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Effects of Soil Burial on Polythene Film Degradation and Associated Bacterial Species

[*1]James, I. I., [1]Bassey, H. O., [1]Ekong, E. A., [2]Akan, O. D., [3]Ufot, U. F., & [2]Iwatt, M. U.
[1]Department of Biological Sciences, Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene LGA, Akwa Ibom State, Nigeria
[2]Department of Microbiology, Akwa Ibom State University, Ikot Akpaden, Mkpat Enin LGA, Akwa Ibom State, Nigeria
[3]Department of Biochemistry, Akwa Ibom State University, Ikot Akpaden, Mkpat Enin LGA, Akwa Ibom State, Nigeria

***Corresponding author email:** jamesinime@gmail.com

Abstract
This study investigated the bacterial degradation of polythene in soil. Polythene bags were obtained from a commercial store, and buried in soil for 45 days (P1) and 90 days (P2). An unburied control (P0) was retained. Soil from the polythene-buried site (S1-S5) and adjacent pristine soil (C1-C5) were obtained for bacteriological analysis. Total heterotrophic bacterial (THB) count of soil samples (C and S) was analyzed using pour plate method on nutrient agar plates. Polythene-degrading bacterial (PDB) counts were obtained after enrichment in mineral salts medium. Polythene degradation was evaluated using Scanning electron microscopy (SEM) and Fourier transform infrared (FT-IR) spectrometry. THB ranged from 1.39 ± 0.02 105 CFU/g to 2.79 ± 0.04 105 CFU/g in pristine soil samples. PDB counts were 1.73 ±0.03 102 CFU/g to 2.67 ±.05102 CFU/g. bacterial genera obtained included: Bacillus, Staphylococcus Enterobacter, Micrococcus Pseudomonas Escherichia, Proteus and Alcaligenes species, with occurrences of (%): 18.9, 9.4, 13.4, 4.7, 12.7, 13.5, 21.7 and 5.7 in pristine soil. Escherichia coli, Proteus and Alcaligenes were not observed in plastic debris soil. Variations in the PDB counts and THB counts suggest that a small but significant population of bacteria in the soil can adapt to exposure to polythene burial, becoming capable of polythene degradation. SEM images revealed deterioration of PE surface after burial, with large with cavities, cracks, and holes, consistent with biodegradation process. FT-IR spectra indicated biodegradation, with the disappearance of functional group peaks suggestive microbial metabolism of polythene additives and pro-oxidant, alongside the emergence of a new peaks suggestive of microbial colonization and attack of the oxidative polymeric metrics.

Keywords: Polythene, Polythene Degradation, Plastic Pollution

Introduction

Plastics are widely distributed today, making their use a direct and continuous source of environmental pollution (PlasticsEurope, 2016). Fossil-based feedstocks are the source of majority of plastics produced today (Ghasemglou, 2022). Commonly used plastics include: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyurethane (PUR), and polycarbonate (Alshehrei, 2017). The characteristics that make plastics enduring and versatile packing materials in the present industrialization, also makes them recalcitrant when polluting the environment. They are highly resistant to corrosion, chemicals, and temperature, with excellent durability, and high mechanical properties (Das & Kumar 2015). Methods fashioned at degrading plastic materials include; thermo-oxidative (Zeenat, et al., 2021), photo-degradation (Ali et al. 2016), and biological degradation, with biodegradation considered cost-effective (Kotova et al. 2021). However, reports for their breakdown in nature attributes success to synergistic actions of biotic and abiotic factors (Restrepo-Florez et al., 2014).

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Capable bacterial species such as *Bacillus cereus* and *Pseudomonas tuomurensis* have been reported to degrade polyethylene (Kopecka et al., 2022). Usha et al. (2011) showed that over the course of six months in liquid (shaker) culture, *Pseudomonas* sp. (37.09 and 28.42 %), *Streptomyces* sp. (46.16 and 35.78 %), and *Aspergillus* sp. (20.96 and 16.84 %) destroyed polythene and other plastic materials, respectively. Kathiresan (2003) reported the degradation of polythene bags by *Pseudomonas* sp. (20.54 %) and *Aspergillus glaucus* (28.80 % of polythene) within 30 days.

Nigeria is Africa's biggest importer of plastics in their primary forms (NESG, 2023). The local consumption of plastics has exceeded 7.5 kg per capita (Brandspur, 2017), generating about 2.5 million tonnes of plastic waste annually. With crude waste collection and management methods, less than 12 % of plastic waste is recycled, increasing its potential as a human and environmental health risk (Ashike, 2024). Microbes offers great potential for plastic pollution remediation without the attendant hazards of physical or chemical methods.

Aim and Objectives

This study aimed to assess the effects of 90-day soil burial on potential degradation parameters of polythene films, and determine bacteria species associated. The specific objectives were to:

i. Bury polythene bags in soil for 90 days
ii. Isolate, enumerate, characterize and identify total heterotrophic bacteria from pristine soil, close to the plastic-polluted soil zone using culture-dependent techniques,
iii. Isolate, enumerate, characterize and identify potential polythene degrading bacteria from plastics-polluted soil using culture-dependent techniques.
iv. Determine potential degradative effect of soil burial on polythene films, using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR).

Materials and Methods

Study area

The study was carried out at the Akwa Ibom State Polytechnic, Department of Biological Sciences Biological Garden, Ikot Osurua, Ikot Ekpene, Akwa Ibom State (GPS coordinates 51°56'64.6" N 76°67'86.3" E)

Soil burial of PE films

Open-air soil test was conducted using the procedure of Mastalygina et al. (2022). Rectangular 1.5 cm × 1.5 cm pieces of PE material, were cut from the polythene bag using scissors. Five PE films each, were buried in three clearly marked plots in natural ground conditions at the Department of Biological Sciences Biological Garden, Akwa Ibom State Polytechnic, Ikot Osurua, at about 5 m depth. The soil had a pH of 4.0 to 5.5. Soil moisture was maintained between 30 and 40 % during the 3 months (August, September and October, 2025) period of the experiment. Average daily temperature within this period was 28.0 ^{O}C

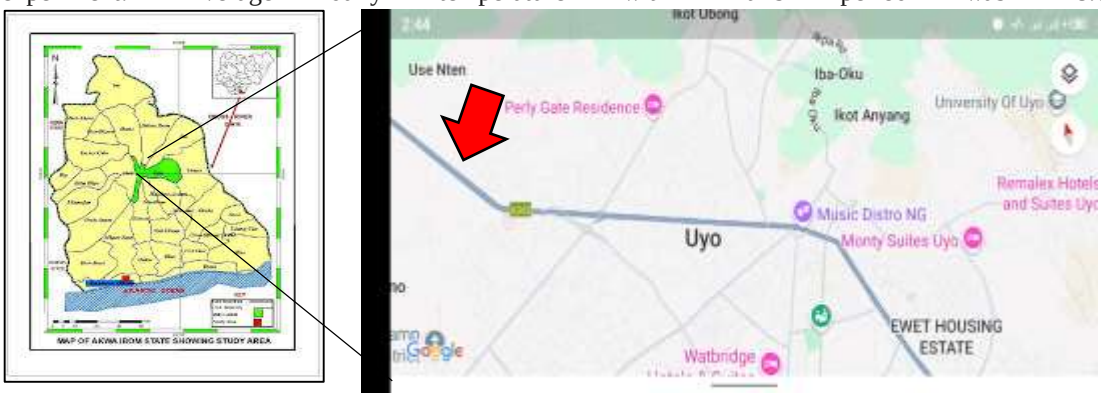

Fig. 1: (a) Map of Akwa Ibom State (b) Map of Uyo showing sampling location

Sample collection

A black polythene bag commonly used in consumer trade was obtained from a retail store in Uyo Metropolis, Akwa Ibom State. LDPE pellets were obtained from Plasticultured Nigeria. LDPE powder was prepared according to Bhatia et al. (2014), by boiling PE granules in xylene and boiling them for 15 min, and washing the ensued powder with 95 % ethanol. Washed LDPE powder was dried overnight in a hot air oven at 50 °C, and stored at room temperature for further use.

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Soil sample collection

Composite soil samples were collected randomly from five selected sampling points of the burial site. Soil was aseptically scooped from and adjacent to buried polyethylene materials. Five composite soil samples (CS 1, CS 2, CS 3, CS 4 and CS 5) unpolluted with plastic waste, about 10 m away from the plastic burial site was obtained at 5 cm depth using a sterile soil auger, and soil from five buried plastic debris (PS 1, PS 2, PS 3, PS 4 and PS 5) were sampled, to identify total heterotrophic bacteria (THB) and polythene-degrading bacteria (PDB) respectively. Buried polythene films, were recovered after 45 and 90 days of burial, washed in sterile water, and in 70 % ethanol and dried after recovery of soil debris. All soil samples were stored in sterile Ziploc bags and transported to the laboratory for further analysis.

Isolation and enumeration of total heterotrophic bacteria (THB) from soil

Exactly 1 ml from the 10^{-3}dilution factor of pre-sieved soil serial dilutions were plated out in Petri dishes containing 15 ml nutrient agar, and incubated at 35 ^{O}C for 24 hours. Colonies developed on the medium were enumerated, and sub-cultured to obtain pure cultures and preserved on nutrient agar at 4 °C.

Visual inspection of colonial appearance of colonies developed on the plates were carried out to identify the characteristics, using the method of Chesbrough, (2006). Biochemical tests were carried out to further characterize bacterial isolates, using the method of Chesbrough, (2006). Identification was done by comparing the colonial and biochemical features of each isolate with that of a known taxonomy in the Bergey's manual of determinative bacteriology (Holt et al., 1994).

Isolation and enumeration of polyethene-degrading bacteria (PDB) from soil

Potential PE-degrading bacteria were isolated and enumerated by plating soil dilutions onto mineral salts medium of Nademo et al. (2023) (g/L): K_2HPO_4, 0.2 g/L KH_2PO_4, 1 g/L $(NH_4)2SO_4$, 0.5 g/L $MgSO_4.7H_2O$, 1 g/L NaCl, 0.01 g/L $FeSO_4.7H_2O$, 0.002 g/L $CaCl_2.2H_2O$, 0.001 g/L $MnSO_4.7H_2O$, 0.001 g/L $CuSO_4.5H_2O$, 0.001g/L $ZnSO_4.7H_2O$ and pH 7.0 and 0.2 % (w/v) LDPE powder). Diluent from the fourth enrichment sample was used to obtain isolates on agar medium.

Evaluation of surface structure of PE films

Scanning electron microscopy (SEM) was used to characterize the surface structure and morphology of the control (P0), and buried polythene films (after 45 (P1) and 90 days (P2)) was characterized by using a Thermo Scientific Axia ChemiSEM integrating a tungsten electron source with an Energy Dispersive X-ray Spectroscopy (EDS) detector at the Ultra-High Technology Laboratory of the African Centre of Excellence, University of Nigeria, Nsukka, Enugu State, Nigeria.

Evaluation of chemical structure of PE films

Changes in chemical structure of the PE films was investigated using Fourier transform infrared spectroscopy (FT-IR) analysis to evaluate quantitatively the carbonyl group content changed during the testing of polythene films (control (P0), and buried polythene films (after 45 (P1) and 90 days (P2)).

Changes in functional groups was investigated using a Nicolet iS20 FT-IR spectrometer at wavelengths of $4000 \leq \nu \leq 400$ cm^{-1} at the Ultra-High Technology Laboratory of the African Centre of Excellence, University of Nigeria, Nsukka, Enugu State, Nigeria. The carbonyl index was using the equation:

$CI = A_{C=O}/A_{CH3}$

Where:

$A_{C=O}$ is the absorbance of the carbonyl stretching vibration,

A_{CH3} is the absorbance of symmetrical bending vibration of the methyl group, where the absorbance is nearly not changed during the test process.

Statistical analysis

Data was obtained in triplicates and analyzed using descriptive statistics, SPSS version 16.

Results

Sample	THB (x 10^5 cfu/g)	Sample	PDB (x 10^2 cfu/g)
CS 1	2.79 ± 0.04	**PS 1**	2.67 ±.05
CS 2	2.02 ± 0.04	**PS 2**	1.88 ±0.02
CS 3	1.80 ± 0.12	**PS 3**	1.99± 0.06
CS 4	1.82 ± 0.02	**PS 4**	2.07 ±0.03
CS 5	1.39 ± 0.02	**PS 5**	1.73 ±0.03

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Table 4.2: Morphological and biochemical identification of bacterial isolates

Isolate	Samples	Colonial morphological	Gram stain	Cell shape	Catalase	Urease	Motility	Citrate	Oxidase	Indole	Nitrate	Coagulase	Sugar fermentation			Probable organisms
													Glucose	Sucrose	Lactose	
1	CS 1, CS 2, CS 3, CS 4, CS 5, PS 1, PS 2, PS 3, PS 4, PS 5	Moderate, circular, creamy, rough, flat, opaque, dry	+	Rods	+	-	+	+	-	-	+	-	A	A	A	*Bacillus* sp
2	CS 1, CS 2, , CS 4, , PS 1, PS 2, PS 3PS 5	Creamy, small, circular, entire, flat, opaque, moist	-	Rods	+	+	+	+	-	-	+	-	A	A	-	*Enterobacter* sp
3	CS 1, , CS 3, CS 4, CS 5, PS 1, PS 5	Milky, moderate, circular, opaque, entire, flat, mucoid	+	Cocci	+	+	-	+	-	-	-	+	A	A	A	*Staphylococcus* sp
4	PS 1, PS 2, PS 4, PS 5,	Creamy, moderate, circular, opaque, entire, flat, dry	+	Cocci	+	+	-	-	+	-	-	-	AG	AG	A	*Micrococcus* sp
5	CS 1, , CS 3, CS 5, PS 1, PS 2, PS 3, PS 5	Creamy, irregular, moderate, rough, flat, dry, opaque	-	Rods	+	-	+	+	+	-	-	-	A	AG	-	*Pseudomonas* sp
6	CS 1, CS 2, CS 4, CS 5,	Whitish, gloss, round, entire, raised, butyrous	-	Rods	+	-	+	-	+	+	+	-	AG	AG	AG	*Escherichia coli*
7	CS 1, CS 2, CS 3, CS 4, CS 5, PS 1,	Irregular, smooth, undulated, convex, butyrous	-	Rods	+	-	+	+	+	+	+	-	AG	-	-	*Proteus vulgaris*
8	CS 1, CS 2, CS 3, CS 4,	Whitish, smooth, opaque	-	Rods	+	+	+	+	+	-	-	-	A	A	A	*Alcaligenes sp*

Table 4.3: Occurrence of bacterial isolates in soil sample.

Isolates	Percentage occurrence (%)	Percentage occurrence (%)
Bacillus sp	18.9	26.8
Enterobacter sp	9.4	11.0
Staphylococcus sp	13.4	17.5
Micrococcus sp	4.7	20.6
Pseudomonas sp	12.7	24.1
Escherichia coli	13.5	-
Proteus sp	21.7	-
Alcaligenes sp	5.7	-

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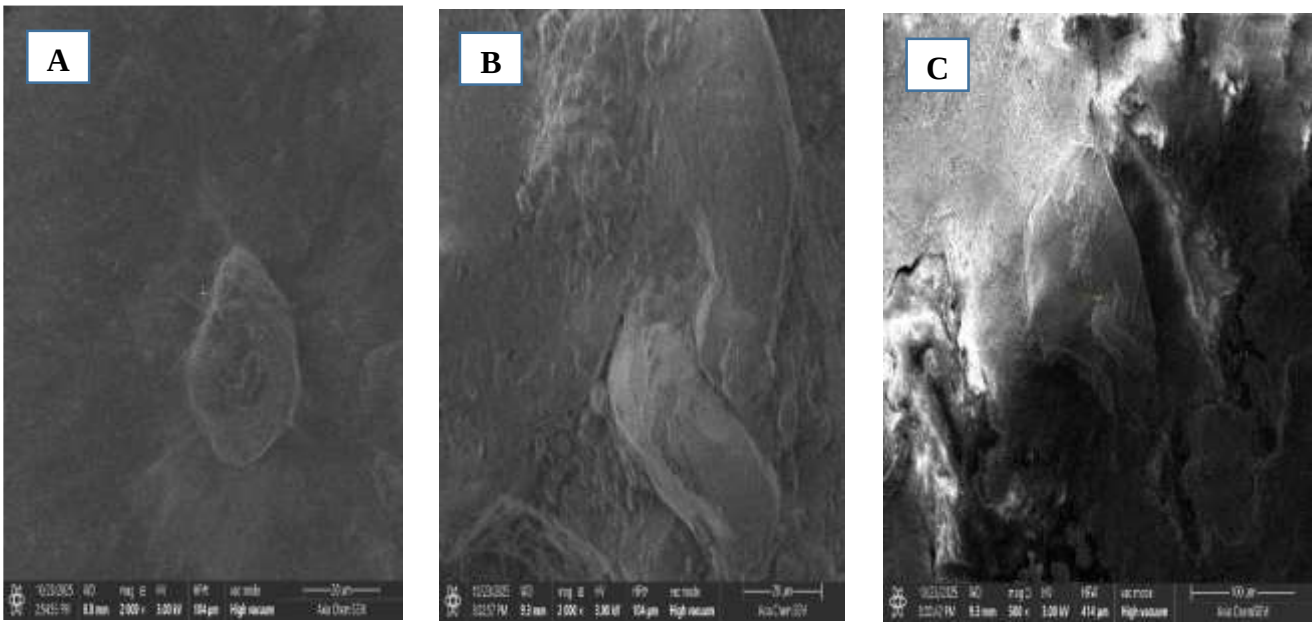

Fig. 1: SEM images of PE films (A) control (P0), (B) after 45-day soil burial (P1), (C) after 90-day soil burial (P2)

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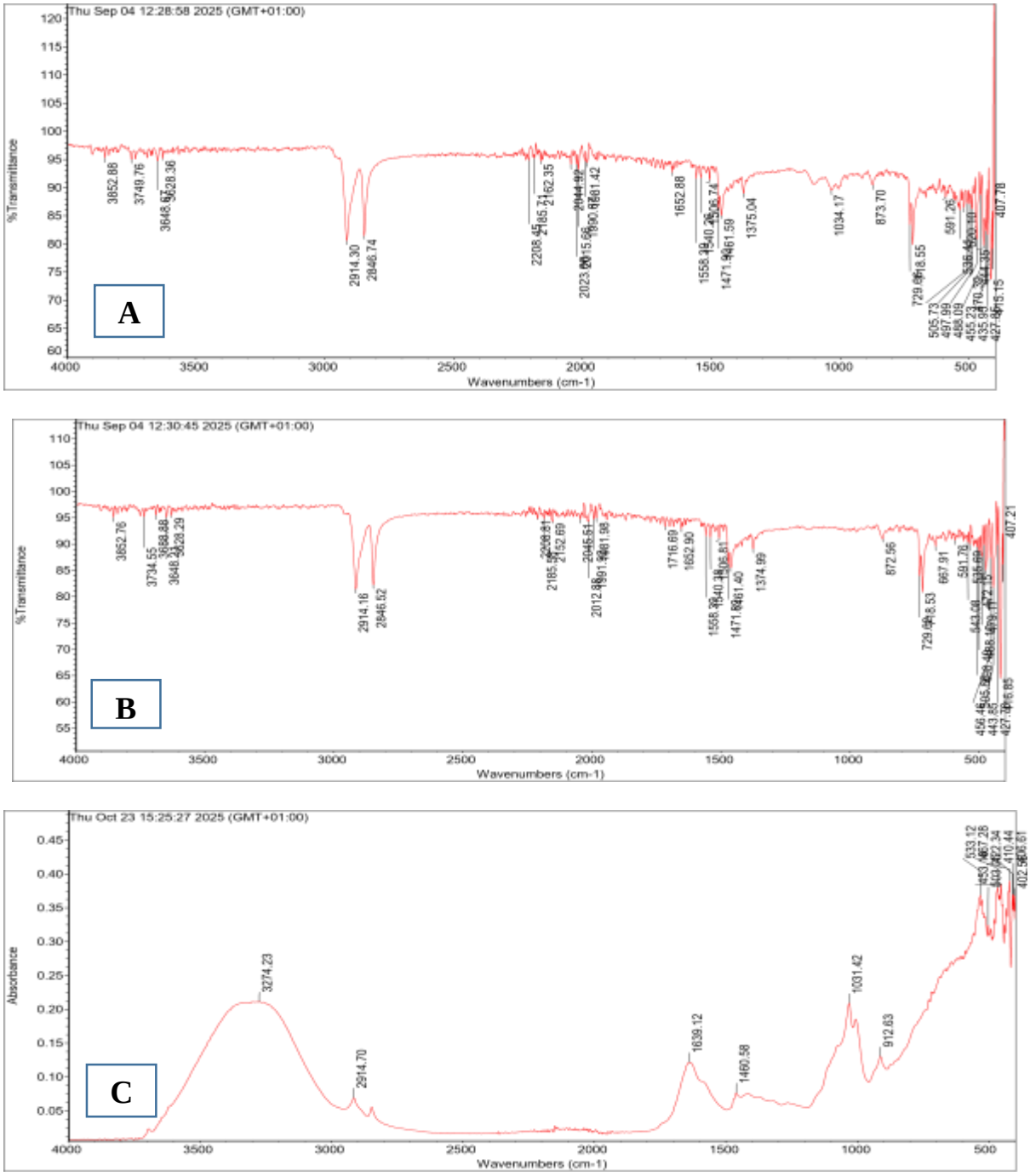

Fig. 1: FT-IR spectra of PE films (A) control (P0), (B) after 45-day soil burial (P1), (C) after 90-day soil burial (P2)

Discussion

Results for total heterotrophic bacterial revealed counts of 1.39 ± 0.02 10^5 CFU/g to 2.79 ± 0.04 10^5 CFU/g in pristine soil samples. Plastic-degrading bacterial numbers were 1.73 ±0.03 10^2 CFU/g to 2.67 ±.0510^2 CFU/g. THB counts were within the normal ranges for soils, suggesting that there was presence of an active microbial population. Usman et al. (2019) have reported total heterotrophic bacteria (THB) ranged from 1.69 x 10^5 CFU/g to 2.94 x 10^5 CFU/g in soil samples. MacLean et al. (2021) reported overall microbial diversity to be higher in reference soils than on plastic debris.

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The presence of polythene-degrading bacteria in the plastic polluted soil evidences the natural adaptation of microbes in the soil to the polyethene material buried in the soil. Variations in the PDB counts and THB counts indicate that a small but significant population are capable of polythene degradation. These result shows that natural bacterial communities in the soil have become adapted from the enrichment of the soil resulting in the potential plastic-degrading bacterial communities in the soil areas in contact with polythene materials. The differences in the PDB counts in the polythene-buried soils may signify different levels of exposure or contact with plastic material, as well as differences in soil parameters (nutrients, oxygen, moisture, etc.). Altogether, the presence of polythene degrading bacteria in the polythene-buried soil samples evidences the natural adaptation of soil microbial communities to the polythene material in soil, and holds promise for selection of polyethene degraders in polythene exposed soils.

Bacterial species isolated include: *Bacillus*, *Enterobacter* sp, *Staphylococcus aureus*, *Micrococcus* sp, Pseudomonas sp., *Escherichia coli*, *Proteus* sp, and *Alcaligenes* sp. Isolates such as *Escherichia coli*, *Proteus* and *Alcaligenes* were not observed in plastic debris soil. This agrees with the findings of Kathiresan, (2003) who reported strains of, *Pseudomonas* sp and *Staphylococcus aureus* amongst other bacteria. SEM analysis showed the structure of the control PE films to be heterogeneous and its surface smooth (Fig. 1a). Surface deterioration occurred in PE films after 45 day-, (Fig.1b) and 90 days-, (Fig.1c) burial in soil. The surface of the PE film after 45 days burial showed initiation of cracks, pits and small holes compared to the control sample. These changes are consistent with biodegradation process. Similar changes in surface structure have been reported in LDPE films by Dang et al. (2018) and Nademo et al., 2023). Intense deterioration of PE surface was noticed in films after 90-days burial, with large with cavities, cracks, and holes Gajendiran et al., (2016) has reported similar surface deteriorations and distortions after 90 days in LDPE films. Dang et al. (2018) have shown that the degradation of plastic can be directly inferred to bacterial colonization of the plastic materials, and utilizing it as the sole source of carbon

In this study, FT-IR was employed to determine changes in chemical structure of the PE material. FT-IR peaks show functional groups present in a compound. The disappearance of peaks has been used as an indication of the loss of functional groups due to transformation of additive oxidants in a substance, and appearance of new peak indicates oxidation and potential microbial actions or evolution of new substances in polythene (Rajendran et al., 2016). Peaks in the soil buried polythene film at 3688.88, 171.69, 667.91 cm^{-1}, where shown to be lost after treatment. These were present in the control sample (P0), but absent in the soil buried sample (P1). This shows that soil burial led to changes in chemical groups at those adsorptions. Peaks at position 3688.9 cm^{-1} are free hydroxyl stretches which occur within 3600-3700 cm^{-1}. So, the peak loss at 3688.88 cm^{-1}suggest removal of adsorbed water or surface hydroxyl contaminants as a result of thermal drying or reduction. After being buried in the soil, this additive was likely leached out (washed out) by soil moisture or degraded by microbial actions. Peak at 1716.17 cm^{-1} indicate very strong and definite carbonyl (C=O) stretching vibration. This indicate that the polythene film has undergone a small amount of oxidation during manufacture. Pro-oxidants are intentionally added to make polythene more susceptible to degradation. The disappearance of this peak suggests that the C=O groups are metabolized by microorganisms. This is a clear sign of biodegradation. Peaks at 667.91 cm^{-1} falls in the fingerprint region and is often associated with the C-H bending vibration. It could have been leached out or degraded. The new peak at 1034.17 cm^{-1} is characteristic of C-O stretching vibration in polysaccharides, very common for cellulose, starch or other complex sugars. This peak does not come from the polythene film itself but from microbial biofilm that have colonize the surface of the polyethene. Microbes (bacteria and fungi) secrete extra polymeric substances (EPS) which are rich in polysaccharides to anchor themselves on the surface of substances. The appearance of this peak is direct evidence of microbial colonization, a necessary step before microbial breakdown of polythene.

FT-IR spectra of P2 showed absence of crystallinity (peaks at 729 and 718 cm^{-1}) which were strong in the control PE film. There was also an abundance of low frequency bands (533, 503, 453, 422, 407, 402 cm^{-1}) in P2 which is often indicative of biodegradation residues, as well as oxidized fragments. This FT-IR result shows the spectrum of peaks as characteristic of polythene film, although containing additives including a pro-oxidant (1716.17 cm^{-1} and possibly 3688 and 667 cm^{-1}). When buried in soil, the film was exposed to moisture minerals and microbial community in the soil. Within a 45-day period pro-oxidants additives facilitated the initial oxidation of polymeric chain creation (carbonyl group) making the polymeric substance more hydrophilic and susceptible to microbial attack. Microorganisms had therefore begun to breakdown the polymeric substances, consuming the easily accessible additives leading to disappearance of peaks at 3688, 667 cm^{-1} in the treated sample (in P1), as well as further loss of crystallinity (in P2). Mastalygina et al. (2022), have however not detected

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any degradation signs of the polymer matrix in carrier bags buried in soil in Russia over a 3-month period. Only degradation of filler material, presumably starch was observed in their FTIR data

Microorganisms have been also been shown to enzymatically clear the oxidant path of the polymeric chain leading to disappearance of peak at 1716.17 cm^{-1}. This FT-IR data therefore offers strong proof, that soil burial processes can contribute to the biodegradation of polythene films, via leaching and degradation of additives through microbial colonization and attack of the oxidative polymeric metrics.

Conclusion

This study revealed the potential for adaptation of microbial communities in soil for plastics degradation upon exposure to plastic waste. SEM and FT-IR analysis show evidence of capability of indigenous soil bacteria in the biodegradation and oxo-biodegradation of polythene films within a 45-90-day period. Polythene films showed more intense deterioration after 90 days of burial in soil. The result holds potential for discovery of potential bacteria capable of polythene degradation, as well as development of more environmentally friendly plastic products.

Conflict of Interest

The study received fundings from the 2024 (Merged) TetFund Intervention in Research Project (RP)

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