Microplastic in Surface Water and Lake Shoreline Sediments of Phewa Lake, Nepal

Rajeshwori Malla-Pradhan

A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Environmental Management

Prince of Songkla University

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Major Program	Environmental Management

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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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Academic Year 2022

ABSTRACT

Microplastic is an emerging environmental pollutant receiving growing concern worldwide. Lakes in Nepal provide a variety of ecosystem services. However, the microplastic pollution in freshwater lakes of Nepal remains unknown. Therefore, this study was carried out to investigate the abundance and characteristics of microplastics along with the spatial and temporal distribution of microplastics in surface water and shoreline sediments of Phewa Lake. In addition, the objective of this study was to analyze the water quality status of Phewa Lake.

Thirty-two water samples and twenty shoreline sediment samples were collected from Phewa Lake from various locations for the uniform distribution of sampling sites. National Oceanic and Atmospheric Administration (NOAA) protocols were followed for the isolation of microplastics with minor changes. The water quality of Phewa Lake was analyzed according to the standard methods for the examination of water and wastewater.

The mean abundance of microplastic for the whole study period (2021) in Phewa Lake was 1.97 microplastics/L and 88.5±50.32 microplastics/kg dry weight for water and sediment samples respectively. The mean abundance in winter season > rainy season > autumn season for both water and sediment samples. Significant spatial variation of microplastic abundance was observed in water and sediment samples which was driven by population density and topographic factors. Seasonal variation in the mean abundance of microplastic was only observed in water samples ($H=22.34$, $p<0.01$). Fibers were the most common type of microplastics accounting for 93.04%, 96.69%, and 85.0% for winter, rainy, and autumn seasons respectively in water samples. Similarly, fibers in sediment samples accounted for 78.11%, 62.03%, and

41.26% for winter, rainy, and autumn seasons respectively. Different types of color were observed in Phewa Lake where transparent was the dominant color in water for all three seasons. But in sediment samples, white color dominated in the autumn season. A maximum abundance of microplastics was found in size class < 1 mm in water and sediment samples for all three seasons in Phewa Lake. FTIR analysis of visible microplastic (size 1-5 mm) reveals polypropylene (PP) and polyethylene (PE) as the main polymer types found in the shoreline sediments of Phewa Lake for all three seasons.

The water quality of Phewa Lake was good with regard to the water quality index but is polluted with heavy metals as indicated by the heavy metal pollution index. The findings of this study demonstrate the presence of microplastics in the water and sediments of Phewa Lake. Moreover, this study provides the first baseline data on microplastics in freshwater lakes of Nepal.

Keywords: Microplastics, Phewa Lake, shoreline sediment, water, abundance

ชื่อวิทยานิพนธ์ Microplastic in surface water and Lake Shoreline Sediments of Phewa Lake, Nepal

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บทคัดย่อ

ในปัจจุบันทั่วโลกให้ความสนใจกับไมโครพลาสติกซึ่งก่อให้มลพิษต่อสิ่งแวดล้อม การศึกษาไมโครพลาสติกในทะเลสาบของประเทศเนปาลยังมีอยู่น้อยมาก ดังนั้นวิทยานิพนธ์นี้จึงได้ดำเนินการศึกษา ตรวจสอบไมโครพลาสติกี่เกิดขึ้น รวมทั้งศึกษาคุณภาพ

และผลกระทบทางสิ่งแวดล้อมของน้ำผิวดินและตะกอนดินในทะเลสาบ Phewa รวมทั้งการกระจายตัวของไมโครพลาสติกอีกด้วย โดยการศึกษานี้เริ่มจากการเก็บตัวอย่างน้ำเป็นจำนวน 32 ตัวอย่าง และตะกอนชายฝั่งอีก 20 ตัวอย่าง รอบ ๆ ทะเลสาบ Phewa เพื่อศึกษากระจายตัวของไมโครพลาสติก ตามมาตรฐาน National Oceanic and Atmospheric Administration (NOAA) เพื่อแยกชนิดของไมโครพลาสติก รวมทั้งการวิเคราะห์คุณภาพน้ำในทะเลสาบ Phewa ตามวิธีมาตรฐานในการตรวจสอบน้ำและน้ำเสีย ปริมาณเฉลี่ยของไมโครพลาสติกนช่วงปี พ.ศ. 2564 อยู่ที่ 1.97 ไมโครพลาสติก/ลิตร และ 88.5±50.32 ไมโครพลาสติก/กิโลกรัม สำหรับตัวอย่างน้ำและตะกอนดินตามลำดับ ความอุดมสมบูรณ์เฉลี่ยในฤดูหนาว > ฤดูฝน > ฤดูใบไม้ร่วง ทั้งตัวอย่างน้ำและตะกอน และพบการเปลี่ยนแปลงเชิงพื้นที่ของปริมาณไมโครพลาสติกอย่างมีนัยสำคัญในตัวอย่างน้ำและตะกอนดินเฉลี่ย ในฤดูหนาว > ฤดูฝน > ฤดูใบไม้ร่วง

ซึ่งเป็นผลมาจากความหนาแน่นของประชากรและปัจจัยทางภูมิประเทศ รวมทั้งความแปรปรวนตามฤดูกาลของปริมาณไมโครพลาสติกเฉลี่ยที่พบในตัวอย่างน้ำ)H=22.34, p<0.01) โดยเส้นใยไฟเบอร์ไมโครพลาสติกเป็นชนิดของไมโครพลาสติดที่พบเจอมาที่สุด คิดปี 93.04% 96.69% และ 85.0% สำหรับฤดูหนาว ฝน และฤดูใบไม้ร่วงตามลำดับในตัวอย่างน้ำ ในทำนองเดียวกัน เส้นใยไฟเบอร์ในตัวอย่างตะกอนคิดเป็น 78.11% 62.03% และ 41.26% ตามลำดับ สำหรับฤดูหนาว ฤดูฝน และฤดูใบไม้ร่วง โดยเส้นใยไฟเบอร์แบบใสถูกพบมากที่สุดตลอดทุกฤดูกาล แต่ในตัวอย่างตะกอนดินพบมากที่สุดคือเส้นใยไฟเบอร์สีขาวโดยเฉพาะในฤดูใบไม้ร่วง ในส่วนของขนาดที่พบของไมโครพลาสติกนั้นจะอยู่ที่ขนาด <1 มม. ในตัวอย่างน้ำและตะกอนดินทั้งสามฤดู การวิเคราะห์ด้วย FTIR ของไมโครพลาสติกที่มองเห็นได้ (ขนาด 1-5 มม.) เผยให้เห็นถึงชนิดของไมโครพลสติก คือ โพลิโพรพิลีน (PP) และโพลิเอทิลีน (PE) ซึ่งเป็นพอลิเมอร์หลักที่พบในตะกอนชายฝั่งของทะเลสาบ Phewa ตลอดทั้งสามฤดู นอกจากนี้ผลการวิเคราะห์คุณภาพน้ำในทะเลสาบ Phewa นั้น พบว่าเป็นไปตามมาตรฐานคุณภาพน้ำของเนปาล แต่ถึงแม้จะเป็นไปตามมาตรฐาน ก็พบว่ายังมีปริมาณของโลหะหนักปนเปื้อนอยู่บ้าง ผลการศึกษานี้แสดงให้เห็นถึงการมีอยู่ของไมโครพลาสติกในน้ำและตะกอนของทะเลสาบ Phewa นอกจากนี้การศึกษาครั้งนี้ยังให้เป็นข้อมูลพื้นฐานครั้งแรกเกี่ยวกับไมโครพลาสติกในทะเลสาบน้ำจืดของประเทศเนปาล

คำสำคัญ: ไมโครพลาสติก ทะเลสาบ Phewa ตะกอนชายฝั่ง คุณภาพน้ำ ความอุดมสมบูรณ์

ACKNOWLEDGMENT

First, I would like to acknowledge the Graduate School, Prince of Songkla University, Thailand for providing a research grant that was partially sponsored to carry out my Ph.D. research work. I would like to extend gratitude to the Nepal Academy of Science and Technology (NAST) for providing laboratory space and facilities to conduct my research work. My sincere gratitude to my advisor Assistant Professor Dr. Khamphe Phoungthong whose support, guidance, and encouragement have been invaluable. I thank him for his quick response to all my messages which helped me to move forward with my research work. I would like to extend my deepest gratitude to my co-advisor research scientist Dr. Tista Prasai Joshi for always being supportive, encouraging me, and reviewing my progress constantly during my entire Ph.D. journey. It's my honor to be her Ph.D. student.

I would like to extend my profound gratitude to the Dean, the faculty of Environmental Management, Prince of Songkla University, Associate Professor Dr. Kuaanan Techato for his valuable guidance, feedback, and motivation during my studies. I want to gratefully acknowledge members of the thesis defense committee of my Ph.D. for their insightful comments and suggestions. I would like to express my gratitude to Associate Professor Dr. Saowalak Roongtawanreongsri, Assistant Professor Dr. Sutinee Sinutok, Dr. Ponlachart Chotikarn, Dr. Saroj Gyawali, and Dr. Bhagwat Rimal for their guidance in the earlier phase of my Ph.D. I would like to thank the faculty members and staff of the Faculty of Environmental Management, Prince of Songkla University for their continuous support. A special thanks to the head of the department, central department of geology, T.U., Professor Dr. Khum Narayan Poudyal, for providing me access to the microscope for capturing the photographs of microplastics. My indebtedness goes to Tribhuvan University (T.U.) and Tri-Chandra Multiple Campus, Nepal for granting me study leave during my Ph.D. I am also thankful to the central department of environmental science, T.U. for forwarding my documents for study leave. I would like to thank Fewa Boat Association for their help during sample collection.

I am very much grateful to my husband, Associate Professor Dr. Bijay Lal Pradhan for his continuous support and encouragement right from sample collection to data analysis and finally the compilation of my work. Without his endless support, this research would not have been completed. I want to take this special opportunity to thank my Dad and Mom for always believing in me and for their unconditional support. I would like to thank my family members, friends, and colleagues for their support. Finally, my very special thanks and love to my two beautiful and loving daughters Ambira and Mabira for their constant support and encouragement which recharged me to accomplish my work.

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%	Percentage
°C	Degree centigrade
µm	Micrometer
µS	Microsiemens
AAS	Atomic absorption spectroscopy
ABS	Acrylonitrile butadiene styrene
Al	Aluminum
APHA	American Public Health Association
ATR	Attenuated total reflection
aWi	Assign weight
BIS	Bureau of Indian Standard
BOD	Biological Oxygen Demand
Ca	Calcium
$CaCl_2$	Calcium chloride
$CaCO_3$	Calcium carbonate
CBS	Central Bureau of Statistics
cm	Centimeter
COVID	Coronavirus diseases
Cu^{2+}	Copper
d	Density
DO	Dissolved Oxygen
EC	Electric Conductivity
EDS	Energy dispersive X-ray spectroscopy
EDTA	Ethylenediaminetetraacetic acid

EDX	Energy dispersive X-ray spectroscopy
ESDO	Environment and Social Development Organization
Fe	Iron
Fe (II)	Ferrous oxide
$FeCl_3$	Ferric chloride
FTIR	Fourier transformed infrared spectroscopy
g	Gram
GPS	Global Positioning System
H	Kruskal Wallis H value
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HPI	Heavy metal pollution index
k	Proportional constant
kg	Kilogram
km	Kilometer
L	Liter
M	Molar
mg	Milligram
$MgSO_4$	Manganous sulphate
mL	Milliliter
mm	Millimeter
Mn	Manganese
mps	Microplastics
N	Normality
n	Number

NaCl	Sodium chloride
NaI	Sodium iodide
NaOH	Sodium hydroxide
Ni	Nickel
nm	Nano meter
NO_3^{-2}	Nitrate
NOAA	National Oceanic and Atmospheric Administration
NTU	Nephelometric Turbidity Unit
p	Significant value
PAN	Polyacrylonitrile
Pb	Lead
PE	Polyethylene
PET	polyethylene terephthalate
PP	polypropylene
PU	Polyurethane
PVC	polyvinyle chloride
Pyr-GC/MS	Pyrolysis–gas chromatography–mass spectrometry
QA	Quality assurance
QC	Quality control
QRi	Quality rating
r	Pearson correlation
RWi	Relative weight
SAARC	South Asian Association of Regional Cooperation
SEM	Scanning electron microscopy
Si	Standard value
SIi	Sub-index of water quality
TDS	Total Dissolved Solid

UV	Ultraviolet
UWi	Unit weight
WHO	World Health Organization
WQI	Water quality index
Zn	Zinc
$ZnCl_2$	Zinc chloride

CHAPTER 1 : INTRODUCTION

1.1 Background of the study

Plastic is a type of synthetic polymer that is widely utilized and has been an essential part of our day-to-day life (Cai et al., 2022; Thompson et al., 2009). As a result, in 2020, 360 million tons of plastics were produced globally (PlasticsEurope, 2021). Plastic is cheap, light, tough, durable, anti-corrosive, and has high thermal and electrical resistance properties so it is widely used in packaging goods, building and construction materials; and automobile parts which together account for 69.7% of all plastic items made. Likewise, polymers used for the manufacturing of plastic products are polypropylene (PP) (19.7%), high and low density polyethylene (PE) (30.3%), polyvinyl chloride (PVC) (9.6%), polyethylene terephthalate (PET) (8.4%) and polyurethane (PU) (7.8%) (PlasticsEurope, 2021). 6.3% of the 8.3 billion tons of manufactured plastic goods were discarded as waste between 1950 to 2015. Overall, only 9% of plastic waste has been recycled and around 79% of this waste generated has made its way to the landfill sites or surrounding habitat (Geyer et al., 2017). As plastic is resistant to degradation, they accumulate in dumping sites or surrounding habitat (Barnes et al., 2009). By 2025, it is expected that 69.14 million tons of mismanaged plastic litter would be discharged globally (Watt et al., 2021) which may end up in different aquatic environments via atmospheric deposition, wave action, rain, flood and storm outflow, industrial effluent, rivers, and sewage disposal (Ryan et al., 2009).

Plastic debris is a global concern due to its impact on economic and environmental sectors. The economic loss due to plastic litter in the marine environment is evaluated to be 22 billion euros (Beaumont et al., 2019). The aesthetic beauty of shoreline and beaches are hampered due to plastic litter lying in these places which in turn affects tourism (Andrady & Neal, 2009). Entanglement and ingestion of plastic debris by marine biota have been reported in more than 690 species (Darmon et al., 2017) which is gaining public and media attention (Thompson et al., 2009).

Microplastic, an emerging environmental pollutant is extensively studied in marine habitats (Thompson et al., 2004). But comparatively limited research has been carried out in the freshwater environments. In Nepal, microplastic pollution was unknown until research was published on microplastic by (Yang et al., 2021a) in the Koshi River. So to explore the possibility of microplastic pollution in the lakes of Nepal, this study was carried out for the first time. The aim of this study was to generate a database on the lakes of Nepal and to fill the knowledge gap in the spatial and temporal distribution of microplastics in small urban lakes. Moreover, the aim of this study was also to make use of facilities available in the country itself which may open doors to a new researcher in the field of microplastics in Nepal.

1.1.1 Microplastic: an overview

Although in the early 1970s, plastic particles were first documented in the marine habitat (Carpenter et al., 1972; Carpenter & Smith Jr, 1972), the term “microplastic” was first used by professor Richard Thompson in the year 2004 to denote plastic fragments of size 20 µm in diameter (Thompson et al., 2004). Microplastics are plastic items smaller than 5 mm as defined by the scientific community at a workshop on “the occurrence, effects, and fate of microplastic marine debris” in the year 2008 (Arthur et al., 2009). Based on their origin, microplastics can be of two main types:

i) Primary microplastics are small size plastic products for direct use or as precursors for other plastic products. Some examples of primary microplastic sources includes industrial plastic preproduction pellets, industrial abrasives for sandblasting (Von Moos et al., 2012), and exfoliates used in body and facial scrubbers (Fendall & Sewell, 2009).

ii) Secondary microplastics are formed by the disintegration of larger plastic items which mainly depends on the nature of the plastic materials, degree of weathering, and the local environmental conditions (Arthur et al., 2009). Some examples of secondary microplastic sources include discarded plastic bottles, carry bags, synthetic textile fibers, household plastic items, and fishnets (Browne et al., 2011; Free et al., 2014).

1.1.2 Occurrence and sources of microplastics

Microplastic is an emerging pollutant of global concern because of their widespread distribution in every environmental component like world’s ocean (do Sul et al., 2013; Goldstein et al., 2013; Lusher et al., 2014; Mendoza & Jones, 2015), Mediterranean sea (Alomar et al., 2016; Panti et al., 2015; Schmidt et al., 2018; van der Hal et al., 2017), costal beach sediments (Veerasingam et al., 2016; Vidyasakar et al., 2018), great lakes (Eriksen et al., 2013; Mason et al., 2016; Zbyszewski et al., 2014), deep sea (Van Cauwenberghe et al., 2013), rivers (Castañeda et al., 2014; Klein et al., 2015; Rodrigues et al., 2018; Sarkar et al., 2019; Treilles et al., 2022), lakes (Alfonso et al., 2020; Faure et al., 2015; Felismino et al., 2021; Free et al., 2014; Kumar et al., 2021; Malygina et al., 2021), Tibet plateau (Jiang et al., 2019; Zhang et al., 2016), snow (Bergmann et al., 2019; Napper et al., 2020), rainwater (Xia et al., 2020), soil (Huerta Lwanga et al., 2017; Liu et al., 2018), polar ice (Kelly et al., 2020) and air (Dris et al., 2015; Jenner et al., 2021; Liao et al., 2021). Therefore, it is a hot research topic in scientific communities as microplastics have been reported from the deep sea (Van Cauwenberghe et al., 2013) to the pristine environment (Allen et al., 2019) and even the highest peak of the world the "Everest" (Napper et al., 2020). In the global context, research on microplastics are on a constant rise but Nepal lacks far behind. Till date, only three research articles have been published to address this emerging pollutant. First on urban road dust (Yukioka et al., 2020), second snow and stream around Mt. Everest (Napper et al., 2020), and third surface and sediment of the Koshi River (Yang et al., 2021a). Microplastics may enter the environment through a wastewater treatment plants (Edo et al., 2020; Talvitie & Heinonen, 2014), agricultural runoff laden with sewage sludge, and disintegrated plastic mulches (Nizzetto et al., 2016; Qi et al., 2020), atmospheric deposition (Cai et al., 2017; Dris et al., 2015), rainwater runoff (Liu et al., 2019a), use of cosmetic and cleansing products (Fendall & Sewell, 2009; Murphy et al., 2016) and accidental spillage of plastic pellets (Thompson et al., 2009).

1.1.3 Environmental risks of microplastics

1.1.3.1 Effects of microplastics on biota (plants and animals)

Potential threats of microplastic exposure on biota, microorganisms, human, and the ecosystem have aroused significant concern among the scientific community and general public (Xiang et al., 2022). Microplastics are adsorbed on vascular plants causing phytotoxic effects including hindrance of plant growth (Pignattelli et al., 2020; Yu et al., 2020b), reduced photosynthetic activities (Gao et al., 2019; Yu et al., 2020b) and oxidative stress (Gao et al., 2019; Yu et al., 2020b). It also inhibit the germination of seeds (Bosker et al., 2019) and the growth of microalgae when exposed to microplastic (Zhang et al., 2017).

Similarly, microplastic can adsorb persistent organic pollutants and heavy metals (Torres et al., 2021; Wang et al., 2021) from the surrounding environment thereby increasing the bioavailability of contaminants (Xia et al., 2021). It may then be taken up by organisms as they mistake microplastics for food (Cole et al., 2013). Once ingested it may cause fullness of the stomach leading to starvation and finally death (Duis & Coors, 2016; Gall & Thompson, 2015). Likewise, it may lead to intestinal obstruction, and mechanical injury of the digestive tract due to the sharp and stiff edge of microplastics (Lei et al., 2018; Song et al., 2019). Similarly, the effects of microplastic ingestion have been linked to oxidative stress and genotoxicity (Avio et al., 2015), inhibition of growth and development (Au et al., 2015; Kaposi et al., 2014) decrease in reproductive output (Au et al., 2015; Cole et al., 2015), neurotoxicity (Luís et al., 2015; Ribeiro et al., 2017), alter immune responses (Köhler, 2010), and modify the composition of intestinal microflora of an organism (Zhu et al., 2018).

1.1.3.2 Effects of microplastics on human health

The human body may be exposed to microplastic via oral intake (ingestion) and skin contact (Chang et al., 2020; Prata et al., 2020) or inhalation of airborne microplastics (Dris et al., 2017; Prata, 2018) which may pose an adverse health risk like impairment of DNA, modification in protein and gene expression, cellular damage, decrease in viable cells, increase in calcium ion, swelling in tissues and oxidative stress (Gallo et al., 2018; Rahman et al., 2021; Yong et al., 2020). In human beings, tiny microplastic is linked with heart and respiratory disease or even cancer in the lungs (Vethaak & Legler, 2021). A review report estimated that human intake of "50 plastic bags (size: 0.04 mm x 250 mm x 400 mm, density: 0.98 g/cm^3)" per year from food consumption (Bai et al., 2022). According to the research, multidrug-resistant human pathogens could be facilitated on the peripheral of microplastic which is polluted with metals and antibiotics (Imran et al., 2019). Likewise, microplastic may be a key factor operating as a potential vector to enrich bacteria that may be resistant to multidrug and antibiotics (Song et al., 2020) which increases the chances of harmful pathogens.

1.1.3.3 Effects of microplastics on soil and ecosystem

The presence of microplastics in the soil can increase the evaporation rate of soil water which may further lead to the drying of the soil forming a cracked soil surface (Wan et al., 2019). In the soil, microplastic acts as a potential vector for harmful chemicals and transfers through the food chain (He et al., 2018).

Microplastic has negative consequences on the overall functioning of the aquatic ecosystem by altering the food chain, disturbing the natural habitat of an organism, changing the microbial assemblage, and hampering the species' development (Ma et al., 2020).

1.1.4 Water

Water is the lifeline of all living organisms. A nation's prosperity is connected with the proper utilization of water. About four billion world's population stays under high water scarcity for nearly a month per year (Mekonnen & Hoekstra, 2016). Water is polluted due to various anthropogenic activities like the directly mixing of domestic and sewage water, agricultural runoff, dumping of municipal wastewater near waterbodies, and industrial effluent. These activities are liable for the aggregation of heavy metals in water (Ahmed et al., 2019) which are also sources of microplastics in freshwater systems (Akdogan & Guven, 2019; Cole et al., 2011; Horton et al., 2017). Studies have shown that microplastics adsorb harmful pollutants such as persistent organic pollutants and heavy metals from the water (Liao & Yang, 2020; Wang et al., 2019b; Yu et al., 2020a) and cause harmful effects on biota (Ashar et al., 2020; Boyero et al., 2020). Therefore, it is necessary to monitor water quality using a rapid and efficient techniques (Chen et al., 2021).

1.1.5 Water quality index (WQI)

The water quality index is a reliable approach that provides a comprehensive overview of the quality of waterbodies (Ravikumar et al., 2013) by providing a single digit score from a complex water analysis data which is easy to understand even by the layman (Uddin et al., 2021). WQI has been widely applied to assess water quality based on the water quality criteria of that place. In general, for WQI calculation four steps are used; these are i) parameters selection ii) determination of sub-indices for each parameter iii) determination of weightage value for each parameter, and iv) computation of single WQI value by aggregating sub-indices and weightage value (Uddin et al., 2021). Then the calculated WQI values are classified as “excellent”, “good”, “poor”, “very poor” and “unsuitable” based on the WQI score. Similarly, water quality can also be measured based on the heavy metal pollution index first used by Mohan et al. (1996) to find the concentration of heavy metals present in waterbodies by arriving at a final single value.

1.2 Rationale of the study

Plastic pollution has occupied every sphere of this planet and smaller plastic so called “microplastics” has gain attention from the scientist. Microplastics are resistant to degradation which can adsorb other harmful contaminants and exert potential effects on biota. Even though lakes act as temporary or long term sink for microplastics endangering biological diversity, food security and human health, very few lakes have been investigated worldwide for microplastic pollution. In Nepal, till date, no data on microplastic is available for freshwater lakes so the pollution load by microplastic in freshwater lake system is unknown. Therefore, this is the first study to examine the occurrence and distribution of microplastic in the second largest lake of Nepal, the Phewa Lake. This research will add data on freshwater lakes of the world. Further, this research will bridge the knowledge gap by providing baseline data of the abundance, characteristics, and spatial and temporal variation of microplastic in the freshwater lake system of Nepal. This study will also try to find the relationship between microplastic pollution with water quality.

1.3 Research questions

The research questions are as follows.

What is the condition of microplastic pollution in Phewa Lake?

How will the concentration of microplastic differ at different locations and seasons within the surface water and its sediments of Phewa Lake?

What is the status of water quality and is there any relationship between microplastic pollution and water quality?

1.4 Objectives of the study

The general objective of the study is to investigate microplastic pollution in Phewa Lake, Nepal and the following are the specific objectives of the study

1. To find the abundance and composition of microplastic in surface water and its shoreline sediment of Phewa Lake.
2. To measure the spatial and temporal variation of microplastic concentration in lake surface water and shoreline sediments.
3. To assess the water quality of Phewa Lake and find the relationship between microplastic pollution and water quality.

1.5 Hypothesis

The following four hypothesis are used in this study

Hypothesis 1: The concentration levels of microplastic at eight different areas of surface water are significantly different.

Hypothesis 2: The concentration levels of microplastic at five different areas of shoreline sediments are significantly different.

Hypothesis 3: The concentration levels of microplastic in three different seasons of surface water are significantly different.

Hypothesis 4: The concentration levels of microplastic in three different seasons of shoreline sediments are significantly different.

1.6 Scope of the study

Pollution has become a great threat to every society in the world. The world is dependent on plastic in every aspect of their daily life. The water of Phewa Lake also has been polluted by microplastic. This study will be the breakthrough in the microplastic pollution status of Nepal, which will elaborate on the descriptive structure of microplastic contamination in Lake Phewa. This study will play a significant role to open up discussion among policymakers, local stakeholders along with researchers on the difficulties posed by plastic debris in Phewa Lake to retain the ecological health and economic benefits. Further, this work will help the local authority to plan conservation measures to minimize the plastic load in Phewa Lake and initiate research to study the impact of microplastic on biota.

CHAPTER 2 : REVIEW OF LITERATURE

2.1 Definition

In the year 2004, the term "microplastic" was used to denote plastic fragments that are ~20 µm in diameter (Thompson et al., 2004) but the size limit was not well defined. In 2008, a group of scientific community proposed to define microplastic as "plastic particles smaller than 5 mm" (Arthur et al., 2009) without denoting the lower limit in size. Maximum size limit of 5 mm was chosen to address the viable ecological impact apart from physical obstruction of gastrointestinal tract (Arthur et al., 2009). But in recent studies, the lower size limit of 1 µm has been used by authors to define microplastic. **Table 2.1** shows a list of researcher's providing definition of microplastic. Yet there is no universally approved definition of microplastic, particles size less than 5 mm has been widely adopted by many researchers.

Table 2.1 Reference studies defining the term microplastics

SN	Size of microplastics		References
	Lower limit	Upper limit	
1	20 µm	-	(Thompson et al., 2004)
2	1.6	-	(Ng & Obbard, 2006)
3	-	5 mm	(Moore, 2008)
4	-	5 mm	(Arthur et al., 2009)
5	-	1 mm	(Costa et al., 2010)
6	-	1 mm	(Browne et al., 2011)
7	-	1 m	(Claessens et al., 2011)
8	-	5 mm	(Faure et al., 2012)
9	1 µm	5 mm	(Duis & Coors, 2016)
10	1 µm	1000 µm	(Hartmann et al., 2019)
11	1 µm	5 mm	(Frias & Nash, 2019)
12	-	5000 µm	(GESAMP, 2015)
13	-	5 mm	(Frias & Nash, 2019)
14	-	5 mm	(Hengstmann et al., 2021)
15	-	5 mm	(Yang et al., 2022)

2.2 Types of microplastics

There are two main categories of microplastics based on origin: primary microplastic and secondary microplastic

i) Primary microplastics are microplastics that are purposely manufactured and are further processed for plastic items production or are added to other products to increase their efficiency (Arthur et al., 2009). For example: pre-production plastic pellets may accidentally be released into the natural environment during manufacturing and transportation via surface run-off (Doyle et al., 2011; Holmes et al., 2012). Microplastics used in personal and beauty care products like exfoliating facial cleanser body scrubbers, hand wash and toothpaste (Auta et al., 2017; Boucher & Friot, 2017; Fendall & Sewell, 2009) have gain public concern in recent years. In 2009, Fendall and Sewell studied 4 facial cleansers in New Zealand and found that the small size of PE microplastics could enter the waterways through wastewater treatment plants. The authors mentioned that small size of microplastics (<100 µm) could be readily ingested by planktonic organisms which should be taken into account (Fendall & Sewell, 2009). Similarly Napper et al. (2020) approximated that a single application of facial scrubber could generated between 4594 to 94,500 microbeads. Likewise, in air blasting, microbeads have been used as an abrasive agent (Boucher & Friot, 2017; Browne et al., 2007).

ii) Secondary microplastics are the result of the fragmentation of larger plastic particles (Thompson et al., 2004) like packaging materials, fishing ropes and gear, soft drink bottles, and daily used plastic household goods (Auta et al., 2017; Browne et al., 2011). The breakdown of plastic particles involve UV degradation (Andrady, 2011), biological disintegration (Eubeler et al., 2010), and mechanical disintegration by frictional force (Duwez & Nysten, 2001) finally resulting into small microplastic fragments (Auta et al., 2017; Browne et al., 2007).

2.3 Microplastic in freshwater systems

In recent years scientists have begun to study microplastic in freshwater. According to Wagner and Lambert (2018), less than 4% of microplastic related studies are reportedly associated with freshwater. In the review paper by Eerkes-Medrano et al. (2015), the author summarizes the recent studies that were carried on microplastic in freshwater and its sediments.

Studies of freshwater microplastics have varied greatly in their magnitude and research area with little standardization in sampling methods and laboratory analysis. Most findings are related to sources, concentration, types, chemical composition, color, and/or size. For comparable and reliable data from different researches, standardized and robust methods for quantification and identification of microplastic should be first developed and verified (Li et al., 2018).

2.3.1 Microplastic in lake surface water and sediments

In 2012, Lake Geneva was studied to investigate the plastic and microplastic pollution load in surface water and sediments by Faure et al. (2012). Two methods were applied for the sampling. In the first method fixed surface area was scraped off to collect plastic fragments from beaches. The second method includes coarse plastic fragments collected from the beaches and also fishermen collected birds and fishes from different location of Lake Geneva. Similarly, microplastic collection and analysis were done based on protocol developed by the 5-Gyre Institute and the Algalita Foundation. Due to adverse sampling condition, only one sample was analyzed and found density of microplastic count as 48,146 parts/km^2. Likewise, very less volume of sand was collected for the analysis which showed 1 to 7 plastic fragments in each sample taken. As no detail sampling technique and fewer samples was taken, the data may have many loop holes. No traces of plastic pieces were found in fish and bird analyzed.

Eriksen et al. (2013) were the first to report on open-water survey of plastic pollution within the Laurentian Great Lakes system of the United States with the collaboration of 5 Gyres Institute and SUNY Fredonia. 21 Samples were collected from three (Lake Superior, Lake Erie and Lake Huron) of the five Great Lakes covering ~1300 km expedition using a 333 µm mesh manta trawl. Using visual analysis followed by scanning electron microscopy (SEM) / energy dispersive x-ray spectroscopy system (EDS) analysis, the authors reported the average abundance of 43,157 plastic particles / km^2 from the studied area. From their observation the sample from Lake Eric were consistently the most concentrated in comparison to Lake Superior and Lake Huron. Plastic particles were categorized into five types (fragment, film, foam, pellet and line) and three size classes where pellets and fragments outnumbered other particle types. 81% of the total particle count lies in 0.355 – 0.999 mm size class. Multi-colored spheres suspected to be microbeads was also found which are believed to be originated from consumer product application. Coal and fly ash particles were also abundant which was confirmed using SEM/EDS analysis that may have originated from nearby urban effluent and coal burning power plants. This study opened doors to freshwater microplastic analysis and to mitigate sources of marine plastic pollution.

Shoreline microplastic and pelagic microplastic was conducted by Free et al. (2014) in Lake Hovsgol, Mongolia in large, remote, mountain lake which is the 19^{th} largest lake in the world. Survey was done using 333 μm manta trawl using visual identification. Finding showed an average abundant of 20,264 particles/km^2.The most abundant type of microplastic were fragments, films and line/fibers. The author pointed out that due to lack of modern waste management system remote areas may also be heavily polluted with low density consumer plastic resulting in microplastic.

Faure et al. (2015) studied six largest lakes of Switzerland to identify the abundance, types and composition of plastic and found average of 1300 plastics per m^2 with the range of 20- 7200 particles per m^2 in beach sediments of Swiss lakes. Similarly, for surface water microplastic average was 91,000 particles per km^2. In the year 2012, (Faure et al.) studied Lake Geneva but it lacks proper information so the data obtained could not be compared in this study which also included Lake Geneva. Fragments, foam, films were the types of microplastic found in the study conducted on rivers and fauna. FTIR-ATR spectroscopy revealed that of the tested particles, only 2% were not plastic indicating reliability if visual sorting is done with care.

In 2016, Taihu lake which is in the most developed area of China was studied for microplastic in water, sediments and an organism by Su et al. (2016). Researchers collected samples from 11 locations within Taihu Lake using nylon plankton net to sample floating microplastic and bulk sample for surface water. Peterson sample was used for sediment and the clams collected by using bottom fauna trawl. Microplastic verification was done by advanced techniques using scanning electron microplastic/ energy dispersive spectroscopy (SEM/EDS) and micro-fourier transformed infrared spectroscopy (μ-FTIR) following the method that was described by Yang et al. (2015).

Their finding showed $0.01x10^6$ -$6.8x10^6$ items per km^2 in plankton net sample for floating microplastic, 3.4 – 25.8 items per liter in surface water as the abundance of microplastic. Similarly, in sediments, the abundance of microplastic was 11.0-234.6 items per kg dry weight and in Asian clams (*Corbicula fluminea*) it was 0.2-12.5 items per gram wet weight. The dominated morphotype was fiber, 100-1000 μm as size and cellophane as the common polymer type. The output of this study indicated that there is microplastic pollution in water, sediments and organism of Taihu Lake. As stated by Barboza and Gimenez (2015); Cole et al. (2011) level of microplastic are correlated with anthropogenic activities. This is true as many researchers found increase level of microplastic in connection to population density, drainage from domestic and industrial areas and wastewater treatment plants with regard to Asian clams, it was found in all the sampling sites of the study area. When the abundance of microplastic was low in lake sediments the result showed higher concentration factor in the clams. Su et al. (2016) also pointed that clams could accumulate microplastic in large quantity making them good indicators of microplastic pollution in freshwater and estuarine system. But further large-scale research is required to verify this.

The possibility put forward by Su et al. (2016) regarding large-scale research to verify clams as bioindicator of microplastic pollution was initiated by Su et al. (2018). With the objective to conduct a large scale survey from 21 sites in the middle lower Yangtze River Basin for microplastic pollution in water, sediments and Asian clams. This objective was similar like in their previous study in Taihu Lake, China. Analysis was also done to draw the relationship between microplastic in the Asian clams to those in sediments and water. Sample collection and isolation of microplastic from Asian clams were done in a similar fashion as their previous work (Su et al., 2016). The result showed concentration of microplastic as 0.5 – 3.1 items per liter in water 15-160 items per kg in sediments. Likewise, in Asian clams, it ranges from 0.3 – 4.9 items per gram (or 0.4 – 5.0 items per individual). The abundance of microplastic in clams significantly depends on microplastic pollution in water and sediments. The average value of microplastic in the clams is approximately equal to the microplastic in the sediments than in water. The fiber was the most dominant type of microplastic in water, sediments, and clams as it was also in case of Taihu Lake, China. Su et al. (2018) highlighted why Asian clam can be a good indicator. First, in freshwater environment internal exposure level of microplastic is represented by Asian clam which is also a benthic organism. Second, as it is an invasive species it is extensively distributed, easy for collection and cost effective for handling. Third, Asian clam can act as a valuable monitoring tool to monitor levels of microplastic where other species in freshwater may be insufficient. Lastly, Asian clam can act as bridge to link microplastic pollution to potential risk for human because soft tissue of Asian clam is eaten along with the digestive tract. The entire above stated sentence makes a strong point why Asian clam can serve as bio indicator of microplastic pollution in freshwater environment.

Spatial distribution of microplastic particles with wind induced changes of particles abundance was carried out by Fischer et al. (2016) in Lake Bolsena and Lake Chiusi, central Italy. Six Manta trawls and 36 sediments samples was analyzed. Mean microplastic in Lake Bolsena accounts for 112 particles per kg dry weight and Lake Chiusi 234 particles per kg dry weight where the dominating concentration occurs in the category < 0.5 mm size. Similarly, 2.68 to 3.36 particles per m^3 (Lake Chiusi) and 0.82 to 4.42 particles per m^3 (Lake Bolsena) was the abundance of microplastic in surface water. Researcher found that on Lake Bolsena there was distinct increase in abundance of fragments but not fiber after the heavy wind just before the day of sampling. Fischer et al. (2016) pointed out that a combination of bulk sampling and volume reduced net-based sampling if used will be helpful to evaluate the missing fraction < 0.3 mm which is possible by using appropriate volume of bulk sampling.

Another work published in 2017 studied microplastic in surface water of 20 urban lakes of Wuhan city, China with regards to the abundance, distribution and morphological characteristics. Teflon pump and 50 µm stainless steel sieve was the method used for collection of microplastics where researchers Wang et al. (2017) found concentration of microplastic in a range from 1660.0 ± 639.1 to 8925 ± 1591 n/m^3 from the studied area. 50.4% to 86.9% were colored particles of the total microplastic in number and more than 80% has a size < 2 mm out of the six class. FTIR analysis of forty-four microplastic sample reveal polyethylene terephthalate (PET) and polypropylene (PP) as the major polymer type of microplastic analyzed.

To fulfil the literature gap around sources and distribution of microplastic in Canadian freshwater ecosystem Anderson et al. (2017) examined Lake Winnipeg using

333 µm manta trawl and found microplastic density as 748,027 particles/km^2 at the outflow of lake Winnipeg in 2014 which is the highest and 52,508 particles/km^2 in the Winnipeg river outflow the lowest. SEM-EDS analysis cleared that 23% of the particles visually identified as plastic were found to be either silicate, iron oxide or paint flakes. Foam was least common type whereas fibers and films identified as the most common type of microplastic. Microbeads which drew media's attention found in surface water in the Great Lakes (Eriksen et al., 2013) was not a significant source of Winnipeg Lake but synthetic textile or atmospheric fallout as a source of microplastic contamination as stated by Anderson et al. (2017).

Qinghai Lake which is the China's largest inland lake was studied for the first time with a board objective to find the microplastic pollution characteristics, distribution, patterns, source and the fate of microplastic by Xiong et al. (2018). Result indicated 0.05×10^5 to 7.58×10^5 items km^{-2} in lake surface water and 0.03×10^5 to 0.31×10^5 items km^{-2} in the inflowing rivers. Similarly, in the lakeshore sediments the microplastic range was 50 to 1292 items $m^{-2.}$ Likewise, in the fish sample it ranged from 2 to 15 items per individual. Lake Hovsgol, Mongolia (Free et al., 2014) had lower microplastic abundance than Lake Qinghai which is also a remote lake. Sources of microplastic is linked with tourism which also proved by Free et al. (2014) and Imhof et al. (2012). Finally, the researcher pointed out that risk assessment of microplastic pollution is needed to protect the lake area.

For the first time west Dongting Lake and south Dongting Lake was investigated for microplastic pollution levels in sediment and surface water emphasizing the distribution, sources and composition of the microplastic by Jiang et al. (2018).The sampling site consists of 14 lakeshore sites each for sediments and surface water and 22 lake center site. Large flow sampler was used to collect the surface water and filtered through 45 µm stainless sieve. Their finding showed microplastic abundance range from 616.67 to 2216.67 items per m^3 in west Dongting Lake and 716.67 to 2316.67 items per m^3 in south Dongting lakeshore surface water. Quadrat method was used to scope the top 0.2 cm sediments and result showed 320 to 480 items per m^3 and 200-1150 items per m^3 in lakeshore sediment of west Dongting Lake and south Dongting Lake respectively. Both the sediment and lakeshore surface water sample showed fiber as the common microplastic found and the main source of fiber was from textile. The dominant color was transparent and the size < 0.5 mm was more prominent. The researchers suggested that the main sources of microplastic in the study area was from rivers that carried industrial effluent and domestic wastewater and concluded that the west and south Dongting Lake has moderate level of microplastic pollution. Further a complete ecological risk assessment model should be developed and research carried out to examine the relationship between distribution of microplastic and effect of hydrodynamic conditions.

A recent study by Yin et al. (2019) has a similar objective as Wang et al. (2017) investigating the abundance, distribution and morphological characteristics but using the SEM and latest technology micro-Raman spectroscopy in the study area of 8 urban lakes in Changsha, China. Their findings noted microplastic concentrations ranged from 2425.0 ± 247.5 to 7050 ± 1060.66 items /m^3 in the surface water of Changsha Lake. 89.5% of microplastic size was found to be smaller than 2000 µm and their finding is similar to published research on Wuhan Urban Lake, China (Wang et al., 2017). Transparent particles were the most dominant when microplastic was categorized by color. Due to the high cost of micro-Raman spectroscopy, 80 randomly selected particles were examined which revealed six kinds of plastic. Polypropylene (PP) accounted for the highest proportion (33.75%) followed by polyethylene (PE), polystyrene (PS) and polyethylene terephthalate (PET).

In 2020, Rawal Lake in the capital city of Pakistan was studied for microplastic presence and concentration in the sediments and surface water by Irfan et al. (2020b). Bulk sampling for surface water and quadrat method for sediments was employed for sampling. Polymer identification was done by FTIR analysis. Their finding showed 0.142 items per 0.1 L as average microplastic abundance for water and 1.04 items per 0.01 kg for sediments. Fibers and fragments were the dominant microplastic and were found to be secondary in origin. The research result showed the significance difference of microplastic concentration of sediments and water. The correlation coefficient of microplastic abundance between water and sediment was found to be moderately negative. The authors suggested source control as the effective way to control microplastic as it is difficult to minimize the level of microplastic once it is discharge to the environment. Continuous monitoring program would be effective to get detail insight of microplastic contamination along with the study on seasonal fluctuation.

Vembanad Lake which is listed in Ramsar site of India was studied for the first time by Sruthy and Ramasamy (2017) to verify the presence and distribution of microplastic in the sediments. Micro Raman spectroscopy was used to detect the polymer type which showed low density polyethylene as the dominant type of polymer found in the sediment samples. Researchers collected sediment samples from 10 sites using Van Veen grab (25 cm^2) and recorded range of 96-496 items per m^2 as abundance of microplastic and the abundance of microplastic significantly varies among the 10 sites. Visual observation with the help of a compound microscope was used for categorizing microplastic on the basis of morphology into fragments, films, foam, fiber/lines and pellets. Study showed no microbeads in Vembanad Lake. The origin of microplastic was secondary as a result of breakdown of larger plastics. Further, this study pointed out those lake sediments is acting as a sink for this microplastic. The authors concluded that well managed effort in monitoring and improving waste management program considering the 3 “R” principle will be effective to bring down the load of microplastic in the lake.

Zhang et al. (2016) carried research on lakeshore sediments from remote lakes in Tibet plateau which is also known as the world’s third pole. Four remote lakes in the Tibet plateau was surveyed with the objective to find the abundance, sources and distribution of microplastics and to contrast the result with data from other developed areas worldwide. Quadrat of size 20 cm x 20 cm was used for sampling the top 2 cm of the sediments. Out of seven sampling sites, microplastic was found in six sampling sites. 8±14 to 563±1219 item per m^2 was the abundance range. There is high degree of heterogeneity in abundance of microplastic in different sites. The researchers pointed out that riverine input as the main source of microplastic and due to the perennial windy weather, atmospheric transport of microplastic was also likely in the study area. Raman spectroscopy revealed polyethylene (PE) and polypropylene (PP) as the dominant type of microplastics. Similarly, surface texture identification by scanning electron microscope (SEM) indicates linear fractures, mechanical pits, and groove occupying the largest proportion indicating mechanical erosion responsible for surface texture. For comparing data from other region Zhang et al. (2016) suggested using items per m^2 as a better unit to express microplastic abundance as sediment weight is affected by its bulk density as well as the sampling depth. The comparison of this study with other research shows that there is variation of abundance of microplastics in different locations worldwide as a result of factors like hydrodynamic condition, source loading and wind direction. Lack of waste management strategy may be the factor of microplastic pollution for inland waters in remote areas also.

Small or urban lake was not studied for the distribution of macro or micro plastics. So Vaughan et al. (2017) investigated the microplastic load in the sediments of Edgbaston Pool which is a shallow eutrophic lake in UK. Using HTH gravity corer sediment samples were collected at 11 sites around the lake perimeter. 2-20 debris items were collected from the sampling area. Microplastic was found at the range of 25-30 particles per hundred grams. Binocular microscope (40 X) discloses plastic fibers and films found in the surface sediments as the most common microplastics. There was no significant relationship between depth and microplastic. Compared to the limited number of studies on freshwater sediments, the concentration of the microplastic is relatively low. Apart from that due to the high load of organic matter and greater discoloration, extraction of microplastic may be difficult and particle concentration may be underestimated.

Liu et al. (2019b) combined field surveys with laboratory analysis to address the poorly quantified levels of microplastic pollution in lakes of China. Sediment sample collected from 10 sites of Poyang Lake indicated 1134 items per kg dry weight as the average abundance of microplastic with a range of 11 to 3153 items per kg dry weight. Like many studies, Liu et al. (2019b) observed that microplastic abundance is related to the accessibility of densely populated areas. Temporal distribution of microplastic in sediments showed higher abundance in December than in April or July. The most abundant fraction of microplastic in sediment was of size < 1 mm which exceeded 50%. In Poyang Lake, fragments were the most abundant types of microplastic indicating that the source is secondary in origin. The surface morphology and oxidative and mechanical weathering texture of microplastic was done with the help of scanning electron microscopy (SEM) connected with an energy dispersive x-ray (EDX). Result showed rough, cracked, porous and badly damaged surface suggesting that microplastic was formed by the breakdown of plastic which was used in daily life. As some site of Poyang Lake showed elevated levels of microplastics, regular monitoring and timely steps should be taken to preserve the ecological and economic importance of the lake. Lastly, Liu et al. (2019b) pointed that further studies are needed to find the effects of microplastic pollution on heavy metals.

In 2019, Poyang Lake, China was studied to know the current status of microplastic in water, sediment and fish by Yuan et al. (2019). 21 sampling site was distributed in three different geographical regions. Surface water sample was collected by steel sample, filtered through 50 µm stainless- steel sieve. Van Veen grab was used to collect 500 g of sediments twice and 11 fish sample collected from market which sold aquatic product in Duchang country. Their findings showed microplastics abundance for surface water as 5-34 items/L, sediment sample as 54-506 items/kg and for wild fish (*Carassius auratus*) as 0 to 18 items per individual. The researchers pointed out that sewage discharge and fishing boats which are more than ten thousand in number is the main cause behind high load of microplastic pollution. The relationship between microplastic abundance in sediment sample and surface water sample was not significant. Micro Raman spectroscopy revealed polypropylene, polyethylene, nylon and polyvinyl chloride as polymer types and fiber as the dominant microplastic found in water, sediment and fish. Further research should be carried out to monitor the impact of consuming fisheries products on human.

Red Hills Lake of Chennai city, Tamil Nadu, India was studied by Gopinath et al. (2020) to find the microplastic pollution status as this lake is the source of drinking water to its vicinity. 32 sediment samples were collected by Van Veen grab whereas for 6 water samples, plankton net with 120 µm mesh size was used. In water sample 5.9 particles /L was the mean concentration of microplastic and in sediment 27 particles /kg. FTIR spectroscopy was used for polymer identification which revealed PE and PP as the main polymer type. Energy dispersive x-ray used for metal presence found Al, Fe, Si and Ca absorbed in microplastics. Water from this lake is supplied for drinking water and the study showed the presence of microplastics. Therefore, further research should be conducted to check if the water treatment facility is capable of removing microplastics before supply.

In 2019, Lake Ulansuhai of the yellow river basin, China was studied to determine the concentration of microplastic in lake sediment by Qin et al. (2020). For the extraction of microplastic 2 step density separation processes were applied. First by saturated NaCl and next by $ZnCl_2$ in order to separate high- density microplastic from sediment. The variation in the data of microplastic was found in sediment with a range of 24 ± 7 to 14±3 n/kg. The researchers pointed out that as this lake is the discharge point for municipal wastewater, industrial effluent, and runoff from agricultural land, the relationship between microplastic and nutrient load should be studied. PE, PET, PP, and PVC were the type of polymers detected by FTIR. The authors concluded that a detail study of microplastic pollution and its fate is needed for overall knowledge about the contamination and its control.

With a broad objective to spot the dissemination patterns of microplastic and identify the hotspot area in near shore, tributary and beach sediments, the Canadian lake were studied by Ballent et al. (2016). Total 50 sampling sites data was collected by applying trap, core and grab sampling techniques. According to the authors, microplastic was found in all sediment samples with a range of 20 to 27,830 / kg. On average microplastic abundance was highest in near shore sediments (980 / kg) and least in beach sediments (140 / kg). Similarly in tributary sediment the average abundance was recorded as 610 / kg. Fragments and fibers with a size < 2 mm were the most common microplastic types. Since very low percentage (1.4%) particles were analyzed through Raman analysis, plastic to non-plastic ratio was not adjusted for the whole data. The researcher's emphasis that future studies should be focused to find the contribution of microplastic by storm water, combined sewer outfalls and wastewater treatment plant to sediments which are near this location. Also find a clear picture of the level of microplastic in sediments with distance from the place of outfall.

Quantity and morphology of microplastic in 9 locations of Lake Mead national recreation area, USA was carried out by Baldwin et al. (2020). The researchers used a hypothesis to test whether with increase in time, the deposition of microplastics also increases. The result showed that there was no significant concentration trend between depth and time but the deepest sediment sample showed the highest microplastic concentration for which the reason is not clear. As the sample was only taken once at each site and small sample size of fish and shellfish, the result is based on limited sample size. Microplastics were present in all studied area like sediments, water and aquatic organism and the concentration was found higher in area with anthropogenic activities.

Uurasjärvi et al. (2020) sampled Lake Kallavesi for surface water by adopting 2 sampling methods and found a total of 495 particles by visual detection. µFTIR verify 34% as microplastic out of which 64% as synthetic fibers and fragments as 36%.It was found that with decreasing filter pore size the microplastic per m^3 increased. PE, PP and PET were the common polymers detected by µ-FTIR. Extensive knowledge of microplastic abundance can be achieved if only spatial and temporal patterns can be studied.

Apart from spatial and temporal distribution of microplastic in Antuã River, Portugal, the degradation of microplastic by UV-B radiation was also studied by Rodrigues et al. (2018). Sampling was done in two different periods –March and October with a total of 12 samples, 6 for sediments and 6 for water sample. 0.055mm mesh net was fitted in a motor water pump for sampling water and Van Veen grab for sediment sampling. According to the authors, the microplastic abundance ranged from 5 to 51.7 mg m^{-3} or 58-1265 items m^{-3} for water sample. The abundance of microplastic was highest in October than in March in the water sample. There was a decreasing trend of microplastic from upstream to downstream in October but no clear result was obtained for March. Similarly, the microplastic abundance in sediment ranged from 2.6-71.4 mg/ kg or 18 to 629 items/ kg. The opposite trend in abundance of microplastic was found in sediment seasonally than in water i.e. highest abundance in March and lowest microplastic in October. ATR-FTIR analysis was conducted for 43 suspected particles out of which 79% was identified as synthetic polymers. More than 50% of the identified microplastic comprises of PE and PP. It was found that both seasons had similar degree of weathering of microplastic and it was based upon the degree of yellowing or darkening. According to the authors, the microplastic abundance of Antutã

River was found higher than rivers and lakes of America, Asia and Europe. Finally, the researchers concluded that rivers are the possible transport system of microplastic.

In a review article by Karim et al. (2020), a brief summary on microplastic and its characteristics, its origin, global events and its effects, remediation and eco toxicological studies are highlighted. The authors have focused on the current situation of microplastic pollution in Bangladesh and future guidance. A study carried out by Environment and Social Development Organization (ESDO, 2016) in three main urban centers of Bangladesh found that sixty most frequent used cleaning and beauty products contain microbeads. Around 800 billion microbeads are released every month into nearby water bodies and land by three urban centers of Dhaka, Chittagong,. and Sylhet. Lack of data regarding microplastic pollution and its impact is missing in Bangladesh. So research in this field is the urgent need which can tackle the actual warning that may be created by the microplastic pollution to the whole ecosystem. Unless proper step is not taken by people and government to cut off and/or minimize microbeads containing products and stop the habit of dumping plastic waste to nearby water bodies, it is difficult to handle the emerging microplastic pollution.

Two reviews on microplastic in sediments have acknowledged that without standardization of the sampling technique it is difficult to assess the microplastic load in various water bodies. Van Cauwenberghe et al. (2015) emphasis that if the researchers report the full detail of the sampling procedure, differences between sampling technique can be overcome so data can be compared between different studies. Hanvey et al. (2017) recommend for planned QA/QC procedure and inter-laboratory comparison to minimize the reported variation in the measurement. Further analytical technique should be adopted in microplastic detection as visual counting solely is liable to human mistake (Hanvey et al., 2017). According to Van Cauwenberghe et al. (2015) for potential effects of microplastic on organisms, the experiment should be conducted in natural environment to get the real facts about the effects instead of lab based where organism are exposed to high level of microplastic concentration.

2.4 Microplastic studies in Nepal

To date, only three works has been reported on microplastic pollution in Nepal. **Table 2.2** summarizes the research work conducted on microplastics from Nepal. In 2020, Yukioka, Tanaka, Nebetani, Suzuki, Ushijima and Fujii studied surface road dust of Kathmandu, Nepal to quantify microplastic. Their study found average microplastic concentration of 12.5±10.1 particles per m^2 in road dust mainly from containers/packaging materials which may be linked to crowded commercial areas. They emphasize that small size microplastics (< 100 µm) should also be incorporated in the study of road dust as substantial amount of microplastic is contributed by clothing fibers and tires of vehicles which are < 100 µm which is not considered in this study (Yukioka et al., 2020).

Napper et al. (2020) explored the highest mountain of the earth, Everest to find out the human impact of microplastic in the remote area facing extreme environmental condition. They collected snow and water samples from the stream along the trail of Mount Everest and found average abundance as 30±11 microplastic per liter in the snow sample. On the other hand, a low concentration of microplastic was observed in stream, just 1±0.3 microplastic/L. Polyester fibres accounted for the highest percentage followed by acrylic, nylon and polypropylene which may have originated from the trekkers and climbers clothing and equipment used (Napper et al., 2020).

Another research published in 2021 looked at microplastic pollution in a remote, trans boundary alpine river. Yang et al. (2021a) samples surface water and sediments of five Koshi River's tributaries and concluded atmospheric transmission and deposition as a key source for microplastic pollution. A mean microplastic abundance of 202±100 items per m^3 and 58±27 items per kg dry weight was found in the surface water and sediments samples of Koshi River respectively. Fibers were the dominant type of microplastic accounting about 98% and the observed polymer type were PE, PET, Polyamide, PP and PS. The authors concluded that long term monitoring and modelling will help to address ecosystem impact for proper management and lowering microplastic pollution of alpine rivers.

Table 2.2 List of microplastic research carried out in Nepal to date

Sample type	Environmental condition	Identification of microplastics	Range / Average microplastic abundance	Particle size	Polymer type	References
Road dust	Urban	Stereoscopic microscope with digital camera; ATR-FTIR	In Kathmandu 12.5±10.1 pieces/m^2	100 µm to 5 mm	PE, PP, PS, PET, PVS, PAK	(Yukioka et al., 2020)
Surface water and sediments	Remote, trans-boundary river	Stereomicroscope with digital camera; µ - FTIR	Water: 202±100 items/m^3 Sediment: 58±27 items/kg dry weight	< 1 mm	PE, PET, Polyamide, PP, PS	(Yang et al., 2021a)
Snow and stream	World's highest mountain	Leica light microscope; FTIR	Snow: 30±11 microplastic/L Stream: 1±0.3 microplastic/L	36-3800 µm	Polyester, acrylic, nylon, poly propylene	(Napper et al., 2020)

2.5 Analytical technique used for quantifying microplastics in freshwater lakes

For the quantification of microplastics in freshwater, sampling is the first essential step. In general, the two common methods of sampling are

i) Volume reduced sampling where the actual volume of the bulk sample is reduced when collecting the sample.

ii) Bulk sampling is where the entire volume or sample is taken without decreasing the content (Hidalgo-Ruz et al., 2012). In sediment studies, mostly bulk sampling is used.

The overview of analytical method used in freshwater lake for microplastic quantification is shown in Error! Reference source not found. and Error! Reference source not found..

Table 2.3 Summary of sample collection and processing for the detection of microplastic in freshwater lakes.

Study area	Collection tool (with size cutoff in µm)	Extraction/Processing	Identification	References
Lake Hovsgol, Mongolia	Manta trawl (333)	H_2O_2 + Fe(II); density separation (NaCl)	Light microscope	Free et al. (2014)
Six Largest Swiss Lake	Manta trawl (300)	H_2O_2 + Fe(II)	Stereomicroscope; ATR-FTIR	Faure et al. (2015)
Lake Bolsena & Lake Chiusi, Italy	Manta trawl (300)	HCl; density separation (NaCl)	UV microscope; SEM	Fischer et al. (2016)
Taihu Lake, China	Plankton net; (333) 5 L Bulk sample (steel sampler)	H_2O_2	Stereomicroscope; µ-FTIR; SEM/EDS	Su et al. (2016)
Lake Winnipeg, Canada	Manta trawl (333)	H_2O_2 + Fe(II)	Dissecting microscope; SEM	Anderson et al. (2017)
20 Major Lake, Wuhan City, China	20 L Teflon pump & stainless-steel sieve (50)	H_2O_2	Stereomicroscope; FTIR; SEM	Wang et al. (2017)
West Dongting & South Dongting Lake, China	30 L large flow sampler & stainless-steel sieve (45)	H_2O_2 + Fe(II); density separation ($ZnCl_2$)	Stereomicroscope; µ-Raman	Jiang et al. (2018)
Qinghai Lake, China	Trawl net (112)	H_2O_2; density separation (Potassium formate)	Stereomicroscope; Raman	Xiong et al. (2018)

8 Urban Lake, China	40 L Bulk sampler & stainless-steel sieve (45)	H_2O_2 + Fe(II)	Stereomicroscope; µ-Raman; SEM	Yin et al. (2019)
Poyang Lake, China	20 L Bulk sampler (steel sampler) & stainless-steel sieve (50)	H_2O_2	Stereomicroscope; µ-Raman	Yuan et al. (2019)
Lake Kallavesi, Finland	Manta trawl Pump filtration (333)	NaOH + Sodium dodecyl sulfate	Stereomicroscope; µ-FTIR	Uurasjärvi et al. (2020)
Red Hill Lake, India	Plankton net (120)	H_2O_2 + Fe(II); density Separation (NaCl)	Stereomicroscope; ATR-FTIR; SEM	Gopinath et al. (2020)
Ox-bow Lake, Nigeria	50 L Teflon pump & stainless-steel sieve	H_2O_2	Stereomicroscope; µ-FTIR	Oni et al. (2020)
Veeranam Lake, India	Plankton net (20)	H_2O_2	Microscope; ATR-FTIR	Bharath et al. (2021)
Lake Guaiba, Brazil	Zooplankton net (60)	H_2O_2 + Fe(II); density separation (NaI)	Stereomicroscope; µ-FTIR & µ-Raman	Bertoldi et al. (2021)
Subalpine Lake, Italy (Maggiore, Iseo, Garda)	Manta trawl (300)	Manual separation	Stereomicroscope; ATR-FTIR	Sighicelli et al. (2018)
Lake Naivasha, Kenya	Plankton net (150)	H_2O_2 + Fe(II)	Dissecting microscope, ATR-FTIR	Migwi et al. (2020)

Lake Ulansuhai, China	20 L Teflon pump & stainless-steel sieve (48)	H_2O_2	Stereomicroscope; FTIR; SEM/EDS	Wang et al. (2019c)
Dongting & Hong Lake, China	20 L Teflon pump & stainless-steel sieve (50)	H_2O_2	Dissecting microscope; Raman	Wang et al. (2018)
Poyang Lake, China	20 L steel bucket & Neustron net (38)	H_2O_2; density separation (NaCl + NaI)	Multifunctional digital microscope; µ-FTIR; SEM	Jian et al. (2020)
Rawal Lake, Pakistan	2 L bulk sample (glass bottle)	H_2O_2	Light microscope; FTIR	Irfan et al. (2020b)
Lake Mead & Mahava, USA	Microplastic net	H_2O_2 + Fe(II); density separation (Lithium metatungstate)	Stereomicroscope	Baldwin et al. (2020)
Wuliangsuhai Lake, China	20 L bulk sample (stainless steel bucket) & sieve (75)	H_2O_2	Metallographic microscope; ATR-FTIR; SEM	Mao et al. (2020)
Gehu Lake, China	Submersible pump (48)	H_2O_2 + Fe(II); density separation (NaCl)	Stereomicroscope; FTIR	Xu et al. (2021)
Nine lakes, Patagonia, Argentina	Trawl (38)	H_2O_2	Stereomicroscope; Raman	Alfonso et al. (2020)
Süreyyabey Dam Lake, Turkey	Microplastic nets (330; 100)	H_2O_2 + Fe(II); density separation (NaCl)	Dissecting microscope; ATR-FTIR	Tavşanoğlu et al. (2020)
Lake Simcoe, Canada	4 L bulk sample (amber glass bottle); Manta net (335)	Manual	Microscope; FTIR/Raman	Felismino et al. (2021)

Crater Lake, Erzurum, Turkey	10 L bulk sample (glass bottle)	Manual	μ-Raman; SEM	Çomaklı et al. (2020)
Lake Sassolo, Switzerland	5 L bulk sample (glass jar) (63)	H_2O_2	Optical microscope; FTIR	Negrete Velasco et al. (2020)
Renuka Lake, India	3 L UWITEC water sample		Dissecting fluorescent microscope; Stereomicroscope; ATR-FTIR and Raman	Kumar et al. (2021)
Lake Erie and Lake Ontario, Canada	Manta trawl (333)	H_2O_2 + Fe(II)	Dissecting microscope; SEM/ EDS	Mason et al. (2020)
Western Lake Superior, Canada	Manta net (333)	H_2O_2 + Fe(II); NaCl	Microscope; pyr-GC/MS; FTIR	Hendrickson et al. (2018)
Six Lakes, Southern Siberia, Russia	5 L bulk sample (glass jar)		SEM/EDS	Malygina et al. (2021)
Songshan Lake, Dongguan, China	Plankton net (112)	Manual	Digital Microscope; μ-FTIR	Tang et al. (2022)
Kodaikanal Lake, India	Manta net (333)	H_2O_2 + Fe; Density separation NaCl	Stereomicroscope; ATR-FTIR	Laju et al. (2022)

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Table 2.4 Summary of sample collection and processing in freshwater lake sediment for the detection of microplastic.

Location	Sampling	Extraction	Identification	Reference
Vembanad Lake, Kerala, India	Van Veen grab (25 cm^2)	Density Separation: NaCl (1.3 g ml^{-1}); H_2O_2; Whatman GF/A 25 mm	Compound microscope; Micro Raman Spectrometer	Sruthy and Ramasamy (2017)
Remote Lakes, Tibet Plateau, China	Shovel; (20 cm x 20 cm quadrat); depth top 2 cm	Density separation: potassium formate (1.5 g /cm^3); Whatman GF/C 1.2 μm	Stereo Microscope; Raman spectroscopy; SEM	Zhang et al. (2016)
Poyang Lake, China	Shovel; (50 cm x 50 cm quadrat) depth top 2 cm	Density separation: saturated NaCl (1.5 g /cm^3); sieved (2 μm mesh size)	Stereo Microscope; SEM/ EDX	Liu et al. (2019b)
Dongting Lake, China	Stainless shovel 14 sites; (0.2 x 0.2 m quadrat); depth top 0-2 cm	Density separation: zinc chlorine solution (1.5 g /cm^3); H_2O_2; Fe(II); GF/C filter (membrane solution)	Stereomicroscope; Micro-Raman spectroscopy	Jiang et al. (2018)
Taihu Lake, China	Peterson sample; 2 kg pooled	Density separation: saturated NaCl (360 g/L); H_2O_2; filtered (5 μm)	Stereomicroscope; μ-FTIR; SEM/EDS	Su et al. (2016)

Lake Bolsena and Lake Chiusi, Central Italy	(.25 m x .25m quadrat); depth top 3 cm	Density separation: NaCl (1.2 g/cm^3); HCl; Filtered (membrane vacuum filtration, 5-13 µm)	UV-microscope; SEM	Fischer et al. (2016)
Qinghai Lake, China	Stainless steel shovel 20 x 20 cm quadrat); depth 2 cm	Density separation: potassium formulate (1.54 g ml^{-1}); H_2O_2; GF/C filtered	Stereomicroscope; Raman	Xiong et al. (2018)
Rawal Lake, Pakistan	stainless steel trowel (15 cm x 15 cm quadrat); depth top 2 cm	Density separation: saturated NaCl(1.2g/mL); H_2O_2; Whatman GF/F 0.7µmx47mm	Light microscope; FTIR	Irfan et al. (2020b)
UK urban Lake	HTH gravity corer (depth 0.5 m to 1.5 m); top 10 cm	Density separated: water	Binocular microscope	Vaughan et al. (2017)
Lake Geneva, Europe	988 cm^2 quadrat; depth 2 cm	Density separated: water	Stereomicroscope	Faure et al. (2012)
Various lakes, Switzerland	(0.3 m x 0.3 m quadrat); depth 5 cm	Density separation: saturated NaCl, 320gL^{-1}; H_2O_2; Fe(II); sieved 300 µm	Stereomicroscope; ATR-FTIR	Faure et al. (2015)
Poyang Lake, China	Van Veen grab (0.25 m^2); 1000 g	Two step density separation (NaCl,; NaI) H_2O_2; GF/F, 45mm	Visually identified; micro-Raman spectrometer	Yuan et al. (2019)

Red Hill Lakes, Tamil Nadu, India	Van Veen grab;150 g	Density separation; NaCl (d=1.2gm/L); H_2O_2; Fe(II); Whatman GF/A 25mm	Stereomicroscope; ATR-FTIR; SEM/EDX	Gopinath et al. (2020)
Lake Ulansuhai of yellow river basin, China	Van Veen grab; depth 10 cm	Two step density separation: NaCl (1.2 gm/cm^3); $ZnCl_2$(d=1.50 g/cm^3); H_2O_2; Whatman GF/F 47mm	Stereomicroscope; FTIR; SEM/EDX	Qin et al. (2020)
Lake Ontario, Canada	Nearshore sediments: Glew gravity cover; Shipek grab samples; acrylic cylinder	Density separation; sodium polytungstate SPT (d=1.5 gcm^{-3}); sieved 0.053 µm sieve; gravity corer samples: decant and filter: 25 µm filter paper	Stereo microscope); Raman	Ballent et al. (2016)
Lake Mead National recreation Area, USA	Superficial sample: Ponar samples; stainless-steel spoon; gravity cores depth; top~3cm sediments : stainless-steel spoon; sediment cores samples: Gravity corer; depth 33 cm	Potassium metaphosphate solution (5.5 g/L); Density separation: lithium metatungstate (1.6 g/mL); H_2O_2+Fenton's reagent; cellulose acetate filter paper, 47 mm	Stereoscope	Baldwin et al. (2020)
Anchar Lake Northwest Himalaya, India	Van Veen grab; 1 kg	Density separation: NaCl; H_2O_2; 0.45 µm cellulose nitrate filter paper, GF/A 25 mm	Stereomicroscope; ATR-FTIR	Neelavannan et al. (2022)

Hawassa Lake, Euthiopia	Top 3 cm	Density separation: $ZnCl_2$ and NaCl; H_2O_2; 0.45 µm cellulose nitrate membrane filter	Optical zoom microscope; ATR-FTIR	Jeevanandam et al. (2022)
Lake Simcoe	Petite Ponar; metal spoon: depth 1.5 to 33, top 1-2 cm	Density separation: $CaCl_2$	Microscope; FTIR/Raman	Felismino et al. (2021)

2.5.1 Sample collection method

In water sample, volume reduced sampling was applied in 18 of the 35 reviewed freshwater studies mostly using manta trawl or plankton net. 14 studies reported bulk sampling whereas in 3 studies, both the sampling technique was used for sample collection. **Figure 2.1** shows different types of sampling tools used for freshwater studies. To capture the microplastics from the water sample, mesh of various sizes from 20 µm to 335 µm has been used. Mesh size of 330 µm are widely used by researchers but it may fail to retain small size microplastics (Löder & Gerdts, 2015). So it is recommended to use smaller mesh size to get representativeness of the sample.

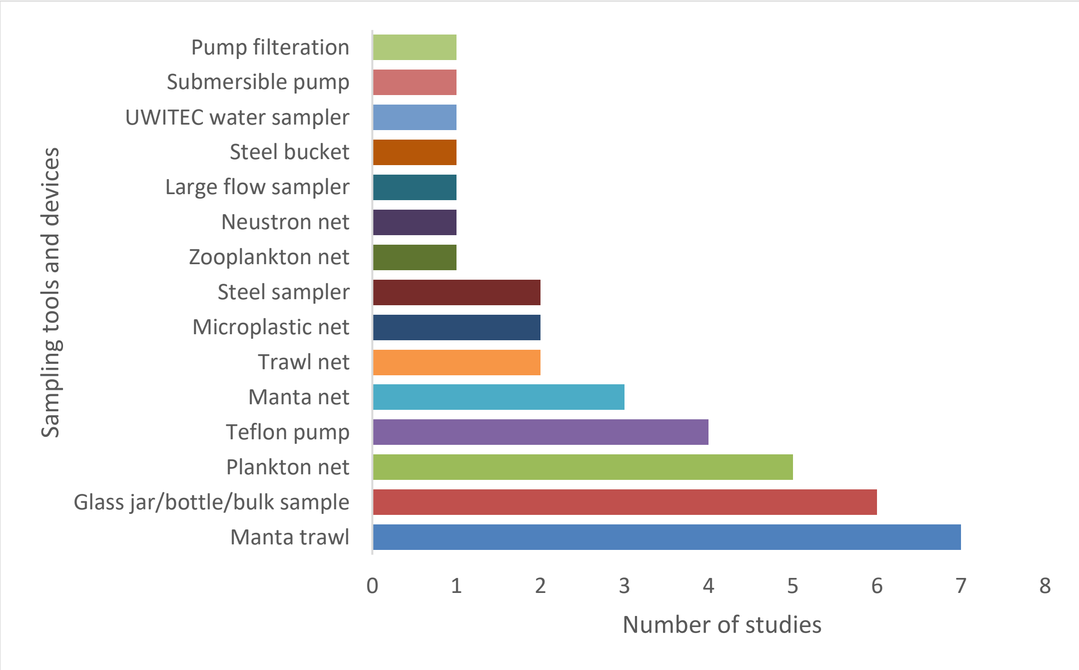

Figure 2.1 Sampling equipment used for collecting water in microplastic studies from freshwater lakes

(Note: The total sampling devices exceed that total number of studies (N=35) because some studies used more than one sampling devices)

Similarly, in sediment samples, sampling equipment were reported in 15 of the 19 reviewed lake sediments papers. Van Veen grab for bottom sediment and hand tool like stainless steel shovel were widely used for shoreline/ littoral sediment sampling. **Figure 2.2** shows the different sampling tools used for freshwater lake sediment studies. The sampling unit is closely linked to the sampling equipment used (Hidalgo-Ruz et al., 2012). Most of the studies use area as sampling unit (25 cm^2 to 998 cm^2) while other sampling unit is weight (1 to 2 kg). Likewise, sampling depth differ between studies. Most studies sample the top 2 cm of the sediment, while some authors take the top 10 cm of the sediment or the sample depth is not mentioned **(**Error! Reference source not found.**).** According to Qiu et al. (2016) the concentration of microplastics is linked to sampling site, depth from where the sample is taken and the distance from human settlement. Moreover, "Guidance on Monitoring of Marine Litter in European Seas" has recommended that the sediment sample to be collected from top 5 cm of the soil with at least five replicates at a distance of five meter apart (Hanke et al., 2013).

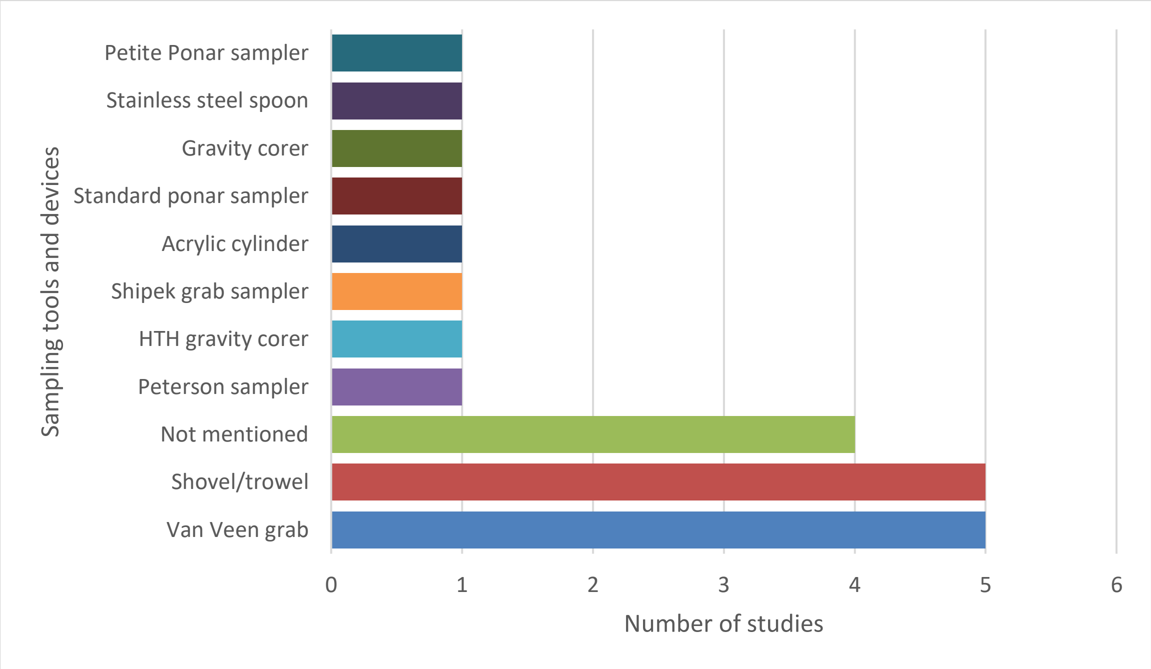

Figure 2.2 Sampling equipment used for collecting microplastics from freshwater lakes sediments.

(Note: The total sampling devices exceed that total number of studies (N=19) because some studies used more than one sampling devices)

Finally, the choice of the sampling techniques relay on researcher's objective of the study, the environmental setting of the study area and the availability of the equipment, and financial resources (Campanale et al., 2020).

2.5.2 Sample preparation/ processing

For the better identification of quantification of the microplastics present in the sample, it should be processed which includes density separation, removal of organic matter and filtration. For the isolation of the microplastic from the matrix (water or sediment), density separation method is commonly used (Sarijan et al., 2021) which was followed in all sediment studies but only 12 of the 35 freshwater studies included density separation method. The difference in density of polymer is utilized to separate microplastics from the samples. Sodium chloride solution is commonly used (both water and sediment samples) by researchers as separating solution due to its low cost, non-toxic nature and readily available (Campanale et al., 2020; Hidalgo-Ruz et al., 2012; Yang et al., 2021b). Other salt solutions used in water and sediment samples are given in **Figure 2.3.**

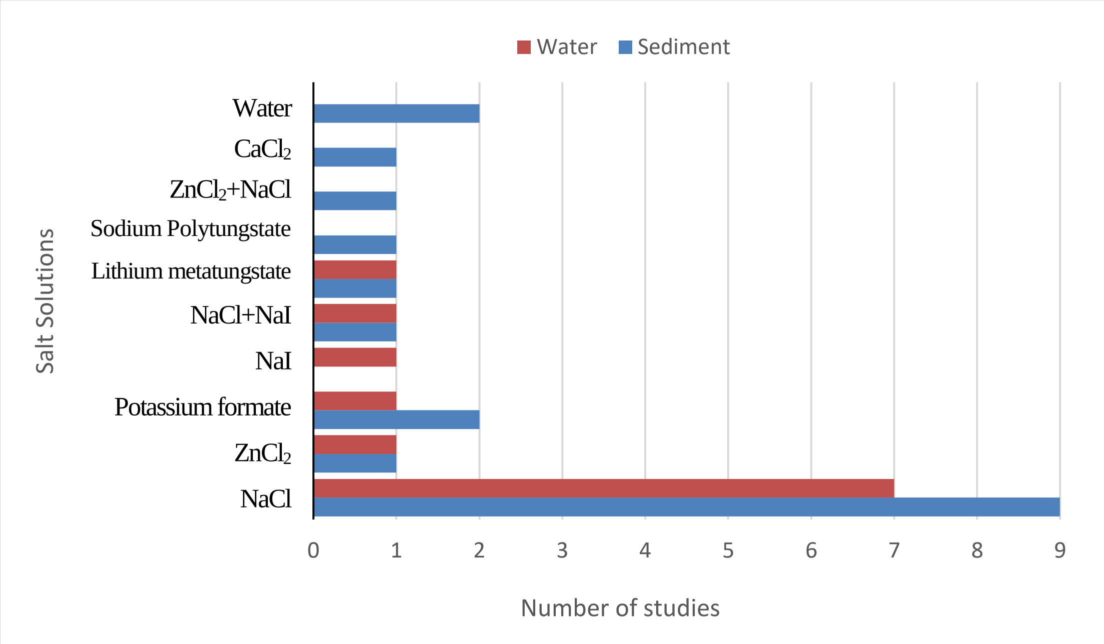

Figure 2.3 Different types of density separating solution used for microplastic studies from freshwater lakes.

As the sample may be loaded with organic matter, chemical or enzymatic digestion need to be performed for organic matter removal. Hydrogen peroxide is used widely as oxidizing agent to remove organic matter. In freshwater, 27 of 35 studies used H_2O_2 and in freshwater lake sediment, H_2O_2 was used by 12 of 19 studies. Acid digestion by HCl was used by Fischer et al. (2016) for both water and sediment sample and alkali digestion (NaOH + Sodium dodecycl sulfate) was used by Uurasjärvi et al. (2020) but none of the researchers used enzymatic degradation. In sediment samples, organic matter removal is a vital step for the quantification of microplastics (Hanvey et al., 2017).

Sample processing is complete after filtration. Sieving and vacuum filtration are commonly applied filtration technique in microplastic analysis. Glass fiber filters are widely used in filtration.

The general steps in microplastic analysis from the reviewed studies include: sieving, digestion, density separation, filtration and identification but the order of steps may vary among studies.

2.5.3 Sample identification

In all 54 reviewed studies of freshwater lakes, visual observation is an obligatory step (Hidalgo-Ruz et al., 2012) mostly under a stereomicroscope. If proper procedure and care is taken during sample observation, visual identification can be a reliable tool up to 500 µm particle size (Renner et al., 2018). Though this method is simple and generally used by researchers (Li et al., 2018), polymer identification cannot be determined (Sarijan et al., 2021). Therefore, the spectroscopic techniques like FTIR and Raman are suggested for polymer identification of microplastics (Hidalgo-Ruz et al., 2012). FTIR is widely used for the polymer identification of microplastics in freshwater (18/35 studies) whereas, in freshwater lake sediment samples, FTIR and Raman are almost equally used methods (7/19, 6/19 respectively). FTIR is a nondestructive based method (Hanvey et al., 2017) where polymer confirmation is acquire by analogizing the acquire spectra with known reference spectra (Mai et al., 2018). In recent years Raman spectroscopy has been used by researchers as this can detect microplastic from 1 to 20 µm in size (Sarijan et al., 2021). The thermoanalytical method like Pyr-GC/MS has only been used by one study of freshwater (Hendrickson et al., 2018). Though this method do not need sample preparation (Elert et al., 2017), it cannot detect particles < 500 µm (Yang et al., 2021b)

Various sampling and processing methods are used for the identification of microplastics from freshwater lakes. Therefore, development of standardized method will help for the uniformity in the study which will be able to give the actual outlook of microplastic pollution globally.

CHAPTER 3 : MATERIALS AND METHODS

3.1 Study area

Pokhara the city of nine lakes is the capital of the Gandaki Province and the most popular tourist destination of Nepal after Kathmandu. It is situated about 200 km west of the capital city Kathmandu. Phewa Lake is the second largest lake of Nepal which lies in Pokhara Metropolitan city. The lake is surrounded by Sarangkot and Kaskikot hills on the northern side. Temple of Barahi is situated in Phewa Lake which is an important place for hindus. On a clear day the mirror image of mount Machhapuchhre and Annapurna range can be reflected on the lake which adds beauty to the lake.

Phewa watershed occupies an area of about 122.53 km^2 at 28°11’39’’ - 28°17’25’’ latitude and 83°47’51’’ - 83°59’17’’ E longitude with elevation ranging from 790 m to 2480 m above sea level at dam outlet and western limit of the catchment respectively (Watson et al., 2019). The water of Phewa Lake is stream fed and the water level of the lake is regulated by the dam. Therefore, the lake is classified as semi-natural freshwater lake (Shrestha & Janauer, 2001). The area covered by Phewa Lake is 5.726 km^2 with 8.6 m as average depth and 23.5 m as maximum depth (Gurung et al., 2010). The water of the lake is used for multiple purpose such as generation of electricity, irrigation, recreation, fishery, and domestic purpose (bathing and washing clothes).

3.1.1 Geology of the area

Pokhara valley is set up in an intermontane fluvial basin by successive deposition of large volume of clastic debris brought from southern glacierised slopes of Annapurna mountain (Yamanaka et al., 1982). The catchment area of the lake consists of weakly-bedded, low to medium grade metamorphic rocks and phyllite schist and gneisis, granites, quartzite and schist (Gautam et al., 2000).

3.1.2 Hydrology of basin

Stream is the source of water for Phewa Lake which feed water in two ways into the lake. Either discharging the water into the lake or discharging the water into the Harpan Khola (stream) and to Phewa Lake. During rainy season, Phirke Khola, Chisapani Khola, Mulabari Khola and Seti directly feed water into the lake. The major inlet to Phewa Lake is Harpan Khola accounting about 70% of the total inflow, Pardi dam located at the southeast end of Phewa Lake is the major outflow where the water is diverted for hydropower generation and irrigation.

3.1.3 Climate of the region

The study area exhibit humid sub-tropical climate. The average minimum temperature is 16.3° C and average maximum temperature is 27.2° C. Likewise, annual precipitation is 3102.1 mm (CBS, 2019).

3.1.4 Land use pattern

The watershed of Phewa Lake constitute the highest portion of forest area (46.93%), agricultural land (39.57%), water body and wet lands (4.95%), built-up area (4.78%), waste land (2.69%). Likewise, bush/shrub and grass land (1.08%) (Regmi et al., 2017).

3.1.5 Biodiversity of the area

Phewa lake basin accounts for rich biodiversity with 104 bird species, 34 mammals, 16 fishes, 14 reptiles and 6 amphibians (IUCN, 1995). Apart from that 39 aquatic macrophytes including 23 hydrophytes and 16 helophytes (Shrestha & Janauer, 2001).

3.2 Sampling locations

There are nine lakes in Pokhara valley. Out of which Phewa Lake, the largest in the lake cluster was chosen for this study. Water and sediment samples were collected for three seasons in the year 2021. February for winter (dry) season, July for rainy (wet) season and October for autumn season. For the equal distribution of the sampling site of the lake's area, 16 sampling locations were selected for water samples and 10 sampling locations for shoreline sediments by global positioning system (GPS) which is given in **Figure 3.1** and **Figure 3.2** for microplastic analysis. Further, for water sample, these locations were divided into eight sections as water inlet (INL-W1), moderately populated area (MPA-W2-W3), densely populated area (DPA-W4-W7), mixing region (MIX-W8), water outlet (OUT-W9), temple area (TEM-W10), lake center area (CPL-W11-W14), and least populated area (LPA-W15-W16).

Similarly, for sediment samples, 10 sampling locations were further divided into five regions. INL-S1 located at the western side of the lake which is the inlet of water to the lake. MPA-S2-S3 lies at the northern side where the population is moderate. DPA-S4-S7 are located in a densely populated eastern side. OUT-S8 represents the outlet of the lake which lies at the southern side and a dam is also located there. Finally, LPA-S9-S10 are located at the least populated southern side of the lake. Likewise, for the water quality analysis, the sampling sites were the same as water samples. Information of the sampling locations is given in the **Table 3.1.**

Table 3.1: Information about sampling locations in Phewa Lake for microplastic analysis

Location	Area Code	Description	Latitude	Longitude
Inlet	INL-W1 INL-S1	Harpan Khola Inlet to Phewa	28.224616	83.9365978
Khapaudi	MPA-W2 MPA-S2	Moderately populated area	28.2276952	83.9389317
Ratmaati Danda	MPA-W3 MPA-S3	Moderately populated area	28.2235502	83.9475607
Bangaladi	DPA-W4 DPA-S4	Densely populated area	28.2203397	83.9570025
Gantavya	DPA-W5 DPA-S5	Densely populated area	28.2168626	83.956998
Hallan chowk	DPA-W6 DPA-S6	Densely populated area	28.2123203	83.955116
Barahi boating area	DPA-W7 DPA-S7	Densely populated area	28.02787	83.95518
Baidam Phirke Khola	MIX-W8	Phirke Khola mixing waste water	28.199668	83.967939
Dam Site	OUT-W9 OUT-S8	Outlet of water (Pardi dam)	28.1964583	83.9688238
Tal Barahi temple	TBA-W10	Temple situated at the center of southeast side	28.2078408	83.9529029
Lake Center area 1	CPL-W11	Located at the center of the lake, less human interference	28.2124572	83.948297
Lake Center area 2	CPL-W12		28.2175817	83.94874
Lake Center area 3	CPL-W13		28.2166645	83.9415426
Lake Center area 4	CPL-W14		28.2221197	83.9412002
Lake House	LPA-W15 LPA-S9	Least populated area	28.211129	83.9420679
Chisapani	LPA-W16 LPA-S10	Least populated area	28.2062436	83.94844087

Note: W represent water sampling site and S represents sediment sampling site

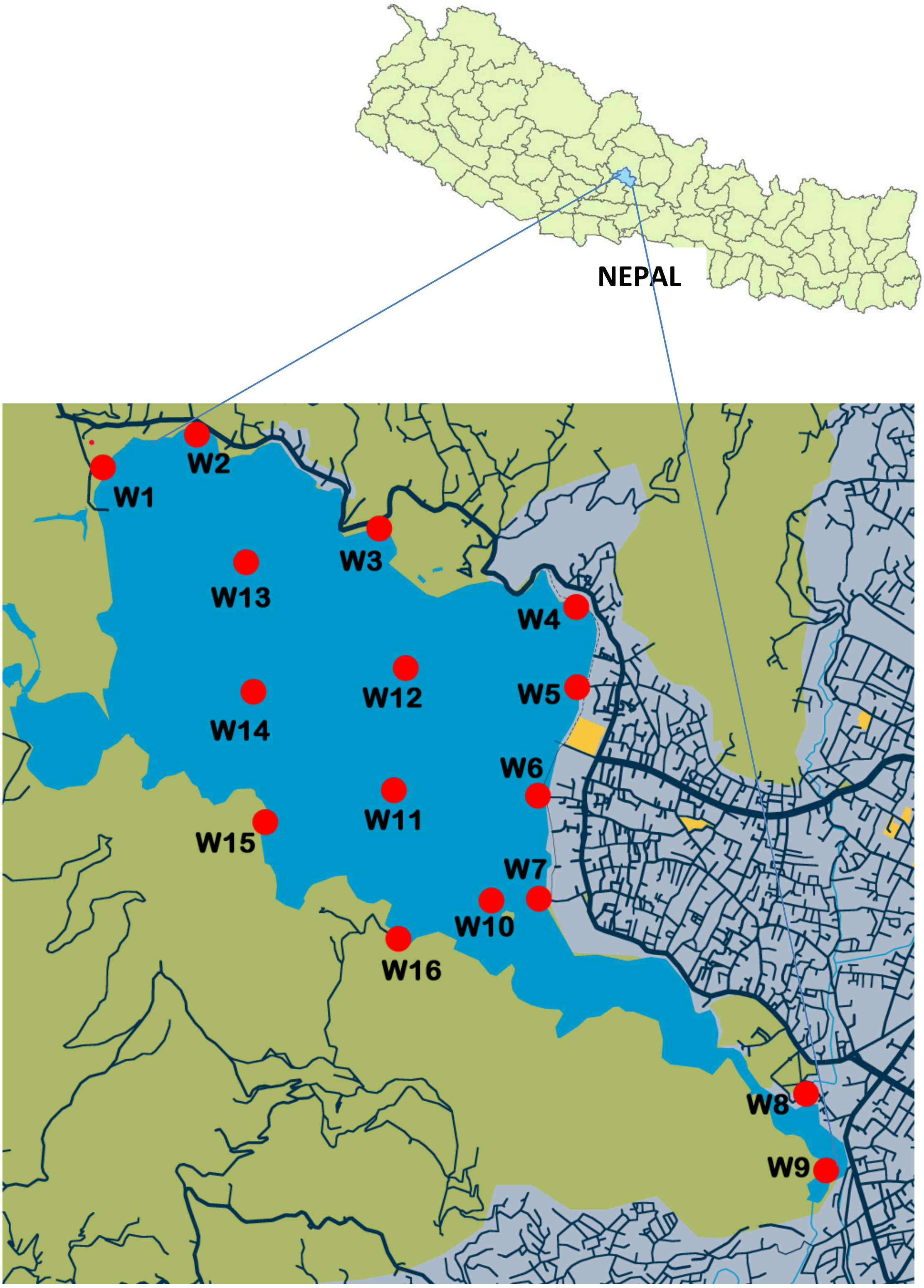

Figure 3.1 Sampling points for water

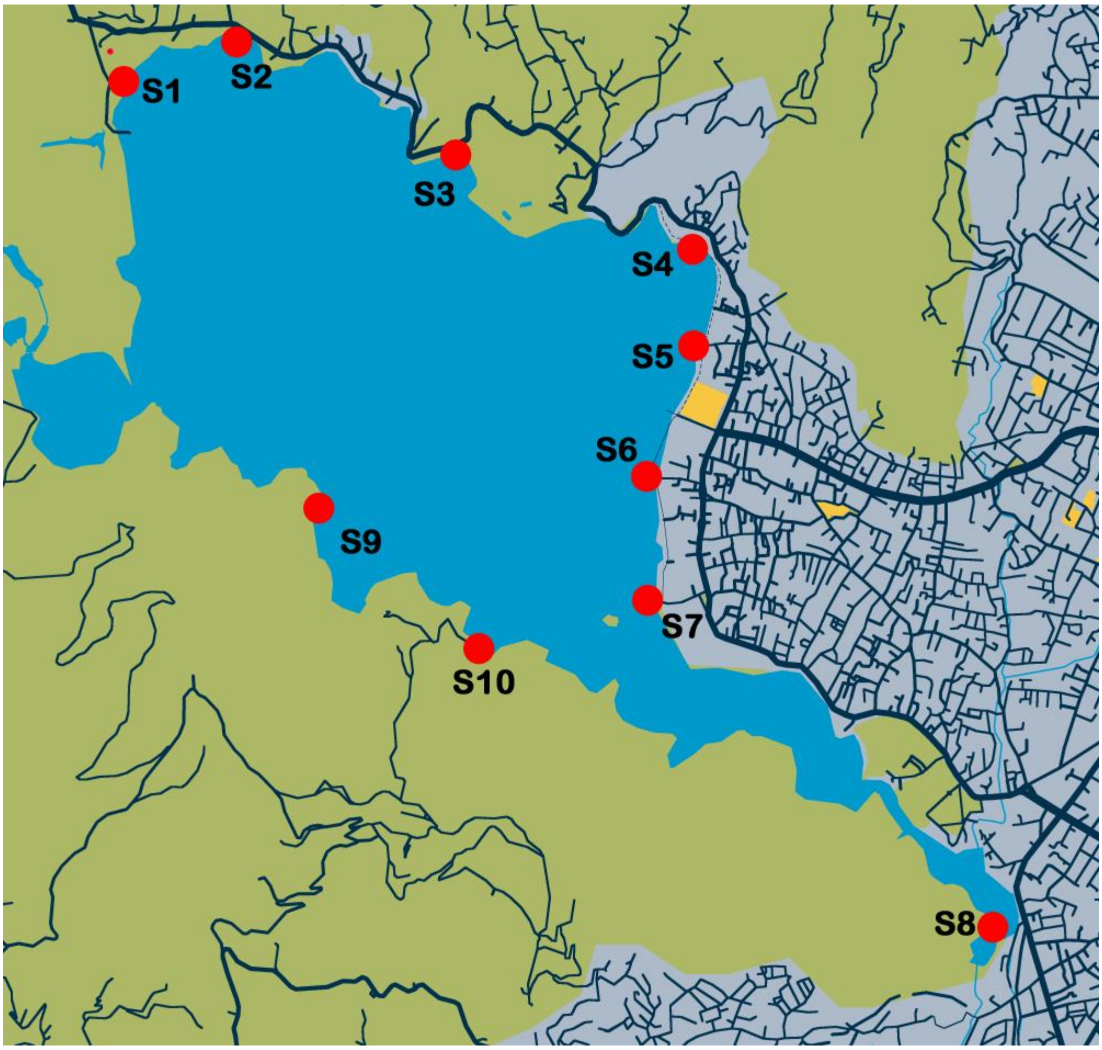

Figure 3.2 Sampling points for sediments

3.3 Sample collection

For quantifying microplastics from freshwater lakes, sample collection is the first crucial step. Bulk and volume-reduced sample collection approach was followed in this study. For water sample, 5 L of bulk surface water (Su et al., 2016) (0-20 cm depth) samples were collected from all 16 locations using cleaned steel bucket (Jian et al., 2020; Mao et al., 2020) The collected water from the bucket was filtered on-site with 75µm brass sieve. All the filtrates remaining on the sieve were rinsed 2-3 times with Milli-Q water and carefully transferred to a pre cleaned 200 mL glass bottles and was stored at 4°C before analysis. Two replicates were collected at each sampling locations. Further, from each site 1 L of water sample was collected in a pre-acid washed polythene bottle and two more water samples in BOD bottles (300 mL capacity) for water quality analysis.

For the sediment sampling the top (0-2 cm) (Jiang et al., 2018; Zhang et al., 2016) of lakeshore sediments was collected from each sampling location using 25 cm x 25 cm quadrat (Fischer et al., 2016) with the help a stainless steel spoon. At each location, three samples were collected about 5 m away from the main sampling location. It was then mixed together and two replicate taken, stored in an aluminium foil container, labelled until further lab analysis.

Figure 3.3 Position of three sampling locations in sediment sampling

3.4 Materials and apparatus required for microplastic analysis

3.4.1 For water sample

- GPS (GARMIN eTrex®10)
- Sieve 1 mm
- Squirt bottle
- 500 – mL glass beaker
- Metal spatula
- Stir bar
- Watch glass
- Steel forceps
- Petri dish
- Aluminium foil
- Whatman filter paper (C Whatman GF/C TM)
- Volumetric flask
- Graduated pipette (10 mL)
- Vacuum filtration
- Needle

- Analytical balance
- Hot air oven
- Hot plate
- Stereomicroscope (40X magnification) (SZ2-ILST, Olympus, Japan)
- Milli-Q water
- 30% H_2O_2
- Iron Fe(II) solution (0.05 M)
  (7.5 g of $FeSO_4.7H_2O$) was added to 500 mL volumetric flask with Milli-Q water and 3 mL of concentrated H_2SO_4)

3.4.2 For sediment sample

Same as above with some additional materials and apparatus like

- Sieve 5 mm, 0.2 mm
- Retort stand
- O – ring
- Glass rod
- 1 L glass beaker
- Density separator (made by fitting latex tubing attached by pinch clamp at the bottom of a glass funnel
- Sodium chloride
- Stereomicroscope (40X magnification) (SZ2-ILST, Olympus, Japan)
- FTIR (IRAffinity, 1S, SHIMADZU, Serial number A221352)

3.5 Methods for microplastics analysis

Several analytical techniques has been used in microplastic studies for the sample processing and identification of microplastics. Microplastics extraction from surface water and sediment samples was carried out in accordance with National Oceanic and Atmospheric Administration (NOAA) (Masura et al., 2015) with some minor changes. For the identification of microplastic, criteria delineated by Hidalgo-Ruz et al. (2012) was followed and for the confirmation of microplastic, hot needle test was performed for small particles < 1 mm. Similarly, Fourier transform infrared spectroscopy (FTIR) was used to confirm polymer type.

3.5.1 Microplastic analysis for water samples

An overview of analytical method for microplastic analysis in surface water is shown in **Figure 3.4.**

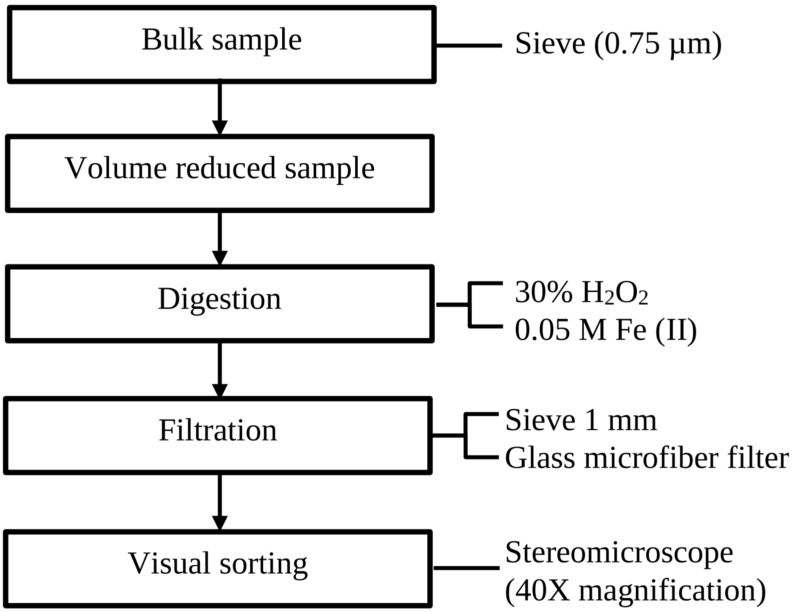

Figure 3.4 Flow diagram for the analysis of microplastics in water samples of Phewa Lake

First, the water sample from the glass jar was transferred to a clean 500 mL beaker. The glass jar was rinsed 2-3 times with Milli – Q water. Then 20 mL of 30% H_2O_2 along with 20 mL of 0.05 M Fe (II) solution was added to the beaker containing the sample water. The mixture was set aside for about five minutes. After 5 minutes, a stir bar was kept inside the beaker and covered with a watch glass and heated on a hot plate at 50° C. As soon as it started to boil, it was set aside until boiling subsided. Then again it was kept on a hot plate for an additional of 15 minutes. The digested sample was kept aside to cool and then filtered through 1 mm sieve. The filtrate was again carefully filtered through a Whatman glass microfiber filter paper under vacuum filtration. To ensure complete removal of microplastics the beaker was rinsed 2-3 times with Milli-Q water. The filter paper was placed in a clean petri dish and allowed to dry at room temperature before visual examination. Any visible microplastic on the 1 mm sieve was carefully picked with forceps, washed with Milli-Q water and kept in a clean petri dish for visual inspection.

Under a stereomicroscope (40X magnification) the petri plate along with filter were visually examined. Identified microplastics were sorted based upon their morphology like shape and color. The shape was further categorized as fiber/line, foam, fragment, and film and two size groups as 1-5 mm and < 1 mm to differentiate plastic from non-plastic, criteria delineated by Hidalgo-Ruz et al. (2012) were followed: absence of cellular structure and particles should have equal thickness and same color throughout. Likewise, the hot needle test described by De Witte et al. (2014) was performed. The plastic piece melted when a sample piece under investigation was brought in contact with a very hot needle. As the size fraction of microplastics were small and the unavailability of the instrument, the FTIR and Raman could not be done. For each examined microplastics, they were counted and noted based on shape, color, and size.

3.5.2 Microplastic analysis for sediment samples

To eliminate particles greater than 5 mm, first, the sediment samples were kept in a hot air oven at 60° C for 24 hours and were sieve through 5 mm mesh (Sruthy & Ramasamy, 2017). Extraction of microplastics from sediment samples was achieved in accordance with Zhao et al. (2018). In each beaker, 100 g of sediment sample was taken and 400 mL of saturated sodium chloride (NaCl d=1.2 gL^{-1}) was added. The mixture was stirred with the help of a glass rod and left to settle for about five minutes. Following settlement, the floatables was carefully poured through 0.2 mm sieve. The separation process was repeated thrice for each sample for the higher recovery and transferred to a clean 500 mL beaker. Sieve was rinsed 2-3 times and the washing was collected in the same beaker. The beaker was kept in hot air oven at 90° C for 24 hours. Then 20 mL each of 30% H_2O_2 and 0.05 M Fe (II) solutions was added to the dried sample to degrade the organic matter and let aside for about five minutes. After 5 minutes a stir bar was kept inside the beaker and covered with a watch glass and heated at 50° C on a hot plate until it starts to boil. As soon as it stared to boil, it was set aside until boiling subsided. Then again it was kept on a hot plate. H_2O_2 was again added if natural organic matter were seen and process was repeated. Then finally about 12 g of NaCl was added to increase the density of the solution. The sample was transferred to the density separator and beaker rinsed 2-3 times with Milli-Q water to ensure all solids has been transferred. The density separator was loosely covered with aluminium foil to avoid contamination and left for about 24 hours to settle. Finally, the supernatant was first passed through 1 mm sieve and the filtrate was again carefully filtered through Whatman glass microfiber filter paper under vacuum filtration.

The visual observation process was same as water sample. The microplastics from sediment samples from 1-5 mm size group were further analysed for polymer composition by FTIR. An overview of analytical method for microplastic analysis in shoreline sediment is shown in **Figure 3.5.**

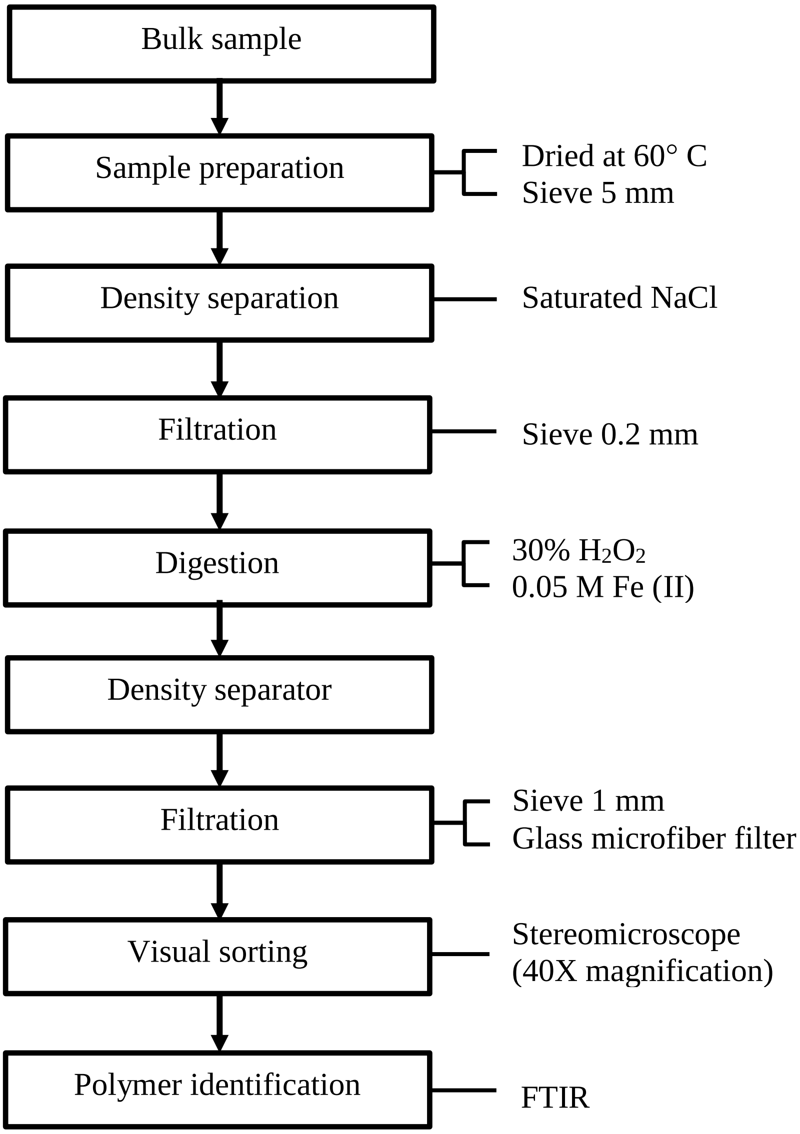

Figure 3.5 Flow diagram for the analysis of microplastics in freshwater shoreline sediments of Phewa Lake.

3.6 Quality assurance and quality control

Measures were adopted at the time of sampling and lab analysis to minimize potential contamination. Cotton laboratory coat and nitrile gloves were worn during laboratory analysis and cotton clothes during sample collection. Glassware were used in the laboratory and rinsed with Milli-Q water before use. Tools and containers were pre-cleaned before sample collection. To avoid possible contamination, samples and glassware were covered with aluminium foil during analysis. Filter papers were quickly kept inside petri dish after filtration process. In order to determine airborne contaminate in the laboratory, first the filter paper was observed with a stereomicroscope and left open on a petri dish for 24 hours. Field blank was carried out at 4 random sample sites. 5 L of Milli-Q water was filtered into 0.75 µm sieve and the filtrate collected on the sieve was transferred into a pre-cleaned glass jar, labelled. Further, processing was same as water sample but digestion process was excluded. For sediment samples, only laboratory air contamination was measured. Field blank results of water samples for winter, rainy and autumn seasons were 0.1, 0.15 and 0.05 microplastics/L respectively and lab contamination were found to be 0.25, 0.25, 0.50 microplastics per filter paper for winter, rainy and autumn seasons respectively. Similarly, for sediment samples the lab contamination were found to be 0.15, 0.20 and 0.10 microplastics per filter paper for winter, rainy and autumn seasons respectively. As negligible amount of contamination were observed, background contamination was not taken in to account (Baldwin et al., 2020).

3.7 Methods for water quality analysis

Water quality parameters like pH, electrical conductivity (EC), total dissolved solid (TDS), turbidity were measured on site. Nitrate was measured using testing kit. Hardness, chloride dissolved oxygen (DO), biological oxygen demand (BOD) and heavy metals like copper, nickel, zinc, manganese, lead and cadmium were determined on the basis of "Standard methods for the examination of water and wastewater (APHA, 2005).

3.7.1 Analysis of physical and chemical parameters

pH

pH of the water sample was directly measure on site by dipping the pH meter into the lake water. The reading was recorded after a constant reading was observed. Pocket size pH meter, HANNA instrument, pH ep®, Romania was used for pH reading.

EC and TDS

EC and TDS of water sample was directly measured by dipping the EC/TDS/Temperature probe on site into the lake water. The reading was recorded after a constant reading was observed. EC/TDS/Temp probe, MILWAUKEE E (59, Europe) was used to record the EC and TDS of each sampling site.

Turbidity

The sample water from the sampling bottle was poured in the beaker on site. Then a small amount of the sample from the beaker was poured in the Nephelometer sample tube and the reading recorded in NTU.

Total hardness

50 mL of sample water was taken in a clean conical flask. Then 1 mL of ammonium buffer and a pinch of Erichrome Black-T indicator was added. The solution was titrated against standard EDTA (0.01 M) solution. The end point is wine red to blue. The process was repeated until same value are obtained and the total hardness of water was calculated in parts of $CaCO_3$ per million parts of water.

Chloride

50 mL of water sample was taken in a clean conical flask. Then 2-3 drops of potassium chromate indicator solution was added. Silver nitrate (0.02 N) solution was added slowly from the burette swirling the liquid constantly until the red color formed by the addition of each drop begins to disappear more slowly. This was an indication that most of the chlorides has been precipitated. Drop wise addition of silver nitrate was continued until a faint but distinct color changes occurred will persist after brisk shaking. The indicator blank correction was determined using the same procedure as above but distilled water was taken in place of the water sample.

Alkalinity

In a conical flask, 50 mL of sample water was taken. The 2-3 drops of phenolphthalein indicator were added. The solution in the conical flask was titrated against 0.02 N H_2SO_4 taken in the burette. The end point is pink to colorless. The process was repeated until the sample value was obtained.

Nitrate

Nitrate in the water sample was measured with the help of nitrate testing kit (Visocolor ® alpha, Germany). First, the test vessel was rinsed several times with the water sample and the water was filled to the ring mark (5 mL). Then 5 drops of NO_3^{-1} was added and mix by swirling. One level measuring spoon of NO_3^{-2} was added and swirl for 30 seconds. Finally, after five minutes the measuring vessel was placed on the color chart to obtain the value of nitrate.

Dissolved oxygen (DO)

Water sample was collected in 300 mL BOD bottle without air bubbles. 2 mL magnesium sulphate followed by 2 mL of alkaline potassium iodide was added and it was stoppered immediately. The sample was mixed well by inverting the bottle 2-3 times and allow the precipitate to settle, leaving about 150 mL clear supernatant. At this stage, 2 mL of concentrated sulphuric acid was added. The bottle was mix well to dissolve the precipitate. Then 50 mL of sample was taken in a clean conical flask and titrated against 0.025 N sodium thiosulphate using starch as an indicator. The end point is pale blue color to colorless. The titration was repeated until the concurrent reading was obtained.

Biological oxygen demand (BOD)

The BOD test is based on the determination of DO. Two BOD bottles was filled with sample water and for one bottle, DO was measured as described above in DO determination. If the DO was 7 or above 7 mg/L then the other bottle was wrapped with carbon paper and was kept in BOD incubator at 20° C for 5 days. After five days, the DO was determined as before and the BOD was calculated by subtracting the initial DO with the DO reading of the fifth day. But if the initial DO was less than 7 mg/L then the sample water was diluted for BOD estimation.

Preparation of dilution water

1 L of distilled water was taken in a clean volumetric flask. Then 1 mL phosphate buffer, 1 mL $MgSO_4.7H_2O$, 1 mL $CaCl_2$ and 1 mL $FeCl_3H_2O$ was added and the solution was mixed thoroughly.

Sample preparation

50 mL of sample water was taken in 2 L bottle and was diluted to 1 L by adding 950 mL of the diluted water. The solution was mix thoroughly. The diluted sample water was aerated thoroughly by bubbling air through a diffusion tube into sample or the sample water is shaken for several minutes.

Now two BOD bottles were taken and was filled with the diluted sample. One bottle was wrapped with carbon paper and was kept inside the BOD incubator at 20° C for 5 days. The second bottle was measured for dissolved oxygen content as described above. After five days of incubation period, the first bottle was measured for DO content and BOD was calculated as done for sample water without dilution but the result was multiplied by the dilution factor.

Iron

50 mL of the filtered sample water was taken in a clean conical flask. Then 2 mL of 35% concentrated hydrochloric acid and 1 mL of hydroxylamine hydrochloride solution was added to the sample water taken. The solution was boiled at 400-450° C on a hot plate until half its volume. The solution was cooled at room temperature and was transferred to 100 mL volumetric flask. Again 10 mL of ammonium acetate buffer and 2 mL of phenonthroline solution was added and the appearance of orange red color was noticed. The volume was adjusted to 100 mL by adding distilled water and was kept for 10 minutes for color development. It was then measured in spectrophotometer at 510 nm and the absorbance recorded. A standard calibration curve was prepared in the range of 0.1 to 3 mg/L with interval of 0.5 mg/L and the concentrated of iron was directly calculated from the standard curve.

3.7.2 Heavy metals (lead, zinc, manganese and nickel)

50 mL of sample water was taken in each conical flask. Then 3 mL of concentrated hydrochloric acid and 1 mL of concentrated nitric acid was added to each conical flask and was heated on a hot plate at 100° C until half its volume. The solution was cooled at room temperature and was transferred to 100 mL volumetric flask. The conical flask was rinsed 2-3 times with Milli-Q water and transferred the washing to volumetric flask. The volume was made up to 100 mL by adding Milli-Q water and was measured for heavy meatal concentration (lead, zinc, manganese and nickel) by flame atomic absorption spectroscopy and the reading was recorded for each heavy metals.

Table 3.2 Analytical methods adopted for water quality analysis in Phewa Lake.

Parameters	**Units**	**Analytical Methods/ Instruments**
pH		Instrumental method, HANNA instrument, pHep®, Romania
EC	µS/cm	Instrumental method, EC/TDS/Temp probe, MILWAUKEE E(59, Europe)
TDS	mg/L	Instrumental method, EC/TDS/Temp probe, MILWAUKEE E(59, Europe)
Hardness (as $CaCO_3$)	mg/L	EDTA titration method
Chloride	mg/L	Argentometric titration method
Nitrate	mg/L	Colorimetric Kit method VISOCOLOR® alpha Nitrate; Macherey-Nagel, Germany
DO	mg/L	Winkler’s idometric method
BOD	mg/L	Winkler’s idometric method
Iron	mg/L	Phenonthroline method, Spectrophotometer (Agilent technology cary UV/V spectrophotometer)
Heavy metals (lead, zinc, manganese, nickel)	mg/L	Spectroscopy method, Flame atomic absorption spectroscopy (Agilent AA5-200 series)

3.7.3 Water quality index calculation

The WQI was calculated using ten physio-chemical water parameters such as pH, TDS, turbidity, EC, chloride, hardness, alkalinity, DO, nitrate, and BOD. The standard guideline values used for this study are given by BIS (2012); CBS (2019); WHO (2011). For calculating WQI four steps applied by Alobaidy et al. (2010) was followed.

i) On the basis of common expert's outlook from the previous research, the parameters were assigned a specific weightage (awi) which ranged from 4.2 to 1.6 (**Table 3.3**). Higher assigned weight stands for more significance and lower for least significant parameters.

ii) For each parameter, relative weight (RWi) was determined using equation. Relative weight and standard value for each parameter is shown in

iii) **Table** 3.4**.**

$$RWi = \frac{awi}{\sum_{i=1}^{n} awi}$$

Where, RWi = the relative weight,

awi = weight assigned of each parameter,

n= number of parameters

iv) For each parameter, a quality rating (QRi) was computed by using equation

$$QRi = \frac{Ci}{Si} \times 100$$

Where, QRi = quality rating scale,

Ci = observed concentration of each parameter

Si = standard value for each parameter

But for pH and DO, the QRi was computed using equation

$$QRi = \frac{Ci - Vi}{Si - Vi} \times 100$$

Where, Vi = ideal value, 7 for pH and 14.6 for DO

v) For the determination of WQI, sub-index (SIi) for each water parameter were calculated as follows

SIi= RWi x QRi

Where, SIi = sub-index of water quality

RWi = relative weight and

QRi = quality rating scale

Finally, WQI was computed by using equation

WQI = $\sum_{i=1}^{n} SIi$

The calculated WQI value were classified according to Sahu and Sikdar (2008); Wu et al. (2020) given in **Table 3.5.**

3.7.4 Analysis of heavy metal pollution index (HPI)

The HPI was calculated using heavy metals such as iron, lead, zinc, manganese and nickel. A method developed by Mohan et al. (1996) was used for HPI calculation which has following three steps.

i) For each heavy metal unit weight (UWi) was calculated using following two equations

UWi $\propto \frac{1}{Si}$ and UWi = $\frac{k}{Si}$

Where, UWi = unit weight

Si = standard value for each heavy metal

k = proportionality constant equal to 1 (Prasad & Bose, 2001)

ii) For each metal, a quality rating (QRi) as computed by using equation

QRi = $\frac{Ci}{Si}$ x 100

Where, QRi = quality rating / sub index value

Ci = observed concentration of each metal

Si = standard recommended value for each metal recommended by CBS (2019); WHO (2011)

iii) Finally, HPI was computed using equation

HPI= $\frac{\sum_{i-1}^{n} QRi*UWi}{\sum_{i=1}^{n} UWi}$

Where, UWi = unit weight

QRi = quality rating or sub index value

n = number of metals used

The HPI value were classified according to Ghaderpoori et al. (2018) as given in **Table 3.6.**

Table 3.3 Assigned weightage estimation from reviewed studies for water quality index.

Parameters	Abrahão et. al. (2007)	Abdul Hameed et. al. (2010)	Ravikumar et. al. (2013)	Xiao et. al. (2014)	Meher et. al. (2015)	Ayvaz & Elçi (2018)	Kükrer and Mutlu (2019)	Mayanglambam & Neelam (2020)	Amiri et. al. (2021)	Maansi et. al. (2022)	Average Assigned weightage (awi)
pH	-	2.1	3	4	4		3	4	2.5	2.54	3.1
EC	4	2.7	3		5	4		3	2.9	3.22	3.5
TDS	-		5	4	4			4	3.4	2.75	3.9
Turbidity	4	2.4							3		3.1
Hardness	1	1.1	3	2			1	2	1.4		1.6
Chloride	-		3	3	3	2	3	3	2.2		2.7
Alkalinity	-	1.6	2		2					3.72	2.3
DO	4	4			5				3.9	4.09	4.2
BOD	3	3							2.8		2.9
Nitrate	2	2.2	5	5		4	5	5		2.57	3.8

Table 3.4 Assigned and relative weight with standard values for WQI computation

Parameters	Standard values (Si)	References	Assigned weight (awi)	Relative weight (RWi)
pH	8.5	CBS (2019)	3.1	0.10
EC	1500 µS/cm	CBS (2019)	3.5	0.11
TDS	1000 mg/L	CBS (2019)	3.9	0.13
Turbidity	5 NTU	CBS (2019)	3.1	0.10
Hardness	500 mg/L	CBS (2019)	1.6	0.05
Chloride	250 mg/L	CBS (2019)	2.7	0.09
Alkalinity	600 mg/L	BIS (2012)	2.3	0.07
DO	5 mg/L	BIS (2012)	4.2	0.14
BOD	5 mg/L	BIS (2012)	2.9	0.09
Nitrate	50 mg/L	CBS (2019)	3.8	0.12

Table 3.5 Classification of water quality index values

WQI values	**Water quality status**
< 50	Excellent water
50 – 100	Good water
100 – 200	Poor water
200 – 300	Very poor water
> 300	Unsuitable for drinking

Table 3.6 Classification of HPI values

HPI values	Status of water
< 100	Low risk water
100	Threshold risk
> 200	High risk water

3.1 Data analysis

The collected data were examined, evaluated and arranged for its accuracy and completeness. All the data were entered in Microsoft Excel for preliminary analysis. The data was further analysed using R v4.2.1 (R Core Team., 2021) with integrated development environment RStudio 2022.07.1+554 (RStudio Team., 2022) and visualized using R library ggplot2 package v3.3.3 (Wickham, 2016). The unit of microplastic abundance in water was reported as number of microplastics per liter, whereas in sediment it was reported as number of microplastics per kg dry weight. The mean and standard deviation of abundance for each sampling point were computed. The frequency and percentage of color, type, size, polymer type of microplastics were also calculated and exhibited in diagrammatic form.

All the data were checked for normality (using Shapiro-Wilk test, > 0.05) and homogeneity (using Leven's test, > 0.05) for comparison purpose. Since most of the analyzed variables exhibited a non-normal distribution, and not having equality of variance, group analysis was conducted using a non-parametric Kruskal-Wallis H test (to test the significant difference among different sites and seasons) with Dunn-Bonferroni post hoc test (pairwise comparison).

In the same way mean and standard deviations of water quality parameters were also determined. The frequency and percentage of WQI and HPI were also analyzed.

Similarly, correlation analysis was done to measure the relationship among water quality parameters with abundance of microplastics in water and sediments.

CHAPTER 4 : RESULTS AND DISCUSSION

4.1 Abundance of microplastics in Phewa Lake

Microplastics were detected in all surface water and shoreline sediment samples collected from different sampling locations of Phewa Lake. During the sampling period (2021), the mean abundance of microplastics in Phewa Lake was 1.97 microplastics/L. As this is the first study on microplastics from freshwater lakes of Nepal, the SAARC countries were selected for comparative studies **(Table 4.1).** The results indicates that the microplastic load of Phewa Lake (1.97 microplastics/L) was almost equal to the microplastic concentration of Rawal Lake, Pakistan (1.42 items/L) (Irfan et al., 2020b) and three times less than the microplastic concentrations seen in Red Hills Lake, India (5.9 particles/L) (Gopinath et al., 2020). Compared to Phewa Lake, the River Ganges (Napper et al., 2021), River Adyar, Kosasthalaiyar and Multhirappuzhayar reported a very low level of microplastics (Lechthaler et al., 2021) **Table 4.1.** As there is much variation in sampling, processing and identification of microplastics, the comparison of microplastics abundance among different studies must be done with prudence (Wang et al., 2018; Yuan et al., 2019). Wang et al. (2018) pointed out that the mesh size used in microplastic sampling is directly related to microplastic abundance. Therefore, worldwide freshwater lakes that used bulk sampling methods with mesh size ≤ 75 µm were selected to compare with Phewa Lake **(Table 4.2).** Studies indicate that Phewa Lake has higher microplastic pollution load than West Dongting and South Dongting Lake, China (Jiang et al., 2018) and lower microplastic concentrations than Poyang Lake, China (Yuan et al., 2019) and Lake Wuliangsuhai, China (Mao et al., 2020). Likewise, the microplastic abundance of Phewa Lake, Nepal is close to Lake Sassolo, Switzerland (2.6 microplastics/L) (Negrete Velasco et al., 2020). Overall, the microplastic pollution in Phewa Lake is low to moderate.

Table 4.1 Comparison of concentrations of microplastic in freshwater environment from SAARC countries.

Compartment	**Location**	**Average/Range**	**Reference**
Lake	Red Hills Lake, India (Tamil Nadu)	5.9 particles/L	Gopinath et al. (2020)
Lake	Veeranam Lake, India (Tamil Nadu)	28 items/ km^2	Bharath et al. (2021)
River	Netravathi River, India (Karnataka)	288 pieces/m^3	Amrutha and Warrier (2020)
Lake	Rawal Lake, Pakistan	1.42 items/L	Irfan et al. (2020b)
River	Ravi River, Pakistan	2074±3651 microplastics/m^3	Irfan et al. (2020a)
River	Koshi River, Nepal	202±100 items/m^3	Yang et al. (2021a)
River	Swat River, Pakistan	192 items/L	Khan et al. (2022)
River	Adyar River, Tamil Nadu, India	0.33 particles/L	Lechthaler et al. (2021)
River	Kosasthalaiyar River, Tamil Nadu, India	0.67 particles/L	Lechthaler et al. (2021)
River	Multhirappuzhayar River, Kerala, India	0.20 particles/L	Lechthaler et al. (2021)
River	Ganges, India/Bangladesh	0.038 particles/L ±0.004 items/L	Napper et al. (2021)
Lake	Renuka Lake, Himanchal Pradesh, India	2-64 particles/L	Kumar et al. (2021)

Table 4.2 Worldwide comparison of concentrations of microplastic in freshwater environment that used bulk sampling methods with mesh size ≤ 75 µm.

Study area	**Collection cut of size (µm)**	**Range/Average**	**References**
20 Major Lake, Wuhan, China	50	1660.0±6391.1 n/m^3 to 8925±1591 n/m^3	Wang et al. (2017)
West Dongting and South Dongting Lake, China	45	616.67 to 2216.67 items/m^3 W. Dongting 416.67 to 2316.67 items/m^3 S. Dongting	Jiang et al. (2018)
8 Urban Lake, China	45	2425 to 7050 items/m^3	Yin et al. (2019)
Poyang Lake, China	50	5 to 34 items/L	Yuan et al. (2019)
Lake Ulanshuhai, China	48	1760±710 to 10120±4090 items/m^2	Wang et al. (2019c)
Poyang Lake, China	38	1064±90 items/m^3	Jian et al. (2020)
Wuliangsuhai Lake, China	75	3.12 to 11.25 items/L	Mao et al. (2020)
Lake Sassolo, Switzerland	63	2.6 microplastics/L	Negrete Velasco et al. (2020)

4.2 Spatial distribution of microplastics in Phewa Lake

Microplastics in Phewa Lake surface water showed marked spatial variability **(Table 4.3)**. The highest microplastic abundance was observed during winter season sampling at site DPA-W7 (6.10 microplastics/L) followed by DPA-W5 (5.8 microplastics/L). Both sites are located at densely populated areas. Previous studies have pointed out that high microplastic concentrations are seen near residential and densely populated locality (Irfan et al., 2020b; Vaughan et al., 2017; Xiong et al., 2018) which may also be in the case of Phewa Lake. The lowest microplastics abundance of

0.60 microplastics/L was recorded from CPL-W14 (autumn season) followed by 0.70 microplastics/L from CPL-W11 (rainy season). The possible reason for low microplastics is that, these sites lies at the center area of the lake where water level is deep so there is less human interference (Kumar et al., 2021). Moreover, the large surface area acts as a dilution factor to reduce the load of microplastic pollution (Yuan et al., 2019).

Table 4.3 Abundance of microplastic location wise (microplastics/L)

	Winter				**Rainy**				**Autumn**			
Area Code	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
INL-W1	3.50	3.25	1.2	5.8	1.60	0.28	1.4	1.8	1.30	0.42	1	1.6
MPA-W2	3.70	0.99	3	4.4	2.60	0.28	2.4	2.8	1.50	0.42	1.2	1.8
MPA-W3	2.20	0	2.2	2.2	1.20	0.57	0.8	1.6	2.10	0.42	1.8	2.4
DPA-W4	3.80	1.70	2.6	5	1.50	0.42	1.2	1.8	1.40	0.28	1.2	1.6
DPA-W5	5.80	3.11	3.6	8	1.40	0.28	1.2	1.6	1.80	1.13	1	2.6
DPA-W6	2.80	0.85	2.2	3.4	1.60	0.28	1.4	1.8	1.40	0.85	0.8	2
DPA-W7	6.10	1.56	5	7.2	2.20	0.28	2	2.4	1.60	0	1.6	1.6
MIX-W8	2.40	0	2.4	2.4	2.70	0.14	2.6	2.8	1.80	0.28	1.6	2
OUT-W9	4.90	1.56	3.8	6	1.60	0.28	1.4	1.8	3.60	1.70	2.4	4.8
TBA-W10	2.40	0.28	2.2	2.6	1.00	0.85	0.4	1.6	1.10	0.14	1	1.2
CPL-W11	1.80	0.85	1.2	2.4	0.70	0.14	0.6	0.8	0.80	0.28	0.6	1
CPL-W12	0.90	0.14	0.8	1	0.90	0.71	0.4	1.4	1.00	0.28	0.8	1.2
CPL-W13	1.40	0.28	1.2	1.6	1.10	0.14	1	1.2	1.00	0.28	0.8	1.2
CPL-W14	1.40	0.28	1.2	1.6	1.40	0.28	1.2	1.6	0.60	0.57	0.2	1
LPA-W15	2.50	0.14	2.4	2.6	1.30	0.42	1	1.6	0.80	0.28	0.6	1
LPA-W16	1.80	0.85	1.2	2.4	1.40	0.28	1.2	1.6	1.10	0.14	1	1.2
Average	2.96	1.83	0.8	8	1.51	0.62	0.4	2.8	1.43	0.83	0.2	4.8

The sixteen sampling locations of the lake was further divided into eight areas as inlet area (INL-W1), moderately populated area (MPA-W2-W3), densely populated area (DPA-W4-W7), Phirke Khola mixing area (MIX-W8), outlet area (OUT-W9), Tal Barahi temple area (TBA-W10), lake center area (CPL-W11-W14) and least populated area (LPA-W15-W16).

Table 4.4 Abundance of microplastic area wise (microplastics/L)

Area	Winter		Rainy		Autumn		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DPA-W4-W7	4.63	2.10	1.67	0.41	1.55	0.57	2.62	1.90
MPA_W2-W3	2.95	1.04	1.90	0.89	1.80	0.49	2.22	0.93
LPA-W15-W16	2.15	0.64	1.35	0.30	0.95	0.25	1.48	0.65
CPL-W11-W14	1.38	0.49	1.02	0.41	0.85	0.33	1.08	0.46
TBA-W10	2.40	0.28	1.00	0.85	1.10	0.14	1.50	0.81
OUT-W9	4.90	1.56	1.60	0.28	3.60	1.70	3.37	1.81
MIX-W8	2.40	0	2.70	0.14	1.80	0.28	2.30	0.43
INL-W1	3.50	3.25	1.60	0.28	1.30	0.42	2.13	1.82
Average	2.96	1.83	1.51	0.62	1.43	0.83	1.97	0.60

During the sampling period (2021), the outlet area (OUT-W9) recorded the highest abundance of microplastics (3.37 microplastics/L) compared to other areas **(Table 4.4)** which was also observed in previous freshwater lake studies from different parts of the world: Rawal Lake (Irfan et al., 2020b), Red Hills Lake (Gopinath et al., 2020) and West Dongting and South Dongting Lake (Jiang et al., 2018). Likewise, in the densely populated area (DPA-W4-W7), the concentration of microplastics was also found to be high (2.62 microplastics/L) as abundance of microplastics is related to population density of that area (Liu et al., 2019b; Nel et al., 2017; Wang et al., 2017). Similarly, the lowest microplastic concentration (1.08 microplastics/L) was found at CPL-W11-W14, followed by microplastics abundance at LPA-W15-W16 (1.48 microplastics/L) **(Figure 4.1).** The first area lies at the central part of the lake and the other lies at the southern side of the lake which is covered by forest area. So it is sparsely populated with very less tourism activities.

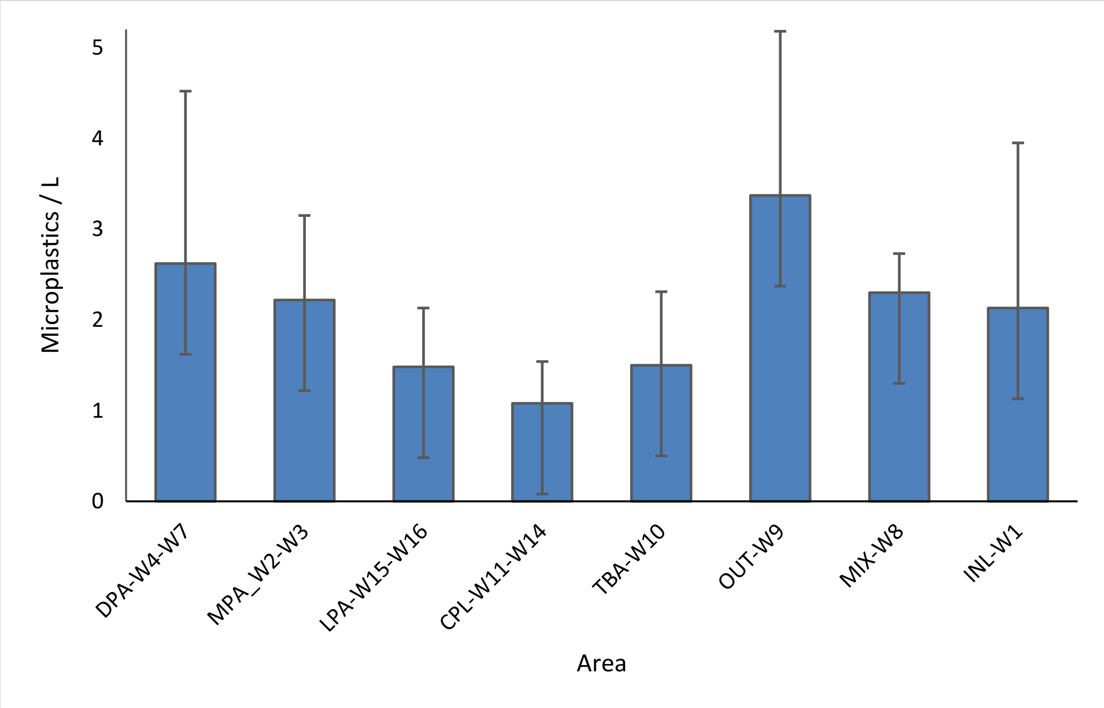

Figure 4.1 Area wise average microplastic abundance

Since the abundance of microplastics did not follow normality and equality of variance, Kruskal-Wallis H test was applied to find out whether there was significance difference in abundance of microplastics in different areas of the lake. The result indicated that the mean abundance of microplastics in different areas was significantly different ($H=35.087$, $p<0.01$). A pair wise comparison was done to find which pair had significance difference in abundance of microplastics. The result showed that the mean abundance of microplastics of lake center area (CPL-W11-W14) and least populated area (LPA-W15-W16) was significantly different with densely populated area (DPA-W4-W7), moderately populated (MPA-W2-W3), Phirke Khola mixing area (MIX-W8) and outlet area (dam site) (OUT-W9). Low concentration at TBA-W10 may be due to close down of temple area for a long period of time during 2021 as a result of COVID-19. Likewise, there was significance difference in abundance of microplastics of Tal Barahi temple area (TBA-W10) with outlet area (OUT-W9). All these areas are influenced by human activities. Studies have pointed out that microplastic concentration is directly related to density of population and land use pattern of that region (Cole et al., 2011; Hendrickson et al., 2018).

4.3 Temporal distribution of microplastics in Phewa Lake

Microplastics were identified in the surface water of Phewa Lake with abundance ranged from 0.8 to 8 microplastics/L for winter (dry) season, 0.4 to 2.8 microplastics/L for rainy (monsoon/wet) season, and 0.2 to 4.8 microplastics/L for autumn (post monsoon) season respectively. Likewise, the average microplastic abundance for winter, rainy and autumn seasons are 2.96 microplastics/L, 1.51 microplastics/L and 1.43 microplastics/L respectively **(Table 4.3)**. To find out the significance difference of microplastic concentrations in different seasons of Phewa Lake, Kruskal-Wallis H test was done. The results indicated that the average concentration of microplastics in different seasons are significantly different ($H=22.34$, $p<0.01$). Pairwise comparison shows that the mean abundance of microplastics in winter season is significantly higher than that of rainy season (test statistics =24.44, $p<0.01$) and autumn season (test statistics=31.11, $p<0.01$). This might be as a result of the recent unexpected flood that took place prior to the sampling event. A study by Schell et al. (2021) also noted increase in the amount of microplastic load related to heavy rainfall before the sampling day. According to studies, storm water and combined sewage overflow may also contribute to increased microplastic load during heavy rain and flood (Blettler et al., 2017; Kataoka et al., 2019).

In winter season, the highest microplastic abundance was observed at OUT-W9 (4.90 microplastic/L) which lies at the south east end of Phewa Lake where a dam is situated. Location of a dam (Zhang et al., 2015) and a long narrow landscape (Yuan et al., 2019) at the outlet area may be a factor for higher microplastic concentrations at this section of the lake. Also the effluent from nearby residential area is directly discharged near the outlet area. Previous studies also reported high microplastic concentrations at the outlet of lake (Gopinath et al., 2020; Irfan et al., 2020b; Jian et al., 2020; Jiang et al., 2018). Similarly, DPA-W4-W7 also reported high mean microplastic abundance (4.63 microplastics/L). This area is surrounded by hotels and restaurants which lies at the eastern side of Phewa Lake, buzzing with tourists. It coincides with high concentrations of microplastics observed at this area. Therefore, tourism is said to be an important source of microplastic pollution in Phewa Lake which is accordance with previous studies (Free et al., 2014; Xiong et al., 2018). Likewise, the lowest average microplastic abundance of 1.38 microplastic was recorded at CPL-W11-W14. This area lies at the lake center where water is deep, so very less recreational boating reaches that point. Moreover, the large area at the center region of the lake acts as sink to dilute microplastic concentrations and decrease the influence of land based waste (Yuan et al., 2019).

In rainy season, the mean abundance of microplastics was almost two times less than that of winter season **(Table 4.3)**. This may be linked with two factors, firstly, due to COVID-19 pandemic, all the tourism activities around the lake were closed down resulting in low microplastic concentrations. Recent studies by Napper et al. (2021) pointed out that quantity of microplastic is closely related to tourist inflow. Likewise, the abundance of microplastics in densely populated area (DPA-W4-W7) was reduced during rainy season as recreational boating activities, hotels and restaurants around this locality was closed down due to lockdown restriction from March-April to the starting of rainy season (2021). Therefore, this study supports that tourism activities is directly connected to microplastic concentrations, secondly, the low microplastic concentrations during the rainy season may be due to flushing effects caused by precipitation (Han et al., 2020) (Napper et al., 2021). Similarly, in rainy season, the highest average microplastic abundance was observed at MIX-W8 (2.70 microplastics/L). This site is where Phirke Khola (stream) carrying domestic wastewater from the surrounding residential and urban area drains into Phewa Lake, thus contributing to a high microplastic abundance at this section (Su et al., 2016; Yuan et al., 2019). Likewise, the lowest mean abundance of microplastic was recorded in Tal Barahi temple area (TBA-W10) (1.00 microplastics/L) followed by lake center area (1.03 microplastics/L). As the temple was closed during COVID-19 pandemic confinement period, it resulted in low concentrations of microplastics.

In autumn season, the highest and the lowest abundance of microplastic were observed in the same area as winter season but the concentration of microplastic was less. This may be due to partial lockdown and restrictions for hotels and restaurants which was just lifted a week before the autumn season sampling.

The temporal variation of mean concentrations of microplastic in different areas of Phewa Lake is shown in **Figure 4.1, Figure 4.2, Figure 4.3, and Figure 4.4.**

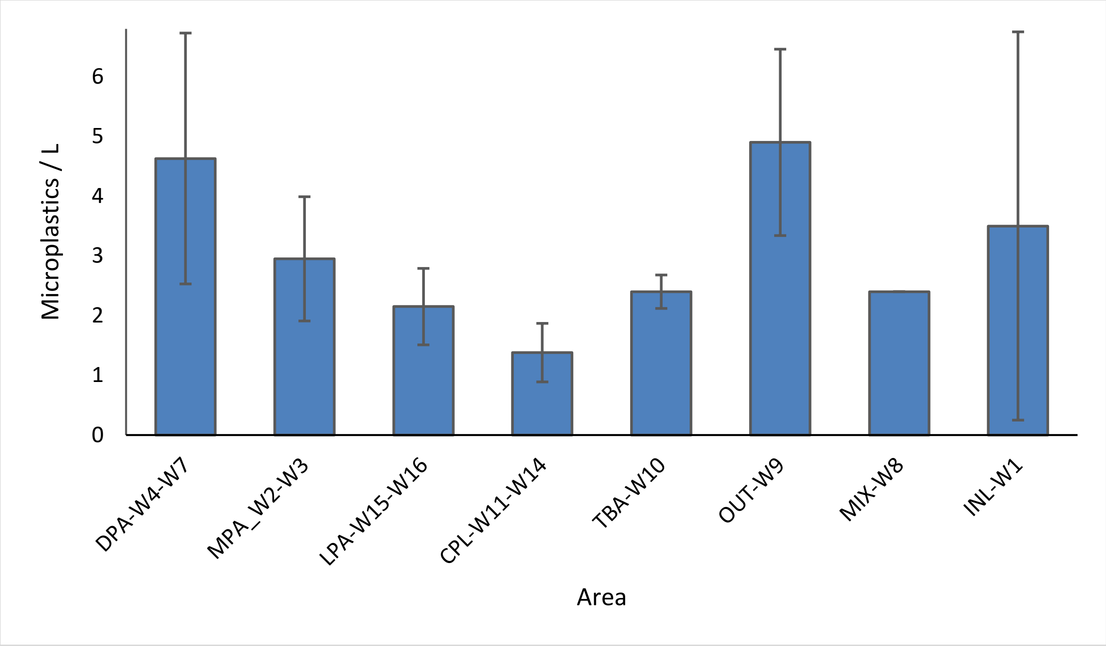

Figure 4.2 Area wise average microplastic abundance in winter season

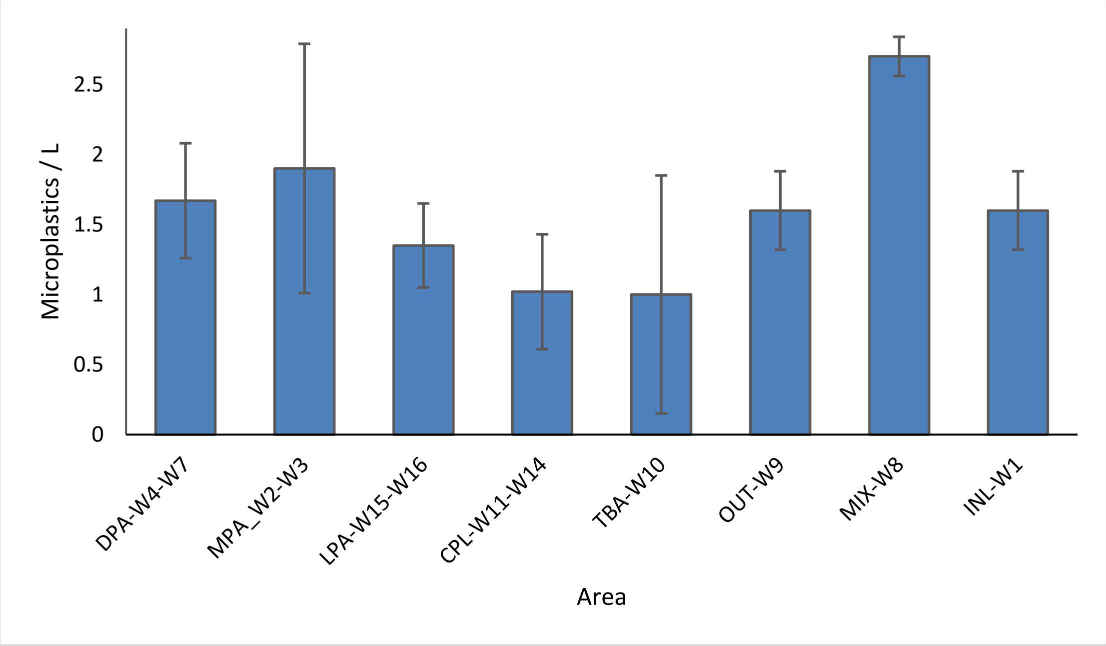

Figure 4.3 Area wise average microplastic abundance in rainy season

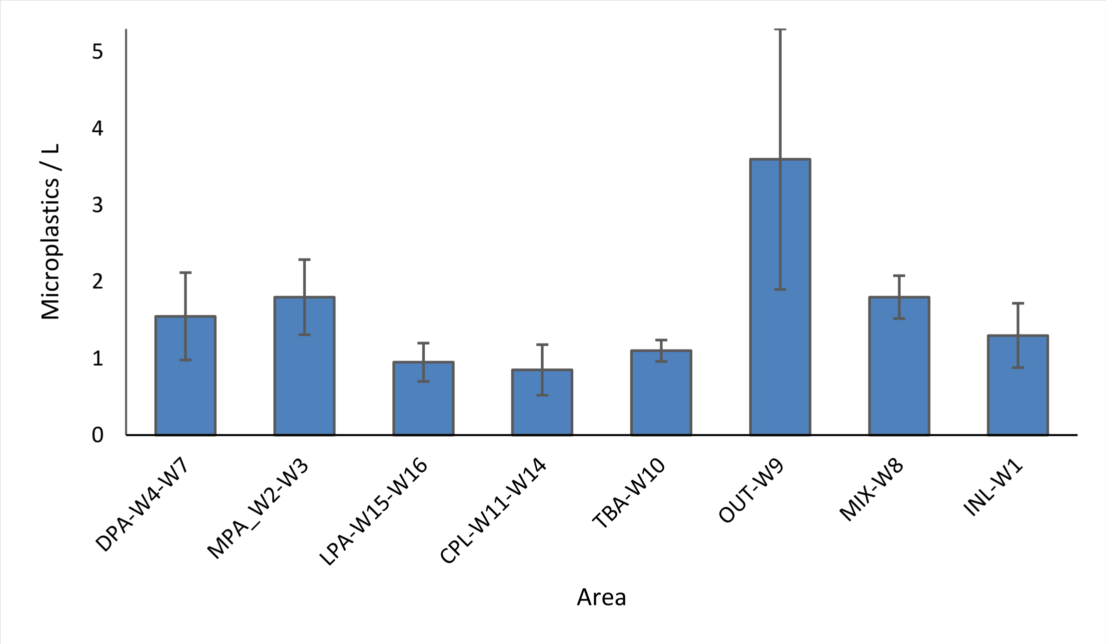

Figure 4.4 Area wise average microplastic abundance in autumn season

The likely sources of microplastic pollution in Phewa Lake is due to untreated domestic sewage and surface runoff from road side which drains near the outlet of lake, discharge of wastewater from hotels and restaurants, unmanaged garbage disposal, abandonment of plastic litter by visitors, fishing activities, laundering, boating activities, agricultural runoff, and most important tourism.

4.4 Morphological characteristics of microplastics in water

4.4.1 Microplastics shape distribution

The identified microplastic of Phewa Lake was classified into four shape categories; fibers/lines, films, foam and fragments. **Figure 4.5** shows photographs of typical microplastics recovered in surface water of Phewa Lake. In winter season, fibers (93.04%) dominated by microplastic type followed by fragments (4.64%) and films (2.32%) (**Figure 4.6**).

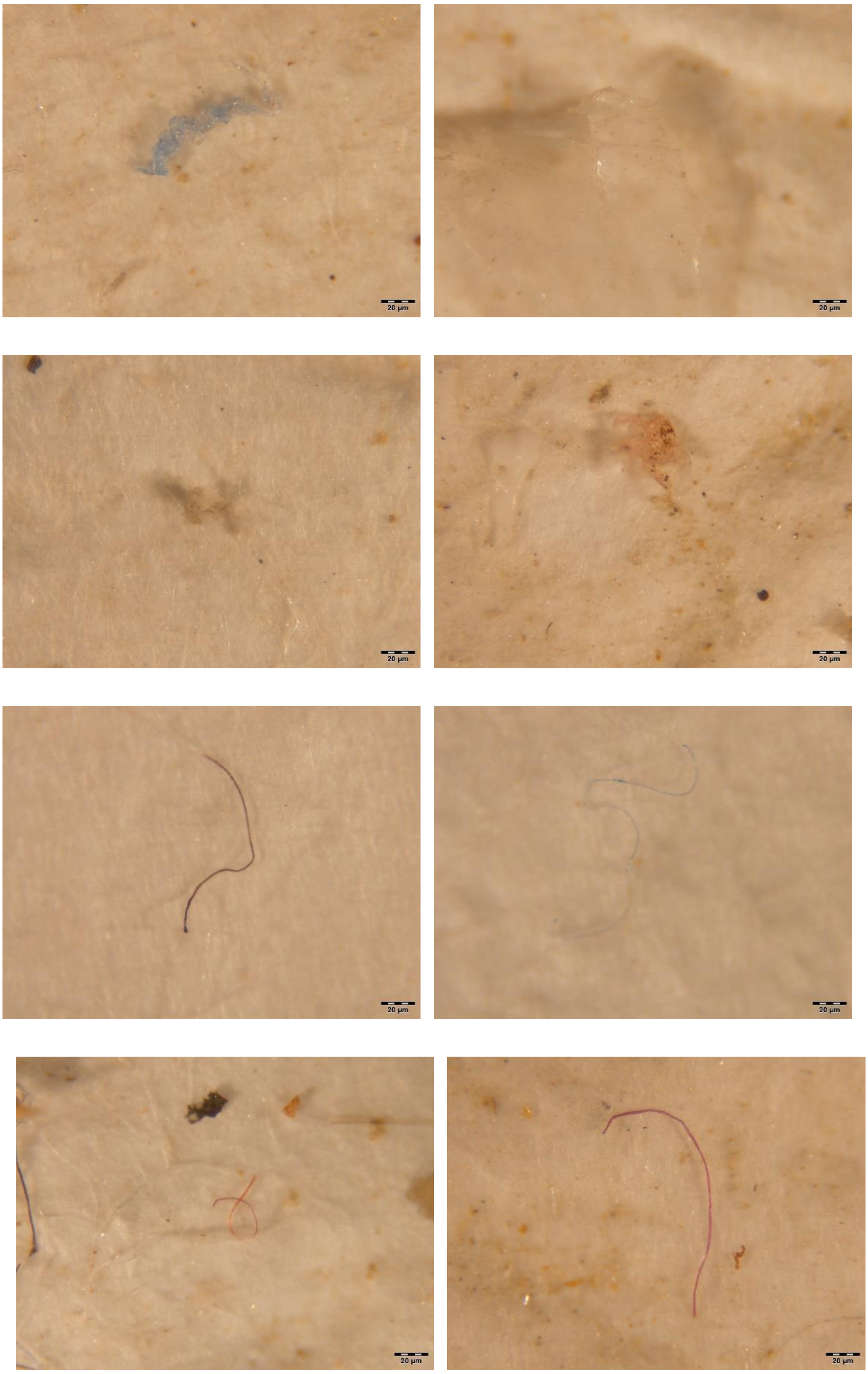

Figure 4.5 Photographs of microplastics recovered from water samples

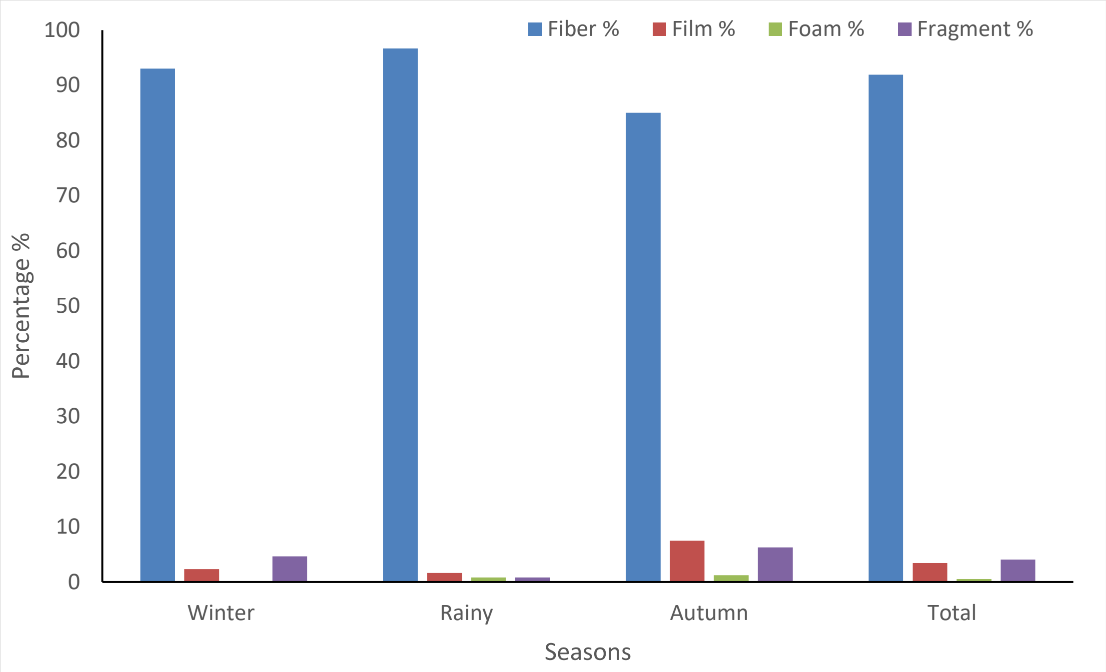

Figure 4.6 Microplastics by type in three seasons.

The microplastic morphology in the rainy season was predominantly fibers (96.69%) followed by films (1.65%) and foam and fragments accounted for 0.83% each **(Figure 4.6).** Similarly, during the autumn season, the fibers was the most abundant shape (85.0%) followed by films (7.5%), fragments (6.25%) and foams (1.25%) **(Figure 4.6).** Fibers were the dominant form of microplastic in all three seasons, which ranged from 0.8 to 5.4 microplastics/L, 0.7 to 2.5 microplastics/L, and 0.6 to 2.8 microplastics/L for winter, rainy and autumn seasons respectively **(Table 4.5)**. Earlier freshwater lake studies such as Lake Mead and Mohave, USA (68.9%) (Baldwin et al., 2020), Lake Simcoe, Canada (82 to 89%), (Felismino et al., 2021), 20 Urban Lakes, Wuhan, China (52.9 to 95.6%) (Wang et al., 2017), Lake Naivasha, Kenya (81%) (Migwi et al., 2020), Dongting Lake (41.9-91.9%) and Hong Lake, China (44.2-83.9%) (Wang et al., 2018), Taihu Lake, China (48-84%) (Su et al., 2016), reported lake water dominated by fibers.

Table 4.5 Seasonal abundance of microplastics according to shape

	Winter season				Rainy season				Autumn season			
	Fiber	Film	Foam	Frag	Fiber	Film	Foam	Frag	Fiber	Film	Foam	Frag
DPA-W7	5.2	0	0	0.9	2.2	0	0	0	1.5	0.3	0	0.2
DPA-W6	2.3	0.2	0	0.3	1.5	0.1	0	0	1.4	0	0	0
DPA-W5	5.4	0.1	0	0.3	1.4	0	0	0	1.4	0.4	0	0.2
DPA-W4	3.3	0.2	0	0.3	1.4	0	0	0.1	1.2	0.1	0	0.1
MPA-W3	2.1	0	0	0.1	1.1	0.1	0	0	2.1	0	0	0
MPA-W2	3.5	0.2	0	0	2.4	0	0.2	0	1.4	0.1	0	0
LPA-W15	2.5	0	0	0	1.3	0	0	0	0.7	0.2	0	0.1
LPAW16	1.8	0	0	0	1.4	0	0	0	1	0.1	0	0
CPL-W11	1.6	0.1	0	0.1	0.7	0	0	0	0.8	0	0	0
CPL-W12	0.8	0.1	0	0	0.9	0	0	0	0.8	0.2	0	0
CPL-W13	1.2	0.1	0	0.1	1.1	0	0	0	0.9	0	0	0.1
CPL-W14	1.4	0	0	0	1.3	0.1	0	0	0.6	0	0	0
TBA-W10	2.4	0	0	0	1	0	0	0	1.1	0	0	0
OUT-W9	4.9	0	0	0	1.6	0	0	0	2.8	0.3	0.2	0.6
MIX-W8	2.2	0.1	0	0.1	2.5	0.1	0	0.1	1.4	0.1	0.1	0.2
INL-W1	3.5	0	0	0	1.6	0	0	0	1.3	0	0	0

The higher proportion of fibers in the water indicates household wastewater discharge (Migwi et al., 2020). As most of the sampling locations are surrounded by hotels and residential areas, domestic effluent discharge into lake water is a source of fibers (Browne et al., 2011) in Phewa Lake. Fibers may also originates from ropes used in boats (Kumar et al., 2021) (around 753 boats are in operation in Phewa Lake), fishing nets (Yuan et al., 2019) and by laundering activities (Browne et al., 2011) by people in the vicinity of the lake. As the concentration of fibers is high in Phewa Lake, risk assessment linked to toxic effects of microplastic fibers on aquatic biota needs further research (Rebelein et al., 2021). During water sampling, plastic wastes were observed at the lake shore and in the water near the sampling sites **(Figure 4.7)**. Therefore, sources of films and fragments in Phewa Lake are the result of the fragmentation of plastic carry bags, plastic wrappers and labels (Nor & Obbard, 2014) and plastic items thrown away by visitors and local people (Eerkes-Medrano et al., 2015). Foam may have likely originated in Phewa Lake due to disintegration of thermocol boxes which are used for fish preservation and transport (Malla-Pradhan et al., 2022). Foam was detected in rainy and autumn season only but not in winter season. Probably as flood occurred prior to the sampling event, it may have flushed the foam as it is lightweight.

Figure 4.7 Plastic wastes at the lake shore and in the water near the sampling sites.

4.4.2 Microplastics color distribution

Seven types of color were observed for microplastics in all three seasons. Transparent was the dominant color for winter, rainy and autumn seasons accounting for 40.5%, 31.40% and 27.92% respectively. It may be due to discoloration during environmental degradation of plastics or digestion of samples while processing for microplastic extraction in the laboratory (Baldwin et al., 2020; Su et al., 2018). The blue color of microplastic is also noted in water samples but the exact sources is unclear though it has been reported in worldwide freshwater studies (Dris et al., 2018; Su et al., 2018). To improve the market appeal of plastic goods, plastics come in variety of colors (Thetford et al., 2003) which may also be the reason for different colors in microplastic particles. The percentage of proportion of various colors observed in Phewa Lake for different season in given in **Figure 4.8.**

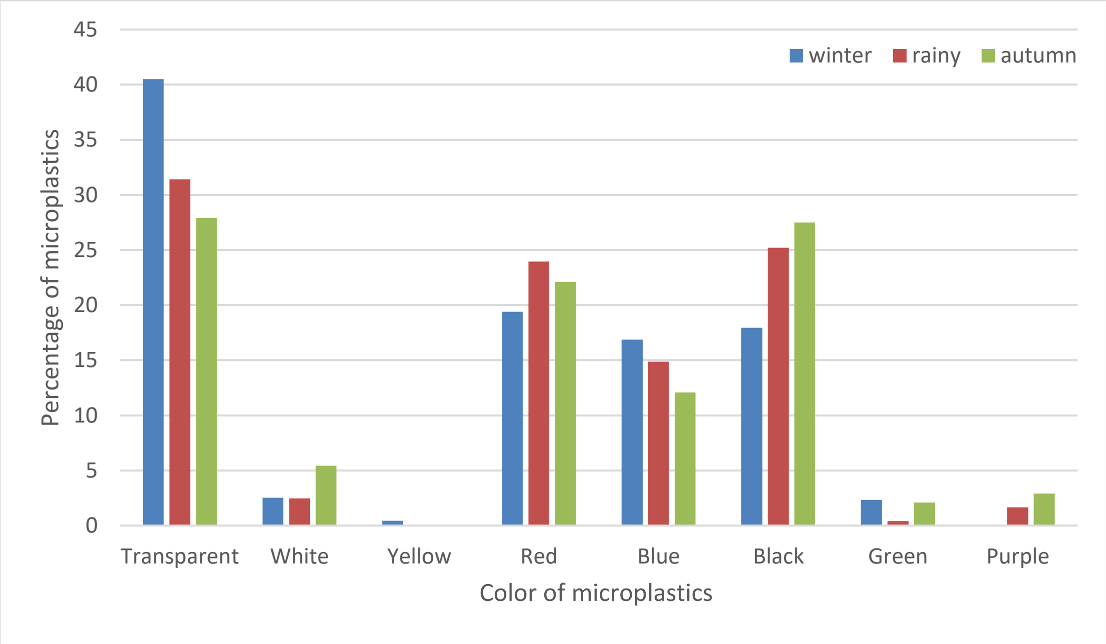

Figure 4.8 Percentage of various colors observed in Phewa Lake for different seasons

4.4.3 Microplastics size distribution

Microplastics were divided into two size categories 1-5 mm and < 1 mm. In winter season, microplastics were found only in size class < 1 mm from all sampling locations. Size spectra < 1 mm was the dominant size classification in all three seasons **(Table 4.6)**. Previous freshwater studies have reported dominance of small sized microplastics in Taihu Lake, China (Su et al., 2016), Qinhai Lake, China (Xiong et al., 2018), Veeranam Lake, India (Bharath et al., 2021) and Rawal Lake, Pakistan (Irfan et al., 2020b). The percentage of microplastic in the size class < 1 mm were 98.76% and 95.42% for rainy and autumn seasons respectively. Microplastics size is influenced by seasonal hydrological condition in which small size microplastic increases in slow discharge condition (de Carvalho et al., 2021). Moreover, the number of microplastics increases with decreasing size of the microplastic (Mao et al., 2020). Biological impact of microplastic is determined by the size of the microplastic (Dhineka et al., 2022).

Table 4.6 Size distribution of microplastics in water sample in percentage

Size	Winter	Rainy	Autumn	Total
< **1** mm	100	98.76	95.42	98.53
1-5 mm	0	1.24	4.58	1.47

4.5 Abundance of microplastics in sediments of Phewa Lake

Microplastics were found in all shoreline sediment samples collected from various sampling locations. The mean microplastic abundance for the whole study period (2021) in Phewa Lake was 88.5±50.32 microplastics/kg. As there is array in the techniques of microplastic sampling, extraction and identification, studies that quantify microplastic using same measurement units in freshwater lake sediments were selected for comparative studies **(Table 4.7)**. Compared with the world's freshwater lake sediments, the microplastic concentrations in the sediment of Phewa Lake was comparable to that of Rawal Lake, Pakistan (104 items/kg) (Irfan et al., 2020b) and Lake Bolsena, Italy (112±32 items/kg) (Fischer et al., 2016). Likewise, microplastic concentrations in the sediment of Songshan Lake, China (244±121 items/kg) (Tang et al., 2022), Lake Chiusi, Italy (234±85 items/kg) (Fischer et al., 2016) and Taihu Lake, China (11-234.6 items/kg) (Su et al., 2016) were one to two order of magnitude higher than that of Phewa Lake. However, the abundance of microplastic in the sediment of Phewa Lake was almost three times higher than that of Red Hills Lake, India (27 particles/kg) (Gopinath et al., 2020), Kodaikanal Lake, India (28.31±5.29 items/kg)

(Laju et al., 2022) and Lake Ulansuhai, China (24±7 to 14±3) (Qin et al., 2020). Overall, the level of microplastic abundance in Phewa Lake is moderate.

Table 4.7 Microplastic abundance measured in items per kg dry weight

Location	Abundance items per kg dry weight	Reference
Dongting Lake, China	West Dongting 320 - 480 South Dongting 200 - 1150	Jiang et al. (2018)
Ox-Bow Lake, Yenagoa, Nigeria	347-4031 dry season 507-7593 rainy season	Oni et al. (2020)
Taihu Lake, China	11-234.6	Su et al. (2016)
Rawal Lake, Pakistan	104	Irfan et al. (2020b)
UK Urban Lake	250-300	Vaughan et al. (2017)
Red Hill Lake, India	27	Gopinath et al. (2020)
Poyang Lake China	1936±121	Jian et al. (2020)
Vesijärvi Lake, Finland	395.5 to 90.7	Scopetani et al. (2019)
Lake Bolsena, Italy	112±32	Fischer et al. (2016)
Lake Chiusi, Italy	234± 85	Fischer et al. (2016)
Taihu Lake, China	460-1380	Zhang et al. (2021)
Lake Mead, USA	87.5-1010	Baldwin et al. (2020)
Renuka Lake, India	15-632	Kumar et al. (2021)
Lake Ulanshuhai, Yellow river basin, China	24±7 to 14±3	Qin et al. (2020)
Anchar Lake, Northwest Himalayan, India	606±360	Neelavannan et al. (2022)
Songshan Lake, Dongguan, China	244±121	Tang et al. (2022)
Kodaikanal Lake, India	28.31±5.29	Laju et al. (2022)
Phewa Lake, Nepal	100.5±58.6	(Present study)

4.6 Spatial variability of shoreline sediments

Microplastic in sediment samples exhibit spatial variability **(Table 4.8)**. The highest amount of microplastic abundance was observed in a sample from location OUT-S8 in winter season (220 microplastics/kg) followed by DPA-S7 in rainy season (175 microplastics/kg). The narrow landscape in the south eastern side may be the reason to concentrate microplastic in the sediment (Yuan et al., 2019) so OUT-S8 recorded the high abundance of microplastics. Likewise, tourism on the eastern side of Phewa Lake may have resulted to a considerable amount of microplastic observed in DPA-S7 sampling site of the lake. The lowest amount of microplastic abundance was observed in a sample from location LPA-S10 (30 microplastics/kg) followed by LPA-S9 (35 microplastics/kg) in autumn season. Both sites are sparsely populated which lies at the southern side of the lake which is covered by forest so relatively less tourism activities compared with the eastern and northern side of the lake.

Table 4.8 Microplastic in sediment samples in three seasons

	Winter season (microplastics/kg)				**Rainy season (microplastics/kg)**				**Autumn season (microplastics/kg)**			
Locations	Mean	SD	min	max	Mean	SD	min	max	Mean	SD	min	max
DPA-S7	80	28.28	60	100	175	7.07	170	180	125	7.07	120	130
DPA-S6	135	77.78	80	190	100	42.43	70	130	100	14.14	90	110
DPA-S5	100	14.14	90	110	150	84.85	90	210	115	7.07	110	120
DPA-S4	135	35.36	110	160	65	21.21	50	80	45	21.21	30	60
MPA-S3	75	35.36	50	100	50	0.00	50	50	45	7.07	40	50
MPA-S2	90	42.43	60	120	120	14.14	110	130	45	21.21	30	60
LPA-S9	65	35.36	40	90	45	7.07	40	50	35	7.07	30	40
LPA-S10	60	42.43	30	90	75	21.21	60	90	30	14.14	20	40
INL-S1	45	21.21	30	60	55	7.07	50	60	45	7.07	40	50
OUT-S8	220	28.28	200	240	100	14.14	90	110	130	14.14	120	140
Total	100.5	57.72	30	240	93.5	48.91	40	210	71.5	40.69	20	140

Based on geographical directions, the ten sampling locations of Phewa Lake was further divided into five areas as western side (INL-S1) which is the inlet to Phewa Lake. Harpan Khola (stream) is the source (about 70%) of water for Phewa Lake which mixes at this sampling site. Northern side (MPA-S2-S3), which is moderately populated where the people in this region are engaged in agriculture and also cage fish culture is located in this part of lake. Similarly, the eastern side (DPA-S4-S7) is densely populated as this is one of the main tourist hub of the lake which is surrounded by hotels and restaurants. Likewise, south eastern side (OUT-S8) is the outlet where the water is diverted for power generation and irrigation. Finally, the southern side (LPA-S9-S10) is sparsely populated area and the periphery is covered with forest**.** During the entire sampling period (2021) the OUT-S8 recorded the highest abundance of microplastic of 150 microplastics/kg followed by DPA-S4-S7 (110.42 microplastics/kg) **(Table 4.9).** Gopinath et al. (2020) also noted high amount of microplastics at the dam area in Red Hills Lake. Moreover, discharge of domestic effluent (Qin et al., 2020) and surface runoff from nearby areas (Yuan et al., 2019) near the outlet region may contribute to high microplastic abundance in the sediments of Phewa Lake at this area. DPA-S4-S7 lies in a densely populated area; as previous studies have pointed out that level of microplastic concentrations is linked to urbanization and population density. Similarly, the lowest microplastics abundance in sediment was found at INL-S1 (48.33 microplastics/kg) which is about 2 order of magnitude lower than the abundance observed at OUT-S8 **(Table 4.9).** Thus, indicating that Phewa Lake receives microplastics primarily from other locality that have more human interference. A similar finding was reported in Qinghai Lake indicating that riverine input was a secondary key source of microplastic compared to other non-point sources (Xiong et al., 2018).

Table 4.9 Microplastic in sediment samples in different areas

	Winter		**Rainy**		**Autumn**		**Total**	
Location	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DPA-S4-S7	112.5	42.68	122.5	58.74	96.25	34.62	110.42	45.73
MPA-S2-S3	82.5	33.04	85.0	41.23	45.0	12.91	70.83	34.23
LPA-S9-S10	62.5	32.02	60.0	21.60	32.5	9.57	51.67	25.17
INL-S1	45.0	21.21	55.0	7.07	45.0	7.07	48.33	11.69
OUT-S8	220.0	28.28	100.0	14.14	130.0	14.14	150	57.97
Total	100.5	57.72	93.5	48.91	71.5	40.69	88.5	50.32

Since the abundance of microplastics in sediments did not follow normality and equality of variance, Kruskal-Wallis H test was applied to find out whether there was significance difference in abundance of microplastics in sediments between different areas of the lake. The result indicated that the distribution of average abundance of microplastics in sediment was significantly different across the different areas with H value 26.96 and p value < 0.01 **(Table 4.10)**. Similarly, a pair wise comparison was done to find which pair had significance difference in abundance of microplastics. The result indicated that the average abundance of microplastic in sediment of inlet (INL-S1) and least populated area (LPA-S9-S10) were significantly different with densely populated area (DPA-S4-S7) and outlet area (OUT-S8) **(Table 4.11)**. DPA-S4-S7 and OUT-S8 are areas with high anthropogenic activities like recreational boating, laundering, tourism activities and visitors littering at the shore with junk food wrappers. Thus, these areas have high microplastic concentrations as anthropogenic activities substantially contribute to plastic pollution (Cole et al., 2011). Likewise, there was a significance difference in abundance of microplastics of moderately populated area (MPA-S2-S3) with outlet area (OUT-S8) as studies have reported an increase of microplastic abundance towards the outlet (Zhang et al., 2015).

Table 4.10 Kruskal-Wallis H Test for area wise significance difference of microplastics in water samples

Null Hypothesis	H-value	Sig.	Decision
The distribution of MPs/kg is the same across categories of Area.	26.963	0.00	Reject the null hypothesis. (Significance Difference)

Table 4.11 Pairwise comparisons of area wise distribution of microplastics

Pair (1-2)	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
INL-S1 - LPA-S9-S10	0.042	8.695	0.005	0.996	1.000
INL-S1 - MPA-S2-S3	9.833	8.695	1.131	0.258	1.000
INL-S1 - DPA-S4-S7	23.583	7.938	2.971	0.003	0.030
INL-S1 - OUT-S8	-33.417	10.041	-3.328	0.001	0.009
LPA-S9-S10 - MPA-S2-S3	9.792	7.100	1.379	0.168	1.000
LPA-S9-S10 - DPA-S4-S7	23.542	6.149	3.829	0.000	0.001
LPA-S9-S10 - OUT-S8	-33.375	8.695	-3.838	0.000	0.001
MPA-S2-S3 - DPA-S4-S7	13.750	6.149	2.236	0.025	0.253
MPA-S2-S3 - OUT-S8	-23.583	8.695	-2.712	0.007	0.067
DPA-S4-S7 - OUT-S8	-9.833	7.938	-1.239	0.215	1.000

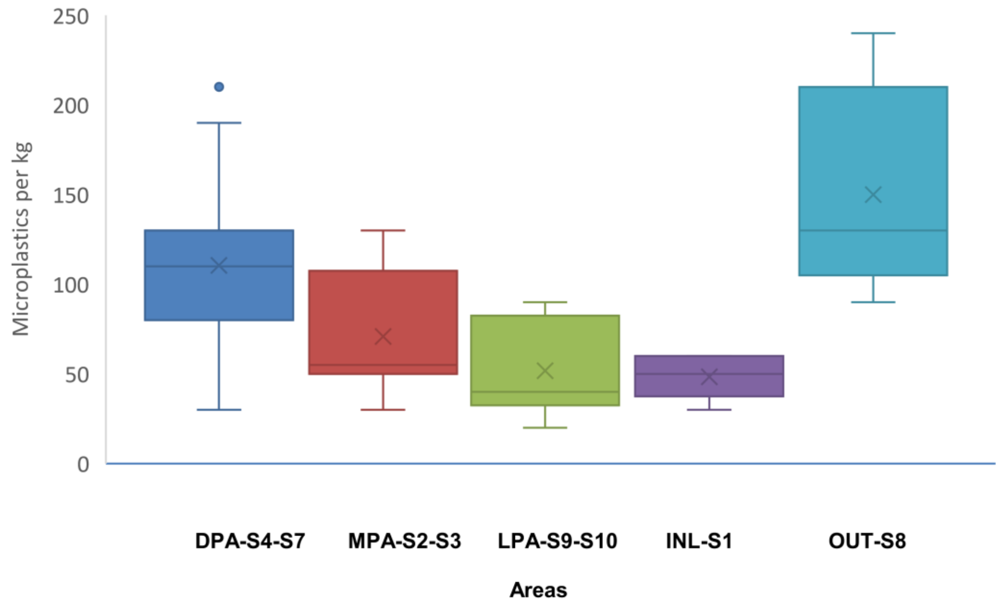

Figure 4.9 Boxplot of area wise distribution of microplastics

4.7 Temporal variability of shoreline sediments in Phewa Lake

Microplastic was identified in the shoreline sediments of Phewa Lake with abundance ranging from 30 to 240 microplastics/kg for winter (dry) season, 40 to 210 microplastics/kg for rainy (monsoon/wet) season and 20 to 140 microplastics/kg for autumn (post monsoon) season respectively. Likewise, the average microplastic abundance in sediment for winter, rainy and autumn seasons are 100.5 microplastics/kg dry weight, 93.5 microplastics/kg dry weight and 71.50 microplastics/kg dry weight respectively **(Table 4.8).** To find the significant difference of microplastic concentrations of sediment in different seasons of Phewa Lake, Kruskal-Wallis H test was done. The results indicated that the mean concentrations of microplastic in different seasons were not significantly different (H=3.17, p value =0.205) **(Table 4.12)** in Phewa Lake shoreline sediments. Contrastingly, Hengstmann et al. (2021) reported significance difference in microplastic concentrations between sampling season in Lake Tollense, Germany for shoreline sediments. However, reliable data for comparison of temporal trends of microplastic concentration in sediment of freshwater lake is scare (Onoja et al., 2022).

Table 4.12 Kruskal-Wallis H Test for area wise significance difference of microplastics in sediment samples

Null Hypothesis	H-value	Sig.	Decision
The distribution of MPs/kg is the same across different seasons.	3.17	0.205	Accept the null hypothesis. (No Significance Difference)

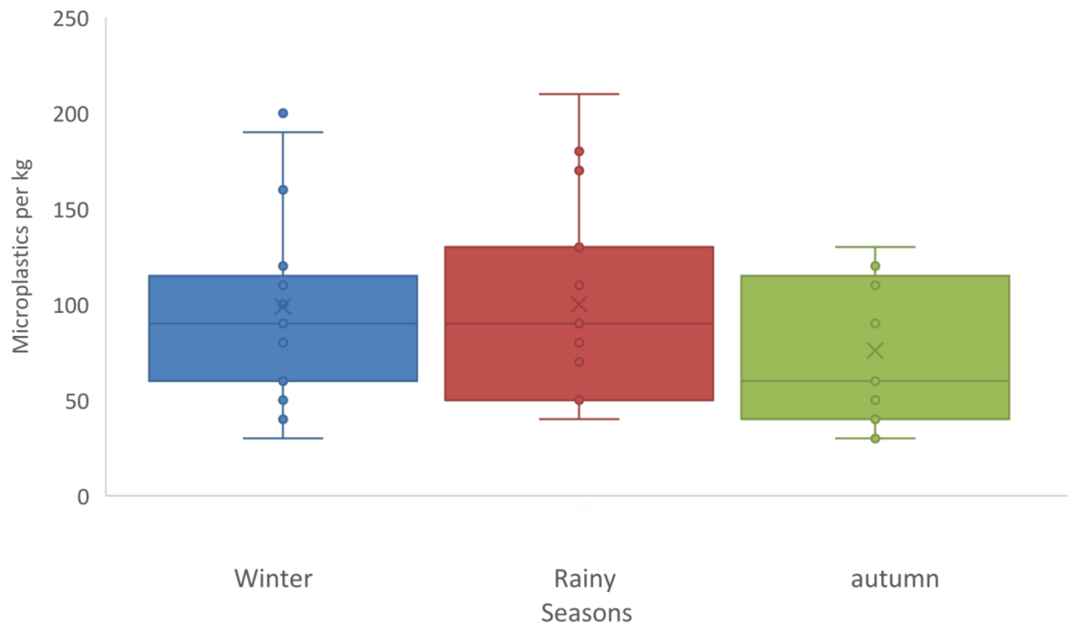

Figure 4.10 Boxplot of season wise distribution of microplastics

In winter season, the highest mean abundance of microplastic was observed at OUT-S8 (220 microplastics/kg) which lies at the south east end of Phewa Lake. A dam controls the outflow of water in Phew Lake which is used for hydropower generation and irrigation. The narrow topographical feature at the outlet area may be a factor to concentrate microplastics in sediments (Yuan et al., 2019) as previous studies have pointed out that shoreline morphology can influence the level of microplastic concentrations (Ballent et al., 2016). Likewise, Phirke Khola (stream) carrying domestic effluent and surface runoff from urban areas drains into Phewa Lake near the outlet area which may increase the load of microplastics at this area (Malla-Pradhan et al., 2022). Similarly, in DPA-S4-S7, the mean abundance of shoreline microplastic was also high (112.50 microplastics/kg). This area lies at the eastern side of Phewa Lake where tourism activities is maximum. Heavy recreational boating and greater tourism inflow in this area resulted in increased plastic waste deposited at the lake shoreline (Kumar et al., 2021). This in turn increased the concentrations of microplastics. Previous freshwater studies pointed that tourism as a source of microplastic pollution (Free et al., 2014; Xiong et al., 2018) which is also in case of Phewa Lake. The lowest mean microplastic abundance of 45 microplastics/kg dry weight was recorded at INL-S1. This area lies at the western side of the lake where human interference is negligible.

In rainy season, the highest mean microplastic abundance was observed at DPA-S4-S7 (122.50 microplastics/kg) which was nearly two times less than the highest concentration recorded at OUT-S8 (220 microplastics/kg) for winter season. Though the recreational activities around the Phewa Lake was closed down due to COVID-19 pandemic from March- April (2021) to the starting of rainy season, the concentration of microplastic in a densely populated eastern side was high. The possible reason may be that during rainy season, surface runoff from the adjacent area may have led to inflow of microplastics from land to water body (Lima et al., 2014). Moreover, previous studies have pointed that concentrations of microplastic in lakeshore sediments are higher in densely populated areas (Baldwin et al., 2020; Faure et al., 2015). Likewise, the rainy season also recorded that lowest microplastic abundance as winter season in the same site (INL-S1) but the concentration was a bit higher (55 microplastics/kg).

In autumn season, the highest mean abundance of microplastic in shoreline sediment was observed in the same area as winter season but the concentration of microplastic was almost two times less (130 microplastics/kg) than that of winter season (220 microplastics/kg). Likewise, the lowest mean microplastic abundance was observed at least populated southern side of the lake, LPA-S9-S10 (32.50 microplastics/kg). This area is sparsely populated with limited tourism activities. Moreover, the periphery of this area is covered with forest. In lake shoreline sediments of Phewa Lake, variation in the mean microplastic abundance were not observed between seasons. The temporal variation of average microplastic concentrations of shoreline sediments in different areas of Phewa Lake is shown in **Table 4.8** and **Table 4.9.**

4.8 Morphological characteristics of microplastics in shoreline sediments

4.8.1 Shape of microplastics

Extracted microplastics from shoreline sediment samples of Phewa Lake were classified into fibers/lines, films, foam and fragments based on morphology through visual sorting under a stereomicroscope. Photographs of typical microplastics recovered from shoreline sediments of Phewa Lake is given in **Figure 4.11.**

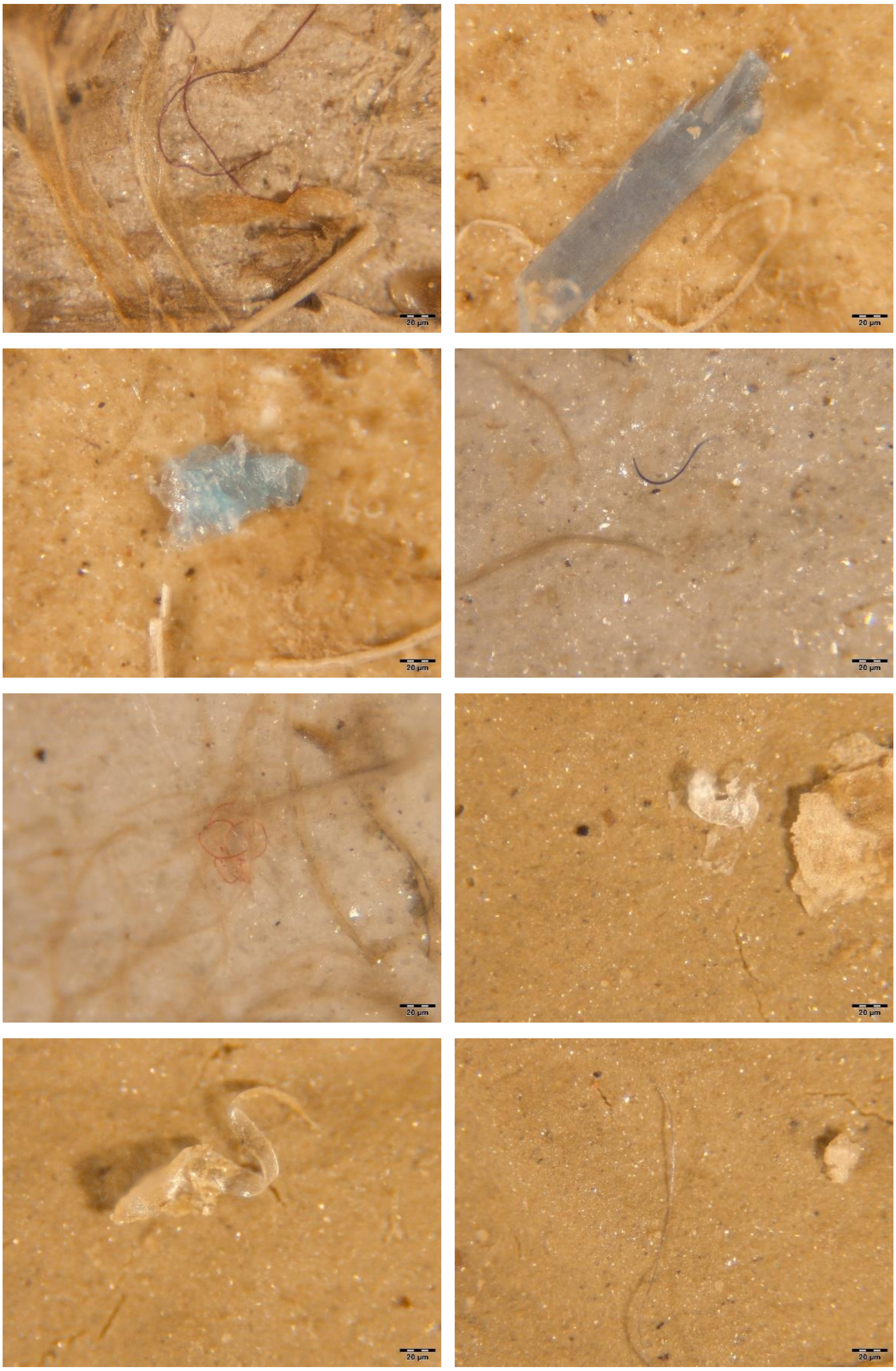

Figure 4.11 Photographs of microplastics recovered from sediment samples

In winter season, fibers (78.11%) were the most abundant type of microplastics in shoreline sediment samples of Phewa Lake, followed by fragments (9.95%), foam (6.97%) and films (4.98%). The microplastic morphology in the rainy season was dominated by fibers (62.03%), followed by fragments (26.20%), films (6.95%) and foam (4.81%). Similarly, in autumn season, fibers (41.26%) was the most abundant microplastic type, followed by films (34.97%), fragments (19.58%) and foam (4.20%) **(Figure 4.12)**.

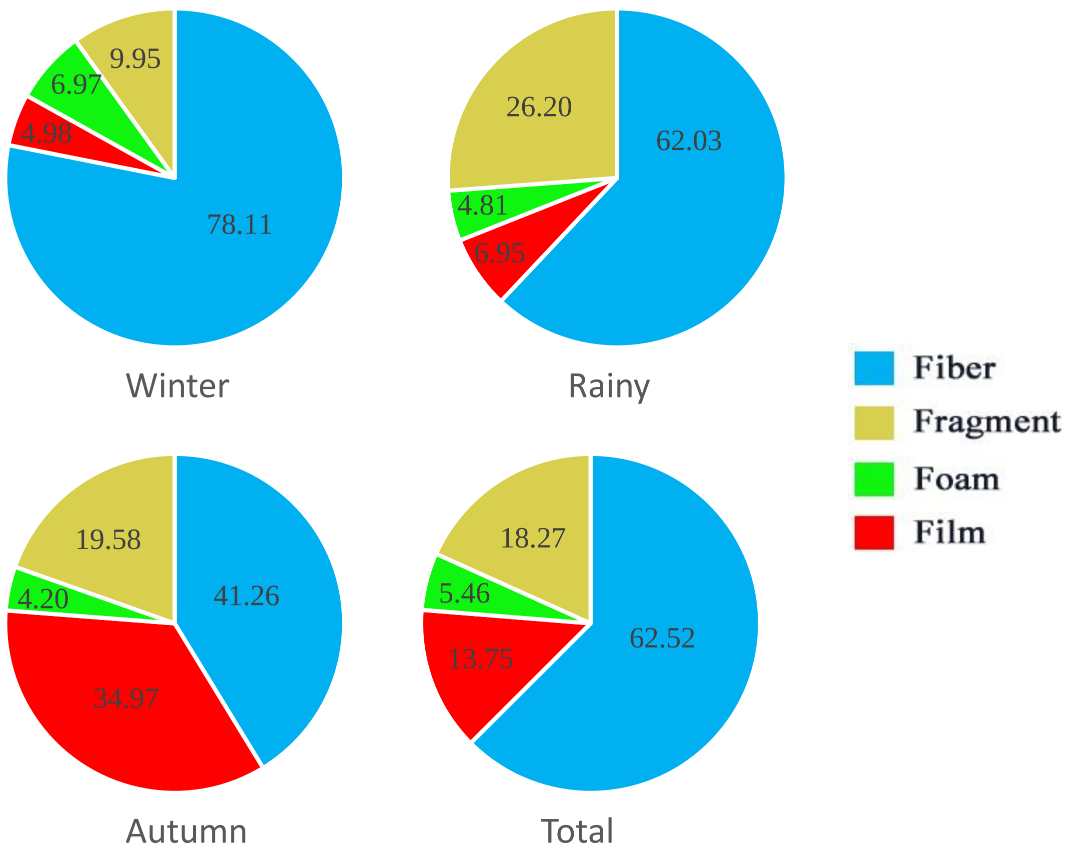

Figure 4.12 Types of microplastics in three different seasons

Like surface water, fibers were also the dominant type of microplastic in sediment for all three seasons in Phewa Lake; which ranged from 60 to 250 microplastics/kg, 60 to 200 microplastic/kg and 30 to 110 microplastics/kg for winter, rainy and autumn seasons respectively **(Table 4.13)**. During the entire sampling period (2021), fibers were the most dominant type of microplastic (62.52%) in shoreline sediments of Phewa Lake, followed by fragments (18.27%), films (15.75%) and foam (5.46%) **(Figure 4.13)**.

Table 4.13 Types of microplastics per kg sediment in three different seasons

	Winter season				Rainy season				Autumn season			
	Fiber	Film	Foam	Frag	Fiber	Film	Foam	Frag	Fiber	Film	Foam	Frag
DPA-W7	140	0	0	20	200	40	20	90	50	50	0	150
DPA-W6	220	0	0	50	140	0	0	60	50	110	0	40
DPA-W5	140	30	0	30	200	10	30	60	90	80	30	30
DPA-W4	240	0	0	30	80	20	0	30	60	20	0	10
MPA-W3	150	0	0	0	90	0	0	10	30	60	0	0
MPA-W2	160	10	10	0	150	0	40	50	30	40	10	10
LPA-W15	100	0	0	30	60	0	0	30	40	20	10	0
LPA-W16	110	10	0	0	120	0	0	30	50	0	10	0
OUT-W9	250	20	130	40	60	50	0	90	110	110	0	40
INL-W1	60	30	0	0	60	10	0	40	80	10	0	0

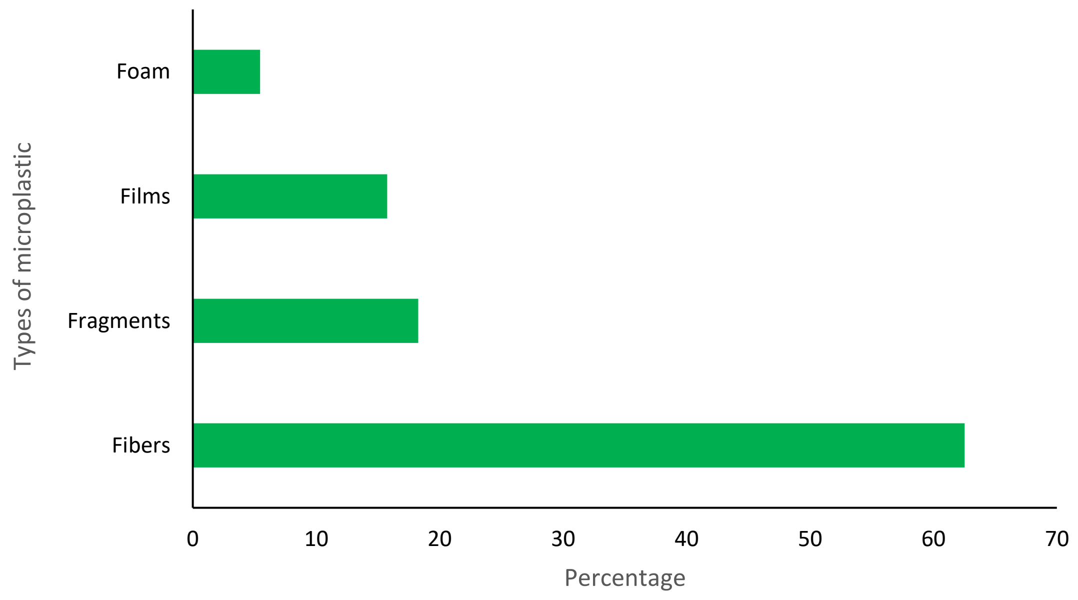

Figure 4.13 Percentage of different shapes of microplastics during sampling period (2021)

Previous freshwater studies also reported lake sediments dominated by fibers (Baldwin et al., 2020; Jiang et al., 2018; Neelavannan et al., 2022; Qin et al., 2020; Su et al., 2016) **(Figure 4.14).**

High proportion of fibers in the sediment may be linked to domestic waste input as studies by Browne et al. (2011) pointed out that a single cloth produces more than 1900 fibers per washing. Fisheries may be a source of fibers (Yuan et al., 2019) to Phewa Lake as the northern side of the lake is flourished with fish cage culture and the “Jalhari” community living at the periphery of Phewa Lake make their living by catching fish. Likewise, another possible pathway of fibers to Phewa Lake is the use of ropes in recreational boating (Davidson & Dudas, 2016; Irfan et al., 2020b; Kumar et al., 2021) and washing clothes (Browne et al., 2011) in the vicinity of the lake. During the sampling events, plastic debris like plastic carry bags, water and soft drinks bottles, plastic wrappers and labels, plastic sacks and other plastic items were observed at the shores of Phewa Lake **(Figure 4.14)** abandoned by visitors. Therefore, those plastic after degradation may have possibly been the source of films and fragments in Phewa Lake. Previous studies have also reported similar findings (Hengstmann et al., 2021; Schell et al., 2021). Sources of foam in Phewa Lake is likely due to disintegration of thermocol boxes which are used for fish preservation and transportation (Malla-Pradhan et al., 2022). Foam were observed in all three seasons from sampling site MPA-S2, as this area is where fish cage culture is located. However, further studies on the potential origin of freshwater microplastics are needed (Jian et al., 2020; Qin et al., 2020).

Figure 4.14 Plastic debris like plastic carry bags, water and soft drinks bottles, plastic wrappers and labels, plastic sacks and other plastic items observed in sampling sites.

Table 4.14 Microplastic abundance measured in shape and polymer type

Location	Shape	Polymer type	Reference
Dongting Lake, China	Fiber	PET	Jiang et al. (2018)
Ox-Bow Lake, Yenagoa, Nigeria	Beads and pellets	PET dry season PVC rainy season	Oni et al. (2020)
Taihu Lake, China	Fibers	Cellophane	Su et al. (2016)
Rawal Lake, Pakistan	Fibers and fragments	PE, PP	Irfan et al. (2020b)
UK Urban Lake	Fibers and films	N/A	Vaughan et al. (2017)
Red Hill Lake, India	Fibers and fragments	PE, PP	Gopinath et al. (2020)
Poyang Lake China	Fragments & fibers	PP, PVC, PE	Jian et al. (2020)
Vesijärvi Lake, Finland	Fibers	Polyamide	Scopetani et al. (2019)
Lake Bolsena, Italy	Fibers	N/A	Fischer et al. (2016)
Lake Chiusi, Italy	Fibers	N/A	Fischer et al. (2016)
Taihu Lake, China	Fragments	PVC, PE	Zhang et al. (2021)
Lake Mead, USA	Fibers	N/A	Baldwin et al. (2020)
Renuka Lake, India	Fragments	PE, PS	Kumar et al. (2021)
Lake Ulanshuhai, Yellow river basin, China	Fibers	PE,PET, PP	Qin et al. (2020)
Anchar Lake, Northwest Himalayan, India	Fiber, fragments, film	Polyamide, PET	Neelavannan et al. (2022)
Songshan Lake, Dongguan, China	Films, fragments	PE, PP	Tang et al. (2022)
Kodaikanal Lake, India	Fragments, films	PE, PP	Laju et al. (2022)
Phewa Lake, Nepal	Fibers	PP, PE	(Present study)

4.8.2 Colors of microplastics

Seven types of color were observed in winter and rainy season and eight types of color in autumn season. Transparent was the dominant color for winter and rainy seasons accounting for 26.37% and 23.53% respectively. Whereas, for autumn season, white color dominated accounting for 36.36% followed by transparent (27.97%). Yellow and purple color were the least encountered color in sediment of Phewa Lake. Yellow color were recovered from site DPA-S7, DPA-S5 only which may be the result of discoloration during environmental degradation (Baldwin et al., 2020; Su et al., 2018). Purple color were observed in INL-S1, LPA-S9-S10 which lies at the western and southern side of the lake. As plastic goods come in various colors (Thetford et al., 2003), it may also be a reason for diverse color seen in microplastics.

The percentage proportion of various colors observed in the shoreline sediments of Phewa Lake for different seasons is given in **Figure 4.15.** Color of microplastics may pose a serious threat to aquatic organism as they may mistake it for food (Wright et al., 2013).

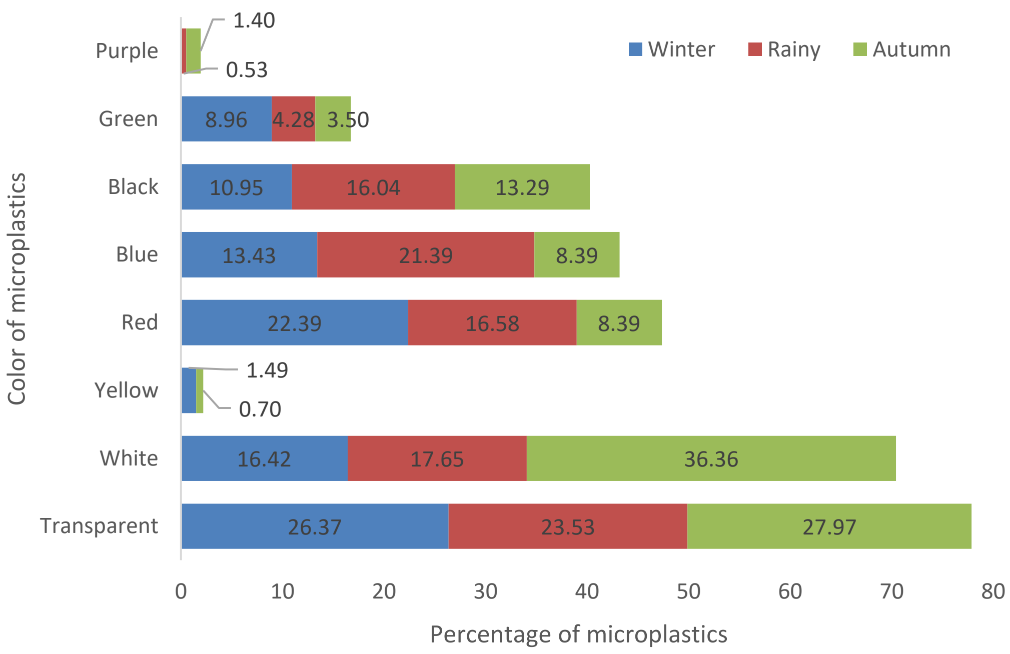

Figure 4.15 Different colors of microplastics observed in shoreline sediments of Phewa Lake in different seasons

4.8.3 Size of microplastics

Microplastic extracted from shoreline sediments were divided into two size categories: 0.2 to 1 mm and 1-5 mm. In winter season, the microplastics was mainly concentrated in the range of 0.2 to 1 mm accounting for 70.65%. In all three seasons the size range of 0.2-1 mm showed the highest abundance of microplastics **(Table 4.15)**. Many freshwater lake studies have reported dominance of small size microplastics in Rawal Lake, Pakistan (Irfan et al., 2020b), Dongting Lake, China (Jiang et al., 2018), Anchar Lake, India (Neelavannan et al., 2022), Lake Bolsena and Lake Chiusi, Italy (Fischer et al., 2016). In rainy and autumn seasons, the percentage of microplastics in sediment samples were 80.75% and 59.44% respectively in the size class 0.2 to 1 mm. During the sampling period of 2021, the highest percentage of microplastic was found in the size class 0.2 to 1 mm (71.19%) **(Table 4.15)**. The number of microplastic increases as the size of microplastics decreases in sediment (Xia et al., 2021) which has higher chances of being ingested by various organisms (Prokić et al., 2019). The microplastic can be ingested by lower trophic organisms in additional to benthic organisms as long as the size of microplastic is similar or even smaller than that of the sediments (Wong et al., 2020).

Table 4.15 Size distribution of microplastics in different seasons

Size	**Seasons**			
	Winter	Rainy	Autumn	Average
0.2 to 1 mm	142 (70.65)	151 (80.75)	85 (59.44)	126 (71.19)
1 to 5 mm	59 (29.35)	36 (19.25)	58 (40.56)	51 (28.81)

*parenthesis indicate the percentage of different size

4.8.4 Polymer composition of microplastics

Due to its high reliability in determining the chemical composition of unknown plastic particles, the FTIR spectroscopy has been extensively used for the microplastic polymer identification (Hidalgo-Ruz et al., 2012), so identification of polymer composition of microplastics from the sediment samples of Phewa Lake was validated using FTIR. In winter season, five types of polymers were identified: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC) based on the appearance of characteristics peaks reported in literature (Chércoles Asensio et al., 2009; Jung et al., 2018; Noda et al., 2007; Verleye et al., 2001). PP made up the highest proportion, accounting for 47.37% in the shoreline sediment samples of Phewa Lake, followed by PE (26.32%) and PS (15.79%) **(Figure 4.16).** The polymer composition in the rainy season was dominated by PP (37.5%), followed by PE (31.25%) **(Figure 4.16)**. Similarly in autumn season, 50% of the sediment samples was dominated by PP polymer, followed by PE (33.33%), PS (11.11%) and PET (5.56%) **(Figure 4.16)**. The above data represents only 31-44% of the total samples collected from size 1-5 mm that was identified using FTIR due to limited cost. In all three seasons, PP and PE dominated in the shoreline sediments of Phewa Lake as plastic production and use is also dominated by PP and PE polymers (PlasticsEurope, 2021). Previous studies also reported PP and PE as common type of polymers found in freshwater sediments (Irfan et al., 2020b; Laju et al., 2022; Tang et al., 2022) **(Table 4.14)**. PP and PE are lower density polymers that floats in water but it can be transported to lakeshore sediments by current and wave actions (Zhang et al., 2016). As polypropylene has good plasticity and stability, it is extensively used in daily life for food packaging, wrappers used in sweet and snack, bottle caps and containers for microwave (PlasticsEurope, 2021). Polyethylene is the main component for the production of carry bags, packaging materials, nets used for fishing and agricultural films (Wang et al., 2019c). Likewise, polystyrene are widely used in packaging, transportation and decoration (Auta et al., 2017). PET is used in the production of beverage bottles and liquid containers (Su et al., 2016) which was seen lying on the shoreline and on the water surface of Phewa Lake during the sampling events **(Figure 4.14)**. PVC are widely used as water supply and drainage pipes, cable insulators and garden hoses (PlasticsEurope, 2021). Acrylonitrile butadine styrene (ABS) are used in decorative works, wheel covers and air conditioning parts (Vishwakarma et al., 2017).

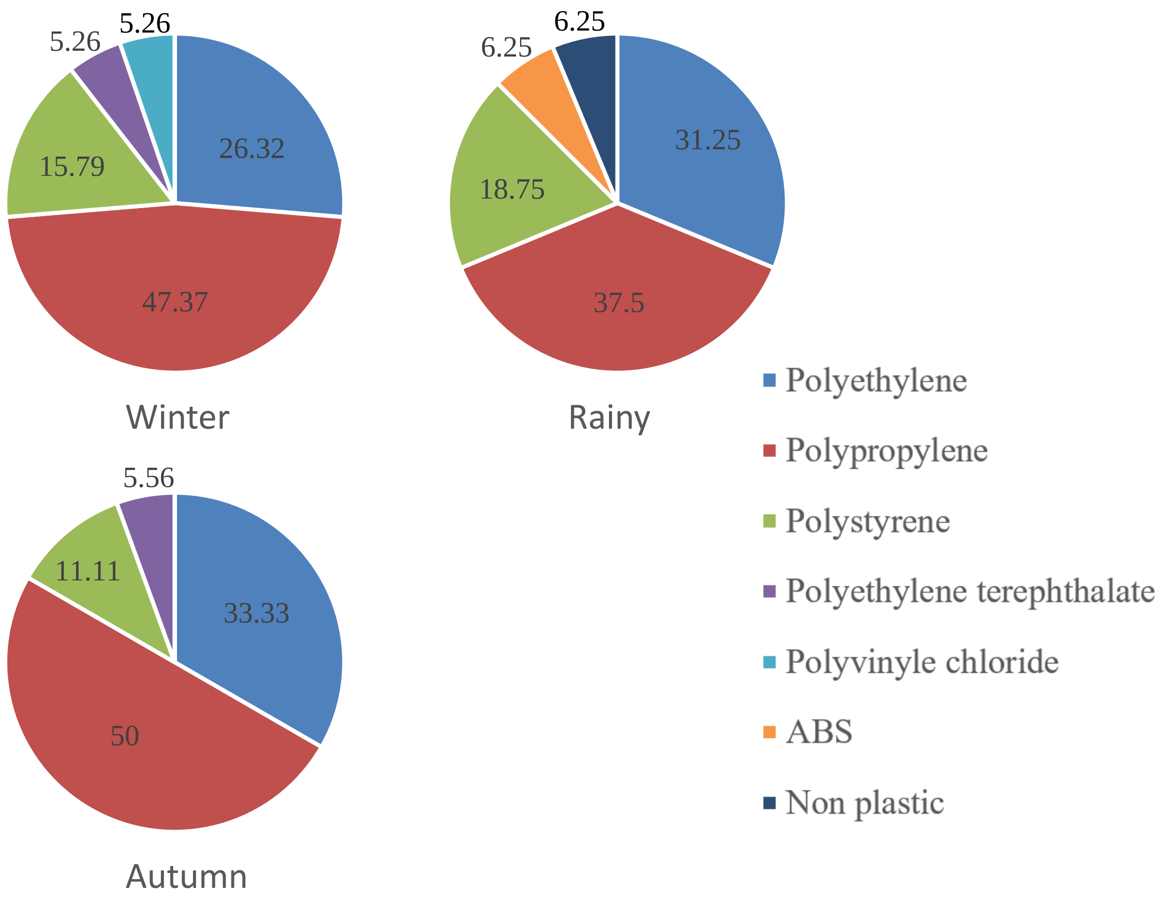

Figure 4.16 Polymer composition of plastics in three seasons

To get the desired characteristics, the chemicals (additives) are added to plastics (Andrady & Neal, 2009) which may further absorb harmful chemicals from the surrounding environment (Velzeboer et al., 2014) posing a serious threat to aquatic organism (Yu et al., 2020c).

4.9 Correlation of microplastic abundance between water and sediments

Microplastics abundance in water is weakly correlated with microplastic abundance in sediment with Pearson's correlation $r=0.355$ (p value<0.01). Red Hills Lake, India (Gopinath et al., 2020) and Poyang Lake, China (Jian et al., 2020) reported no correlation between the microplastic abundance in water and sediments. River erosion and sediment precipitation can transfer microplastic from water to sediments. Likewise, re-suspension of microplastics from sediments to water column may also take place (Wu et al., 2019).

4.10 Physicochemical analysis of water quality

Due to their major impact on the water quality status, the physicochemical parameters are regarded as an essential attributes as aquatic life directly depends on it (Rahman et al., 2021). pH is a mathematical expression denoting the extent to which the water is acidic or alkaline. The highest pH value observed in rainy season (8.18) and lowest pH was observed in autumn season (7.67) reflecting almost neutral to slightly alkaline in nature. Similar observation were also noted by Dadwal et al. (2014); Ongom et al. (2017); Saturday et al. (2021) in Lake Bunyonyi, Lake Kyoga and Lake Sukhna respectively. A low pH makes water corrosive and a high pH value may affect skin and eyes and taste complain (Rao & Nageswararao, 2010).

Electrical conductivity (EC) is a measure of the total concentration of soluble salts and is directly related to the total dissolved salts in the solution (Ravikumar et al., 2013). The average EC values in three seasons ranged from 52.63 µS/cm to 83.56 µS/cm which is well within the permissible limit **(Table 4.16)**. The water of Phewa Lake was not substantially ionized as a result, comparatively less ionic concentration level was noticed.

Total dissolved solids (TDS) measures the amount of inorganic salts and organic matter dissolved in water (WHO, 2017). The TDS varied between 26.38 mg/L and 41.94 mg/L recorded in rainy and winter season respectively. The TDS value of Phewa Lake were below the permissible level 1000 mg/L recommended by (CBS, 2019) showing no effects on aquatic habitat.

Nitrate is a vital plant nutrient found naturally in the surrounding and may enter surface water through agricultural runoff, sewage disposal laden with human and animal excreta (WHO, 2017). The mean nitrate in winter season was 1.13 mg/L, which was more than the other two seasons but the observed concentration indicate less pollution load in Phewa Lake.

Dissolved oxygen (DO) provides on overview of the quality of water which relay on factors like activities of microorganism, temperature of the water body, load of organic matter and the time of sampling (Das Kangabam & Govindaraju, 2019). DO values ranged from 6.58 mg/L in autumn and 7.91 mg/L in winter and mean of 7.2 mg/L. If DO decreases to less than 5 mg/L, it may bring stress to aquatic life.

Alkalinity is the capability of water to neutralize acids. Alkalinity values varies between 44.13 mg/L and 72.63 mg/L obtained in winter and autumn seasons respectively. Autumn season observed high alkalinity but Tyor and Chawla (2012) reported high alkalinity in rainy season in Lake Sukhna.

Hardness is the result of calcium and magnesium in water. In Phewa Lake, hardness ranged from 23 mg/L to 38.50 mg/L in rainy and winter seasons respectively with a mean of 31.08 mg/L. Lower hardness was reported by Usman et al. (2018) in winter season than other season from urban lakes of Mumbai but in Phewa lake, winter recorded higher value than autumn and rainy seasons.

Chloride is found in low concentration in lakes and rivers. High chloride in water is the result of pollution by human activities and discharge of sewage (Chatterjee et al., 2010). The mean value of chloride in autumn season was 20.06 mg/L which was twice the average value recorded than winter and rainy seasons. Corrosion rate of metals increases when the concentration of chloride is higher.

Turbidity is the cloudiness of water resulting from suspended matter, chemical precipitates and debris of plant and animals (WHO, 2017). In winter season, the turbidity was lowest (6.36 NTU) and highest was seen during the rainy season (17.23 NTU) as a result of rain water carrying debris mixes into Phewa Lake. The mean turbidity value of 10.71 NTU was observed in Phewa Lake during the sampling period which is twice the permissible value 5 NTU **(Table 4.16)**. High turbid water cause staining of clothes and hampers water treatment process (WHO, 2017).

Biological oxygen demand (BOD) is an important indicator of contaminated water bodies with organic debris. BOD varied distinctly between sampling months. The values of BOD in winter (1.35 mg/L) < rainy (6.53 mg/L) < autumn (12.40 mg/L) seasons. BOD concentration shows an inverse relationship with DO level. Lower BOD value is the indicator or good water quality.

Table 4.16 Mean and standard deviation of the physiochemical parameters at the study area

SN	Winter		Rainy		Autumn		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH	7.95	0.32	8.18	0.67	7.67	0.34	7.93	0.51
EC	83.56	14.57	52.63	27.25	65.13	60.68	67.10	40.56
TDS	41.94	7.57	26.38	13.53	32.31	30.42	33.54	20.35
Nitrate	1.13	0.50	0.25	0.45	0.06	0.25	0.48	0.62
DO	7.91	0.54	7.13	0.94	6.58	1.43	7.20	1.16
Alkalinity	44.13	9.16	58.75	14.12	72.63	20.25	58.50	18.96
Hardness	38.50	4.29	23.00	12.69	31.75	22.22	31.08	16.00
Chloride	10.03	1.21	10.47	1.36	20.06	4.37	13.52	5.39
Turbidity	6.36	6.84	17.23	11.11	8.54	10.72	10.71	10.65
BOD	1.35	1.86	6.53	8.49	12.40	9.93	6.76	8.74

4.11 Water quality evaluation based on WQI

In the year 2021, the WQI of sixteen sampling locations was calculated from Phewa Lake for winter, rainy and autumn seasons. The computed WQI ranges from 20.74 to 70.65 in winter season, 36.32 to 126.06 in rainy season and 28.70 to 167.11 in autumn season **(Table 4.19)**. The mean WQI values of 33.64, 66.94 and 58.71 were obtained for winter, rainy and autumn seasons respectively (**Figure 4.17)**. Based on WQI classification, the water of Phewa Lake during the sampling period fall under ‘good’ category with WQI value of 53.1**.**

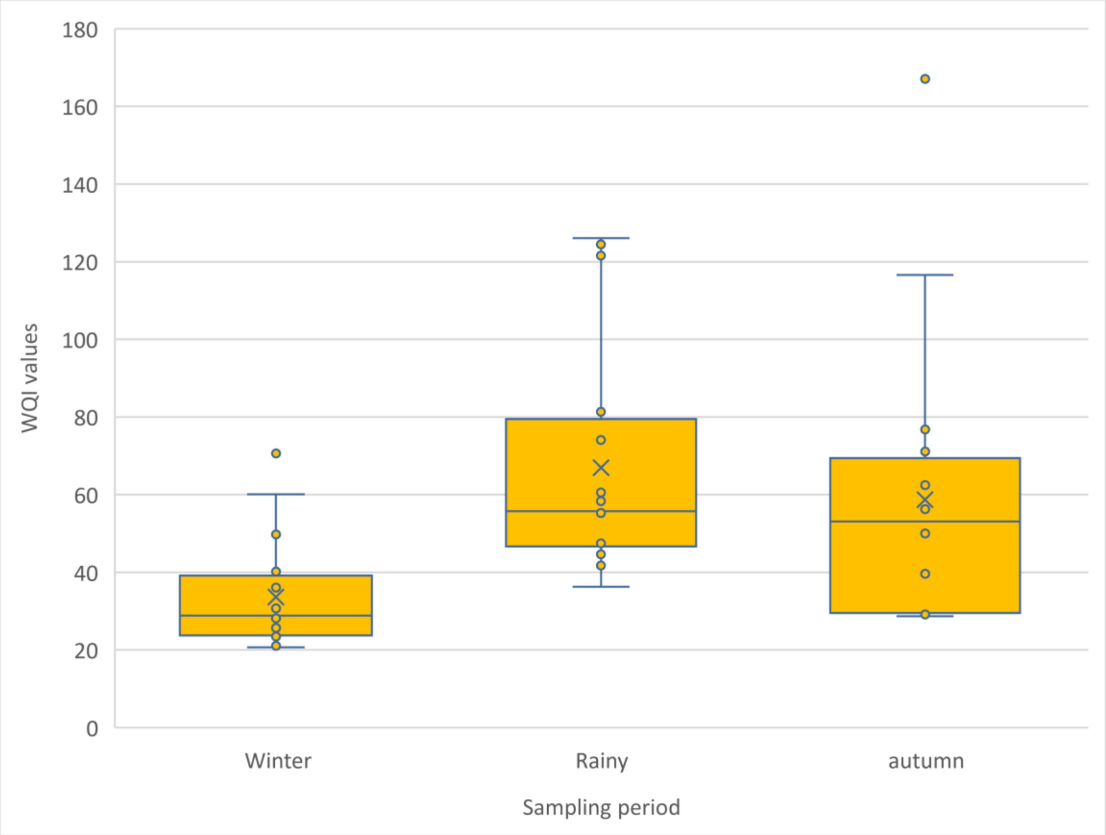

Figure 4.17 Seasonal WQI value of Phewa Lake.

Similar result was also reported by Wu et al. (2020) in Beiyun River, China. The water quality of Phewa Lake during the sampling period (2021) was ‘excellent’ accounting for 58.33%. ‘Good’ quality accounted for 31.25% while ‘poor’ water quality was just 10.42%. The proportion of water quality status for different seasons is given in **Figure 4.18 and Table 4.20.**

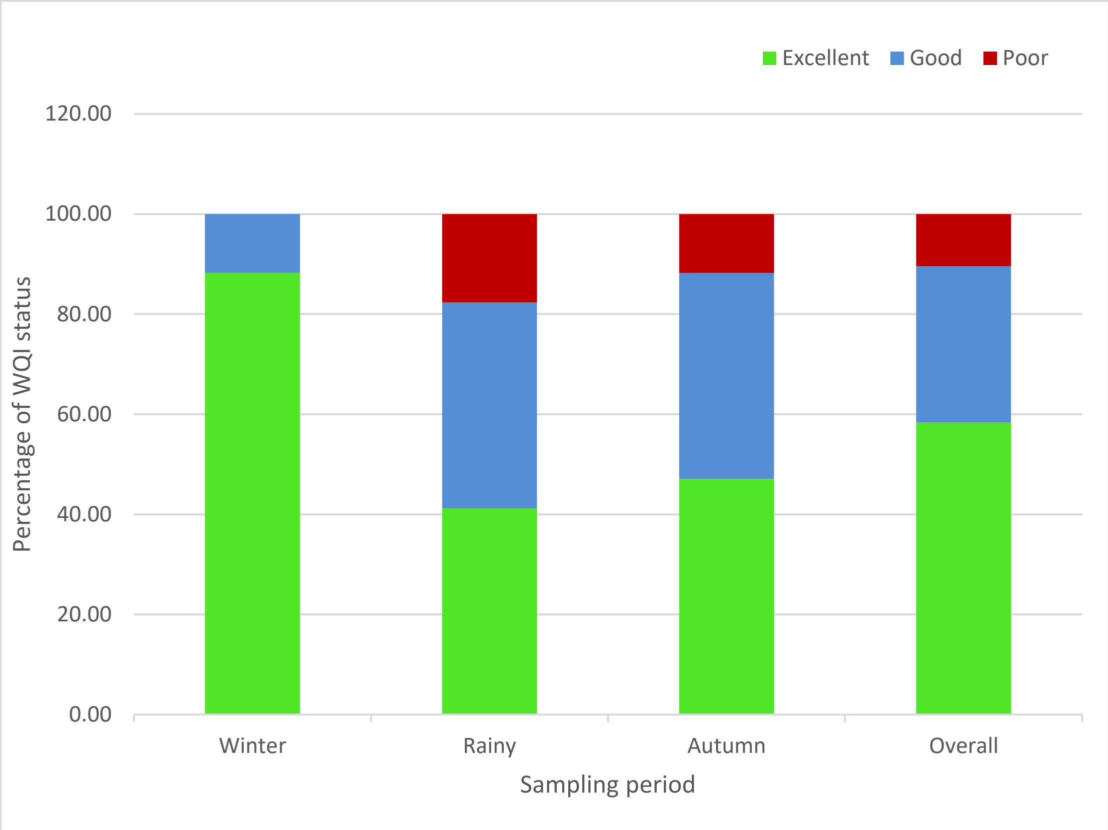

Figure 4.18 Percentage of different water quality categories in three seasons of Phewa Lake

There is a distinct seasonal variation in water quality of Phewa Lake. Winter season having the best water quality followed by autumn and rainy seasons. Kruskal Wallis H test also indicates that there is significant variation in water quality of Phewa Lake seasonally (H=15.138 with $p<0.01$) **(Table 4.17)**. Since data indicated significant difference, pair wise comparison was applied to determine which pair has the difference. **Table 4.18** indicates significant pairwise comparison.

Table 4.17 Kruskal-Wallis H Test of WQI for seasonal significance difference

Null Hypothesis	H-value	Sig.	Decision
The water quality index is the same across different seasons.	15.138	.000	Reject the null hypothesis. (Significance Difference)

Table 4.18 Pairwise comparison of WQI in different seasons

Seasons	Test statistics	SE	P value	Adjusted p value*
Autumn-winter	13.375	4.95	0.007	0.021
Rainy-winter	18.687	4.95	0.000	0.000
Rainy-autumn	5.313	4.95	0.283	0.849

*adjusted using Bonferroni correction

Table 4.18 and **Figure 4.17** indicates that winter season has significantly less WQI than those of rainy and autumn seasons.

A lower WQI was noticed by Maansi et al. (2022) for winter season than other seasons in Sukhna Lake but water of Phewa Lake was found to be of ‘excellent’ category. This may be directly linked to COVID-19 pandemic confinement by the government. Hotels, restaurants and tourism activities came to a halt resulting in low pollution of Phewa Lake. Drastic improvement of Ganga River water quality was reported by Rupani et al. (2020) during COVID-19 lockdown. Debata et al. (2020) also reported similar findings that water quality of Yamuna and Ganga Rivers improved significantly during lockdown period. Remote sensing technique applied by Wagh et al. (2020) also proved improvement in water quality of Hussain Sagar Lake during confinement period. As sampling was done just after the upliftment of lockdown restriction, the water quality of Phewa Lake was ‘excellent’ regarding WQI value in winter season.

In lake center (SW11, SW12, SW13, SW14), the WQI values were ‘excellent’ for all three seasons. These sites are located almost at the center area of a lake where human intervention is minimal. Wu et al. (2021) mentioned that human interference and spatial water quality condition is closely related. Samples from Hallan Chowk (DPA-SW6), Barahi boating area (DPA-SW7), and Tal Barahi temple (TEM-SW10) was ‘excellent’ for winter and rainy seasons but turned to ‘good’ category during autumn season. In sites LPA-SW16, LPA-SW15, MPA-SW2, the water quality was ‘excellent’ than 'good' and back to 'excellent' in winter, rainy and autumn seasons respectively (**Table 4.19).**

The periphery of these sampling sites are surrounded by low to moderate population. These was no change in the WQI of DPA-SW4 for all three seasons which was 'good' throughout. Similarly, the sample water from Ratmaati Danda (MPA-SW3) was 'good' during winter season but were of 'poor' category in rainy and autumn seasons **(Table 4.19).** It may be due to Seti Khola carrying pollutants from nearby regions directly entering Phewa Lake during rainy season near the sampling site.

The water sample from OUT-SW9 (dam site) was 'excellent' during winter season but were of 'good' category in rainy and autumn seasons. Likewise, the water sample of Baidam, Phirke Khola (MIX-SW8) was of 'excellent' category in winter season which changed to 'good' category in rainy season and further degraded in autumn season with 'poor' water quality (**Table 4.19).**

Phirke Khola carry domestic effluent and sewage from nearby residential areas which directly drains into Phewa Lake at this section of the lake, degrading the water quality of that site. In Lake Wuli and Taihu, a low water quality were noticed during rainy season (Wang et al., 2019a) linked to agricultural activities (Wu et al., 2020). Site INL-SW1 of Phew Lake also observed 'poor' water quality during rainy season because of rice cultivation in the periphery of this site which may have degraded the water quality in rainy season.

Table 4.19 Spatial and temporal water quality indices values in Phewa Lake

	Winter		Rainy		Autumn	
Area Code	WQI	Status	WQI	Status	WQI	Status
INL-SW1	49.78	Excellent	124.47	Poor	71.14	Good
MPA-SW2	30.71	Excellent	56.26	Good	39.63	Excellent
MPA-SW3	70.65	Good	126.06	Poor	116.62	Poor
DPA-SW4	60.10	Good	74.07	Good	62.47	Good
DPA-SW5	36.10	Excellent	121.54	Poor	58.13	Good
DPA-SW6	25.4	Excellent	36.32	Excellent	76.80	Good
DPA-SW7	40.22	Excellent	47.46	Excellent	64.23	Good
MIX-SW8	29.67	Excellent	58.34	Good	167.11	Poor
OUT-SW9	28.18	Excellent	55.28	Good	49.98	Excellent
TBA-SW10	25.69	Excellent	48.54	Excellent	56.24	Good
CPL-SW11	20.74	Excellent	48.04	Excellent	30.5	Excellent
CPL-SW12	21.10	Excellent	41.73	Excellent	29.11	Excellent
CPL-SW13	22.29	Excellent	44.67	Excellent	29.28	Excellent
CPL-SW14	23.49	Excellent	46.40	Excellent	28.70	Excellent
LPA-SW15	29.46	Excellent	60.56	Good	30.32	Excellent
LPA-SW16	24.59	Excellent	81.33	Good	29.12	Excellent

Table 4.20 Percentage of different water quality categories in three seasons of Phewa Lake

Quality of water	**Winter**	**Rainy**	**Autumn**	**Overall**
Excellent water	87.5	43.75	43.75	58.33
Good water	12.5	37.50	43.75	31.25
Poor water	-	18.75	12.50	10.42

4.12 Water quality analysis based on HPI

To find the status of heavy metal contaminations in Phewa Lake, Heavy metal pollution index (HPI) was computed for three sampling seasons which is given in **Table 4.21**. The calculated HPI ranges from 0.03 to 1193.22 in winter season, 0.03 to 510.93 in rainy season and 0.02 to 598.94 in autumn season. The mean HPI value of 367.31, 208.61 and 182.14 were obtained for winter, rainy and autumn seasons respectively.

Table 4.21 Heavy metal pollution index (HPI) in three seasons in Phewa Lake

Area	**HMPI-S1**	**HMPI-S2**	**HMPI-S3**
INL-W1	0.12	510.93	0.15
MPA-W2	255.45	255.45	0.02
MPA-W3	681.40	85.21	0.35
DPA-W4	596.02	85.18	0.39
DPA-W5	1193.22	0.09	0.08
DPA-W6	340.58	255.44	425.75
DPA-W7	0.04	255.44	510.86
MIX-W8	0.14	170.31	0.46
OUT-W9	1021.69	431.12	85.19
TBA-W10	255.44	425.75	183.33
CPL-W11	0.07	85.19	340.60
CPL-W12	170.30	0.03	427.45
CPL-W13	0.03	170.30	255.43
CPL-W14	510.89	11.17	598.94
LPA-W15	681.13	255.46	85.16
LPA-W16	170.35	340.61	0.03

Based on HPI classification, the water of Phewa Lake during the sampling period (2021) fall under 'high risk' category **(Figure 4.19)**. Agricultural runoff (Hong et al., 2020) and urban sewage discharge (Sharma et al., 2020) may lead to high HPI value. Of the total, 58.33% of the sampling site water fall under 'high risk' and 'low risk' accounted for just 41.67%. The proportion of HPI status for different season is given in **Figure 4.19**.

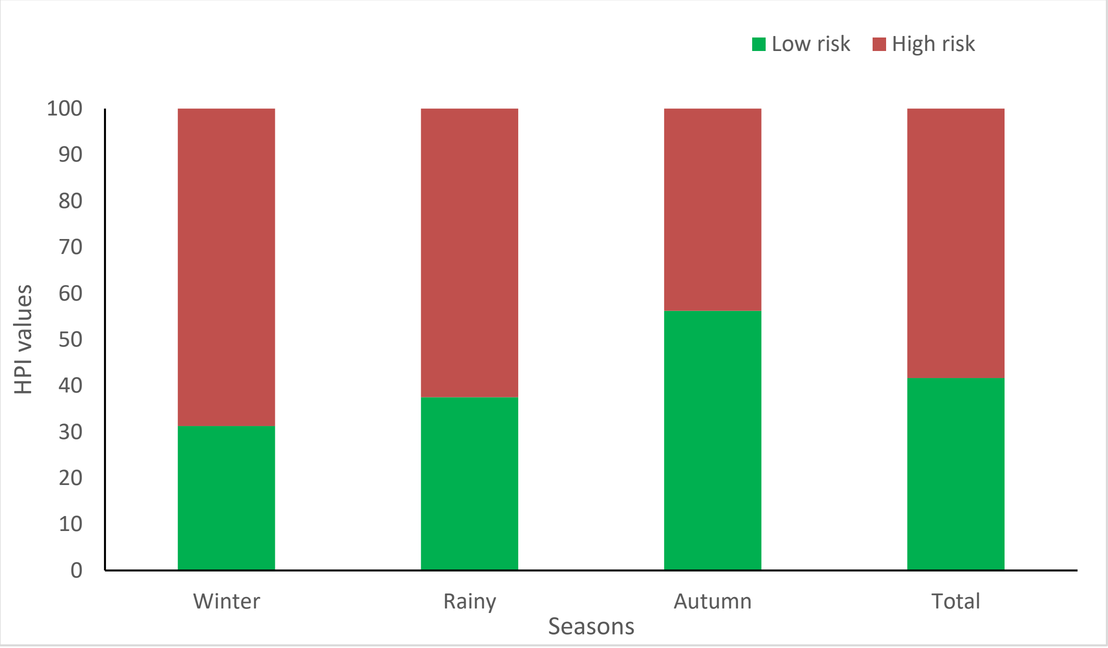

Figure 4.19 Percentage distribution of HPI categories during sampling period in Phewa Lake

Hallan chowk (DPA-SW6) and Tal Barahi temple (TEM-SW10) sampling sites observed 'high risk' HPI value for all three sampling seasons. This may be due to domestic waste discharge at DPA-SW6 site and the floating toilet located at temple area. So wear and tear of metal structure and pipelines may be reason for heavy metal contamination in that area. Likewise, the lake center area 4 (CPL-SW14) was found to be at 'high risk' category for winter and autumn seasons but HPI value in rainy season was at 'low risk'. Abraham and Susan (2017) pointed out that wear and tear of old water pipe may be the reason for lead contain in water. The supply of drinking water to the other side of Phewa Lake pass through the center so there is high possibility of wear and tear of pipeline. As lead contain was also found high in this section, it may be linked but further research is needed to verify this.

4.13 Correlation analysis

Correlation between microplastic abundance with WQI and HPI was tested with Pearson correlation analysis. The results indicate that there were no statistically significant correlation between microplastic abundance in water and sediments with WQI. Whereas, microplastics in water and sediments were weakly correlated with heavy metal pollution index **(Figure 4.20)** with r value 0.322 and 0.380 respectively (both p values < 0.01). As heavy metals can easily adsorb on the surface of microplastics, studies on toxicological interaction of heavy metals with microplastics have started recently (Lin et al., 2020; Wang et al., 2020). For example Wang et al. (2020) pointed out that microplastic and cadmium brings negative influence on soil biodiversity and agro ecosystem. Likewise, Lin et al. (2020) found out that the combined impact of polyacrylonitrile (PAN) polymer and Cu^{2+} decreases the content of chlorophyll a and b in Chlorella pyrenoidosa (*C. pyrenoidosa*). Thus, further research are needed to investigate the toxic effects of microplastics and heavy metals on aquatic biota of Phewa Lake.

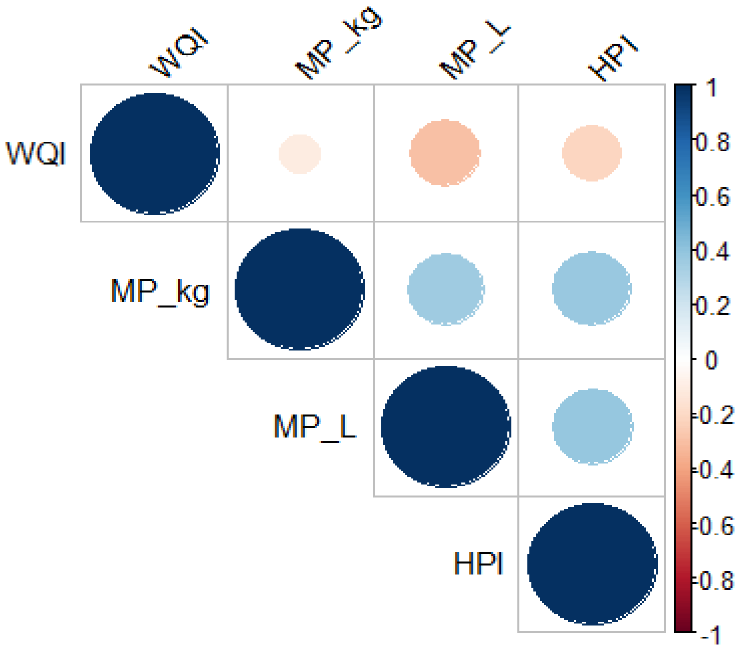

Figure 4.20 Correlation plot

CHAPTER 5 : CONCLUSIONS

Though microplastic pollution is capturing the attention of the world, no such data on freshwater lake microplastic exits in Nepal. This study provides data on the spatiotemporal distribution and characteristic of Phewa Lake for the first time. The results showed that

- The mean abundance of microplastics vary significantly among sampling location (spatial variation) in water and sediments.
- Seasonal variation was only observed in water samples.
- In general, densely populated area and outlet recorded the highest abundance of microplastics in both water and sediment samples.
- During the sampling period (2021), fibers were the most dominant type of microplastics in water and sediment samples accounting for 91.95% and 62.52% respectively and transparent as frequently encountered color.
- The particles size < 1 mm showed the maximum abundance of microplastics in water samples and particles size 0.2 to 1 mm showed the maximum abundance of microplastics in sediment samples for all three seasons.
- Polymer identification by FTIR reveal PP and PE as the main plastic type in the shoreline sediments of Phewa Lake.
- Water quality of Phewa Lake fall under 'good' category with respect to WQI value but 'high risk' category with regard to HPI.
- Weak correlation between microplastic abundance in water and shoreline sediment samples (r=0.355), p value < 0.01) were observed. But there was no correlation between microplastic abundance in water and sediment with WQI whereas, HPI showed weak correlation.

Compared to other freshwater lake studies, the level of microplastic concentrations in Phewa Lake is currently at moderate levels. This lake is an important tourist destination that provides livelihood support to the local people which help to boost the economic growth of that region. Therefore, the occurrences of microplastics in Phewa Lake and their influence on aquatic ecosystem are crucial issues that must be addressed.

The morphological characteristics of the microplastics in this study area indicates that their main source is from the secondary source as a result of fragmentation or degradation of larger plastic debris laying on the shoreline, domestic sewage, fishery, and washing clothes in the vicinity of the lake. In addition to tourism activities, the distribution pattern of microplastic contaminations of Phewa Lake may be affected by topographic factor. Distribution and impact of microplastics can be minimized if microplastics are controlled at source because once it is released into freshwater habitat, little can be done to bring down the level of microplastics contamination. Therefore, domestic waste management around the lake needs improvement. Moreover, public awareness and education on the need for environmental protection which is a powerful tool must be implemented immediately.

5.1 Future research perspective

Microplastic pollution is a new dimension of research field in Nepal. This study has generated a database of microplastic for the first time in fresh water lake system (Phewa Lake) of Nepal which may be a basis for managerial action. Moreover, it has provided insights for future research which are outline below.

- Ingestion of microplastics by aquatic organisms and trophic transfer of microplastics
- Characterisation and risk assessment of plastic additives and environmental contaminants on aquatic organisms.
- Risk assessment of microplastic pollution in Phewa Lake.
- Analysis of microbial biofilms on the surface of microplastic that determines the fate and ecotoxicity of microplastic.
- Interaction of microplastic with heavy metals and its toxic effects on biota.
- Role of topographic factor in transportation and movement of microplastics.
- Estimation of microplastics influx into Phewa Lake by domestic waste input.

5.2 Recommendations

- Launching awareness programs about the impact of microplastic pollutions.
- Ban of single-use plastic around the periphery of Phewa Lake
- Participation of locals (owners of hotels and restaurants around Phewa Lake) in weekly shoreline clean-up campaign of Phewa Lake.
- Continuous research and monitoring programs in initiation of local authorities.

5.3 Limitations

- Sampling of spring season (pre-monsoon) was not done due to COVID-19 pandemic lockdown.
- All the microplastics recovered from size class 1-5 mm was not identified for polymer composition with FTIR due to limited cost.
- For small size microplastic (0.2-1 mm), polymer identification was not done due to unavailability of micro-Fourier Transform Infrared Spectroscopy (µ-FTIR) and Raman Spectroscopy in Nepal.

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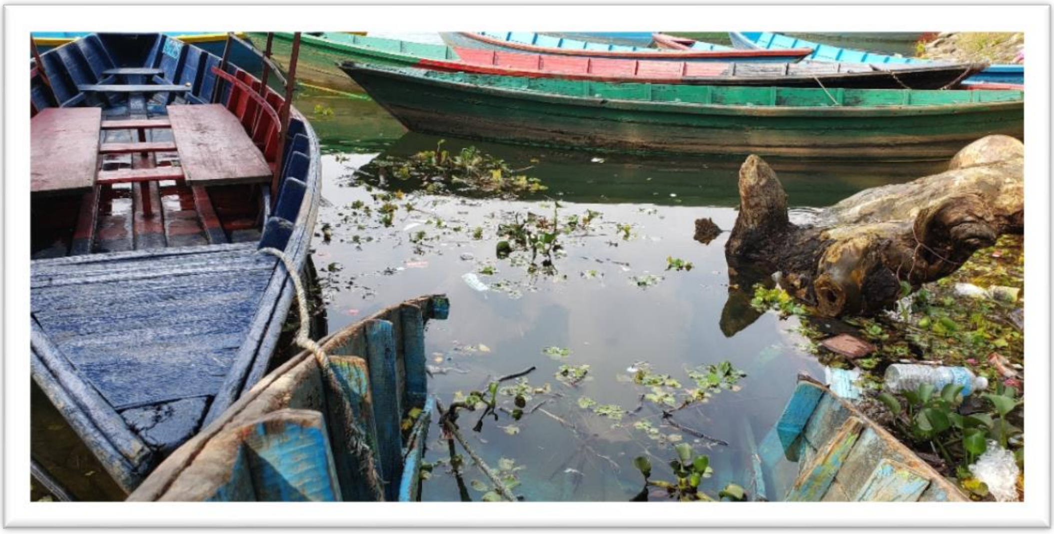
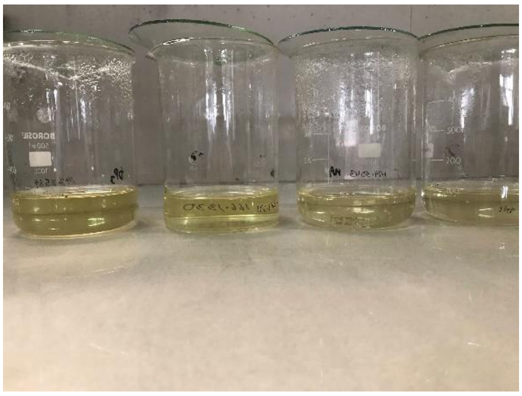
ANNEX-I (PHOTOGRAPHS)

Microplastic seen in water and shoreline during sampling



Microplastic seen in water and shoreline during sampling

Photo taken during sample collection



Digestion by hydrogen peroxide (water and sediment sample)

During lab analysis of water sample

Vacuum filtration

Microplastic extracted from sediment samples

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List of Publication and Proceeding

- Malla-Pradhan, R., Suwunwong, T., Phoungthong, K., Joshi, T.P., Pradhan, B.L., Microplastic pollution in urban Lake Phewa, Nepal: the first report on abundance and composition in surface water of lake in different seasons. *Environ Sci Pollut Res* 29, 39928–39936 (2022). https://doi.org/10.1007/s11356-021-18301-9
- Malla-Pradhan, R.,Pradhan, B.L.,Phoungthong, K., Joshi, T.P., Occurrence and distribution of microplastics from Nepal's second largest lake. *Water Air Soil Pollut.* 233, 423 (2022). https://doi.org/10.1007/s11270-022-05896-z
- Malla-Pradhan, R., Pradhan, B., Phoungthong, K. et al. Microplastic in freshwater environment: A review on techniques and abundance for microplastic detection in lake water. Trends in Sciences (TiS) in Volume 20, 2023. **(accepted for publication)**

Conference Paper

- “Microplastic Research in Freshwater Lake Sediments from Asian Countries: A Review on Methods and Abundance” paper presented on International Conference on Sustainable Energy Management, with the theme “Sustainable Energy for Mitigation & Adaptation of Climate Change and Global Warming”. May 28 –31, 2021, Kathmandu, Nepal.
- “Microplastic Concentration and Characterization in the Surface Water of Phewa Lake, Nepal” paper presented on 9th National Conference on Science and Technology “Science for Society and Innovation for Prosperity”, June 26-28, 2022, Kathmandu, Nepal.