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### Research Article

## Isolation and Identification of Low Density Polyethylene Degrading Bacteria from Selected Dumpsites in Maiduguri, Borno State

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### ABSTRACT

Low density polyethylene (LDPE) along with other synthetic plastic is non-biodegradable and poses a significant threat to the environment. This study aimed to screen and identify indigenous bacterial isolates capable of degrading LDPE. The study involved determining some physicochemical components (organic matter, moisture content, pH, electrical conductivity and temperature) of soil samples collected from five selected dumpsites within Maiduguri Metropolis, Borno State. A total of 12 bacterial isolates were identified using morphologic and biochemical test according to standard protocols. The percentage of weight loss and structural changes in the LDPE was determined using weight loss experiment and Fourier Transform Infrared (FTIR) analysis respectively. From the result of the study, bacterial strains including *Pseudomonas aeruginosa*, *Providencia rettgeri*, *Flavobacterium* sp. and *Bacillus* spp., were the common isolates that showed LDPE degradation abilities. In addition, *Pseudomonas aeruginosa* and *Providencia rettgeri* exhibited significant (25%) while *Bacillus* sp. and *Flavobacterium* sp. exhibited (9%) LDPE degradation capabilities and the FTIR analysis that showed peaks shifting, indicates bond stretching, bond bending, and new bond formation. It was concluded therefore, that the high LDPE degradation by *Pseudomonas aeruginosa* and *Providencia rettgeri* may partly reflect their potentials in bioremediation strategies for plastic waste in land fields and dumpsites found in Maiduguri metropolis.

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## INTRODUCTION

Polyethylene is one of the most abundant commercially-produced synthetic plastic materials. It is a polymer of ethylene, ( $\text{CH}_2\text{-CH}_2$ ), having the formula  $(\text{-CH}_2\text{-CH}_2\text{-})_n$ , where “n” is the number of carbon atoms (Sandhu *et al.*, 2019). Plastic materials have grown to become indispensable in all aspects of human endeavors in the last 50 years, replacing materials such as glass, metals and wood in different applications as a result of their low cost, durability and high strength. Hence, global plastic production has tripled in the last twenty five years (Feil and Pretz, 2020). At least, half of the mostly produced plastic is used for short term applications such as packaging or producing single used products that are disposed immediately or several weeks after their application (Kumar *et al.*, 2021). Among the polyethylene family, Low-density polyethylene (LDPE) accounts for 60% of the total production of plastic bags, and it is the major component of municipal solid waste (Gajendiran *et al.*, 2016). Due to its high molecular weight, long carbon chain backbone, three-dimensional structures, hydrophobic nature, and lack of functional groups recognizable by microbial enzyme systems, LDPE is very resistant to biodegradation (Emmanuel *et al.*, 2022). The extensive usage of LDPE posed severe environmental threat to terrestrial and marine ecosystems. Plastic waste disposed of into the environment entangles the animal or bird's body, thereby causing the mortality of the organism (Kumar *et al.*, 2021). It also blocks the sewage system and creates a breeding ground for mosquitoes (Ahmedgajen *et al.*, 2014). Improper disposal of plastic waste can result in lost revenue from tourism by deteriorating the natural beauty of the environment (Ahmedgajen *et al.*, 2014). Plastic materials dumped into the earth prevent the production and mobilization of nutrients in the soil (Gharahi and Zamani, 2022). Some plastic products cause human health problems by causing immune and enzyme disorders, hormonal disruption, carcinogenicity and even infertility (Gasperi *et al.*, 2018). Currently, little efforts have been embraced to ameliorate the pollution caused by plastics especially in less developed countries (Jambeck *et al.*, 2015). Even the little efforts in place are

not without setbacks including environmental pollution. However, biodegradation is the best method as compared to the others because it is cheap and does not generate harmful pollutants into the environment (Prather, 2020). Biodegradation is a process whereby microorganisms break down organic substances into smaller compounds through metabolic or enzymatic processes (Emmanuel and Akinyemi, 2022). Organic substances that can be biodegraded include polystyrene, polyethylene, polyvinylchloride, and other plastic products (Bakht *et al.*, 2020). Through the process of degradation, hazardous toxic materials are made less toxic or non-toxic (Patil, 2018). Microorganisms have been isolated from various sources showing the ability to degrade polyethylene waste (Azeko *et al.*, 2015). Some of the important bacteria reported for the biodegradation of LDPE includes, *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp., *Rhodococcus* sp., *Acinetobacter* sp., *Brevibacillus* sp., *Flavobacterium* spp., *Ralstonia* spp., *Staphylococcus* spp., *Stenotrophomonas* spp., *Micrococcus* spp., *Microbacterium* sp. and *Nocardia* sp. (Harshvardhan *et al.*, 2013). However, the success rate is not satisfactory for the biodegradation of polyethylene. Accordingly, exploring microorganisms from different environments is essential to discover strains with strong potential for polyethylene degradation (Singh and Gupta 2016). The complex physical, chemical and biological conditions present in solid waste environments suggest that such site hold great promise for isolating bacteria capable of degrading low density polyethylene (Harshvardhan and Jha 2013). Therefore, This study aimed to isolate and identify bacteria from selected dumpsites in Maiduguri that have the potential to biodegrade LDPE, thereby contributing to sustainable waste management practices and mitigating the environmental impact of plastic pollution.

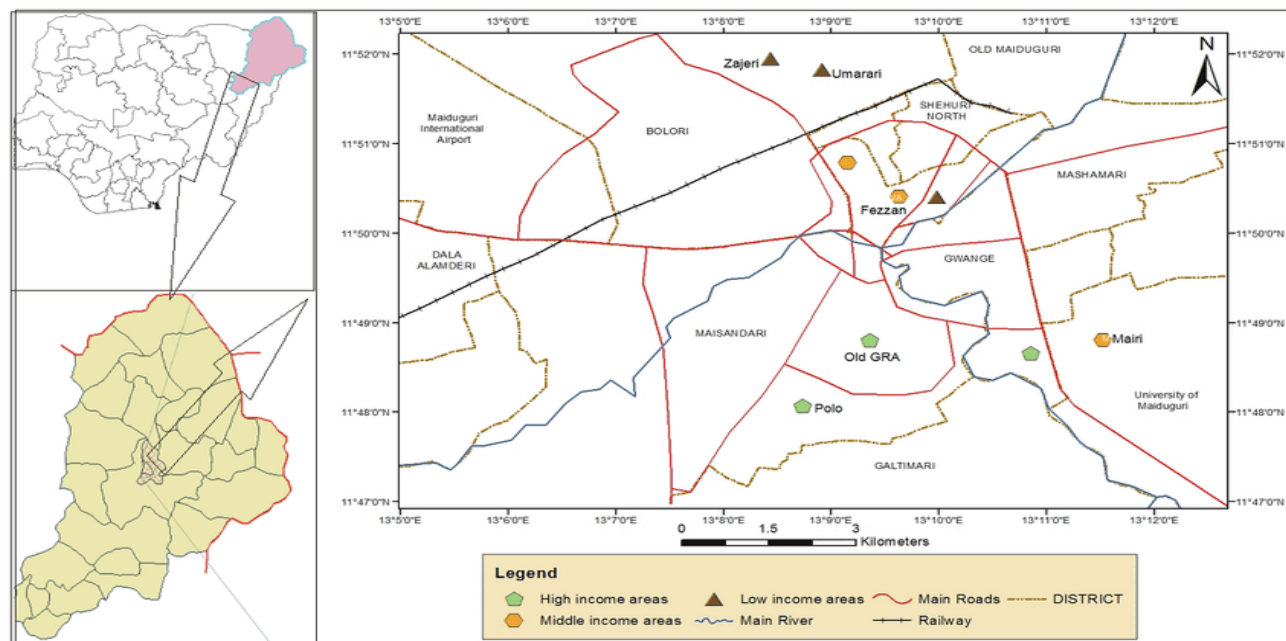
## MATERIALS AND METHODS

### Study Area

The study was conducted in Maiduguri Metropolitan Council, a Local Government Area in Borno State, Nigeria. Maiduguri has a hot climate with two distinct seasons: wet (June-September) and dry (October-May). The area is

mostly flat plain land with some sparse trees and grasses. As of 2006, the population of Maiduguri Metropolitan Council was 540,016 (Nkwocha *et al.*, 2019). Figure 1 shows the study area for sample collection. Soil samples were collected from five different sites: Shehuri dump site, 600282 (Lat 11.856242°N Long 13.16341°W),

Bolori dump site, 600282 (Lat 11.852478°N Long 13.131928°W), Mafoni dump site 600282 (Lat 11.84662°N Long 13.144604°W), Gwange dump site 600211, (Lat 11.838301°N Long 13.163396°W) and Hausari dump site 611107 (Lat 11.84334°N long 13.15704°W).



**Figure 1:** Map of Maiduguri Metropolitan showing the Collection Dumpsites

**Source:** Friday *et al.*, 2020

### Sample Collection

A total of five soil samples were collected at different locations in Maiduguri Metropolitan council (MMC), samples were collected with a well sterilized ziploc container using a clean augur at the depth of 10 cm. The samples were collected in quadruplets to minimize error and enhance accuracy, samples were transported to soil science, University of Maiduguri pending analysis.

### Low density polyethylene pieces and powder

Low density polyethylene pieces and powder were obtained from Borno Plastics (Boplas). Both polyethylene pieces and powder were collected using a sterilized forceps and a sterile spatula respectively. The samples were placed into a clean airtight container to avoid contamination. They were then transported to

the Microbiology laboratory, University of Maiduguri. Samples were cleaned using 70 % ethanol to remove any surface contaminants. The samples were dried and kept under sterile conditions pending bacterial screening and subsequent biodegradation analysis.

### Physicochemical Analyses

The physicochemical analyses were carried out on the soil samples to determine the level of acidity/ alkalinity of the soil, organic matter, moisture content and electrical conductivity.

The physicochemical analyses were carried out on the soil samples to determine the level of acidity/ alkalinity of the soil, organic matter, moisture content and electrical conductivity. For pH determination, the electrode of the pH meter (BANTE-510, China) was calibrated with standard buffer solutions. Twenty gram of the

soil sample was poured into 100 ml of distilled water, stirred for 10 min and the pH value was recorded according to method described by Corwin *et al.* (2020). Organic matter was determined according to Oyeyiola *et al.* (2013), by oven drying twenty crucibles at 105 °C for 24 h. One gram of soil sample was placed at each crucible and combusted at 550 °C for 30 mins in a furnace with occasional stirring. They were transferred into desiccators to cool down, and the weight was recorded. For moisture content, twenty crucibles were again dried at 105 °C for 24 h, one gram of the soil sample as added and dried at 105 for 24 h, after cooling, the weight of each crucible was then recorded as described by (Bhuwal *et al.*, 2014). To determine the electrical conductivity, 100 grams of soil was mixed with 200 ml of distilled water, stirred for 5 mins and allowed to settle for 30 mins. The mixture as filtered, and the filtrate was analysed using a conductivity meter (BANTE-510, China) following the method of Corwin *et al.* (2020)

#### **Isolation and Screening of Bacteria**

A total of 1 g soil of each sample is transferred into a flask containing 99 ml of sterile distilled water. These flasks were shaken and serially diluted up to 5-fold dilutions. From the fifth dilution, 0.1 ml was inoculated onto a nutrient agar and mineral salt medium. The mineral salt medium (MSM) was prepared and autoclaved in a flask by adding K<sub>2</sub>HPO<sub>4</sub> (0.1g/L), KH<sub>2</sub>PO<sub>4</sub> (3.0 g/L) NaCl (5.0 g/L), NH<sub>4</sub>Cl (2.0 g/L), MgSO<sub>4</sub> (0.2 (g/L), CaCl<sub>2</sub>.2H<sub>2</sub>O (0.1 g/L), KCl (0.15 g/L) and agar powder (15 g/L) in distilled water as prepared by Obire *et al.* (2008), and polyethylene powder was added at the final concentration of 0.1 % (w/v) as sole carbon and energy source. . The pH of the medium was adjusted to 7.0 ±0.2 using 1N NaOH for 15 minutes. The medium was sonicated for 1 hour at 120 rpm in a shaker and autoclaved at 121 °C and 15 kPa for 20 minutes. The medium was poured into sterile plates and allowed to solidify. Samples were inoculated onto MSM and then incubated at 37 °C for a period of 7 days. Isolates from nutrient agar were sub-cultured onto Blood agar (BA), MacConkey (MAC) agar, Eosin Methylene Blue (EMB) agar, Mannitol Salt agar (MSA) for the isolation of *Bacillus*, *Providencia*, *Escherichia coli* and *Staphylococcus*. Afterwards, 0.1 ml from each

sample was spread on Petri plates and incubated at 37 °C for 18-24 hours. After growth, developed colonies were isolated and sub-cultured to obtain pure isolates.

#### **Bacterial Identification**

Gram staining was performed according to Oyeleke and Manga (2008): smears were prepared, air-dried and heat-fixed, and sequentially treated with a drop of crystal violet (30-60 seconds), Gram's iodine (30-60 seconds), and two drops of 80% acetone (10 seconds for decolorization), and safranin (45 seconds). After each step, slides were rinsed with distilled water. Finally, slides were blot-dried and examined under an oil immersion microscope. For the Catalase Test, a drop of 3% (v/v) hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added to a clean glass slide, and a fresh bacterial colony was mixed in. Immediate effervescence indicated a positive reaction (Cheesebrough, 2006). The Indole Test was carried out by inoculating isolates into a tube containing tryptophan broth, incubating for 24 hours, and then adding 5 drops of 1% Kovac's reagent. The tubes showing the formation of pink colour were marked positive as explained by Cheesebrough (2006). Triple Sugar Iron Agar slants were inoculated with isolates and observed after 24 hours for sugar fermentation, black precipitation indicates H<sub>2</sub>S production, and bubbles indicate hydrogen gas production (Cheesebrough, 2006). For the Methyl Red Test, isolates were inoculated into sterile peptone water and incubated for 24 hours. Five drops of methyl red were then added, and the broth was observed for color change (Cheesebrough, 2006). The Voges-Proskauer Test was done by transferring 1ml of bacterial culture broth to a clean test tube. 0.6 ml of Barrett's reagent A as then added, followed by 0.2ml of Barrett's reagent B. Pink to red coloration was noted within 30 minutes (Oyeleke and Manga, 2008). Simmon's citrate agar slants were inoculated with selected isolates, and a change in color from green to blue after 24 or 48 hours indicated positive citrate utilization (Cheesebrough, 2006). The Oxidase Test was performed by placing a clean filter paper on a sterile petri dish, adding 3 drops of fresh oxidase reagent and emulsifying colonies onto the paper; a blue-purple color within 10 seconds indicated a positive result

(Oyeleke and Manga, 2008). Lastly, for the Urease Test, urea media slants were inoculated by streaking 24-hour old bacterial isolates, and a change in coloration from pale brown to red after 24 hours indicated urease production (Oyeleke and Manga, 2008).

#### **Biodegradation Assay**

All the bacterial isolate were used for the plastic biodegradation assay for the periods of 60 days using the method described by Lou *et al.* (2020). The plastic pieces were cleaned with tap water and surface sterilized with 70% (v/v) ethanol. They were then pre-weighed. The sterile pre-weighed plastic pieces were taken using a micropipette and aseptically transferred into series of 13 flasks (for each bacterial isolate including control) containing 100 ml of mineral salt broth. Exactly 1 ml of 24 hours-old cultures of each bacterial isolate was taken using a micropipette and individually inoculated into the different MSM flasks containing 0.1g of the

$$\text{Weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \dots\dots\dots \text{Eqn (1)}$$

#### **Fourier Transform Infrared (FT-IR) Analysis**

The structural change in the LDPE surface was investigated using the Fourier transform infrared (FT-IR) spectrometer. FT-IR analysis detects any change in the functional groups. Spectrum was recorded at 450–4000 wave numbers  $\text{cm}^{-1}$  for all LDPE samples (Gajendiran *et al.*, 2016).

#### **Statistical Analysis**

For the physicochemical analysis of the soil, four replicate samples were collected from each of the five locations of the five selected dumpsites, making a total of 20 soil samples. The mean and standard deviation (SD) were calculated for each parameter per site. The data were subjected to statistical analysis using one-way analysis of variance (ANOVA) (SPSS version 25) to assess whether there were statistically differences among the five locations and the significance was set at ( $p < 0.05$ ).

### **RESULTS**

#### **Physicochemical Properties of the Soil Samples from Selected Dumpsite Soils in Maiduguri Metropolis**

The predominant color of the soil samples collected from various dumpsites in Maiduguri

plastic pieces. Control flask was containing 0.1 g of pieces of LDPE plastic without bacterial suspension. All the flasks were incubated at 37 °C for 60 days.

#### **Turbidometric Analysis**

The ability of the isolates to degrade the plastic was monitored by observing change in the media turbidity manually at the interval of 20 days. The turbidity in each conical flask was compared to that of the control in order to discern any difference.

#### **Gravimetric Analysis**

After 60 days, plastic pieces were removed from each flask using a sterile forceps, washed thoroughly with tap water, and shade-dried. The plastic pieces were then weighed for final weight, which was recorded. The percentage of degradation (%) was determined through calculating the percentage of weight loss of the plastic materials using the formula of Ogunbayo *et al.* (2019):

is dull brown. There is a notable similarity in the soil texture, which is predominantly fine. This fine texture is observed in the two main soil samples present in the area: sandy and clay soils. Samples from Shehuri, Bolori and Gwange had fine-textured sandy soils whereas; those of Mafoni and Hausari were fine-textured clay soils (Table 1)

Table 2 presents the moisture content and organic matter contents of the soil samples obtained from selected dumpsites in Maiduguri. The results show that unlike soils from Shehuri which had the highest moisture content ( $1.84\% \pm 0.2$ ), soil from Mafoni had the lowest moisture content ( $0.58\% \pm 0.64$ ) followed by Gwange and Hausari with  $1.05\% \pm 0.44$  and  $1.52\% \pm 0.04$  respectively. There was no significant difference in the moisture content of the soil samples ( $p = 0.0605$ ). Similarly, the results also showed that soils from Shehuri had the highest organic carbon contents ( $1.58\% \pm 0.04$ ) while soil from Bolori had the lowest carbon content ( $1.48\% \pm 0.03$ ). There is also no significant difference in the organic matter content of the sampled soil ( $p = 0.9351$ ). This suggest that despite numerical

differences, the moisture content and organic carbon across sites is relatively uniform and unlikely to be a major factor influencing microbial activity or LDPE degradation

Table 3 presents the p value of electrical conductivity, pH and temperature of all soil samples is 0.1566, 0.7238 and 0.6374

respectively. This shows there is no significant difference in the electrical conductivity, pH and temperature of the soils sampled. These stable physicochemical conditions indicate that environmental differences among the sites were minimal and may not account for variation in bacterial presence or degradation efficiency

**Table 1:** Physical Properties of some Selected Dumpsites Soil Samples in Maiduguri

Sample Location	Texture	Soil Type	Colour
Shehuri	Fine	Sandy	10YR 6/4 Dull brown
Bolori	Fine	Sandy	10YR 5/8 Dull brown
Mafoni	Fine	Clay	10YR 6/8 Dull brown
Gwange	Fine	Sandy	10YR 6/8 Dull brown
Hausari	Fine	Clay	10YR 6/8 Dull brown

**Table 2:** Physicochemical Components (Moisture and Organic) in Soil Samples Obtained from some Selected Dumpsites in Maiduguri

Sample Location	Moisture content (%)	Organic carbon (%)
Shehuri	1.84 ± 0.2	1.58 ± 0.04
Bolori	1.55 ± 0.24	1.48 ± 0.03
Mafoni	0.58 ± 0.64	1.56 ± 0.02
Gwange	1.05 ± 0.44	1.55 ± 0.13
Hausari	1.52 ± 0.04	1.55 ± 0.20
P value	0.0605	0.9351

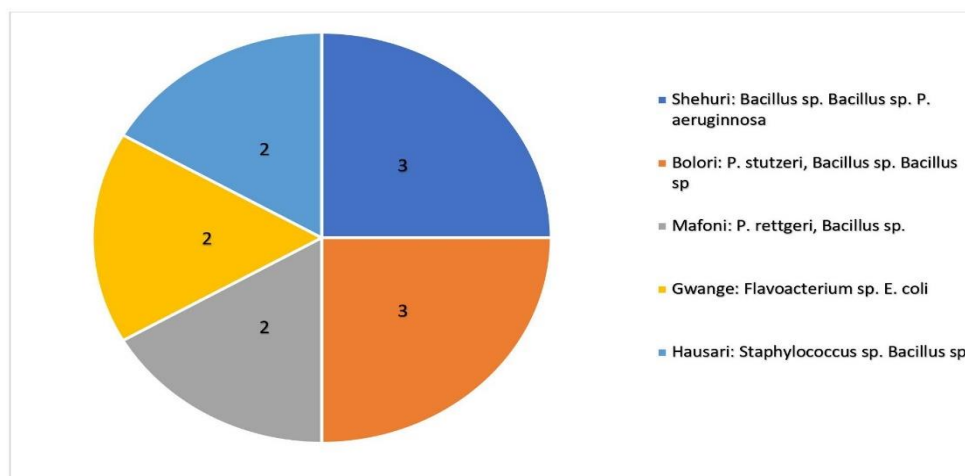
**Table 3:** Physicochemical Components (Moisture and Organic) In Soil Samples Obtained from some Selected Dumpsites in Maiduguri

Sample Location	pH	Electrical Conductivity (µS/cm)	Temperature (°C)
Shehuri	6.32 ± 0.82	0.37 ± 0.38	25.66 ± 0.57
Bolori	6.42 ± 0.82	0.31 ± 0.33	26.00 ± 1.00
Mafoni	5.00 ± 0.66	0.14 ± 0.03	25.66 ± 0.57
Gwange	5.64 ± 0.59	0.15 ± 0.06	26.33 ± 1.15
Hausari	6.08 ± 0.55	0.39 ± 0.37	26.66 ± 1.15
p value	0.1566	0.7238	0.6374

#### Isolation of Plastic Degrading Bacteria from Soil Samples at Some Selected Dumpsites in Maiduguri Metropolis

Out of the 5 soil samples collected, a total of 12 bacterial isolates were recovered after incubation for the period of seven days as illustrated in figure 2. Each colored segment represents a

sampling location, Shehuri and Bolori had the highest number of isolates (3 each), while 2 isolates were isolated from Mafoni, Gwange and Hausari. These isolates were phenotypically characterized and their occurrence rate is presented in Figure 2.



**Figure 2:** Distribution of LDPE Degrading Bacterial Isolates Isolated from Soil Contaminated with Plastic Waste

### Identification of Bacterial Isolates from the Soil Sampled at some Selected Dumpsites in Maiduguri

The bacterial isolates were identified by integrating morphological observations (Gram stain reaction, shape, and colony color) with a biochemical tests including catalase, indole, methyl red (MR), Voges-Proskauer (VP), urease, citrate, oxidase, and Triple Sugar Iron (TSI) agar analysis, with reference obtained from PIBWIN as shown in Table 4. This method was used for the differentiation of various bacterial isolates. *Bacillus* spp. isolates (S1A, S1B, B2B, B2C, M3B, and H5B) were identified as Gram-positive rods, consistently positive for catalase, MR, citrate, and TSI (A/A), while being negative for indole, VP, urease, and H<sub>2</sub>S. *Pseudomonas aeruginosa* (S1C), *P. stutzeri* (B2A), and *P. rettgeri* (M3A) were characterized as Gram-negative rods, all positive for catalase, citrate, and oxidase with AK/AK TSI reactions, but differentiated by their distinct biochemical test profiles and other minor variations as presented in Table 4. *Flavobacterium* sp. (G4A) was identified as a Gram-negative rod, oxidase-positive, and negative for other tests like indole, MR, VP, and citrate, distinguishing it from the *Pseudomonas* species. *E. coli* (G4B) was definitively identified as a Gram-negative rod by its characteristic IMViC profile (positive Indole and MR, negative VP and Citrate), positive catalase, and

A/A TSI reaction with gas, coupled with a negative oxidase test. Lastly, *Staphylococcus* sp. (H5A) was recognized as Gram-positive cocci, positive for catalase, MR, urease, citrate, and A/A on TSI, yet negative for all other tested biochemical reactions.

**Table 4:** Morphological and Biochemical Characteristics of the Isolates

Sample Identity	GR	Shape	Color	Cat	In	MR	VP	Urease	Cit	TSI	Oxi	Gas	H <sub>2</sub> S	Suspected Organism
S1A	+	Rod	Purple	+	-	+	+	-	+	A/A	-	-	-	<i>Bacillus</i> sp.
S1B	+	Rod	Purple	+	-	+	+	-	+	A/A	+	-	-	<i>Bacillus</i> sp.
S1C	-	Rod	Pink	+	-	-	-	-	+	AK/AK	+	-	-	<i>P. aeruginosa</i>
B2A	-	Rod	Pink	+	-	+	-	-	+	AK/AK	+	+	-	<i>P. stutzeri</i>
B2B	+	Rod	Purple	+	-	+	+	-	+	A/A	-	-	-	<i>Bacillus</i> sp.
B2C	+	Rod	Purple	+	-	+	+	-	+	A/A	-	-	+	<i>Bacillus</i> sp.
M3A	-	Rod	Pink	+	+	-	-	+	+	A/AK	+	+	-	<i>P. rettgeri</i>
M3B	+	Rod	Purple	+	-	+	+	-	+	A/A	-	-	-	<i>Bacillus</i> sp.
G4A	-	Rod	Pink	+	-	-	-	-	-	AK/AK	+	-	-	<i>Flavobacterium</i> sp.
G4B	-	Rod	Pink	+	+	+	-	-	-	A/A	-	+	-	<i>E. coli</i>
H5A	+	Cocci	Purple	+	-	+	-	+	+	A/A	-	-	-	<i>Staphylococcus</i> sp.
H5B	+	Rod	Purple	+	-	+	+	-	+	AK/AK	+	-	-	<i>Bacillus</i> sp.

KEY: + positive: - negative, GS: Gram's reaction, Cat: Catalase, In: Indole, MR: Methyl Red, Ci: Citrate Oxi: Oxidase, TSI: Triple Sugar Iron, VP: Voges Proskauer



### Degradation of Polyethylene Film

After the period of 60 days, the bacterial growth (turbidity) and direct polymer degradation (weight loss) by various bacterial isolates was and compared to the control. Turbidity, indicated by '+' (slight) and '++' (significant), reflects bacterial proliferation in the medium. The control, with no bacterial inoculation, showed no turbidity or weight loss, confirming that observed changes in test groups were due to microbial activity. A majority of the isolates, including *Bacillus* spp. S1A, S1B, B2B, B2C, M3B, *P. stutzeri* B2A, *E. coli* G4B, and *Staphylococcus* sp. H5A, showed no turbidity or corresponding LDPE weight

reduction after 4 and 8 weeks (0.00% degradation), indicating their inability to effectively degrade LDPE under the experimental conditions. In contrast, four isolates demonstrated varying degrees of degradation activity. *P. aeruginosa* S1C and *P. rettgeri* M3A exhibited the highest degradation, showing significant turbidity (++) by 60 days and achieving a substantial 25.00% LDPE weight loss after 8 weeks, suggesting robust growth and efficient polymer breakdown. *Flavobacterium* sp. G4A, and *Bacillus* sp. H5B with slight turbidity (+) throughout, resulted in a 9.00% degradation indicating a slower but still active degradative capability.

**Table 5:** Initial and Final Weight of Plastics Pieces after 4 and 8 Weeks.

Bacterial isolates	Turbidity (days)			Initial weight	Final weight after 4 weeks	Final weight after 8 weeks	Parentage of degradation (%)
	20	40	60				
<i>Bacillus</i> sp. S1A	-	-	-	0.10	0.10	0.10	0.00
<i>Bacillus</i> sp. S1B	-	-	-	0.10	0.10	0.10	0.00
<i>P. aeruginosa</i> S1C	-	+	++	0.10	0.09	0.08	25.00
<i>P. stutzeri</i> B2A	-	-	-	0.10	0.10	0.10	0.00
<i>Bacillus</i> sp. B2B	-	-	-	0.10	0.10	0.10	0.00
<i>Bacillus</i> sp. B2C	-	-	-	0.10	0.10	0.10	0.00
<i>P. rettgeri</i> M3A	+	+	++	0.10	0.10	0.08	25.00
<i>Bacillus</i> sp. M3B	-	-	-	0.10	0.10	0.10	0.00
<i>Flavobacterium</i> sp. G4A	-	+	+	0.10	0.09	0.09	9.00
<i>E. coli</i> G4B	-	-	-	0.10	0.10	0.10	0.00
<i>Staphylococcus</i> sp. H5A	-	-	-	0.10	0.10	0.10	0.00
<i>Bacillus</i> sp. H5B	-	-	+	0.10	0.10	0.09	9.00
Control	-	-	-	0.10	0.10	0.10	0.00

### Fourier Transform Infrared Spectrophotometer (FTIR) Peaks of LDPE Plastics Treated with Bacterial Isolates

Fourier Transform Infrared Spectrophotometer (FTIR) peaks of LDPE plastics treated with bacterial isolates indicated that different groups ranging from carbonyl, hydroxyl, alkenes, nitro compounds and aliphatic amines are found to undergo oxidative degradation, biodegradation and dehydrogenation at different wave numbers for *P. aeruginosa*, *P. rettgeri*, *Bacillus* sp. and *Flavobacterium* sp. Peaks are observed from new to no peaks across all the bacterial isolates (Table 6).

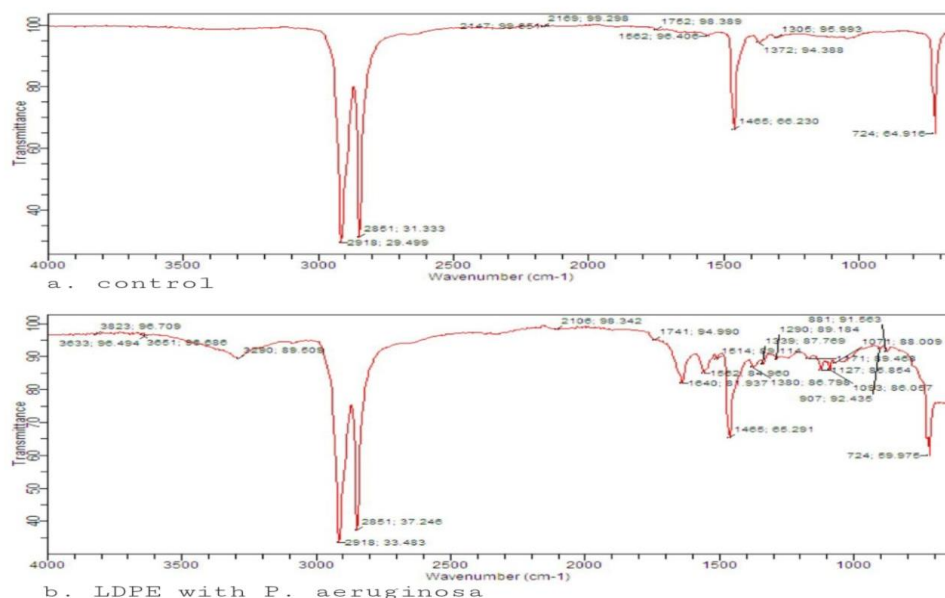
The chemical bonds detected include C–H stretch ( $2915.38\text{ cm}^{-1}$  and  $2851.37\text{ cm}^{-1}$ ), and bends ( $1465.66\text{ cm}^{-1}$   $1372.94\text{ cm}^{-1}$  and

$724.64\text{ cm}^{-1}$ ) representing the presence of alkanes (hydrocarbon) as the prominent chemical functional group present in the control LDPE sheet. Whereas, in LDPE sheet treated with *Pseudomonas aeruginosa* S1C, *Providencia rettgeri* M3A, and *Flavobacterium* sp. G4A treated LDPE sheet, functional groups such as carboxylic acids (O–H stretch at  $3290.89\text{ cm}^{-1}$ ); esters (C=O stretch at  $1741.94\text{ cm}^{-1}$ ); alkenes (C=C stretch at  $1640.81\text{ cm}^{-1}$ ); nitro compounds (N–O asymmetric stretch at  $1582.84\text{ cm}^{-1}$ ) and aliphatic amines (C–N stretch at  $1093.86\text{ cm}^{-1}$ ) were present in addition to those in control LDPE sheet. Therefore, the presence peaks presented in table 6 such as carboxylic acids, amino groups, nitro-compounds, and esters in

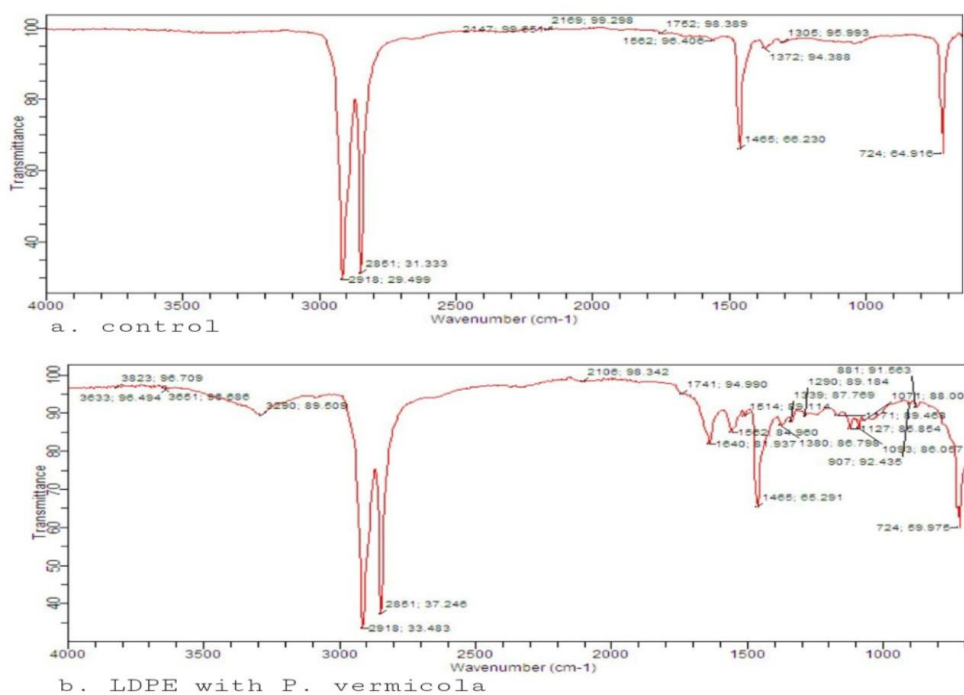
the degraded LDPE sheet represents the isolates (Figure 3 to 6).  
utilization of polyethylene by the bacterial

**Table 6:** Fourier Transform Infrared Spectrophotometer (FTIR) Peaks of LDPE Plastics Treated with Bacterial Isolates

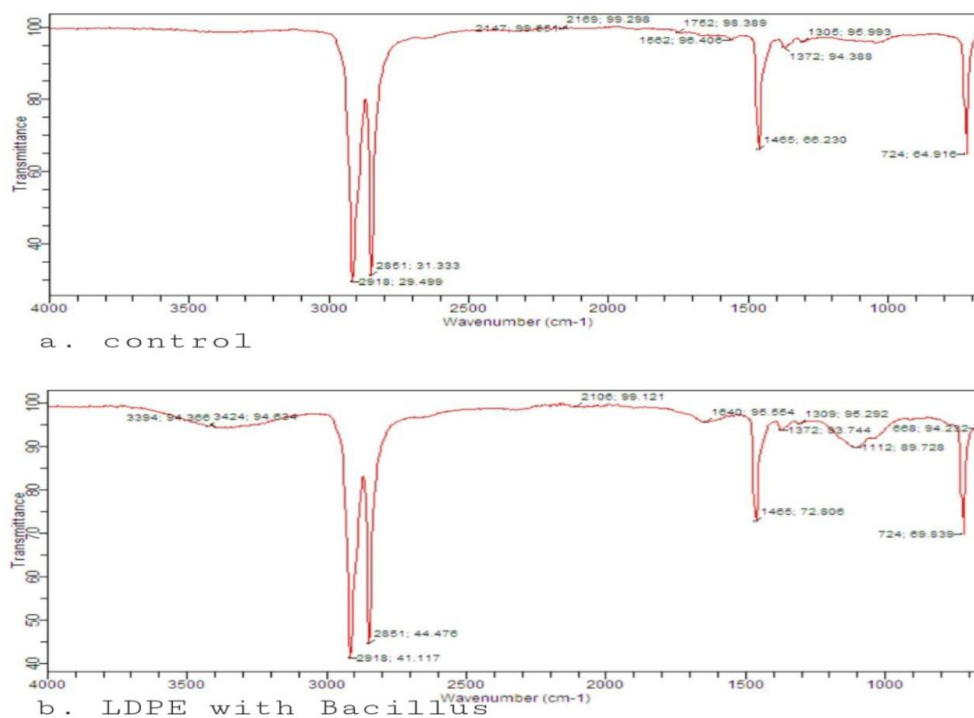
Wave number (cm <sup>-1</sup> )	Functional groups	<i>P. aeruginosa</i>	<i>P. Rettgeri</i>	<i>Bacillus</i> sp.	<i>Flavobacterium</i> sp.	Control	Interpretation
2918, 2851	CH <sub>2</sub> stretching	Present	Present	present	Present	Present	Backbone of LDPE
1465, 1372, 724	CH <sub>2</sub> bending	Present	Present	present	Present	Present	Backbone of LDPE
17441	C=O stretching (Carbonyl)	New peak observed	New peak observed	New peak observed	No peak observed	No peak observed	Carbonyl formation indicating oxidative degradation
3290	O-H stretching (hydroxyl)	Broad peak observed	Broad peak observed	Minor peak observed	No peak observed	No peak observed	Introduction of hydroxyl groups suggesting biodegradation
1640	C=C Stretching (alkene)	New peak observed	New peak observed	New peak observed	No peak observed	No peak observed	Indicating dehydrogenation or chain incision
1582	N-O Stretching (nitro compound)	New peak observed	New peak observed	No peak observed	New peak observed	No peak observed	Indicates nitrogen containing products
1093	C-N Stretching (aliphatic amines)	New peak observed	New peak observed	No peak observed	New peak observed	No peak observed	Indicates presence of aliphatic amines from microbial activity



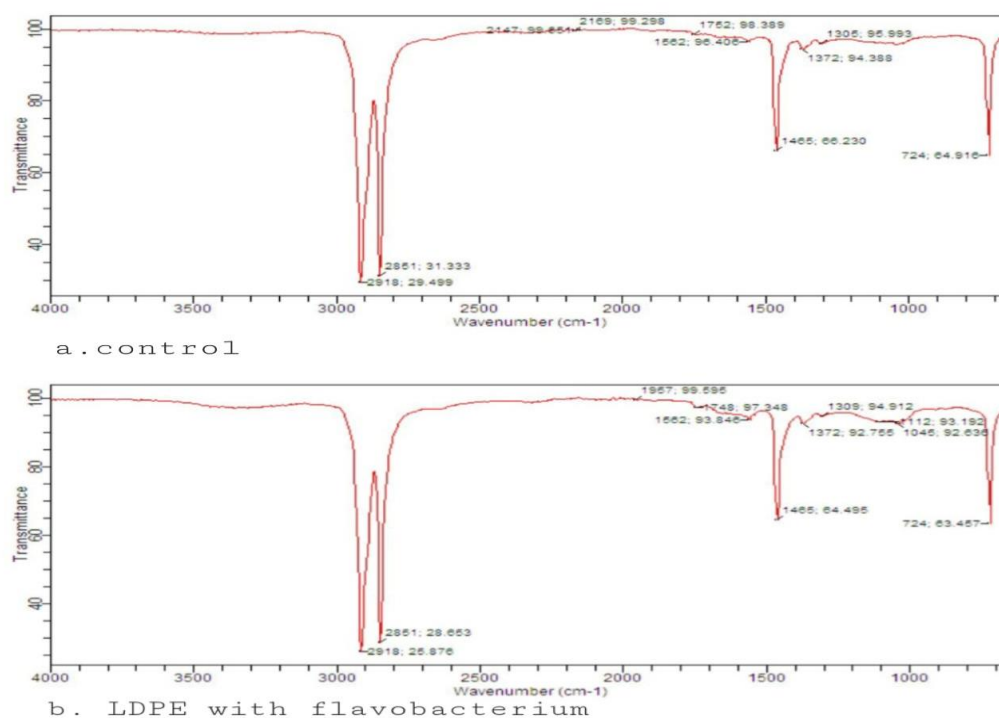
**Figure 3:** FT-IR spectrum of LDPE control and LDPE treated with *Pseudomonas aeruginosa*



**Figure 4:** FT-IR spectrum of LDPE control and LDPE treated with *Providencia rettgeri*



**Figure 5:** FTIR spectrum of LDPE control and LDPE treated with *Bacillus* sp.



**Figure 6:** FT-IR spectrum of LDPE control and LDPE treated with *Flavobacterim* sp.

## DISCUSSION

This research focused on the process of isolating and identifying microorganisms found in soil that have the ability to break down low density polyethylene. Despite the ability of these microorganisms to degrade synthetic plastics, the process typically takes approximately 8 years or more due to the complex structure of the plastics (Emmanuel *et al.*, 2022). The result of this study showed that the predominant color of the selected dumpsites in Maiduguri is dull brown with fine texture. The findings in this report show that Shehuri had the highest moisture content of  $1.84 \pm 0.2\%$  and organic matter of  $1.58 \pm 0.04\%$ . Mafoni had the lowest mean of moisture content of  $0.58 \pm 0.68\%$ , while Bolori had the lowest organic matter of  $1.48 \pm 0.03\%$ . These findings differ from those reported by Priyanka and Archana (2011), who observed higher moisture content of 0.606% and organic matter of 0.9857% in soils collected during a different season. The seasonal variation in sample collection could explain these disparities. Additionally, the measured pH 0.7238, temperature 0.6374 (°C) and electrical conductivity 0.1566 (μS/cm) are consistent with the findings of Emmanuel *et al.* (2022)

on the physicochemical assessments of soils from dumpsite for plastic degrading microorganism. There were no significant differences observed across sampling sites, and the values were not statistically supported through ANOVA at ( $P < 0.05$ ) Several bacterial species were isolated from the soil samples, including *Pseudomonas* spp., *Bacillus* spp., *Flavobacterium*, *Staphylococcus* sp., *Providencia rettgeri* and *Escherichia coli*. Many of these species, such as *Pseudomonas* sp., *Bacillus* sp., *E. coli*, *Providencia rettgeri*, and *Staphylococcus* sp., have been previously reported in solid waste dumpsites across Nigeria and other regions (Bassey *et al.*, 2015; Mwaikono *et al.*, 2015; Williams and Hakam, 2016; Auta and Paul, 2020; Beatrice *et al.*, 2022). The presence of these bacteria in various geographic locations suggests their widespread distribution and potential role in waste degradation. However, the identification in this study was based solely on gram stain and biochemical characterization which may not provide definitive species confirmation. Molecular techniques such as 16S rRNA are recommended for accurate identification.

In the biodegradation study, only *Pseudomonas aeruginosa* S1C, *Providencia rettgeri* M3A, *Flavobacterium sp.* G4A, and *Bacillus sp.* H5B demonstrated the ability to degrade polyethylene film. The identification of these species was confirmed using Bergey's Manual of Systematic Bacteriology. *Pseudomonas aeruginosa* and *Providencia rettgeri* exhibited the highest LDPE degradation rates at 25%, while *Flavobacterium sp.* and *Bacillus sp.* showed lower rates of 9%. This observation aligns with the findings of Singh Singh and Gupta (2016), who determined the degree of degradation by calculating the weight loss over a 40-day period. Of the 15 bacteria isolated from various soil samples, only three strains of *Staphylococcus sp.*, *Pseudomonas sp.*, and *Bacillus sp.* demonstrated the highest percentage of degradation 20%, 25%, and 9% respectively. However, discrepancies in degradation rates compared to other studies, such as Bakht *et al.* (2020), who reported a 6.6% degradation by *Pseudomonas sp.*, could be due to variations in bacterial strains or differences in the expression of polyethylene-degrading genes. Ojiego *et al.* (2020) also reported that bacterial strain such as *Providencia spp.* (*Providencia vermicola* strain M4 14.96%, and *Providencia rettgeri* strain 11 12.20%) possesses varying degree of biodegradation capacity. In this study, the rate of plastic biodegradation ability of *Providencia rettgeri* is relatively higher than the one reported by Ojiego *et al.* (2022). This variation might be attributed to the differences in the bacteria strain and molecular weight of the LDPE used for biodegradation study. Although this study focused on single strain, future research should explore consortia performance and their enzymatic interaction for more efficient LDPE degradation.

The biodegradation of LDPE by *Pseudomonas aeruginosa*, *Providencia rettgeri*, *Flavobacterium sp.* and *Bacillus sp.* was further confirmed through Fourier-transform infrared (FTIR) spectroscopy after 60 days of incubation. The FTIR spectra

showed new peaks indicative of bond breakdown and the formation of functional groups such as esters, carbonyls, and ethers. These changes suggest microbial activity altering the polymer structure, as reported by Rajandas *et al.* (2012). The results are consistent with those of Gupta and Devi (2020), who observed similar spectral changes due to microbial degradation. The increase in carbonyl groups in LDPE after microbial incubation suggests the action of oxidoreductases, which form polar bonds that destabilize the polymer structure (Novotný *et al.*, 2018).

## CONCLUSION

Bacterial strains including *Pseudomonas aeruginosa*, *Providencia rettgeri*, *Flavobacterium sp.*, and *Bacillus sp.* were identified as being capable of degrading LDPE. Notably, *Pseudomonas aeruginosa* and *Providencia rettgeri* exhibited significant degradation capabilities, degrading up to 25% of LDPE. While *Flavobacterium sp.* and *Bacillus sp.* also showed degradative potential, their activity was comparatively lower, resulting in 9.00% LDPE degradation. The observed weight loss was supported by Fourier-transform infrared (FTIR) spectroscopy analysis, which revealed structural modifications in the treated LDPE films, indicative of polymer breakdown due to microbial activity. These findings highlight the potential of these bacterial strains for biodegradation strategies in managing plastic waste in landfills and dumpsites. To further enhance biodegradation efficiency, it is recommended that future studies investigate the potential of using mixed cultures of *Pseudomonas aeruginosa*, *Providencia rettgeri*, *Flavobacterium sp.*, and *Bacillus sp.* to achieve higher biodegradation rates than single strains. Additionally, further research involving molecular characterization of these bacterial isolates should be carried out

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this research.

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