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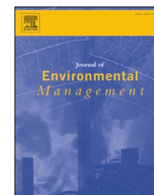

Enhanced microbial degradation of PET and PS microplastics under natural conditions in mangrove environment

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ABSTRACT

In-situ bioremediation of mangrove soil contaminated with polyethylene terephthalate (PET) and polystyrene (PS) microplastics was investigated using indigenous microbial consortium with adequate capacity to degrade the plastics. Eight (8) bacteria were isolated from plastic/microplastic-inundated mangrove soil and screened for the ability to degrade PET and PS microplastics. Optical density at 600 nm and colony forming unit counts were measured to evaluate the growth response of the microbes in the presence of PS and PET microplastics at different times of exposure. Structural and surface changes that occurred post biodegradation on the microplastics were determined through EDS and SEM analysis. The obtained results demonstrated the elongation and disappearance of peaks, suggesting that the microbial consortium could modify both types of microplastics. The overall results of the microplastic degradation showed varied degrees of weight loss after 90 experimental days, with the treated plot recorded 18% weight loss. The augmented soil was increased in the concentrations of Si S, and Fe and decreased in the concentrations of C, O, Na, Mg, Al, Cl, and K after bioremediation.

1. Introduction

Plastics are pervasive and slow-degrading polymers in environmental waste, whose application has been augmented over the past few years due to their combined features of light weight, strength, flexibility, low cost and easy production (Wilkes et al., 2017; Stagner, 2016). With extensive application of plastics, around 49% of gross manufacture is devised for single-use packaging (Regusa et al., 2021), thus accentuating the importance of plastic disposal, primarily low- and high-density polyethylene (LDPE and HDPE), polystyrene (PS), and polyethylene terephthalate (PET) (Peixoto et al., 2017). Recycling of polyethylene is not economically viable as the cost associated with the production of plastics is lesser than the cost associated with recycling (Tolinski, 2012). Therefore, a large amount of such plastics is discarded after use, thus increasing the quantity of plastic wastes in the environment (Álvarez-Barragán et al., 2016).

More than 250,000 tons of plastic debris (over 5 million plastic items) have been discovered afloat at sea (Eriksen et al., 2014), with a considerable amount entering the marine environment from different sources, including industrial and urban effluents, sewer overflows, atmospheric deposition, rivers, run-offs, direct inputs, and the uncontrolled disposal of waste (Tolinski 2012). Plastics are moved by currents, and they aggregate in areas of low water movements. Considerable aggregations of floating plastic debris were first reported in the North Pacific Ocean (Moore, 2008; Moore et al., 2005; Eriksen et al., 2014). Similar scenarios have been reported in other oceans, including the North Atlantic (Lusher et al., 2014), South Pacific, and Indian Ocean (Eriksenet al., 2014). Among plastic debris, microplastics (plastic items that are less than 5 mm in diameter) are of particular concern in terms of the environment, human health, and animal health (Barboza*et al.*, 2018).

Microplastics are global contaminants of special concern that have

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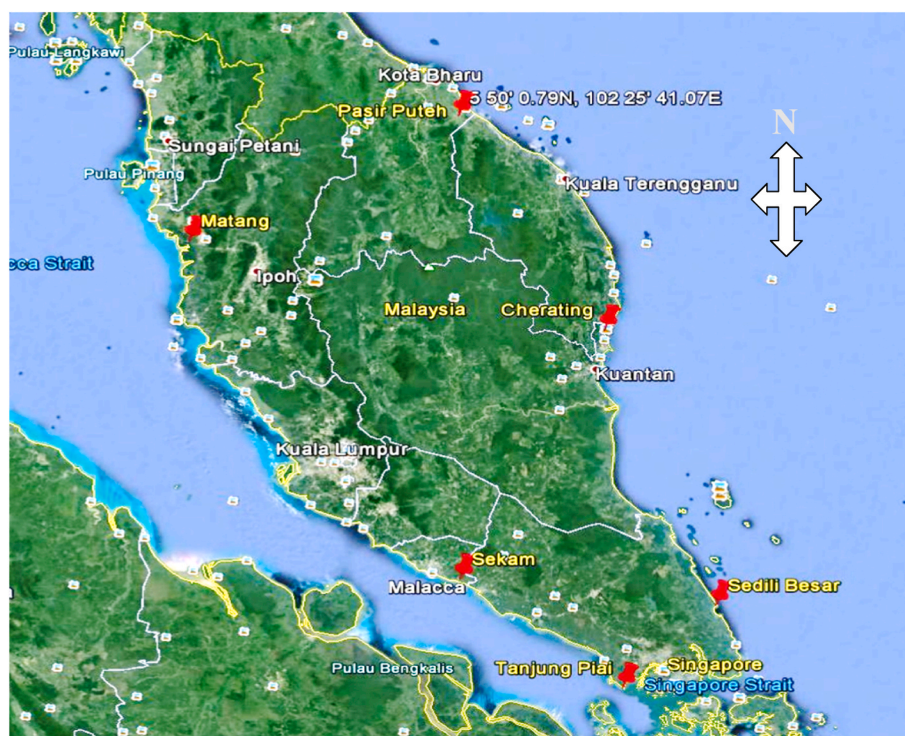

Fig. 1. A geographical map showing the sampling sites (marked red) and locations. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

been ubiquitously detected in sediments (Zamprogno et al., 2021), water, sea salt (Kosuth et al., 2018), marine biota (Berlino et al., 2021), food stuff (Barboza et al., 2018), atmosphere (Brahney et al., 2021), and sewage sludge. The microplastics present in the aquatic environment result from the breakdown of microplastic debris (secondary microplastics) or from primary microplastics (those intentionally manufactured for use).

Microplastic problem has received increased attention over the last few years, and while a significant number of studies have documented the distribution, quantification, fate, sources, and pathways and the ingestion of microplastics by aquatic biota has increased, few studies have been carried out on the degradation of microplastics. The landfill disposal of plastics and incineration release a huge quantity of CO_2 and increase global warming (Eriksson and Finnveden, 2009). Bioremediation using biological agents, such as bacteria, fungi, and algae, has been reported to be the best method to reduce plastic wastes in an eco-friendly manner (Pathak, 2017). Synthetic biodegradable polymers (such as polyesters and starch-based polymers) are associated with major problems, including higher cost and durability than synthetic polymers, such as polyethylene, polypropylene, PS, and PET (Leaversuch, 2002; Leja and Lewandowicz, 2010). Polyethylene is non-biodegradable due to its hydrophobic character, which limits the diffuseness of water and other enzymes, acids, and bio-surfactants produced by microorganisms. The use of additives, such as antioxidants and stabilizers, during the production process and higher molecular weight makes polyethylene non-biodegradable (Albertsson and Banhidi, 1980; Zheng et al., 2005; Koutny et al., 2006; Krueger et al., 2017). Otake et al. (1995) observed partial biodegradation of polyethylene film in moist soil over a period of 32 years. Tribedi and Sil (2013) reported that polyethylene persists in the environment for a longer period as it is not susceptible to microbial attack due to the absence of functional groups. Abiotic factors, such as temperature, UV, and chemical treatments, must be considered prior to the biodegradation process for highly resistive materials, such as polyethylene, because of their hydrophobic nature and large molecular weight (Koutny et al., 2006).

Biostimulation popularly called indigenous bioremediation is mainly related to the addition of nutrients to stimulate biodegradation by the indigenous microorganisms. Studies reported that the slow biodegradation rate of microplastics leads to their breakdown in smaller size hence increasing their persistence in the environment. On the other hand, the usage of stimulants and inducers could help in increasing the enzyme activities to enhance the microplastics degradation rate. For instance, Satti et al. (2018) stimulated the native microbial community using 0.2% sodium lactate (Satti et al., 2018). The author reported the increase in the mineralization rate of PLA (24%) in the soil at ambient temperature for 150 days. Moreover, the author also suggested further optimization with a stimulant to further increase the degradation rate and reduce the degradation time. In general, bioaugmentation is mainly involved in using pure and/or consortia polymer-degrading cultures and the addition of genetically engineered microorganisms to increase the biodegradation activities (Kalogerakis et al., 2015). Recently, efforts to isolate microbial consortia and/or pure cultures from microplastics have occurred in marine/terrestrial habitats to design possible site-specific tailored bioaugmentation strategies for increasing microplastics biodegradation. Interaction between the microorganisms and the surface of microplastics and the biochemical changes confirmed the potential use of bioaugmentation.

In the present study, microbes formulated in the treatment were indigenous mangrove bacteria that were enhanced in the laboratory and therefore expected to thrive well and provide better degradative performance when introduced back into the mangrove environment. This study is the first report of a project that aimed to investigate the degradation of microplastics in natural marine environments and gain information on the behavior of the tested microplastics in the presence of physical strength due to tidal inundation and waves. *In-situ* bioremediation of mangrove soil that was artificially contaminated with PET and PS microplastics was investigated using indigenous microbial consortium with adequate capacity to degrade plastics. This manuscript is of great scientific importance for combining laboratory and *in-situ* remediation of plastics with biological technologies. It deals with a real

Fig. 2. Soil collection points (a) Sekam mangrove, Melaka (b) Tanjung Piai mangrove, Johor.

environmental problem in this region. For having been carried out in a region where few reports exist on the bioremediation of microplastics in the mangrove and few studies in the international literature in such environment are available, this study could arouse the interest of the scientific community.

2. Materials and methods

2.1. Polymer characterization

For degradation experiments, microplastics were obtained by grating/cutting commercial plastic materials obtained from plastic producing industries made of PET and PS by using a bastard-cut hand file and scissors. The grated plastics obtained were passed through sieves (mesh sizes of 2 mm and 5 mm; mesh no. 60; Chunggye Industrial Mfg., Co., Seoul, Republic of Korea) to screen off larger debris. Each was irradiated for 2 days under ultraviolet rays and stored for further use. The sizes of the prepared plastic debris were measured using an optical microscope (IX71, Olympus, Japan) equipped with 4 × lens (Olympus) (Auta et al., 2018).

2.2. Soil sample collection and characterization

The mangrove sites were randomly selected in this study to collect sediment to provide indigenous microbes for the biodegradation and bioremediation study. The soil samples were collected bi-monthly from different locations at mid tidal zones through a period of 12 months to observe the seasonal variations in the diversity of the microbes. Samples were collected from Matang mangrove in Perak (4°50′25.80″ N, 100°38′9.60″ E), Cherating mangrove in Pahang (4°7′36.15″ N, 103°23′29.46″ E), Tanjung Piai in Johor (1°16′5.20″ N, 103°30′31.36″ E), Sekam mangrove in Melaka (1°19′37.84″ N, 103°26′30.61″ E), Sedili Besar in Johor (1°55′54.39″ N, 104°7′27.25″ E), and PasirPuteh mangrove in Kelantan (5°50′0.79″ N, 102°25′41.07″ E), in Peninsular Malaysia. The map of different sampling locations and geographical coordinates is presented in Fig. 1, and some of the sampling environments are presented in Fig. 2. Soil samples were collected from 0 cm to 4 cm depths in the sediment from three different points, with a quadrat of 0.5 m × 0.5 m placed 2 m apart from high tide in undisturbed areas, as described by Nor and Obbard (2014), filled into sterile containers, and transported to the laboratory for further analysis. All samples were collected in replicates to accommodate variability and ensure homogeneity. The excavated samples were analyzed for pH, salinity dissolved oxygen (DO), and temperature by using a multi probe meter (YSI Professional Plus, USA). All assessments were carried out in triplicates. Sequel to sample collection, microbial study became a routine component of the study. Preliminary investigation and assessment of the mangrove sites, including visual observation, topographical outlay, and soil testing, determined the degree of heterogeneity and siting of the sampling spots.

2.3. Microbial isolation and identifications

Bacteria species were isolated by mixing 1 g of soil samples from Matang, Cherating, Tanjung Piai, Sekam, Sedili Besar, and Pasir Puteh mangroves with 10 mL of normal saline water (0.9% NaCl) as stock. The mixture was vigorously shaken for 3 h at 150 rpm using Lab-Line 3521 orbit shaker (LabLine Instruments, Inc., Maharashtra, India). The resulting suspension was subjected to 20 times serial dilution. 0.1 mL dilutions were dispensed on freshly prepared nutrient agar (NA) under aseptic conditions (Kauppi et al., 2011). The inoculated media plates and associated replicates were incubated at 37 °C for 24 h. Colonies that developed were further sub-cultured on freshly prepared NA in triplicates to obtain discrete individual pure cultures and ensure the purity of the samples prior to identification.

2.3.1. Identification of microbes

Isolated bacteria were identified using the Biolog GEN III microplate protocol. A standardized micro-method to profile and identify a broad range of Gram-positive and -negative bacteria was implemented using 94 biochemical tests, as provided by the GEN III MicroPlate test panel (Bochner, 1989).

For identification purpose, the cells were freshly regrown on agar to avoid loss of viability and metabolic vigor, which is typical of most organisms when in the stationary phase. Using the inoculum fluid (IF), inoculums of each cell were prepared using protocols A (IF-A catalog no. 72401) and B (IF–B catalog no. 72403) at a turbidity range of 95%–98% T. The preparation was conducted using a cotton-tipped inoculator swab (catalog no. 3321) to pick up 3 mm diameter area of cell growth from the surface of the agar plate and eventually dipping it into the desired IF. Any cell clump was carefully crushed against the tube wall to ensure uniform suspension. The resulting cell suspensions were poured into a multichannel pipette reservoir.

An eight-channel automated pipettor was used to dispense 100 μL of

the suspension into each of the wells in the MicroPlate (Catalog no. 1030). The wells contained 71 carbon source utilization assays (columns 1–9) and 23 chemical sensitivity assays (columns 10–12). Thus, the isolates could be identified at the species levels on the basis of the "phenotypic fingerprint" of the microorganisms provided by the test panel. All the wells started out colorless when inoculated. During incubation, increased respiration was observed in the cells, where they could utilize a carbon source and/or grow. Increased respiration also causes reduction in tetrazolium redox dye, forming a purple color. Negative wells remained colorless, as did the negative control well (A-1) with no carbon source. A positive control well (A-10) was also present and used as reference for the chemical sensitivity assays in columns 10–12. After incubation, the "phenotypic fingerprint" of the purple wells was compared with Biolog's extensive species library. If a match was found, a species level identification of the isolate was made. These MicroPlates were placed in Omnilog reader, which identified the bacterial species contained in Biolog's Microbial Identification Systems software. The identified microbes were recorded. The microbes isolated/identified were then formulated for biodegradability tests.

2.4. Screening of bacterial isolates for PET and PS microplastic degradation

Isolates were screened for microplastic degradation by using BH media as described by Kannahi and Sudha (2013) and Harshvardhan and Jha (2013), with modifications. The media contained all nutrients except a carbon source necessary for bacterial growth. All bacteria isolated were assayed for the potential to utilize PET and PS microplastics as source of carbon and energy for growth. The isolated bacteria were aseptically re-grown by inoculating each species onto NA. All pure colonies obtained were transferred unto freshly prepared BH agar plates supplemented with 0.5 g plastic powder at pH 7 and incubated for a period of 4 weeks at room temperature. Control sets were maintained (inoculation on media without polymer) for each sample simultaneously, and the media were observed for growth. All experiments were carried out in triplicates. The polymer degrading activity of the isolates was screened by formation of clear halo zones around the colonies. The diameter of clear zones was measured and recorded after 9 days.

2.5. Biodegradation experiment setup with blended isolates

Ten percent (10 %v/v) of the blended isolates having approximately 1.76 × 107 CFU/mL cells were inoculated into 270 mL Bushnell-Haas broth (BHB) in flasks containing 0.5 g of PET and PS microplastics. The flasks containing non-inoculated BHB media supplemented with PET and PS microplastics served as control (negative control). Triplicates were maintained for all experiments and were left on a shaker (rpm 150) for a period of 40 days. The weight loss of PET and PS microplastics were determined (Auta et al., 2018).

2.6. Determination of dry weight of residual microplastics

After 40 days of incubation, all microplastics were recovered from BHB through filtration and sieving. The bacterial films colonizing the microplastics were removed by washing the microplastics with 70% ethanol (four-step washing, with incubation time of 2 min for each step). These were kept on a filter paper and then dried in hot-air oven at 50 °C overnight prior to weighing. Residual microplastic weight was determined to measure the extent of degradation by using Sartorious analytical balance ENTRIS 224-1 S (accuracy of ±0.0001 g) (Auta et al., 2018; Mohan et al., 2016). The initial weights of the pre-incubated microplastic samples were also measured following the same technique mentioned above. The degradation of the microplastic polymers was evaluated in terms of percentage weight loss by using Eq. (1) as follows:

$$\%\text{weight loss} = \frac{w_0 - w}{w_0} \times 100, \tag{1}$$

where W_0 and W are the initial and residual weights of microplastics (g), respectively.

2.7. Determination of reduction rate of PET and PS microplastics

Data were further processed to determine the rate constant of PET and PS microplastic reduction by using the first-order kinetic model on the basis of the parameters assessed: initial and final weights along specific intervals (10 days) (Alaribe and Agamuthu, 2015), in Eq. (2) as follows:

$$K = -\frac{1}{t}\left(\ln\frac{W}{W_O}\right), \tag{2}$$

where k refers to the first-order rate constant for polymer uptake per day, t denotes time in days, W is the weight of residual microplastics (g), and W_O is the initial concentration of microplastics (g).

Following the generation of the microplastic removal rate constant, half-life ($t_{1/2}$) was calculated in accordance with Eq. (3).

$$\frac{(t_{1/2}) = \ln(2),}{k} \tag{3}$$

where t refers to time, 1n (2) = 0.69, and k and is the rate constant.

2.8. Analytical methods for monitoring biodegradation

2.8.1. Scanning electron microscopy (SEM) of PET and PS microplastics

The morphology of the degraded microplastic particles were monitored after 40 days of incubation with bacterial isolates by using SEM (Leica EM SCD005, Austria) at 100–10,000 × magnification to obtain insights into the small-scale changes in the microplastic surfaces. The samples were removed from the culture medium and gently washed with distilled water to remove excess medium and most of the biofilms without damaging the microplastic surfaces. Subsequently, they were washed with 70% ethanol and then rewashed with distilled water to eliminate most surface-adhered cells. The samples were dried and sputter-coated with a gold layer at 25 mA under Argon (Ar) atmosphere at 0.3 MPa and visualized via SEM at 3500 × magnification (Auta et al., 2017; Sekhar et al., 2016).

2.8.2. Microbial inoculum preparation for consortium microorganisms

Each strain was grown as a pure culture. The isolates were inoculated in nutrient broth and grown to a stationary phase in a rotating shaker at 29 °C at 150 rpm. Individual suspensions at the same physiological phase ($1.75OD_{600}$) were pooled in equal proportions to set up inoculums for biodegradation. The cell densities of the inoculums were adjusted to 1.76×10^{11} CFU/mL for the biodegradability experiment.

2.8.3. Site selection and design

Given that the study was based on remediating microplastic-contaminated mangrove environments, Sementa mangrove located in Klang (2°54′38 N, 101°21′06″ E), Selangor State, was selected for field tests and bioremediation setup. A preliminary visit to the mangrove was conducted to identify a suitable site based on the tidal zones. The experiment was designed to investigate the degradation of microplastics buried under the sand that was kept wet with sea water. Thus, a mid-tidal zone was selected for the experiment. The mangrove soil used in the field biodegradation tests had the following characteristics: total organic carbon (3.8%), total alkalinity (12 ppm), organic matter content (11.4%), percentage of chlorides (0.02%), moisture content (51.6%), total nitrogen (15 ppm), salinity (1.99 ppm), and cation exchange (12.2 meq/100 g). The pH of soil was 7.4. This pH was found to be near optimal for hydrocarbon biodegradation and thus assumed to favor

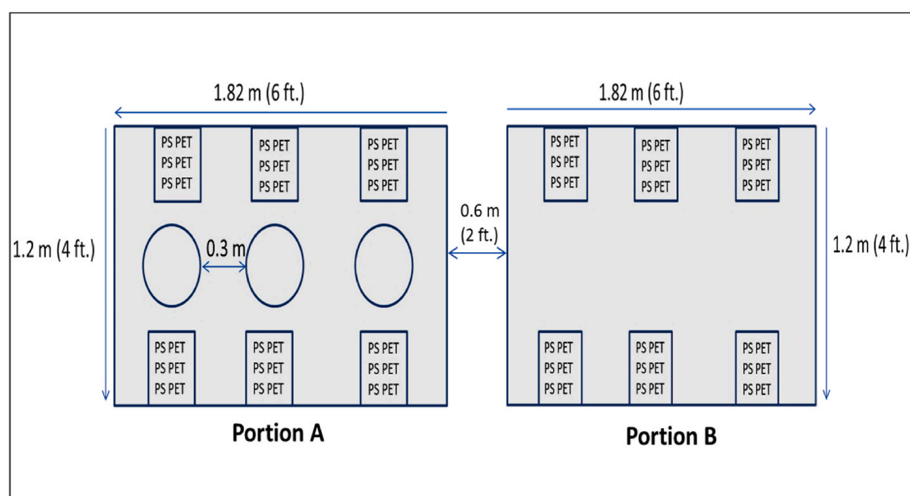

Fig. 3. Schematic diagram of in situ experiment design.

microplastic degradation (Yabannavar and Bartha, 1994).

The plain selected for the experimental setup was divided into two portions: portion A (amended portion) and portion B (non-amended control portion), with dimensions of 1.82 m (6 ft) × 1.2 m (4 ft) each, and a gap of 0.6 m (2 ft), as illustrated in Fig. 3. Injection wells made of perforated PVC pipes were installed (at 30 cm depth) linearly at 30 cm intervals in portion A. No installation of PVC pipes was conducted in portion B.

Individual suspensions at the same physiological phase (1.163 OD_{600}) were pooled in equal proportions to set up inoculums for bioremediation. Inoculums (18 L) were generated for application. The study assumed this volume to be approximately relative to the soil compartment of the selected areas. Therefore, the microbial cell concentration used for remediation was 3.49×10^{11} CFU/mL.

2.8.4. In-situ bioremediation of PET and PS

For amended and non-amended portions, microplastics were sewn into small bags made to prevent eventually forming microplastic fragments from falling apart. The material was non-biodegradable, with a 1.8 mm × 1.6 mm mesh. The bags were buried in triplicates at 10 cm depth in the mangrove soil, with a distance of approximately 2 cm between each bag and covered with soil (Tolinski, 2012). Consequently, the prepared inoculum was introduced into the injection wells (9 L), and the other 9 L was applied directly to the soil surface and allowed to penetrate/percolate the soil core. No inoculum was applied in the control portion (portion B). Monitoring activities were carried out at 15-day interval for a period of 90 days. For sampling, the mesh bags with microplastic specimens were taken out (in triplicates) to monitor biodegradation. Basic soil environmental properties (such as pH, salinity, temperature, and redox potential) were monitored. The microbial population in both portions was measured every 15 days. The microbial consortium formulated was introduced to the amended portion on each monitoring day.

2.9. Statistical analysis

Statistical analysis of all data was carried out using ANOVA in SPSS software 21.0, with the LSD post-hoc test at $p = 0.05$, to compare the means of variance, that is, to test the differences between the means.

3. Results and discussion

3.1. Isolation and identification

A total of Twenty bacteria were isolated from the different mangrove environments. The list demonstrated diverse genera of microbes that included aerobic gram-positive and gram-negative bacteria. The growth patterns were distinctive enough to enhance identification and differentiation into individual isolates. The isolated species belonged to 16 genera of *Bacilli*, 5 genera of Proteobacteria, and one genera of Actinobacteria. The microbes isolated reflected the native bacteria community found in mangrove environments (Basak et al., 2016; Saimmai et al., 2012). Akpan-Idiok and Solomon (2012), Behera et al. (2014), and Behera et al. (2014), also isolated *Bacillus* sp., *Pseudomonas* sp. (sulphate-reducing bacteria), and *Staphylococcus* sp. from mangrove soils. *Bacillus* was the most abundant genus isolated in this investigation. The result corroborates the results obtained by Castro et al. (2014) who reported the predominance of *Bacillus* sp. in their study of the bacterial diversity of Brazilian mangrove ecosystem, and the results obtained by Ando et al. (2001) who isolated vast number of *Bacillus* sp. from mangrove sediments in Japan. The microbes isolated were halophilic bacteria hence, their presence in mangrove soil was not surprising. The microbes have been reported to possess useful antibiotics, proteins, enzymes and salt tolerant genes, all of which have biotechnology significance (Castro et al., 2014; Thatoi et al., 2013). However, in order to identify the strains that possess degradation/deterioration potential on microplastics, bioassay screening on the isolates was carried out.

3.2. Screening of individual isolates for microplastic degradation

Nine bacterial isolates out of twenty two were capable of growing on BH media and utilizing the PET and PS polymers as carbon source. The isolates grew and indicated significant clear zones on the media within 5–9 days after incubation. This finding indicated that the microbes could depolymerize the polymer, which is usually the first step of biodegradation, as reported by (Shah et al., 2008). The clear zones observed could have been due to the hydrolysis of the polymer materials by the microbes as a result of the extracellular enzymes excreted by the microbes, which diffused through the agar and degraded the polymers into water soluble materials. The screening assay for microplastic degradation is presented in Table 2. As shown in Table 2, *B. cereus*, *A. faecalis*, *B. sonorensis*, *S. epidermidis*, *B. vietnamensis*, *R. ruber*, *B. flexus*, *S. globispora*, and *B. gottheilii* demonstrated significant clear zones. Thus, they were selected for the biodegradation study. The ability of *B. gottheilii* to degrade all four different microplastic types compared with other isolates was also observed. All microbes with the ability to degrade microplastics in this study are Gram-positive organisms, except for *A. faecalis*, which is a Gram-negative organism. Most of these bacterial species have been reported to be potential producers of biosurfactants that facilitate the assimilation of pollutants, especially those

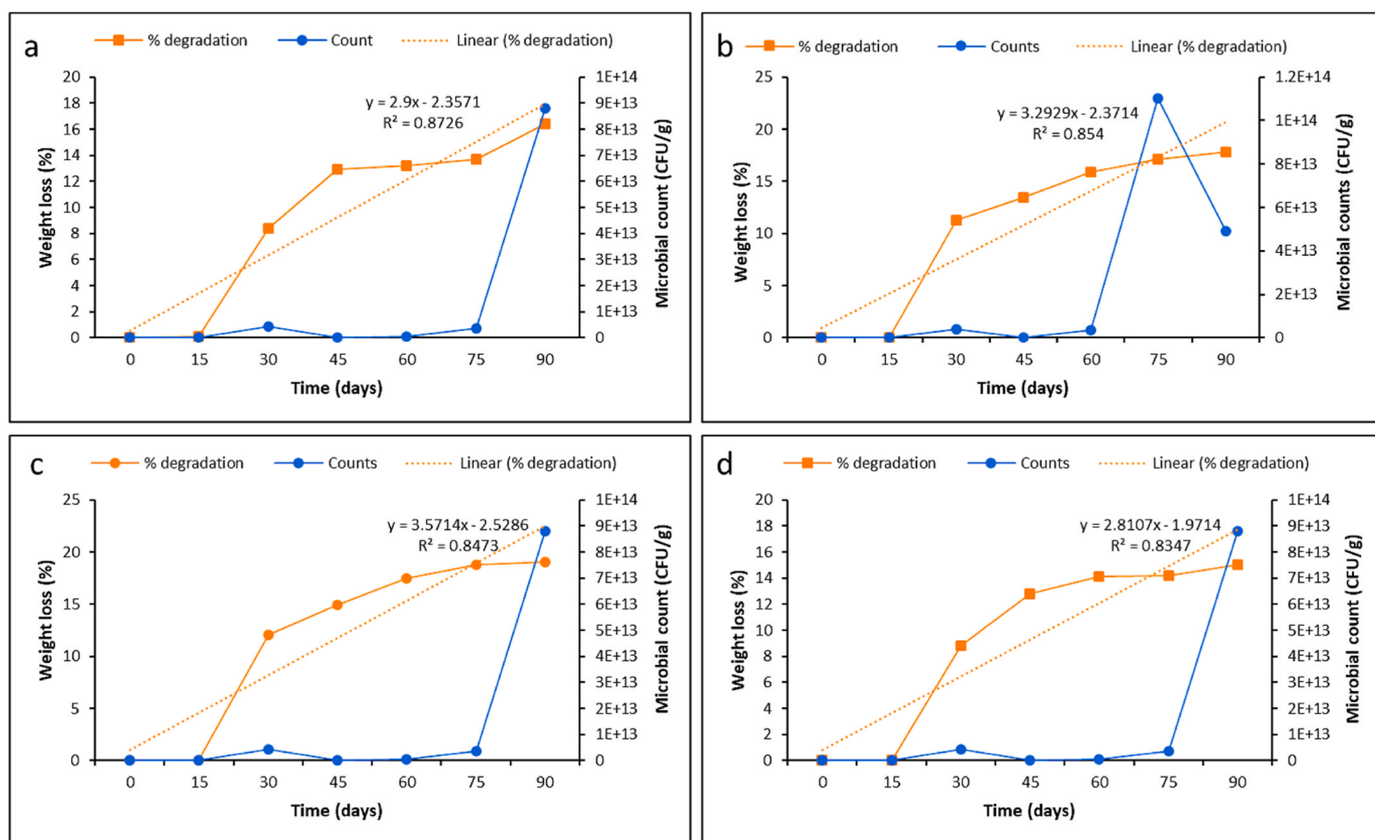

Fig. 4. Relationship of microplastic degradation (%) and bacterial cell numbers in each treatment during the 90 days bioremediation study. (a) PET microplastics in control (unamended) soil, (b) PET microplastics in amended soil, (c) PS microplastics in control (unamended) soil, (d) PS microplastics in amended soil.

of hydrocarbon origin (Saimmai et al., 2012). As such, their utilization in polymer biodegradation was expected to show metabolic potential.

3.3. Bioremediation of PET and PS microplastics

3.3.1. Determination of weight loss of PET and PS microplastics

The overall results of the microplastic degradation at the marked plots showed varied degrees of weight loss after 90 experimental days (Fig. 4). Even the control experiment showed bio-reduction for PET and PS, demonstrating that the Sementa mangrove soil exhibited a natural ability to remediate itself from microplastic pollution. The degradation trend exhibited by the microplastics differed between PET and PS under *in-situ* conditions.

For PET microplastic assessment, the weight loss for control was 16.4%, while that for treated plot was 18%. The reduction was 2.0% higher than that in control. Despite the 1.6% difference, a significant difference existed ($P < 0.05$). Such reductions could have probably been induced by the microbial interaction with the microplastics. Considering the augmentation performed on the experimental plot, some synergy between the introduced consortia and indigenous microbes seemed to have induced a degradation effect on PET compared with the level found in the control experiment. Similarly, the isolates used to formulate the consortia were originally isolated from mangrove soil. This finding may justify the reason for the reduction in PET microplastics recorded in the control plot. However, in some cases, microbes in the polluted environment may not have enough metabolic strength to remedy the ecosystem, except when amended with respect to diversity and concentration (Brenner et al., 2008). Furthermore, some other environmental factors possibly influenced the results, especially pH.

A similar evaluation of bio-reduction in PS microplastics under the same environment did not yield exact results for PET microplastics, possibly due to microbial specificity with environmental conditions. With respect to the overall weight loss, the control experiment showed better performance in PS microplastics degradation. It recorded 19%, while only 15% was recorded in the amended portion, implying a 4% difference in weight loss. Therefore, environmental conditions may clearly influence microbial concentration in a unit area but differently direct their metabolic complex interactions, especially with pollutants. The reason for this finding is subjected to the fact that PET microplastics were buried in the same plot with PS and expected to experience the same effect from the prevalent microbial concentration. Therefore, the lesser degradation recorded for PS suggested the influence of some other factors. The observed weight loss within the control plot could be attributed to the prevalent microbial community that was indigenous to

Table 1
Rate constant (k) in each treatment at different periods of the bioremediation experiment, n = 3.

Treatment	K value at certain periods of time (day^{-1})						Half life
	0–15 days	0–30 days	0–45 days	0–60 days	0–75 days	0–90 Days	($t_{1/2}$) after 90 days
Control (C) (PET)	0.000001	0.001	0.0018	0.0016	0.00163	0.0019	365
Bioaugmented (T) (PET)	0.0	0.0013	0.0016	0.0019	0.0021	0.0022	315
Control (C) (PS)	0.0	0.0014	0.0018	0.0021	0.00231	0.0024	289
Bioaugmented (T) (PS)	0.0	0.0010	0.0015	0.0016	0.0017	0.0018	385

Table 2
Screening test results for different microplastic degradation by bacterial isolates.

Organism	Control (C)	PS	PET
B. cereus	–	+++	+++
B. sonorensis	–	+	–
B. vietnamensis	–	+	+
S. globispora	–	++	++
A. faecalis	–	–	–
S. epidermidis	–	+	++
B. flexus	–	++	++
R. ruber	–	–	–
B. gottheilii	–	+++	+++

Strong (+++) = diameter ≥2.5 mm, moderate (++) = diameter between 1.0 and 2.5 mm, weak (+) = diameter <1.0 mm, no growth (–).

the mangrove environment. The population distribution during the 90 days showed fluctuation, often due to some other associated environmental factors, including pH, salinity, DO, and other factors that were not monitored in this study, such as nutrient enrichment, tidal distribution, and fauna capacity. The existing indigenous microbes probably had more selective activity on the carbon content of PS microplastics and thus were utilizing more PS microplastics. A notable detail that the Sementa mangrove environment had the ability to degrade PET and PS microplastics naturally over the 3 months of the study. This high intrinsic ability may have been caused by the environmental conditions, the possible presence of microplastic degrading microbes in the mangrove soil, and the microplastic characteristics.

The bioaugmentation employed in the degradation of PET microplastics was faster and higher in the treated plot than in the control experiment. This finding was demonstrated by the reduction rate, which was expressed as k value (Table 1), and the weight loss of PET microplastics. The k value on the 90th day of bioremediation studies was 0.0022/day higher than the 0.0019/day of PET in the control. On the contrary, the bioaugmentation treatment recorded lower k values (0.0018/day) than that recorded by the control in PS degradation (0.0024/day), demonstrating that the natural degradation of PS microplastics was higher. The calculated microplastic removal rate constant (K) and the corresponding half-life further supported the degree of activities within the aqueous medium. Results depicted that 0.0024 g of PS microplastic (Table 1) was removed or taken up by the isolates on a daily basis. Additionally, 315 days is required to reduce the PET microplastic polymer to its half while PS required 385 days to reduce to half. This removal rate might be from the genetic make-up of the isolate, which could discretely possess considerable polymer degradation capacity.

PET is hygroscopic, meaning that it absorbs water from its surrounding, and when heated, the water hydrolyzes the polymer, thus decreasing its resilience. This unique property of PET may have enhanced its degradation in mangrove soil, as demonstrated by the yellowing of the microplastics due to the formation of chromophoric systems (Adhikari et al., 2015). The high microbial counts observed in the present study may have also favored PET microplastic degradation. Similarly, the pH conditions of the soil observed in both portions during the *in-situ* experimental period may have played a role in promoting enhanced degradation of PET microplastics in mangrove soil. Though the control experiment indicated almost neutral pH, it recorded higher pH that was directed towards alkalinity. Thus, this could have promoted the metabolism of the pollutants in the experimental portion, leading to the production of high concentrations of potential degrading enzymes (not measured in this study) and eventually increased PET microplastic reduction. A positive correlation existed ($r^2 = 0.64$) when the pH level was compared with the weight loss of PET microplastics in the experimental portion. This finding could possibly justify the higher reduction in *in-situ* PET microplastic remediation than the laboratory experiment with aqueous system.

However, PS microplastic degradation was higher under laboratory conditions. Approximately 30.1% of PS microplastics was reduced in aqueous medium under laboratory conditions against 15% recorded in situ upon exposure to the same consortia. This study found that the pH level attained under laboratory experiment was more alkaline (pH 9), whiles the maximum pH recorded during the *in-situ* experiment was 7.78, which dropped to 7.41 after 90 days.

3.4. Microbial growth

Bacterial population was measured at every monitoring day during the bioremediation period. The counts across the monitoring days are presented in Fig. 4.

The results revealed that the microbial population density in both treatments varied during the experimental period. The total cell counts in the control portion (1.2×10^{10}–8.8×10^{13} CFU/g) were lower than the counts in the bioaugmented portion (1.8×10^{10}–1.1×10^{14} CFU/g). A lag phase was observed in both treatments in the first 15 days, probably indicating a period of acclimatization of the microbes and their inability to adhere and colonize the PET and PS microplastics. Thus, no weight loss was recorded at this period, except for PET in the control portion, which recorded a weight loss of 0.1%. An exponential phase of growth occurred between 15 and 30 days in treatments. At this period, a reduction in the weight of PET and PS microplastics was observed, indicating that after acclimatization, utilization of the microplastics by the microbes began, and this allowed the synergy between the microbial consortium and the existing indigenous microbes. A higher count (4.4×10^{12} CFU/g) was recorded in the control plot than in the augmented plot, indicating that the microbes were probably utilizing the microplastics for growth. In addition, despite the addition of inoculum, the microbes were still trying to establish a defined interaction with the existing indigenous microbes. The growth of the bacterial cells was concomitant with the reduction in PET and PS microplastics. The correlation between PET and PS microplastic degradation and the population density of the microbes in the control and augmented soil is presented in Fig. 4. The results revealed a strong positive correlation between the degradation of PET and PS microplastic and the microbial population density in the control and augmented soil during the experimental period, with R^2 values ranging from 0.83 to 0.87. Highest correlation ($R^2 = 0.87$) was recorded between PET microplastic degradation and microbial growth in the control soil, indicating that the percentage correlation was approximately 87%. On day 45, the cell densities from both treatments were reduced to 1.1×10^{10} and 1.8×10^{10} CFU/g for control and augmented portions, respectively, possibly due to changes in the environmental conditions in the mangrove soil.

The bacterial population in the augmented and control treatments exhibited the highest numbers of colonies on days 75 and 90, respectively (Fig. 4). These counts were higher than those recorded by Kumar et al. (2007) and Kathiresan (2003) in mangrove soils from Suva, Fiji Islands, and in India, respectively. However, such variations could occur between different geographical locations owing to differences in the environmental parameters. The high cell load reported in the present study is possible as the Sementa mangrove soil was rich in nutrients and organic matter, the content of which was approximately 11% and may have contributed to the proliferation of the microbes. The organic matter in soil has been reported to influence the activity of microbes and enzyme production (Nowak et al., 2016; Salazar et al., 2011).

3.5. Environmental conditions

The temperature of the mangrove soil across the monitoring days ranged from 25.9 °C to 28.4 °C in the control plot and 26.5 °C–28.4 °C in the bioaugmented plot, while the salinity ranged between 1.72–3.4 and 1.99–3.4 ppt, respectively. The availability of oxygen in soil declined during the experiment, not only in the bioaugmented treatment mesocosm but also in the control mesocosm. However, the depletion rate in the augmented plot was lower than that in the control plot.

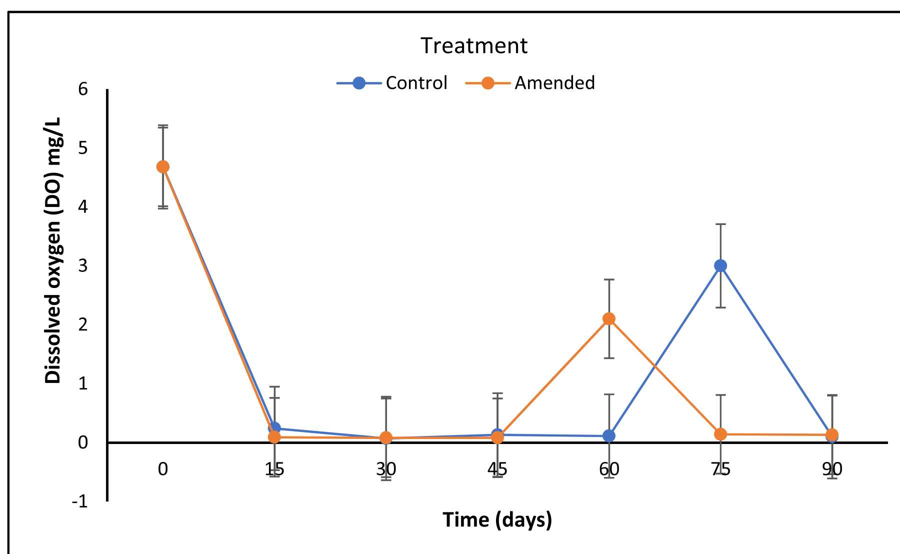

Fig. 5. Changes in dissolved oxygen (DO) content in each treatment across the experimental monitoring days (n = 3 for each sampling time).

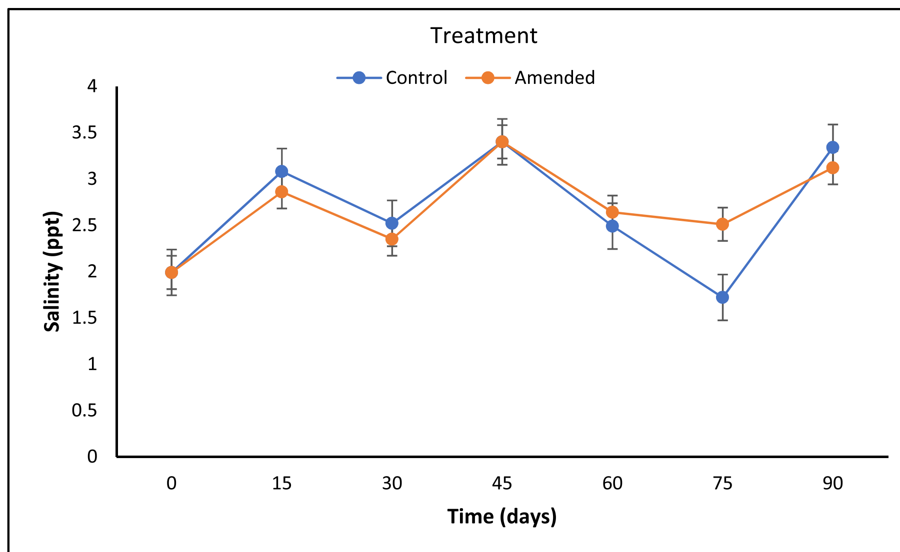

Fig. 6. Changes in mangrove soil salinity across days in amended and un-amended (control) mangrove soil during bioremediation studies (n = 3 for each sampling time).

Fluctuation in DO values in the control and augmented soil plots was observed throughout the experimental period. The DO became stable (0.08 mg/L) in the augmented plot on days 30 and 45. The control soil recorded the lowest average DO value of 0.07 mg/L on day 30, while the augmented soil had its lowest DO values (0.08 mg/L) recorded on days 30 and 45 (Fig. 5).

DO is necessary for the survival and proliferation of microbes in the aquatic environment (Spietz et al., 2015). The DO values of the control and augmented soils increased on days 75 and 60, with DO values of 3.0 and 2.1 mg/L, respectively.

The increase in DO values on these monitoring days may have been the result of wave action and contributed to the increase in cell counts recorded on similar days. Darmayati et al. (2015) recorded similar fluctuation in DO in their study of the effect of biostimulation and biostimulation-bioaugmentation on oil degradation on sandy beaches by using mesocosms.

The salinity of soil in the control and amended portions varied during the experiment, with ranges of 1.72–3.4 and 1.99–3.4 ppt, respectively (Fig. 6). The lowest salinity value was observed on day 75 for the control, while the augmented soil recorded lowest salinity levels on the initial day of the experiment. This low salinity values may have resulted from rainfall. Higher salinity levels in both treatments were recorded on day 45. This increase in salinity may have been responsible for the decrease in bacterial growth observed in the growth profile (Ryan, 2017). However, a decrease did not affect PET microplastic degradation, as the rate of degradation of the microplastics in the augmented plot increased on day 45 compared with that in the control. The variations in salinity observed during the experimental period could have resulted from the relative amount of precipitation (by rain) or evaporation in the mangrove environment. Other factors that may have contributed to the variations include tidal fluctuations and freshwater runoff into the mangrove environment. The salinity changes in this study were under the tolerable level for marine bacteria, as explained by the significant growth demonstrated by the microbes during the experimental period.

pH is one of the major selective environmental factors that affect microbial growth and activity, nutrient availability, transport process,

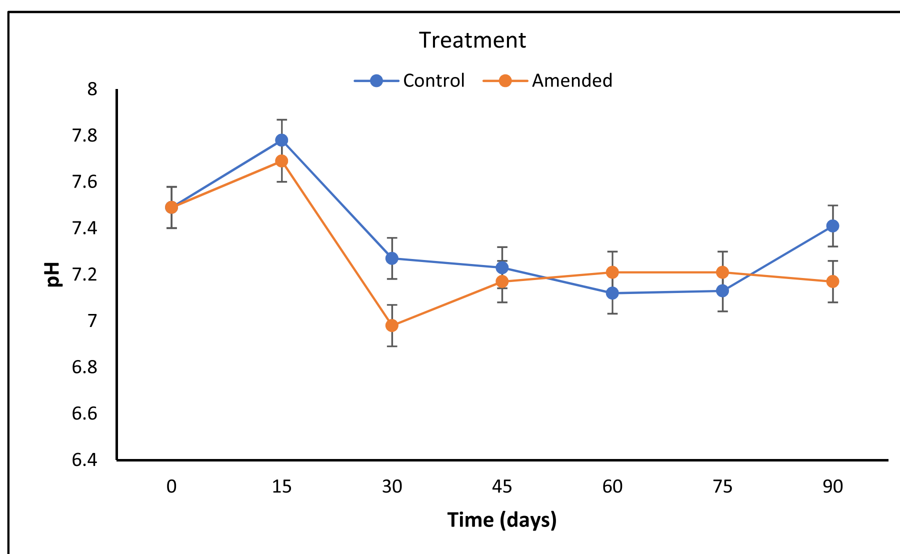

Fig. 7. pH changes across days in amended and un-amended (control) mangrove soil during bioremediation studies.

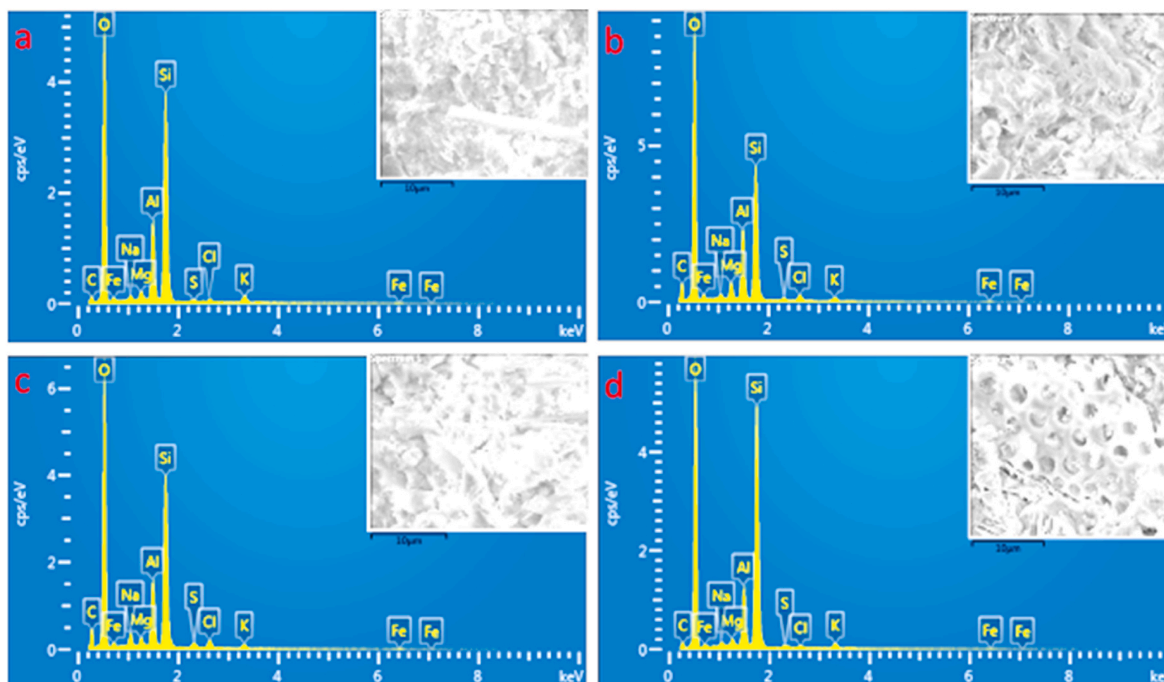

Fig. 8. a, b, c, d: SEM photograph and corresponding EDS spectrum of (a) control soil before bioremediation, (b) control soil after bioremediation, (c) microbially amended soil before bioremediation, (d) microbially amended soil after bioremediation.

and enzyme activity (Dhote et al., 2010). The periodic pH changes that occurred in the control and augmented soil across the monitoring days during the bioremediation experiment are presented in Fig. 7.

After 15 days, the pH values of both treatments increased (pH 7.49–7.78) for control soil and (pH 7.49–7.69) amended soil without a corresponding change in the weight of the buried microplastics. The changes in pH may have been the result of the ammonification of nitrogenous components present in the soil by the microbes (Esmaeili et al., 2013). Day 30 witnessed a drop in pH values and a significant weight loss of PET and PS microplastics in both treatments, with the highest loss of PET recorded in the augmented plot. The decrease in pH values could be attributed to the production of organic acids during microplastic degradation.

The rate of PET hydrolysis has been reported to be higher under acidic or basic conditions and results in the formation of alcohol functional groups and carboxylic acid end groups (Gewert et al., 2015). Darmayati et al. (2015) recorded similar changes in pH values in their study of the degradation of oil in oil-polluted sandy beaches by microbial consortium. The decrease in pH values may have also favored microbial proliferation, as the number of microbes in both treatments increased on day 30, depicting that the pH attained was possibly optimum for the degradation of the microplastics in both treatments. The pH in both treatments became stable on days 60 and 75, after which the pH in the control soil increased (pH 7.41), while that in the augmented soil decreased towards neutrality (pH 7.17). For the microbes to degrade PS microplastics, they require a more alkaline condition as opposed to PET microplastics, which appeared to have been responding to neutrality. This finding could be justified by the fact that a higher rate of PS

Table 3
EDS analysis of mangrove soil before and after bioremediation.

Elemental composition	Control soil		Amended soil	
	Before bioremediation (Wt %)	After bioremediation (Wt %)	Before bioremediation (Wt %)	After bioremediation (Wt %)
C	7.18	17.34	10.47	6.42
O	48.08	42.72	43.99	43.79
Na	0.75	0.69	1.46	1.37
Mg	0.91	1.32	0.99	0.64
Al	10.54	9.05	9.59	9.29
Si	26.16	24.70	24.26	28.67
S	0.57	0.50	0.93	3.82
Cl	0.75	1.09	2.21	0.53
K	2.26	1.98	1.97	1.81
Fe	2.82	2.05	4.14	4.42

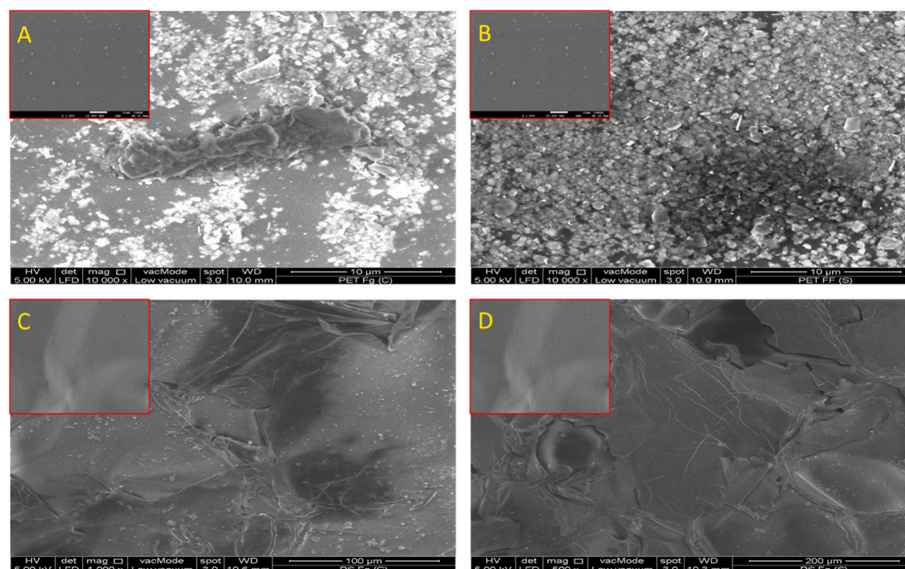

Fig. 9. SEM micrographs of PET and PS microplastics before and after bioremediation in mangrove soil. (a) Un-amended (control) PET microplastics (b) amended PET microplastic (c) un-amended PS microplastics (d) amended PS microplastics.

microplastic degradation was observed on days 15–45, when the pH values drifted towards alkalinity, while a higher rate of PET degradation was observed when the pH declined towards neutrality.

SEM coupled with energy-dispersive X-ray spectroscopy (EDS) was used to analyze the elemental composition of the mangrove soil before and after bioremediation. The EDS spectra showing peaks corresponding to different elements in the control and treated soil samples before and after bioremediation are presented in Fig. 8, while the elemental composition in terms of weight is presented in Table 3. The analysis showed that both soils before treatment were enriched with organic and inorganic elements, such as carbon, oxygen, silicon, aluminum, potassium, magnesium, sodium, chlorine, sulfur, and iron. Fig. 8a and b demonstrate that when the elemental compositions were compared before and after bioremediation, the concentrations of C, Mg, and Cl in the control soil increased, whereas those of O, Na, Al, Si, S, K, and Fe decreased at the end of the experiment.

3.6. Condition of augmented soil after bioremediation

The augmented soil showed increased concentrations of Si, S, and Fe, while the concentrations of C, O, Na, Mg, Al, Cl, and K decreased after bioremediation (Fig. 8d) compared with those recorded before bioremediation (Fig. 8c). The decreased Si and O content possibly indicated increased microbial biomass and provides insights into the ability of the microbes in changing the elemental composition of microplastic-contaminated soils during biodegradation. The concentrations of alkaline earth metals, such as Na and K, decreased in both treatments after bioremediation, while the concentration of Mg in the control soil increased compared with that in the augmented soil. Conclusively, the study demonstrated a slight change in the concentration of most of the elements in both soils after the bioremediation process.

The SEM micrographs of PET and PS microplastics before and after 90 days of *in-situ* bioremediation are presented in Fig. 9. Before the experiment, the samples had smooth surfaces, with no defects observed (Fig. 9a–d intercepts). However, after 90 days of bioremediation, cavities, distortions, cracks, irregularities, surface erosion, and fissures were observed on the surface of the microplastics, revealing the disruption of the surface texture of both microplastics. The micrographs similarly demonstrated the occurrence of several non-uniformly-scattered whitened and eroded areas (Fig. 9a and b), illustrating the surface erosion mechanism involved in the degradation of microplastics, which may have been due to the catalytic action of the enzymes produced by the microbes. SEM characterization by Bhatia et al. (2014) also revealed a similar result in their study of LDPE degradation.

The weight reduction took place in PET and PS microplastics, indicating degradation activity. The reduction could have been due to the oxidative or hydrolytic cleavage of the ester or amide bonds. This finding suggested the ecological nature of mangrove soil in comparison with liquid synthetic medium. It also revealed that mangrove soil could be a source of factors responsible for good PET microplastic degradation. Such factors may include moisture, heat, microbes, and salinity. Mangrove soil becomes heated during low tides upon exposure to

sunlight and due to the exothermic reactions of biological compounds in soil; it maintains moisture by tidal water flooding during high tides (Kathiresan, 2003).

3.7. Conclusion

The indiscriminate and absence of limited land spaces for safe disposal of plastic wastes makes plastic waste pollution a serious problem in recent years. A safe and eco-friendly approach called biodegradation is a necessary to degrade plastic waste. This study showed a change in PET and PS microplastic composition by using EDS and SEM. These changes occurred in the structure and chemical composition of the microplastics in the mangrove environment. Similarly, the weight reduction in PET and PS microplastics showed variations on their surface and biofilm formation. SEM also showed a decrease in the carbon content of plastic in comparison with that in controls. With respect to future research through stimulation and engineered microbial augmentation, these organisms could be a potential solution for *in-situ* plastic remediation.

Author contribution

This work was carried out in collaboration between all authors. Author HSA anchored conceptualization, data curation, formal analysis and funding acquisition. Authors OPA, JDB and SAA handled the Investigation, Methodology and Writing -original draft, writing-review and editing and Project administration while authors VIC, AAH, AZ and SHF took care of resources, software, supervision, validation and visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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