Assessment of Microplastics in Hanumante River of Kathmandu valley

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Abstract

Plastic debris is one of the most significant organic pollutants in the aquatic environment. Researchers are currently focusing on the impact of micro and nano-scale plastic waste on aquatic systems. In this study, we investigated the distribution of plastic pellets and fragments present in the freshwater ecosystem. The goal was to assess microplastic (MP) abundance in the Hanumante River, a tributary of Bagmati river and analyze their properties. Sample collection involved the bottle sampling method. Filtration, wet peroxide oxidation, density separation, gravimetric analysis, and microscopic examination were performed to study the characteristics of microplastics. The study was conducted by following the guideline of National Oceanic and Atmospheric Administration (NOAA) protocol. Gravimetric analysis was applied to calculate the reduced mass of the sample after total organic carbon reduction. Results showed that maximum amount of reduced sample was obtained from sample taken from sample taken from Madhyapur Thimi area (~3.593g) and minimum amount of reduced sample was obtained from the sample taken from Shiva temple Jagati area (~2.130g). Microscopic inspection showed that samples taken from different locations were composed of an average of 14–23 microplastics per liter of sample. FT-IR analysis was performed to analyze the characteristics of micro plastics and the type of polymers present in the sample which showed the abundance of polymer materials like polyethylene, polypropylene, and polycarbonates. The findings imply that appropriate plastic waste management measures be implemented in the communities to safeguard the ecosystem benefits derived from the river.

Keywords: River; Microplastics; Pollution; Density separation; Hanumante River
Introduction

Plastic is a synthetic material made from organic polymers that is widely used in modern life for clothing, packaging, etc. Global challenge with harmful impacts on the environment, economy, and health. Pollution, especially macro-sized debris, has become a concern to researchers due to its extreme durability and buoyancy, which threaten aquatic ecosystems [1]. Due to its lightweight, durable nature and impressive ratio of cost to performance, plastic has become an indispensable tool in sustaining and delivering the quality, comfort, and safety of modern lifestyles [2]. Microplastics can be categorized into two types. Primary microplastics are manufactured by industry, for example, microbeads in cosmetics, toothpaste, and face wash, or microfibers from synthetic clothing and rope, whereas secondary microplastics are the result of the breakdown of larger plastic fragments by erosion, solar radiation, water currents, and freezing and thawing [3]. As microplastics may contain problematic plasticizers and can also adsorb other organic pollutants, the long-term effects are unpredictable and responsible for various hazardous health disorders [4]. Nowadays, it is believed that more than 250 species, including fish, sea birds, turtles, and marine mammals, are affected by the ingestion of these micropollutants [5]. Plastic has been popular in public since the 1950s and has been tremendously produced and utilized in people’s daily lives due to its relative cheapness, convenience, and versatility [6].

Plastics are comprised of hydrocarbons derived from petroleum, organic materials such as cellulose, coal, natural gas, salt, and crude oil through a polymerization or poly-condensation process and are hydrophobic in nature [7]. In spite of several benefits offered by plastics in our daily lives, they simultaneously generate the problem of increasing environmental pollution. In particular, the small-sized plastic particles that originate from manufacturing units and the degradation products of large items are prone to accumulate in the environment and may pose a serious effect on the ecosystem [8–10].

Plastics are categorized in accordance with their size due to their extensive range of sizes, from larger to millimeters to micrometers. Particles smaller than 5 mm are referred to as microplastics, so about the size of the grain of rice, although a subdivision into large microplastics (1–5 mm) and small microplastic particles (1 μm–1 mm) has been introduced [11]. A very diverse group differing in size, shape—fragments, pellets, cosmetic beads, lines, fibers, films, foams, chemical composition—polypropylene, polyethylene, polystyrene, polyester, polythene, etc. Those fragments persist due to the extremely slow degradation process and stability of plastic, which have been estimated to have been in the range of hundreds of years [12]. When water creatures ingest contaminated microplastics, they can enter the food web and then get exposed to the human body through the consumption of seafood [13]. Considering the potential threats to aquatic organisms, birds, mammals, and even humans via
mistaken ingestion, microplastic contamination is attracting increasing attention worldwide [14]. Moreover, microplastics serve as carriers for heavy metals and synthetic organics, generating complex contaminants in living systems [15–17]. Numerous studies have been conducted to assess microplastic pollution in the marine environment; however, freshwater environments such as rivers and lakes have been understudied [18–20]. Nevertheless, studies have shown that freshwater bodies play an important role in pollution with microplastics, as they act as carriers of plastic and microplastics into the marine environment [21–23]. Although Nepal is rich in water resources, microplastics research in Nepal has received less attention and is in an early stage. So far, in Nepal, very few studies on microplastics and microplastic pollution have been conducted in freshwater environments [24–26]. As a result, there is an urgent need to assess this emerging pollutant in freshwater systems more adjacent to terrestrial points and directly affected by human activities.

The Hanumante River, an important tributary of the Bagmati River, located in the Bagmati province of Nepal, was chosen as the study site for microplastic research due to its proximity to commercial and residential areas as well as its agricultural uses [27,28]. Additionally, there is a lack of understanding regarding microplastic pollution. Moreover, the cultural value of the river has been affected by the degradation in water quality due to pollution. Hence, the aim of the current study was to contribute to a better understanding of microplastic abundance and distribution in the water sample of the Hanumante River so that baseline information may be imparted for further work.

Materials and Methods

Study area and sampling

The study area, the Hanumante River, is almost entirely located in the district of Bhaktapur, Nepal. The river originates from Mahadev Pokhari, Nagarkot, flows through rural and urban areas across Bhaktapur and Thimi municipalities, and confluences the Manohara River at Jadibuti, in Kathmandu District. The river possesses several tributaries, whose average width ranges from 10 m to 20 m. The water quality of the Hanumante River, with a total stretch of 23.5 km, is deteriorating with increased urbanization [27].

The sampling was done in January 2021. A total of six sampling sites were selected along the length of the Hanumante River to represent the entire area of the river. These sampling sites include: Tikathali area (SA), Lokhanthali area (SB), Suryabinayak area (SC), Shiva temple-Jagati area (SD), Radhe-Radhe area (SE), and Madhyapur Thimi area (SF), respectively. In order to improve the accuracy and precision of the analysis, each sample was replicated. The bottle sampling method was employed to collect water samples from each location along the Hanumante River. At each site, water samples were collected and filtered by utilizing three mesh sieves with pore sizes in the range of 2.36 mm, 1.18 mm, and 75 μm. After filtration, the samples...
were washed with distilled water to remove any residuals and sediments remaining on the sieve. The samples were then stored in a glass container and kept in the refrigerator at 4 °C for laboratory analysis [29].

Analysis

For analysis, the standard protocol of the National Oceanic and Atmospheric Administration (NOAA) was followed [30]. Each sample was oven-dried at 90 °C for 24 hours. After drying, the mass of the total solids was determined by subtracting the mass of the empty conical flask from the mass of the conical flask with dried solids. Masses were precisely measured using an analytical balance.

Wet peroxide oxidation and density separation

Organic matter in the residue was digested by employing the wet peroxide oxidation (WPO) method [30]. For this, 20 mL of the 0.05 M Fe(II) solution was added to the sample along with 20 mL of 30% hydrogen peroxide, and the mixture was kept aside for 5 minutes. Then the content was heated at 75°C on a hot plate [31]. As soon as the bubbles appeared, they were removed from the hot plate and left aside for 2–3 minutes. Again, the content was digested for the next 30 minutes to remove organic matter. The process was continued until all the organics were removed. Following WPO, all the samples were processed for density separation. The density separation was carried out using 250 mL of saturated zinc chloride and ZnCl2 solution to float the microplastics from the river. The content was heated at 60 °C for some time to dissolve the salt completely. The solution was transferred to the density separator, which allowed the solids to settle down overnight for better separation.

Vacuum filtration

The process was carried out using a Buchner funnel, where a vacuum was created through water pressure. Glass microfiber filter paper was employed to filter the sample. The supernatant containing the floating microplastics from the density separator was filtered. Finally, the filter paper containing the filtered microplastics was transferred to petri dishes, labeled, and dried for further examination [32].

Contamination control

As preventive measures, cotton laboratory coats and nitrile gloves were used during sampling and analysis. To avoid any possible contamination, all equipment and glassware were cleaned three
times with distilled water before use. Glassware and samples were wrapped with aluminum foil to minimize contamination during sample processing. Procedural blanks were also performed, which shows it is negligible to check background contamination. Hence, the background was not subtracted from the total count [33].

**Sample analysis for assessment**

Finally, the dried mass of each sample was examined under a fluorescence microscope at 40X magnification. The suspected microplastic particle was confirmed by a break test [34]. Further insight into the microplastic was obtained by FTIR spectroscopy. The FTIR spectra were recorded within 400–4000 cm$^{-1}$ in the attenuated total reflection mode using a spectrophotometer (IR Tracer-100, SHIMADZO, Japan).

**Results and Discussion**

**Gravimetric analysis**

Fresh samples collected from different sites were subjected to gravimetric analysis, which provides information about the total mass of organic compounds decomposed by wet peroxide oxidation and the amount of analytes (microplastic particles) left for further analysis. The results from the weight of a freshly taken sample before complete dryness are summarized in Table 1. The results demonstrated that sample SE (Radhe-Radhe area) exhibited the highest value of 5.41 g, indicating a large mass of solids present in the water sample. In contrast, the sample SD (Shiva temple-Jagati area) exhibited the lowest value of 2.30g with the least load of solids.

**Table 1.** Gravimetric analysis of fresh sample from the source

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Wt. of conical flask with sample in g (b)</th>
<th>Wt. of empty conical flask in g (a)</th>
<th>Mass of solid in water sample in g (M = b-a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA</td>
<td>139.66</td>
<td>136.18</td>
<td>3.48</td>
</tr>
<tr>
<td>SB</td>
<td>139.49</td>
<td>142.49</td>
<td>3.0</td>
</tr>
<tr>
<td>SC</td>
<td>140.28</td>
<td>136.89</td>
<td>3.39</td>
</tr>
<tr>
<td>SD</td>
<td>116.32</td>
<td>114.02</td>
<td>2.30</td>
</tr>
<tr>
<td>SE</td>
<td>115.53</td>
<td>110.12</td>
<td>5.41</td>
</tr>
<tr>
<td>SF</td>
<td>101.58</td>
<td>97.87</td>
<td>3.71</td>
</tr>
</tbody>
</table>

**Table 2.** Reduced mass of the sample

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Initial mass of sample in g (x)</th>
<th>Final mass of sample after WPO in g (y)</th>
<th>Reduced mass of the sample in g R=(x-y)</th>
<th>Reduced mass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA</td>
<td>3.48</td>
<td>0.219</td>
<td>3.261</td>
<td>93.70%</td>
</tr>
<tr>
<td>SB</td>
<td>3.0</td>
<td>0.153</td>
<td>2.847</td>
<td>94.9%</td>
</tr>
</tbody>
</table>
Results of the reduced mass of the sample that has been obtained after complete dryness in an oven at 90°C are summarized in Table 2. The maximum reduced mass (96.85%) was observed for the sample SF, i.e., sample taken from Madhyapur Thimi area and similar result was observed with the sample SE, i.e., sample taken from Radhe-Radhe area. However, minimum value (92.60%) of reduced mass was observed in the case of sample SD collected from Shiva temple-Jagati area.

### Microscopic examination

Based on the relative level of pollution and human population density, we have categorized our entire samples into three classes: Tikathali (SA) and Lokhanthali area (SB); Radhe-Radhe (SE); Madhyapur Thimi area (SF); Suryabinayak (SC); and Shiva temple-Jagati area (SD) for microscopic examination. Microscopic observations were performed just after the laboratory analysis. Most of the colored and transparent objects observed in the images were found to be microplastic particles; further, they were confirmed by a needle-piercing test.

Figure 2. Microscopic images of microplastics along the Tikathali (SA) and Lokhanthali (SB) area.

Figure 2 represents the microscopic images of the samples taken from the Hanumante river along the Tikathali and Lokhanthali areas. Approximately 175–185 microplastic fragments were estimated from eight liters of sample taken in the study. This indicated a microplastic load of 22–23 microplastics per liter of sample. Since the area is located at the junction point of Kathmandu, Bhaktapur, and Lalitpur districts, drainage and household waste have been directly supplied into the riverine system, so the level of pollution in the river flowing through this area is very high.
Figure 3. Microscopic images of microplastics along the Suryabinayak (SC) and Shiva temple-Jagati (SD) area.

Figure 3 displays the images of the samples collected from the Suryabinayak and Shiva temple-Jagati area. These samples were composed of large amount of organic matters. Approximately 120–130 microplastics were observed in the recovered water samples during microscopic examination. This demonstrated that nearly 15–16 microplastics were present per liter of sample in this area. The microplastic contamination in these samples were relatively less as compared to those from Tikathali and Lokhanthali area.

Figure 4. Microscopic images of microplastics along the Radhe-Radhe (SE) and Madhyapur Thimi (SF) area.

The images presented in Figure 4 show that approximately 110–115 microplastics were observed from the samples recovered from the Radhe-Radhe and Madhyapur Thimi areas for microscopic examination. This demonstrated that nearly 13–14 microparticles per liter of water sample were collected from the area

FT-IR analysis for potential microplastics

FT-IR spectra of potential microplastics were identified in the three categorized representative samples.

Figure 5. FT-IR spectrum of representative sample from Tikathali and Lokhanthali area.

Figure 5 shows the FTIR spectra of the microplastics samples extracted from the Tikathali and Lokanthali areas. This area was observed to be intensely polluted. Prominent, tiny plastic fragments were observed during sample extraction. Therefore, this sample is expected to have lots of microplastic fragments. Several spectral peaks can be observed in the spectrum. C-H stretching was observed at 2916.37 cm^{-1} and 2846.93 cm^{-1}, and methylene deformation were seen at 1450.47 cm^{-1} and 649.69 cm^{-1} which are very similar to the data provided by the free
Therefore, the most possible polymers found in the sample above are polyethylene, polychloroprene, and some of the nitrile rubber particles [36].

![Figure 6. FT-IR spectrum of a representative sample from Suryabinayak and Shiva Temple-Jagati areas.](image)

An intense peak can be observed with a wavelength of 2916.37 cm\(^{-1}\). This value matches the 2920 cm\(^{-1}\) and 2850 cm\(^{-1}\) for methylene stretch, as suggested by free library FT-IR data [35,37]. All of this evidence indicates the presence of polyethylene in the sample under observation. The presence of a methyl peak and methyl deformation at 1458cm\(^{-1}\) indicates the spectrum of polypropylene rubber in the sample as described by the free library data.

![Figure 7. FT-IR spectrum of a representative sample from Radhe-Radhe and Madhyapur Thimi areas.](image)

Methyl stretch can be observed at wave numbers of 2916.37cm\(^{-1}\) and 2846.93 cm\(^{-1}\). On the basis of the above interpretation suggested by free library data, it can be deduced that the sample from Radhe-Radhe and Madhyapur Thimi areas must be composed of polymers such as polyethylene, high-density polyethylene, vinyl acetate, and polycarbonate [38–40].

**Conclusions**

The study showed the abundance and distribution of microplastics across the Hanumante River. It is a unifying river, and it has been considered one of the most polluted tributaries of the Bagmati River. Through the microscopic observations, transparent as well as intensely colored microscopic fragments, generally with various shades such as red, brown, blue, and black, were observed in the refined sample. In the sample from Tikathali and Lokanthali areas, approximately 23 microplastics per liter of sample were observed, whereas the sample from Suryabinayak and Shiva Temple-Jagati areas was found to be composed of nearly 16 microplastics per liter of sample taken, and the number of microplastic fragments observed in the sample from Radhe-Radhe and Madhyapur Thimi areas was nearly 14 microplastics per liter. The FT-IR analysis of the sample examined from the Tikathali and Lokanthali areas was observed to consist of rubber, polyethylene, polychloroprene, and fibrous materials as major polymer components. Similarly, the most abundant types of polymers observed in the samples from Suryabinayak and Shiva Temple-Jagati were...
polyethylene and polypropylene. The most dominant types of polymers observed in the samples taken around the Madhyapur Thimi area were polyethylene, high-density polyethylene, vinyl acetate, and polycarbonate-containing compounds. The work will provide an understanding of the impact of urbanization on microplastic pollution, thereby contributing to the conservation of riverine ecosystems.

Acknowledgements

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