

Occurrence and Polymer Types of Microplastics from Surface Sediments of Molawin Watershed of the Makiling Forest Reserve, Los Baños, Laguna, Philippines

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ARTICLE INFO

Received: 2 Jun 2020

Received in revised: 11 Sep 2020

Accepted: 5 Oct 2020

Published online: 9 Nov 2020

DOI: 10.32526/ennrj/19/2020114

Keywords:

Microplastics/ Molawin Creek/
Surface sediments/ Fourier-transform
infrared spectroscopy

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ABSTRACT

Microplastic pollution is an emerging topic in environmental science. However, information about its prevalence in the freshwater ecosystems is still scarce. This study quantified and identified microplastic form and polymer types from surface sediments of the Molawin River. Sediment samples were collected from the upstream, midstream, and downstream stations of the river. Isolation of microplastics was performed through a modified granulometric approach, density separation, and filtration. Stereoscopic microscopy and Fourier-transform infrared spectroscopy (FTIR) were conducted to quantify and describe microplastics and identify the polymer types based on the infrared spectrum of absorption, respectively. The highest concentration of microplastics was found in the downstream station, with an average number of 97 ± 12 items/100 g and 47.33 ± 11.39 items/100 g sediment dry weight in the bank and channel, respectively. The isolated microplastics were dominated by ≥ 100 to $\leq 200 \mu\text{m}$ size range. Based on stereoscopic microscopy, microfragments and microfibers were the most common microplastic type, while polyethylene (PE) and polypropylene (PP) were the polymer types identified based on FTIR analyses. This study revealed the presence of microplastics and confirmed the microplastics polymers present in the Molawin Watershed of Makiling Forest Reserve.

1. INTRODUCTION

Microplastic pollution is an emerging contaminant, and it is considered as one of the most discussed topics in the field of environmental science (Wagner et al., 2014; Eerkes-Medrano et al., 2015; Anderson et al., 2017). These micropollutants are divided into two categories: primary and secondary. Primary microplastics are manufactured raw "minute" plastic materials and move directly into water bodies (Browne et al., 2007; Andrade, 2011). Secondary microplastics are derived from various types of materials (meso- and macro-plastics) that degrade into smaller particles which are not readily detected (Thompson et al., 2004; Browne et al., 2007; Andrade, 2011). Generally, less than 5 mm plastics are considered microplastics (GESAMP, 2015). While some other authors used other size classifications.

Blair et al. (2017) divides the size class into large microplastics, small microplastics, microdebris, and microplastic. Large microplastics are microplastics ranging from 1-5 mm (Faure et al., 2012), small microplastics (<1 mm) (Vianello et al., 2013), microdebris (<2 mm) (Lechner et al., 2014), small microplastics (<1 mm) and microplastics (<0.5 mm) (Thompson et al., 2004; Fendall et al., 2009; Sanchez et al., 2014; Corcoran, 2015). At present, however, there is no standard definition of microplastics in terms of size range (Hidalgo-Ruz et al., 2012).

Although there is no standard microplastics size classifications, the study of Lehtiniemi et al. (2018) showed that fish and mysid shrimp uptake $<200 \mu\text{m}$ size microplastics. Moreover, the smaller sizes of microplastic could be a great concern because it could be ingested by planktonic organisms and entrained by

Citation: Limbago JS et al. Occurrence and polymer types of microplastics from surface sediments of Molawin Watershed of the Makiling Forest Reserve, Los Baños, Laguna, Philippines. Environ. Nat. Resour. J. 2021;19(1):57-67. (<https://doi.org/10.32526/ennrj/19/2020114>)

settling detritus (Cole et al., 2013; Botterell et al., 2019; Ballent et al., 2013). The entry of smaller size microplastics and nanoplastics in the planktonic food web could lead to further bioaccumulation and biomagnification in higher vertebrates (Saley et al., 2019; Akhbarizadeh et al., 2019). Hence, microplastics size are crucial nominators on determining the impact of microplastics on environment fauna. On top of that, microplastics are also vectors of highly hydrophobic contaminants and endocrine-disrupting chemicals such as polycyclic aromatic hydrocarbon, polychlorinated biphenyls, and polybrominated diphenyl ethers (Chen et al., 2018; Chen et al., 2019). This emerging concern has brought the microplastic research on the international spotlight since widespread plastic littering is a pronounced issue, however, degradation and its possible entry into the food web has long not been taken into account.

The increasing prevalence of microplastics in our aquatic ecosystems can be attributed to the continuous production and patronage of synthetic plastics coupled with poor solid waste management (Ang and Sy-Changco, 2007; Magalang, 2014). Recent literature has brought to light the abundance of microplastics in freshwater systems that are comparable to that of coastal and marine environments (Anderson et al., 2017; Blettler et al., 2017; Peng et al., 2018). For example, the studies of Sadri and Thompson (2014), Gallagher et al. (2016), and Vendel et al. (2017) reported acute microplastic pollution in estuaries indicating river input to coastal litters. Despite terrestrial water being considered as a significant transport vector of microplastics towards coastal environments, studies on its prevalence in freshwater ecosystems are still lacking to date-highlighting the need to focus on investigating its presence and distribution in the freshwater ecosystem (Wagner et al., 2014; Li et al., 2020).

Molawin Creek is one of the watersheds of the Makiling Forest Reserves under the administration of the University of the Philippines-Los Baños and a minor tributary of Laguna de Bay (Liogson et al., 2005). The watersheds of the reserve is habitat to diverse and abundant freshwater fish populations, including one endemic fish species *Leiopotherapon plumbeus*, and diminutive fish species such as *Glossogobius celebius* and *Hippichthys heptagonus* which are prone to extinction (Paller et al., 2011). Towards protecting its fauna and flora, Molawin has been declared as Biopark in 2010 (Casila et al., 2019). However, despite being a forest reserve and declared

as a Biopark, anthropogenic micropollutants from university facilities, commercial, and residential communities that may affect the aquatic organisms have received limited attention, considering biological sustainability is highly dependent on the physical, chemical, and biological viability of a particular habitat. On top of that, damaging biological diversity will eventually affect the ecological services that a watershed provides. Hence, from an ecological standpoint, there is a need to obtain a baseline study that will fill the data gap identified.

The objectives of this work were to (i) identify and characterize microplastics from surface sediments of Molawin Creek; (ii) determine the distribution of microplastics from surface sediments of different stations of the Molawin Creek; and (iii) identify the microplastics polymers isolated from surface sediments of Molawin Creek. The hypotheses that were defined to validate the objectives of the study are as follows: (i) the number of microplastics is higher in the downstream station of the Molawin Creek than in the midstream, and upstream stations; (ii) fibers and fragments are the most abundant type of microplastics present in Molawin Creek; and (iii) polyethylene is the most abundant type microplastic polymer in Molawin Creek. The result of this study will reveal the occurrence and will confirm the polymer types of microplastics in Molawin Watershed of the Makiling Forest Reserve. And eventually will contribute to the international data gap of the presence of microplastic prevalence in a freshwater body.

2. METHODOLOGY

2.1 Description of the study site

Three sampling stations within the river system of Molawin Creek, one of the major watersheds of the Makiling Forest Reserve at Los Baños, Laguna, Philippines, were identified in the study-upstream, midstream, and downstream stations (Figure 1). The upstream station located in Flat Rocks (14.147700°N, 121.229260°E) is inside the University of the Philippines Los Baños campus along the Mt. Makiling trail. The general area of the upstream station is not adjacent to any built-up infrastructure nor human settlement and is heavily forested. The midstream station designated at Molawin Biopark (14.162320°N, 121.244440°E), also inside the campus, is primarily surrounded by University establishments. On the other hand, the downstream station is in Barangay Bayog (14.189360°N, 121.259830°E) and is mostly surrounded by built-up areas, particularly residential

areas along the riverbank, and annual crops, forms a confluence with Maahas Creek. These sampling stations were selected to compare the concentration of

microplastics in different depositional environments and varying anthropogenic activities. Banks and channels were considered as a substation in the study.

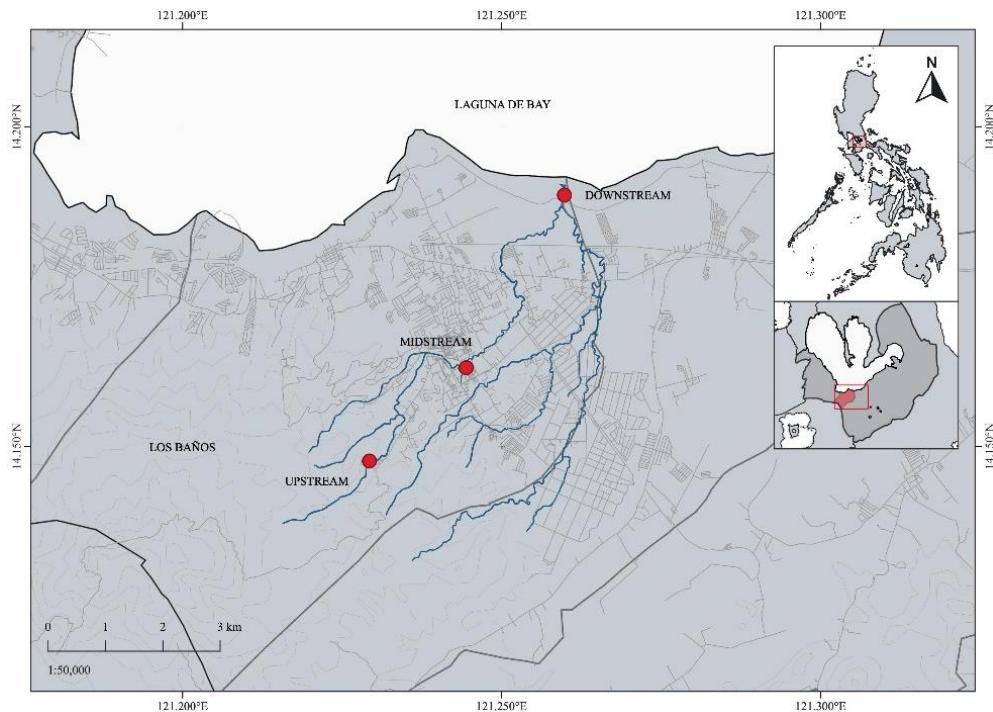


Figure 1. Land use map of the general study site with three sampling stations. The red points indicate upstream (Flat Rocks), midstream (Molawin Biopark), and downstream (Bayog) stations along Molawin Creek, Los Baños, Laguna, Philippines.

2.2 Sediment sampling

One day field sampling was conducted in October 2018. The collection of sediment samples from three stations, with two substations, was carried out along the Molawin Creek. Along the banks of each substation, a 50 m transect line was laid down haphazardly. While in channels of each substation, the transect line was laid down to areas satisfying these criteria: (i) should be in a straight reach of 50 m; and (i) should not be adjacent to hydraulic structures. Then three replicates were randomly collected along the transect line following the bank and the channel of the creek. Surface sediments (0-5 cm) were collected in a modified 15 cm × 15 cm quadrat laid on the substrate using a metal trowel with gradations. However, a different sample collection method was employed in the channel of the downstream station. In the downstream station's channel, a box corer (15 cm × 15 cm) was used to collect the sediments. Samples were placed in glass containers and then sealed to avoid contamination during transport. All obtained samples were stored at 4°C for subsequent laboratory analysis.

2.3 Processing of sediment samples and microplastic isolation

The isolation of microplastics was conducted according to the methods prescribed by the National Oceanic and Atmospheric Administration (NOAA) and a modified granulometric approach (Masura et al., 2015; Thompson et al., 2004; Kedzierski et al., 2016; Whitmire et al., 2017). Briefly, sediment samples were weighed (~1000 g wet weight) and were oven-dried at 60°C for 48 h. Dried samples were sifted through a nested set of standardized sieves with progressively smaller openings (2 mm - 0.63 µm) (López, 2017).

Sediments with less than 0.5 mm size were then weighed. Because of differences in sediment dry weight, a standardized aliquot of 100 g dry weight of sediments was used in subsequent analysis (Peng et al., 2017). Samples were poured with 500 mL of concentrated saline solution (200 NaCl g/L) in 1,000 mL glass jars (Thompson et al., 2004). After settling the samples overnight, the supernatants were sifted through Whatman filter No. 2 with the aid of a vacuum pump. The tube of vacuum pumps was then rinsed

with Milli Q water to minimize cross-contamination between samples. Samples were then placed in Petri plates and were oven-dried at 60°C for an hour.

2.4 Stereoscopic microscopy and microplastic quantification

Samples of microplastics in filter paper were photographed and documented using a stereomicroscope at 40X magnification. Isolated particles were counted, measured for maximum length (relative to a 5 mm scale bar), and classified based on its general form-microfibers, microfragments, microfilms, and microbeads. Microplastics size of <0.5 mm as early defined and used by some authors (Thompson et al., 2004; Fendall et al., 2009; Sanchez et al., 2014; Corcoran, 2015) was considered in this study because this size has higher ingestibility by aquatic organisms and were entrained by settling detritus (Lehtiniemi et al., 2018; Cole et al., 2013; Botterell et al., 2019; Ballent et al., 2013). Suspected microplastic particles were submitted for Fourier-transform infrared spectroscopy (FTIR) analyses for validation and identification of plastic polymer types.

2.5 Fourier-transform infrared spectroscopy

Polymer types of microplastics were determined separately using the FTIR spectrometer (Bruker, United States). Wave numbers were recorded in transmission mode with 4,000-6,000/cm range and a spectral resolution of 4/cm. A total of 24 scans were co-added for every spectrum. The background measurements were conducted with the same settings: against air for samples that have not adhered to the filter paper, and against the filter paper for adhering samples. The FTIR instrument was administered by OPS IR software V7.5. Post-processing of the spectra was also implemented using the same software.

2.6 Data analyses

Results were expressed as mean±standard errors (SE) from three sample replicates. Data analyses were performed using MS Office Excel 365, and histogram of microplastics size distribution were plotted using Paleontological Statistics (PAST) software version 2.17.

3. RESULTS

3.1 Microplastic quantity

The results of the study showed that microplastics were present in all sampling stations (Figure 2). The highest number of microplastics was

found in downstream sampling stations (47.33 ± 11.39 items and 97.00 ± 12.34 items/100 g sediment dry weight in channel and bank, respectively), followed by midstream stations (1.33 ± 0.88 items and 6.33 ± 1.20 items/100 g sediment dry weight in channel and bank, respectively) and the least number of microplastics were isolated from upstream stations (1.00 ± 0.58 items/100 g sediment dry weight in both substations).

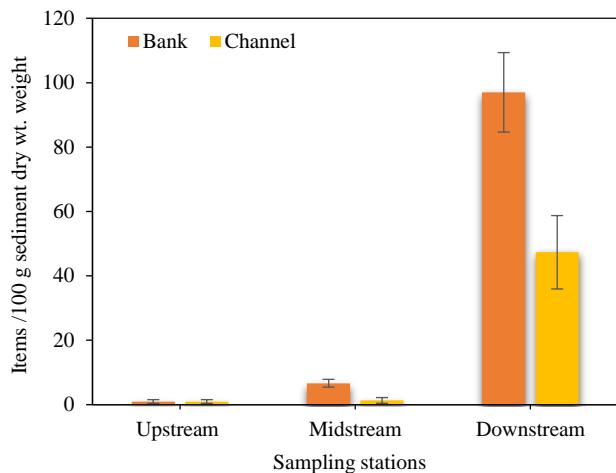


Figure 2. Mean number±standard error (n=3 per substation) of microplastics identified from surface sediments of three sampling stations of the Molawin Creek (bank and channel).

3.2 Microplastic types

All types of microplastics were isolated and identified from the Molawin Creek continuum (Figure 3). As shown in Table 1, the most collected microplastics type was microfragments in all stations with the exception for the midstream channel substation (Table 1). As shown in Table 1, the highest number of microfragments was isolated from the bank (71.33 items/100 g sediment dry weight) and in the channel (30 items/100 g sediment dry weight) of the downstream station. Microfibers isolated from the bank (20 items/100 g sediment dry weight) is higher than the microfibers identified from the channel (5 items/100 g sediment dry weight) of the downstream station. In contrast, microfilm (11.67 items/100 g sediment dry weight) in the channel is higher than microfilms identified from the bank (5 items/100 g sediment dry weight) of the downstream station. On the other hand, only microfragment in the channel of midstream station has a notable number (4 items/100 g sediment dry weight). Microbeads are the least identified microplastic from the Molawin Creek continuum. In terms of size range, microplastics with ≥ 100 to ≤ 200 μm length dominated the isolated particles (Figure 4).

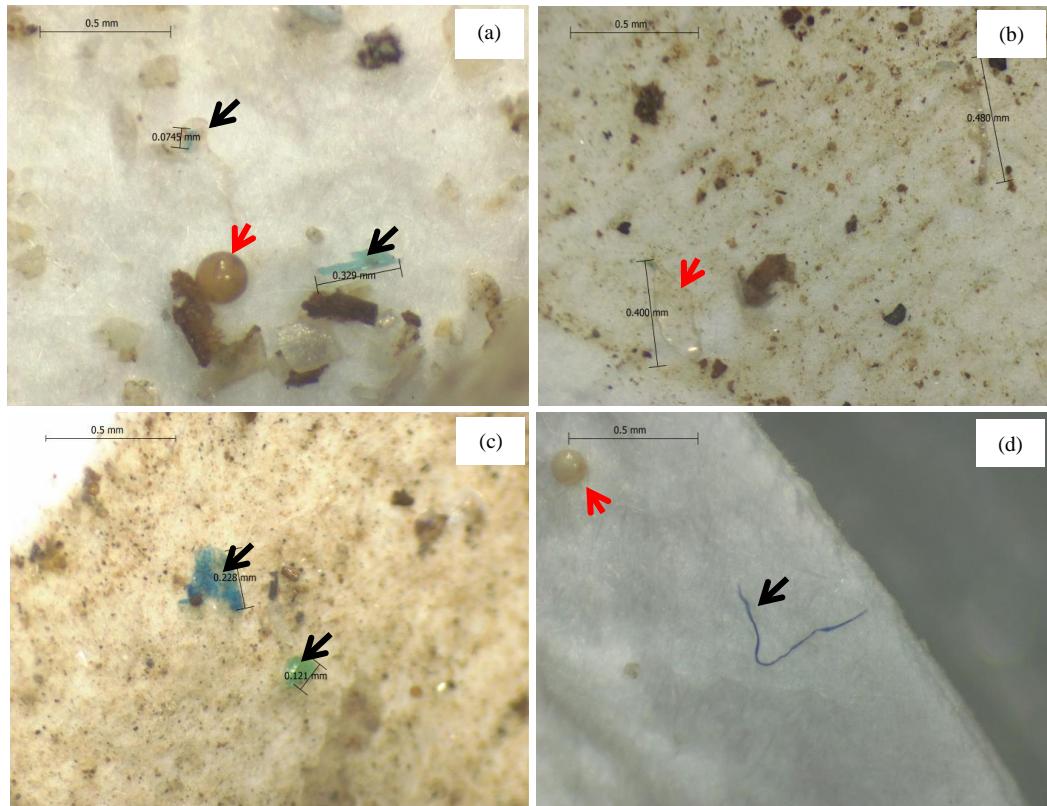


Figure 3. Microplastic types identified from Molawin Creek continuum: (a) black arrows - microfragments; red arrow - microbeads; (b) black arrow - microfiber; red arrow - microfilm; (c) microfragments; (d) black arrow - microfiber; red arrow - microbeads. Scale bar=0.5 mm.

Table 1. The average number of microplastic types obtained from sediment samples of bank and channel of Molawin Creek, Los Baños, Laguna, Philippines.

Station	Average number of microplastics types				
	Fragment	Beads	Films	Fiber	Total
Upstream channel	1.00	0.00	0.00	0.00	1.00
Upstream bank	0.67	0.00	0.00	0.33	1.00
Midstream channel	0.33	0.00	0.00	1.00	1.33
Midstream bank	4.00	0.67	1.00	0.67	6.33
Downstream channel	30.00	0.67	11.67	5.00	47.33
Downstream bank	71.33	0.67	5.00	20.00	97.00

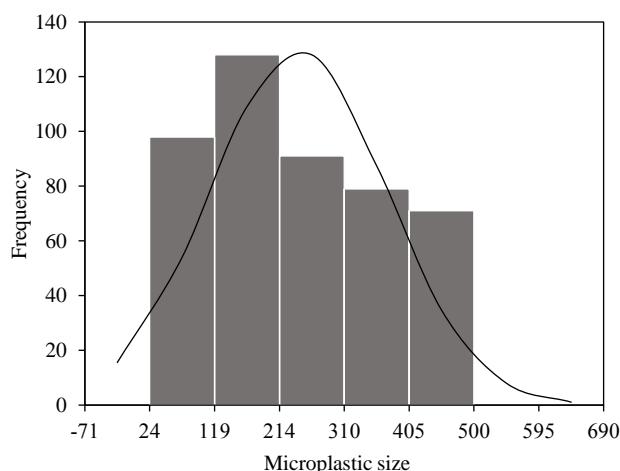


Figure 4. Microplastic size distribution along Molawin Creek continuum.

3.3 Sediment granulometry

Sediment grain size distribution in the Molawin Creek is shown in [Figure 5](#). It is observed that upstream and midstream sampling stations are composed of coarse sediments. In terms of larger grain size (≥ 2 mm), upstream substations are composed of 70.56% (bank) and 76.04% (channel), midstream substations are composed of 63.79% (bank) and 46.01% (channel) while downstream substations are composed only of 26.78% (bank) and 20.15% (channel). Grain sizes of downstream stations are typically composed of smaller grains and moderately sorted according to grain sizes in comparison to upstream and midstream stations.

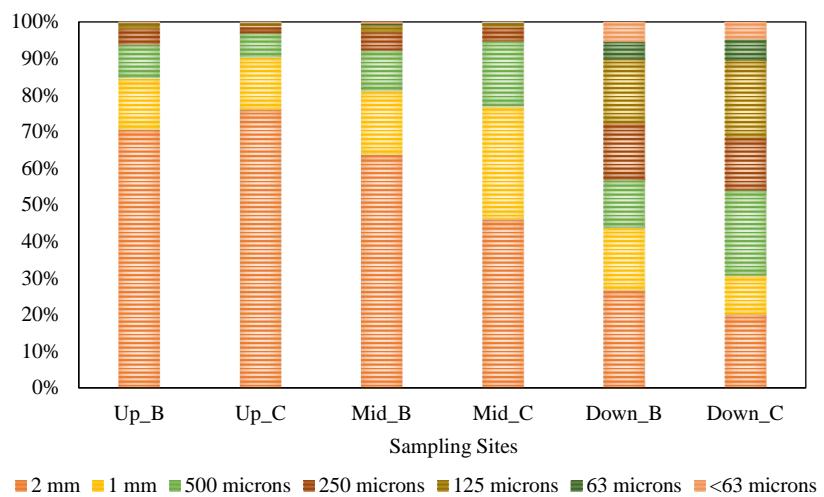


Figure 5. Sediment grain sizes distribution of Molawin Creek. Upstream-Bank (Up_B); Upstream-Channel (Up_C); Midstream-Bank (Mid_B); Midstream-Channel (Mid_C); Downstream-Bank (Down_B) and Downstream-Channel (Down_C).

3.4 Fourier-transform infrared spectroscopy (FTIR)

Previous studies on polymers using FTIR analyses have established the absorption bands used for the identification of high-density polyethylene (HDPE), low-density polyethylene (LDPE), and polypropylene (PP) spectra. The stretching of vibration bands of CH_2 in polyethylene and CH_2/CH_3 in polypropylene was observed within the range of 3,000-2,800/cm, while the bending vibrations of CH_2 and CH_3 groups fall in the range of 1,500-1,350/cm, and CH_2 rocking vibration between 1,200-700/cm (Käppler et al., 2015). Fourier-transform infrared spectroscopy (FTIR) analysis (Bruker, United States) presented the spectra of microplastic samples that were obtained from three stations in the Molawin Creek. The samples from the bank and channel of the

downstream station exhibited peaks within the range of 3,000-2,800/cm (Figures 6(a) and (b)). In the midstream station, only samples collected from the bank registered peaks with a similar range (Figure 7(b)). No significant peaks were observed in the samples acquired in the channel of midstream station (Figure 7(a)). Furthermore, results from the spectra of microplastics in the upstream station for both bank and channel substations were negligible (Figures 8(a) and (b)). Polypropylene (PP) particles were identified for both samples obtained in the bank and channel of the downstream station. Polyethylene (PE) polymers were the only samples that were determined from the bank of the midstream station. Lastly, no microplastic polymers were recorded in the channel of midstream station, and for both the bank and channel of the upstream station.

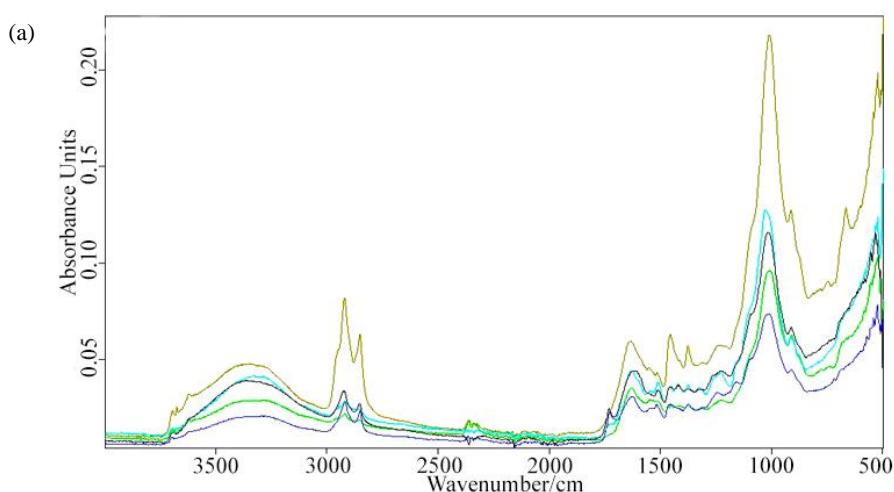


Figure 6. Fourier-transform infrared spectroscopy (FTIR) spectra of microplastic samples obtained from surface sediments of the downstream stations of Molawin Creek. (a) channel; (b) bank.

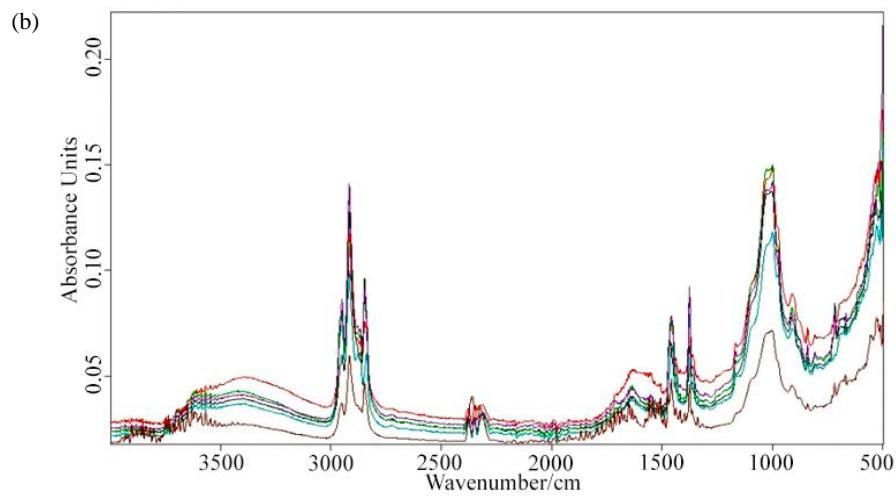


Figure 6. Fourier-transform infrared spectroscopy (FTIR) spectra of microplastic samples obtained from surface sediments of the downstream stations of Molawin Creek. (a) channel; (b) bank (cont.).

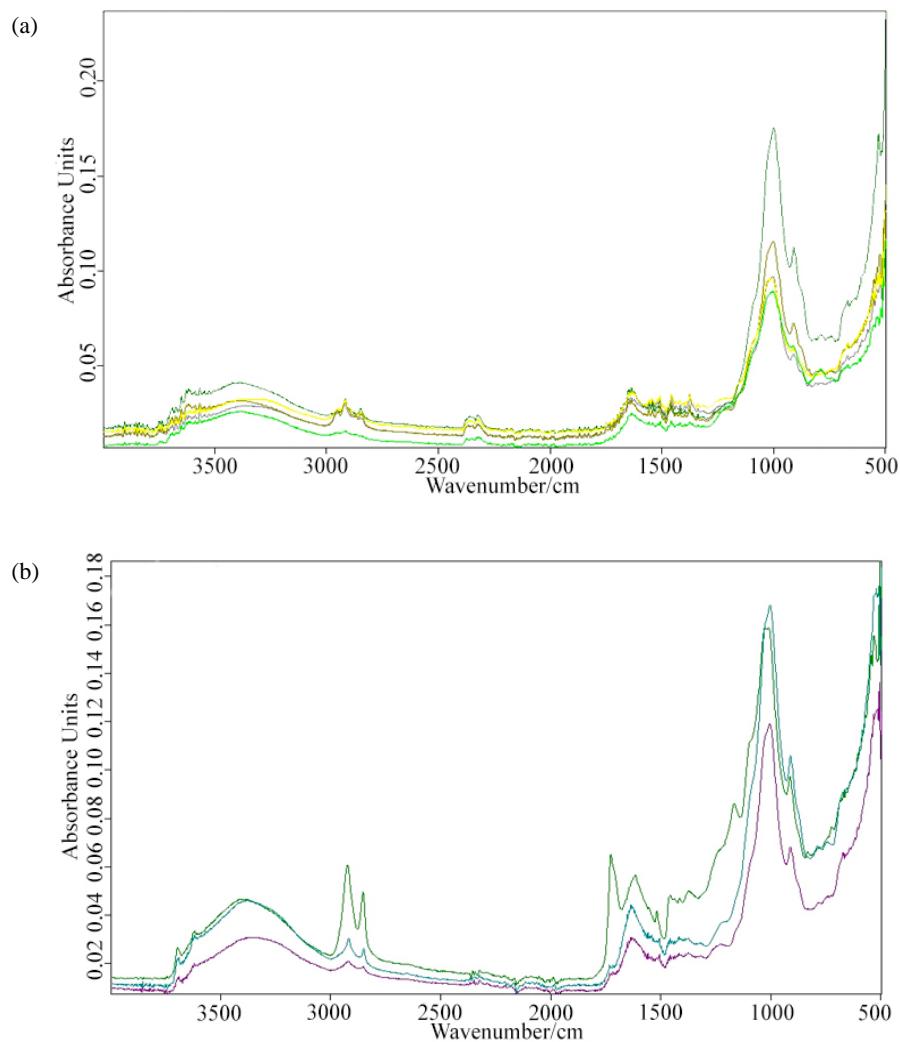


Figure 7. Fourier-transform infrared spectroscopy (FTIR) spectra of microplastic samples obtained from surface sediments of the midstream stations of Molawin Creek. (a) channel; (b) bank.

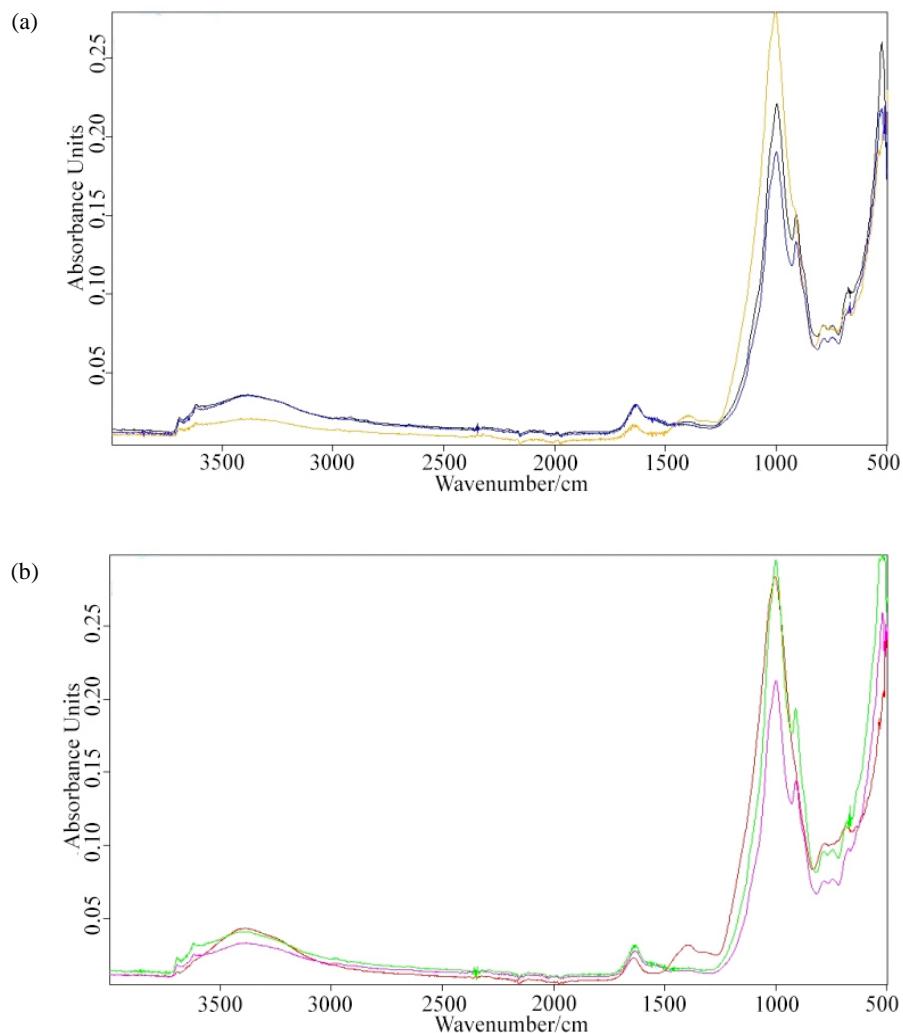


Figure 8. Fourier-transform infrared spectroscopy (FTIR) spectra of microplastic samples obtained from surface sediments of the upstream stations of Molawin Creek. (a) channel; (b) bank.

4. DISCUSSION

Environmental scientists, globally, have put increasing attention on microplastics research (Guzzetti et al., 2018). The issue raises concern since microplastics are considered vectors of endocrine-disrupting compounds (EDCs) in the aquatic environment (Chen et al., 2018; Chen et al., 2019). However, the focus seemed limited to the marine ecosystem, where microplastic prevalence in the freshwater ecosystem has an immense data gap (Wagner et al., 2014; Li et al., 2020). In this study, we assessed the occurrence of microplastics in the Molawin Creek continuum using a modified granulometric approach. Microplastic physical and polymer types were further identified using light microscopy and Fourier-transform infrared spectroscopy (FTIR).

The results were consistent with the first hypothesis of the study, which followed a decreasing

trend of microplastic abundance from the upstream to downstream stations. Microplastics were prevalent in sediment samples from both the bank and channel of the Molawin Creek downstream station, where a confluence with the Maahas Creek is formed (Liongson et al., 2005). Through stereoscopic identification, only one microplastic type has been isolated from the upstream stations and in the channel of the midstream station. Additionally, six microplastic types were isolated from the bank of the midstream station. The isolated microplastics were dominated by ≥ 100 to ≤ 200 μm in terms of size. Differences in sizes of microplastics may provide insights into their sources and unknown weathering transport effects. On the other hand, minimal anthropogenic activities in the upstream and midstream could be attributed to low microplastic counts, contrary to downstream stations where residential areas are located along the riverbanks. The

presence of microplastic in Molawin Biopark, which is inside the University Campus, is an indicator that waste from the University are drained in the watershed, eventually affecting the habitat.

Population density is not a sole factor affecting the microplastics abundance in the freshwater ecosystem (Klein et al., 2015; Tibbetts et al., 2018). Other factors that could affect the abundance of microplastics in sediments include microplastic polymer density, river hydrodynamics, weather conditions, and heteroaggregation of microplastics rendering higher riverbed retention (Corcoran, 2015; Kowalski et al., 2016; Hurley et al., 2018; Nizzetto et al., 2016; Besseling et al., 2017). The downstream station of the Molawin Creek has fine-grained sediments as compared to upstream and midstream stations. The lower velocities in the downstream station of rivers are known to be sinks for fine-grained sediments. Fine-grained sediments have higher retention, which provides an explanation to the microplastics abundance variation within upstream, midstream, and downstream stations (Nizzetto et al., 2016). Hence, the potential for microplastic settlement is higher (Corcoran, 2015; Vaughan et al., 2017; Botterell et al., 2019). The river hydrodynamics could also explain the greater abundance of microplastics in banks than in channels. This result is in accordance with the study of Tibbetts et al. (2018) where the abundance of microplastics in low velocity environments was recorded. Results of this study, moreover, implies that low velocity environments like banks, floodplains, lakes, meander cut-offs are areas for accumulation of microplastics.

In congruence with the studies of Deocaris et al. (2019), Mani et al. (2015), and Tibbetts et al. (2018) microfragments are the predominant microplastic type in Molawin Creek. Fragmentation or abrasion and degradation of larger plastic items result in microplastic fragments and fibers (Wagner et al., 2014). This suggests that microplastic pollution from the Molawin Creek is from the degradation of larger plastic material and is derived from land-based litters. However, the results did not conform with the second hypothesis of the study, where fibers and fragments were hypothesized to be the dominant microplastic type in the Molawin Creek. Presently, there is no adequate literature explaining the prevalence of fibers over fragments or vice versa. Some authors that published reports on the dominance of fibers over fragments are studies of Horton et al. (2017) and Vermaire et al. (2017).

Interestingly, polyethylene (PE) and polypropylene (PP) were detected using the FTIR spectra, consistent with the third hypothesis of the study. The FTIR spectra also confirm that microplastic is more abundant in banks than in channels. PE and PP were detected from the bank and channel of the downstream station and only PE polymers from the bank of midstream station. The presence of PE could be attributed to the widely used PE-based plastic bags (Yurtsever and Yurtsever, 2017). The current use of oxo-biodegradable type PE plastic bags also contributes to the abundance of microfragments since these materials are easily degraded by UV radiation or heat into smaller fragments (Eyheraguibel et al., 2018). While there are microplastics that were determined in physical identification and microscopy, polymer types were not detected in FTIR analyses. This underscores the importance of the chemical-based identification techniques such as FTIR, and Raman spectroscopy (Jung et al., 2018; Simon et al., 2018; Song et al., 2015; Lenz et al., 2015). Physical identification would lead to the misidentification of microplastics since there is no standard method for physical identification and quantification (Hidalgo-Ruz et al., 2012; Shim et al., 2017; Song et al., 2015). However, it should be noted that the approximate density of 200 g NaCl/L water will only allow recovery of polystyrene, polypropylene, high-density polypropylene, and nylon (Gray et al., 2018). Denser polymers were possibly not recovered by the protocol and methodology of this study.

While this study is one of the few attempts to record the presence of microplastics in the Philippine freshwater bodies, several limitations should be acknowledged. The protocol followed was designed for marine sediments, and density separation procedures could not separate denser microplastic particles. This study, hence, likely underestimated the microplastic counts in the Molawin Creek continuum. Moreover, there is no standard, manual for microplastic visual identification rendering errors in isolation and quantification. Further studies should be implemented to establish a more standardized technique for quantifying and identifying microplastics in the freshwater ecosystem.

5. CONCLUSION

The present study revealed four primary results: (i) microplastics are present in Molawin Watershed of the Makiling Forest Reserve; (ii) microplastics in Molawin Creek were dominated by ≥ 100 to ≤ 200 μm

size range; (iii) microplastic is more prevalent in the downstream station of the creek compared to upstream and midstream stations; and (iv) polyethylene and polypropylene microplastic polymers are present in Molawin Creek. These data indicate that downstream station is an accumulation zone of microplastics and highlights the need to study its impact on aquatic fauna and flora of Molawin Watershed and pollution contribution on Laguna de bay.

ACKNOWLEDGEMENTS

We would like to acknowledge the Department of Science and Technology-Accelerated Science and Technology Human Resource Development Program (DOST-ASTHRDP) of Republic of the Philippines for scholarships grants and financial supports.

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