RESEARCH ARTICLE

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Preliminary observation of bacterial biofilm communities on plastic litters and their surface degradation in two coastal areas of Tuticorin, India

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Abstract: The accumulation of plastic litter in the marine environment is a growing ecological concern. Microorganisms can create a biofilm on the surface of plastic litters making them more hazardous. Although plastics are difficult to biodegrade, they can act as substrate for microorganism attachment. To investigate this problem, biofilm coated plastic litters such as covers, films and ropes were collected randomly from Muthunagar and Inigonagar in Tuticorin coastal areas and analysed using FTIR-ATR. The spectra obtained demonstrate the presence of Polyethylene (PE), Polypropylene (PP), Polystyrene (PS) and Polyamide (PA). The concentration of biofilm formed on the surface of plastics is higher in Inigonagar compared to Muthunagar coastal area. Investigations were also conducted into the bacterial development on the plastic surface and in the surrounding water and sediment. Several bacterial communities including human pathogens namely Faecal coliform, E. coli, Salmonella sp., Streptococcus sp., Staphylococcus sp., Pseudomonas sp., Bacillus sp., Klebsiella sp., and Vibrio sp. were found to be associated with the collected plastic litters. We confirmed the weathering of plastic litters by carbonyl and vinyl peaks formation. According to the Carbonyl Index values plastic litters collected from Inigonagar exhibit higher degree of degradation compared to Muthunagar coastal areas. The present study could provide significant baseline information for both plastic pollution and biofilm composition in Muthunagar and Inigonagar coastal areas.

Keywords: Plastic Litters, Biofilm, Bacteria, Degradation, Water, Sediment

Introduction

Over the past three decades, the usage of non-degradable plastic materials has dramatically increased from 1.7 million tonnes to 359 million tonnes [1-3], but no equivalent

processes have been developed for properly disposing or decomposing them. The widespread use of plastic and the improper disposal of its trash are now understood to be the primary causes of ocean plastic pollution [4]. The risks posed by plastics to marine ecosystems are many and include animal ingestion, transfer of bonded organic pollutants such as polychlorinated biphenyls, and animal entrapment [5-9]. These plastics have the potential to not only adsorb various hazardous elements, but also act as vectors for microbes, endangering both the environment and human health. The capacity of bacteria to assemble and get attached to surfaces to form tiny colonies known as biofilms is the main mechanism by which bacteria can survive in the environment. Biofilms formed on the surface of plastic litters are called "plastisphere" [10-14]. The development of biofilms offers the bacteria defence against harmful environments and facilitates their dispersal [15]. Thus, it is thought that bacterial biofilms represent a hotspot for the interspecies spread of antibiotic resistance [17]. Bacterial biofilm communities are recognised as having a significant role in degradation and biogeochemical cycles and capable of influencing the settlement of numerous invertebrates during the larval stage [17].

Additionally, unlike in the surrounding habitats such as sediments and water, bacteria can survive longer in association with marine plastics [18, 19]. Gamma proteobacteria have been demonstrated to be the predominant class of bacteria in the early phases of plastic colonisation. This is a matter of concern because this group of bacteria contains numerous species harmful to humans, including Escherichia coli, Vibrio cholerae, Salmonella enterica, and many more [20]. As several earlier researches show, many bacteria colonise the surface of plastic debris, including some pathogenic bacteria that are not detected in the water, and these bacteria might be moved from the initial site to a new place where they are usually not found [21, 22]. Accordingly, it has been proposed that marine plastic particles act as global transporters of microorganisms, which could aid in the spread of human infections and antimicrobial resistance [23]. Though the entire scope of this phenomenon has not yet been fully examined, it would depend on how long different species might survive on the marine plastic particles [24].

Diverse microbial communities can be found in the plastisphere, which also contains microorganisms capable of degrading plastics [25]. Plastic degradation takes place in a variety of ways, including thermal, chemical, optical, and biological degradation. By the action of environmental elements such light, heat, moisture, chemical conditions, or biological activity, polymers undergo physical or chemical changes that lead to the degradation of plastics [26]. The weathering of macroplastics is the main source of microplastics (<5mm) and nanoplastics (1-100nm). Biodegradation in marine environment occurs via biotic (enzymes) and abiotic factors (UV, pH and salinity). Biofilm generation by the microbial community in the coastal environments favours the degradation of polymers [27]. Microorganisms can deteriorate plastic polymers largely by the action of the endoenzymes and exoenzymes they secret. Biofilm adherence to plastic surfaces is strengthened by exopolysaccharides (EPS) produced by microorganisms. The biodegradation of polymer is significantly influenced by EPS. These enzymes by disrupting the carbon backbone of the polymer break it down into oligomers,

dimers, and monomers. Monomers that are released by depolymerisation are readily assimilated by microbes as a carbon source, which enhance microbial biomass [28]. Numerous studies have shown that enzymes such amidases, oxidases, laccases, and peroxidases are involved in the breakdown of polymers [29]. This process results in full degradation of polymers and release of end products including carbon dioxide, water and methane [30]. So, taking into consideration the above-mentioned aspects, the current study was planned to screen the biofilm-associated bacterial species isolated from plastic litter from two different sources like tourist and residential coastal areas and to assess the degradation level of plastic litter.

The objectives of this study are:

i) to determine the polymer composition of collected plastic litters,

ii) to quantify the amount of biofilm formed on the surface of the plastic litters,

iii) to isolate the bacterial species associated with the biofilm, surrounding water and sediment from the study sites, and

(iv) to measure the degradation level of the plastic litters.

Materials and Methods

Study area

In this study, two coastal areas namely Muthu nagar and Inigo nagar were selected in Tuticorin district was shown in Figure 1. Muthu nagar coastal area (8°48'26"N, 78°9'42"E) is one of the largest beaches in Tuticorin. The enormous quantity of plastic litters left by the tourists thronging this coast is a matter of concern. Fishing is the major occupation of the 275 families living on the coast of Inigo nagar (8°47'26.87"N, 78° 9'40.56"E). Dumping of untreated domestic waste on the shore and the release of untreated sewage into the sea increases the pollution impact.

Figure 1: The above image shows the study sites, Inigo nagar (left) and Muthu nagar (right) was contamainated with plastic litters

Sample collection

Plastic litters such as plastic covers, films and ropes were collected from the coastal areas of Muthu nagar and Inigo nagar. At each site, litters coated with biofilm were identified by on visual observation and collected randomly using sterile forceps from the beach shoreline during low tide. Sampling was done in the month of October 2022. The collected samples, roughly of 10-25 cm2 in size, were divided into several pieces for various analyses. Separate Plastic zip lock bags were used to store the collected plastic litters. Further, samples of surrounding seawater (1 litre) and sediment (1 kg) were collected in sterilised glass bottles, taken to the laboratory, kept at 5° C and analyzed within 18 hours.

In the laboratory, the plastic litters were washed with sterile seawater in an ultrasonic cleaner for 10 minutes to remove the loosely attached materials [31]. The washed plastic samples were cut into pieces of 4 X 4 cm and used for further analysis. Following standard procedures [32], the collected water samples were analysed for environmental factors such as temperature, pH, salinity, electrical conductivity (Ec), total dissolved solids (TDS) and Turbidity. Physical parameters such as pH, TDS and Ec were measured using handheld digital meter with an accuracy level of 1 µS/cm and 1ppm. Turbidity was measured with turbidometer and salinity with salinometer. Sediment samples were analysed for sediment texture and organic matter content. By using the Pipette method, the percentages of sand, silt, and clay compositions were determined [33]. The loss-on-ignition (LOI) method was used to calculate the amount of organic matter (OM) [34].

Chemical composition of plastic litter

In this study we used Fourier Transform Infrared (FTIR-ATR) spectroscopy to determine the polymeric nature of the plastics. FTIR-ATR is a well-established, quick, easy, and efficient method, by which the polymer is identified based on the infrared spectroscopy absorption bands by particular frequency areas [35, 36]. Attenuated Total Reflection (ATR) mode, with an acceptance rate of 90%, was used to examine all samples. The composition of the polymer was ascertained by comparing the spectra to FT-IR references database. Spectra were collected within the range from 400 to 4000 cm^{-1}.

Biofilm assay

Biofilm development on the plastic litters was evaluated using the quantitative biofilm assay following an existing protocol [21]. Plastic samples (n=21) were washed three times with sterile seawater and air-dried in sterile Petri plates for 45 minutes. After drying, the plastics underwent a 45-minute staining procedure with crystal violet (1% w/v) and three sterile seawater washes. After the washes, the stained samples were air-dried for another 45 minutes and transferred to a 2 ml Eppendorf tube with 1 ml of ethanol (95% v/v). The ethanol was then 100-fold diluted and was measured by a UV-VIS spectrophotometer for the optical density at 595

nm. The amount of biofilm per surface area on the plastic is directly proportional to the optical density.

Isolation and identification of bacterial species

The plastic samples were thoroughly rinsed with sterile seawater to remove loosely bound microorganism and were inoculated, with the help of sterile forceps, onto the surface of different selective agar media namely Zobell marine agar for total viable count, Salmonella agar for E. coli & Salmonella sp., thiosulphate citrate bile salts agar (TCBS) for Vibrio spp., mFC agar for faecal coliform, Pseudomonas agar for Pseudomonas sp., Mannitol Salt Agar for Streptococcus sp. and Staphylococcus sp., and MacConkey agar for Bacillus sp. & Klebsiella sp. All plates were inverted and incubated at 37o C for 24-72 hrs. The grown colonies were subcultured frequently onto another fresh medium to get pure culture, and the bacterial Colony Forming Units (CFU) were enumerated [31]. Each water sample (10mL) and sediment sample (5g) was added to 10 mL sterile seawater and shaken for 15 minutes and allowed to settle for a few minutes. From each sample, 1 mL was taken and serially diluted to 10−1, 10−2, 10−3, 10−4 and 10-5 using sterile seawater. The samples were inoculated on the same media as plastic samples. Following incubation, the growing colonies were counted, and the values were determined using the dilution ratios and expressed in the units of CFU/ml or CFU/g. Using Bergey's Manual, various biochemical assays were carried out to identify the bacterial isolates. The main biochemical tests used were Triple sugar ion test, Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test, Urease test, Nitrate reduction test, Oxidase test, Catalase test, and Hydrogen sulphide production test (H2S).

Degradation level of plastic litters

Carbonyl Index

When polymers are exposed to oxygen-containing environments, the principal driver of degradation is oxidation, which can occur under thermal, photo-oxidative, radioactive, biological or mixed circumstances. Analysing and quantifying carbonyl production in polymers by IR spectroscopy is the analytical technique that is most frequently used to measure oxidation levels. The degree of oxidation of PE, PP, PA and PS can be expressed using the carbonyl index (CI), which was calculated in the present study using the equation

$$\text{Carbonyl Index (CI)} = \text{AC} = \text{o/AC} - \text{H}$$

where AC=O is the absorbance of carbonyl peak at about 1719/1637cm-1, which is the characteristic peak of carbonyl group for PE/PP/PA/PS [38, 39] and AC-H is the absorbance of asymmetric stretching vibration of CH2 at 2914/2915/2916 cm-1, the reference peak [40].

Vinyl Index

The vinyl index was determined by comparing the peak intensity of the vinyl group (910-900 cm^{-1}) to that of the methylene group (2914 cm^{-1}). Vinyl index for PE was calculated using the equation [40].

$$\text{Vinyl Index (VI)} = A_{909cm\text{-}1} / A_{2914cm\text{-}1}$$

Results and Discussion

Identification of environmental plastic litters

Using FTIR-ATR, the polymer types of the collected plastic litters were determined and classified according to the primary components. A total of 21 plastic litters were collected which consisted of four different types of polymers. Of the 21 samples, ten were collected from Muthu nagar coastal area, which contained four PE, three PS and three PA. Eleven samples were collected from Inigo nagar coastal area, which included four PE, three PP and four PA. These are some of the most prevalent types of plastic polymers used in consumer goods [41].

Biofilm formation on the surface of plastic litters

Biofilm development was clearly visible on the plastic litters. Previous works have demonstrated that microorganisms stick to surfaces that are more hydrophobic such as plastic [42,43]. This may be one reason for the capacity of these bacteria to build biofilm in large quantities on plastic surface, as adhesion is the initial stage in the intricate process of biofilm development [44].

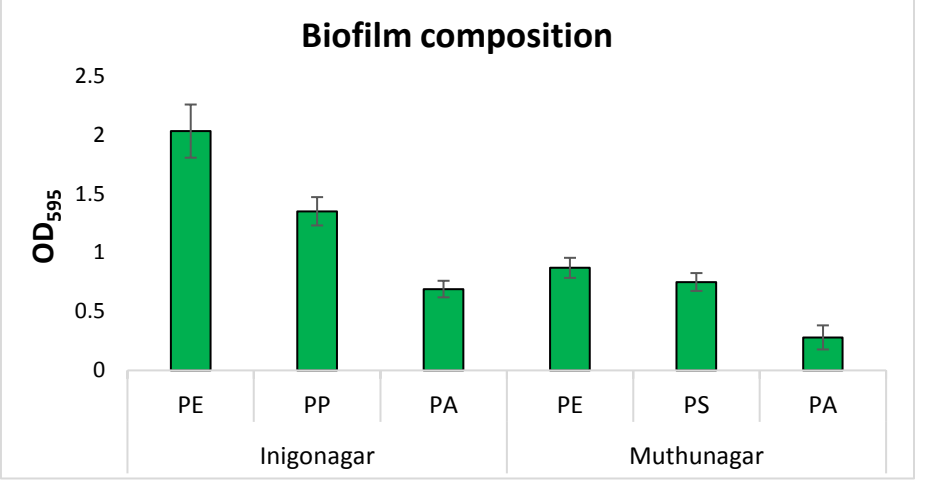

Figure 2: Biofilm composition on different types of plastic litters in Inigo nagar and Muthu nagar

Biofilms formed on the plastic litters were not removed during the washing process, which shows that biofilms are firmly attached to the plastic surface. The staining method was used to quantify the adherent biofilm on the plastic litters and the stained samples was measured using UV-Vis spectroscopy (OD595). A comparison of the amounts of biofilm formed on various plastic litters from Inigo nagar coastal area indicates that PE has higher amount (OD595: 1.58-2.03) than PP (OD595: 1.11-1.35) and PA (OD595: 0.55-0.69). In Muthu nagar coastal area, PE again has higher amount of biofilm (OD595: 0.71-0.87) than PS (OD595: 0.61-0.75) and PA (OD595: 0.09-0.28) which was given in Figure 2. Plastic litters collected from Inigo nagar show higher amount of biofilm formation on the plastic litters than Muthu nagar coastal area. This might be because Muthu nagar is cleaned frequently, which allows only a short time for plastic litter to reside in the environment to form biofilm. On the other hand, domestic sewage and waste from fishing ropes are deposited in the coastal area of Inigo nagar, where cleaning is also scarce. Therefore, the plastic items settle in the environment for several days resulting in heavier colonization of microorganism on the surface of plastics.

Previous studies show that environmental factors like temperature, salinity, pH, and nutrients affect biofilm formation. Environmental parameters of seawater and sediment of this study are given in Table 1a and 1b. This study finds not much variation in the water quality and sediment texture between the two sites [45].

Table 1a: Water quality parameters in Inigo nagar and Muthu nagar coastal area

Water Quality	Inigo nagar	Muthu nagar
Temperature	29.6 ± 0.15	29.3 ± 0.15
pH	7.50 ± 0.02	7.35 ± 0.03
Ec (mS/cm)	51.81 ± 0.05	50.15 ± 0.04
Turbidity (NTU)	3.6 ± 0.10	2.46 ± 0.04
TDS (mg/l)	32.32 ± 0.05	30.15 ± 0.03
Salinity (ppt)	34 ± 1	33 ± 1.53

Table-1b: Sediment texture in Inigonagar and Muthunagar coastal area

Sediment Texture	Inigo nagar	Muthu nagar
Sand (%)	98.7	98.1
Slit (%)	0.5	0.8
Clay (%)	0.2	0.3
Organic matter (%)	0.6	0.8

show biofilm growth to be most prominent at temperatures 25 to 42° C and pH levels 6.0 to 8.0. In the present study, the temperatures of 29.3 ± 0.15° C and 29.6 ± 0.15° C and pH

values of 7.35± 0.03 and 7.50± 0.02 observed in the sites may be considered to be optimum for biofilm formation. In the case of sediment, the percentage of sand is higher, which shows that the grain fissures of sand grains offer a place for attachment as well as access to nutrients and carbon [46, 47]. Sand grains provide a huge surface area with cracks and crevices, creating a potentially favourable habitat for microbial survival and growth [48]. These results show that biofilm formation is not affected by the environmental factors in both the sites.

Bacterial community associated with plastic litter and environmental samples

The abundance of heterotrophic bacteria cultured is expressed as number of Colony Forming Units of the sample (CFU/g or CFU/mL). In this study, the microbial populations on plastics were compared to communities in seawater and sediment. All the three kinds of samples (seawater, sediment and plastic) were placed on Zobell marine agar plate. The result shows that the plastic samples have more bacterial colonies than the surrounding water or sediment (Table 2). The bacteria isolated were identified by biochemical test (Table 3). The ranges of total viable counts on plastic litters are 3.2×10^{5}-5.6×10^{5} CFU/g (Inigo nagar) and 1.9×10^{5}-3.4×10^{5} CFU/g (Muthu nagar). Several species were isolated from plastic litters including faecal coliform, *E. coli, Salmonella* sp., *Streptococcus sp., Staphylococcus* sp., *Pseudomonas sp., Bacillus sp., Klebsiella sp.*, and *Vibrio sp.* in Muthu nagar and Inigo nagar. Species such as faecal coliform, *E. coli*, *Pseudomonas sp., Klebsiella sp.*, and *Vibrio sp.* show higher abundance on plastic litters than the other species. It is clear that plastic litter has the ability to affect microbial loading and water quality because the concentration of pathogenic bacteria colonising plastic litter are higher in this study. Species such as *Salmonella* sp., *Staphylococcus* sp., and *Bacillus sp.* are less abundant in plastic litters. Several factors such as plastic properties, duration and environmental parameters affect the colonisation of microorganisms on plastic litters [49]. A comparison between water and sediment discloses that bacterial density is higher in sediment than in water. This may be due to the fact that bacteria survive in sand for longer period due to their protection in biofilms, since sand particles promote adhesion and contain nutrients and carbon in the grain pores [46, 47]. Additionally, sand provides a more effective barrier to harmful UV rays than water does [50]. Further, the concentration and distribution of indicator bacteria in the sand may potentially be influenced by the

movements of people on the beach [51-53]. According to sediments as osmoprotectors counteract the effects of high salinities [54]. Studies by and others have demonstrated that intertidal sand can contain more faecal indicators than the water which correlates with our study [55-58].

Several investigations have found the microorganisms on plastics to be more varied and distinctive from those in the surrounding water [14, 59].

Table 2. Bacterial community isolated from water, sediment and plastic litters from Inigo nagar and Muthu nagar coastal area

Microbiological Parameters	Inigo nagar					Muthu nagar				
	Water (CFU/mL)	Sediment (CFU/g)	PE (CFU/g)	PP (CFU/g)	PA (CFU/g)	Water (CFU/mL)	Sediment (CFU/g)	PE (CFU/g)	PS (CFU/g)	PA (CFU/g)
TVC	$4.7X10^4$	$5.3\ X10^4$	$5.9\ X10^4$	$4.8\ X10^4$	$3.2\ X10^4$	$3.5\ X10^4$	$4.2\ X10^4$	$5.4\ X10^4$	$4.8\ X10^4$	$4.1\ X10^4$
FC	$2.8\ X10^4$	$4.1\ X10^4$	$5.2\ X10^4$	$4.7\ X10^4$	$4.3\ X10^4$	$1.9\ X10^4$	$2.7\ X10^4$	$3.1\ X10^4$	$4.3\ X10^4$	$2.9\ X10^4$
E.coli	$2.7\ X10^4$	$3.8\ X10^4$	$4.0\ X10^4$	$4.9\ X10^4$	$3.2\ X10^4$	$1.6\ X10^4$	$2.2\ X10^4$	$2.8\ X10^4$	$2.5\ X10^4$	$2.0\ X10^4$
Salmonella sp.	$1\ X10^3$	$1.5\ X10^3$	$0.8\ X10^3$	$0.5\ X10^3$	$0.6\ X10^3$	$1.1\ X10^3$	$1.4\ X10^3$	$0.4\ X10^3$	$0.2\ X10^3$	$0.5\ X10^3$
Pseudomonas sp.	$1.7\ X10^4$	$2.3\ X10^4$	$4.0\ X10^4$	$3.7\ X10^4$	$3.1\ X10^4$	$1.5\ X10^4$	$1.9\ X10^4$	$3.1\ X10^4$	$2.3\ X10^4$	$2.9\ X10^4$
Vibrio sp.	$0.9\ X10^4$	$1.6\ X10^4$	$2.7\ X10^4$	$2.9\ X10^4$	$2.1\ X10^4$	$1\ X10^4$	$1.5\ X10^4$	$2.4\ X10^4$	$1.6\ X10^4$	$1.9\ X10^4$
Staphylococcus sp.	$0.4\ X10^3$	$0.7\ X10^3$	$0.1\ X10^3$	$0.5\ X10^3$	$0.3\ X10^3$	$0.1\ X10^3$	$0.7\ X10^3$	$0.3\ X10^3$	$0.1X10^3$	$0.1\ X10^3$
Steptococcus sp.	$0.1\ X10^3$	$0.3\ X10^3$	-	-	-	$0.2\ X10^3$	$0.4\ X10^3$	-	-	-
Klebsiella sp.	$1.2\ X10^4$	$1.7\ X10^4$	$2.6\ X10^4$	$2.0\ X10^4$	$1.8\ X10^4$	$0.9\ X10^4$	$1.4\ X10^4$	$2.4\ X10^4$	$1.6\ X10^4$	$1.9\ X10^4$
Bacillus sp.	$0.5\ X10^3$	$1.9\ X10^3$	$0.3\ X10^3$	$0.7\ X10^3$	$0.2\ X10^3$	$0.1\ X10^3$	$0.3\ X10^3$	-	-	-

Table 3. Biochemical observation of different isolated bacteria

Microbes Identified	Catalase Test	Oxidase Test	Citrate Utilization Test	Triple Sugar Iron Test	Hydrogen Sulphide Production (H_2S)	Methyl Red Test	Voges-Proskauer Test	Nitrate Reduction Test	Indole Test	Urease Test	Motility Test
Salmonella sp.	+	-	+	K/A with H_2S	+	+	-	+	-	-	+
E.coli	+	-	-	A/A	-	+	-	+	+	-	+
Vibrio sp.	+	+	-	A/A	-	-	+	+	+	-	+
Pseudomonas sp.	+	+	+	K/K	-	-	-	+	+	+	+
Klebsiella sp.	+	-	+	A/A	-	-	+	+	+	+	-
Bacillus sp.	-	+	+	K/A	-	+	-	-	-	-	+
Streptococcus sp.	-	-	-	A/A	-	+	-	-	-	-	-
Staphylococcus sp.	+	-	-	A/A	-	+	-	+	-	+	-

(A=acid production; K=alkaline reaction; H2S = H2S production)

In the present study, variations occur among the bacterial compositions of surrounding water, sediment and plastic surface. The presence of the pathogenic bacterium Streptococcus sp. in surrounding water and sediment and its absence on the plastic litters is one of the deviations encountered by our study. In Muthu nagar, Bacillus sp. is present in water and sediment but not on the plastic litters. Despite the differences among the samples of water, sediment and plastics, no unique species were discovered in this investigation. This study demonstrates that all bacterial groups adhering to the plastic surface could be found in the surrounding seawater or sediment, which supply the microbes that colonise the plastic surface. Strain complexity and biofilm dynamics render our results to be occasionally inconsistent with the prior findings [60].According to, the local environment significantly influences the biofilm communities. Microbial communities from plastic debris tend to be influenced more by their surrounding environment than by their probable coastal or terrestrial origins [61].

Pathogenic bacteria associated with different polymers isolated from different study areas

The relative abundance of pathogenic bacteria on different types of polymer was evaluated to assess the ecological effects of bacterial communities on plastics. Although the kind of species and the concentrations of pathogenic bacteria on various types of plastic litter may not be the same, it is generally believed that plastic debris is a good carrier of pathogenic bacteria, transforming them into hitchhikers [20, 62, 64]. This study found pathogenic bacteria, including E. coli, Pseudomonas sp., Klebsiella sp., Vibrio sp., Staphylococcus sp., and Bacillus sp. on the surface of plastic litters. The relative abundance of most pathogenic bacteria on the plastic litters shows significant difference among the polymer types though to a lesser extent. In both the sites PE shows higher abundance of bacterial community, followed by PP, PS and PA, the last having the lowest abundance of bacterial community. Among the pathogenic bacteria, Pseudomonas sp. (4.0×104), Klebsiella sp. (2.6 ×104), and Vibrio sp. (2.7 ×104) are abundant in PE, whereas E. coli (4.9 ×104) is abundant in PP. On plastic litters, Vibrio sp. has been extensively reported in many studies [62, 64], which is also confirmed by the presence of Vibrio sp. in our study. Several Vibrio sp. are capable of harming vertebrates and invertebrates and some of them are also harmful to humans causing diarrhoea or extra testicular infections [65].

In this study, potential pathogens like E. coli, Pseudomonas sp., Klebsiella sp., Vibrio sp., Staphylococcus sp., and Bacillus sp. are found on the surfaces of plastic litter, highlighting not only the dangers that these pathogens pose to human health but also the significance of studying the bacterial plastisphere. Despite the fact that harmful pathogens may grow on all surfaces, including wood and stones, plastic litter can act as a unique vector for human exposure due to specific human contact [66, 62, 67]. There is rarely any focus on the potential threat of plastic transferring pathogens during clean-up initiatives when locals gather plastic litter manually. In addition, due to the deposition of pathogenic microorganisms on plastic waste, the bacterial plastisphere may also be employed as an indicator of microbial contamination of the marine environment.

Comparatively, the coastal areas of Inigo nagar and Muthu nagar display significant variation among the bacterial communities present on the three types of samples namely water, sediment and plastics. Most of the bacterial colonisation is higher in Inigo nagar than Muthu nagar. Total viable counts in Inigo nagar are 4.7×10^4 CFU/mL (water) and 5.3×10^4 CFU/g (sediment), which are higher than the respective Muthu nagar values of 3.5×10^4 CFU/mL and 4.2×10^4 CFU/g. Heterotrophic bacteria grow more quickly in seawater due to the influx of untreated wastewater and harbour outfalls that contain high levels of nutrients [68, 69]. This might be the reason for the increased presence of bacteria in Inigo nagar, where untreated wastewater and industrial waste are discharged into the seawater, and the increase in sediment may be attributed to the dumping of domestic and fishing waste on the seashore. As for faecal coliform in water and sediment, the highest level is found in Inigo nagar (2.8×10^4 CFU/mL and 4.1×10^4CFU/g) and the lowest in Muthu nagar (1.9×10^4CFU/mL and 2.7×10^4CFU/g). In the current investigation, the faecal coliform levels at both the sites are significantly above the limits of 200 CFU/100 mL allowed by USEPA's and the national regulation levels (CPCB, 1993) for bathing and recreational activities in natural marine habitats [70, 71]. Untreated sewage discharge plays a significant role in scaling up the levels of coliform bacterial contamination of coastal ecosystems. Human and animal faecal waste on the shore of Inigo nagar might be another cause of the high faecal coliform contamination. In addition, sewage effluents contain *E. coli, Salmonella* sp., *Streptococcus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Bacillus* sp., *Klebsiella* sp., and *Vibrio* sp. [72, 73] which were also detected in both the study areas. But Inigo nagar shows higher abundance than Muthu nagar due to the untreated wastewater that flows into the coastal area of Inigo nagar. Among the species of microorganisms in water and sediment, *E. coli* has the highest occurrence of 2.7×10^4 CFU/mL and 3.8×10^4CFU/g in Inigo nagar and 1.6×10^4 CFU/mL and 2.2×10^4CFU/g in Muthu nagar. At both the sites, the *E. coli* concentrations exceed 126 CFU/mL the regulatory limit prescribed for bathing water (USEPA, 1986) [48]. *Bacillus* sp. is observed in Inigo nagar but not found in Muthu nagar. In the case of plastic samples, all the bacterial species show greater abundance in Inigo nagar due to the dumping of domestic waste on the seashore, longer period of retention and insufficient cleaning activities, in contrast to Muthu nagar where frequent cleaning is undertaken in view of tourism.

As the findings show, the standard acceptable limits for seawater environment are consistently exceeded, and the greatest mean variance is exhibited by faecal coliform and *E. coli.* The presence of pathogens in open sea may not be a serious threat due to the dilution caused by water. Plastics, however, would promote the formation of biofilm by pathogenic microbes [74]. Among the bacterial types isolated from all samples in the present study, some vital ones like *E. coli, Salmonella* sp., *Streptococcus* sp., *Staphylococcus* sp., *Pseudomonas sp., Bacillus sp., Klebsiella* sp., and *Vibrio* sp. emerge as potentially pathogenic and hazardous to public health in view of the spread of antibiotic resistance patterns within the bacterial community in marine environment.

Degradation level of plastic litters

The chemical compositions of all plastic litters were determined using FTIR-ATR spectroscopy. FTIR spectra show that the plastic litters collected are made of PE, PP, PS and PA which was shown in Figure 3. The spectra reveal different absorption peaks for PE with wave numbers 2846 cm^{-1} and 2958 cm^{-1} due to symmetric and asymmetric CH_2 stretching, peaks of 1460-1471 cm^{-1} to CH_2 bending and peak of 716 cm^{-1} refers to CH_2 rocking deformation. PP shows peaks at 2846-2914 cm-1 corresponding to symmetric and asymmetric CH_2 stretching, peak at 1460cm^{-1} to CH_2 bending and peak at 716 cm^{-1} to CH_2 rocking deformation. For PS and PA, several peaks are presented between 3904-530 cm^{-1}, peak at 2916 cm^{-1} corresponding to asymmetric CH_2 stretching, and 1380 cm^{-1} due to methyl (C-H). New peaks for PE, PP, PS, and PA at 1735 cm^{-1} due to ester carbonyl (-COO-), at 1719 cm^{-1} due to ketone (C=O), at 1635 cm^{-1} corresponding to carbonyl group and at 1044 cm^{-1} due to ester linkage (C-O-C) are formed due to the photo oxidation, thermal oxidation and biodegradation [75].

In the marine environment, plastics are continuously exposed to oxygen in both air and water, as well as to microorganisms, sun radiation, and other environmental factors [76]. Following the exposure to the environmental factors, carbonyl and vinyl groups are introduced into the polymer chain, which indicates the weathering of polymer [77, 78].

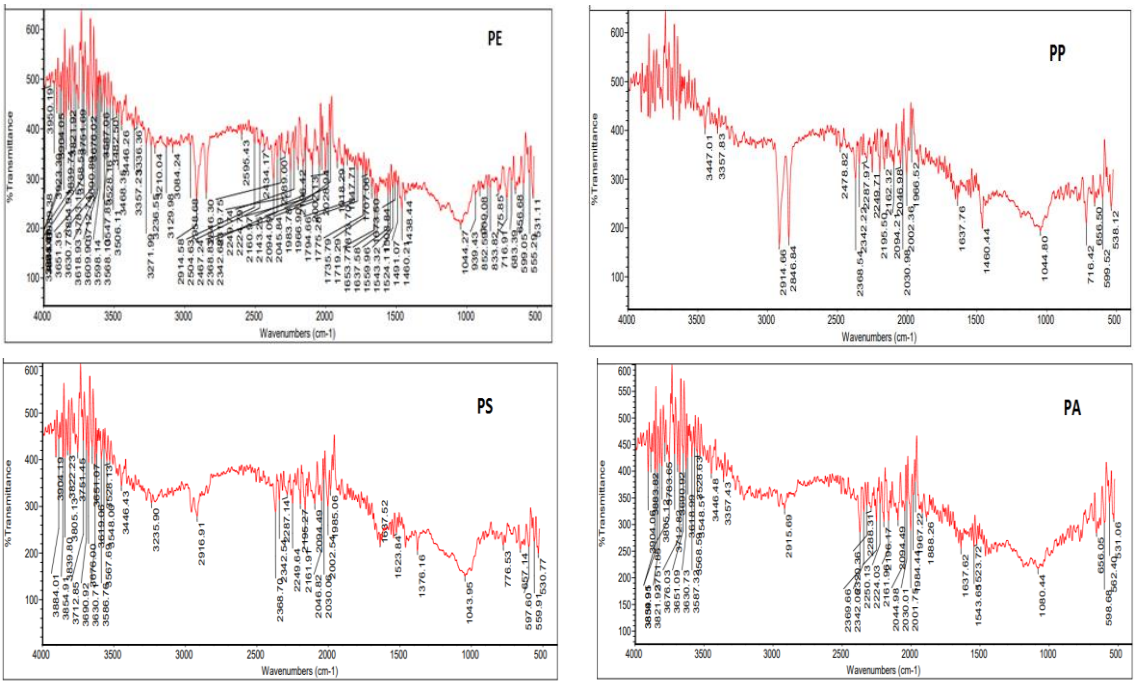

Figure 3: FTIR spectrum of PE, PP, PS and PA

The formation of carbonyl and vinyl groups yields the peaks at 1637 cm^{-1}, 1719 cm^{-1} and 909 cm^{-1}. Carbonyl index and Vinyl index are the most commonly used metrics to assess the degree of polymer degradation. The calculated carbonyl index values for plastic samples collected from Inigo nagar coastal area are 1.28-1.57 for PE, 1.01-1.17 for PP, and in Muthu nagar are 0.43-0.87 for PS and 0.11-0.57 for PA. The result shows that plastic litters collected from Inigo nagar have undergone higher rate of degradation than those from Muthu nagar beach. Similarly, vinyl index values for PE collected from Inigo nagar are 1.06-1.59.

Identifying the microbes responsible for the biodegradation of plastic is one of the key goals of research on microbial communities in marine environments. When microbes adhere to a polymer, they secrete enzymes that cause the polymer structure to break down through a hydrolysis process [79]. Typically, the formation of a microbial biofilm on the substrate increases the substrate's degradation efficiency. The metabolic activity of microbial populations that form biofilms is higher than that of planktonic microorganisms. Marine organisms such as Pseudomonas sp., Bacillus sp., and Vibrio sp. are reported to degrade polymers [80-86].

These species are isolated from plastic surface in the current investigation too. The degree of biofilm formation on the surface of plastic litters is in the following order: PE > PP > PS > PA. Similar polymers with high biofilm formation exhibit a higher rate of degradation. Pseudomonas sp. and Vibrio sp. are most abundant in plastic litters. Particularly, PE colonised abundantly by both the species exhibits higher degradation rate. These microbes use polyethylene as their only supply of carbon, which causes some polymers to partially degrade. They establish colonies on the polyethylene surface, generating a biofilm. The formation of a biofilm on the surface of polyethylene is found to be influenced to a significant extent by the hydrophobic nature of the cell surface of these organisms, which accelerates the degradation of the polymer [87]. Since the plastic debris is randomly collected from the marine environment, the exact degradation process of plastic litters is unknown. We assume that the degradation of plastic litters may be accelerated by microorganism due to the formation of biofilm associated with various bacterial communities including some plastic-degrading bacteria that use plastics as their carbon supply, which in turn results in the degradation of plastics.

Conclusion

Focussing on the differences in microbial contamination indicators in the coastal ecosystems of Tuticorin, this study investigated the bacterial community associated with the biofilm on randomly collected plastic litters, and compared the variation of bacterial community among the samples of plastic litter, surrounding seawater and sediment. The results show that the bacterial community on plastic litters are from the surrounding environment. The parameters of the bacterial community on plastic litters vary according to the substrate type and the location. A particularly important finding of major concern is the presence of great populations of many pathogenic organisms on the plastic surface. This may be due to the untreated sewage and industrial discharge of wastewater into the marine ecosystem. The degradation rates of plastics

are higher in Inigo nagar, as measured in this study. This might be due to the high abundance of pathogens on plastics in Inigo nagar. It may be concluded that monitoring plastic pollution on a regular/periodic basis helps in reducing pollution sources around the shoreline region of the coastal marine ecosystems.

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