronds) obtained through the Carolina Biological Supply Company.

Each sample was then tested to quantify the copper sulfate concentration at three days, six days and nine days. During the nine-day treatment period all samples were housed in a ventilated growth chamber (four foot by two foot by two foot) with a sixteen hour “on” and eight hour “off” light cycle produced by two 105 watt (7,600 lumens) “Full Spectrum Eco-Plus Fluorescent Grow Lights” (See Appendix A for photos). Temperature was maintained at a constant 70 degrees Fahrenheit throughout the experiment. At the end of the nine days the total nitrogen and phosphate were also retested along with the copper concentration. Final copper concentration was subtracted from initial concentration and any difference was assumed to be taken up by the Lemna minor.

Data was imported and subjected to statistical analysis using the freely available “R” 2.11.1 Statistical analysis software (The R Foundation for Statistical Computing,

2010) to perform linear regression analyses, standard deviations, confidence levels, etc. A 95% confidence level ($\alpha=0.05$) was used for all tests to determine if there was a statistically significant reduction in the copper concentration due to the presence of the Lemna minor.

RESULTS AND DISCUSSION

The mean concentration of copper is shown for each group at each time interval in Table 1. The treatment group, containing Lemna minor, shows a significant decrease in the mean concentration of copper for all time intervals. The loss of copper from solution (attributed to absorption by Lemna minor) appears to be cumulative over time with the greatest decrease of 55.0% in mean concentration by day nine (9). The Control groups shows no significant decrease in mean concentration of copper (only 4.16% over 9 days).

Mean Concentration of Copper

Group	Day	Mean Concentration (mg/L)	Standard Deviation
Control	0	8.000	±0.000
Control	3	7.933	±0.258
Control	6	7.733	±0.458
Control	9	7.6670	±0.489
Treatment	0	8.000	±0.000
Treatment	3	7.400	±0.632
Treatment	6	5.067	±0.704
Treatment	9	3.600	±1.121

Table 1: Mean Concentration of copper

Figures 1 and 2 show the clear decline in copper concentrations for the Treatment group while no significant change in the concentration is seen in the Control group.

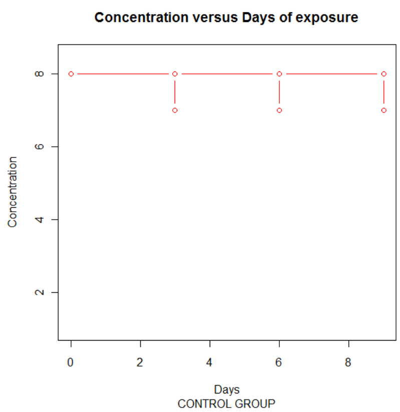

Figure 1 - Control group concentration

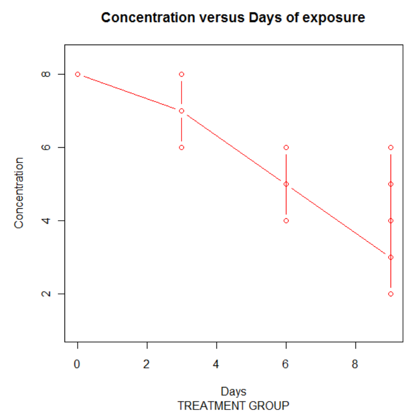

Figure 2 - Treatment group concentration

Figures 3 and 4 show the same trend in concentration levels while also showing that most data points are well within the expected deviation. The greatest deviation occurs in the treatment group at 9 days (±1.121 mg/L). This deviation may be attributed to the number of fronds in each treatment sample or actual absorption rate variations in the plants themselves. The variation appears to be compounded over time.

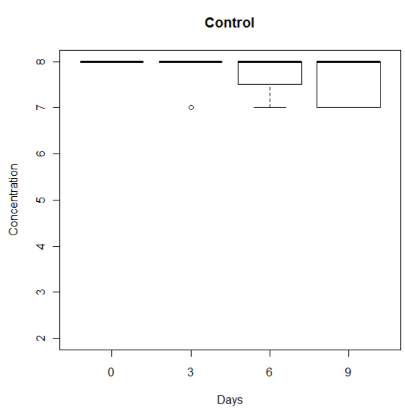

Figure 3 - Control group concentration distribution

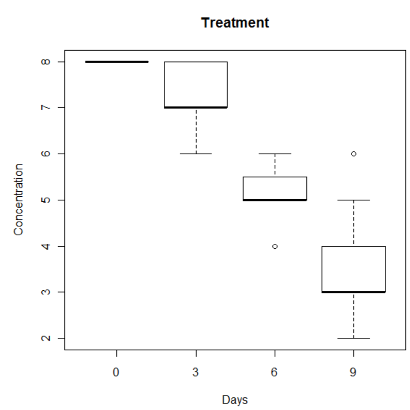

Figure 4 - Treatment group concentration distribution

In order to determine if there is a true statistical difference between the control groups and the treatment groups, the data must be statistically analyzed. The student's t-test attempts to determine whether the means of two normally distributed populations are statistically equal (the null hypothesis). The data generated in this study, however, is not guaranteed to have the same variance (how far the individual values lie from the mean) among each test group. Since the assumption that the variance is the same must be dropped, the null hypothesis was tested using the Welch's t-test (no variance assumption) for differences in means according to the following formula:

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$

where $\overline{X}_i$ is the sample mean, s_i^2 is the sample variance and N_i is the sample size.

Table 2 displays the results of this statistical testing. The mean copper concentrations for the treatment group and the control group were compared to determine if a truly statistical difference exist for each time intervals of 3, 6, and 9 days. The p-values (probability of observing a pattern equal or greater to the data given the null hypothesis is true), as determined by the Welch's test, were less than $\alpha = 0.05$ ("α" represents the arbitrarily assigned "significance level"), for all time intervals. These data fail to confirm the null hypothesis (that all differences are equal to zero) with 95% confidence. Table 2 also includes the 95% confidence intervals for each time interval. The data shows that all "differences in means" consistently fall within the expected range based on a 95% confidence level.

Results of Hypothesis Testing

Days	Difference in means	Confidence Interval at 95%	p-value	Difference not equal to zero
3	0.533	0.164 , 0.903	0.007	TRUE
6	2.666	2.219 , 3.114	7.249e-12	TRUE
9	4.067	3.406 , 4.727	7.134e-11	TRUE

Table 2: Results of Welch's t-test at 95% confidence (α=0.05)

The data supports the idea that Lemna minor acts as a bio-accumulator of the heavy metal copper (Cu). Data shows that a statistically significant decrease in the concentration of copper did occur among the treatment group, but not the control group which had no Lemna minor. Not only did the Lemna minor appear to decrease the copper concentration, it appears to do so rather effectively with a total decrease of 55% in only 9 days. Under the scope of this study, there is also no apparent reduction in the Lemna minor's ability to absorb copper over the time frame used (9 days).

While it is clear that Lemna minor is effective in the reduction of copper sulfate at low concentrations, this study does not provide evidence that Lemna minor would be effective in a larger scale setting or with greater concentrations of copper sulfate. The study was run only in small quantities of 100 ml and over a relatively short period of 9 days. The copper sulfate solution used in this experiment had an extremely low concentration of copper, which may be lower than would typically be found in many environmental sites of concern for copper toxicity. Additionally, many of the Lemna minor fronds showed signs of significant stress by the end of the 9 days (yellowing of fronds).

CONCLUSION

Under the scope of this research study, Lemna minor was shown to be effective at remediating copper from an aqueous solution of municipally generated waste water. Lemna minor may be a suitable flora species for phytoremediation of copper contaminated waste water. The positive results obtained indicate that the use of Lemna minor could be used as a cost effective method for phytoremediation in-situ at a water reclamation facility. Some concerns have been expressed as to the use of Lemna minor (or phytoremediation in general) for large scale remediation. The suggestion has been made that phytoremediation may be limited with respect to size of treatment area and time needed for proper treatment (Cunnungham, 1996). Lemna minor is a rapidly growing plant and may be suitable for large areas, but further research would be required to asses this concern.

Specifically, research is needed to determine the maximum uptake values and rates of Lemna minor in relationship to copper as well as the effectiveness of Lemna minor under uncontrolled environmental conditions (e.g. light, temperature, predation). Maximum uptake values could likely be determined by expanding the length of the study until copper concentrations appear to no longer decline. The uptake rate could then be calculated using the graph data. If the rate and maximum values are deemed sufficient than in-situ studies may be warranted. Additional studies may also be advisable to determine if other species of Lemna (L. gibba, L. minuta, L. trisulca or L. valdiviana) are more suitable (or at least as efficient) for the removal of copper contaminated water. Ultimately in-situ studies would need to be conducted to determine if Lemna minor is suitable beyond the highly controlled laboratory setting. The results of this experiment provided a solid foundation for further research on Lemna minor and copper remediation.

LITERATURE REVIEW

The following is a list of sources used for background information related to the above research topic. A brief summary is given and the relevance of the source when needed.

Agency for Toxic Substances and Disease Registry. (March 1, 2010). *Background and Environmental Exposure to Copper in the United States.* Retrieved 2/19, 2010, from http://www.atsdr.cdc.gov/toxprofiles/tp132-c2.pdf

Reference provides a comprehensive overview of sources and health impacts from copper.

Antonovics, J., Bradshaw, A. D., & Turner, R. G. (1971). Heavy metal tolerance in plants. *Advances in Ecological Research, 7*(1), 1.

Early work on the ability of plants to bioaccumulate otherwise toxic levels of heavy metals. Provides a comprehensive review of the literature that was available at the time. While somewhat out dated it does provide a nice overview of the basics on heavy metal phytoremediation and the biology of plant accumulation without detremental toxicity.

Antonovics, J., & Wu, L. (1975). Zinc and copper uptake by agrostis stolonifera, tolerant to both zinc and copper. *New Phytologist, 75*, 231.

Researchers determined that little or no significant relationship is seen in the uptake potential of the heavy metals Zinc and copper when remediated independently or together. This data is important in limiting the impact of other metals that may be found in wastewater. It may be assumed that the uptake potential of the copper is uneffected by other contaminants that may be present.

Bennicelli, R, et.al. (2004). The ability of *Azolla caroliniana* to remove heavy metals (Hg(II), Cr(III), Cr(VI)) from municipal waste water. *Chemosphere*, 55(1), 141-146.

Brooks, R. R. (1977). Copper and cobalt uptake by haumaniastrum species. *Plant Soil, 48*, 541-544.

Author investigates the use of metal accumulating plants in relationship to copper and cobalt mining. He concludes that the Haumaniastrum species is well suited for diagnostic mining of the two metals due to the concentration levels found naturally in the plant. Copper and cobalt were both found to naturally exist in concentration 10+% greater then expected and the plant is only found to exist in areas of high copper and cobalt mineralization.

Cunnungham, S. D., & Ow, D. W. (1996). Promises and prospects of phytoremediation. *Plant Physiology, 110*, 715.

Article describes the current understanding of phytoremediation in relationship to both organic and inorganic compounds. Further details are given on the future of phytoremediation through agronomic enhancements and genetic modification.

Concerns for limitation are given with respect to size and time needed for proper remediation techniques.

Eisazadeh, H. (2008). Removal of arsenic in water using polypyrrole and its composites. *World Applied Sciences Journal, 3*(1), 10-13.

The author Investigates the use of surface absorption for the removal of heavy metals. Specific focus is on Arsenic. Article is of relevant to this thesis due to its relationship with the waste water (reclamation) stream.

Garbisu, C., & Alkorta, I. (2001). Phytoextraction; a cost-effective plant-based technology for the removal of metals from the environment. Unpublished Instituto Vasco de Investigacion y Desarrollo Agrario, Derio, Spain.

Very detailed paper describing the various methods of phytoremediaiton with a brief look at the necessary requirements of a plant to be used a phytoextractor. Further details methods that may be used to improve extraction of heavy metals from contaminated soils.

Huang, J.W., Poynton, C.Y., Elless, M., Kochian, L.V. 2004. Phytofiltration of arsenic from drinking water using an arsenic-hyperaccumulating fern. Environmental Science and Technology. 38:3412-3417.

Kara, Y., Basaran, D., Kara, I., Zeytunluoglu, A., & Genci, H. (2003). Bioaccumulation of nickel by aquatic macrophyta Lemna minor (duckweed). *International Journal of Agriculture and Biology, 5*(3), 281-283.

Investigation sought to determine the accumulation capabilities of Nickel (Ni) by

Lemna minor. Results showed that Lemna minor is capable of concentrating high levels of Nickel in the plant fronds. Laboratory produced solutions of Nickle Cloride were prepared at various concentrations. Atomic Absorption Spectrophotometry was used to analyze initial and final concentration. Results show Lemna minor to be a hyperaccumulator plant and likely suitable for uptake of other heavy metals.

Keith, C., Borazjani, H., Diehl, S. V., Prewitt, M. L., Su, Y., Han, F., et al. (April 24-27, 2007). Aquatic Phytoremediation of CCA and Copper Contaminated Water. *37 Annual Mississippi Water Resources Conference,* Mississippi State University. 23. Author describes the results from experimentation involving both Limna Minor and Myriophyllum Aquaticum to remove Chromeated copper Arsenate from stock sollutions. Study showed promising results with 85% reduction of copper concentration from solution.

Khellaf, N., Zerdaoui, M., Faure, O., & Leclerc, J. C. Tolerance of Heavy Metals in the duckweed, Lemna Minor. Unpublished Universiti Badji Mokhtar,
Researches in Algeres investigated the tolerance of Limna minor in relationship to various heavy metals. Conclusions stated that Limna minor, while efficient in the uptake of copper, may be limited to concentrations below 15 mg/L.

Lasat, M. M. *The use of plants for the removal of toxic metals from contaminated soil* American Association for the Advancment of Science: Environmental Science and Engineering Fellow.
Publication produced under U.S. E.P.A. Grant No. CX824823. Article provides complete review of phytoremediation in relationship to heavy metals. While the

article focuses on soil contamination as opposed to water contamination the information background on phytoremediation methods and plant biology is very useful.

M., G., & S.P., S. (2005). A review of phytoremediation of heavy metals and utilization of its byproducts. *Applied Ecology and Environmental Research, 3*(1), 1. Provides a comprehensive overview of current knowledge related to phytoremediation. Reviews 90 contemporary journal articles and publications. Article provide significant insight for this thesis in relationship to the uptake mechanisms and environmental benifits/concerns related to phytoremediation and accumulatioin.

Marschner, H., *Mineral Nutrition in Higher Plants*. Academic Press, New York, 1995.

Merck, Sharp and Dohme Corp. (2010). The Merck Manual Online Medical Library. Merck Publications, Whitehouse Station, NJ. Sect. 1, Ch. 5

National Institute of Health. (2003). *TOXNET - national library of medicine's toxicology data network. hazardous substances data bank (HSDB)* (NLM. Bethesda, MD: U. S. Department of Health and Human Services.

Organisation of Economic and Co-operation Development. (2006). *Guidlines for the testing of chemicals. lemna sp. growth inhibition test* No. Guidline 221) Report provides detailed overview of testing and analyzing methods for working with Lemna minor. Overview indicates that Lemna minor is highly suitable for toxicity testing and remediation due to its small size, rapid growth rate, simple structure, and wide range of tolerance to environmental conditions such as pH and temperature.

Owen, C. A. 1981. *Copper deficiency and toxicity: acquired and inherited, in plants, animals, and man.* Noyes Publications, New Jersey.

Prasad, M. N. V., Greger, M., & Aravind, P. (2006). Biogeochemical cycling of trace elements by aquatic and wetland plants: Relevance to phytoremediation. In Taylor & Francis Group, LLC (Ed.), *Trace elements in the environment* (pp. 451)
Section describes the biology of metal uptake (as currently understood) in aquatic plants. Also describes the difference mechanisms and usages of free floating plants versus submerged aquatic plants. Provides reference for the conclusion that many hyperaccumulators increase their uptake rate as concentration increases due to changes in metal ion concentration and charge.

Raskin, K., P.B.A.N, Dushenkov, S., & Salt, D. (1994). Bioconcentration of heavy metals by plants. *Current Opinion in Biotechnology, 5*, 285.

Salt, D., Blaylock, M., Kumar, N., Dushenkov, V., Ensley, B., Chet, I., et al. (1995). Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. *Nature Biotechnology, 13*, 468.
Discusses the three main types of phytoremediation including the methods for implementation, biological mechanisms used by plants, and suggestions for improved remediation. Of specific interest to this study in the idea of Rhizofiltration by which plants accumulate toxic material in the roots from polluted waters.

Scherr, C., Simon, M., Spranger, J., & Baumgartner, S. (2007). Duckweed (Lemna gibba) as a test organism for homeopathic potencies. *The Journal of Alternative and Complementary Medicine, 13*(9), 931.

Investigation attempted to determine if Duckweed variety Lemna gibba could be used as a bioassay to study homeopathic potencies. Screening included 12 substanvces including copper sulfate. Researchers used frond count and photometric measurements to evaluate the substance. Article provided possible duckweed measurement and analysis tool produced by LemnaTec, Aachen, Germany.

Tripathi, B.D. and Shukla, S.C., Biological treatment of wastewater by selected aquatic plants, *Environ*. *Pollut*., 69, 69, 1991.

Tront, J. M., Reinhold, D. M., Bragg, A. W., & Saunders, F. M. (2007). Uptake of halogenated phenols by aquatic plants. *Journal of Environmental Engineering, October*, 955.

Investigates the uptake of Halogenated Phenols by Lemna minor in wastewaters. Study was intended to determine maximum rate coefficient for uptake of persistant organic compounds. Application suggestions relate to artificially constructed lagoons for biological reclamation of wastewater. Results indicate that uptake is less effected by Phenol structure and more so by internal enzymatic transformation of plant.

Tu, C., **L.Q. Ma**, and B. Bondada. 2002. Arsenic accumulation in a hyperaccumulator fern (*Pteris vittata L*.) and its utilization potential for phytoremediation. *J. Environ. Qual*. 31:1671-1675.

Yamada, Y., Bucovac, M.J., and Wittwer, S.H., Ion binding by surfaces of isolated cuticular membranes, *Plant Physiol*., 39, 978, 1964.

APPENDIX A

Experimental Photos

Photo 1 - Growth Chamber with Treatment group to the left, Control group to the right

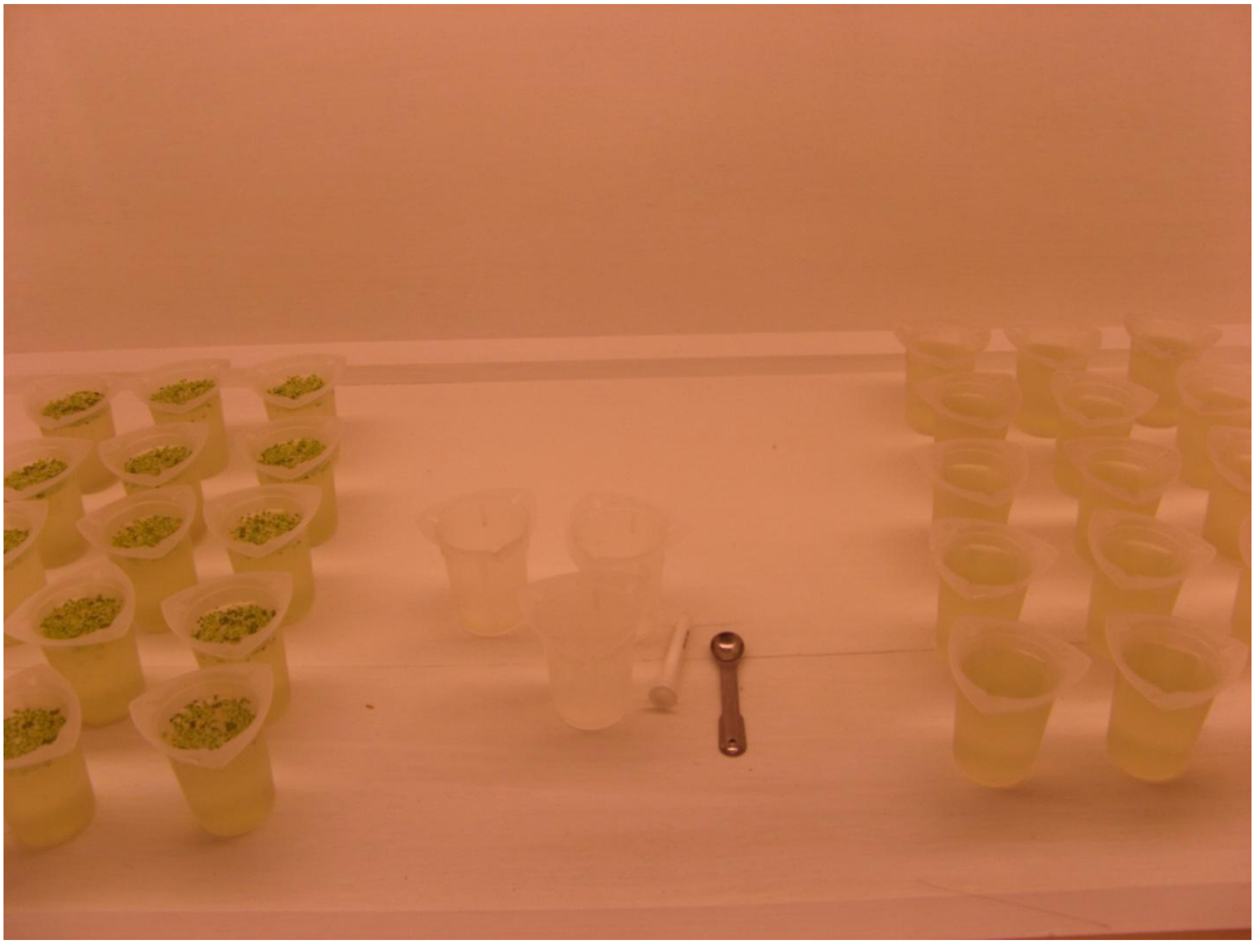

Photo 2 - Treatment Group and Control Group with light on

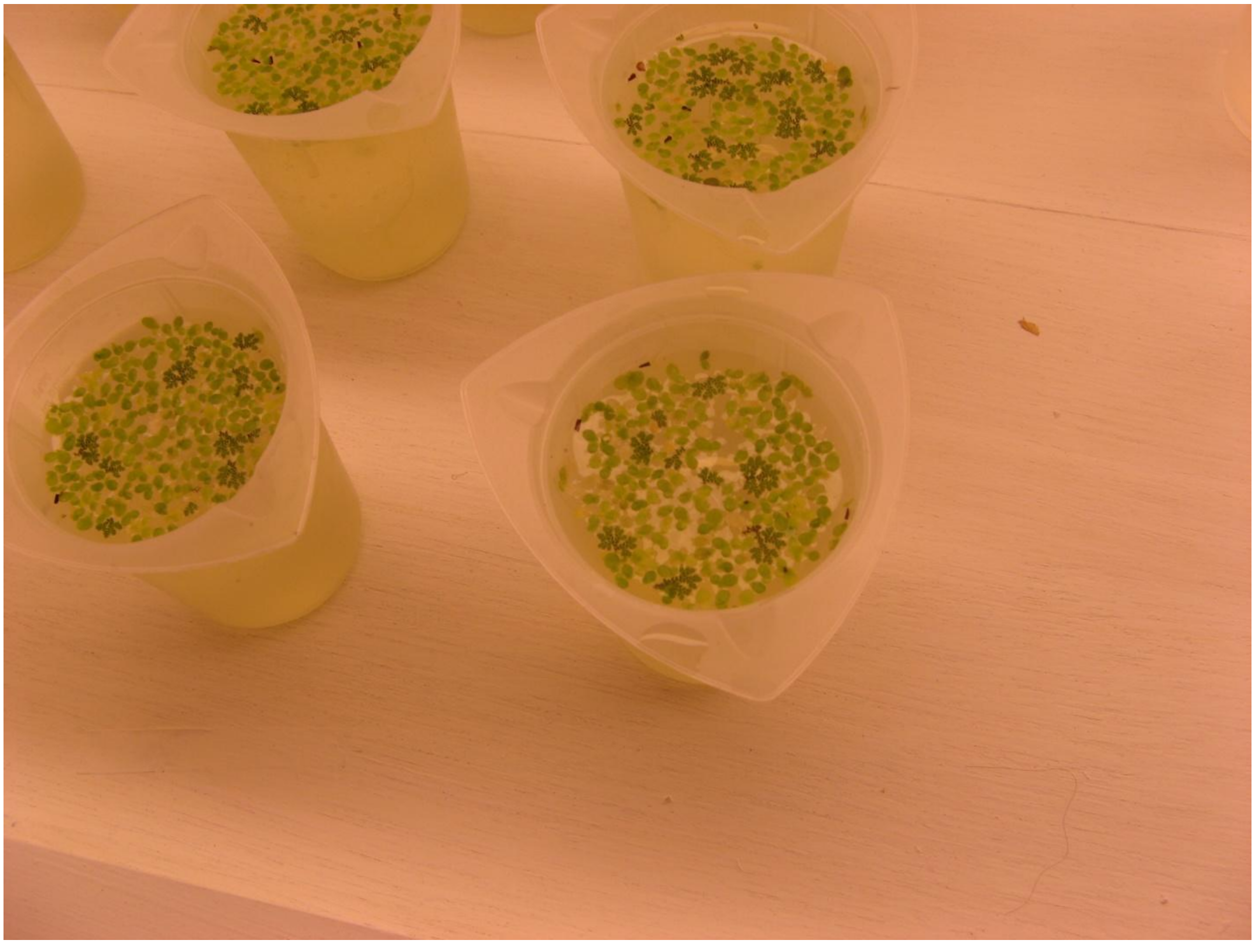

Photo 3 - Close up of Treatment group showing individual Lemna fronds

VITA

Mark R. Apelt was born on October 26, 1977, in Norfolk, Virginia and graduated from Granby High School in 1996. He attended the University of Virginia where he received his Bachelor of Arts in Environmental Studies. He spent several years in Seattle, Washington prior to moving to Richmond, Virginia with his wife. He worked in the environmental research and engineering field for many years and has subsequently taught in the public schools in Henrico County for the past five years.