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Detection and Characterization of Microplastics in Two Major Lakes of Nepal: Begnas and Phewa

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Abstract
The widespread use and poor management of plastics are harming the environment. As plastics degrade, they break down into tiny particles smaller than 5mm, known as microplastics (MPs), which have become a growing environmental concern. In Nepal, very few studies have focused on MPs.
This study investigates the sources, identification, and quantity of MPs, including their color and shape, in Begnas Lake and Phewa Lake, Nepal, using a slightly modified National Oceanic and Atmospheric Administration (NOAA) protocol. Visual microscopy confirmed the presence of fibers and fragments in samples collected from both lakes. Overall, in Phewa Lake, MPs concentrations ranged from 0.56 ± 0.17 to 2.46 ± 1.66 particles/L, while in Begnas Lake, they ranged from 1.07 ± 0.27 to 1.33 ± 0.51 particles/L. Further studies on exposure and potential health risks are needed. Thus, this study indicates the prospective baseline data of MPs research in Nepal's lake water.
Keywords: Abundance, Color, Morphology, Visual Microscopy, Lakes

1. Introduction
MPs are smaller plastic particles less than 5mm in diameter (Lin, 2016), and they have been detected in a wide range of environments, including oceans (Amelia et al., 2021), rivers (McCormick et al., 2016), lakes (D'Avignon, Gregory-Eaves, & Ricciardi, 2022) and agricultural fields (Corradini et al., 2019), as well as in our food and drinking water (Koelmans et al., 2019). Both Macroplastics (>5mm) and MPs (<5mm) are now recognized as emerging pollutants in marine and aquatic environments (Imhof et al., 2016).

MPs are categorized as primary when plastics are manufactured in small particles and secondary when fragmented from larger plastic items (Lehtiniemi et al., 2018). According to the report, primary MPs accounted for 15% to 31% of plastic pollution, with abrasion of synthetic textiles while washing and abrasion of tires while driving contribute to nearly two-thirds of the total (Boucher & Friot, 2017).

MPs degrade very slowly in the environment (Krueger et al., 2015) leading to primary and secondary MPs building up and persisting in the environment. These particles can affect the health of plants and animals, ultimately disrupting the food chain. Although some MPs are intentionally designed to degrade in the open environment, but they do not biodegrade. In marine ecosystems, MPs may settle as a result of greater density induced by biofouling, a process in which organisms attach to plastic particles (Kaiser et al., 2017). As biofouling progresses, the density of the plastic material increases, and once it reaches a density greater than that of water, the plastic items sink to the bottom of the water bodies. It is estimated that 80% of aquatic litter originates from land-based sources before entering streams and other aquatic ecosystems (Bellasi et al., 2020). Identifying MPs in the environment remains

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challenging, and there is no defined standardized methodology for the identification of MPs (Koelmans et al., 2019).

Begnas Lake is renowned for its stunning landscapes and offers various services, including fisheries. Fishing serves as the primary livelihood for the communities residing around the lake, with approximately 40 fisher families (Jalari) living near Begnas Lake. In 2016, the total fish catch from Begnas Lake was recorded at 15 tons (Husen & Sherpa, 2017). MPs in lakes come from various sources, including human activities, fisheries, and plastic waste carried by runoff and atmospheric transportation, and other means (Wang et al., 2022; Sharma et al., 2024; Dahal and Babel, 2025). These MPs include nearly all plastic types, with polypropylene (PP) and polyethylene (PE) being the most common (72.67%), followed by polyethylene terephthalate (PET) (18.03%) and polystyrene (PS) (3.19%) (Pan et al., 2023). MPs have been detected in the digestive tracts and gills of fish, likely due to accidental ingestion or being trapped while they breathe (Galafassi et al., 2021). These particles can cause tissue damage, trigger oxidative stress, and disrupt the immune system, leading to problems such as neurotoxicity, stunted growth, and unusual behavior in fish (Bhuyan, 2022). When humans consume contaminated fish, they may also experience harmful health effects.

The objectives of this study were to determine the abundance of MPs in Begnas and Phewa Lakes, and to identify their shapes, such as fibers or fragments. The study also aimed to observe the colors of the MPs and compare the pollution levels between the two lakes. Another goal was to identify possible sources of the MPs, such as fishing, tourism, or waste.

2. Materials and Methods

2.1 Study Area

Pokhara Valley is located about 198 km west of Kathmandu at an elevation of 945 m above sea level, with coordinates 28.2096° N latitude and 83.9856° E longitude. The study was conducted at Begnas Lake, Phewa Lake, and the Phewa Powerhouse in Pokhara Valley. Begnas Lake is the third-largest lake in Nepal, while Phewa Lake is the second-largest lake and a semi-natural freshwater lake managed by a dam for irrigation and hydropower. Approximately 1.5 km south of Phewa Lake, the Powerhouse generates 1 MW of electricity. These locations were selected for their ecological significance and the potential risks associated with water and plastic pollution. Table 1 presents the key attributes of the study sites, and Figure 1 illustrates the study area along with the sampling points. As Pokhara is rapidly urbanizing, it is gradually emerging as a key tourism hub (Rimal et al., 2015). The city's development of infrastructure, hotels, museums, transportation networks, and its expansion in tourism-related services such as travel and trekking agencies, are contributing to plastic pollution both directly and indirectly (IFC World Bank Group, 2018). In Pokhara Metropolitan, around 200 metric tons of waste are generated daily, with 20% (approximately 40 metric tons) made up of plastic. However, only 20% of this plastic is recycled, leaving the remaining 80% (about 32 metric tons) to accumulate

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in the environment (Dhungana, 2023; World Bank, 2020). This excess plastic contributes significantly to environmental issues, with much of it ending up in waterways or landfills, intensifying the pollution problem.

Moreover, fish-rearing industries in these lakes further add to plastic contamination, as discarded fishing gear and aquaculture-related waste. Fishing nets and equipment contribute significantly to MPs accumulation in oceans since they release microfibers, are reused, and get accidentally lost or abandoned. MPs from fishing nets and equipment primarily originate from synthetic materials such as nylon, PP, and polyester used in fishing lines, ropes, and nets (Sharma et al., 2024).

Table 1: Attributes of two study sites (Ministry of Forests and Environment, 2018)

Attribute	Phewa Lake	Begnas Lake
Latitude (N)	28.1943 - 28.2902	28.1621 - 28.2167
Longitude (E)	83.8004 - 83.9898	84.0814 - 84.1332
Elevation (m)	763 - 2482	647 - 1447
Catchment Area (km^2)	119.39	18.6
Water Body (km^2)	4.33	3.13
Total Population	63,128	18,710

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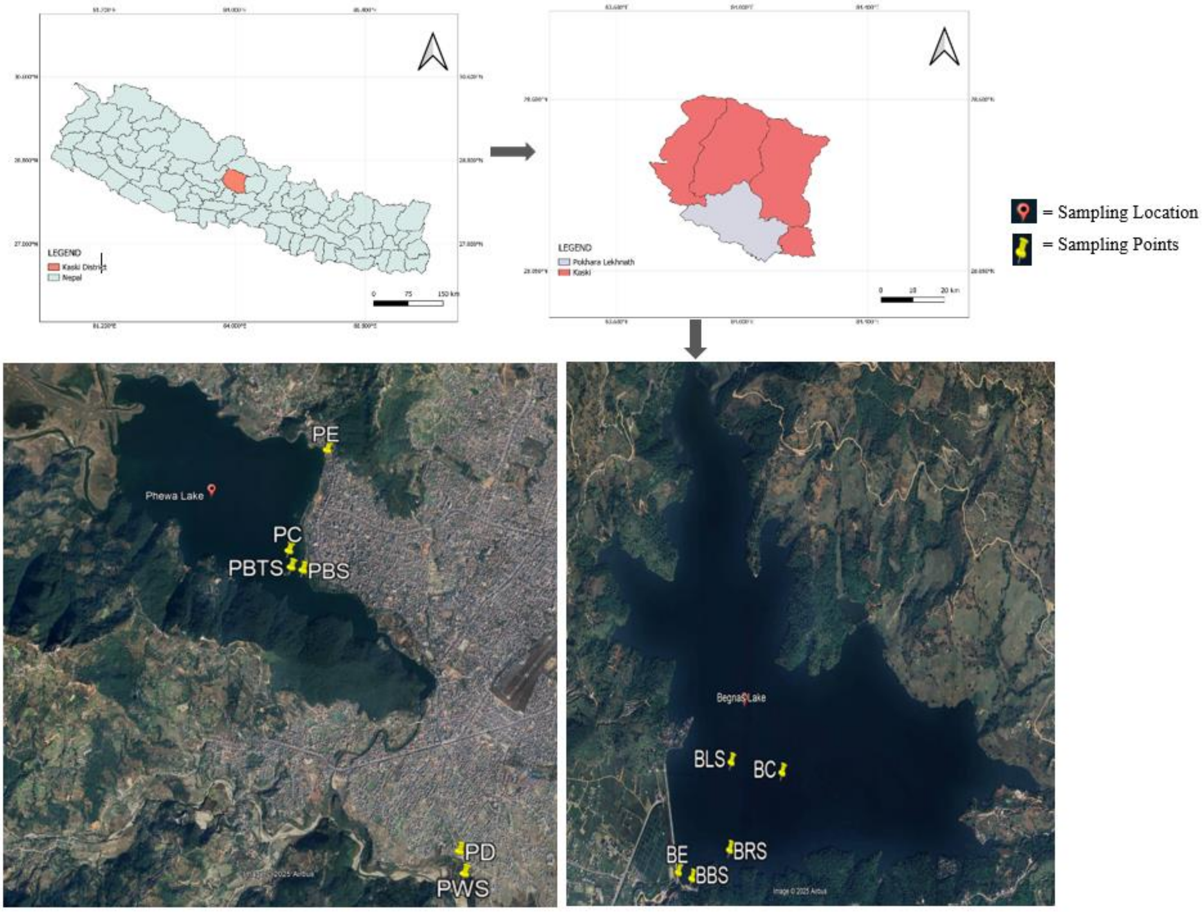

Figure 1: Study area and sampling point

2.2 Sampling Site and Sampling Collection

The study included five sampling points from Begnas Lake and six from Phewa Lake, with two near the Phewa Powerhouse Station. These sites were selected based on their proximity to water and plastic pollution. The specific locations of these points are listed in Table 2**.**

1 liter of surface water samples was collected twice at each sampling point from Begnas Lake and Phewa Lake. A total of 44 samples were collected, with 22 collected in early November 2021 to represent the pre-winter season and another 22 samples in late December 2021 to capture winter-season conditions. Given that MPs concentrations can fluctuate due to weather and sampling conditions, samples were collected in the pre-winter season and winter season from each location to capture these variations. All sampling sites were selected for grab sampling, and the water samples were kept at 4°C until further analysis.

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Table 2: Location of Sampling Stations

Site Name	Site Code	Geographical Co-ordinates (X = Easting, Y = Northing)	Elevation (m)
Begnas Right Side	BRS	214868.59, 3119138.18	688
Begnas Effluent	BE	214521.38, 3119041.79	680
Begnas Left Side	BLS	214885.87, 3119553.14	687
Begnas Boat Side	BBS	214612.00, 3119010.00	686
Begnas Central	BC	215229.17, 3119495.89	686
Phewa Central	PC	789918.00, 3123887.00	797
Phewa Effluent	PE	790285.00, 3125035.00	797
Phewa Boat Side	PBS	790077.00, 3123803.00	797
Phewa Barahi Temple Sample	PBTS	789949.00, 3123709.00	798
Powerhouse Dam	PD	791721.00, 3120900.00	787
Powerhouse Waterfall Site	PWS	791770.00, 3120690.00	700

2.3 Extraction of MPs from water sample

The NOAA was followed with some minor adjustments for the extraction of MPs from surface water (Masura, Baker, Foster, & Arthur, 2015). First, the sample was passed through a 1mm sieve to filter out larger particles. Digesting procedure dissolves organic matter without damaging plastic polymers, and is essential for extracting and identifying MPs in biological samples. To properly extract and identify MPs from biological samples, the organic matter needs to be digested without damaging the plastic. If organic material isn't removed, tiny MPs, especially microscopic ones, could be missed (Vandermeersch et al., 2015). For this process, the water sample was mixed with 20 mL of 30% hydrogen peroxide (H_2O_2) and 20 mL (0.05 M) of an iron (Fe (II)) solution, which helped break down the organic material. The mixture was then heated on a hot plate at 75°C until it started boiling, which took about 5–10 minutes. After that, it was left to cool for another 15 minutes before further testing (Masura, Baker, Foster, & Arthur, 2015).

The digested sample was carefully filtered through a 47 mm diameter (Whatman GF/C™, 0.45 µm) glass microfiber filter. To make sure all MPs were fully removed, the beaker was rinsed 2-3 times with Milli-Q water to ensure the complete removal of MPs. The filter paper was immediately kept in a clean petri dish wrapped in aluminum foil and kept for drying at room temperature for further examination.

2.4 Sample Analysis and Quantification

The filter papers were analyzed under a stereomicroscope with at least 10X magnification (Olympus U- CMAD3, Tokyo Japan). Many studies have utilized stereo microscopy to

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examine MPs contamination (Hossain et al., 2023; Makhdoumi et al., 2021; Mercy, Alam, and Akbor, 2023; Praveena, Ariffin, & Nafisyah, 2022). Replicate samples from each site during the two seasons were systematically scanned in a row-by-row pattern to ensure no plastic particles were missed. The particles were identified as MPs based on the guidelines established by Kommunernes International Miljøorganisation (KIMO) (Sweden, 2007). According to these criteria: (1) no organic structures should be visible, (2) fibers must have a uniform length, and (3) colors should be distinguishable. Identified MPs were categorized based on their physical characteristics, i.e., morphology and color. They were classified into thirteen colors: Black, White, Yellow, Red, Blue, Transparent, Green, Purple, Grey, Orange, Pink, Silver, and Brown. As for the morphology, they were classified into fibers and fragments. Fragments are broken-down pieces of larger plastics and tend to have rough, angular, or sharp edges, whereas fibers are long and thread-like, with their length being much greater than their width (Masura, Baker, Foster, & Arthur, 2015). Their concentration was measured in (particles/L), and processed in Microsoft Excel 2019 to calculate the mean and standard deviation.

2.5 Squeeze test

When identifying environmental samples, natural materials such as grass sheaths, pine needles, diatom tests, and salt crystals can sometimes resemble plastic. To differentiate between them, forceps are used to rub or squeeze the particles. If the material powders or crumbles, it is not plastic. However, if the particle retains its shape, it is likely plastic (Masura, Baker, Foster, & Arthur, 2015). The squeeze test, a widely used method for identifying MPs particles, was conducted following the guidelines outlined in the MPs Sampling and Processing Guidebook (Sartain, Wessel, and Sparks, 2018) and further applied in a study by (Savitz , 2021).

2.6 Statistical Analysis

To determine whether seasonal differences in MPs abundance were statistically significant, a paired t-test was conducted for each lake using site-level mean concentrations from the pre-winter and winter seasons. To evaluate whether one lake exhibited higher MPs pollution than the other, independent t-tests were applied to the site-level mean MPs concentrations for both sampling seasons.

3. Results and Discussion

3.1 Abundance of MPs

MPs were detected in all 44 samples collected from 11 sites around Phewa Lake and Begnas Lake, with samples collected twice during both the pre-winter and winter seasons. At Begnas Lake, the mean concentration of MPs in pre-winter samples ranged from 1.04 ± 0.21 to 2.46 ± 1.66 particles/L, while in winter samples, it ranged from 0.56 ± 0.17 to 1.16 ± 0.25 particles/L, whereas at Phewa Lake, the mean concentration of MPs in pre-winter samples ranged from 1.07 ± 0.27 to 1.33 ± 0.51 particles/L, while in winter samples, it ranged from 1.10 ± 0.32 to

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1.30 ± 0.62 particles/L. The abundance of MPs found in Begnas and Phewa Lakes is presented in Table 3 and Table 4, respectively.

Across Begnas and Phewa Lake, the mean concentration of MPs varied. The overall mean decreased from 1.57 ± 0.81 particles/L in pre-winter to 0.99 ± 0.22 particles/L in winter in Begnas Lake. While the mean remained relatively stable, with a slight increase from 1.15 ± 0.35 particles/L in pre-winter to 1.18 ± 0.41 particles/L in winter in Phewa Lake. The MPs found in the water samples of both lakes are shown in Figure 2.

The highest concentration of MPs was observed at the Begnas Boat Side (BBS) site in Begnas Lake, measuring 2.46 ± 1.66 particles/L, and in Phewa Lake the Phewa Central (PC) site, which recorded 1.33 ± 0.51 particles/L, both during the pre-winter season. MPs concentrations in the surface water of Phewa Lake varied by season, averaging 2.96 ± 1.83 particles/L in the winter (dry) season and 1.51 ± 0.62 particles/L in the pre winter (rainy) season. The significantly lower concentration in the rainy season was likely due to a recent unexpected flood, which may have flushed MPs out of the lake (Malla-Pradhan et al., 2022). In a study conducted in Bangladesh MPs levels in water ranged from 8 to 36 items/L, with Gulshan Lake having the highest (36 items/L), followed by Hatir Jheel Lake (33 items/L) (Mercy, Alam, and Akbor, 2023). In Taihu Lake, China, MPs were detected in surface water at concentrations ranging from 3.4 to 25.8 items/L (Su et al., 2016).

Table 3: Abundance of MPs (Particles/L) in Begnas Lake

	BEGNAS LAKE			
	SAMPLE 1 (Pre-Winter)		SAMPLE 2 (Winter)	
Site code	MEAN	S. D	MEAN	S. D
BRS	1.5	0.54	1.13	0.36
BE	1.66	1.21	1.13	0.35
BLS	1.04	0.21	1.00	0.00
BBS	2.46	1.66	1.16	0.25
BC	1.20	0.44	0.56	0.17

Table 4: Abundance of MPs (Particles/L) in Phewa Lake

	PHEWA LAKE			
	SAMPLE 1 (Pre-Winter)		SAMPLE 2 (Winter)	
SITE CODE	MEAN	S. D	MEAN	S. D
PC	1.33	0.51	1.14	0.32
PE	1.07	0.27	1.21	0.43
PBS	1.15	0.36	1.30	0.62
PBTS	1.22	0.44	1.10	0.32
PD	1.07	0.27	1.22	0.44
PWS	1.09	0.30	1.12	0.35

Note: S. D = Standard Deviation

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For Begnas Lake, MPs concentrations showed a statistically significant decline from pre-winter (mean = 1.57 particles/L) to winter (mean = 0.996 particles/L), $t(4) = 2.78$, $p = 0.0499$. This indicates that Begnas Lake is more polluted in pre-winter than in winter. In contrast, Phewa Lake did not exhibit a significant seasonal shift in MPs abundance. The paired t-test yielded $t(5) = -0.437$, $p = 0.680$, demonstrating that MPs concentrations remained relatively stable across the two sampling periods.

For the pre-winter season, the independent t-test yielded $t(7.9) = 1.66$, $p = 0.168$, indicating that the difference in average MPs abundance between Begnas and Phewa Lakes was not statistically significant. Similarly, during the winter season, the difference between the two lakes remained statistically non-significant, $t(7.8) = -1.59$, $p = 0.177$. In this season, Phewa Lake exhibited marginally higher MPs concentrations, but again, the difference was not statistically meaningful. Overall, the results suggest that Begnas and Phewa Lakes do not differ significantly in their MPs pollution levels in either season.

Figure 2: Representative images of MPs (fibers and fragments) found in surface water samples from Begnas and Phewa Lakes.

3.2 Color and Morphology of MPs

3.2.1 Morphology

Two types of MPs were found in the surface water samples from our study sites. Overall, fragments were the most abundant. However, during the pre-winter season, fibers were more common in Begnas Lake, accounting for 61.25% of the MPs, which was the highest proportion of that season; the fragments made up 38.75%. During winter, this pattern shifted, with fragments increasing to 53.57% and becoming the dominant type, while fibers decreased to 46.43%. Whereas in Phewa Lake, fragments were consistently higher in both seasons. During pre-winter, fragments made up 66.25%, which was the highest percentage of any category in Phewa Lake, while fibers were 33.75%. In winter, fragments remained more common at 57.66%, while fibers increased slightly to 42.34% but still stayed lower.

The two samples from Begnas, Begnas Effluent (BE) during winter and Begnas Left Side (BLS) during pre-winter, contained only fibers. MPs in Phewa Lake are driven by human

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activities and unchecked waste disposal. Many hotels, resorts, and restaurants along the lakeside release wastewater directly into the lake, while activities like washing clothes, fishing, tourism, and recreation add to the contamination (Malla-Pradhan et al., 2022).

Fibers represented the dominant form of MPs in Taihu Lake, China, making up 48% to 84% of the total (Su et al., 2016). In a study by Malla-Pradhan et al. (2022), fibers were found to constitute the majority of MPs, making up 93.04% in winter and 96.69% during the rainy season. A total of 168 MPs were identified among them, 64% were synthetic fibers, and 36% were fragments (Uurasjärvi et al., 2020).

3.2.2 Color

The most common colors observed in both lakes were black, yellow, red, blue, white, and transparent. Black was the most dominant color across all samples, followed by transparent and yellow in Begnas Lake. In Phewa Lake, yellow was most dominant, followed by black and transparent. The reason behind the dominance of yellow color in Phewa Lake may be due to higher tourism inflow, which increases the quantity of MPs resulting from Personal care products (PCPs) like liquid soap, shower gel, and facial scrub (Rodrigues, Almeida, & Ramos, 2020; Sun et al., 2020). The primary sources of black fibers in Begnas Lake include fishery nets, improper solid waste disposal (Horton et al., 2017), and laundry activities, which release microfibers into the water (Browne et al., 2011). Figures 3 and 4 show the distinguished color found in Phewa and Begnas Lakes.

A study conducted by Malla-Pradhan (2022) observed MPs of seven colors in winter and rainy seasons, while eight types of color were noted in autumn. Transparent was the dominant color in winter (26.37%) and rainy (23.53%) seasons, whereas white dominated in autumn (36.36%), followed by transparent (27.97%). Overall, MPs were mostly transparent, while black, white, red, and blue were the most common among the colored ones (Cai, Chen, Huang, & Lu, 2022). In Taihu Lake, China, the blue color was the most abundant in the surface water, followed by the transparent color (Su et al., 2016). Regarding particle shape, fragments were irregular, three-dimensional, and appeared in colors such as white, blue, green, and red. In contrast, plastic fibers were identified in a range of colors, including white, blue, red, brown, black, and green (Uurasjärvi et al., 2020).

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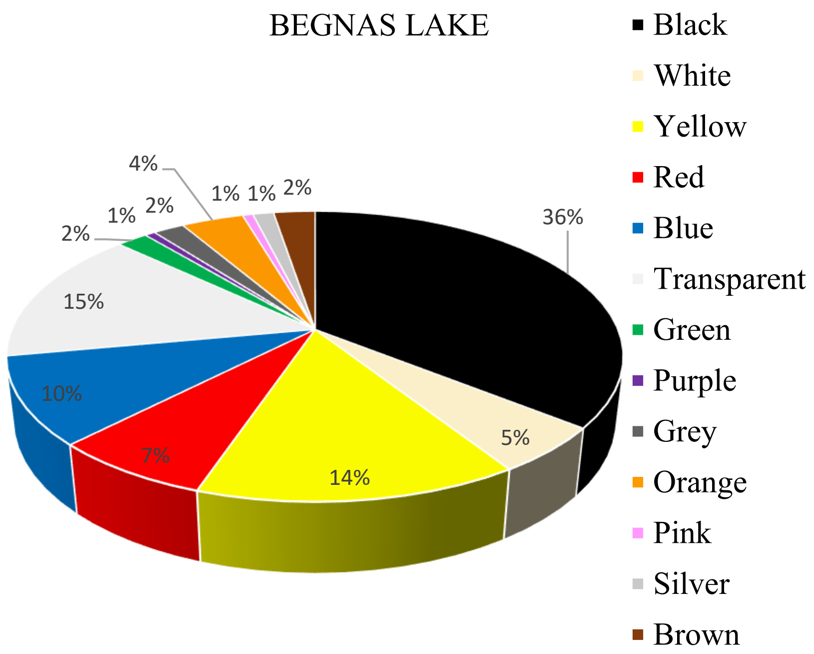

Figure 3: Percentage composition of MPs by color in the Begans Lake

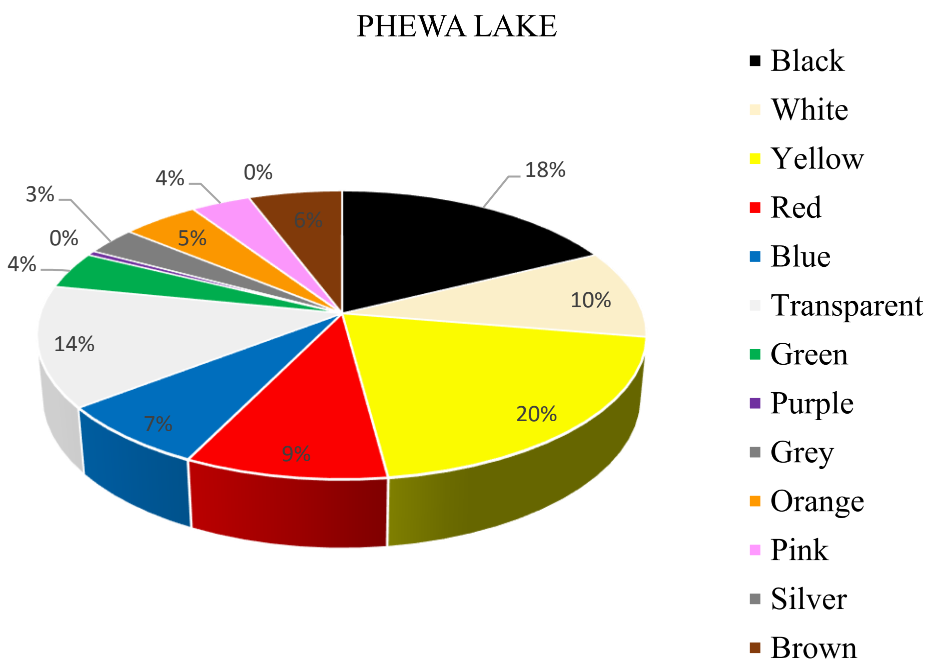

Figure 4: Percentage composition of MPs by color in the Phewa Lake

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4. Conclusion

MPs were detected in every sample collected from both lakes, with fragments being the most common morphology. Begnas Lake had higher concentrations of MPs compared to Phewa Lake. Interestingly, during the pre-winter season, fibers were more abundant in Begnas Lake, but by the winter season, fragments took the lead. In Phewa Lake, fragments were consistently more dominant, with a slight rise during the winter months. The color analysis showed that black MPs were most frequently found in Begnas Lake, likely because of fishing nets and laundry activities. In contrast, yellow MPs were more common in Phewa Lake, which can probably be attributed to the large number of tourists and the use of PCPs in the area. This highlights how human activities such as waste disposal, tourism, and fishing play a significant role in the MPs pollution seen in both lakes. Thus, this study underscores the urgent need for better waste management practices, especially in tourist-heavy areas like Phewa Lake, to prevent further contamination of the ecosystem with MPs. It also highlights the importance of conducting more research to fully understand the sources and impacts of MPs in these lakes, so that more effective and targeted strategies can be developed to tackle this growing environmental issue.

However, the present study should only be regarded as a preliminary study. Some of the recommendations after analysis for future work:

1. Future research should focus on obtaining more precise data on the properties and concentrations of MPs in water bodies, keeping in mind the challenges posed by the limited availability of advanced detection technologies in Nepal.
2. Further explore the presence and concentration of MPs in fish from these lakes to gain a deeper understanding of their ecological impact.
3. In areas where unmanaged wastes dominate plastic releases, implementing better waste management and practices should be prioritized, boosting recycling rates (e.g., by increasing the value of plastic waste) and reducing the amount of plastic waste generated (e.g., optimizing, minimizing, and reusing packaging) could both be viable strategies.
4. Future studies should aim to use visual identification tools in combination with suitable techniques for identifying the polymer types of MPs. This approach will allow researchers to gain a clearer understanding of the composition, shape, size, and other characteristics of MPs.
5. For the analysis, a better understanding of the mechanisms that drive MPs in lake water is required.

5. Limitation

1. Filtering and isolating MPs is challenging and risky due to the transparency of many plastics and their wide range of sizes.

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2. Given that MPs research is still in its early stages, there is a lack of consistent data on their abundance and characteristics, making comparisons difficult.
3. Nepal currently lacks advanced technology, such as micro-FTIR and Raman spectroscopy, to effectively detect and analyze MPs.

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Authors' Contributions: The authors jointly conducted all research activities i.e., concept, data collecting, drafting and final review of manuscript and second author contributes for feedbacks ad correction in each steps of research and final review of manuscript.

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