

Investigating the effects of PVC microplastics on the growth of *Chlorella vulgaris*

Avi Beaubien^{1*}, Matthew Jacoby^{1*}, Arlene Spevak², Stanley Shmia³

¹ Jack M. Barrack Hebrew Academy, Bryn Mawr, Pennsylvania

² Science Department, Jack M. Barrack Hebrew Academy, Bryn Mawr, Pennsylvania

³ STEAM Department, Jack M. Barrack Hebrew Academy, Bryn Mawr, Pennsylvania

* These authors contributed equally

SUMMARY

Microplastic pollution is an increasing environmental concern because microplastics can accumulate in freshwater ecosystems and disrupt aquatic organisms. As primary producers that form the foundation of freshwater food webs, algae are particularly important to study when assessing the ecological impacts of microplastics. We hypothesized that increasing concentrations of polyvinyl chloride (PVC) microplastics would reduce the growth of *Chlorella vulgaris* algae through chemical stressors associated with PVC exposure. This study aimed to investigate how different concentrations of PVC microplastics affect the growth of *C. vulgaris* in freshwater conditions. We cultured *C. vulgaris* under six PVC concentrations: 0, 20, 50, 100, 250, and 600 mg/L. We manually measured viable and non-viable cell densities using a hemocytometer before and after 15 days of growth. While water quality tests showed no measurable changes in chemical elements such as iron, zinc, or lead across groups, cell count data revealed a clear trend: Higher PVC concentrations were associated with nearly monotonic decreases in cell density after 15 days. At our highest concentration, we found a 65.58% growth inhibition rate. A Spearman correlation test confirmed the statistical significance of this effect. These findings support our hypothesis: PVC microplastics impaired algal growth. However, the physical or chemical stressors that caused this reduction in growth were not detected by basic water-quality assays. This study highlights the potential ecological risks posed by increasing microplastic contamination in freshwater ecosystems.

INTRODUCTION

Microplastics, or plastic debris < 5 mm resulting from the physical and biological degradation of disposable plastic products, are increasingly problematic for the environment (1). Microplastics have been detected in organisms across all major trophic levels, including zooplankton, fish, seabirds, and marine mammals (2). This is a result of the degradation of the millions of tons of plastic added to aquatic ecosystems each year (3). This has a devastating impact on the ecosystems and wildlife. In larger marine mammals, microplastics can cause a microbial imbalance, inflammation, and insufficient oxygen provided to tissues (4). Research on microplastics has explored a wide range of environmental and biological impacts, including effects on plankton, sediments, vertebrates, invertebrates, and chemical pollution (5). Research has also explored

microplastics' effect on algae; however, toxic effects are without consensus (6).

Algae are vital to aquatic ecosystems because they are primary producers, converting sunlight into energy through photosynthesis and forming the base of most aquatic food webs (7,8). Many organisms, such as zooplankton and small fish, depend directly on algae for food, while larger predators rely on those organisms. As a result, unhealthy algae populations can disrupt the entire food web and affect ecosystem stability (6). Therefore, if algae are affected by the increased amount of microplastics in the environment, it could have a substantial effect on the rest of the food web.

PVC has been listed as the second most harmful microplastic polymer due to its waste generation, size distribution, density, and degradation (9). With approximately 15 million tons of PVC waste produced per year, polyvinyl chloride (PVC) is of particular concern due to the presence of stabilizers and plasticizers (9,10). While all commercially produced plastics have plasticizers and stabilizers, PVC is particularly dependent on plasticizers such as diethylhexyl phthalate (DEHP) and bisphenol A (BPA), which are necessary to increase the longevity and flexibility of PVC (11). However, because these additives are not chemically bound to the polymer, they leach into the surrounding water, posing an environmental concern by poisoning aquatic food chains (11,12). Moreover, PVC is a vector for the introduction of heavy metals such as lead and cadmium into the water, as these are occasionally included as additives or incorporated into the dyes and stabilizers that go into PVC (13). Microalgae absorb these metals, which, in sufficiently high quantities, cause heavy metal stress by disrupting metabolism and inducing toxic cellular effects (14).

Chlorella vulgaris is a widely studied green microalga used as a model organism due to its ecological relevance, rapid growth, and ease of cultivation, making it an accessible and practical model organism for our study (15,16). Laboratory studies have reported biologically meaningful effects from microplastic exposure: PVC caused growth inhibition on the order of tens of percent in *C. vulgaris* (17). In another *Chlorella* species, exposure to PVC at concentrations in the hundreds of milligrams per liter reduced chlorophyll a, indicating adverse effects on photosynthetic function (18). While current microplastic concentrations in polluted freshwater environments are on the order of 1-2 mg/L, these are still important studies in understanding the effects of microplastics, as they are a growing ecological concern (19).

Our study aimed to expand on these results and investigate the growth impact of various concentrations of PVC microplastics on *C. vulgaris*. We hypothesized that

increasing concentrations of PVC microplastics would reduce the growth of *C. vulgaris* algae through chemical stressors associated with PVC exposure.

RESULTS

To test the effects of PVC exposure on *C. vulgaris*, we grew the algae for 15 days in separate flasks with varying concentrations of PVC microplastics. We incubated samples, which started with 90,640 viable cells/mL and 19,176 non-viable cells/mL, under a growth light for 15 days and then counted viable and non-viable cells manually with a hemocytometer. In addition to this, we used test strips to check whether PVC microplastic leaching resulted in any testable change in heavy metal or harmful chemical concentrations, but found no detectable difference in standard water chemicals such as iron, mercury, lead, and zinc at day 15, despite the expectation that higher PVC microplastic loads might leach harmful chemicals (**Table 1**).

In contrast, algal growth varied with PVC concentration. Using a cell count, all flasks began at the same initial density (90,640 viable cells/mL and 19,176 non-viable cells/mL). By day 15, endpoint cell densities varied across the PVC gradient, suggesting that PVC concentration was negatively associated with *C. vulgaris* growth. Flasks with higher PVC concentrations appeared as a lighter green, whereas flasks containing less PVC were darker and foggier (**Figure 1**).

In the experiment, we calculated Spearman rank correlations between viable, non-viable, and total cells to determine the relationship between PVC concentration and cell population. Spearman's rank correlation coefficients and corresponding *p*-values were calculated on each of these three series ($\rho = -0.9076$, $p = 0.000113$; $\rho = -0.7183$, $p = 0.012770$; $\rho = -0.9051$, $p = 0.000127$, respectively). Therefore, our data suggest a statistically significant, negative monotonic correlation between microplastic concentration and viable, non-viable, and total cell densities. It is worth noting that the negative correlation between PVC concentration and non-viable cell count does not suggest that PVC is somehow preventing cell death. Rather, this is likely a consequence of there being fewer total cells.

Finally, we quantified the effect size of this relationship by calculating growth inhibition. We found a maximum growth inhibition of 65.58% at a PVC concentration of 600 mg/L. Interestingly, 20 mg/L had a negative growth inhibition, although this is possibly due to noise causing the average cell counts to be slightly higher than the control at low concentrations (**Figure 2**).

DISCUSSION

Our study consisted of two components: assessing water chemical content through routine chemical test strips and evaluating the growth of algae. Interestingly, none of the standard water chemicals we tested for varied across PVC concentrations. This can likely be attributed to two factors: either our tests were not sensitive enough to detect a slight increase in measured chemicals, or the impact on growth was due to other chemicals or biological mechanisms, such as PVC additives or physical effects on algal cells.

Nonetheless, our study suggests that PVC microplastics have a negative correlation with our *C. vulgaris* algae growth. While our data is highly limited and we therefore cannot accurately predict an effect size for current

concentrations of microplastics in the environment, any reduction in growth could be a cause for concern. Given that algae are primary producers, other parts of the ecosystem could potentially be affected by a change in algae growth. Therefore, this study highlights the ecological risk of increasing microplastic concentrations in freshwater environments.

Other studies examining the impact of PVC microplastics on the growth rate of *C. vulgaris* have reported a maximum growth inhibition of approximately 29%, whereas ours suggests a much larger 65.58% (17,20). There are several reasons for this discrepancy. First, PVC exhibits a positive correlation between surface area and maximum growth inhibition (20). Xie et al., for example, used particles of PVC ranging from 1 to 10 μm (20). Due to limitations in our budget and equipment, we are unable to determine the size of the PVC particles used in our experiment, so a direct comparison is not possible between our experimental value and other studies. Additionally, our plastic was not tested for purity, meaning there is potential for other harmful contaminants in our PVC.

Our study had three principal limitations. First, as discussed above, the PVC particles used were not well characterized for size or purity. This limited comparison with existing literature and prevented contamination from being ruled out. Second, manual cell counting and measurements taken only at the beginning and end of the experiment

Parameter	Unit	Detectable Range	Results across all concentrations
Total Hardness	ppm	0-425	50
Free Chlorine	ppm	0-10	0
Iron	ppm	0-100	0
Mercury	ppm	0-0.03	0
Total Chlorine	ppm	0-10	0
Copper	ppm	0-10	0
Lead	ppm	0-400	0
Zinc	ppm	0-50	0
Manganese	ppm	0-5	0.1
QAC/QUAT	ppm	0-80	10
Fluoride	ppm	0-200	10
Sodium Chloride	ppm	0-250	250
Hydrogen Sulfite	ppm	0-5	0
Total Alkalinity	ppm	0-240	240
Carbonate	ppm	0-240	<240
pH	—	6-8.2	<8.2

Table 1. Water tests for various concentrations of PVC at 15 days of exposure. Water quality was measured via test strips and showed the levels of elements and factors across all six concentrations of PVC at the end of the 15 days of algae growth. This measurement method is semi-quantitative, meaning it places each test at one of a few discrete values. With this, every concentration of microplastics yielded the exact same water test results. Note that QAC/QUAT refers to quaternary ammonium compounds, a broad class of sanitizers.

reduced measurement precision and prevented detailed analysis of growth dynamics. Third, the limited number of replicates and concentration groups reduced confidence in the estimated magnitude of the effect.

To address these limitations, we propose a number of methodological improvements to our study. First, purchasing standard characterized microplastics would be a significant improvement. This would allow us to rule out potential contamination, and it would inform us of microplastic size distributions. Using an automated hemocytometer would also be worthwhile because it would increase the efficiency of gathering data as well as its accuracy. An automated hemocytometer would also allow for more frequent data collection and more precisely tracking the development and health of *C. vulgaris* over time. Finally, increasing the number of replicates and concentration levels would increase the confidence in our findings.

If these methodological adjustments prove successful, our next step would be to expand the scope of the study by testing different combinations of microplastics and algal species. For instance, additional microplastics such as polyethylene (PE), polypropylene (PP), and polystyrene (PS) could be examined. Likewise, other freshwater algal species, such as *Chlorella pyrenoidosa* or various members of the *Chlorophyta* phylum, would offer useful comparative data. These variations would help determine whether the effects observed are species-specific or indicative of broader

ecological patterns. After these have been investigated, another method to expand our study would be to analyze how microplastics affect higher trophic levels. For example, incorporating copepods (zooplankton that feed on algae) into these experiments would allow us to analyze how an algal reduction affects other lifeforms.

This experiment aimed to investigate whether PVC microplastics negatively affect the growth of *Chlorella vulgaris* algae in freshwater environments. After culturing algae under controlled conditions with varying PVC concentrations and analyzing water chemistry and cellular growth data, we observed a clear pattern: as PVC concentration increased, algal growth consistently decreased. Statistical tests confirmed the monotonicity and significance of this effect. Given that algae are primary producers in freshwater ecosystems, any reduction in their growth could have cascading ecological effects. As microplastic contamination continues to rise, understanding these impacts becomes increasingly relevant. This reinforces the need for further experimentation, such as our suggested extensions above.

MATERIALS AND METHODS

Study Overview and Experimental Design

We tested whether PVC microplastics suppress the growth of *C. vulgaris* in freshwater culture. Twelve 50 mL Erlenmeyer flasks were assigned to six PVC concentrations (0, 20, 50, 100, 250, and 600 mg/L), with two biological replicates per concentration; however, one 100 mg/L flask was lost during handling, leaving 11 endpoint observations used for statistical analyses and regression. All groups and replicates were handled identically, and the primary study outcome was viable cell density at the study endpoint. We collected final measurements on day 15 of the algae growth process.

Media and PVC Preparation

Culture medium, algae, growth salts, and algal nutrients were obtained from Algae Research and Supply using the “Algae Culture Kit for *C. vulgaris*” (21). Following the kit guidance, reverse osmosis (RO) water was supplemented with algal nutrients and growth salts at 8.98 g/gallon. A single 1 L batch of standardized medium was prepared and distributed across all flasks; both salts and nutrients came from the kit. Each flask received exactly 50 mL of medium. To approximate environmental microplastics, PVC dust was generated by sanding approximately 4 inches of PVC pipe (outer diameter 0.84 in; wall thickness 0.11 in) using ~100-grit sandpaper on a belt sander. PVC dust particle size could not be measured with available equipment. Dust was collected in a sealed bag, and PPE (including KN95 masks and gloves) was used to minimize exposure.

Dosing and Growth Conditions

PVC was added to each 50 mL flask to match the target concentration. For the 600 mg/L and 250 mg/L treatments, PVC was weighed directly (0.030 g for 600 mg/L; 0.0125 g for 250 mg/L). For the 100, 50, and 20 mg/L treatments, a 1 mg/mL PVC stock was prepared by suspending 0.10 g PVC in 100 mL of medium, and aliquots were added to flasks as follows: 5.0 mL (5 mg) for 100 mg/L, 2.5 mL (2.5 mg) for 50 mg/L, and 1.0 mL (1 mg) for 20 mg/L. The 0 mg/L control

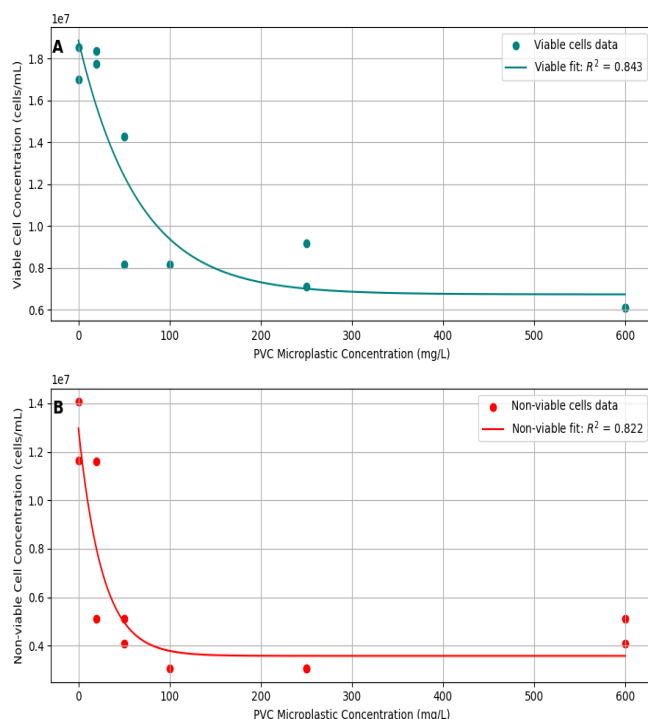


Figure 1. Cell concentration vs. PVC microplastic concentration after 15 days of exposure. *C. vulgaris* was grown at varying concentrations of PVC microplastics, all with identical growth medium and light, for 15 days before cells were counted. Exponential regressions with offsets were then fitted to this data. This resulted in $C_{\text{viable}} = 12,112,916.48 \times e^{-0.015198 \times P} + 6,743,315.67$ being plotted against viable cell counts **A** and $C_{\text{non-viable}} = 9,377,562.44 \times e^{-0.038127 \times P} + 3,585,827.34$ being plotted against non-viable cell counts in **B**. Here, *C* is cell concentration and *P* is PVC concentration.

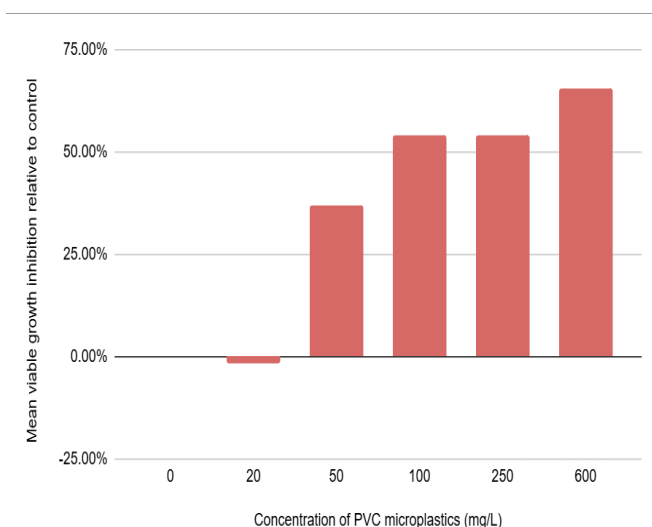


Figure 2. Mean viable growth inhibition relative to control by PVC microplastic concentration. *C. vulgaris* was grown for 15 days with varying concentrations of PVC microplastics before cell counts. Then, growth inhibition was calculated using $\% \text{ inhibition} = (\text{control} - \text{treatment}) / \text{control} \times 100$. Growth inhibitions were averaged within each concentration and plotted. The maximum growth inhibition recorded was 65.58% at 600 mg/L of PVC.

received no PVC. Each flask was then brought to a final volume of 50 mL using the standardized medium.

Flasks were positioned 17.8 inches below an LED grow light and maintained at $\sim 20^\circ\text{C}$. Illumination was nearly continuous (on weekdays, ~ 17 – 20 hours/day due to handling; on weekends, 24 hours/day). A brief manual aeration using a pipette was performed twice a day to refresh dissolved gases, and all flasks were handled identically.

Outcome Measurement, Water Checks, and Statistical Analysis

To distinguish viable from non-viable cells, methylene blue, a dye that stains the inside of compromised cells, was used. Then, a light microscope and hemocytometer were used to perform these viable and non-viable cell counts. Samples were gently mixed before subsampling, and counts from defined grid areas were converted to cells/mL using standard hemocytometer geometry.

Baseline and periodic test-strip measurements were used to verify comparable chemical conditions across all flasks. We used Bestprod test strips to measure total hardness, free chlorine, iron, mercury, total chlorine, copper, lead, zinc, manganese, quaternary ammonium compounds (QAC/QUAT), fluoride, sodium chloride, hydrogen sulfite, total alkalinity, carbonate, and pH.

For data analysis and statistical tests, SciPy version 1.16.3 was used (22). Specifically, 'spearmanr' from scipy.stats was used to run a Spearman correlation test on our raw cell count data against PVC concentrations. Three separate, two-sided Spearman rank correlations were calculated for viable, non-viable, and total cell density. This produced our values for ρ and p . A significance threshold of $\alpha=0.05$ was used. Then, for figure creation, Matplotlib version 3.10.0 was used with SciPy's curve fit to run the

regressions (23). Finally, to calculate growth inhibition, we used the standard formula: $\% \text{ inhibition} = (\text{control} - \text{treatment}) / \text{control} \times 100$.

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