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Contact: Prof. Bassam Z. Shakhashiri

UW-Madison Department of Chemistry

bassam@chem.wisc.edu

www.scifun.org

Detection of Low Micrometer Microplastics and Nanoplastics in Freshwater by Coupling Raman Spectroscopy, Membrane Sensors, and Machine Learning Algorithms

By

Ziyan Wu

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The dissertation is approved by the following members of the Final Oral Committee:
Mohan Qin, Assistant Professor, Civil and Environmental Engineering
Haoran Wei, Assistant Professor, Civil and Environmental Engineering
Sarah Janssen, M3 Laboratory Lead, U.S. Geological Survey
Christy Remucal, Professor, Civil and Environmental Engineering
Erica Majumder, Assistant Professor, Bacteriology

Appendix A. A Macro Environmental Issue Caused by Micro-pollutants: Unveiling the World of Low-Micrometer Microplastics and Nanoplastics

– A chapter for the general audience

One of my goals in writing this chapter was to open a window into my doctoral research for readers without a scientific background. I wanted to communicate not only what I studied, but why it matters, in a way that is accessible to a wider audience. I am sincerely grateful to the Wisconsin Initiative for Science Literacy (WISL) at UW–Madison for supporting this effort and providing the opportunity to create this chapter. My heartfelt thanks also go to Professor Bassam Shakhashiri, Elizabeth Reynolds, and Cayce Osborne for their guidance, feedback, and encouragement. I also want to thank my advisors, Dr. Mohan Qin and Haoran Wei, along with all co-authors and collaborators, for making this research possible.

What are low micrometer microplastics (LMMPs) and nanoplastics (NPs)?

We have long worried about plastic bags choking sea turtles or bottles piling up in landfills. But a quieter, more pervasive environmental threat has been building in the shadows, one measured not in feet or inches, but in millionths of a meter. These are microplastics and nanoplastics. Microplastics and nanoplastics (NPs) are often defined as plastic debris smaller than 5 millimeters. In this study, low micrometer microplastics (LMMPs) are defined as plastic debris smaller than 0.1 millimeters and NPs smaller than 1 micrometer.

LMMPs and NPs (LMMNPs) may sound tiny, and they are: they are on a scale where a human hair is enormous. The U.S. Environmental Protection Agency notes that a strand of hair is about 0.08 millimeters wide. Compared to a human hair, LMMPs are a similar size, and NPs are 80 times smaller. Collectively, the size definition of LMMNPs covers a broad range of plastic debris from those that are visible to the naked eye to those at the nanoscale that are only visible with advanced microscopy methods. Besides the broad size range, the chemical composition of LMMNPs also encompasses a wide range of plastic types that are commonly found in our daily life, such as polyethylene terephthalate (PET) used for plastic water bottles and polyethylene (PE) used for food containers.

Where are LMMNPs from in the natural environments?

Where do LMMNPs come from? The answer is: nearly everything that is made of plastic.

When a car drives down the road, its tires shed microscopic rubber and plastic particles with every rotation. Those particles drift into the air, settle onto roads, wash into storm drains, and eventually reach rivers and oceans. Studies estimate that tire wear alone contributes hundreds of thousands of metric tons of microplastics to the environment each year, making it one of the largest single sources globally.

Synthetic clothing is another major contributor. A single load of laundry can release hundreds of thousands of tiny plastic fibers from fabrics like polyester and nylon. These fibers pass through washing machine filters, through wastewater treatment plants — which are not designed to catch particles this small — and into waterways (Figure A.1).

Floating plastic trash in the natural environment is another source for LMMNPs. Ultraviolet (UV) radiation from direct sunlight relentlessly bombards the discarded plastic, initiating a chemical process known as photo-oxidation. This process fundamentally weakens the chemical bonds in the plastic. Simultaneously, environmental mechanical forces, such as the violent churning of ocean waves, the grinding action against coastal rocks and beach sand, and the stress of fluctuating seasonal temperatures, shatter the chemically weakened plastics into progressively smaller and more brittle fragments.

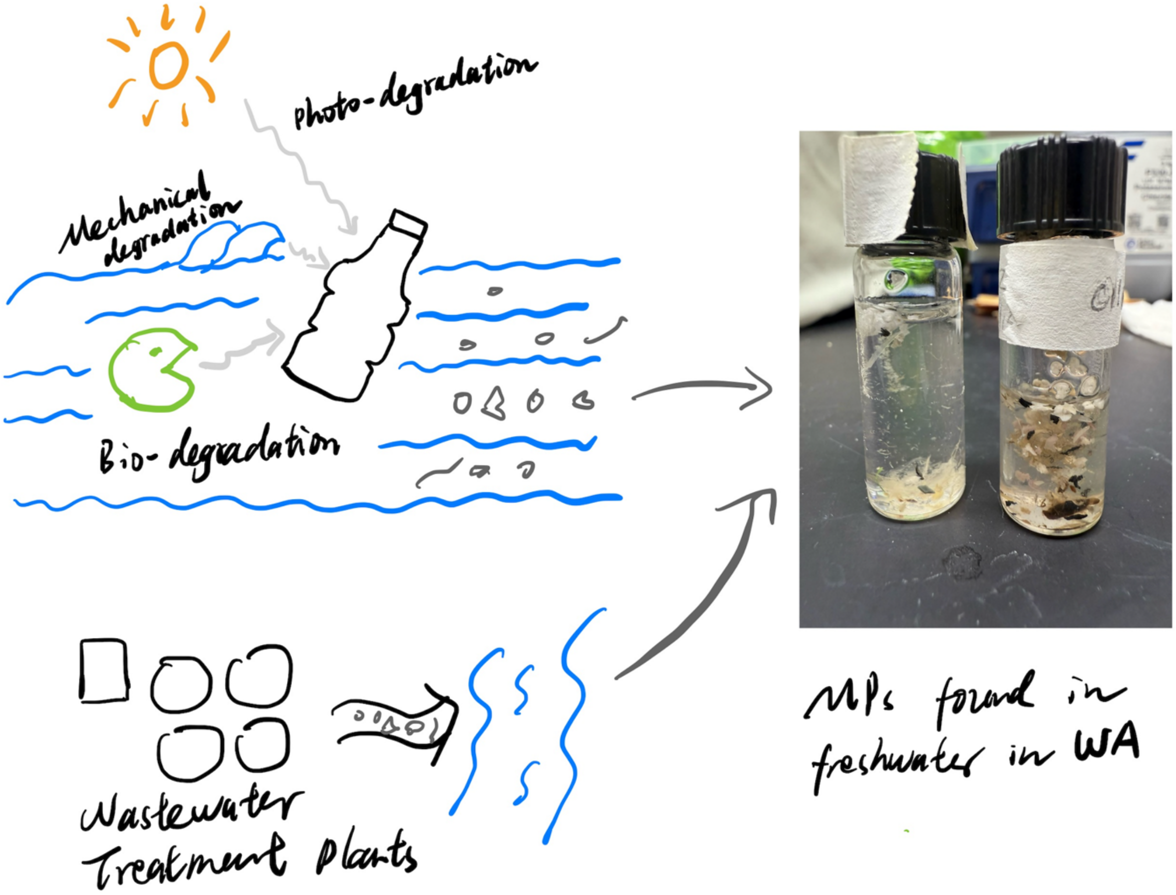

Figure A. 1. The pathways of LMMNPs into the natural environment.
LMMNPs in the natural environment can originate from the degradation of floating plastic trash (classified as secondary LMMNPs) or from wastewater treatment plant discharge (which contains both primary and secondary LMMNPs). The picture on the right-hand side shows the large microplastics found in a freshwater reservoir in the State of Washington.

Why do we care about LMMNPs in freshwater environments?

LMMNPs are not just tiny pieces of litter. For aquatic life, their small size can make them more dangerous than larger plastic fragments. LMMNPs are small enough to be taken up by organisms such as microalgae, zooplankton, and fish. Previous studies show that these particles can reduce survival, growth, and reproduction, threatening species such as algae, sea urchin, and mussels.

A fish that mistakes a plastic particle for food does not just consume the plastic. It may consume pollutants that the particle has absorbed on its journey through the environment.

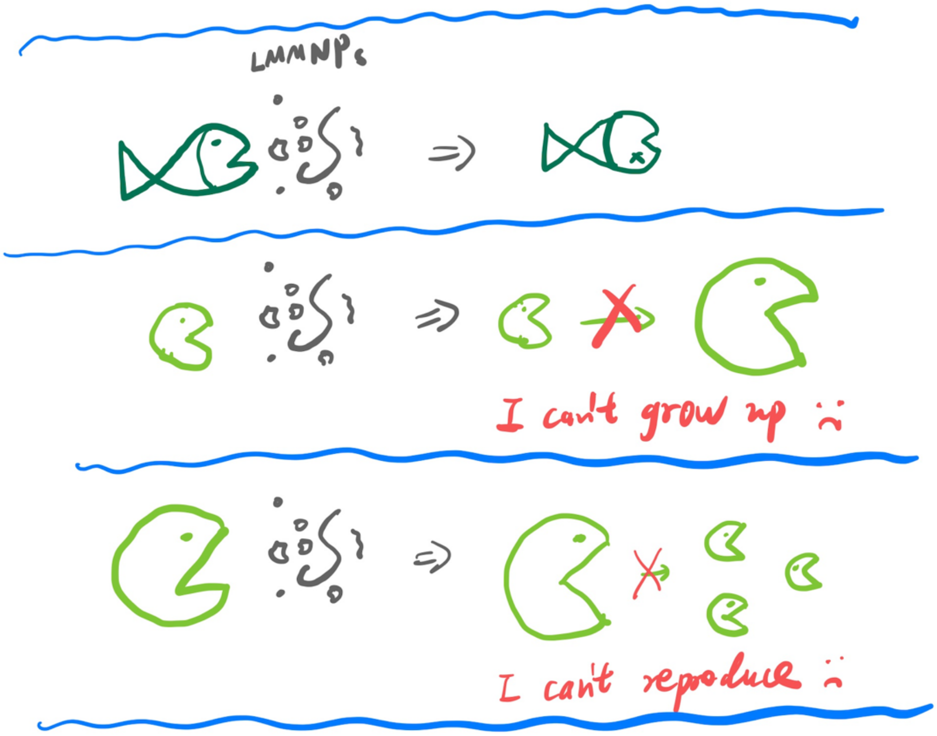

Figure A. 2. Potential health risks of LMMNPs on aquatic species.

Because LMMNPs are so small, they also have a larger surface area relative to their size, which means they can more easily carry other harmful chemicals, such as toxic organic pollutants or heavy metals, and potentially make their effects worse. Smaller LMMNPs also tend to remain longer in tissues and accumulate more readily than larger plastic pieces.

NPs raise even greater concern. Compared with larger LMMNPs, they are generally easier to be mistakenly eaten by aquatic species and more toxic. Once ingested, NPs can clump in the gut, move into tissues and cells, and interfere with normal cellular functions. As plastic particles shrink, they become easier for living things to take up and harder for those organisms to avoid.

What are the challenges to detecting LMMNPs?

Detecting LMMNPs, especially NPs, in freshwater is often like trying to count and identify a few specific grains of sand in a jar of mixed spices. Finding grains, in this case, may be even easier compared to detecting LMMNPs in freshwater, since we probably know the number and type of grains, but the particle concentrations of LMMNPs can vary by orders of magnitude, and their shape, size, and types are extremely diverse.

More specifically, these challenges dominate in LMMNP detection:

They are tiny. The size scale of LMMNPs is reaching the limit of optical instruments. The smaller you go, the harder it is to see, focus, and confidently identify these particles.

Natural water is messy. Freshwater, such as lake and river water, carries algae bits, mineral grains, and organic matter that can look like plastics in a microscope and can interfere with spectroscopic signals. In addition, the water composition in each lake, each river, and each pond is different. This water variation leads to different interferences during spectroscopic examination, making the detection of LMMNP challenging.

The act of measuring can contaminate the sample. Laboratory materials, such as plastic laboratory products and lab coats, and sample processing steps can introduce contaminations to the samples. Without proper quality control during these procedures, the unintentional contamination can lead to misidentification of LMMNPs.

Standards and definitions are still evolving. LMMNPs, or even microplastics research in general, has historically lacked consensus on definitions and categorization, creating confusion and slowing progress. Standards are now emerging for larger microplastics, but smaller size classes, including LMMNPs, remain particularly challenging and can fall outside some monitoring methods intended for larger microplastics.

How do I address these challenges in this work?

My work is built around a simple idea to address these challenges for LMMNP detections in freshwater:

If LMMPs and NPs are hard to detect because they are small, rare, and easily confused, then I should utilize capable and practical instruments and redesign the surface of the analytical platform they land on and the way the signal is processed, so the tiny plastic signature stands out.

I chose *Raman spectroscopy* to reveal the LMMNP world because of its fast speed, operation simplicity, and its potential for detecting nanoscale LMMNPs. Raman spectroscopy is a way of identifying chemicals by using light, much like reading a molecular fingerprint. In simple terms, a laser is shone onto a sample, and most of those light bounces back unchanged. But a very small portion interacts with the chemical bonds inside the material and returns with slightly different energy. Those tiny energy shifts create a pattern that reflects how the atoms in the material are connected. Because different substances produce different patterns, Raman spectroscopy can help tell one chemical from another without destroying the sample. In this work, that makes Raman especially useful for studying LMMNPs in water, because it can reveal not only that a particle is present, but also what kind of plastic it is.

With Raman, my work centers around the following four themes to develop practical and rapid detection methods to reveal the LMMNP world in freshwater environments:

Fractionated membrane filtration (sorting by size before looking): Previously, researchers usually measured microplastics of all sizes at once. However, larger microplastics may overwhelm the signals from smaller ones, leading to the underestimation of their concentrations. One of my earlier studies uses fractionated membrane filtration to separate LMMPs into narrower size bins (reported as 1–5 micrometers and 5–10 micrometers fractions), which improves the reliability of Raman spectroscopic imaging and LMMP quantification because each filter captures a more uniform particle-size class.

Engineered plasmonic membranes for LMMPs (a "filter that is also a sensor"): When analyzing LMMPs in the previous studies, LMMPs were usually separated from water by membrane filters. A membrane filter is a very thin material with tiny pores that catch small particles from water while letting the water pass through. The separated LMMNPs were then transferred to a specific surface for Raman analysis. This may sound simple, but it involves multiple steps during the sample handling process, which may introduce contaminants to the sample during these steps. To simplify this process and reduce the potential sample contaminants, I came up with *membrane sensors*.

I developed gold-coated plasmonic membrane sensors that act as both a membrane filter and a Raman surface to simplify sample separation and analysis. This membrane sensor also enhances Raman intensity (48% ± 25% compared to unmodified membrane filters), reduces background interference, and enables a detection limit of 1 microgram per liter (µg/L) with an ultrafast 0.01-second scan time for individual LMMPs in eutrophic lake water. Eutrophic water is water that is rich in nutrients, which often leads to large amounts of algae and plant growth and can potentially create difficulties for LMMP analysis. Despite this, LMMPs are visualized and identified on the membrane sensors. The detection limit of 1 µg/L is comparable to the environmentally relevant concentration of LMMPs in freshwater environments that were reported previously, demonstrating the potential of deploying my membrane sensors for the rapid and reliable detection of LMMPs in freshwater.

AAO membrane sensors for NPs (low-background, reusable surfaces): For NPs, I used anodic aluminum oxide (AAO) membrane sensors as both filters and Raman imaging substrates because AAO membrane sensors would not interfere with the analysis for NPs. AAO membrane sensors are flat, homogeneous, and suitable for distributing and imaging individual NPs on the surface. I also demonstrated that the AAO membrane sensors can be regenerated and reused after multiple reusing cycles by baking the AAO membrane sensors under 550 °C and reusing the baked ones for NP analysis. This regenerable AAO membrane sensor provides a more sustainable and economically feasible sensing tool for NP detection.

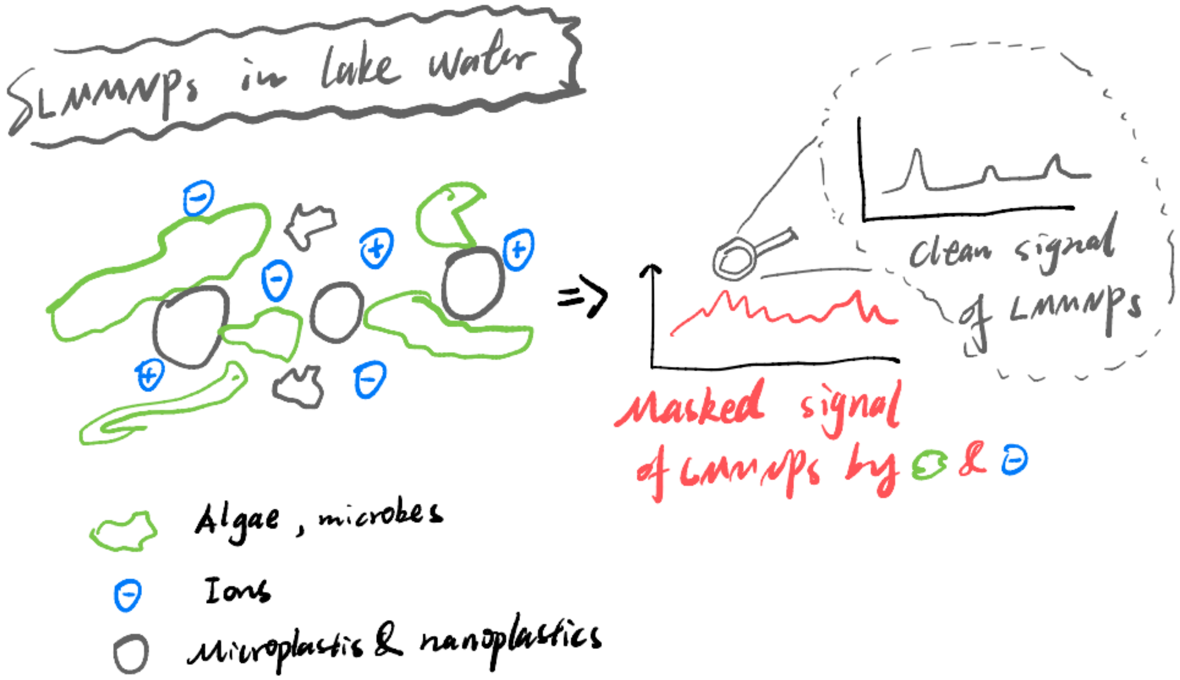

Figure A. 3. In lake water, there could be algae, organic matter, sediments, or ions that cover LMMNPs and complicate the Raman spectra of LMMNPs (red spectra). Methods are needed to clean up the sample and make the signals of LMMNPs easier to recognize (grey spectra).

Data-driven preprocessing for LMMNP detection (separating LMMNP signals from "spectral clutter"): When detecting LMMNPs in real freshwater samples, background interference from algae, organic matter, and sediments often generates a complex and disordered set of Raman spectra that can overwhelm the signals from LMMNPs. To better sort out and identify LMMNP signals, especially the weak signals of NPs, among thousands of Raman spectra, I turned to data-driven algorithms and artificial intelligence (AI).

I introduced Pre_seg, a streamlined Raman spectral processing algorithm that uses baseline correction, peak detection, and denoising based on signal-to-noise ratio, a measure of how strong and clear a useful signal is compared with unwanted background noise, and peak width to reduce false positives. It reports 93.5% prediction accuracy for NPs, and ≥90.4% rejection accuracy for non-NP spectra, with mixed NPs quantified down to 0.5 microgram per liter.

Optimizing the sale pretreatment process (cleaning up the messy matrix of freshwater): When scientists get samples of LMMNPs in freshwater, the first thing they need to do is clean the sample and isolate LMMNPs from the water samples for later analysis. Currently, there is no standardized sample pretreatment process for LMMNPs. Inappropriate sample pretreatment may lead to LMMNP loss, damage, or contamination during the handling processes. I systematically tested one of the commonly used pretreatment methods, oxidative digestion, to find out the interaction between the oxidant (hydrogen peroxide), NPs, and water matrix (everything present in a water sample, such as salts, natural organic matter, algae, minerals, and other particles, that can affect how the sample behaves during testing) to guide the optimization process for sample pretreatment. Oxidative digestion uses oxidants (such as hydrogen peroxide) to break down unwanted natural material. It is a common method to clean a sample.

I sampled four types of freshwater in and around the Great Lakes Basin. The study demonstrated that the amount of hydrogen peroxide needed for pretreatment and the necessary digestion time both depend on the type of water being analyzed. When the freshwater is relatively clean, like Lake Michigan water, a little bit of oxidant and a short digestion time (1.5% hydrogen peroxide with 2 hours of digestion) will be sufficient to clean up the water matrix for NP detection. However, for more complicated freshwater systems with higher organic content, like one of the Great Lakes tributaries in the St. Louis River, more oxidant and a longer digestion time (6% hydrogen peroxide with 24 hours of digestion) is needed to clean up the sample matrix. My results also indicate that if the samples are digested for too long or with too much oxidant, NPs will be damaged, leading to the underestimation of NPs in the sample.

How does my work affect society?

Since the discovery of small plastic debris in the ocean in the 1970s, attention from the scientific community has surged around microplastics and nanoplastics in the natural water environment. Microplastics and nanoplastics have permeated environments ranging from surface waters and groundwater aquifers to the deep sea and remote mountains. However, our understanding of their occurrence, fate, and toxicity is still largely limited. This limitation primarily stems from challenges in their detection. Microplastics and nanoplastics include a wide range of sizes and chemical compositions of plastic debris, making it challenging to accurately identify and detect them, especially for smaller ones like LMMNPs. Even though LMMNPs potentially have higher toxicity and may be present in higher concentrations in freshwater environments, they are much more difficult to detect than larger microplastics.

My work tries to address this challenge, aiming to advance our understanding of LMMNPs across freshwater systems. The *membrane sensors* I developed demonstrate a detection limit of LMMNPs at 1 μg/L, which is comparable to the environmentally relevant concentration of LMMPs in freshwater environments. The Raman spectral processing algorithms I created offer over 99% accuracy to detect nanoplastics and reject non-nanoplastics signals, which largely advances the detection limit of nanoplastics across freshwater systems compared to the existing Raman-based detection methods. By investigating the interaction between hydrogen peroxide and nanoplastics, my work provides some insights to better guide the design of hydrogen peroxide concentration and digestion length to clean up water samples for better NP detection. Overall, my work provides fast tools and algorithms for the reliable detection of LMMNPs in freshwater at environmentally relevant conditions, providing a solid foundation to advance our understanding of the abundance, fate, and toxicity of LMMNPs across freshwater environments.