

Microplastics in coastal sediments of Pakistan: Site-specific patterns and biodegradation by native bacterial isolates

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ABSTRACT

The widespread use of materials harmful to the biosphere has escalated environmental challenges, with microplastics emerging as a critical issue. While global research has advanced in this area, data from regions like Pakistan remain scarce. This study somehow addresses this gap by examining the occurrence and characteristics of microplastics and exploring the degradation potential of bacterial species isolated from Pakistan's coastal areas. Beach sediment samples were collected from various locations along the 850 km coastline. Microplastics were separated using density-separation method and visually inspected under a microscope for enumeration and physical characteristics. Additionally, site characteristics were also analyzed in relation to microplastic abundance. For studying microbial degradation, microorganisms from sediment samples were isolated via serial dilution, identified through rRNA gene analysis and cultured on 3 different polymers viz. Poly vinyl chloride (PVC), poly ethylene terephthalate (PET) and polyethylene (PE). Polymer weight loss was measured to assess degradation efficiency by various microbial species. The results showed that all sites were infested with microplastics, having different colors and shapes. However, locations showed natural variation in abundance of microplastics based on territory, site characteristics, population, industry and tourism etc. It was observed that urban areas of Karachi showed maximum abundance of microplastics, whereas western part of coastal line in Baluchistan province was less infested. Clifton (760 particles/kg,) and Manora (607 particles/kg) areas of Karachi city were found most abundant in microplastics. Kund Malir (107 particles/kg) and Jiwani (167 particles/kg) in Baluchistan province were found to be least abundant in microplastics. Black and transparent were the most prevalent colors with a mean percentage of 23% and 22% respectively. Fibers and fragments were most prevalent shapes with a mean percentage of 47% and 23% respectively. 7 bacterial species from 5 genera were identified as plastic degraders. *Pseudomonas aeruginosa* showed the highest degradation potential for PET, recording mean percentage weight loss of 30.06%. *Bacillus flexus* demonstrated the highest average PVC degradation potential (27.53%), whereas *Pseudomonas azotoformans* exhibited the highest average potential for PE degradation at 37.97%. This study sets the foundation for further research in addressing this pressing environmental issue.

Keywords: polymers, coastal, bacterial degradation, microplastics

INTRODUCTION

The rate of hazardous compounds being released into the environment is escalating at an alarming rate. Rising concerns and awareness about toxic materials have compelled the scientific community to investigate the origins, fate and consequences of these compounds. Plastics are increasingly being recognized as persistent

and enduring pollutants in the environment. Plastic materials account for a significant portion of long-term environmental pollution, making up approximately 80% of the waste found in agricultural fields, landfills, and water bodies (Monkul and Özhan, 2021; Shen et al., 2021). Plastics serve as the foundation for a multitude of everyday products that are indispensable in modern life. These include automobile parts,

electrical appliances, plastic furniture, defense materials, agricultural pipes, packaging materials, sanitary products, plumbing fixtures, tiles and flooring, synthetic leathers, bottles, jars, shoes and numerous household items. The ubiquity of plastic products underscores their central role in contemporary society, but it also highlights the growing environmental challenge posed by their persistence in ecosystems. Plastics, mostly from households and industry, after being discarded, are washed away through wastewater systems and accumulate in the sludge of wastewater treatment plants. From there, these plastics are transported to agricultural soils, where they continue to build up over time, resulting in a concerning increase in plastic presence in our environment (Nizzetto et al., 2016). The widespread accumulation of these resilient polymers in the soil has serious consequences, including the potential spread of invasive species and the risk of harmful organisms being transported across different ecosystems (Zhang et al., 2024).

Microplastics are smaller plastic fragments (< 5 mm), which have been part of our ecosystems for more than a decade (Arthur et al., 2009). These tiny particles are either released into the environment directly and referred to as Primary Microplastics or are produced as a result of natural weathering and breakdown of larger plastic particles and referred to as Secondary Microplastics. Both types are expected to have equivalent persistence and effects once released into the environment. Microplastics may also be classified based on their shapes, chemical structure etc. Microplastics are part of our daily lives with diversified sources and serious impacts. In a broader sense, major sources of microplastics include industrial operations, clothing, and cosmetics. These tiny particles have huge impacts on the wellbeing of fish, invertebrates and other life forms (Cannon et al., 2025; Nadal et al., 2016). Different studies have also revealed that microplastics pose significant health issues to humans in terms of physiological setbacks. These effects include damage to DNA, malfunctioning of organs, oxidative stress, poor immunity, disorders in metabolism and other issues (Li et al., 2023; Wei and Zimmermann, 2017). Additionally, microplastics have indirect impacts through trophic transfer and adsorption to heavy metals, organic pollutants, antibiotics as well as other harmful pollutants. This worsens and widens the impacts of microplastics, beyond marine ecosystems (Dong et al., 2021).

Once released into the environment, the recovery of microplastics is impossible, as they are tiny particles and are dispersed at a larger scale. However, the degradation of these tiny particles through physical factors and through microbial metabolism is viable and possible. Therefore, to mitigate the microplastic pollution, microbial species with substantial capacity to degrade microplastics need to be identified for their subsequent introduction in the most infested areas. Generally, the degradation of microplastics is by two methods, viz. Physical degradation or weathering and biodegradation through microbes. Physical degradation is by means of physical factors, such as light, heat, moisture, etc. (Dey et al., 2024), whereas biodegradation involves degradation by the action of living organisms, mostly microbes. The enzymatic activities that result in a chain cleavage of the polymer into monomers are the root cause of the degradation of plastics that occurs as a result of microbe attacks.

Owing to their drastic effects on ecosystems and life, the possible avoidance, degradation and remediation pathways of microplastics is naturally, a subject of interest for the scientific community. Further, it is essential to have a complete understanding about quantity, composition and geographic distribution of microplastics on international, national as well as regional scale in order to provide assistance in the management of marine litter made of plastic. On the other hand, there is a paucity of information regarding the microplastic contamination of shorelines at national level in some areas including Pakistan. Yet, the role of local microflora in degradation of microplastics remains completely untapped, to date.

The objective of this study was to investigate the abundance of microplastics in sediment samples along the coastal line of Pakistan and study their characteristics. Additionally, site characteristics were also analyzed in relation to microplastic abundance. Further, bacterial species screened out from these samples were studied for their potential to degrade different microplastics. Standard methods were followed for isolation of microplastics, bacterial isolation and degradation studies. Microplastics were separated using density-separation method (Besley et al., 2017). The extracted microplastics were inspected under a microscope for enumeration and physical characteristics (Lots et al., 2017). For studying microbial degradation, microorganisms from sediment samples were isolated via serial dilution, identified through rRNA

gene analysis and cultured on 3 different polymers viz. Poly vinyl chloride (PVC), poly ethylene terephthalate (PET) and poly ethylene (PE). Polymer weight loss was measured to assess degradation efficiency by various microbial species (Kannahi and Sudha, 2013)

MATERIALS AND METHODS

Study area and site selection

A total of 15 locations were selected along Pakistan's 850-kilometer coastline, taking into account such criteria as accessibility, population density, tourism value and proximity to industrial zones. The selected locations also represent a wide range of coastal ecosystems.

Sample collection

Standard method by Besley et al. (2017) was used to collect samples. Briefly, high tide zones were selected for sampling. Sampling was done in the year 2022. A total of 3 samples were collected separately from each location, with a minimum distance of 20 meters maintained between sampling points. Using a metallic quadrat measuring 0.25 m², top 5 cm layer of sand sediment was carefully extracted using a metal spoon. Before being packed into non-plastic containers, the sediment samples were sieved using a 5 mm sieve to eliminate larger debris.

Extraction

The extraction of microplastics from the collected sediment samples was performed in Ecotoxicology Lab, International Islamic University Islamabad Pakistan, using a density separation method, as described by Besley et al. (2017). Initially, 100 grams of wet sand was oven-dried at 60 °C for two days to remove moisture. After drying, 50 grams of sand was mixed with 200 mL of fully saturated sodium chloride (NaCl) solution (358.9 g salt per liter) in a conical flask prior to combining and agitated at 900 revolutions per minute (RPM) for 2 minutes to ensure adequate mixing. After stirring, the sand was settled for 8 hours. The supernatant was collected by vacuum filtration with a 0.45 µm filter (Whatman glass filtration assembly with 0.45 µm membrane) and vacuum pump (Rocker 400) after settling. Once

filtration was complete, the filter paper containing the microplastics was carefully placed in a clean Petri plate for further processing. This extraction method was repeated three times for each sample to achieve maximum recovery of microplastics. Special precautions were taken to prevent contamination throughout the procedure.

Enumeration and observation

The filter papers were examined under a stereo-microscope (Olympus SZ61) at 40× magnification and microplastics were counted and observed for physical characteristics (Lots et al., 2017). During the identification process, the filter paper was divided into four quarters, with each quarter being inspected sequentially in a clockwise direction. Each sample was quantified by two independent researchers to reduce human error.

Bacterial isolation

Isolation of bacteria was done in a lab in Department of Bio-Sciences, Comsats University, Islamabad, by mixing 1 g sediment sample with 0.9% NaCl Solution. The resulted mixture was vortexed for 3 hours at an RPM of 180 making in orbital shaker (IKA KS 4000 i control). A suspension was therefore formed which was serially diluted, plated on Nutrient Agar (NA) (Sigma-Aldrich) and incubated at 37 °C for 24 h. Finally, to obtain distinct pure cultures, single colonies were sub-cultured on freshly prepared NA. All experiments were carried out in triplicates.

Bacterial identification

On the basis of high level microplastic degradation, the potential microbes were selected for identification only. Bacterial Identification was carried out by observing the 16S rRNA gene traits. The 16S rRNA gene sequencing was performed by the services of Macrogen, Korea. The obtained sequences were analyzed aligned and submitted in Gen Bank (Database of National Center for Biotechnology Information – NCBI). The accession numbers were obtained for each sequence.

Screening of bacteria for microplastic degradation

To assess the potential of bacterial species in degrading microplastics, a method described by

Kannahi and Sudha (2013) was applied. This approach utilized mineral salt media (MSM), a medium that provides all the essential nutrients required for bacterial growth, with the exception of a carbon source. The bacteria were then tested for their ability to utilize specific polymers – PVC, PET and PE – as their sole source of carbon and energy. Each bacterial isolate was cultured individually in MSM, supplemented with 0.5 grams of one of the selected polymers and incubated for a period of four weeks at standard room temperature. In parallel, a control experiment was conducted, where MSM was inoculated without the addition of any polymer to observe the bacterial growth under these conditions. Each experiment was repeated three times with independent replicates.

Preparation of microbial inoculum and biodegradation

Once the bacterial species with significant microplastic degradation potential were identified, they were grown on NA plates at a temperature of 33 °C for up to 24 hours to obtain pure cultures. To further optimize their growth, the same bacteria were cultured in a stationary phase in an orbital shaker at 29 °C and 150 RPM, ensuring consistent conditions for the experiment. The use of alternating temperatures is a common practice in bacterial isolation experiments where slightly elevated temperatures encourage culture initiation and moderate temperatures are employed for long-term activity to avoid stress-induced inhibition. The bacterial suspensions were collected at the same physiological phase, characterized by an absorbance of 1.09 at 600 nm, to standardize the inoculum for the biodegradation experiments. Equal volumes of these bacterial suspensions were prepared for inoculation in biodegradation trials. Pure cultures of the plastic-degrading bacteria, with a cell concentration of approximately 3.8×10^8 CFU/mL, were then inoculated into 270 mL conical flasks containing MSM broth and 0.5 grams of microplastics (PE, PET, or PVC). Control flasks were also prepared, containing MSM with polymers but without bacterial inoculation, to evaluate the natural degradation process in the absence of microbial activity. Similar to the screening procedure, all experiments were performed in triplicate to ensure accuracy and minimize experimental variability. The flasks were placed on a shaker set at 150 RPM and the samples were regularly monitored at 10-day intervals for up to 40 days. Key parameters, such as optical

density, pH and microbial counts, were recorded to assess the bacterial growth and the degradation of microplastics over the study period.

Determination of weight loss in microplastic particles

After completion of six-week incubation period, the broths inoculated with microplastics and without microplastics were processed for extraction using filtration. Next, 70% ethanol was used to wash the extracted microplastics, in order to remove any contaminants. Then, the microplastic particles were kept at 50°C overnight in a hot air oven (Memmert UN55) for drying. Completely dried polymers were checked for residual weight for quantification of weight loss during the experiment. The percentage weight loss of polymers was calculated using the formula presented below:

$$\text{Percentage weight loss} = \frac{\text{Initial weight of polymer} - \text{Final weight of polymer} \times 100}{\text{Initial weight of polymer}}$$

Statistical analysis

Multifactor analysis of variance (ANOVA) was applied for analysis in both parts of the study, viz. Correlation of site characteristics with microplastic abundance and correlation of bacterial strain and microplastic type.

RESULTS

Sediment samples collected from 15 locations along 850 km coastal line of Pakistan were analyzed for presence of microplastics along with physical characterization. Microbial isolation and screening for microplastic degradation was also part of the study. Site names and locations are shown in Figure 1.

Occurrence of microplastics

In all 15 locations, there was significant variation in the abundance of microplastics. Microplastic concentrations exhibit considerable variation among the 15 sites, with urbanized and industrialized locations such as Clifton (mean: 760 particles/kg) and Manora (mean: 607 particles/kg) demonstrating elevated counts relative to less

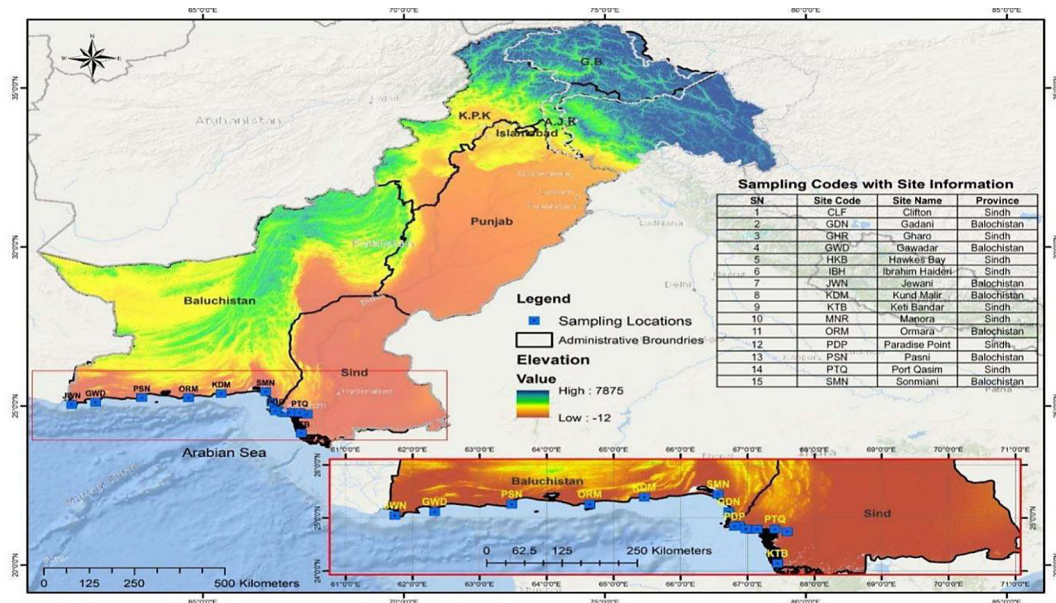


Figure 1. Map showing 15 locations of sediment sampling along coastal line of Pakistan

urbanized sites like Kund Malir (mean: 107 particles/kg) and Jiwani (mean: 167 particles/kg). Table 1 provides detailed data for microplastic abundance for all 15 locations.

Locations identified as sandy beaches, especially those with elevated human activity, demonstrated increased concentrations of microplastics. Clifton, a densely populated sandy beach with considerable recreational and urban impact, exhibited the highest microplastic abundance (760 particles/kg).

Data analysis for microplastic abundance

Before conducting two-way ANOVA, Shapiro–Wilk test and Levene’s test were used to check the assumptions of normality of residuals and homogeneity of variances respectively. Both assumptions were satisfied, indicating that the data met the criteria for ANOVA. Using ANOVA, comparison of microplastic abundance with other parameters such as location, landscape type and human activity were also done. The results revealed that Sindh province had statistically significant ($P = 0.017$) higher microplastic pollution (mean: 442 particles/kg) compared to Baluchistan (mean: 230 particles/kg). Further, Sindh also showed greater variability due to extremes like Clifton (760 particles/kg) and Keti Bandar (200 particles/kg). Sandy beaches and mudflats had nearly identical mean abundance of microplastics (~330–340 particles/kg), therefore no statistically

significant variation was seen due to landscape type. Further, areas with high human activity showed notably higher levels of microplastics. However, despite this clear trend, the variation was high, so the result was not statistically significant. Some of the observed trends, tally with Ahmed et al. (2021). Figure 2 shows mean microplastic abundance plotted against province, landscape type and human activity.

Physical characteristics of microplastics

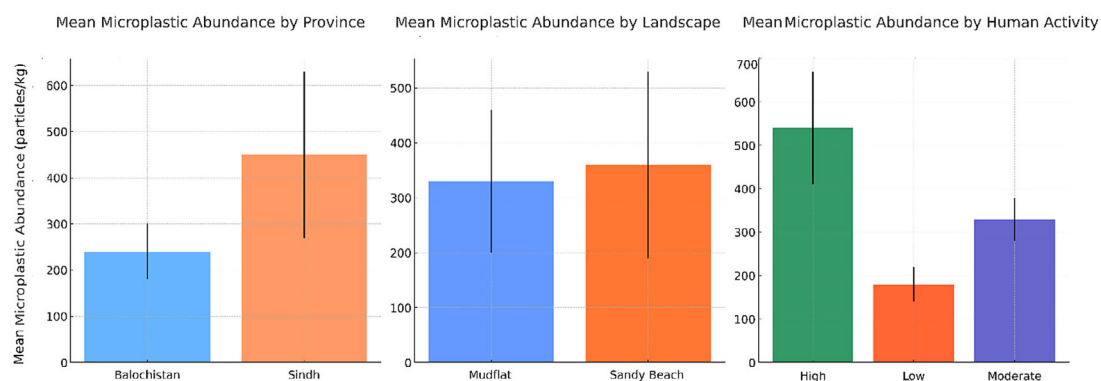
Physical characterization of microplastics was done in terms of colour and shape. A general trend of occurrence of various colors and shapes of microplastics was also studied and compared with similar studies. Detailed data and discussion are discussed below:

Characterization by color

Black, green and transparent microplastics were the most common across all sites. The prevalence of these colors indicates shared origins, including packaging materials, synthetic textiles and fishing equipment (Hidalgo-Ruz et al., 2012). Colors such as red, yellow, green, blue, and brown are seldom yet evident, signifying several origins including industrial pellets, personal care items and deteriorated plastic waste. Figure 3 shows mean percentage values for different colors of microplastics among all sites.

Table 1. Site information and microplastics abundance data from 15 locations along coastal line of Pakistan

Site name	Location coordinates	Province	Landscape type	Human activity (Population, Industry & Tourism)	Mean microplastic abundance particles per Kg	Minimum value (per Kg)	Maximum value (Per Kg)	SD value
Clifton	24° 47' 16.02" N 67° 2' 29.40" E	Sindh	Sandy Beach	High	760	700	760	60.00
Gadani	25° 07' 08" N 66° 43' 17.15" E	Baluchistan	Sandy Beach	Moderate	273	240	280	30.55
Gharo	24° 44' 25.96" N 67° 35' 28.36" E	Sindh	Mudflat	High	380	340	420	40.00
Gawadar	25° 07' 06" N 62° 19' 47.05" E	Baluchistan	Sandy Beach	High	327	320	340	11.55
Hawke's bay	24° 51' 21.93" N 66° 52' 24.53" E	Sindh	Sandy Beach	High	453	380	500	64.29
Ibrahim hyderi	24° 47' 25" N 67° 08' 46.13" E	Sindh	Mudflat	High	507	407	560	50.33
Jewani	25° 02' 48" N 61° 44' 15.14" E	Baluchistan	Sandy Beach	Low	167	140	200	30.55
Kund malir	25° 23' 28.35" N 65° 27' 32.81" E	Baluchistan	Sandy Beach	Low	107	60	160	50.33
Keti bandar	24° 08' 33" N 67° 26' 56.21" E	Sindh	Mudflat	Low	200	160	240	40.00
Manora	24° 47' 36.58" N 66° 58' 29.45" E	Sindh	Sandy Beach	High	607	560	640	41.63
Ormara	25° 15' 34" N 64° 38' 42.21" E	Baluchistan	Sandy Beach	Low	220	180	220	52.92
Paradise point	24° 50' 34.5" N 66° 48' 24.21" E	Sindh	Sandy Beach	Moderate	360	320	400	40.00
Pasni	25° 15' 38" N 63° 28' 44.25" E	Baluchistan	Sandy Beach	Moderate	293	240	360	61.10
Port qasim	24° 47' 23.79" N 67° 24' 33.05" E	Sindh	Mudflat	High	387	360	420	30.55
Sonmiani	25° 26' 58.87" N 66° 33' 35.89" E	Baluchistan	Mudflat	High	227	200	260	30.55

**Figure 2.** Comparisons of mean microplastic abundance by territory, landscape and human activity for 15 locations along coastal line of Pakistan

Characterization by shape

Fibers constituted 75.35% of micro plastics. The second most common micro plastic was fragments (12.02%) followed by films (6.47%) and pellets (6.16%). Figure 4 shows MP shape percentages. The obtained findings match with those of Ahmed et al. (2021).

Biodegradation experiments

In total, 7 bacterial species belonging to 5 genera were studied for degradation potential against 3 microplastic species viz. PVC, PET and PE during degradation experiments. The data in Table 2 implies that *Pseudomonas azotoformans* showed the highest degradation for PE (37.97%), followed

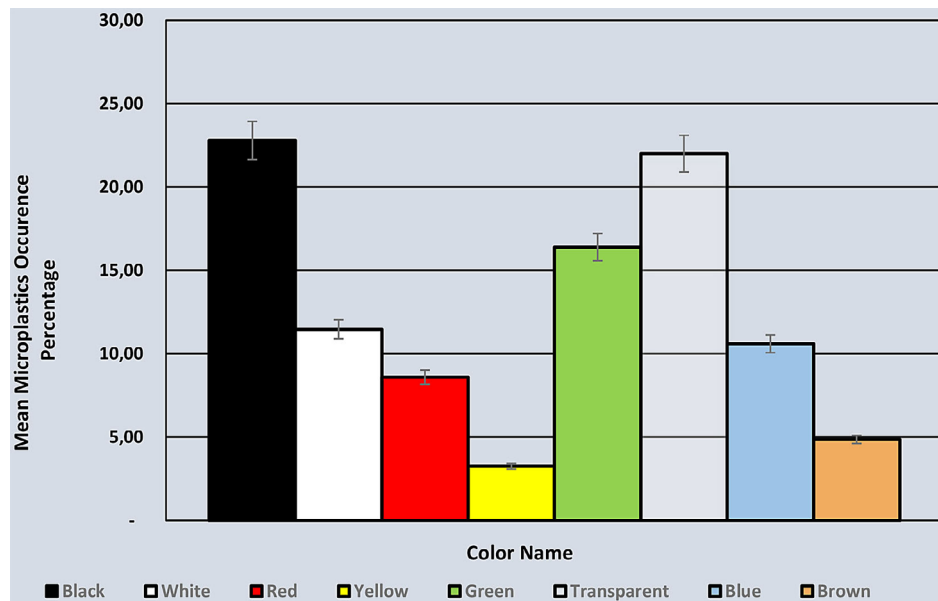


Figure 3. Mean microplastic occurrence percentage by color for 15 locations along coastal line of Pakistan

by PET (29.77%) and the least for PVC (18.48%). *Bacillus flexus* showed the highest degradation for PVC (27.53%), while its activity was lower for PET. *Halomonas campaniensis* performed much better on PVC (24.57%) than on PET and PE. Variance and standard deviations indicate that *Micrococcus luteus* has the most variation in degradation percentages. Figure 5 highlights the variations in degradation potential of all species in a clearer way.

Data analysis for microbial degradation

Before conducting two-way ANOVA, Shapiro–Wilk test and Levene’s test were used to

check the assumptions of normality of residuals and homogeneity of variances respectively. Both assumptions were satisfied, indicating that the data met the criteria for ANOVA. The 2 Factor ANOVA suggested that the bacterial strain factor had an F value of 96.08, which was quite large, indicating significant variation due to bacterial strain. The microplastic factor had an F value of 33.35, indicating significant variation due to microplastic type. The interaction (bacterial strain: microplastic) had an F value of 72.57, showing that the interaction between bacterial strain and microplastic type was highly significant. Pr (>F): The p-value is

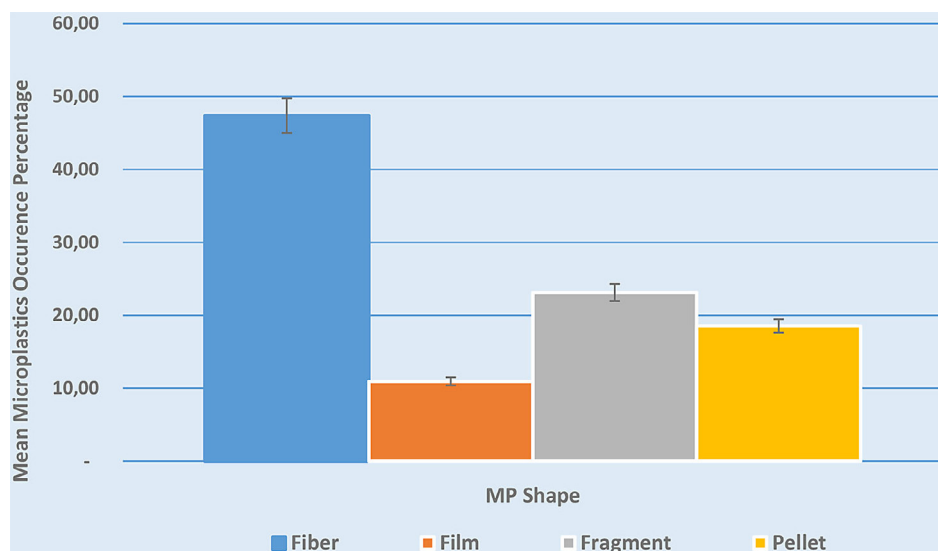
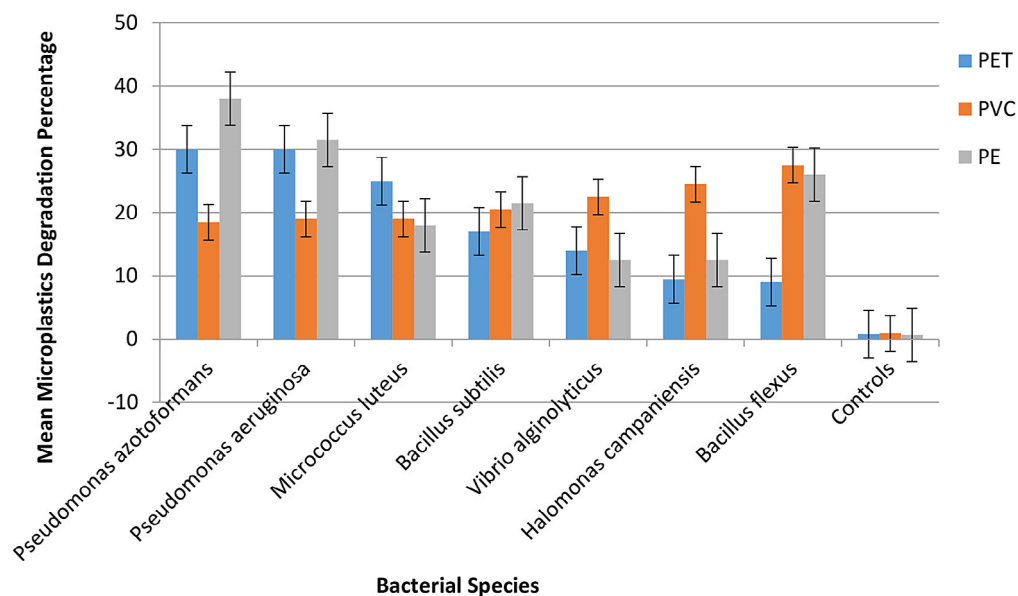


Figure 4. Mean microplastic occurrence percentage by shape for 15 locations along coastal line of Pakistan

Table 2. A tabular representation of mean microplastic degradation percentages, CI values and standard deviations of 7 microbial species for 3 plastic types – PVC, PET and PE

Microbial Species	PET Mean (95% CI)	PET SD	PVC Mean (95% CI)	PVC SD	PE Mean (95% CI)	PE SD
<i>Pseudomonas azotoformans</i>	29.77 (27.56–31.98)	1.95	18.48 (17.37–19.59)	0.98	37.97 (35.97–39.97)	1.77
<i>Pseudomonas aeruginosa</i>	30.07 (27.73–32.41)	2.07	19.08 (18.07–20.09)	0.89	31.48 (29.61–33.35)	1.65
<i>Micrococcus luteus</i>	24.85 (21.72–27.98)	2.77	19.33 (18.2–20.46)	1	18.0 (16.87–19.13)	1
<i>Bacillus subtilis</i>	16.99 (14.7–19.28)	2.02	20.48 (19.24–21.72)	1.1	21.48 (20.35–22.61)	1
<i>Vibrio alginolyticus</i>	13.87 (12.44–15.3)	1.26	22.5 (21.37–23.63)	1	12.51 (12.4–12.62)	0.1
<i>Halomonas campaniensis</i>	9.51 (9.0–10.02)	0.45	24.57 (22.15–26.99)	2.14	12.58 (11.23–13.93)	1.19
<i>Bacillus flexus</i>	8.95 (7.87–10.03)	0.95	27.53 (25.59–29.47)	1.71	26.17 (24.73–27.61)	1.27

**Figure 5.** A graphical representation of mean microplastic degradation percentages of 7 microbial species for 3 plastic types – PVC, PET and PE

associated with the F-statistic. The p-values for all main effects (bacterial strain, microplastic) and the interaction (bacterial strain: microplastic) were extremely small, much less than 0.05, indicating that all of these factors had statistically significant effects on the removal rates. Specifically, p-values $< 2e^{-16}$ indicated highly significant results. Therefore, it is evident that both bacterial strain and microplastic type significantly affect microplastic removal. A highly significant interaction between bacterial strain and microplastic type, suggests that the effect of strain on microplastic removal depends on the type of microplastic.

DISCUSSIONS

Microplastic occurrence and physical characteristics

Microplastic concentrations in coastal sediments worldwide exhibit significant variability, ranging from less than 10 particles/kg in rural regions to over 1000 particles/kg in severely polluted metropolitan beaches (Eriksen et al., 2014). Asian rivers, including the Yangtze, Indus and Ganges, have been identified as significant conduits for the transportation of plastic garbage to the oceans (Lebreton et al., 2017) coastal and

marine pollution research in nations such as China, India, and Indonesia have documented elevated levels of microplastics in coastal waters, sediments, and biota.

In coastal regions of India, microplastic concentrations ranging from 200 to 500 particles/kg have been reported (Banik et al., 2022). The obtained results of Keti Bandar (mean 200 particles/kg) align with these values, suggesting similar infestation level. However, the microplastics pollution level of Keti Bandar does not match with those of heavily polluted Asian sites like Bohai Sea, China (270–1450 particles/kg). In the conducted study, data from 15 locations in Pakistan indicates considerable heterogeneity in microplastic concentrations. Metropolitan regions such as Clifton (mean: 760 particles/kg) and Manora (mean: 607 particles/kg, when compared to less urbanized sites such Kund Malir (mean: 107 particles/kg) and Jiwani (mean: 167 particles/kg) show considerable variation and diversification of pollution levels. This pattern is in line with global trends and implies that sites in proximity to industrial as well as urban areas have elevated microplastic pollution resulting from intensified human activities and vice versa (Geyer et al., 2017). A territorial comparison, based on classification of sites by province also reveals the same pattern. Sindh Province (mean = 442 particles/kg) on account of more population and industrial activity has more pollution level than Balochistan (230 particles/kg). The same trend has already been reported by Ahmed et al. (2021).

At gross level, the microplastic pollution levels in Pakistan identified through this study are in line with those documented in other south Asian nations. Several studies in south Asian countries have reported microplastic pollution levels having mean values comparable to those in this study. Previously, the research conducted in Bangladesh and Pakistan has indicated mean microplastic concentrations between 100 and 1000 particles/kg in coastal sediments (Ahmed et al., 2021; Banik et al., 2022). Principal sources of microplastic pollution in Pakistan may be industrial effluents and untreated urban wastewater.

The most abundant microplastic types by color were black, green and transparent. The presence of these colors indicates shared origins, such as fishing Equipment, packaging materials and synthetic textiles (Hidalgo-Ruz et al., 2012). Few other colors viz. red, yellow, green, blue and brown were less prevalent but evident. This

indicates several origins, including but not limited to personal care items, industrial pellets and degraded plastic waste. The color prevalence pattern of our study is line with other studies in the region (Hosseini et al., 2020).

The ingestion of microplastics by marine life results in several adverse effects including physical damage, chemical exposure and bio-accumulation (Rochman et al., 2015). Colored microplastics, particularly transparent and black being resembling to natural prey are more likely to be ingested by marine life. Further, microplastics have also been reported to have linkage with altering the habitat quality for benthic organisms and therefore affect the natural functionality of ecosystems (Browne et al., 2011).

Biodegradation of microplastics

Biodegradation of polymers has always been a subject of interest for researchers. In this study, 3 plastic species viz. PET, PVC and PE were tested for biodegradation by different species. This section attempts to discuss the degradation of all 3 types separately.

PET degradation

PET degradation by 2 *Pseudomonas* species was observed to have best results among all 7 bacterial species. Mean percentage weight loss of PET by *Pseudomonas aeruginosa* and *Pseudomonas azotoformans* was 30.06% and 29.76%, respectively. Earlier studies have already indicated similar results and they attribute PET degradation by *Pseudomonas* species towards their ability to synthesize extracellular enzymes such as esterases and lipases, capable of degrading PET into smaller monomers (Urbanek et al., 2018; Yoshida et al., 2016). *Micrococcus luteus* degraded PET by an average value of 24.85%, showing comparatively lesser ability to degrade PET. Yet, the efficacy of the species is still considerable for inclusion in a bioremediation program for degrading PET (Shah et al., 2008). *Bacillus subtilis* displayed a mean weight loss of 16.98%. Studies indicate that *Bacillus* species have enzymes, notably cutinases, capable of hydrolyzing PET (Ronkvist et al., 2009). *Vibrio alginolyticus*, *Halomonas campaniensis* and *Bacillus flexus* showed relatively lesser degradation capacities, with average weight losses of 13.87%, 9.51%, and 8.95%, respectively. Optimization or

genetic modification of these species may improve their PET-degrading capabilities (Wei and Zimmermann, 2017). To sum-up, *Pseudomonas* species turn out to be the most suitable candidate for further investigation and inclusion in PET bioremediation. The medium level degradation of *Micrococcus luteus* and *Bacillus subtilis* for PET breakdown may be useful especially under optimized conditions or within mixed microbial communities. Ester bonds in PET make it more suitable for degradation, compared to PVC and PE. Ester bonds can be hydrolyzed by microbial enzymes like esterases, cutinases and lipases (Ronkvist et al., 2009; Urbanek et al., 2018; Yoshida et al., 2016).

PVC degradation

PVC degradation by all 7 species was also studied. *Bacillus flexus* exhibited the best degradation capacity for PVC, having mean degradation percentage of 27.53%. Studies indicate that the species has a robust enzymatic system, capable of decomposing PVC (Shah et al., 2008). *Halomonas campaniensis* (24.57%) and *Vibrio alginolyticus* (22.50%) displayed considerable PVC degradation capability. These species are known for their adaptability to adverse conditions, which perhaps supplements their polymer-degrading abilities (Urbanek et al., 2018). *Bacillus subtilis* (20.48%) and *Micrococcus luteus* (19.34%) were found to degrade PVC moderately, on account of established enzymatic activities by esterases and lipases (Ronkvist et al., 2009). *Pseudomonas aeruginosa* (19.08%) and *Pseudomonas azotoformans* (18.48%) showed relatively diminished PVC degradation in contrast to having significant PET degradation potential. This indicates substrate-specific enzymatic activity (Yoshida et al., 2016). The obtained findings imply that *Bacillus flexus* and *Halomonas campaniensis* are viable species for PVC bioremediation, on account of their greater degradation efficiency. Actually, PVC resists microbial degradation because of its strong carbon-chlorine bonds and its overall chlorine contents.

PE degradation

The degradation potential of all 7 species was also studied for PE. Once again, *Pseudomonas azotoformans*, which has already been assessed as best degrader of PET in this study, was recorded as the best degrader species for PE degradation

as well, having a mean degradation percentage of 37.97%. Naturally, this superior degradation capacity relates to the enzymes including lipases and esterases, capable of degrading hydrophobic polymers such as PE (Urbanek et al., 2018; Yoshida et al., 2016). *Pseudomonas aeruginosa* (31.48%) and *Bacillus flexus* (26.17%) also demonstrated much considerable PE breakdown. These species are known to have enzymatic flexibility and therefore have the capacity to breakdown complex polymers (Shah et al., 2008). *Bacillus subtilis* (21.48%) and *Micrococcus luteus* (18%) had relatively less, yet considerable polyethylene breakdown. Their performance relates to enzymes such as cutinases and other hydrolytic enzymes (Ronkvist et al., 2009). *Vibrio alginolyticus* (12.51%) and *Halomonas campaniensis* (12.58%) showed relatively reduced polyethylene degradation capacity. These species may require optimization or genetic modification for improvement in PE Degradation capacity (Wei and Zimmermann, 2017). So, conclusively, *Pseudomonas azotoformans* and *Pseudomonas aeruginosa* remain the most feasible options for PE and PET bioremediation on account of excellent degradation efficacy for both plastic types. It is worthwhile to mention that PE is hydrophobic and possesses a high molecular weight, making it a tough material for microbes to degrade. However, certain bacterial species can oxidize PE surfaces, resulting in partial degradation.

CONCLUSIONS

The study confirmed the presence of microplastics along Pakistan's entire coast line. The level of microplastics contamination in sediments was higher in the areas where the sampling stations were located in close proximity to populated, commercial and touristic centers. Further, the research on discovery of new potential plastic-degrading microorganisms has been a remarkable achievement of the study and has revealed drastically significant results. The degradation of microplastics through microbes seems to be a practical solution for remediation of microplastics pollution. In this study, bacterial strains isolated from Pakistan's coastal region were checked for their ability to biodegrade 3 plastic species PET, PVC and PE. These strains seemed to have significant potential for bioremediation of different plastic types. Studies such as this one highlight the

significance of undertaking additional research, particularly when taking into consideration the design of procedures with tests conducted under genuine environmental settings. It is essential that this be done in order to ensure that, in the future, the interaction of bacteria with microplastics will have significant practical and biotechnological value on a broad scale. The available data is significant to necessitate immediate action by the scientific community, the industry, as well as policy and civil societies, in order to limit the constant flow of plastics and their toxic additives into marine environments.

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