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Variation of microplastics in the shore sediment of high-altitude lakes of the Indian Himalaya using different pretreatment methods

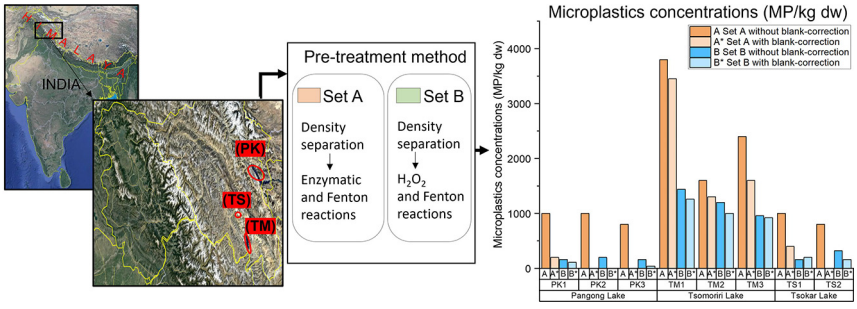
Tenzin Tsering [a,*], Mika Sillanpää [b], Mirka Viitala [a], Satu-Pia Reinikainen [a]

[a] LUT School of Engineering Sciences, Lappeenranta-Lahti University of Technology LUT, Sammonkatu 12, 50130 Mikkeli, Finland
[b] Department of Chemical Engineering, School of Mining, Metallurgy and Chemical Engineering, University of Johannesburg, P. O. Box 17011, Doornfontein 2028, South Africa

HIGHLIGHTS

- With differently pretreated sediment samples, microplastics concentrations vary between sediment samples from the same site.
- The microplastics concentrations were higher in enzymatic pretreated samples than hydrogen peroxide pretreated samples.
- PP and PE fragments are the most commonly found microplastic types in the studied lakes in Ladakh.
- The high-altitude mountainous lakes in Indian Himalaya can potentially act as microplastics sinks.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics pollution is a growing environmental concern. However, microplastics studies in high altitude remote lakes are scarce. In this study, microplastics pollution was assessed in the shore sediment of three high altitude lakes in Ladakh of the Indian Himalaya, namely Pangong Lake, Tsomoriri Lake and Tsokar Lake.

Sampling of lakes shore sediment was performed in August 2019. Two different pretreatment methods were implemented with sediment samples from same sites, resulting two sets of samples. One set of samples was pretreated utilizing enzymatic degradation together with Fenton reactions. Another set of samples from the same sites were pretreated with 30 % hydrogen peroxide (H_2O_2) and Fenton reaction. Enzymatically pretreated samples resulted in higher microplastics concentrations than the set of H_2O_2 pretreated samples, which indicated that microplastics concentrations in sediment samples varies even among samples from the same site and that the pretreatment procedure may impact on the reported microplastics concentrations. Considering both sets of samples, microplastic concentration was 160–1000 MP/kg dw in Pangong Lake, 960–3800 MP/kg dw in Tsomoriri Lake, and 160–1000 MP/kg dw in Tsokar Lake. Blank correction based on the limit of detection and the limit of quantification indicated that microplastics concentrations at some sites of the studied lakes are higher than the limit of detection and the limit of quantification.

The findings of this study indicated that the studied lakes in the Indian Himalaya are contaminated with microplastics. In addition, the comparison of microplastics using different pretreatment methods illustrated the importance of harmonization of microplastics studies to enable a reliable comparison among microplastics data. Therefore, this study contributes towards an assessment of microplastics in the high-altitude lakes in Indian Himalaya. The findings attributed towards clearer understanding regarding the need of harmonization of pretreatment methods and demonstrated the importance of reporting complete information in microplastics research.

* Corresponding author.
E-mail addresses: tenzin.tsering@lut.fi, tenziitserin@gmail.com (T. Tsering).

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1. Introduction

Plastics pollution is a global challenge. Plastics are indispensable materials for various purposes and their production steadily increases (Plastics Europe, 2021). Microplastics are plastic particles with size range from 1 μm to 5 mm (Frias and Nash, 2019). However, a clear consensus on definition of microplastics is lacking including the lower size limit varies among different studies (Viitala, 2021). Microplastics originate from various sources such as industrial production, textile, packaging, and plastics products. The predominant sources of microplastics are the fragmentation of larger plastics. Researches indicated that microplastics are omnipresent in different environmental compartment (Allen et al., 2022).

A global review on microplastics in freshwater by Talbot and Chang (2022) indicated that the number of publications on microplastics in running water, such as rivers, is higher than in still water, such as lakes. A review on microplastics pollution of worldwide lakes by Dusaucy et al. (2021) demonstrated the lack of data from rural lakes. Moreover, fewer microplastics studies in freshwater systems have been reported for developing than developed countries (Blettler et al., 2018). Furthermore, Asia is facing higher microplastics pollution than other continents due to its economic structure and local development levels (Yang et al., 2021b).

Among freshwater sources, lakes are relatively closed water systems with long retention times, which can potentially become long-term sinks of microplastics (Pastorino et al., 2022). Microplastics research reported that microplastics are found in lake water (Abbasi and Turner, 2022), lake bottom sediment (Li et al., 2022) and lake shore sediment (Jeevanandam et al., 2022). Furthermore, the extent of microplastics pollution in a lake system largely depends on its geographical condition such as depth, altitude, wind current, precipitation, and population density (Koutnik et al., 2021).

A review by Zhang et al. (2020b) concluded that climate change will directly impact on microplastics pollution due to the increased amount of plastics entering the lakes through the increased precipitation and surface runoff. Furthermore, due to limited or lack of access to proper waste disposal conveniences in the remote areas, the microplastics are discharged into freshwater and eventually settle in sediment (Yang et al., 2021a). High-altitude remote lakes have potential to act as sinks of microplastics and to play an important environmental compartment of microplastics monitoring research (Yang et al., 2021a; Zhang et al., 2020a). However, field based microplastics studies in high altitude lakes are lacking in comparison with microplastics studies in rivers (Padha et al., 2022). In such scenario, freshwater in Himalaya regions are important sites for microplastics research due to the lack of field-based microplastics data and the regions vulnerability to the repercussion of climate change. To our knowledge, microplastics pollution in lakes in the Indian Himalaya has not been investigated except in the bottom sediment of Anchar Lake in Kashmir valley (Neelavannan et al., 2022). Thus, microplastics studies on the Indian Himalaya are crucial due to the sites being prone to impacts of climate changes and the lack of microplastics studies.

According to current state of microplastics research, direct comparison among microplastics abundance is difficult mainly due to different methods applied during sampling, pretreatment, and analysis. However, towards a holistic understanding of microplastics pollution and environmental monitoring, microplastics data with reliable comparability is important. Thus, the need for the harmonization of methods is widely recommended in microplastics research (Brander et al., 2020; Cowger et al., 2020; Lu et al., 2021).

In this light, three brackish lakes on the Ladakh Himalaya were studied to investigate the distribution and abundance of microplastics in lake shore sediments. Our hypothesis was that microplastics accumulate in the mountainous lakes of Indian Himalaya, particularly in the shore sediment of lakes in Ladakh. To contribute towards understanding the complexity of microplastics in environment, microplastics abundances in the sediment samples from the same sites were investigated by using two different pretreatment methods including an enzymatic pretreatment method and hydrogen peroxide pretreatment method accompanied with Fenton reactions in both methods. Additionally, the study aims to address towards the harmonization of the microplastics research by applying a strict blank correction as suggested by Horton et al. (2021).

2. Material and methods

2.1. Sampling sites

The sampling sites of three lakes, namely Pangong Lake, Tsomoriri Lake and Tsokar Lake, are located in Ladakh, North West Indian Himalaya (Phartiyal et al., 2021). Ladakh is situated in trans-Himalayan Mountain range. It is the highest plateau in the northern India with an average elevation of 2987 m above sea level (Ahmad et al., 2021). The average temperature in Ladakh during winter drops down to −15 °C in January, while the average temperature in summer reaches up to 25 °C in July (Hussain et al., 2015).

Ladakh receives an annual rainfall of around 100 mm, and the area is characterized by an arid and semi-arid climate with dry steppe vegetation (Joshi et al., 2021; Phartiyal et al., 2021). However, due to seasonal fluctuation, the intensity of the precipitation varies across the year.

Pangong Lake is a 134 km long and 5 km wide endorheic lake situated at an elevation of 4250 m. It is one of the largest brackish lakes in the Himalayas that remains frozen during winter for about three months (Rathour et al., 2020). Tsomoriri Lake is an endorheic lake at an elevation of 4522 m with surface area around 147 km^2 (Leipe et al., 2014). Tsomoriri Lake is approximately 29 km long and up to 8 km wide. From January to April, the lake remains mostly frozen. Tsokar Lake is a hyper saline lake at an elevation of 4572 m with an area of 16.7 km^2 (Wünnemann et al., 2010).

All three lakes are located in remote area with large influx of tourists during May to September. In addition, there are few small villages nearby all three lakes. Sampling locations at respective lakes are shown in Fig. 1 and detailed locations are reported in supplementary information (SI), Table S1.

2.2. Sampling

The sediment collection was performed similarly as in Tsering et al. (2021). At each sampling location, sediments were collected from five random sites, approximately 2 m along the lake shoreline. Sediment from five sites were mixed in a composite sample of around 1 kg and stored in a steel container. The 2–3 cm of the sediment was collected with a metal spoon. Samples were transported to the laboratory and then stored at −21 °C until pretreatment.

2.3. Sample pretreatment

Prior to sample pretreatment, sediment samples were dried in oven for four days at 40 °C. Then, subsamples of each sediment sample were subjected to two different pretreatment methods, which were named as set A and set B. The pretreatment steps for both sets of samples are summarized in Fig. 2.

The samples pretreated using a modified method of universal enzymatic purification protocol (UEPP) as suggested by Löder et al., 2017 were named as set A. The treatment with hydrogen peroxide was replaced by a Fenton reaction with addition of sulfuric acid for dissolution of iron precipitates (Al-Azzawi et al., 2020). First, 10 g of dry sediment sample was weighed in a centrifugation tube for density separation with sodium tungstate dihydrate solution ($Na_2WO_4{\cdot}2H_2O$, 41% w/v; 1.4 g/cm^3; from Merck, Darmstadt, Germany). Density separation was performed three times. A control shaker (KS 4000 ic, IKA, Germany) for mixing the solution with sediment samples was utilized for 5 min at 200 rpm at the beginning and between centrifugation steps. The supernatant from centrifugation steps was stored in the same glass bottle for each sample and filtered on stainless-steel mesh with aperture of 20 μm (Ø5 cm). The mesh was stored in a glass bottle for incubation with sodium dodecyl sulphate solution (SDS, 10%; from VWR chemicals), subsequent enzymes (from ASA Spezialenzyme GmbH, Germany) and buffer solutions, Fenton reactions

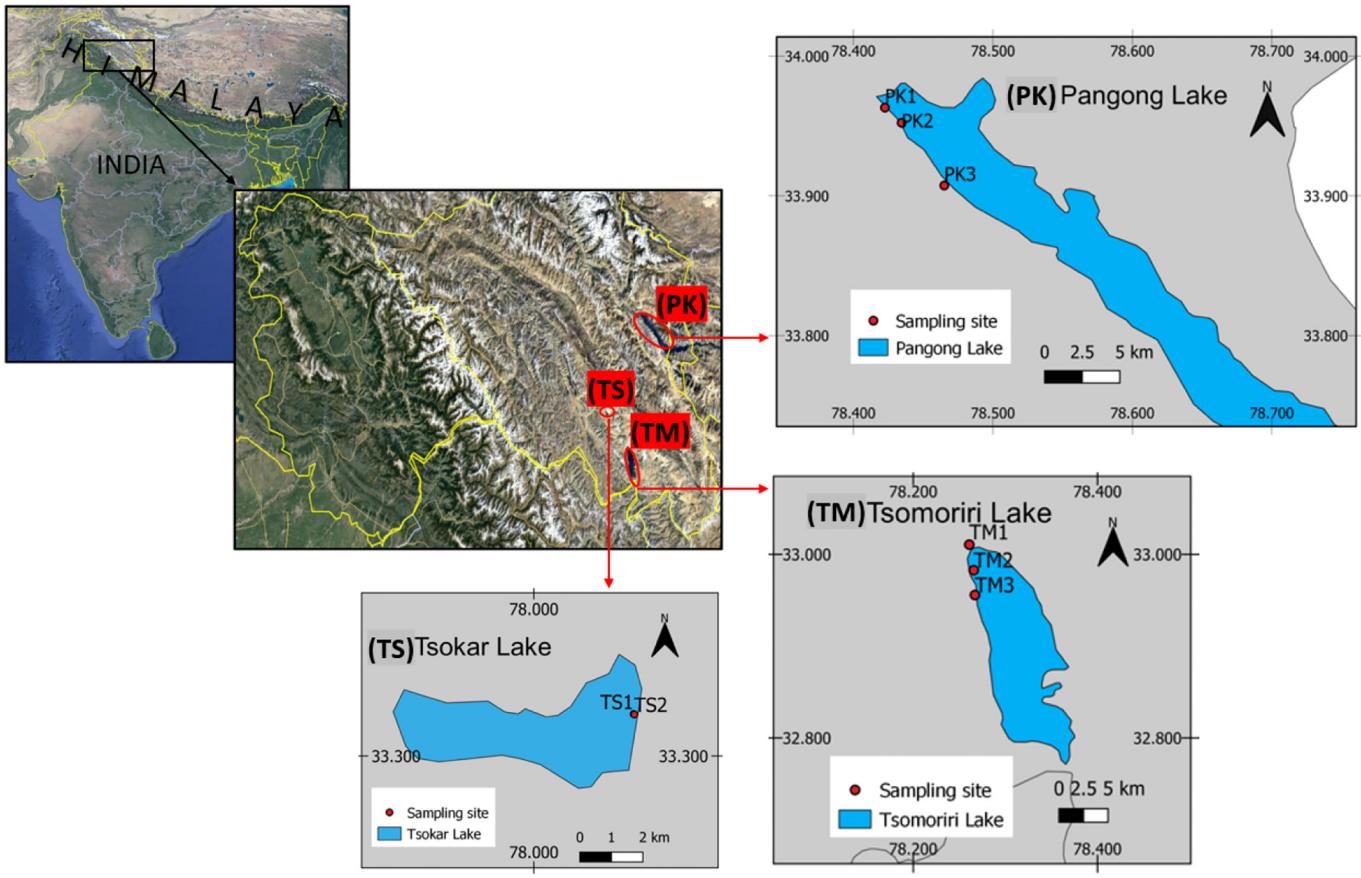

Fig. 1. Sampling location of lakes in the Ladakh Himalaya. Maps were drawn using Google earth Pro and QGIS.

with ferrous solution ($FeSO_4{\cdot}7H_2O$, from Sigma-Aldrich) and hydrogen peroxide (H_2O_2, from VWR chemicals). Initially, 5 mL of $FeSO_4{\cdot}7H_2O$ (20 g/L) and 10 mL 30% H_2O_2 were added to the sample. Then, 2.5 mL of 30% H_2O_2 per each minute for total reaction time of 10 min was added. Finally, the sample was cooled down for around 10 min and H_2SO_4 addition, until sample looks clear. Between each addition of different enzymes and solutions, the same metal mesh was used for filtration and rinsed thoroughly with ultrapure water for the next pretreatment step. The preparation of the solutions is reported in the SI.

Set B consisted of samples that were pretreated similarly as Tsering et al. (2021) with an additional step of Fenton reaction with addition of sulfuric acid for dissolution of iron precipitates. The same metal mesh and the same glass bottle were used throughout the pretreatment steps, as with the set A. First, 50 g of sediment samples were weighed, and density separation was performed in a 500-mL separatory funnel. The supernatant was filtered on a stainless-steel metal mesh, rinsed, and stored in a glass bottle. Then removal of organic material was performed with hydrogen peroxide followed by Fenton reaction in the same glass bottle with stainless steel mesh.

After all the pretreatment steps, the final samples were filtered on gold coated PC membranes (0.8 μm pore size). For both set of samples, subsampling of the pretreated samples was necessary before the final filtration due to the high number of particles for the feasible analysis of microplastics. The subsampling of pretreated sample was performed in two 500 mL graduated cylinders. Prior to the distribution of the pretreated sample into the two cylinders, the sample in the glass bottle was shaken manually to divide the sample uniformly in both cylinders. Then the sample was equally distributed into both cylinders. Finally the glass bottle was rinsed three times with ultrapure water, which was similarly distributed into the two cylinders.

2.4. Sample identification

Microplastics identification was performed with Raman imaging microscope (Thermo Scientific, DXR3xi with OMNICxi software) (Tsering et al., 2022). For each sample, a 110-μm thick terrain mosaic of the effective filtration area was created by 9-μm increment with 10× objective. Particle selection was utilized and set to detect particles larger than 100 μm, which were then scanned with 785 nm laser, with 10 mW power, 0.1 s exposure time with 10 scans, 50 μm confocal pinhole and photobleaching of 0.01 s. Resulted spectra were compared within spectral range of 600–1800 cm^{-1} against four libraries including two polymer libraries, a non-plastics library, and a mineral library. The spectral libraries of polymer and non-plastics were self-made and mineral library was a commercial library (RRUFF Raman Minerals Broad Scan by Thermo Fisher Scientific). The polymer libraries included 13 polymers, namely high density-polyethylene (PE-HD), low density-polyethylene (PE-LD), polypropylene (PP), polystyrene (PS), polyamide 6 and 12 (PA6, PA12), polyvinylchloride (PVC), polyethylene terephthalate (PET), polycarbonate (PC), polytetrafluoroethylene (PTFE), polyvinyl alcohol (PVA), polyoxymethylene (POM) and poly (methyl methacrylate) (PMMA). The non-plastics library included spectra of glass, cotton and a gold coated PC membrane. Particles with spectral correlation >30 % with the reference spectra of polymers were identified as plastics. The terrain mosaic of samples with a mark on each particle with accompanied result was obtained. In addition, to minimize the underestimation of microplastics count in the samples, all the unidentified spectra (with a spectral correlation <30 % with the reference spectra) were checked manually against the four set of previously used libraries and spectra identified with same peaks as reference spectra were added to the microplastics count. The color, shape, and size of microplastics were manually identified from the terrain mosaic.

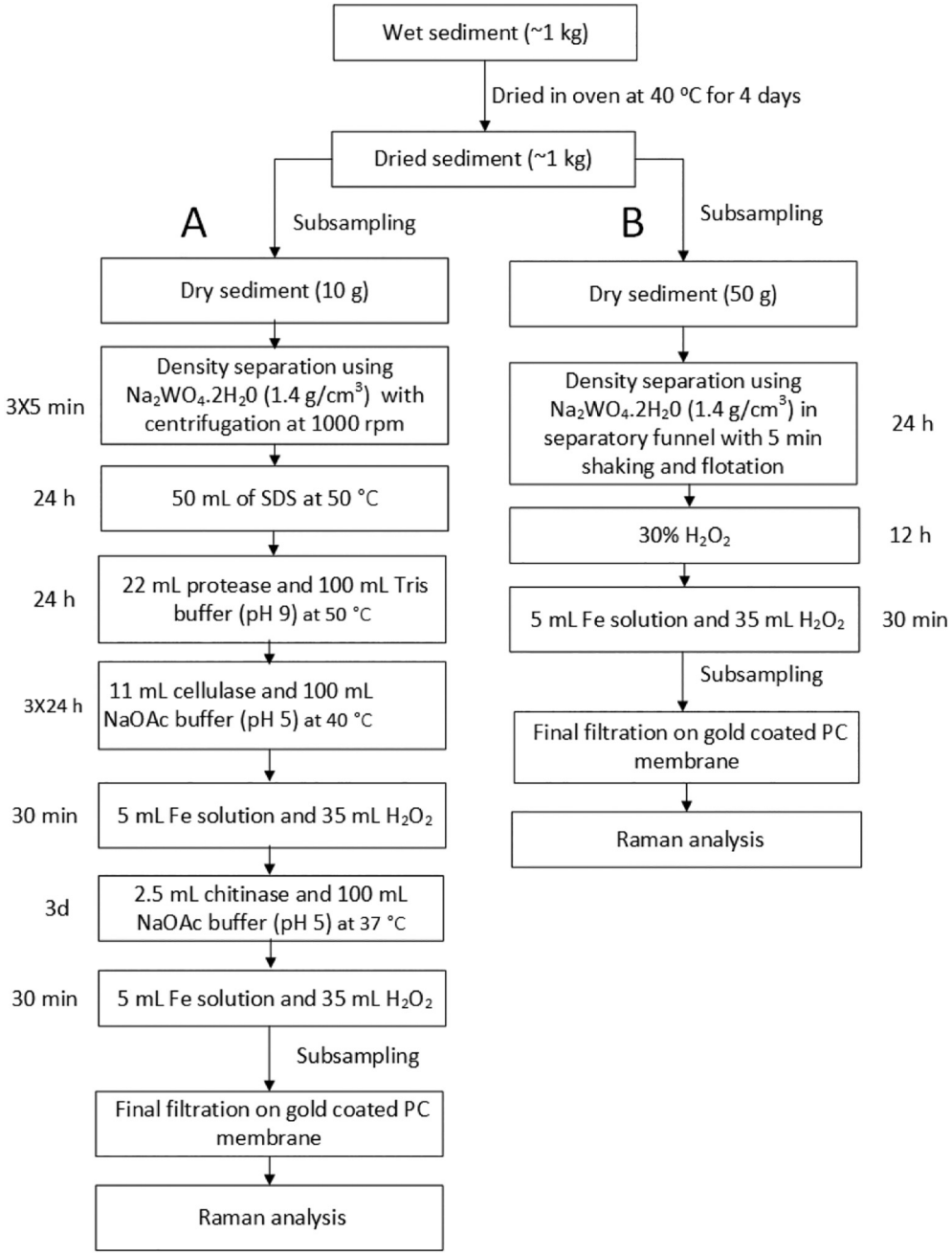

Fig. 2. The different steps used to pretreat samples for set A and set B.

2.5. Statistical analysis

For data analysis, *t*-test was applied to compare the detected microplastics concentrations of different sets of samples. The t-test was performed in Microsoft Excel. The *p*-values (two tail) lesser than 0.05 were considered as significant. However, normality of distribution could not be tested due to small number of samples that might increase the uncertainty of the statistical analysis.

2.6. Quality assurance (QA) and quality control (QC)

Strict measures for contamination control were implemented throughout the study. All enzyme solutions were filtered through stainless-steel meshes (20 μm, Ø 50 mm) and chemical solutions were filtered through a precombusted GF/C filters (Whatman GF/C, 1.2 μm, Ø 47 mm) except for concentrated sulfuric acid due to safety reason. Stainless metal meshes were checked under stereomicroscope prior to pretreatment. Cotton lab coats and nitrile gloves were used from the sample handling to analysis. Glassware, steel containers and spoons were pre-cleaned and pre-rinsed thrice with ultrapure water. Pretreatment steps were performed in a laminar flow hood and in fumehood to minimize aerial contamination from lab environment. Similarly, all solutions and final samples were covered with aluminum foil and glass petri dishes, respectively.

The recovery rates of the different pretreatment methods were assessed with positive control samples. For set A, the recovery of PE, PS and PVC fragments as well as PET, PA and PP fibers was assessed in our previous study with three spiked reference sediment samples (Tsering et al., 2022). The total recovery of spiked microplastics was 57%. Overall, fragments were recovered and detected more efficiently than fibers. The recovery rates for pretreatment method of set B samples were considered similar as performed in Tsering et al. (2021). The overall recovery rate was 91% and details on recovery rates can be referred from Tsering et al. (2021).

3. Results and discussion

For the samples from set A, a total of 3745 particles were analyzed and out of them 62 were identified as microplastics. For set B, a total of 3565 particles were analyzed and 46 were identified as microplastics. A stringent blank correction was assessed on both sets of the samples and its impacts were discussed. Potential sources, pathways, and fate of microplastics were discussed with two sets of samples pretreated with different methods. In addition, microplastics concentrations obtained in this study were compared with other studies from high-altitude lakes.

3.1. Assessment of blank correction

To avoid reporting of false positive microplastics and over estimation of microplastics counts, conducting procedural blanks and correction of blank contamination in the samples are highly recommended. The study by Horton et al. (2021) on stringent application of blank correction on complex samples indicated that for the samples that have initially high microplastics concentrations such as sludge, the blank corrected concentrations are more likely above LOQ. Whereas, the possibility to report microplastics concentrations above LOD and LOQ can be challenging for samples that have initially low microplastics concentrations.

To assess the procedural contamination, two replicates of procedural blanks were conducted for each pretreatment method. Even with strict contamination control measures, microplastics were found in both sets of procedural blanks. For set A with modified UEPP, the detected microplastic contamination per sample (mean with 95% confidence interval (±95% CI)) consisted of PS (0.5 ± 1.0), PE (1.5 ± 1.0) and PP (1.5 ± 1.0). For set B (H_2O_2 and Fenton reaction), the detected microplastic contamination per sample consisted of PE (1.0 ± 2.0), PET (0.5 ± 1.0) and PP (2.0 ± 2.0). Microplastics in the procedural blanks could likely originate from plastics part of the equipment used during pretreatment, including PE cap of centrifugation tubes, cover of Raman imaging microscope, incubator and shaker, wash bottles with PE cap and PP body. Based on the above-mentioned contamination in the procedural blanks, limits of detection (LOD) and limits of quantification (LOQ) were calculated for both sets of samples and reported in Table S2. Microplastic concentrations of the sediment samples from three studied lakes were reported and further discussed both without and with blank correction based on LOD and LOQ of procedural blanks.

Blank correction on the basis of LOD and LOQ of the procedural blanks was applied in this study on both sets of samples (Horton et al., 2021). LOD and LOQ values derived from procedural blanks and blank corrected concentrations in both set of samples are reported in Tables S2 and S3. In set A, blank corrected concentrations above LOD were detected for PS and PE at TM3, for PE at TM2, for PP at TM2, for POM and PMMA at PK1 and TS1, respectively. PE value at TM1 was above LOQ (Table S3). In set B, blank corrected concentrations higher than LOD were detected for PS at PK3, for PE at TM1, for PET at PK1, for PP at TM2 and TM3, and for PMMA at TM2 (Table S3).

However, many sites in both sets had blank corrected values lower than LOD. Thus, the finding is in line with the study by Horton et al. (2021) that for relatively clean samples with less microplastics counts, the application of blank correction on microplastics concentration can make significant differences. Nevertheless, reporting complete information including necessary details of sampling to identification and contamination control measures are crucial in microplastics research for reliable comparison among microplastic studies.

In addition, the dilemma of microplastics detected in the procedural blanks might not be exactly same in the sediment samples. Similarly, the contamination in the sediment samples might not be precisely captured in the procedural blanks. Thus, both microplastics concentration with and without blank correction must be reported.

Nevertheless, blank corrected microplastics concentrations in three lakes were above LOD particularly at the Tsomoriri Lake.

3.2. Abundance, shapes, colors and sizes of microplastics

Similarly with other high-altitude lakes, such as Gangtang Co of Tibet (Liang et al., 2022), and Lake Sassolo in Switzerland (Velasco et al., 2020), microplastics were also found in the shore sediments of all sites around the three studied lakes in the Ladakh Himalaya. The detected microplastics concentrations in sediment samples of sets A and B with and without blank correction are presented in Fig. 3. The blank-corrected

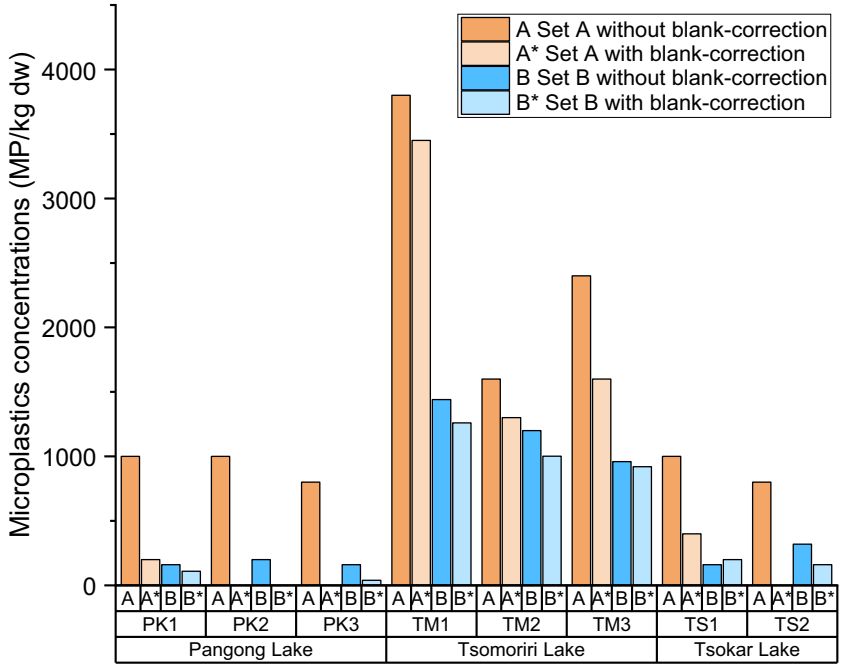

Fig. 3. Microplastics concentrations (MP/kg dw) in Pangong Lake (PK1, PK2 and PK3), Tsomoriri Lake (TM1, TM2 and TM3) and Tsokar Lake (TS1 and TS2) with and without blank correction. Samples for set A were pretreated with modified UEPP and samples for set B with H_2O_2 and Fenton reaction. Only microplastics larger than 100 μm were considered.

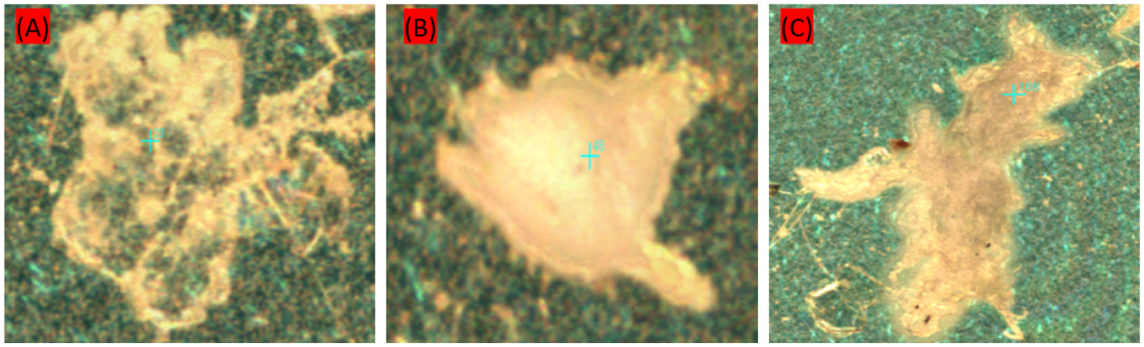

Fig. 4. Images of microplastics found in studied lakes with different color as (A) transparent (B) white and (C) off white (black).

microplastics concentrations above LOD or LOQ were included in Fig. 3. In this study, only microplastics larger than 100 μm were considered and microplastics concentrations discussed in this and subsequent sections are not blank-corrected concentrations.

In set A, total microplastics concentrations ranged from 800 to 1000 MP/kg dw in Pangong Lake, 1600–3800 MP/kg dw in Tsomoriri Lake, and 800–1000 MP/kg dw in Tsokar Lake. In set B, total microplastics concentrations were 160–200 MP/kg dw in Pangong Lake, 960–1440 MP/kg dw in Tsomoriri Lake, and 160–320 MP/kg dw in Tsokar Lake. The samples in set B, which were pretreated with H_2O_2 and Fenton, had lesser microplastics abundance than samples in set A, which were pretreated with enzymes and Fenton reaction.

In both sets A and B, the highest abundance of microplastics among three studied lakes was observed in Tsomoriri Lake. Even though all three lakes are tourist destinations during summer, Tsomoriri Lake and Pangong Lake are more popular sites with greater number of visitors.

The microplastics concentrations were lower in all samples of set B than set A (Fig. 3). The *t*-test result indicated that the microplastics concentrations between samples of two sets were significantly different ($p = 0.04$).

The main reasons for the differences in microplastics concentration between sets A and B could be the different pretreatment method utilized in each set, namely enzymatic reactions followed by Fenton reaction, and reaction with 30 % H_2O_2 followed by Fenton reaction, respectively. Even though Fenton reaction was utilized in both sets, it was performed two times in set A. Thus, pretreatment of samples was likely more efficient for set A than for set B. Secondly, the contamination caused during different pretreatment steps can be different, as samples in set A were pretreated with a multistep procedure in laminar flow hood, whereas samples in set B were pretreated with lesser steps, including reaction with H_2O_2 in fume hood and Fenton reaction in laminar flow hood. However, microplastics were found in all procedural blanks, including PS, PP, PET, and PP as discussed in Section 2.5. As the amount of contamination did not vary between sets A and B, higher microplastics concentrations of set A cannot be explained by contamination. Thirdly, the heterogenic composition of the studied shore sediment can explain part of the difference between sets A and B. Even though the primary samples in respective sets were from the same site, the secondary sediment subjected to pretreatment may have been different.

Overall, this finding confirmed that direct comparison of microplastics results among different studies, even with similar sample types, should be done with a high precaution, because various factors impact on detected microplastics concentrations. In addition, in case of the samples that have low microplastics concentrations, even a small variation in microplastics counts in subsamples can lead to significant differences in reported microplastics concentration, when the results are extrapolated for the whole site.

All detected microplastics were fragments in both sets of samples. Images of some microplastics extracted from the terrain mosaic created with the Raman imaging microscope are shown in Fig. 4. Intense UV radiation results in oxidation and fragmentation of plastics that causes fragment shaped microplastics in high altitude lakes (Dusaucy et al., 2021). Nevertheless, fiber shaped microplastics were likely underestimated due to analysis method used in this study. The lower size limit of 100 μm utilized in this study may have caused underestimation of narrow and small microplastic fibers.

Color of microplastics was classified into three broad types as transparent, white (including lighter color, such as yellow) and black (including darker color such as off white). White and transparent were most commonly found in both sets followed by black in set A (Fig. S1). However, microplastics color is not a reliable characteristic for interpretation of their source since color can fade and change under different environmental conditions and even due to the pretreatment of the samples. In addition, focus of the microscopic light on microplastic could impact on its color. Nevertheless, according to their color, transparent, and white microplastics could be from water bottles and food packaging items. Black colored microplastics could be from the clothes, vehicles, and trekking items such as tent.

In this study, microplastics sizes ranged from 100 to 5000 μm were considered. The longest dimensions of microplastics were measured and classified in respective size ranges (Fig. 5). Majority of the detected microplastics were smaller than 200 μm in both sets of samples, which indicate either

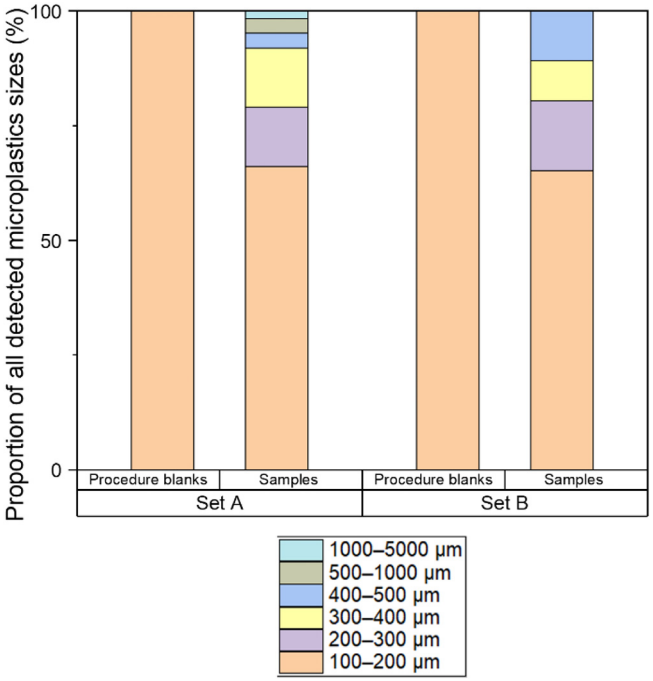

Fig. 5. Proportion of all detected microplastics sizes in the procedure blanks and samples of set A (samples treated with enzymes and Fenton reactions treated) and set B (samples treated H_2O_2 and Fenton reaction).

high fragmentation of larger plastic items or transportation of especially small microplastics via air or water to the studied sites. Furthermore, the high occurrence of smaller microplastics indicates the need for future microplastics studies to focus on microplastics smaller than 100 μm.

The detected microplastics from procedural blanks in both sets A and B ranged from 100 to 200 μm. The microplastics in samples of both sets A and B ranged from 100 to 5000 μm size with the majority microplastics within 100–200 μm size (Fig. 5). The microplastics detected in the procedural blanks indicate the importance of conducting procedural blanks.

3.3. Potential sources of microplastics

Microplastics in a lake environment could originate from two key sources; as fragmentation of larger plastics and microplastics that has been intentionally manufactured for various purposes such as in cosmetic products (Dusaucy et al., 2021). In rural and remote areas, such as in the lakes in Himalaya region, improper plastic waste treatment from various tourism activities can lead to fragmentation of those wastes that could be the most prominent source of microplastics.

In this study, PE and PP were most commonly found polymers, followed by PS, PA, PET, POM, and PMMA in set A and set B samples (Fig. 6).

Furthermore, PP and PE are most extensively used in plastic products. PE is utilized for plastic bags and bottles, while PP is commonly utilized for plastic packaging. A review on microplastics in high mountain lakes by Pastorino et al. (2022) summarized that PE and PP are most commonly found polymers, which is in line with the results of this study. The studied sites in Ladakh Himalaya are remotely situated with no vicinity industrial activities, so the microplastics are likely caused by fragmentation of larger plastics. Environment impact assessment on mountain tourism in Ladakh Himalaya by Geneletti and Dawa (2009) indicated that the lack of proper infrastructure and planning tools with growing tourism development poses a high threat to fragile ecosystem due to improper waste dumping. Improper waste dumping also allows plastic waste to enter the aquatic environment, which can further lead to higher levels of microplastic pollution.

Detected polymers were also considered for each site separately (Fig. 7). In addition to PP and PE in the samples of set A, POM was observed at site PK1, PS in TM3, and PA and PMMA in TS1. In set B, PET was observed at PK1 and PK2, and PMMA in TM2. PA and PET could originate from different trekking items from tourists, POM and PMMA could source from parts of vehicles. Mass human settlement are absent at these study sites. However, nearby areas have few human localities that could have also contributed microplastics into these sites. The differences in microplastics concentration

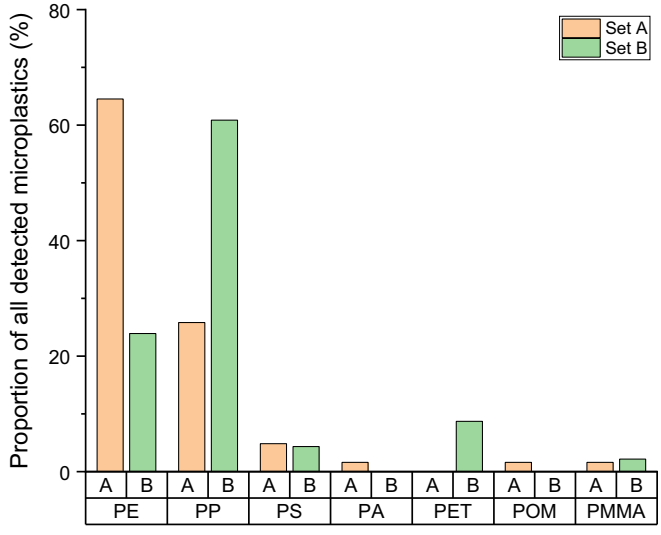

Fig. 6. The proportions of different polymers detected for two sets of samples treated with A) Enzymes and Fenton reactions B) H_2O_2 and Fenton reaction.

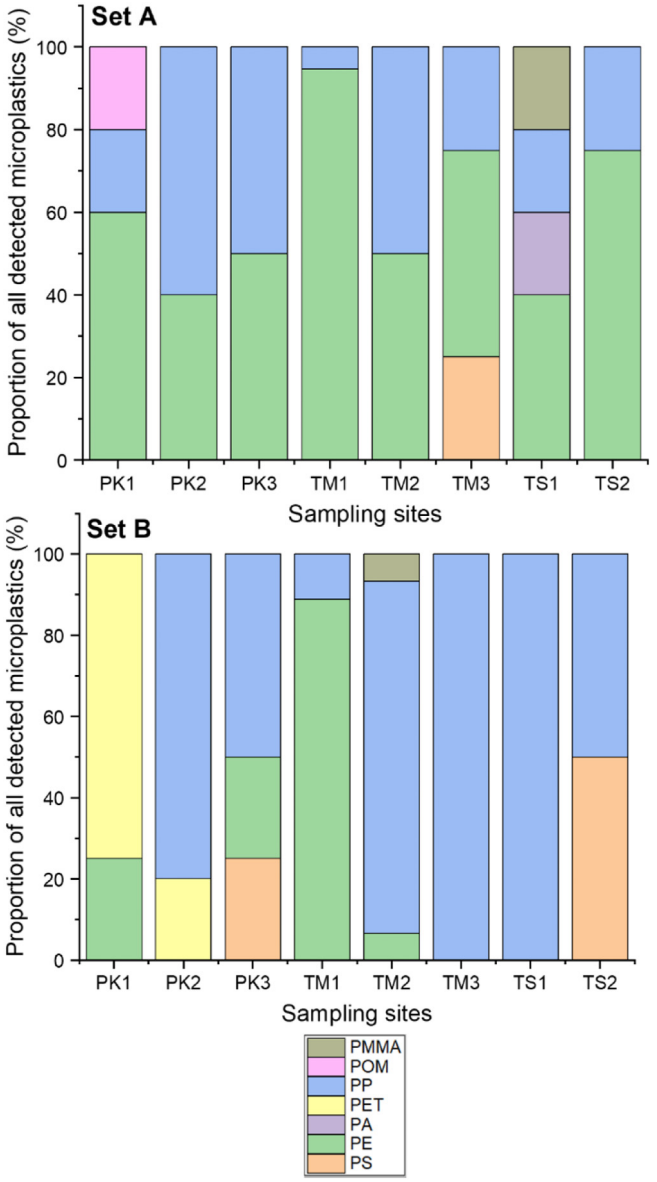

Fig. 7. The proportions of different polymers detected for each site in samples of set A and B.

and composition of the samples from the same sites indicate the complexity of sediment matrix and heterogenous nature of microplastics in environment.

3.4. Pathway and fate of microplastics

This study did not focus on detailed assessment of pathways and fate of microplastics. However, potential pathways and fate of microplastics in the studied lakes were discussed.

A review by Padha et al. (2022) on the microplastics pollution in mountain terrains concluded that glacial retreat contributes to microplastics in rivers and lakes. Furthermore, microplastics were found in glaciers such as Mount Everest at elevation around 8850 m (Napper et al., 2020). Similarly, lakes in this study are located at elevation above 4000 m and lake water is formed from the glaciers. Thus, glaciers retreat could contribute microplastics in studied lakes (Majeed et al., 2021).

Microplastics study by Allen et al. (2019) in French Pyrenees, a remote pristine mountain catchment, indicated that dry and wet deposition can be a potential source of microplastics in remote areas. Precipitation on the Ladakh Himalaya is relatively low with annual rainfall around 100 mm, but seasonal fluctuation due to climate change impacts on frequency of

precipitation over the years. Thus, precipitation could be also a pathway of microplastics into the studied lakes. In addition, aerial transport via wind could be another possibility of microplastics pathway (Zhang et al., 2019).

Surface runoff caused by melting of snow, washing away plastic litters by rain, cleaning of vehicles along the shore particularly in Pangong Lake and Tsomoriri Lake could add microplastics into the Lake shore sediment. Anthropogenic activities, including tourism such as trekking, vehicles, clothes with synthetic fibers, tents, and disposal of plastics litters like drinking water bottles and food packaging, around the lake sites could be among the major contributors of microplastics in studied lakes. Thus, proper waste management and environmentally sustainable tourism activities should be practiced.

Microplastics act as a vector for toxic contaminants such as heavy metals and organic pollutants that could intensify the negative impact of these contaminants on organisms, humans and surrounding environment (Gao et al., 2022). Furthermore, lakes on the Ladakh Himalaya are enclosed with high retention time of water with water exchange mainly through evaporation. The retention of microplastics in the studied lake sediment can be long and these lakes can act as a potential sink of microplastics.

3.5. Comparison with other studies

Even though, direct comparison among different microplastics studies is not reliable. Herein, some studies on high altitude lakes sediments are summarized in Table 1.

Based on this study, microplastic concentration in the lake shore sediments in Indian Himalaya were significantly higher than in the other high-altitude lakes such as Lake Sassolo (Velasco et al., 2020). Microplastics abundance in 12 lakes of Tibet (Liang et al., 2022), Lake Victoria (Egessa et al., 2020) and Anchar lakes (Neelavannan et al., 2022) were roughly similar ranging in 0–3800 MP/kg dw. However, in direct comparison among the lakes, microplastics abundance in lakes in Indian Himalaya were higher than the other lakes.

In most of studies, oxidation for removal of organic materials has been performed with 30 % H_2O_2 and in some studies with ferrous solution (Table 1). In this study, enzymes were utilized in addition to 30 % H_2O_2 with ferrous solution. Enzymatically pretreated samples had higher concentration in comparison with other lakes. Especially enzymatically pretreated Tsomoriri Lake sediments had the highest abundance of microplastics among all the lakes.

Thus, direct comparison of microplastics result among same type samples may provide some information. However, challenges on reliability of the comparison persist because various factors differ among different studies such as size limits, pretreatment methods, analysis techniques, and contamination sources. As observed in this study, microplastics concentrations vary even among the samples from the same site.

According to the comparison among the lakes in Table 1, lakes in the Indian Himalaya require rigorous attention to microplastics pollution. Booming tourism on Ladakh regions, including lakes, urgently needs an environmentally sustainable strategy and practices in combating the plastics pollution. On the basis of findings in this study, microplastics pathways and fate in the remote lake ecosystems, such as in Indian Himalaya, should be further studied in the future.

4. Conclusions

According to this study, the shore sediments of lakes in the Indian Himalaya, including Pangong Lake, Tsomoriri Lake and Tsokar Lake, have microplastics concentrations ranging from 160 to 3800 MP/kg dw. The maximum microplastics concentrations were observed in Tsomoriri Lake. The most dominant polymer compositions in all the studied sites were PE and PP. Fragmentation of larger plastic materials were potentially the main sources of microplastics in the studied sites.

A significant difference in microplastics concentrations of sediment samples from same site pretreated with two different methods demonstrated both the heterogeneity of microplastics in sediment matrix and

Table 1
A summary of microplastics studies in high-altitude lake sediments.

Lake	Elevation (m)	Sample type	Sampling depth	Microplastics size (μm)	Microplastics pretreatment method	Microplastics analysis method	Microplastics concentration (MP/kg dw)	Reference
12 lakes of Tibet	4469–4872	Shoreline	0–5 cm	50–5000	$ZnCl_2$; 30% H_2O_2	Stereomicroscope, FTIR	17.22–2643.65	(Liang et al., 2022)
Victoria Lake, Uganda	1140	Shoreline	0–5 cm	300–5000	NaCl; Fenton	Microscope, FTIR	0–1102	(Egessa et al., 2020)
Anchar lake, Northwest Himalaya	1583	Bottom sediment	Top of sediment 0–6 cm	300–5000	NaCl; 30% H_2O_2	Stereomicroscope, FTIR	233–1533	(Neelavannan et al., 2022)
Sassolo lake, Switzerland	2074	Bottom sediment	–	125–5000	NaI; 30% H_2O_2	Optical microscope, FTIR	0–60	(Velasco et al., 2020)
Dimon Lake, Northeast Italy	1872	Bottom sediment	–	10–5000	NaCl	Stereomicroscope, FTIR	Not detected	(Pastorino et al., 2021)
Pangong Lake, Indian Himalaya	4250	Shoreline	0–3 cm	100–5000	$Na_2WO_4{\cdot}2H_2O$; Enzymes and Fentons $Na_2WO_4{\cdot}2H_2O$; H_2O_2 and Fenton	Raman	800–1000 160–200	This study
Tsomoriri Lake, Indian Himalaya	4522	Shoreline	0–3 cm	100–5000	$Na_2WO_4{\cdot}2H_2O$; Enzymes and Fentons $Na_2WO_4{\cdot}2H_2O$; H_2O_2 and Fenton	Raman	1600–3800 960–1440	This study
Tsokar Lake, Indian Himalaya	4572	Shoreline	0–3 cm	100–5000	$Na_2WO_4{\cdot}2H_2O$; Enzymes and Fentons $Na_2WO_4{\cdot}2H_2O$; H_2O_2 and Fenton	Raman	800–1000 160–320	This study

the effect of pretreatment method on the resulted microplastics concentrations. Furthermore, the findings highlighted the importance of reporting complete information in microplastics studies especially during comparison among different studies of same sample type. The study also demonstrated the importance of conducting procedural blanks and reporting microplastics concentrations with and without blank correction.

Overall, this research indicated that lakes in the Indian Himalaya are contaminated with microplastics. The lack of environmental sustainability strategy with growing tourism activities worsens the microplastics pollution specially through improper waste treatment. Thus, strategies to combat the plastic pollution in these regions must be implemented. This study further demonstrated that the high-altitude mountainous lakes can potentially become long-term sinks of microplastics.

CRediT authorship contribution statement

Tenzin Tsering: Conceptualization; Methodology; Formal analysis; Investigation; Methodology;

Validation; Visualization; Roles/Writing - original draft; Writing - review & editing.

Mika Sillanpää: Investigation; Project administration; Funding acquisition; Supervision; Writing - review & editing.

Mirka Viitala: Methodology; Writing - review & editing.

Satu-Pia Reinikainen: Supervision; Validation; Writing - review & editing.

Data availability

Supplementary Information submitted.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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