The Effect of *Spirulina major* and *Oscillatoria* on Reducing the Acidity of Freshwater Containing Antibacterial Liquid and Bar Soaps .

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Personal care products are major pollutants in freshwater environments that are often difficult to mediate due to their fluid-like properties. Hand and bar soaps often cause the most pollution in freshwater environments due to their pH level, which often jeopardizes the livelihood of surrounding wildlife. With the prospect of algae such as *Spirulina major* and *Oscillatoria* as potential viable sources of bioremediation, the toxic pH level and effect of hand and bar soaps can possibly be reduced. The purpose of the research is to test the effectiveness of *Spirulina major* and *Oscillatoria* in reducing the pH level of personal care products (PPCPs) in freshwater environments. The hypothesis is that the algae *Spirulina* and *Oscillatoria* will reduce the pH level of a Dial Gold Antibacterial Bar Soap and Clorox Antibacterial Liquid Hand Soap mixture by 2 pH levels given that algae has been effective in reducing the pH level of personal care products in marine environments. The method of the research included four groups, two of which belonged to *Spirulina* and *Oscillatoria*, and the other two as control groups. Both algae were separated into 20 mg samples, and the pH level of all samples were recorded every day for ten days and averaged for each day. The research finds that both the *Spirulina major* and *Oscillatoria* groups became more acidic through an 19.9% and 48.83% increase in pH, respectively. The results of the research suggest that the algae did not aid in reducing the pH level of the PPCPs.

Introduction

In recent years, algae has been proven as a viable and significant option for reducing pollutants in aquatic environments. Not only is algae an environmentally-friendly method of bioremediation, the introduction of algae into environments is often non-invasive and conducive to the livelihood of the wildlife around it. Algae's impact on reducing pollutants has been most evident in wastewater, industrial wastewater, and saltwater environments (Pacheo et al., 2020). Understanding algae's role in reducing pollutants can play a role in gaining insight into environmentally friendly ways of reducing harmful pollutants such as personal care products, or PPCPs. PPCPs as pollutants in natural environments are often found in areas of high human population, and are often difficult to mediate due to their liquid-like properties. With the rise of algae as a potential widespread source of environmental remediation, it is important to view its effect on reducing the influence of PPCPs on natural environments.

In regards to aquatic environments, Imran Ahmad, Norhayati Abdullah, Imran Koji, Ahmad Yuzir, and S.E. Mohammad of BCREC in Malaysia found that algae has proven successful in reducing and even degrading pollutants such as plastics, heavy metals, pharmaceuticals, and personal care products, otherwise known as PPCPs (2021). Furthermore, algae successfully indoctrinate themselves into environments through their quick growth, which is a result of high absorption of light to conduct photosynthesis (Ahmad et al., 2021). Ahmad et al. (2021) explain that algae degrades solids like plastics through the enzymes that are released by the algae themselves, using the polymers of the plastics as a source of carbon, which aids the production of the enzymes released. Algae successfully reduces the acidity and toxicity of heavy metals and pharmaceuticals in aquatic environments through physical adsorption, in which the cell surface of the algae opens up to essentially consume the heavy metal or pharmaceutical (Dwivedi, 2012). Dwivedi (2012) explains that algae tends to reduce almost 90% of heavy metal and plastic pollutants, and significantly reduces the toxicity and acidity of pharmaceuticals as pollutants in the environment. More specifically, algae like *Spirulina major* and Oscillatoria have been deemed effective in reducing the impact that plastics, heavy metals, and pharmaceuticals have on freshwater ecosystems (Ahmad et al., 2021).

Currently, gaps in knowledge exist in regards to the effect of personal care products (PPCPs) in the environment, much less in freshwater environments. The potential implications of PPCPs in the environment poses a threat to the livelihood of the organisms in the environment; research conducted by Manganelli (2015) indicates that the presence alone of PPCPs in aquatic environments can adversely affect aquatic wildlife. Both *Spirulina major* and *Oscillatoria* have potential in reducing the acidity of PPCPs in freshwater environments given their effectiveness in reducing the acidity of heavy metals in natural environments (Ahmad et al., 2021). *Spirulina* effectively detoxifies heavy metals such as copper, chlorine, and fluoride that are detrimental to human health and the environment, while Oscillatoria can reduce the acidity of heavy metals like cadmium up to 60% (Dwivedi, 2012). Evidently, the impact that both *Spirulina major* and *Oscillatoria* have in reducing acidity of pollutants can play a major role in reducing the acidity of PPCPs.

Essential PPCPs used by humans in everyday life, such as bar soaps and liquid soaps, pose significant danger to aquatic wildlife due to their inorganic composition and acidity (Manganelli, 2015). Additionally, with the rise of new innovative PPCPs coined as "antibacterial," it is important to view how algae can possibly reduce the acidity of these different and new pollutants. The acidity of antibacterial bar soaps such as Dial or Dettol ranges from 4-6 pH, which is acidic compared to the pH level of freshwater environments where the average pH is around 9-10 pH (Jett, 2021). The acidity of antibacterial liquid soaps, such as Clorox antibacterial liquid hand soap is often 5-6 pH, which is once again quite acidic compared to the pH of freshwater environments (Jett, 2021). These soaps are viable PPCPs in freshwater environments, and can disrupt wildlife given their high acidity level in respect to the environment that they can pollute.

The purpose of the research is to view the effectiveness of algae in reducing the acidity of personal care products as pollutants in freshwater environments. Exploring algae's role in bioremediation as well as algae's role in bioremediation in regards to reducing the acidity of personal care products is vital to reducing pollution and gaining insight on viable bioremediation options. The hypothesis is that the algae will reduce the pH by two levels in the antibacterial bar soap and liquid soap mixture over the span of 10 days, thus being a viable source of bioremediation in freshwater environments in regards to reducing PPCPs. Samples will be divided into four groups; one group consisting of samples with *Spirulina major* and another group consisting of samples with *Oscillatoria*, both of which have an even .5 g mixture of Dial Gold Antibacterial Bar Soap and Clorox Antibacterial Liquid Hand Soap. These two groups will contain 20 mg of algae in each sample. The other two groups are the control groups, one group with freshwater and soaps and the other group with only freshwater. All four groups will have lake freshwater in each sample cup, and will be measured for acidity every day for 10 days.

Methods

Samples were divided into four groups, group 1 belonging to *Spirulina major*, group 2 belonging to *Oscillatoria* and group 3 and 4 as control variables. Both of the experimental groups had 20 mg of algae in each of the samples. 20 mg of algae in the experimental groups was decided due to a lack of samples of *Oscillatoria*. The amount of 20 mg would allow for there to be a significant amount of data points for *Oscillatoria*; as a result, this became a constant amount in each sample for both algae to be able to better compare both algae. The algae were separated through a process the vials were placed in a weigh boat and divided into 20 mg among all of the samples. This restriction would yield 16 samples in the *Oscillatoria* group, and 36 samples in the *Spirulina major* group.

All of the samples in groups requiring soaps contained an even .50 g mixture of both the Dial Gold Antibacterial Bar Soap and Clorox Antibacterial Liquid Hand Soap. The group that did not require soaps was the control group that measured the acidity of only the freshwater. The even mixture of the bar and liquid soap remained constant throughout all samples of the research in all groups except the one control group that only measured the acidity of freshwater. These control groups were made to measure the acidity of the given freshwater environment as well as the freshwater environment with the soaps and no intervening algae.

All four sample groups contained .5 ounces of lake freshwater in each sample cup and were measured for acidity every day for 10 days. The acidity was measured for each sample in every group using a pH meter. The average acidity of each group was subsequently transcribed onto the tables on the respective day. Safety considerations taken into account were the inhalation or possible consumption of the algae. To reduce the risk of these hazards, masks were worn at all times and hands were washed before and after the experimental set up and data collection.

Results

Figure 2 displays the average pH of each group over ten days. This information displays the trends of each group over the ten day period of data collection.

Table 1 displays the mean acidity of each of the groups including *Spirulina major* and *Oscillatoria* as well as the control groups on day one compared to day ten. The percent change was subsequently calculated using these values.

The following changes were observed from day one to day ten: a 19.90% decrease for *Spirulina major*, a 48.83% decrease for *Oscillatoria*, a 4.59 increase for the control group with soaps, and lastly a 0.68% increase for the control group with only freshwater.

The mean, range, and standard deviation of the groups are represented through the data in Table 2.

The means of the data represent the average pH level of each group over the span of the ten days; the *Spirulina major* group has a mean of 9.02, the *Oscillatoria* group has a mean of 8.119, the control group with soaps has a mean pH level of 9.442, and the control group with only freshwater has a mean of 8.829. Evidently, *Oscillatoria* has more variation in average acidity over the 10 day span, whereas *Spirulina major* had significantly lower variability in average acidities. This is indicative of the strength/impact that both algae had on their samples respectively.

The data shown in Table 3 rejects the null hypothesis that the means of observations in the groups are the same, when they instead are different along with the other data presented.

The p-value of the *Spirulina major* group was 0.004019, and the p-value of the *Oscillatoria* group was 0.09985. Evidently, the data of *Spirulina major* was significant due to it being less than the alpha value of 0.05. On the contrary, the data of *Oscillatoria* is not significant due to its p-value being significantly higher than the alpha value of 0.05.

Discussion

The purpose of the research was to test the effectiveness of *Spirulina major* and *Oscillatoria* in reducing the pH level of personal care products (PPCPs) in freshwater environments. It was hypothesized that *Spirulina* and *Oscillatoria* would increase the pH level of Dial Gold Antibacterial Bar Soap and Clorox Antibacterial Liquid Hand Soap by making the samples more alkaline by 2 pH levels given that algae had been effective in reducing the acidic pH levels of personal care products in marine environments. However, the results of the research do not support the hypothesis, as the pH levels of the samples with algae became increasingly acidic compared to the pH level of a regular freshwater environment.

Figure 2 accurately shows the trend of each group's pH level over the ten day period. In this figure, it is apparent that the pH levels of both algae continued to trend downwards instead of becoming similar to the pH level of the regular freshwater environment, or even to that of the freshwater environment with soaps. Compared to *Spirulina major*, *Oscillatoria* is seen to have a more significant decrease in pH level, which is notable considering the minimal difference between both algae in regards to their role in bioremediation evident through previous literature.

The information presented in Table 1 accurately demonstrates the effect that 20 mg of *Spirulina major* and *Oscillatoria* had on the pH level of the samples over the span of ten days through the percent change. There is a 19.90% increase in acidity for the *Spirulina major* group and a 48.83% increase in acidity for the *Oscillatoria* group. Evidently, both algae had minimal impact on the acidities of their samples, as both algae made significantly drastic changes from the acidity of a regular freshwater environment, which is normally around 7-8 pH. Evidently, *Oscillatoria* made a more significant decrease in regards to the average pH level over the 10-day span compared to *Spirulina*.

Table 2 accurately shows the behavior of the data for each group over the course of ten days. Most notably, the standard deviation of the groups show that *Spirulina major* had low standard deviation and *Oscillatoria* had high standard deviation. This shows the difference in pH levels for both algae; *Oscillatoria* had more drastic changes in its data compared to *Spirulina major*. This is vital to take into consideration, as it shows how both algae are different in reaction to the same environment.

Table 3 shows the differences between both the *Spirulina major* and *Oscillatoria* groups. Though the sum of squares for both groups are relatively similar, the p-values are different, which is notable in understanding the behavior of both groups' data. At 0.09985, the p-value of *Oscillatoria* is significantly higher than the value of *Spirulina major*, which indicates that the data collected within the *Oscillatoria* group is not significant. This is due to the p-value being significantly higher than the alpha value, which is 0.05. On the contrary, the data within the *Spirulina major* group is significant due to its p-value being 0.004019, which is less than the alpha value of 0.05.

The primary purpose of the research was to test *Spirulina major* and *Oscillatoria* as possible sources of reducing pollution from PPCPs, specifically soaps in freshwater environments. The research conducted indicates that the algae cannot be a possible source of reducing PPCP pollution if it is overpowered by the PPCP present in the environment. Additionally, the prospect of algae used in environmental bioremediation still needs to be heavily researched upon for viability as well as feasibility in the real world.

Ahmad et al. (2021) explains that algae still needs time to be studied before it is used as an extensive and viable source of bioremediation. Additionally, there are issues in regards to algae being used to degrade pollutants such as PPCPs in water. Once again, this is due to algae not being extensively researched as a potential source of remediation in natural environments as a whole (Ahmad et al., 2021). However, Dwivedi (2012) explains that algae's simple cultivation can lead to sustainable environments, which means that algae could be a viable and widespread source of bioremediation if extensively researched. On the contrary, the simple cultivation of algae could lead to algae not being properly used, thus causing inverse effects on the environment (Dwivedi 2012).

Manganelli (2016) states pollutants can oftentimes overpower the natural bioremediation tactics set in place if the pollutants accumulate at an extremely high rate. Research conducted by Manganelli (2016) displayed altering amounts of algae with a constant level of heavy metal mixture. The degradation of the heavy metal mixture in the research was proportional to the amount of algae that was introduced into each group. Similarly, patterns most likely occurred in this research as well. Due to the unprecedented lack of algae growth, each of the samples for both algae groups received 20 mg of algae compared to the .5 g mixture of Dial Gold Antibacterial Bar Soap and Clorox Antibacterial Liquid Hand Soap, thus being significantly overpowered by the PPCPs. This significant difference could have contributed to the lack of accurate data in this research.

Furthermore, Jett (2021) explains that more research needs to be done in regards to the impact of PPCPs on wildlife in general before bioremediation methods are implemented. Jett (2021) cites that the main bulk of information regarding the impact of PPCPs in the natural environment surrounds its impact on the human life surrounding the natural environment. This magnified lack of information in regards to the impact of PPCPs on wildlife further obscures the potential effectiveness of algae as a source of bioremediation as well as the correlation between PPCPs and algae.

Additionally, Boxall et al. (2012) explain that algae is often used in bioremediation at industrial wastewater sites or biorefineries, where there is no concern for external factors. Furthermore, research conducted by Boxall et al. (2012) significantly implicates the effectiveness of the algae in degrading heavy metals with the lack of wildlife disturbance on the cultivation of algae. This conclusion can jeopardize the feasibility and effectiveness of algae should they be implicated into freshwater ecosystems, as they can potentially not be effective in reducing pollutants such as PPCPs.

These conclusions magnify the infancy of algae in bioremediation as a whole. Algae as a source of bioremediation has not been researched enough to conclude its significant impact in reducing pollutants in the natural environment. Additionally, the lack of concrete information on the effect of PPCPs on wildlife obscures its validity as a potential pollutant that can be bioremediated. With these conclusions in mind, it is easy to say that algae has potential in some form of pollutant reduction, but tough to say how applicable it can be to reducing PPCPs as well as the natural environment in the near future.

The biggest setback in the research was due to a severe lack of algae growth in the weeks leading up to the ten day span of research. Despite placing the vials of algae under light, they did not grow significantly. As a result, the algae used in the research was a very small amount and did not truly represent the potential impact of algae in a natural environment with a pollutant. This could be improved next time through meticulous observation of algae growth to ensure large enough samples.

To further explore algae's potential bioremediation role, it would be important for scientists to view algae's applicability to the natural environment. Most literature as of date has viewed the effect of algae in reducing pollutants in controlled environments, if not controlled marine environments. Additionally, more research surrounding the impact of PPCPs as pollutants on wildlife rather than human life is important to understand the environmental danger of PPCPs. Putting the impact of PPCPs in perspective of environmental pollutants as a whole is important in prioritizing the fight against pollution. In general, it is important for scientists to understand and pursue algae as a source of bioremediation, given its promising potential in the literature that displays its impact on reducing pollutants.

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Tables and Figures

Figure 1. *Experimental Design Diagram*

Title of the Experiment
The Effect of *Spirulina major* and *Oscillatoria* on Reducing the Acidity of Freshwater containing Antibacterial Liquid and Bar Soaps

Hypothesis
Spirulina major and *Oscillatoria* will reduce the acidity of the .50 g mixture by 2 pH levels given that algae has been effective in reducing the acidity of personal care products in marine environments.

Independent Variables
Spirulina major (mg)
Oscillatoria (mg)

Levels of Independent Variable	***Spirulina (20 mg)***	***Oscillatoria (20 mg)***
Number of Repeated Trials	*16*	*36*

Dependent Variable
Acidity of freshwater environment (pH)

Control Groups
Freshwater with soaps
Freshwater (negative control)

Constants
Temperature of water
Temperature of setting samples are in
Source of water
Sample cup type/size
Type of pH meter
Amount of light each sample receives

Figure 2. *Average pH level of every group over 10-Day span*

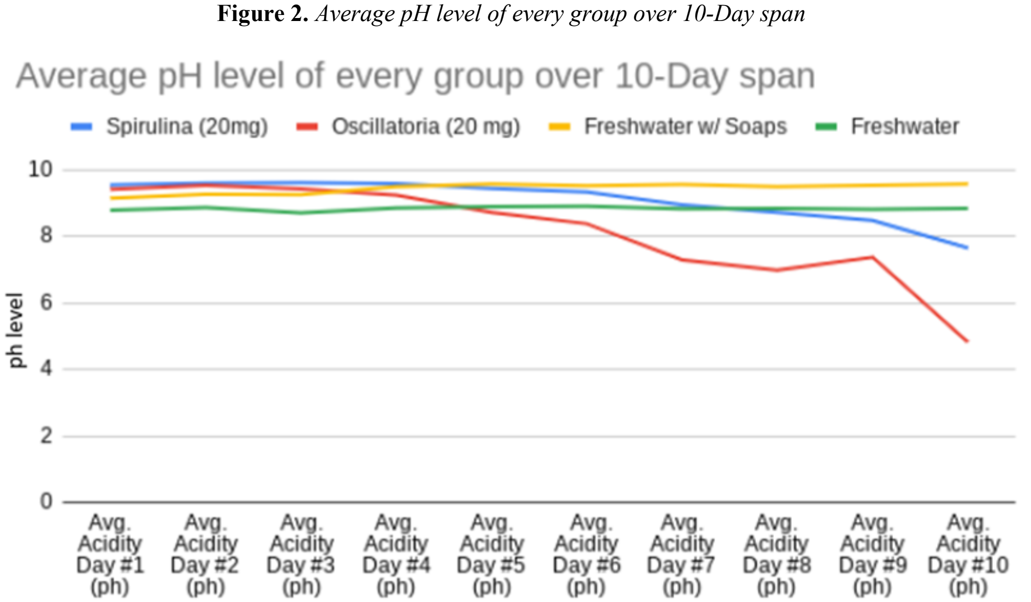

Figure 2 displays the general trends of the pH level as well as the average pH level of each day for the groups which contributes to their overall means.

Table 1. *The Effect of Algae on the Acidity of Freshwater Environments Contaminated by Soaps*

	Spirulina major	*Oscillatoria*	Control (freshwater with soaps)	Control (freshwater)
Mean acidity of group on day #1 (pH)	9.55	9.42	9.15	8.78
Mean acidity of group on day #10 (pH)	7.65	4.82	9.57	8.84
% change in acidity	-19.90%	-48.83%	4.59%	0.68%

Table 1 displays the mean pH level on day one and ten for each group as well as the percent change calculated using $\frac{(day\ \#10\ acidity\ -\ day\ \#1\ acidity)}{day\ \#1\ acidity}\ x\ 100$

Table 2. *Mean, Range, and Standard Deviation of pH levels of the Groups (pH)*

	Spirulina major	*Oscillatoria*	Control (freshwater with soaps)	Control (freshwater)
M	9.02	8.119	9.442	8.829
Range	1.96	4.72	0.43	0.2
SD	0.297	1.51	0.157	0.0576

Table 2 displays the mean, range, and standard deviation of the pH levels of each group.

Table 3. *Two-way ANOVA Summary Table (a = 0.05)*

Source	*DF*	*SS*	*MS*	*F Statistic (df1, df2)*	*p-value*
Spirulina major	3	9.4215	3.1405	5.6081 (3,27)	0.004019
Oscillatoria	9	9.4504	1.05	1.8751 (9,27)	0.09985
Error	27	15.1199	0.56		
Total	39	33.9918	0.8716		

Table 3 displays the values calculated during a two-factor ANOVA.

APPENDIX

Table A1. *Average pH level of all groups over 10 days; Raw Data*

Day #	*Spirulina major**	*Oscillatoria*9*	Control (freshwater with soaps)	Control (freshwater
1	9.55	9.42	9.15	8.78
2	9.6	9.54	9.27	8.86
3	9.61	9.43	9.25	8.7
4	9.59	9.24	9.5	8.85
5	9.44	8.72	9.58	8.89
6	9.33	8.38	9.52	8.9
7	8.95	7.29	9.56	8.82
8	8.72	6.98	9.49	8.84
9	8.48	7.37	9.53	8.81
10	7.65	4.82	9.57	8.84

* Includes .50 mg of Dial Gold Antibacterial Bar Soap and Clorox Antibacterial Liquid Hand Soap as Depen Variable