An Acute 72hr Growth Inhibition Test using Ampicillin on a mixed population of Microalgae (*Pseudokirchneriella subcapitata., Chlorella spp., Scenedesmus spp.*)

Primary Investigator: Cole Aquila

Submitted in partial fulfillment of the requirements for BIO 489

April 15th, 2016

Supervisors: Dr. Sheri Dalton & Dr. Xin Chen

Department of Biological and Environmental Sciences, Concordia University of Edmonton

Abstract

Increased use of pharmaceuticals such as antibiotics has lead to the presence of biologically active antibiotics (i.e ampicillin) in surface waters, causing awareness of possible human and environmental health concerns. These non-target effects could cause effects on aquatic ecosystems and ecological communities. One of the main components of an aquatic ecosystem is their microalgae communities which are the main primary producers for aquatic ecosystems. Microalgae species and communities could be affected by antibiotic presence in the water bodies leading to possible shifts of populations in the communities. Using Pseudokirchneriella subcapitata, Chlorella spp., and Scenedesmus spp. an acute 72hr growth inhibition test was performed on three individual species, and as a community. Toxicity was assessed by measuring absorbance at 415nm to find algae density changes and by observing population changes in different concentrations of ampicillin.

Keywords: *Pseudokirchneriella subcapitata., Chlorella spp., Scenedesmus spp.,* Ampicillin, Toxicity, Absorbance, Cell density, Growth inhibition.

Introduction

Environmental contamination in the modern world has become a significant problem posing risk to both human health and environmental health. Of these risk water contamination is one of the largest concerns for the environment, one such contaminant posing risks to water contamination is pharmaceuticals. These pharmaceuticals are being released through waste treatment facilities, and for the majority will be transported to water ways (Crane et al 2006). These pharmaceuticals are released into the water system in major cities, and farm land (Papageorgiou et al 2016; Crane et al 2006), being used for both human and veterinary medicine (Magdaleno et al 2015). Human antibiotic use is a typical treatment techniques while antibiotics are used for farming are given to animals as a precaution (Halling-Sorensen 2000). Once pharmaceuticals are released they can make their way to the sewage treatment plants, which cannot remove all of the contamination before reaching the surface waters (Magdaleno et al 2015). Once in the waters the pharmaceuticals can undergo biodegradation, environmental persistence, or are partially degradable (Richardson and Bowron 1985; Crane et al 2006).

Both human and veterinary medicine results in contamination, but of the two sewage treatment plants result in higher exposure rates causing direct point source contaminations causing continuous contamination (Crane et al 2006). All leading to contamination of surface waters, sea waters, ground water and fresh water, the ecological effects of these contaminants are still unknown (Fent et al 2006). Pharmaceuticals persistence is largely due to high polarity which influences their ability to spread (Crane et al 2006). The unknown effects have led to the current concern being developed along with the rise in occurrence of pharmaceutical use, industrial runoff amount, and the synergistic effects of these chemicals on the aquatic environment. These effluents are typically treated to remove as much of the toxic chemicals as possible (Sun et al

2014), but this does not necessarily remove all of the harmful pharmaceuticals. Pharmaceutical contamination is still largely unknown in many areas of the world, most research looks at the contamination under set temperatures and treatment characteristics, but further research must be performed to understand the difference during seasonal changes, changing temperatures and climates can alter the way a pharmaceutical reacts in an environment, possibly deterring degradation and increasing harm (Sun et al 2014).

Pharmaceuticals such as antibiotics are biologically active compounds, and are being used at higher rates. Since their introduction in the 1930's, environmental health from a human perspective has increased, helping improve human, animal and agricultural health (Roose-Amsaleg and Laverman 2016). The increase rate of use has also increased the rates of antibiotic exposure in the environment, considering ~70% of antibiotics are released from urine and feces are non-metabolized, meaning these antibiotics are still active and can interact in their aquatic environment, especially since most are water soluble, in similar rates that they would in the body (Magdaleno et al 2015; Roose-Amsaleg and Laverman 2016; Kemper 2008; Halling-Sorensen 2000). Their release has resulted in significant concentrations of antibiotics in aquatic water and sediment.

Antibiotics typically target bacteria by inhibiting growth, or killing the bacteria, targeting cell wall, protein synthesis or DNA replication (Roose-Amsaleg and Laverman 2016). Once introduced into an environment, antibiotics can influence the ecological functions of the environment, affecting ecosystem stability, causing an effect in population dynamics (Ding and He 2010). This will have an effect on microbial community structure, along with the planktonic community. Microbial species are highly tolerant and adaptive, while planktonic species are much more susceptible to the toxic effect of antibiotics (Ding and He 2010). While microbial

communities have been shown to exhibit tolerance due to their natural abilities, helping resistance develop, this can further influence the microbial community structure and influence the overall community dynamics (Roose-Amsaleg and Laverman 2016). These communities adapt quickly due to their high reproduction rates, and can cause considerable damage to community function due to it (Roose-Amsaleg and Laverman 2016).

Current research tends to focus on the effect of antibiotics relative to resistance acquired by biological organisms and tends to shy away from the toxicological effects caused, especially the effects of antibiotics on organisms that are not of bacterial (Roose-Amsaleg and Laverman 2016). With detection of antibiotics in surface water being found worldwide, and in certain places having concentrations of ng/L to ug/L (Lee et al 2008; Magdaleno et al 2015), new research must be performed to test for the toxicological effect within aquatic ecosystems looking at more than just bacterial resistance.

β-lactam's are among one of the major antibiotic groups used for treating bacterial infections. Among them are ampicillin and amoxicillin are most commonly used for both human health and animal health concerns (Magdaleno et al 2015). These penicillin antibiotics target the peptidoglycan layer on bacterial cell walls of gram negative and positive bacteria (Magdaleno et al 2015), causing a leaky cell, interfering with the cell structure and osmoregulation abilities causing lysis of the bacterial cell. These antibiotics can also have effects on non-target organisms; Halling-Sorensen found that they have different effects on prokaryotic algae in comparison to eukaryotic algae in 2000. Antibiotics have shown to cause inhibition in prokaryotic protein synthesis, while eukaryotic algae shows to be sensitive and to the antibiotics having an effect on their chloroplasts (Halling-Sorensen 2000). There is however conflicting data, Halling-Sorensen wrote in 2000 that members of the β-lactam antibiotics class would only

affect prokaryotic algae (cyanobacteria). While Matsumoto and others found in 2012, that ampicillin ended up inhibiting chloroplast division, but not the number of chloroplasts present in *Closterium*. This suggests that the antibiotic ampicillin target (the peptidoglycan wall) has a relationship to chloroplast development which could effectively alter the microalgae's fitness, possibly resulting in inhibited growth or alterations to the community structure of algae (Matsumoto et al 2012).

Freshwater water bodies are diverse and precious resources; they are the homes to diverse, abundant and delicate ecosystem communities. Freshwater is not a renewable resouce and so sources of it around the world must be cared for, being a requirement for life. Within these ecosystems are communities of organism's that rely on each other to maintain their community structure, phytoplankton communities being one of the most significant parts of the food chain. Within the phytoplankton communities are bacterial and microalgae species make up the bases of most primary producers in aquatic ecosystems. These communities, along with individual populations of planktonic species do experience toxicological effects from the toxins in their environment. Exposure to types of pharmaceuticals and antibiotics could affect the entire ecosystem (Cole 1982). This relationship is the main interaction controlling the energy flow and nutrient cycle in the aquatic environment, without it the entire ecosystem could collapse (Cole 1982).

Microalgae populations are extremely diverse, being found in most water bodies worldwide. This allows communities, populations and individuals to change in size (1um-250um) and abundance (Porter 1977). Most species are single celled, some cluster and chain, while cells divide for reproduction typically having a division rate of 1-3 time/day (Porter 1977). Individual species tend to vary in shape, size and arrangement. Population sizes being influenced

by density dependent factors, and independent factors, in this toxicity. Freshwater green algae use photosynthesis for energy and other nutrients for reproduction. Typically, these communities have high amounts of biodiversity within a community (Wang et al 2011), suggesting that toxicity tests should be performed on individual species and in a mixed culture setting to receive the best possible estimation of toxicity effect.

For this experiment three widely found freshwater microalgae species were used to test the antibiotic ampicillin toxicity both on an individual organism level and a community level to properly assess the ecologicall effect, *Pseudokirchneriella subcapitata, Chlorella spp.,* and *Scenedesmus spp*. Microalgae represents unicellular and multicellular photosynthetic microbes, containing chloroplast a and b, which end up representing a large amount of their body composition, dividing through cell division roughly every 12 hours (Spoehr 1976). *Pseudokirchneriella subcapitata* (previously known as *Selenastrum capricornutum* and *Raphidocelis subcapitata* before that) is a unicellular organism heavily used for toxicity test because it is non-motile, replicate at a rapid rate for algae, and are sensitive to stressors in the environment, making them a bioindicator species (Environment Canada 2007).*Chlorella spp.* a microalgae genus that can asexually replicate via autospores causing rapid growth (Myers 1976), is unicellular and is not considered sensitive to environmental stressors (Spoehr 1976). *Scenedesmus spp.* are incredibly variable, they are typically unicellular and can form colonies off 4 to 8 cells (Lampert et al 1994;Trainor 1996). Cells are typically formed within the parents wall and then released, they are non-motile , and when stress occurs, they typically form colonies (Lampert et al 1994;Trainor 1996). These microalgae species have been the subject of many experiments over the years.

Methods

Methodology for this experiment were adapted and modified from "Environment Canada, Growth Inhibition Test Using a Freshwater Algae" (Environment Canada 2007).

To complete this research cultures of *Scenedesmus spp., Chlorella spp.,* and *Pseudokirchneriella subcapitata.* were established, these cultures were subcultures of previously established cultures from Boreal scientific, and the Canadian Phycological Culture Center (CPCC). These cultures were established in 150mL sterile culture flask's, in 50ml of 100% Bold's Basal Modified Medium (BBMM), using BBM obtained from the CPCC. Once these cultures were established a mixed culture was created of all three species, this mixed culture was created using even ratios of all species of microalgae, and allowed to establish its community dynamics for 3weeks. If the cultures became over grown then a new culture would be created. These cultures were then used for the bases of this experiment, where these three freshwater microalgae would be exposed to treatments of the antibiotic ampicillin.

All cultures and tests were grown in a standing refrigerator style growth chamber, being inoculated at 12D:12L light cycle, with a light intensity of 4000 lux, a temperature of 24^{o}C, and being agitated on a shaker at 60rpm. Equipment and lab space for this experiment were provided by Concordia University of Edmonton (CUE) Department of Biology and Environmental Science.

Treatments and Techniques

Treatment solutions were created, using ampicillin salt from Fisher Scientific, at a concentration of 2000mg/L being 100%, decreasing in a logarithmic scale; 80%, 60%, 40%, 20%, 10%, 5%, 1%, 0% (negative control) and a NaCl (100mg in 50ml Sterile distilled water)

positive control at a concentration of 2g/L. All treatment solutions were created using sterile distilled water.

Toxicity tests were performed on the three freshwater algae species individually, using 96-well plates. One well would contain 200uL of the test solution, 10ul of 100% BBMM, and 10ul of algae inoculums. The algae inoculums were recounted and diluted down to ~250,000 cells/mL to result in ~2500cells within one well. Mixed cultures treatments were performed in 6 well plates, allowing the treatments wells to be counted prior and after treatments were finished. To do this the ratios were established to match the ratios created within the smaller 96-well plates. These ratios resulted in 3.6mL of treatment solution, 0.2mL of 100% BBMM, and 0.2mL of algae inoculums at a concentration of ~4.5million cells/mL.

Growth inhibition was measured after 24, 48, and 72hrs using a Bio-rad 96-well plate reader at an absorbance of 415nm for the 96 well plates, and a multi-sample spectrophotometer for the 6 well plates, plates were agitated for 20 seconds for both plate types before readings. In total there were 8 replicates of 96 well plates for each individual species and 3 replicates for the mixed culture, each with 4 wells of each treatment performed, along with 6 replicates of 6 well plates for the mixed culture. The 6 well plates were also counted using a hemocytometer prior to being inoculated, and once the 72hr treatment period had concluded, treatments were fixed using a standing refrigerator, cooling down the solutions to help prevent microalgae growth. As a side note, all three species were tested using the 6 well plates as well, to observe the difference in growth in the different well sizes, 2 replicates each. The cultures used were in growth phase ~7-14 days after initial inoculation. This experiment was carried out between January 1st – March 24th 2016.

Analysis

Results were anazlyzed looking for the change in average absorbances between 24hrs and 72 hrs of exposure, to do this effectively the data was put on a log scale (base of 10^1), along with a single factor ANOVA being done to find statistical significance on the averages of the absorbances in relation to the concentration of treatments over time, in both the 96 well treatments and the 6 well treatments. Change in absorbance directly correlates to growth inhibition in of microalgae present in a well. In the 6 well treatments the cell densities were calculated as well, and cell count allowed for growth inhibition calculations to be conducted. Slope of the trendlines were used to assess the sensitivity among the microalgae. This effectively allows growth inhibition and sensitivity to ampicillin to be observed.

Results

After allowing the cultures of microalgae in different concentrations of ampicillin and NaCl as a positive control to incubate for 72hrs, results were collected and then able to be analyzed. To do this analysis was separated into three sections; 96 well plates, 6 well plates and cell densities in 6 well plates.

96 Well Plates

In figure 1, *Chlorella spp.* showed no distinct trends of change in absorbances were seen, this suggests that the ampicillin has no noticeable effect on the species ability to grow, and is verified by the slope having a -0.0043 value, reflecting 0 change in slope. The averages of absorbances over time was found to be statistically significant using a single factor ANOVA , $F(2,27,0.05) = 3.35, p = 0.015$. This suggests that there was a significant difference in the between

the concentrations of absorbances over time. Figure 2, shows that *Pseudokirchneriella subcapitata* exhibits positive growth as treatment concentrations increase. This was confirmed by the slope value of 1.4361 a positive slope, showing increase in absorbance as concentrations of ampicillin increase after 72 hrs of exposure. Data was again significant F(2,27,0.05)= 3.35,p=0.018. *Scenedesmus spp.* shown in figure 3, showed that as concentrations of ampicillin increased that the absorbances decreased at a rate of -0.2683, this suggests that it is the most sensitive of the algae, F(2,27,0.053.35,p<0.05. A mixed culture was also tests, in figure 4 that all three species show sensitivity to ampicillin as the concentration increased being most prevalent at 100%, F(2,27,0.05) =3.35,p=0.76 .

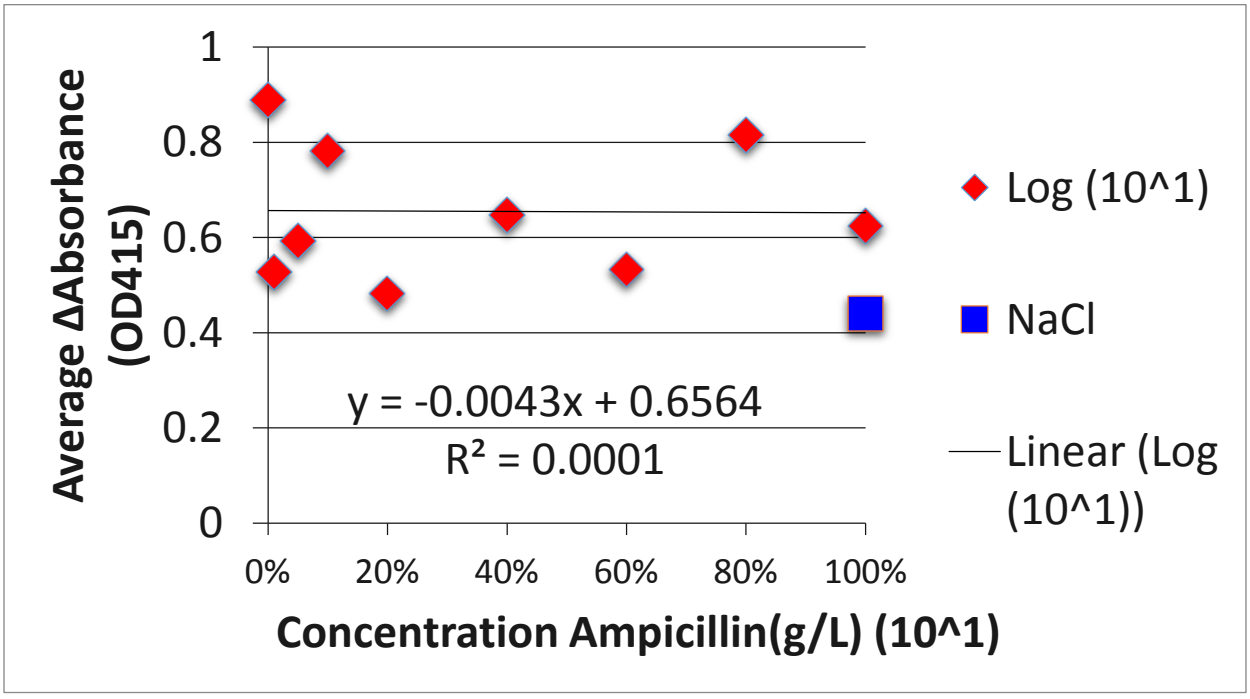

Figure 1. Average Absorbance (OD415) of *Chlorella spp.*to the Log (10^1) in relation to the concentration of ampicillin in 96 well plates, F (2,27,0.05) =3.35,p=0.015.

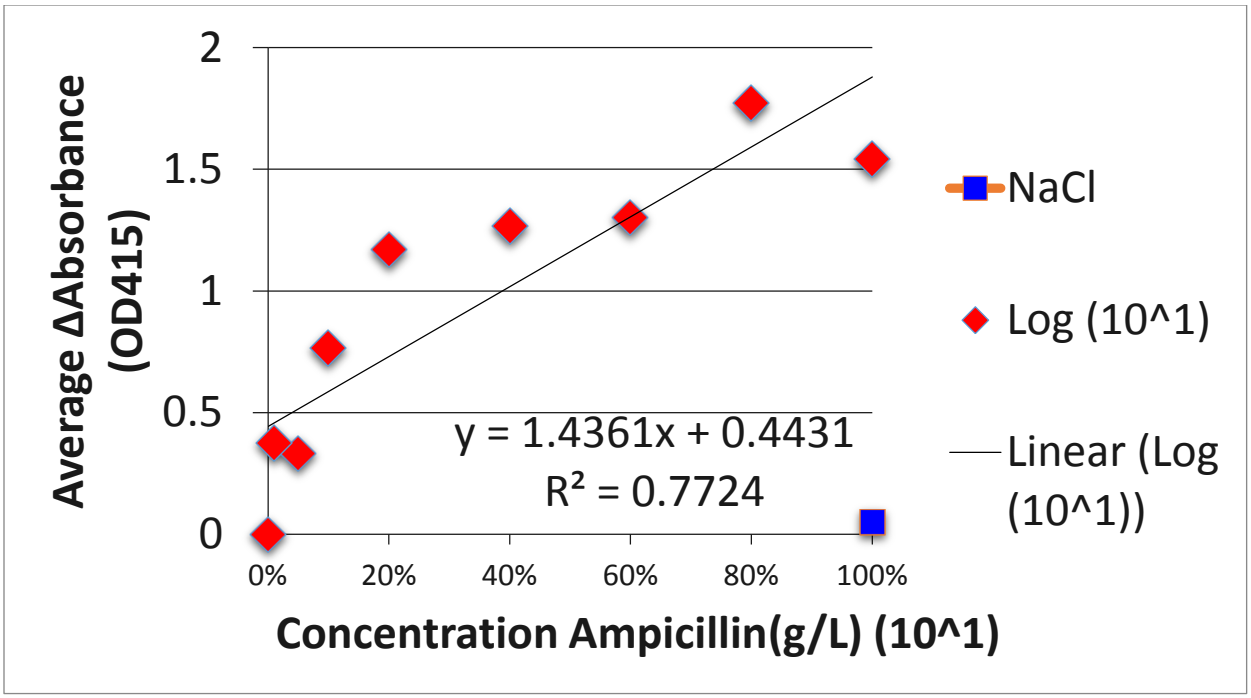

Figure 2. Average ΔAbsorbance (OD415) of *Pseudokirchneriella subcapitata* to the Log (10^1) in relation to the concentration of ampicillin in 96 well plates, F (2,27,0.05)= 3.35,p=0.018.

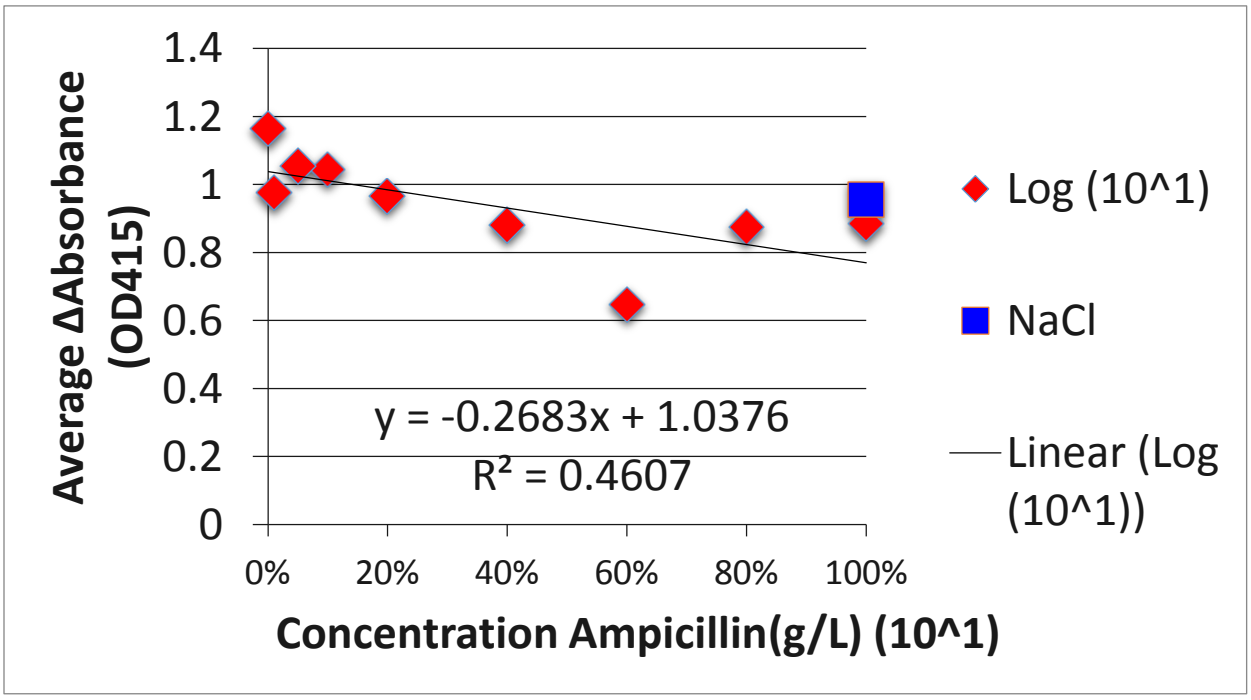

Figure 3. Average ΔAbsorbance (OD415) of *Scenedesmus spp*. to the Log (10^1) in relation

to the concentration of ampicillin in 96 well plates, F (2,27,0.05) 3.35,p<0.05.

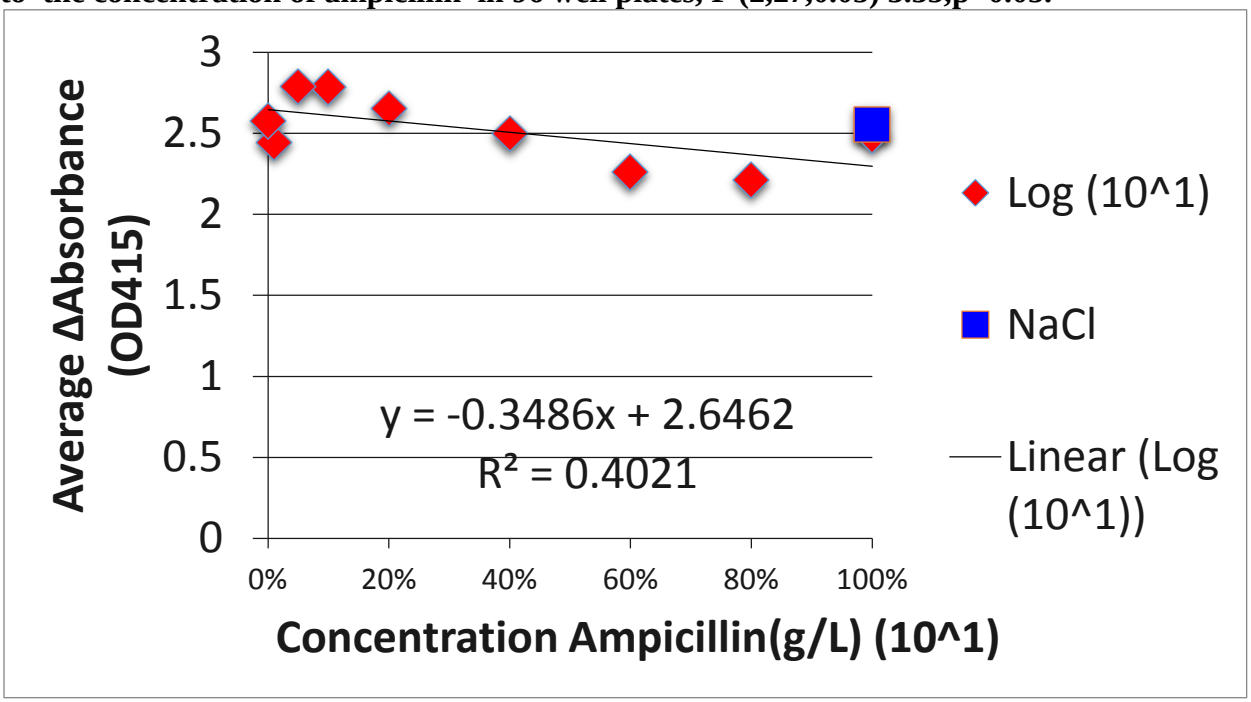

Figure 4. Average ΔAbsorbance (OD415) of mixed species to the Log (10^1) in relation to the concentration of ampicillin in 96 well plates, F (2,27,0.05) =3.35,p=0.76 .

6 Well Plates

Figure 5, shows *Chlorella spp* 's average change in absorbance after 72hrs of exposure in different concentrations of ampicillin, this log graph shows that as concentration increases the change in absorbance decreases, this indicates that the growth of *Chlorella* is inhibited by ampicillin. The slope being the highest, rate value so far for sensitivity and the data was found to be statistically significant , F (2,27,0.05) =3.35, p=0.00056. In figure 6, shows similar trends but for *Pseudokirchneriella subcapitata*, but is much less sensitive overall to ampicillin, F (2,27,0.05)= 3.35, p=<0.05. Trends continure in figure 7, but *Scenedesmus* shows the least amount of sensitivity within the 6 well plates. The mixed culture experiment showed again that

overall growth was inhibited, at the second highest sensitivity of the tests for the 6 well plate treatments, showing statistical significance as well, F (2,27,0.05) =3.35, p=0.001092.

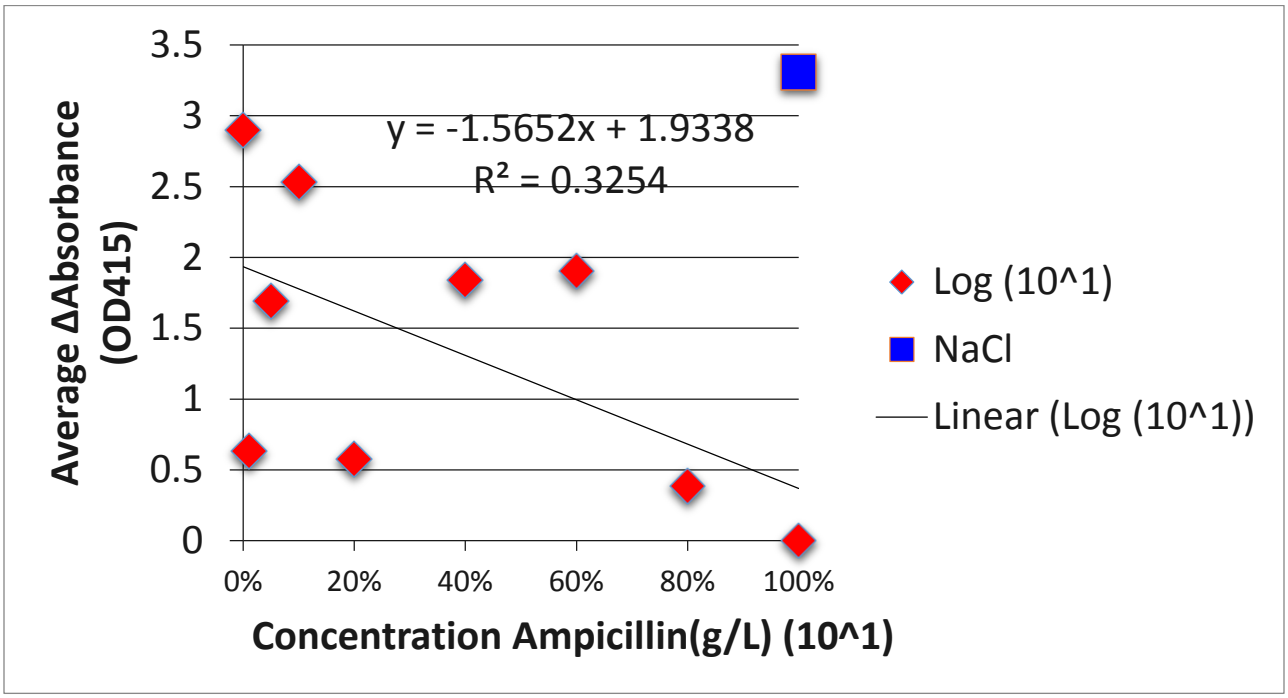

Figure 5. Average ΔAbsorbance (OD415) of *Chlorella spp* to the Log (10^1) in relation to the concentration of ampicillin in 6 well plates, F (2,27,0.05) =3.35, p=0.00056.

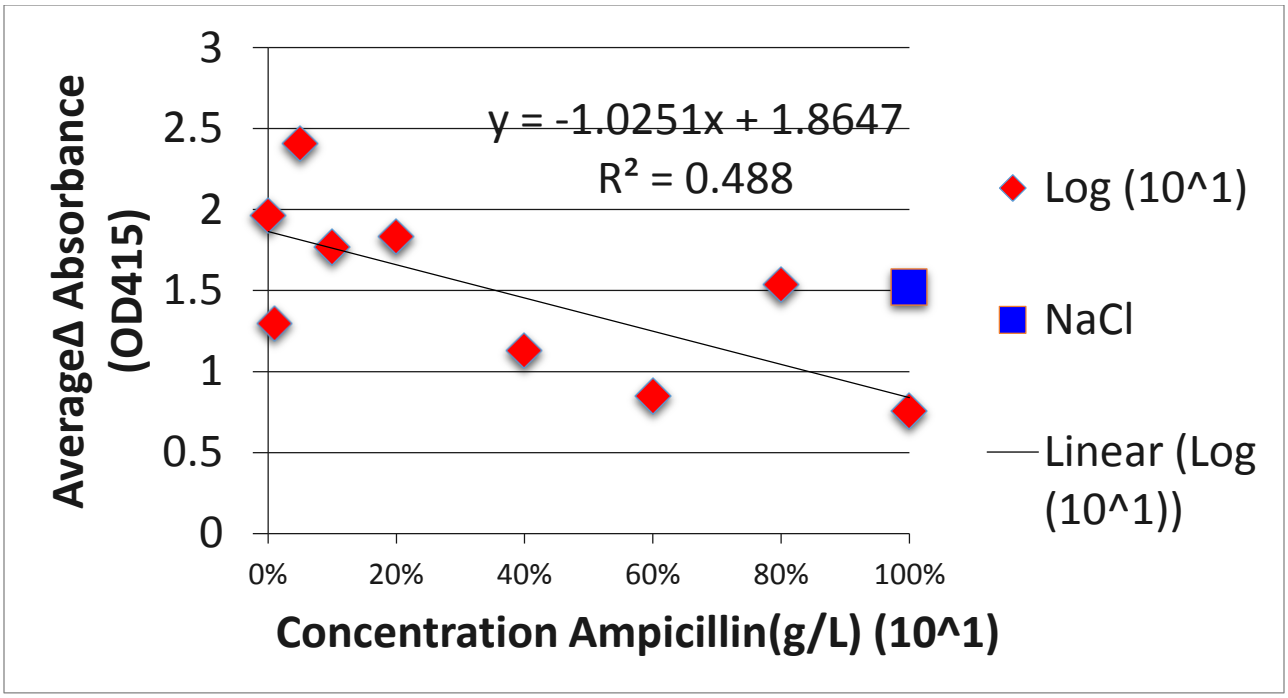

Figure 6. Average ΔAbsorbance (OD415) of *Pseudokirchneriella subcapitata* to the Log (10^1) in relation to the concentration of ampicillin in 6 well plates, F (2,27,0.05)= 3.35, p=<0.05.

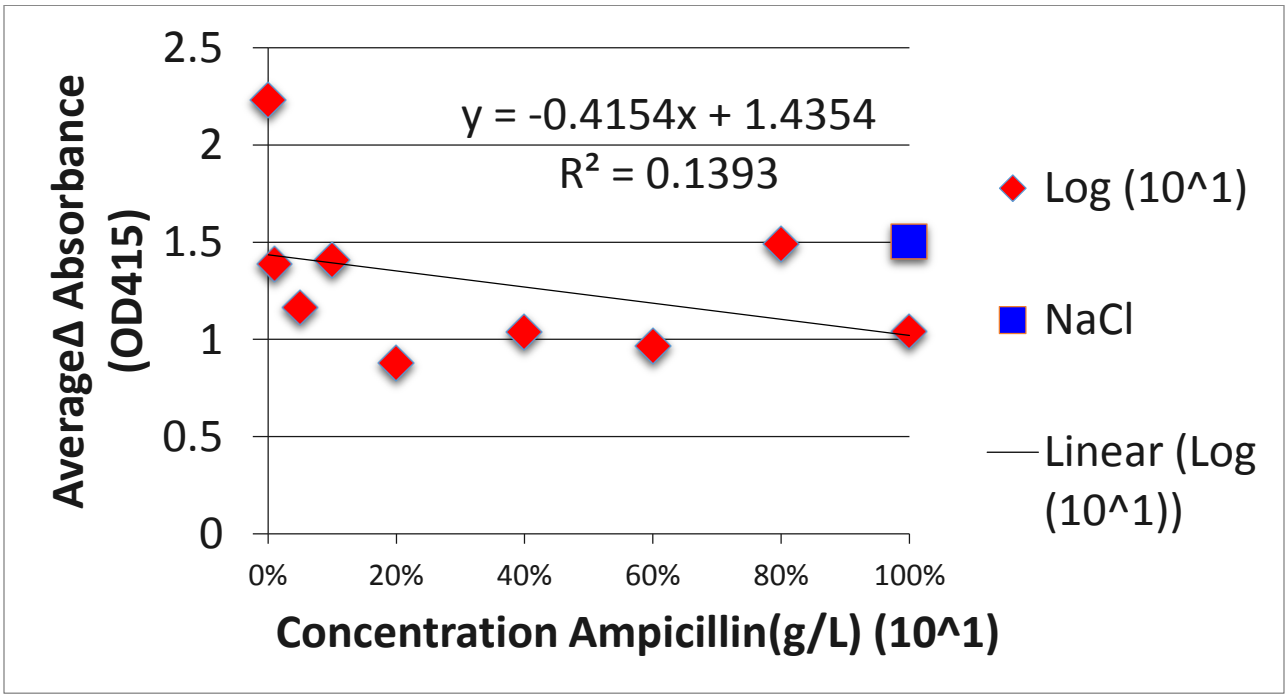

Figure 7. Average ΔAbsorbance (OD415) of *Scenedesmus spp* to the Log (10^1) in relation to the concentration of ampicillin in 6 well plates, F (2,27,0.05) 3.35, p<0.05.

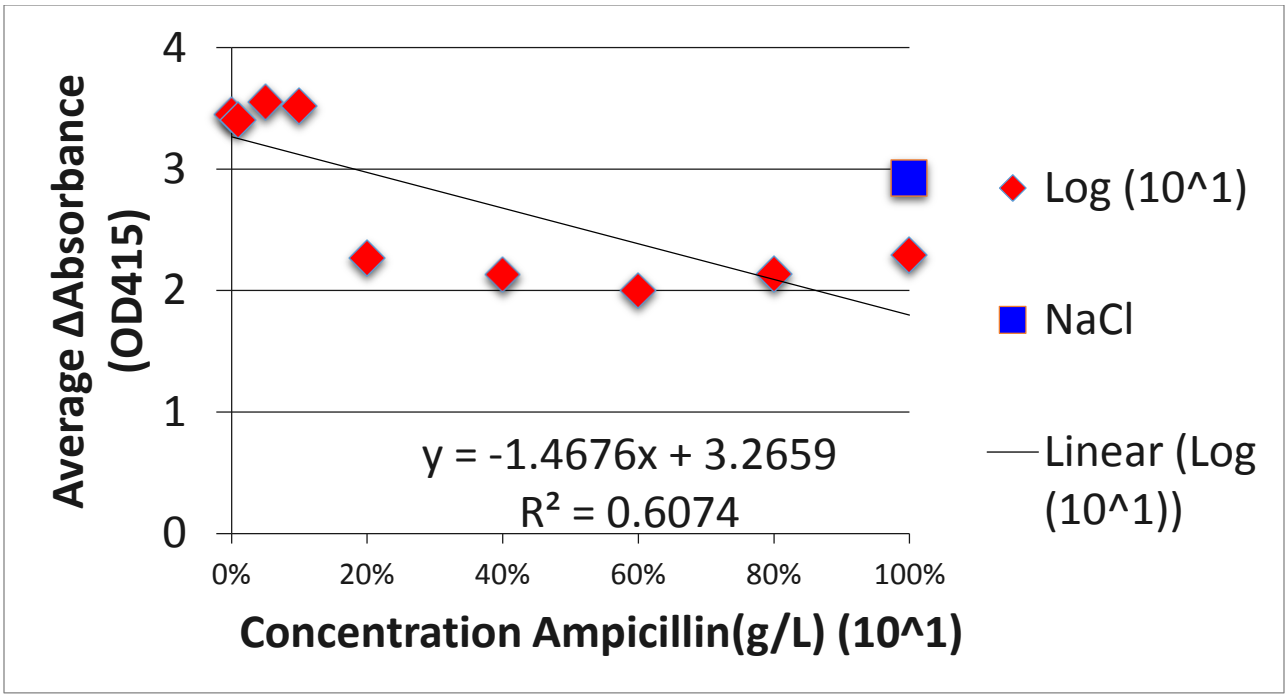

Figure 8. Average ΔAbsorbance (OD415) of mixed algae to the Log (10^1) in relation to the concentration of ampicillin in 6 well plates, F (2,27,0.05) =3.35, p=0.001092.

Cell Densities in 6 Well Plates

Using the previous 6 well plate treatments cell densities were counted using a hemocytometer chambers. These counts were performed initially at 0 hrs of exposure and at 72hrs of exposure, then were averaged. In figure 9, a) and b) represent the same data but allow the trends to be viewed differently. Figure 9. a) shows the overall cell densities of the community within their well, allowing the population trends to be viewed together. From this data we can see that the initial community cell densities at equilibrium had *Pseudokirchneriella subcapitata* as the most prominent species, and the *Scenedesmus* and *Chlorella spp's* representing a small part of the community. After 72hrs the community establishes a new equilibrium that has *Chlorella* become the most prominent species. As concentrations increase all species decrease in cell densities, and *Scenedesmus* seems most affected. When we observe figure 9.b) we see that

Psuedokirchneriella subcapitata is actually the most sensitive species, but maintains large presence within the culture, and that *Chlorella* has a similar sensitivity to *Scenedesmus* but does remains more prominent in the culture. These results are confirmed by individual growth inhibition found in figure 9,c) showing that any positive value represents inhibition in a species relative to concentration of ampicillin. Also being confirmed in the by the individual species trails in figure 10,a) and 10,b).

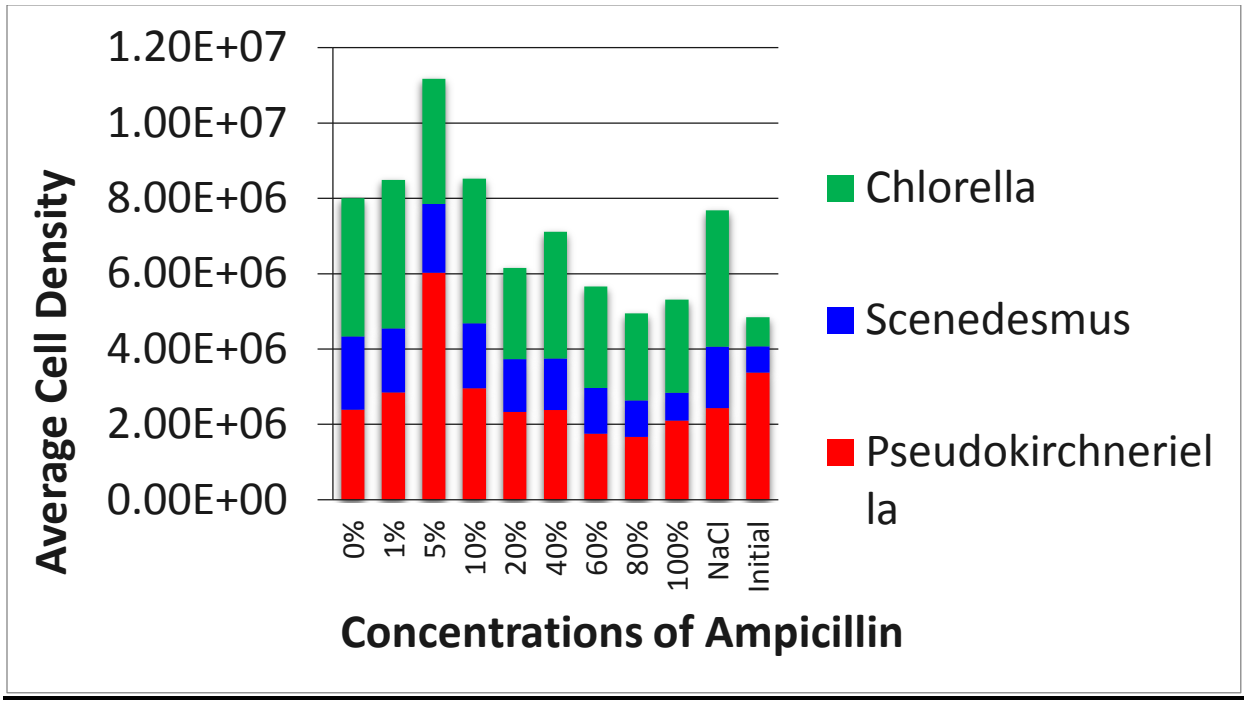

Figure 9.a) Mixed 6 well plate, cultures and the population dynamics of different species cell densities in varying concentrations of ampicillin after 72 hrs, with initial 0hr of exposure counts for comparison, F(9,20,0.05)=2.39,p=0.47. A decrease of 2.69E+06cells.

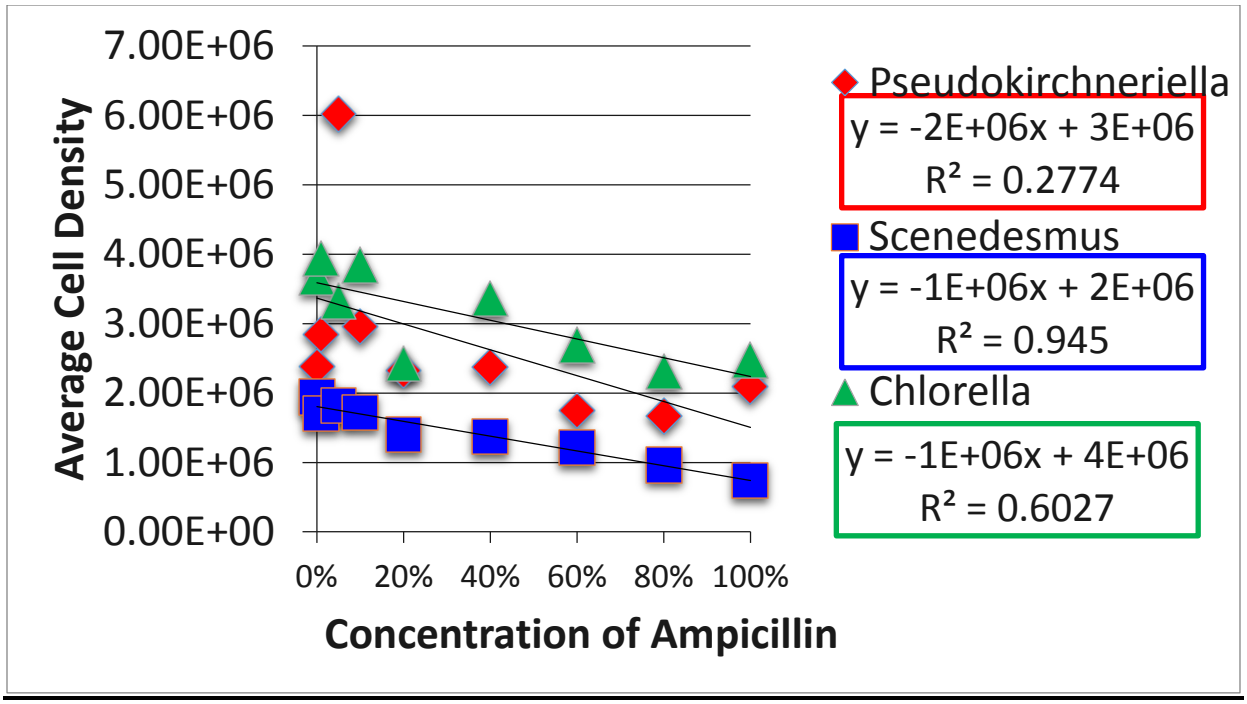

Figure 9.b) Mixed 6 well plate, cultures and the population trends of different species cell densities in varying concentrations of ampicillin after 72 hrs, F(9,20,0.05)=2.39,p=0.47.

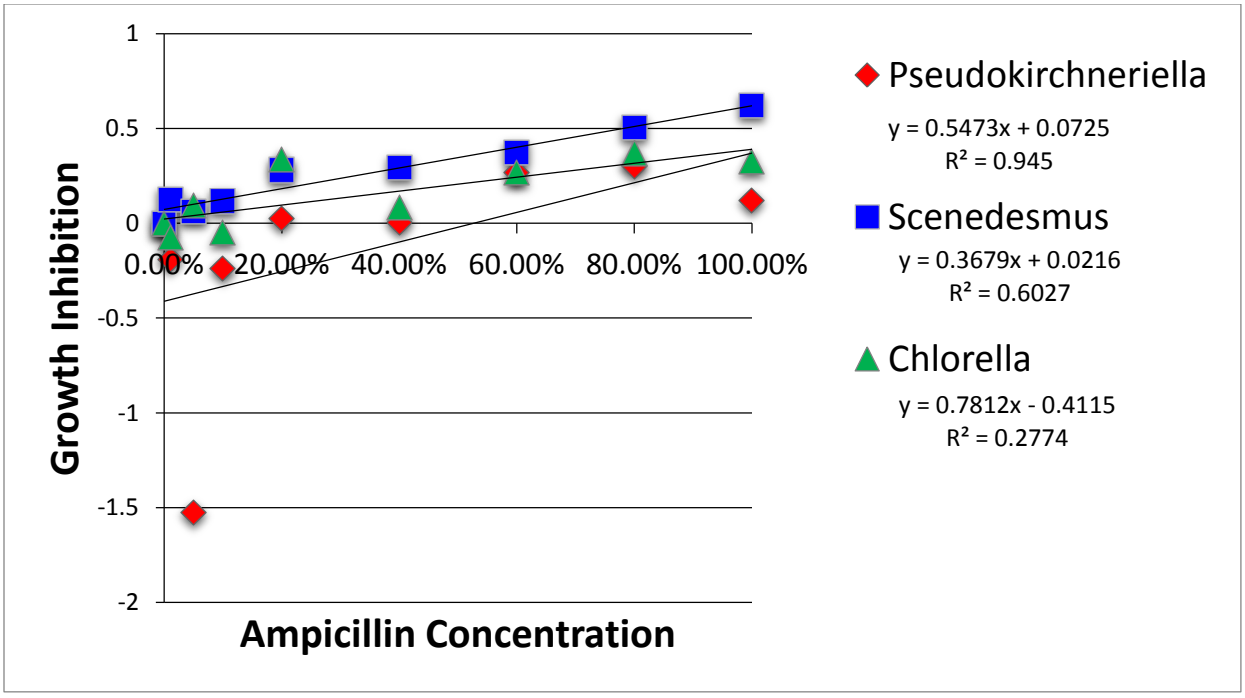

Figure 9.c) Mixed 6 well plate, population growth inhibition per species in varying concentrations of ampicillin after 72 hrs, F(8,18,0.05)=2.51,p=0.13.

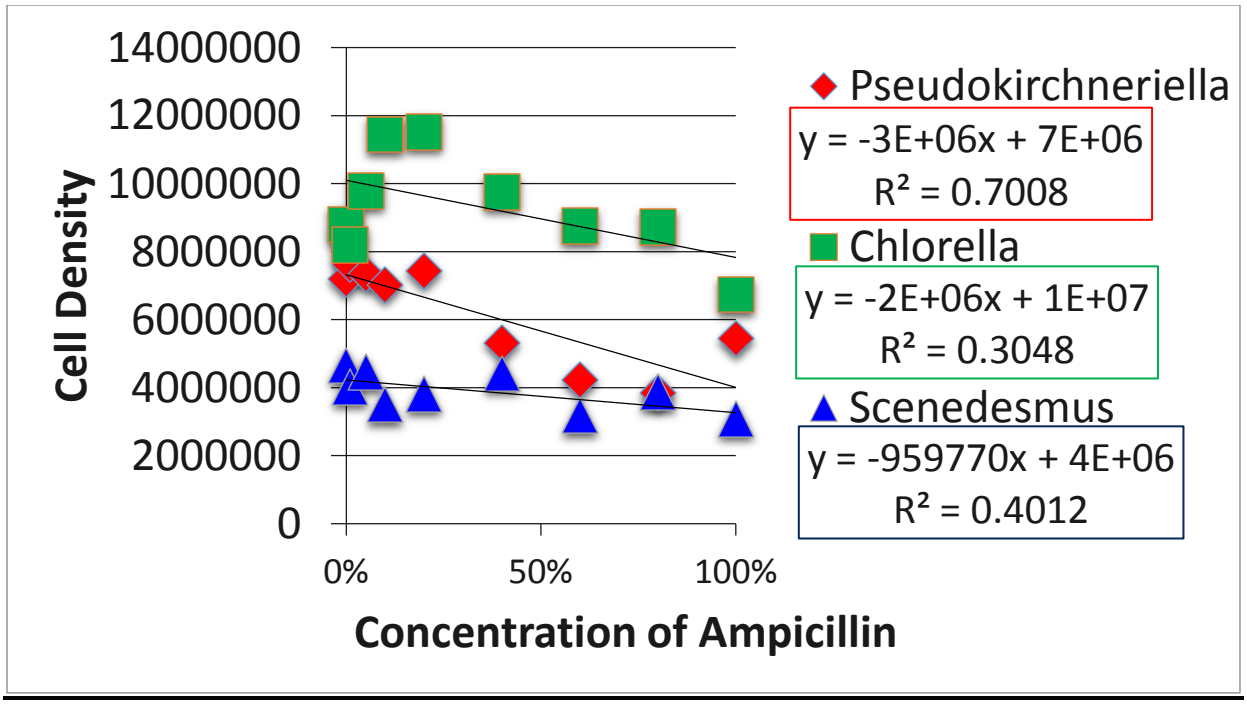

Figure 10,a). Individual microalgae cultures cell densities in varying concentrations of ampicillin after 72 hrs, F(9,20,0.05)=2.39,p=0.967.

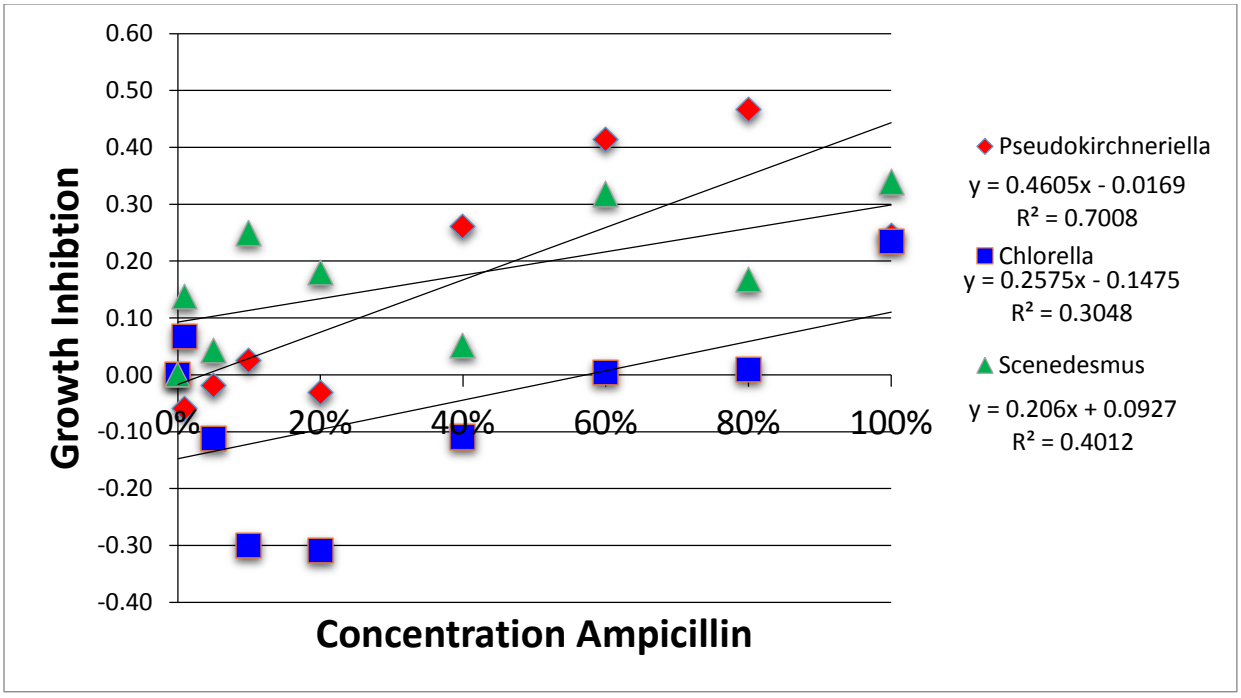

Figure 10,b) Individual microalgae cultures growth inhibition in varying concentrations of ampicillin after 72 hrs, F(8,18,0.05)=2.51,p=0.13.

Discussion

The purpose of this experiment was to observe the possible effect of ampicillin on *Pseudokirchneriella subcapitata, Chlorella spp.,* and *Scenedesmus spp,* both individually and in a community to assess possible ecological effects.

In the 96 well plate cultures there was a variety of results, *Chlorella spp* (figure 1) seems to suggest that ampicillin would have no effect on the algae, and shows no sensitivity. While *Pseudokirchneriella subcapitata* showed positive growth, by having an overall increase in absorbance at 100% ampicillin. *Scenedesmus spp,* was the only microalgae to exhibit growth inhibition as concentrations increase. The positive control provided stable results showing either no change correlating to inhibition or slightly above the 100% treatment. And finally the mixed culture showed growth inhibition with all species present, and was the most sensitive exhibiting the largest slope, all three had statistically significance.

This suggests that there is no observable effect concentration on *Chlorella*. *Chlorella* is known to be a hardy microalgae that can replicate quickly, this would potentially allow the species to have maintained a constant growth in all concentrations. Potential building a tolerance to the ampicillin within the well. *Pseudokirchneriella subcapitata* (figure 2) however showed positive growth, which would suggest that the *Pseudokirchneriella subcapitata* was able to develop a tolerance to the antibiotic. Previous experiments have shown the ability of many microalgae to acclimate to the toxic environment their in and adjust to, such as copper (Bossuyt and Janssen 2004), or even other pharmaceutical mixtures. It is also possible that the microalgae species were effectively able to biodegrade the ampicillin. Considering ampicillin is biodegradable in the environment and has a fragile structure (Hirsch et al 1999).

Scenedesmus (figure 3) and the mixed culture (figure 4) both showed growth inhibition, this suggests that *Scenedesmus* is sensitive to the ampicillin at higher concentration, showing decreased change in absorbance at 40% concentration, while as the mixed culture decreased absorbance was visualized at the 60 % concentration. This suggests that it is possible that as a community there would be a much larger effect on all the algae species, resulting in the overall decline of cells. The mixed community effect however was not statistically significant, suggesting it might not be that reliable.

The 6 well plate trail provided slightly different results, showing growth inhibition in all individual culture, and all of them being significant (figure 5,6,7). This suggests that the previous 96 well plate trail did not provide the most reliable results and that the 96 well plate read might have been faulty, but this is not likely so because all well plates were randomized an specific trends were witnessed. In the 6 well trials, the *Chlorella* had the largest slope, suggesting it was the most sensitive species, and right behind that was the mixed trial (figure 8) showing that a community effect is occurring. This is further supported by the cell counts.

In figure 9,a) the initial equilibrium community dynamics are seen, this shows that *Pseudokirchneriella subcapitata* is the dominate species, and makes up over 50% of the community structure, once grown in the well plate the dynamics shifted, creating a new equilibrium. This new community was quite a bit smaller than the previous 50mL culture that was already established. As concentrations of ampicillin increase the total cell density in a well decreases, but *Chlorella* has effectively created more competition, leaving less resources for the other species to survive. Ampicillin is also present and seems to be having a larger effect on *Scenedesmus*, than other species. Figure 9,b) shows the trends of each population, showing that *Pseudokirchneriella subcapitata* is actually the most affected by ampicillin, confirmed by the

individual treatment plates in figure 10,a. Growth inhibition (figure 9,c) however showed the true inhibition by concentration showing that *Scenedesmus* is inhibited in the mixed and individual cultures at concentrations as low as 1%, this could be caused by crowding within the lower concentrations. *Chlorella* showed growth inhibition at concentrations of 60% in the mixed culture and individually. While *Pseudokirchneriella subcapitata* showed inhibition at 60% in the mixed culture but 30% for the lowest observable effect for the individual culture. These results indicate that provided ampicillin is present in the aquatic environment at concentrations of 1000mg/L then there could be potential effects on the composition of microalgae.

Currently literature has mixed results suggesting that ampicillin has little to no effect. Magdaleno et al (2015) found that ampicillin even at 2000mg/L had no effect on green algae *Chlorella vulgaris* and *Pseudokirchneriella subcapitata*. While Kviderova and Henley (2005) found that ampicillin and streptomycin have only a slight effect on growth rate, and a significant inhibition effect on photosynthesis, in one species but not another. That being said, β-lactams have been known to cause chloroplast irregularities in higher species of plants such as moss, and liverwort (Kasten and Reski 1997; Matsumoto et al, 2012). But is typically caused by pepitidoglycan presence within the plastid walls, hence allowing the ampicillin to influence the plant cell. This is most likely not the case for why ampicillin caused growth inhibition within *Pseudokirchneriella subcapitata, Chlorella spp.,* and *Scenedesmus spp.*

Considering most studies do not observe for the overall ecological effect (Roose-Amsaleg and Laverman 2016). The change in population dynamics caused by ampicillin is hard to veryify, considering population dynamics can be influenced by so many other external factors. Considering the algae were grown in optimal conditions we can assume they would try to maximize growth. Supported by the changes seen in figure 9.a). They could have also been

inhibited by crowding in the environment and intra/interspecific competition and changing ecosystem stability (Ding and He 2010).

Conclusion

From these results we can conlude that ampicillin does cause growth inhibition in *Pseudokirchneriella subcapitata, Chlorella spp.,* and *Scenedesmus spp,* in both individual populations and mixed population. Population dynamics can result in many different ways, it is possible that the 96-well plates, population dynamics were altered due to the size of the wells, the community grew in. This indicates that the effects of ampicillin might be more prevalent to community structure on a larger scale, and that the ampicillin can influence the competitive advantage of microalgae within a community, possibly leading to shifts in the community structure. Further studies should be performed on mixed communities of aquatic organisms, to develop a possible model to observe possible effects of antibiotics on individual species and ecosystems.

Acknowledgments

I would like to thank both Dr. Sheri Dalton and Dr. Xin Chen for providing amazing support, guidance and supervision in analysis, design and follow through. Thank you to both Ms. Emily Pollock and Mr. Devin Hughes for direction and guidance of the lab equipment, for allowing the use of the lab space and support. I would also like to thank the Department of Biological and Environmental Sciences for providing funding, lab space and equipment which without would not have led to the completion and success of this project. The Department and Institute have been very supportive and have provided excellent learning opportunities.

References:

Bossuyt B, Janssen C. 2004. Long-term acclimation of Pseudokirchneriella subcapitata (Korshikov) Hindak to different copper concentrations: changes in tolerance and physiology. Aquatic Toxicology. 68(1):61-74.

Cole J. 1982. Interactions between bacteria and algae in aquatic ecosystems. Ann Re. Ecol. Syst. 13: 291-314.

Crane M, Watts C, Boucard T. 2006. Chronic aquatic environmental risks from exposure to human pharmaceuticals. Science of the Total Environment. 367: 23-41.

Ding C, He J. 2010. Effect of antibiotics in the environment on microbial populations. 87: 925-941.

[EC] Environment Canada. 2007. Biological Test Method: Growth Inhibition Test Using a Freshwater Alga. Canada (Ottawa): Environment Canada; [Accessed Feburary 12, 2016]. https://www.ec.gc.ca/faunescience-wildlifescience/AFA6529F-02C5-429C-A507-C2CF6EADEB0A/RM25%202nded-AlgaeEnglish.pdf

Fent K, Weston A, Caminada D. 2006. Ecotoxicology of human pharmaceuticals. Aquatic Toxicology. 76:122-159.

Halling-Sorensen B. 2000. Algal toxicity of antibacterial agents used in intensive farming. Chemosphere. 40:731-739.

Hirsch R, Ternes T, Haberer K, Ludwig Kratz K. 1999. Occurrence of antibiotics in the aquatic environment. The Science of the Total Environment. 225: 109-118.

Kasten B, Reski R. 1997. β-Lactam antibiotics inhibit chloroplast division in a moss (Physcomitrella patens) but not in tomato (Lycopersicon esculentum). J Plant Physiol. 150: 137-140

Kemper N. 2008. Veterinary antibiotics in the aquatic and terrestrial environment. Ecological Indicators.8:1-13.

Lampert W, Rothhaupt K, Elert E. 1994. Chemical induction of colony formation in green alga (*Scenedesmus acutus*) by grazers (*Daphnia*). Limnol. Oceanogr. 39(7):1543-1550.

Lee Y, Lee S, Lee D, Kim Y. 2008. Risk assessment of human antibiotics in Korean aquatic environment. Environmental Toxicology and Pharmacology. 26: 216-221.

Liu B, Nie X, Liu W, Snoeijs P, Guan C, Tsui M. 2011. Toxic effects of erythromycin, ciprofloxacin and sulfamethoxazole on photosynthetic apparatus in *Selenastrum capricornutum*. Ecotoxicology and Environmental Safety. 74: 1027-1035.

Magdaleno A, Saenz M, Juarez A, Moretton J. 2015. Effects of six antibiotics and their binary mixtures on growth of pseudokirchneriella subcapitata. Ecotoxicology and Environmental Safety. 113: 72-78.

Matsumoto G, Takechi K, Sato H, Takio S, Takano H. 2012. Treatment with Antibiotics that Interfere with Peptidoglycan Biosynthesis Inhibits Chloroplast Division in the Desmid Closterium. PLoS ONE. 7(7): 1-6.

Papageorgiou M, Kosma C, Lambropoulou D. 2016. Seasonal occurrence, removal, mass loading and environmental risk assessment of 55 pharmaceuticals and personal care products in a municipal wastewater treatment plant in Central Greece. Science of the Total Environment. 543: 547-569.

Porter K. 1977. The Plant-Animal Interface in Freshwater Ecosystems: Microscopic grazers feed differentially on planktonic algae and can influence their community structure and succession in ways that are analogous to the effects of herbivores on terrestrial plant communities. American Scientist. 65 (2): 159-170.

Ramanan R, Kim B, Cho D, Oh H, Kim H. 2016. Algae-bacteria interactions: Evolution, ecology, and emerging applications. Biotechnology Advances 34: 14-29.

Richardson M, Bowron J. 1985. The fate of pharmaceutical chemicals in the aquatic environment. J. Pharm. Pharmacol. 37 : 1-12.

Roose-Amsaleg C, Laverman A. 2016. Do antibiotics have environmental side-effects? Impact of synthetic antibiotics on biogeochemical processes. Environ Sci Pollut Res. 23: 4000-4012.

Spoehr H. 1976. The Need for a New Source of Food. In: Algal Culture: From Laboratory to Pilot Plant. Burlew John, editor. Carnegie Institution of Washington Publication. 4th ed. Washington (D.C.) p. 24-40.

Sun Q, Lv M, Hu A, Yang X, Yu C. Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in a wastewater treatment plant in Xiamen, China. Journal of Hazardous Materials. 277: 69-75.

Trainor F. 1996. Reproduction in *Scenedesmus*. Algae. 11(2): 183-201.

Wang Z, Nie X, Yue W. 2011. Toxicological effects of cypermethrin to marine phytoplankton in a co-culture system under laboratory conditions. Ecotoxicology. 20: 1258-1267.

Kviderova J, Henley W. 2005. The effect of ampicillin plus streptomycin on growth and photosynthesis of two halotolerant chlorophyte algae.Journal of Applied Phycology. 17: 301-307.