# OPTIMIZING THE *IN SITU* TOXICTY IDENTIFICATION EVALUATION SYSTEM: AMMONIA AND ZINC AS EXAMPLES

by

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A thesis submitted
in partial fulfillment of the requirement
for the degree of
Master of Science
Natural Resources and Environment
at the University of Michigan
April 2016

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**Abstract**

Anthropogenic influences on the environment have increased the levels of contaminants and pollution in aquatic ecosystems humans and wildlife depend on for water resources. It is especially important to be able to accurately and efficiently evaluate riverine contamination. The United States Environmental Protection Agency developed toxicity identification evaluation (TIE) protocols for evaluating sediment and surface water toxicity in a laboratory setting. Following this framework, Burton and Nordstrom developed an *in-situ* toxicity identification evaluation (iTIE) system, which allows for the toxicity analysis to be done in the field to avoid artifacts introduced when samples are transported to the laboratory.[19] The tests presented in this paper provide data to further optimize the iTIE system through the evaluation of different resin (chelex, charcoal, and zeolite) and pollutant or contaminant (ammonia, zinc, nickel, and vanadium) combinations. The experimental results indicate that at least 3-5 grams of resin is needed for significant contaminant removal, the system flow must be maintained below 14 ml/min, and the iTIEs can transition successfully into Phase II of the TIE protocol by allowing for specific contaminant characterization.

**Acknowledgements**

I would like to thank the members of my thesis committee, Allen Burton and Paul Drevnick, for their support and advisement; Gus Steigmeyer for his generous assistance with iTIE experiments and knowledge; Anna Harrison, Shelly Hudson, and Sara Nedrich for their advice and assistance; and the University of Michigan's Rackham Graduate School, University of Michigan's School of Natural Resources and Environment, Water Environment Research Foundation (WERF) CEC6R12 and Strategic Environmental Restoration and Demonstration Program (SERDP) 14ER03-025 for funding support.

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## Introduction

Aquatic ecosystems are increasingly subject to anthropogenic influences. The resulting ecosystem impacts range from chemical to physical to biological, and stem from urban, industrial, agricultural and pharmaceutical sources. Freshwater ecosystems, particularly riverine environments, are vital resources for human and environmental health. Maintaining healthy river environments is important for future water security, so identifying the type of and source of the stressor is key to addressing each situation.

For chemical stressors, the sources that contaminate surface waters are classified as either point or non-point sources.[3] Point sources are specifically traceable, such as sewage treatment facilities, while non-point sources include urban and agricultural runoff and are more difficult to exclusively detect.[3] Different stressors are more commonly associated with different source types. Nitrogen (N) and phosphorous (P) are two common nutrients associated with aquatic ecosystem stress. Although certain levels of these nutrients are required for natural biological function in the ecosystem, the high influx of nutrients from fertilizers, agricultural runoff and wastewater treatment plants lead to eutrophication problems.[1] Eutrophication is the result of nutrient loading and leads to excess macrophyte and algal growth (potentially toxic and harboring bacteria), hypoxia (decreased oxygen levels), and fish kills.[1] In the Midwestern United States, fertilizers, agricultural runoff and wastewater treatment plants are major contributors to excess nutrients entering aquatic environments. Ammonia entering aquatic systems through the discharge of wastewater treatment plants is of growing concern, as ammonia is an extremely toxic form of N.[2]

Common metal contaminants in riverine systems include copper, zinc, cadmium, chromium, nickel, and lead. The contamination by these trace heavy metals stems from urban runoff and industrial waste outflows.[4] These metals can accumulate in sediments, plants and organisms, causing detrimental ecosystem health effects. The physicochemical conditions of the river determine the toxicity of the metals in both the sediments and the water as they flux between oxidized and reduced states. These fluctuations, in turn, influence how long the metal is in a toxic form and the resultant ecosystem and human health effects.

Pharmaceuticals and other organic compounds are also contaminants of concern in aquatic systems. These contaminants stem from personal care product disposal and industrial wastes from urban runoff. There is concern that these contaminants can cause significant effects at low levels of exposure.[6] Pharmaceuticals, particularly endocrine disrupters that alter hormone levels in organisms, can get into aquatic systems via wastewater treatment plants and storm runoff. Exposure to these chemicals can cause significant reproductive effects and result in fish and other organism population crashes, which are detrimental to the aquatic food chain and ecosystem.[5] Legacy synthetic organics, such as polychlorinated biphenyls (PCBs) and DDT, are also of concern in freshwater ecosystems. The bioaccumulation of these organics in aquatic organisms, such as fish, can lead to greater impacts up the food chain and affect human health, too.[7] There is an urgent need to stop the influx of these organics and mitigate the effects of those currently present in aquatic environments to protect the ecosystem health and the health of humans, who depend on the ecosystems for recreation, freshwater, and food.

Biological stressors also impact riverine environments. These stressors include invasive species and other pathogens that can take advantage of the absence of predators to outcompete native fauna and lead to detrimental effects on the ecosystems. Human actions are the main source for the introduction of many invasive species, such as zebra mussels in the Great Lakes

through trading ship ballast waters or the dumping of aquarium organisms into river systems.[8] The presence of invasive species exerts stress on ecosystem structure and balance, as exemplified by the proliferation of hydrilla and water hyacinth choking waterways, altering nutrient cycles and thus altering fish and benthic species in Florida rivers and lakes.[9] Warming temperatures and increased flooding induced from climate change are also further facilitating the spread of pathogens and invasive species.[10]

Alterations at the landscape scale are also a major threat to the ecological integrity of river ecosystems.[11] The resulting physical stressors lead to changes in stream sedimentation, water flow, and contaminant levels and thus affect ecosystem equilibrium and cause organism stress. Sundermann et al. (2013) found that catchment-related physical factors, along with water quality, displayed overriding importance in shaping benthic invertebrate biodiversity assemblages, thus supporting the need to address physical changes when evaluating the health of aquatic ecosystems.[12] Industrial impacts, such as dams changing flow patterns or power plants altering the temperature regime in river systems, are also of physical concern. Although often overlooked, power plant cooling systems release thermal discharges that increase water temperatures enough to potentially impact aquatic life and need to be considered when evaluating sources of ecosystem stress.[13]

The two stressors evaluated in this study were ammonia and zinc. While ammonia pollution has some point sources, such as fertilizer and coke manufacturing plants and wastewater treatment plants, the majority of ammonia contamination in aquatic ecosystems is from non-point sources.[21] Agricultural runoff is the main source of ammonia pollution into riverine environments.[22] Studies done examining the causes of hypoxia in the Mississippi River basin, as well as high N levels in agricultural prairie river systems, for example, linked these events stemming from high N concentrations to agricultural runoff.[22, 23] Fertilizers rich in N and P are used in agricultural fields, where rain events result in runoff of excess N and P entering streams, increasing their concentrations from the background levels.

In aqueous solutions, total ammonia exists in two main forms – ammonium ion (NH4+) and unionized ammonia (NH3) – the concentrations of which are pH dependent.[26]As expected, Ankley et al. found that a greater fraction of total NH3 in the water remained in the unionized form at higher pH.[25] Although unionized NH3 is commonly considered the more toxic form, Borgmann (1993) found that toxicity of ammonia to *H. azteca* was a function of total NH3 (not just unionized NH3).[27] This follows with Erickson's model conclusion in which unionized NH3 and NH4+ are jointly toxic, with toxicity predominately due to ammonium ion at low pH and unionized NH3 at high pH.[26] With North American riverine environments typically maintaining a fairly neutral pH, the balance between unionized NH3 and NH4+ is likely in a continual state of flux in the water column. The toxicity of both forms to *H. azteca*, therefore, is beneficial for a laboratory study.

Laboratory toxicity studies have been performed on aquatic test species ranging from macroinvertebrates to fish. The range provided for 7-14 day old *H. azteca* was 39.8 mg TAN/L (total NH3) to 105 mg TAN/L for a 96-hour acute exposure test.[24] These NH3 concentrations were based on a *H. azteca* toxicity study performed by Ankley et al. (1995).[25] The ambient water quality acute criterion (1-hour average) for NH3 is 17 mg/L TAN, while mean acute values for test species range from 70.22 mg TAN/L for sunshine bass to 1029 mg TAN/L for *Chironomus riparius*.[24] Field comparison tests have been completed in tandem with laboratory toxicity tests in support of the acute and chronic NH3 concentrations for common test species.[28]

Zinc is a heavy metal used for industrial purposes including steel galvanization and paint and alloy production.[29] Although Zn can occur naturally in the environment, such as through the weathering of some igneous bedrock material, it can reach concentrations toxic to aquatic organisms in areas where runoff from mining and other industrial processes is not controlled.[29] In aqueous solutions, Zn has a +2 oxidation state; and when Zn (II) complexes with common ligands in surface waters, it is soluble in neutral and acidic conditions.[29] These conditions are common in natural freshwater, making Zn one of the most mobile heavy metals.[29] The toxicity of Zn is influenced by water hardness, as Zn competes with calcium and magnesium ions for binding sites in biological tissues, and the pH affects Zn solubility.[29]

Similar to N, Zn is essential for metabolic functions of living organisms, but concentrations can reach toxic levels.[30] The USEPA's national recommended water quality criteria for aquatic life identifies 120 ug/L as Zn's acute aquatic toxicity.[29] This criteria takes a range of aquatic organism toxicity levels (juvenile rainbow trout, for example have an LC50 of over 1,000 ug/L Zn; while *D. magna* have an LC50 of 100 ug/L Zn) into account.[29] A regional environmental risk assessment of Zn for nine European river basins found no deterministic risk associated with the current use patterns of Zn when aquatic Zn concentrations ranged from 20-40 ug/L in the systems.[30] Findings such as this suggest that natural Zn levels support healthy aquatic ecosystems, but it is a concern when point and non-point source pollution increases Zn levels, as the toxicity can lead to ecosystem risk.

The chemical, biological, and physical stressors to riverine ecosystems have significant effects individually, but they also interact to have even greater impacts on aquatic health. Understanding the effects of these stressors on ecosystem health and connecting these effects with their source are ways to start addressing the problems. There are assessment approaches researchers are currently employing to evaluate the effects of multiple stressors on the environment, including chemical criteria, biological surveys, habitat assessment, laboratory toxicity studies, and in situ toxicity studies.

The U.S. Environmental Protection Agency (USEPA) generally follows three approaches for aquatic ecological assessment of pollution and contaminant exposure: comparing chemical concentration data in water to chemical criteria guidelines, ambient water toxicity assessments, and bioassessments of biotic assemblages.[14] Chemical criteria guidelines are established through laboratory toxicity tests on cultured freshwater macroinvertebrate indicator species, such as *Hyalella azteca* and *Daphnia magna*. Tests exposing these organisms to a range of aquatic chemical contaminant concentrations are used to determine the endpoint effects, including mortality, reproduction and growth.[14] These tests identify the chemical concentration above which adverse effects are frequently observed and are compiled in the USEPA ECOTOX Database.[14] To perform these evaluations, source water is tested to see if chemical criteria are exceeded. If the chemical concentration exceeds the criteria in the source water, there is a need to address the contaminant and its source to improve ecosystem health. Ambient water toxicity studies are performed in a laboratory using test organisms and site water.[14] The organisms are exposed to the site water for a specific time period (commonly 28 days) and the chosen endpoints are evaluated. Laboratory toxicity studies are similar, but use laboratory spiked water instead of site water.

Biological surveys and habitat assessments are done in the field at specific sites. The USEPA introduced rapid bioassessment protocols for wadeable streams and rivers to provide indications of cumulative impacts of multiple variables, not just water quality, on the biological community.[15] The protocols are based on comparing habitat, water quality, and biological

measures (such as macroinvertebrates and vegetation) of a given stream with an expected stream reference condition that would be present without human disturbance.[15] The Ohio EPA established a qualitative habitat evaluation index (QHEI) with a scoring system.[16] The six metrics of this evaluation index include substrate type, instream cover, channel morphology, riparian zone and bank erosion, pool/glide and riffle-run quality, and map gradient.[16] This evaluation system can provide evidence of ecosystem health and human impact through a user-friendly scorecard system, and can help expose the presence of multiple stressors in an aquatic ecosystem.

In situ experiments are also used to evaluate multiple stressors in riverine environments. Semi-controlled field tests are favorable for accurately elucidating stressors in aquatic ecosystems because they provide more realistic exposure environments of variables such as pH, temperature, flow changes, ultraviolet light, and dissolved oxygen, while avoiding the artifacts associated with sample collection and manipulation in laboratory experiments.[17] Current in situ approaches include colonization studies, marking studies, mesocosm experiments, and caged studies using native species.[17] These approaches increase environmental realism, but decrease experimental control.[17] With the current abundance of laboratory test data, as exemplified in the ECOLOG database, the field accuracy is extremely important because understanding the characteristics of the actual environments is key to addressing current aquatic ecosystem degradation.

USEPA developed a toxicity identification and evaluation (TIE) experimental approach to identify components responsible for toxicity effects in effluents and pore waters.[18] The TIE uses physical/chemical manipulation of a sample to isolate or change the potency of different groups of toxicants potentially present in the sample.[18] The manipulation is done through the use of resins that adsorb specific compounds (i.e., metals, organics, NH3) and remove or reduce the presence of those compounds in the sample water.[18] Through the evaluation of endpoints for organisms exposed to the post-resin sample, the stressor levels can be deduced. USEPA divided the TIE process into characterization (Phase I), identification (Phase II) and confirmation (Phase III).[18] The purpose of the TIE is to build a weight-of-evidence case against specific chemicals, but the sample manipulation in the TIE protocols can potentially alter toxicity.[19] Understanding the difficulties in trying to create realistic field toxicity conditions in the laboratory and aiming to eliminate confounding variables introduced into sample toxicity that inevitably occur when samples are transferred from the field to the laboratory, Burton and Nordstrom (2004) developed an in situ TIE (iTIE) method.[19]

The iTIE method can characterize ammonia and metals based on their selective attraction to zeolite and Chelex, respectively.[19] With the sample solution pumped through a resin chamber before entering an organism chamber, containing common aquatic toxicity test organisms such as *D. magna* and *H. azteca*, the iTIE stystem has been tested in both laboratory and field experiments.[19, 20] Previous experiments have confirmed the ability of the iTIE resins to remove the specified contaminants and allow for the evaluation of *D. magna* and *H. azteca* endpoints (i.e., survival) over a 24-hour exposure period.[19, 20] The iTIE system, however, is still undergoing refinement before it is more widely integrated into environmental risk assessment procedures. Energy source requirements for pumping capabilities, as well as managing pumping rates, resin capabilities, and improving the ease of deployment are still being addressed.

The study objectives were to further validate the iTIE system through laboratory surface water tests that gather data for optimizing the iTIE system. With the goal of increasing iTIE feasibility in support of further implementation, it is important to consider the cost effectiveness

of each resin. To address this, the first set of experiments tested the removal capabilities of different amounts of resin. The use of multiple resins in the same iTIE chamber was also tested to see if multiple contaminants could be removed in one treatment, therefore allowing for the evaluation of different contaminants to organisms in the chambers. Experimenting with the ability of the iTIE to address USEPA's Phase II (identification) via resin use was also explored.

## Methods

### *Overall approach*

A series of experiments were conducted to investigate the ability of different amounts of three iTIE resins (zeolite, chelex, charcoal and no resin) to adsorb the following contaminants at a variety of concentrations in solutions of various compositions: NH3, Zn, Ni, and V. (Table i). These were short term, two-hour tests focused on optimizing the pumping system, resin selectivity and sorption capacity.

### *iTIE System Design*

The iTIEs used for this study were of the same design as those used by Steigmeyer et al.[31] Both the resin chamber and the organism exposure chamber were constructed from acrylic, with rubber o-rings seals to connect the two pieces.[31] (Appendix A – Image 1) The inflow port, sealed to the resin chamber with rubber o-rings, was fitted with an acrylic extender piece to help facilitate water intake.[31] (Appendix A – Image 1) Tubing was connected at the outflow port of the iTIE using nylon 1/8" hose-to-threaded male pipe adapters for 1/4" ID tubing (McMaster-Carr).[31] The interior outflow port in the organism chamber was covered with 0.25mm nylon mesh to prevent organisms from flowing through.[31]

Water was drawn through the iTIE chambers using 12V DC peristaltic dosing pump heads.[31] The rotation of each pump head was regulated individually with a custom-made circuit board with LM2496 voltage switching regulators (DROK) to raise or lower the voltage delivered to each pump individually and tightly control pump speed.[31] (Appendix A – Images 2, 3) The pump circuit was powered by an AC/DC converter plugged into the main laboratory electricity during the experiments.

Water samples drawn through each iTIE were pumped into 500 ml polyethylene bottles via PVC tubing. If a water sample exceeded the capacity, the water was able to overflow into the bottle stand. The pumps and flow were monitored throughout each experiment. The resins were removed and MilliQ water was run through the iTIEs for about two hours to rinse them between each experiment.

### *Resins*

The resins used for this study are commercially available and included zeolite for NH3 (API FilStar Zeolite Ammonia Remover, Item#AP7345); Chelex (Sigma-Aldrich, CAS 11139-85-8) for metals; carbon, in the form of activated charcoal, for metals and other compounds

(Marineland Black Diamond Media Premium Activated Carbon, product no. PA0370); and glass wool (Sigma-Aldrich) for the control resin. The zeolite and carbon were ground slightly using a mortar and pestle to increase the surface area of the resins before being weighed for each experimental run. The carbon and zeolite were also wetted with MilliQ to remove interstitial space prior to being put into the iTIEs. Glass wool was used to sandwich the resin in the resin chambers. Electrostatic register filtration vent filter material was cut to cover both ends of the resin chamber to reduce resin movement during the experiments. The control resin chambers only contained glass wool or the vent filter material.

*D. magna NH3 test*

A laboratory toxicity test, with an established reproduction endpoint, was done using cultured 4-day-old *D.magna*. There were four treatments of NH3 concentrations: 0 mg/L NH3, 20 mg/L NH3, 40 mg/L NH3, and 60 mg/L NH3, with 10 replicates per treatment. All of the solutions were made with flume water. The NH3 concentrations were made with 1000 mg/L N as NH3 liquid standard (Thermo Scientific Orion 951007) and flume water. One organism was randomly placed into each of the 100 ml Nalgene bottles containing the different NH3 concentrations. The Nalgene bottles were sealed to reduce atmospheric exposure and chemical changes during the test.

The reproduction test followed Lewis and Horning's daphnia short-term chronic toxicity test procedure.[32] One exception was that the test was carried out until three broods were observed (or extreme daphnia mortality occurred). The pH, dissolved oxygen and conductivity of the bottles were recorded to make sure that those parameters did not influence daphnia reproduction and survival. The NH3 concentrations were checked via water collection from one randomly selected bottle of each concentration every other day for the first week. This was done to confirm whether the NH3 concentration in the sealed bottles was changing overtime.

*NH3 measurement*

An Orion High-Performance Ammonia Electrode (Thermo Scientific) was used to determine the NH3 concentrations in the water samples for all of the experimental tests. The procedure provided in the electrode user manual was followed. The membrane was changed for the evaluation of each new experiment and allowed to soak in the NH3 storage solution for a minimum of overnight before usage.

A standard curve was created using 100 ml each of prepared 0 mg/L, 5 mg/L, 10 mg/L, 25 mg/L, 50 mg/L and 75 mg/L NH3 test solutions (prepared with 1000 mg/L N as NH3 liquid standard - Thermo Scientific Orion 951007). Before reading the NH3 concentration, 2 ml of NH3 pH adjusting ISA (Thermo Scientific Orion 951211) was added to each 100 ml test solution. For the iTIE tests, only 50 ml of test water was collected for each replicate, in which case 1 ml of the NH3 pH adjusting ISA was used (this ISA amount was similarly adjusted for the sample volume when necessary). The measurements were taken in millivolts (mV) to establish a standard curve for the known NH3 standard concentrations. A new standard curve was created to evaluate the samples during each individual test when the NH3 electrode probe was used.

*NH3 iTIE tests*

The first experiment tested four different amounts of zeolite in the resin chamber. There were three replicate iTIEs for each of the following treatments: glass wool, 1 gram zeolite, 3 grams zeolite, 5 grams zeolite. The iTIEs were placed in a custom-made circular fitted iTIE holder in a five-gallon bucket with a test solution containing 30 mg/L NH3 solution. This test solution was created using a 1000 mg/L N as NH3 liquid standard (Thermo Scientific Orion 951007) and MilliQ ultrapure water. When necessary, more of the test solution was mixed and added to the bucket throughout the experiment to maintain an adequate level of water in the bucket for iTIE function.

The test was run for 2 hours. The flow rate for the iTIEs was monitored throughout the experiment, with the flow rates around 20 ml/min. The peristaltic pumps were stopped at 20 minutes, 40 minutes, 60 minutes, 90 minutes, and 120 minutes after the start of the test. At each stopping point, water samples were taken from the collection bottles for each iTIE. These water samples were transferred to sealed 50 ml centrifuge tubes. There was not a concern about the NH3 concentrations changing significantly between sample collection and analysis based on results from the previous *D.magna* NH3 toxicity test. The samples were tested for NH3 concentrations within 24 hours of collection following the NH3 measurement protocol discussed above.

This test was repeated. The same procedure was followed, except the flow rates were monitored to maintain a slower flow. During this second NH3 iTIE test, the flow rates ranged from 2-14 ml/min.

*Zn iTIE tests*

An 80 ug/L solution of Zn was created using 1000 mg/L ZnCl and MilliQ. The iTIE set up was moved to a 20-gallon aquarium to allow for easier experimental transitions. There were three replicate iTIEs for each of the following treatments: glass wool, 1 gram chelex, 3 grams chelex, 5 grams chelex. Samples were taken every 30 minutes during a two-hour period. When the samples were taken, the pumps were stopped. The water remaining in the collection bottle was disposed of between collection times. When necessary, more of the test solution was mixed and added to the aquarium throughout the experiment to maintain an adequate level of water in the aquarium for iTIE function. The iTIE flow rates were kept in the 2-15 ml/min range.

Water samples were collected from the collection bottles of each iTIE replicate using a 10 ml syringe. 10 ml of each water sample was filtered through a 25 mm non-sterile solvent-resistant PTFE 45 μm nylon filter unit (Fisher Scientific) into a 15 ml centrifuge tube. The samples were acidified with 400 μl of 8M HNO3. The samples were analyzed for [Zn] via inductively-coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer). Seven well used and confirmed Zn standards were used for the ICP analysis: 0 mg/L, 0.025 mg/L, 0.050 mg/L, 0.100 mg/L, 0.500 mg/L, 1 mg/L, 2 mg/L, 3 mg/L Zn.

Following the same basic procedures for Zn outlined above, a second test was done using 40 mg/L Zn, but this solution was created using 1000 mg/L ZnCl and flume water. A third test using 40 mg/L Zn in flume water used charcoal resin, with 3 replicates each of glass wool, 1 gram charcoal, 3 grams charcoal, and 5 grams charcoal. These chelex and charcoal treatment samples were diluted 40% with MilliQ before being analyzed via ICP-OES.

*NH3 and Zn iTIE test*

A flume water solution with 30 mg/L NH3 and 4 mg/L Zn was used to evaluate multiple resins in the iTIE resin chamber. Three replicates of each treatment were used: vent filter paper only (control), 4 grams zeolite, 4 grams charcoal, 4 grams zeolite + 4 grams charcoal. Both NH3 and metal analysis water samples were collected following the same protocols described above. In order to create more space in the resin chamber for the multiple resin treatments, glass wool was not used.

*Multiple metals iTIE test*

A flume water solution with 4 mg/L Zn, 4 mg/L Ni and 4 mg/L V was created using Ni(II) chloride hexahydrate, zinc chloride, and sodium metavanadate. The metal solutions were made individually and mixed into the flume water to obtain desired concentrations. Following the above outlined procedure for metals analysis, the water samples were collected every 30 minutes over a two-hour period, filtered, and acidified. The Zn standard was maintained for the ICP-OES analysis. The Ni standards used were 0 mg/L, 0.01 mg/L, 0.05 mg/L, 0.1 mg/L, 0.15 mg/L, 0.25 mg/L, 1 mg/L, and 5 mg/L Ni. The V standards used were 0 mg/L, 0.025 mg/L, 0.05 mg/L, 0.1 mg/L, 0.5 mg/L, 1 mg/L, 1.5 mg/L, and 3 mg/L V.

*Diffuse Oxygen Tubing for iTIE Pore Water Tests*

Initially, these tests were planned to evaluate sediment pore water toxicity. The diffuse tubing used by Burton and Nordstrom, however, had been discontinued.[19] Different tubing options were evaluated by connecting the tubing to the iTIEs, following Burton and Nordstrom's method, and purging MilliQ water of oxygen with nitrogen gas.[19] Oxygen was then run through the tubing on the iTIE and the dissolved oxygen was measured overtime with a DO probe. This was done in a 100 ml glass beaker sealed with parafilm during the test. The tubing tested was Zeus's PTFE, Extruded Sub-Lite-Wall 0.118 ID and 0.003 Wall (Part No. 172326); 1/8" Cole Parmer Masteflex L/S Peroxide-Cured Silicone Tubing 1/8 x 1/4 (Fischer Scientific 13-310-108); TEF Tubing 1.5mm, ID 0.3W (Ace Glass Incorporated 12684-17); PTFE Tubing, Light Wall (Component Supply Co. Part#/STT-08-50.)

*Data analysis*

Analysis of Variance (ANOVA) tests were used to analyze differences in concentration means between various treatments and time periods, with evidence against the null hypothesis

defined as $p<0.05$. Treatment, time, and interactions between both of those dependent variables on the resulting contaminant concentration (dependent variable) were evaluated. Significance was considered at the 0.05 alpha-level. If the ANOVA provided evidence against the null hypothesis, a post-hoc Tukey Honest Significant Difference test was used for multiple comparisons to identify which groups had significant differences. RStudio statistical software was used for this analysis.

**Results**

ANOVA statistical analysis was performed for each of the contaminants in the experiments. The effects of treatment, time, and interactions between both of those independent variables were compared to contaminant concentrations (dependent variable).

*D. magna Test*

The *D.magna* NH3 toxicity test was not successful, as the daphnia did not produce adequate brood numbers over the 21-day period, The test did, however, allow for the evaluation of the changes in NH3 concentrations in test solutions overtime. The NH3 concentrations in the sealed Nalgene bottles maintained the initial ammonia concentrations, 0 mg/L, 20 mg/L, 40 mg/L, or 60 mg/L NH3, respectively for 48-72 hours. (Figure 1) [Appendix B –Table 11]

*NH3 Tests*

During this first NH3 test, the pump speeds were maintained between 4.5-5.0 volts for a flow of 20-24 ml/min for each of the iTIEs. Most of the test water 30 mg/L NH3 remained in the flow water of the iTIEs with none, 1 gram, and 3 gram zeolite treatments. (Figure 2) [Appendix B – Tables 1, 2] The iTIEs containing 5 grams of zeolite had significantly less mean NH3 in their flow water than the other treatments ($p<0.05$). [Appendix C – Tables 20-22]

During this second NH3 test, the pump speeds were maintained between 3.0-4.0 volts for a flow of 2-14 ml/min for each of the iTIEs. The zeolite adsorbed more of the NH3 in this second, slower flow ammonia test. The iTIEs containing 5 grams of zeolite had significantly less mean NH3 in their flow water than the other treatments ($p<0.05$). [Appendix C – Tables 23-25] During analysis, the 30 minute water sample from the iTIE with 1 gram of zeolite, replicate B, was not included in the statistical analysis because it had an uncharacteristically high NH3 concentration from the electrode reading, this was due to a probe problem while reading that sample. [Appendix B – Tables 3, 4] (Figure 3)

*Chelex Test*

The iTIEs containing 5 grams of chelex had significantly less mean Zn in their flow water compared to each of the other resin amounts ($p<0.05$). There was also a significant difference between iTIEs containing no resin and 1 gram of chelex and both 3 grams and 5 grams, respectively ($p<0.05$). [Appendix C – Tables 26-28]. The iTIEs containing 5 grams of

chelex had the highest Zn adsorbance, while the iTIEs containing no resin had the lowest Zn adsorbance. [Appendix B – Tables 12, 13] (Figure 4).

*Charcoal Test*

The [Zn] were highest in the iTIEs containing no charcoal and 1g of charcoal, compared to the iTIEs containing 3g and 5g of charcoal overtime. [Appendix B – Tables 18, 19] The iTIEs containing 5g of charcoal had significantly less Zn overtime, compared to the iTIEs containing no resin, 1g of charcoal, and 3g charcoal ($p<0.05$). [Appendix C – Tables 29, 30] There was also a significant difference between Zn in the iTIEs containing 3g of charcoal and no charcoal ($p<0.05$). [Appendix C – Table 30]. Time alone did not have a significant effect on the Zn concentrations. [Appendix C – Table 31]. But, when analyzed as a blocking variable, there was a significant difference between mean Zn concentrations from samples taken during the first sampling period and those taken at 90 minutes, and 120 minutes, respectfully. [Appendix C – Table 34] (Figure 10)

*Ni, Zn, and V Multiple Metals Test*

ANOVA statistical analysis was performed for the datasets of each individual metal. For each of the metals, there was no significant interaction between treatment and time ($p>0.05$). Treatment was the only independent variable with significant effect on the resulting contaminant concentrations. For this test, the water pH was 7.43. The flow rates on the iTIEs ranged from 2-10 ml/min. During the third sampling period, one of the chelex treatment iTIEs stopped functioning due to a faulty pump and could not be restarted. For this reason, the chelex treatment only has two iTIE replicates for the 90 minute and 120 minute sampling times.

The Ni concentrations were highest in the iTIEs containing no resin. The iTIEs containing both charcoal and chelex had the lowest Ni concentrations in the analyzed flow water. [Appendix B – Tables 5, 6] There was a significant difference between the iTIEs without resin and the chelex, charcoal, and both treatments, respectively ($p<0.05$ for each comparison). [Appendix C – Tables 38, 39]. There was also significantly lower average Ni concentrations in the flow water of the iTIEs containing both resins, as compared to the iTIEs containing charcoal only ($p<0.05$), but not in comparison to the iTIEs containing only chelex. [Appendix C – Tables 38, 39] (Figure 5)

The iTIE flow water Zn concentrations were significantly different between treatments. The Zn concentrations were highest in the iTIEs containing no resin. The iTIEs containing chelex had the lowest Zn concentrations in the analyzed flow water. [Appendix B – Tables 7, 8] There was a significant difference between the iTIEs without resin and the iTIEs with chelex, charcoal, and both treatments, respectively ($p<0.05$ for each comparison). [Appendix C – Tables 43, 44] The Zn concentrations in the iTIE flow through water were lower than the Ni concentrations in the same analyzed water samples. [Appendix B – Tables 6, 8]

The iTIE flow water V concentrations were significantly different between treatments. The V concentrations were highest in the iTIEs containing no resin. [Appendix B – Tables 9, 10] There was a significant difference between the iTIEs with no resin treatment and the iTIEs

with chelex, charcoal, and both treatments, respectively ($p<0.05$ for each comparison). [Appendix C – Tables 48, 49] The V concentrations in the iTIE flow through water remained relatively high throughout the experiment for the iTIEs with chelex, charcoal, and both resin treatments. (Figure 7).

*NH3 and Zn Test*

iTIES containing both zeolite and charcoal had less NH3 in the sample solutions compared to the other three treatments. [Appendix B – Tables 14, 15] There was a significantly lower NH3 concentration in the iTIEs containing both zeolite and charcoal when compared to the other three treatments ($p<0.05$). [Appendix C – Tables 50, 51] Sampling time also was a significant variable. The NH3 concentrations collected in the samples at 45 minutes were significantly lower than the NH3 concentrations in the samples collected at the latter two collection times ($p<0.05$). [Appendix C – Tables 52, 53]. (Figure 8)

iTIEs containing both zeolite and charcoal had the lowest average Zn compared to the other treatments. [Appendix B – Tables 16, 17]. There was a significantly lower Zn concentration in the iTIEs containing both zeolite and charcoal when compared to the iTIEs containing no resin and just zeolite overtime ($p<0.05$). [Appendix C – Tables 54, 55] There was not a statistically significant difference between Zn concentrations in the iTIEs containing both resins and only charcoal ($p>0.05$). [Appendix C – Table 55]. There was not a significant difference between the Zn concentrations in the iTIEs containing no resin and the iTIEs containing just zeolite, either ($p>0.05$). [Appendix C – Table 55]. Time did not have a significant effect on the mean Zn concentrations during the treatments, although it did change through time for the iTIEs with both resins and the iTIEs containing only charcoal. [Appendix C – Table 56]. (Figure 9).

*Diffuse Oxygen Tubing for iTIE Pore Water Tests*

The Zeus PTFE tubing, upon arrival, was clearly not going to work in the iTIE system. The tubing was too thin and folded harshly. Although not as thin, the harsh fold also occurred in the Ace Glass Inc. and Component Supply Co. tubing. The pore water tubing needs to curve to connect to the iTIE port system, so this tubing did not work. The MasterFlex tubing was not porous enough to reoxygenate the MilliQ. After an hour long testing period, the oxygen level in the test water had not increased.

**Discussion**

*Overall*

The general conclusions drawn from these iTIE experiments are that at least 5 grams of resin is necessary to observe statistically significant contaminant removal, and it is important to maintain slow pump flow-through speeds in order to allow for adequate resin-contaminant interaction time. Based on the results from the experiments presented in this paper, the iTIEs have the potential to accomplish Phase II of the U.S. EPA's three phase TIE system. The use of

different resins with different contaminant affinities will help facilitate this further expansion of the iTIE system.

*D.magna test*

The *D.magna* reproduction test evaluated the change in NH3 concentrations through time. The NH3 solutions were maintained in sealed Nalgene bottles, which prevented loss of NH3 via volatilization. The NH3 concentrations did not change significantly over the course of 48 hours, which suggests iTIE NH3 water samples can be evaluated within 24-hours of collection without concern regarding altered NH3 concentrations.

*NH3 Tests 1 & 2*

Results from the first 30 mg/L NH3 test indicate 5 grams of zeolite is required to significantly remove NH3 in solution. However, the low removal efficiency of the zeolite was unexpected, as the ability of zeolite to remove NH3 with the iTIE system was previously confirmed by Burton and Nordstrom.[19] This result was possibly due to the high pump flow of around 20-24 ml/min for each pump. Another explanation is that this brand of zeolite differed in its characteristics from those used by Burton and Nordstrom, as their test used Pond Care (Aquarium Pharmaceuticals) and SIR-600 (Resintech) for their zeolite tests.[19]

To examine this discrepancy, a second iTIE test was conducted using 30 mg/L NH3 and following the same protocols as before except pumps were closely monitored during this test to ensure they maintained a slower speed of 2-14 ml/min. It was difficult to maintain similar flow rates across the pumps as each resin type provides different resistances to the flow. Based on the results of this second NH3 experiment, it is clear pump speed is an important variable and should be monitored.

The second NH3 iTIE test had the same general result, that 5 grams of zeolite significantly remove the most NH3; however, 20-25 mg/L NH3 was removed as compared to only 2-5 mg/L NH3 by the final sampling point. The iTIEs containing 5 grams did not become saturated, as in the first test. In conclusion, lower pump speeds, which result in lower flow rates, are needed to ensure resins are not saturated prematurely.

*Chelex Test*

iTIEs with 5 grams of chelex removed significantly more Zn from the test solutions as compared to iTIEs with 1 and 3 grams. The iTIEs containing 3 grams of chelex also had significantly less average Zn compared to the iTIEs containing 1 gram of chelex. The iTIEs with 1 gram were near saturation by the end of the two hour sampling period. iTIEs containing chelex all had significantly lower average Zn concentrations during the first two sampling periods. The chelex became saturated overtime, as seen in the increasing concentrations during the course of sampling (although the time variable did not result in statistically significant mean Zn concentration differences). These Zn tests also indicate that chelex can adsorb high

concentrations of metals before becoming saturated. Initially dosed with 40 mg/L zinc, approximately 35 mg/L was removed from the solution.

The importance of water pH was observed. When using flume water (pH~7) instead of MilliQ (pH~5) there were improved results. The chelex chemical structure is influenced by pH, losing carboxyl binding sites, necessary for binding cationic metals, as the pH decreases. In order to maintain the efficiency of chelex as an anionic resin, the pH must remain closer to that of natural waters.

*Charcoal Test*

iTIEs with 5 grams of charcoal removed significantly more Zn from the test solutions as compared to those iTIEs containing no charcoal, 1g, and 3g of charcoal. 5g of charcoal can remove the greatest amount of Zn, while taking longer to reach saturation overtime. iTIEs containing 1g of charcoal reached saturation by the second sampling period, while those containing 3g of charcoal reached saturation by the end of the two hour test. The iTIEs containing 5g showed evidence of saturation overtime, but had not yet reached complete saturation by the end of the experiment. This test again supports that more resin (5g) is best for more Zn removal. Comparing the charcoal results to the chelex test, it is clear that chelex has a stronger affinity for Zn, as the iTIEs containing 1g, 3g, and 5g of chelex resulted in sample water with lower Zn concentrations compared to the iTIEs containing the same amounts of charcoal.

*Ni, Zn, and V Multiple Metals Test*

The iTIEs containing no resin had significantly higher average Ni concentrations in their flow-through water compared to the iTIEs containing chelex, charcoal, and both chelex and charcoal. Although time did not have a significant effect, evidence of chelex and charcoal saturation was observed. Charcoal appears to have a lower saturation point for Ni than chelex. The iTIEs containing both chelex and charcoal had the highest adsorption of Ni. The iTIEs containing both resins also had significantly lower average Ni compared to charcoal only. Based on these results, chelex is a better resin for Ni than charcoal but the combination of both resins is best for adsorbing Ni.

The iTIEs containing no resin, similarly, had significantly higher average Zn compared to chelex, charcoal, and both chelex and charcoal Compared to Ni, Zn has a higher saturation point. Charcoal, however, again had a lower saturation point for Zn as compared to chelex. The combination of charcoal and chelex did not have improved Zn removal compared to either chelex or charcoal.

These resins also removed V, but the removal was lower than for Ni and Zn. Only ~1 mg/L of V was removed on average for all three resin treatments. The poor adsorption of V can be attributed to the fact that V most frequently takes the form of an anionic vanadate oxide, which would not bind to the negative binding sites of the charcoal and chelex.[33]

These findings support the use of the iTIEs for Phase II evaluations, as the resins can be used to separate specific metals, and, in theory, specific contaminants from water sources. Different resins can be used to target specific contaminants and will have low affinities for other

contaminants. The use of multiple resins allows for testing the effects of multiple contaminants on organisms in the iTIE chambers, which can improve the efficiency of the iTIE tests. If different resins remove different contaminants, combining the resins in one chamber can allow for these multiple contaminants to be removed and the resulting effect to be observed, which further supports Phase II characterization.

*Zn and NH3 Test*

The iTIEs containing both charcoal and zeolite had significantly lower NH3 compared to the iTIEs containing charcoal, zeolite, or no resin. Overall, however, the resins did not remove a significant amount of NH3 from the test solution. Perhaps this is due to the co-occurrence of Zn, which may outcompete NH3 for binding sites on charcoal. This type of situation should be taken into account when designing site-specific iTIE resin combinations.

The iTIEs containing both zeolite and charcoal had significantly lower mean Zn concentrations compared to iTIEs containing no resin and only zeolite. Based on these results, zeolite does not have a high affinity for Zn as the iTIEs containing only zeolite had similar Zn concentrations as those iTIEs containing no resin. The iTIEs containing only charcoal did not have a significantly different mean Zn concentration compared to the iTIEs containing both resins, which provides evidence that suggests that the charcoal is the main resin adsorbing Zn in this test.

*Diffuse Oxygen Tubing for iTIE Pore Water Tests*

The iTIE pore water tubing tests were not successful. However, they did rule out a couple of tubing options. Despite investigation, the original tubing used by Burton and Nordstrom could not be found.[19] It is key for the tubing to be porous and have a balance between flexibility and sturdiness to account for the necessary curvature in the iTIE pore water set-up.

*Recommendations for Future iTIE Experiments*

The current design of the iTIEs can be improved by optimizing the resins to be highly selective for specific contaminants, and by attaining accurate, low-flow pumping rates. The pumps require immediate attention. During experiments, pumps would on occasion stop working and had difficulty maintaining low flow rates. The resin comparisons showed that TIE Phase II characterizations are possible but need further validation of contaminant removal efficiency and specificity. Testing with other key metals, such as Pb, Cd, Cu should also be conducted.

The current iTIE tubing system does not seem feasible for field tests (unless there is adequate space between the pumps and the iTIEs), so testing experiments to see how flow rates are affected by the shortening of tubing between iTIE, pump, and collection bottle might be helpful for field implementation.

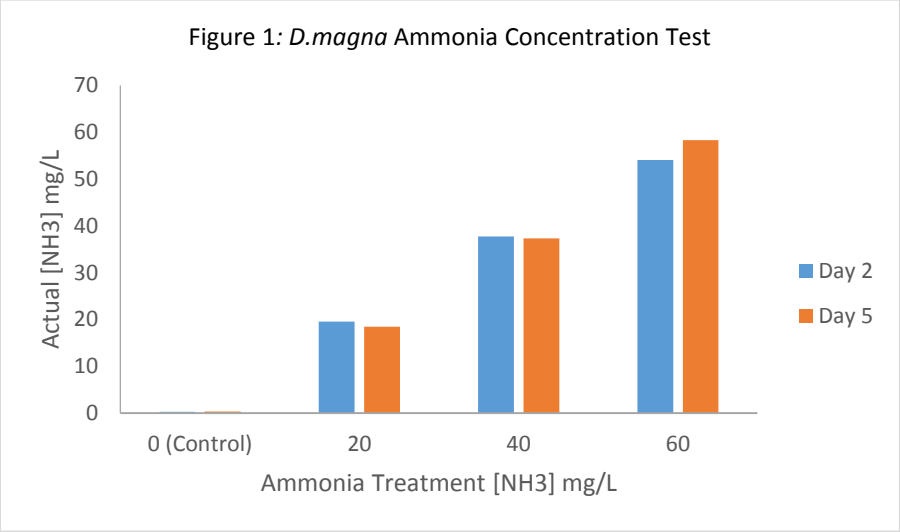
It is possible that the iTIE spike design needs to be reevaluated. Returning to a smaller resin and chamber size similar to the original version (Burton and Nordstrom 2004) might allow for improved suction and flow.[19] The size of the resin chamber may also need optimizing, as

only 8 grams of courser resins such as zeolite and charcoal could fit in the chamber. Since larger quantities cannot be used for some resins, the threshold of resins at 3 or 5 grams should be determined for a range of contaminants. These resin tests should also be further validated through 24 hour tests that mimic the timeline of field exposure experiments.

Determining the detection point limits of the resins to specific contaminants is also an important next experimental option. If contaminants cannot be detected from resin extracts or in the ambient waters, then the usefulness of the iTIE is lost. It is important for the iTIEs to be able to detect contaminants at their acute and chronic toxicity concentrations. This should be evaluated with field testing where a broad range of chemicals are monitored.

Future tests could continue to sample the iTIE flow-through water until the 3 gram and 5 gram resin reach saturation. This would allow for better conclusion about whether 3 grams of chelex is sufficient for a typical 24-hour iTIE field test depending on the saturation time of the two resin amounts. The ability to reduce the amount of chelex used in iTIE experiments could make the system more economically feasible, as chelex is one of the most expensive resins.

## Figures




*Daphnia magna test:* NH3 concentrations of the test solutions two and three days, respectively, after refilling the bottles with fresh NH3 solution. [Appendix B – Table 11]

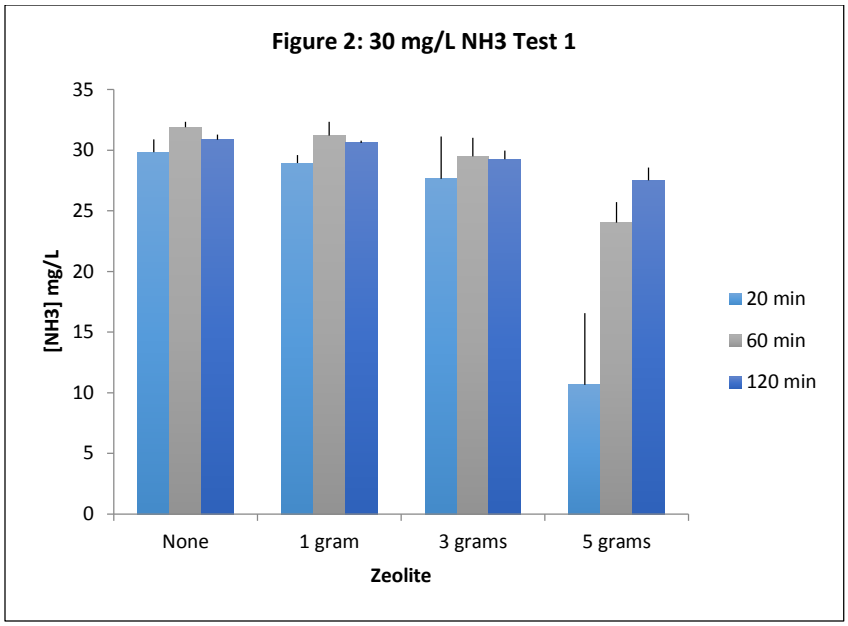



Figure 2: NH3 Test 1: Removal of NH3 by zeolite through time. Error bars are Standard Deviation. [Appendix B – Tables 1, 2]

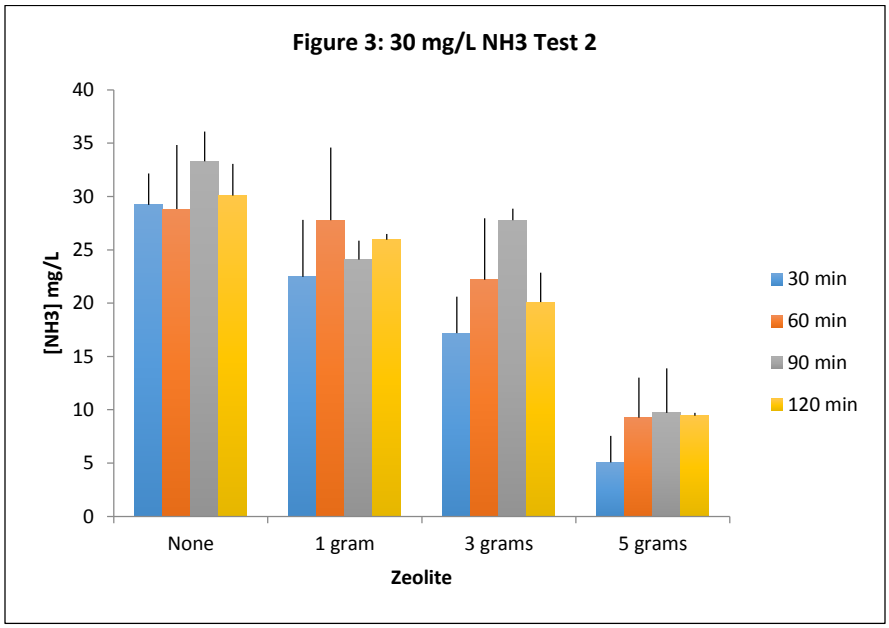



Figure 3: NH3 Test 2: Removal of NH3 by zeolite through time. Error bars are Standard Deviation. [Appendix B – Tables 3, 4]

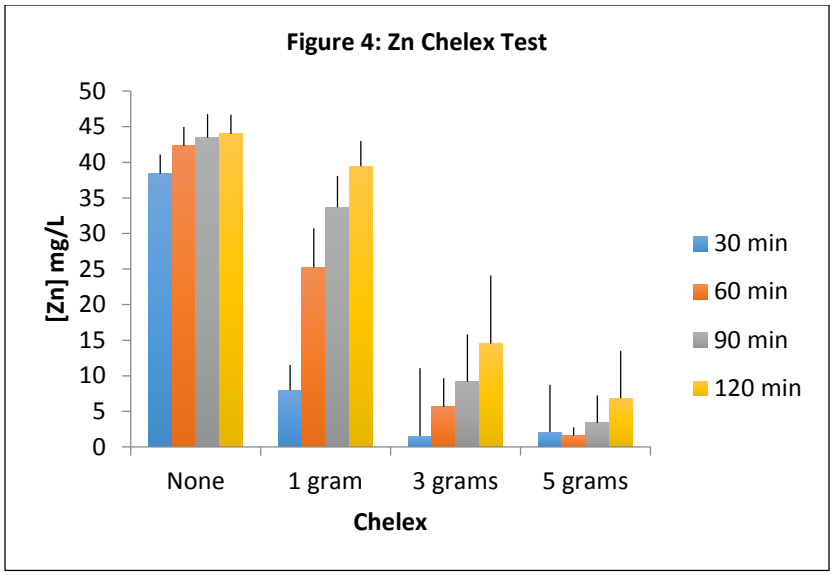



Figure 4: Chelex-Zn test: Removal of Zn by chelex through time. Error bars are Standard Deviation. [Appendix B – Tables 12, 13]

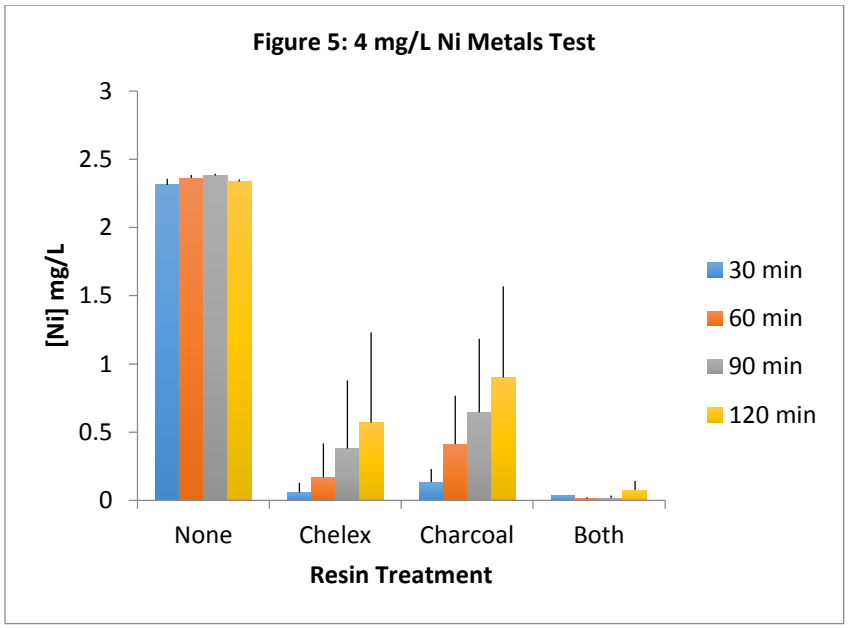



Figure 5: Multiple Metals Ni Results: Removal of Ni by chelex, charcoal, and both resins through time. Error bars are Standard Deviation. [Appendix B – Tables 5, 6]

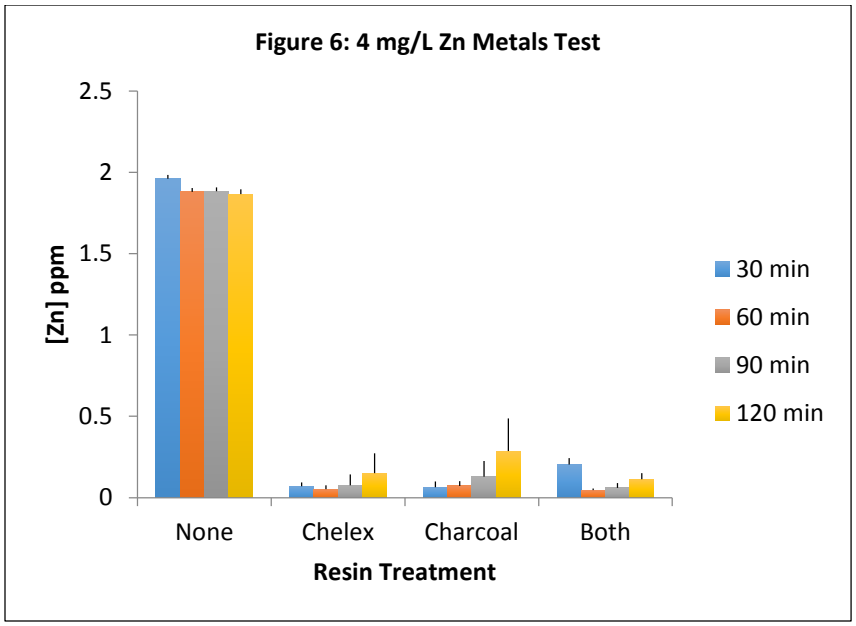



Figure 6: Multiple Metals Zn Results: Removal of Zn by chelex, charcoal, and both resins through time. Error bars are Standard Deviation. [Appendix B – Tables 7, 8]

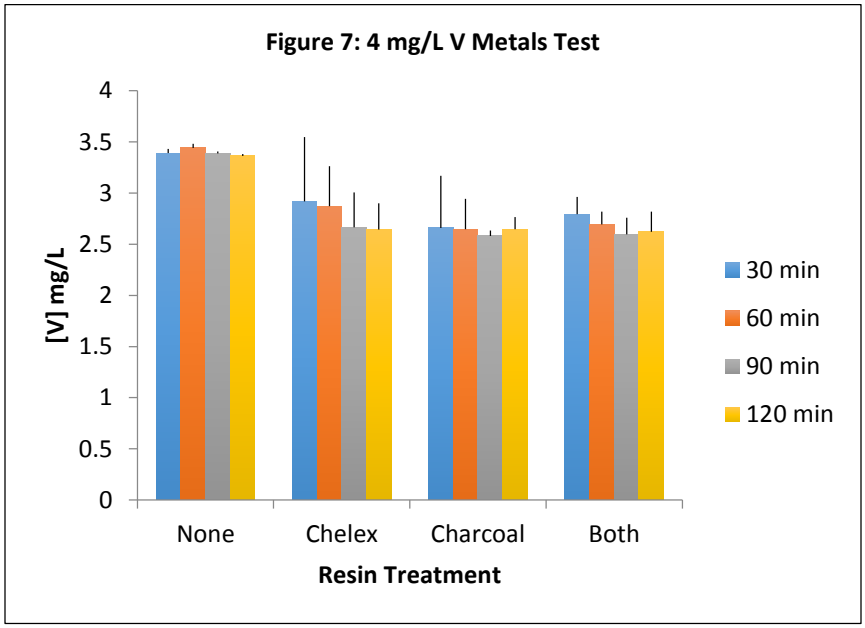



Figure 7: Multiple Metals V Results: Removal of V by chelex, charcoal, and both resins through time. Error bars are Standard Deviation. [Appendix B – Tables 9, 10]

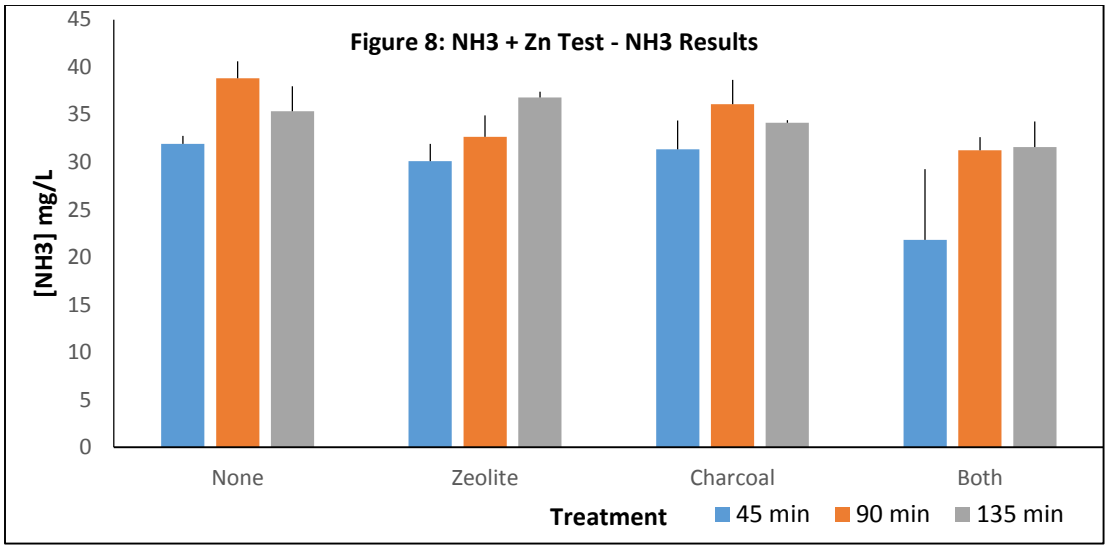



Figure 8: NH3 and Zn Results: Removal of NH3 by zeolite, charcoal, and both resins through time. Error bars are Standard Deviation. [Appendix B – Tables 14, 15]

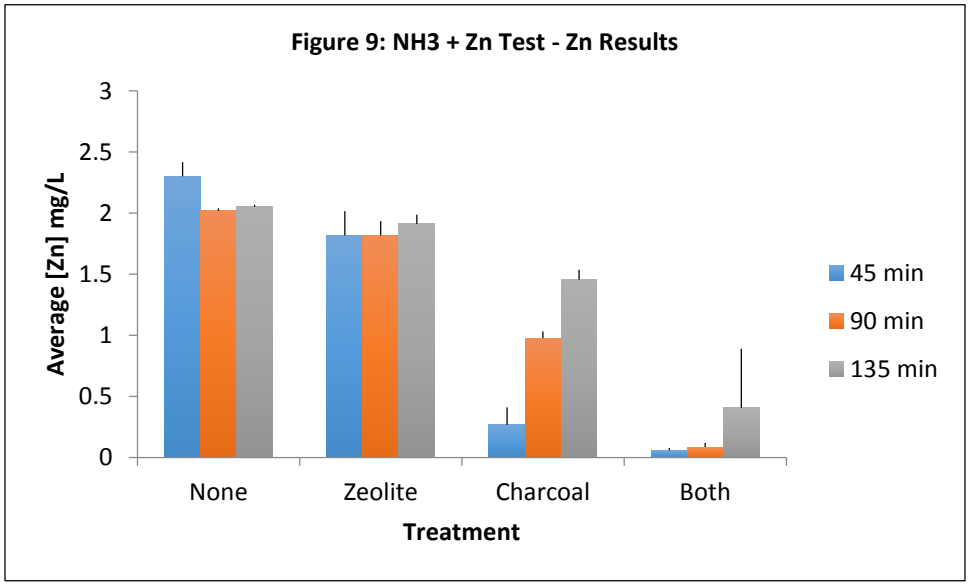



Figure 9: NH3 and Zn Results: Removal of Zn by zeolite, charcoal, and both resins through time. Error bars are Standard Deviation. [Appendix B – Tables 16, 17]

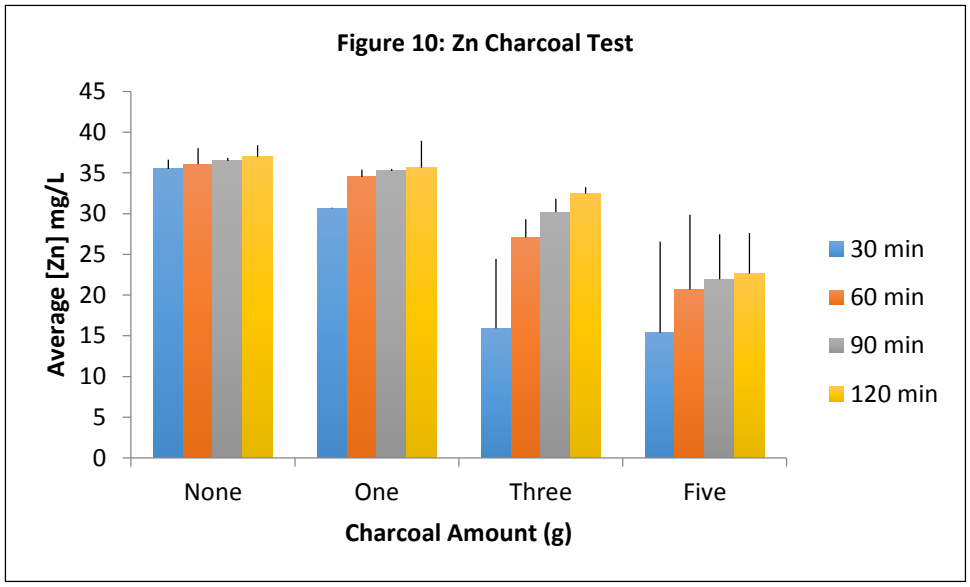



Figure 10: Charcoal - Zn Results: Removal of Zn by different amounts of charcoal through time. Error bars are Standard Deviation. [Appendix B – Tables 18, 19]

## Appendix A

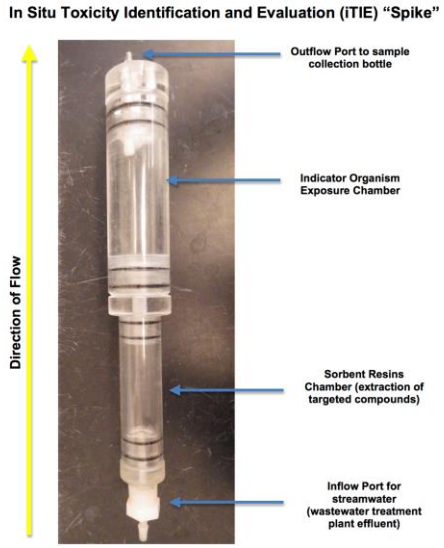



Image 1: iTIE spike design, with resin chamber and organism chamber (Gus Steigmeyer, 2015).

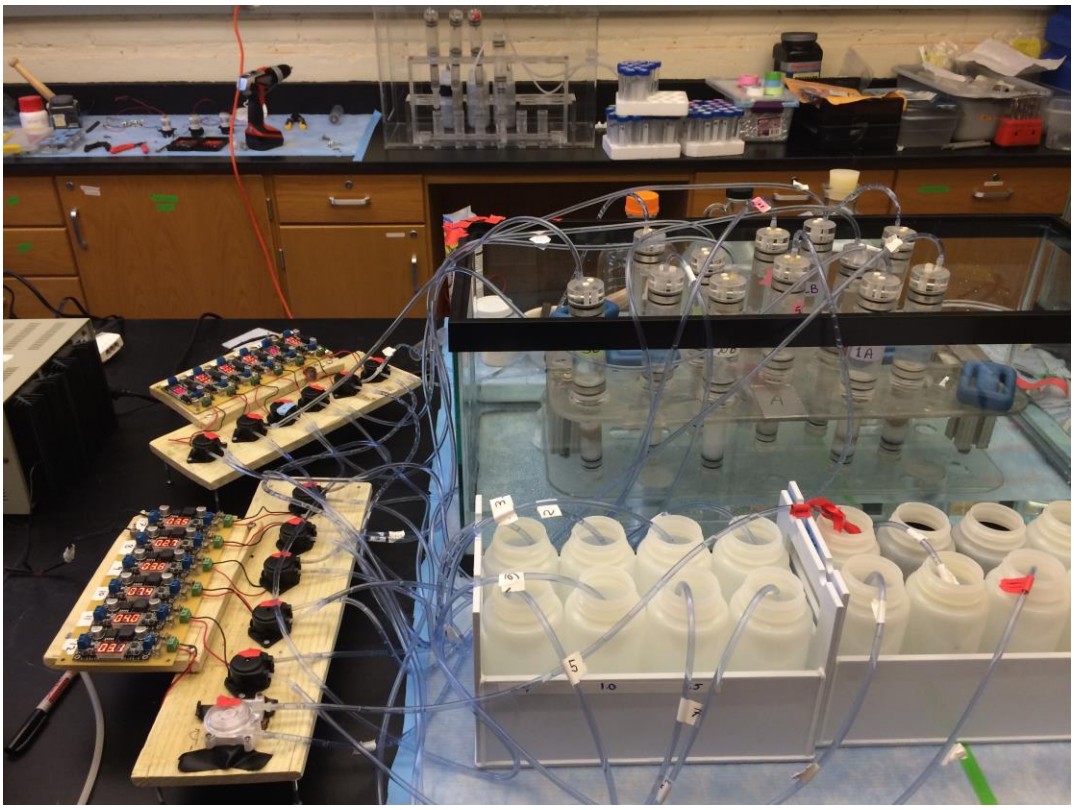


Image 2: iTIE laboratory system set up, with view of the iTIEs in the aquarium with test water, the peristaltic pump system, and the collection bottles, all connected via tubing (Kathryn Meyer, 2016)

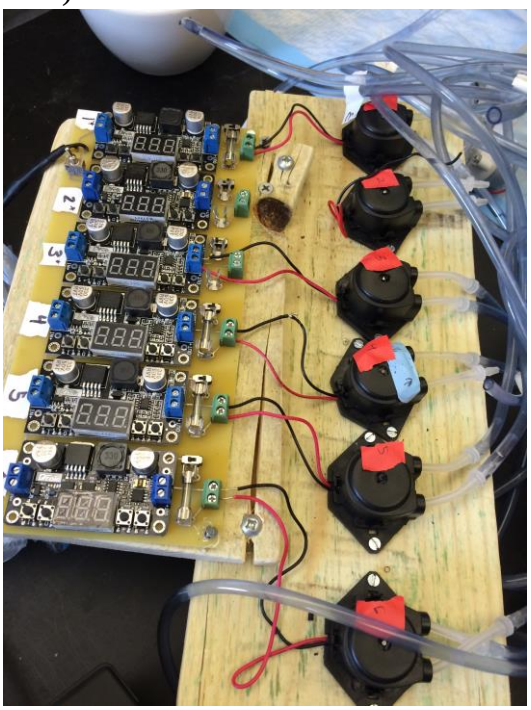


Image 3: Custom-made circuit board with LM2496 voltage switching regulators (DROK) and pump heads (Kathryn Meyer, 2016)

## Appendix B

Table i: List of Conducted iTIE Experiments

| Test(s) | Resin(s) | Pollutant/Contaminant(s) |
|---|---|---|
| NH3 #1 | Zeolite (1g, 3g, 5g,) | 30 mg/L NH3 |
| NH3 #2 | Zeolite (1g, 3g, 5g,) | 30 mg/L NH3 |
| Zn | Chelex (1g, 3g, 5g,) | 80 µg/L Zn |
| Zn | Chelex (1g, 3g, 5g,) | 40 mg/L Zn |
| Zn | Charcoal (1g, 3g, 5g,) | 40 mg/L Zn |
| Zn | Chelex (10g), Charcoal (8g), Chelex+Charcoal (4g each) | 4 mg/L Zn |
| Zn, Ni, V | Chelex (10g), Charcoal (8g), Chelex+Charcoal (4g each) | 4 mg/L Zn, 4 mg/L V, 4 mg/L Ni |
| Zn and NH3 | Zeolite (5g), Charcoal (5g), Zeolite+Charcoal (5g each) | 30 mg/L NH3, 4 mg/L Zn |

Table 1: NH3 Test 1 NH3 Electrode Raw data and log analysis (Figure 2)

| Treatment | Replicate | Time (min) | mV | log[NH3] | [NH3] mg/L |
|---|---|---|---|---|---|
| Standard 0 | N/A | N/A | 224 | N/A | 0 |
| Standard 5 | N/A | N/A | 55.4 | 0.69897 | 5 |
| Standard 10 | N/A | N/A | 34.8 | 1 | 10 |
| Standard 25 | N/A | N/A | 11.7 | 1.39794 | 25 |
| Standard 50 | N/A | N/A | -6.5 | 1.69897 | 50 |
| Standard 75 | N/A | N/A | -17.2 | 1.875061 | 75 |
| None | A | 20 | 6 | 1.491926 | 31.04028 |
| None | B | 20 | 7.7 | 1.464111 | 29.11461 |
| None | C | 20 | 7.5 | 1.467383 | 29.33481 |
| 1 gram | A | 20 | 7.3 | 1.470656 | 29.55668 |
| 1 gram | B | 20 | 7.8 | 1.462475 | 29.00513 |

| | | | | | |
|---|---|---|---|---|---|
| 1 gram | C | 20 | 8.5 | 1.451022 | 28.25022 |
| 3 grams | A | 20 | 6.7 | 1.480473 | 30.23239 |
| 3 grams | B | 20 | 7.8 | 1.462475 | 29.00513 |
| 3 grams | C | 20 | 13.2 | 1.374123 | 23.66588 |
| 5 grams | A | 20 | 25.6 | 1.17124 | 14.83337 |
| 5 grams | B | 20 | 47.6 | 0.811286 | 6.475692 |
| None | A | 40 | 7.6 | 1.465747 | 29.2245 |
| None | B | 40 | 6.3 | 1.487017 | 30.69143 |
| None | C | 40 | 5.4 | 1.501743 | 31.74991 |
| 1 gram | A | 40 | 5.8 | 1.495198 | 31.27504 |
| 1 gram | B | 40 | 8 | 1.459203 | 28.78741 |
| 1 gram | C | 40 | 8 | 1.459203 | 28.78741 |
| 3 grams | A | 40 | 7.1 | 1.473928 | 29.78022 |
| 3 grams | B | 40 | 8.5 | 1.451022 | 28.25022 |
| 3 grams | C | 40 | 8.5 | 1.451022 | 28.25022 |
| 5 grams | A | 40 | 14.3 | 1.356125 | 22.70518 |
| 5 grams | B | 40 | 31.9 | 1.068162 | 11.69936 |
| 5 grams | C | 40 | 14.4 | 1.354489 | 22.6198 |
| None | A | 60 | 5.2 | 1.505483 | 32.02453 |
| None | B | 60 | 5 | 1.508756 | 32.26682 |
| None | C | 60 | 5.7 | 1.4973 | 31.42675 |
| 1 gram | A | 60 | 6.3 | 1.48748 | 30.72413 |
| 1 gram | B | 60 | 4.8 | 1.512029 | 32.51094 |
| 1 gram | C | 60 | 6.6 | 1.48257 | 30.37873 |
| 3 grams | A | 60 | 6.2 | 1.489116 | 30.84013 |
| 3 grams | B | 60 | 7.1 | 1.474386 | 29.81167 |
| 3 grams | C | 60 | 8.9 | 1.444926 | 27.85649 |

| | | | | | |
|---|---|---|---|---|---|
| 5 grams | A | 60 | 13.5 | 1.36964 | 23.42286 |
| 5 grams | B | 60 | 14.3 | 1.356547 | 22.72724 |
| 5 grams | C | 60 | 10.8 | 1.41383 | 25.93163 |
| None | A | 90 | 7.1 | 1.473928 | 29.78022 |
| None | B | 90 | 7.3 | 1.470656 | 29.55668 |
| None | C | 90 | 6.8 | 1.478836 | 30.11871 |
| 1 gram | A | 90 | 7.6 | 1.465747 | 29.2245 |
| 1 gram | B | 90 | 7.1 | 1.473928 | 29.78022 |
| 1 gram | C | 90 | 8.1 | 1.457566 | 28.67916 |
| 3 grams | A | 90 | 7.9 | 1.460839 | 28.89606 |
| 3 grams | B | 90 | 7.3 | 1.470656 | 29.55668 |
| 3 grams | C | 90 | 8.6 | 1.449386 | 28.14399 |
| 5 grams | A | 90 | 14.8 | 1.347944 | 22.28149 |
| 5 grams | B | 90 | 11.5 | 1.401937 | 25.23116 |
| 5 grams | C | 90 | 11.5 | 1.401937 | 25.23116 |
| None | A | 120 | 6.4 | 1.485381 | 30.57602 |
| None | B | 120 | 5.9 | 1.493562 | 31.15744 |
| None | C | 120 | 6.4 | 1.485381 | 30.57602 |
| 1 gram | A | 120 | 6.5 | 1.483745 | 30.46105 |
| 1 gram | B | 120 | 6.2 | 1.488653 | 30.80727 |
| 1 gram | C | 120 | 7.3 | 1.470656 | 29.55668 |
| 3 grams | A | 120 | 7.1 | 1.473928 | 29.78022 |
| 3 grams | B | 120 | 8.3 | 1.454294 | 28.46388 |
| 3 grams | C | 120 | 10.2 | 1.423207 | 26.49764 |
| 5 grams | A | 120 | 9.2 | 1.439569 | 27.51495 |
| 5 grams | B | 120 | 8.2 | 1.45593 | 28.57132 |

Table 2: NH3 Test 2 Mean Concentration values and standard deviation (Figure 2)

| Zeolite | [NH3] mg/L | Time (min) | Stnd deviation |
|---|---|---|---|
| None | 29.8298999 | 20 | 1.05398363 |
| 1 gram | 28.9373417 | 20 | 0.65586321 |
| 3 grams | 27.6344674 | 20 | 3.49124924 |
| 5 grams | 10.654529 | 20 | 5.90976801 |
| None | 30.5552816 | 40 | 1.2681962 |
| 1 gram | 29.6166181 | 40 | 0.65586321 |
| 3 grams | 28.7602177 | 40 | 0.88334843 |
| 5 grams | 19.0081136 | 40 | 6.32971009 |
| None | 31.9060348 | 60 | 0.43238737 |
| 1 gram | 31.2045958 | 60 | 1.14442864 |
| 3 grams | 29.5027613 | 60 | 1.51561805 |
| 5 grams | 24.027242 | 60 | 1.68552311 |
| None | 29.8185359 | 90 | 0.28296879 |
| 1 gram | 29.2279607 | 90 | 0.55053978 |
| 3 grams | 28.8655761 | 90 | 0.99892256 |
| 5 grams | 24.2479352 | 90 | 1.70299396 |
| None | 30.8667299 | 120 | 0.41112344 |
| 1 gram | 30.6147812 | 120 | 0.1763382 |
| 3 grams | 29.2669255 | 120 | 0.70438335 |

| | | | |
|---|---|---|---|
| 5 grams | 27.5279679 | 120 | 1.03689926 |

Table 3: NH3 Test 2 NH3 Electrode Raw data and log analysis (Figure 3)

| Treatment | Replicate | Time (min) | mV | [NH3] mg/L | log[NH3] |
|---|---|---|---|---|---|
| Standard 0 | N/A | N/A | 190 | 0 | N/A |
| Standard 5 | N/A | N/A | 55.9 | 5 | 0.69897 |
| Standard 10 | N/A | N/A | 40.1 | 10 | 1 |
| Standard 25 | N/A | N/A | 14.3 | 25 | 1.39794 |
| Standard 50 | N/A | N/A | -2.6 | 50 | 1.69897 |
| Standard 75 | N/A | N/A | -15.1 | 75 | 1.875061 |
| Flow | N/A | 0 | 17.9 | 22.10212 | 1.344434 |
| Flow | N/A | 120 | 23.1 | 18.13043 | 1.258408 |
| None | A | 30 | 7.7 | 32.59694 | 1.513177 |
| None | B | 30 | 12.4 | 27.25353 | 1.435423 |
| None | C | 30 | 11.9 | 27.77758 | 1.443694 |
| One Gram | A | 30 | 13.4 | 26.2349 | 1.418879 |
| One Gram | B | 30 | -1.5 | 46.27818 | 1.665376 |
| One Gram | C | 30 | 22.3 | 18.69145 | 1.271643 |
| Three Grams | A | 30 | 28.1 | 14.98619 | 1.175691 |
| Three Grams | B | 30 | 27.3 | 15.44991 | 1.188926 |
| Three Grams | C | 30 | 19.1 | 21.11455 | 1.324582 |

| | | | | | |
|---|---|---|---|---|---|
| Five Grams | A | 30 | 78.1 | 2.231106 | 0.34852 |
| Five Grams | B | 30 | 51.5 | 6.145753 | 0.788575 |
| Five Grams | C | 30 | 48.7 | 6.83749 | 0.834897 |
| None | A | 60 | 10 | 29.86256 | 1.475127 |
| None | B | 60 | 17.6 | 22.35615 | 1.349397 |
| None | C | 60 | 6.4 | 34.25179 | 1.534683 |
| One Gram | A | 60 | 5.4 | 35.5817 | 1.551227 |
| One Gram | B | 60 | 16.8 | 23.04792 | 1.362632 |
| One Gram | C | 60 | 15 | 24.68368 | 1.39241 |
| Three Grams | A | 60 | 11 | 28.74641 | 1.458584 |
| Three Grams | B | 60 | 20.6 | 19.94191 | 1.299767 |
| Three Grams | C | 60 | 23.4 | 17.92442 | 1.253445 |
| Five Grams | A | 60 | 50.1 | 6.482401 | 0.811736 |
| Five Grams | B | 60 | 45.4 | 7.75336 | 0.88949 |
| Five Grams | C | 60 | 30.8 | 13.52147 | 1.131024 |
| None | A | 90 | 4.8 | 36.40431 | 1.561153 |
| None | B | 90 | 9 | 31.02204 | 1.49167 |
| None | C | 90 | 7.8 | 32.47301 | 1.511522 |
| One Gram | A | 90 | 16.6 | 23.22418 | 1.36594 |
| One Gram | B | 90 | 17 | 22.873 | 1.359323 |
| One Gram | C | 90 | 13.5 | 26.13515 | 1.417225 |

| | | | | | |
|---|---|---|---|---|---|
| Three Grams | A | 90 | 12.9 | 26.73936 | 1.427151 |
| Three Grams | B | 90 | 11.9 | 27.77758 | 1.443694 |
| Three Grams | C | 90 | 10.9 | 28.85612 | 1.460238 |
| Five Grams | A | 90 | 49.1 | 6.734096 | 0.828279 |
| Five Grams | B | 90 | 44.8 | 7.932608 | 0.899416 |
| Five Grams | C | 90 | 29 | 14.48112 | 1.160802 |
| None | A | 120 | 8.4 | 31.73923 | 1.501596 |
| None | B | 120 | 8.3 | 31.86037 | 1.503251 |
| None | C | 120 | 13 | 26.6377 | 1.425497 |
| One Gram | A | 120 | 13.6 | 26.03578 | 1.415571 |
| One Gram | B | 120 | 14.3 | 25.35072 | 1.40399 |
| One Gram | C | 120 | 13.2 | 26.43553 | 1.422188 |
| Three Grams | A | 120 | 18.1 | 21.93438 | 1.341125 |
| Three Grams | B | 120 | 18.7 | 21.43874 | 1.331199 |
| Three Grams | C | 120 | 24.9 | 16.92895 | 1.22863 |
| Five Grams | A | 120 | 40.8 | 9.23825 | 0.96559 |
| Five Grams | B | 120 | 39.4 | 9.744296 | 0.98875 |
| Five Grams | C | 120 | 40.6 | 9.3089 | 0.968898 |

Table 4: NH3 Test 2 Mean Concentration values and standard deviation (Figure 3)

| Zeolite | Average [NH3] mg/L | Std Deviation | Time (min) |
|---|---|---|---|
| None | 29.20935157 | 2.94541585 | 30 |
| 1 gram | 22.46317163 | 5.33402393 | 30 |
| 3 grams | 17.18354764 | 3.41223591 | 30 |
| 5 grams | 5.071449615 | 2.48400665 | 30 |
| None | 28.82349823 | 6.01550508 | 60 |
| 1 gram | 27.7710993 | 6.81344554 | 60 |
| 3 grams | 22.20424582 | 5.75477686 | 60 |
| 5 grams | 9.252410093 | 3.75133061 | 60 |
| None | 33.29978449 | 2.78475488 | 90 |
| 1 gram | 24.0774424 | 1.79065774 | 90 |
| 3 grams | 27.79102158 | 1.05844108 | 90 |
| 5 grams | 9.715940142 | 4.17004578 | 90 |
| None | 30.07910033 | 2.98095586 | 120 |
| 1 gram | 25.94067782 | 0.548624 | 120 |
| 3 grams | 20.10068847 | 2.75796203 | 120 |
| 5 grams | 9.430481975 | 0.27405728 | 120 |
| START FLOW | 22.10212175 | N/A | 0 |
| END FLOW | 18.13043367 | N/A | 120 |

Table 5: Multiple Metals Test Ni ICP data values (analyzed values used in analysis, as corrected for ICP standard curve) (Figure 4)

| Treatment | Replicate | Time (min) | Intensity | ICP Reported [Ni] mg/L | Analyzed [Ni] mg/L |
|---|---|---|---|---|---|
| FLOW | N/A | 0 | 3625.4368 | 3.519 | 2.396452286 |

| | | | | | |
|---|---|---|---|---|---|
| NONE | A | 30 | 3556.9782 | 3.442 | 2.351200439 |
| NONE | B | 30 | 3513.3281 | 3.392 | 2.32234725 |
| NONE | C | 30 | 3427.7021 | 3.296 | 2.265747532 |
| Chelex | A | 30 | 211.24457 | -0.339 | 0.139634909 |
| Chelex | B | 30 | 22.40828 | -0.552 | 0.014812111 |
| Chelex | C | 30 | 27.4314 | -0.546 | 0.018132447 |
| Charcoal | A | 30 | 234.29325 | -0.313 | 0.154870333 |
| Charcoal | B | 30 | 327.38778 | -0.207 | 0.216406806 |
| Charcoal | C | 30 | 47.506856 | -0.524 | 0.031402537 |
| Both | A | 30 | 52.928669 | -0.517 | 0.034986413 |
| Both | B | 30 | 52.933048 | -0.517 | 0.034989308 |
| Both | C | 30 | 52.958801 | -0.517 | 0.035006331 |
| NONE | A | 60 | 3610.464 | 3.502 | 2.386555147 |
| NONE | B | 60 | 3568.104 | 3.454 | 2.358554722 |
| NONE | C | 60 | 3542.256 | 3.425 | 2.341468888 |
| Chelex | A | 60 | 687.8732 | 0.2 | 0.454691505 |
| Chelex | B | 60 | 57.202677 | -0.513 | 0.037811578 |
| Chelex | C | 60 | 23.206249 | -0.551 | 0.015339577 |
| Charcoal | A | 60 | 709.45582 | 0.224 | 0.468957848 |
| Charcoal | B | 60 | 1112.0278 | 0.679 | 0.735062217 |
| Charcoal | C | 60 | 48.11024 | -0.523 | 0.03180138 |
| Both | A | 60 | 7.7340579 | -0.569 | 0.005112295 |
| Both | B | 60 | 25.704745 | -0.548 | 0.01699111 |
| Both | C | 60 | 32.015694 | -0.541 | 0.021162714 |
| NONE | A | 90 | 3622.3189 | 3.516 | 2.394391311 |
| NONE | B | 90 | 3603.445 | 3.494 | 2.381915484 |
| NONE | C | 90 | 3582.7269 | 3.471 | 2.36822057 |

| | | | | | |
|---|---|---|---|---|---|
| Chelex | A | 90 | 1109.6535 | 0.677 | 0.733492758 |
| Chelex | B | 90 | 38.594501 | -0.534 | 0.025511376 |
| Charcoal | A | 90 | 1176.7518 | 0.752 | 0.777845485 |
| Charcoal | B | 90 | 1671.8968 | 1.312 | 1.105141586 |
| Charcoal | C | 90 | 76.188651 | -0.491 | 0.050361509 |
| Both | A | 90 | 16.602798 | -0.559 | 0.010974626 |
| Both | B | 90 | 1.8185662 | -0.575 | 0.001202092 |
| Both | C | 90 | 56.462941 | -0.513 | 0.037322605 |
| NONE | A | 120 | 3563.8566 | 3.45 | 2.355747101 |
| NONE | B | 120 | 3523.6241 | 3.404 | 2.329153007 |
| NONE | C | 120 | 3522.3634 | 3.403 | 2.328319678 |
| Chelex | A | 120 | 1569.2823 | 1.196 | 1.037312287 |
| Chelex | B | 120 | 154.70011 | -0.402 | 0.102258417 |
| Charcoal | A | 120 | 1621.5755 | 1.255 | 1.071878636 |
| Charcoal | B | 120 | 2220.0374 | 1.931 | 1.467468361 |
| Charcoal | C | 120 | 242.27478 | -0.304 | 0.160146207 |
| Both | A | 120 | 13.409555 | -0.562 | 0.008863858 |
| Both | B | 120 | 100.40222 | -0.464 | 0.066366934 |
| Both | C | 120 | 222.86193 | -0.325 | 0.147314106 |

Table 6: Multiple Metals Test Ni Mean Concentration values and standard deviation (Figure 4)

| Treatment | Average [Ni] mg/L | Time (min) | Stdeviation |
|---|---|---|---|
| None | 2.313098407 | 30 | 0.04347074 |
| Chelex | 0.057526489 | 30 | 0.07112735 |
| Charcoal | 0.134226559 | 30 | 0.09421395 |
| Both | 0.034994017 | 30 | 1.0762E-05 |
| None | 2.362192919 | 60 | 0.02276225 |

| | | | |
|---|---|---|---|
| Chelex | 0.169280887 | 60 | 0.2474281 |
| Charcoal | 0.411940482 | 60 | 0.35508053 |
| Both | 0.01442204 | 60 | 0.00832791 |
| None | 2.381509122 | 90 | 0.0130901 |
| Chelex | 0.379502067 | 90 | 0.50061844 |
| Charcoal | 0.644449527 | 90 | 0.53989454 |
| Both | 0.016499774 | 90 | 0.01868337 |
| None | 2.337739929 | 120 | 0.01560023 |
| Chelex | 0.569785352 | 120 | 0.66118293 |
| Charcoal | 0.899831068 | 120 | 0.67042753 |
| Both | 0.074181633 | 120 | 0.06955516 |

Table 7: Multiple Metals Test Zn ICP data values (analyzed values used in analysis, as corrected for ICP standard curve) (Figure 5)

| Treatment | Replicate | Time | Intensity | Reported [Zn] mg/L | Analyzed[Zn] mg/L |
|---|---|---|---|---|---|
| FLOW | N/A | 0 | 3509.52 | 3.244 | 2.007822605 |
| NONE | A | 30 | 3430.384 | 3.164 | 1.962547855 |
| NONE | B | 30 | 3384.302 | 3.117 | 1.936183997 |
| NONE | C | 30 | 3467.458 | 3.201 | 1.98375838 |
| Chelex | A | 30 | 71.01253 | -0.226 | 0.040626792 |
| Chelex | B | 30 | 148.1175 | -0.148 | 0.084739134 |
| Chelex | C | 30 | 143.6733 | -0.153 | 0.082196541 |
| Charcoal | A | 30 | 83.87879 | -0.213 | 0.047987674 |
| Charcoal | B | 30 | 62.72293 | -0.234 | 0.035884253 |
| Charcoal | C | 30 | 184.5279 | -0.111 | 0.105569768 |
| Both | A | 30 | 294.243 | -0.001 | 0.16833859 |
| Both | B | 30 | 429.4538 | 0.136 | 0.245693689 |

| | | | | | |
|---|---|---|---|---|---|
| Both | C | 30 | 347.166 | 0.053 | 0.198616243 |
| NONE | A | 60 | 3309.994 | 3.042 | 1.893671938 |
| NONE | B | 60 | 3308.906 | 3.041 | 1.893049786 |
| NONE | C | 60 | 3237.496 | 2.969 | 1.852195356 |
| Chelex | A | 60 | 138.3315 | -0.158 | 0.079140478 |
| Chelex | B | 60 | 68.56575 | -0.228 | 0.039226973 |
| Chelex | C | 60 | 66.23735 | -0.231 | 0.037894875 |
| Charcoal | A | 60 | 119.4788 | -0.177 | 0.068354712 |
| Charcoal | B | 60 | 178.9218 | -0.117 | 0.102362499 |
| Charcoal | C | 60 | 82.95545 | -0.214 | 0.047459424 |
| Both | A | 60 | 64.01969 | -0.233 | 0.036626138 |
| Both | B | 60 | 76.50922 | -0.22 | 0.043771488 |
| Both | C | 60 | 98.81146 | -0.198 | 0.056530766 |
| NONE | A | 90 | 3334.831 | 3.068 | 1.907881268 |
| NONE | B | 90 | 3297.704 | 3.03 | 1.886640926 |
| NONE | C | 90 | 3252.915 | 2.985 | 1.861016795 |
| Chelex | A | 90 | 213.9938 | -0.082 | 0.122427444 |
| Chelex | B | 90 | 48.95706 | -0.248 | 0.028008698 |
| Charcoal | A | 90 | 188.3605 | -0.107 | 0.107762461 |
| Charcoal | B | 90 | 410.2323 | 0.116 | 0.234696954 |
| Charcoal | C | 90 | 74.92363 | -0.222 | 0.04286436 |
| Both | A | 90 | 77.45329 | -0.219 | 0.0443116 |
| Both | B | 90 | 79.42024 | -0.217 | 0.045436904 |
| Both | C | 90 | 163.0052 | -0.133 | 0.093256453 |
| NONE | A | 120 | 3287.474 | 3.02 | 1.880788147 |
| NONE | B | 120 | 3205.559 | 2.937 | 1.833924 |
| NONE | C | 120 | 3300.431 | 3.033 | 1.888201211 |

| | | | | | |
|---|---|---|---|---|---|
| Chelex | A | 120 | 413.5762 | 0.12 | 0.236609986 |
| Chelex | B | 120 | 113.4026 | -0.183 | 0.064878446 |
| Charcoal | A | 120 | 384.2321 | 0.09 | 0.219822008 |
| Charcoal | B | 120 | 894.9278 | 0.605 | 0.511994815 |
| Charcoal | C | 120 | 220.8296 | -0.075 | 0.12633827 |
| Both | A | 120 | 139.0551 | -0.157 | 0.079554443 |
| Both | B | 120 | 189.936 | -0.106 | 0.1086638 |
| Both | C | 120 | 265.5913 | -0.03 | 0.151946747 |

Table 8: Multiple Metals Test Zn Mean Concentration values and standard deviation (Figure 5)

| Treatment | Average [Zn] mg/L | Time (min) | Stdeviation |
|---|---|---|---|
| None | 1.960830077 | 30 | 0.023833664 |
| Chelex | 0.069187489 | 30 | 0.024766938 |
| Charcoal | 0.063147232 | 30 | 0.037234082 |
| Both | 0.204216174 | 30 | 0.038980409 |
| None | 1.879639027 | 60 | 0.023768951 |
| Chelex | 0.052087442 | 60 | 0.023438081 |
| Charcoal | 0.072725545 | 60 | 0.02771128 |
| Both | 0.045642797 | 60 | 0.010083398 |
| None | 1.885179663 | 90 | 0.023466384 |
| Chelex | 0.075218071 | 90 | 0.066764136 |
| Charcoal | 0.128441258 | 90 | 0.097573796 |
| Both | 0.061001652 | 90 | 0.027939143 |
| None | 1.867637786 | 120 | 0.029431325 |
| Chelex | 0.150744216 | 120 | 0.121432537 |
| Charcoal | 0.286051697 | 120 | 0.201177836 |
| Both | 0.11338833 | 120 | 0.03642667 |

Table 9: Multiple Metals Test V ICP data values (analyzed values used in analysis, as corrected for ICP standard curve) (Figure 6)

| Treatment | Replicate | Time (min) | Intensity | ICP Reported [V] mg/L | Analyzed [V] mg/L |
|---|---|---|---|---|---|
| FLOW | N/A | 0 | 415343 | 3.409 | 3.418794628 |
| NONE | A | 30 | 413577.6 | 3.395 | 3.404263586 |
| NONE | B | 30 | 415841.8 | 3.413 | 3.422900772 |
| NONE | C | 30 | 406428.9 | 3.336 | 3.345420561 |
| Chelex | A | 30 | 265912.4 | 2.182 | 2.188793327 |
| Chelex | B | 30 | 396114.3 | 3.251 | 3.260518279 |
| Chelex | C | 30 | 401339.7 | 3.294 | 3.303529769 |
| Charcoal | A | 30 | 279865.6 | 2.296 | 2.303645475 |
| Charcoal | B | 30 | 295014.8 | 2.421 | 2.428342318 |
| Charcoal | C | 30 | 394247.2 | 3.236 | 3.245149681 |
| Both | A | 30 | 316132.1 | 2.594 | 2.602164491 |
| Both | B | 30 | 351197.1 | 2.882 | 2.890793295 |
| Both | C | 30 | 351421.8 | 2.884 | 2.892642804 |
| NONE | A | 60 | 412753.2 | 3.388 | 3.397477555 |
| NONE | B | 60 | 418695.7 | 3.437 | 3.446391644 |
| NONE | C | 60 | 422491.6 | 3.468 | 3.477636948 |
| Chelex | A | 60 | 294239.3 | 2.414 | 2.42195942 |
| Chelex | B | 60 | 378674.7 | 3.108 | 3.11696898 |
| Chelex | C | 60 | 373829.8 | 3.068 | 3.077088909 |
| Charcoal | A | 60 | 290636.4 | 2.385 | 2.392303085 |
| Charcoal | B | 60 | 313147.7 | 2.57 | 2.577599072 |
| Charcoal | C | 60 | 360964.7 | 2.963 | 2.971192808 |
| Both | A | 60 | 309915.2 | 2.543 | 2.550991359 |

| | | | | | |
|---|---|---|---|---|---|
| Both | B | 60 | 335973 | 2.757 | 2.765480106 |
| Both | C | 60 | 336202.6 | 2.759 | 2.767370223 |
| NONE | A | 90 | 414439.8 | 3.402 | 3.411360265 |
| NONE | B | 90 | 410287.5 | 3.368 | 3.377181478 |
| NONE | C | 90 | 409832.6 | 3.364 | 3.373437428 |
| Chelex | A | 90 | 294679.2 | 2.418 | 2.425579929 |
| Chelex | B | 90 | 353089.8 | 2.898 | 2.906372838 |
| Charcoal | A | 90 | 306536.1 | 2.515 | 2.523177261 |
| Charcoal | B | 90 | 315717.5 | 2.591 | 2.598751791 |
| Charcoal | C | 90 | 318637.2 | 2.615 | 2.62278474 |
| Both | A | 90 | 297324.5 | 2.44 | 2.447354164 |
| Both | B | 90 | 313100.5 | 2.569 | 2.577210955 |
| Both | C | 90 | 336231.8 | 2.759 | 2.767610646 |
| NONE | A | 120 | 410542.8 | 3.37 | 3.379283048 |
| NONE | B | 120 | 408983.6 | 3.357 | 3.366448918 |
| NONE | C | 120 | 406040.8 | 3.333 | 3.342225782 |
| Chelex | A | 120 | 298995.2 | 2.453 | 2.461106001 |
| Chelex | B | 120 | 343264.9 | 2.817 | 2.825501457 |
| Charcoal | A | 120 | 305960.6 | 2.511 | 2.518440105 |
| Charcoal | B | 120 | 324445.2 | 2.662 | 2.670591362 |
| Charcoal | C | 120 | 334222.9 | 2.743 | 2.751074447 |
| Both | A | 120 | 300010.2 | 2.462 | 2.469460876 |
| Both | B | 120 | 310559.8 | 2.548 | 2.556297496 |
| Both | C | 120 | 345314.6 | 2.834 | 2.842372824 |

Table 10: Multiple Metals Test V Mean Concentration values and standard deviation (Figure 6)

| Treatment | Average [V] mg/L | Time (min) | Stdeviation |
|---|---|---|---|

| | | | |
|---|---|---|---|
| None | 3.39086164 | 30 | 0.04044138 |
| Chelex | 2.917613791 | 30 | 0.63154331 |
| Charcoal | 2.659045825 | 30 | 0.51139576 |
| Both | 2.795200197 | 30 | 0.16717638 |
| None | 3.440502049 | 60 | 0.04040294 |
| Chelex | 2.872005769 | 60 | 0.39026131 |
| Charcoal | 2.647031655 | 60 | 0.29562475 |
| Both | 2.694613896 | 60 | 0.12438436 |
| None | 3.38732639 | 90 | 0.02089796 |
| Chelex | 2.665976383 | 90 | 0.33997193 |
| Charcoal | 2.581571264 | 90 | 0.05197875 |
| Both | 2.597391922 | 90 | 0.1610792 |
| None | 3.362652583 | 120 | 0.01881806 |
| Chelex | 2.643303729 | 120 | 0.2576665 |
| Charcoal | 2.646701972 | 120 | 0.11814276 |
| Both | 2.622710399 | 120 | 0.19512518 |

Table 11: *D. magna* NH3 Concentration Moderation Test (Figure 1)

| Sample | mV | log[NH3] | [NH3] mg/L | Day |
|---|---|---|---|---|
| Standard 0 | 169 | N/A | 0 | 2 |
| Standard 5 | 57.4 | 0.69897 | 5 | 2 |
| Standard 10 | 41.6 | 1 | 10 | 2 |
| Standard 25 | 16.8 | 1.39794 | 25 | 2 |
| Standard 50 | -3.6 | 1.69897 | 50 | 2 |

| | | | | |
|---|---|---|---|---|
| Standard 75 | -11.4 | 1.875061 | 75 | 2 |
| Control | 131.2 | -0.51394 | 0.30623965 | 2 |
| 20 | 22.7 | 1.291837 | 19.5810765 | 2 |
| 40 | 5.6 | 1.576433 | 37.7079902 | 2 |
| 60 | -3.8 | 1.732878 | 54.0602964 | 2 |
| Standard 0 | 180.2 | N/A | 0 | 5 |
| Standard 5 | 56.7 | 0.69897 | 5 | 5 |
| Standard 10 | 38.4 | 1 | 10 | 5 |
| Standard 25 | 14 | 1.39794 | 25 | 5 |
| Standard 50 | -1.6 | 1.69897 | 50 | 5 |
| Standard 75 | -13.9 | 1.875061 | 75 | 5 |
| Control | 123 | -0.42342 | 0.37720623 | 5 |
| 20 | 22.7 | 1.267095 | 18.4967222 | 5 |
| 40 | 4.6 | 1.572163 | 37.339023 | 5 |
| 60 | -6.9 | 1.765991 | 58.3432741 | 5 |

Table 12: Chelex Zn ICP data values (analyzed values used in analysis, as corrected for ICP standard curve and ICP initial sample dilution) (Figure 7)

| Chelex | Replicate | Time | Intensity | ICP Reported [Zn] mg/L (diluted) | Analyzed [Zn] mg/L (diluted) | Actual [Zn] mg/L |
|---|---|---|---|---|---|---|
| None | A | 30 | 1490.062 | 0.915027332 | 0.915 | 36.60109327 |
| None | B | 30 | 1796.739 | 1.103353578 | 1.104 | 44.13414311 |
| None | C | 30 | 1406.617 | 0.863784907 | 0.864 | 34.55139627 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 gram | A | 30 | -1.18484 | 0 | 0 | 0 |
| 1 gram | B | 30 | 335.3394 | 0.207819877 | 0.216 | 8.312795079 |
| 1 gram | C | 30 | 630.3732 | 0.390661164 | 0.405 | 15.62644655 |
| 3 grams | A | | 98.2535 | 0.060890638 | 0.064 | 2.435625521 |
| 3 grams | B | 30 | 43.77982 | 0.027131664 | 0.029 | 1.085266561 |
| 3 grams | C | 30 | 41.34182 | 0.025620763 | 0.027 | 1.024830539 |
| 5 grams | A | 30 | 151.3871 | 0.092964766 | 0.094 | 3.718590644 |
| 5 grams | B | 30 | 27.12035 | 0.016654241 | 0.017 | 0.666169643 |
| 5 grams | C | 30 | 75.71555 | 0.046495905 | 0.047 | 1.8598362 |
| None | A | 60 | 1601.069 | 0.992230082 | 1.027 | 39.68920329 |
| None | B | 60 | 1811.906 | 1.1228922 | 1.162 | 44.91568798 |
| None | C | 60 | 1710.917 | 1.060306581 | 1.097 | 42.41226325 |
| 1 gram | A | 60 | 1151.695 | 0.713739639 | 0.739 | 28.54958555 |
| 1 gram | B | 60 | 761.9602 | 0.472209558 | 0.489 | 18.88838231 |
| 1 gram | C | 60 | 1138.19 | 0.705370253 | 0.73 | 28.21481011 |
| 3 grams | A | 60 | 47.3305 | 0.02933213 | 0.031 | 1.173285181 |
| 3 grams | B | 60 | 359.8097 | 0.222984851 | 0.231 | 8.919394045 |
| 3 grams | C | 60 | 276.2522 | 0.171201773 | 0.178 | 6.848070931 |
| 5 grams | A | 60 | 68.73 | 0.04259404 | 0.045 | 1.703761598 |
| 5 grams | B | 60 | 16.2976 | 0.01010011 | 0.011 | 0.404004409 |
| 5 grams | C | 60 | 108.1199 | 0.067005129 | 0.07 | 2.680205162 |
| None | A | 90 | 1768.242 | 1.095832589 | 1.134 | 43.83330355 |
| None | B | 90 | 1879.129 | 1.164552724 | 1.205 | 46.58210896 |
| None | C | 90 | 1615.326 | 1.001066086 | 1.036 | 40.04264345 |
| 1 gram | A | 90 | 1500.712 | 0.93003588 | 0.962 | 37.20143519 |
| 1 gram | B | 90 | 1159.634 | 0.718659774 | 0.744 | 28.74639098 |
| 1 gram | C | 90 | 1415.034 | 0.876939015 | 0.908 | 35.07756062 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 grams | A | 90 | 82.85741 | 0.051349217 | 0.054 | 2.05396868 |
| 3 grams | B | 90 | 610.4622 | 0.378321687 | 0.392 | 15.13286749 |
| 3 grams | C | 90 | 417.2784 | 0.258599888 | 0.268 | 10.34399554 |
| 5 grams | A | 90 | 63.11008 | 0.039111209 | 0.041 | 1.564448341 |
| 5 grams | B | 90 | 28.9707 | 0.017954008 | 0.019 | 0.718160338 |
| 5 grams | C | 90 | 315.7234 | 0.195663224 | 0.203 | 7.826528956 |
| None | A | 120 | 1806.003 | 1.109042081 | 1.109 | 44.36168324 |
| None | B | 120 | 1892.049 | 1.161881693 | 1.162 | 46.47526773 |
| None | C | 120 | 1674.505 | 1.028291156 | 1.029 | 41.13164624 |
| 1 gram | A | 120 | 1704.209 | 1.056149428 | 1.093 | 42.24597712 |
| 1 gram | B | 120 | 1430.867 | 0.886750937 | 0.918 | 35.47003747 |
| 1 gram | C | 120 | 1639.297 | 1.015921502 | 1.051 | 40.63686007 |
| 3 grams | A | 120 | 144.3923 | 0.089484246 | 0.093 | 3.579369849 |
| 3 grams | B | 120 | 856.3301 | 0.530693423 | 0.549 | 21.2277369 |
| 3 grams | C | 120 | 758.4969 | 0.470063207 | 0.487 | 18.8025283 |
| 5 grams | A | 120 | 172.9002 | 0.106175668 | 0.107 | 4.247026722 |
| 5 grams | B | 120 | 76.85616 | 0.047196336 | 0.048 | 1.887853438 |
| 5 grams | C | 120 | 586.9065 | 0.360411409 | 0.361 | 14.41645635 |
| FLOW | N/A | 0 | 1742.241 | 1.069886746 | 1.07 | 42.79546984 |

Table 13: Chelex Zn Mean Concentration values and standard deviation (Figure 7)

| Chelex | Time (min) | Average [Zn] mg/L | Std Dev |
|---|---|---|---|
| None | 30 | 38.42887755 | 5.046073 |
| 1 gram | 30 | 7.97974721 | 7.818545 |
| 3 grams | 30 | 1.515240874 | 0.797649 |
| 5 grams | 30 | 2.081532162 | 1.538239 |
| None | 60 | 42.33905151 | 2.614011 |

| | | | |
|---|---|---|---|
| 1 gram | 60 | 25.21759266 | 5.483812 |
| 3 grams | 60 | 5.646916719 | 4.010315 |
| 5 grams | 60 | 1.59599039 | 1.141921 |
| None | 90 | 43.48601865 | 3.283536 |
| 1 gram | 90 | 33.67512893 | 4.398529 |
| 3 grams | 90 | 9.176943903 | 6.617092 |
| 5 grams | 90 | 3.369712545 | 3.882842 |
| None | 120 | 43.9895324 | 2.691179 |
| 1 gram | 120 | 39.45095822 | 3.540214 |
| 3 grams | 120 | 14.53654502 | 9.566356 |
| 5 grams | 120 | 6.850445504 | 6.657688 |

Table 14: NH3 and Zn Test Ammonia Electrode Raw data and log analysis (Figure 8)

| Treatment | Replicate | mV | log [NH3] | [NH3] mg/L | Time (min) |
|---|---|---|---|---|---|
| None | A | 10.1 | 1.501156 | 31.7070813 | 45 |
| None | B | 9.2 | 1.51646 | 32.844283 | 45 |
| None | C | 10.5 | 1.494355 | 31.2143792 | 45 |
| Zeolite | A | 10.5 | 1.494355 | 31.2143792 | 45 |
| Zeolite | B | 13.3 | 1.446744 | 27.9733031 | 45 |
| Zeolite | C | 10.6 | 1.492654 | 31.0924046 | 45 |
| Charcoal | A | 13.4 | 1.445043 | 27.8639935 | 45 |
| Charcoal | B | 8.8 | 1.523261 | 33.3627123 | 45 |
| Charcoal | C | 9.2 | 1.51646 | 32.844283 | 45 |
| Both | A | 28.8 | 1.183183 | 15.2469555 | 45 |
| Both | B | 11.6 | 1.47565 | 29.8985688 | 45 |
| Both | C | 21.5 | 1.307312 | 20.2913846 | 45 |
| None | A | 3.7 | 1.609981 | 40.7362733 | 90 |
| None | B | 6 | 1.570872 | 37.2282225 | 90 |
| None | C | 5.1 | 1.586176 | 38.5634447 | 90 |
| Zeolite | A | 7.5 | 1.545366 | 35.1047944 | 90 |
| Zeolite | B | 9.7 | 1.507958 | 32.2075604 | 90 |
| Zeolite | C | 10.9 | 1.487553 | 30.7293332 | 90 |
| Charcoal | A | 4.9 | 1.589577 | 38.8666046 | 90 |
| Charcoal | B | 7.1 | 1.552168 | 35.6589047 | 90 |

| | | | | | |
|---|---|---|---|---|---|
| Charcoal | C | 8.5 | 1.528363 | 33.7568974 | 90 |
| Both | A | 9.2 | 1.51646 | 32.844283 | 90 |
| Both | B | 11 | 1.485853 | 30.6092541 | 90 |
| Both | C | 11.3 | 1.480752 | 30.2518245 | 90 |
| None | A | 9.2 | 1.51646 | 32.844283 | 135 |
| None | B | 5.4 | 1.581075 | 38.1131327 | 135 |
| None | C | 7.5 | 1.545366 | 35.1047944 | 135 |
| Zeolite | A | 6 | 1.570872 | 37.2282225 | 135 |
| Zeolite | B | 6.1 | 1.569172 | 37.082748 | 135 |
| Zeolite | C | 6.8 | 1.557269 | 36.0802196 | 135 |
| Charcoal | A | 8.2 | 1.533464 | 34.1557398 | 135 |
| Charcoal | B | 8.4 | 1.530063 | 33.8893247 | 135 |
| Charcoal | C | 8 | 1.536864 | 34.4242493 | 135 |
| Both | A | 11.4 | 1.479051 | 30.1336113 | 135 |
| Both | B | 7.8 | 1.540265 | 34.6948697 | 135 |
| Both | C | 11.6 | 1.47565 | 29.8985688 | 135 |

Table 15: NH3 and Zn Test Mean NH3 Concentration values and standard deviation (Figure 8)

| Treatment | Time | [NH3] mg/L | Std Dev |
|---|---|---|---|
| None | 45 | 31.9219145 | 0.83592 |
| Zeolite | 45 | 30.0933623 | 1.837038 |
| Charcoal | 45 | 31.3569963 | 3.036115 |
| Both | 45 | 21.8123029 | 7.443275 |
| None | 90 | 38.8426468 | 1.770613 |
| Zeolite | 90 | 32.6805627 | 2.22575 |
| Charcoal | 90 | 36.0941356 | 2.582508 |
| Both | 90 | 31.2351205 | 1.404988 |
| None | 135 | 35.3540701 | 2.643255 |
| Zeolite | 135 | 36.7970633 | 0.625052 |
| Charcoal | 135 | 34.1564379 | 0.267463 |
| Both | 135 | 31.5756833 | 2.70385 |

Table 16: NH3 and Zn Test Zn Raw and Analyzed ICP data

| Treatment | Replicate | Time | Intensity | [Zn] Reported mg/L | [Zn] mg/L analysis |
|---|---|---|---|---|---|
| None | A | One | 2961.002 | 2.314 | 2.353609 |
| None | B | One | 2727.106 | 2.131 | 2.167692 |
| None | C | One | 2994.04 | 2.34 | 2.37987 |

| | | | | | |
|---|---|---|---|---|---|
| Zeolite | A | One | 2199.371 | 1.72 | 1.748212 |
| Zeolite | B | One | 2567.797 | 2.007 | 2.041062 |
| Zeolite | C | One | 2091.293 | 1.635 | 1.662304 |
| Charcoal | A | One | 518.3594 | 0.408 | 0.412028 |
| Charcoal | B | One | 155.518 | 0.125 | 0.123616 |
| Charcoal | C | One | 330.5493 | 0.262 | 0.262743 |
| Both | A | One | 71.85316 | 0.06 | 0.057114 |
| Both | B | One | 98.9746 | 0.081 | 0.078672 |
| Both | C | One | 55.93585 | 0.047 | 0.044462 |
| None | A | Two | 2537.73 | 1.984 | 2.017163 |
| None | B | Two | 2511.653 | 1.963 | 1.996435 |
| None | C | Two | 2567.051 | 2.007 | 2.040469 |
| Zeolite | A | Two | 2204.908 | 1.724 | 1.752613 |
| Zeolite | B | Two | 2455.061 | 1.919 | 1.951452 |
| Zeolite | C | Two | 2200.181 | 1.72 | 1.748856 |
| Charcoal | A | Two | 1242.402 | 0.973 | 0.987546 |
| Charcoal | B | Two | 1150.807 | 0.902 | 0.914741 |
| Charcoal | C | Two | 1287.896 | 1.009 | 1.023709 |
| Both | A | Two | 106.7783 | 0.087 | 0.084875 |
| Both | B | Two | 148.1669 | 0.119 | 0.117773 |
| Both | C | Two | 62.9261 | 0.053 | 0.050018 |
| None | A | Three | 2558.424 | 2 | 2.033612 |
| None | B | Three | 2594.034 | 2.028 | 2.061918 |
| None | C | Three | 2590.763 | 2.025 | 2.059317 |
| Zeolite | A | Three | 2348.287 | 1.836 | 1.86658 |
| Zeolite | B | Three | 2515.896 | 1.967 | 1.999808 |
| Zeolite | C | Three | 2347.484 | 1.835 | 1.865942 |
| Charcoal | A | Three | 1851.414 | 1.448 | 1.471632 |
| Charcoal | B | Three | 1718.347 | 1.344 | 1.365861 |
| Charcoal | C | Three | 1920.845 | 1.502 | 1.52682 |
| Both | A | Three | 98.30396 | 0.081 | 0.078139 |
| Both | B | Three | 1210.451 | 0.948 | 0.96215 |
| Both | C | Three | 218.5711 | 0.174 | 0.173735 |

Table 17: NH3 and Zn Test Mean Zn Concentration values and standard deviation (Figure 9)

| Treatment | Time | Average [Zn] mg/L | Std. Dev |
|---|---|---|---|
| None | One | 2.30039032 | 0.11566761 |
| Zeolite | One | 1.817192675 | 0.198577782 |

| | | | |
|---|---|---|---|
| Charcoal | One | 0.266129233 | 0.144235521 |
| Both | One | 0.060082458 | 0.017297206 |
| None | Two | 2.018022471 | 0.022029497 |
| Zeolite | Two | 1.817640121 | 0.115899496 |
| Charcoal | Two | 0.975332083 | 0.055501257 |
| Both | Two | 0.084222021 | 0.033882356 |
| None | Three | 2.051615444 | 0.015645814 |
| Zeolite | Three | 1.910776809 | 0.077103571 |
| Charcoal | Three | 1.454771015 | 0.081793712 |
| Both | Three | 0.404674686 | 0.485148077 |

Table 18: Charcoal Zn Test Zn Raw and Analyzed ICP data

| Sample | Replicate | Time | Intensity | [Zn] Reported (ICP) | [Zn] mg/L analyzed (diluted) | [Zn] mg/L (not diluted) |
|---|---|---|---|---|---|---|
| None | A | One | 1142.013 | 0.895 | 0.907751 | 36.31003 |
| None | C | One | 1091.238 | 0.855 | 0.867391 | 34.69564 |
| One | A | One | 967.0501 | 0.758 | 0.768678 | 30.74713 |
| One | B | One | 961.5603 | 0.754 | 0.764315 | 30.57258 |
| One | C | One | 963.0695 | 0.755 | 0.765514 | 30.62057 |
| Three | A | One | 215.9674 | 0.172 | 0.171666 | 6.866633 |
| Three | B | One | 749.1247 | 0.588 | 0.595456 | 23.81824 |
| Three | C | One | 537.6134 | 0.423 | 0.427332 | 17.09329 |
| Five | A | One | 688.9971 | 0.541 | 0.547662 | 21.9065 |
| Five | B | One | 684.7093 | 0.538 | 0.544254 | 21.77017 |
| Five | C | One | 75.36011 | 0.063 | 0.059901 | 2.396057 |
| None | A | Two | 1145.993 | 0.898 | 0.910915 | 36.43658 |
| None | B | Two | 1189.646 | 0.932 | 0.945613 | 37.82452 |
| None | C | Two | 1068.706 | 0.838 | 0.849482 | 33.97926 |
| One | A | Two | 1080.871 | 0.847 | 0.859151 | 34.36605 |
| One | B | Two | 1060.509 | 0.831 | 0.842966 | 33.71863 |
| One | C | Two | 1115.543 | 0.874 | 0.886711 | 35.46842 |
| Three | A | Two | 793.1982 | 0.623 | 0.630489 | 25.21955 |
| Three | B | Two | 930.3352 | 0.73 | 0.739495 | 29.57979 |
| Three | C | Two | 831.8064 | 0.653 | 0.661177 | 26.44708 |
| Five | A | Two | 783.9364 | 0.615 | 0.623127 | 24.92507 |
| Five | B | Two | 848.3637 | 0.666 | 0.674338 | 26.97352 |
| Five | C | Two | 319.4506 | 0.253 | 0.253921 | 10.15685 |
| None | A | Three | 1140.858 | 0.894 | 0.906833 | 36.27331 |

| | | | | | | |
|---|---|---|---|---|---|---|
| None | B | Three | 1161.414 | 0.91 | 0.923172 | 36.92689 |
| None | C | Three | 1137.048 | 0.891 | 0.903804 | 36.15218 |
| One | A | Three | 1117.689 | 0.876 | 0.888416 | 35.53666 |
| One | B | Three | 1101.912 | 0.864 | 0.875876 | 35.03504 |
| One | C | Three | 1105.033 | 0.866 | 0.878357 | 35.13427 |
| Three | A | Three | 891.6046 | 0.699 | 0.708709 | 28.34836 |
| Three | B | Three | 969.7012 | 0.76 | 0.770785 | 30.83142 |
| Three | C | Three | 988.3582 | 0.775 | 0.785615 | 31.42461 |
| Five | A | Three | 671.8931 | 0.528 | 0.534067 | 21.36268 |
| Five | B | Three | 872.5305 | 0.685 | 0.693548 | 27.7419 |
| Five | C | Three | 526.5172 | 0.415 | 0.418512 | 16.74049 |
| None | A | Four | 1165.043 | 0.913 | 0.926057 | 37.04227 |
| None | B | Four | 1207.061 | 0.946 | 0.959455 | 38.37821 |
| None | C | Four | 1118.199 | 0.876 | 0.888822 | 35.55286 |
| One | A | Four | 1185.884 | 0.929 | 0.942623 | 37.70491 |
| One | B | Four | 1000.295 | 0.784 | 0.795104 | 31.80414 |
| One | C | Four | 1175.987 | 0.921 | 0.934756 | 37.39024 |
| Three | A | Four | 1020.295 | 0.8 | 0.811001 | 32.44005 |
| Three | B | Four | 995.6742 | 0.781 | 0.791431 | 31.65722 |
| Three | C | Four | 1046.392 | 0.82 | 0.831744 | 33.26977 |
| Five | A | Four | 606.7833 | 0.477 | 0.482313 | 19.29253 |
| Five | B | Four | 892.0504 | 0.7 | 0.709063 | 28.36253 |
| Five | C | Four | 639.0717 | 0.502 | 0.507978 | 20.31913 |

Table 19: Charcoal Zn Test Mean Zn Concentration values and standard deviation (Figure 10)

| Treatment | Time | Average [Zn] mg/L | Std. Dev |
|---|---|---|---|
| None | One | 35.50283774 | 1.141548 |
| One | One | 30.6467584 | 0.090174 |
| Three | One | 15.92605544 | 8.535871 |
| Five | One | 15.357575 | 11.22521 |
| None | Two | 36.08012129 | 1.947253 |
| One | Two | 34.51770037 | 0.8847 |
| Three | Two | 27.08214005 | 2.248419 |
| Five | Two | 20.68514922 | 9.175117 |
| None | Three | 36.45079279 | 0.416739 |
| One | Three | 35.23532191 | 0.265639 |
| Three | Three | 30.20146386 | 1.632015 |

| | | | |
|---|---|---|---|
| Five | Three | 21.94835727 | 5.524041 |
| None | Four | 36.99111591 | 1.41337 |
| One | Four | 35.63309551 | 3.3197 |
| Three | Four | 32.45568101 | 0.806388 |
| Five | Four | 22.65806375 | 4.966808 |

## Appendix C Raw Statistical Results

### *30 mg/L NH3 Test 1*

Table 20: ANOVA: Treatment (independent variable) and log [NH3] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Sample | 3 | 0.2793 | 0.09309 | 11.6 | 5.61E-06 |
| Residuals | 54 | 0.4332 | 0.00802 | | |

Table 21: Tukey HSD for ANOVA: Treatment (independent variable) and log[NH3] (dependent variable)

| Zeolite | diff | lwr | upr | p adj |
|---|---|---|---|---|
| 1g-5g | 0.163382 | 0.07515 | 0.25162 | 5.1E-05 |
| 3g-5g | 0.146474 | 0.05824 | 0.23471 | 0.00029 |
| 0g-5g | 0.172856 | 0.08311 | 0.2626 | 2.6E-05 |
| 3g-1g | -0.01691 | -0.1036 | 0.06979 | 0.95467 |
| 0g-1g | 0.009474 | -0.0788 | 0.09771 | 0.99187 |
| 0g-3g | 0.026382 | -0.0619 | 0.11462 | 0.8575 |

Table 22: ANOVA: Time (independent variable) and log[NH3] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Time | 4 | 0.0675 | 0.01687 | 1.386 | 0.251 |
| Residuals | 54 | 0.645 | 0.01217 | | |

### *30 mg/L NH3 Test 2*

Table 23: ANOVA: Treatment (independent variable) and log [NH3] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Sample_2 | 3 | 2.5262 | 0.8421 | 54.27 | 1.14E-14 |
| Residuals | 43 | 0.6672 | 0.0155 | | |

Table 24:Tukey HSD for ANOVA: Treatment (independent variable) and log[NH3] (dependent variable)

| Sample_2 | diff | lwr | upr | p adj |
|---|---|---|---|---|
| ONE-FIVE | 0.51361 | 0.37466 | 0.65256 | 0 |
| THREE-FIVE | 0.443088 | 0.30719 | 0.57899 | 0 |
| ZERO-FIVE | 0.594184 | 0.45829 | 0.73008 | 0 |
| THREE-ONE | -0.07052 | -0.2095 | 0.06843 | 0.53305 |
| ZERO-ONE | 0.080574 | -0.0584 | 0.21953 | 0.41766 |
| ZERO-THREE | 0.151097 | 0.0152 | 0.287 | 0.02407 |

Table 25: ANOVA: Time (independent variable) and log[NH3] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Time_2 | 3 | 0.1874 | 0.06248 | 0.894 | 0.452 |
| Residuals | 43 | 3.006 | 0.06991 | | |

***Chelex Test***

Table 26: ANOVA: Treatment (independent variable) and [Zn] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Chelex | 3 | 3816 | 1271.9 | 22.08 | 3.55E-05 |
| Residuals | 12 | 691 | 57.6 | | |

Table 27: Tukey HSD for ANOVA: Treatment (independent variable) and [Zn] (dependent variable)

| Chelex | diff | lwr | upr | p adj |
|---|---|---|---|---|
| None-Five | 38.58645 | 22.6547 | 54.5182 | 5.7E-05 |
| One-Five | 23.10644 | 7.17469 | 39.0382 | 0.00487 |
| Three-Five | 4.244491 | -11.687 | 20.1762 | 0.85718 |
| One-None | -15.48 | -31.412 | 0.45174 | 0.0578 |
| Three-None | -34.342 | -50.274 | -18.41 | 0.00017 |
| Three-One | -18.8619 | -34.794 | -2.9302 | 0.01928 |

Table 28:ANOVA: Time (independent variable) and [Zn] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Time..min. | 3 | 409 | 136.4 | 0.4 | 0.756 |
| Residuals | 12 | 4097 | 341.5 | | |

***Charcoal Test***

Table 29: ANOVA: Treatment (independent variable) and [Zn] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 649.4 | 216.45 | 12.31 | 0.000566 |
| Residuals | 12 | 210.9 | 17.58 | | |

Table 30: Tukey HSD for ANOVA: Treatment (independent variable) and [Zn] (dependent variable)

| Treatment | diff | lwr | upr | p adj |
|---|---|---|---|---|
| None-Five | 16.093931 | 7.291976 | 24.8959 | 0.00076 |
| One-Five | 13.845933 | 5.043978 | 22.6479 | 0.00263 |
| Three-Five | 6.254049 | -2.547906 | 15.056 | 0.2049 |
| One-None | -2.247998 | -11.04995 | 6.55396 | 0.87145 |
| Three-None | -9.839882 | -18.64184 | -1.0379 | 0.02718 |
| Three-One | -7.591884 | -16.39384 | 1.21007 | 0.09991 |

Table 31: ANOVA: Time (independent variable) and [Zn] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr>F |
|---|---|---|---|---|---|
| Time | 3 | 136.7 | 45.55 | 0.755 | 0.54 |
| Residuals | 12 | 723.6 | 60.3 | | |

Table 32: 2-way ANOVA: Treatment and Time independent variables, [Zn] dependent variable; independent variable interaction

| | Df | Sum Sq | Mean Sq |
|---|---|---|---|
| Time | 3 | 136.7 | 45.55 |
| Treatment | 3 | 649.4 | 216.45 |
| Time: Treatment | 9 | 74.3 | 8.25 |

Table 33: 2-way ANOVA: Treatment and Time independent variables, [Zn] dependent variable; Time as blocking variable

| | Df | Sum Sq | Mean Sq | F value | Pr>F |
|---|---|---|---|---|---|
| Time | 3 | 136.7 | 45.55 | 5.519 | 0.0199 |
| Treatment | 3 | 649.4 | 216.45 | 26.224 | 8.81E-05 |
| Residuals | 9 | 74.3 | 8.25 | | |

Table 34: Tukey HSD for 2-way block ANOVA: Treatment and Time (independent variable) and [Zn] (dependent variable)

| Time | diff | lwr | upr | p adj |
|---|---|---|---|---|
| One-Four | -7.576182 | -13.91808 | -1.23429 | 0.02017 |
| Three-Four | -0.975505 | -7.3174 | 5.36639 | 0.9616 |
| Two-Four | -2.343211 | -8.685106 | 3.998683 | 0.6683 |
| Three-One | 6.6006773 | 0.2587826 | 12.94257 | 0.04128 |
| Two-One | 5.2329711 | -1.108924 | 11.57487 | 0.11318 |
| Two-Three | -1.367706 | -7.709601 | 4.974188 | 0.9046 |

| Treatment | diff | lwr | upr | p adj |
|---|---|---|---|---|
| None-Five | 16.093931 | 9.7520359 | 22.43583 | 0.00011 |
| One-Five | 13.845933 | 7.504038 | 20.18783 | 0.00037 |
| Three-Five | 6.254049 | -0.087846 | 12.59594 | 0.05336 |
| One-None | -2.247998 | -8.589893 | 4.093897 | 0.6947 |
| Three-None | -9.839882 | -16.18178 | -3.49799 | 0.00415 |
| Three-One | -7.591884 | -13.93378 | -1.24999 | 0.01994 |

***Multiple Metals iTIE Experiment Test***

*Ni Statistical Results*

Table 35: 2-way ANOVA: Treatment and Time independent variables, [Ni] dependent variable; Time as blocking variable

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Time..min. | 3 | 0.252 | 0.084 | 3.317 | 0.0708 |
| Treatment | 3 | 13.266 | 4.422 | 174.573 | 2.72E-08 |
| Residuals | 9 | 0.228 | 0.025 | | |

Table 36: 2-way ANOVA: Treatment and Time independent variables, [Ni] dependent variable; independent variable interaction

| | Df | Sum Sq | Mean Sq |
|---|---|---|---|
| Time..min. | 3 | 0.252 | 0.084 |
| Treatment | 3 | 13.266 | 4.422 |
| Time..min: treatment | 9 | 0.228 | 0.02 |

Table 37: ANOVA: Time (independent variable) and [Ni] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Time..min. | 3 | 0.252 | 0.084 | 0.075 | 0.972 |
| Residuals | 12 | 13.494 | 1.125 | | |

Table 38: ANOVA: Treatment (independent variable) and [Ni] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Treatment | 3 | 13.27 | 4.422 | 110.5 | 5.24E-09 |
| Residuals | 12 | 0.48 | 0.04 | | |

Table 39: Tukey HSD for ANOVA: Treatment (independent variable) and [Ni] (dependent variable)

| Treatment | diff | lwr | upr | p adj |
|---|---|---|---|---|
| Charcoal-Both | 0.487588 | 0.13627 | 0.83891 | 0.00842 |
| Chelex-Both | 0.258999 | -0.0923 | 0.61032 | 0.16876 |
| None-Both | 2.313611 | 1.96229 | 2.66493 | 0 |
| Chelex-Charcoal | -0.22859 | -0.5799 | 0.12273 | 0.24607 |
| None-Charcoal | 1.826023 | 1.4747 | 2.17735 | 3E-07 |
| None-Chelex | 2.054611 | 1.70329 | 2.40593 | 1E-07 |

*Zn Statistical Results*

Table 40: 2-way ANOVA: Treatment and Time independent variables, [Zn] dependent variable; Time as blocking variable

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Time..min. | 3 | 0.02 | 0.007 | 1.523 | 0.274 |
| Treatment | 3 | 9.598 | 3.199 | 744.012 | 4.26E-11 |
| Residuals | 9 | 0.039 | 0.004 | | |

Table 41: 2-way ANOVA: Treatment and Time independent variables, [Zn] dependent variable; independent variable interaction

| | Df | Sum Sq | Mean Sq |
|---|---|---|---|
| Time..min. | 3 | 0.02 | 0.007 |
| Treatment | 3 | 9.598 | 3.199 |
| Time..min: treatment | 9 | 0.039 | 0.004 |

Table 42: ANOVA: Time (independent variable) and [Zn] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Time..min. | 3 | 0.02 | 0.0066 | 0.008 | 0.999 |
| Residuals | 12 | 9.637 | 0.803 | | |

Table 43: ANOVA: Treatment (independent variable) and [Zn] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Time..min. | 3 | 9.598 | 3.199 | 657.9 | 1.42E-13 |
| Residuals | 12 | 0.058 | 0.005 | | |

Table 44: Tukey HSD for ANOVA: Treatment (independent variable) and [Zn] (dependent variable)

| Treatment | diff | lwr | upr | p adj |
|---|---|---|---|---|
| Charcoal-Both | 0.031529 | -0.1149 | 0.17792 | 0.91719 |
| Chelex-Both | -0.01925 | -0.1656 | 0.12714 | 0.97886 |
| None-Both | 1.792259 | 1.64587 | 1.93865 | 0 |
| Chelex-Charcoal | -0.05078 | -0.1972 | 0.09561 | 0.73583 |
| None-Charcoal | 1.76073 | 1.61434 | 1.90712 | 0 |
| None-Chelex | 1.811512 | 1.66512 | 1.9579 | 0 |

*V Statistical Results*

Table 45: 2-way ANOVA: Treatment and Time independent variables, [V] dependent variable; Time as blocking variable

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Time..min. | 3 | 0.0534 | 0.0178 | 4.44 | 0.0355 |
| Treatment | 3 | 1.512 | 0.504 | 125.77 | 1.15E-07 |
| Residuals | 9 | 0.0361 | 0.004 | | |

Table 46: 2-way ANOVA: Treatment and Time independent variables, [V] dependent variable; i ndependent variable interaction

| | Df | Sum Sq | Mean Sq |
|---|---|---|---|
| Time..min. | 3 | 0.0534 | 0.0178 |
| Treatment | 3 | 1.512 | 0.504 |
| Time..min: treatment | 9 | 0.0361 | 0.004 |

Table 47: ANOVA: Time (independent variable) and [V] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Time..min. | 3 | 0.0534 | 0.01779 | 0.138 | 0.935 |
| Residuals | 12 | 1.5481 | 0.12901 | | |

Table 48: ANOVA: Treatment (independent variable) and [V] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Treatment | 3 | 1.512 | 0.504 | 67.62 | 8.68E-08 |
| Residuals | 12 | 0.0894 | 0.0075 | | |

Table 49: Tukey HSD for ANOVA: Treatment (independent variable) and [V] (dependent variable)

| Treatment | diff | lwr | upr | p adj |
|---|---|---|---|---|
| Charcoal-Both | -0.04389 | -0.2251 | 0.13735 | 0.88766 |
| Chelex-Both | 0.097246 | -0.084 | 0.27849 | 0.41811 |
| None-Both | 0.717857 | 0.53661 | 0.8991 | 3E-07 |
| Chelex-Charcoal | 0.141137 | -0.0401 | 0.32238 | 0.14968 |
| None-Charcoal | 0.761748 | 0.5805 | 0.94299 | 2E-07 |
| None-Chelex | 0.620611 | 0.43937 | 0.80185 | 1.6E-06 |

***NH3 and Zn Test***

Table 50: ANOVA: Treatment (independent variable) and log [NH3] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Treatment | 3 | 0.06224 | 0.020747 | 4.493 | 0.00967 |
| Residuals | 32 | 0.14776 | 0.004618 | | |

Table 51: Tukey HSD for ANOVA: Treatment (independent variable) and log[NH3] (dependent variable)

| Treatment | diff | lwr | upr | p adj |
|---|---|---|---|---|
| Charcoal-Both | 0.090121 | 0.00333 | 0.17691 | 0.03944 |
| None-Both | 0.108636 | 0.02185 | 0.19543 | 0.00959 |
| Zeolite-Both | 0.080863 | -0.0059 | 0.16765 | 0.07506 |
| None-Charcoal | 0.018515 | -0.0683 | 0.1053 | 0.93795 |
| Zeolite-Charcoal | -0.00926 | -0.096 | 0.07753 | 0.99143 |
| Zeolite-None | -0.02777 | -0.1146 | 0.05902 | 0.8217 |

Table 52: ANOVA: Time (independent variable) and log [NH3] (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| Time..min. | 2 | 0.06147 | 0.030737 | 6.829 | 0.0033 |
| Residuals | 33 | 0.14853 | 0.004501 | | |

Table 53: Tukey HSD for ANOVA: Time (independent variable) and log[NH3] (dependent variable)

| Time..min. | diff | lwr | upr | p adj |
|---|---|---|---|---|
| three-one | 0.086578 | 0.01937 | 0.15378 | 0.00917 |
| two-one | 0.088704 | 0.0215 | 0.15591 | 0.00751 |
| two-three | 0.002125 | -0.0651 | 0.06933 | 0.99669 |

Table 54: ANOVA: Treatment (independent variable) and [Zn] mg/L (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 7.146 | 2.3821 | 22.62 | 0.000291 |
| Residuals | 8 | 0.843 | 0.1053 | | |

Table 55: Tukey HSD for ANOVA: Treatment (independent variable) and [Zn] mg/L (dependent variable)

| Treatment | diff | lwr | upr | p adj |
|---|---|---|---|---|
| Charcoal-Both | 0.7157511 | -0.1328 | 1.56434 | 0.10143 |
| None-Both | 1.9403497 | 1.09176 | 2.78894 | 0.00038 |
| Zeolite-Both | 1.6655435 | 0.81695 | 2.51413 | 0.00107 |
| None-Charcoal | 1.2245986 | 0.37601 | 2.07319 | 0.0074 |
| Zeolite-Charcoal | 0.9497924 | 0.1012 | 1.79838 | 0.02934 |
| Zeolite-None | -0.274806 | -1.1234 | 0.57378 | 0.73394 |

Table 56: ANOVA: Time (independent variable) and [Zn] mg/L (dependent variable)

| | Df | Sum Sq | Mean Sq | F value | Pr>F |
|---|---|---|---|---|---|
| Time | 2 | 0.247 | 0.1234 | 0.143 | 0.868 |
| Residuals | 9 | 7.742 | 0.8603 | | |

Table 57: 2-way ANOVA: Treatment and Time independent variables, [Zn] dependent variable; independent variable interaction

| | Df | Sum Sq | Mean Sq |
|---|---|---|---|
| Time | 2 | 0.247 | 0.1234 |
| Treatment | 3 | 7.146 | 2.3821 |
| Time: Treatment | 6 | 0.596 | 0.0993 |

Table 58: 2-way ANOVA: Treatment and Time independent variables, [Zn] dependent variable; Time as blocking variable

| | Df | Sum Sq | Mean Sq | F value | Pr>F |
|---|---|---|---|---|---|
| Time | 2 | 0.247 | 0.1234 | 1.243 | 0.353584 |
| Treatment | 3 | 7.146 | 2.3821 | 23.987 | 0.000968 |
| Residuals | 6 | 0.596 | 0.0993 | | |

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