ANNEXURE III

MICRO PLASTIC ASSESSMENT REPORT

Table of Content

Micro-plastics and their assessment in Ganga and Yamuna Basin – Snapshots from Agra and Allahabad

1.1 Micro-plastics and indicated key policy concerns

Micro-plastics are tiny fragments of plastic smaller than a few millimeters, such as micro-beads used in exfoliators and injection moulding, or plastic debris resulting from the fragmentation of larger plastic objects. Micro-plastics are not a specific kind of plastic, but rather any type of plastic fragment that is less than 5 mm in length according to the U.S. National Oceanic and Atmospheric Administration (NOAA).

The term "micro-plastic" coined by Thompson et al. in the year 2004 basically represents heterogeneous mixture of smaller plastic fragments in the size range of 0.001-5 mm.

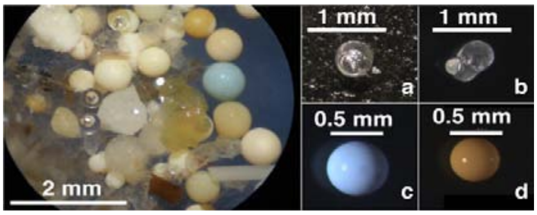
It has been indicated that essentially there are two categories of micro-plastics:

Primary: – Primary micro-plastics are any plastic fragments or particles that are already 5.0 mm in size or less before entering the environment. These include micro-fibers from clothing, micro-beads, and plastic pellets. These are purposely manufactured to fulfil a function. These also include microplastics from cosmetics, detergents, paints, cleaning products, pharmaceuticals (nano-capsules), fertilizers etc.

Secondary: – Secondary micro-plastics are those that are generated from the degradation process and resulting from wear and tear or fragmentation of larger plastic products once they enter the environment through natural weathering processes. Sources of secondary micro-plastics include water and soda bottles, fishing nets, plastic bags and many others, including from wearing of tyres, synthetic textiles, pellet losses, plastic dust from shredders or dust from handling of plastics in landfills etc.

Both types are recognized to persist in the environment at high levels, particularly in aquatic and marine ecosystems.

Types of microplastics by shape are reflected in the figure below.

<table>
<tr><td></td><td></td></tr>
<tr><td>Fibres</td><td>Pellets</td></tr>
<tr><td></td><td></td></tr>
<tr><td>Films</td><td>Fragments</td></tr>
<tr><td></td><td>
</td></tr>
<tr><td>Foams</td><td>Microbeads</td></tr>
<tr><td colspan="2">Figure 1 : Various Types of Microplastics by Shape</td></tr>
</table>

In view of the attention on microplastics that are reaching and being found in aquatic environments, it is important to reflect on the polymer types and their tendency to float or sink in aquatic systems and table below is a reflection of the same.

Table 6.1: Specific Gravity of various polymer types and general behaviour in aquatic environment (adapted from GESAMP 2016)

Polymer	Common Applications	Specific Gravity	Behavior
Polystyrene (expanded)	Cool boxes, floats, cups	0.02 – 0.64	Float
Polypropylene	Rope, bottle caps, gear, strapping	0.90-0.92	
Polyethylene	Plastic bags, storage containers	0.91-0.95	
Styrene-butadiene (SBR)	Car tyres	0.94	
Average seawater		1.03	
Polystyrene	Utensils, containers	1.04-1.09	Sink
Polyamide or Nylon	Fishing nets, rope	1.13-1.15	
Polyacrylonitrile (acrylic)	Textiles	1.18	
Polyvinyl chloride	Thin films, drainage pipes, containers	1.16-1.30	
Polymethylacrylate	Windows (acrylic glass)	1.17-1.20	
Polyurethane	Rigid and flexible foams for insulation and furnishings	1.20	
Cellulose Acetate	Cigarette filters	1.22-1.24	
Poly (ethylene terephthalate) (PET)	Bottle, strapping	1.34-1.39	
Polyester resin+glass Fibre	Textiles, boats	>1.35	
Rayon	Textiles, sanitary products	1.50	
Polytetrafluoroethylene (PTFE)	Teflon, insulating Plastics	2.2	

2.1 Key policy concerns regarding Micro-plastics

The policy concerns and implications regarding microplastics are several and some of the key aspects are enumerated below (UN SDGs 2030 include Marine Litter/pollution concerns and the density of floating plastic litter is reflected as an Indicator 14.1).

Abundance of marine litter in seas under national jurisdiction: Type and origin of marine litter.

Identification of hotspots

Data points for GIS applications setting target for reduction measures Impact on:

- Biodiversity and animal welfare Human health issues and injuries Seafood safety
- Food security-ghost fishing Tourism and recreation Maritime safety (navigation)

It is indicative in the literature and ongoing research that a substantive focus has been to identify and understand nature of microplastics in marine environments and in sediments also of inland waterways in various countries and oceanic regions by researchers and various institutions. The exploration of microplastics is accordingly being further undertaken in the Ganga and Yamuna river stretches towards obtaining snapshots from Allahabad and Agra cities of India as well and to reflect parallel with the nature of macroplastics being found to be disposed and / or leaking from identified hotspots in these cities, as being explored and studied in the hotspots particularly near the river banks or on the river beds here.

The existence of Microplastics and related concerns: – Key insights from literature

Micro-plastics are being found almost everywhere. These can be located at remote mountain top glaciers and also the human gut. Indeed tiny pieces of plastic appear in the most unexpected places. The identification of microplastics in the environment has been reflected since 1970s as a pollutant found via studies when in large quantities traced as synthetic fibers and plastic fragments in the North Atlantic Ocean (Barbuzano, 2019). Explorations are ongoing to understand how and where these particles originate.

It is indicated that micro-plastics are carried and dispersed throughout the world's oceans; at shorelines, beaches, in seabed sediments, and on surface waters from the Arctic to the Antarctic where they concentrate at remote locations (IMO, 2015). Further, assessments have been made that the distribution in the marine environment is influenced by the density of the particles, location of the sources and conveyance with ocean currents and waves (Kukulka et al., 2012; Magnusson et al., 2016). It has been indicated that the buoyant and persistent nature of micro-plastics allow them to become easily and widely dispersed via hydrodynamic processes and ocean currents (Carvalho and Baptista Neto, 2016).

It is important to note that investigations on the presence of micro-plastics in the marine environment started in the year 2000 onwards. Recently, research has shown that micro-plastics have ubiquitously permeated the aquatic ecosystem, and even the Polar Regions are not left out (Lusher et al., 2015a,b; Barnes,)

The sources of micro-plastic in river are considered as likely to be:

- Mismanaged plastic waste
- Wastewater discharge
- inland navigation and
- industrial activities

As per available research and literature, Rivers are viewed as highways that enable micro-plastics generated inland to reach the ocean (Barbuzano, 2019). It has been estimated that at a rate of 3.5 particles per cubic meter of water, the Ebro River dumps 2.2 billion pieces of micro-plastic into the Mediterranean Sea every year. The origin of marine plastic debris is emphasized to be from land-based sources and rivers (Schmidt, et al, 2017). It is also indicated that plastic debris related loads, including micro-plastic (particles <5 mm) and macro-plastic (particles >5 mm) are found to be positively related to the mismanaged plastic waste (MMPW) generated in the river catchments (Schmidt, et al, 2017). Further, Micro-plastics have been identified in mangrove sediments in some locations, regarding which it has been also indicated there is little if any data on meso-litter in this habitat (Mohamed Nor and Obbard 2014, GESAMP, 2019).

The estimates being made also state that the 10 top-ranked rivers transport 88–95% of the global load into the sea (Schmidt, et al, 2017) and that the Yangtze River in China contributes 55 percent of the estimated 2.75 million metric tonnes of plastic waste going into oceans each year. As regards efficiency of transportation, it is indicated that Micro-plastic is more efficiently transported than macroplastic (Schmidt, et al, 2017). The estimates also as reflected in a IUCN report of year 2017 that Micro-plastics could contribute up to 30% of the Great Pacific Garbage Patch polluting the world's oceans and, in many developed countries, are a bigger source of marine plastic pollution than the visible larger pieces of marine litter.

2.1.1 Concerns and impacts of micro-plastics on aquatic eco-system

Some of the key concerns regarding microplastics that have been highlighted include the following.

Micro-plastic is ingested by marine organisms (Wright et al. 2013), and may impact their physiological processes (von Moos et al. 2012; Cole et al. 2013, 2015; Rochman et al. 2013, 2014b; Wright et al. 2013; Watts et al. 2015; Lu et al. 2016; Sussarellu et al. 2016).

Micro-plastic may also contain harmful chemicals such as flame retardants, plasticizers, or dyes (Browne et al. 2013; Fries et al. 2013; Rochman et al. 2013, 2014a, b).

Micro-plastics may provide a substrate for the adsorption of other harmful chemicals in the ocean, like PCBs and DDT (Teuten et al. 2007).

And Micro-plastics with additional harmful chemicals then may be transferred up the food chain (e.g., Farrell and Nelson 2013; Rochman et al. 2014a; Setala et al. 2014)

2.1.2 Micro-plastics in marine sediments

It has been highlighted that Micro-plastics with density greater than that of sea water sink down in sediments and here they accumulate (Alomar et al., 2016; Woodall et al., 2014), and that those which have low density float on the sea surfaces (Suaria and Aliani, 2014).It is also indicated that an increase in density can occur via biofouling by organisms in the marine environment and this can result in sinking of micro-plastics as well, since density of the aggregated mass rises due to biofouling and once this becomes greater than that of sea water, the plastic material sinks to the bottom of the sea (Andrady, 2011; Reisser et al., 2013; Jorissen, 2014). Studies have been made to reflect on the potential of marine sediments to accumulate micro-plastics (Nuelle et al., 2014), and that these are appearing as long-term sinks for micro-plastics (Cozar et al., 2014).

The estimates that are arising from studies with regard to marine sediments are also indicative. It has been reflected that significant concentrations of micro-plastics being found within marine sediments; and that plastics can make up 3.3% of sediment weight on heavily impacted beaches (Van Cauwenberghe et al., 2015a, 2015b; Boucher et al., 2016). Indeed deep sea areas, and submarine canyons, besides marine coastal shallow sediments are being considered as sinks for micro-plastics (Alomar et al., 2016; Pham et al., 2014).

2.1.3 Micro-plastics in mangrove sediments

A mangrove is a tree or shrub which grows in tidal, chiefly tropical, coastal swamps, having numerous tangled roots that grow above ground and form dense thickets. The term is also used for tropical coastal vegetation consisting of such species. Mangroves accumulate carbon, nutrients and sediments; hence, it is often referred to as "enhancer of sedimentation" (Valiela and Cole, 2002). The deposition of sediments into mangroves occur from different sources; allochthonous sediments - these are sediments that come from external sources such as terrestrial or oceanic sources, and the autochthonous sources which are sediments that are re-suspended in the same region (Adame et al., 2010). As with sediments in other aquatic environments, micro-plastics similarly accumulate in mangrove sediments.

In a study conducted by Nor and Obbard (2014) to study the prevalence of micro-plastics in mangrove habitats of Singapore, micro-plastic particles were extracted using the floatation technique and then counted and categorized according to particle shape and size. And polymer identification was done using Attenuated Total Reflectance- Fourier Transform Infrared (ATR-FTIR) spectroscopy. The plastic particles extracted were smaller than 20 μm and contained polypropylene, polyvinyl chloride, nylon and polyethylene. The concentration of the micro-plastics ranged from 12.0–62.7 particles per dry sediment sample taken.

2.1.4 Microplastics and the interaction with Marine Biota

As the abundance of micro-plastics grows, its bioavailability to marine organisms is indicated to be increasing. Concerns about the transfer of micro-plastics and harmful chemicals between trophic levels have resulted in laboratory studies being carried out to demonstrate the impacts of micro-plastics on marine biota. It is emphasized that the color, density, shape, size, charge, aggregation and abundance of these tiny plastic particles affect their potential bioavailability to marine organisms (Wright et al., 2013; Van Cauwenberghe et al., 2015a,b). Indeed, the ingestion of micro-plastic particles has been observed in oceanic regions globally in a wide range of marine organisms as presented in studies and assessments (Ferreira et al., 2016; Setälä et al., 2015; Devriese et al., 2015; Green, 2016). Ingestion of micro-plastics by marine organisms in most cases is indicated to be accidental because the particle is often mistaken for food, although some microplastics can be specifically targeted by some organisms (Lönnstedt and Eklöv, 2016).

It is indicated that when micro-plastics are ingested by marine organisms, these cause chemical and physical harm. These clog the digestive tract, or the effect could be chemical such as inflammation, hepatic stress, decreased growth (Setala et al., 2016). It has been reflected that the larger plastics, in the form of fishing lines and nets cause entanglement of the marine biota and organisms and hinders their mobility.

It has been shown that the consumption of micro-plastics is common to a wide range of marine organisms representing different trophic levels and that these are entering the food chain towards higher trophic levels. Microplastics are being ingested by invertebrates, including especially lugworms (Green et al., 2016; Besseling et al., 2012), barnacle; sea cucumbers, amphipods and zooplankton (Rehse et al., 2016; Cole et al., 2013; Goldstein and Goodwin, 2013), mussels (von Moos et al., 2012; Avio et al., 2016), and fish-eating birds, fishes, turtles, and mammals (Ferreira et al., 2016; Batel et al., 2016; Fossi et al., 2016; Caron et al., 2016), and accordingly, microplastics are being found to interfere with the food chain, as micro-plastics ingested by organisms in the lower trophic level including zooplankton and copepods, could pass up the food chain when lower trophic organisms are consumed by the higher ones.

2.1.5 Micro-plastics in fish

Several studies have also been undertaken to prove that micro-plastics are a peril for fish as mortality is prevalent before reaching maturity due to micro-plastic ingestion. Studies have reported the presence of chemicals in fish tissues which are the same chemicals that form plastics. Predator-prey interaction enhances the transfer of the toxic chemicals in greater concentrations since toxic chemicals from multiple sources can accumulate in the body (Andrady, 2011; Wang et al., 2016). Batel et al. (2016) investigated the transfer of micro-plastics and potential harmful substances between different trophic levels in the marine environment. The study clearly proved that micro-plastics and associated harmful substances can be transferred along food chains across various trophic levels. Figure 2 highlights the issue.

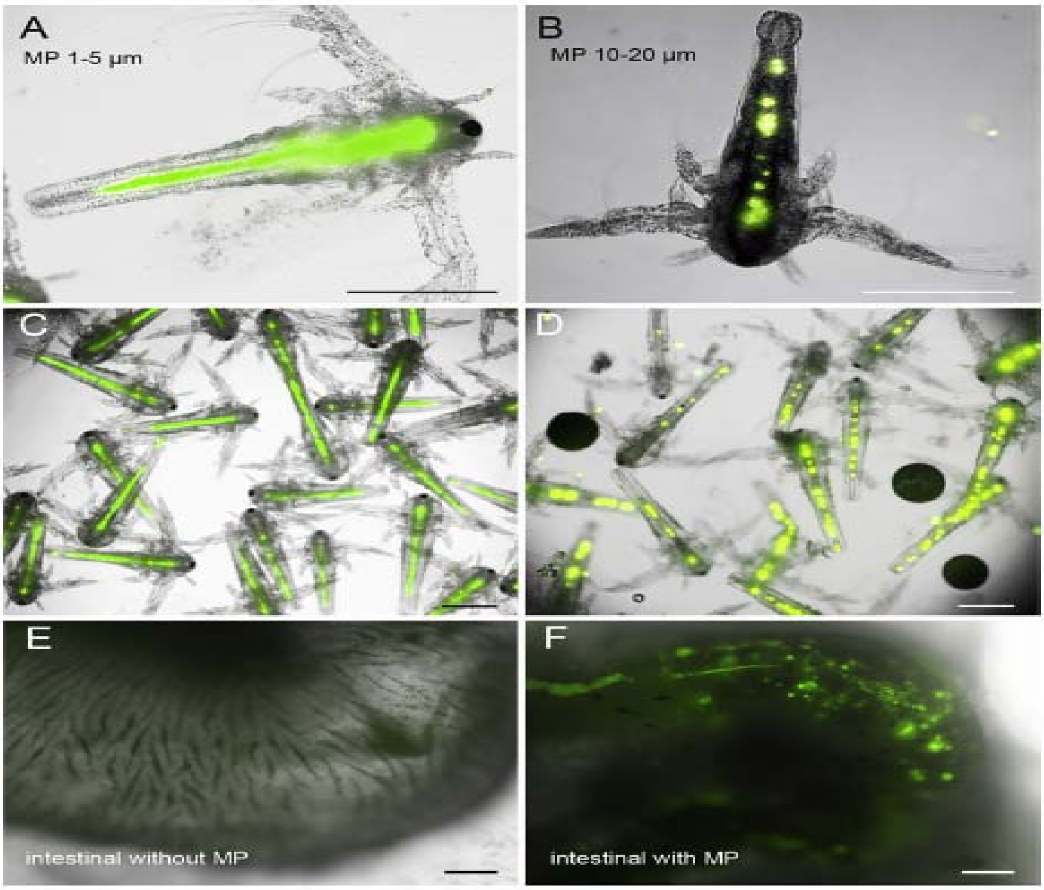


Figure 2: Uptake and transfer of fluorescently labeled micro-plastic particles from Artemia nauplii (instar II) to zebrafish (Danio rerio).
Source- A. Batel et al., Environ Toxicol Chem 35, 2016

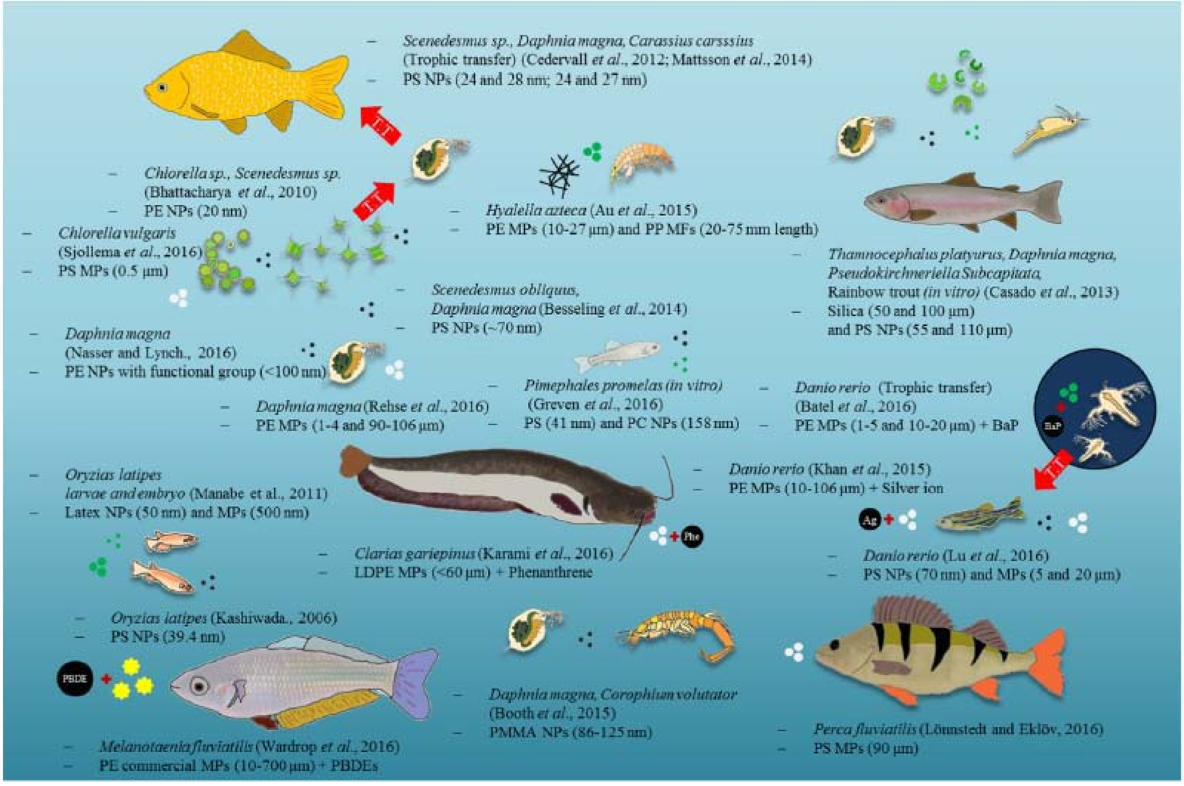


Figure 3: Studies on the toxicities of micro-plastics and nanoplastics to freshwater organisms and ecosystems. Source-Y. Chae et al. In Marine Pollution Bulletin (2017)

2.1.6 Micro-plastics in other marine biota

The issue of micro-plastic ingestion has gone beyond fishes and zooplanktons and sea turtles are also found to be susceptible to micro-plastics. Outdoor mesocosm studies were carried out on the effect of micro-plastics on the health and biological functioning of the European flat oyster (Ostrea edulis) and on the structure of associated macrofauna. The organisms were subjected to low and high doses (0.8 µg-1 L and 80 µg L-1) of biodegradable and conventional micro -plastics for a 60 day period. After exposure, it has been indicated that it was observed that the respiration rates of Ostrea edulis were elevated in response to high doses of polylactic acid (PLA) which indicated that the oysters were under stress. It has been also indicated that similarly the abundance and biomass of associated benthic organisms which included periwinkles (Littorina sp.), isopod (Idotea balthica), and the peppery furrow shell clam (Scrobicularia plana) reduced, and that the reduction was attributed to reduced reproductive output and mortality due to micro-plastic ingestion and reduced feeding (Green, 2016).

Further, it has been cited that Desforges et al. (2015) investigated micro-plastic ingestion by two ecologically important zooplankton in the North Pacific marine food web; the calanoid copepod (Neocalanus cristatus), and the euphasiid (Euphasia pacifica) using acid digestion method to assess the ingestion of micro-plastics by the zooplankton. In another study Cole et al. (2016), demonstrated the effect of polystyrene microbeads on the feeding, function and fertility of the marine copepod; Calanus helgolandicus. The copepods were exposed to 75 mL-1 of polystyrene beads and 250 µg CL-1 of cultured algae. It was observed by the researchers that the copepods exposed to the micro-plastics ingested fewer algal cells which resulted in 11% reduction in algal cells and a significant reduction in carbon biomass (40%). They found that the prolonged exposure resulted in death of some of the copepods, fewer egg productions, and decreased reproductive output which affected hatching. The study highlighted that copepods exposed to micro-plastics suffered energy depletion overtime, and that impeded feeding in copepods. The results have been indicated to be comparable with Kaposi et al. (2014) and Lee et al. (2013) who in their research and analysis proved that the survival of zooplankton may be impacted by exposure to high concentrations of micro-plastics.

3.1 Research on micro-plastic assessment in Indian Rivers and comparisons

Research work on river Ganga (Sarkar, etal, 2019) indicated that the plastic pollution in river Ganga in eastern parts is mainly from Polyethylene terephthalate (PET) (39%) followed by polyethylene (PE) (30%). It was also found that level of micro-plastic in river Ganga is lower as compared to other rivers of the world.

Table 6.2: Micro-plastics assessments in rivers - Research insights

Rivers	Mass Fraction	Number Fraction
River Ganga, India	37.56 ± 16.50 ng/g as mass fraction	210.25 ± 124.65 items/kg
River Antuã, Portugal	2600 to 71,400 ng/g	
Rhine river		228–3760 items/kg
Beijiang river		178 to 544 items/kg,
Thames river		185–660 items/kg,

Source: Rodrigues et al., 2019, Klein et al., 2015; Wang et al., 2017; Horton et al., 2017.

In a study by Anju, etal, 2019, it was found that polypropylene was the most abundant polymer type followed by polyethylene and polyvinyl aldehyde in sediments of river Ganga. They found that middle zone of river Ganga has more micro-plastics in the sediments.

4.1 Methodology for Micro-plastic Assessment

An outline of the micro-plastics assessment process is presented in Figure 4. The sequence essentially involves sampling, extraction, identification and quantification, with specific features for water, sediment and biota samples testing. Suitable equipments are developed for the purpose. There are further efforts ongoing to standardize the ongoing efforts for making the procedures and results comparable across studies and regions, for informing suitable policy making and developing and implementing interventions and countermeasures to address the growing macro and microplastics problem.

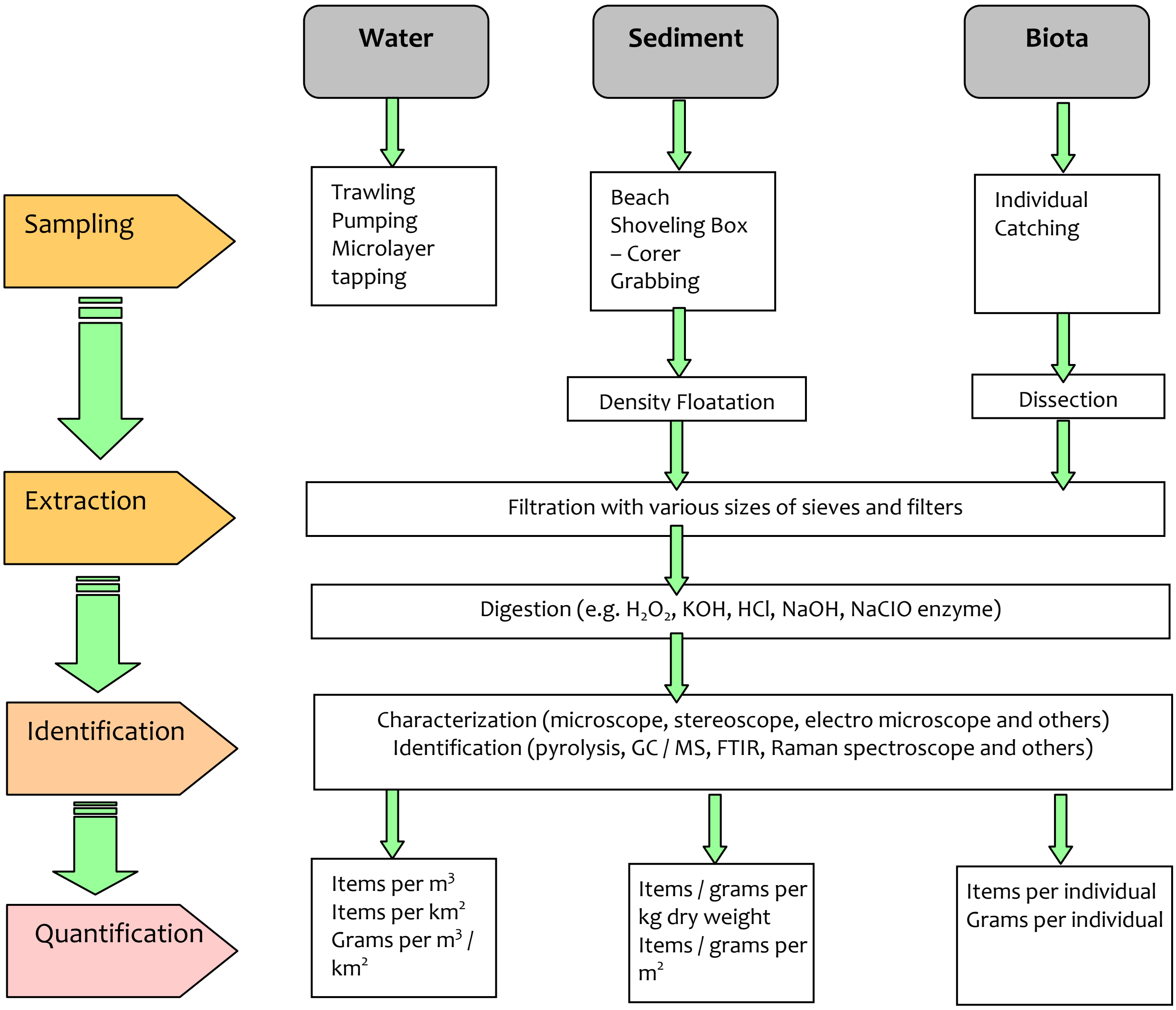


Figure 4: Overall methodological features of microplastics assessments

4.1.1 Microplastics assessment– Focus on sampling

The analysis of microplastics in the environment starts with sample collection. Selection of an appropriate technique is essential as it will determine the types of microplastics that are collected, separated, identified and subsequently reported. The method of sample collection is influenced by many factors. However, primarily the matrix to be sampled (water, sediment, soil, air or biota) will determine the abundance, size and shape of the microplastics obtained.

There are three main methods of sampling:

Selective sampling

Items visible to the naked eye are directly extracted from the environment, such as on the surface of the water or sediment.

This collection method is adequate in situations where different microplastics of similar morphology and of a size greater than 1mm are present, such as primary microplastics pellets and similarly shaped secondary microplastics.

However, the main disadvantage of this technique is that the less obvious, more heterogeneous items are often overlooked, particularly when they are mixed with other contaminants

Volume reduced sampling

The volume of the bulk sample is reduced until only the specific items of interest for further analysis remains. Thus, the majority of the sample is discarded.

Consequently, this method is typically utilized to collect samples from surface water because it has the advantage that large areas or quantities of water can be sampled.).

Bulk sampling

The entire sample is taken without reducing its volume. Although there are practical limitations to the amount of sample that can be collected, stored and processed, the advantage of this method is that in theory, all the microplastics in the sample can be collected, regardless of their size or visibility.

4.1.2 Equipment used as reflected by the researchers

The most common method of sampling floating micro-plastics is to use a towed net, such as a manta trawl with a fixed mesh size, usually 330 µm. This means that any particle < 330 µm in diameter will be under-sampled. One way of overcoming this problem would be to pump a water sample through a 1 µm filter, to provide a measure of all the particles in the micro-plastic size range (1 µm – 5 mm). However, this is not feasible for routine monitoring. This is an operational constraint but it should not detract from the utility of using towed nets for monitoring purposes, to detect trends in space and time (GESAMP, 2019)

Table 6.3: Key methods in microplastics sampling / observations and their advantages and limitations

Method	Explanation	Advantage	Limitations	Sources
Net tows (Manta Trawl, Neuston Net)	Fine-mesh net attached to a large rectangular frame developed for sampling surface and water column waters for plankton, insects and other small biota. Manta trawl with floating wings to keep it on the surface.	Can be deployed from small to large vessels. Use of flow meter to estimate volume. And flow over time.	Use is weather dependent Care needed to minimize contamination from sampling vessel and tow ropes. Can only estimate volume of water filtered when flow meter is used and the frame completely immersed Towing speed and time must be limited to avoid clogging.	Virsek et al. (2016)
Visual observation	Visual survey of floating	Easy to do from	Limited to water	Ryan (2013

Method	Explanation	Advantage	Limitations	Sources
from a ship	marine litter from the surface of a vessel at sea. Use either fixed width transects (assumes all items seen) or distance sampling (corrects for decrease in detection probability with distance from the vessel).	vessels of opportunity. Low cost, needs only binoculars (but ideally also a good quality digital SLR camera and telephoto lens).	adjacent to the ship (up to 50 m typically). Bias against dark items and subsurface items white and buoyant items easier to spot.	
Photographic and aerial surveys	Visual survey of floating marine litter from an airplane or drone.	Cover large area, ideal for mega litter.	High cost to charter, expensive photography equipment.	Lebreton (2018)

Figure 5 (a and b): Schematic and plate of Microplastics sampling device by Pirika

This device consists of a battery and a screw, a filtered water counter and plankton net. It sucks in water with driving screw, and collects solid that contains plastics. To estimate volume of sampled water, recording of values of the filtered water counter are made before submerging and after being pulled up.

4.1.2.1 Protocols followed for sampling

NOAA Protocol for water samples (Masura et al., 2015):

This method can be used for the analysis of plastic debris as suspended solids in water samples collected by a surface net. Plastics include hard plastics, soft plastics (e.g. foams), films, line, fibers, and sheets. The method involves the filtration of solids obtained in a 0.335 mm surface sampling net (e.g. a manta net for surface water tows) through 5.6-mm and/or 0.3-mm sieves to isolate the solid material of the appropriate size. The sieved material is dried to determine the solids mass in the sample. The solids are subjected to wet peroxide oxidation (WPO) in the presence of a Fe(II) catalyst to digest labile organic matter. The plastic debris remains unaltered. The WPO mixture is subjected to density separation in NaCl(aq) to isolate the plastic debris through flotation. The floating solids are separated from the denser undigested mineral components using a density separator. The floating plastic debris is collected in the density separator using a custom 0.3-mm filter, air-dried, and plastic material is removed and weighed to determine the micro-plastics concentration. Figure 6 below depicts the protocol for micro-plastic analysis.

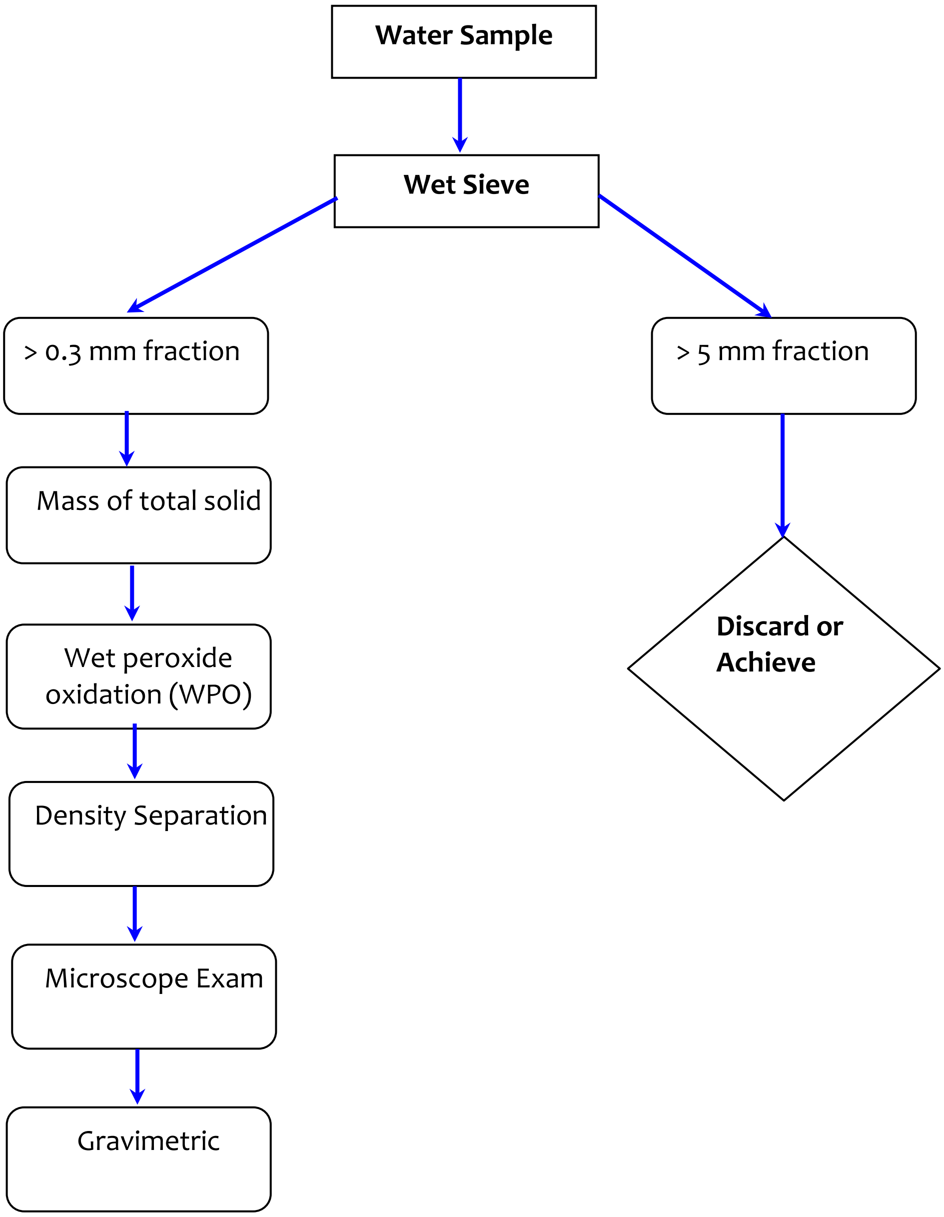


Figure 6: Protocol / Flow Diagram for the analysis of Micro-plastics in water samples

4.1.2.2 Environmental Condition

In general, wind speed and wave heights are known to influence the degree of vertical mixing of the ocean/river surface layer and affect the amount of micro-plastics collected. It has been indicated that Quantity of micro-plastics at the ocean surface greatly decreased in situations where both wind speed and wave height increased during sampling. It had been observed that density of Micro-plastics in the same sampling area changed by about one order within several hours, as sea conditions, including wind speed and wave height, changed. (CMSM 2018)

Table 6.4: Indications for preferred Sea Condition in literature and actual river condition observed for microplastics sampling at Allahabad and Agra

Parameter	Standard	Reference	Standard followed during collection and sampling of Micro- plastics in rivers in selected cities.
Sea Condition	Collection of Micro-plastics at the Ocean / river surface should be conducted under mild sea conditions. To avoid unfavorable timing and conditions for sampling, such as high concentrations of natural particles or organisms.	Guidelines for Harmonizing Ocean Surface Micro-plastic Monitoring Methods Version 1.0, May 2019 Ministry of the Environment, JAPAN May, 2019.	Collection of Micro- plastics done under calm condition.

4.1.2.3 Sampling Equipment

Generally, to collect Micro-plastics particles floating at the river/sea surface, most researchers use nets that can efficiently filter a large mass of water (Neuston or Manta nets). (Ref. Ministry of the Environment, JAPAN May, 2019, "Guidelines for Harmonizing Ocean Surface Microplastics Monitoring Methods - Version 1.0"). The features are reflected below and comparison of advantages and disadvantages indicated in Table 6.5.

(a) Neuston Net

Neuston nets can capture the ocean surface layer even in wavy conditions, but it is difficult to estimate the volume of water filtered accurately because the net's immersion depth changes constantly. It can operate in rough waters.

Figure 7: Neuston Net

(b) Manta Net

Manta nets can maintain a constant immersion depth under the sea surface and thus filtered water volume can be estimated fairly accurately providing there are no waves on the sea surface. If the wave height exceeds a certain level, the net tends to jump and skip on the water surface.

Figure 8: Manta Net

Table 6.5: Microplastic sampling Nets and relative advantages / disadvantages

Net	Advantage	Disadvantage	Reference
Manta net	Remains in surface water except in rough water.	Tends to jump and skip on rough water	Guidelines for harmonizing ocean surface micro plastic monitoring methods version 1.0 May 2019, Ministry of Environment Japan May 2019
Nueston net	Operates relatively in rough water	Needs some efforts to maintain the stable net immersion depth	

4.1.2.4 Key parameters for sampling equipment

Mesh Openings

"Mesh openings" as used in Japanese Guideline is expressed as the side length of a quadrangle separated by mesh thread and through which sea water passes (① in figure on right), but in some cases the length of the diagonal line (② in figure on right) is used as the mesh opening.

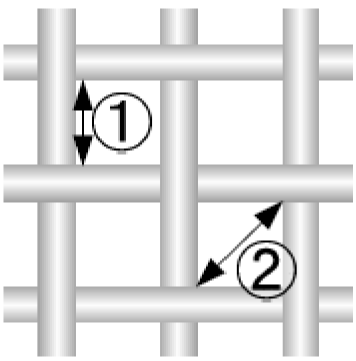


Figure 9: Mesh Opening

From a broader, global perspective, the use of the most common mesh opening (0.3 mm) is considered desirable.

- **Tow Duration**

Tow duration has most commonly been set at about 10 to 30 minutes in past surveys.

- **Vessel Speed**

Vessel speeds at the time of towing were reported as approximately 1 to 3 knots in earlier surveys.

- **Sweep area and filtered water volume**

Micro-plastics observed at the ocean surface are often reported as quantity of particles or weight per unit area (/m2, /km2) and/or as quantity of particles per unit water volume (/m3). Therefore, it is necessary to obtain the swept area of the net tow and/or the amount of filtered water volume, as calculated by the following equations:

Swept area = net width × tow distance

Filtered water volume = (net width × net immersion depth) × tow distance

* Net width is the horizontal dimension of the net aperture

- **Tow Distance**

There are three methods for obtaining tow distances, as follows:

- Calculate from ground speed obtained from position information measured by GPS, etc.
- Calculate from the relative speed of the vessel to seawater (log speed), measured with a current meter.
- Calculate using the rotation count of a flow meter installed in the net mouth and its calibration value.

In the case of utilization of flow meter set at the net mouth for various purposes, it needs to be suitably calibrated. A calibrated and suitably functioning flow meter can facilitate accurate assessment of concentration of micro-plastics per swept area and also concentration of micro-plastics per filtered water volume. While calibration of the flow meter is important, Location / vessel position at the start and end of each tow should be accurately recorded for the distance assessments.

- **Tow Position**

In general, a sampling net is towed at one side of the vessel. However, in some cases it may be towed at stern by angling a rope to divert the net from the center line of the vessel and avoid its wake. It is desirable to conduct sampling at the side of the vessel with less influence from its turbulence.

- **Net Immersion depth**

Recording immersion depth of the net during sampling is important as the section area of the net mouth under the sea surface is multiplied by the tow distance to estimate the filtered water volume. Net immersion depths have been recorded between 10 cm and 100 cm. Manta net immersion depth is measured as the height of the net's mouth, whereas a Neuston net is often set at about 1/2 to 3/4 of the height of the net's mouth.

Net depth as normal immediately after tow starts (net immersion depth normal)

Net immersion depth 13 min. after tow starts (net immersion depth increased).

Sample inside net with increased immersion depth (a large amount of sea buckthorn (Zostera marina) has been caught).

Figure 10: Indicative aspects of net immersion depth – A Case example with photographs

- **Recording Meta Data**

To ensure comparability, associated information to link to meteorological data for each sampling event should be recorded where possible through in situ observations or onboard instruments. Data required include:

- Time of day and date (to account for seasonality), as well as environmental variables (e.g., weather conditions, wind speed, wind direction, wave height, Beaufort scale index, etc.)

- Sampling parameters (net type and dimensions, measured sampling water volume, vessels movements —heave, pitch, roll, vessel speed, etc.).

- **Implementation of Blank Tests**

A blank test is recommended to be conducted for at least one of several nets to be used for a survey, as it can confirm whether sampling procedures such as washing have been carried out properly without contamination. When towing multiple times, it would be desirable to periodically conduct blank tests to ensure particle contamination has been sufficiently controlled.

5.1 Laboratory Analysis of Micro-plastics

In general, analysis of samples that include micro-plastics obtained by trawling a net through the ocean/river surface layer is carried out in the following order.

- Pretreatment (separation of non-plastic material other than micro-plastics), picking out micro-plastics, counting and measurement, and material identification.

- Pretreatment process are selected based on purpose of the study

5.1.1 Biological Digestion

When there are many non-plastic materials such as plankton (in the sample), pretreatment to digest organic substances with chemicals or enzymes is performed in many cases to remove the non-plastic material as well as biofilms that have formed on the surface of the sampled plastic particles. The intent is to minimize the possibility of misidentifying plastic particles, improving the accuracy of the picking out process and overall work efficiency. If improperly conducted, however, it may lead to deterioration (deformation and/or weight reduction) of plastic particles from chemicals added or from heating.

Add solution (H2O2 or formalin) React in Covered Beaker Reaction in Beaker

Figure 11: Photographs depicting digestion of organic matter through Biological digestion

5.1.1.1 Different Methods for Biological Digestion

The methods being utilized for biological digestion and their advantages / disadvantages are reflected in Table 6.

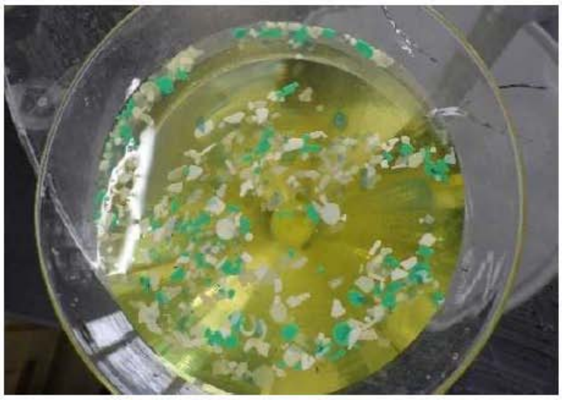
Table 6.6: Purification / Digestion methods and their advantages / disadvantages

Purification method	Advantages	Disadvantages	Reference
Oxidative digestion	Inexpensive Temperature needs to be controlled	Several applications may be needed	Masura et al. (2015)
Acid digestion	Rapid (24 h)	Can attack some polymers	Claessens et al. (2013)
Alkaline digestion	Effective Minimal damage to most polymers	Damages cellulose acetate	Dehaut et al. (2016)
Enzymatic digestion	Effective Minimal damage to most polymers	Time-consuming (several days)	Löder et al. (2017

5.1.2 Density Separation

Density separation may be performed to remove non-plastic material in the sample. Density separation is an effective method of fractionating low-density plastic particles and high-density natural particles of inorganic matter. In general, density separation is conducted by mixing the sample into a solution with a higher specific gravity than that estimated for the collected plastic particles, letting high-density inorganic substances settle out and recovering and fractionating the floating low-density plastic particles.

Density Separators



Floating plastic particles in a density separator. Plastics with lower specific gravity than the solvent float on the surface

Figure 12: Density Separation equipment / process

Table 6.7: Solutions commonly used for the density separation of micro- plastics (reproduced from GESAMP, 2019)

Salt	Density (g / cm3)	Reference
Sodium Chloride (NaCl)	1.2	Hidalgo-Ruz et al. 2012
Sodium Polytungstate (PST)	1.4	Hidalgo-Ruz et al. 2012
Sodium Iodide (NaI)	1.6	Claessens et al. 2013
Zinc Chloride ($ZnCl_2$)	1.7	Imhof et al. 2012
	1.6.	Zobkov & Esiukova, 2017

5.1.3 Sample Splition

Sample splitting before counting is often performed in analyses for zooplankton, especially where the quantities sampled are large, but it is not common in the analyses of Micro-plastics.

5.1.4 Picking out Micro-plastics

Picking out particles is an important process that greatly affects the accuracy of micro-plastics analysis. There are several methods of separating plastic particles from a sample, such as picking plastic particles out after fractionating the sample by size using sieves of various sieve mesh opening sizes such as 5 mm, 1 mm, and 0.3 mm, and picking the plastic particles from the filter paper after directly filtering the sample. Stereomicroscopes are commonly used to facilitate picking out micro- plastics.

The accuracy of picking out particles greatly affects micro-plastic analysis results, as plastic particles picked out from the sample, whether pretreated or not, are used for subsequent measurement and analysis.

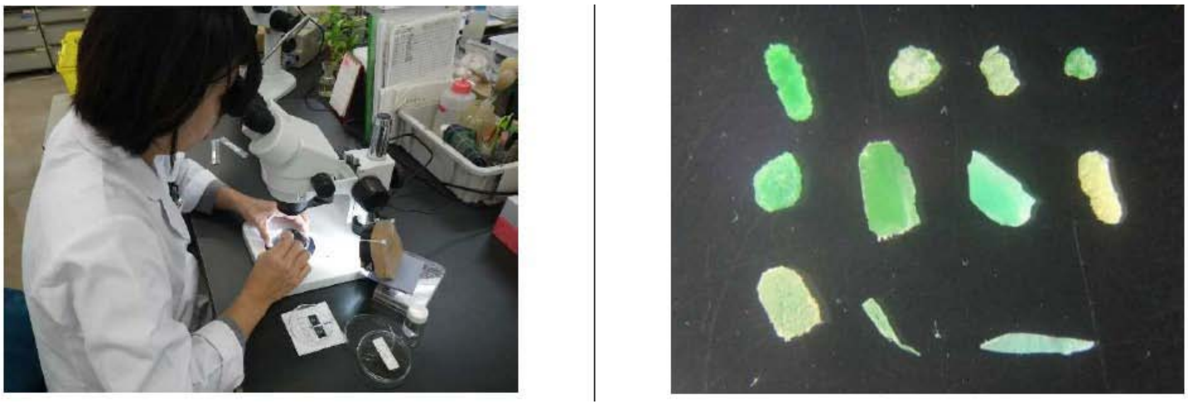


Figure 13: Separating Microplastics by picking and using the stereomicroscope (CMSM, 2018) Density

5.1.5 Counting and measuring sizes of Micro-plastics

There are two common methods for counting the quantity of particles by size-

- Directly measuring the longest diameter (maximum Feret's diameter) of separated particles individually, and
- Counting the quantity of particles remaining in the sample after fractionating by size using sieves of various mesh opening sizes.

Measurement of Micro-plastics	Multi-stage sieve with various mesh openings

Figure 14: Counting and Measuring sizes of microplastics

5.1.5.1 Feret's Diameter

The microplastic particles size is measured as Feret's diameter* that is generally defined as the distance between the two parallel planes restricting the object perpendicular to that direction. It is essentially the longest diameter!!

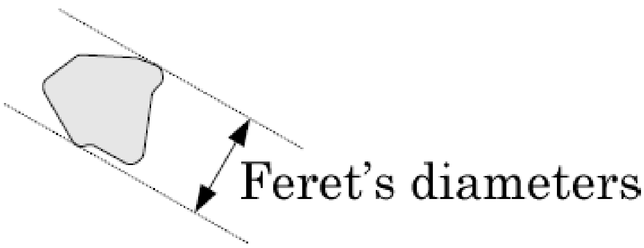


Figure 15: Depiction of Feret's Diameter

Among those measured Feret's diameter values, such that the area of the rectangle enclosing the particle outline becomes a minimum is called Minimum Feret's diameter" and the dimension perpendicular to it is called Maximum Feret's diameter" (Pabst et al., 2017).

5.1.6 Identifying the Micro-plastics

Micro-plastics are first identified visually, before an identification of the polymer type is undertaken. Larger particles can be identified with the naked eye, whereas small micro-plastics are identified by spectroscopic identification methods.

i. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectrometer coupled with an Attenuated Total Reflection (ATR) accessory.

The ATR allows the IR spectrum of a material to be obtained simply by pressing the sample against a transparent crystal, commonly diamond. The infrared light passes through the crystal into the sample where energy is absorbed by the sample, and the light is reflected back into the crystal to generate a spectrum.

Figure 16: Nicolet iS5 FTIR Spectrometer with the ID7 ATR accessory in the sample compartment.

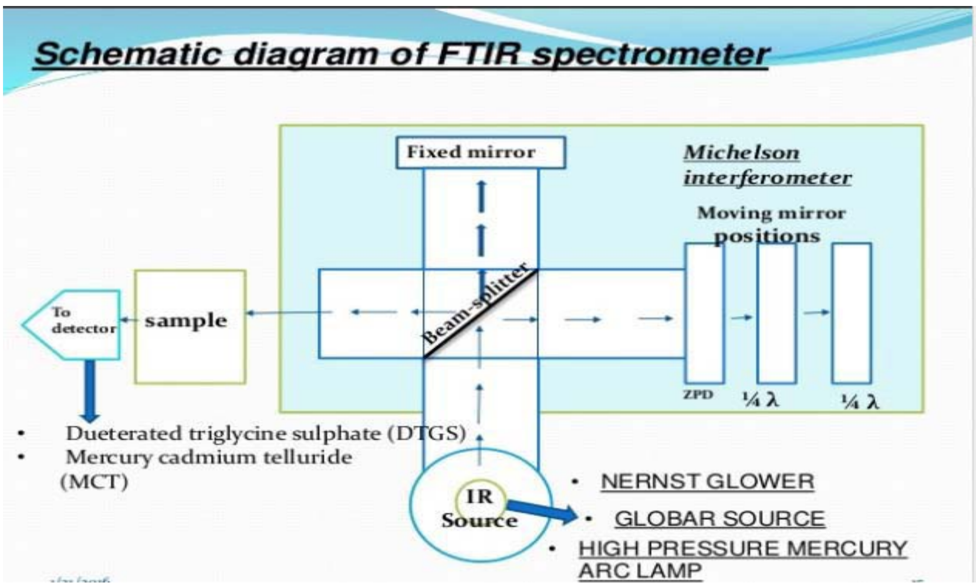


Figure 17: Schematic Diagram of FTIR spectrometer

ii. Raman Spectroscopy

Raman spectroscopy uses sub-micron wavelength lasers as its light source and, as such, is capable of resolving particles down to 1 micron and less.

Raman microscopes are built around research-grade, white-light microscopes, which facilitate easy viewing of the particles.

The Raman system laser is focused on the sample, and the spectrum is simply acquired by collecting the scattered light.

Figure 18: DXR2 Raman Microscope for analysis of Micro-plastics

iii. Thermo-analytical methods

Thermo-analytical methods are also routinely used in synthetic polymer analytics. Based on known thermo-analytical methods (thermogravimetry, differential scanning calorimetry etc.

Table 6.8: Micro-plastic characterization methods, including identification of polymer types (reproduced from Shim et al. (2017)).

Identification method	Advantages	Disadvantages
Microscopy	Simple	No chemical information for confirming composition
	Low cost	High possibility of false positives
	Color and morphological information	High possibility of missing small and transparent particles
Microscopy + spectroscopy (sub-set)	Polymer composition of a subset of the sample	Possibility of false positives
		Possibility of missing small and transparent particles
		Sub-set may not be representative
		Potential bias in sub-set selection
Microscopy + FTIR spectroscopy	No false positives – confirmation of all plastic-like particles	Manual selection of particles means some plastic may be missed
	Reduction in false negatives	Expensive instrument
	Non-destructive	Laborious and time-consuming for identification of all particles
	20 µm particle detection limit	Requires expertise in spectral interpretation
		Contact analysis (ATR)
		Need to transfer particles from filter paper to metal plate
		Removal of organic material a prerequisite
Microscopy + Raman spectroscopy	No false positives – confirmation of all plastic-like particles	Manual selection of particles means some plastic may be miss ed

Identification method	Advantages	Disadvantages
	Reduction in false negatives	Expensive instrument
	1 µm particle detection limit	Laborious and time-consuming for identification of all particles
	Non-destructive analysis	Requires expertise in spectral interpretation
	Non-contact analysis	Interference by pigments
		Risk of laser damage to particles
		Removal of organic material a prerequisite
		Exact focusing required
Semi automated spectroscopy (mapping based)	No manual particle selection error	No visual image data on single particles
	High automation potential	Production of a large volume of data
	In principle no false negatives	Long post-processing time
		Still requires expertise in spectral interpretation
		Efficient removal of interfering particles a pre-requisite
		Still lacks validation for smaller particles
		Expensive instrument
Semiautomated spectroscopy (image Analysis directed point analysis)	High automation potential	Production of a large volume of data
	Fewer false negatives	Long post-processing time
	Potential for faster sample throughput	Still requires expertise in spectral interpretation
	Size and morphology of single particles	Efficient removal of interfering particles a pre-requisite
		Still lacks validation for smaller particles
		Expensive instrument
Thermal analysis	Simultaneous analysis for polymer type and additive chemicals (Pyro-GC/MS)	Destructive analysis
	Mass-based information	No quantity or size-based information
		Limited polymer type identification (DSC)
		Complex data (Pyro-GC/MS)

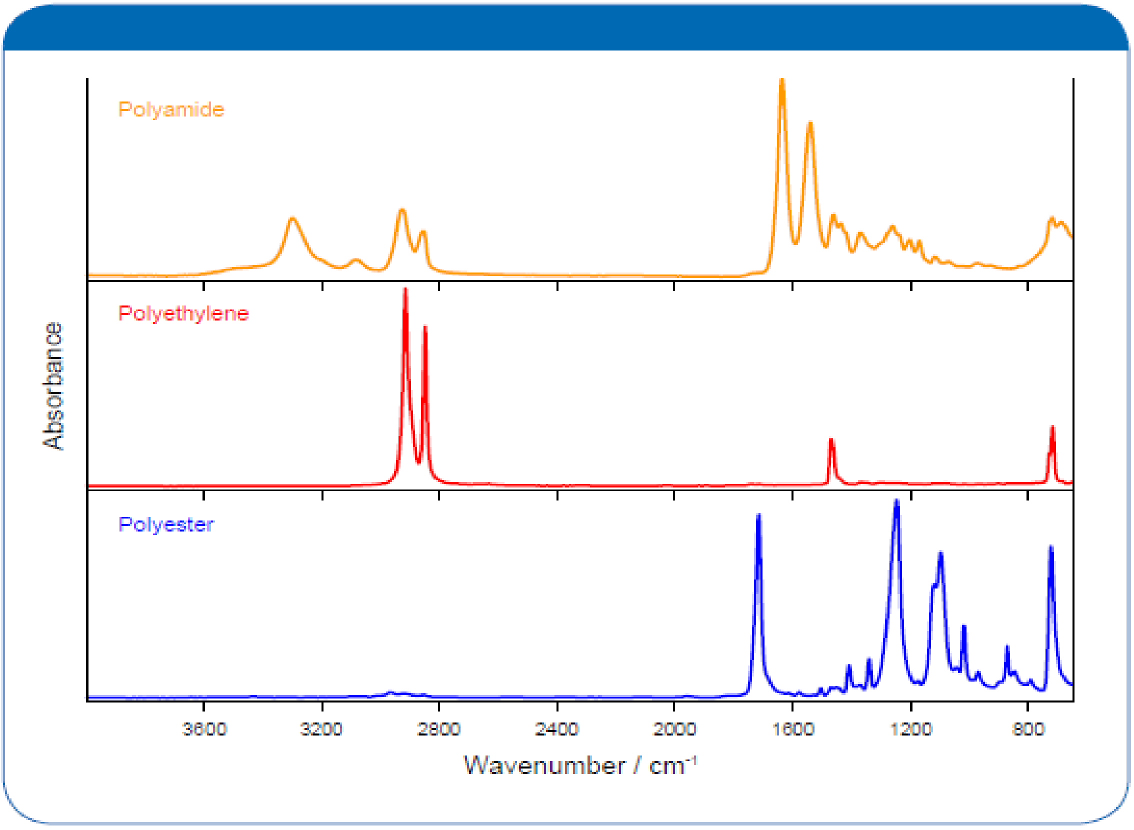


Figure 19: IR spectra of different polymer types (Bruker Optics Inc (2016), "Application Note AN M144 Analysis of Microplastics using FTIR and Raman-Microscopy)

5.1.7 Weight measurement

Weight measurement is carried out because it is important to understand the mass balance and also due to the difficulty of estimating the actual abundance of micro-plastics from the quantity of the particles only, because even if the same amount of micro-plastics exists at the ocean surface by weight, the quantity of particles may differ depending on fragmentation processes.

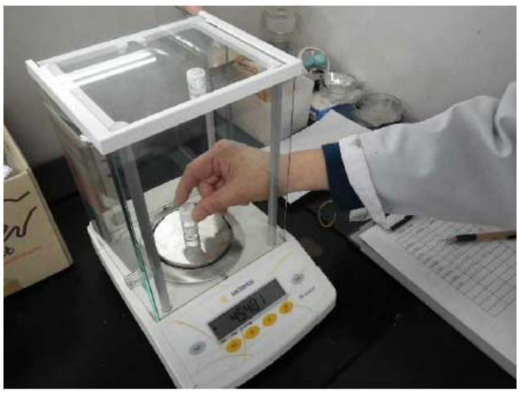


Figure 20: Weight measurement

5.1.8 Laboratory analytical process quality control

In laboratory analysis, countermeasures, for preventing predictable airborne contamination such as with fibrous matter and contamination from washing water in the fractionation and filtration processes, are important consideration to be avoided, and accordingly practice is made such as conducting blank tests in the laboratory or using filtered water to wash the equipment (EC, 2013, Masura et al, 2015).

5.1.8.1 Prevention measure to controlling contamination in micro-plastics (modified from Lusher et al. (2018)).

All sample containers should be prewashed with filtered distilled water before use. Samples should be kept covered as much as possible using aluminum foil or glass lids.

All equipment used in the processing and analysis stages should be rinsed and checked under a microscope for any micro-plastic particles adhering to them. The vacuum filtering apparatus should be rinsed with filtered water between each sample. All reagents should be vacuum filtered through Whatman GF/D filter papers immediately prior to use.

Sample processing should be performed in a sterile cabinet.

Several procedural blanks should be performed as negative control samples through the sample processing and analytical stages in order to test for laboratory contamination.

Table 6.9: Examples of contamination risks and preventive measures

Contamination risks	Preventive measures
Contamination with plastic particles adhering to analytical instruments/ apparatuses	Pour purified water into the apparatus used for analysis beforehand and conduct the same analytical process as for sample treatment to confirm the presence or absence of micro-plastic particles
Contamination with fibrous micro-plastics during operations	Wear clothing that is not plastic-derived and remove any loose fibers from clothing with a lint roller before sampling and analysis. For example, wear clothing of a unique and visible color so that the fiber can be distinguished even if it contaminates the sample.
Contamination with plastics from air	Use of clean benches and clean rooms. Implementation of blank tests in the laboratory

6.1 Micro-plastic assessment in Ganga and Yamuna Basin

Micro-plastic assessment has been carried out in Ganga and Yamuna basin in Prayagraj and Agra as described below:

6.1.1 Need

- River Ganga travels almost 2,525 kilometers from its origin and almost 625 million people live in the Ganga River basin. An estimated 11,625 tons of solid waste is generated in cities laying along the Ganga River and its tributary the Yamuna River.

- Lebreton et al. (2017) highlighted the River Ganga was in second most polluted, among the top 20 polluted rivers in the world based on global model with geospatial data of population density. However there was no real time data available to validate their study and confirm the position of Ganga River. Hence the proposed study is extremely important to get real time data on the River Ganga, not only as regional study but also as a national study.

- Method standardization is needed in order to obtain comparable data from different environmental compartments and sites. This includes sampling strategies (at spatial and temporal scales), sample treatment (taking into consideration high levels of rganic matter and suspended solids) and reliable analytical methods.

- Implementing mitigation strategies requires an understanding and quantification of marine plastic sources, taking spatial and temporal variability into account.

6.1.2 Study Area

In Agra, Micro-plastic survey was carried out in Yamuna River on dated 21.02.2020 & 12.02.2020 the following are the locations and Map of micro-plastic sampling depicted below;

- Dussera Ghat (AGYD2102)
- Kailash Ghat (AGYU1202)

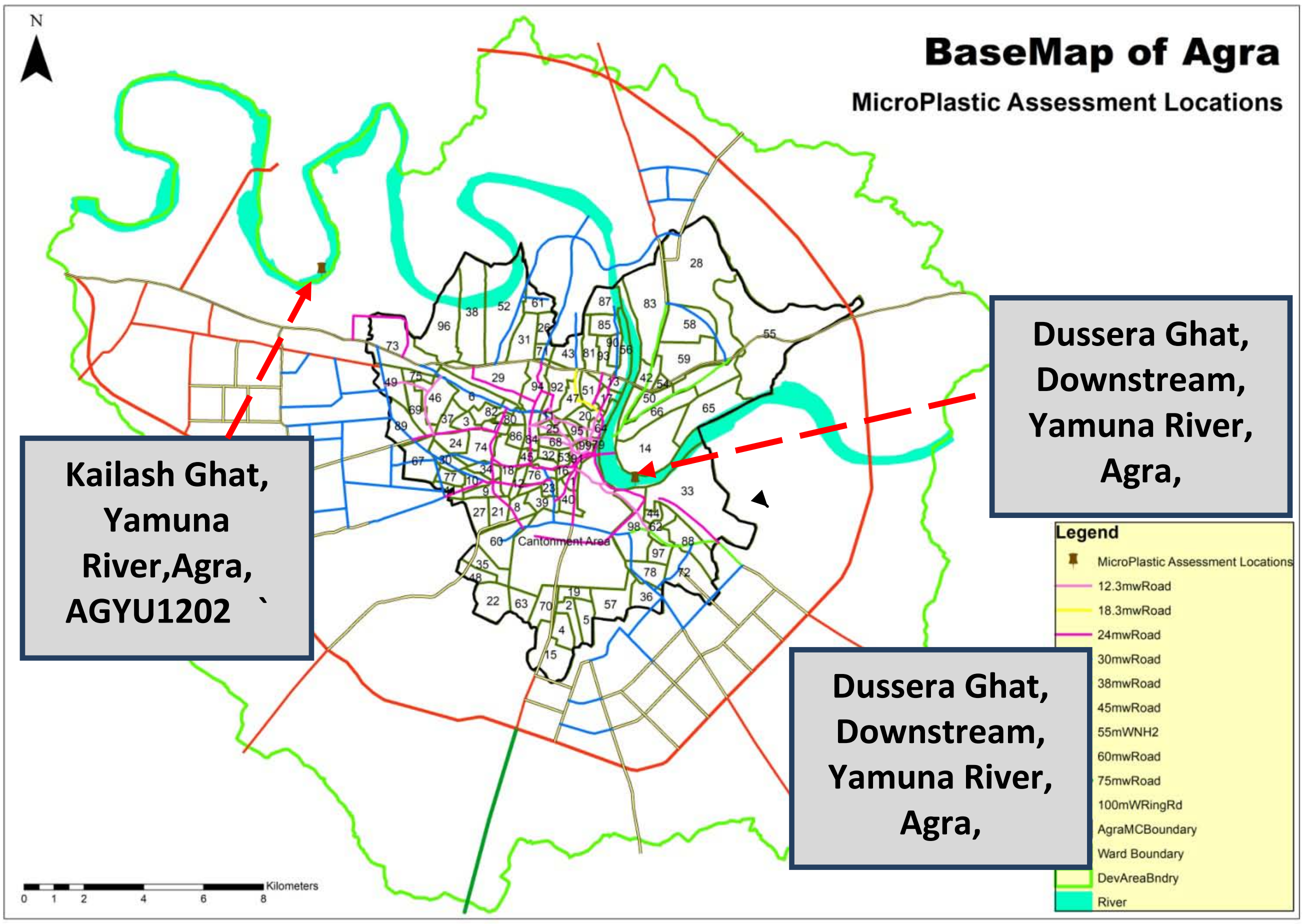


Figure 21-Location of Micro-plastic sampling in Agra

Location Name	Sample Code	Start point	End point	Remarks
Kailash Ghat	AGYU1202	27°14’12.39”/ 77°55’59.19”	27°14’31.47”/ 77°56’14.5”	Upstream location: Many domestic wastewater discharge outlets were , Shallow depth
Dussera Ghat	AGYD2102	27°10’33.9”/ 78°02’25.46”	27°10’13.08”/ 78°02’40.92”	Downstream location: One Industrial wastewater stream into river was observed, some macroplastics were also found floating in the river

Prayagraj

In Prayagraj, Micro-plastic survey was carried out in Ganga, Yamuna and Sangam on dated 08.02.2020 the following are the locations and Map of micro-plastic sampling depicted below;

- River Yamuna, Praygraj (ALYU0802)
- River Ganga, Prayagraj (ALGU0802)
- River Ganga, Prayagraj (ALGD0802)
- Sangam, Prayagraj (ALLSD0802)

Location Name	Sample Code	Start point	End point	Remarks
River Yamuna: Yamuna boat club, Naini bridge	ALYU0802	25°25’25.39”/ 81°51’19.9”	25°25’28.6”/ 81°51’4”	Upstream location: Factories located near this location - ITI, Triveni, Reliance, Cement industries, Cotton Mill. Many Wastewater discharges were also observed.
River Ganga	ALGU0802	25°25’39.77”/ 81°53’20.38”	25°25’21.33”/ 81°53’14.64”	Upstream location: Ganga river full of organic matter(flowers, plants), sandy
River Ganga	ALGD0802	25°30’05.32”/ 81°51’07.5”	25°25’54.33”/ 81°53’13.56”	Downstream location: Sandy water, shallow depth
Confluence point (Sangam)	ALLSD0802	25°25’28.14”/ 81°53’18.6”	25°25’17.9”/ 81°53’18.6”	Downstream location Bathing point, silty content was high

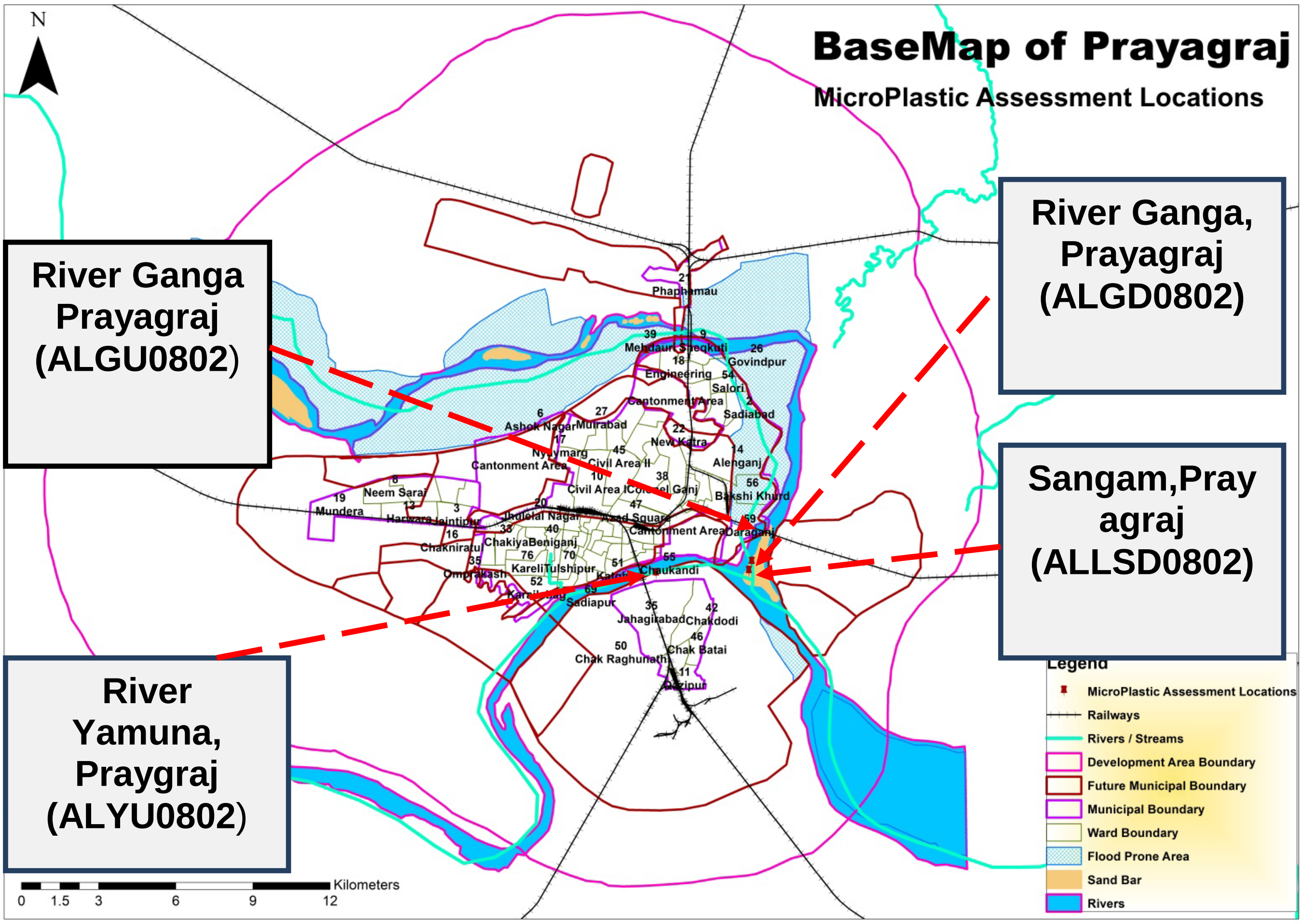


Figure 22 -Location map of microplastic sampling in Prayagraj

6.2 Methodology for Micro-plastic Assessment

An outline of the micro-plastics assessment process is presented in Figure 23.

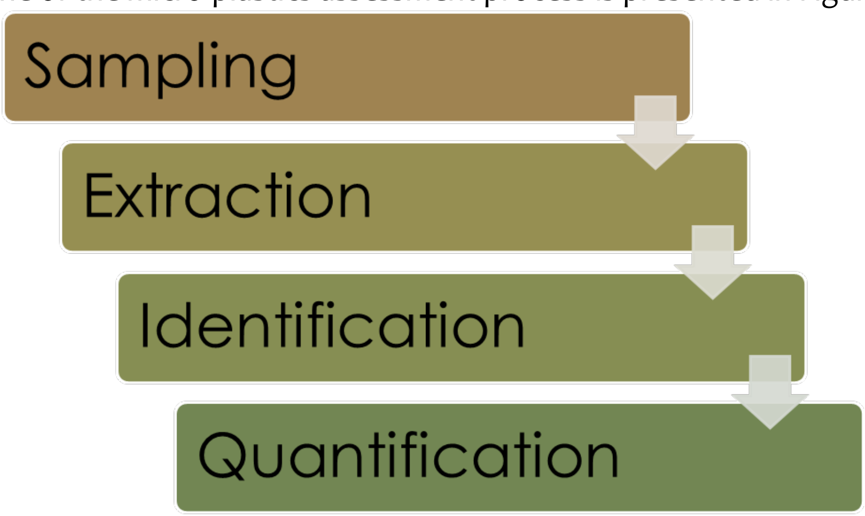


Figure 23- Outline of the micro-plastics assessment process

6.2.1 Sampling

Volume reduced sampling method was used during micro-plastic sampling in Agra and Prayagraj. The water samples were collected using a neuston net (25cm i.d. and 1.8m length) of 300µm mesh size attached with a small receiver which was deployed from the side of boat and towed for around 10-20 minutes (based on the water system). After trawling the net was brought back to the boat and the water retained in the receiver was collected in separate cleaned and sterilised glass bottles, and then taken to the laboratory for further analysis. A flow meter was attached to the net to allow estimation of total water volume sampled and the expression of results by m^3.

6.2.2 Key parameters of sampling

Following is description of sampling equipment and key parameters of sampling:

Table 6.10-Key parameters of sampling used during microplastic assessment

Tow Duration	**About 10 to 30 minutes**
Tow distance	500 to 1500 meter
Vessel Speed	~1 to 3 Nautical
Sweep area	**500 – 900 sq. metre**
Filtered water volume	900 – 1500 ml
Tow Position	sampling net was towed at one side of the vessel with less influence from its turbulence.
Net Immersion depth	About 1/2 to 3/4 of the height of the net's mouth.
Meta Data recorded	Time of day and date, latitude, longitude, intial and final flowmeter reading

6.2.3 Sampling Equipment Used

Neutson Net with mesh size 300 micrometer, flow meter at the top of the net and sample collection bottle at the bottom showing in below photoplates.


The step in microplastic sampling is depicted below:

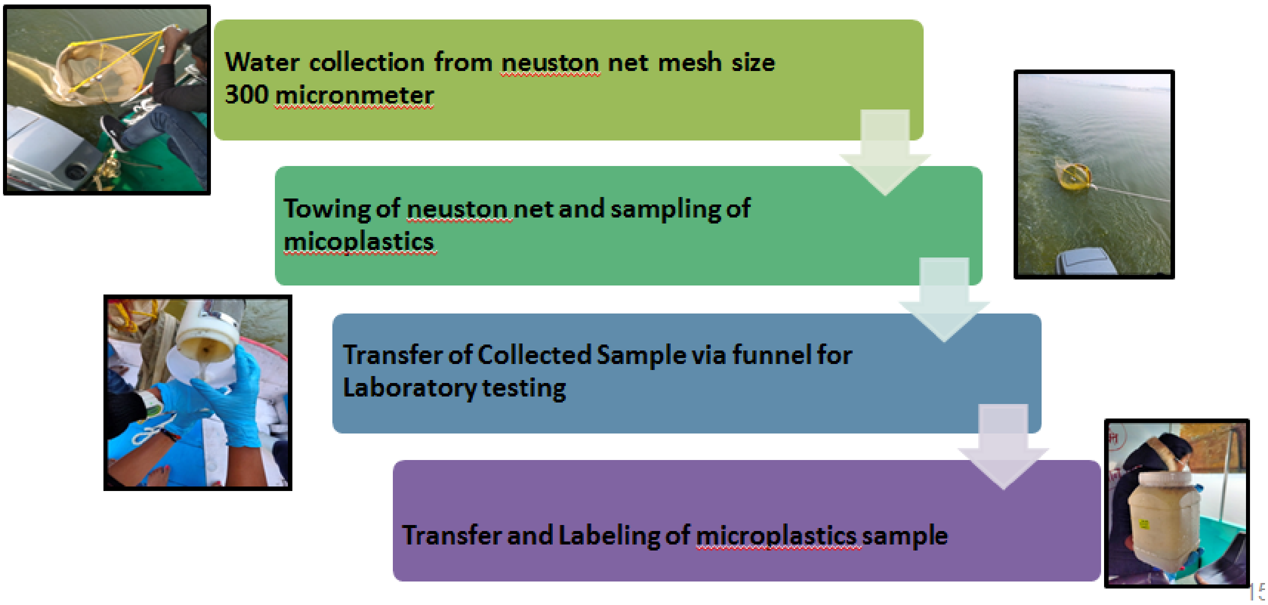


Figure 24: Flow chart of Microplastic sampling

6.2.4 Extraction

In general, analysis of samples that include micro-plastics obtained by trawling a net through the ocean/river surface layer was carried out in the following order.

- Pre-treatment- biological digestion-formalin was added to degrade the organic substance.
- Picking out Micro-plastics
- Counting and measuring sizes of Micro-plastics

The volume passed through formerly filtered by pouring into a 5mm,1mm steel mesh and 300µm nylon net which was staked from top to bottom, attached with a steel receiver at the end. In order to prevent the loss of particles the sample bottle and the lid was rinsed with distilled water. The water collected in the steel receiver was then filtered using vacuum filtration unit containing a filter paper of 47mm diameter and 5µm pore size. Finally the filter papers were transferred into the labelled petriplates and dried in the oven at 40º until it fully dried.

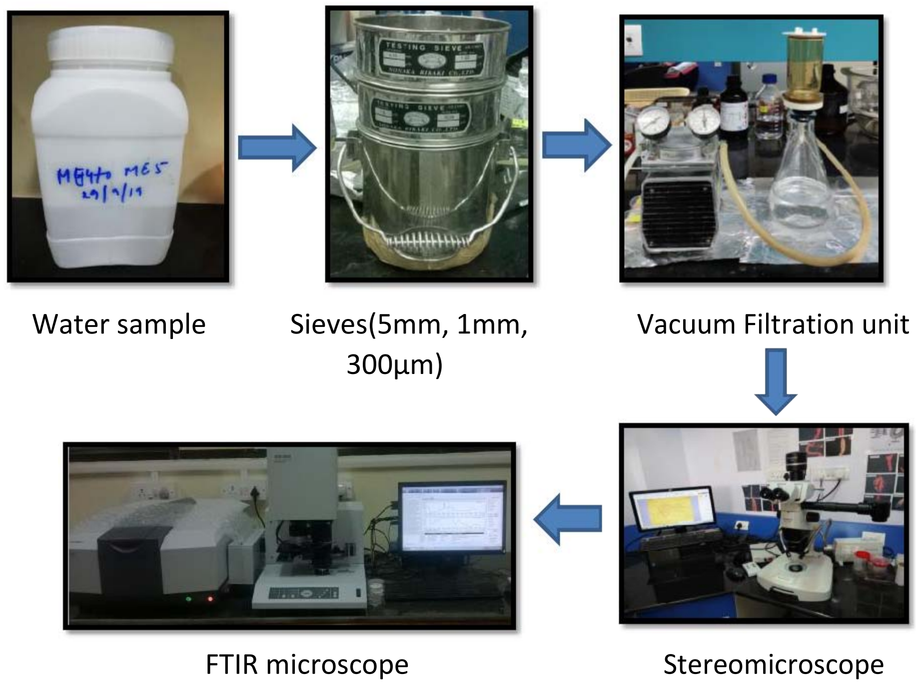


Figure 25-Pictorial flow chart of micro plastic sampling

6.2.5 Identification

Identification of microplastic was undertaken using Steromicroscope with OLYMPUS SZX10 attached with OLYMPUS DP7 camera.

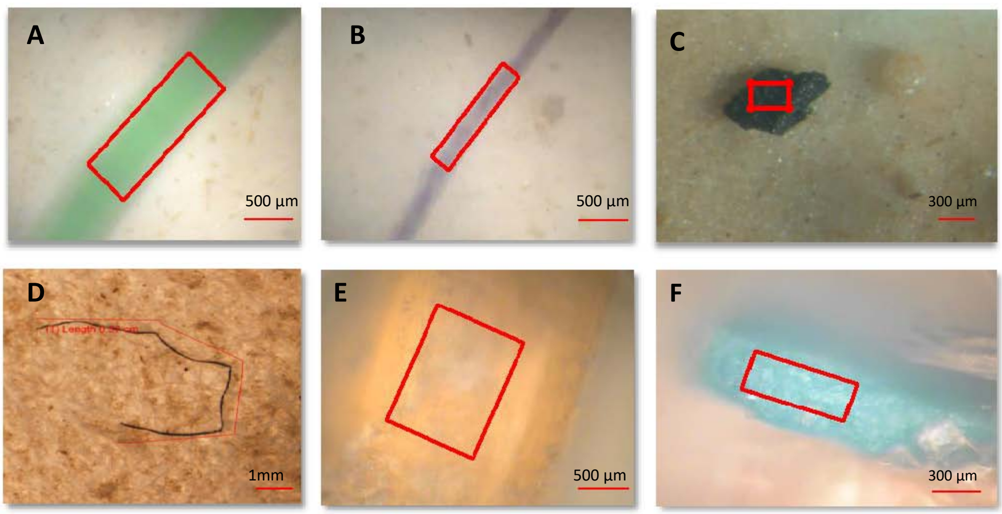


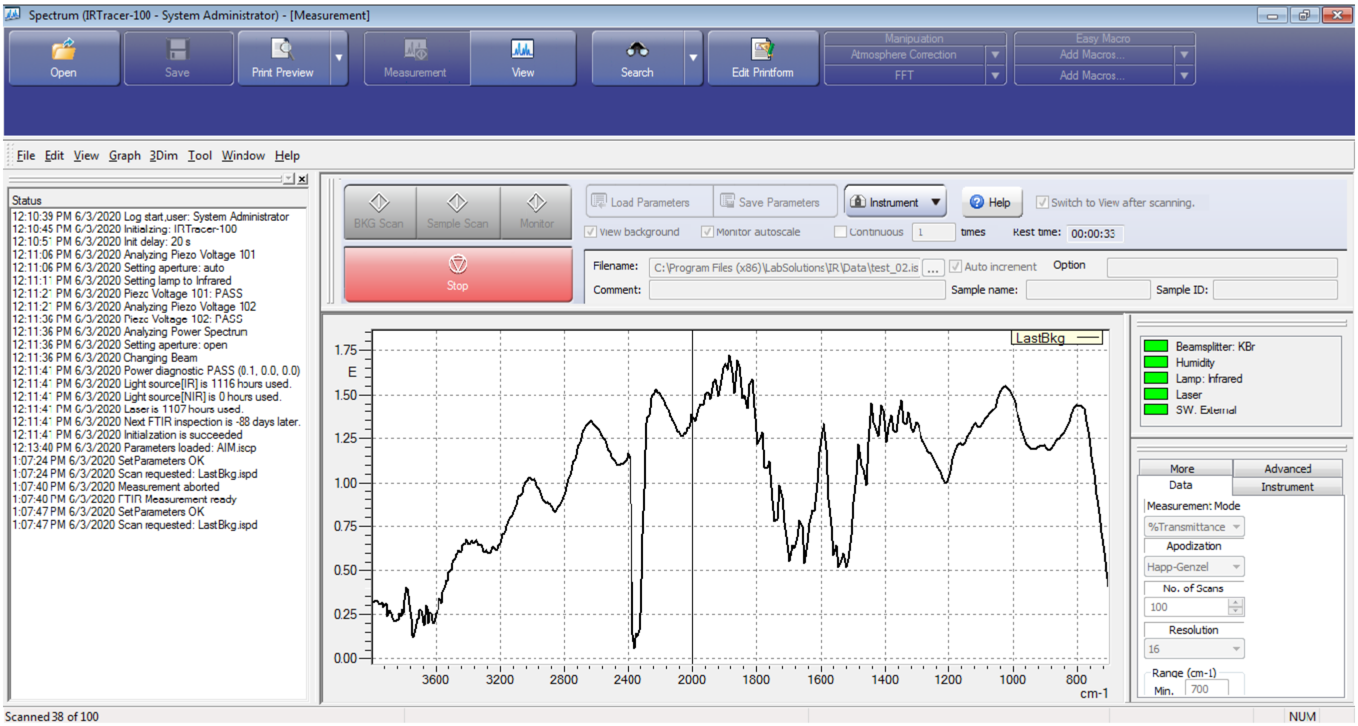
Figure 26: Microplastics found in surface water of Ganga and Yamuna River. Film (A, E), fibers (B, D) and fragments (C, F)

6.2.6 Quantification

Polymer types were identified using FTIR-Microscope (AIM-3800 made of Shimadzu) AIM-View software with Spectrum resolution: 16 cm-1; number of scans: 100 (400-4000) hz. The composition of MPs in each filter paper was identified by using Micro-Fourier transform infrared spectroscopy (μ-FTIR) with advanced imaging and microscopy (AIM). The specification of FTIR were as follows; Made of Shimadzu, IR tracer and AIM view software, spectrum resolution 16cm^{-1}; number of scans:100 per a sample; mirror used for background correction and advanced AIM correction. Blank filter were examined to check the air-born contaminations.

6.2.6.1 Calibration procedure before sample analysis

Background scan before analysis of MP samples in FTIR (IR Tracer- 100)

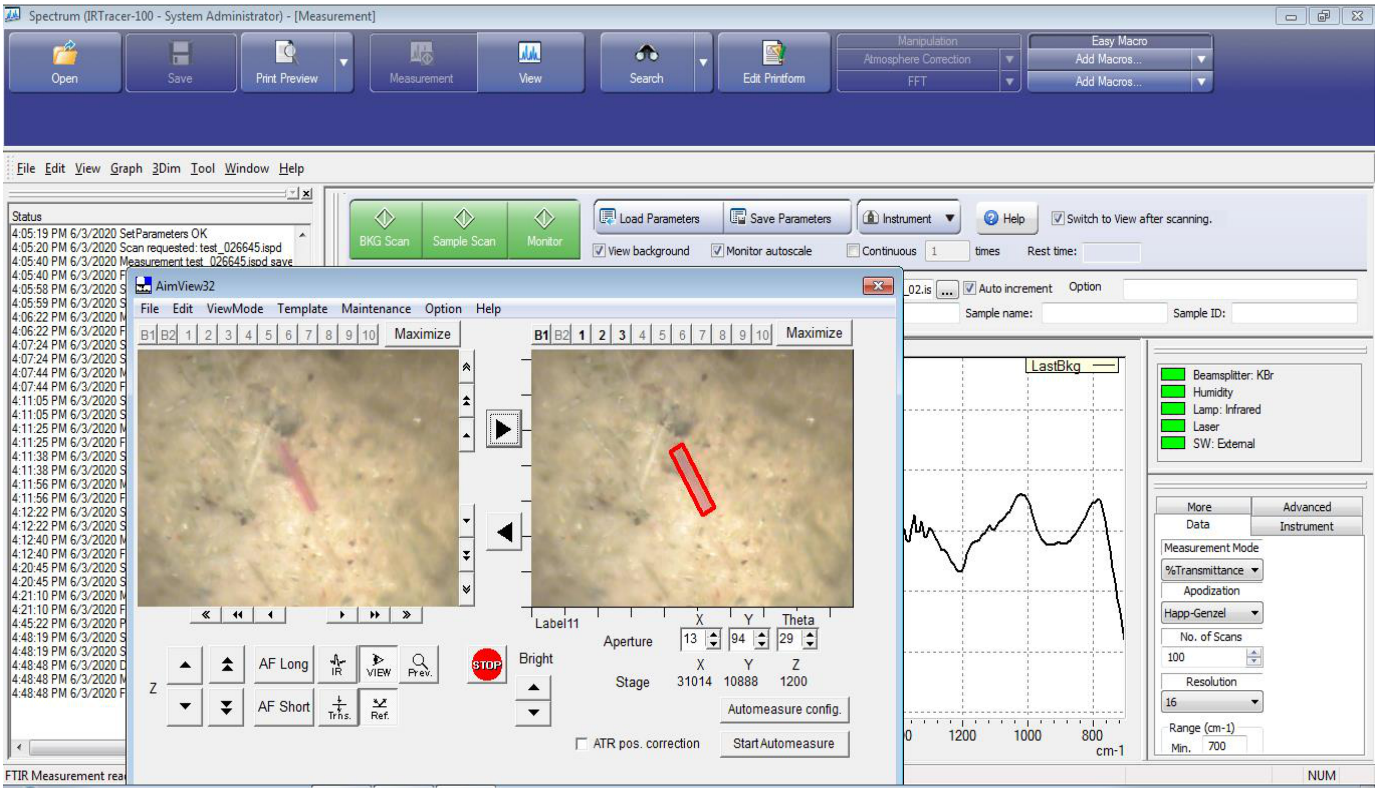


A

Figure 27-Background scan

The samples were analyzed by using Micro-Fourier transform infrared spectroscopy (FTIR, Model: Shimadzu) attached with Advanced Imaging & Microscopic in the reflectance mode. Mirror was used for the background correction (using Lab solution software) before the particle polymer detection. The IR light hits the sample from above and reflects back to the detector thus, spectra are produced for that particular MP sample. It is observed between the mid infrared regions i.e. 700- 4500 cm^{-1} with 100 scans per sample with resolution 16 cm^{-1}.

µ- FTIR analysis of MPs in AIM viewer software for the polymer detection



B

Figure 28- MPs in AIM viewer software for the polymer detection, Selected spectra of most found polymers

Here the samples are analyzed in for the polymer identification using AIM software. First we select the aperture for the selected MP particle and a background scan is run which is followed by sample scan of the selected aperture shown in the red box in the figure.

Major Polymer spectra found in MPs

After the spectras are produced for the MP particles the spectra obtained are matched with the FTIR polymer library for the confirmation of the specific polymer in the particle which is already mentioned below the spectra.

The figures, given below also show the polymer library where other possible matchings spectra with their respective scores and thus we chose the highest matching score for our final results.

(C) Ethylene vinyl alcohol (EVOH)

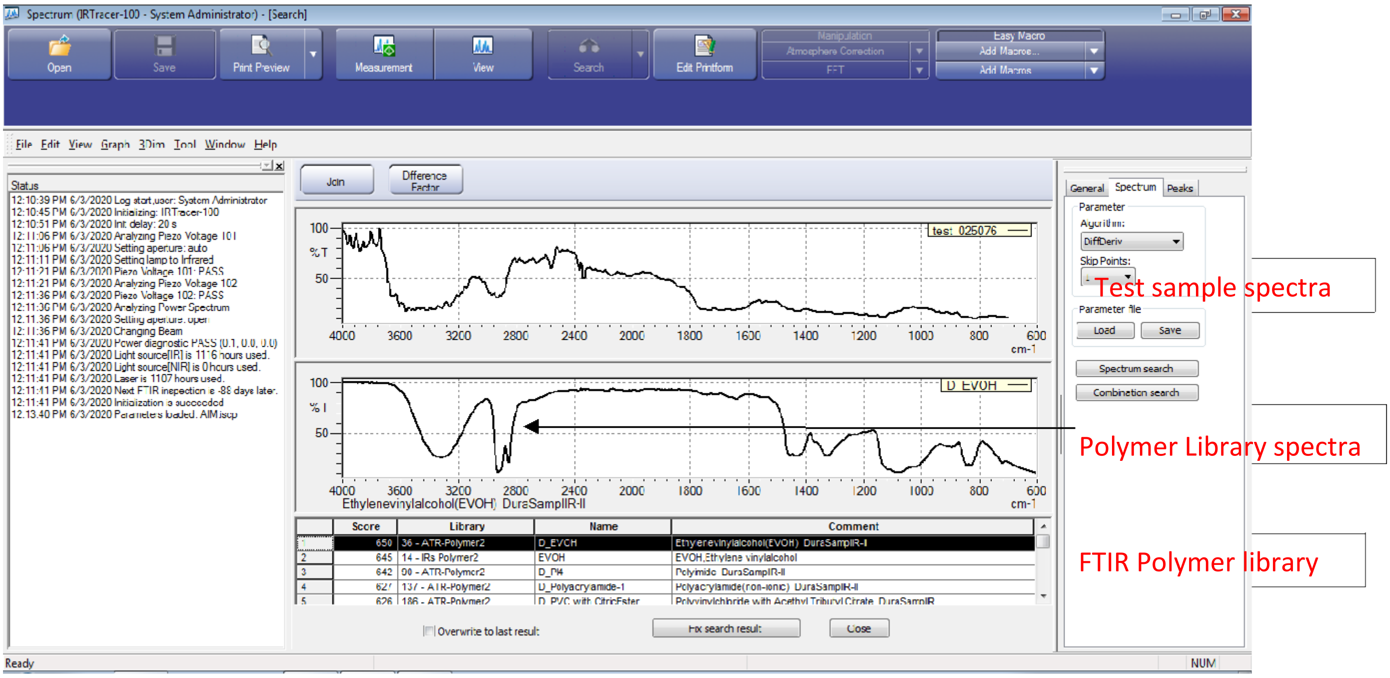


Figure 29- Polymer detection ofEthylene vinyl alcohol (EVOH)

(D)Polyvinyl chloride (PVC)

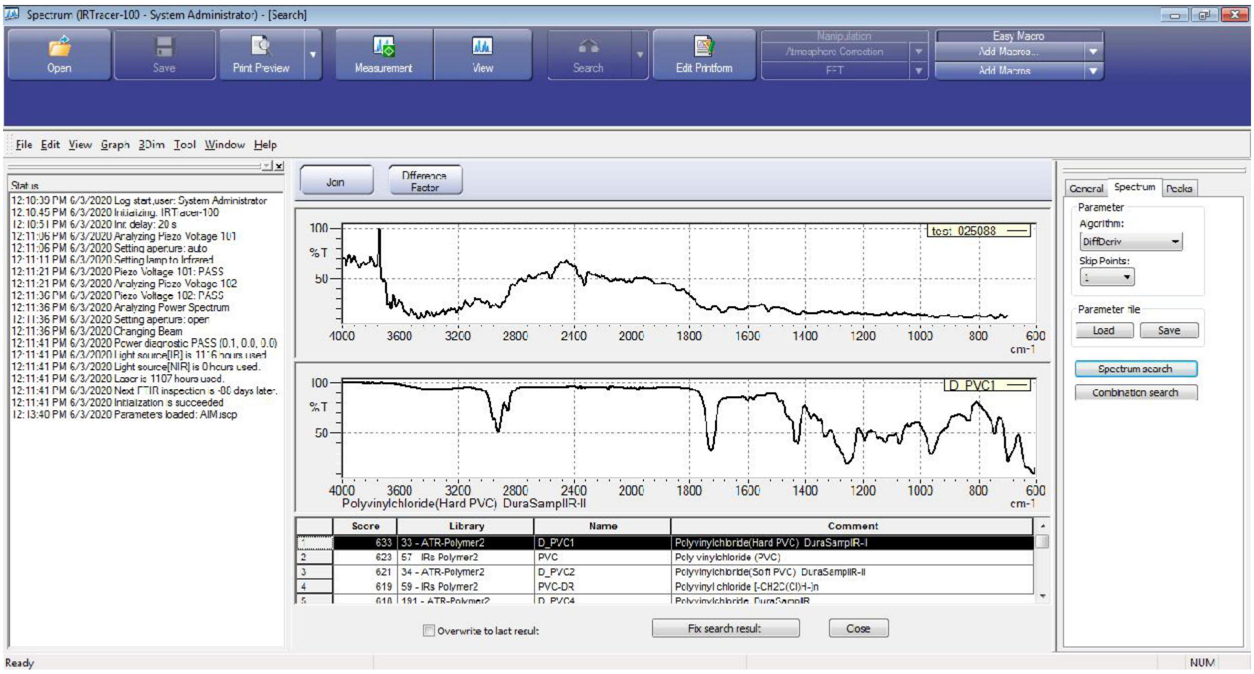


Figure 30- Polymer detection of Polyvinyl chloride (PVC)

(E) Polyurethane (PU)

Figure 31- Polymer detection of Poly Polyurethane (PU)

(F) Polyamide (PA)

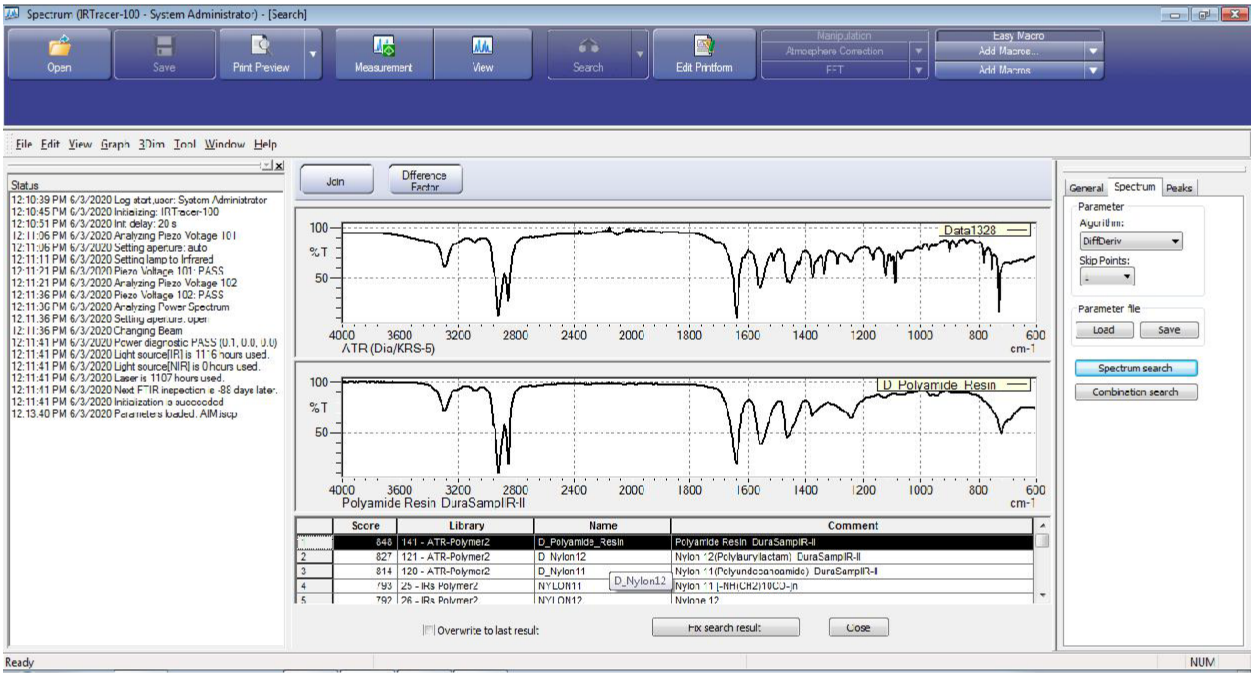


Figure 32- Polymer detection of Polyamide (PA)

(G) Polyester Film

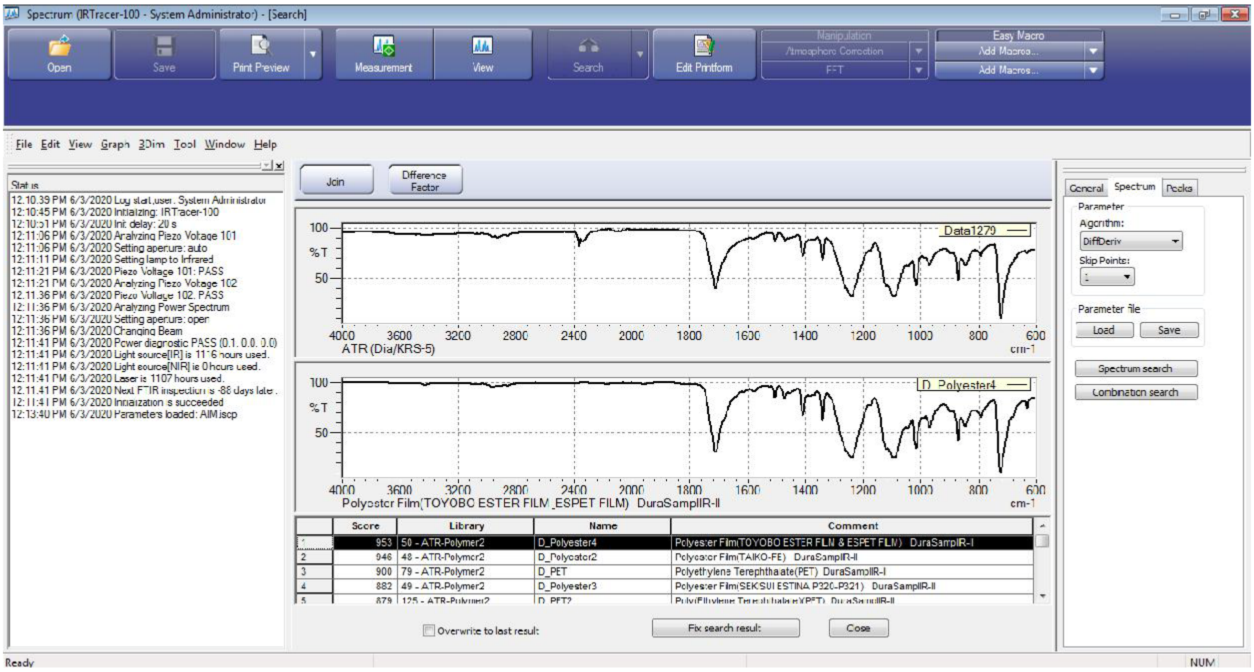


Figure 33- Polymer detection of Polyester Film

(H) Polyacetylene

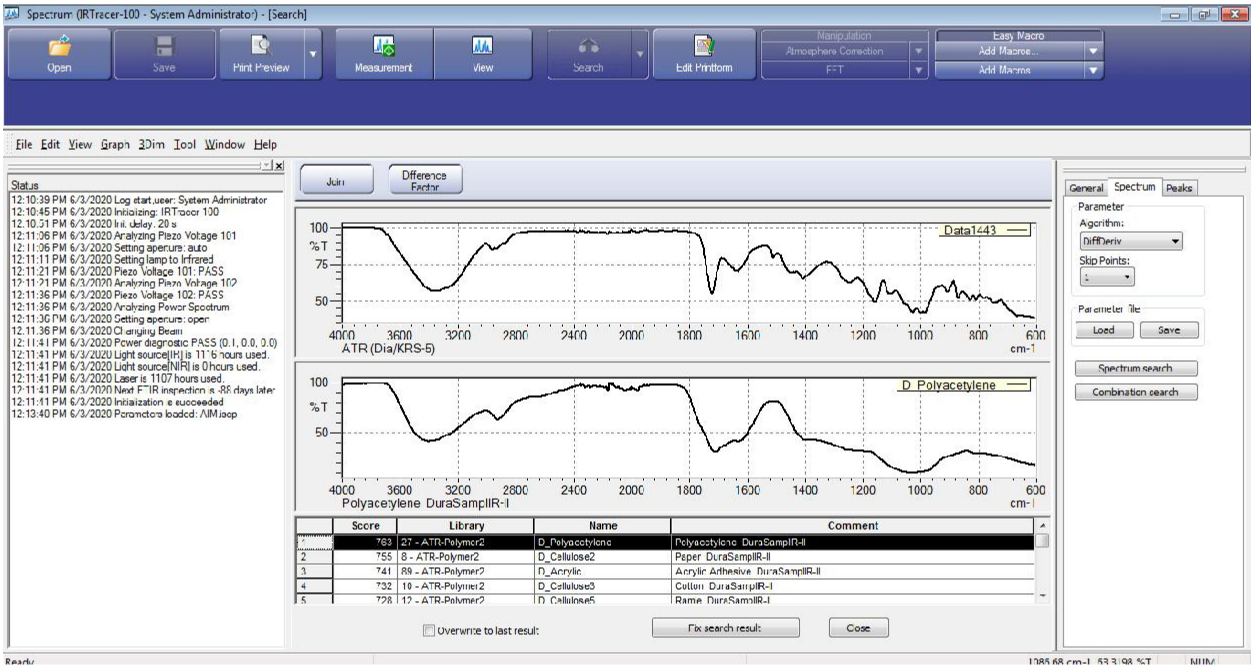


Figure 34- Polymer detection of Polyacetylene

(I) Poly Vinyl Pyrrolidone (PVP)

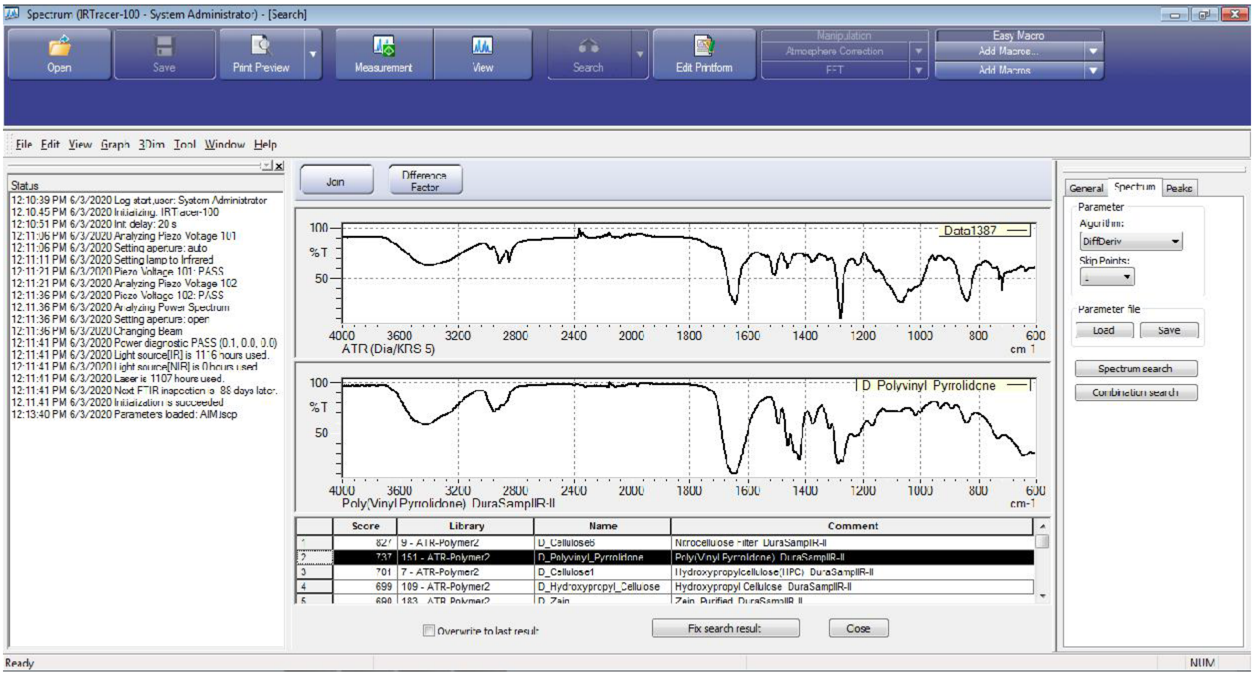


Figure 35- Polymer detection of Poly Vinyl Pyrrolidone (PVP)

(J) Ethylene vinyl acetate

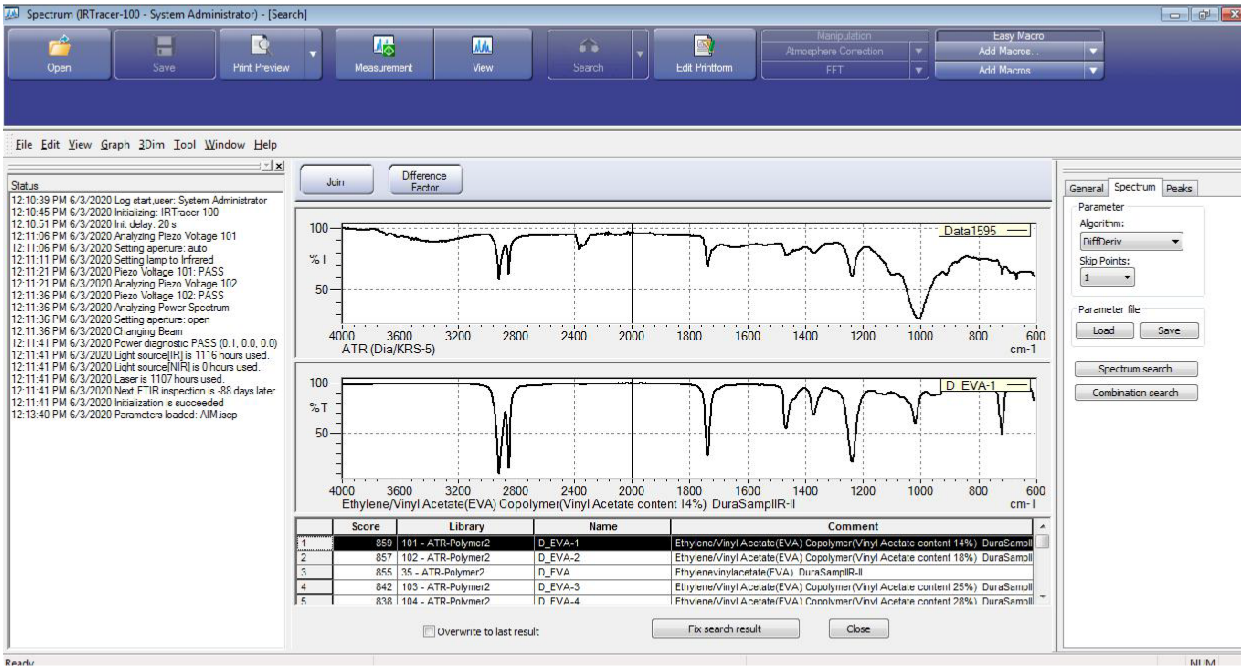


Figure 36- Polymer detection of Ethylene vinyl acetate

6.3 Abbreviations used

Following are the abbreviation used in results of microplastic assessment.

Table 6.11- Abbreviation used in result of microplastic assessment

EVOH	**Ethylene vinyl alcohol**
PP	**Polypropylene**
HDPE	**High Density Polyethylene**
LDPE	**Low Density Polyethylene**
PVC	**Polyvinyl Chloride**
EVA	**Ethyl vinyl alcohol**
ABS	**Acrylonitrile butadiene styrene**
PET	**Polyethylene Tetrathalate**
PIP	**Poly Isoprene**
PES	**Polyether sulfone**
PVAL	**Poly vinyl alcohol**
PVDC	**Polyvinylidene chloride**
PVB	**Poly vinyl butyral**
PMMA	**Polymethyl methacrylate**

6.4 Result and Discussion

The results of micro-plastic sampling are discussed below have been revised. The data earlier reported was estimated using different calculation as was done under pressure during Corona. The data has been now processed with proper water volume.

i. Abundance and distribution of MPs in surface water of Ganga and Yamuna River

The river waters selected for the study was polluted with plastic waste mainly single use and secondary plastic products Accumulation of plastic litter was found in and around the river banks and its negative impact was observed in river as floating litter. The river collects all the urban wastes and sewage wastewater discharging directly into the river. The number of microplastics detected in River Ganga A8 (5.69 MPs/m^3) was significantly higher among all the other locations followed by Yamuna River in Agra (AG4 and AG3) where it was found as 4.62 and 4.00 MPs/ m^3 respectively. The MPs concentration was found to be lowest in Sangam in Prayagraj A5 (1.23 MPs/ m^3) followed by another Ganga sample A7 in Prayagraj (1.47 MPs/ m^3), whereas Yamuna in Prayagaraj (A1) shown moderately high concentration 2.43 MPs/ m^3, given in Fig. 37 and Table 2.

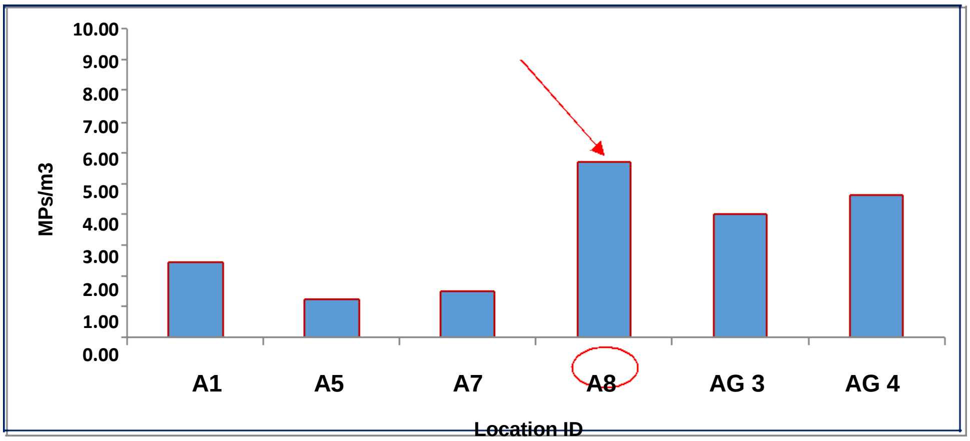


Figure 37: Distribution of MPs per m3 in different riverine water (A: Prayagraj and AG: Agra sample)

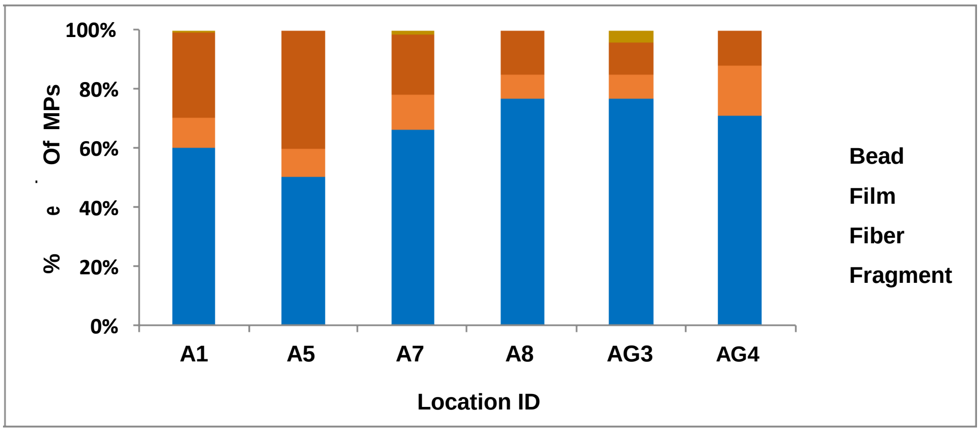
Location	Distance trawled (m)	Duration of trawling (minutes)	Cylindrical Area covered (m^2)	Total volume passed through the net (m3)	Total volume Filtered (ml)	Total no. of MPs found in filtered water (number)	Total MPs/ m3
ALYU0802 (Yamuna)	459.08	12	721.15	59.728	1200	145.2	2.43
ALSD0802 (Sangam)	347.82	10	546.47	52.618	950	64.66	1.23
ALGU0802 (Ganga)	632	16	992.63	79.638	1450	117.45	1.47
ALGD0802 (Ganga)	545.09	15	856.18	75.371	1600	428.8	5.69
AGYU1202 (Yamuna)	517.34	13	812.62	72.527	1300	289.9	4.00
AGYD2102 (Yamuna)	623.17	15	978.77	78.216	1500	361.5	4.62

Table 6.12- Relative distribution of MPs/m^3 in different riverine water

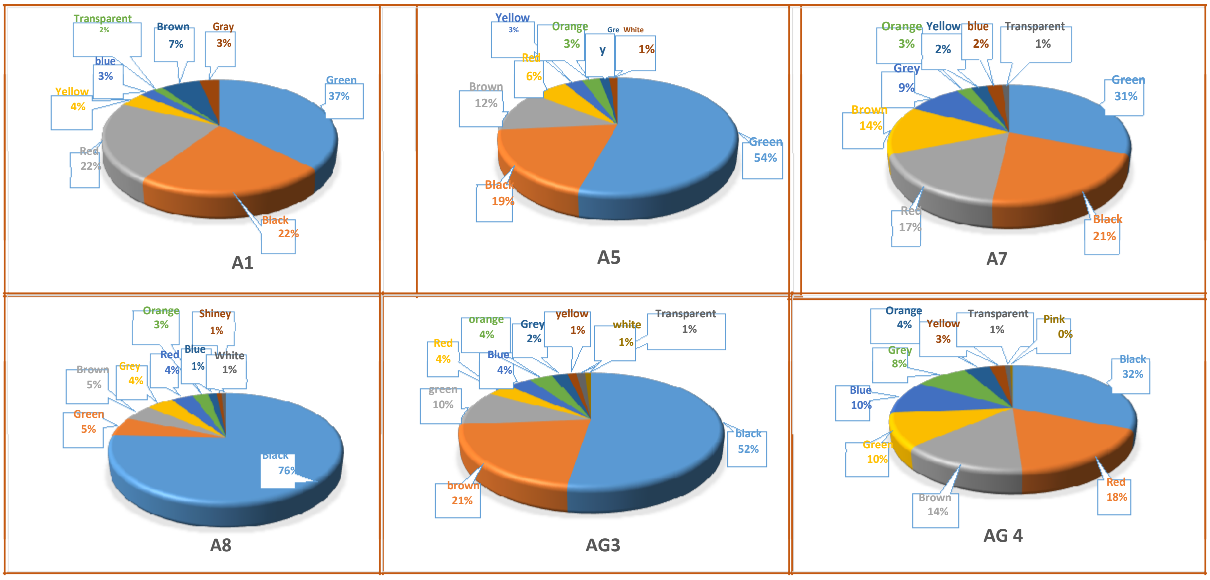
ii. Characterization and Identification of MPs

The shapes of the observed particles were sorted into fibers, fragments, films and beads (Fig 38). Fragments were the most abundant shape of microplastics in all the locations accounting for 65.78±10.79 followed by films (20.95±11.3) and fibers (10.67±3.32) whereas beads were found to be in less concentration (0.93±1.55).

(A)



(B)



(C)

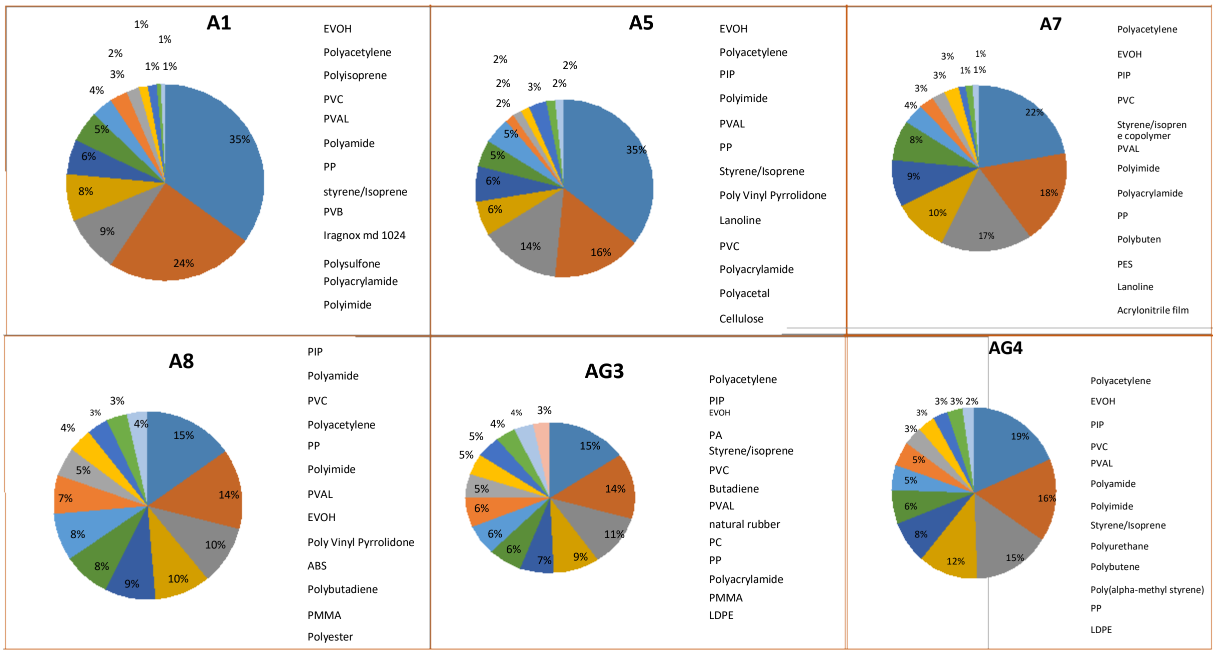


Figure 38 . Microplastics Shape (A), colour (B) and composition (C) in different riverine water

Location Name	Sample Code	Location Code in above figure
River Yamuna: Yamuna boat club, Naini bridge, Prayagraj	ALYU0802	A1
River Ganga, Prayagraj	ALGU0802	A7
River Ganga, Prayagraj	ALGD0802	A8
Confluence point (Sangam), Prayagra	ALLSD0802	A5
Kailash Ghat, Agra	AGYU1202	AG3
Dussera Ghat, Agra	AGYD2102	AG4

Very few particles were observed in the range of 1-5mm, but MPs (20-300 µm) constituted more than 90% by number of items. Green, black, red and brown coloured plastics comprised the majority of the particles. In this study we assessed the distribution of MP in surface waters of Ganga and Yamuna River. The abundance varied from 1.2-5.7 MPs/m^3 in water samples of different riverine system (Table. 2). Globally, between 0 and 1.3 MP m^{-3} (median 2.75 MP m^{-3}) were reported in river surface waters (**Koelmans et al., 2019;)**

Mintenig et al., 2020). The concentration of MPs in this study is comparable to other riverine system (Table. 3) as we have used same sampling methods with a 300µm plankton net and µ- FTIR technique for the identification of polymers upto 20µm in size. Further, Many studies has reported very few polymers but in present study we identified more than 30 polymers (Figure.5) interestingly, several types of rubbers (butadiene, polyisoprene, natural rubber) were highly abundant, which has not yet been reported for riverine surface waters in earlier studies. Several studies reported that they have analysed only 10-25% of filter paper, here we have analysed all filter papers in the µ-FTIR and found rare polymers which might be the reason of high diversity in the polymeric composition of MPs. In our study two of the location each in Prayagraj (A1) and Agra (AG4) detected particles ranging between 1-5mm showed less denser and common polymers viz PE, PP and HDPE which has less concentration in smaller sized MPs. From the studies **(Haave et al., 2019; Lorenz et al.,**

2019; Mani et al., 2019; S.M. Mintenig et al 2020) confirmed that smaller size particles <1mm had a diverse polymers than >1mm which were almost exclusively made from PE or

PP. Some particles (black, opaque with rubber like consistency) are probably from vehicle tires, from both natural wear-down process during driving and shredding of used tires for recycling purposes (Heloisa Westphalen and Amira Abdelrasoul., 2018). As quantitative and qualitative information on the occurrence of Water soluble polymers (e.g. PVP, Polyacrylamide) is very limited, covering only a few polymer types and chain lengths (**Petrović *et al.,* 2000; Huppertsberg et al., 2020**). This may be attributed to a general lack of awareness in the scientific community (**Arp *et al.* 2020; Huppertsberg et al., 2020**) and some severe analytical challenges.

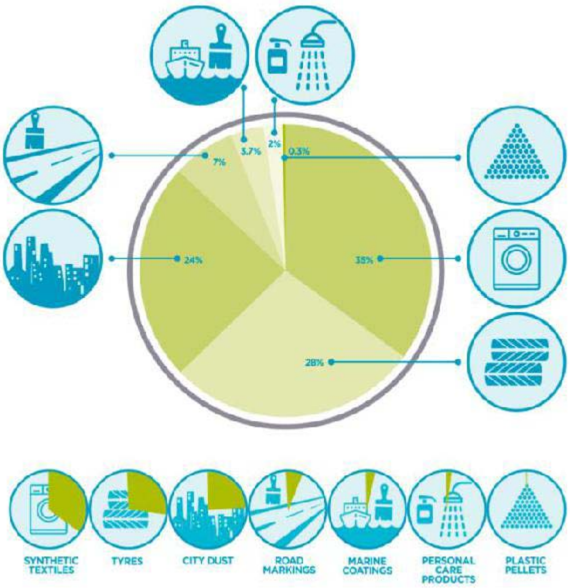


Figure 39: Global release of Primary microplastics to the world's ocean (*sourcenhttps://images.app.goo.gl/wgARYv9gsdvpUeJk7)*

A 2017 study by Pieter Jan Kole at The Open University of The Netherlands, published in the *International Journal of Environmental Research and Public Health*, estimated that tires account for as much as 10 percent of overall microplastic waste in the world's oceans. A 2017 report by the International Union for Conservation of Nature put that number at 28 percent. Synthetic rubber, made from a variant of plastic, makes up around 60% of the rubber used in tyres. Fragments were mainly attributed to the breakdown of larger plastics (plastic bags, plastic bottles, plastic cups etc) due to physical factors like fragmentation and weathering. Films were second most abundant shape found in all the locations which may due to the fact that films could promote MPs to suspend in waters rather than deposit in sediments. Fibers may be originated from WWTPs which constitutes fibers from textiles and washing machine effluents from household and laundry and other domestic and industrial runoff which directly discharge their waste into this holy river. In addition mismanaged plastic can be more rapidly fragmented on land and enter in river as microplastics. Moreover litter inputs in the river banks include poor waste management practices, both in the land and on the river bank, where recreational activities is one of the major reasons for this pollution.

Discussion based on Macroplastic assessment studies in Prayagraj and Agra

Agra

Further, based on macroplastic assessment studies, land based waste sources have been correlated with microplastic survey as depicted below table:

Table 6.13- Land based waste sources have been correlated with microplastic survey

Types of Plastic found in Macroplastic assessment study in Agra	Possible microplastic polymer escaping into river
Multilayer Large and Medium Size packets of snacks, chips, Namkeen, biscuits etc. 	EVOH, PVAL, EVA, PVC, Polyimide, PP, LDPE, Polyacrylamide, Acronitrile film, PE/PP, Polyester film, HDPE, Poly ethylene oxide
Monolayer Plastic Packaging used for food, Detergent etc 	Polyethylene, Polypropolyene
Synthetic woven bags used for cement packaging etc.	Polyester

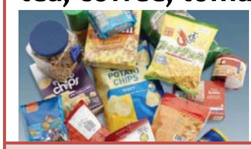
	
Polythene bags (colored white, black)  	Polyethylene
Disposable plastic Cups/Glasses, take away food containers 	Polyamide, Styrene/Isoprene, PIP, PES, Polyester film, HDPE
Packing used for water, milk etc 	Polyethylene
Ritual Material 	Polyethylene, PP, PIS
Plastic Sheet & other thicker plastic bags. Color-Black & White 	Polyethylene
Tobacco, Pan Masala Sachet/Wrappers 	EVOH, PVAL,PET, Polyethylene, PVC, PS, PVDC

This photo plate depicts site representation of micro-plastic assessment in Agra

Figure27-Site representation of micro-plastic assessment in Agra

Prayagraj

Table 6.14- Land based waste sources have been correlated with microplastic survey

Types of Plastic found in macroplastic assessment	Possible Polymer types escaped into river
Food packaging material for snacks, chips, namkeen, biscuits etc. 	EVOH, PVAL, PP, PVB(Polyvinyl butyral), LDPE, Polyethylene, polycarbonate, Polyamide, PVC
Multilayer Sachets for Shampoo, Tobacco, tea, coffee, tomato sauce etc. 	EVOH, PVAL, PP, PVB(Polyvinyl butyral), LDPE
HDPE bottles, tray, PVC etc. 	Polyethylene, PVC
Polythene bags (colored white, black) 	Polythene

Disposable paper cups coated with plastic film, Take away food containers, disposable cup& plates 	Polyamide, Styrene/isoprene copolymer, styrene/Isoprene, polysulfone
Packing used for water, milk etc 	Polyethylene
Shopping Bags/ Grocery Bags 	Polyethylene
Synthetic Clothes 	Polyester
Tobacco, Pan Masala Sachet/Wrappers 	EVOH, PVAL, Polyethylene, PVDC, PVC, PP, PS, PET
others	PE, PP,PVC

The following photoplate depicts leakage hotspots identified in Prayagraj during the microplastic survey.

Figure 40 - Site representation of microplastic assessment in Prayagraj

The sources of most of these polymers are land based except polyacetylene as indicated below table:

Table 6.15- Major Polymer found during microplastic Assessment

Polymer	Origin	Remarks
EVOH	EVOH is a polymer used in Multilayer Packaging which binds with Oxygen molecule thereby trapping it in the packaging layer and maintaining hygiene inside the packet	Macro plastic assessment studies indicate maximum Multi layer packets in the litter. Primary survey in the city also indicates presence of Multi layer packets in almost every hotspot.
Polyacetylene	Polyacetylene is a organic conducting polymer which is a secondary metabolite of plant (E.G.Cosio, et al, 1988) and has no commercial use).	Lot of plant material was found in the microplastic sample collected in Prayagraj (both Ganga and Yamuna).
Polyisoprene	Polyisoprene is found in rubber bands, rubber material, footwear, adhesives used in leather and footwear industry etc	This is probably coming from footwear clusters as industrial waste. Agra has one of the largest leather footwear cluster. Kanpur which is hub of shoe industry whether safety shoe or other is located along Ganga before Prayagraj.

PVC	PVC is also used in footwear. It is used for medicine packaging, Multilayer packaging in combination with polyethylene or polypropolyene.	PVC has many uses but that land up in the litter includes blister used for medicine packaging. PVC is also part of Multilayer packets.
PVAL	Polyvinyl alcohol	Polyvinyl alcohol has same functionalities as that of EVOH in Multilayer packaging indicated above

Types of plastic product waste found in microplastic sampling but not found in macroplastic samples are as given below:

River Ganga & Yamuna, Allahabad

Waste	Polymers leached out
Rubber material waste	Polyisoprene
Other packaging waste	PVC, PP, PVB(Polyvinyl butyral), LDPE
Laminated films such as silver foil, laminated disposable plates	Polysulfone

River Yamuna, Agra

Waste	Polymer
Other packaging waste including medicine packaging,	Poly(alpha-methyl styrene), PVC, Polyimide, PP, LDPE, PVB, PE/PP, Polyester film, HDPE, PVDF, Styrene/Butadiene, Styrene/Maleic anhydride
Laminated films such as silver foil, laminated disposable plates	PET, LDPE
Foams	Polyurethane
Toys	polybutene
other plastic pieces such making basins, bath room fixtures, window glass, sinks, etc	PMMA (Polymethyl methacrylate)
Automobile parts,	Polycarbonate, Polysulfone, Polyetheretherketone (PEEK), PC/PBT
Nonstick cookware coating	PTFE
skin care product	Poly 1-butene

6.5 Challenges faced during the study

- While collecting surface water, due to high water flow and turbulence it is difficult to tow the net which provide a demanding environment, especially when handling large-sized net.
- High organic matter (plant leaves, flower and other natural waste) can stuck in to the net, which can hamper the movement of flowmeter.

- For collecting measurements in medium- and large-sized natural streams is keeping the device stable at the required points in the water column is very difficult some times.
- Towing time of net should be selected based on the water system (River, estuary, ocean), because there will be high chance of eructation of net due to phytoplankton blooms and other natural phenomenon.
- In the shallow water specially when the water depth is very low less than 3m, towing the net is not possible as sediment load can prevent the flow of net horizontally.

6.6 Lesson learnt

- Around 40-47 types of polymers have been found in the microplastic samples. Considering so many varieties of polymers in water, a detailed investigation of all sources needs to be undertaken.
- Another source of Microplastic is wastewater both domestic as well as industrial which is drained into the river. Therefore, Microplastic survey also needs to be undertaken in wastewater falling into river
- Microplastic survey results can be a very good source of information in developing plastic leakage scenario.
- The analytical data will also be helpful to develop the risk assessment and mitigation strategies.

6.7 Way ahead

- There is a requirement to make a comprehensive microplastic monitoring plan in water, sediments, fishes and other aquatic plants to understand its impact
- Microplastic survey has to be validated through detailed macroplastic assessment studies leading to identifying the polymers in macroplastic being leaked into the natural environment
- This study has to be undertaken in other major rivers along the major plastic waste generating cities in the country

6.8 Conclusion

In Microplastic sample**,** Black (may be part of tire and electronic industries) fragment and Green film (Packaging material) majorly accounted in this water. **EVOH** is known for having some of the best barrier resistance to gases such as oxygen, nitrogen, and carbon dioxide which makes it particularly suited for packaging food, drugs, cosmetics, and other perishable products. When compared to other common films, EVOH is considered to have superior barrier properties. **Polyacetylene,** which is a conductive polymer, has no commercial application but use as a doping agent to manufacture electronic parts and thin films (Ron dagani 1981), found very commonly. Few studies suggest that it is plant based polymer so the amalgamation of plastic and organic particles in the river may also be a reason of high concentration of this polymer. **PP,** very commonly use in Packaging, Plastic sheets, fiber and fabrics, tape, rope. **PIP**, mainly use in footwear, baby bottle nipples. **Polyamide (PA)**, commonly known as nylon, used as a natural fibers and metal wires in clothing and industry, also in disposable curtlery**. PVC**, used in flexible packaging, pipes, wire and cables, medical and automotive. Polyacrylamide use as a fiber in fishing net. **PVP**, majorly use in cosmetics and Pharmaceutical industry. In this connection, we believe that river microplastics in Ganga and Yamuna waters are not only come from the industrial waste but also from the anthropogenic activities (degradation of larger plastic debris into small fragments).

Table6.16 -Comparison between this study and other studies for suspended microplastics (Particles/m^3) in surface water

Sr. No	Region	River	MP's Abundance (particles/m^3)	References
1	Indonesia	Surabaya	21.16	Prieskarinda Lestari et al.,2020
2	Europe	European River	5.57	Christian Scherer et al.,2020
3	Europe	Mediterranean	18.8±28.1	Mel Constant et al.,2020
4	China	Yangtze	4137.3 ± 2461.5	Zhao et.al 2014
5	Europe	Seine	3 to 36 (may) 4-108 (April)	Dris et al., 2015
6	Europe	Danube	0. 316.8± 4.664	A. Lechner et al. 2014
7	Europe	Po	1-12.2	Vianello et al., 2015
8	Europe	Rhine	1.85-4.92	Van der Wal et al., 2015
9	North America	Patapsco	0.399-8.72	Yonkos et al., 2014
10		Magothy	0.240-1.73	
11		Rhode	0.124-0.880	
12		Corsica	0.0369-0.617	
13	South America	Elqui	0.129	Rech et al.2015
14		Maipo	0.64	
15		Biobio	0.05	
16		Maule	0.74	

17	Asia	Ganga and Yamuna	1.2- 5.7	Present study

Further, based on linkage with macroplastic assessment, the study indicated presence of polymers that are likely present in Multi-layers packaging and Tobacco, Pan Masala sachet (EVOH, PVAL, PE,PP,PVC, PET), thermo packaging material (Polyamide), thin polybags and plastic bags (LDPE, HDPE). However, in Allahabad, polyethyelene was not found in microplastic water samples (surface water). In Sarkar et al, 2019, polyethylene was found in sediments along Ganga river. It is likely that polyethylene microplastics due to biofouling are not carried away by river to a long distance but get settled in the sediments. Microplastic analysis validates our primary studies and macro assessment studies in Allahabad and Agra.

Acknowledgements

Government of Japan
UNEP Bangkok
UNEP India
NIO, Goa, India
Taj Security, Agra, India
Central Water Commission, Agra, India

Appendix-I

List of polymer found in Agra

Kailash Ghat, River Yamuna, Agra (AGYU1202)	
Polymer	**Total no**
Polyacetylene	24
PIP	22
EVOH	17
PA	14
Styrene/isoprene	10
PVC	10
Butadiene	10
PVAL	10
natural rubber	8
PC	7
PP	7
Polyacrylamide	6
PMMA	6
LDPE	5
Styrene/butyl/methacrylate	5
Styrene/acrylic alcohol	4
Lanoline	4
Pvdf	4
PEG	4
Sanol 770	4
EVA	4
PU	4
Iragnox 1010	3
Polysulfone	3
PVDC	3
HcBR	3
vinyl choride/vinyl acetate	3
Polybutene	3
Epoxy resin	2
Ethylene/vinyl acetate	2
PTFE	2
Styrene/ethylene/butyene	2
Polyester film	2
Nylon66	2
Poly 4-methyl 1- pentene	2
PVS	2

Dussera ,Ghat Yamuna, Agra (AGYD2102)	
Polymer	**Total no**
Polyacetylene	32
EVOH	27
PIP	26
PVC	20
PVAL	14
Polyamide	11
Polyimide	8
Styrene/Isoprene	8
Polyurethane	6
Polybutene	6
Poly(alpha-methyl styrene)	5
PP	5
LDPE	4
PVB	4
Polyvinyl pyrolidene	4
Polyvinyl pyrolidone	3
PES	3
Polybuten	3
Polyacetal	3
PMMA	3
Sanol IS770	3
Polyacrylamide	2
Acronitrile film	2
PE/PP	3
Polyester film	2
Ethylene/Acrylic acid	2
PAR	2
PVDF	2
Pinene	2
Polybutadiene	2
Styrene/Maleic anhydride	2
HDPE	2

Appendix-II

List of polymer type found in Prayagraj

River Ganga, Prayagraj (ALGU0802)	
Polymers	**Numbers**
PIP	30
Polyamide	27
PVC	20
Polyacetylene	19
PP	17
Polyimide	16
PVAL	16
EVOH	13
Poly Vinyl Pyrrolidone	10
ABS	8
Polybutadiene	7
PMMA	7
Polyester	7
Polyurethane	6
Epoxy Resin	6
Nylon (6/10)	6
Polyacrylamide	6
PES	5
Poly (α-Methylstyrene)	4
Polybutene	4
PS	4
PVDF	4
Iraganox 1010	3
Polyacrylic Acid	3
Polyarylate	3
PC	3
PVFM	3
PP/PE	3
1,2- Polybutadiene	2
PVDC	2
Polyphenylene Oxide	2
Styrene/Acronitrile	1
Polyarylate	1

River Ganga, Prayagraj (ALGD0802)	
Polymer Types	Total no of polymer types
Polyacetylene	17
EVOH	14
PIP	13
PVC	8
Styrene/isoprene copolymer	7
PVAL	6
Polyimide	3
Polyacrylamide	2
PP	2
Polybuten	2
PES	2
Lanoline	2
Acrylonitrile film	2
Polysulfone	2
Polyamide	2
PVDF	2

River Yamuna, Praygraj (ALYU0802)	
Polymer Types	**Total no of Polymers**
EVOH	49
Polyacetylene	34
Polyisoprene	13
PVC	11
PVAL	8
Polyamide	7
PP	5
styrene/Isoprene	4
PVB	3
Iragnox md 1024	2
Polysulfone	2
Polyacrylamide	1
Polyimide	1
Pinene	1
PET	1
ABS	1
Ethylene/Propylene coplolymer	1
LDPE	1
Poly (alpha- methyl styrene)	1
Polymer additive Sanol LS770	1
Styrene/Isoprene	1
Lanoline	1
Styrene/Ethylene/Butylene	1
Poly Acylic acid	1
Iragnox 1010	1
Ethylene/Propylene	1
polybutene	2
PMMA	1
Polyetherimide	1
HDPE	1
Polybuten	2
Chimassorb 944LD (Polymeradditive)	1
Styrene/ allyl alcohol copolymer	1
Ethylen/acrylic acid coplymer	1
EVA	1
PVDC	1
Polyacetate	1

Sangam,Prayagraj (ALLSD0802)	
Polymers	**Numbers**
EVOH	22
Polyacetylene	10
PIP	9
Polyimide	4
PVAL	4
PP	3
Styrene/Isoprene	3
Polyacrylamide	2
Poly Vinyl Pyrrolidone	1
Lanoline	1
PVC	1
Polyacetal	1
Cellulose	1
HcBr	1
PEMA	1
Polyamide	1
Pulybuten	1
Styrene/Butadiene	1
Polybutene	1
Total	68

PES	1
PS	1
Polmer additive	2

Appendix-III

Polymers used in Flexible MLP

Plastic polymer	Functions in multilayers	Applications
Polyethylene (PE)	heat-sealable food contact layer moisture barrier can be combined with gas/aroma barriers (e.g. PA, EVOH)	breathable packaging for fresh produce (LDPE, HDPE) carton liners (LLDPE)
Polypropylene (PP)	moisture barrier to provide mechanical strength can be coated with heat seal coatings (PVDC, acrylate) can be combined with gas/aroma barriers (e.g. PVDC coatings, PA, EVOH)	modified atmosphere packaging thermoformed containers for microwavable packaging, hot-filled packaging
Polyamide (PA)	gas/aroma barrier to provide mechanical strength heat resistance can be used as outside layer of a heat seal film ¨ film will not stick to the sealing bar surface	boil-in-bag packaging thermoformed packaging
Polyethylene terephthalate (PET)	gas/aroma barrier moisture barrier to provide mechanical strength heat resistance	plastic bottles for carbonated softdrinks, meat and cheese packaging, snack food wrapper boil-in-bag, sterilisable pouches, ovenware containers
Polystyrene (PS)	gas permeability printability can be combined with gas/aroma barriers (coextruded or laminated) ¨ commercially available structures: e.g. PS/PVDC/PS, PS/PVDC/PE, PS/EVOH/PE, PS/EVOH/PP	breathable packaging for fresh produce (e.g. fresh-meat packaging) printable outside layers
Ethylene vinyl alcohol (EVOH)	oxygen barrier needs to be protected from moisture ¨ often sandwiched (coextruded) between PE or PP, in some applications also sandwiched between PET, PA or PS	modified atmosphere packaging packing of oxygen-sensitive food
Polyvinylidene chloride (PVDC)	gas/aroma and/or moisture barrier to protect the surface from scratches and abrasion heat-sealable food contact layer often copolymers of vinylidene chloride and ester-type monomers (e.g. ethyl acrylate)	modified atmosphere packaging applied as coating or coextrudedfilm
Ethylene vinyl acetate (EVA)	moisture barrier adhesion layer (tie layer) for co-extrusion of polar (e.g. PA, PET-G) and non-polar (e.g. PE) polymers heat-sealable food contact layer; heat-sealable extrusion coatings on PET or BOPP films	modified atmosphere packaging applied as coating or coextrudedfilm
Polycarbonate (PC)	heat resistance mechanical strength moisture barrier	microwavable packaging, hot-filled packaging modified atmosphere packaging barriers for fruit juice cartons
Polyvinylchloride (PVC)	gas/aroma barrier mechanical strength	fresh food packaging (e.g. PVC/PE films) modified atmosphere packaging (e.g. PVC/EVOH/PE films)
Polyethylene naphthalate (PEN)	gas/aroma and moisture barrier heat resistance	for hot refills, rewashing, reuse beverage bottles (e.g. beer)

Glycol modified polyethylene terephthalate (PET-G)	heat-sealable food contact layer	
Ethylene acrylic acid (EAA)	extrusion coating tie layer between aluminium foil and other polymers heat-sealable food contact layer	

Source: European Commission, JRC Technical Reports , Guidance for the identification of polymers in multilayer films used in food contact materials

Appendix-IV

List of polymer type found in other applications

Plastic polymer	Functions	Applications
PMMA	transparent hard plastic	used for aquarium, automobile sector
Polycarbonate		making baby feeding bottle which leaches out BPA, boil in bag packs, microwave cookware
Polyamide	PVDC coated nylon offers very good Oxygen, water vapour and UV resistant barrier	used in pouches
PVC	Inerts in its chemical behavior being iself extinguishing when exposed to a flame	Used in supermarkets for the stretch wrapping of trays containing red fresh meat and produce.
Polystyrene	when heated, • polycarbonate which leaches Bisphenol A, • Polystyrene which leaches styrene • or poly vinyl chloride (PVC) which break down in vinyl chloride and sometimes pthalates which can leach in to various chemicals	Used in Disposable curtleries

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