Microplastic Detection in Lawaye River, San Juan, Batangas City, Philippines Using Front-Face Fluorescence Spectroscopy

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Abstract: The Lawaye River in San Juan, Batangas plays a crucial role in sustaining the very complex local ecosystem within the city. As it is utilized for irrigation for agricultural activities, household needs, fishing, and tourism. However, increasing human activities pose significant threats to these vital bodies of water. Pollution, particularly from microplastics, is a major concern due to its detrimental impacts on aquatic life and potential human health risks. Studies have demonstrated the widespread presence of microplastics in various organisms and their ability to accumulate in vital organs, including the brain. This study investigated the presence of microplastics in the Lawaye River. Surface water samples (50 cm depth) were collected and subjected to initial debris removal. Subsequently, samples were treated with a KOH solution to dissolve organic matter and filtered through a 0.3 mm glass filter. Microscopic examination revealed the presence of microplastics in various forms, including fragments, fibers, and films. Further fluorescence spectroscopy analysis, based on known excitation-emission wavelengths of different plastics, suggested the potential presence of microplastics, specifically Polypropylene (PP) and potentially Polystyrene (PS) which is commonly used on single-use plastics.

1 INTRODUCTION

The Lawaye River is a freshwater class B type of River located in San Juan, Batangas plays a crucial role in sustaining the complex local ecosystem within the city. As it is being utilized for irrigation for agricultural activities from farming, rice paddies, crop cultivation, household needs, fishing, and tourism, it faces increasing pressure from human activities such as agricultural runoff, pollution, being near the town's public market, and due to the growing population in Batangas (Rochman, Hoh, Kurobe,, Teh, & & Teh, 2023) This intensified human activity has resulted in a steady increase in pollution, posing a serious threat to the aquatic ecosystems and the organisms that inhabit them. Recent studies, such as that of Ziani on Microplastics have documented the alarming presence of microplastics in various animal species. These tiny plastic particles can accumulate in vital organs, including the liver, spleen, heart, lungs, and even brain, due to their ability to cross the blood-brain barrier (Ziani, et al., 2023). Given the persistence of plastics in the environment – taking centuries to fully degrade while readily fragmenting into smaller, more easily ingested microplastics (Andrady, 2011) – this creates a pathway for microplastics to enter and ascend the marine food web.

Consequently, the presence of microplastics in aquatic ecosystems poses a significant and ongoing threat to the entire food chain. Addressing this concern requires efficient and cost-effective analytical techniques that can readily identify their presence in the environment, such as fluorescence spectroscopy. This technique offers a powerful yet affordable alternative to methods in identifying the

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presence of microplastic as well as characterizing them like Scanning Electron Microscopy (SEM), which can be costly and destructive to the sample. Fluorescence spectroscopy works by sending off light to the plastic particles with a specific wavelength of light, causing them to excite and then emit a fluorescent at a different, and usually longer wavelength. The excitation, emission light, or fluorescence is unique to each and different types of plastic that can be used to differentiate them from one another. By analyzing the fluorescence spectrum, researchers can determine the types of plastics present in a sample (Syed, Aisha, Murugesan, Hill, & Rozhin, 2024). This technique is particularly useful in identifying and quantifying the number of microplastics present in a sample due to its high sensitivity and ability to detect even the smallest particles (A. L. Lusher, N. A. Welden, P., & M., 2016). To assess the extent of microplastic contamination and to test the detection capacities of fluorescence spectroscopy presence of microplastic in filtered specimens in the Lawaye River, the study employed fluorescence spectroscopy accompanied by Microscopy to check for the presence of microplastic and to identify and characterize the types of microplastics present in surface water samples.

2 MATERIALS AND METHODS

The surface water samples were collected from the bridge that connects 2 roads in Lawaye River near the public city market in Batangas City, Philippines, with the coordinates 13°49'20"N 121°23'48"E as indicated in Figure 1.

2.1 Collection of Samples

The collection of water samples and sample preparation follows the methodology described by Gabriel, et al. (2023) with minimal changes. Water samples were collected using a stainless-steel bucket, taking a 10-liter sample at a depth of up to 50cm, and subsequently filtered using a 0.3 mm metal sieve to remove large debris before being transferred to amber bottles for storage.

2.2 Sample Preparation

To prepare the sample for an initial investigation using Olympus BX51 Microscope, the samples were prepared by mixing them with a 2:1 sample ratio to a 20% concentration of KOH and water solution. KOH

** Coordinates: 13°49'20"N 121°23'48"E*

Figure 1: Location of Lawaye River Samples.

is an effective chemical that digests organic matter without damaging other components. The samples were then stirred for an hour. Due to the uneven heating capabilities of the magnetic stirrer present, the samples were not heated and just maintained at room temperature for 1 hour while stirring to prevent the degradation of the physical and chemical properties of the microplastic. The resulting mixture containing the microplastics was then filtered using a 0.3 mm Whatman GF/C glass filter on a vacuum filtration setup consisting of a Glass filter between the Buchner funnel and the Buchner flask held together by a clamp the vacuum is connected to the Buchner Flask to create a negative pressure at the bottom of the setup as shown in Figure 2. After the sample were passed through the filter, the glass filter was carefully removed using clean tongs placed in a clean petri dish, and airdried inside a vacuum chamber for 2 days.

2.3 Microscopy

Using an Olympus BX51 with an eyepiece magnification of 10x and an objective lens magnification of 4x, giving us a total of 40X magnification, each dried filter was visually examined to identify and categorize the types of microplastics present, such as fibers, fragments, and others. The length and colors were also noted through visual inspection using NIS ELEMENTS D 4.6.000 software of NIKON.

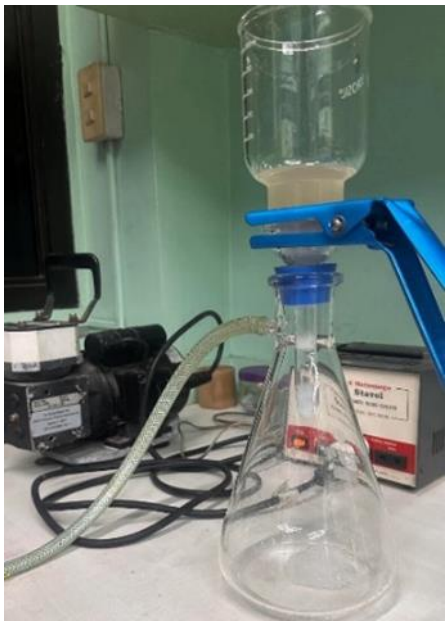


Figure 2: Water Filtration Setup.

2.4 Fluorescence Spectroscopy

Due to the limitations of access in the laboratory, the next analysis was done after 2 weeks from the digestion of organic matter within the sample. Fluorescence spectroscopy was performed in the filter sample. The filter sample is prepared by cutting the filtered sample into a strip where the suspected microplastic is located as shown in Figure 3 (left).

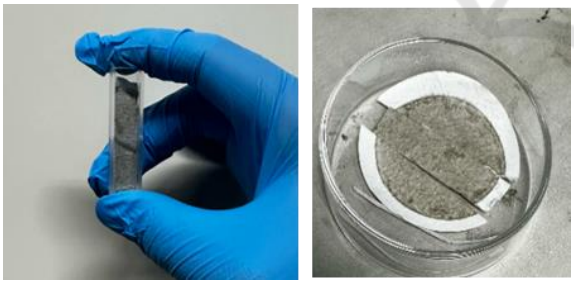


Figure 3: Cutting of the sample (Left) and Sample in the Cuvette (Right).

A front-face-fluorescence spectroscopy (FFFS) was performed on the air-dried filter. The block diagram of the FFFS system is shown in Figure 4. The light source is a broadband Xenon lamp with a spectral range of 120 to 2000 nm. Light from the lamp was guided by an optical fiber to a scanning monochromator (MonoScan 2000) for wavelength selection. From the monochromator, an optical fiber directs the beam to the filter that was placed inside the cuvette in such a way that the incident light is at a right angle to the emitted light that goes through another optical fiber that is connected to the spectrometer, which is an Ocean Optics 2000+ XR1-ES, to measure the fluorescence emission spectrum. A fluorescence excitation-emission (FLE) map was then obtained by following the known excitation-emission peaks from Table 1 for the filter with known microplastics.

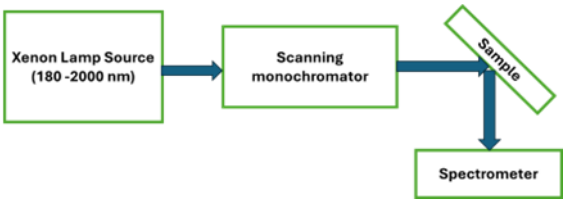


Figure 4: Block diagram of the FFFS set-up.

Table 1: Plastic Fluorescence Excitation - Emission Pairings (Syed, Aisha, Murugesan, Hill, & Rozhin, 2024).

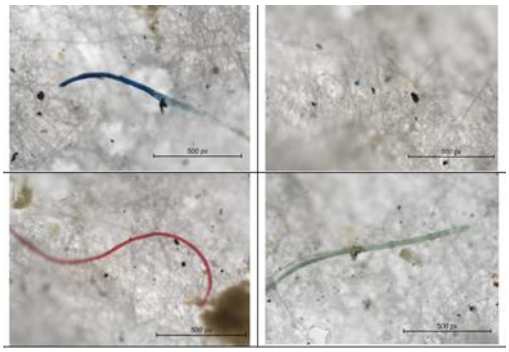
Plastic	**Excitation (Peak)**	**Emission (Peak)**
Polystyrene (PS)	300–400 nm (360 nm)	350–450 nm (380 & 405)
Polyethylene terephthalate (PET)	330 and 380nm & 380 and 485 nm (360nm)	370 and 510nm & 400 to 530 nm (390)
Polypropylene (PP)	360 and 380 nm & 385 and 430 nm (370)	400 and 550 & 425 and 550 (455)

3 OBSERVATIONS

Microscopic examination of surface water samples from the Lawaye River revealed the presence of microplastics ranging in size from 0.5 µm to 1000 µm, consistent with the generally accepted definition of microplastics as particles between 1 µm and 5000 µm (Syed, Aisha, Murugesan, Hill, & Rozhin, 2024).

The observed microplastics exhibited known morphologies of microplastics, including fragments and fibers, and a range of colors, with blue, red, and green being the most common as illustrated in Figure 4.

The sample underwent fluorescence spectroscopy to identify the type of plastic detected within the film. To identify the type of plastic, the excitation-emission pairings were analyzed.



*Legend: *500 px = 500 um*

Figure 5: Sample Microphotograph.

Fluorescence analysis within the visible light spectrum (400-420 nm) revealed a broad emission peak between 490-550 nm. Figure 6. This emission profile is characteristic of Polypropylene (PP) plastics as it is compared with the emission-excitation peak of PP plastic in Table 1, suggesting their presence within the sample.

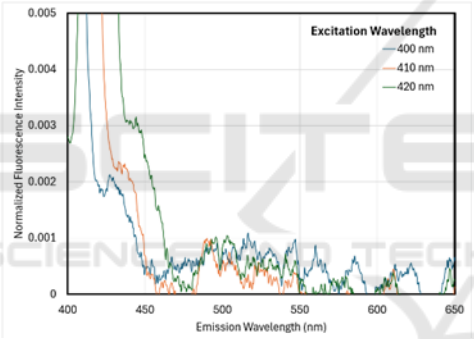


Figure 6: Visible Light Fluorescence Analysis.

UV fluorescence (300-320) Figure 7 Reveals a broad peak between 370-420 nm. A prominent peak is observed at an excitation wavelength of 310 nm, with emission peaking around 373 nm. This spectral profile strongly suggests the presence of Polystyrene (PS), as it exhibits greater similarity to the characteristic PS emission-excitation wavelengths (Table 1) compared to Polyethylene Terephthalate (PET), which typically displays a broader emission spectrum from 370-510 nm with a peak at the UV range.

The presence of microplastics, specifically Polypropylene (PP) and potentially Polystyrene (PS), in the Lawaye River highlights the significant impact of human activity on aquatic ecosystems. These plastic types, commonly found in household items like diapers, napkins, and disposable food ware, underscore the pervasive nature of plastic pollution (Dayrit, 2019).

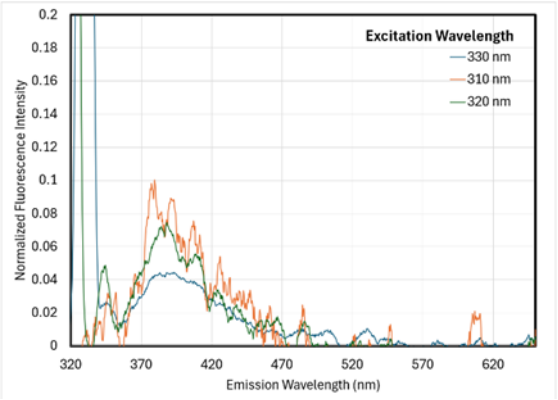


Figure 7: Ultraviolet Fluorescence Analysis.

As plastic consumption continues to rise, the development of rapid, non-destructive, and label-free methods for microplastic analysis across various environments becomes crucial. This study demonstrates the effectiveness of fluorescence spectroscopy in characterizing microplastics, offering a promising approach for future investigations into the extent and impact of plastic pollution.

4 RECOMMENDATIONS AND SUGGESTIONS

For future research, we recommend expanding the use of Excitation-Emission Matrix (EEM) spectroscopy to comprehensively map the excitation and emission spectra of various microplastic polymers. By establishing a robust spectral library of common microplastic types, we can deconvolute complex EEMs and accurately identify and quantify the prevalent microplastic types within riverine environments. Furthermore, incorporating an internal standard and refining data processing methods to minimize spectral interferences will enhance the accuracy and reliability of these analyses.

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