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Human Activities Increased Microplastics Contamination in the Himalaya Mountains

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Abstract: Microplastic pollution is an emerging environmental concern, and has been found in remote regions, including the high Himalaya mountains. However, the abundance and sources of microplastics in the region are not well documented. This research investigated the abundance, types, and potential sources of microplastics in the Sagarmatha National Park (SNP), a rural and sparsely populated region of Nepal on the southern side of the Himalaya mountains. Water samples were collected from streams and tributaries in SNP in May of 2022. The average microplastic concentration among all samples was 2.0 ± 1.7 pieces/L, similar to that of water samples collected in other high mountain areas and is in the lower range of that found in water samples across the globe. Microplastic abundance is higher in water samples collected near settlements than in streams far from human settlements, indicating the impact of human activities. The presence of microplastics in all samples, including headwaters immediately beneath glaciers, illustrates the widespread distribution of microplastics and suggests the potential for airborne sources. While the concentration of microplastics does not change dramatically from upstream tributaries to downstream rivers, the total load of microplastics increases due to higher discharge downstream. This research demonstrates the anthropogenic and air-borne influences on microplastics contamination on the southern side of the Himalayan range and contributes to filling the data gaps towards a better understanding of the global fate and transport of microplastics.

Keywords: Sagarmatha National Park; microplastics; Nepal; anthropogenic influences

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1. Introduction

Microplastics (MPs) are plastic particles with the longest dimension being less than 5 mm [1,2] and can be categorized as primary or secondary plastics. Primary MPs are intentionally manufactured in small sizes for various applications, such as microbeads in cosmetics. Secondary MPs are created by fragmentation and degradation of primary MPs and include fibers from synthetic textiles [2–4]. MPs pose a growing environmental and public health concern due to their potential for mechanical fragmentation, chemical degradation, bioaccumulation, and biomagnification in the marine, freshwater, and terrestrial environments [5,6]. Moreover, MPs may serve as a carrier of other pollutants and microbes, thereby posing a risk to ecosystems and habitats [7,8].

MPs have been observed across the globe, including remote mountains, polar regions, and the ocean [9–14]. They are widely spread across continents due to human littering and transportation and deposition via wind and accumulate in water bodies and the ocean through runoff. Some MPs, especially those lighter and smaller ones as well as thin MP fibers, can be transported via atmospheric circulation to very remote regions, such as the Earth's poles and the Himalayan mountains [15,16]. For example, an average of

393 ± 457 items/kg dry mass of MPs were observed in the sediments of Qinghai Lake [17], and 5–340 items/kg dry mass of MPs were observed in soils across the Tibetan Plateau [18]. Up to 58 items/s of MPs were discharged from the Northern Dvina River into the Arctic Ocean during peak flooding seasons [19]. Furthermore, MPs have been found in snow and glaciers, which are highly likely to be transported and deposited via atmosphere circulations [20]. However, due to the remoteness and limited accessibility of these remote mountains, studies are still rare in those areas [10]. The distance MP particles can travel is currently unknown and further event-based research is needed to identify the source and transport vectors of atmospheric MP particles [16].

The Himalayan mountains are the world's highest mountain range and are treated as a sacred area by many people around the world, especially the indigenous population living there. However, in recent years, population growth, land use/land cover change, global warming, glacier melt, and tourism have together changed the hydrology and water quality in the region [21]. Glaciers and seasonal snow pack are an important part of the hydrological cycle, and the southern Himalayas form the headwaters for many hydrologic systems and are a key source of fresh water for more than one billion people. Hence, the recession of the Himalayan glaciers will cause hydrologic changes, including a reduction in dry-season water discharge, an increase in peak discharge, and a general decrease in water resources [22–24]. The degradation of water quality, including the newly found contaminants of emerging concern such as MPs, impacts the environment and public safety within the region and further downstream. Unfortunately, little is known about the occurrence, fate, transport, and sources of MPs in the Himalayan mountains. In limited studies, most have focused on the northern side of the mountain while studies on the southern side remain sparse [18,20,21]. MPs have been detected in the Koshi River basin, mainly coming from urbanization, agriculture, traffic, and tourism [25]. Studying MPs in the Himalayan region is important to reveal the current abundance of these contaminants of emerging concern and the extent of their impact on the area. This study contributes to the current literature and data gaps on MPs in remote regions and lays the groundwork for next step studies related to the fate and transport of MPs on a global scale.

2. Study Area

Water samples were collected from the Sagarmatha National Park (SNP), Nepal, because of its unique location and the mixture of human and natural factors that influence hydrologic regime change and potential contaminants [26,27] (Figure 1). SNP is situated on the southern side of Mount Everest, a sacred area with a population of about 6000 people, predominantly Sherpa who are indigenous to the region [26–28]. Despite being remote and having a low population density, the park attracts thousands of tourists each year, especially during the pre-monsoon season, because of its proximity to Mt. Everest and other high mountain peaks and an abundance of trekking routes [26]. The place has a high altitude gradient, ranging from 2610 m at Phakding near the southern border of the park to 8849 m at the peak of Mount Everest, allowing for the investigation of spatial changes across various altitudes. The SNP was established in 1976 with an area of 1148 km^2. It was then declared as a World Natural Heritage Site in 1979. In 2002, a protective buffer zone of ~275 km^2 was added (SNP Buffer Zone) to the park [26]. The park allows access to both moderate- and higher-altitude areas that have not been affected by major human populations as well as to areas that have been affected by human populations with intensive agricultural practices, which allows for comparative analyses of contaminant sources.

The SNP is a critical zone for global climate, water resources, and water contaminant research, as it serves as the headwater region for major rivers such as the Koshi and Ganges rivers. The area experiences abundant snow and glacier accumulation and melting, especially during the pre-monsoon and monsoon seasons, making the area famous for the "Asian Water Tower" that supplies water to downstream major rivers [21]. However, the area is very sensitive to climate change and human interruptions. Studies have shown that temperatures in the Himalayan region are increasing faster than the global average,

with even higher rates at higher elevations [29]. This rapid warming has led to accelerated glacier melting and alterations of hydrologic regimes [21]. Along with a warming climate, the SNP has observed increased water contamination in recent years due to land use and land cover (LULC) change, local development, and popular tourism, which continues to impact local residents and downstream users [26,27,30]. Despite its vulnerability to climate change, the southern side of the Himalayan Mountains has received less research attention and generated fewer data compared to the northern and eastern sides, especially in the assessment of water quality and pollution sources, including the contamination of MPs [31,32].

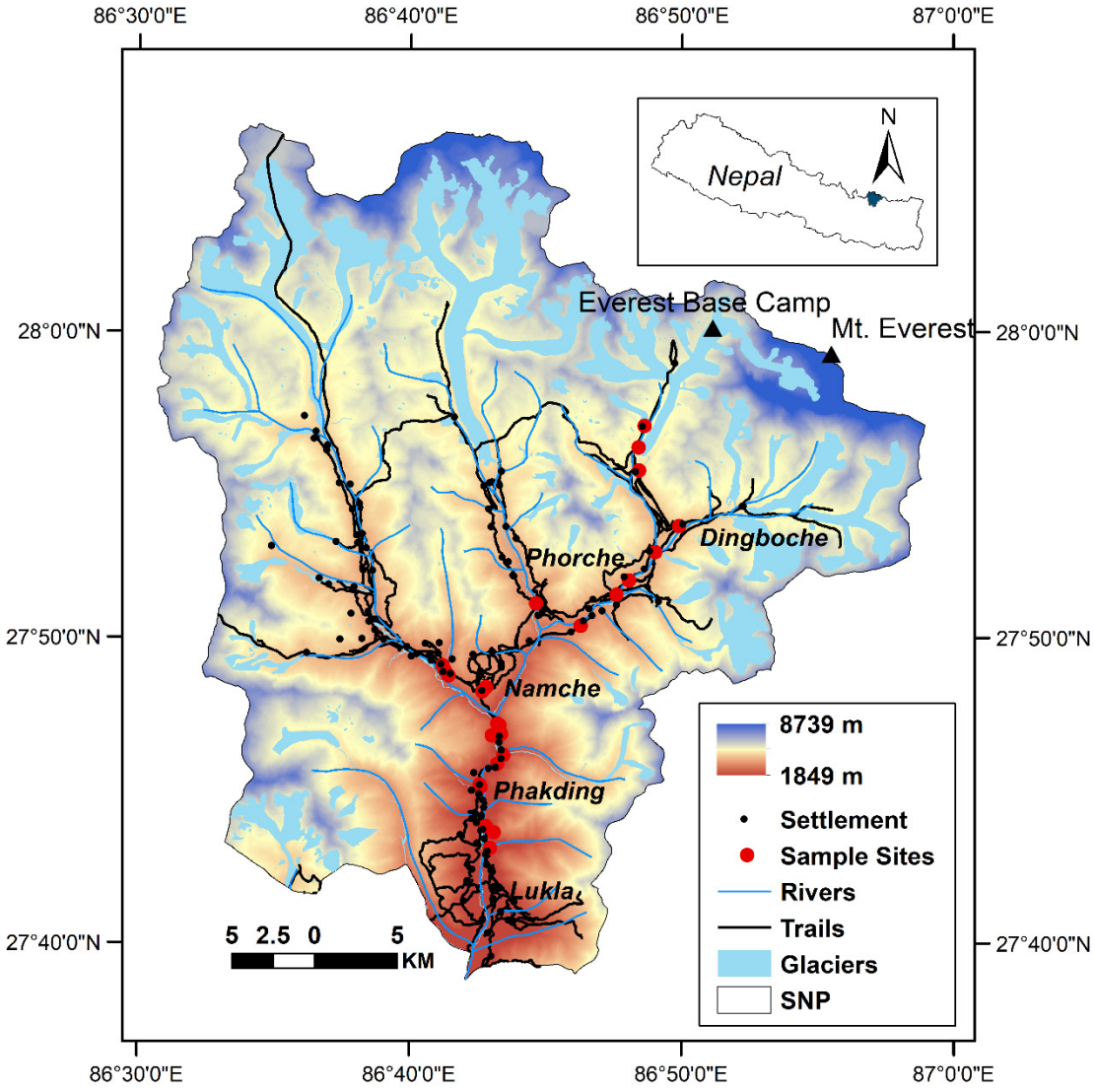


Figure 1. The location of SNP, DEM, settlements, major trails, MP sampling sites, rivers, and glaciers in SNP. Major settlement places along the sampling route are also marked. Sampling sites were mostly along major trails and rivers due to inaccessibility to other areas. Whenever possible, samples were taken from waterfalls and small tributaries before they entered main river stems when the sites were accessible. Figure was created using ArcGIS 10.7.1 https://www.arcgis.com/index.html (accessed on 1 May 2023).

3. Methods

3.1. Sample Collection

Twenty-five water samples were collected along the major trekking routes across a variety of altitudes, from 2610 m in Phakding to 5000 m near Mount Everest Base Camp

(EBC), during May 2022 [26,27] (Figure 1). Samples were collected along the main stem of the Koshi River, its tributaries, and residential water sources around settlements. We further separated headwaters with low discharge from tributaries and marked them as small tributaries, aiming to analyze the differences of MP concentration in streams of different orders. This water sampling strategy allows us to identify the MP concentration from upstream to downstream of the area and also isolate the impacting factors from anthropogenic sources (e.g., human activities near settlements) to natural sources (e.g., air-borne sources).

Water samples were all collected during daytime while trekking. Weather conditions at the sampling time were noted as references. Samples were collected using a net sieve with 85 µm mesh size attached to the end of a flow meter (Figure 2). The flow meter captured the flow velocity of the water entering the net sieve and was submerged into the river flow for one minute per sample. The volume of water filtered through the net sieve was then calculated using the radius of the flow meter end (i.e., the area allowing water to pass through), the duration of the sampling time (i.e., one minute), and the flow velocity [33–35], as shown in Equation (1).

$$V = 1000 \times 60\pi v r^2 t \quad (1)$$

where V is the volume of water flowing through the filter in L, v is the flow velocity caught with the flow meter in m/s, r is the radius of the flow meter end wrapped with the net sieve in m, and t is the sampling time in minutes.



Figure 2. Design illustration of the MP collection set attached to a flow meter for discharge measurement.

After the plastic and other residuals were captured in the net sieve, the outside and inside of the net sieve were thoroughly rinsed using a squirt bottle with river water at the site into a stainless-steel cup. The residuals were then transferred into 12 mL glass vials. The samples were stored in a clean, sealed box until the end of the trip. Temperature was not specifically controlled and was generally the same as the environmental temperature throughout the trip. The samples were then transported to the lab for further analysis [36]. These steps ensured that a large volume of water flux was filtered to collect MPs in these pristine areas with relatively low pollutant concentration as in many freshwater sites. On-site filtration also eliminates the burden of bringing a large volume of water to the lab from remote regions [37]. For samples from source waters (e.g., headwater streams) with discharge that was too tiny to submerge the flow meter, five liters of water was collected with a steel cup, filtered through the same net sieve, and then rinsed into small vials following the same procedure.

3.2. Laboratory Analysis

Water samples were analyzed in the lab to identify MPs following commonly adopted methods with slight adjustments [37,38]. Major steps included density separation to elimi-

nate particles that were denser than plastics and visual characterization and identification of polymer types. Due to the low organic matter content in headwaters and samples collected in high mountains, the organic matter degradation step typically involved in MP analysis was skipped in this study. For density separation, the samples were transferred into 50 mL centrifuge tubes. Sodium iodide (NaI) was then added to the samples to attain a concentration of 5 M. This increased density of the solution ensured that plastics floated on the surface layer. Samples were then centrifuged at 3900 rpm for 10 min. The supernatant was vacuum filtered through Whatman No.42 filter papers with pore size of 2.5 µm. Then, a rose bengal solution (4,5,6,7-tetrachloro-20,40,50,70-tetraiodo-fluorescein, Sigma-Aldrich, 95% dye content) was added to ensure that natural micro-scale particles such as natural fibers were stained pink and removed prior to identification so that visual inspection was less time consuming and less biased. Once filtered, each filter paper was loosely covered with aluminum foil and dried in the oven at 60 °C for 15 min. The samples then were visually inspected under a stereo-microscope at 20× to identify MP particles [39,40]. Size, shape, and categories of each particle in each sample were recorded.

3.3. Data Analysis

Descriptive statistics, including mean, maximum, minimum, and standard deviation, were computed for all data collected using R. ArcGIS (10.0, Esri, Redlands, CA, USA) was used to describe geographic locations and map the sites.

Sources of MPs were explored based on plastic morphology and type and by examining the relationship of MPs with sample collection sites and altitudes, which could be connected to community water consumption patterns and tourists' activities. The results were also compared with recent studies conducted in similar areas and freshwater bodies in other remote regions.

3.4. Quality Assurance and Quality Control

It is important to identify potential sources of uncertainties and to be cautious and ensure that every step follows the best operational procedures currently adopted. This is especially critical for MP studies because this is a contaminant of emerging concern without a standard testing method yet. In field and laboratory work, quality assurance and quality control measures were used throughout the processes, including testing field and laboratory blanks and taking precautions at each step of sampling and analysis. Samples were completely sealed in glass vials during the transportation processes. All samples were processed carefully and covered by aluminum foil in the lab when not being analyzed. All equipment, such as containers and sampling tools, were covered by aluminum foil as well and washed using ultrapure water before analysis to avoid cross-contamination. During lab analysis, reagent blanks were tested along with randomly picked samples. The filter for the blank was saved in a Petri dish and inspected at the end of the analysis to account for potential contamination inside the lab. Glass or stainless-steel sample collection cups, funnels, Petri dishes, centrifuge tubes, and stirrer rods were used throughout the analysis to avoid sorption of MPs by plastic materials. White cotton lab coats were worn during analysis and sample handling. All chemical operations were performed under a fume hood to avoid cross-contamination and for the safety of the researchers. Detailed field notes and photographs were taken at each sampling location as a reference. A total of two fiber pieces were found in the field and laboratory blank samples, indicating minimal contamination during the collection, transportation, and analysis procedures.

As a training process for visual identification and a validation process of the visual inspection, selected particles that were suspected to be plastics collected from environmental samples (soil and water) were manually picked out and tested with FTIR-ATR spectroscopy. Spectrums of all the suspected particles were analyzed to determine if they are plastic materials. However, due to the limit of FTIR-ATR, only particles that are close to or larger than 1 mm can be manually picked out for this process. Nonetheless, the morphology of bigger particles mimics those smaller particles under a microscope. As such, this training

and validation process, despite its own limitations, enhanced the confidence of the visual inspection based on morphological characteristics. The validation is a compromise of the limit of FTIR-ATR that smaller size particles were not manually movable so they could not be tested under FTIR [41]. This may be a feasible approach for researchers without access to advanced instruments such as μ-FTIR or Raman to adopt.

4. Results and Discussion

4.1. Abundance of MPs in the SNP Water Bodies

A total of 467 pieces of MP, including 212 pieces of fiber, 56 fragments, and 199 pieces of film were detected from 25 samples collected in SNP (Figure 3). No pellets or foams were observed in any sample. Fiber was the dominant type of MP, accounting for 45% of all the MPs found. Fibers are frequently reported as the dominant MP type in water samples due to their wide application in synthetic clothing and other applications such as ropes [25,42,43]. Film was the second most dominant type of MP, accounting for 43% of all MPs found, and fragments accounted for 12%.

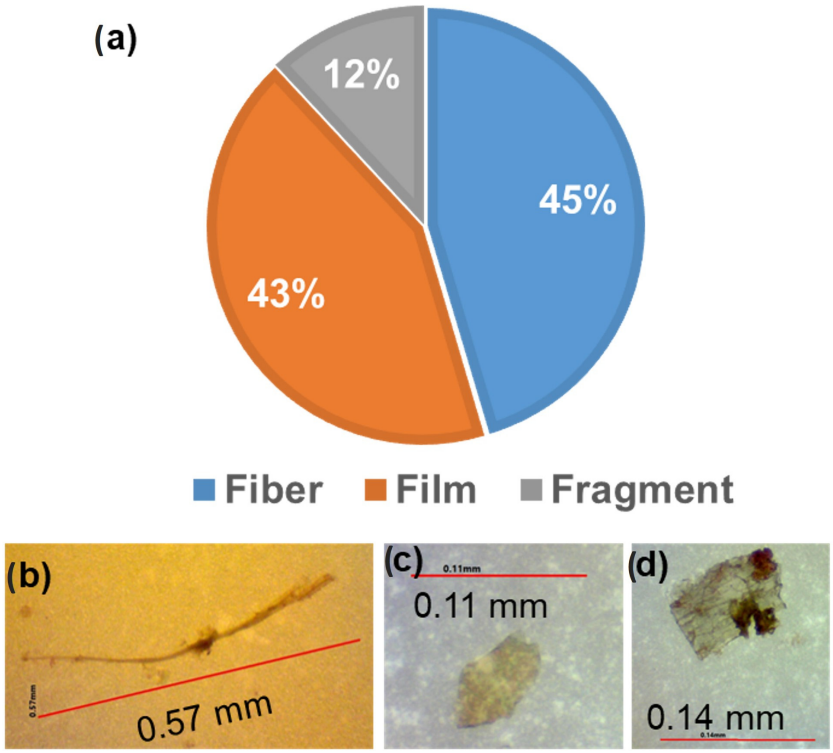


Figure 3. (**a**) The percentage of each MP category found in all the samples, and (**b**) some example pictures of fibers, (**c**) films, and (**d**) fragments.

The concentration of MPs ranged from 0.4 pieces/L in some tributaries to 6.4 pieces/L in some samples near settlements, with an average of 18.7 pieces/sample or 2.0 pieces/L (Appendix A Table A1; Figure 4). These findings align closely with observations from a recent study conducted in the Koshi River Basin, which spans the boundaries of China and Nepal [25]. The value was evaluated to be in the lower range among MP pollutants in water samples collected in the other areas of the globe [25]. Note that this study used an 85-micron mesh size, which is smaller than the 100 micron mesh used in Yang et al. (2021) [25]. This exemplifies the complexities involved in comparing MP research, as inconsistencies in sampling method, definition of MPs, analytical protocols, and measuring units can impact the results [44].

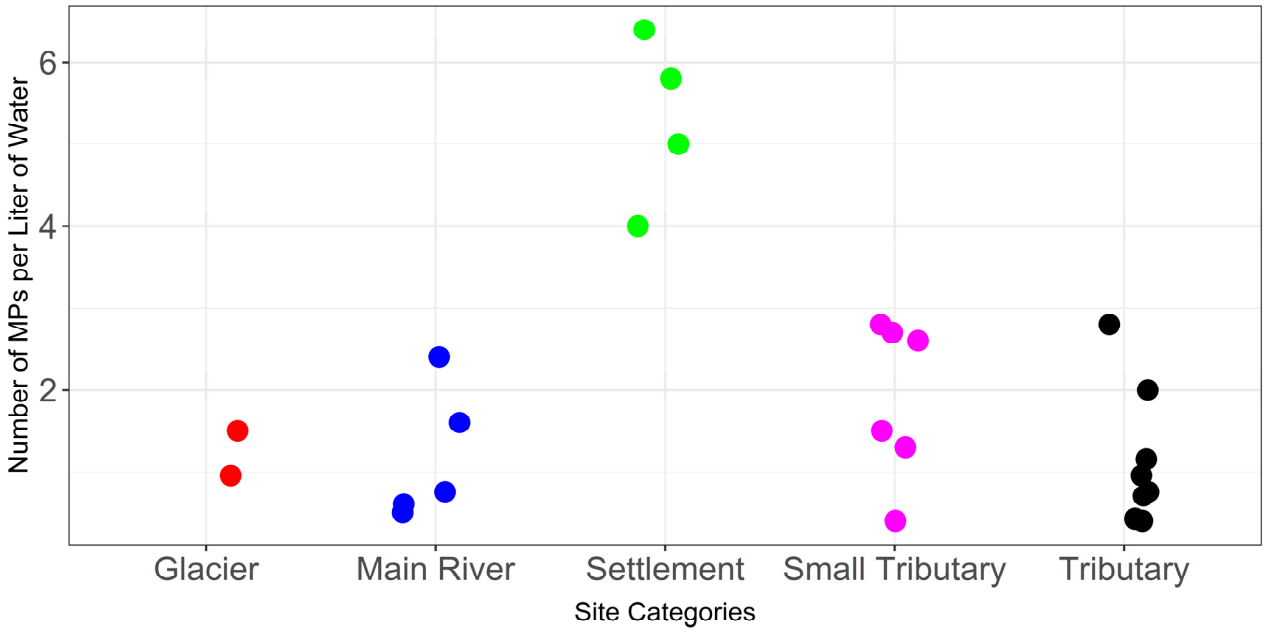


Figure 4. Distribution of MP concentration observed in samples from different categories of water sources. River samples are divided into main river, (main) tributaries, and small tributaries. Settlement includes samples collected from villages, tap water, and ditches flowing across towns.

4.2. Spatial Distribution and Potential Sources of MPs in the SNP Water Bodies

There were remarkable differences in the concentration of MPs among the different water samples (Figure 4). The samples collected near settlements exhibited the highest mean MP concentration (5.3 ± 1.0 pieces/L), as these areas were exposed to human activities including agricultural practices, lodging, and trashing of primary plastics. In contrast, the samples collected near glaciers had the smallest mean concentration (1.2 ± 0.4 pieces/L), which was characterized by glaciers nearby, cold temperature, strong sunlight with intensive UV radiation, high altitude, and less exposure to human activities. However, the differences in MP concentration among samples collected in different tributaries and the main stem of the Koshi River were less pronounced. Note that while the concentration of MPs in tributaries did not vary significantly, the total load of MPs increased at downstream sites when the river accumulates with large discharge downstream. Overall, it can be deduced that human activities are major contributions of significant levels of MP pollution in SNP.

The concentration of MPs exhibited higher variability in lower altitudes, except for those near settlement sites (Figure 5). We analyzed the MP concentration change with altitude among sites except at those at settlements and found that while MPs at the sites of different altitude bands had similar low concentrations, the variability was much higher in lower altitudes than higher altitudes. Considering the remote features of the sites, even places with lower altitudes are mostly remote pristine regions inaccessible to the public. However, due to relatively higher population densities and human activities in lower altitudes, certain samples that are immediately impacted by human activities may contain higher amounts of MPs. On the other hand, areas in high altitudes typically are mainly impacted by tourists and a limited number of tea houses, so the MP abundances found there were relatively low with lower variances [15,16].

Atmospheric transmission and deposition may be a major pathway of MP occurrence in these remote areas [10,15,16]. Considering MPs were found in all the samples, even near glaciers, it is suggested that atmospheric deposition is a likely source of MPs in the region (Figure 6). Similar conclusions have been drawn in other remote regions, including European mountains [10], the Tibetan Plateau [18,45], Arctic Sea [46], and Koshi River Basin of southern Asia [25]. Global simulations of atmospheric transport of MP particles

produced by road traffic further illustrated this potential pathway and demonstrated a similar magnitude of contribution from air-borne sources to direct and riverine transport of MP particles to the remote ocean [15,16,47].

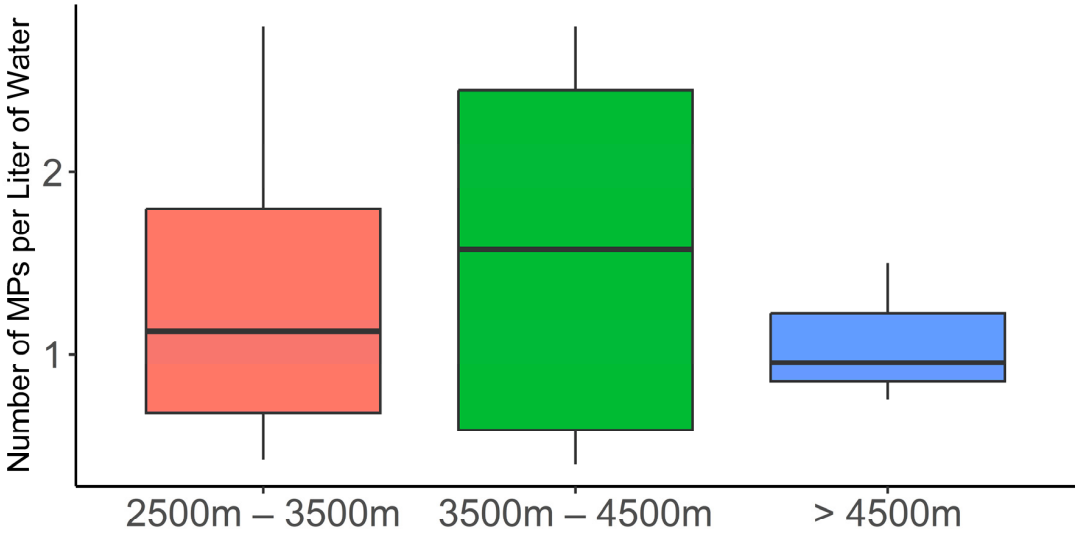


Figure 5. Concentration of MPs (number of MPs found per liter of water sample) at different bands of altitudes. Samples at settlements were excluded from this analysis to focus on the unknown anthropogenic impacts. The variability was higher in lower altitudes than higher altitudes and was highest in the medium range of altitude (3500–4500 m).

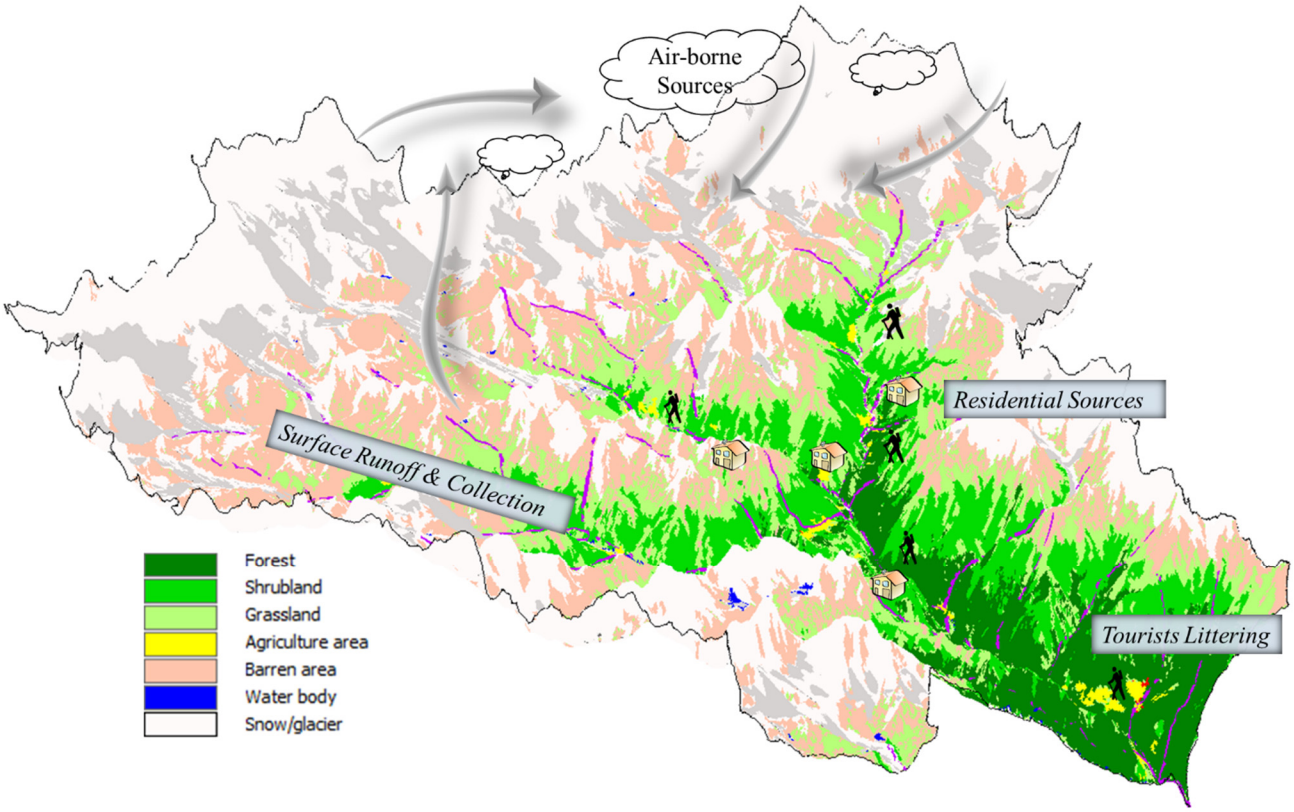


Figure 6. An illustration of potential sources of MPs in the SNP, including residential and agricultural sources from settlements, littering from tourists, surface collection via runoff, and air-borne sources from atmospheric transportation. Figure was created using ArcGIS 10.7.1 https://www.arcgis.com/index.html (accessed on 1 May 2023).

It is worth noting that, due to the small sample size, statistical significance of the differences was not comprehensively analyzed in this current study. However, a simple one-way ANOVA test indicated that the differences in MP abundance among different sampling sites and tributaries were significant ($p < 0.05$). A Tukey HSD test of differences between each category pair indicated that the differences in MP concentration among samples collected in different tributaries and the main stem of the Koshi River were insignificant ($p > 0.05$), while the differences among settlements and other categories were significant ($p < 0.05$).

4.3. Limitations and Future Directions

Abundant samples are required to gain a deep understanding of the MPs' sources, fate, and transportation mechanisms in remote regions. However, due to the inaccessibility of the region, samples collected in the study are limited and are restricted to the areas along the major trekking routes and below the Everest Base Camp. Future studies may benefit from a wider range of sampling sites beyond the trekking routes to better understand the distribution of MPs in soils of various land covers that are not directly disturbed by human activities.

Analysis of MPs relies on the accurate collection of samples. MP distribution in water bodies is non-uniform due to the three-dimensional and mixing nature of water bodies. In addition, accessibility of water bodies vary significantly, e.g., from small creeks to large rivers to the ocean. Various sampling strategies have been developed and adopted in different studies, including using Neuston and Manta nets, direct grabbing, pumps, onsite sieving, etc. [48]. The onsite sieving method with a flow probe used in this study only applies to water that is relatively clean; otherwise, the nylon sieve will be prone to clogging from residuals.

This study relies heavily on visual identification of MPs under a stereo-microscope. While this approach is widely adopted and pre-treatment has been conducted to facilitate better visualization [49–51], it may still introduce human-induced errors because of the similarities of morphological characteristics of MPs with some other particles. However, advanced technologies, such as μ-FTIR, Raman, and simultaneous optical photothermal infrared (O-PTIR), have been employed in various situations to characterize the chemical characteristics of polymers, which provide more confidence and are critical for identifying sources [52,53]. However, these methods are expensive and instruments are not available to many researchers. This study used FTIR-ATR to train researchers and enhance the confidence of visual inspection. Considering that FTIR is more accessible, this may be a viable approach to adopt for researchers with limited resources.

5. Conclusions

This study collected MPs from water samples along major trekking routes in the SNP of Nepal's Himalaya mountains in the pre-monsoon season of 2022. The objective was to investigate the abundance, distribution, and sources of MP particles. Among the types identified MPs, fibers and films were dominant, while pellets or foams were not observed. The average MP concentration in the collected water samples was 2.0 pieces/L, falling within the lower range reported in existing literature on MPs in global water bodies. MPs are most concentrated in samples collected from settlements and least concentrated in those collected near glaciers, which indicates a significant contribution from anthropogenic sources and widespread contribution from air-borne sources. The concentration at lower and medium altitudes also observed higher variances which is indicative of increased human impacts in those areas. Considering that SNP is a UNESCO heritage site with a pristine environment, the findings of MPs in SNP are noteworthy, calling for further investigations towards a better understanding of MP fate, transport, and impact on the environment and public health.

Author Contributions: B.H.: conceptualization, field work and sample collection, methodology and analysis, and original draft. M.Y.: sample laboratory analysis. A.L.: data analysis and visualization. K.N. (Kirsten Nicholson): resources, conceptualization, funding, and field support. J.G.: editing and field work assistance. K.N. (Klaus Neumann): funding and field trip support. S.S.: travel support and local logistics. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available in Appendix A.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Pieces of MPs observed in water samples. S. Tributary indicates small tributaries that are headwaters just emerged from springs or waterfalls, which distinguishes them from bigger tributaries that are indicated as "Tributary" in the table.

Sample#	Lat	Lon	Altitude (m)	Volume (L)	Total MPs	MP Density (Pieces/L)	Fiber	Film	Fragment	Site Category
1	27.7185	86.7161	2554	19.9	19	1.0	3	13	3	Tributary
2	27.7531	86.7097	2735	10.0	28	2.8	11	8	9	Tributary
3	27.7854	86.7218	2871	10.0	27	2.7	5	17	5	S. Tributary
4	27.7864	86.7205	2847	10.0	24	2.4	13	7	4	Main River
5	27.8039	86.7112	3468	5.0	25	5.0	13	11	1	Settlement
6	27.8126	86.6904	3497	5.0	32	6.4	21	7	4	Settlement
7	27.8181	86.6869	3450	19.9	15	0.8	5	8	2	Tributary
8	27.8156	86.6881	3509	5.0	20	4.0	3	13	4	Settlement
9	27.8520	86.7442	3583	49.8	20	0.4	5	6	9	Tributary
10	27.9488	86.8104	4908	19.9	19	1.0	11	8	0	Glacier
11	27.9371	86.8068	4856	10.0	15	1.5	9	3	3	Glacier
12	27.9245	86.8071	4626	19.9	15	0.8	6	7	2	Main River
13	27.8942	86.8317	4349	5.0	29	5.8	16	11	2	Settlement
14	27.8800	86.8171	4163	10.0	20	2.0	11	9	0	Tributary
15	27.8646	86.8011	4000	5.0	13	2.6	7	5	1	S. Tributary
16	27.8568	86.7934	3946	19.9	23	1.2	10	13	0	Tributary
17	27.8398	86.7714	3739	5.0	14	2.8	8	6	0	S. Tributary
18	27.8062	86.7135	3553	29.9	12	0.4	4	8	0	S. Tributary
19	27.7800	86.7171	3220	19.9	14	0.7	8	3	3	Tributary
20	27.7808	86.7228	2821	10.0	16	1.6	8	7	1	Main River
21	27.7693	86.7242	2816	19.9	10	0.5	2	5	3	Main River
22	27.7643	86.7205	2742	10.0	15	1.5	9	6	0	S. Tributary
23	27.7517	86.7102	2679	39.9	17	0.4	10	7	0	Tributary
24	27.7304	86.7135	2578	10.0	13	1.3	10	3	0	S. Tributary
25	27.7271	86.7184	2913	19.9	12	0.6	4	8	0	Main River

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