Macroplastics are Tomorrow's Microplastics: a study of debris collect around Lake James, NC

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Abstract

Plastic pollution has been a growing concern in the topic of environmental conservation. As industry expands, the need for single-use products has exponentially grown in the past century. Many researchers have questioned what threats plastic could pose to ecosystems. One main concern is microplastics. When a macroplastic such as a water bottle gets into the lake, it can break down overtime into smaller and smaller particles. These particles are known as microplastics. Microplastics can pose many threats to the ecosystem but this research focuses on sediment testing. When microplastics get into the sediment it can affect soil health, leach into the groundwater, get taken up by plant roots, eventually getting passed along the food chain. There has been growing concern about plastic getting into Lake James. Sediment was collected and density separation methodologies were explored in order to see if there was a presence of microplastics in Lake James. The results indicated that there is accumulation of microplastics in Lake James however does not relate to the amount of macroplastics collected. Suggesting that microplastics are from an earlier time period and more action needs to be taken to prevent future macroplastics getting into the surrounding watershed.

Introduction

In recent years, plastic pollution has been a topic of discussion. Over 8.3 billion metric tons of plastic have been produced globally since the 1950s (Cashman et al, 2020). As time passes, plastics break down into small particles that range from 5 mm to 0.1 µm, known as microplastics (MP). Many marine biologists examined plastics in our oceans and beaches. In a study to see if there was a presence of microplastics in the estuaries within the Gulf of Mexico, it was found that MP were abundant in all locations they studied (Wessel et al, 2016). More MP was found at higher salinity and the most prevalent type was polyethylene and polypropylene. The increase in discarded trash, along with very slow degradation rates, is leading to the gradual increase of marine litter found at sea, on the ocean floor, and along the shore (Wessel et al, 2016). Marine debris has even been found broken down in sediments (Claessens et al, 2011). Different areas of sediment deposits such as harbors, beaches and seafloor were compared. Harbor exhibited the highest MP concentrations and an analysis of sediment cores suggest increasing concern of microplastic concentrations in sediment (Claessens et al, 2011). Over time, the build up of MP in the ocean and sediment poses a threat to the ecosystem.

One potential threat that MP causes to the sediment, is it can affect soil carbon storage (Yang et al, 2021). As seen in figure 1,contaminants combination and interaction with MP results in absorption which may affect soil health and function eventually, migrating along the food chain and into groundwater(Yang et al, 2021). There are a variety of materials that can be considered as MP, from microfibers to textiles or plastic fragments to rope pieces. It was only just recently that scientists identified the concern of MP in freshwater environments (Campanale, 2020).

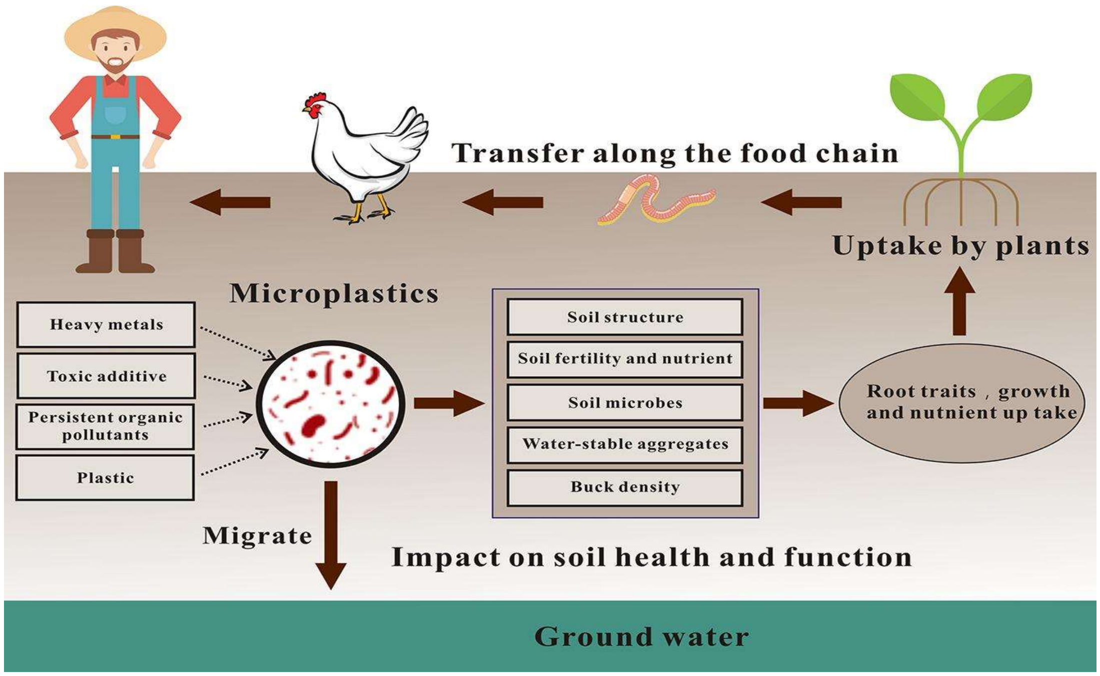

Figure 1. A diagram showing how microplastics influence soil health and function. The figure is from a study conducted by Yulan Zhang, 2021. The research found that when microplastics get into soil it can cause variation in soil structure and eventually leach into the groundwater (Yang et al, 2021).

MP is a crisis to the ecosystem because it can give off toxic chemicals (Coppock,2017). Plastics can act as an absorbent for toxic organic contaminants which can be ingested by marine species affecting their digestive tract (Sartain et al 2018, Willis 2017). A heavy presence of MP causes a change in the heat transfer and water movement of sediment (Claessen et al 2011). With this in mind, MP in riverine environments is becoming a growing problem.

In Lake James there is a concern about the pollution that regularly washes into the lake. Many pollutants get filtered into the lake, such as fertilizer run off or trash left on the side of the road. After a heavy rainfall, a significant amount of debris gets deposited into the lake from the surrounding 386 square mile watershed. In order to better understand the level of plastic pollution in the lake, the Lake James Environmental Association (LJEA) has decided to examine the presence of microplastics in the lake. By looking at if plastic is breaking down at a molecular level, LJEA determines if the amount of marine debris pollution getting into the lake is of significant concern. MP in sediments is going to be examined because plastic often settles to the bottom and LJEA wants to gather an accurate representation of MP pollution in Lake James.Furthermore, a correlation between macroplastics and microplastics is another assessment that is going to be conducted. Various methodologies have been enacted for MP testing. For this study, density separation methodologies are going to be used.

Image 1. Photograph of Linville boat ramp. A depiction of what Lake James looks like.

Methods

Samples were collected from eight sites around Lake James. The sites were selected based on the relevance of the site. The majority of the sites are utilized for fishing, swimming and other recreational purposes. Sediment was gathered for the purpose of this study. Using a posthole digger, sediment was collected at the surface (Up) and slightly below the surface

(Down) to analyze microplastics using density separation methodologies targeted at 1.2 g/mL and 1.6 g/mL. The densities were selected based on table 1 (Radford et al, 2021). The range of microplastic density is 0.015-1.58. The reasoning behind selecting densities of 1.2 g/mL and 1.6 g/mL is to target a range of microplastic based on previous methodologies used (Radford et al, 2021). Litter within a 5 m radius of the sample site was collected to analyze macroplastics. The litter was broken down into six categories to decipher the types of materials found within the watershed.

Recycling Code	Type of Plastic	Density (g/mL
1	PET	1.36-1.45
2	HDPE	0.93-0.97
3	PVC	1.16-1.58
4	LDPE	0.91-0.92
5	PP	0.9-0.91
6	PS	0.015-0.03

Table 1. Research conducted by Radford et al, 2021. The table is a summarization of polymer type and density of results from using density separation methodologies to identify types of plastics.

Collection of samples

Various sites around Lake James were analyzed. These sites included Linville boat ramp (1), White Creek (2), Lake James State Park (LJSP) Paddy's creek (3), LJSP Catawba (4), Linville River above NC126 (5), Black Bear Boat Ramp (6), North Fork (7) and Marion greenway Catawba river (8). Sites 1 and 6 were selected because they are the main boat ramps in the area. Sites 3, 4, 5, 7 and 8 are primarily used for swimming, fishing and other recreational activities. Site 2 is the most remote site. To view sample sites look at figure 2 or use this link for an interactive map to see each sample site **https://arcg.is/05vvmj**

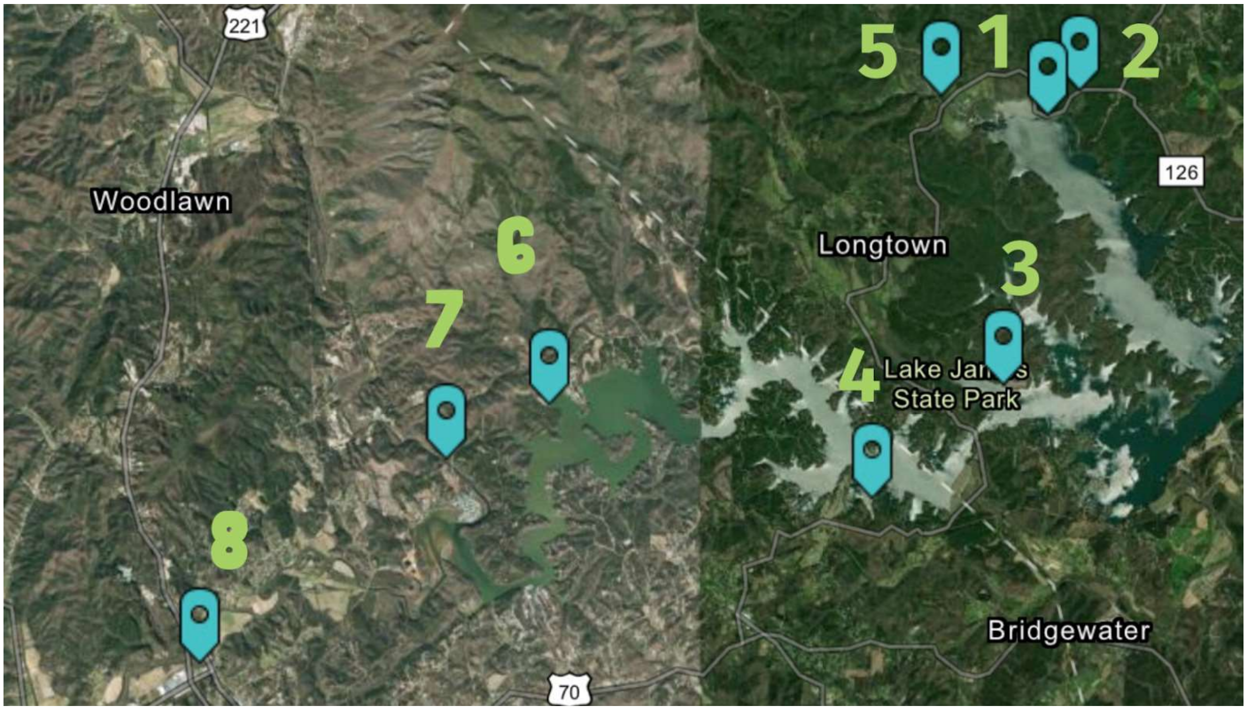

Figure 2. Map of field sample sites. Location of each sample site is (1) -81.87N 35.79E, (2) -81.86N 35.80E, (3) -81.88N 35.75E, (4) -81.90N 35.733E, (5)-81.89N 35.80E, (6) -81.97N 35.75E, (7)-81.99N 35.74E, (8)-82.03N 35.71E

For sediment collection

Two sediment samples are collected at each site. The first sample is collected from the surface using a posthole digger. The sample is placed into a bag with a label of site number, the word "Up", the site name, and GPS coordinates. The second sample is also collected with a posthole digger in the same hole. Looking at sediment deeper than the surface collection, placing the sample into a bag and labeling the site number, the word "Down", the site name and GPS coordinates. Transported to lab facilities at WWC for further examination and sediment preparation.

For Shoreline Debris Collection

Using gloves and trash pickers to collect debris around sediment sample collection. Measure a 5m radius around the sample collection and pick up surrounding litter. After collecting debris, label the sample site of the bag and set aside for sorting and counting.

Image 2. An image of site 1 debris collection with the help of Marshall Taylor from LJEA science committee

Preparing Samples for Density Separation

Samples were taken to Warren Wilson College (WWC) for further analysis. To prepare the sediment samples, each sample was placed into a tin muffin and set to dry at 50 °C for approximately 72 h to remove water. Once dried, take 100 mL of each sample to sieve. Using

Geotech Sand Shaker Portable Handheld Sieve/Grain-Size Analyzer separate soil to the finest grade of sediment. Standard US sieve used was OPN 40 (1160µm), OPN 09 (229 µm), OPN 046 (117 µm), OPN 021 (53µm). After shaking for 2 minutes, set aside the smallest sieve size and weigh 0.2g of the sample. Place the sample into a vile and set aside for density separation. Refer to the figure 2 below for further explanation of sample preparation.

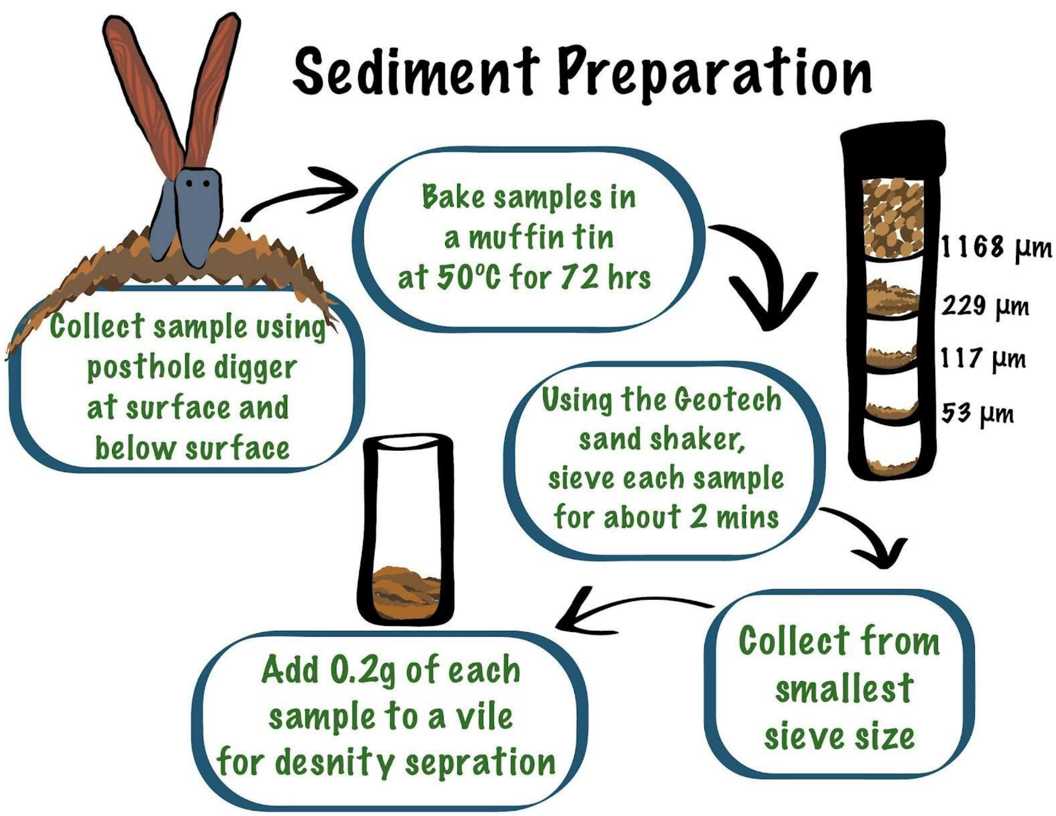

Figure 2. Description of how to prepare sediment samples to be used for density separation.

Density Separation Methodology Exploration

Plastics have a density ranging from 0.9-1.5 g/ml. For the purpose of this experiment, a 1.2 g/mL and 1.6 g/mL was the target range to extract microplastics from sediment samples.

Methodology using density separation was adapted from (Yang et al, 2021), (Sartain et al, 2018), and (Maes et al, 2017). Each research explored density separation methodologies using NaCl and ZnCl2. For the purpose of this study, NaCl (1.2g/ml) and ZnCl2 (1.2g/mL and 1.6g.mL) were used to target microplastic for extraction.

Preparing Solutions for Density Separation

NaCl solution- Saturated NaCl, was prepared for trial 1. Deionized water was added to 36 g of NaCl. Using a specific gravity hydrometer, a 1.2 g/mL was recorded. The solution was then a filter to reduce microplastics within the stock solution.

ZnCl2 solution-The Concentration of ZnCl2 was prepared for all trials and experiments. After trial 1, a density of 1.2 g/mL was created due to problems using NaCl. Two different solutions were created. For a density of 1.2, deionized water was added to 100.22g of ZnCl2 concentrate. A hydrometer recorded a 1.2 g/mL and the solution. For a density of 1.6, deionized water was added to 314.07 g of ZnCL2. Using a hydrometer, a 1.6 g/mL concentration was recorded and the solution was then filtered with cellulose filter paper grade 1 (11μm). The purpose of filtration of both stock solutions was to reduce microplastics within the solution being added to the sediment solutions

Trial 1

25mL of Canola oil was added with 175mL of water to a 200mL beaker with 0.5g of soil. Using a separatory funnel, the solution was added and sat for two hours. Water was separated from the canola oil. Then canola oil was added to a Petri dish and placed under a microscope for analysis. Methodology technically worked however, an overabundance of sediment made it difficult for microplastic identification. Other density separation methodology was adapted using NaCl and ZnCl2

Trial 2

Using a 50 mL beaker, 0.2g of site 1 down samples was added. One beaker received 25mL of NaCl at 1.2 g/mL, the other beaker received 25 mL of ZnCl2 at 1.6 g/mL. The solution sat overnight to allow for density separation. Two extraction methods were used for filtration. The first was using a glass pipet. The other method was a pour-over method by dumping the surface liquid into the filter. Both methods work, however the pour-over method is not effective. Glass pipette extraction methodology is adapted.

Trial 3

Solutions of 1.2 g/mL and 1.6g/mL of ZnCl2 are made to test funnel separation methodology. To set up funnel separation, a long-stem funnel was placed in an iron ring on a ring stand. The funnel was clipped with a rubber tube. Two funnels were used to test methodology at each density. 25 mL of each density was added to a funnel with 0.2g of site 7 down. Foil was placed over the funnel and remained overnight. The sediment was separated from the solution by releasing a clip on the rubber tube. Extraction methodology is not very effective because too much sediment got on the filter paper. Made it difficult for counting and identifying microplastics.

Trial 4

Vials and glass pipette extraction were used for the final density separation methodology test. 0.2g of site 2 was added to two vials. One vial received 12.5 mL of 1.2g/mL of $ZnCl_2$ and the other received 12.5 mL of 1.6 g/mL of $ZnCl_2$. A cap was placed on the vial and the samples sat overnight. Using a glass pipette, the surface liquid was extracted for filtration. Starting with the edges then going towards the center reduces microplastics sticking on the side of the vial. The filter paper was labeled and placed aside for identification.

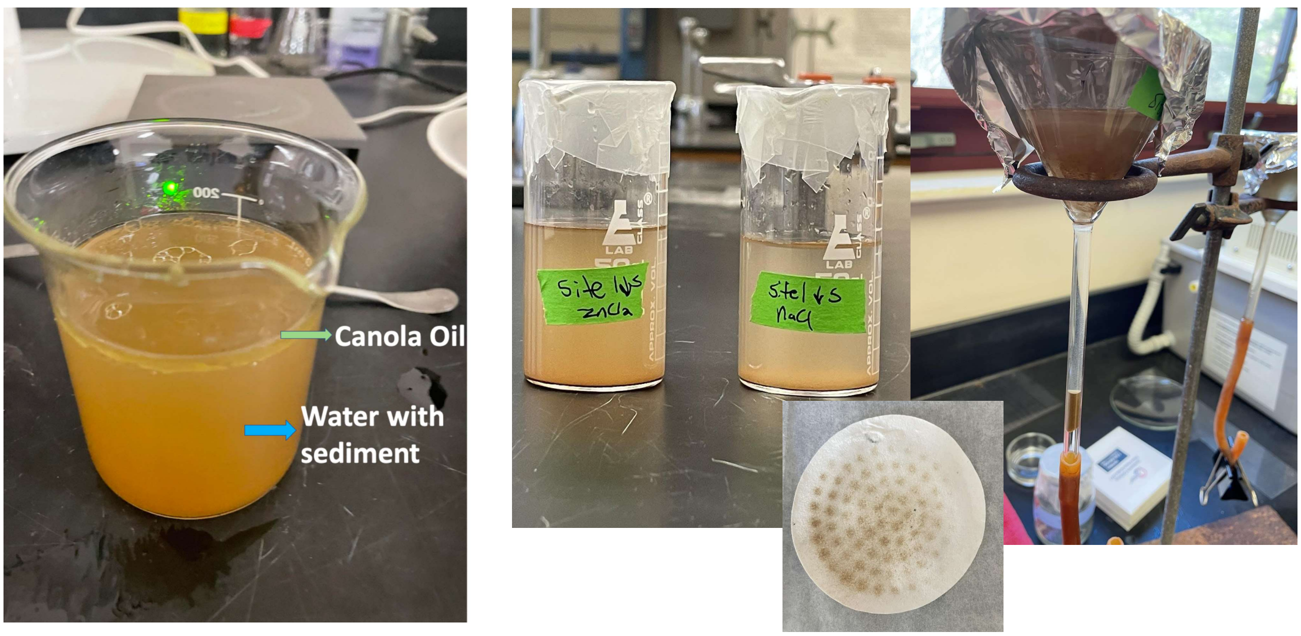

Image 3. Images of trial runs. trial 1 (left), canola oil and water. trial 2 (Center), 1.2 g/mL saturated NaCl and 1.6g/mL $ZnCl_2$. trial 3 (right), Funnel method and filter paper with 1.2 g/mL and 1.6 g/mL $ZnCl_2$.

Experiment 1

Each sample site was examined for experiment one. Two samples were created for up and down. Each site received both densities. For the experiment, 0.2g of sediment is added to a vial. 12.5 mL of 1.2 g/mL and 1.6 g/mL of $ZnCl_2$ was added to two separate vials. Two drops of Nile red dye were added to each in order to test for blue light fluorescence. A cap was added to each vial and sat overnight. Glass pipette was used for filtration by starting on the edges and moving to the center during surface filtration. The sample was labeled and set aside for identification.

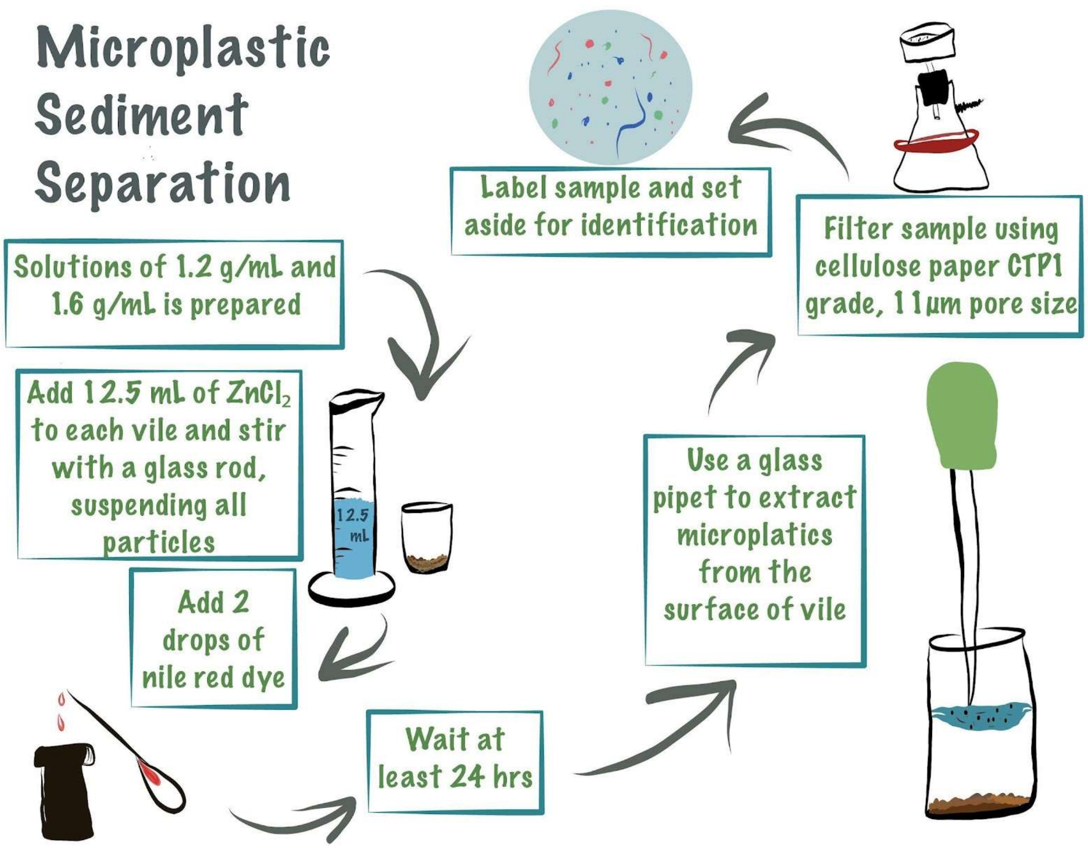

FIgure 3. A description on density separation methodology used for extracting microplastics.

Experiment 2

To further analyze samples, three sites were selected to see if there is a correlation between microplastics and macroplastics. Figure 3 shows the final process used to analyze microplastics in sediment using density separation. Site 1 and 7 was selected from having the most trash and being located in two different counties. Then site 2 was selected because no trash was collected at the site and it is the most remote location. Three vials of 1.6 g/mL $ZnCL_2$ and one vial 1.2g/mL. Repeat steps in diagram 2 for each sample site up and down. The samples were all filtered and set aside for microplastic identification.

Counting and identifying samples

Macroplastics identification

The litter collected was categorized into six different categories; metal, glass, fishing gear, cloth, plastic and paper. Each site was counted, except site 2 because there was no litter found at the sample site. The litter was categorized and weighed for each site. The trash collected was photographed for archival purposes and disposed of properly.

Image 4. Categorization of sites 1 and 7 debris. Site 1 and 7 had the most litter.

Microplastics Identification

Each filter paper was placed under a microscope. Using a 0.5 x 0.5 cm grid, each sample was counted. A square was selected in 20 magnification and 40 magnification was used to count the square. A total of 15 random squares were selected to count and identify microplastics. The microplastics were then categorized as green fragment, blue fragment, red fragment, blue fiber and red fiber. Each sample was counted and identified

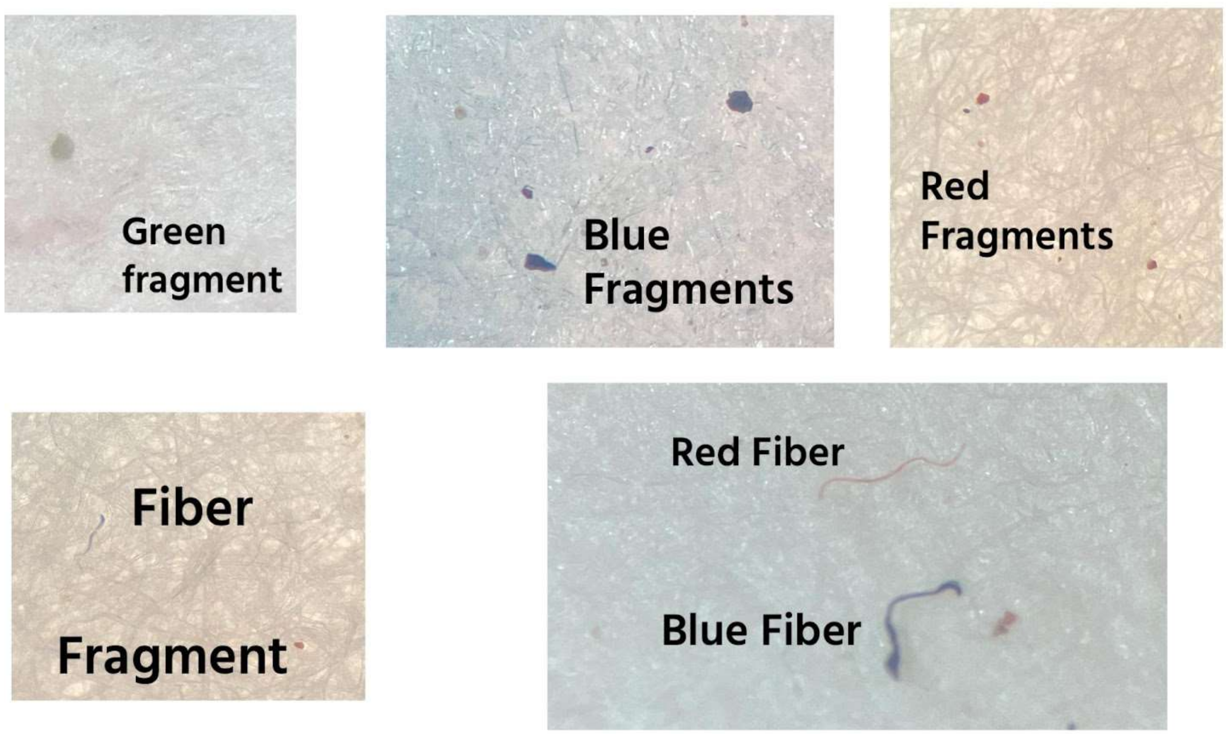

Image 5. Types of microplastics found. One the top, there are images showing the different type of fragments found. On the bottom left there is an image showing the difference between a fiber and a fragment. Then, on the bottom right, image shows the difference between a red and blue fiber

Results

Based on the methodologies used, the best substance to use for extraction of microplastics is ZnCl2 because ZnCl2 can be made to a range of densities. It is suggested that one uses a small beaker or vile to sit overnight to allow for density separation. Also, the best extraction method is using a pipet, going around the rim of the vile, to reduce sediment disturbance.

Extraction methods	NaCl	ZnCl2
1.2 g/mL	Yes, however saturation caused for crystallization on the filter paper	yes
1.6 g/ml	n/a	Yes
Funnel/ draining method	No, too much sediment on filter paper	No, too much sediment on filter paper
Beaker/vile, waiting overnight and pipet extraction	yes	yes

Table 2. A breakdown of different density separation methodologies used.

Macroplastic results

Distribution of debris across sample sites was disproportionate. Sites 1 and site 7 have about 30% of litter compared to the other sites (figure 4). In relation to the total of macroplastics found, all sample sites had distribution ranging from 11-19% (figure 5). The distribution of macroplastics across the sample sites are proportionally distributed.

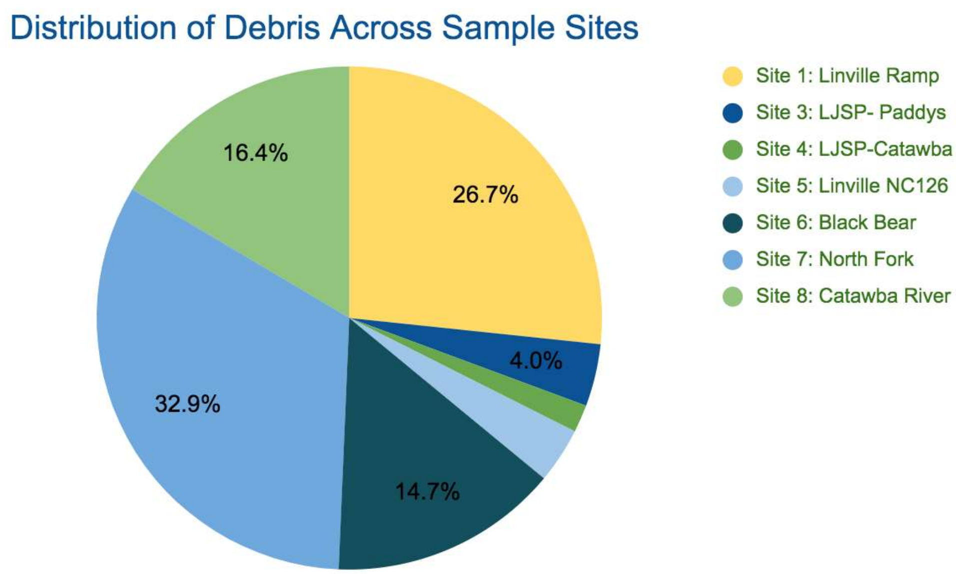

Figure 4. A breakdown of total litter found across all sample sites. Site 2 had no litter. Site 1 and site 7 have approximately 30% of all debris.

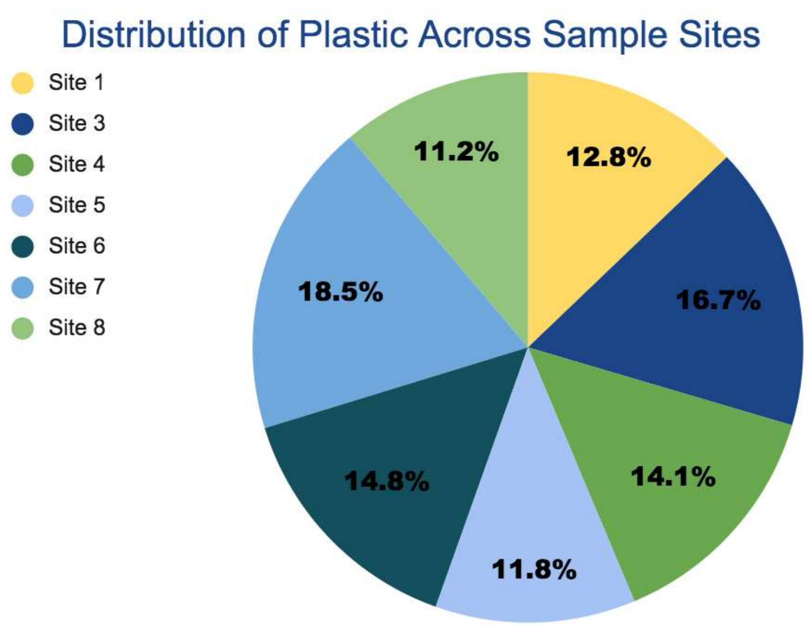

Figure 5. A breakdown of total plastics found across all sample sites. Site 2 had no litter. All sample sites range from about 11-19% in relation to debris collected.

Microplastic results

Microplastics were found at all sites including site 2 where no litter was collected. All samples did not react with nile red dye. Majority of microplastic material found was approximately 50% blue fragments. Green fragments and the majority of fibers were found at the surface .

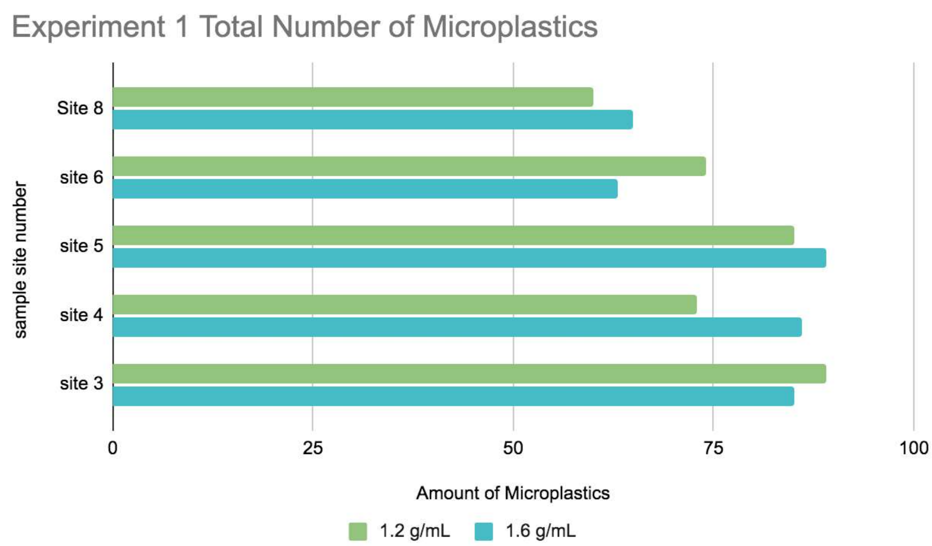

Figure 6. Total Number of Microplastics found during experiment 1.

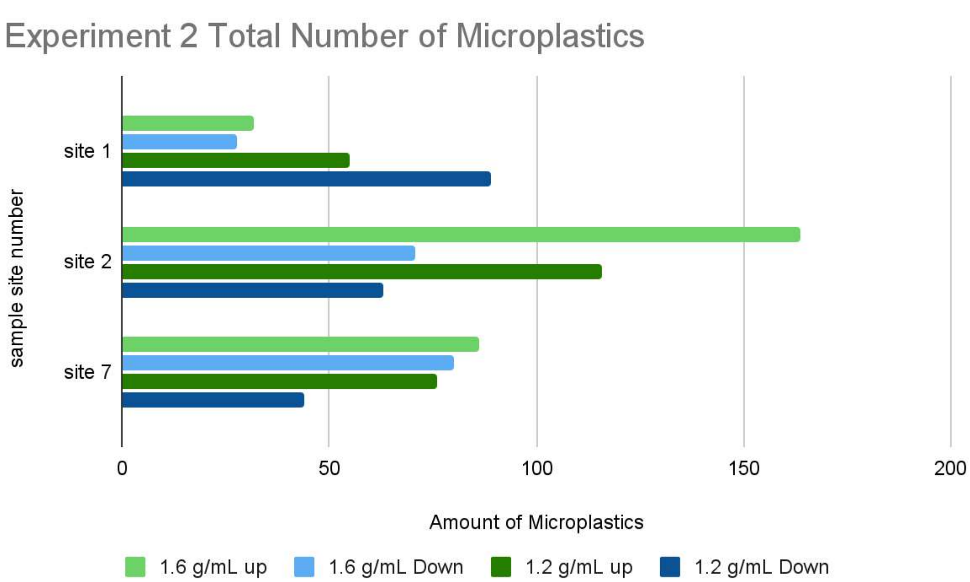

Figure 7. Total number of microplastics for experiment 2

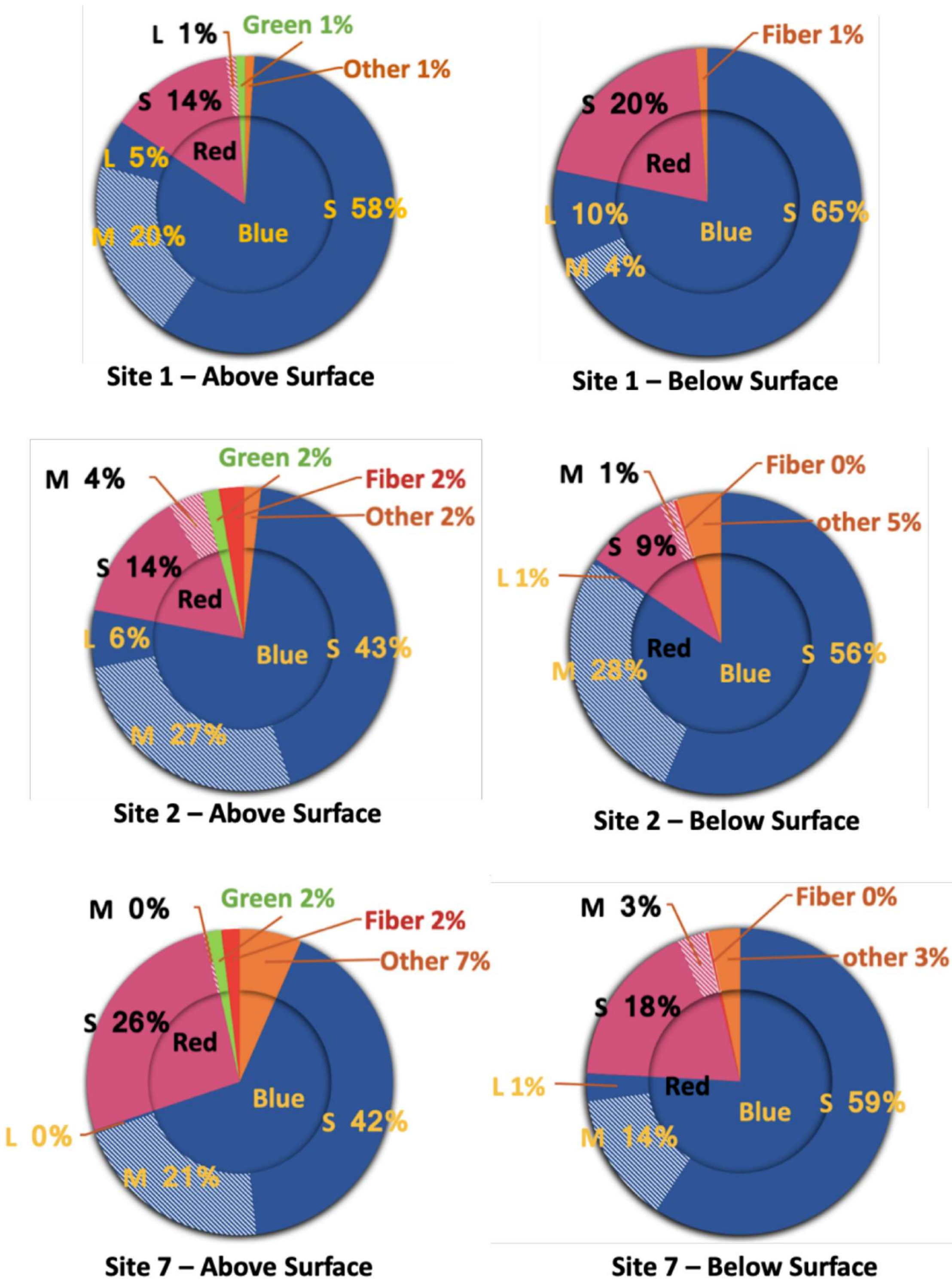

Figure 8. A breakdown of different types of microplastic material found using 1.6g/mL ZnCl2.

Discussion

Microplastics exist in Lake James and there is not much correlation between macroplastic and microplastics. It is best to use ZnCl2 with viles and pipet surface extraction to filter the samples. The majority of microplastics found were blue fragments. Microplastics are non-reactive with nile red dye, so it is possible the type of plastic found is polyester, polyimide or acrylic (Maes et al, 2017). The microplastic found are most likely from an earlier time period. Based on the history of manufacturing in the counties, the area produced a significant amount of the nation's hosiery. The area converted their cotton mills to produce nylon products during WWII. After WWII, the plastic manufacturers decided to create consumer products. Based on this history the microplastics found are most likely from the 1960s-1980s. It is difficult to exactly date these particles but based on the ideas of particles breaking down one can assume the microplastics are the result of having nylon manufacturing. The main plastic found was possibly polyimide which is a form of nylon. Even though the macroplastics collected do not fully relate to microplastics found, it is important to reduce the amount of trash getting in the watershed.From a report by OECD, only 9% of plastic is actually recycled. Lake James already has a presence of microplastics by adding macro debris to the watershed, which could result in the breakdown of plastic over time. It is important to be aware of the trash getting into the lake because the recent development of more chains in the area such as fast-food, could cause more litter to get into the lake.

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Plastic pollution is growing relentlessly as waste management and recycling fall short, says OECD Available at: https://www.oecd.org/environment/plastic-pollution-is-growing-relentlessly-as-waste-management-and-recycling-fall-short.htm.