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The influence of anthropogenic pollutants on filamentous green algae, a vital bioindicator of freshwater ecosystem health

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Abstract

Freshwater algae are necessary and important living organisms contributing to the ecological and biological cycle of aquatic ecosystems. Nitrogen and phosphorus play a significant role in the growth of freshwater algae, as they use and thrive on these nutrients. Anthropogenic pollution is a major threat to aquatic biodiversity and sustainability because of its nutrient and chemical composition. Exposure to pollutants containing high levels of nitrogen and phosphorus can result in rapid algal growth, which blankets the surface waters and limits oxygen availability for other organisms. Chlorophyll-*a* abundance is a well-known method used to measure true algal growth in the form of photosynthetic output. We measured the chlorophyll-*a* abundance of freshwater, filamentous algae, and water quality when exposed to different pollutants: motor oil, sunscreen, insecticide, and shampoo. Algae samples were individually treated with 1 mL of pollutant, and their chlorophyll-*a* abundance was measured before and after 0.1N HCl acidification. Mean chlorophyll-*a* abundance was highest in motor oil (4,006.13 $\mu g\ g^{-1}$) and lowest in shampoo (272.114 $\mu g\ g^{-1}$). Results for both nitrate and phosphate levels showed no significant association between treatment type and water quality ($P > 0.05$). While most research focuses on pollution effects in marine habitats, freshwater habitats are especially vulnerable to pollution due to close proximity to human activity and their use as direct sources of potable water. The protection of freshwater ecosystems from anthropogenic pollution warrants further research on algae-pollutant effects, with access to freshwater resources essential for the continued sustainability of human populations.

Keywords: anthropogenic activity, chlorophyll-a, bioindicator, eutrophication, photosynthesis

Introduction

Human activity is one of the largest and most impactful sources of pollution in natural ecosystems (Rhind, 2009). Anthropogenic pollution is an environmental threat affecting all types of ecosystems and the diverse organisms living within them. Such pollution is introduced to these systems by both natural and artificial processes in the form of geochemical cycles, flooding, stormwater runoff, industrial effluent, and littering (Céréghino *et al.,* 2007; Schmeller *et al.,* 2018). Most research on anthropogenic pollution focuses on marine habitats, even though freshwater habitats are more likely to become contaminated due to their accessibility, closer proximity to heavily populated urban cities and their agricultural and industrial runoff, and smaller volumes of water (Fu-Liu 1999; Zhang *et al.,* 2011). The protection of freshwater ecosystems from anthropogenic pollution is necessary for maintaining aquatic biodiversity, ecosystem health, and access to natural resources such as freshwater animals and drinking or irrigation water sources (Schmeller *et al.,* 2018). Continued loss of freshwater habitats and biodiversity would have major consequences on human health, survival, and sustainability as access to natural resources dwindles due to an ever-growing human population and the resulting increase in pollution rates (Fu-Liu, 1999; Zhang *et al.,* 2011). To preserve the health of and prevent the disappearance of freshwater habitats, active biomonitoring of these aquatic systems must be conducted to identify the locations most in need of environmental management.

Filamentous algae are primary producers, important organisms contributing to the ecological, biological, and chemical processes of aquatic ecosystems by providing the necessary foundation for sustainable freshwater food webs (Zhang *et al.,* 2011; Vadeboncoeur & Power, 2017). One method of measuring aquatic ecosystem health involves using the presence of algae as bioindicators of overall habitat quality (Lourenço *et al.,* 2019). As bioindicators, the absence or abundance of freshwater algae can be used to determine whether a body of water has been polluted (Lourenço *et al.,* 2019) and can indicate the need for immediate action to protect endangered habitats and organisms. In conditions of high algal concentration, dead zones and eutrophication can occur, resulting in the loss of biodiversity through fluctuating water conditions and loss of oxygen and light availability important for the process of photosynthesis (Yao *et al.,* 2019). Algal concentrations are heavily impacted by the nutrient composition of the aquatic body and any pollutants that may be contributing to excess nutrient availability. The anthropogenic pollutants of particular concern are those containing high levels of nitrogen and phosphorus, which are direct catalysts for harmful algal blooms. These algal blooms involve rapid growth which blankets the surface waters and reduces nutrient availability for other organisms living within the same habitat. Thus, further research is needed to understand the ecological impacts of different anthropogenic pollutant types and compositions on freshwater algae and its chlorophyll-*a* abundance (Dou *et al.,* 2019; Eullaffroy & Vernet, 2003). Chlorophyll-*a* is a major blue-green pigment essential for photosynthetic ability and the survival of algal organisms (Hosikian *et al.,* 2010). The measurement of chlorophyll-*a* is an effective method used to quantify algae biomass through its oxygenic photosynthesis output (*APHA,* 1999; Hosikian *et al.,* 2010).

In this research project, the abundance of chlorophyll-*a* pigment and the nutrient composition of freshwater algae were used as an indication of pollution and impact in freshwater ecosystems. The biological questions of most interest included: 1) does anthropogenic pollution impact freshwater algae growth, 2) are the nitrogen and phosphorus levels in the freshwater affected by the presence of anthropogenic pollutants, and 3) is there an association between the freshwater algae abundance and water quality measured? We aimed to observe and quantify the impact of various anthropogenic pollutants (insecticide, motor oil, sunscreen, and shampoo) on freshwater algae growth (*i.e.,* chlorophyll-*a* abundance), to determine whether nitrogen and phosphorus levels of the freshwater change before and after contamination occurs, and to establish whether there is a relationship between chlorophyll-*a* abundance and the resulting water quality measured. These specific pollutants were chosen based on their contrasting nutrient compositions and polluting aspects: agricultural use, vehicular use, human recreational activity, and wastewater pollution from home activity, respectively. Insecticides, often used in conjunction with fertilizers by farmers (*e.g.,* agrochemicals), may readily contaminate nearby freshwater bodies in the form of unmanaged agricultural runoff, and are thus expected to have the highest growth compared to motor oil, sunscreen, and shampoo. Wijewardene *et al.,* 2021 demonstrates variation in the effects of pesticides on freshwater phytoplankton, with the resulting negative or positive impacts dependent on whether nutrient-rich fertilizers were used simultaneously. As such, these algal-pollutant interactions are often complex and may involve exposure to different pollutants and habitat conditions, such as variation in temperatures, invasive species, and *etc.,* within a single freshwater ecosystem.

Environmental transport and exposure of diverse, anthropogenic pollutants can occur across the rural-urban interface, resulting in an additive or synergistic toxicity to nearby freshwater systems. This study is focused on providing a baseline understanding of varying pollutant types and their individual impacts on freshwater algae upon exposure. Farmers often apply a mixture of chemicals upon cultivated cropland to reduce crop loss via pests and weeds, while also improving crop quality (*e.g.*, nutrient-rich fertilizers used in conjunction with insecticides). The observed effects (*e.g.,* additive, synergistic, or antagonistic) of different combinations of pollutants can be better understood or explained by first evaluating the toxicity of each pollutant separately. For example, without the addition of fertilizers or other nutrient-rich pollutants, the introduction of insecticides to freshwater algae is expected to be a limiting factor on chlorophyll-*a* abundance because of the lack of nutrient enrichment needed for eutrophication and excessive algal abundance to transpire (Wijewardene *et al.,* 2021). These research findings will help identify the respective risks of specific anthropogenic pollutants and the association between freshwater algae abundance and water quality. By contributing to the overall understanding of anthropogenic pollution on freshwater filamentous algae, we can better identify, monitor, and prevent the loss of our natural freshwater habitats and organisms to human-induced eutrophication.

Methods

Site selection and algal sampling

A single field collection of filamentous, freshwater algae was conducted at an easily accessible koi pond located at California State University, Bakersfield (35.3487° N, 119.1033° W; Fig. 1) in October of 2019. The presence of filamentous green algae was confirmed macroscopically and broadly identified based on known characteristics, with a large mat of green threadlike algae collected from an area preferentially free of organic debris and away from a nearby pond aerator. Similar composition and environmental conditions for all samples were maintained by immediately transferring the collected algae into small containers of off-gassed tap water to prevent desiccation and cell death from prolonged exposure to dry conditions. All algal samples were collected, processed, and treated on the same day. The experiment was kept on the dry side (*i.e.,* no sprinklers) of the greenhouse on campus with ambient lighting and a day and night cycle to simulate the natural conditions experienced in its freshwater ecosystem.

Figure 1: The koi pond site at California State University, Bakersfield (CSUB) used for the sample collection of filamentous algae

Algal and pollutant treatments

The experiment occurred during a 3-week period, where observations were made on the appearance of the algae and water twice a week. Containers (500 mL) for the experiment were labeled on the bottom with a sticker indicating type and treatment number. Treatments were replicated and named after the type of pollutant used:

motor oil, sunscreen, control (no pollutant added), insecticide, and shampoo (Brands: Pennzoil© SAE 5W-30, Coppertone© Sunscreen Ultra Guard 50 SPF Lotion, ECOSMART© Organic Ant & Roach Killer Insecticide Spray, and V05© Extra Body Volumizing Shampoo with Collagen; *n* = 8 containers/treatment; Table 1, Fig. 2). Tap water was outgassed of chlorine for 24-hrs and used to fill containers with 250 mL of water. The collected algae were cleaned by hand of all visible organic debris (*e.g.,* pine needles and flowers from nearby trees) and lightly pat-dried with paper towels. The cleaned, lightly-dried algae were divided into 1 g per container, and 1 mL of pollutant (concentrated) was added immediately after using a disposable pipette. The control treatment contained only algae (1 g) and outgassed tap water (250 mL), with no added pollutant. Water quality of the tap water, specifically nitrate and phosphate, was tested before the addition of algae and at the end of the experiment using freshwater quality strips and a phosphate liquid tester. An additional 1 g of the collected algae was set aside and kept in a dark container filled with 100 mL of 90% absolute ethanol solution for chlorophyll-*a* analysis; extraction of chlorophyll-*a* occurred for 24-hrs at 4ºC and was transferred to the freezer until ready for absorbance analysis using a spectrophotometer (*APHA,* 2019).

Pollutant Type	**Brand**	**Composition/Ingredients**	**Ingredient Source**
Motor Oil	Pennzoil© SAE 5W-30	Ingredients: highly refined mineral oils (contains <3% dimethyl sulphoxide (DMSO) extract) and additives (zinc alkyl dithiophosphate)	MSDS No. 11501, Ver. 1.1
Sunscreen	Coppertone© Sunscreen Ultra Guard 50 SPF Lotion	Active: Avobenzone (3%), Homosalate (13%), Octisalate (5%), Octocrylene (7%), Oxybenzone (4%) Other: Water, Sorbitol, Aluminum Starch Octenylsuccinate, VP/Eicosene Copolymer, Stearic Acid, Triethanolamine, Sorbitan Isostearate, Benzyl Alcohol, Dimethicone, Tocopherol (Vitamin E), Polyglyceryl-3 Distearate, Fragrance, Methylparaben, Carbomer, Propylparaben, Disodium ethylenediaminetetraacetic acid (EDTA)	Container Label
Insecticide	ECOSMART© Organic Ant & Roach Killer Insecticide Spray	Active: Rosemary Oil (5%), Cinnamon Oil (3%), (Water, Wintergreen Oil, Mineral Oil, Oleic Acid, Canola Oil, Nitrogen, Vanillin, Lecithin - 92%)	Container Label
Shampoo	V05© Extra Body Volumizing Shampoo with Collagen	Ingredients: Water (Aqua, Eau), Sodium Lauryl Sulfate, Cocamidopropyl Betaine, Ammonium Chloride, Sodium Laureth Sulfate, Fragrance (Parfum), Hydrolyzed Collagen, DMDM Hydantoin, Citric Acid, Tetrasodium EDTA, Benzyl Salicylate, Butylphenyl Methylpropional, Linalool, Yellow 5 (Cl 19140), Red 40 (Cl 16035), Sodium Chloride, Polysorbate 20, Blue 1 (Cl 42090), Tocopheryl Acetate, Panthenol, Ascorbic Acid, Niacinamide, Biotin.	Container Label

Table 1: A list of the four pollutants used (Pennzoil© SAE 5W-30, Coppertone© Sunscreen Ultra Guard 50 SPF Lotion, ECOSMART© Organic Ant & Roach Killer Insecticide Spray, and V05© Extra Body Volumizing Shampoo with Collagen) and their ingredients (active and/or other) provided via container label or Material Safety Data Sheet (MSDS)

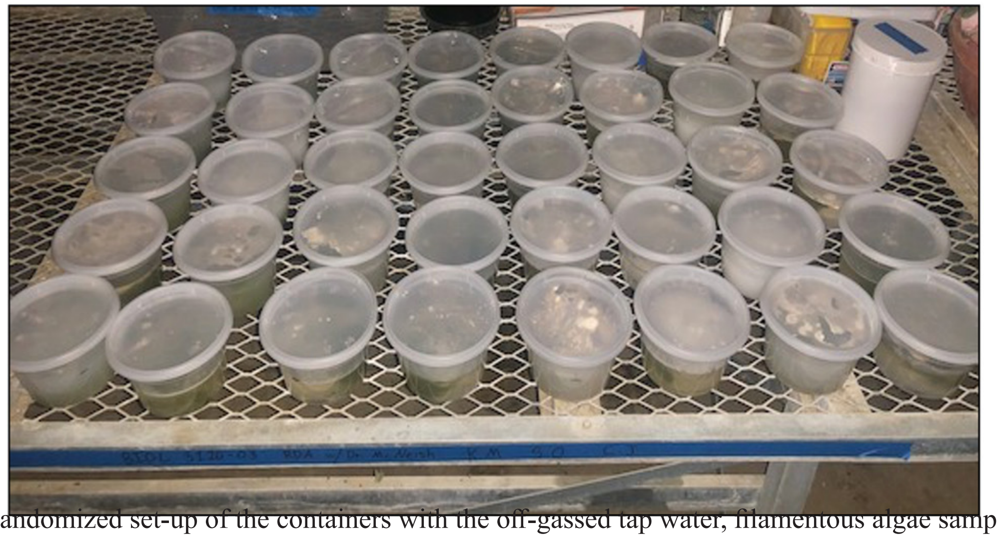

Figure 2: The randomized set-up of the containers with the off-gassed tap water, filamentous algae sample, and 4 pollutant treatments (motor oil, insecticide, sunscreen, and shampoo) kept at the CSUB greenhouse

Isolation of chlorophyll-*a*

During absorbance analysis, lights were dimmed to prevent the excitement of chlorophyll-*a*.

Acidification using 100µl 0.1N HCl was conducted for each algal sample to denature other pigments and to isolate chlorophyll-*a*. Three mL of each algae sample were measured before acidification at wavelengths of 750 nm and 664 nm and after acidification at 750 nm and 665 nm. An additional 3 samples of 1 g of algae were weighed, placed in a drying oven for 24-hrs, re-weighed again, and averaged as the initial dry mass of algae for all treatments. The measurement process of chlorophyll-*a* abundance applied established procedures determined by the APHA (1999) and in Hosikian *et al.,* 2010. This process was repeated for all the algae samples at the end of the experiment.

Controls and limitations

Container placement within the greenhouse was generated randomly, ensuring the treatments were in mixed order to prevent potential environmental bias. The containers were also covered with lids to prevent evaporation and contamination of the water and to allow adequate conditions for any potential algal growth. The volume of pollutants and tap water added, type of container, temperature, and light availability were all kept consistent among treatments to support the credibility and accuracy of our findings. Chlorophyll-*a* abundance (µg g^{-1}) was measured for only three samples of each pollutant due to limited resources, with the calculated means imputed to replace missing chlorophyll-*a* data. The three samples for chlorophyll-*a* analysis were chosen randomly. Nitrate levels (mg L^{-1}) of the water were measured for all containers. Phosphate levels (mg L^{-1}) of the water were measured for three random containers of each pollutant, also due to limited resources, with the calculated means imputed to replace missing phosphate data.

The filamentous green algae were only macroscopically identified (not taxonomically identified) based on known identifiable characteristics such as free-floating, slimy, and stringy or threadlike large mats that cover the surface of freshwater habitats (*e.g.*, pond scum); thus, the presence of specific (filamentous or other) green algal types was not established via microscopy. Additionally, the water quality of the koi pond was not tested at the time of algal collection, which may have provided further insight into the initial quality of the collected algae prior to pollutant exposure. The koi pond was chosen based on proximity and ease of accessibility (*i.e.,* no permits required); however, due to its location (*i.e.,* a university) and the presence of anthropogenic structures (*i.e.*, bridge and multiple benches for public use), it can experience heavy foot traffic with no barriers to prevent against anthropogenic litter or activity from entering. Therefore, the filamentous, freshwater algae may have already been previously exposed to various anthropogenic pollutants at the time of sample collection. Pollutants were also not rinsed off from the treated algal samples prior to the experiment and the chlorophyll-*a* analyses, which may have influenced our results. Additionally, the nutrient compositions of each pollutant were not measured at the time of experiment. The provided limitations of this study are important to note and suggest further research is needed to support or expand our findings.

Statistical analyses

To perform all statistical analyses of the data, the R program version 3.6.1 and Excel version 16.29.1 were used. Kruskal-Wallis One-Way Analyses of Variance tests, using *kruskal.test()*, were conducted to compare the impact of anthropogenic pollutants on water quality and algal growth. Normality tests were also conducted for each treatment with *shapiro.test()* to determine what type of test was appropriate to run. Non-parametric analyses were performed due to non-normality of the data, and pairwise post-tests were conducted to identify which treatments were significantly different using *pairwise.wilcox.test()* with a parametric Bonferroni correction. Correlation analyses, with *cor.test()* and the Spearman correction, were also conducted for the chlorophyll-*a* abundance and both nitrate and phosphate levels to measure the amount of association between each dependent variable.

Results

Chlorophyll-*a* abundance levels

Chlorophyll-*a* abundance (µg g^{-1}) was significantly different among the five treatments ($X^2 = 37.59$; $df = 4$; $P < 0.001$) and ranged from 4,006.13 µg g^{-1} (±554.765) and 272.114 µg g^{-1} (±124.314; Table 2; Fig. 3). Chlorophyll-*a* abundance was measured for three out of five replicates in each treatment and averaged, with motor oil containing the highest amount of chlorophyll-*a* (4,006.13 µg g^{-1}) and shampoo containing the lowest amount (272.114 µg g^{-1}; Table 2). The pairwise comparisons showed a significant difference between all treatment types (all *P*-values < 0.05). The patterns in the 95% confidence interval show a decreasing chlorophyll-*a* abundance trend for motor oil, sunscreen, control, insecticide, and shampoo treatments, respectively.

Treatment	***n***	**Mean**	**SD**	**SEM**	**UCL**	**LCL**	**95% CI Value**
Motor Oil	8	4,006.13	663.577	234.610	4,560.89	3,451.36	554.765
Sunscreen	8	2,086.21	465.277	164.500	2,475.19	1,697.23	388.981
Insecticide	8	982.635	79.6362	28.1556	1,049.21	916.058	66.5775
Shampoo	8	272.114	148.698	52.5726	396.429	147.800	124.314
Control	8	3,424.11	479.965	169.693	3,825.37	3,022.85	401.260

Table 2: Descriptive statistics of chlorophyll-*a* abundance (µg g^{-1}) of freshwater algae for different pollutant treatments (all $n = 8$ samples treatment^{-1}). n = sample size, SD = standard deviation; SEM = standard error of the mean; UCL = upper 95% confidence limit; LCL = lower 95% confidence limit; 95% CI = 95% Confidence Interval value

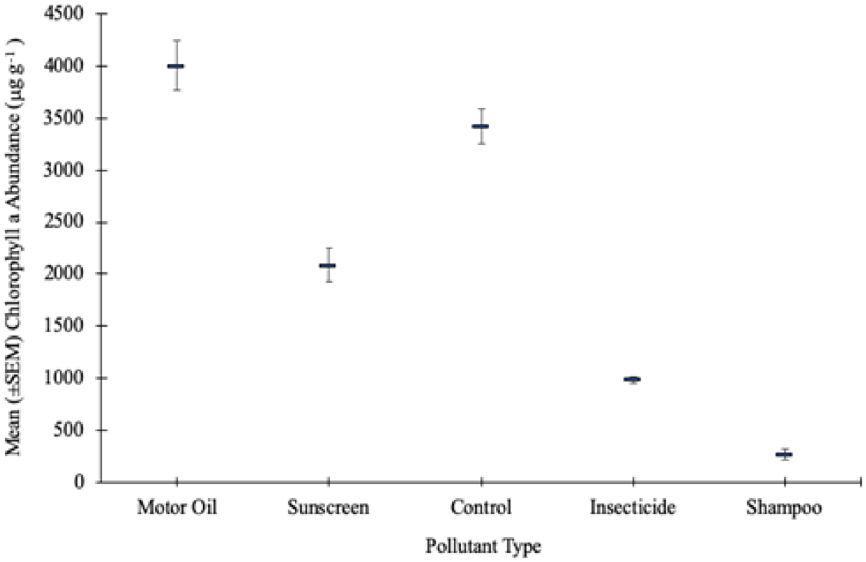

Figure 3: Mean (±95% CI) chlorophyll-a abundance (µg g^{-1}) of freshwater algae for each pollutant treatment (all n = 8 samples $treatment^{-1}$). Error bars represent the 95% confidence interval with the central symbol representing the mean. CI = Confidence Interval

Chlorophyll-a and water quality

The strength and amount of association between the two dependent variables—algal abundance and water quality—were measured from the chlorophyll-a, nitrate, and phosphate data of all treatments. The initial water quality prior to pollutant exposure was 0.00 mg L^{-1} for both the nitrate and phosphate levels of all treatments. After the 3-week treatment period, all treatments resulted in a mean nitrate and phosphate concentration of 0.00 mg L^{-1}, except for the shampoo treatment (Nitrate concentration = 2.5 mg L^{-1}) and the sunscreen treatment (Phosphate concentration = 0.25 mg L^{-1}; Table 3; Fig. 4 & 5). Correlations between the chlorophyll-a abundance, nitrate, and phosphate levels of each treatment were not statistically significant, and graphical patterns show no linear relationship (all P-values > 0.05; Table 4; Fig. 6 & 7).

Nutrient	Pollutant	Mean	*n*	SD	SEM
Nitrate	Motor Oil	0	8	0	0
	Sunscreen	0	8	0	0
	Control	0	8	0	0
	Insecticide	0	8	0	0
	Shampoo	2.5	8	4.63	1.67
Phosphate	Motor Oil	0	8	0	0
	Sunscreen	0.25	8	0	0
	Control	0	8	0	0
	Insecticide	0	8	0	0
	Shampoo	0	8	0	0

Table 3: Descriptive statistics of nitrate and phosphate levels (µg g^{-1}) of the water containing the freshwater algae for different pollutant treatments (all *n* = 8 samples $treatment^{-1}$). *n* = sample size, SD = standard deviation; SEM = standard error of the mean

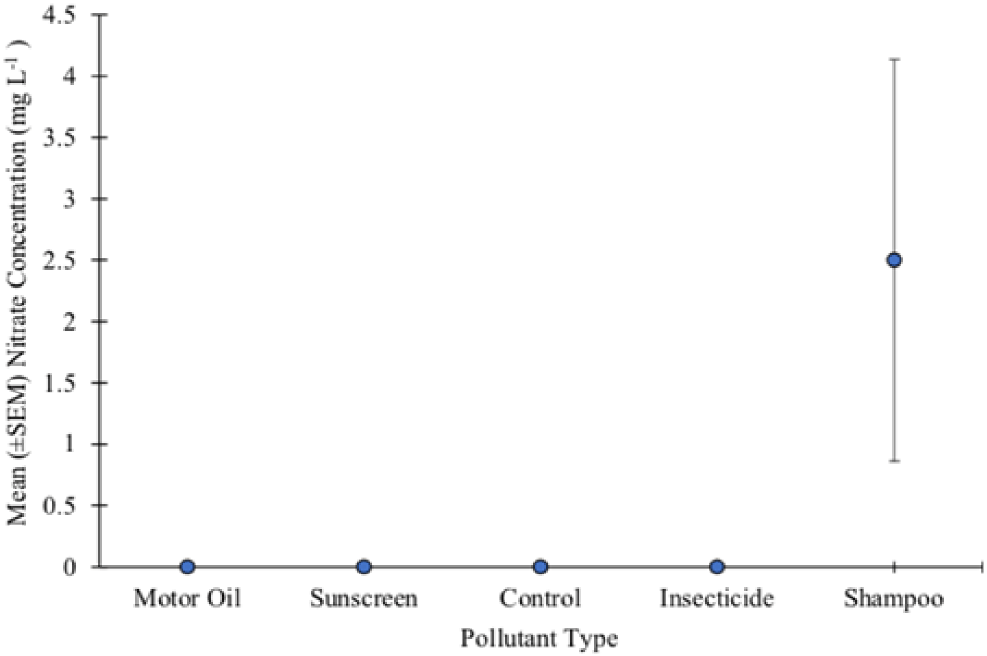

Figure 4: Mean (±SEM) nitrate concentration (mg L^{-1}) of the water containing the freshwater algae for each pollutant treatment (all n = 8 samples treatment^{-1}). Error bars represent the standard error of the mean with the central symbol representing the mean.

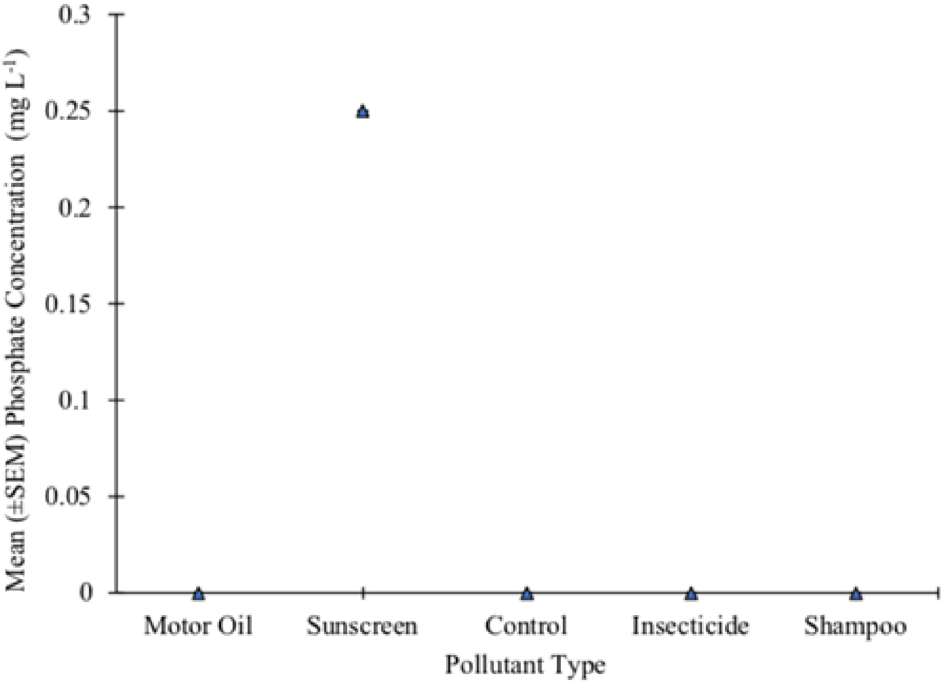

Figure 5: Mean (±SEM) phosphate concentration (mg L^{-1}) of the water containing the freshwater algae for each pollutant treatment (all n = 8 samples treatment^{-1}). Error bars represent the standard error of the mean with the central symbol representing the mean.

Nutrient	*n*	*r*	s-Statistic	*P*-value
Nitrate	8	-0.280	13,640	0.0806
Phosphate	8	0	10,660	1

Table 4: Correlation analysis statistical results for filamentous, algal chlorophyll-*a* abundance (µg g^{-1}), nitrate levels (mg L^{-1}), and phosphate levels (mg L^{-1}) for each pollutant treatment (all *n* = 8 samples $treatment^{-1}$). *n* = sample size, *r* = correlation coefficient

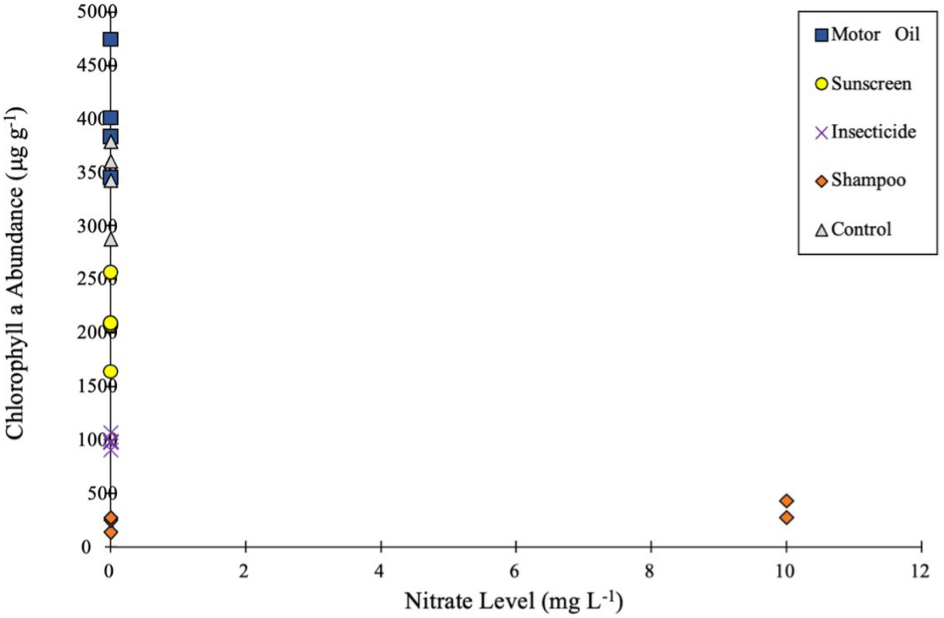

Figure 6: Correlation analysis between the chlorophyll-*a* abundance (µg g^{-1}) of freshwater algae and nitrate levels of the water (mg L^{-1}) for each pollutant treatment (all *n* = 8 samples $treatment^{-1}$).

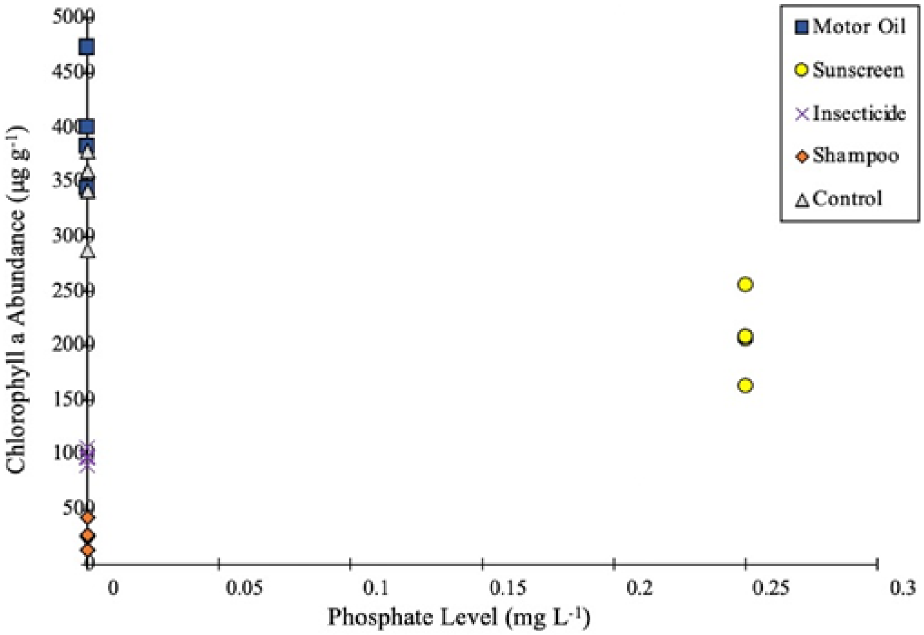

Figure 7: Correlation analysis between the chlorophyll-*a* abundance (µg g^{-1}) of freshwater algae and phosphate levels of the water (mg L^{-1}) for each pollutant treatment (all *n* = 8 samples $treatment^{-1}$).

Discussion

Implication of chlorophyll-*a* abundance levels and pollutant type

The focus of this study was investigating the individual effects of specific anthropogenic pollutants (insecticide, motor oil, sunscreen, and shampoo) on algal biomass measured as chlorophyll-*a* abundance. Results from this research project demonstrated the significance of anthropogenic pollutants and their ability to influence algal growth and ultimately the ecological health of the contaminated freshwater system. Particular insight was achieved on how a specific pollutant may harm freshwater habitats when introduced and can be used towards understanding the combined interactions within more complex freshwater habitats under multiple stressors when exposed to other pollution types. The introduction of anthropogenic pollution with and without nutrient enrichment can have a profound consequence on the health of natural ecosystems by either limiting or supporting the growth of filamentous freshwater algae. Because algae are aquatic primary producers, many organisms along the trophic cascade rely on their presence. Monitoring pollutant contamination of aquatic systems is necessary to preserve algal communities, habitat biodiversity, and habitat sustainability, which humans rely on for accessible resources. Understanding the factors contributing to anthropogenic pollution in freshwater ecosystems is critical for developing and implementing quality environmental risk assessments to ensure effective preservation and management processes against identified pollutants.

The research questions involved understanding the interaction between the anthropogenic pollutant and the exposed freshwater algae and how nitrogen and phosphorus levels of the freshwater were affected by each pollutant type. Results support that an association exists between the freshwater algae abundance and water quality measured from the experiment. Based on the statistical results, the effects of different anthropogenic pollutants on algal growth via chlorophyll-*a* abundance statistically differed between one another ($P < 0.001$), with the highest algal growth observed in the motor oil treatment and the shampoo treatment as the most inhibitive pollutant on chlorophyll-*a* abundance (Table 2). As suggested by Wijewardene *et al.,* 2021 and supported by the findings of this study, the insecticide pollutant was growth-inhibiting of freshwater algae in the absence of fertilizer commonly utilized together for agricultural crop maintenance. Without the nitrogen- and phosphorus-rich fertilizer, the insecticide was successful in preventing the growth of algae and aquatic plants (Dou *et al.* 2019; Yao *et al.* 2019; Wijewardene *et al.,* 2021). The only pollutant that resulted in algal growth was the motor oil treatment (Fig. 3), as the rest of the pollutants (insecticide, shampoo, and sunscreen) ended with lower chlorophyll-*a* abundances compared to the control treatment. Explanations for why the motor oil treatment may have been the most supportive towards filamentous algal growth includes the low concentration administered via treatment (1 mL pollutant per 250 mL of water) and the potential ability to cause biofouling of aquatic organisms, suggesting the introduction of potentially desirable, nutritive ingredients (Pennzoil© SAE 5W-30 MSDS No. 11501, 2013); however, its nutrient composition was not tested when the experiment was conducted, and no correlation between the motor oil treatment, algal abundance, and nitrate or phosphate was supported in the experiment (Table 3; Figs. 6 & 7). One relevant study conducted on used motor oil effects in aquatic systems shows opposite findings, where aquatic macrophyte growth was significantly limited by oil exposure of different concentrations ($P < 0.001$; Özbay 2016). Used motor oil differs from non-used motor oil, because it contains more heavy metals, hydrocarbons, 0.05-18% nitrogen, and 80-32,000 $\mu g\ g^{-1}$ phosphorus (Özbay 2016). Thus, the complexity of algal-pollutant interactions is further supported: anthropogenic pollutants can affect the growth of other aquatic organisms differently (*e.g.,* algae and aquatic macrophytes), as well as pollutants exposed in different forms and compositions (*e.g.,* burned oil vs. non-burned oil). Additionally, distinct types of pollution (*e.g.,* motor oil and shampoo) may interact with filamentous algae in contrasting behaviors, with some either acting as a limiting or promoting factor (Fig. 3). Further research is needed to elaborate on the effects of different forms, ingredients (inactive and active), compositions, types of anthropogenic pollutants (exposed both individually and mixed), and the resulting interactions with freshwater algae.

Based on the correlation analyses for both nitrate and phosphate levels and their association with chlorophyll-*a* abundance, we found that there was no positive association and no statistical differences between the dependent variables (chlorophyll-*a* and nitrate: r =

-0.2795531 and P-value = 0.08064; chlorophyll-*a* and phosphate: $r = 0$ and P-value = 1; Table 4). Thus, the association between chlorophyll-*a* abundance and water quality was not statistically significant. This could be caused by both instrumental error and lack of resources due to the use of water quality strips instead of accurate water testing instruments (*e.g.,* YSI meter). Possible improvements of this research project include access to higher quality materials and an incubation site for samples to control for environmental variation and contaminants. For future projects, better equipment and an incubation site can improve the strength and accuracy of the results. Increasing the range of pollutant concentrations would provide an improved understanding of pollutant interactions within freshwater ecosystems at different scales of pollution. Introducing other types of pollutants based on their known nutrient and chemical composition would also elucidate why a specific pollutant induces or reduces algal growth, as well as testing their active and inactive ingredients individually. In-depth evaluations of each pollutant through their active and inactive ingredients (Table 1) and nutrient compositions would improve the findings of this study. As anthropogenic pollution continues to impact aquatic habitats worldwide and worsen due to growing human populations and commercial industrialization, it remains imperative to conduct further studies on distinguishing and understanding overall ecological impacts to freshwater systems and organisms.

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