JNCS
Journal of Nepal Chemical Society
February 2024, Vol. 44, No. 1, 95-107
ISSN: 2091-0304 (print)
DOI: 10.3126/jncs.v44i1.62684
RESEARCH ARTICLE

Assessment of Microplastics in Hanumante River of Kathmandu valley

Khageshwari Bhatta[1], Gauri Devi Sharma[1], Ziliang Li[3], Khagendra Prasad Bohara[2*], Mahesh Kumar Joshi[1,2,3]*

[1]Department of Chemistry, Tri-Chandra Multiple Campus, Kathmandu, Nepal

[2]Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu, Nepal

[3]School of Materials Science and Engineering, Liaocheng University, Liaocheng City, China.

**Corresponding Email: joshimj2003@gmail.com, khagendraprasadbohara@gmail.com*

Submitted: 07/07/2023, revised: 01/08/2024, accepted: 01/10/2024

Abstract

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

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

Introduction

Plastic is a synthetic material made from organic polymers that is widely used in modern life for clothing, packaging, etc. Global challenge with harmful impacts on the environment, economy, and health. Pollution, especially macro-sized debris, has become a concern to researchers due to its extreme durability and buoyancy, which threaten aquatic ecosystems [1]. Due to its lightweight, durable nature and impressive ratio of cost to performance, plastic has become an indispensable tool in sustaining and delivering the quality, comfort, and safety of modern lifestyles [2]. Microplastics can be categorized into two types. Primary microplastics are manufactured by industry, for example, microbeads in cosmetics, toothpaste, and face wash, or microfibers from synthetic clothing and rope, whereas secondary microplastics are the result of the breakdown of larger plastic fragments by erosion, solar radiation, water currents, and freezing and thawing [3]. As microplastics may contain problematic plasticizers and can also adsorb other organic pollutants, the long-term effects are unpredictable and responsible for various hazardous health disorders [4]. Nowadays, it is believed that more than 250 species, including fish, sea birds, turtles, and marine mammals, are affected by the ingestion of these micropollutants [5]. Plastic has been popular in public since the 1950s and has been tremendously produced and utilized in people's daily lives due to its relative cheapness, convenience, and versatility [6]. Plastics are comprised of hydrocarbons derived from petroleum, organic materials such as cellulose, coal, natural gas, salt, and crude oil through a polymerization or poly-condensation process and are hydrophobic in nature [7]. In spite of several benefits offered by plastics in our daily lives, they simultaneously generate the problem of increasing environmental pollution. In particular, the small-sized plastic particles that originate from manufacturing units and the degradation products of large items are prone to accumulate in the environment and may pose a serious effect on the ecosystem [8–10].

Plastics are categorized in accordance with their size due to their extensive range of sizes, from larger to millimeters to micrometers. Particles smaller than 5 mm are referred to as microplastics, so about the size of the grain of rice, although a subdivision into large microplastics (1–5 mm) and small microplastic particles (1 µm–1 mm) has been introduced [11]. A very diverse group differing in size, shape—fragments, pellets, cosmetic beads, lines, fibers, films, foams, chemical composition—polypropylene, polyethylene, polystyrene, polyester, polythene, etc. Those fragments persist due to the extremely slow degradation process and stability of plastic, which have been estimated to have been in the range of hundreds of years [12]. When water creatures ingest contaminated microplastics, they can enter the food web and then get exposed to the human body through the consumption of seafood [13]. Considering the potential threats to aquatic organisms, birds, mammals, and even humans via mistaken ingestion, microplastic contamination is attracting increasing attention worldwide [14]. Moreover, microplastics serve as carriers for heavy metals and synthetic organics, generating complex contaminants in living systems [15–17].

Numerous studies have been conducted to assess microplastic pollution in the marine environment; however, freshwater environments such as rivers and lakes have been understudied [18–20]. Nevertheless, studies have shown that freshwater bodies play an important role in pollution with microplastics, as they act as carriers of plastic and microplastics into the marine environment [21–23]. Although Nepal is rich in water resources, microplastics research in Nepal has received less attention and is in an early stage. So far, in Nepal, very few studies on microplastics and microplastic pollution have been conducted in freshwater environments [24–26]. As a result, there is an urgent need to assess this emerging pollutant in freshwater systems more adjacent to terrestrial points and directly affected by human activities.

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

Materials and Methods

Study area and sampling

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

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

Figure 1. Map indicating the sampling locations, and river network in the Hanumante river of Kathmandu, Nepal

Analysis

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

Wet peroxide oxidation and density separation

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

Vacuum filtration

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

Contamination control

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

Sample analysis for assessment

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

Results and Discussion

Gravimetric analysis

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

Table 1. Gravimetric analysis of fresh sample from the source

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

Table 2. Reduced mass of the sample

Sample Code	Initial mass of sample in g (x)	Final mass of sample after WPO in g (y)	Reduced mass of the sample in g R=(x-y)	Reduced mass (%)
SA	3.48	0.219	3.261	93.70%
SB	3.0	0.153	2.847	94.9%

SC	3.39	0.254	3.136	93.5%
SD	2.3	0.17	2.13	92.60%
SE	3.41	0.126	3.284	96.84%
SF	3.71	0.117	3.593	96.85%

Results of the reduced mass of the sample that has been obtained after complete dryness in an oven at 90°C are summarized in Table 2. The maximum reduced mass (96.85%) was observed for the sample SF, i.e., sample taken from Madhyapur Thimi area and similar result was observed with the sample SE, i.e., sample taken from Radhe-Radhe area. However, minimum value (92.60%) of reduced mass was observed in the case of sample SD collected from Shiva temple-Jagati area.

Microscopic examination

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

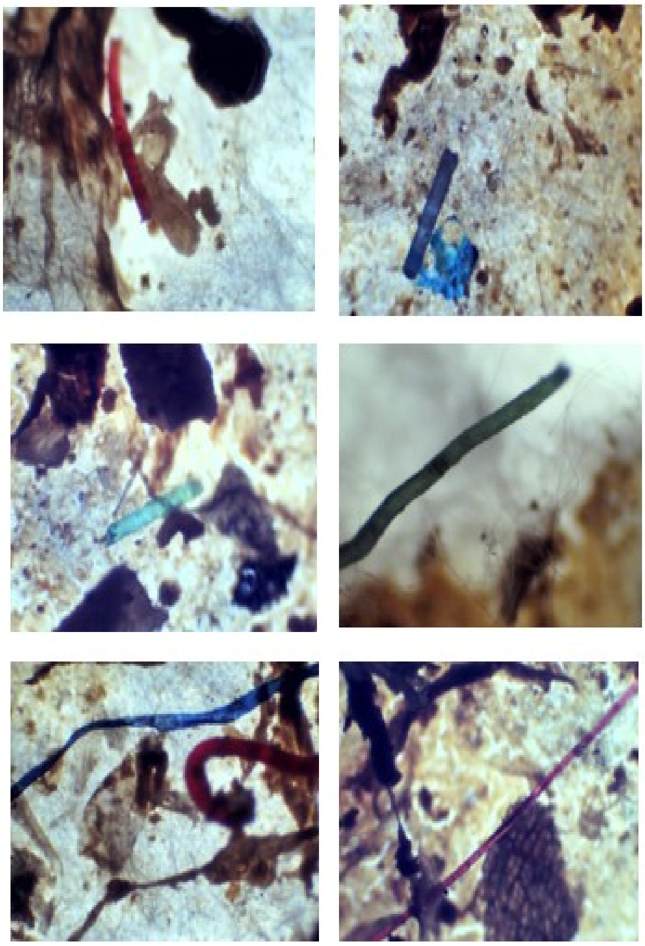

Figure 2. Microsopic images of microplastics along the Tikathali (SA) and Lokhanthali (SB) area.

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

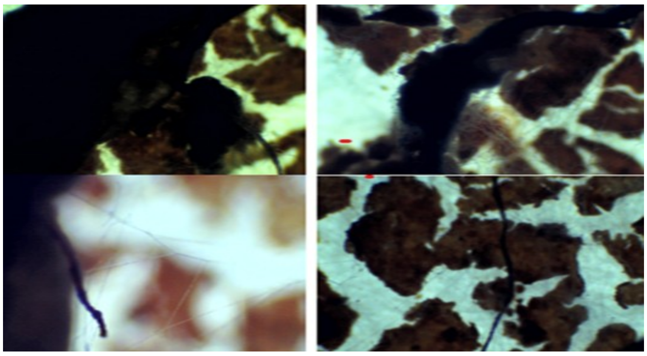

Figure 3. Microsopic images of microplastics along the Suryabinayak (SC) and Shiva temple-Jagati (SD) area.

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

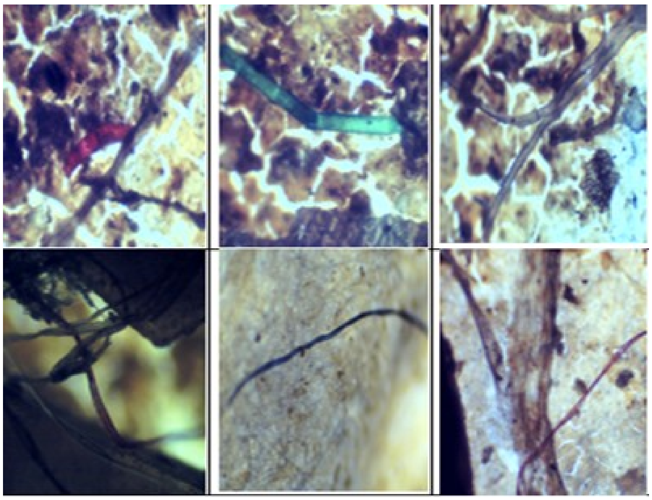

Figure 4. Microsopic images of microplastics along the Radhe-Radhe (SE) and Madhyapur Thimi (SF) area.

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

FT-IR analysis for potential microplastics

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

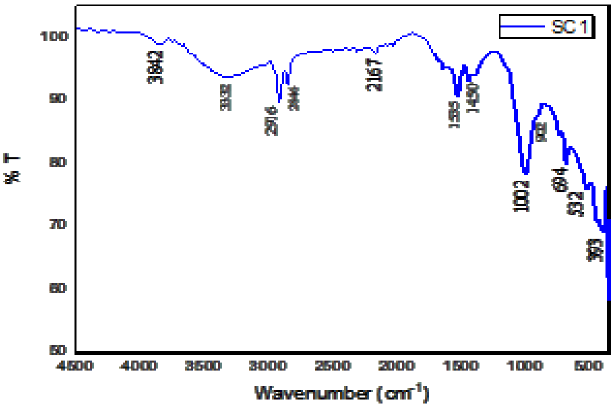

Figure 5. FT-IR spectrum of representative sample from Tikathali and Lokhanthali area.

Figure 5 shows the FTIR spectra of the microplastics samples extracted from the Tikathali and Lokanthali areas. This area was observed to be intensely polluted. Prominent, tiny plastic fragments were observed during sample extraction. Therefore, this sample is expected to have lots of microplastic fragments. Several spectral peaks can be observed in the spectrum. C-H stretching was observed at 2916.37 cm^{-1} and 2846.93 cm^{-1}, and methylene deformation were seen at 1450.47 cm^{-1} and 649.69 cm^{-1} which are very similar to the data provided by the free library [35]. Therefore, the most possible polymers found in the sample above are polyethylene, polychloroprene, and some of the nitrile rubber particles [36].

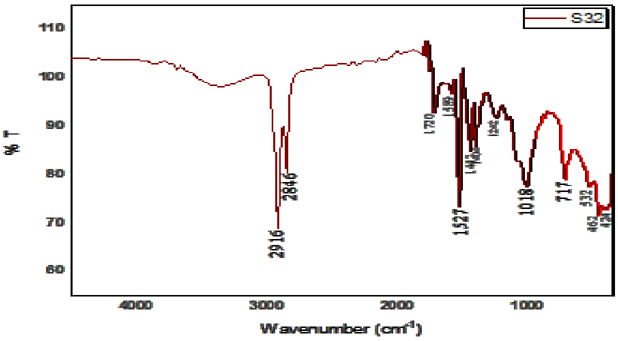

Figure 6. FT-IR spectrum of a representative sample from Suryabinayak and Shiva Temple-Jagati areas.

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

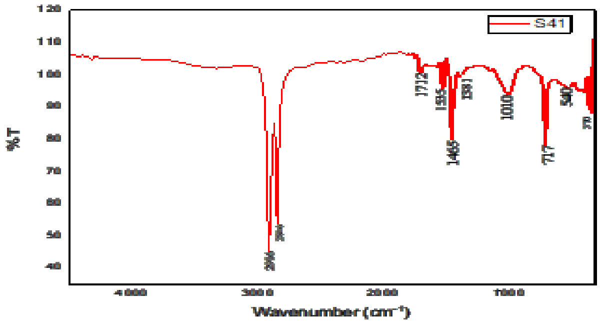

Figure 7. FT-IR spectrum of a representative sample from Radhe-Radhe and Madhyapur Thimi areas.

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

Conclusions

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

Acknowledgements

We would like to thank the University Grants Commission for the research grant for the work Support from Tribhuvan University is highly appreciated.

References

1. S. Klein, I. Dimzon, K. Eubeler, J., & T. P. Knepper. Analysis, occurrence, and degradation of microplastics in the aqueous environment. *Freshwater microplastics: emerging environmental contaminants?* (2018) 51-67. DOI 10.1007/978-3-319-61615-5

2. N. Phuong, A. Zalouk-Vergnoux, L. Poirier, A. Kamari, A. Châtel , C. Mouneyrac , & F. Lagarde, Is there any consistency between the microplastics found in the field and those used in laboratory experiments? *Environmental pollution*, (2016) 211, 111-123. DOI: 10.1016/j.envpol.2015.12.035

3. D. K. Barnes, F. Galgani, R. C. Thompson, & M. Barlaz. Accumulation and fragmentation of plastic debris in global environments. *Philosophical transactions of the royal society B: biological sciences*, (2009) 364(1526), 1985-1998. **https://doi.org/10.1098/rstb.2008.0205**

4. I. E. Napper, & R. C Thompson. Release of synthetic microplastic plastic fibers from domestic washing machines: Effects of fabric type and washing conditions. *Marine pollution bulletin, (*2016) *112*(1-2), 39-45.

5. I. E. Napper, B. F. Davies, H. Clifford, S. Elvin, H. J. Koldewey, P. A. Mayewski & R. C. Thompson (2020). Reaching new heights in plastic pollution—preliminary findings of microplastics on Mount Everest. *One Earth*, *3*(5), 621-630. https://doi.org/10.1016/j.oneear.2020.10.020

6. S. Dudas, G. Covernton, M. R. Miller, C. Pearce, H. Gurney-Smith, & J. Dower. Does habitat influence bivalve microplastic concentration? (2018)

7. W. J. Shim, Y. K. Song, S. H. Hong, & M. Jang. Identification and quantification of microplastics using Nile Red staining. *Marine pollution bulletin*, (2016) 113(1-2), 469-476. DOI: 10.1016/j.marpolbul.2016.10.049

8. M. Cole, P. Lindeque, C. Halsband, & T. S. Galloway. Microplastics as contaminants in the marine environment: a review. *Marine pollution bulletin, (*2011) 62(12), 2588-2597. https://doi.org/10.1016/j.marpolbul.2011.09.025

9. S. L. Wright, R. C. Thompson, & T. S. Galloway, The physical impacts of microplastics on marine organisms: a review. *Environmental pollution*, (2013) 178, 483-492. DOI: 10.1016/j.envpol.2013.02.031

10. A. A. Koelmans, N. H. M. Nor, E. Hermsen, M. Kooi, S. M. Mintenig, & J. De France, . Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. *Water research, (*2019) 155, 410-422. doi: 10.1016/j.watres.2019.02.054

11. M. Sighicelli, Pietrelli, F. Lecce, V. Iannilli, M. Falconieri, L. Coscia, & G. Zampetti. Microplastic pollution in the surface waters of Italian Subalpine Lakes. *Environmental Pollution*, (2018) 236, 645-651. DOI: 10.1016/j.envpol.2018.02.008

12. D. K. Barnes, F. Galgani, R. C. Thompson, & Barlaz M. Accumulation and fragmentation of plastic debris in global environments. *Philosophical transactions of the royal society B: biological sciences*, (2009) 364(1526), 1985-1998. **https://doi.org/10.1098/rstb.2008.0205**

13. J. Teng, Zhao, C. Zhang, B. Cheng, A. A. Koelmans, D. Wu, ... & Q. Wang. A systems analysis of microplastic pollution in Laizhou Bay, China. *Science of the Total Environment, (*2020) *745*, 140815. DOI: 10.1016/j.scitotenv.2020.140815

14. W. Wang, A. W. Ndungu, Z. Li, & J. Wang. Microplastics pollution in inland freshwaters of China: A case study in urban surface waters of Wuhan, China. *Science of the Total Environment*, (2017) *575*, 1369-1374. DOI: 10.1016/j.scitotenv.2016.09.213

15. S. L. Wong, B. B. Nyakuma, K. Y. Wong, C. T. Lee, T. H. Lee, & C. H Lee. Microplastics and nanoplastics in global food webs: A bibliometric analysis (2009–2019). *Marine pollution bulletin*, (2020) *158*, 111432. DOI: 10.1016/j.marpolbul.2020.111432

16. Y. Tang, Y. Liu, Y. Chen, W. Zhang, J. Zhao, S. He, ... & Z. Yang. A review: Research progress on microplastic pollutants in aquatic environments. *Science of The Total Environment*, (2021) *766*, 142572. DOI: 10.1016/j.scitotenv.2020.142572

17. T. van Emmerik, Y. Mellink, R. Hauk, K. Waldschläger, & L. Schreyers . Rivers as plastic reservoirs. *Frontiers in Water*, (2022) *3*, 212. https://doi.org/10.3389/frwa.2021.786936

18. D. Eerkes-Medrano, & R. Thompson, Occurrence, fate, and effect of microplastics in freshwater systems. In *Microplastic contamination in aquatic environments* (2018) (pp. 95-132). Elsevier. DOI:10.1016/B978-0-12-813747-5.00004-7

19. M. C. Blettler, E. Abrial, F. R. Khan, N. Sivri, & L. A. Espinola. Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. *Water research*, (2018) 143, 416-424. https://doi.org/10.1016/j.watres.2018.06.015

20. N. L. Fahrenfeld, G. Arbuckle-Keil, N. N. Beni, & S. L. Bartelt-Hunt. Source tracking microplastics in the freshwater environment. *TrAC Trends in Analytical Chemistry*, (2019) 112, 248-254. DOI. 10.1016/j.trac.2018.11.030

21. L. C. M. Lebreton, J. Van Der Zwet, J. W. Damsteeg, B. Slat, A. Andrady, & J. Reisser. River plastic emissions to the world's oceans. Nat Commun (2017) 8: 15611. DOI: 10.1038/ncomms15611

22. R. Hurley, J. Woodward, & J. J. Rothwell. Microplastic contamination of riverbeds significantly reduced by catchment-wide flooding. *Nature Geoscience, (*2018) *11*(4), 251-257. https://doi.org/10.1038/s41561-018-0080-1

23. A. Ockelford, A. Cundy, & J. E. Ebdon. Storm response of fluvial sedimentary microplastics. *Scientific Reports, (*2020) *10*(1), 1865. | https://doi.org/10.1038/s41598-020-58765-2

24. K. Amrutha, V. Unnikrishnan, S. Shajikuma, & A. K. Warrier. Current state of microplastics research in SAARC Countries—a review. *Microplastic Pollution*, (2021) 27-63. DOI:10.1007/978-981-16-0297-9_2

25. L. Yang, W. Luo, P. Zhao, Y. Zhang, S. Kang, J. P. Giesy, & F. Zhang. Microplastics in the Koshi River, a remote alpine river crossing the Himalayas from China to Nepal. *Environmental Pollution, (*2021) *290*, 118121. https://doi.org/10.1016/j.envpol.2021.118121

26. R. Malla-Pradhan, T. Suwunwong, K. Phoungthong, T. P. Joshi, & B. L. Pradhan. Microplastic pollution in urban Lake Phewa, Nepal: the first report on abundance and composition in surface water of lake in different seasons. *Environmental Science and Pollution Research*, (2022) *29*(26), 39928-39936. DOI: 10.1007/s11356-021-18301-9

27. R. Sada, (2012). Hanumante River: Emerging Uses, Competition & Implications. DOI:10.3126/jsce.v1i0.22489

28. J. Diwakar, & J. K. Thakur. Environmental system analysis for river pollution control. *Water, Air, & Soil Pollution*, (2012) *223*, 3207-3218. DOI 10.1007/s11270-012-1102-z

29. W. S. Choong, T. Hadibarata, A. Yuniarto, K. H. D. Tang, F. Abdullah, M. Syafrudin, ... & A. M. Al-Mohaimeed. Characterization of microplastics in the water and sediment of Baram River estuary, Borneo Island. *Marine Pollution Bulletin, (*2021) *172*, 112880. DOI. 10.1016/j.marpolbul.2021.112880

30. J. Masura, J. Baker, G. Foster, & C. Arthur. Laboratory Methods for the Analysis of Microplastics in the Marine Environment: Recommendations for quantifying synthetic particles in waters and sediments (2015). http://hdl.handle.net/11329/1076 http://dx.doi.org/10.25607/OBP-604

31. M. R. M. Zaki, P. X. Ying, A. H. Zainuddin, M. R. Razak, & A. Z. Aris. Occurrence, abundance, and distribution of microplastics pollution: evidence in surface tropical water of Klang River estuary, Malaysia. *Environmental Geochemistry and Health*, (2021) 1-16. DOI: 10.1007/s10653-021-00872-8

32. W. Yuan, X. Liu, W. Wang, M. Di, & J. Wang. Microplastic abundance, distribution and composition in water, sediments, and wild fish from Poyang Lake, China. *Ecotoxicology and environmental safety*, (2019) 170, 180-187. DOI: 10.1016/j.ecoenv.2018.11.126

33. A. K. Baldwin, A. R. Spanjer, M. R. Rose, & T. Thom. Microplastics in Lake Mead national recreation area, USA: occurrence and biological uptake. *PloS one*, (2020) 15(5), e0228896. https://doi.org/10.1371/journal.pone.0228896

34. R. Egessa, A. Nankabirwa, H. Ocaya, & W. G. Pabira. Microplastic pollution in surface water of Lake Victoria. *Science of the Total Environment*, (2020) *741*, 140201. DOI: 10.1016/j.scitotenv.2020.140201

35. D. Miller, "The Free Library," 2014. [Online]. Available: https://www.thefreelibrary.com/Identification+of+polymers+by+IR+spectroscopy.-a0119376722 .

36. K. Berry, M. Liu, K. Chakraborty, N. Pullan, A. West, C. Sammon, & P. D. Topham . Mechanism for cross-linking polychloroprene with ethylene thiourea and zinc oxide. *Rubber Chemistry and Technology, (*2015) *88*(1), 80-97. DOI.10.5254/rct.14.85986

37. R. Chércoles Asensio, M. San Andrés Moya, J. M. De la Roja, & M. Gómez. Analytical characterization of polymers used in conservation and restoration by ATR-FTIR spectroscopy. *Analytical and bioanalytical chemistry*, (2009) *395*, 2081-2096. DOI: 10.1007/s00216-009-3201-2

38. M. R. Jung, F. D. Horgen, S. V. Orski, V. Rodriguez, K. L. Beers, G. H. Balazs, ... & J. M. Lynch. Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by

marine organisms. *Marine pollution bulletin, (*2018) *127*, 704-716. https://doi.org/10.1016/j.marpolbul.2017.12.061

39. K. Yamada-Onodera, H. Mukumoto, Y. Katsuyaya, A. Saiganji, & Y. Tani. Degradation of polyethylene by a fungus, Penicillium simplicissimum YK. *Polymer degradation and stability, (*2001) *72*(2), 323-327. https://doi.org/10.1016/S0141-3910(01)00027-1

40. P. Vermeiren, C. Muñoz, & K. Ikejima. Microplastic identification and quantification from organic rich sediments: A validated laboratory protocol. *Environmental Pollution, (*2020) 262, 114298. https://doi.org/10.1016/j.envpol.2020.114298