Separation and Analysis of Microplastics In Freshwater Lakes, Glacial Lakes, and Ocean Systems Around the Lower Mainland

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Abstract:

Microplastic accumulation in our oceans and lakes could have a great impact on our ecosystems and human health. Knowing where the highest accumulation of microplastics is can help inform future environmental and health-related plans. The goal of this paper was to quantify the amount of microplastics found in lakes, glacial lakes, and the Pacific Ocean in the Lower Mainland. Sediment samples were obtained from six different locations with three replicates each, with two being freshwater lakes, glacial lakes, and the ocean respectively. The sediment samples were washed with ethanol and treated with hydrogen peroxide to remove organic matter. Finally, the purified sediment and microplastics were separated by density with a salt solution, and the microplastics were counted in a 450 mm diameter viewing field. There was no statistical difference between the amount of microplastics found in freshwater lakes, glacial lakes, or the Pacific Ocean ($p=0.3402$). The trends suggest that the Pacific Ocean has the highest accumulation of microplastics present, while freshwater lakes have the least, thus, pointing to the Pacific Ocean to be prioritised in terms of future environmental and health-related plans and policies.

Introduction:

Global plastic growth from 1950 to 2012 has increased to 300 million tons and between 22 and 43 percent of plastics are disposed of in landfills (Gourmelon, 2015). When larger plastic items degrade, it results in the formation of microplastics, which are plastic particles under 5 mm

in diameter (Law & Thompson, 2014). These degrading plastics originate from a variety of sources not limited to sewage, clothing fibres and beads, litter, automobile tire wear, and atmospheric particles (Chae & An, 2018). Landfills have been suggested to be a notable source of microplastics, as they store a large amount of global plastic waste and microplastics may be leaching out through water leaks (He et al., 2019).

Microplastics accumulate in aquatic ecosystems and affect almost all trophic levels. Due to the small size of microplastics, they can be ingested by organisms as small as zooplankton because they may mistake the plastics for food (Cole et al., 2013). These organisms are then ingested by others higher on the food chain resulting in a larger accumulation of microplastics in organisms at the top of the food chain as the microplastics accumulate through trophic levels through ingestion (Vandermeersch et al., 2015). As zooplankton are low on the food chain, their ability to transfer microplastic contamination to organisms higher up could cause widespread health consequences (Cole et al., 2013). Microplastic accumulation in zooplankton can negatively impact their health by reducing their feeding capacity (Cole et al., 2013) and plastics have been shown to affect reproduction and hormone systems in other organisms (Oehlmann et al., 2009).

Microplastic contamination in aquatic ecosystems is widespread in oceans, particularly the North Pacific Ocean. This ocean was found to have the highest levels of microplastic contamination in the world (Eriksen et al., 2014) and the presence of these microplastics have been found on the coast of British Columbia (Desforges et al., 2014). Lakes have also been affected, as microplastics from wastewater and agricultural land (Eriksen et al., 2013) are driven

into lake bottoms by the movement of freshwater systems (Anderson et al., 2016). As such, the threat of microplastics poses a problem for Canadian food consumption and drinking water as Canadian food and water are sourced from local aquatic systems. While plastics have the possibility of posing a risk to our health, the toxic effects of microplastics on food safety are not fully known and further research needs to be conducted to understand their effects on humans (Rainieri & Barranco, 2019).

In order to better create an environmental plan to combat the accumulation of microplastics in British Columbia's bodies of water, a greater understanding of where the most accumulation occurs is needed. To do this, using a combination of methods to isolate microplastics from sediment samples, we compared sediment from the ocean, freshwater lakes, and glacial lakes to identify which type of water source has the highest accumulation of microplastics. It was hypothesised that the lowest microplastic count would be in glacial lakes, followed by freshwater lakes, and lastly the Pacific Ocean. Glacial lakes form through the continuous melting of glaciers (Fujita et al., 2001) and would have less contact and time to accumulate microplastics through landfill leaching and from other sources. As such, they will have the lowest accumulation. With the immense accumulation of microplastics observed in the North Pacific Ocean (Eriksen et al., 2014), the ocean will have the highest accumulation of the three types of water sources.

Methods and Materials:

Sample Collection

First, samples of sediment were collected in small plastic containers from the shores of six different locations: Deep Cove, Abbey Falls, Deer Lake, Como Lake, Kitsilano Beach, and Crescent Beach. By doing so, we made sure that the samples were taken from across the Lower Mainland rather than focusing on just Vancouver itself. Three different environments were chosen to compare the concentration of microplastics: beaches (ocean water), freshwater lakes, and glacial lakes. At each of the six locations three samples were collected (n=18 total). The samples were collected on the outskirts of the body of water by individual team members on different days.

Treatment and Density Separation

Next, the samples were transferred into a plastic weighing boat to be dried in the oven. They were placed in an oven at 60 °C overnight (24 hrs). The dried samples were taken out of the oven the next day and 15 grams per sample were weighed into eighteen 50 mL falcon tubes. In total there were 6 locations and each location had 3 replicates. After weighing out the samples into falcon tubes, 1.5 times the mass of the sediment (32.5 mL or 25.05 grams) of 95% ethanol was added to each sample. They were then centrifuged for 7 minutes to have the less dense organic material float to the surface and subsequently be removed. The ethanol was then poured out into a waste beaker and the samples were air-dried on the counter overnight.

The following day, a 2:1 ratio of hydrogen peroxide (H_2O_2) mass to sediment mass, which amounted to 30.55 grams of H_2O_2, was poured into separate falcon tubes to remove any remaining organic matter in the samples. The samples were mixed twice after the pour, one at the start and the second at the 15-minute interval. The samples were treated for a total of 30 minutes

with H_2O_2. The H_2O_2 layer was then poured into a waste beaker. The samples were again placed back in the oven at 40 °C overnight to dry.

Lastly, for density separation of microplastics, a fully saturated salt bath was prepared by adding 360 grams of salt to 1 L of deionized water. The salt bath was mixed using a magnetic stirrer to ensure all the salt was able to dissolve into the water while the water was being heated up. The samples were transferred into 50 mL beakers and a 2:1 ratio of salt solution to sediment sample, which amounted to 37.5 mL of the salt solution, was poured into each sample and mixed with a magnetic stirrer for 4 minutes. The samples were left to settle for 15 minutes to allow for the microplastics to rise to the surface. Finally, 30 μL from the top layer of each salt bath solution containing the microplastics were pipetted and placed onto a slide for further analysis using a compound light microscope.

Data Analysis

All sample slides were viewed in the centre with microscopes to keep the results consistent. Pictures were taken of each sample at 400X magnification in a viewing field of 450 mm in diameter and were analysed to see how many particles of microplastics were found in each (Supplement 3). Particles resembling anything other than salt crystals (Supplement 4) were counted as microplastics. The means of each water source were compared using a one-way ANOVA test.

Results:

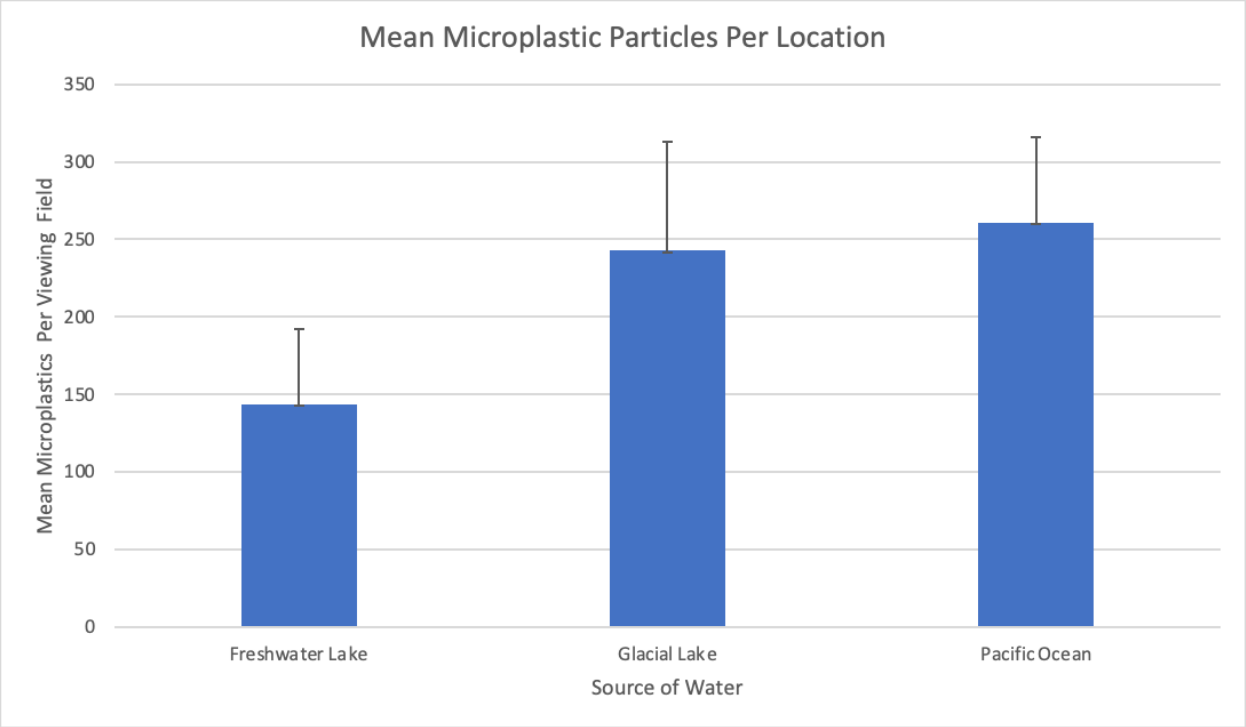

Figure 1. Mean microplastic count per viewing field for each water source. Each water source had 6 replicates from 2 different locations (n=6 for each water source). Microplastics were counted from a viewing field with a diameter of 450 mm and mean values for each water source were calculated. The bars represent the mean number of microplastic particles per viewing field with 95% error bars.

Saltwater had the highest mean number of microplastics per viewing field with 260. The second highest mean was glacial water with a mean of 243 microplastics per viewing field. Lastly, freshwater lakes had the lowest mean with 143 microplastics per viewing field. Using a one-way ANOVA, there was no statistical significance between the mean microplastics per viewing field ($p=0.3402$).

Discussion:

The goal of this experiment was to better understand the quality of our environment and quantify the pollution of microplastics in the environment. It is no hidden fact that oceanic environments are flooded with plastics and debris which impact aquatic and terrestrial life (Wang et al., 2020). Our inspiration to analyse microplastics in sediment on shores of water lakes and oceans came from (Lau et. al 2019), who looked at microplastics in freshwater and saltwater ecosystems. Previous research on the isolation of microplastics has been exploratory as well and there hasn't been a method developed to show a precise separation of microplastics. Our experiment followed a density separation approach using a fully saturated salt solution.

There was no significant difference in microplastics dispersal from location to location. As such, the null hypothesis, that the water source does not affect the amount of microplastics present in a sample, could not be rejected. While the difference in means was insignificant, trends suggest that freshwater lakes have the least amount of microplastics, while saltwater beaches have the highest amount of microplastic. These trends only partially supported our hypothesis as the glacial lakes did not have the lowest number of microplastics, but freshwater lakes did. Instead, the freshwater lakes, Como Lake and Deer Lake, had the lowest number of microplastics. This could be a result of lower public traffic in those areas and protection put in place via municipalities. Similarly, a rise in count at Deep Cove could be due to its connectivity with the North Pacific Ocean at a distance and also having high tourism attractions. For glacial waters, we see the second-highest count (243), however, the site in Abbotsford at Cascade Falls had the least mean value of all sites (94), and Deep Cove on the other hand had the most of all sites (291). Based on these trends, future studies looking into similar questions should compare

the difference in the accumulation of microplastics in bodies of water based on visitation popularity and accessibility to the public.

Since there is no exact standardised method of microplastic separation, we made assumptions on what to include as part of our microplastic count from materials viewed under the microscope. Our pre-treatment of the samples with ethanol and hydrogen peroxide resulted in the separation and removal of organic matter from the sediment such as tree materials and organisms. Our first treatment with ethanol resulted in a minimal separatory effect. Thus we opted to treat samples with hydrogen peroxide as well, which had a better result in removing the organic matter. In future studies, the ethanol treatment should be removed in favour of only a hydrogen peroxide treatment. Given the assumptions that the hydrogen peroxide treatment broke down all the organic matter and the density separation with salt baths brought microplastics to the top layer, we counted all observed particles as microplastics other than salt grains (Supplement 4). It would also be important to use denser salt solutions like NaI or ZnCl2 (Herrera, A. 2018) to compare separation efficiency, since our sampling technique for samples was to scrape sediment at the edge of the water and not at depth. This would allow us to capture plastics as they are washed off to the shore and filtered from water and then trapped in the sediment.

The measure of microplastics in our environment is of paramount importance as it helps us understand the greater implications of microplastics in food chains, climate change, and human health. More studies need to be done, similar to this one, to find where the greatest concentration of microplastics is. This way, we can prioritise those areas in future environmental

plans to address the accumulation of plastics as the rise of microplastics in our oceans and rivers is at an all-time high (Sham et al., 2015). As well, future research can help inform new human health policies as new developments in this field of study are emerging every day. During the time of our study, a discovery was published showing the first-ever detection of microplastics in human blood (Leslie et al., 2022). This shows that plastics are bioavailable for uptake and creates a need for the future to understand the risks of plastic particles on human health, adding another reason for microplastic research to gain more prominence. Some potential solutions are being explored, like research into plastic-degrading bacteria (Urbanek et al., 2018) and a push towards ending plastic use.

Our research confirmed the methods of density separation using home-based materials like the salt solution, which was also used in Aiken et al. (2017). Given our experience and timeframe, we were able to produce a viable experiment, however, some improvements can be made for future studies. A different study by Thompson et al. (2004) showed better results when mixing peroxide solutions, treating the samples with peroxide solution for longer, and also resting the density separating solution for longer to allow more separation. Other areas for improvement or investment include technological advances, allowing better equipment availability to observe the samples at depth. Aiken et al. (2017) also suggest multiple density extraction of microplastics for optimal results. As well, in future studies, it would be appropriate to have multiple trials of sampling and include more locations to get more powerful results. Measuring the number of microplastics in a sample of deionised water as controls would also be useful too. All in all, given the difficulty surrounding this area of research of microplastic separation, we have produced expected results and hope that in the future a fixed method can be

developed. We are optimistic that the focus and research in this area will only flourish given that microplastics appear to be impacting humans more than ever, as microplastics have now appeared in human blood (Leslie et al., 2022).

Conclusion:

Our research on the concentration of microplastics around lakes, ocean beaches, and glacial sediment on the Lower Mainland found insignificant results. The trends suggest that the Pacific Ocean has the highest accumulation of microplastics, but also that the highest number of microplastics were seen at ocean beaches and glacial lakes with high tourism. Our extraction method of density separation using salt baths inspired by previous research was successful in achieving visuals under a microscope. This experiment allows for further inspiration in this emerging field relating to the health and wellbeing of ecosystems given the rise of plastics.

Acknowledgements:

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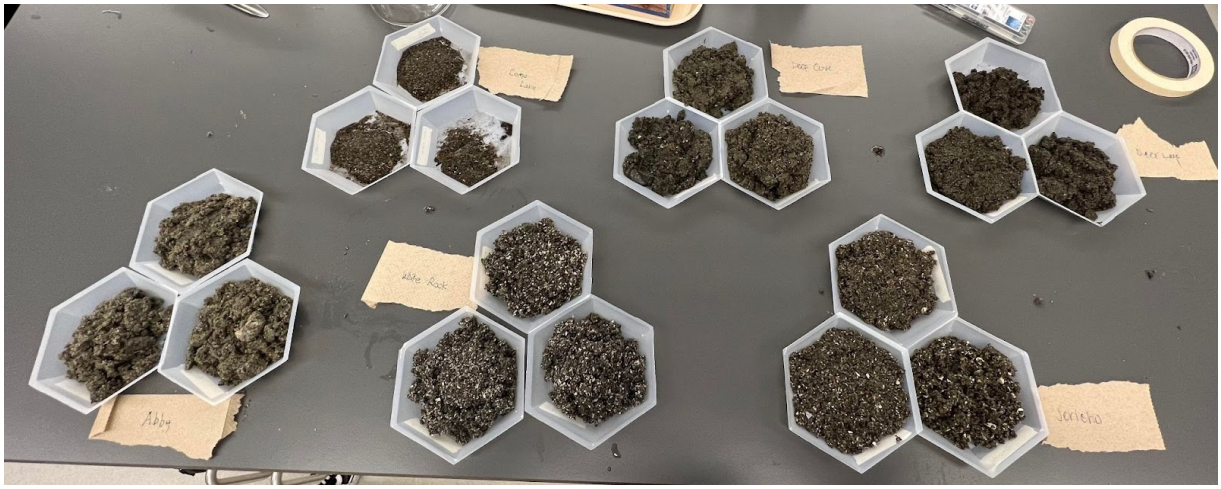
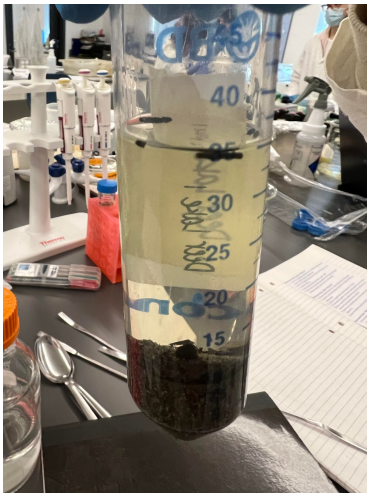
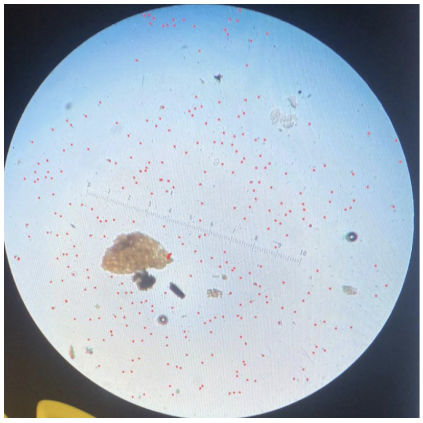
Supplementary



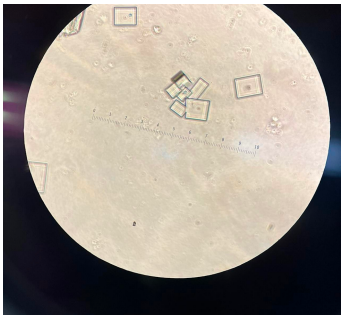
Supplement 1: Microplastic samples before treatment and density separation at each of the six locations. Of the six locations, two locations came from each of the three water sources: freshwater lakes, glacial lakes, and the ocean.



Supplement 2: Sample of Deer Lake during ethanol treatment. The ethanol treatment was used to separate organic matter from the sediment.



Supplement 3: Example of microplastic count. Microplastics were counted in this way in each of the 18 views counted.



Supplement 4: Salt at 400x magnification. This reference image was used to more accurately count microplastics in each viewing field, and disregard particles looking like salt.