Abundance of microplastics in a river-estuary along a rural-urban gradient pathway

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Abstract

Microplastics are rapidly accumulating in quantity. Exposure to microplastics can be a threat to ecosystems as mammals ingest 0.1 to 5 grams of microplastics per week. This study sought to determine the abundance of microplastics found along a rural-urban gradient in Iloilo River. Samples were gathered from five sites of differing parameters, treated to remove non-microplastic substances, and identified through a compound microscope. Results showed that the abundance along the river ranged between 1 to 4 particles per liter. The unmonitored rural sites had the highest abundance and the monitored urban sites had the lowest abundance. Additionally, most of the microplastics gathered were in the size range of 150 to 500 µm, transparent in color, and are film morphological type. Investigation of additional parameters like area usage may be needed to understand microplastic abundance patterns as there was no strong correlation between urbanization and microplastic abundance.

Introduction. - Plastic is one of the most widely accessible and mainstream production resources. It is reported that the Philippines uses roughly 59.7 billion sachets and 20.6 billion shopping bags annually [1]. The staggering amount of single-use plastic consumed yearly is one of the greatest obstacles in current efforts in waste management [1].

Microplastics are a type of plastic debris that is less than five millimeters (5 mm) in length, and they originate from the degradation of macro plastics and manufacturing runoffs [2, 3]. Its existence has been seen in the air, waterways, sediments, and in the digestive system of animals [4, 5]. The threat of microplastics has prompted research on the material and its effects on ecosystems and human life [6]. Humans have been heavily exposed to microplastics through ingestion, inhalation, and dermal contact [7, 8]. Ingestion is thought to be the main pathway, due to humans' high trophic level in the food chain [2]. Ingestion allows humans to consume 0.1 to 5 grams of microplastics per week, or 1,000 to 3,000 microplastic particles per year into the body via food products [7]. This may lead to chromosome alteration, cancer, and increased vulnerability to neuronal disorders [8].

Abundance is the measure used to determine how much microplastics are present in the water, usually measured per m^3 or liter [9]. Understanding the microplastic abundance along a river's course is a critical factor in identifying the key sources of microplastic and in developing management interventions. Moreover, the morphological type and the physical characteristics of the microplastics are also important in identifying the possible sources of the microplastics. They are one of the many ways to tell the origins of the plastic as certain morphological types retain their characteristics despite degradation. A rural-urban gradient pathway is a river with one continuous pathway that crosses through a mixture of rural and urban areas. Rivers in urban areas can contribute to the microplastic concentrations in the ocean, specifically the microplastics found in the surface water since they can easily be transported by tides.

Iloilo City (10.7202° N, 122.5621° E), the provincial capital of Iloilo, Philippines, has a population density of 5,842 people per km^3 [10]. Due to its high population density, the presence of microplastics can pose a hazard to humans and animals alike [11]. The Iloilo River is a river-estuary landmark that spans over 10 km in length [12]. It starts in the municipality of Oton where the surrounding barangays have less than 5,000 inhabitants and thus are classified as rural [13]. The river's surroundings transition from rural to urban as it enters the populated city, thus making it a rural-urban gradient pathway. There is a need for microplastic studies in freshwater bodies, specifically in South East Asian countries, where there is a trend of rapid urban development but poor waste management [14].

This study aims to provide information regarding the abundance of microplastics in the surface water of a rural-urban gradient pathway. The microplastics were identified through sample collection, filtration of microplastics, microscopy, and classification. Additional data such as microplastic physical characteristics and morphological type were also collected. It specifically aims to:

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(i) To quantify the number of microplastics found per area via visual counting;

(ii) To calculate the abundance (in particles per liter) of microplastics found per site;

(iii) To determine the most prevalent physical characteristics (size and color) among the microplastics found;

(iv) To determine the most prevalent morphological type among the microplastics found; and

(v) To determine the trend line in the abundance of microplastics in different sections of the Iloilo River.

Methods. - The data gathering procedure was divided into four groups: sample collection, sample treatment, sample identification, and data analysis.

Sampling Site. The research study took place in the Iloilo River in Iloilo, Philippines, which is approximately 14.5 km in length. The river's starting point is located in the municipality of Oton. It stretches across the Iloilo city districts and ends at the Iloilo Port located between the Iloilo City Proper and the Lapuz district.

There are three categories for a barangay to be considered urban [13]. The first category of an urban barangay should have at least 5000 inhabitants. The second category requires at least one establishment with a minimum of 100 employees in the barangay. The third category states that there should be five or more establishments with 10 to 99 employees within the two kilometer radius of the barangay hall [13]. Meanwhile, a barangay is considered rural if it does not fit any of the urban criteria.

The Iloilo River is considered a rural-urban gradient pathway. A rural-urban gradient pathway is a body of water that is surrounded by both rural and urban areas. The river's origin point, Oton, is a rural zarea because it has less than 5000 inhabitants. Meanwhile, the Iloilo Port area fits all of the criteria, and is thus labeled as urban. On the other hand, there is a mixed gradient of urban and rural barangays surrounding the area from the start to the end of the river.

Table 1. Sampling Sites Details

Site	Code	Distance	Coordinates	State of Area
1	U_MR1	1.46 km	10.69479°N, 122.49888°E	Unmonitored Rural Area
2	U_MR2	4.54 km	10.69632°N, 122.52166°E	Unmonitored Rural Area
3	U_MU3	7.76 km	10.70032°N, 122.54170°E	Unmonitored Urban Area
4	MU4	9.67 km	10.70167°N, 122.55886°E	Monitored Urban Area
5	MU5	12.08 km	10.69618°N, 122.57207°E	Monitored Urban Area

Sample Collection. Systematic sampling was used to determine the five general sites, with equal distance between each site as shown in Table 1. Ten liters of water samples were collected from each of the five sites from Iloilo River, Philippines as shown in Figure 1.

The sampling area proper is in the center of the bodies of water: each site was at least 2.4 km away from the other, at least 5 meters away from the riverside, and 0 to 12 centimeters below the surface for each site [15]. Moreover, the weather, time, and pin location of the collection was documented for each site.

The water samples were wet-sieved using stacked mesh sieves (5 mm, 2 mm, 1 mm, 0.3 mm, and 0.15 mm) in order to obtain the particle samples [15, 16]. These were then transported to the lab.

Sample Treatment. Microplastics are removed from the sieve using a vacuum pump with a 0.47 µm pore size filter paper [16]. Next, samples underwent Wet Peroxide Oxidation (WPO). The samples were

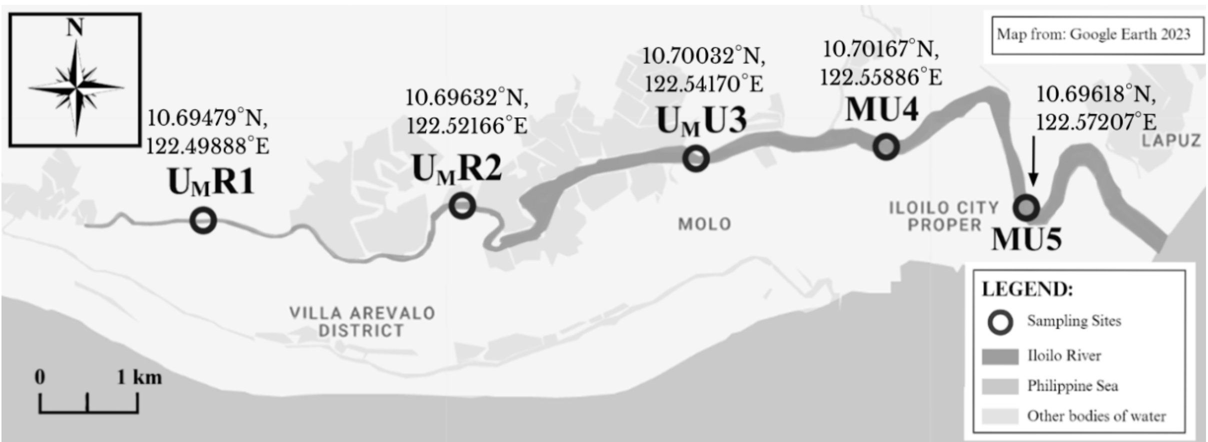

Figure 1. Map of the Iloilo River, Philippines and the five encircled sites (from Google Earth)

mixed with 5 mL of aqueous 0.05 M iron (II) solution and 5 mL of 30% hydrogen peroxide (H_2O_2), and then heated to 75 °C and stirred for 45 minutes at 200 rpm to remove organic material [5, 15].

The remaining particles underwent density separation to remove the inorganic particles with a higher density than plastics. This was done using a 10 mL sodium iodide (NaI) (1.55 to 1.6 g per mL) brine solution and left overnight to settle [17]. Next, a peristaltic pump with a 0.47μm pore size filter paper was used to drain the water out of the mixture and left to air dry overnight [5, 15].

Sample Identification. The treated samples were individually isolated with tweezers and placed on glass slides for identification. For the smaller samples, the filter paper was placed under the microscope at full brightness in order to observe the particles. A compound microscope at 40x magnification [4] was used to identify its physical characteristics as shown in Figure 2. Free et al. 's [18] table, Lusher et al. 's [19] description, and Dehghani et al.'s [4] study served as libraries for confirmation. This process was done for each microplastic.

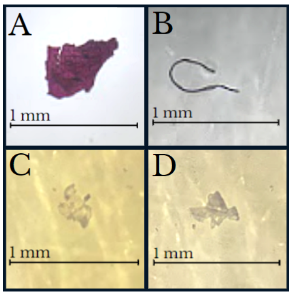

Figure 2. Photographs of different microplastics;
(A) Violet fragment, (B) Black fiber, (C&D) Transparent film

The software *ImageFocus4 Version 3.1.2* [20] was used to photograph the particles and used to measure the size of each particle sample. This was done using a 1 mm / 100 div calibration slide for calibration. Samples below 150 μm were disregarded due to difficulty in identifying their characteristics [21]. The background color of the photos was caused by the reagent (NaI) during density separation. These shades of yellow are from the differing I2 amounts in the solution due to possible amounts of chlorine in the freshwater.

Data Analysis. The data gathered was analyzed by calculating the abundance per area and determining the trend line. The characteristics and morphological types of the microplastics were placed in bar graphs, while the abundance was shown in a table. A scatter plot was used to show the trend line of the data. No specific statistical analysis test was performed due to the descriptive nature of the study.

The abundance of the microplastics was calculated using the following equation:

$$Abundance = \frac{\#\ of\ particles}{10\ L}$$

Safety Procedure. Face masks, lab gowns, and nitrile gloves were worn for all laboratory procedures with adult supervision.

Results and Discussions - The results show that the number of microplastics found in the river ranged from 9.7 to 38 samples per 10L. Meanwhile, the microplastic abundance has a range of 0.97 to 3.80 par/L. The microplastic abundance average of the rural areas was 3.4 par/L and the average of the urban areas was 1.85 par/L.

Table 2. Microplastic Results Per Sampling Site

Site	U_MR1	U_MR2	U_MU3	MU4	MU5
Number	38	30	30	9.7	13.3
Abundance	3.80	3	3	0.97	1.33
Most Abundant Size(in μm)	150 to 500	150 to 500	150 to 500	150 to 500	150 to 500
Most Abundant Color	Black	Black	Trans-parent	Black	Black
Most Abundant Morphological Type	Film	Film	Film	Film	Film

Legend: U_M for Unmonitored, M for Monitored. R for Rural, U for Urban. The number indicates the site number.

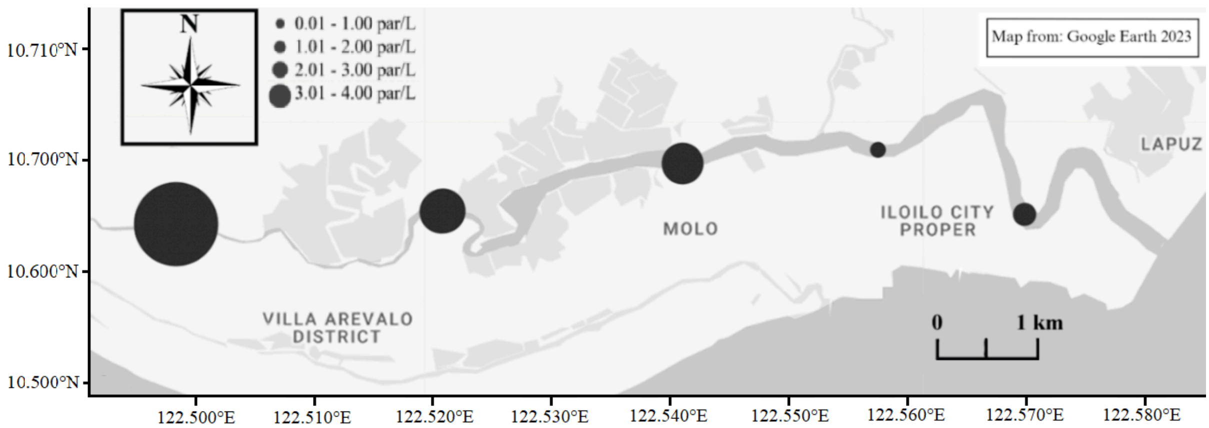

Figure 3. Iloilo River Map with Microplastic Spatial Distribution

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Observations suggest that the site's monitoring status affects the microplastic abundance in the area. The abundance of the Iloilo River has similar values to that of Cañas River (1.58 par/L), Pasig River (3.41 par/L), and Parañaque River (5.02 par/L) [15]. These similarities may be due to their shared history and surroundings. In the study conducted by Osorio et al. (2021), the Cañas river with the least microplastics was far from sources of plastic emissions, while the rivers with higher abundance values were heavily polluted and surrounded by plastic manufacturing industries [15]. On the other hand, the Pasig River, despite being in a polluted area, had a lower abundance due to a rehabilitation program, similar to Iloilo River [15]. However, there are other studies that have lower microplastic ranges than this study; which could have been affected by the difference in water sample amount [5, 15]. The counting of microplastics was limited to microplastics within the size range of 150 µm to 5 mm because the available equipment is not able to handle microplastics less than 150 µm.

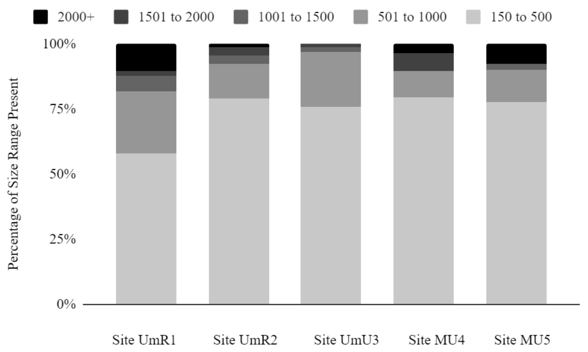

Figure 4. Bar Graph of Microplastic Sizes per site

As seen in Figure 4, the majority of microplastics were characterized within the size range of 150 to 500 µm. It is to be noted that this software provides low quality images, and can lead to inaccurate particle measurements. The results were triangulated to eliminate such limitations. It is hypothesized that the abundance of 150 to 500 µm sized microplastics originated from severe degradation and fragmentation of waste in the surrounding areas. These results align with microplastic findings and observations in the Parañaque River, where their most abundant size range is 250 to 500 µm [15]. It contains a public seafood market - this meant that its main source possibly had more degradable plastic products. The results also align with microplastic findings in the Chi River Basin, which has their most abundant size range at 51 to 500 µm [22]. One of the Chi River Basin sampling sites was surrounded with light industry plants.

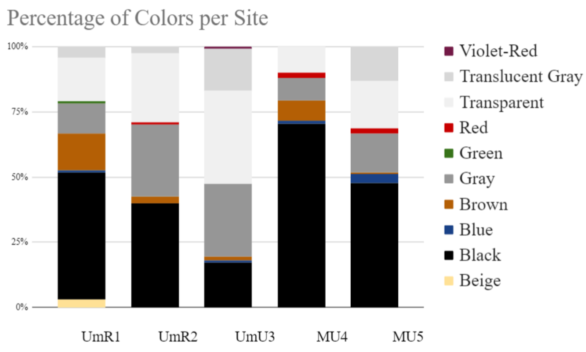

Figure 5. Bar Graph of Microplastic Colors per site

Figure 5 shows that the most prevalent colors among the microplastics found were black, transparent, and gray. This suggests that the majority of the microplastics found can be traced back to plastics of those colors. Although the transparent opacity was also prevalent in other studies, the majority of the surface water microplastic studies had blue and white as their most abundant colors [23, 24].

For studies with vibrant colors common in their findings, it was found that these studies were conducted in rivers lined with industrial and commercial areas where colored plastic is commonly used. This description does not fit Iloilo River's surroundings, and asserts the small percentage of vibrant plastics in the study. Only three out of the five river sites were near industrial and commercial areas (U_MR1, U_MU3, and MU5). U_MR1 had the most diversity in color. U_MR2, a site surrounded by rice fields and the least industrialized, had the least color diversity. Additionally, the abundance of transparent and non-vibrant colored microplastics is due to the microplastic degradation that causes the color to fade overtime [3, 15]. However, the degradation rate of the microplastics found is not constant, as the age of the particles and their duration in the river are uncontrollable variables. Additionally, because the samples are in the surface water, there is a lack of ultraviolet B-rays to initiate the extreme degradation process which can cause the microplastics to compile in the river [3].

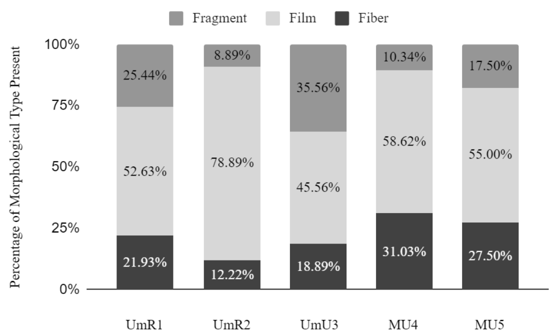

Figure 6. Bar Graph of Microplastic Morphological Types per Site

Only three morphological types were identified across the five sites (film, fragment, and fiber), with film being the most abundant. This aligns with a study in a Mongolian lake; however, their results showed that fiber and fragments were more prevalent [11, 15, 18]. Their findings were caused by waste dumping and plastic manufacturing industries within the area. The Iloilo River, especially in urban protected areas, is mostly free from plastic waste and has little fishery activity. The only macro plastic waste encountered were sachets and plastic bags, which are film types [17]. This suggests that the waste surrounding the area influences the prevalence of morphological types found. It also suggests that wrappers, plastic bags, and other film plastics are the main cause of pollution along the Iloilo River. The microplastics found in the river differ from previous microplastic studies in abundance and morphological type due to undetermined factors such as the waste management in the sampling site areas.

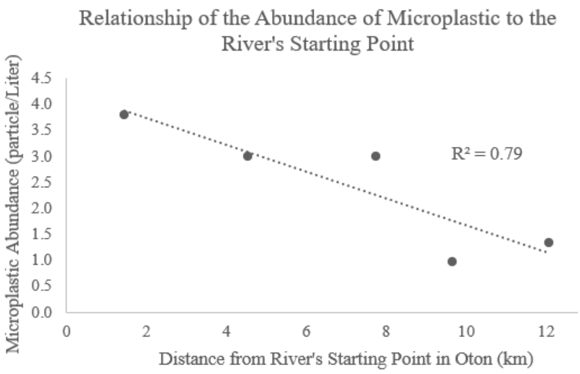

Figure 7. Relationship of the Abundance of Microplastic to the River's Starting Point

There is a negative trendline suggesting an inverse correlation between the microplastic abundance and the distance from the origin point. This indicates that rural areas have less microplastic abundance. This corresponds to a study indicating a positive correlation between high microplastic abundance and lack of solid waste management despite population density [11, 18]. However, the same studies also stated that there is a high positive correlation between microplastic abundance and population density [22, 23]. This has the opposite results to our study, as the urbanized and densely populated areas of the Iloilo River are rehabilitated [12]. However, this rehabilitation did not apply to the rural areas, especially in the UMR1 site, where the area was a mix of populated and heavily vegetated. This suggests that the state of urbanization itself may not be a factor in the increase of microplastic abundance in the Iloilo River, but rather the human activities in the affected areas. It can be observed that the unmonitored areas had higher values in comparison to the monitored areas. It is to be noted that the number of sites used might have affected the trendline of such phenomena as this study was limited to having only five sites. Additionally, only the population density and visual observation of surroundings were taken into account. Other possible factors such as waste management practices and regulations of the barangays were not taken into account as they were outside the scope of the study.

Limitations. The flow rate of the water's current during the collection and its area could not be measured. The water volume (L) was used as a replacement for the collection area measurement. Only ten liters of water were collected per site, and only microplastics within the size range of 0.15 mm to 5 mm were investigated. Due to limited chemical supplies, the reagents used in the Wet Peroxide Oxidation method used in the sample treatment were scaled down; thus, no blank samples were used during the study.

Conclusion. - The abundance of the microplastics throughout the river is 0.97 to 3.8 par/L, with an inverse correlation between microplastic abundance and the distance from the starting point. Microplastics commonly are in gray colors, a size range of 150 to 500 μm, and a film morphological type. There is a weak correlation between rural-urban and microplastic abundance, but a higher correlation with monitoring status, and thus its lack of waste management.

Recommendations. - A higher quality microscope camera lens is recommended to accurately count a larger microplastic size range. For abundance, future studies should have a higher water volume quantity per site for a more detailed data set. For the color, size, and morphological type, high quality software is recommended for more accurate measurements and identification of the characteristics. It is also noted that the opacity of the microplastic may also be investigated as it could be related to the other characteristics. More sampling sites are needed to provide a more accurate and detailed trendline. Further investigation is needed regarding possible correlations between the abundance in the river and other influencing factors, such as the surroundings' waste management.

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