RESEARCH PAPER
Microplastics in Our Water; a Study of Minnesota Lakes Indicated by *Dreissena polymorpha*

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Abstract
Microplastics, non-biodegradable particles made from polyethylene, are contaminating Earth's water and food supply. They have been found in bottled water, fish and even beer.[2] The pollution levels of the Great Lakes and the oceans have been tested extensively but those of Minnesota lakes have not. To understand the level of pollution and potential impact of microplastics to the Minnesota marine ecosystem, microplastics found in zebra mussel samples from three major Minnesota lakes were tested, measured and compared to those found in Lake Superior, which has significant microplastic pollution.[4] It was hypothesized that these three lakes are as contaminated as Lake Superior. Samples were collected from Pelican Lake and Lakes Superior, Minnetonka and Mille Lacs. Mussels from each lake were dissolved in a potassium hydroxide solution (10% KOH) which broke down the biological tissue but left microplastics intact and floating on the surface. Samples were viewed at 20x on a microscope using a hemocytometer. Microplastics were counted from each sample and an average per lake was taken and compared to that of Lake Superior. Lakes Mille Lacs and Minnetonka showed higher concentration of microplastics than Lake Superior and Lake Minnetonka was the highest with an estimated 25,290 pieces of microplastic per square km. This research shows that the microplastic pollution has accumulated over time and is negatively impacting Minnesota lakes. Even though water flows out of the lakes, microplastics can be ingested by fish or other wildlife and can sink to the bottom and get lodged in the sediments.[7]

The Problem
Microplastics are contaminating the water and food supply. Microplastics are defined as plastic pieces that are less than five mm in width. They can come from such things as microbeads, which are tiny, non-biodegradable polyethylene particles found in cosmetics, glitter, industrial products, cleansers and toothpaste. When people rinse away these products, the microbeads go down the drain. They also come from plastic microfibers washed away from clothing. By 2015, approximately eight trillion of these microbeads were introduced into U.S. waterways each day. In addition, larger plastic debris physically breaks up over time into smaller and smaller pieces and eventually turns into microplastics. By 2050, the ocean is expected to contain more plastic than fish.

These microplastics go right through the water treatment systems and end up in lakes, rivers and oceans. These microplastics have ended up in tap water, bottled water and in beer.[2] One study showed that 93% of bottled water from eleven different brands are contaminated with microplastics.[5] Also, scientists think that fish "stuff themselves" with these plastics because they are coated in bacteria and algae and so mimic their food.[1] The problem for humans is that a lot of these microplastics stay in the guts of the fish and end up in human food supply. The plastics contain chemicals that can migrate from the digestive tract into the flesh of the fish, which is what is consumed by humans. In the case of some sea creatures, like mussels and oysters, humans eat the entire organism so all the chemicals and the microplastics would be consumed. There are also other harmful chemicals in the marine environment that can get absorbed by the microplastics that then get leached out into the flesh of the fish. Some of these include PCBs (polychlorinated biphenyl), the pesticide DDT and other endocrine disruptors that can cause cancer. The extent of the damage to our waterways and the impact to human health is still unknown.

President Barack Obama signed legislation banning the production of products with microbeads starting in 2017 and phasing completely out by 2019. The problem with this is that the legislation only covers wash-off products and doesn't cover other products like cosmetics, detergents, clothing or industrial products. There are currently no FDA requirements that these ingredients be disclosed in products.[8]

Minnesota contains numerous lakes and waterways. People fish year-round and the level of microplastic contamination is unknown since no previous studies have attempted to evaluate the level of plastic pollution in three major Minnesota water bodies.

Current Evidence

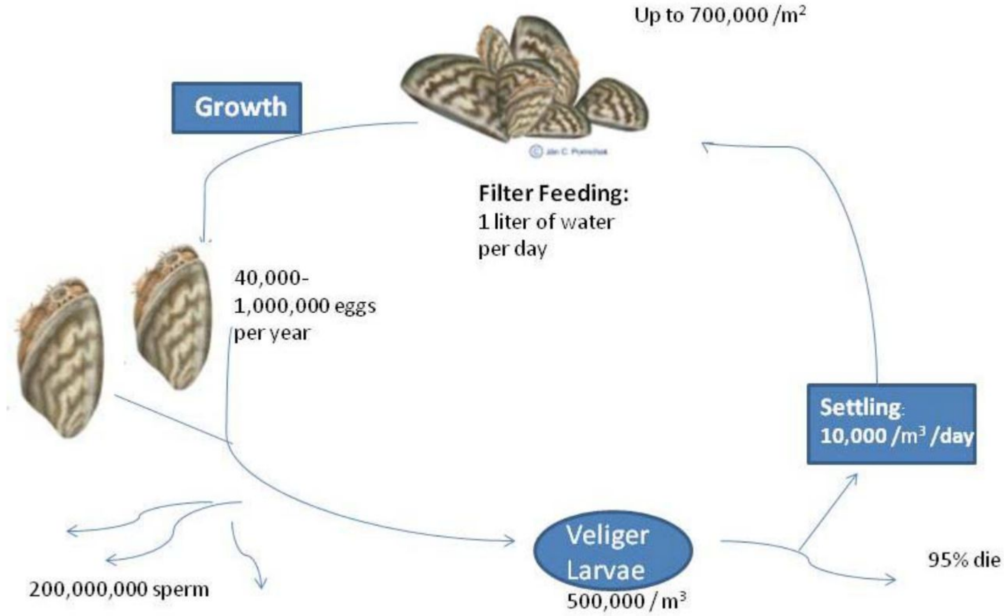
There is evidence that microplastics have contaminated the Great Lakes including Lake Superior. These plastics have also been found in tap water, bottled water and beer brewed with water from the Great Lakes. One recent study from the University of Minnesota took water samples from areas around the Great Lakes and found that eight out of nine tap water samples contained plastics.[2] Another study tested water treatment plants nationally and found that wastewater treatment facilities "are releasing over four million microparticles per facility per day."[3]

In 2013, Eriksen et al. found relatively high levels of microplastics in the surface waters of the Great Lakes. The study determined that Lake Superior contained up to 12,645 pieces of microplastic per km^2 (Eriksen et al.2013).[4] While the Great Lakes have been tested extensively, no testing was found regarding other Minnesota lakes, such as Minnetonka, Mille Lacs and Pelican Lake.

About *Dreissena polymorpha*

Dreissena polymorpha, commonly known as Zebra Mussels, are a freshwater mussel native to the lakes of Russia and the Ukraine. *Dreissena polymorpha* are small, fingernail-sized mollusks.This species has invaded many of the Minnesota lakes. These mussels tend to colonize hard substrates and surface in high densities, with as many as tens of thousands living in a square yard. *Dreissena polymorpha* were transported to the Great Lakes region through ballast water discharge from ocean going ships. It is an invasive species that is a filter feeder and can each filter up to one liter of water per day. These creatures filter pollution out of the water but do end up as food for some bottom-feeding fish. The lifespan of a zebra mussel is four to five years and, during that time, it can filter a significant amount of water in a lake and would be a good indicator of the amount of microplastics in the lake.

Zebra Mussel Life Cycle



Source: Cary Institute of Ecosystem Studies https://www.caryinstitute.org/sites/default/files/public/downloads/curriculum-project/zebra_mussel_fact_sheet.pdf

About Microplastics

Microplastics are tiny pieces of plastic that are less than 5.0 mm in size. They can be any type of plastic and come from many sources such as personal care products, clothing and industrial processes. They enter the environment and end up in water supplies as pollution. There are two classifications of microplastics, primary and secondary. The primary microplastics are those that are manufactured to the 5.0 mm or smaller size prior to entering the environment such as those found in exfoliating personal care products, microfibers from clothing and others. The secondary microplastics come from the breakdown of larger plastic pieces after they enter the environment. Plastic doesn't biodegrade; rather it breaks up into smaller pieces over time through weathering.

Unfortunately, microplastics are common. In 2014, there were an estimated 15 to 51 trillion individual pieces of microplastics in the oceans alone. This equates to 93 to 236 metric tons. Because plastic does not biodegrade, the microplastics can accumulate in the bodies and tissues of organisms such as fish after being ingested. Research is underway to investigate the cycle of movement of microplastics throughout the global environment but its full impact is not yet known.

Research Questions Addressed – Going Beyond Current Research

The main goal of this study was to understand the level of pollution and potential impact of microplastics to the Minnesota aquatic ecosystem. To do that, the amount of microplastics found in the biomass of zebra mussel samples taken from the following lakes was tested, measured and compared:

- Lake Superior
- Lake Minnetonka
- Lake Mille Lacs
- Pelican Lake

As the contamination to Lake Superior is already known, the levels of microplastic pollution in the other three lakes were compared to that of Lake Superior. It was hypothesized by the authors that these three lakes would be as contaminated as Lake Superior.

Methodology
The authors conducted a field study at each lake to collect several *Dreissena polymorpha* samples from rocks near the shoreline of each lake. The samples were left sealed for two to four days in water from the lake from which they were obtained. Initially the samples were stored in 10 ml Scientific Plastic Test Tubes Screw Top Tube Caps Vial Clear Seal Cap Pack Containers, Cylindrical Bottom. Then, three mussels of approximately the same size from each lake were transferred to 10 ml Scientific Plastic Sealable test tubes with ten milliliters of a ten-percent potassium hydroxide (10% KOH and 90% distilled H_2O) solution and left to sit at room temperature for a seven days. This solution broke down the mussels' biological tissues but left any microplastic contaminants intact. This method was used by A. L. Lusher, N. A. Welden, P. Sobral and M. Cole. to dissolve biological tissue in search of microplastics in 2017.[9] After seven days, the biological tissue was dissolved and the microplastics had floated to the surface for collection.

Experimental design:
A sample from each of the three zebra mussels from each lake was reviewed under a microscope on a hemocytometer slide (microscopic slide with a grid). Four cells within the grid were selected randomly from each sample and microplastics from these cells were counted and recorded. A total of twelve samples were taken.

Lake 1											
Zebra Mussel 1				Zebra Mussel 2				Zebra Mussel 3			
Cell 1	Cell 2	Cell 3	Cell 4	Cell 1	Cell 2	Cell 3	Cell 4	Cell 1	Cell 2	Cell 3	Cell 4

Microplastics were classified into five types:

1. Fibers
    - Usually curved/bent and uniform in thickness
    - Could be tangled
    - Thread-like
    - Commonly clear or of unnatural color
2. Fragments
    - Jagged ends
    - White or of unnatural color
3. Pellets/beads
    - Perfectly spherical and unbreakable
4. Foam
    - Springy / squishy
    - White or unnatural colors
5. Film
    - Looks like saran wrap

Counting microplastics under the microscope: Methods for plastic identification were adapted from those used for “Analysis of Microplastics in Water and Sediment,” *(Baker et al. 2011).*

1. One datasheet was used for each sample to record findings for each type of microplastic.
2. One microliter of the floating surface debris from each sample was placed on a hemocytometer and viewed under a microscope at 20x.
3. Microplastics were counted and classified according to the five types listed above and their descriptions.
4. Microplastic density was measured and recorded using grid lines on the hemocytometer as a boundary for each measurement.
5. Observed microplastics were counted one cell at a time, sorted into one of five categories (Fiber, Fragment, Pellet/Bead, Foam or Film) and recorded.
6. Four random cells indicated within the gridlines of the hemocytometer were inspected for microplastics for each sample.

The density of the plastics in each group were compared to that in Lake Superior (the control that has already been extensively tested) as a basis of comparison.

Risk and Safety

There was adult supervision during every stage of the experimentation and data collection. Lakeshore regulations and water safety rules were followed. Life jackets were worn when collecting all samples from lakes. The potassium hydroxide solution was used with adult supervision in a well-ventilated area (garage). All safety precautions were met and all necessary personal protection equipment (apron, gloves, goggles) were worn if required.

Data Analysis

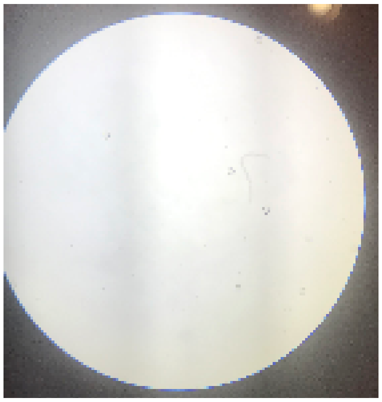


Image 1: Microscopic view of sample from Lake Superior

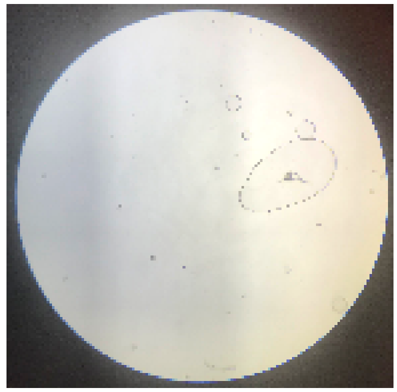


Image 2: Microscopic view of sample from Lake Minnetonka

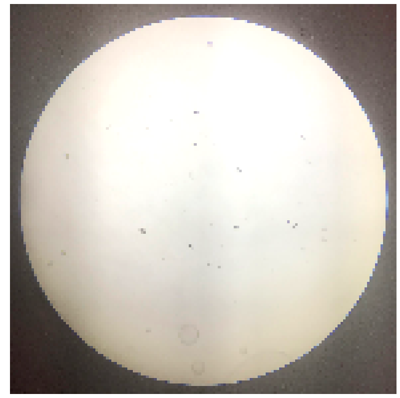


Image 3: Microscopic view of sample from Lake Mille Lacs

The density of the plastic found in each lake was calculated by averaging the count per sample (*Images 1, 2 and 3*). An example of the data collection can be found in Table 1, below. The results were compared by sample type for the purpose of drawing conclusions about the pollution levels of the other three lakes relative to those of Lake Superior. The microplastic contamination levels of Lake Superior have been estimated by Eriksen et al. and their study determined that Lake Superior contained up to 12,645 pieces of microplastic per square km (Eriksen et al.2013).[4] These estimates were used to extrapolate estimates for the total pollution for the other lakes.

Lake Minnetonka		Microbeads	Fibers	Filaments
Mussel 1	Cell 1	5	7	1
	Cell 2	5	6	0
	Cell 3	6	1	0
	Cell 4	29	3	2
Mussel 2	Cell 1	7	5	0
	Cell 2	7	2	2
	Cell 3	13	4	0
	Cell 4	7	11	1
Mussel 3	Cell 1	6	7	2
	Cell 2	8	5	1
	Cell 3	4	2	1
	Cell 4	6	7	2

Table 1: Results from samples taken from Lake Minnetonka

Results

Taking the average of the count from each of the four cells for each of three samples from each lake provides the results shown in Table 2. Lakes Mille Lacs and Minnetonka both show higher numbers of microplastics per sample, on average, than Lake Superior which has already been shown to have significant microplastic pollution. Using estimates of Lake Superior from Eriksen et al. 2013,[4] the number of pieces of microplastic per km^2 were estimated for each lake (Table 3). Lake Minnetonka is estimated to have 25,290 pieces of microplastic per km^2. This estimate was obtained by using the following ratio:

$$\frac{\text{Sum of microplastics found in each lake}}{\text{Estimated \#of pieces of microplastic per km}^2} = \frac{\text{Sum of microplastics found in Lake Superior}}{\text{\# in Lake Superior estimated by Eriksen et al. 2013}^4}$$

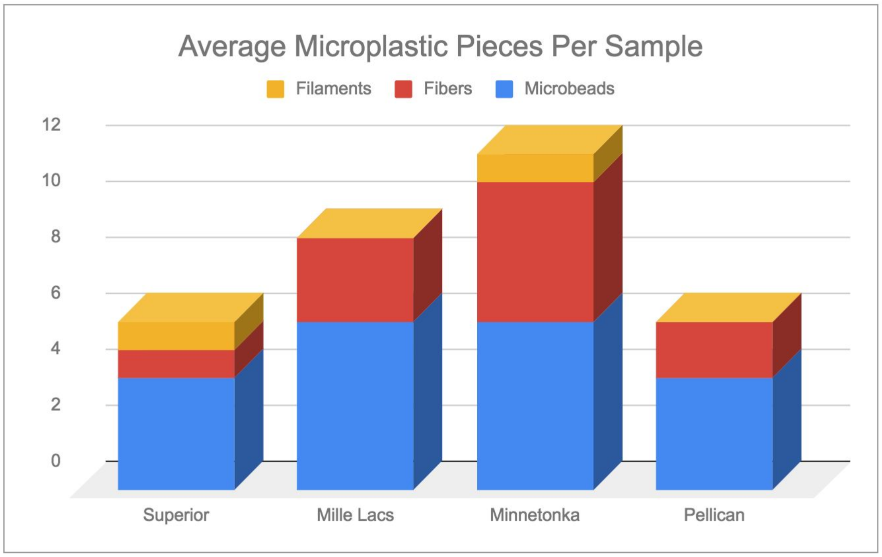


Table 2: The average number of microplastics found per cell in each of three samples from each lake

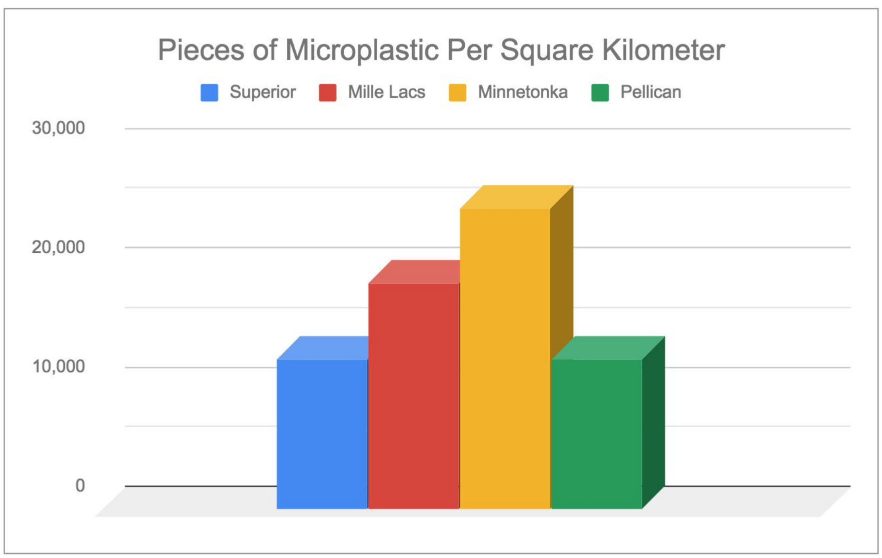


Table 3: The estimated number of microplastics per square km in each lake based on the count from the samples

Conclusions

By calculating the average of the three samples from each lake, it was found that all three of the lakes that were tested had greater than or equal the amount of microplastic contamination as Lake Superior. Lakes Minnetonka and Mille Lacs both contain more plastic contaminants than Lake Superior with Lake Minnetonka showing the highest degree of contamination. The contamination in Pelican Lake was found to be similar to that of Lake Superior.

This research shows that the microplastic pollution has accumulated in Minnesota lakes. Even though water flows out of the lakes, microplastics can be ingested by fish or other wildlife and can sink to the bottom and get lodged in the sediments.[7]

Plastic pollution in the Great Lakes areas generally comes from sources including pollution from microplastics found in consumer and industrial products as well as from trash on shorelines being left behind by people. The plastic travels through watersheds from beaches into lakes. Then it flows out of the lakes into streams and rivers and flows into the ocean.[6] Lake Minnetonka, for example, has a problem with holiday-makers and summer beachgoers polluting the shores by leaving significant amounts of trash behind (Image 4).

Image 4: Lake Minnetonka 4th of July trash clean-up

Source: https://www.google.com/search?q=lake+minnetonka+trash&tbm=isch&source=iu&ictx=1&fir=C9tNxd513YFE6M%253A%252Ch-ItZTYAtBYpkM%252C_&usg=AI4_-kS_NjI6_GK0JyM_OXufjSbn-MhfMg&sa=X&ved=2ahUKEwjm8ab36I7gAhUOtIMKHXRsBlkQ9QEwA3oECAAQBA#imgrc=C9tNxd513YFE6M

Limitations and Next Steps

There are some limitations to this study and additional research should be performed. Some additional factors to include could be the following:

- More samples per lake could be tested
- Location and depth of mussels sample collected could be more varied. For example, getting some mussels close to share, some from deeper in the lake and collect from various points around the lake
- Lake turnover rates could be considered. For example, the lakes that turnover their water faster may have less contamination than those that hold the water longer before it goes downstream.
- Flow from treatment plants could be considered. Those lakes that have a water treatment plan in the path of their source would likely have more contamination
- Size and estimated age of zebra mussel samples should be considered. Older (larger) zebra mussels would have filtered more water during their life and would likely contain more microplastics.

Preventing the pollution from entering the lakes in the first place is the best way to prevent this from continuing. Finding out which types of plastic pollutants are the most damaging would also help target what products to target to help fix the problem. The authors of this experiment would like to conduct further research into the impact of microplastic contamination to the sediment in the lakes as well as to fish and other living organisms.

President Barack Obama signed legislation banning the production of products with microbeads starting in 2017 and phasing completely out by 2019. Since this legislation only covers wash-off products and doesn't cover other products like cosmetics, detergents, clothing or industrial products, the microplastic pollution will likely continue.

Bibliography

- [1]*The Guardian,* **Fish for dinner? Your seafood might come with a side of plastic, access 6/15/18,** https://www.theguardian.com/lifeandstyle/2016/aug/31/fish-plastic-pollution-ocean-environment-seafood.
- [2]*Inforum* (May, 2018), **Plastic found in most Great Lakes tap water, beer,** https://www.inforum.com/news/science-and-nature/4443850-plastic-found-most-great-lakes-tap-water-beer.
- [3]*ResearchGate* (August, 2016), **Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent,** https://www.researchgate.net/publication/307510890_Microplastic_pollution_is_widely_detected_in_US_municipal_wastewater_treatment_plant_effluent.
- [4]Clemson University (2017), **Quantification of Microplastic Pollution on National Park Beacher,** https://marinedebris.noaa.gov/sites/default/files/publications-files/Quantification_of_Microplastics_on_National_Park_Beaches.pdf.
- [5]Wikipedia**. Microplastics.** https://en.wikipedia.org/wiki/Microplastics
- [6]Alliance for the Great Lakes. (2018). **5 Ways Plastic Pollution is Different in the Great Lakes**. https://greatlakes.org/2018/06/5-ways-plastic-pollution-is-different-in-the-great-lakes/
- [7]The Conversation. **Tons of plastic trash enter the Great Lakes every year – where does it go?** http://theconversation.com/tons-of-plastic-trash-enter-the-great-lakes-every-year-where-does-it-go-100423
- [8]Huffpost. 2016. Alexander C. Kaufman. **Obama's Ban On Plastic Microbeads Failed In One Huge Way.** https://www.huffingtonpost.com/entry/obama-microbead-ban-fail_us_57432a7fe4b0613b512ad76b.
- [9]Royal Society of Chemistry. A. L. Lusher, N. A. Welden, P. Sobral and M. Cole. 2017. **Sampling, isolating and identifying microplastics ingested by fish and invertebrates.** https://pubs.rsc.org/en/content/articlepdf/2017/ay/c6ay02415g
- Clearwater News and Bulletins. **What Are The Human Health Effects Of PCBs?** https://www.clearwater.org/news/pcbhealth.html.
- Chemistry World. 2016. **US bans microbeads from personal care products.** https://www.chemistryworld.com/news/us-bans-microbeads-from-personal-care-products/9309.article.
- Wikipedia. **Zebra Mussels.** https://en.wikipedia.org/wiki/Zebra_mussel.
- Carey Institute. **Zebra Mussel Fact Sheet**. https://www.caryinstitute.org/sites/default/files/public/downloads/curriculum-project/zebra_mussel_fact_sheet.pdf.
- Kim, Lisa, McNeish, Rachel, Hoellein, Timothy. (June 2016). **Analysis of Microplastics in Water and Sediment**. A paper provide by Timothy Hollein, Loyola University, Chicago. This paper is not published and is available upon request.
- Journal of Great Lakes Research. 2014. **Abundance and environmental drivers of anthropogenic litter on 5 Lake Michigan beaches: A study facilitated by citizen science data collection**. *https://www.sciencedirect.com/science/article/pii/S0380133014002767.*