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Analyzing the Impact of Road Salt in Stormwater on Phosphorus removal by Duckweed (Lemna minor)

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Analyzing the Impact of Road Salt in Stormwater on Phosphorus removal by Duckweed (*Lemna minor)*

A Thesis Presented by Jenna Fracasso
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Thesis Examination Committee:

Matthew Scarborough, Ph.D., Primary Advisor

Courtney Giles, Ph.D., Secondary Advisor

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Abstract

Water and nutrients are vital resources to all life forms on Earth. Excess nutrients, however, can have detrimental impacts on aquatic ecosystems. In many watersheds, high phosphorus (P) levels can lead to eutrophication and harmful algal blooms. Stormwater runoff is a major contributor for introducing P into natural water bodies from sources such as agricultural fertilizers and yard waste. Another contaminant that makes its way into stormwater runoff is road salts. In cold climates, various salts are distributed onto roads and sidewalks in large quantities to lower the melting temperature of snow and ice. This study analyzed the intersection of phosphorus contamination and road salt contamination. There are many treatment measures to remove phosphorus from water bodies, such as chemical, biological, and physical methods. *Lemna minor*, more commonly known as duckweed, is an aquatic plant species that is known to uptake phosphorus from water bodies and incorporate it into its biomass. In this study, a synthetic stormwater media was created, and the phosphorus uptake of duckweed was tested under sodium chloride road salt conditions ranging from 0.5 g L^{-1} up to 10 g L^{-1}. Results for biomass increase as well as physical observations were taken under each salt condition. As the road salt concentration increased, the phosphorus removal by the duckweed was ultimately less successful, the duckweed grew by a lower percentage of its original biomass, and showed physical signs of degradation.

Introduction

Water is one of the few resources on Earth that is constantly being recycled. As the quantity of water has remained unchanged for millions of years, it has been impacted time and time again by the activity of various life forms on our planet. As water goes through its natural cycles, it is impacted by pollutants. This has been exacerbated immensely by humans, as development and urbanization have increased impervious surface areas and the use of chemicals that have a negative impact on aquatic ecosystems. With impervious surfaces comes an increased rate of surface runoff, limiting the opportunity for natural treatment processes by soil and vegetation to remove pollutants. Stormwater is increasingly being examined for its role in degrading natural aquatic ecosystems as treatment and retention of stormwater is becoming standard practice in many parts of the United States.

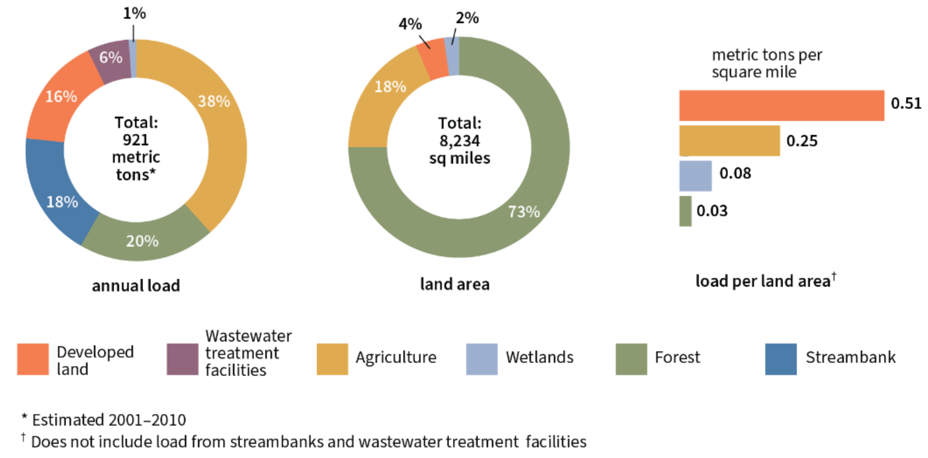
Phosphorus (P) is a vital macronutrient when it comes to many aspects of plant growth and crop yield; it aids with essential development processes in plants (Hasan et al. 2016). Yet once in excess, P is also a leading pollutant that can become harmful to an aquatic environment. P enters stormwater runoff through human activities such as fertilizers, pet waste and yard waste (US EPA 2013). This has compounded with the increased number of impervious surfaces in urbanized regions to cause a massive decrease in the quality of stormwater runoff. When P finds its way into stormwater, this water then runs off into the surrounding water bodies in an area. There is minimal natural occurrence of P in surface water bodies, so when it gets introduced through external sources the ecosystem becomes incredibly disturbed (Mylavarapu, 2009). This happens through eutrophication, when P Luckily, there are naturally occurring plant species that can remove P from the environment. Lemna minor, more commonly known as duckweed, is the plant that I will be using for my study.

Another contaminant that will make its way into stormwater runoff is salt. This can come in different forms, often dependent on climate and location. When looking at areas with cold climates that experience ice and snow in the winter, the introduction of salt in stormwater comes primarily through the use of road salts. In winter road maintenance, salt is often used as the primary way to melt snow and ice on impervious surfaces. Therefore, dissolved salts enter the environment in runoff. This becomes an even greater issue in cold climates as once ground cover freezes, the liquid runoff resulting from road salting is unable to be absorbed into the soil which further increases its rate of movement. When this salt-contaminated water enters the environment, it will typically find its way into nearby waterways such as rivers and lakes. This is increasingly relevant in Vermont, where Lake Champlain shows direct results of being impacted by polluted stormwater runoff. Further, salt can dissolve in the environment into Na^+ and Cl^- ions. In high concentrations, Chloride is known to be a hazardous material to natural ecosystems and can cause degradation in aquatic plants and animals.

There are known strategies to remove P from stormwater; one method is through the natural uptake of P by aquatic plants. It is unclear, however, how this P mitigation by plants may be impacted when salt is introduced into their aquatic environment. Overall, there is not enough literature or general discussion of the impact of road salting on stormwater management. This research will bridge that gap by assessing the impacts of varying salt concentrations on the removal of P by Lemna minor, a naturally abundant floating plant species commonly known as duckweed. The impact will be assessed through reactive phosphorus concentration in the stormwater, as well as measuring the change in duckweed biomass throughout the incubation period. The hypothesis created at the start of this research anticipated that the increase in the salinity of a synthetic stormwater will decrease the phosphorus uptake of the L. minor, indicating that the use of road salts is indirectly contributing to nutrient enrichment and eutrophication by limiting growth and P uptake by aquatic plants.

Background & Literature Review

The issues of phosphorus inundation in natural environments have been addressed for years. While phosphorus is a nutrient that is necessary for the growth of all living things, it is often a limiting nutrient and excess amounts can be detrimental to the health of aquatic ecosystems. The introduction of phosphorus happens through both point sources and non-point sources, largely through agricultural fertilizers (Mylavarapu 2009). Point sources are easy to track; what is more difficult are the non-point sources of pollutants. Figure 1 (below) shows some of the sources of Phosphorus in the Lake Champlain Basin (*Phosphorus Sources*, n.d.).



* Estimated 2001–2010
† Does not include load from streambanks and wastewater treatment facilities

DATA SOURCES: Lake Champlain Long-Term Monitoring Program;
2016 Phosphorus TMDLs for Vermont Segments of Lake Champlain

Figure 1. Phosphorus Sources in the Lake Champlain Basin

With excessive amounts of phosphorus entering natural water systems, there are many negative impacts such as eutrophication and algal blooms. This will alter the wildlife that is able to grow in these conditions, as well as impact the potential for recreation and human use of natural water bodies. When the source of the pollutant is essentially untraceable, it is up to a treatment system to then mitigate these unavoidable and detrimental effects.

Stormwater treatment systems often involve some type of stormwater retention pond. In a retention pond, floating and standing vegetation can remove dissolved pollutants such as phosphate and any suspended particles. In a study by Karine E. Borne from the University of Auckland, the efficiency of a floating treatment wetland (FTW) was analyzed. This study showed significant decrease in total phosphorus levels between the inlet and outlet of the retention pond after the addition of this FTW (Borne 2014). In my study specifically I will be working with duckweed, which is a plant commonly used in FTWs but also one that exists in natural environments all over the world. There have been many studies of duckweed regarding its P removal capabilities. Tripati et al. conducted a study in 1991 placing duckweed into ponds of different chemical characteristics to observe its removal of inorganic natural pollutants. Within each variation of P concentrations, the duckweed showed similar removal characteristics. This indicates that P concentration alone does not necessarily impact the rate at which each plant is able to remove it. Temperature had the greatest effect on P removal in the warmer summer months duckweed was not as effective compared to the other plants in the study, while under cooler temperatures it increased in its relative efficiency.

Salinity is another issue that presents itself in stormwater. Salt enters stormwater through the means of road salts as well as seawater intrusion in coastal communities (Szota et al. 2015).

Once salt makes its way into stormwater it has many environmental implications, particularly on the processes of biological communities. Road salts in particular have been found to create a conductivity gradient in stormwater ponds, increasing TDS and chloride concentrations greatly (Marsalek 2003). Marsalek also explains that there is the potential of increased chloride enhancing the toxicity of urban pollutants, furthering their impacts before they can be removed by vegetative systems. Regarding chloride concentrations, the current benchmark levels for drinking water and aquatic life toxicity are 250 mg/L and 230 mg/L respectively (LCBP, 2021). Considering this is a concentration for the entirety of Lake Champlain, it is safe to assume that the concentration in stormwater itself would prove much higher. Chloride concentrations in stormwater ponds are highly variable based on size of pond and volume of de-icing salt used per season; a typical range can be anywhere from 500 mg/L to 5,000 mg/L (Marsalek 2003).

There is some existing knowledge on the salt tolerance of duckweed. It was found in a 2021 study by Ullah et al. that higher salt concentrations in water caused a decline of protein synthesis, lipid content, and carbohydrate content. At salt concentrations of 2 g/L and 4 g/L there were less drastic effects, but after 6 g/L more changes were present (Ullah et al. 2021). When comparing to the concentration range stated above, the concentration of 6 g/L (or 6,000 mg/L) lies just above the threshold of 5,000 mg/L. With the understanding that salt concentrations have been steadily increasing in Lake Champlain over previous years, it is important to see how vegetation may act over these high concentrations.

With these findings, it is clear that there are some visible impacts of dissolved salts on duckweed. However, it is unclear how salt impacts the process of phosphorus removal at concentrations expected in storm water. Only one piece of literature could be found regarding this issue, a 2017 study by Liu et al titled *Potential of duckweed (Lemna minor) for removal of nitrogen and phosphorus from water under salt stress*. This study was conducted relative to water bodies off the coast of China, and found that P removal was inhibited by salt stress. At the highest experimental concentrations, the duckweed began to release P into its environment (Liu, Dai, and Sun 2017). Due to what is known about salt tolerance, it is expected that the duckweed will be impacted in more complex ways than just inhibiting the growth of the plant. Salinization brings great environmental stressors to plants, bringing both osmotic and ionic stress (Xiao and Zhou 2023). The balance of sodium ions will become disordered and cells can dehydrate, which could also cause an increase in the impact of P in a surrounding stormwater environment. Overall, the impact of environmentally relevant levels of salt on phosphorus removal is largely unexplored.

Research Objectives

The goal for my thesis was to investigate the impact of road salt on our engineered stormwater treatment systems as well as naturally occurring stormwater treatment processes. Stormwater treatment is a vital part of keeping our world sustainable by protecting the health of natural water bodies. Human activity causes eutrophication in our waterways; in Vermont, eutrophication is driven by phosphorus runoff. This is an issue that has been widely addressed, but there is less discussion regarding how our use of road salts may be furthering eutrophication by impacting natural biological P removal processes. For my thesis, I investigated the link between salt contamination and the natural uptake of phosphorus by aquatic plants. This involved numerical results in the form of reactive phosphorus removal and duckweed biomass growth, as well as qualitative observations of the physical health of the duckweed plants.

Materials and Methods

Synthetic Stormwater

I began my laboratory work by creating the recipe for a synthetic stormwater medium that was used throughout the entirety of my study. The overarching goal for this medium was to contain a Phosphorus source, other elements that reflect what can be found in natural stormwater, and nutrients that would foster growth of the duckweed plants. The following chemicals were decided through researching the makeup of synthetic stormwaters seen in previous studies, as well as the chemical makeup of typical biological growth media (Sims and Hu 2013), (Davis et al. 2001). The concentrations of these chemicals had to be high enough to impact the duckweed and be detectable in future testing, but also remain in the range of a typical stormwater. The final makeup of this medium is outlined in Table One (below).

Table 1. Chemical Makeup of Synthetic Stormwater Solution

Chemical	**Concentration (mg/L)**
Dibasic Potassium Phosphate	1 (as P)
Potassium Nitrate	2 (as N)
Glycine	4 (as N)
Calcium Chloride	120
Humic Acid	15
Magnesium Sulfate	100

Due to the low concentrations of each chemical in the stormwater medium, a 10X solution was created and stored in the fridge in a 2L glass bottle. In order to create the final synthetic stormwater for each trial, the 10X solution was diluted using de-ionized water. Each trial utilized a final total volume of 2.25L of synthetic stormwater.

Incubation Experiments

A series of incubation experiments were conducted from January to March 2024. For each trial, 2.25L of synthetic stormwater was split into three 1L beakers with 750 mL of stormwater each beaker. In each 750 mL batch of stormwater, the different road salt concentrations were mixed. The salt used throughout the experiments was a sodium chloride rock salt provided by UVM custodial services that is regularly used to de-ice sidewalks and roads around UVM's campus. Each 750 mL batch was finally split into three 600 mL beakers, each containing 250 mL of the final stormwater and salt combination. This allowed for the use of biological replicates in each analysis, with three duckweed colonies for each salt concentration.

Trial one consisted of a 0 g/L control, 0.5 g/L NaCl and 1 g/L NaCl. All beakers started out with approximately 1.2 mg of duckweed, and the incubation period was a total of eleven days. In trial two, the road salt concentrations were 2 g/L, 10 g/L, and a negative control which contained no road salt and no duckweed. For the 2 g/L and 10 g/L tests, approximately 2 mg of duckweed was added to each beaker, and the incubation period was eight days. In trial three, the salt concentrations were 4 g/L, 6 g/L and 8 g/L. Again, approximately 2 mg of duckweed was added to each beaker and they were left for a total testing time of eight days.

Each beaker was placed on a stir plate and mixed at a constant speed of 50 rotations per minute (rpm) using a magnetic stir rod to prevent particulate settling. The system was also placed underneath two LED grow lamps. The physical setup of the experiment is shown in Figure Two (below).

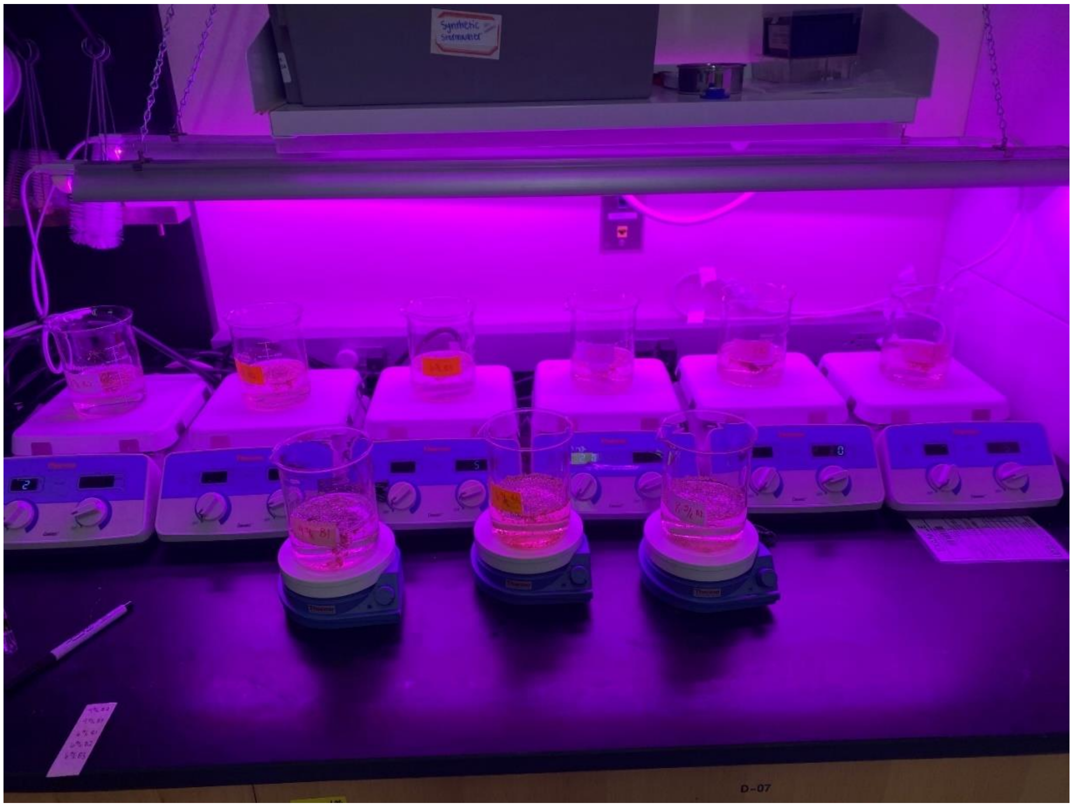


Figure 2. Experimental Setup

As seen in this image, the three stir plates at the front of the experimental setup were different than the other six, and therefore did not allow for the consistent 50 rpm stirring. This caused these systems to either be spun at a higher rate than the other six, or remain stagnant.

Analysis of Phosphorus Concentration

Stormwater samples were taken at various times throughout each incubation period. In trial one, samples were taken at day four, day seven and day eleven. In trials two and three, samples were taken at days two, four, six and eight. The beakers experienced some evaporation through the duration of the trial, so with each sample collection the water level in the beaker was brought back up to 250 mL using de-ionized water to maintain accuracy in the phosphorus concentration. A 10 mL sample of stormwater was collected from each beaker, filtered through a 10 nm syringe filter. Samples were stored in the freezer after collection and thawed in the fridge before testing.

The HACH Phosphate Test Kit was used to measure the PO_4-P levels in each sample. This method uses spectrophotometry to indirectly measure reactive phosphorus. At the start of testing, a calibration curve was created using dilutions of known phosphate concentrations, shown in Figure Eleven (Appendix 1). This was done by measuring the absorbance value of each standard dilution at 890nm in a spectrophotometer and plotting the absorbances against phosphate concentration. Using a linear regression analysis, the relationship between absorbance units (A_{890}) and reactive phosphorus (mg/L PO_4-P) was found. This relationship was used throughout the rest of the trials to find the concentration of reactive phosphorus in each stormwater sample.

Results and Discussion

PO4-P Removal

The following graphs depict the concentration of reactive phosphorus present in the stormwater throughout each trial. Figures three, four and five show the results separated for each trial. It is important to note that a few changes were made to the experimental protocol after trial one. A new batch of duckweed was used for the final two trials, which was larger and appeared to be much healthier. The incubation period was shortened to eight days with more frequent sample collection, as the results from trial one showed that phosphorus removal was happening very quickly, within the first few days of treatment.

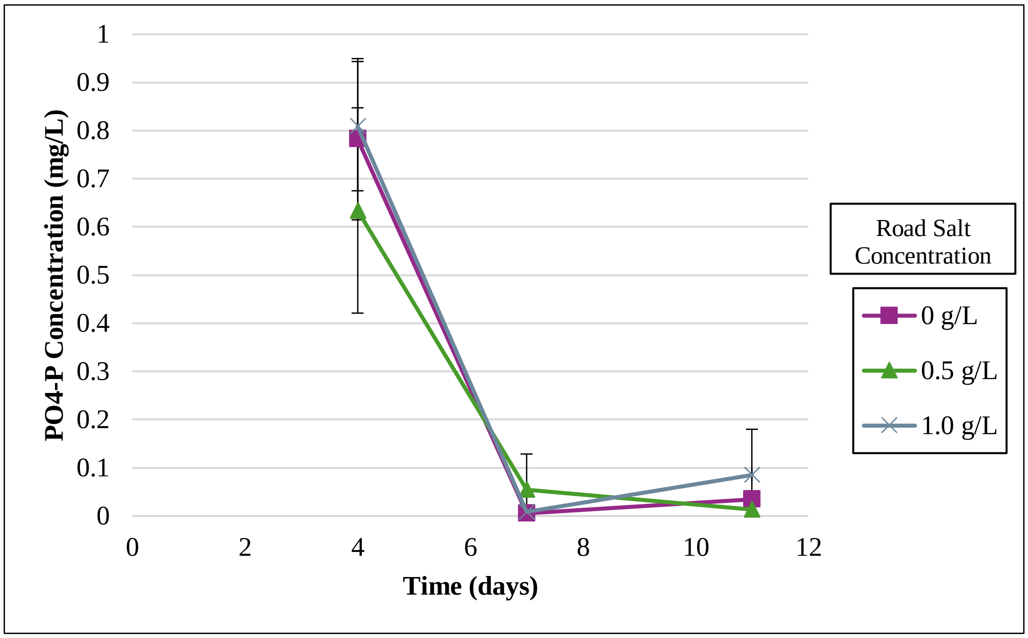


Figure 3. Trial One PO_4-P Concentrations over Time

In this trial, each batch of duckweed was able to remove just about all of the phosphorus in the stormwater. On day four, the 0.5 g/L NaCl sample had removed the most out of the three, but by day seven all phosphorus levels in all replicates were under 0.1 mg/L PO_4-P.

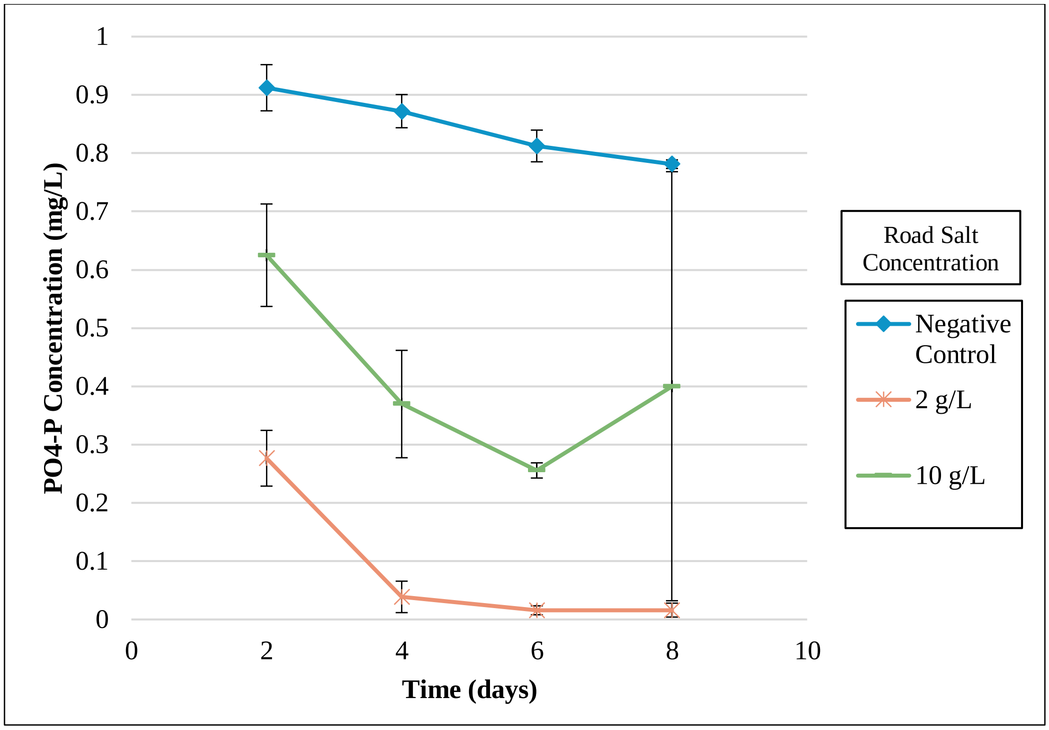


Figure 4. Trial Two PO_4-P Concentrations over Time

This trial consisted of three replicates over a broader range of salt concentrations. The negative control had no salt or duckweed, so shows that there was some natural degradation of phosphorus in the stormwater. This could be due to internal reactions such as the phosphorus absorbing to the Humic Acid in the stormwater, or any original phosphorus from the K_2HPO_4 converting from a reactive form to organic phosphorus.

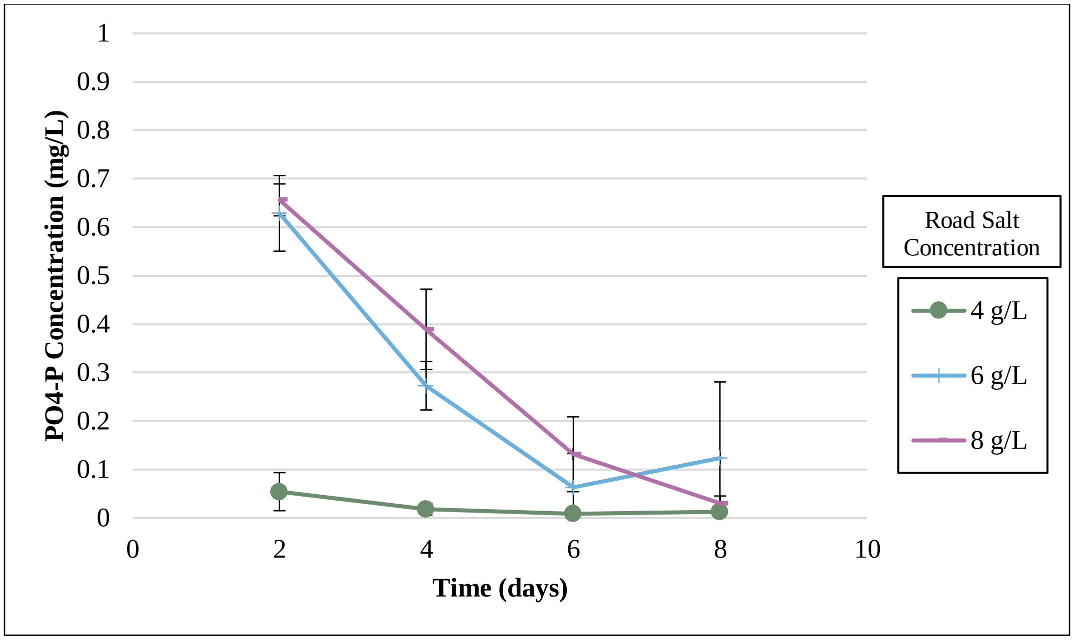


Figure 5. Trial Three PO_4-P Concentrations over Time

This final trial was done to bridge the gap in the data between the 2 g/L NaCl sample and 10 g/L NaCl sample. These results show that in these mid-range salt concentrations the duckweed was still able to successfully remove reactive phosphorus. The 4 g/L sample removed the phosphorus most quickly, which is difficult to explain as it seemed to be more successful than the 2 g/L sample in Trial Two. This could be due to human error in the reactive phosphorus measurement, or biological variability in the duckweed samples.

Figure Six (below) then combines the results from every trial to show a comprehensive comparison of phosphorus removal across all salt concentrations studied in this experiment.

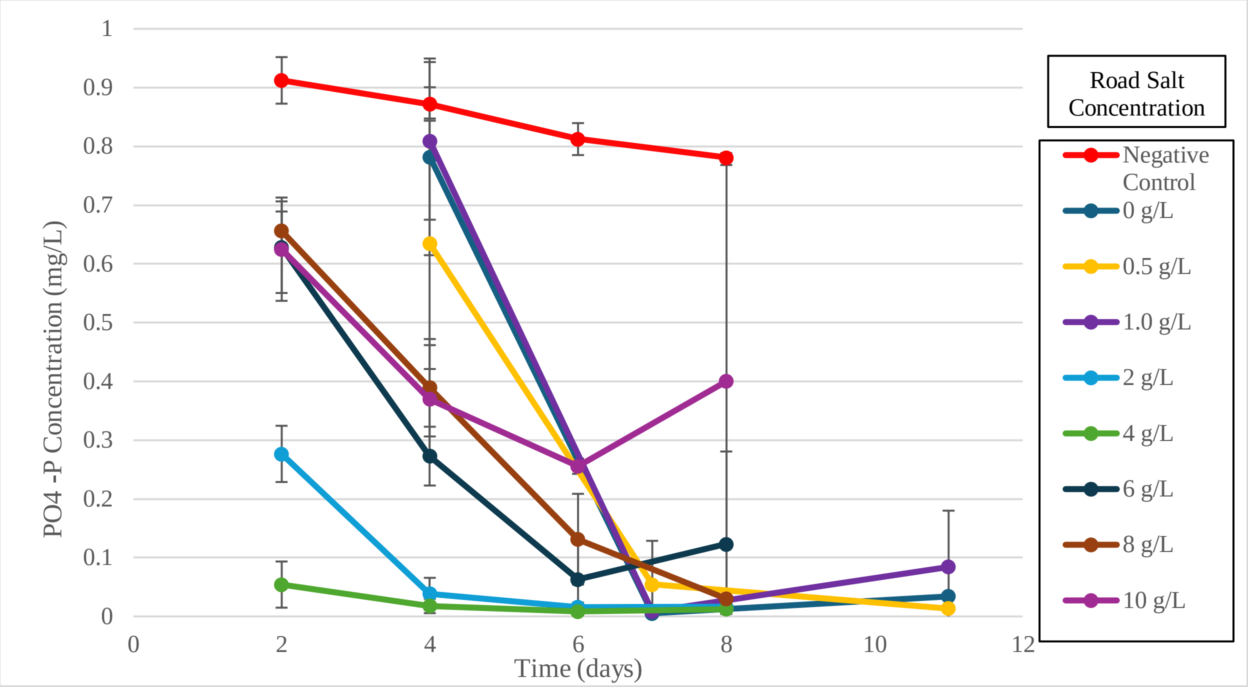


Figure 6. PO_4-P Removal by Duckweed Across NaCl Concentrations in Synthetic Stormwater

All trials began at the same phosphorus level of 1 mg/L K_2HPO_4 (as P). Starting with the negative control, these results show the natural activity of the synthetic stormwater; the P levels decreased to a final level of about 0.8 mg/L P on day 8. This natural degradation does have an impact on our experimental results, but remains consistent through every trial. In the lowest concentrations, there is a clear drop in P levels from day four to day seven. This prompted the change in procedure for trials two and three, where water samples were collected for P measurement on days two, four, six and eight.

Across all trials, the P levels were decreased to their lowest point between days six and eight. In the 10 g/L trial, the P level decreased to 0.25 mg/L on day six, and increased back up to

0.40 mg/L on day eight. This shows that not only did the road salt inhibit the duckweed from effectively removing all the phosphorus from the stormwater, but there was also eventually an increase in P due to degradation of the duckweed that sent P back into the water.

The rate of removal of reactive phosphorus was calculated for each trial and summarized in the table below.

Table 2. PO_4-P Removal Rates for each Road Salt Concentration

NaCl Conc. (g/L)	**P-Removal Rate (mg/L-day)**
Negative Control	0.031
0	0.332
0.5	0.315
1	0.330
2	0.481
4	0.491
6	0.364
8	0.305
10	0.315

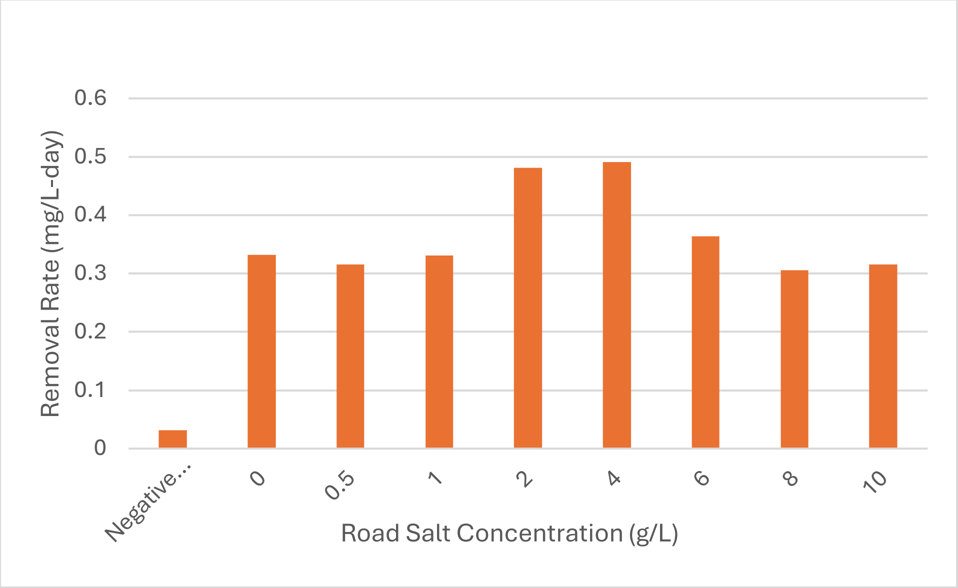


Figure 7. PO_4-P Removal Rate in mg(L-day)$^{-1}$ across Road Salt Concentrations

These rates were calculated over the first couple days of each trial period, which can be considered the moment of initial removal of phosphorus. These rates are among the same magnitude, between 0.3 and 0.5 mg/L-day. However, the rate follows a downward trend as the road salt concentration increases. It can also be anticipated that with more time, the duckweed may begin to re-introduce phosphorus back into its environment as it breaks down.

Impacts on Plant Biomass

Each trial began with equal amounts of duckweed biomass in each biological replicate. The final mass was then taken at the end of each incubation period, and the percent growth was calculated. These values are summarized below:

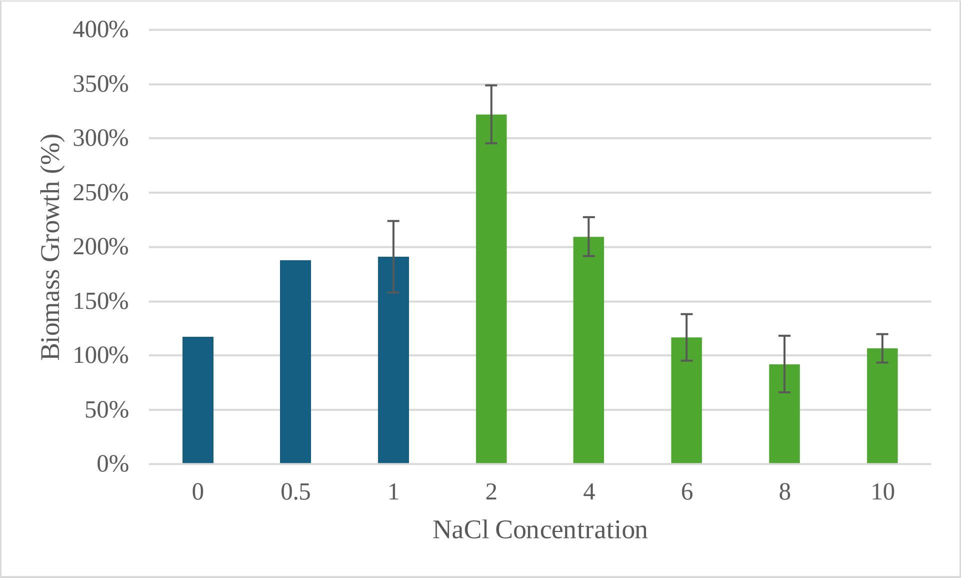


Figure 8. Percent Growth of Duckweed Fresh Weight versus NaCl concentration*

*Blue bars correspond to Batch 1 of Duckweed, Green bars correspond to Batch 2 of Duckweed

When looking at Figure 6, the Batch 2 duckweed results show a downward trend of biomass growth as you increase the concentration of road salt in stormwater. Each sample was able to increase its fresh weight by over 100%, indicating that road salt does not completely deplete the duckweed's ability to grow. However at lower road salt levels, the duckweed was able to replicate much more abundantly.

Qualitative Assessment of Plant Growth

Throughout experimentation, physical characteristics of the duckweed plants were observed. Duckweed is mainly comprised of two components; the upper leaf, seen floating on the water's surface, and the lower stem. Throughout this study, it was observed that the health of the duckweed plant was more accurately represented by the root structures underneath the surface than the surface area of the leaves. Figure nine (below) shows the duckweed colonies on day eight of trial two. In this comparison, it is clear to see the difference in physical structure of each duckweed colony.

Figure 9. Comparison of Duckweed Grown in 2 g/L NaCl (left) and 10 g/L NaCl (right)

At 2 g/L of road salt, the duckweed was able to grow solid root structures, with a bright green coloring and minimal signs of degradation. At 10 g/L road salt, the duckweed was a darker brown color, with no solid roots and a higher amount of small leaves.

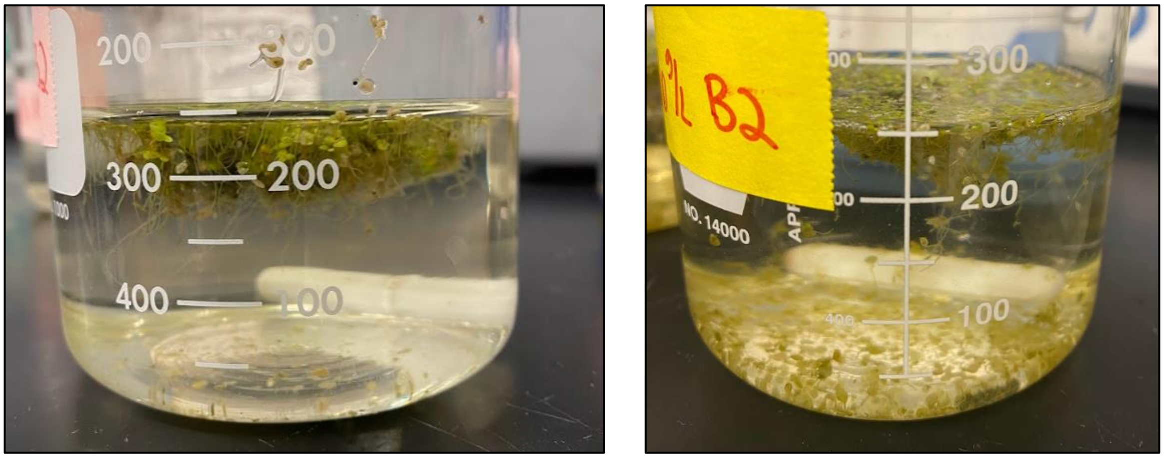


Figure 10. Duckweed Plants in 4 g/L NaCl (left) and 6 g/L NaCl (right) on Day 8

At the middle road salt concentrations, the duckweed was able to grow and appeared to grow solid root structures. However by day eight, the plants in higher salt concentrations began to show more signs of degradation. The roots turned a dark brown color, and leaves began to sink to the bottom of the stormwater, as seen in Figure ten (above). This displays signs of a decrease in overall health of the duckweed plant and indicates that after this point the organism would no longer be able to remove P from its environment. When duckweed is used in wastewater treatment plants, it is a common practice to harvest the duckweed from the water's surface once it has completed P removal to avoid overcrowding. With the duckweed sinking to the bottom, this would become difficult and potentially contribute to the issues of eutrophication as the duckweed continues to break down.

Conclusion

Summary of Findings

Overall, duckweed is an arguably salt tolerant plant. When exposed to road salt conditions in synthetic stormwater, the duckweed was still able to grow and remove Phosphorus over the course of about one week. Yet there were clear differences in the activity of the duckweed when exposed to different road salt concentrations, ranging from 1 g/L to 10 g/L. The concentration with the highest P removal was 1.0 g/L of NaCl, taking the phosphorus concentration in the stormwater down from 1.0 mg/L PO_4-P to 0.009 mg/L PO_4-P on day eleven of incubation. With a concentration of 10 g/L of road salt in the stormwater the duckweed was still able to remove phosphorus, but only to a level of 0.4002 mg/L PO_4-P. The rates of P removal over the first few days of incubation were also found to decrease as road salt concentrations got above 6 g/L. The highest removal rate was found by the duckweed in 4 g/L NaCl at 0.491 mg/L-day, while the duckweed in 8 g/L NaCl removed P at a rate of 0.305 mg/L-day, and eventually began to reintroduce P back into the system at the end of incubation. When exposed to higher road salt concentrations, the duckweed would still remove phosphorus from its stormwater environment, but less effectively and at a lower rate.

The health of the duckweed plants was also observed, and it was clear that the plants were able to reproduce more easily when exposed to lower road salt conditions. At 2 g/L NaCl the duckweed fresh weight increased by 322%, while at 8 g/L NaCl it increased by 92%. There were also visible changes to the duckweed plants. With higher salt presence, the duckweed began to degrade and sink to the bottom of their environment. At concentrations up to 4 g/L NaCl, the duckweed was a light, bright green with solid root and leaf structures. At concentrations above 6 g/L, the leaves and stems of the plants were less bright green and instead a darker brown color, with thinner roots that caused the plants to break apart more easily.

Error Discussion

When working with plants, there is always the unpredictability that comes along with living organisms. This was addressed through the use of biological replicates, having three beakers for each salt concentration with equal starting masses of duckweed. These different colonies could have behaved differently in terms of their P removal, causing variations in results that prompted large standard deviations in the data. Although samples were filtered after collection, there is also still the chance that some organic matter got through the filter, which could have had an impact on the phosphorus levels in the stormwater. Another variation in testing materials was the stir plates; in each trial, three stir plates were different than the other six. This caused them to either spin at a faster rate or be off and not agitating the system at all. This would cause the duckweed to be disturbed, or allow particles to settle, respectively. Finally, taking day zero measurements would have been advisable; the assumption was made that each system was starting out with 1.0 mg/L PO_4-P, but after observing the activity of the stormwater through the negative control it is clear that this was not the case, and the phosphorus levels were starting at a lower level.

Recommendation for Future Work

Future work could involve bridging the gap between this lab-based study and our real, natural environment. This could be fieldwork that studies how much road salt is actually making its way into our waterways from year to year, how long it remains in the environment, and if there are any potential methods of mitigation for existing salt in our freshwater systems. Other road salts could be studied, to observe if they have a different impact on phosphorus removal. Alternatives to road salt could be tested and compared for their environmental impact on natural ecosystems. A study could also be done on other phosphorus removal methods, such as biological removal through microbial communities, chemical removal, or other plants commonly used in FTWs.

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Appendices

Appendix 1: Supplemental Tables and Figures

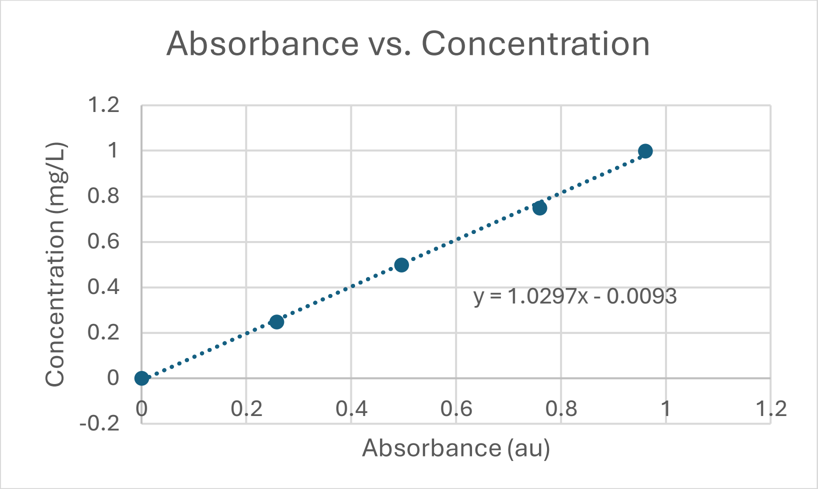

Figure 11. Calibration Curve for PO_4-P Testing

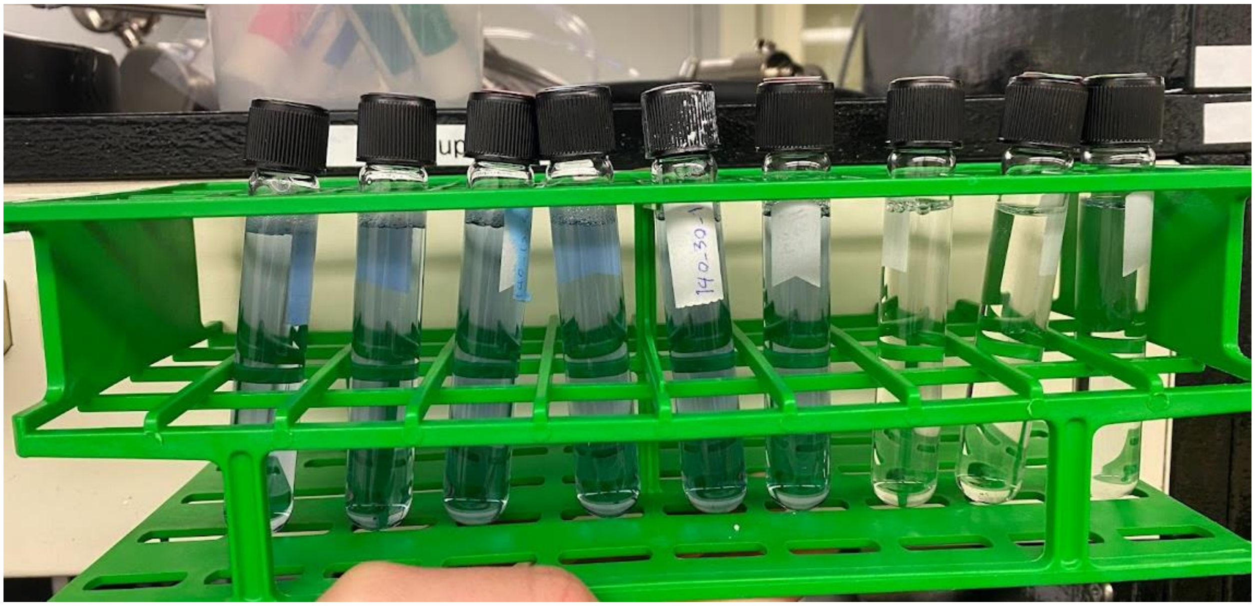

Figure 12. Test Tubes During HACH Reactive Phosphorus Testing

Appendix 2: Raw Data

Table 3. Trial One P-Testing Data

NaCl Concentration		1/29 au	1/29 mg/L	2/1 au	2/1 mg/L	2/5 au	2/5 mg/L
0 g/L	B1	0.955	0.974064	0.008	-0.0010624	0.041	0.032918
	B2	0.695	0.706342	0.012	0.0030564	0.044	0.036007
	B3	0.656	0.666183	0.023	0.0143831	n/a	
0.5 g/L	B1	0.864	0.880361	0.145	0.1400065	0.022	0.013353
	B2	0.506	0.511728	0.024	0.0154128	n/a	
	B3	0.505	0.510699	0.017	0.0082049	n/a	
1.0 g/L	B1	0.952	0.970974	0.014	0.0051158	0.01	0.000997
	B2	0.8	0.81446	0.022	0.0133534	0.042	0.033947
	B3	0.633	0.6425	0.016	0.0071752	0.221	0.218264

Table 4. Trial Two P-Testing Data

NaCl Concentration		2/25 au	2/25 mg/L	2/27 au	2/27 mg/L	2/29 au	2/29 mg/L	3/2 au	3/2 mg/L
Negative Control	B1	0.891	0.908	0.817	0.832	0.775	0.789	0.78	0.789
	B2	0.944	0.963	0.878	0.895	0.784	0.798	0.77	0.784
	B3	0.85	0.866	0.873	0.8890	0.835	0.851	0.758	0.771
2.0 g/L	B1	0.326	0.326	0.08	0.073	0.028	0.020	0.015	0.006
	B2	0.215	0.212	0.016	0.007	0.031	0.023	0.041	0.033
	B3	0.292	0.291	0.044	0.036	0.014	0.005	0.018	0.009
10 g/L	B1	0.633	0.643	0.4	0.403	0.242	0.240	0.154	0.149
	B2	0.711	0.723	0.458	0.462	0.257	0.255	0.136	0.131
	B3	0.504	0.510	0.246	0.244	0.273	0.272	0.903	0.921

Table 5. Trial Three P-Testing Raw Spectrophotometer Data in Absorbance Units

NaCl Concentration		21-Mar	23-Mar	25-Mar	27-Mar
4 g/L	B1	0.115	0.043	0.025	0.014
	B2	0.042	0.02	0.015	0.024
	B3	0.028	0.016	0.012	0.025
	MEAN	0.061667	0.02633333	0.0173333	0.021
	STD DEV	0.038143	0.01189771	0.0055578	0.004967
6 g/L	B1	0.558	0.275	0.166	0.345
	B2	0.726	0.333	0.027	0.022
	B3	0.574	0.214	0.018	0.019
	MEAN	0.619333	0.274	0.0703333	0.128667
	STD DEV	0.075707	0.04858669	0.0677463	0.152976
8 g/L	B1	0.691	0.496	0.216	0.059
	B2	0.618	0.361	0.158	0.026
	B3	0.63	0.304	0.036	0.03
	MEAN	0.646333	0.387	0.1366667	0.038333
	STD DEV	0.031962	0.08051087	0.075017	0.014704

Table 6. Trial Three P-Testing Data in mg/L

NaCl Concentration		2	4	6	8
4 g/L	B1	0.109116	0.034977	0.016443	0.005116
	B2	0.033947	0.011294	0.006146	0.015413
	B3	0.019532	0.007175	0.003056	0.016443
	MEAN	0.054198	0.017815	0.008548	0.012324
	STD DEV	0.039276	0.012251	0.005723	0.005114
6 g/L	B1	0.565273	0.273868	0.16163	0.345947
	B2	0.738262	0.33359	0.018502	0.013353
	B3	0.581748	0.211056	0.009235	0.010264
	MEAN	0.628428	0.272838	0.063122	0.123188
	STD DEV	0.077956	0.05003	0.069758	0.157519
8 g/L	B1	0.702223	0.501431	0.213115	0.051452
	B2	0.627055	0.362422	0.153393	0.017472
	B3	0.639411	0.303729	0.027769	0.021591
	MEAN	0.656229	0.389194	0.131426	0.030172
	STD DEV	0.032911	0.082902	0.077245	0.015141