Microplastic pollution in urban Lake Phewa, Nepal: the first report on abundance and composition in surface water of lake in different seasons

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Abstract

Microplastics are man-made pollutants which have been detected in surface water and groundwater. Research on microplastic concentration in aquatic environment is attracting scientists from developing countries, but in Nepal no information regarding microplastic in freshwater system is available. Therefore, this study investigates the presence and abundance of microplastic in lake surface water of Phewa Lake, the second largest lake in Nepal. The average concentration of microplastic for surface water was 2.96 ± 1.83 particles/L in winter (dry) season and 1.51 ± 0.62 particles/L in rainy (wet) season. Significant difference with $t = 4.687$ ($p < 0.01$) in microplastic concentration was observed in two different seasons. Fibers (93.04% for winter and 96.69% for rainy season) were the commonly found microplastic type in lake water and transparent as the dominant color for the two seasons. Almost all the detected microplastic were found to be < 1 mm in size. Due to the small size of microplastic and unavailability of micro-Fourier transform infrared spectroscopy (μ -FTIR) and Raman spectroscopy in Nepal, polymer identification was not done. The findings from this study can provide a valuable baseline data on microplastics for the first time in Nepal's freshwater lake environment.

Keywords Microplastic · Lake Phewa · Surface water · Seasons · Fibers

Introduction

Plastics are versatile materials and have become an integral part in peoples' daily lives across the globe (Xia et al. 2021). Extensive use of plastic during the outbreak of COVID-19 pandemic in healthcare sector and public health safety highlighted the essential role of plastic in modern society (Parashar and Hait 2021). As plastics are lightweight, flexible and durable, global plastic production has increased drastically to 368 Mt in 2019 (PlasticsEurope 2020) and by 2050, it is predicted that around 12,000 Mt of plastic debris will end up in dumping ground and in the surrounding environment (Geyer et al. 2017). With the widespread indiscriminate use of plastic products and improper disposal of plastic litter (Silva et al. 2020) during COVID-19 pandemic, it has intensified plastic pollution crisis that must be addressed by a powerful and effective plan of action (United Nations Environmental Program 2021). A recent research predicted that around 14.4 Mt of microplastic have made its way into the world's ocean floor (Barrett et al. 2020).

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Microplastics are plastic fragments with a diameter < 5 mm as defined by USA National Oceanic and Atmospheric Administration in 2008 (Arthur et al. 2009). In the natural environment, microplastics are released as primary or secondary microplastics. Primary microplastics are discharged into the environment in their original form mainly from textiles and personal care products as microbeads. Likewise, secondary microplastics are formed by the fragmentation of macroplastic as a result of weathering of plastic debris or disintegration of car tire (United Nations Environmental Program 2018). Microplastics are ubiquitous in the aquatic environment. This is of increasing concern as it is associated with various ecotoxicological effects (Akdogan and Guven 2019) once inhaled or ingested by human which include oxidative stress, inflammatory lesions, increased internalization or translocation (Rahman et al. 2021; Yong et al. 2020), and even cellular damage, immune reactions, and DNA damage (Yong et al. 2020). Likewise, organophosphate esters used as plasticizer are potential carcinogens and endocrine disruptors that have been linked to severe toxicity in freshwater biota (Onoja et al. 2021). Even in vascular plants, phytotoxic effects like changes in plant growth, photosynthesis, and antioxidant defense system are seen when exposed to microplastics and nanoplastics (Yin et al. 2021). Similarly, aging of microplastics increases the risk of ingestion by organism, enhances interaction with pollutants, and the release of microplastic-derived organic compounds (Liu et al. 2021).

Microplastics enter the freshwater environment through domestic wastewater effluent (Browne et al. 2011), runoff from agricultural lands, and overflow of drains during storm or rain event (Eriksen et al. 2013; Galgani et al. 2015), factory spillage (Gasperi et al. 2014), busy traffic (Klein and Fischer 2019), and atmospheric fallout (Allen et al. 2019; Negrete Velasco et al. 2020). Once released into the aquatic ecosystem, microplastics form a dynamic recycling process for its transport in the environment (Xiang et al. 2021), which depends on the type of plastic waste and the local environmental conditions (Schwarz et al. 2019). Microplastics is an emerging pollutant which has been intensely studied in marine environment (Thompson et al. 2004). However, in freshwater environment comparatively less research has been done (Fahrenfeld et al. 2019). Freshwater bodies act as a carrier of microplastics and transport network of microplastic to the marine environment (Jiang et al. 2018). Among the countries from South Asia, significant research on microplastic has been carried out by India. A few studies have been reported by Pakistan, Bangladesh, Maldives, and Sri Lanka, whereas, Bhutan and Afghanistan have not yet reported studies on microplastics in their environment (Amrutha et al. 2021). In Nepal, microplastic research is in the initial stage where only one research on microplastics

has been reported on road dust (Yukioka et al. 2020) and the other in the river (Yang et al. 2021).

The country Nepal is blessed with rich water resources which have been providing important services such as drinking water source, generation of hydropower, irrigation, aquaculture, and recreation. So, there is an urgent need to address this emerging pollutant.

The study area, Phewa Lake, is the second largest lake and one of the main tourist destinations of Nepal. The panoramic view of Manaslu and Annapurna Mountain range and the reflection of Machhapuchhre Mountain on the lake attract tourists. The lakeshore is encroached by hotels and restaurants to capture the scenic beauty of the lake which has increased the pollution load of the lake. Till date, no study on microplastic has been reported previously from the proposed study area. Therefore, the objective of this study is to compare the microplastic concentration in different seasons for Phewa Lake with regard to surface water. Thus, this study will bridge the gap to generate abundance and spatial distribution of microplastic from lake surface water, for the first time. Furthermore, this study will provide a baseline data on microplastics so the local authority can take action to protect the lake ecosystem before it is too late.

Materials and methods

Study area and sample collection

Pokhara valley consists of nine lakes among which Phewa Lake is the largest in Lake Cluster. Its elevation ranges from 763 to 2482 m (Ministry of Forests and Environment 2018). The area of Phewa Lake covers 5.72 km² with 122.53 km² as the watershed region. The average depth of the lake is 8.6 m and maximum depth recorded as 23.5 m (Gurung et al. 2010). Phewa Lake is fed by Harpan Khola (stream), the main inlet of water to lake whereas at the outlet, a dam is situated through which the water flows out. The lake has multipurpose use such as drinking water source, fishery and cage culture, irrigation, hydropower generation, and boating. However, the lake is threatened by siltation, pollution from nearby city area, and eutrophication by water hyacinth (Ministry of Forests and Environment 2018).

Surface water samples were collected in the beginning of February, 2021 for winter (dry) season and in the third week of July, 2021 for rainy (wet) season. A total of 16 sampling sites were located by global positioning system (GPS) (GARMIN eTrex® 10) for uniform distribution of the lake area (Fig. 1). The sites were divided into 8 sections as inlet and outlet areas of the lake, dense, moderate, and less populated areas, center lake, temple area, and mixing area where drainage from the city is discharged. At each location, 5 L of surface water (0–20 cm in depth) was collected (Negrete

Fig. 1 Sampling points of study area with possible sources of microplastics

Velasco et al. 2020; Su et al. 2016) by using a steel bucket. Then, it was filtered through a 75- μm brass sieve on site and carefully transferred into the glass bottle (Mao et al. 2020) using 18.2-M Ω water (Millipore, Milli-Q). At each site, 2 replicates of a sample were collected and were stored at 4 °C before analysis. Furthermore, 1 L of surface water was collected from each site to measure the physical, chemical, and biological water quality parameters.

Extraction of microplastics from water sample

Extraction of microplastics from the surface water was performed with reference to National Oceanic and Atmospheric Administration (NOAA) (Masura et al. 2015) with a slight modification. Briefly, the water sample was treated with 20 mL of 30% H_2O_2 and 20-mL Fe solution as a catalyst to degrade the organic matter. After about 5 min, it was heated on a hot plate at 50 °C until it boiled and kept for additional 15 min. Then, the digested sample was filtered through a 1-mm sieve. The filtrate collected in a beaker was again carefully filtered through a 47-mm diameter glass microfiber filter (Whatman GF/C™) under a vacuum. The beaker was rinsed 2–3 times with Milli-Q water to ensure complete removal of microplastics. The filter paper was immediately kept in a clean petri dish and dried at room temperature for further examination (Yuan et al. 2019). If any microplastic was visible in the sieve, it was picked with

a tweezer, washed, and kept in a petri dish for visual identification separately.

Identification of microplastics

The petri dish along with filters was visually inspected under a stereomicroscope (SZ2-ILST, Olympus, Japan) at 40 $\times$ magnification. Microplastics were identified according to their morphological characteristic into shape and color. Based on their shape, microplastics were divided into 4 categories such as film, fiber/line, foam, and fragment (Liu et al. 2019). Criteria described by Hidalgo-Ruz et al. (2012) were followed to identify microplastics. In addition, hot needle test was done to confirm whether the suspected particles were plastic or non-plastic (De Witte et al. 2014). A plastic piece melted when a very hot needle was brought in contact, if the sample piece under examination was plastic. This test was carried out due to the small size of the collected microplastics. Number, shape, and color were also recorded for each microplastic.

Control of contamination

Preventive measures were taken during sampling and laboratory analysis to avoid possible contamination. Cotton laboratory coat and nitrile gloves were worn during sampling and processing of the sample. As far as possible, glassware was

used in the laboratory; and before use, it was rinsed with Milli-Q water. Samples and glassware were covered with aluminum foil to minimize contamination during sample processing (Fig. S1). After filtration, filter paper was immediately kept inside a petri dish to avoid contamination. To find out airborne contamination, filter paper was first examined under a stereomicroscope; and then kept open on a petri dish in the working environment for 24 h. Field blanks were also conducted in four randomly selected sampling locations. Briefly, on each location, 5 L of Milli-Q water was filtered through a 0.75- μ m brass sieve. Further treatment was similar to the water sample without the digestion process as mentioned above. The results of field blanks for winter and rainy seasons were 0.1 particles/L and 0.15 particles/L respectively. Similarly, 0.25 particles per filter paper were found in air contamination of lab for both seasons. Results indicated a negligible amount of contamination. Therefore, the background contamination was not subtracted from the total microplastic count (Baldwin et al. 2020).

Data analysis

The mean abundance and standard deviation of microplastics were measured at different areas. The abundance of microplastics was expressed as the number of particles/L. The distribution of abundance according to different color and type of microplastics was also expressed. To test the significance difference among the various sections of the lake; first, the normality and homogeneity test was done using Shapiro–Wilk test for normality and Levene’s test for homogeneity. Since the data did not follow the normality and homogeneity, Kruskal–Wallis H test was adopted to test the significance abundance among the different sections. Similarly, Dunn–Bonferroni post hoc test was used for comparison of different pairs. To test the significance difference of

mean abundance between two seasons, Students’ *t* test was adopted.

Results and discussion

Abundance and spatial distribution of microplastics in Phewa Lake

Microplastics were present in all surface water collected from 16 sampling locations of Phewa Lake for winter and rainy seasons. The abundance of microplastics ranges from 0.8–8 particles/L for winter season and 0.4–2.8 particles/L for rainy season. The mean abundance of microplastic in rainy season (1.51 particles/L) was significantly lower than that in winter season (2.96 particles/L) with $t=4.687$ ($p<0.01$). This could be due to the recent unexpected flood that occurred before the sampling event. Schell et al. (2021) also reported increased microplastic concentration linked to heavy rain prior to the sampling day. Studies have shown that heavy rain and flood events may result in increased microplastic loads from non-point sources like combined sewer outflow and stormwater discharge (Blettler et al. 2017; Kataoka et al. 2019). Studies have indicated that urban sewage drainage, solid waste disposal (Cole et al. 2011; Horton et al. 2017), runoff from agriculture land (Akdogan and Guven 2019), and laundry activities (Browne et al. 2011) are sources of microplastics in the freshwater system. Phewa Lake is polluted by the above mentioned sources (Pokharel 2009), which may be the cause for microplastic pollution of the lake. Fishery is another source of microplastics to the lake (Gopinath et al. 2020; Yuan et al. 2019). For comparative study, the freshwater lakes from SAARC countries (Afghanistan, Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, and Sri Lanka)

Table 1 Comparison of microplastic abundance in freshwater from SAARC countries

Study area	Sample type	Average/range	Shapes	Reference
Red Hills Lake, India	Water, sediment	Water: 5.9 particles/L Sediment: 27 particles/kg	Fibers, fragments Fibers, fragments	Gopinath et al. (2020)
Veeranam Lake, India	Water, sediment	Water: 28 items/ km ² Sediment: 309 items/kg	Film, fibers	Bharath et al. (2021)
Vembanad Lake, India	Sediment	252.80 $\pm$ 25.76 particles/m ²	Film, foam	Sruthy and Ramasamy (2017)
Ganga River, India	Sediment	107.57–409.86 items/kg	Fibers	Sarkar et al. (2019)
Netravathi River, India	Water, sediment	Water: 288 pieces/m ³ Sediment: 96 pieces/kg dry weight	Fibers, film Fragments, fibers	Amrutha and Warriar (2020)
Rawal Lake, Pakistan	Water, sediment	Water: 1.42 items/L Sediment: 1.04 items/0.01 kg	Fibers, fragments Fibers, fragments	Irfan et al. (2020b)
Ravi River, Pakistan	Water, sediment	Water: 2074 $\pm$ 3651 MPs /m ³ Sediment: 3726 $\pm$ 9030 MPs/m ²	Fragments, fibers Fragments, fibers	Irfan et al. (2020a)
Koshi River, Nepal	Water, sediment	Water: 202 $\pm$ 100 items/m ³ Sediment: 58 $\pm$ 27 items/ kg dry weight	Fibers Fibers	Yang et al. (2021)

were selected (Table 1). This result showed that Phewa Lake has twice as much microplastic load (2.96 particles/L) than Rawal Lake (1.42 items/L) in winter (dry) season (Irfan et al. 2020b). Similarly, the concentration of microplastics in Red Hills Lake was nearly four times higher (5.9 items/L) (Gopinath et al. 2020) than the microplastic concentration in Phewa Lake (1.51 particles/L) during the rainy (wet) season. Furthermore, we reviewed studies that used bulk sampling method and mesh size < 100 µm and compared them with Phewa Lake (Table S1). The microplastic pollution level of this study was low to moderate. The microplastic abundance in Wuliangsuhai Lake ranged from 3.1 to 11.25 items/L (Mao et al. 2020) and 5 to 34 items/L for Poyang Lake (Yuan et al. 2019); which was both higher than the microplastic abundance observed in Phewa Lake for both seasons: (2.96 items/L in winter and 1.51 items/L in rainy season). Compared to Phewa Lake, West Dongting Lake, and South Dongting Lake have less microplastic load (Table S1) (Jiang et al. 2018).

The whole lake was divided into 8 sections as inlet (IN-S1), outlet (OU-S2), mixing area (MI-S3), temple area (TE-S4), dense populated area (DE-S5-S8), moderate populated area (MO-S9-S10), less populated area (LE-S11-S12), and center lake area (CL-S13-S16). To find out the significance of abundance in different sections of the lake, Kruskal-Wallis H test was adopted. It was found that the average abundance of microplastics in different sections was significantly different ($H=18.727$ with $p<0.01$) in winter season while it was not significantly different in rainy season ($H=13.825$ $p=0.054$). This could be attributed to two factors. First, as tourism activities were totally closed due to the COVID-19 pandemic, it may have resulted in low microplastic concentration in all sites. Recent studies have shown that, higher tourism inflow increases the quantity of microplastics in nearby water body (Napper et al. 2021). Second, probably due to the flushing mechanism caused by precipitation (Han et al. 2020; Napper et al. 2021). In order to confirm which pair had a significance difference in winter season, Dunn-Bonferroni post hoc test was adopted (Table S2). The result showed that there was a significance between the mean abundance of dense populated area with center lake area ($p<0.01$) and outlet with center lake area ($p<0.1$). DE-S5-S8 which lies at the eastern part of the lake has high tourism flow; which coincides with high abundance of microplastics in this section of the lake. Therefore, tourism is considered as an important source of microplastics to the lake as reported by previous studies (Free et al. 2014; Xiong et al. 2018). Likewise, factors responsible for high microplastic abundance in the OU-S2 section of Phewa Lake may be linked to long narrow landscapes (Yuan et al. 2019) and dam structure (Zhang et al. 2015) located on the south-east side.

In winter season, the lowest mean abundance of microplastic was found at the center lake area (1.375 ± 0.494

items/L) where human activities are negligible followed by less populated area (mean = 2.15 ± 0.640 items/L) as shown in Table 2. This section is located on the southern side of the lake which is covered by forest area with sparse rural settlement. Similarly, at the lake outlet, mean microplastic concentration was found to be the highest (5.3 ± 0.989 items/L). This is probably due to direct discharge of untreated sewage from urban areas near the outlet of the lake; and also by Phikre Khola (stream) which drains huge loads of sewage and solid waste close to the outlet of Phewa Lake. Likewise, wind may also have transferred microplastics from nearby residential area (Liu et al. 2019). Hence, this could be the reason for higher concentration of microplastics at the dam outlet of Lake. Irfan et al. (2020b) reported a similar finding of Rawal Lake where the concentration of microplastics was highest at the outlet. Gopinath et al. (2020), Jian et al. (2020), and Jiang et al. (2018) also noted higher microplastic concentration at the lake outlet compared to the other areas of the lake.

In the same way in rainy season, a slight variation in the mean concentration of microplastic was observed than in winter season in different locations of lake (Table 2). Temple area (TE-S4) which lies in the center of the lake, at the southeast site, was found to have the lowest concentration of microplastics. This could be due to the close down of the temple area as a result of the COVID-19 pandemic. Furthermore, studies have shown that the concentration of microplastics is related with anthropogenic factors such as population density and the land use pattern (Cole et al. 2011; Hendrickson et al. 2018). Similarly, previous studies have shown that a wide area of the lake acts as a factor to dilute the abundance of microplastics and decrease the impacts of land-based plastic pollution (Yuan et al. 2019). Therefore, this could be the reason for low abundance of microplastics in the center lake area. In rainy season, the concentration of microplastics in a dense population section of the lake was reduced as all the hotels and restaurants around the lake were closed due to lockdown from summer season to the

Table 2 Areawise distribution of microplastics (particles/L)

Area-code	Winter season		Rainy season	
	Mean	SD	Mean	SD
DE-S5-S8	4.63	2.10	1.68	0.41
MO-S9-S10	2.95	1.04	1.90	0.89
LE-S11-S12	2.15	0.64	1.35	0.30
CL-S13-S16	1.38	0.49	1.03	0.41
TE-S4	2.40	0.28	1.00	0.85
OU-S2	4.90	1.56	1.60	0.28
MI-S3	2.40	0.00	2.70	0.14
IN-S1	3.50	3.25	1.60	0.28
Overall	2.96	1.83	1.51	0.62

beginning of rainy season. Therefore, this study supports that densely populated locality and residential area close to the lake have high microplastic concentration (Xiong et al. 2018). Similarly, the highest mean abundance of microplastic concentration in rainy season was recorded in a mixing area (MI-S3); which may be linked with direct discharge of untreated domestic effluent and surface run-off carrying microplastics from the road side by Phikre Khola (stream) which drains into the Phewa Lake at this section of the Lake. During field observation (sampling events), plastic wastes were observed at the periphery of the lakeshore and directly in the water at some places which may be a possible source of microplastic fragments (Schell et al. 2021) to the lake as fragments were also found during lab analysis of water samples. Therefore, the sources of microplastics in the Phewa Lake is due to sewage and drainage discharge near the dam outlet, dry deposition, and unmanaged sewage flow from hotels and restaurants surrounding the lake, fishing and fish cage culture, laundry activities, tourism, and agricultural run-off from nearby land.

Morphological characterization of microplastics

Photographs of typical microplastic items observed in surface water of the Phewa Lake are given in Fig. 2. Fibers, films, and fragments were the types of microplastics identified from the Phewa Lake in winter season whereas foam was also detected in rainy season. Foam probably originated from thermocol

boxes which are used for fish storage and transportation. The reason behind the detection of foam only in rainy season is not clear, but we speculate flood event during winter season to effectively flush the foam due to its lightweight. Therefore, foam was not detected in winter season. Fibers were the most dominant shape in both seasons accounting for 93.04 and 96.69% of the total microplastics observed in the lake surface water in winter and rainy seasons, respectively (Fig. 3). This finding is similar to the previous freshwater studies like 20 major lakes, Wuhan, China (52.9–95.6%) (Wang et al. 2017), Taihu Lake, China (48–84%) (Su et al. 2016), Dongting Lake China (12.17–77.42%) (Jiang et al. 2018), Lake Kallavesi, Finland (64%) (Uurasjärvi et al. 2020), Lake Naivasha, Kenya (81%) (Migwi et al. 2020), which also reported surface water dominated by fibers. Therefore, fibers in the lake surface water

Fig. 3 Percentage distribution of microplastics by shape in Phewa Lake

Fig. 2 Photographs of typical microplastics found in surface water of Phewa Lake **a** fiber, **b** fragment (after melt test), **c** film, and **d** foam

Fig. 4 Percentage distribution of microplastics by color in Phewa Lake

Table 3 Size distribution of microplastics

Abundance size	Winter season	Percentage	Rainy season	Percentage
1.0–5.0 mm	0	0.00	3	1.24
0.2–1.0 mm	474	100.00	239	98.76

may be associated with domestic wastewater (Browne et al. 2011), which is directly discharged into the lake, aging fishing rods, and nets (Yuan et al. 2019) and washing clothes (Browne et al. 2011) at the lake bank. However, further extensive researches are required to disclose the toxicity of microplastic fibers in the risk assessment (Rebelein et al. 2021). Films and fragments are produced by the fragmentation of plastic debris thrown away by tourist and local residents (Eerkes-Medrano et al. 2015), which was also confirmed by field observations as plastic wastes were littered around the periphery of the lake.

Microplastics were identified and classified into seven colors in winter season and eight colors in rainy season as shown in Fig. 4. Transparent was the predominant color of microplastics in both seasons accounting for 40.51 and 31.41% in winter and rainy seasons, respectively. In winter season, all the microplastics detected were found to be less than 1 mm in size from sixteen sampling locations. At the same time in rainy season, 98.78% of the microplastics were of size less than 1 mm and 1.2% were of size 1–5 mm (Table 3). Size of microplastics is driven by seasonal hydrological conditions where smaller microplastics increase in low discharge condition (de Carvalho et al. 2021). In addition, concentration of microplastics increases with decreasing size of microplastics (Su et al. 2020). Color and size of microplastics are linked with ingestion by aquatic organisms if it resembles prey (Wright et al. 2013).

Conclusion

This study determines the first report on microplastic occurrence in the lake system of Nepal and fills the gap by providing data on microplastic pollution. This further attracts

researchers to explore this emerging environmental pollutant with the limited resources available in the country as water resources acts as a driving force for overall development and economic progress of the country. The Phewa Lake serves as an important tourist site. Therefore, high concentration of microplastic in surface water is raising an ecological concern that must be addressed. Large number of hotels, resorts, and restaurants around the lakeside, direct disposal of drainage into the lake, activities like washing clothes, fishing, tourism, and recreation are the cause of microplastic concentration of the Phewa Lake. Future research should focus to assess the risk of microplastic on aquatic organism to protect the lake diversity.

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Author contribution Conceptualization, visualization, and writing—original draft were done by Rajeshwori Malla-Pradhan. Writing—reviewing and editing were done by Thitipone Suwunwong and Tista Prasai Joshi. Supervision and validation were done by Khamphue Phoungthong. Data curation and formal analysis were performed by Bijay Lal Pradhan. All the authors read and approved the final manuscript.

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Declarations

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