Distribution and quantification of micro-plastic concentrations in aquatic system in Indian subcontinent

Ajay Kumar

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Certificate of Examination

This is to certify that the dissertation titled "**Distribution and quantification of micro-plastic concentrations in aquatic system.**" submitted by **Ajay Kumar** (Reg. No. MS14091) for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Sharmila Bhattacharya   Dr. Sunil Patil   Dr. Anoop Ambili

Thesis Supervisor

Dated: November 22, 2019

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Anoop Ambili at the Indian Institute of Science Education and Research Mohali. This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Ajay Kumar

(Candidate)

Dated: November 22, 2019

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Anoop Ambili

(Supervisor)

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Abstract

The microplastics (MPs, particle size less than 5 mm) have been identified as environmentally recalcitrant and complex contaminants of growing societal concern. The available data on MPs concentrations have been overwhelmingly focused on marine realm, whereas there is a dearth of studies that have quantified microplastics in lacustrine ecosystems. In the present study ecologically important (Ramsar site) freshwater ecosystem in Central Himalaya – Renuka lake have been selected to investigate the concentrations of MPs in sediments. To examine the occurrence of microplastic particles (MPs) in the Renuka Lake, the collected lake water and sediment samples were processed for microplastic extraction through density separation. The microplastics were retrieved from all sediment and water samples in the entire lake basin, indicating their extensive distribution in the lake basin. The abundance of MPs recorded from the lake surface sediment samples is in the range of 5-86 particles/500gm and 5-65 particles/litre are recorded for water samples. The Raman spectra of microplastic indicated that low density polypropylene as the dominant polymer component in the MPs of Renuka Lake.

Contents

Chapter 1

Introduction

Introduction

1.1. Background

Anthropogenic contaminants in aqueous systems have been a crucial and longstanding environmental issue with far-reaching effects to the ecosystems. Limnic systems are vulnerably exposed to a large array of contaminants as a consequence of intense anthropogenic activities and are introduced into the freshwaters through atmospheric and terrestrial interactions. The lacustrine systems shelter a plethora of organic contaminants which include both naturally occurring pollutants (organic metabolites and byproducts) as well as synthetic chemicals that include industrial discharges, agricultural run-off, combustion related polyaromatic hydrocarbons, municipal sewage waste discharges from waste water treatment plants (Baldwin et al., 2016). The discharge of these organic contaminants into aqueous systems affect surface water which in turn compromises the drinking water sources; unfortunately, a large portion of the micro pollutants cannot be removed through the standard purification processes from water (Momepelat et al., 2009). The removal mechanism and efficiency are guided by the properties of the pollutant compounds (Su et al., 2017 and references therein).

In aqueous environments, plastic litter due to its high durability and infinitely slow decaying properties is extremely detrimental to the environment and the consistent accumulation over decades is imposing serious threats to the terrestrial environments. Plastics could be classified according to the polymer types which in turn determine the density of the debris (Driedger et al., 2015) including polyvinylchloride, polyethylene, polypropylene, polystyrene and polyacrylates (Vert et al., 2012). The plastic production has steadily increased worldwide over the last 60 years (0.5 million tons/yr in 1960 to almost 300 million tons in 2013) (Plastics Europe, 2015) with about 10% of it making its way into the aqueous domains (Anderson et al., 2016). These are extremely damaging class of chemicals occurring in the environment having extremely recalcitrant structures and also retard biodegradation of other organic contaminants adsorbed onto the debris particles. These also act as vectors for persistent organic contaminants and heavy metals. The breakdown of plastics encompasses a huge time window and a series of processes like oxidative degradation by ultraviolet radiation, physical disintegration and microbial degradation. Microplastics (defined as <5 mm size) derived from atmospheric oxidation, hydrolysis in water and mechanical disintegration of larger plastic material fragments (Claessens et al., 2013) have been marked as emerging contaminant of concern in the environment (Thompson et al., 2004; Andrady, 2011; Lebreton et al., 2017; Barboza et al., 2018). The research related to microplastics (MPs) in the aquatic environment has gained sufficient momentum since the first published reports on the dramatic increase of microplastic contamination in marine sediments (Thompson et al., 2004). In the last decade, various research have provided evidence of microplastic as an emerged threat, with the urgent need to better assess their distribution and concentration in the marine and terrestrial environments, as well as the ecological risks that these particles pose to the ecosystems (Klein et al., 2015; Anderson et al., 2016; Machado et al., 2018). The available research on microplastics has been mostly focused on the occurrence, distribution, characterization as well as its adverse effect on ecosystem in the marine environments (Thompson et al., 2004; Moore, 2008; Van Cauwenberghe et al., 2015; Gewert et al., 2017; Barboza et al., 2018). The studies demonstrated the propensity of fish, birds, and vertebrates ingesting plastics (Laist, 1997; Denuncio et al., 2011; Poppi et al., 2012). The plastic debris readily absorbs harmful chemicals such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) increasing their concentration in sediments (Andrady, 2011). The adsorbed pollutants particularly have toxic effects which might conveniently propagate through food chain (Barnes et al., 2009; Baulch and Perry, 2014). Despite its importance, the global data on microplastic concentration from terrestrial, fresh water and marginal environments is limited (Rillig, 2012; Wagner et al., 2014). The dataset generated from studies of the different realms of the environment in the Indian subcontinent is also inadequate (Reddy et al., 2006; Jayasiri et al., 2013; Sruthy and Ramasamy, 2017).

With this background in mind, we have designed our experiment to study in detail the microplastic pollutants in surface and water samples from lake system Renuka Lake in Himachal Pradesh. The fundamental goals of the proposed research work include precise qualitative and quantitative assessments of the plastic pollutants in the selected lake system. Water quality parameters highlighting the inorganic constituents have been investigated from Renuka (e.g., Das et al., 2001; Singh and Jain, 2013 and references therein). The future demands an urgent necessity to probe the microplastics contaminants with resilient structures and physico-chemical properties which are accumulating at an alarming rate in the lakes primarily due to human interactions. The research work aims to provide the first comprehensive record of the distribution and quantitative estimation of microplastic, including the detailed structures, source and fate of these plastic pollutants in the lake waters and sediments. The research data will enhance the existing dataset of microplastic contamination from India.

1.2. Objectives

The major objectives of the present work are understanding the below mentioned processes;

1. To identify the extent and distribution of microplastics pollution in aquatic system in Indian subcontinent.

2. Quantify the type and size of microplastics in the sediment and water samples.

3. The identification of polymer composition of all microplastic particles to address source input.

4. Understanding the factors controlling spatial variability of microplastic concentration in aquatic system.

5. Utilization of microplastic concentration from the Indian lake system as a baseline for future monitoring.

1.3. Study area

The lesser Himalaya is characterized by numerous aquatic ecosystems of great ecological and economic importance. The fresh water lake Renuka of Himachal Pradesh have important multistage components like source of drinking water, irrigation, agriculture, socio economic development, recreation and immense religious importance. The Renuka lake situated in Sirmaur district, Himachal Pradesh has a water spread of 670 ha bound within geographical co-ordinates of 30° 36’ 30” N latitude and 77° 27’ 6” E longitude. The lake is oval shaped lying at an altitude of 620 m above msl and is surrounded by dense forests and vegetation (Das et al., 2001). The maximum depth of the lake is 14 m and is a monomictic type (Zutshi and Gopal, 2000).

The Renuka lake follows a riparian course between two steep hill slopes with forests along the abandoned course of Giri River which got separated due to tectonic upliftment. The outcrop of the region has Jaunsar, Blaini, Infra-Krol and Krol rock formations with catchment rocks comprises of slates, calcareous shales and limestones. The region is tectonically active due to its proximity to Krol Thrust (Rao, 1975). Renuka wetland is a perennial water body fed by 21 seasonal streams which are vigorous particularly during the monsoon season. Internal springs of the wetland are a perennial source of water. The underground network of channels in the Limestone and Dolomite formations of the area is possibly conduit to discharge groundwater to the wetland. In view of its rich biodiversity and uniqueness of the area this wetland was declared as a RAMSAR SITE in the year 2005.

Numerous limnological studies have been conducted on diverse lake systems of lesser Himalayas but the study on Renuka lake system has been limited to water geochemistry, bacterial analysis and eutrophication status (Das et al., 2008; Singh and Jain, 2013; Sawan et al., 2018). The investigations reveal changes in the water geochemistry and biological characteristics of the lake system. The various water quality parameters indicate hard water nature of lake due to dominance of major cations (Mg and Ca) that has been brought by runoff from carbonate catchment rocks (Das and Kaur 2001). The reported trace elements (e.g. Fe, Mn, Pb, Zn, Cu, Cd) data from the region shows their presence has exceeded desirable limit in drinking water as per BIS norms (Das et al., 2008; Singh et al., 2013). The heavy metal enrichment in lake sediments is due to enhanced human activities as huge amounts of domestic effluents and untreated solid wastes are disposed in the lake system that has strikingly degraded the water quality (Das et al., 2008). The TDS (total dissolved solids) and anion concentration of fluoride, sulphate has intensified and crossed permissible limits in the lake system (Singh and Jain, 2013).

Bacterial analysis indicates high rates of contamination by total coliform and fecal coliform in the lake system (Singh and Jain, 2013). The Renuka lake reported presence of Chlorophycean algae, Cyanophycean, Euglenopycean which indicates eutrophication that leads to high gross primary production (GPP) values of the lake system (Jindal and Prajapat, 2005). Numerous lake pollution indices involving Shannon-wiener diversity index and Palmer index suggest that lake is highly polluted due to high human indulgence in the form of boating, bathing and assemblage of tourists at the religious spot (Sawan et al., 2018). Other parameters like Trophic State Index, Carlson Trophic Index based on phosphate data also demonstrate that water nutrient levels have been enriched which has turned lake hyper-eutrophic recently (Singh and Jain, 2013). The high accumulation of silt in eastern part of lake is caused by deforestation, soil erosion and agricultural practices on catchment area of the lake (Reddy and Char., 2006).

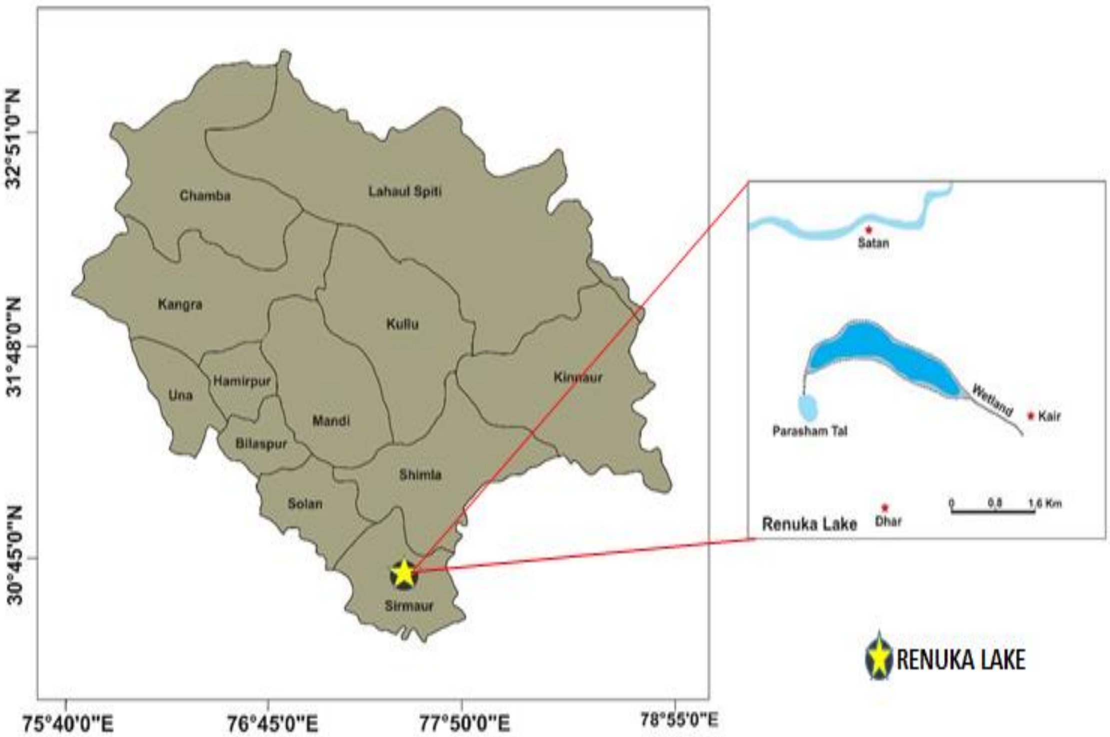

Fig.1. Geographical Location of Renuka lake in Himachal Pradesh, India

1.4. Climate

The region lies within the sub-tropical zone and is influenced by monsoonal precipitation. The average precipitation at Renuka wetland area is 150-199.9 cm per annum with an average maximum temperature of 30°C. The winter is mild in low-lying areas, whereas it snows on higher altitudes. The rainy season is the wettest part of the year and is characterized by high humidity. Summers are prickly hot during monsoon months.

1.5. Geology

The catchment rocks of Renuka are dark carbonaceous shales and slates, closely inter bedded with thin slaty quartzite belonging to Infra Krol Group. The slaty quartzite, or clay slates are frequently calcareous and consists of quartz, carbonate, sericite and pyrite and in some cases chlorite. The green-gray calcareous shale and argillaceous limestone with variable gradation are of Krol A Group. The purple red shales with intercalation of green-shales and thin dolomitic cherty limestone belong to Krol B Group. The well bedded grayish white limestone with shale represents Krol C. The soil composition of the region also shows a heterogeneous mixture of carbonate rocks, sandstones, shales, siltstones in various proportions. The soil cover is thin due to weathering-limited conditions.

Chapter 2

Methodology

Methodology

2.1. Sampling

The bathymetric survey over the study area was carried out using a portable GARMIN echo-sounder (model no. GPSMAP 585 plus). Sediments and water samples (n=25) were collected along the east-west transect covering the entire basin of Renuka Lake. The sediment samples were collected using Van-Veen grab sample, whereas the water samples were collected using Uwitec water sampler. All the sediments and water samples were analyzed for microplastic concentration.

2.2. Grain Size Analysis

The grain size was measured for the sediment's samples (n=25) using Malvern Mastersizer Particle size Analyzer (3000E) coupled to a Hydro 2000S sample dispersion accessory at Indian Institute of Science and Education Research (IISER)-Mohali (India). Prior to grain size analysis, all the sediment samples were weighed and dried using hot air oven at a temperature of 60 º C. For the analysis, the samples were pre-treated with 30% v/v hydrogen peroxide to remove the organic carbon followed by carbonate removal with 0.5 N hydrochloric acid (HCl). The solution was then further washed with milli-Q water and centrifuged 5 times to remove excess acid fraction from the sample solution. Treated samples were stirred at 3500 rpm and sonicated for 1 min prior to measurement and the average value of five consecutive runs were considered as the final output. De Brouckere mean diameter (D[4,3]) of the samples was observed with the instrumental measurements and sand, silt and clay fraction in each of the samples were calculated.

Image1. Van-Veen garb sampler used for surface sediment sampling

Image2. GARMIN GPSMAP 585 plus

Image3. MALVERN Mastersizer particle analyzer

2.3 Microplastic Extraction

2.3.1. Water samples

All the water samples (n=25) were homogenized by shaking the container and 1000ml of sample was measured using graduated measuring cylinder. Measured water samples were filtered using glass fibre filter paper having a diameter of 47 mm and pore size of 0.2 micron. Each of the filter paper was divided into 4 quadrants and samples were successfully filtered using filtration unit (Merck-millipore XI1504700) shown in image 4.

Image4. Merck-millipore filtration unit

2.3.2. Sediment samples

Wet sediment samples (n=10) were and dried with hot air oven at a temperature of 60ºC. The dried samples were weighed (ca. 500 gm) and subsequently wet peroxide oxidation (WPO) was performed to remove the organic matter from the samples. The WPO solution was sieved using a sieve set having a mesh size of 300 micron to 4.75 mm and later the solution was transferred to saturated sodium chloride solution(1.5 Litre) for density separation of microplastics from the sediment samples (Claessens et. al 2011). The solution was left to settle down and supernatant was filtered once the sediment debris is settled in the separating funnel. Separating funnel was further rinsed with sodium chloride solution to transfer all the solids to the collection cup of the filtration unit as shown in image 6. All the filter papers were kept in glass petri dishes and left overnight to air dry.

Image5. Wet peroxide oxidation- WPO (Left) and SIEVEs set (Right)

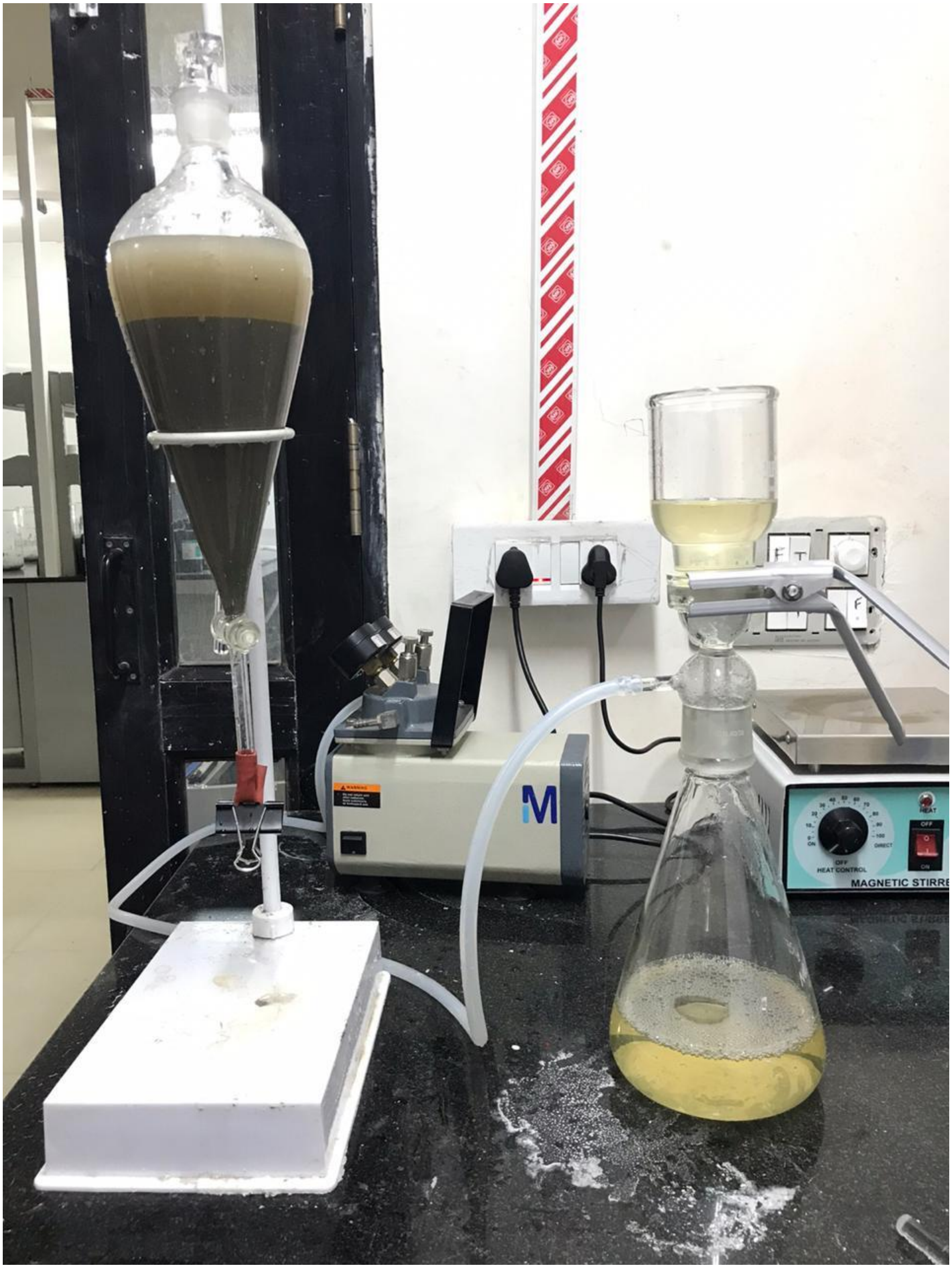

Image6: Density separation unit

2.4. Microplastic Analysis

2.4.1. Microscopic investigation

The detection and identification of microplastics (MPs) is a combinational approach combining optical and spectroscopic tools to quantify the extent of these contaminants in an aquatic environment. The most common approach for the detection of MPs includes visual inspection of extracted plastic particles under dissecting microscopes. The examination of MPs extracted from the water and sediments of Renuka lake were performed by visual observations and filter papers were visually examined under a dissecting fluorescent microscope (Nikon SMZ 18) coupled with illuminator (Nikon INTENSILIGHT Epi-fluorescence illuminator) and images (image xx-xx) of all the potential microplastics were recorded. Furthermore, Microplastics were morphologically characterised into: fibres, fragments, film, foam and pellet (beads).

Image7. NIKON SMZ18 Stereo microscope (Left), NIKON Intensilight C-HGFI (Right).

2.4.2. Raman Spectroscopy

Raman Spectroscopy is one of the most commonly used spectroscopic techniques for the characterization of various polymers. The spectroscopic technique is a non-destructive technique applied frequently to characterize the MPs, even those less than 1μm. The extracted MPs from the sediments were analyzed using the Renishaw InVia Raman spectrometer coupled with three different lasers having wavelengths of 514, 635 and 785 nm. The microplastics from Renuka lake were analyzed under the 785 nm laser having an exposure time of 10 seconds and an intensity of 0.001 % scanning over a spectral range of 400 to 4000 cm^{-1}. The Raman spectra's were baseline corrected.

Image8. RENISHAW InVia Raman Spectrometer.

Chapter 3

Results and Discussions

3.1. Bathymetry

Renuka (the largest natural lake system of Himachal Pradesh) lake is important hydrological entities in Himachal Pradesh providing source of drinking water, irrigation, fishery, agriculture, socioeconomic development and immense religious importance. The bathymetric survey of Renuka lake shows two major depressions along the east-west transect of the basin. The maximum depth of the lake was 14 m (Fig. 2). The eastern part of Renuka lake is a part of a wild life sanctuary, while the western part has a temple and boating point.

3.2. Grain Size

The grain-size distribution of the surface sediments from Renuka lake is characterized with the dominance of clayey-silt with higher fraction of clay observed at deeper part following the classical depositional pattern (Hjulstorm, 1978) (Fig. 3 and Fig. 4). The grain size distribution showed that silt and clay fraction having percentage variation of 45-67 (wt %) and 6-30 (wt %) respectively appears to be the principal sediment type for the investigated sediment samples (Fig. 5 and Fig. 6). The grain size distribution in Renuka Lake exhibits a spatial distribution coupled to hydraulic conditions, water depth and size of the lake. These observations provide the feasibility to establish a model linking the MPs concentration to sedimentary components.

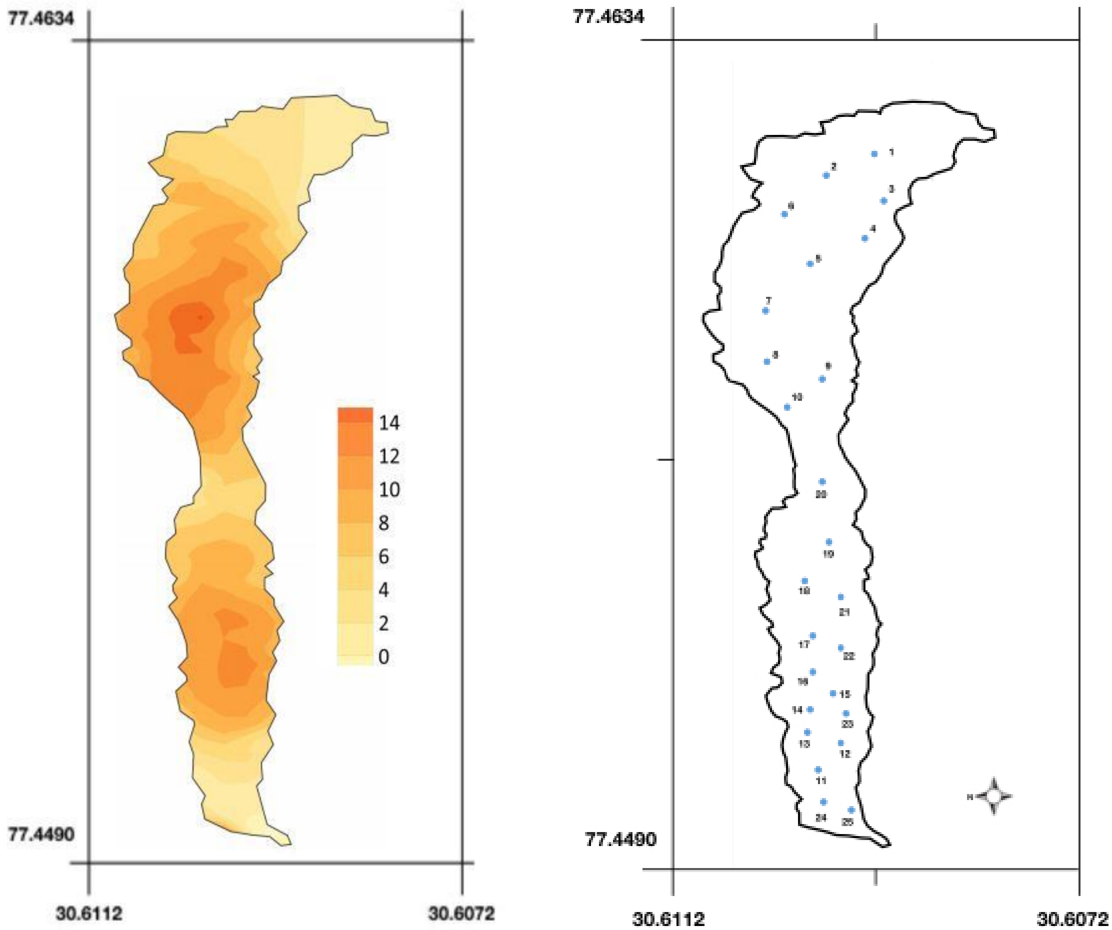

Fig 2. Bathymetry and sample collection sites of Renuka Lake.

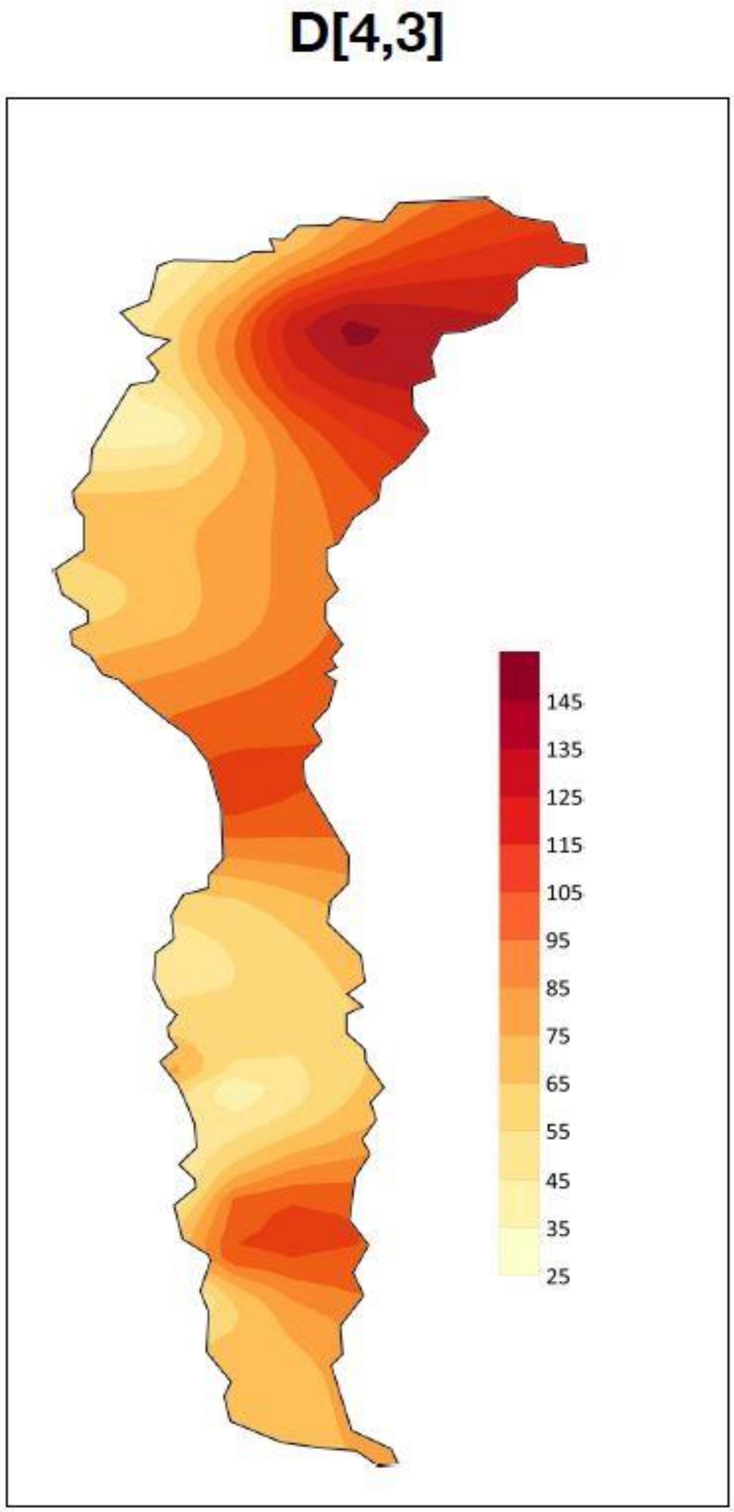

Fig.3. De Brouckere mean diameter (D[4,3])

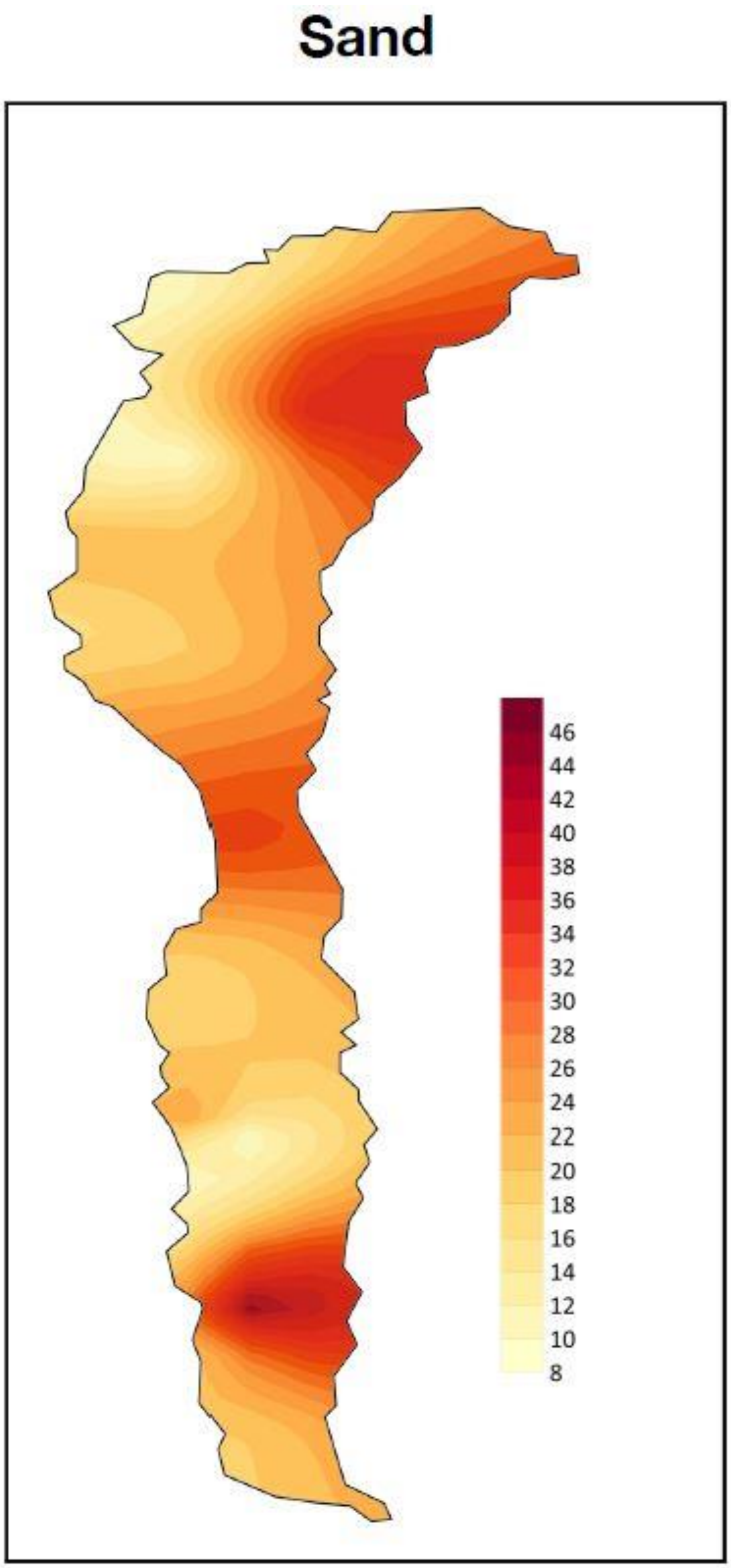

Fig.4. Spatial distribution of sand (wt%) in lake sediments.

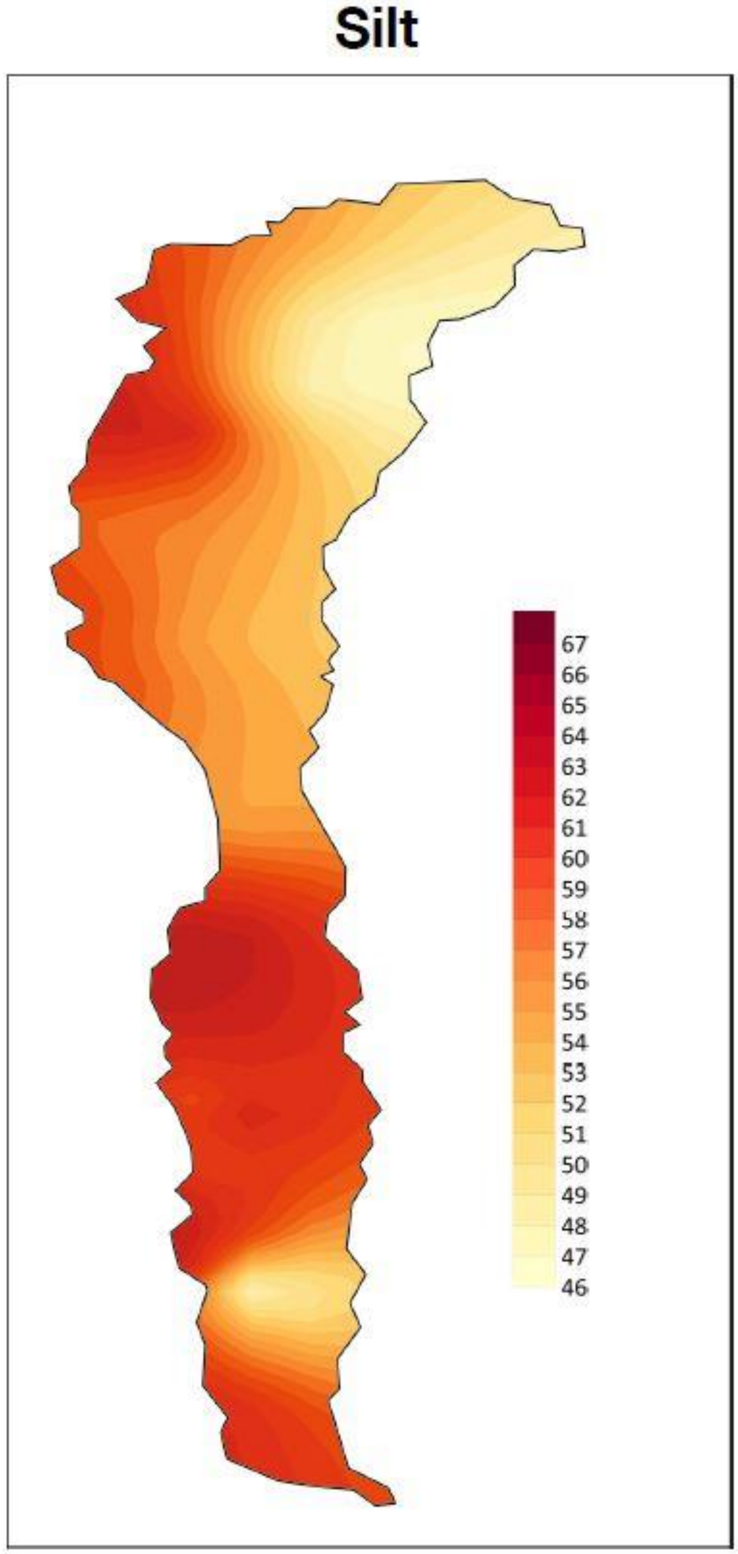

Fig.5. Spatial distribution of silt (wt%) in lake sediments.

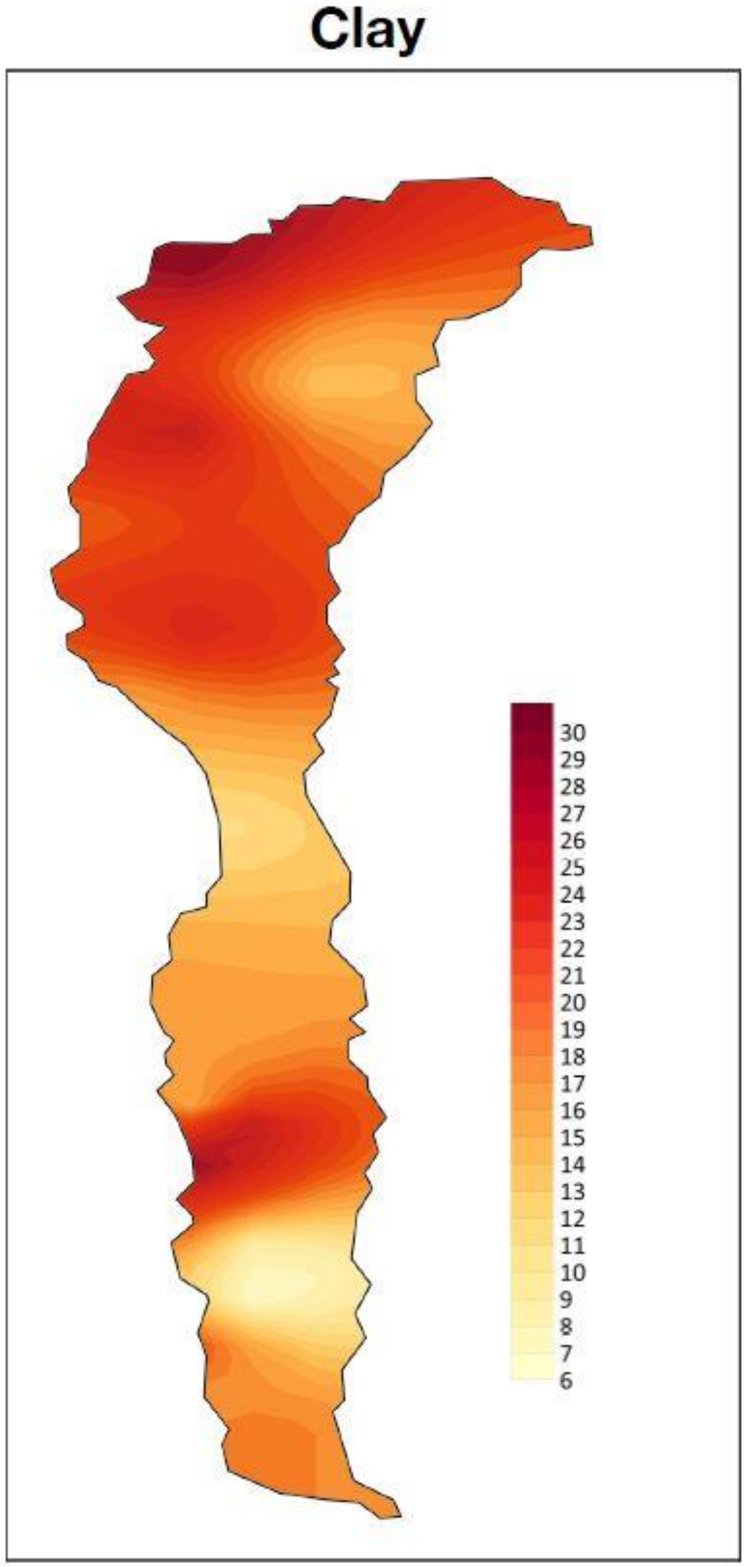

Fig.6. Spatial distribution of clay (wt%) in lake sediments.

3.3. Microplastic Abundance and Characterization

The average concentration of MPs in water samples is 20 particles/litre and 40 particles/500 gm. Microplastics were identified in all water and sediment samples with abundances varied between 5-86 particles/litre in water samples and 5-65 particles/500gm in sediment samples of Renuka lake (Fig. 7 and Fig. 8). The abundance of MPs in water samples was high in sample RNK-24 in the proximity of the temple and boating site, subsequently high abundance of MPs in sediment was also observed in sediment sample RNK-13 (Fig. 9). The concentration of morphologically characterized microplastics (Image 9-13) within the surface water samples and sediment samples are given in Table 1 and Table 2 respectively. The fragments were dominant morphologies of MPs present in lake surface sediment and water samples (Fig. 7 and Fig. 8). The other morphologies present in lesser abundance include fiber, film, foam and pellet (beads). Fragments were most abundant in the sample RNK-24, RNK-11and RNK-14 close to the sites with high anthropogenic activities in the basin.

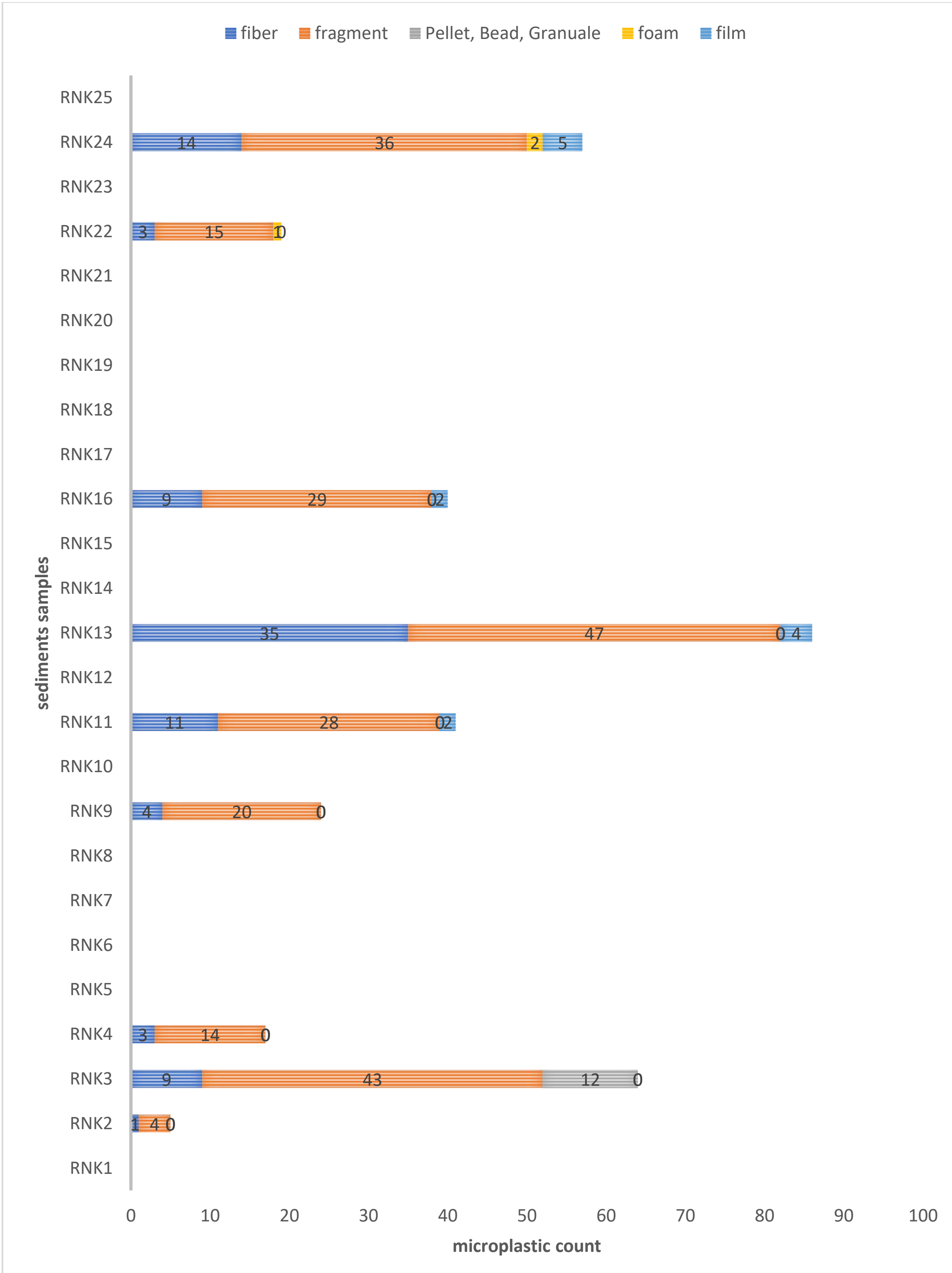

Fig.7. Stacked bar representation of morphologically characterized microplastics in sediment samples

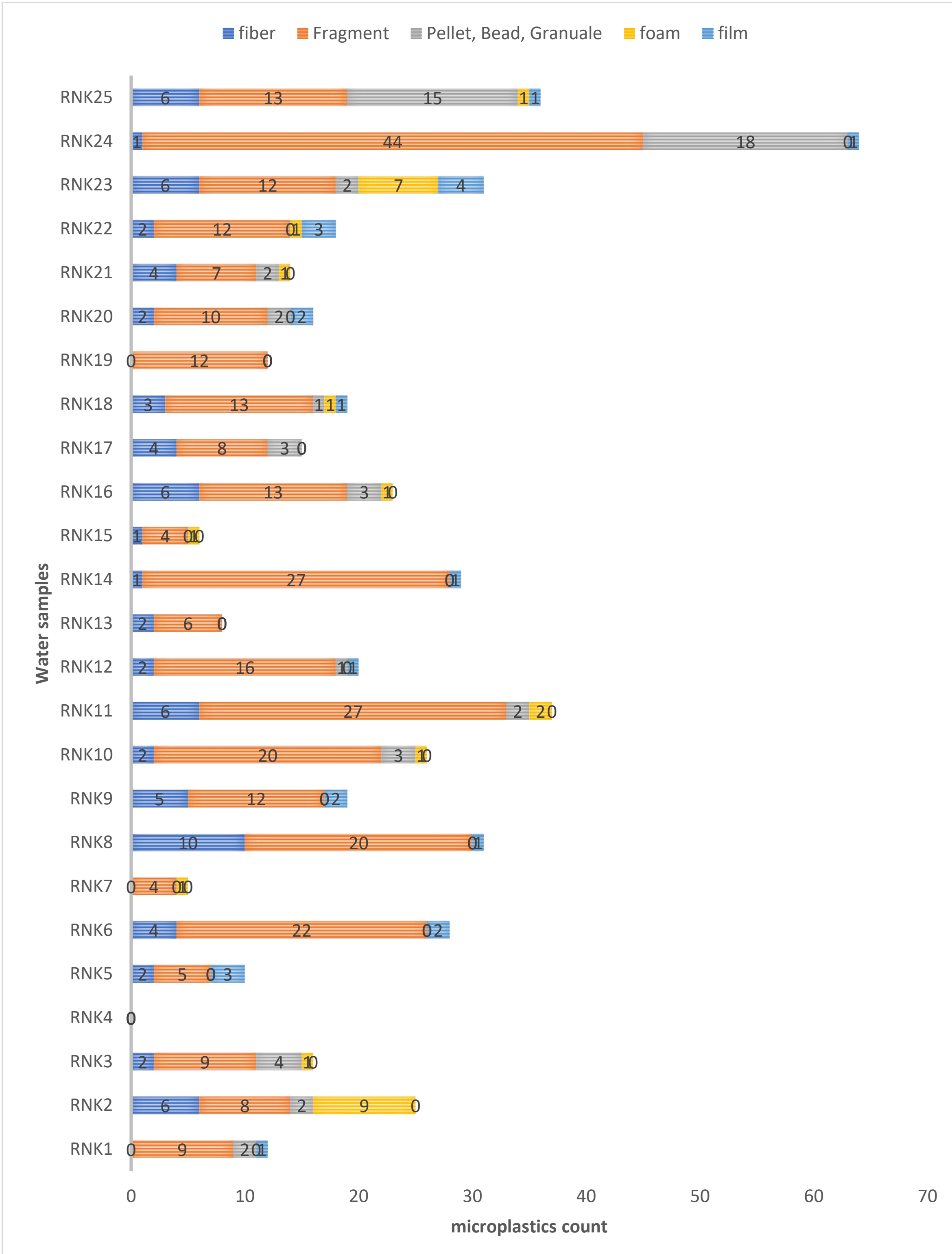

Fig.8. Stacked bar representation of morphologically characterized microplastics in water samples.

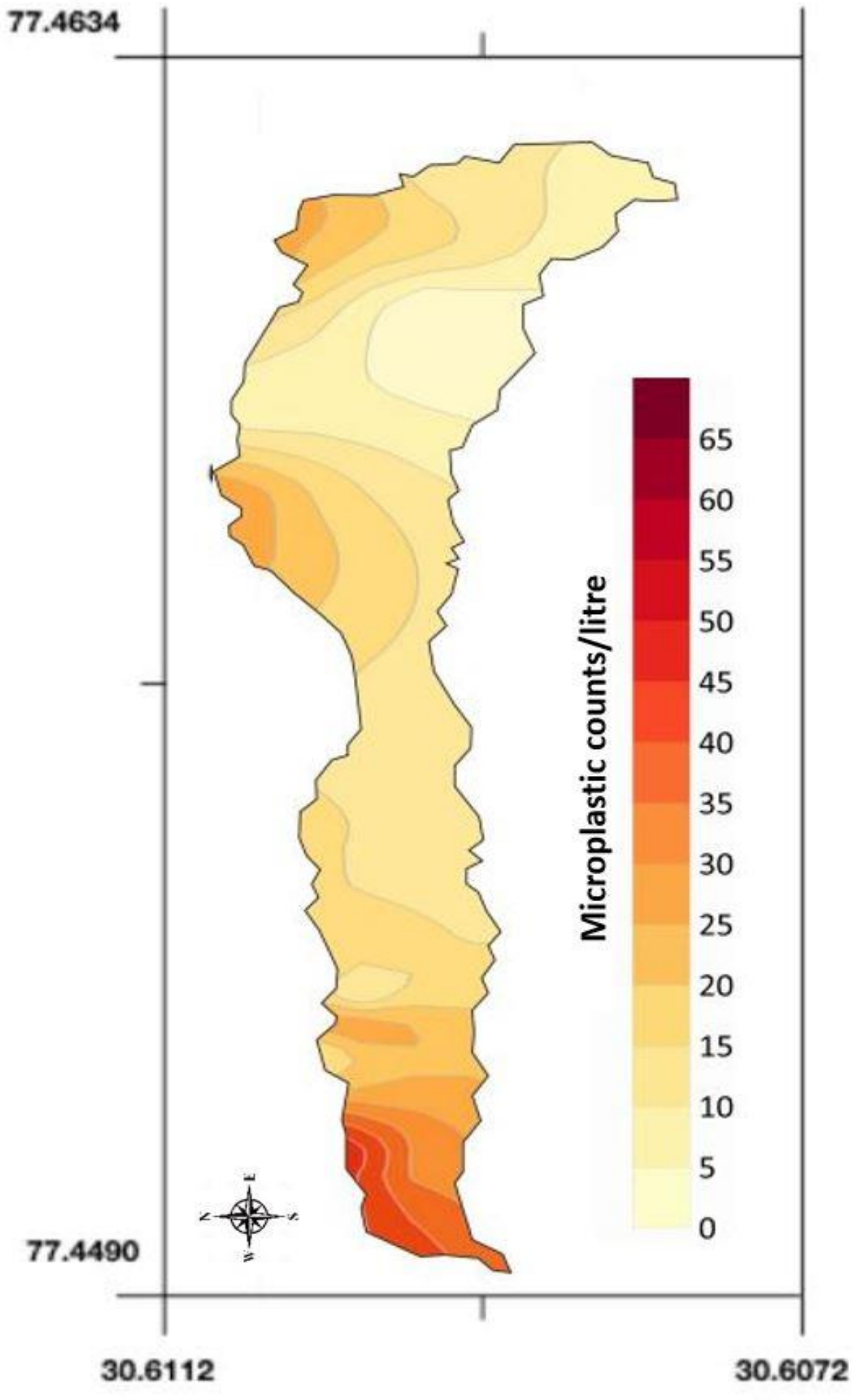

Fig.9. Spatial distribution of total microplastic counts in water samples.

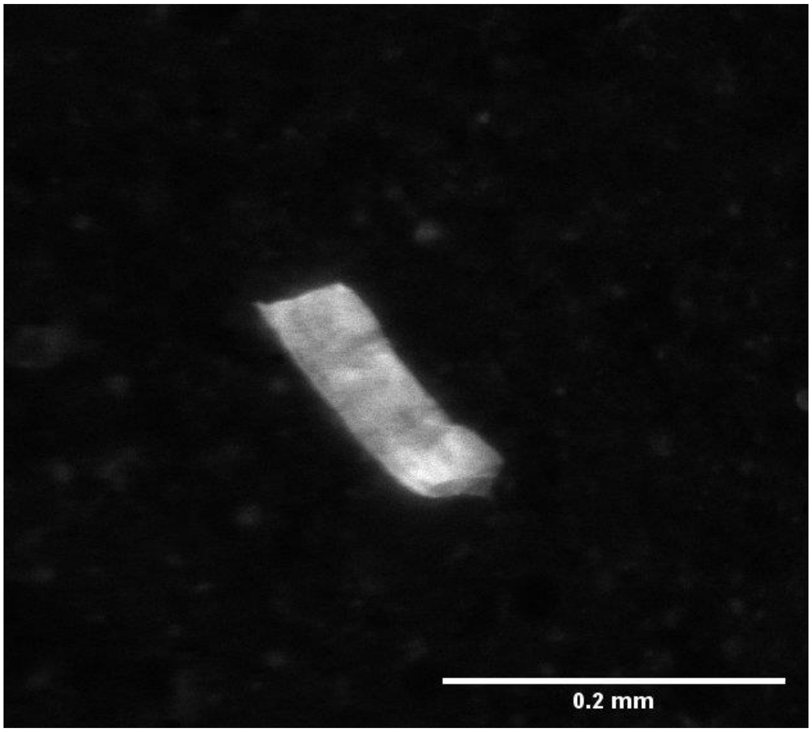

Image9: Film shaped microplastic.

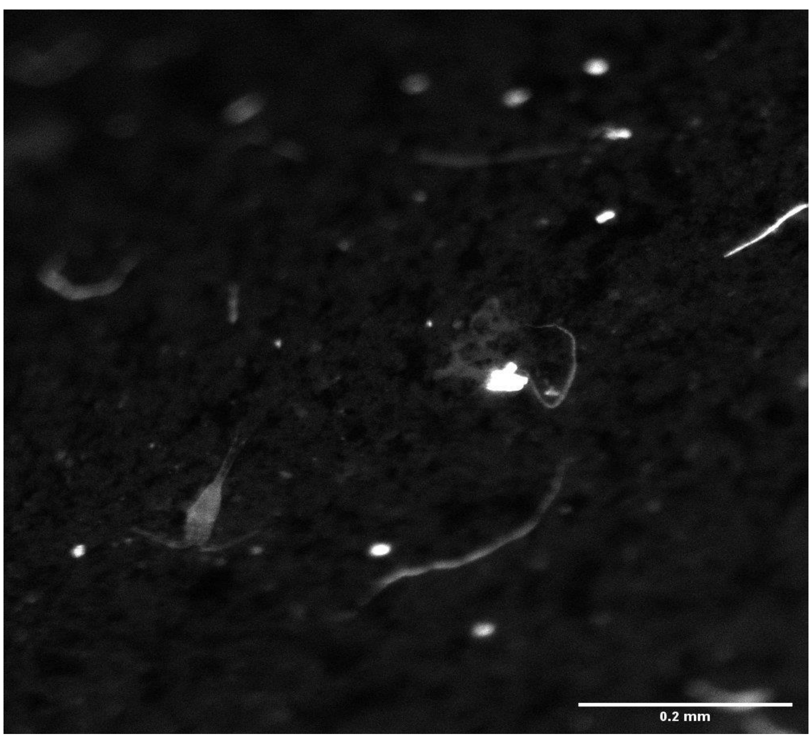

Image10: Pellet (Beads) shaped microplastic.

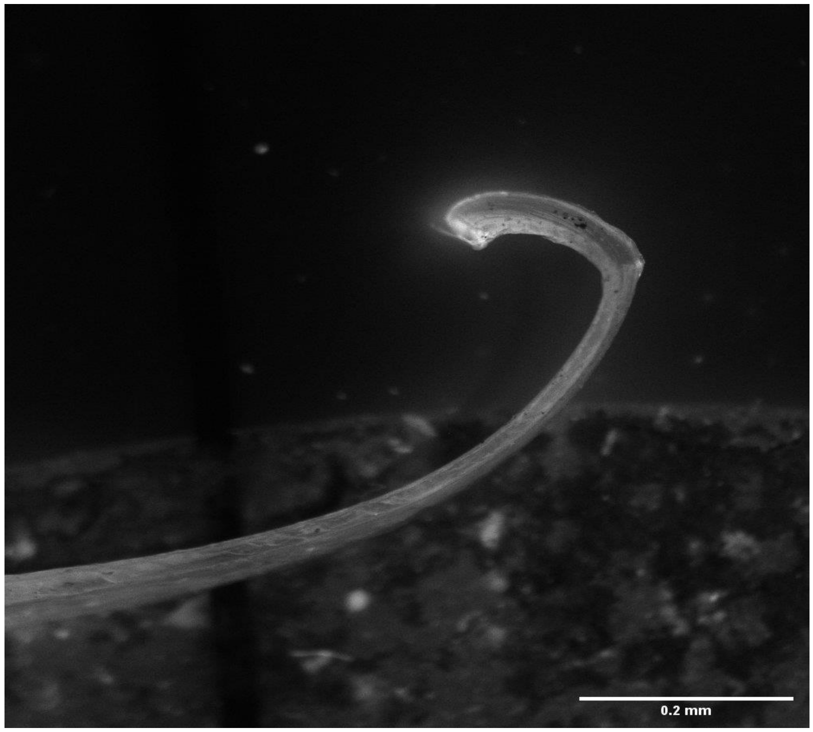

Image11: Fibre shaped microplastic.

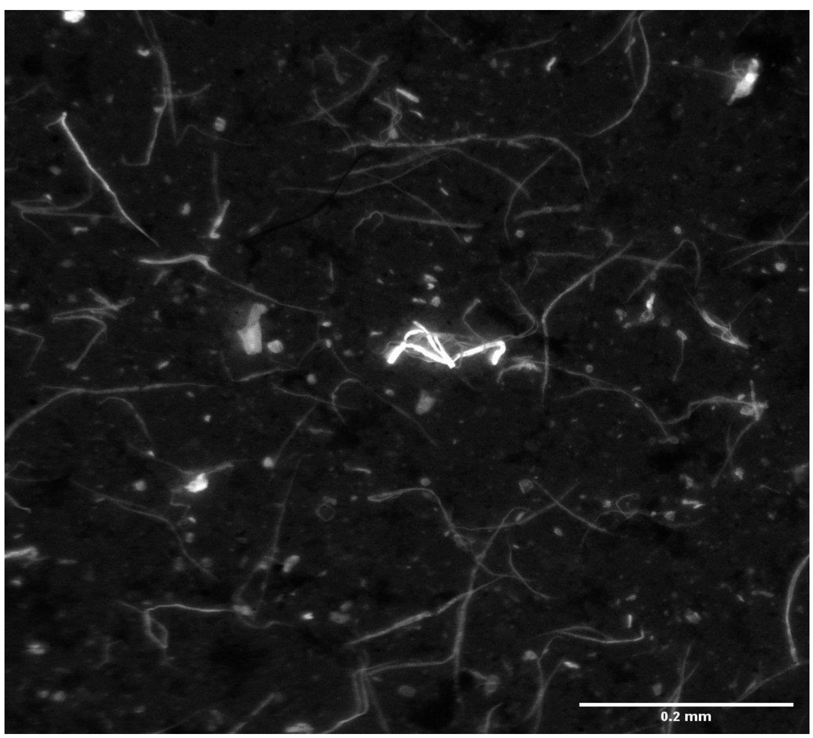

Image12: Fragment shaped microplastic.

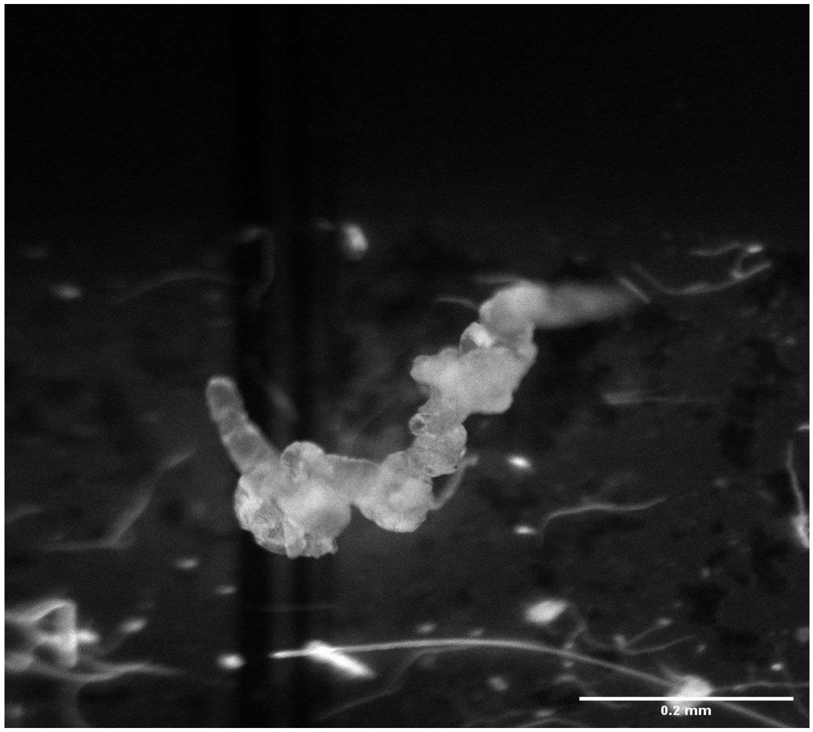

Image13: Foam shaped microplastic.

Compositional analysis of microplastics was performed using Renishaw InVia Raman spectrometer (Fig. 10) (Fischer et al. 2015; Löder et al., 2015). The identified microplastics were characterised as polypropylene based on the characteristic Raman shifts (Fig. 11 and Fig. 12). Among the observed Raman lines for the investigated microplastics, frequency of 1637 cm^{-1} can be ascribed to C=C frequency of styrene while the symmetric and antisymmetric CH2 valency vibrations can be attributed to the frequency range of 2953 cm^{-1} to 2885 cm^{-1}. The Raman spectra of polypropylene peaks are observed between 500- 1500 cm^{-1} and from 2600- 3000cm^{-1} characteristic to C-C backbone stretching, deformation and stretching vibrations of -CH2- and -CH3 groups present in the polymeric structure. The C-H stretching vibration mode in methyl group present in PP is represented with 2905 cm^{-1}.

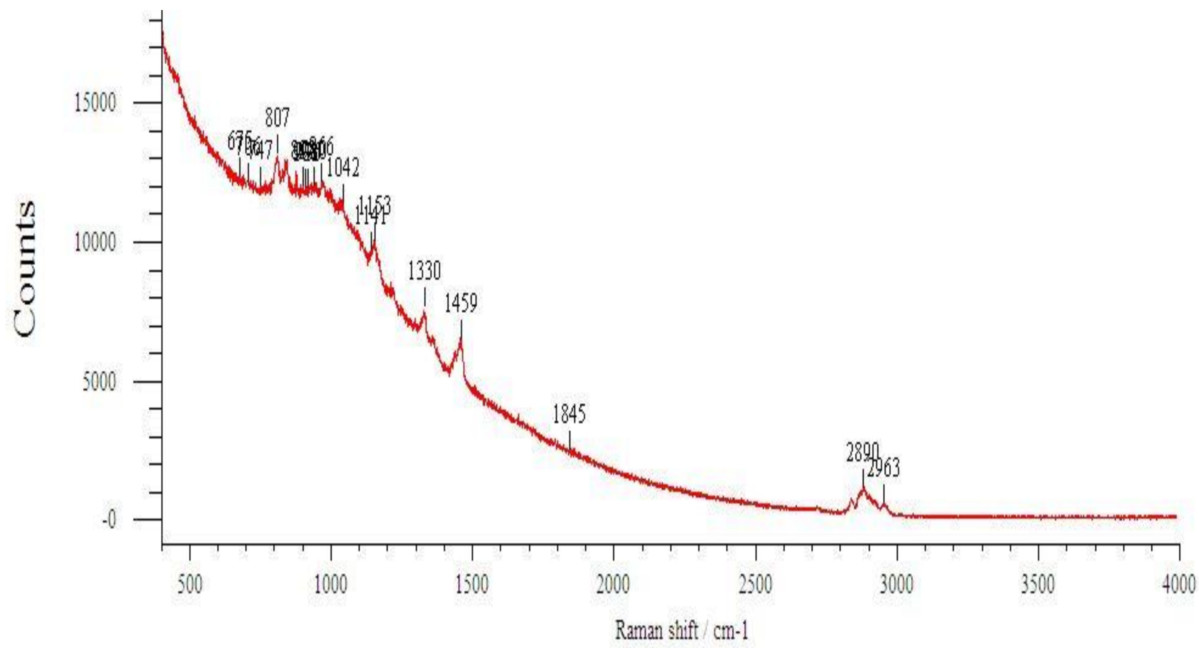

Fig.10. Raw spectra of Raman scattering.

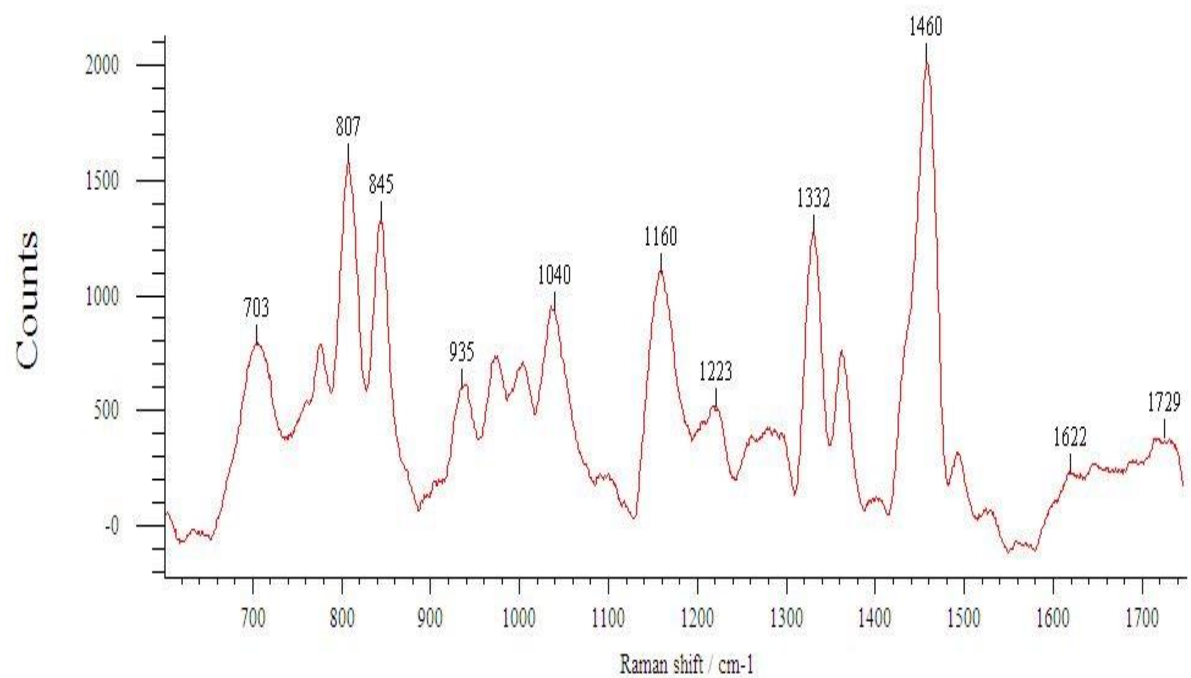

Fig.11. Baseline corrected Raman spectra of polypropylene microplastic fragment.

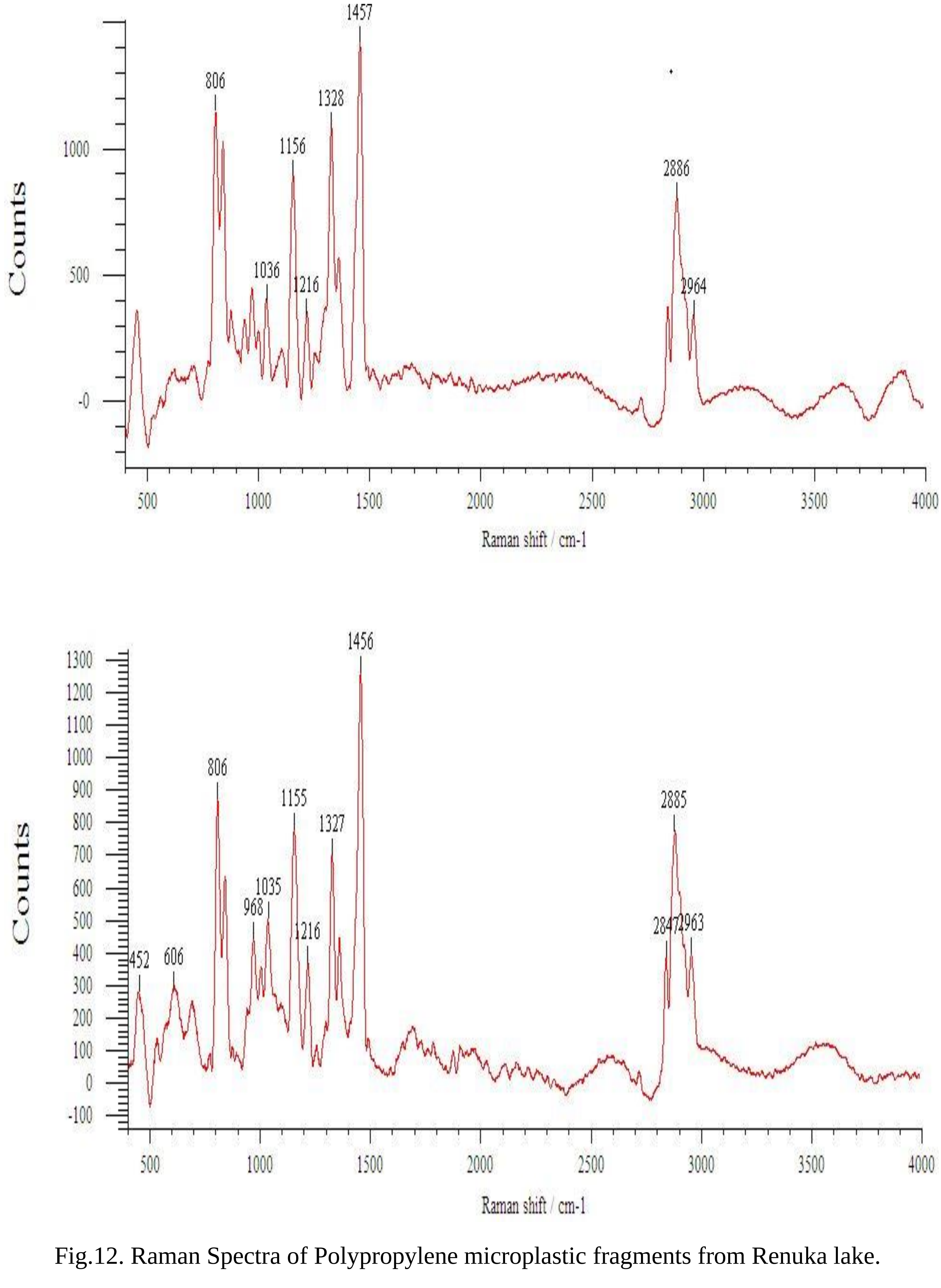

Fig.12. Raman Spectra of Polypropylene microplastic fragments from Renuka lake.

Chapter 4

Conclusions & Highlights

4.1. Conclusions

The data on extent of microplastic contaminant in a freshwater system lacks across Indian subcontinent. This study reports for the first time, the concentration of microplastics from a lake system in Indian subcontinent. Microplastics extraction and quantification was performed on sediment and water samples from a freshwater lake system to assess the anthropogenic influence of these contaminants into the environment. We evaluated the applicability of standard procedures including density separation and wet peroxide oxidation to extract MPs from the sediment's samples collected from Renuka lake. The microplastic concentration in the water and sediment samples clearly demonstrates a shift in microplastic concentration in the anthropogenic affected catchment of the lake system. The identification of investigated MPs using Raman spectrometer characterizes these particles as Polypropylene. Furthermore, this data will serve as baseline for the policy makers and waste management committees to draft policy decisions.

4.2. Highlights

- MPs were recovered from all sediment and water samples, indicating their extensive distribution in the lake.
- The abundance of MPs recorded from the sediment samples is in the range of 5-86 particles/500gm and for water samples is in the range of 5-65 particles/litre.
- Polypropylene has been identified as the dominant type of polymer component of the MPs.
- Investigation shows first report of microplastics in lake sediments and water samples from northern India.

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