The Effect of Tire Wear Micro-Particles on The Photosynthetic Function and Morphology of Chlorella Vulgaris

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Abstract

The Earth's environment constantly changes as humans make technological advancements. The development of plastics produces many benefits for humanity but is harmful to the environment because plastics break down into small particles and take hundreds of years to decompose fully. One of the most common microplastics is tire wear particles that wash into waterways. Once in the freshwater or saltwater environments, the particles interact with living organisms such as algae. A common unicellular freshwater alga named Chlorella vulgaris uses photosynthesis to create energy. The algae utilize this energy to grow and reproduce. Through testing the optical density and examining the algae under a microscope, the effects of tire wear particles on *C.vulgaris* were investigated. When TWPs are present in the environment of *C. vulgaris*, the morphology and photosynthetic function are negatively impacted.

1.0 Introduction

Tire dust particles are common microplastics polluting the oceans (Järlskog et al., 2022). Whenever a driver brakes, accelerates, or turns, their car's tires wear down releasing potentially toxic microplastics into the environment. While tire dust particles are one of the most prevalent microplastics in waterways and impact many microorganisms, there remains a lack of research in this area. Understanding these pollutants' effects on organisms and the environment is crucial because algae is a major contributor to the oxygen humans breathe and the foundation of the food chain.

The first level in every food chain starts with a primary producer such as algae. These organisms capture the sun’s energy and create their food, glucose, through photosynthesis. Algae produces roughly seventy percent of the oxygen in the atmosphere (University of California Museum of Paleontology, 2022) and is the primary food for aquatic herbivores. When an alga adsorbs tire dust particles, the particles adhere to the algae. Once consumed by a herbivore, the concentration of particles increases at each level of the food chain. Eventually, the bioaccumulation, or an increase in the concentration of the particles over time, spreads up the food chain until consumed by an apex predator. Every year the average human consumes approximately 39,000 to 52,000 microplastic particles due to bioaccumulation (Gibbens, 2019). With the constant increase of tire and road wear particle contamination in aquatic ecosystems, understanding the impact on microorganisms will help scientists gauge how these pollutants will ultimately impact human health.

1.1 Background

The single-celled microalgae *C. vulgaris* belongs to the Trebouxiophyceae division and Chlorophyta phylum (*ACIR Community*, 2023). The name, Chlorella, comes from a combination of the Greek “chloro’", which means green, with the Latin conjugation “ella”, which means small. Vulgaris means common or not special. So by name, Chlorella vulgaris is just a common little green cell. The genus of Chlorella is identified by its single large chloroplasts. *C. vulgaris* is commonly used as a dietary supplement or protein-rich food additive. (Chlorella vulgaris, 2023). This algae has also been studied to provide benefits for treating cancer and other acute illnesses (Panahi et al., 2015) and is being experimented with as a possible biofuel (*C.Vulgaris*, 2023). *C. vulgaris* are easy to culture, have short growth periods, and are highly sensitive to environmental pollution (Oukarroum et al., 2012).

1.1.1 Typical Living Environments and Trophic Relationships of *C.Vulgaris*

C. vulgaris is a common eukaryotic microalgae species that lives in inland freshwater environments (Aigner et al., 2020). *C. vulgaris* exists in water temperatures ranging from 20-35°C, with an optimal temperature for growth of 25°C (Josephine et al., 2022). This algae prefers freshwater pH levels between 6.5 to 9 with the highest growth rate at a pH level of 8 (Josephine et al., 2022). The average salinity of the water remains near 0 practical salinity unit (PSU) in typical environments, but optimal growing conditions are at 30 PSU. The typical environmental nitrogen level for *C. vulgaris* is less than 4mg/L. *C. vulgaris* is mixotrohic. In low-light conditions or the presence of carbon sources, it can switch to heterotrophy, obtaining energy and food from carbon in the water.

1.1.2 Properties of *C. vulgaris*

Once matured, the *C. vulgaris* cell membrane is a unilaminar (single-layered), 100-200nm thick rigid layer (Joël Fleurence & Levine, 2018). The outer cellular layer is UV resistant and protects against desiccation, because of the protein sporopollenin produced in the cell wall (He et al., 2016). The thickness and composition of the cell wall are variable depending on the growth phase or environmental conditions. The microalgae cell contains a cytoplasm with water, soluble proteins, minerals, and organelles. When nitrogen is abundant in the environment, algae fall into nitrogen stress. Nitrogen stress can affect the morphology and the biochemical composition of microalgae. While stressed, there is an increase in starch around the chloroplast and lower levels of lipids. Once the stress is removed the starch degrades, and lipids accumulate in the chloroplast and cytoplasm for energy storage (Zhu et al., 2023). Each cell contains one chloroplast with a double enveloping outer membrane. The chloroplast is where photosynthesis occurs. Algal cells also contain mitochondria to metabolize sugars and a nucleus to store genetic material.

1.1.3 Important Algae Morphologic Features

Figure 1A shows healthy *C. Vulgaris* cells under the microscope. Figure 1B shows damaged S.quadricauda cells under the microscope. Clear-looking and expanded cells are likely damaged cell walls or the remains of dead cells (black arrow *Figure* 1B). A bigger “bubble” in algae cells illustrates an expansion of the vacuole (red arrow *Figure* 1B).

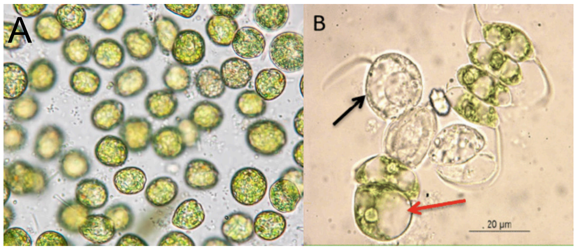

Figure 1: Healthy *C.vulgaris* cells (Barron, 2023) (A) and damaged *S.quadricauda* cells (Chu et al., 2015) (B)

1.1.4 Structure and Chemical Composition of Tire Dust

There are two types of tire dust particles, tire wear particles (TWP) and tire and road wear particles (TRWP). The TRWP are particles that originate from tire tread and result from mechanical abrasion between car tires and road surfaces. TWP consists solely of tire particles, not road and asphalt particles. TRWP is a broader category that embodies road particles and tire wear particles. The term “road wear particles" encompasses all the particles in the road environment, including minerals and road dust (Redondo-Hasselerharm et al., 2018). Two primary tire pollutants have been researched: tire leachate and tire particles. Tire leachate refers to the contaminated liquid that percolates through the tire dissolving the tire chemicals with it. Tire toxicity research on aquatic systems has concentrated on the component's leachate-related toxicity (Cunningham et al., 2022). Particle distribution based on mass is distinct from particle distribution based on count. TWP greater than 0.1 μm in size can be directly observed and massed. These larger particles comprise the majority of the mass of the particles. Nanoparticles sized 0.001-0.01µm occur in far greater numbers but don't contribute a large mass due to their size (Figure 2).

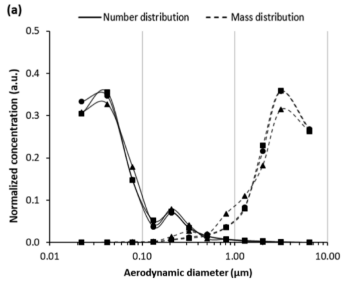

Figure 2: Particle size distribution according to mass vs. number (A. Beji et al., 2020)

1.2. Literature Review

The influence of particle size on the content of road dust has been explored in recent research, with a study in the Spanish suburbs of Barcelona (Dídac Navarro-Ciurana et al., 2023). Potentially toxic elements (PTEs) such as Fe, Cr, Cu, Zn, Ni, and REE were shown to be more prevalent in fine particles, especially those smaller than 10 µm. The study's findings indicate that assessing pollution and health risks using fine dust particles (<45 µm) is more appropriate than coarser particles, as these particles could directly interact with living organisms. This study provides a baseline size for a particulate matter that poses a health risk to living organisms.

An experiment conducted by researchers at Alzahra University, the University of Chemistry and Technology, and Dalhousie University looked at the specific toxic effects polystyrene nanoplastics (particles sized 1-1000nm) had on *C.vulgaris* (Mehdi Khoshnamvand et al., 2021). The study looked at biomass, morphology, and photosynthetic pigment (chlorophyll a) of algae when placed into solutions of 90, 200, and 300 ng/L of microplastic particles. The experiment found abnormalities such as decreasing algal density. Although in this experiment the concentrations and types of microplastics differ from our experiment, we expect similar results for our algae. Their experiment suggests that polystyrene nanoplastics affected Chlorella vulgaris by inhibiting the reproductive rate. We hypothesize that the microparticles of tire wear particles will also impede the division of Chlorella vulgaris by decreasing the cells' ability to photosynthesize and ultimately slowing growth and reproduction.

When algae interact with vehicle-emitted particles, Pikula et al. found either a general enlargement of the algae or cell death (Pikula et al., 2019). Treatment of 100 mg/L of TLC80 particles caused a size increase of 91–93% in algae cells in the 6-—10 μm size range, and death in almost all the cells below the size range. This demonstrates that larger algae cells tend to survive the interaction with particulate matter while smaller ones do not. One explanation for the enlargement in algae size is adsorption. Extracellular polymeric substances (EPS) of algae: polysaccharides, proteins, nucleic acids, and lipids are secreted and released when algae interact with outside materials (Xiao & Zheng, 2016). The EPS will adsorb the particulate matter by surface adhesion of particles onto the biofilm generated by EPS. The EPS serves a function similar to a shield as it prevents the absorption of molecules (*Figure* 3), with the EPS accounting for the larger cell size.

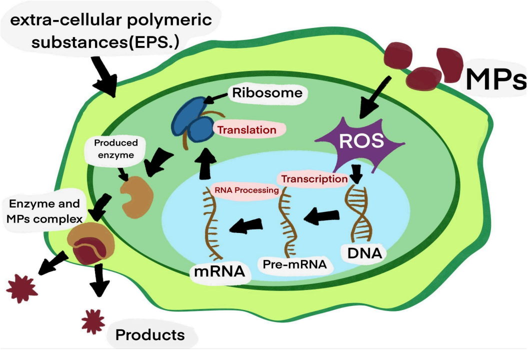

Figure 3: Diagram demonstration of EPS structure and interaction with MPs

1.3. Project Statement

As the levels of microplastics (specifically tire wear particles -TWPs) in aquatic environments continue to increase, the question remains: how do these particles impact the ecosystem? The effects of microplastics on freshwater ecosystems are important because understanding the potential impacts TWPs have on algae will facilitate an understanding of how microplastics may impact humanity. This project will investigate how TWPs affect the photosynthetic rate of *C. vulgaris*. Specifically, measuring optical density and microscopic cell size will be used to evaluate the potential effects of TWPs. Concentrations of TWPs used will be consistent with those currently in the environment. If TWPs and algae interactions are similar to other microplastic interactions studied, we expect a decrease in photosynthetic function and an increase in cell size and damage.

2.0. Method

2.1. Algae Culture

Nutrient media consisted of a 1% BBM solution that has been pH balanced with a 5mM HEPES buffer and NaOH to reach pH 7.2. To evaluate the stability of the nutrient media, pH was taken at the start and conclusion of the experiment. The HEPES solution helped the algae media maintain a stable pH: neither crashing nor blooming. The nutrient media was filtered before use and an aseptic technique was used throughout the experiment. The algae cultures were grown in a constant temperature environment (25°C) and a light system set for a 12-hour light to 12-hour dark cycle. Culture flasks were kept in continuous motion and rotated twice a week ensuring even lighting.

2.2. Tire Wear Particle Collection and Classification

A wire brush on a rotary tool abraded a car tire and the resulting particles were collected in a petri dish. A 40g/L solution was created with TWP suspended in ethanol. The ethanol helped break down the self-adhesion of the particles and maintain sterilization. Particle size classification was achieved by viewing a sample of the 40g/L solution under 40x magnification. The smallest particles that could be resolved were ~1µm and measurements of irregularly shaped TWPs were classified according to the longest side (*Figure* 4).

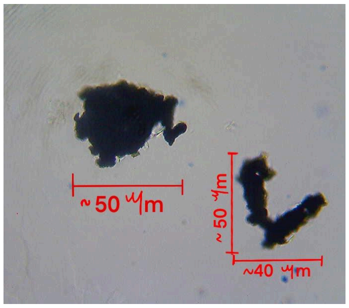

Figure 4: TWP in 40x magnification

2.3. Experimental Setup

Experiment cultures were created using a mature culture as a 20% seed stock in fresh media. After 7 days of growth, the culture was subcultured again and divided into 10 flasks. Each flask received different masses of TWPs to create 0.1mg TWP/L, 1mg TWP/L, 10 mg TWP/L, and 100mg TWP/L concentrations. Each concentration was duplicated to have A and B flasks growing for fifteen days each as a backup in case of contamination.

2.4. Measurement

2.4.1 Optical Density Measurement

Optical Density measurements were taken with a ThemoMax absorbance microplate reader set to a 650nm wavelength. For each reading, three samples of each flask were used to confirm the reliability of the samples, and five consecutive readings were recorded to test the repeatability of the reading. Data from all samples and readings were averaged and the error was found using the standard deviation of all measurements.

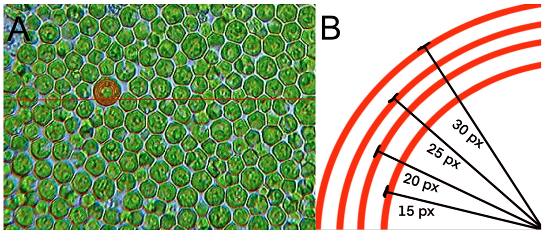

Figure 5: Algae slide for cell size assay (A) and circular scale (B)

2.4.2 Microscopy Observations/Measurement Method

Observations were made under a compound microscope using the magnification 40x and 100x. Different experimental groups of algae were sampled and examined on the final day. A camera captured images for size analysis of the algae cells using a circular scale as the reference (*Figure* 5).

3.0. Results

3.1. Tire Dust

The overall size distribution of microscopic TWP indicated a much higher abundance of smaller particles, particularly under the groups that were less than 10 µm in diameter (*Figure* 6). The inverse relationship between tire dust diameter and tire dust abundance showcases a similar graph trend as *Figure* 2.

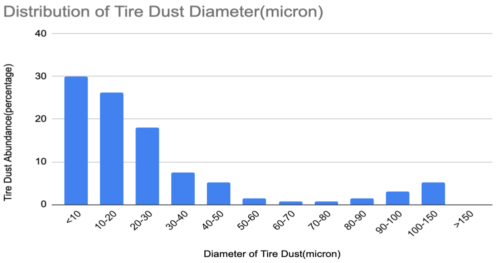

Figure 6: Distribution of tire dust diameter

3.2. Optical Density

The decreasing trendline (*Figure* 7) shows a slight decrease in the growth of algae samples at higher concentrations of TWPs.

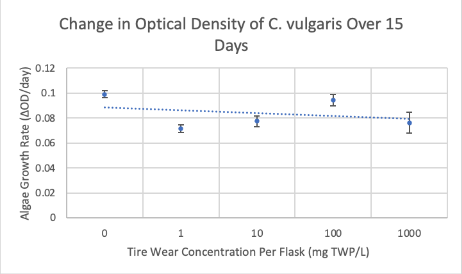

Figure 7: Optical density trendline

3.3. Algae Cell Size Distribution

Increased concentration of TWP resulted in a higher percentage of larger algae cells. The 1000 mg TWP/L experimental group had the largest average cell size, whereas the control group had the lowest average (*Figure 8)*.

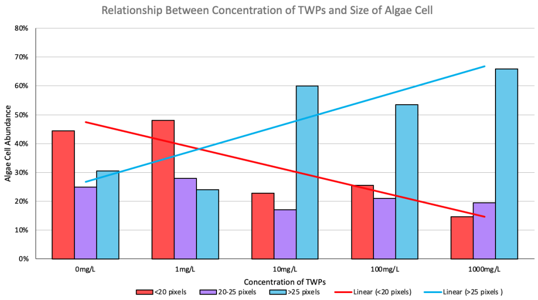

Figure 8: Relationship between the concentration of TWPs and Size of Algae Cell

3.4. Algae Cell Damage

The control group algae (*Figure* 9A) are spherical and evenly distributed. The 1000mg TWP/L algae group (*Figure* 9B) shows clumping, abnormal shapes, visibly deformed chloroplasts, and scattered dead cell membranes. In *Figure* 9C, the transparent-looking cells indicated by the red arrow suggest a leakage in the cytoplasm. Some cells also showed the possibility of an enlarged vacuole presented by the blue arrow. The clear-looking cell matter in the yellow circle is damaged cell walls or dead algae cells. Stacked algae cells were observed in areas adjacent and isolated to visible TWPs.

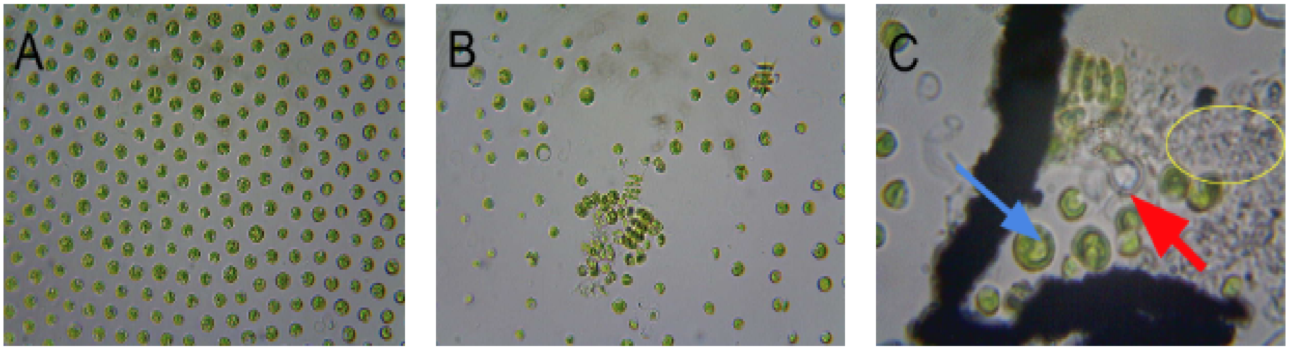

Figure 9: Control group algae (A) and 1000mg TWP/L (B) under 40x magnification. Deformed algae of 1000mg TWP/L (C) under 100x magnification

4.0. Discussion

TWP contamination negatively affects algae. Additionally, cell deformation and enlargement were observed in microscopy displays.

4.1. Optical Density

The declining growth rate of the algae samples indicates that the experimental groups with higher concentrations of TWPs had a lower photosynthetic function and reproduced slower (see *Figure* 6). The deviation between the trendline and the data raises questions about the algae sample procured for optical density measurements. Before retrieving an algae sample, the flask was shaken to ensure uniform distribution. It was noted that some algae was clumped to the bottom of the container after shaking and did not become mixed into the sample. Since the trendline does not overlap with the error bars, additional data collection would be helpful to answer if the uncertainty results from algae clumping.

4.2. Microscopy

With the microscopes available in our facility, only TWP down to 1µm size was quantified and categorized. However, Beji showed in 2020 (see *Figure* 2) that the particle size of TWP increased exponentially to approximately 50nm. While this project did not attempt to differentiate between the effects of microplastic and nanoplastic particles, we expect these unobservable nanoparticles to contribute to cell damage and algae growth slowing (see *Figure* 9B).

The direct relationship between larger cell size and higher TWP concentration has two possible explanations. First, as observed from the quantitative data, the larger cell size might be due to the expansion of the vacuole of the algae cell (*Figure* 9A). The expansion of the vacuole in this experiment can be explained by the increase in nanoscale TWP molecules around the algae cell environment, resulting in an accumulation of chemicals within the cell's cytoplasm and stimulated osmosis. Also, as referenced earlier in section 1.2.3, the particulate matter molecules can be absorbed by algae cells and harm the inner structure of algae, thereby altering its functionality and shape, similar to the previously observed effects of nitrogen stress (Zhu et al., 2021). In Figure 9B, dead cell matters are scattered around large tire dust particles, implying a potential intake of nanoscale TWP for the algae cells. The increase in algae's cellular size and abnormal cell shapes suggest a clear interaction with particulate matter.

5.0 Conclusion

As plastic remains a heavily relied upon material, comprehending the impacts of microplastics and nanoplastics is crucial to prevent further environmental damage. Microplastics persist for hundreds of years; the microparticles can influence the environment during this period. One common plastic that runs off into waterways is TWPs. These particles interact with vital organisms like algae. In this experiment using *C. vulgaris*, measurements recording optical density, microscopic cell size, and microscopic readings were used to evaluate the effects of TWPs. The results showed a slower growth rate and increased algae cell size. This experiment concludes that TWPs negatively affect *C. vulgaris* impacting their morphology and photosynthetic function.

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