

## Microplastics in water, sediment and seafood as an emerging threat to the marine environment: A case study in Goa, west coast of India

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### Highlights

- First evaluation of microplastics (MPs) in Sal estuary, Goa in three different matrices (water, sediments and biota)
- MPs found in shellfish and finfish species closely reflected those in surrounding environment (water column and sediments)
- Fibres with different colours were most abundant MPs in all matrices
- Smaller sized MPs (10-300µm) were found most significantly in biota
- Major polymers, found in all three matrices were polyacrylamide, polyacetylene and ethylene vinyl alcohol

### Abstract

The present study exhibits the occurrence of MPs in different matrices (water, sediments and biota) from the Sal estuary, Goa, situated on the central west coast of India. The average numbers of MPs in the water column and sediment were  $48 \pm 19$  MP particles/L (MPs/L) and  $3950 \pm 930$  MP particles/kg (MPs/kg), respectively. In shellfish (whole soft tissue), the average concentrations of MPs were  $4 \pm 2$  (*Crassostrea* sp.),  $3.2 \pm 1.8$  (*Pernaviridis*) and  $0.7 \pm 0.3$  (*Paphiamalbarica*) MPs/g body weight (bw), respectively. The highest MPs were recorded in finfish (gastro-intestinal tract) (*Mugilcephalus*)  $7.8 \pm 4$ , followed by (*Gerresfilamentosus*)  $5.3 \pm 4.9$ , (*Arius jella*)  $4.6 \pm 2.6$ , and (*Etroplussuratensis*)  $1.4 \pm 0.3$  MPs/g bw. MP fibres were predominant in all matrices. Interestingly, a dominance of small sized (10-300µm) MPs was recorded in biota. Among the 37 polymer types identified by µ-FTIR, the most prevalent ones were, polyacrylamide, polyacetylene, ethylene vinyl alcohol (EVOH), polyvinyl chloride (PVC) and polyamide (nylon). Notably, the polymers dominant in the gut of finfish and in whole shellfish were equally prominent in sediment and the water column. This study highlights the presence of MPs in commercially important shellfish and finfish samples from the Sal estuary. This study clearly shows the presence of MPs in various types of marine organisms in the Sal estuary. As shellfish is locally consumed as a delicacy and plays a major role in the seafood industry, the MPs may pose a hazard for human health. There is also an ecological risk as MPs are also found in water and sediments and in the digestive tract of finfishes.

**Keywords:** microplastics, biota, water, sediments, estuary, µ-FTIR

## 1. Introduction

Plastics are synthetic polymers that are typically made from non-renewable resources, some of which are chemically inert materials. These are not easily degraded in the marine environment, resulting in plastic littering in the oceans. The consistent increase in the production and usage of plastics along with the increasing world population and a very limited plastic waste management causes serious societal and health concern. Microplastics (MPs), plastic particles of <5 mm size, have been recognized as an emerging threat to the marine environment, due to their persistence, ubiquity and toxicity ([Wang et al., 2016](#); [Rezania et al., 2018](#)). They accumulate at relatively high rates in several ecological systems ([Cózar et al., 2017](#); [Waller et al., 2017](#)). Depending on chemical composition and usage, MPs can be classified as primary and secondary MPs. Primary MPs are particles that are produced intentionally at small sizes for various applications and can reach coastal and marine habitats by release of intermediate plastic feedstock, i.e. pellets or micro-beads from personal care products ([Anderson et al., 2017](#); [Fu and Wang, 2019](#)) Secondary MPs are the tiny fragments, which are formed after breakdown of larger plastic items that undergo weathering, mainly photo-degradation by ultraviolet radiation from sun light, and cleaving bonds through oxidation processes ([Thompson et al., 2004](#)). They are potentially released from plastic packaging material, agricultural plastic film and are also generated from tire wear and tear and marine paints ([Galafassi et al., 2019](#); [Peng et al., 2020](#)). MPs can also absorb pollutants, viz. persistent organic pollutants (POPs), hydrophobic pollutants, heavy metals and also pathogenic microbes ([Dehghani et al., 2017](#)). The presence of MPs and larger pieces of plastics have been recorded in almost every type of marine habitat ([Eriksen et al., 2013](#); [Zhang et al., 2017](#)). Precise characterization of MPs in estuarine ecosystems is, however, still lacking. The coastal zone acts as a hot spot for MP pollution, as the majority of the world's population lives near the coasts ([Zhang et al., 2017](#)) and apparently almost all MPs are terrestrially derived ([Jambeck et al., 2015](#)), transported by inland freshwaters, entering estuaries and ultimately the open sea ([Lebreton et al., 2017](#)). Discharges from urban, industrial and agricultural areas too directly drain into estuaries and lakes ([Eriksen et al., 2013](#)), while less-efficient sewage treatment plants (STPs) and direct domestic runoff can be other point sources. Many marine invertebrates tend to ingest MPs that have a similar size to plankton ([Browne et al., 2008](#)). Furthermore, MPs are ultimately deposited in sediments ([Thompson et al., 2004](#)), wherein the benthic community is consuming them. It is estimated that by the end of 2025, if proper waste management systems are not employed, the total amount of plastic waste expected to enter the oceans will increase by an order of magnitude ([Jambeck et al., 2015](#)), leading to a further increase of their accumulation in the marine environment ([Thompson et al., 2009](#); [UNEP, 2014](#)). Ingestion of MPs by commercially important seafood, mostly fish (e.g. Atlantic cod, Atlantic horse mackerel, red

mullet and, European sea bass) (Lusher et al., 2013; Bessa et al., 2018), bivalves (e.g. mussels and oysters)(Van Cauwenberghe and Janssen, 2014;Devriese et al., 2015), and crustaceans(e.g. brown shrimp)(Barboza et al., 2018)has been reported in several studies worldwide. Bivalves are benthic filter feeders or suspension feeders and have a tendency to accumulate MPs(VanCauwenberghe and Janssen, 2014;Ample evidence was found for ingestion of MPs byzooplankton (Cole et al., 2014;Setala et al., 2014),seabirds (Codina-Garcia et al., 2013)andcetaceans (Fossi et al., 2016).

Toxins leaching from MPs such as phthalates, polychlorinated biphenyls (PCBs) and other persistent organic pollutants (POPs), and metalspose an additionalthreat to the health of the organisms(Rochman et al., 2014). The hazardous chemicals that percolate into the gastrointestinal tract caneventually be transported to the edible tissue (Rochman et al., 2013).Consequently, humans consuming seafood encounter a double risk: that of the MPs and an extra load of chemical contaminants (Lassen et al., 2015). Ingestion of MPs in higher organisms/humans can cause alteration in chromosomes, whichcan lead to infertility, obesity and cancer (Kershawand Rochman, 2015). Furthermore, studies have also discussed the effects of nanoplastics on human body such as cardiopulmonary responses, alterations of endogenous metabolites, genotoxicity, inflammatory responses, oxidative stress, effects on nutrient absorption, gut microflora, and reproduction (Smith et al.,2018).

With 7517 km of coastline, one third of India's population lives and sustain on oceanic resources (Senapati and Gupta, 2014)with considerable consumption of finfishand shellfish. According to (Anon., 2018), annual per capita fish consumption in India lies in the range of 5-10 kg, with about 56% of the population being fish consumers. In Goa, situated on the central west coast of India, fish is extensively consumed,making up 1.85% of the total marine and estuarine in fish landing of India (CMFRI, 2015). Being resourceful, several industrial clusters as well as townships prefer coastal areas. Goa, being an international tourist destination, has complex and intense human-environment interactions. Indiscriminate plastic usage and discard has so far gonemostly unreported, as very few studies have been carried out in thesecoastal waters of India (Naidu et al., 2019; Dowarah et al., 2020).The present study aims to provide critical information on (i) the accumulation of MPs in shellfish (whole body soft tissue) and their role in transfer directly to humans via consumption, (ii)ingestion of MPs in finfish (in gastrointestinal tract) and its ecological implications, (iii) thedetection, occurrence and distribution of MPs in the surrounding environment, which includes the water column and sediments, and (iv) correlation of the amount of MPs found in the surrounding environment with that ingested by shellfish/finfish.

## 2. Materials and methods

### 2.1 Study area and sample collection

Goa has a coastline of 160 km with nine major estuaries. The Sal estuary is one of them with a depth of 2-3m. The state Goa is a known international tourist destination of India, where this estuary is a medium sized river with considerable urbanisation due to thriving tourism. The river originates from three springs on the plateau in Verna and is saline from Kharebandar onwards. It flows into the Arabian Sea west of India. This estuary is about 40 km long and has a basin size of 300 km<sup>2</sup>. Since many years, it is being used indiscriminately for release of sewage and untreated solid and domestic waste. This estuary is rain-fed and is subject to tidal influence to a great distance inward from its mouth. Sewage discharge by urban population, cattle washing, and solid waste dumping are major issues the estuary is facing, which are all related to the dense habitation across the estuary bank. The Sal estuary has been categorized as the most polluted estuary of the state (Goa State Pollution Control Board, GSPCB, 2019) based on two parameters, i.e. Biological Oxygen Demand (BOD) and total coliforms.

### 2.2 Water and sediment sampling

Water and sediment samples were collected during the post-monsoon (January) 2019 from four locations in the Sal estuary, Goa. The sampling locations are shown in Fig 1. A Niskin sampler with a length of 692mm and capacity of 5L was used to collect the water from a depth of 1-3m. In total 5L volume of water was collected from four locations (S1, S2, S3 and S4) and carefully transferred to pre-cleaned and labeled dried glass bottles so as to avoid decaying of organic content present in the sample. Sediment samples were collected from the same four locations (S1, S2, S3 and S4) using a Van Veen grab (0.1m<sup>2</sup>) from depths of 2-3m. The sediment in the Sal estuary showed a more or less uniform sediment textural distribution, wherein three stations were sandy in nature with 90 or more weight% of sediment having a grain size >63µm. Most of the sediment samples contained 0 to 5.0% silt and ~2.0 to 16% clay (Anon, 2019-I). The top 2 cm of the sediment were carefully collected with a pre-cleaned stainless steel scoop in a clean aluminum foil pouch. Once brought to laboratory, samples were stored at -20°C, till further analysis.

### 2.3 Biota sampling and morphometric analysis

Four species of finfish samples, *Mugil cephalus*, *Arius jella*, *Gerres filamentosus* and *Etroplus suratensis* were collected from two different sites marked as B1 and B2, shown in Fig 1. Three species of shellfish samples, *Perna viridis*, *Paphiagalbarica*, *Crassostrea* sp., were collected from the local fishermen, harvesting at the same sampling locations on January 2019. The samples

were brought to the laboratory in an icebox, and were stored at -20°C for subsequent analysis. The morphometric data of each individual shellfish were taken by measuring its length, height, width and umbo length ([Lusher et al., 2013](#)) and for finfish by measuring its total length, standard length, head length, eye orbit length and body width. Out of all these species only *E. suratensis* was benthopelagic whereas the other species were benthic. All shellfish species were filter feeders and amongst the finfishes, *M. cephalus* and *E. suratensis* were omnivores, whereas *A. jella* and *G. filamentosus* were carnivores. Details of the species and their morphometric data are given in Table 1.

#### 2.4 Sample treatment and extraction of MPs from water and sediments

Once brought to the laboratory, the entire 5L of each water sample were filtered (without organic matter) using a vacuum filtration unit with the help of a 47mm diameter and 5µm pore size filter paper and dried further in an oven at 40°C for 24 h. The apparatus used were thoroughly rinsed with deionized water before the commencement of the next sample filtration.

10 g of freeze-dried sediment were analyzed for MPs with modification after [Matsuguma et al. \(2017\)](#). 200 mL of 30% H<sub>2</sub>O<sub>2</sub> was added to the sediment in a glass beaker to remove biofilms. Further, samples were continuously stirred and were thereafter kept undisturbed for 2-3 days. When the reaction ceased, the sediment was passed through a 300µm nylon sieve net. Particles retained in the sieve as well as the residual filtered sediment sample were suspended in 500mL of a 5.3M NaCl solution and were allowed to settle down for 3-4 days. The supernatant was carefully collected in tubes and further centrifuged at 3000rpm for 15 min. The residues were vacuum filtered using 5µm filter paper and were stored in glass Petriplates. The filter papers were oven dried at 40°C for further analysis.

#### 2.5 Microplastic extraction from shellfish and finfish

In this study in total 39 shellfish samples of three different species *Crassostrea* sp. (n=14), *P. malbarica* (n=15) and *P. viridis* (n=10) and 12 finfish samples of four different species *M. cephalus* (n=4), *Arius jella* (n=4), *G. filamentosus* (n=2) and *E. suratensis* (n=2) have been analysed. The 14 oyster samples (*Crassostrea* sp.) and 10 green mussels (*P. viridis*) were analyzed individually for quantification of MPs and named as OS and GS respectively. On the other hand, the 15 clam samples (*P. malbarica*), referred to as CS, were analyzed after combining five large, medium and small sized clams and marked as LCS, MCS and SCS, respectively. All shellfish samples were thoroughly rinsed with filtered distilled water before dissection. The soft tissues of nearly equal weight were placed in 1L conical flask individually for further digestion in an oscillation incubator as the conical flasks have a larger surface area, which enables the organic matter to digest properly without spillage.

Subsequently, 200mL of 30% H<sub>2</sub>O<sub>2</sub> per 5g of the body weight was added to each conical flask, which was then placed in an oscillation incubator at 65 °C at 80 rpm for 24 h and then at room temperature for 24-48 h depending on its digestion status ([Digka, 2018](#)). Termination of digestion was done once the solutions appeared clear. The finfish samples were marked as MFS (*M. cephalus*), CFS (*A. jella*), WFS (*G. filamentosus*), and PFS (*E. suratensis*), respectively. The individual fish samples were cleaned with distilled water before dissection. The digestive tract was removed from each fish sample by longitudinal incision and was placed in a 1L conical flask for further processing. 200mL of 10% KOH per 5g of the gastrointestinal tract (GIT) was added to the flask. The samples were kept in an incubator (10 days) for complete digestion ([Tanaka, 2016](#)). All the digested samples of shellfish and finfish were filtered using a vacuum filtration unit with a 5µm filter paper and were stored in pre-cleaned glass Petriplates, which were kept for 24 h oven drying at 40-45°C (24 h) until dryness.

### *2.6 Identification and quantification of microplastics*

The dried filters of all the samples were examined under a stereomicroscope (Olympus SX10) for identification and quantification of plastics resembling MPs based on their size, colour, shape and structure. A digital camera (Olympus DP73) and the CELL SENS ENTRY software were used for quantification and identification of MPs like particle. MPs were photographed, counted and categorized based on their length, colour and its type (e.g. fibre, fragment, film, beads, etc.) following the guidelines produced by the MSFD technical group and marine litter ([Galgani et al., 2013](#)) (Fig. 2). The number of MPs was recorded separately for each filter and then summed for total MPs in each individual. The filter papers were then kept at room temperature for further confirmation and identification of polymer type by using µ-FTIR spectroscopic analysis.

### *2.7 Detection of polymers by using µ-FTIR*

The polymers of each MP particle, were identified by Shimadzu IR TracerTM-100 Fourier Transform Infrared Spectrophotometer (FTIR) and AIM-3800 Infrared Microscope with 16cm<sup>-1</sup> resolution operating in mid infrared range of 700-4000cm<sup>-1</sup>. Lab solutions and AIM viewer software were used and 100 scans were observed. Since the samples were adhered to filter paper, we used a high-sensitivity reflection method for the detection of MPs. The samples were scanned on the selected object by passing IR and resultant graphs were compared with the standard graphs of polymers in the AIM library to identify the polymer type. The µ-FTIR method for identification of polymers was standardized and successfully applied for the first time in Indian water, sediments and biota samples. The polymer identification of particles is shown in SI Fig 3.

## 2.8 Precautions and quality control

The microplastics extraction from all the three matrices (water, sediment and biota) and the subsequent examination of the filter papers was performed in compliance with the recent analytical MP methodologies (Woodall et al., 2015; Wesch et al., 2017). Before starting the experiments, glassware was rinsed thoroughly with distilled/deionised water. The students and scientists wore lab coats of non-polymer nature. The whole experimental setup was done in a sterile area. Furthermore, filter papers were not exposed to air and were kept in a clean air laminar flow hood and maintained in clean Petridishes. After dissection samples were covered with aluminium foil. Glass lids were used during microscopic work to avoid contamination. Moreover, non-existence of any airborne micro-fibre was confirmed by checking three replicates of blank filter paper in  $\mu$ -FTIR, to eliminate the probability of MP contamination by air.

## 2.9 Data and statistical analysis

To analyze similarity and dissimilarity among different matrices, non-metric multi-dimensional scaling (n-MDS) was implemented on a Bray-Curtis similarity matrix with transformed data (log) using PAST software. Besides ANOVA, an n-MDS method was used for ordination purpose. The differences between the abundances of the different matrices (water, sediment, shellfish and finfish) were analyzed with a two-factor analysis of variance without replication, created from transformed data (square root; ANOVA;  $p < 0.05$ ).

## 3. Results

### 3.1 Abundance of MPs in water, sediments and biota samples

The present study detected the presence of MPs in *Crassostrea* sp. (Oysters), *P. malbarica* (Clams), *P. viridis* (Mussels), *M. cephalus* (Silver/Grey Mullet), *A. Jella* (Catfish), *G. filamentosus* (Whipfin silver-biddy) and *E. suratensis* (Pearl spot cichlid) from the Salestuary. By using a stereozoom microscope in total 363 MPs particles were found in 14 *Crassostrea* sp. (OS) samples, 110 MPs in 15 *P. malbarica* (CS) and 214 MPs in 10 *P. viridis* (GS), all whole body samples. In finfish (gut content), 325 MPs were found in 4 *M. cephalus* (MFS), 162 MPs in 4 *A. jella* (CFS), 12 MPs in two *E. suratensis* (PFS) and 77 MPs in 2 *G. filamentosus* (WFS) were found (SI Table 1a). The highest mean abundance was found in *Crassostrea* sp. ( $4.0 \pm 2.1$  MPs/g) followed by *P. viridis* ( $3.2 \pm 1.8$  MPs/g) and *P. malbarica* ( $0.7 \pm 0.3$  MPs/g) (Table 2; SI Fig 1). In case of all 12 finfish gut samples, the predominant MP presence was found in *M. cephalus* ( $7.8 \pm 4.4$  MPs/g) followed by *G. filamentosus* ( $5.3 \pm 4.9$  MPs/g), *A. jella* ( $4.6 \pm 2.6$  MPs/g) and *E. suratensis* ( $1.4 \pm 0.3$  MPs/g) (Table 2; SI Fig 1).

The average MP concentration in water was  $48 \pm 19.4$ MPs/L, whereas  $3950 \pm 933$  MP/kg in sediment samples (Table 2). In accordance with statistical analysis highly significant difference was observed for abundance of MPs in the surrounding environment (sediment and water,  $p$ -value, 0.003) whereas biota (shellfish and finfish  $p$ -value, 0.030) depicted significant difference ( $p < 0.05$ , SI Table 2a, b). Interestingly, no significant difference was found in all types of MPs for the surrounding environment ( $p$ -value, 0.1,  $p > 0.05$ ) while significant difference was observed in biota ( $p$ -value, 0.0002,  $p < 0.05$ , SI Table 2a, b).

### 3.2 Characterization of MPs (shape, size, color)

MP characterization was done by categorizing it as fibres, fragments, films or other plastics (having irregular distorted shape and not falling in any of the above categories) based on the stereomicroscope analysis (Fig 2). Water samples showed a slight dominance of fibres (56%) over fragments (25%), whereas other plastics(11%) and films (8%) had the lowest occurrence (Fig 3). In sediments, fibres (77%) were much more abundant than films (13%), followed by fragments (8%) and other plastics (3%) (Fig 3). In terms of shape, interestingly, fibres were also the dominant MPs in all shellfish and finfish species (Fig 3), followed by fragments, films and others. Among the shellfish, almost all species show a similar percentage of fibres(OS:72.7%, CS 69.1 % and GS 73.4%) and fragments (OS:14.6 %; CS 20.9 % and GS 20.6%) (Fig3). Whereas films were recorded significantly higher in *Crassostrea* sp. (OS: 5.5%) followed by *P. viridis*(GS: 1.4%) and significantly lowest in *P. malbarica* (CS: 0.91%). Other plastics were predominantly found in *P. malbarica* (9.1%), followed by *Crassostrea* sp. (7.2%) and least in *P. viridis* (4.7%). Similarly, the foremost types of MPs found in the fish guts are fibres (MFS: 72.3%; CFS: 67.9%; PFS: 66.7% and WFS: 89.6%), followed by fragments (MFS: 24.3%; CFS: 22.2%; PFS: 16.7% and WFS: 5.2%), films (MFS: 0.62%; CFS: 2.5%; PFS: 16.7% and WFS: 5.2%) and others (MFS: 2.8%; CFS: 7.4%; PFS: 0% and WFS: 0%) (Fig 3). In *G. filamentosus*(PFS) and *E. suratensis*(WFS) other plastic particles were not found.

The predominant size ranges of MPs found in water and sediment were 100-300 and 300µm-1mm, respectively. The percentage of the size range <100 µm MPs in water was 21.7%, 41.8% for 100 µm-300 µm, 29.3% for 300 µm-1mm and 7.2% for 1-5mm (Fig. 4), whereas in sediment 300 µm-1mm MPs were found in the highest quantities (33.3%), followed by 100 µm-300 µm (25.9%), 1mm-5mm (24.4%), and 20 µm -100 µm (16.3%) (Fig. 4). The size variations of the different types of MPs found in all shellfish and finfish species are given in (Table 2). The length of the three major categories of MPs, i.e. fibres, fragments and films in shellfish were in a range of 10-428µm, 10-202µm and 15-331µm, respectively, whereas in fish gut samples, these were 10-582 µm, 10-58 µm



and 12-71  $\mu\text{m}$ , respectively (Table 2). MPs of smaller size ( $<50 \mu\text{m}$ ) were found in both shellfish and finfishes (58.7% and 63.3%), followed by MPs with size ranges 51-100 $\mu\text{m}$  (30.1% and 27.1%), 101-300 $\mu\text{m}$  (9.7% and 8.5%) and 301-1000 $\mu\text{m}$  (1.5% and 1.1%) (Fig 4).

Different colours of MP particles including black, blue, green, red, orange, pink, yellow, brown, white and transparent were observed in both water and sediments. Black coloured MPs were the most common in both water (43.9%) and sediments (38.2%) (SI Fig 2). Brown and pink coloured MPs (0.6%) were found least among water samples whereas yellow colour MPs (1.5%) were found to be the lowest in sediment samples. The most prominent colours of MPs observed in all shellfish and finfishes were blue followed by red, green, black, orange and transparent (SI Fig 2). Among the seven species of seafood, finfish *M. cephalus* (MFS) showed a higher percentage of MPs of blue colour (58.8%), whereas the percentage of MPs having a transparent colour was the lowest (1.8%) in the shellfish species *P. malbarica* (SI Fig 2). Among the rest of the coloured MPs, two other dominant MPs were green and red in a range of (8.3-24.7%) and (11.7%- 24.5%), respectively.

### 3.3 Polymer identification of MPs in the water, sediment and biota samples

$\mu$ -FTIR analysis was successfully carried out for 350 MP particles in water and sediment samples and for 374 particles in seven commercially important species. Utterly 37 types of polymers have been detected in all three matrices. The total numbers of MPs analysed by  $\mu$ -FTIR microscopy in water and sediments were 192 (19 types of polymers) and 158 (12 types of polymers), respectively. In shellfish such as *Crassostrea* sp., *P. malbarica* and *P. viridis*, 155 (37 types of polymers), 42 (17 types of polymers) and 72 (12 types of polymers), respectively were found, whereas in finfish (*M. cephalus*, *A. jella*, *G. filamentosus* and *E. suratensis*) 63 (16 types of polymers), 32 (8 types of polymers), 4 (4 types of polymers) and 6 (5 types of polymers) were identified, respectively. Polyacrylamide (27.8%), polyacetylene (23.1%), polyimide (14.2%) and ethylene vinyl alcohol (EVOH) (12.6%) were most common in water (Fig 5 shows the five dominant polymers), whereas in sediment samples polyacetylene (36.1%), polyacrylamide (32.9%), EVOH (10.3%), and polyimide (9.0%) were abundant (Fig 5). Polymer types identified in both shellfish and finfish were of almost identical. EVOH was found to be predominant in shellfish (10-35%). Other major polymers, found in both shellfish and finfishes were polyacetylene, polyacrylamide, ethylene vinyl alcohol (EVOH), polyvinyl chloride (PVC), polyvinyl alcohol and other plastics, in all species (Fig 5; SI Table 3). No MP particles were found on the blank filter papers, which ensures that airborne contaminants have not contributed to the total count of MPs, during filtration or the further process. The spectra of the most abundant polymers and blank filters are given in SI Fig. 3.

A non-metric multi-dimensional scaling (n-MDS) test showed the dimensional distance separating shellfish from other matrices (water, sediment and finfish), based on their polymer composition (SI Fig 4). Water and sediment showed a similarity and were characterized by the high presence of polyacetylene and polyacrylamide, whereas biota samples were characterized by the high abundance of EVOH and polyacetylene.

## 4. Discussion

### 4.1 Abundance of MPs in all matrices from the Sal estuary

The Sal estuary is a valuable source for local and artisanal fisheries, mainly for oysters (*Crassostrea* sp.) but also for finfish and shellfish. However, the fishing activities and fishing vessels may also contribute to the MP pollution. The average abundance of MPs in the Sal estuary ( $48 \pm 19.3$  items/L in water and  $3950 \pm 930$  items/kg dw in sediment) is significant (Table 2, SI Table 4), which is comparable to the MP abundance in water from the Netherlands and China and in sediment in China and Nova Scotia (SI Table 4). This is primarily the influence of anthropogenic activities but there may also be an influence of hydrodynamic factors, viz. tidal influence and freshwater influx (Kazour et al., 2019). Additionally, a relative change in the density of the particles due to biofouling, flocculation or ingestion may alter the MPs into finer particles. If that happens, MPs may remain in suspension or may settle in low-energy environment sediment traps, that can be quite efficient (Harris P.T., 2020). This could result in an increase in the concentration (Patterson et al., 2019) and corresponding tendency to settle down to the bed, making the particle bioavailable for pelagic and benthic/pelagic organisms (Brandao et al., 2011). The Sal estuary has both tidal and rain fed fluxes. It is subjected to tidal influence to a great distance (18 km) inward from the mouth. The nature of sediment is known to influence the distribution of MPs. In the present study, the majority of the locations consisted of sandy silt sediments which may not influence the distribution and abundance of MPs as in agreement with the study (Blašković et al., 2017). The difference in abundance of MPs in water and sediment can be influenced by several estuarine hydrodynamic forces, mainly by wave and tide induced turbulence (Vermeiren et al., 2016; Krelling et al., 2017). Re-suspension of the epibenthic layer during high tide builds the probability for MPs to interact with highly suspended organic and inorganic matter, leading to an increased aggregation and deposition (Besseling et al., 2017).

Overall, the total number of MPs was higher in finfish gut (total MPs range 6-107 particles/individuals) compared to shellfish (whole body) (total MPs range 3-54 particles/individuals) (SI Table 1). Among the shellfish the highest numbers of MPs were noted in *Crassostrea* sp.

( $4.0 \pm 2.1$ MPs/g) followed by *P. viridis* ( $3.2 \pm 1.8$ MPs/g) and *P. malbarica* ( $0.7 \pm 0.3$ MPs/g) (Table 2). This differential accumulation can be justified by the size of bivalves, since oysters (*Crassostrea* sp.) were the largest (avg. length 8.2- 14.5cm). The surface area of the oyster is also higher and the oyster will, therefore, consume more MPs from the water or sediment, compared to the green mussel (*P. viridis*; length 6.1-8.9cm) and clams (*P. malbarica*; length 2.3- 4.6 cm). These results are comparable to previous research work (SI Table 5). Dowarah et al. (2020) demonstrated that green mussels (*P. viridis*) tend to retain fewer MPs than clams (*Metretrix meretrix*). This may be due to the relation of body size and weight, since *M. meretrix* is larger than *P. viridis* (SI Table 5). However, in both studies larger and heavier bivalves accumulated more MPs. In this study, the clam (*P. malbarica*) was examined for the first time and was found with very low amounts of MPs, which is justified by their much smaller size compared to green mussels and oysters. It may be inferred that, increase in body size tends to increase the volume of water filtered and thus allowing more MPs to be accumulated.

The MP content in *P. viridis* in the present study was  $3.2 \pm 1.8$ MPs/g, which is higher compared to *P. viridis* analysed from east coast of India (Dowarah et al., 2020):  $1.8 \pm 0.54$ MPs/g and (Naidu, 2019):  $0.1 \pm 0.03$ MPs/g (SI Table 5). Similarly, finfishes like *M. cephalus* depicted a much higher accumulation of MPs ( $7.8 \pm 4.4$ MPs/g) in the present study than in the same species from China ( $4.3$  items/individual) (Cheung et al., 2018) (SI Table 1a and SI Table 5). Previous studies hypothesised that *Mugil cephalus* may change their diet as they grow. As a result of that, it may lead to increasing amounts of detritus and sediment in the guts of larger fish (Cheung et al., 2018), which in turn may affect the type and quantity of plastic ingested. Additionally, higher MP contents in the species from the Sal estuary might be due to the direct effect of the intensity of environmental pollution (Anon, 2019 (I); Sun et al., 2019).

#### 4.2 Characterization /Morphology of MPs in all matrices from the Sal estuary

The presence of significant amount of MPs clearly indicates that the biota species in the Sal estuary are interacting with various types of pollutants. This is most likely due to the similarity of their food with MPs and due to the high density of MPs in the aquatic environment (Oryetal, 2017; Dantas et al., 2020). Interestingly, MPs found in all the three matrices, water, sediment and biota from the Sal estuary were dominated by fibres (55.3%, 76.6% and 72.9%, respectively), followed by fragments, films and other plastics (Fig3). The prominent ubiquity of fibres in all three matrices suggests a variety of sources of MPs, most likely including domestic sewage, effluents from industries and laundry (Renzi et al., 2019b; Dantas et al., 2020). In the Sal, the biota seem to be chronically exposed to anthropogenic discharges, especially to fibres from fabrics, persistently observed in wastewater discharges, domestic sewage and in solid waste dumping locations. The

presence of a fishing jetty at the mouth of this estuary as well as traditional fishing practices (gill nets and cast nets) may contribute to the microfiber load from discarded old nets in the surrounding waters. Large amounts of small fragmented old nets originate from net repairs and rope emerging from net mending operations. This may gradually flush into the estuary during the monsoonal runoff (Browne et al., 2011; Sillanpaa and Sainio, 2017). Fragments were the second most abundant micro-debris in water (27%) and biota (16.6%). They mostly originate from the degradation/weathering of larger plastic pieces such as packaging materials, plastic bottles and other macro-plastic litter, which are often directly discarded into the estuarine environment (Goswami et al., 2020). Remarkably, beads like spheres, mainly transparent, were also found in water (11%) and sediments (3%) with a varying polymer composition (polypropylene, polyamide, polyacrylamide). These could originate from personal care products, textiles and wastewater treatment plants. Notably, no such beads were found in the shellfish and finfish samples.

The size of MPs is a factor significantly influencing the distribution and ingestion and, consequently, the harmful effects on biota (Ziajahromi et al., 2017). In case of water, the most abundant size appeared to be 100-300 $\mu\text{m}$  (~42%), whereas in sediments the largest particles were 300 $\mu\text{m}$  -1mm (~33.3%). However, the size classes 100-300 $\mu\text{m}$  (~26%) and 1-5mm (24%) were also present in substantial amounts (Table 2; Fig.4). Once the particles sink, they are less susceptible to physical degradation and weathering processes. Bivalves showed a high accumulation of smaller size MPs (size 10-50 $\mu\text{m}$ ) (Fig.4), which can be explained by the resemblance of smaller size MPs with their prey size and filter feeding habit (Botterell et al., 2019; Patterson et al., 2019). In this study, 10-50 $\mu\text{m}$  (64.2%) and 51-100 $\mu\text{m}$  (23.7%) particles were present in *Crassostrea* sp. (Fig.4), similar as in mussels (*P. viridis*) reported by Naidu et al. (2019). 50% of MPs were in size range of 10-50 $\mu\text{m}$ . Mussels are known to ingest much smaller ranged MPs like 3-10 $\mu\text{m}$  particles (Browne et al., 2008). Therefore, the likely pathway of accumulation for these plankton sized MPs resembles a trophic transfer. The clams (*P. malbarica*) accumulated (61%) MPs in the range of 10-50 $\mu\text{m}$ , probably due to smaller size of these organisms. Weathered MPs developing biofilms or algal layers may give out false signatures of the presence of DMS (dimethyl sulphate), which might be one of the reasons for ingestion (Savoca, 2017). Notably, the filter feeding branchial apparatus of all the bivalves sort particles efficiently (Collard et al., 2017), but often MPs are mistaken for food due to false cues given out due to bio-fouling (Harrison et al., 2018, Harris, 2020). Such signals are accepted by the bivalves as phytoplankton rather than expelling it as pseudo-faeces. Smaller sized *Crassostrea* sp., *P. malbarica* and *P. viridis* tend to accumulate more MPs compared to their larger

counterparts, probably due to narrow gut diameter with convoluted alimentary canal not allowing the MPs to pass through.

Consumption of large sized MPs (101-300  $\mu\text{m}$ , i.e. 60.3%) was observed for finfishes (Fig. 4) unlike shellfish from the same study area. Although in the present study both shellfish and finfish are benthic, the difference in feeding habits could be the reason for varied size of MPs consumed by shellfish and finfishes. Usually, finfishes engulf additional sediment and water where shellfish are filterfeeders. Hence the chance of incorporation of higher size MPs is more in finfishes compared to shellfish. MPs in fishes were dominated by fibres that have a tendency to aggregate in the gut, which was proven experimentally (Woods et al., 2018). Fibres are known to stay longer in the gut, causing entanglements due to the long convoluted fish gut (Goswami et al., 2020). MP fibres can cause physiological stress on marine organisms by reducing feeding rates and body mass as these fibres have a tendency to intertwine and retain in the gut for longer periods (Murray and Cowie, 2011; Welden and Cowie, 2016). Further, this prolonged retention time has detrimental health impacts due to leaching of plastic-associated pollutants (Nobre et al., 2015; Khan et al., 2017) such as plasticizers, flame retardants, antioxidants, colorants, heat stabilizers and bisphenol-A. Limited growth, energy reserves and depletion in nutritional value have been noted experimentally for *Sebasteschlegelii* (Yin et al., 2018) after exposure to MPs. This can further cause alterations in growth rate, impacting fish yield and ecosystem functioning. Although few experimental studies have shown little or no impact from microplastic ingestion by fish, the majority of these studies used smooth globular beads, which are easily egested by organisms due to their structure (Batel et al., 2016 and Naidoo et al., 2019).

The colour of MPs is imperative in source detection (Veerasingam et al., 2016; Koongolla et al., 2018) and is an important ingestion cue for few organisms that visually detect food (Maharana et al., 2020). The proportion of black coloured plastics (43.9% and 38.1%) were abundant in water and sediments, respectively, followed by green (16%) in water whereas blue in sediments (29.0%) (SI, Fig. 2). The black MPs mainly might have come into the environment due to abrasion of tires on the road surfaces as regular wear and tear (Wik and Dave, 2009). In contrast, blue coloured MPs (44.0%) were most abundant in the biota followed by red, green and yellow (SI, Fig. 2). Prevalence of blue fibres in *Crassostrea* sp. has been well noted in the previous study as well (Jamila et al., 2019; Dantas et al., 2020). These might originate from fishing nets or the use of other local products. Blue MPs were predominantly found in *M. cephalus* (58.8%). They are invariably dominant in fishes across the world's oceans (Peters et al., 2017; Compa et al., 2018). This reveals the degree of marine microplastic pollution and its ingestion by organisms directly and/or trophic transfer (Au et al., 2017).

### 4.3 Characterisation of polymers in all matrices from the Sal estuary

The present study reports a wide range of commercially applied and water soluble polymers (WSPs) viz; polyacrylamide (PAM), polyvinylpyrrolidone (PVP), polyacrylic acid (PAA), polyethylene oxide (PEO), polypropylene oxide (PPO), polyethylene glycol (PEG), and polyethylene imide (PEI). These polymers are produced in large quantities and have applications that finally lead to their discharge into the environment (Schmiege et al. 2020). There is very limited information on WSPs, covering only a few polymer types and chain lengths (Petrović et al., 2000), while their fate and impact remain mostly unclear.

Each of the three sample types had a specific dominant polymer, wherein PAM (33%) was most abundant in water and polyacetylene (39%) in sediments. In shellfish, EVOH was the most abundant polymer (41%), whereas in finfish it was PAM (32%) (Fig 5). PAM, a polymer with fibrous morphologies which includes round and branched fibres and flat ribbons with solution properties has a wide range of biomedical and industrial applications because of its dual organic solvent and aqueous media compatible fibrous membranes. These membranes are used for solid-supported catalysis and diagnostics (Lu and Hsieh, 2009). However, in this study particles are mainly fibres containing PAM, which may have their origin from clothes and other textiles (Novillo et al., 2020). Fewer studies have mentioned the finding of polyacrylamide and acrylic polymers in cetaceans (Neves et al., 2015; Nelms et al., 2018). These are also widely used in ophthalmic operations, drug treatment, food packaging products, water purification like in hydrogels and as coagulants in wastewater treatment plants (Tangri, 2014). Polyacetylene, an organic polymer is mainly used as a doping agent in electronic devices due to its thin, stretchable film making ability to induce conductivity (Dagani, 1981). EVOH has applications in food packaging, drugs, cosmetics, and other perishable products because of its superior barrier properties. It is often used in a multilayer co-extruded film structure with materials such as HDPE, PP and PET. Polyamide (nylon) was also a commonly occurring polymer in all the three matrices although in biota, the abundance was higher because of the fibres and microfibers that originate mainly from the domestic sewage containing laundry wastewater together with fishing activities could be some possible sources for fibrous MPs in the environment (Browne et al., 2011), which further highlights the untreated discharge of wastewater in the Sal estuary. Nylon is also used in fishing nets. PVC has replaced building materials, viz; wood and metal. It is mainly used in pipelines, domestic appliances, wallpapers, medical equipment, toys and footwear, pharmaceuticals packaging and garments, etc. (Dowarah et al., 2020). The presence of polymers in water and sediments of the Sal, viz. EVOH, polyacrylamide, polyacetylene, PVC, polyamide (nylon), LDPE and PP (Fig.5) shows the similarity in the polymer

type consumed by biota and the polymer types present in the water column. This indicates that the mechanism of high volume filter feeding is important in MP accumulation. The polymer types in water and sediment were quite similar and also finfish reveals a fairly similar polymer composition (SI Fig. 4). Interestingly, although the shellfish species are sedentary, they showed a completely different polymer composition compared to water and sediment (SI Fig 4).

This study was an attempt to understand the abundance of MPs in the estuarine environment (water and sediment) and in addition its channelling into seafood, through which humans may also be exposed. An average Indian consumes 10 kg/annum of seafood (Anon, 2019 II). This includes 3109.3 g of bivalves, which are consumed per capita per annum in the eastern coastal zone of India (Dowarah et. al., 2020). The average number of MPs in shellfish found in the present study is 2.6 MPs/g. Accordingly, the estimated annual intake of MPs from shellfish alone per capita for Goa would be 8084.1 particles/year per person. Shellfish therefore poses a possible threat in terms of consumption as it is a local delicacy and also consumed by the many tourists in the area. Usually the whole soft body is consumed either raw or cooked. This is different for finfish of which the gut is discarded prior to consumption.

## **5. Conclusion**

This study provides baseline and preliminary information on the quantities and types of MPs found in water and sediment from the Sal estuary and in commercially important fishery products (shellfish and finfish). The frequent occurrence of MPs in substantial concentrations can harm the sustainability of the ecosystem. In this study, a similar signature of polymers in water and sediment, and in seafood suggests that human activities in the densely populated area around the estuary was the leading source of microplastic contamination throughout the estuarine environment. The MPs found in all species mark the physical relationship between the estuarine environment and the local fish and shellfish. The significant presence of MPs in the gastrointestinal tract of finfish may pose an additional threat to these fishes due to the leaching of chemicals from the MPs (Rochman et al., 2013). The profound abundance of various MPs in bivalves directly contributes to the human health risk, since bivalves are mostly consumed as a whole.

This study also showed the presence of very small-sized MPs with 37 different types of polymers which were rarely reported by previous studies in the Indian coastal environment. Hence this study overall adds to the increasing evidence that ubiquitous contamination by plastic particles is seriously impacting the coastal marine biota. Robust management strategies have to be taken, to stop further addition of MPs into ecosystems. Moreover, further work should be carried out comparing the contamination of MPs in biota belonging to different zones like demersal, pelagic, sediment dwelling

groups *viz*; polychaetes and gastropods. In fish further studies are needed on the possible presence of MPs in muscle tissue to better estimate the risk for human health.

### **CRedit authorship contribution statement**

**MahuaSaha**: Supervision, Project administration, Data curation, Writing- original draft, Writing review and Editing. **AkshataNaik**: Methodology, Data curation, Formal analysis, Writing-review and Editing. **Aniket Desai**: Sample collection, Methodology, Statistical analysis, Writing review and Editing. **MandarNanajkar**: Visualization, Statistical analysis, Writing review and Editing. **ChayanikaRathore**:Sample collection, Methodology, Data curation, Writing review and Editing. **Manish Kumar**: Methodology, Formal analysis. **Priyansha Gupta**: Formal analysis.

### **Declaration of Competing Interest**

This manuscript has not been published previously, that it is not under consideration for publication elsewhere, that its publication is approved by all the authors and implicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere, in English or in any other language, including electronically without the written consent of the copyright holder.

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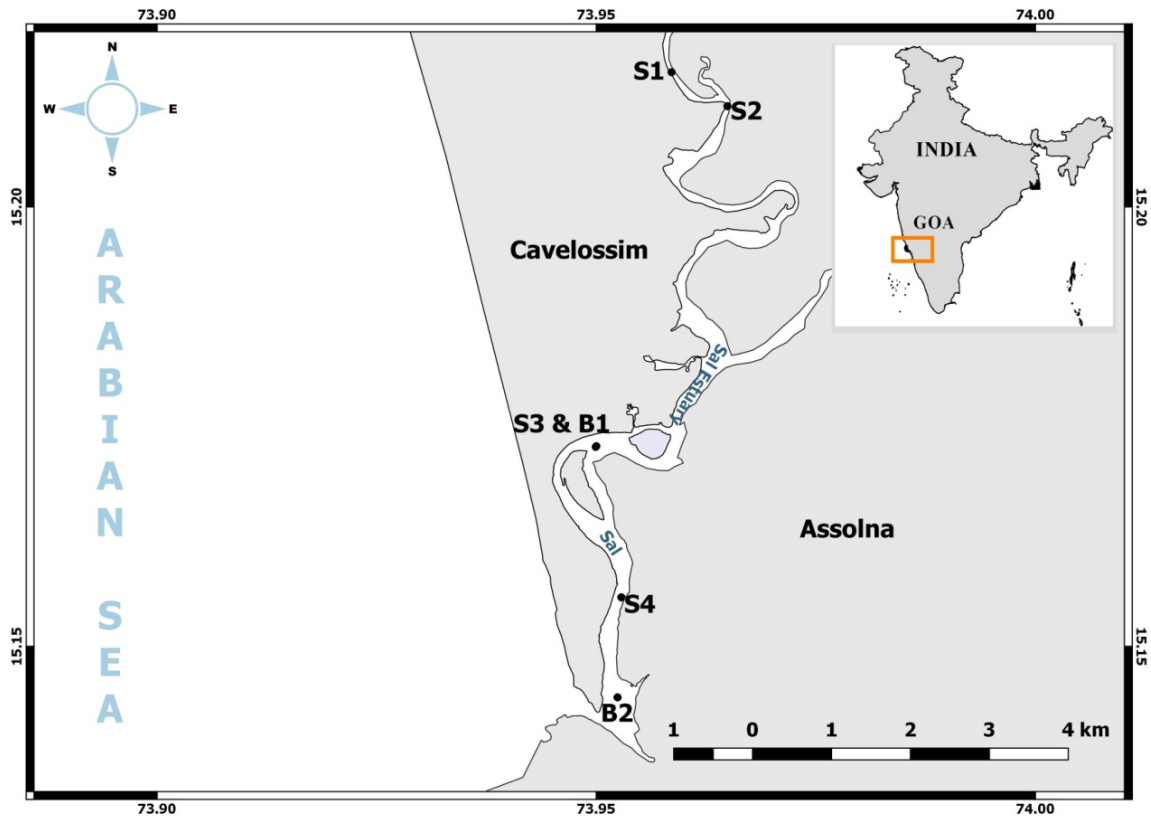
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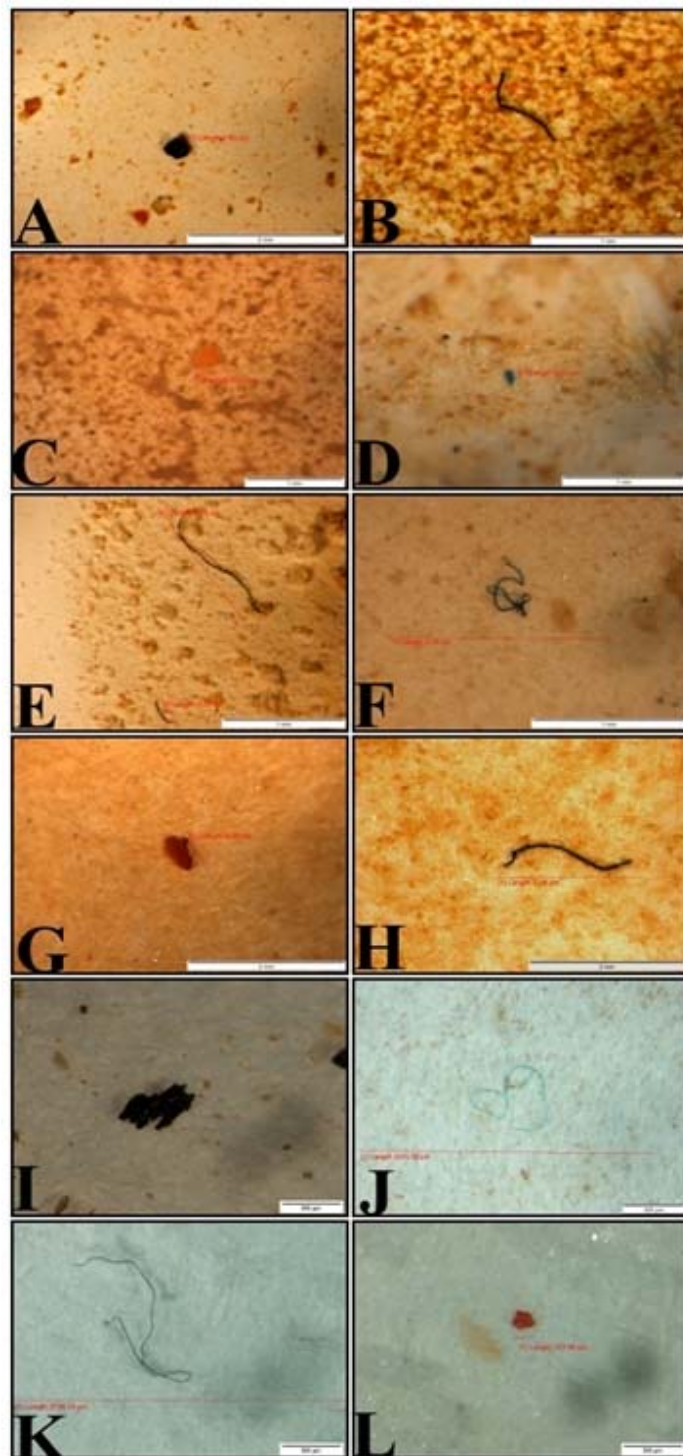
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## **Figures**

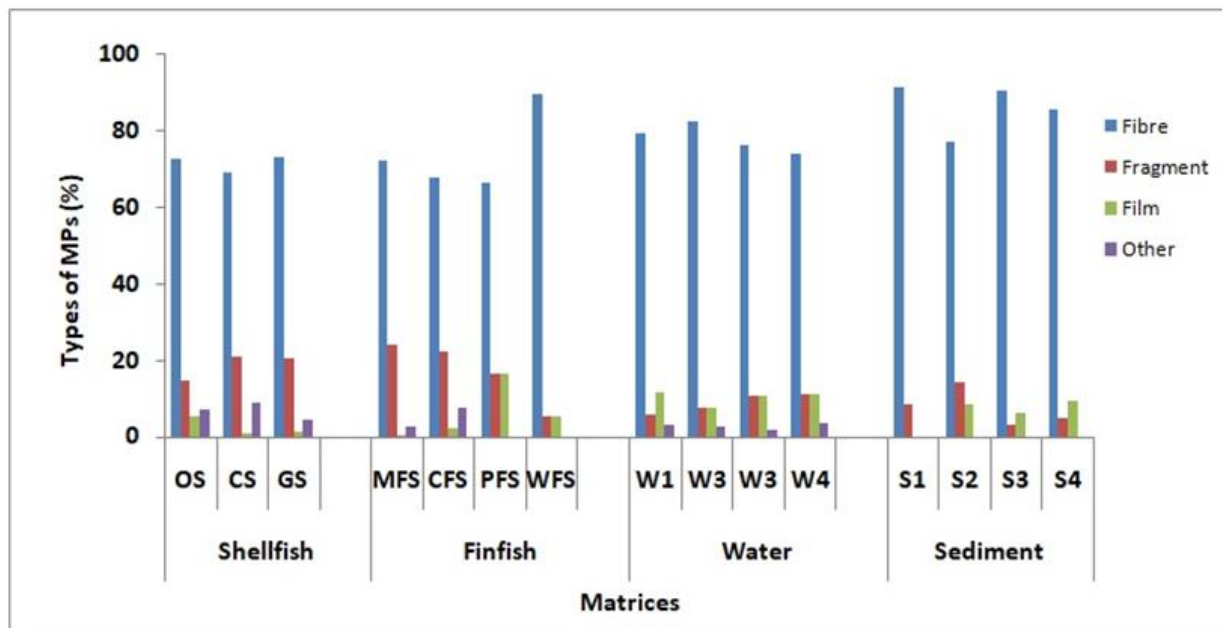


**Fig 1:** Sampling sites in the Sal estuary, Goa. S1, S2, S3 and S4 represent the water and sediment sample collection points and B1 and B2 are biota collection sites.

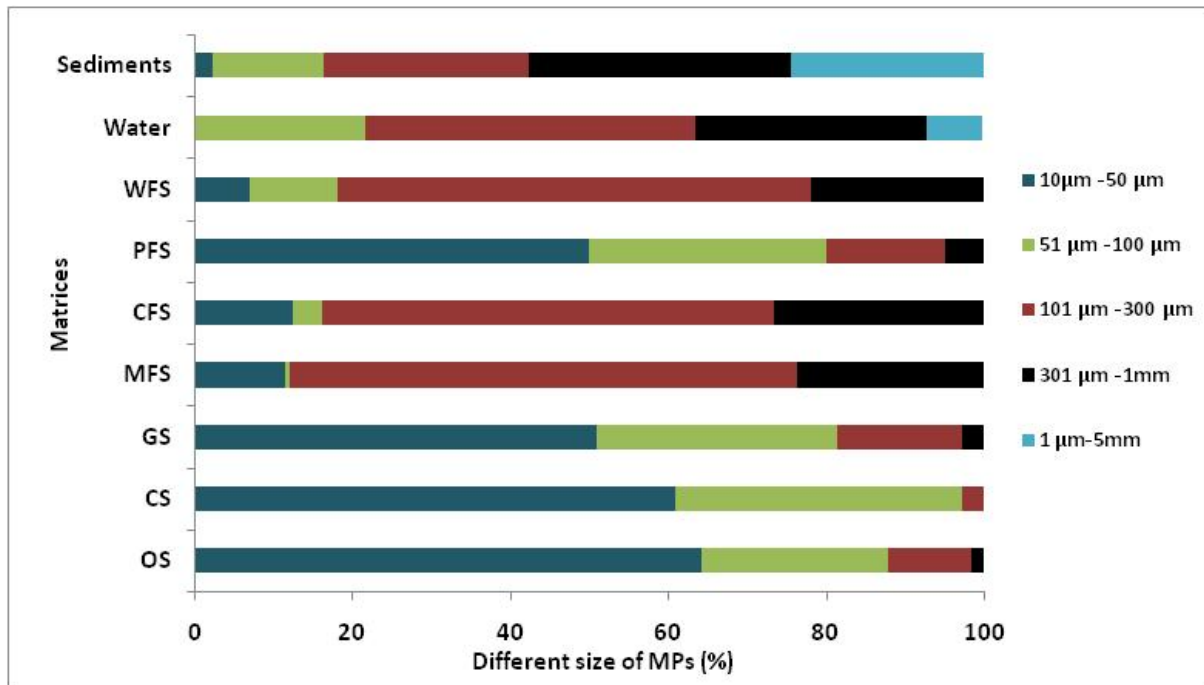




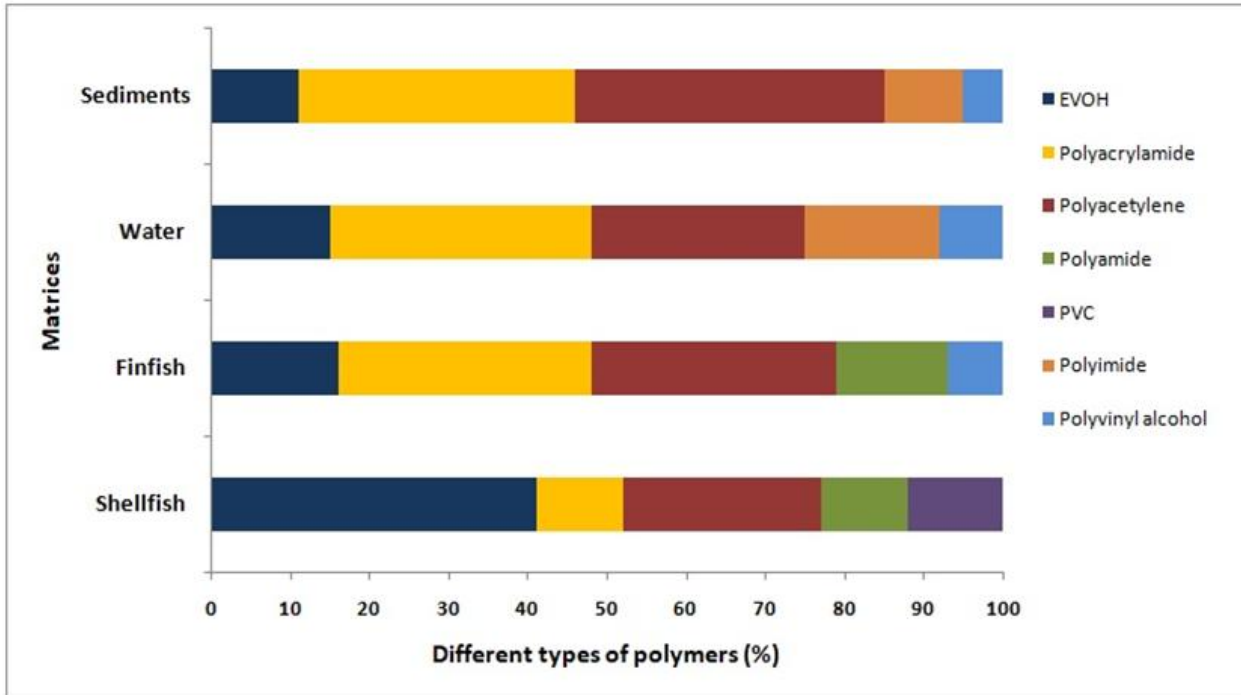
**Fig 2:** Stereoscopic images of MPs in shellfish, finfish, water column and sediment samples. Fragments (A,C and D) and fibre (B) in shellfish, fibres (E,F and H) and fragment (G) in finfish, fibre (J) and fragment (I) in sediment and fibre (K) and fragment (L) in water.



**Fig 3:** Percentage composition of different shape of MPs (fibres, fragments, films and other plastic particles) in stereoscopic analysis recovered from all matrices (shellfish, finfish, water and sediment).



**Fig 4:** Percentage of different size of MPs detected in all matrices (shellfish, finfish, water and sediment).



**Fig 5:** Percentage of predominant polymers in all matrices (shellfish, finfish, water and sediment).

**Table 1:** Trophic level, feeding habit, habitat and morphometric measurements (length and wet weight) of shellfish and finfish collected from different locations in the Sal estuary.

Scientific Name (species)	Common name	Trophic level	Feeding habit	Habitat	Morphometric measurements	
					Length (cm)	Wet weight (g)
<i>Crassostrea</i> sp.	Oysters	Primary consumers	Filter feeder	Benthic	8.2-14.5	4.9-14.5
<i>P. malbarica</i>	Clams	Primary consumers	Filter feeder	Benthic	2.3-4.6	5.1-18.4
<i>P. viridis</i>	Green mussels	Primary consumers	Filter feeder	Benthic	6.1-8.9	4.6-13.1
<i>M. cephalus</i>	Grey Mullet	Secondary consumers	Omnivorous	Benthic	21.7-26.0	92.3-152.9
<i>A. jella</i>	Catfish	Secondary consumers	Carnivorous	Benthic	23.1-25.5	127.9-141.7
<i>G. filamentosus</i>	Whipfin silver-biddy	Secondary consumers	Carnivorous	Benthic	20.2-22.2	148.9-155.2
<i>E. suratensis</i>	Pearlspot	Secondary consumers	Omnivorous	Benthopelagic	14.2-19.0	121.3-132.4

**Table 2:** Range and mean ( $\pm$ SD) of MP abundance, no. of samples analyzed, types of MPs and their size range ( $\mu$ m) in water, sediment and biota from the Sal estuary.

Species	Range of total MPs/ individual	Abundance of MPs (mean $\pm$ SD)	No. of samples	Size range ( $\mu$ m)/median (M) of different types of MPs				Range of total no. polymers
				Fibres	Fragments	Films	Other plastics	
Shellfish (MPs/g)								
OS	1-10	4.0 $\pm$ 2.1	14	37-428/49	29-202/41	31-74/38	12-52/28	1-16
CS	2-5	0.7 $\pm$ 0.3	15	36-364/50	10-93/36	15-71/32	10-86/26	1-12
GS	1-6	3.2 $\pm$ 1.8	10	10-252/32	13-47/22	20-331/48	14-55/25	1-25
Finfish(MPs/g)								
MFS	3-14	7.8 $\pm$ 4.4	4	20-566/105	10-58/20	13-71/27	23-130/109	1-13
CFS	2-7	4.6 $\pm$ 2.6	4	26-582/155	18-53/28	22-42/35	19-65/51	1-9
PFS	1-2	1.4 $\pm$ 0.3	2	10-420/45	21-40/27	12-35/30	12-58/33	1-2
WFS	2-9	5.3 $\pm$ 4.9	2	15-330/105	17-53/43	18-39/32	11-112/102	1-4
Water column(MPs/L)	23-69	48 $\pm$ 19.4	4	97-4932/300	88-247/171.5	102-1350/300	110-223/195	1-53
Sediments(MPs/kg)	300-500	3950 $\pm$ 930	4	89-4750/705	40-507/100	32-300/100	100-220/160	1-56