Best Practices for Marine Microplastic Sampling and Analysis

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Abstract

Plastic pollution in the marine environment has received increasing attention over the last decade. Marine plastics are distributed throughout the ocean surface, subsurface, water column and seabed sediments, because of the surface currents carries floating plastics into the other places and bio-fouling in the plastic particles tend to sink into the ocean floor. The large plastic particles undergo degradation that has form as a microplastics and microfiber which will affects the marine organisms. Hence, it is necessary to monitor and assess the microplastic to have possible mitigation methods to reduce the plastic inputs into the ocean. This paper describes the best practice for the methodology of sample collection, analysis and identification of plastic particles in the marine environment. The purpose of this best practice is to provide recommendations, and practical guidance, for establishing a programme to monitor and assess the distribution and abundance of plastics in the ocean.

1. Introduction

Marine plastics (MPs) are recognized as an emerging contaminant of growing concern in the marine environment, with direct and indirect impacts on marine wildlife and the local economy. In the early 1970s, Carpenter and Smith reported the first evidence of small pieces of floating plastics in the surface ocean (Carpenter and Smith, 1972), but the term 'microplastics' was only coined in 2004 to describe the accumulation of microscopic plastics from environmental samples. The fragmentation of larger items results in numerous plastic pieces of ever smaller dimensions, known as 'microplastics' (Thompson et al., 2004) (João Frias et al., 2019). The definition has been further refined and MPs have been classified as either i) primary: microscopic particles released directly into the environment (e.g. industrial resin flakes, microbeads, etc. or (ii) secondary:

fragments arising from the degradation of larger plastic components (Cole et al., 2011). Microplastic fragments and fibers are commonly found in the water surface, water column, and sediment samples, nearly almost the ecosystem. It is known that after the 1950s, plastics began to reach the ocean in significant quantities and are still the most abundant recorded material (Arthur et al., 2009). Some plastics are extremely durable and stable materials in the marine environment, and some estimates suggest hundreds of years of potential life in the environment. (Arthur et al., 2009; JRC, 2013). MPs may move long distances, with ocean currents and winds depending on physical characteristics such as density (e.g. polyethylene, polypropylene); and sink (e.g. polyvinyl chloride) in the seabed (Olga Carretero et al., 2017). Microplastics can act as a transport for chemicals and organisms in the ocean, being a potential threat to ecosystems and human health. For example, micro-plastics that can contain persistently adsorbed organic contaminants can also provide a route to transmitting harmful chemicals to the network of the food chain (Shim et al., 2017). Plastics present in the environment are majorly due to mismanaged waste and littering (Barnes et al., 2009) this plastic waste enters the ocean via Rivers (Figure 1), waste water outflows and transport by wind or tides, the total quantity entering in the ocean is 4.8 to 12.7 million MT (Jambeck et al.,2015) in these 80% of plastics debris are from land-based sources and 18% of marine plastics in the ocean are contributed from fishing industry (Hinojosa and Thiel, 2009). Plastic material enters into marine environment undergoes various forms of degradation for converting larger particles to small particles, like thermal oxidation, photo-oxidative degradation, biodegradation and Hydrolysis. (Andrady et al., 2011, Dharani et al., 2003). Smaller particles of diameter less than 5mm are termed as Microplastics (Arthur et al., 2009).

The current status of distribution and quantity of microplastics in the ocean is a matter of urgency. It is important for policy planning promotion to be based on concrete scientific knowledge while getting a head start with preventive measures against plastic litter in the ocean. It is expected that more monitoring will be conducted in the future, but as different sampling and analytical methods are used, depending on the purpose of the surveys of each country and research

institution, there is a lack of comparability among currently available data (Yutaka Michida et al., 2019). There are numerous and different sources of plastics and microplastics to the ocean, but the actual volumes remain largely unknown. There is currently no accurate statistical distinction between input loads of macroplastics and microplastics, their origins, their sectors of production and consumers, and this is a significant knowledge gap. Marine Plastics in the open ocean and their drift, fragmentation, remaining afloat in the mid-water column and settling down on the seabed are still not clearly understood. Hence, there is a need to ensure that good quality data are available that will allow comprehensive analyses of the nature and sources of marine plastics in the open ocean and how these vary through time and in response to management interventions. This vision should start with "best practices" by developing a working protocol on microplastics that includes sampling, processing and characterization of isolated microplastics. This best practice for marine plastic sampling and monitoring will tackle accurate measures of plastic volumes, particle weights and chemical additive concentrations in dynamic matrices that are not simple and are needed to track plastic waste to have the best evidence to educate business and policymakers to better solve this that issue. Such best practice for surveying and recording marine plastics will provide accurate measures of plastic volumes, particle weights, product origin, and chemical additive rates in complicated matrices that are not readily accessible and include plastic waste tracking to include the best evidence for educating business to policymakers about the crisis.

A baseline data on temporal trends (Maximenko et al., 2019) and any seasonal distribution patterns of plastics in the oceanic regions have to be created for better management of plastics pollution. Periodic sampling in selected stations in both coastal and offshore waters of the ocean is necessary to achieve this objective. However, the extensive field campaign required for the data collection is often constrained by limited ship-time availability and the costs involved. Hence, it is imperative to utilize ships of opportunity for monitoring plastic pollution in the oceans (Montoto-Martínez et al., 2020). The research vessels involved in the servicing of moored buoys frequently revisit stations, and hence

could be utilized in periodic sampling for marine plastics. This document covers the sampling protocol used by the National Institute of Ocean Technology, India to document microplastics in selected stations in the north Indian Ocean during the moored buoy servicing operations.

2. Materials and Methods

2.1 Selection of ocean sampling stations

The sampling stations for the analysis are selected with an objective to document the distribution pattern of plastics and accessibility of stations. The study requires a coastal station and open ocean stations to figure out the distribution of plastics. A model coastal station is identified near river mouth/estuary having significant discharge into the open ocean. The open ocean stations are identified along the major shipping routes and to study possible gyres from the ocean circulation pattern. The survey should be conducted along a transect normal to the coast at depth contours of 40 m, 200 m and 800 m. Stations near river mouths in the continental shelf region were identified for periodic analysis of surface and sub-surface seawater, seabed sediments. Surveys in island stations will also be carried out similar to coastal stations. Micro Plastics in the surface and sub-surface seawater of open ocean stations will be analyzed using the procedure illustrated.

2.2 Pathways of Plastics debris in Marine Environments

Drifters are an important source of information relevant to transport processes as well as distribution patterns of floating marine litter (FML) on a regional to a global scale. The low-cost and robust design of a satellite-tracked drifter applicable in studying complex pathways and sub-mesoscale dynamics of floating litter in tidally influenced coastal and estuarine systems (Meyerjürgens et al., 2019). An increasingly popular technique for measuring the flow patterns in nearshore currents is using GPS-tracked Surf zone drifters. We have used buoyant, PVC tubes equipped with on-board GPS data loggers (Figure 2) which record the drifter’s position in the surf zone. Recent surface drift tests have significantly improved our understanding of complicated surface current

systems, such as the floating marine litter (FML) pathway and buildup prediction (Van Sebille et al. 2012), improved search and recovery methodologies, and oil spill dispersion modeling and prediction. (Reed et al., 1994).

3.3 Estuary Sampling

Rivers are a major source of ocean plastic pollution. The model-based measurements are with large uncertainties and lack of field measurements to estimate the quantity of plastic reaches the ocean from river (Eerkes-Medrano et al., 2015, Jambeck et al., 2015). An accurate method to quantify the plastic in the river is scarce (Lechner et al., 2014 Lebreton et al., 2017). We identified a pilot site to quantify plastics reaching the ocean. The method adopted is during Flood and Ebb tide, Manta net is positioned in backwaters by boat with Current meter sensor and Conductivity Temperature (CT) Sensor attached to it. The sample is collected after completion of one complete Flood or Ebb Tide. The simultaneous measurement of current speed profile, temperature and salinity measurements along with the microplastics data is used to quantify the macro plastics and microplastics reaching the Ocean from river mouth. The method can also be used to estimate the amount of Macro plastics and Microplastics reaching the off surf zone and accumulation of microplastics in seabed sediment in river mouth and off surf zone (Figure 3).

3.4 Sampling methodology

Plastic contamination is observed on the sea surface, sub-surface water column and sediments. The sampling procedure adopted for the north Indian Ocean region covers all three cases. Plastics in the surface seawater are studied using samples collected using manta trawl. Vertical haul of zooplankton net and water samples from discrete depth levels will be used to study microplastics and microfibers in the water column. Sediment samples collected using a Grab sampler could be used to document microplastics in the seabed (Figure 4). Repeating the horizontal trawl and vertical haul of net sampling increases the reliability of the result.

3.5 Sampling of microplastics on the sea surface

A significant proportion of the plastic pollution on the surface of the ocean consists of particles with a diameter below 1 cm (Hidalgo-Ruz et al. 2012). One of the widely used tools for documenting microplastics floating at the sea surface is Manta Trawl as shown in Figure 5(a)&(b) (Peter G. Ryan et al. 2009, Eriksen et al. 2013, Gago et. al. 2018, Qiqing Chen et al. 2018). The Manta Net is an enhanced version of the Neuston Net and can capture microplastics on the ocean's surface.

3.5.1 Procedure:

The manta trawl is towed horizontally along the side of the ship (KovačViršek, et al.,2016) using a rotatable spinnaker boom assembly as shown in Figure 5(a). It is advisable to deploy the trawl outside the wake zone (about 4 m away from ship hull). The turbulence in the wake zone could momentarily submerge the floating plastics thereby lead to an underestimation of plastics concentration.

The ship has to sail at speed less than 2 knots and the net shall be towed for 20 minutes. A flow meter is attached to the net before deployment for quantitative analysis. The distance towed by net can be calculated by noted GPS coordinates during the initial and final trawl. After 20 minutes of sampling, the manta net is brought to the deck and thoroughly washed with seawater on the outside of the net to collect the complete sample on the cod end. The large plastic pieces are collected separately in the zip bag and all large living organisms in the net shall be discarded. Use filtered seawater to rinse all items removed from the net to collect microplastic on their surface. Rinse the cod end of the net and funnel with filtered water and transfer the contents to a glass bottle. Label station details on sample bottles and zip bags/envelopes. Record all metadata on data sheets (Appendix 1). At each station, samples should be replicated by using two consecutive trawls. Alternatively, the manta net can be towed on both sides of the ship simultaneously as shown in Figure 5(a).

The density of plastics is reported per unit area of the sea surface which is calculated by

Area of water filtered:

$$A = \alpha W N_R$$

Where:

A = Area of water filtered (m^2)

α = Flow meter calibration factor

N_R= Number of revolutions (read from the flow meter dial)

W = Width of the mouth of the net (m)

The formula for flow meter calibration is as follows:

$$\alpha = \frac{d_1}{\mathrm{NR1}} + \frac{d_1}{\mathrm{NR2}} + \cdots + \frac{d_n}{\mathrm{NRn}}$$

Where:

$d_1, d_{2,\ldots,} d_n$ = distance of trawling of the net for n number of tows

$\mathrm{NR1}, \mathrm{NR2} \ldots\ldots\ldots, \mathrm{NRn}$= Number of flow meter counts, for n number of calibration tows

3.5.2 Best Practice:

The manta net shall be towed in sufficiently illuminated condition so that an observer on deck can continuously monitor the net for any clogging. Sampling should be conducted in a calm sea state with a wave height <0.5m, high sea state causes nets to rise above and below water surface which results in an error. Avoid sampling while paint chips from the vessel deck or hull. Avoid using plastic containers and tools during the sampling procedure to prevent contamination and the sample should be stored in metal or glass containers. Cloths made out of synthetic fibers can also potentially contaminate the samples. The specific details of the ropes used for towing should be documented.

3.6 Sampling of microplastics on the subsurface seawater column

Plastics can be also neutrally buoyant and float in a certain depth in the water column. Some studies have confirmed the presence of plastics at different depths of the ocean. The vertical integral of plastics in the top 100 m of the

water column is estimated by the vertical haul of the zooplankton/multiplanton net (Lattinet al., 2004).

3.6.1Procedure:

Zooplankton net/Multiplankton Net (MPN) is lowered from the ship using the winch as shown in Figure 5(d). A flow meter is fixed at the center of the aperture of the net before lowering to the water for measuring the quantitative volume of water flowing through the net. After lowering the plankton net to 100 m depth, hoist it back to deck at a slow speed (10-20 meter per minute). After surfacing, the net is brought to the deck. Rinse the samples on net as thoroughly as possible from outside of the net using filtered seawater and collect all samples at the cod end. The large plastic pieces shall be stored separately in the zip bag and all large living organisms in the net shall be discarded. Use filtered seawater to rinse all items removed from the net to collect plastic on their surface. Rinse the cod end of the net and funnel with filtered water and transfer the contents to the glass bottle. Label station details on sample bottles and zip bags/envelops. The procedure is repeated at the same location which can be compared to have more accurate results.

Record all metadata on data sheets (Appendix 2).

The number of plastics per cubic meter is calculated by Volume of water filtered:

$$V = \alpha \, A \, N_R$$

Where:

V = Volume of water filtered (m^3)

α = Flow meter calibration factor

N_R= Number of revolutions (read from the flow meter dial)

A = Area of the mouth of the net (m^2) = 0.4536 m^2 for 0.76m diameter net

The formula for flow meter calibration is same as give in section 3.5.1.

3.7 Specific depth levels sampling using CTD Instruments

The particles in the size range 0.1mm -1.5mm constitute the majority of plastics samples documented along coast (79%) and Open Ocean (74%) (Barrows et al.,

2018). Microplastics and microfibers of much smaller size range were also recoreded as in important issue in the marine environment. The presence of microplastics and microfibers dispersed discrete depth levels can be identified by analyzing water samples. The standard depth levels identified for the seawater sampling are surface, 10m, 100m and 500 m.

3.7.1 Procedure:

The CTD rosette equipment is used to collect the water sample from the desired depth in the ocean as shown in Figure 5(c) (Zhenfei Dai., et al 2018). The glass bottles should be used for storing the water sample to avoid contamination and then collect water samples from different depth levels surface, 10m, 100m and 500 m using the CTD rosette and record the temperature and salinity at designated depths. Water samples should be transferred to glass bottles. Label station details on sample bottles. Record all metadata on the datasheet (Appendix 3). The collected water samples shall be filtered anad analyzed as described in section 4.2.

3.7.2Best Practice:

The glass bottles should be properly covered by lids and placed in a safer place to avoid leakage. Gloves should be used while collecting the water sample. Care should be taken to avoid any contamination.

3.8 Sampling of microplastics in the seabed sediments

Plastics settled in the seabed are identified by analyzing the sediment samples collected from the seabed (Strand and Tairova, 2016, João Frias et al., 2018). Plastic fibers in surface marine sediment can be collected using Van Veen Grab sampler as shown in Figure 5(e) (Michiel Claessens et al.,2011). The bed sample should be collected from different depth levels.

3.8.1Procedure:

Lower the grab sampler using a winch and collect a sample from the seabed. The sediment sample should be stored in a stainless-steel vessel (Olga et al.,

2017) and closed properly to avoid contamination. Before storing the sample, the stainless-steel vessel should be cleaned thoroughly using distilled water. Measure the area and volume of sediment sampled, to calculate the abundance. Record all metadata on the datasheet (Appendix 4)

3.8.2 Best Practice:

The gloves should be worn while transferring samples to the stainless-steel vessel. Replicate sediment samples need to be collected possibly a minimum of three samples.

3.9 Mobile application for Visual microplastics Sampling

A mobile app by name MarPlast; compatible with IOS and Android platforms has been developed for carrying out Marine Plastic Study in the coastal waters where mobile signal exists. The MarPlast app includes multiple pre-loaded lists of commonly found marine debris items and the user has to just select the type of plastics observed upon during the survey. For items that are not on the list, the item can be logged as "Other" and description can be given in the remarks. The item details along with the latitude and longitude, amount of debris found and its approximate weight can be recorded along with the photo. The app works offline and once logs into the home network, the recording along with images are uploaded to the central database.

4. Laboratory analysis of Marine plastics

Materials:

1. Glass beakers 2. Brass/ stainless-steel sieves 3.Glass Petri dishes 4.vaccum filter pump 5.glass fiber filters 6. Temperature controlled oven 7. Hot plate 8.Aluminium foil 8. Pincette

Reagents:

1. Hydrogen peroxide 30% solution 2. Sodium chloride 3. Aqueous 0.05M Ferrous compound (Fe –II catalyst) 4. Sodium hexametaphosphate

4.1 Analysis of Microplastics in Surface and Sub-surface Samples

The net samples collected after a 20-minute horizontal trawl and vertical haul from 100m water depth are transferred to the Brass/stainless-steel sieve of mesh size 150 μm. Rinse the sample with distilled water and transfer all solids into the sieve. (Masura, J., et al. 2015) Transfer all solids into the clean and dry 500 ml beaker and place the beaker in the oven for at 90ºC for over 24 hrs to dry the sample (Figure 6). Digestion is performed by adding 20 mL of aqueous 0.05M Ferrous compound (Fe –II catalyst) and 20 mL of 30% hydrogen peroxide solution to the sample. The sample should be heated on a hotplate at 75ºC. If natural organic is visible add another 20 mL of 30% hydrogen peroxide. Repeat until no organic material is visible.

To increase the density of the aqueous solution adds ~6 g of salt (NaCl) per 20 mL of sample. Heat mixture until the salt dissolves. Transfer the solution to density separator and cover it loosely with aluminium foil. Allow the solids to settle overnight. Drain settled solids from the separator and discard it. Floating particles are passed through filter paper using a vacuum filter pump. Finally, the Microplastics can be quantified using a Stereomicroscope and characterized using Fourier-transform infrared spectroscopy.

4.2 Analysis of Microplastics in Sub-surface CTD water samples

Water samples are filtered using a Glass fiber filter paper with a pore size of 2.7 μm and changed filter paper for every 5 litres of the water sample. A glass filter (Whatman GF/D) with a pore size of 2.7 μm, diameter 47 mm, was placed in a vacuum filtration assembly with a glass collection flask, attached to a vacuum pump (Carney et. al., 2018). Water from one sample at that time was carefully poured through the filter, and the flasks were rinsed with ultra-pure water to ensure that no fibers were lost on the sides. Until a microscopic examination, the filter was transferred to a thoroughly rinsed glass Petri plate with cover. The filter papers are dried with an oven and placed in a petri dish at a lower temperature. The visual analysis may be carried out with a microplastic sample in the filter paper. The FTIR analysis indicates that the identified microplastics present in the samples are characterized.

4.3 Analysis of microplastics in seabed sediments

After collecting the sample, it should be dried at 90°C until the sample gets dried completely. The dispersing solution for disaggregation of solid is prepared by dissolving 40gm of sodium hexametaphosphate in one litre of distilled water. 400 gm of dried sediment is mixed in 400 mL of dispersing solution and stirred continuously to disaggregate the sediment particles. The disaggregated sediments are sieved using 150-micron sieve and the density separation is carried out using sodium chloride. The sample is filtered again using 150-micron sieve and the collected sample is dried in an oven at 90^0C.

The dried sample is subjected to wet peroxide oxidation (WPO) in the presence of Fe (II) catalyst to digest organic matter. Plastic debris remains unaltered during this procedure. The WPO mixture is subjected to density separation in NaCl (aq) to isolate the plastic debris through flotation, (Julie Masura et al., 2015). Drain and discard the settled solids from the separator. Floating particles are passed through a 2.7 µm filter paper using a vacuum filter pump and kept in the glass Petri dish. The microplastic particles present in the filter paper can be counted under a microscope by visual examination. The FTIR analysis is suggested to characterize selected microplastics found in the samples.

4.3.1 Precaution to minimize contamination while analyzing

- The preliminary filtering of water samples and net samples should be carried out immediately after the sample collection to avoid contamination and inconvenience due to the decay of organic materials.
- The glass bottles should be properly covered by lids and placed it in a safer place to avoid leakage.
- Gloves should be used while analyzing the water sample
- Keep all storage containers as clean as possible and properly closed to avoid airborne microplastics.
- The sampling team should avoid using synthetic cloths as it could contaminate while doing the procedure.
- The repeated transferring of the samples must be avoided.

- All laboratory materials used in this experiment were thoroughly rinsed to reduce the risk of contamination including the workplace.

5. Identification

Physical (size, shape, color) and chemical properties are critical factors to be taken into account during identification. Classifications for these criteria have been taken from the most widely recorded microplastic colors in peer-reviewed publications (Pham et al., 2017).

The most common colors identified are Red, Blue, Green, Yellow, Black, and others-transparent. Visual identification of Microplastics based on physical appearance was identifying using a Stereomicroscope. The types of microplastic are characterized such as Pellet, Fragment, Fibre, Film, Rope and filaments, Microbeads (perfect spheres), Sponge/foam (Gago et al., 2018). The size ranges from > 5 to 0.1 mm, large microplastics (1 – ≤5 mm) & small microplastics 1µm – ≤ 1000 µm, it is accepted globally that the lower size value for small microplastics is 1 µm (Gago et al., 2018).

In chemical properties polymer type can be identified through the following techniques: 1) micro-Fourier Transform Infrared spectroscopy (in brief micro- or µ-FTIR); 2) Attenuated Total Reflection Fourier Transform Infrared spectroscopy (ATR-FTIR); 3) micro-Raman spectroscopy (µ-RAMAN); 4) Pyrolysis gas chromatography-mass spectrometry (PyGCMS); and 6) Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Analysis (EDX). The use of micro FTIR, ATR FTIR, to identify the polymer type is recommended from the above-mentioned techniques. These are economical and easier to access than others. (Gago et al., 2018).

6. Reporting results

Reporting units are extremely important for making comparisons between studies possible (Olga Carretero et al., 2017). The proposed microplastics monitoring units derived from the samples are:

1. Number microplastics per area (# particles km^{-2} | # particles m^{-2})
2. Number microplastics per volume (# particles m^{-3})

3. Mass of microplastics per volume (gl^{-1} | gm^{-3})

4. Mass of microplastics per area (gkm^{-2} | gm^{-2})

Observed higher concentrations of ocean surface microplastics are usually stated in terms of density, or particle quantity or weight per unit area ($/m^2$, $/km^2$) or water volume ($/m^3$). Studies on the distribution of microplastics on the ocean surface should include not only their quantity or weight per unit area or volume of water but also their particle size and materials and metadata at the time of sampling (Yutaka Michida et al., 2019, Kanhai et al., 2018).

7. Summary

Marine plastic sampling on the surface of seawater is performed by Manta Net of mesh size 0.3 mm (300micron) is widely used as specified in the sampling methodology because of the ability to filter a large volume of water through it and the collection of relevant number plastic pieces in the cod end of the Net. The horizontal position of net in the sea surface is properly maintained by connecting from the rotatable spinnaker boom present in the deck of ship and flow meter is attached at the center of Manta Net to document the volume of water filtered. It should be away from the wake zone to reduce turbulence in the flow of water passing through the Net. The salinity and temperature profile varies with various water column depths and to study different types of plastics, using CTD rosette connecting by Niskin bottles is lowered down for collecting water samples. The subsurface sampling at 100m depth is collected by lowering CTD winch with attaching zooplankton net of size 200 to 300microns mesh size and attaching flow meter at the center of the net. The winch lowered to the desired depth and the sample is collected at the cod end of the net and lowering the winch 20-30m per minute for accuracy in the collection of samples. The van Veen grab sampler is used for collecting sediment from the seabed top layer. The sample collected from the surface to subsurface and seabed sediment we are collecting for the minimal area the spatial extent of the sea is very large for accuracy of the sampling method it has to repeat (replicate to be made for improving accuracy) for the large area.

At present in the Indian Ocean region, not many estimates made for sampling marine plastic debris in deep-sea and has no noted garbage patches as of now. Though there are increasing studies on marine debris in several parts of the world, as the second populous country of the world, the data on marine debris particularly marine plastics are limited, not extensive, and require further studies in the Indian scenario. So, the best practices of sampling methods are useful for estimating the marine plastic debris with high accuracy.

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Figures

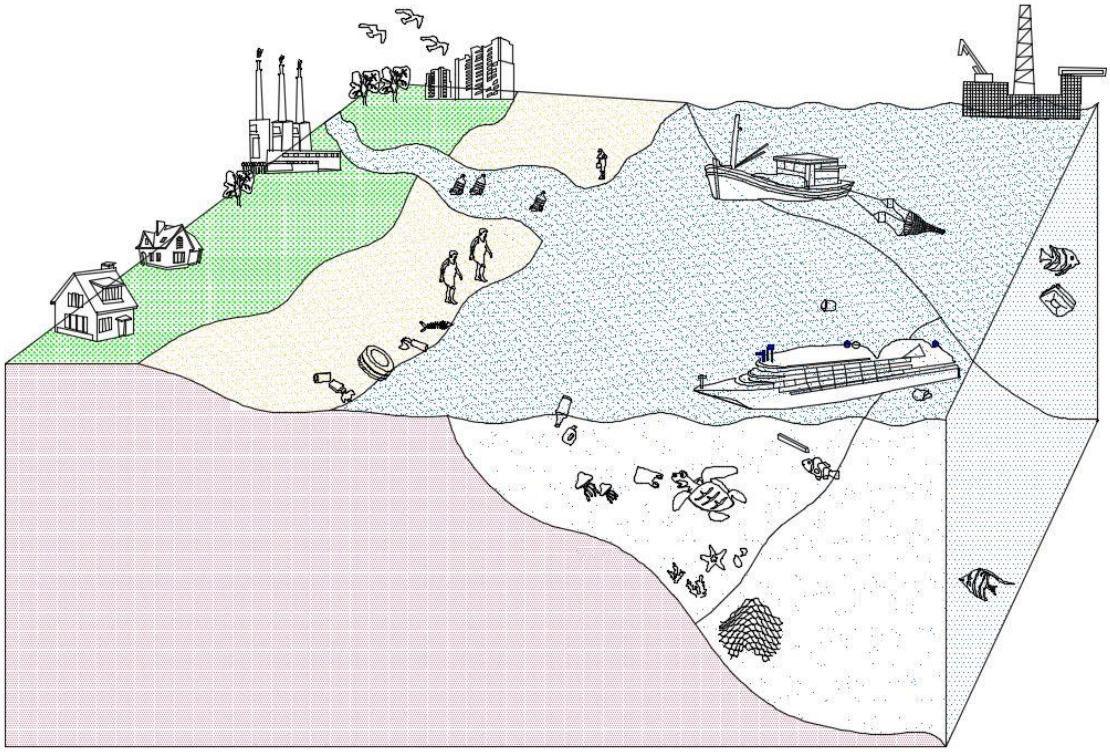

Figure 1: Source of marine plastic

Figure 2. Tracker Study for Plastic debris in Marine Environment

Figure 3. Micro plastic Sampling in Estuary

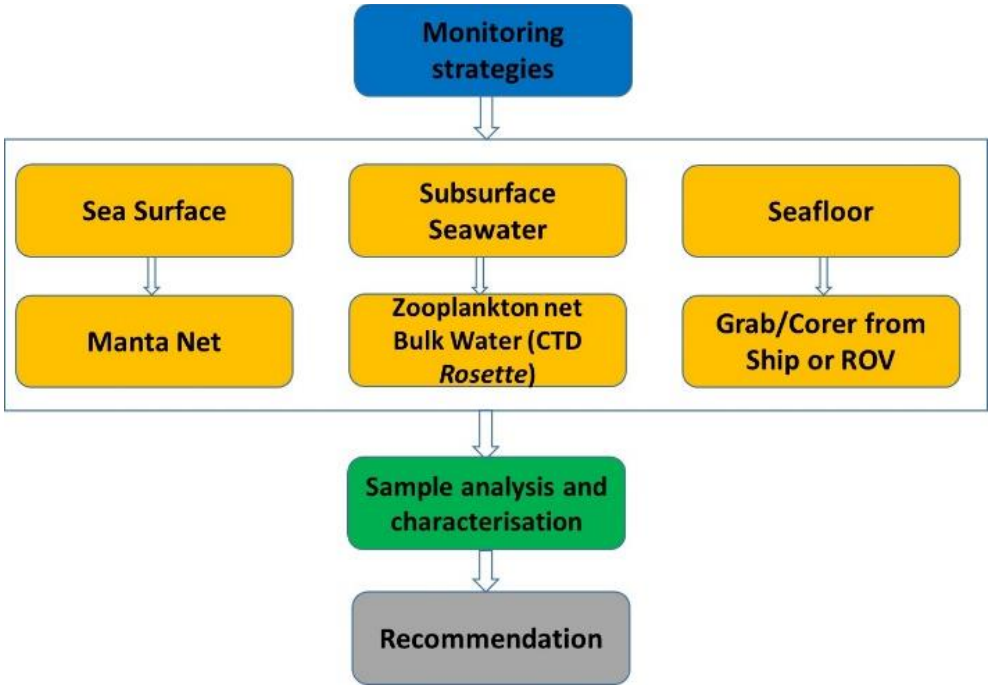

Figure 4: Best practice in the Microplastic sampling

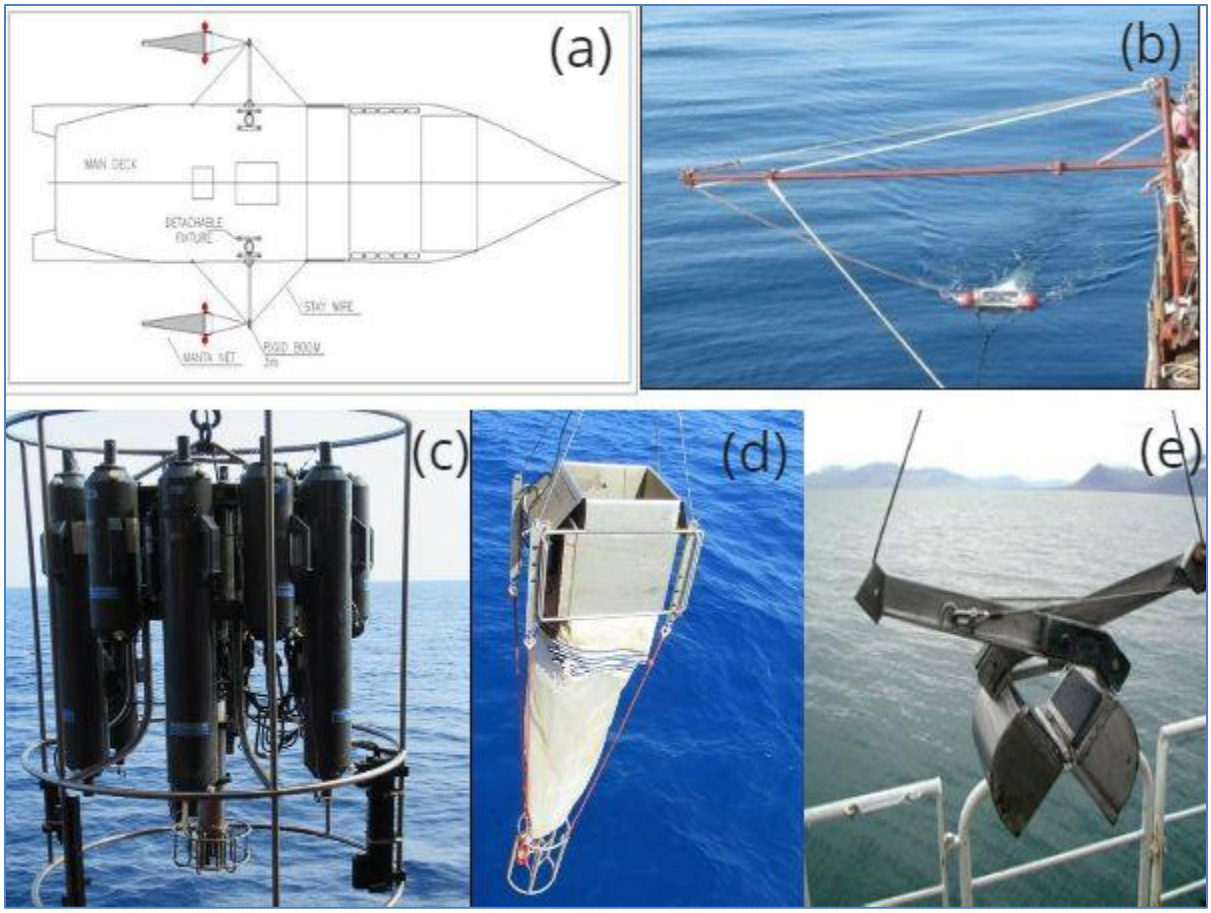

Figure 5: (a)&(b) Procedure for trawling manta net with the use of a rigid boom, (c) CTD Rosette, (d) Multiplankton net, (e) Sediment Grab

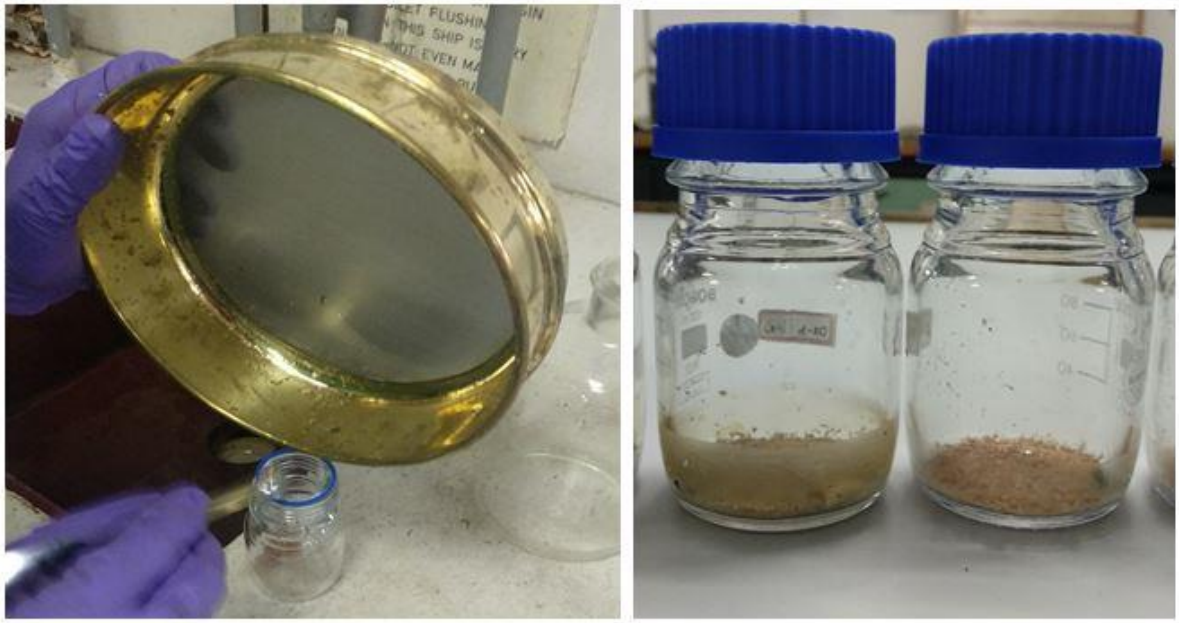

Figure 6: Filtered and Oven dried samples at 90^0c

APPENDIX

Appendix 1: Data sheet for horizontal trawling of Manta trawl.

Start Data	Trawl#	Time start	Latitude (N) start			Longitude (E) Start	
Stop Data	Date	Time end	Latitude (N) stop			Longitude (E) Stop	
Sea and ship conditions	Start Sea State / End Sea State	Start Speed / End Speed m/sec	Start Direction/ End Direction	Average ship Speed (Knot)	Start Wind Direction/ End Wind Direction	Start Wind Speed/ End Wind Speed m/sec	Flow meter reading
							Start:
							Stop:

Appendix 2: Data sheet for vertical haul of Zooplankton net

The diameter of zooplankton net:

Mesh size:

Haul#	**Date**	**Time**	**Latitude (N)**	**Longitude (E)**	**Sea State**	**Flowmeter reading**		**Winch hoist speed m/sec**	**Depth up to which Plankton net was lowered m**
						Start	StoP:		

Appendix 3: Data sheet for water samples from the coastal stations.

Location ID	Depth Contour m	GPS		Water Samples: Bottle Number				Remarks
		Latitude N	Longitude E	Surface	10 m	100 m	500 m	
(Stn#1)	40							
	200							
	800							
(Stn#2)	40							
	200							
	800							
(Stn#3)	40							
	200							
	800							

Appendix 4: **Data sheet for water samples from the open ocean stations.**

Location ID	GPS Location		Water Samples: Bottle Number				Remarks
	Latitude N	Longitude E	Surface	10 m	100 m	500 m	

Appendix 5: Data sheet for sediment samples from the coastal stations

Location ID	Depth Contour m	GPS		Sample Number	Remarks
		Latitude N	Longitude E		
(Stn#1)	40				
	200				
	800				
(Stn#2)	40				
	200				
	800				
(Stn#3)	40				
	200				
	800				