Profiling microplastics in the Indian edible oyster, Magallana bilineata collected from the Tuticorin coast, Gulf of Mannar, Southeastern India

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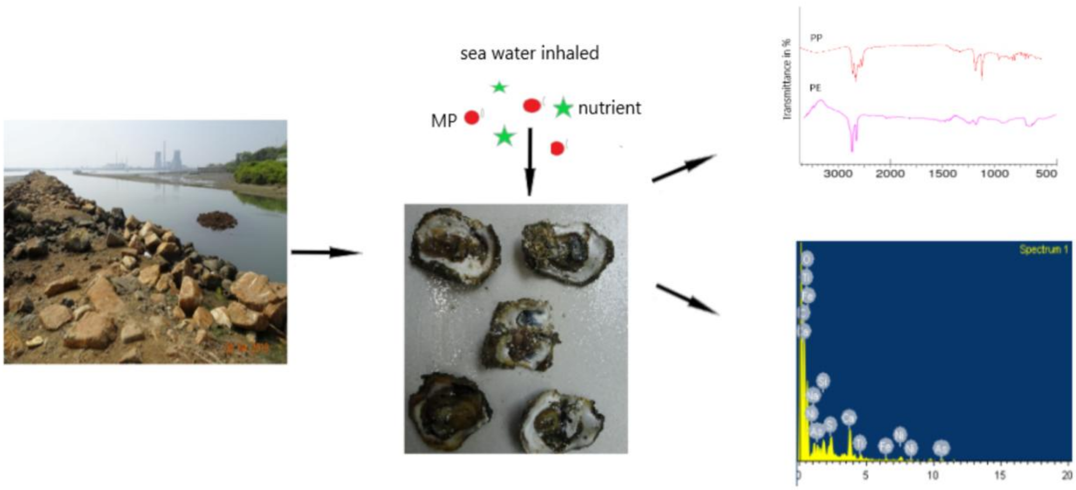
**Graphical Abstract*



Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)

Highlights

- MP distributions in oysters closely reflected those in surrounding seawater.
- Mean abundance of 6.9±3.8 MP/individual and concentration of 0.81±0.45 MP/g detected.
- MP abundance and concentration increases with increasing size of oysters.
- PE fibers ranging from 0.25-0.5 mm were the most common MP in oysters.
- Sediments contained a broader range of MP than seawater or oysters.

Abstract

The objective of this study is to quantify the extent of microplastic (MP) contamination in the Indian edible oyster (*Magallana bilineata*) and to understand how this relates to the MP contamination in its surrounding marine environment. Samples of water, sediment and oysters of different sizes were collected from three sites along Tuticorin coast in Gulf of Mannar in Southeast India. The mean abundance of MP in oysters was found to be 6.9±3.84 items/individual and the mean concentration to be 0.81±0.45 items/g of tissue. Polyethylene (PE) and polypropylene (PP) fibers were the dominant MP types in oysters (92% and 4%, respectively) and in seawater (75% and 25%, respectively), with PE fibers, ranging from 0.25 to 0.5mm, being the most common. Both PE and PP are low-density polymers which are slow to sediment to the seafloor. This increases the potential of their availability in the environment and ingestion by the oysters. The largest oysters (14-16cm) contained the highest abundance and concentrations of MP, suggesting a greater proportion of MP in the water column is ingested with increasing size. The calculated microplastic index (0.02 to 0.99) also indicates that MP bioavailability increases with increasing size of oysters. The distribution patterns of MP abundance, shape and size in oysters more closely resemble those in water than in sediment. The surface morphology of the MPs reveals the characteristic pits and cracks which result from partial degradation through the weathering processes. Energy-dispersive X-ray spectroscopy analysis shows the presence of Ni and Fe in association with MP, and this probably indicates the fly-ash pollution and the petroleum-related activities in the surrounding area. Being sessile animals the oysters are good candidates for use as sentinel organisms for monitoring MP in specific marine environments.

Keywords: Microplastic index; water; sediment; organism size; SEM-EDAX.

1. Introduction

Microplastics (MPs), defined as plastic materials <5 mm in size, are the most abundant form of plastic debris in the global environment (Law and Thompson, 2014). MP in the environment can be derived from the successive breakdown of larger plastic pieces through UV-induced, mechanical and biological degradation processes (Strungaru et al., 2018; Guzzetti et al., 2018; Booth et al., 2018; Gewert et al., 2015). However, these processes are very slow under most environmental conditions, and the significant proportion of MP in the marine environment is deemed to have been directly transported from various terrestrial and industrial processes and consumer products (Peixoto et al., 2019; Booth et al., 2018). More than 10% of plastics end up in oceans due to the combination of large-scale use of plastic products and their poor management by consumers (Thompson et al., 2009). MPs have been reported in all environment matrices from air to groundwater and from the tropics to the poles (Panno et al., 2019; Gasperi et al., 2018). In the marine environment, MPs have been ubiquitously observed in beach sediment, surface waters, the water column, and coastal and deep-sea sediments (e.g. Sathish et al., 2019; Zhang et al., 2017; Cincinelli et al., 2017; Peng et al., 2017; Bergmann et al., 2017). Owing to their small size, MPs are easily mistaken for food and their ingestion has been documented in a wide range of marine organisms at different trophic levels, including zooplankton, bivalves, fish and crustaceans (Savoca et al., 2019; Piarulli et al., 2019; Cole et al., 2019; Sun et al., 2018; Li et al., 2016; Welden and Cowie, 2016). Importantly, bivalves, fish and crustaceans are the main sources of seafood for human consumption (Davidson and Dudas, 2016), and so the presence of MP represents not only a health risk to the organism but a potential economic risk to us if the organisms are of commercial importance.

According to the United Nations Environment Programme, India dumps nearly 0.6 million tons of plastic waste into the ocean annually (UNEP, 2018). As far as the southeast coast of India is concerned, there have been several reports on MP contamination in beach sediment (Karthik et al., 2018; Vidyasakar et al., 2018), in mussels (Naidu, 2019) and in fish (Kumar et al., 2018). Oysters are benthic marine species inhabiting near-shore areas, shallow waters, bays, and estuaries, and they are widely distributed throughout the tropical and subtropical zones. A number of different oyster species inhabit the coastal waters around India. The Indian edible oyster (*Magallana bilineata*) is a common species found in Gulf of Mannar in Southeast India. *M. bilineata* is one of the oyster species previously classified as C*rassostrea* but recently renamed as *Magallana* (Bayne et al., 2017). Filter-feeding organisms like oysters strain particulate matter from the water column to ingest nutrients. Previous studies have shown that oysters, including their larval stages, readily ingest MP and nanoplastic particles present in the water column (Capillo et al., 2018; Sussarellu et al., 2016; Cole and Galloway, 2015; Li et al., 2015). As oysters are sessile filter feeders, they are excellent candidates for studying the exposure to and uptake of dissolved and particulate pollutants present in a specified area. They are therefore an ideal choice for use in the environmental monitoring of MP in biota (Xie et al., 2016).

This study investigates the presence, distribution and properties of MP in whole oysters collected from the Indian coast of Gulf of Mannar (Southeast India). The aim of the study is to estimate the abundance of MP in the oysters and to investigate the relationship between the MP content in oysters and that in the water and sediment samples collected from the oysters' environment. The collected oysters were divided into 4 groups on the basis of size so that the bioavailability of different types of MP could be linked to organism size. MPs extracted from oysters, water and sediment samples were used to quantify abundance and to characterize individual particles according to their size, shape, polymer composition and particle surface morphology. The present study is the first to report MP pollution in filter-feeding organisms from the Indian coastline and it represents an opportunity to investigate the possible correlations between the high levels of emission and exposure of plastics reported in India and the potential for MP uptake in this group of marine organisms.

2. Materials and methods

2.1. Study area

In Roche Park, Tuticorin, there is a 250-meter groin built in 2009 using rock boulders to reduce erosion and sedimentation at the mouth of the backwater. Live samples of *M. bilineata* attached to the submerged rock boulders were collected from a depth of 20-30 cm from three sites (Fig. 1). Site 1 is located at the southeast end of Roche Park, where the backwater opens into the sea. On the other side of the groin, fishermen anchor their boats and clean their nets. At Site 2, 900 meters away from Site 1, the backwater is blocked by deploying rock boulders to prevent erosion. Site 3 is a water channel located 1.3 km from Sites 1 and 2, and it is the continuation of backwater flow from Site 1. At the times of flood the excess water is discharged into Karappad Bay near Site 3. There is also the Sengulam Odai bringing in the waste water from the salt pans. At this site, oyster samples were collected from the submerged rock boulders of an old damaged bridge.

2.2. Sample collection

A total of 180 specimens of Indian edible oyster *Magallana bilineata* were collected from the three sites and placed immediately into aluminum foil bags. These were then placed in an ice box and transferred to the laboratory, where they were stored at -20° C for subsequent analysis. All of the experiments were carefully performed with the aim of preventing MP contamination in the laboratory. The oysters were taken out of the freezer and allowed to thaw for 1 h before the shells were rinsed thoroughly with water. The shell length and weight of each individual oyster was determined to calculate MP index. After measurement, the samples were sorted according to their size and placed under four categories: Type 1 (2 - 4 cm), Type 2 (5 - 9 cm), Type 3 (10 - 13 cm) and Type 4 (14 - 16 cm). The soft tissue of individual oysters was removed from the shell and the wet weight of tissue per individual was determined with a balance (Table 1).

In addition to the live specimens, samples of water and sediment were also collected from the study sites. One liter of surface water from the top 20 cm was collected in triplicate at each site by stainless steel manta trawls plankton net with 333 µm mesh size and pooled together into a steel container as a sample. The water sample was preserved with 5% formaldehyde solution and transferred to the laboratory for further analysis. Approximately 1 kg of sediment from the top three centimeters was sampled in triplicate using a Van Veen grab, placed in a 1 L glass jar and stored at 4° C until analysis.

2.3. Sample analysis

The soft tissues of five individuals for each Type (1 to 4) of oyster were combined to produce each sample and three replicate samples (each comprising 5 oysters) were prepared for each sampling site. Five individuals of the same oyster type collected from each site were combined for each of the three replicate samples. The processes of digestion, separation and collection of MPs from oysters were conducted using the protocol of Li et al., (2015). In brief, the soft flesh of oyster was rinsed with filtered, distilled water to remove any MP present on the outside. The tissue samples were then placed in a 1 L glass bottle to which 180 mL of 10% KOH was added for digestion. The bottles were covered with aluminum foil and placed in incubator at 50° C for 72 hours until a clear solution had formed. After the digestion procedure was complete, 500mL of saturated sodium iodide (NaI; 1.6 gmL^{-1} density at 3.3M) was added to each bottle to make the MP particles float. After allowing the samples to stand at room temperature for 24 hours, the upper part of the solution was filtered in Millipore Filtration Unit using 0.8μm nitrate cellulose filter papers. The filter papers were then dried at room temperature in individual Petri dishes with lids.

In case of water (1L) and sediment (200g), 30 mL of 10% H_2O_2 solution was added to the samples to digest any biological material present in them. The digestion was allowed to proceed for 72 h at room temperature. A density separation solution (supersaturated NaI, 1.6 gmL^{-1} density at 3.3M) was added at a ratio of about three times the volume of the sample to make the MPs float. After allowing the samples to stand at room temperature for 24 hours, the upper part of the solution was filtered on 0.8 cellulose nitrate filter papers, and the papers were dried at room temperature in individual Petri dishes with lids.

2.4. Microplastic identification

The dried filter papers were first observed under 40x magnification stereomicroscope. MPs were tentatively identified on the basis of their small size, absence of cellular structure, homogeneous color and equal thickness. Tweezers were used to check whether individual particles break apart readily, and those which did were discarded as non-plastic. The remaining particles were then subjected to the hot needle test (De Witte et al., 2014) to complete the primary MP identification step. The MP particles on the filters were then characterized in terms of their abundance, size, shape and color, as described by Li et al., 2015. The MPs were classified into five different size groups: 0.005-0.25 mm, 0.25-0.5 mm, 0.5-1 mm, 1-3 mm, and 3-5 mm. Based on their individual morphology, MPs were described as fibers, films or fragments. MP color was assigned and recorded according to the dominant surface color. A total of 60 suspected MP particles were randomly selected from the filters representing the different sample types and sampling sites and analyzed by FTIR-ATR to verify the polymer composition. The FTIR-ATR analysis (Thermo Nicolet model iS5) gave spectra ranging from 4000cm^{-1} to 750cm^{-1}. The spectra obtained were compared with reference library spectra, and matches with confidence levels of 80% or greater were accepted. The MP abundance in oysters was recorded as number of items per individual (wet weight). The MP abundance in surface water was recorded as number of items/L and in sediment as number of items/ kg.

2.5. SEM-EDAX analysis

A selection of MPs present in different samples was examined under a scanning electron microscope (SEM; Carl Zeiss EVO 18) to produce high-resolution images of their surface morphology. During the SEM observation, the qualitative elemental composition of particles was confirmed using an energy-dispersive X-ray spectroscopy (EDAX; X-Act, Oxford).

2.6. Quality assurance and quality control

During each step of the sampling and sample-handling procedures precautions were taken to minimize background contamination. The highest risk is associated with airborne contamination, such as synthetic fibers from clothing, equipment, and general atmospheric deposition. Therefore, strict control measures were implemented during the laboratory analyses to avoid airborne and laboratory contamination. Sources of contamination were reduced by cleaning all equipment prior to sampling. Non-plastic materials were used wherever possible and all glassware was cleaned using ultrapure water before use. Samples were covered as soon as and whenever possible. Contamination from research personnel was minimized by their wearing polymer-free (cotton) clothing and gloves. The stereomicroscope area was cleaned prior to samples analysis. Once the filtration was performed, the filters were kept in Petri dishes made of glass until the FTIR analysis. The movement of people was minimized in the laboratory, and the lab windows were closed throughout the experiments. Blank experiments were also conducted without sample to determine the level of background contamination derived from the air. This value was subtracted from the value of the field samples to remove the error due to air contamination.

2.7. Microplastic index

Microplastic bioavailability is measured by microplastic index (MPI), which is calculated as under:

$$MPI = MPSB \times (TW/SW)$$

Where MPSB is the concentration of MP in soft body tissue (No of MP/g), TW is the soft tissue wet weight (g) and SW is the shell weight (g).

2.8. Data analysis

A one-way analysis of variance (ANOVA) was completed to determine the variation in the number of MP between the different sampling sites and to investigate the distribution of MP with regard to their shape, size, color and type. A significance level of 0.05 was chosen. A linear regression analysis was done to find out the significant relations, if any, among the abundance of microplastics in oyster and water. All statistical analyses were performed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA).

3. Result and Discussion

3.1. Abundance of microplastics

The abundance of MP is presented as mean ± SD of items/individual (Table 1, Fig.2). MP was detected in all oyster samples collected from the three sites. The highest abundance of MP was found in oysters from Site 2 (9.74±8.92 items/individual) and the lowest was recorded in Site 1 (5.21±4.85 items/individual). However, analysis of variance indicates that the difference between the sites was not statistically significant ($p>0.05$). This may reflect the relatively close proximity of the different sampling sites and potential differences might be elucidated through the use of additional replicates. The mean tissue concentrations of MP in the oyster samples vary from 0.1 to 1.73 items/g depending on the mussel size (Type 1- 4) and collection site. Across the three sampling sites and across all oyster sizes collected, the mean abundance of MP is 6.9±3.84 items/individual and the mean concentration is 0.81±0.45 items/g, which corresponds well to an average concentration of 0.62 items/g reported in oysters from China (Teng et al., 2018). However, the mean abundance of MP found in oysters in this study are lower than those documented in mussels from England (12.6 items/individual) (Catarino et al., 2017) but higher than the values estimated for mussels and oysters from the French Atlantic coast (0.61 ± 0.56 and 2.10 ± 1.71 items/individual, respectively) (Phuong et al., 2018). It is important to note that differences in the methods of sampling, extraction and analysis employed across the different studies may also contribute to the observed differences in results (Thiele et al., 2019).

The MP abundance in water varies from 12.14±3.11 to 31.05±2.12 items L^{-1} and in sediment it varies from 8.22±0.92 to 17.28±2.53 itemskg^{-1} (Table S1, SI). Analysis of variance indicates ($p>0.05$) slightly higher concentrations of MP in the water samples than in the sediment samples, which suggests either a significant transport of MP away from these sites or rapid burial of MP due to accumulating sediment, or a combination of both processes.

A comparison of the MP abundance in oysters of different sizes (Fig. 2) shows significant differences between Type 1 and Type 2 oysters ($p=0.01$) and between Type 2 and Type 3 oysters ($p=0.016$), with smaller organisms containing fewer MP particles. However, there is no significant difference in MP abundance between Type 3 and Type 4 oysters, indicating all particles that can be considered as MP (<5 mm) are readily ingestible by oysters at these larger sizes and that their capacity to ingest is not significantly different. Interestingly, there are significant differences in the concentration of MP in tissue between Type 1 and Type 2 oysters ($p=0.01$), while Type 2, 3 and 4 oysters show insignificant variations ($p>0.05$). This suggests that larger oysters are capable of ingesting larger quantities of MP per gram of tissue weight. However, it may also reflect smaller oysters not being able to ingest larger MP particles and therefore being effectively exposed to a lower concentration of MP than larger oysters.

The microplastic index (MPI) offers a useful tool for the estimation of MP bioavailability in the environment. The MPI is calculated based on the MP uptake per individual and the corresponding weights of the soft tissue and shell. In the current study, the MPI was determined for each of the oyster Types (1- 4) collected from each of the sampling sites (Table 1). MPI was used to evaluate and compare the degree of contamination between sites and oyster types. The calculated MPI values vary from 0.02 to 0.99, where lower values indicate a low degree of MP contamination and higher values indicate a high degree of contamination. The lowest MPI was determined at Site 1 and the highest value was found at Site 2. During the field observation, a lot of macro debris was observed at Site 2, and blockage of backwater due to the deployed rock boulders to prevent erosion might be the reason for the higher MPI at Site 2 than the other sites. Microplastic pollution in small stagnant water bodies is more serious than in the continuously flowing estuarine and coastal waters (Luo et al., 2019). The highest MPI value was observed for Type 4 oysters at all sites, and this indicates that MP bioavailability increases with increasing organism size and that larger oysters can ingest a broader range of MP types and sizes. The ready bioavailability of MP to *M. bilineata* suggests that this species of oyster is good indicator for assessing the broader MP pollution in the marine environment. Moreover, the edible oyster *M. bilineata* is abundant in the coastal waters off Tuticorin (Kannaiyan and Venketraman, 2008) and is widely distributed throughout the central western Pacific region. This fact lends further support to the status of *M. bilineata* as a good bioindicator in the study of microplastic pollution in the marine environment.

3.2. The relationship of MP in oyster, water and sediments

The MP concentration in the surrounding water and sediment (Table S1, SI) was compared to the MP concentration present in the oysters from different sites (Table 1) to determine if uptake is directly influenced by exposure or if it is dependent only on the size of the oysters. Analysis of variance indicates that there are significant differences between MP in the surrounding water and the abundance of MP in Type 1, 2 and 3 oyster samples ($p<0.05$). However, there was no significant difference between MP concentrations in water and those in Type 4 oyster samples from any site ($p>0.05$). Correlation analysis was conducted for the MP concentration in each oyster Type against the mean MP concentrations in the surrounding water at each site (Fig. S1, SI). The correlation increased with increasing size of the oysters, with Type 4 oysters having the best correlation with MP concentration in water (R^2= 0.9847; Fig S1d). These results are consistent with those of the study conducted by Qu et al., (2018), which reported a positive relationship between MP levels in mussels and waters. This primarily relates to the smaller oysters not being able to ingest the larger items of MP. In contrast, the larger oysters are able to ingest any plastic particle in the water column that can be classified as MP (i.e. up to 5 mm in size).

Analysis of variance indicates significant differences between the MP concentrations present in the sediment at the different sampling sites and the abundance of MP in Type 1 and 2 oysters ($p < 0.05$), but not with Type 3 and 4 oysters ($p>0.05$). There is a greater correlation between MP concentrations in sediment and MP concentrations in oyster with increasing organism size (Fig. S2). The strongest correlation was observed in Type 4 oysters (R^2 = 0.9736; Fig. S2d). Su et al., (2017) reported that the process of sediment re-suspension could transfer MP from the surface of the sediment to the overlying water, making them bioavailable once more to filter feeding organisms. In a high energy coastal environment re-suspension of MP is likely to occur, and this is supported here by the relatively similar MP concentrations determined for the water and sediment samples collected from the 3 sites. The results of this study show that Type 4 oyster samples contain the highest abundance and concentrations of MP, indicating that an increasing proportion of suspended MP particles are ingestible by the organisms at this size. Larger oysters could therefore be used as bio-indicators of MP pollution in the marine environments (Teng et al., 2018).

3.3. Physical properties of the microplastics

MP particles present in the water, sediment and oyster samples were categorized by shape into the following: fibers, fragments or films (Fig.3). At all sampling sites, fibers constitute the major proportion of MP in the oyster (61%) and water (63%) samples ($p<0.05$). Fragments are the next most common form of MP in the same samples, followed by films. In contrast, 50% of the MP present in the sediment is in the form of fragments, followed by fiber and then film. Generally, each form of MP was found in increasing abundance with increasing size of oyster. These data further help to refine the outcome of the analysis of variance and correlation studies for total MP, which indicate that MP concentrations in Type 4 oysters reflect those in both the water and the sediment samples. The result further suggests that oysters are primarily influenced by the MP content and distribution in the water column rather than by that in the sediments. This also suggests that true uptake and bioaccumulation of MP does not occur in *M. bilineata* and that the ingested MP is excreted relatively quickly. The observation of high levels of fibers in the water and oysters samples relative to the other types of MP is consistent with the results of several other studies (Piarulli et al., 2019; Gago et al., 2018; Li et al., 2015).

The individual MP particles present in the water, sediment and oyster samples are categorized into five size classes (0.005 - 0.25 mm, 0.25 - 0.5 mm, 0.5 - 1 mm, 1 - 3 mm and 3-5 mm) as shown in Fig. 4. In all oyster samples, with the exception of Type 4 oysters from Site 3, the most abundant size class is 0.25-0.5mm, with an average abundance across all samples of 44.6%. This is followed by 0.005-0.25 mm (25%), 0.5-1 mm (17.4%) and 1-3 mm (13%). As expected, the 3-5 mm class of microplastic is not present in oyster samples as particles of this size are too large to be ingested. In the water samples, the most abundant size class is 0.5-1mm, with an average abundance across all samples of 26%, followed by 0.25-0.5mm (20%). In the sediment samples, 36% of MP belongs to the 1-3mm class, followed by 0.5-1mm (27%). The proportion of 0.25-0.5 mm sized MP is significantly higher in oysters (44.6%) than in the surrounding water (20%). This evidences particle selection by the oysters, with a preference for smaller particles which are more similar in size to their natural food items. Figure 4 also indicates that larger oysters (Type 3 and 4) are able to ingest a greater range of variously sized particles (smaller and larger particles and fibers), while MP ingestion by smaller oysters (Type 1 and 2) is limited to smaller-sized particles. Larger oysters have larger gills and labial palps, which facilitate their taking in larger particles (Cognie et al., 2003). The gill and palp size increases with increasing shell size, with the relative palp size varying between 1.1 and 5.3 mm and the relative gill size varying between 24.5 and 39 mm (Evseev and Yakovlev, 1996). Teng et al., (2018) also observed MP of size <500μm in cultured oysters and other bivalves from China, suggesting that large MP particles are bioavailable and ingestible. In this study, MP particles of several colors were observed in the oyster, water and sediment samples. Of these white ($p<0.05$) is the predominant color in both oyster and water samples (data not shown), confirming that color plays no role in the selection and ingestion of particulates by oysters.

3.4. Identification of microplastics

A preliminary characterization was conducted using a combination of visual identification under a light microscope, a fragmentation test with tweezers and the application of the hot needle test (De Witte et al., 2014). From the remaining particles, 60 particles of size >0.5mm were selected for the FTIR-ATR analysis. For Site 1, 12 particles were selected (7 fibers, 3 films, 2 fragments), for Site 2, 31 particles were selected (16 fibers, 8 films, 7 fragments) and for Site 3, a total of 17 particles were selected (9 fibers, 4 films, 4 fragments). This study notes that fibers constitute the major proportion of MP in the oyster and water samples from all three sampling sites. The fibers in oyster, water and sediment samples may originate from commercial fisheries, laundry and domestic wastewater and other local human activities in the Tuticorin region. However, the FTIR analysis reveals that the polymers present in MP (percentage contribution shown in parentheses) from the water samples are polyethylene (PE; 75%) and polypropylene (PP; 25%); in sediment samples PE (60%), PP (20%), polyester (12%), polyamide (4%) and paint (4%); and in oyster samples PE (92%), PP (4%), and unidentified particles (4%). The analyses indicate that PE is the most common form of MP studied across the three sites. From a qualitative point of view, fibers identified as PE and PP are highly predominant among the identified MP particles in all samples, suggesting that a high proportion of fibers originate from fisheries (ropes and lines) rather than textiles (which are dominated by polyester, nylon and acrylic fibers). The results are consistent with the widespread use of PE and PP ropes in fisheries activities, which have been shown to lose 0.39-0.45% of their mass per month in the marine environment (Welden and Cowie, 2017; Huang et al., 2014). PE is more abundant in oyster (92%) than in water (75%), whereas PP is less abundant in oysters (4%) than the water samples (25%). This suggests a preferential ingestion of PE over PP by *M. bilineata* and may reflect differences in the size distribution of the two polymer types. The most common size of PE MP obtained in this study ranges from 0.25-0.5 mm, whereas PP is the most abundant in the size range of 0.5 mm, indicating PE to be more abundant in ingestible sizes. Furthermore, the results of a study on MP fibers in marine life originating from fragments of polyethylene, polypropylene, polyamide, and knotted polyester (Murray and Cowie, 2011) are consistent with the results of the current study.

Depending on their polymer composition and corresponding density, MPs either sink into water or remain floating at the surface. PE and PP are low-density polymers and are therefore expected to be buoyant, while polyester and polyamide are high-density plastics and are more likely to sink. The dominance of PE and PP in water samples is consistent with their low density and tendency to remain in the water column longer than the rapidly sinking MP particles comprised of denser polymers. This is borne out by the higher proportion of polyester, polyamide and paint flakes observed in the sediment samples. Sedimentation of low density MP particles is known to occur due to a combination of biological processes (e.g. biofilm formation/biofouling, egestion-excretion by biota), physicochemical processes (oxidation and degradation) and hetero-aggregation with other particles (Booth et al., 2018; Galloway et al., 2017; Chubarenko et al., 2016; Long et al., 2015). The PE MP undergoing oxidative weathering processes is evidenced by the FTIR spectra produced in the present study and is confirmed by the formation of extra peak (due to hydroxyl, carbonyl and alkene group) with the characteristic peak [2919 and 2850 cm^{-1} (-CH), 1460 cm^{-1} and 1470 cm^{-1} (-CH) and approximately 720 –730 cm^{-1} (-CH)] in the spectra (Fig. S3). This suggests a relative change in the density of the particle, which would result in an increase in the density and a corresponding tendency to settle down to the seafloor making the particle bioavailable to the benthic organisms.

Polymer distribution in the oyster body more closely resembles polymer distribution in water column than that in the sediment. Oysters being sessile benthic filter-feeders, this clearly indicates that most MP exposure derives from the water column than from the sediment. Oysters have protractile mouth and feed using suction pressure. They feed by sucking in the surrounding water to maximize predation efficiency (Cyrus and Blaber, 1982). During this process, MP may be ingested if they are present in the surrounding water. The predominance of PE has also been reported in three sessile invertebrates (*Saccostrea forskalii, Balanus amphitrite,* and *Littoraria sp*.) from the eastern coast of Thailand and in two sessile bivalves (*Mytilus edulis, Crassostrea gigas*) from the French Atlantic coast (Phuong et al., 2018; Thushari et al., 2017). Indeed, the results of the current study are consistent with those of Kumar et al., (2018), who reported widespread contamination of the marine environment around Tuticorin with PE and PP. The presence of denser polymer MP in sediment samples indicates a broader range of the sources of plastic pollution. Polyamide (nylon) and polyester are known to originate from both the fisheries industry and from synthetic textiles (Browne et al., 2011). Paint particles are common pollutants in the coastal marine environment, originating from boat washing, abandoned structures and grounded ships (Andrew et al., 2009).

3.5. Surface morphology and elemental composition of microplastics

SEM-EDAX is a useful tool for imaging the surface morphology of MP particles and it provides an insight into the inorganic elements present on the surface of MP particles. SEM-EDAX analysis was conducted on a small sub-set of PE particles representing the different particle types (viz. fiber, fragment, film) from the Type 4 oysters samples (Fig. S4 a-f). SEM images of MP in Type 4 oyster from all sites showed surface pitting and cracking/striations suggesting that the particle has undergone some degree of degradation. EDAX analysis identified the presence of a broad range of inorganic elements on the surface of the MP. While many elements (like Ca, Si, Na, S,K, Mg, Cl, Ti) commonly occur naturally in the marine environment, others such as Al, Fe and Ni may represent the existing environmental contaminants that have become associated with the MP or the additive chemicals present within the MP polymer matrix. A huge number of organic and inorganic additive chemicals are known to be used as polymer additives to impart specific visual, physical and chemical properties (e.g. colorants, flame retardants, softeners, UV stabilizers). For example, the observed Ti might have been derived from the TiO_2-based white pigments used in plastics (Wang et al., 2017), but it also occurs naturally in the environment. Since the sampling sites are situated in the backwater zone adjacent to the thermal power station, Fe in the MP might have been derived from the fly ash from the nearby plant (Baskaran et al., 2002), while Ni may be indicative of inputs from petroleum-related activities in the surrounding areas (Muthu Raj and Jayaprakash, 2008).

4. Implications

The current study highlights the widespread ingestion of a broad range of MP types and sizes by the oyster *M. bilineata*, with the accumulated MP reflecting distributions in the water column more closely than those in the surrounding sediment. Although ingestion of MP does not lead to acute toxicological responses, sub lethal effects have been reported in a number of sessile invertebrates (reviewed by Barboza et al., 2019). Furthermore, additive chemicals present in ingested MP particles and environmental pollutants adsorbed to their surface may also be bioavailable and impact the health of oysters. As humans consume the whole soft tissue of oysters (along with the digestive tract), any MP present in them is potentially transferred to humans as well as to the other higher-level organisms (Seltenrich, 2015). Wright and Kelly (2017) report that ingestion of MP with associated chemical pollutants may cause a series of inflammatory and immune responses in humans. Such health risks have the potential to cause a decline in consumer confidence in seafood, which may lead to reduced consumption and a corresponding economic effect on those associated with the seafood industry.

5. Conclusion

This study illustrates a strong relationship between MP concentrations and distributions in the water column and those observed in oysters collected from the same location. The MP particles in both water and oysters are predominantly fibers comprising PE and PP. PE fibers ranging from 0.25 to 0.5 mm in size are commonly found in oyster samples. Both PE and PP are low density plastics and they are slow to sediment to the seafloor. This property potentially increases their exposure and ingestion. The relatively high level of PE and PP fibers also suggests that a high proportion of the MP load in the water column is derived from PE and PP ropes used in the fisheries industry. The MP profile in the sediment samples indicates a broader range of sources, but they contain relatively higher proportion of polyester, polyamide and paint, which indicates that MP of these dense polymers has a lower exposure and bioavailability potential to oysters. The size of the oyster also plays a key role in the bioavailability of MP, with both MP abundance and concentration being the highest in the largest oysters (Type 4). This is supported by the calculated MPI, which shows that the bioavailability of MP is higher in Type 4 oyster than in the smaller oysters, indicating that a greater proportion of MP in the water column is ingested by larger oysters. The sessile nature of oysters enables them to act as sentinel organisms for monitoring MP in specific marine environments, and therefore they can be used in the study of MP uptake and accumulation. Regular quantification of MPs in various sea products and aquatic environments may become necessary to ensure food quality and maintain consumer confidence in seafood products.

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