**Original Research Article**

**Isolation and Identification of Bacteria from Microplastic Polluted Soil from Three (3) Geopolitical Zones in Osun State, Nigeria.**

**Abstract**

Plastic pollution has become a pressing environmental concern due to its widespread presence and persistence in terrestrial ecosystems. This study isolated and identified the microplastic-degrading bacteria from plastic-polluted soils in Osun State, Nigeria. Soil samples were collected from six zones, and their physicochemical properties, pH and temperature, were analyzed. Microbial isolation and identification were carried out using morphological and biochemical tests. The pH ranged from 8.2 to 8.8. and the temperature ranged from 25°C to 29.1°C. Microbiological assessments revealed high bacterial counts, with total viable bacterial counts ranging from 2.5 x 106 to 4.0 x 106 CFU/g. Four bacterial strains were isolated: Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliticus, and Streptococcus spp. The isolated bacteria can be further studied for their enzymatic activities, offering eco-friendly solutions for plastic degradation. Further research and policy development are recommended to support bioremediation efforts in Nigeria and other regions affected by plastic pollution.

**Keywords:** Microplastic, Pollution, Soil.

**Introduction**

Microplastic pollution is increasingly recognized as an escalating environmental threat because of its extensive distribution, durability, and negative ecological consequences. Microplastics are defined as tiny plastic particles measuring less than 5 mm in size, and their buildup in both land and water environments represents a significant obstacle for waste management and ecological sustainability (Da Silva et al., 2024). This problem is especially severe in developing nations like Nigeria, where insufficient waste management systems and growing plastic consumption have led to rising levels of plastic pollution (Ololade *et al.,* 2023).

In recent times, research has begun to concentrate on discovering biological techniques to address microplastic pollution, especially through the involvement of microorganisms in plastic degradation (Yalwaji, 2022). This method, referred to as microbial bioremediation, provides a sustainable and environmentally friendly option compared to traditional plastic waste management approaches. Microorganisms that can break down plastics have specific enzymes, including hydrolases and oxidases, which can decompose complex polymer structures into simpler molecules, thus aiding the degradation process (Onyekachi and Chukwuemeka, 2018).

Numerous studies have uncovered plastic-degrading microorganisms from a variety of settings, including soil, water, and marine environments (Yalwaji, 2022). For example, research conducted by Yoshida et al. (2016) identified bacterial strains from soil contaminated with plastic that exhibited significant potential for polyethylene degradation. Likewise, Da Silva et al. (2024) discovered fungal species capable of breaking down polypropylene sourced from municipal waste. These results affirm the wide variety of microorganisms available for plastic degradation and highlight the necessity of investigating local microbial communities for possible bioremediation uses.

As one of the rapidly growing economies in Africa, Nigeria has seen fast-paced urbanization and industrial growth, resulting in considerable plastic waste production. Osun State, situated in southwestern Nigeria, is no exception. Many of its key cities, such as Iwo, Ara, Osogbo, Ikirun, Ife, and Ilesa, encounter issues related to plastic pollution, exacerbated by population increase and insufficient waste management infrastructure (Onyekachi and Chukwuemeka, 2022). These cities exemplify broader environmental challenges facing the area and serve as optimal sites for examining plastic-degrading microorganisms. By concentrating on Osun State, this research tackles a vital environmental concern that impacts both human health and ecosystem integrity. The buildup of microplastics in soil can lead to extensive repercussions, including the contamination of food chains and the disturbance of soil microbial populations (Ololade et al., 2023). Consequently, identifying and utilizing microorganisms with the ability to degrade plastics presents a promising approach to alleviating the effects of plastic pollution. This study isolated and identified bacteria with the capability to degrade microplastics from dumpsites in six (6) locations within three (3) geopolitical zones of Osun State, Nigeria.

**MATERIALS AND METHODS**

**Study Area and Soil Sample Collection**

Soil samples were collected from six locations within three (3) geopolitical zones in Osun State, namely Iwo, Ara, Osogbo, Ikirun, Ife, and Ilesa, with coordinates of 7040’14.5”N & 4013’38.1”E, 7046’22.1”N & 4026’31.6”E, 70’46.25.6”N & 4033’52.9”E, 7055’11.3”N & 4040’12.2”E, 7028’59.9”N & 4033’17.6”E, and 7040’52.2”N & 4047’15.7”E, respectively.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S/N** | **Location** | **Gps Location** | **Local Government Area** | **Senatorial****District** |
| 1 | Iwo | 7040’14.5”N & 4013’38.1”E | Iwo | Osun West |
| 2 | Ara | 7046’22.1”N & 4026’31.6”E | Egbedoore | Osun West |
| 3 | Osogbo | 70’46.25.6”N & 4033’52.9”E | Olorunda | Osun Central |
| 4 | Ikirun | 7055’11.3”N & 4040’12.2”E | Ifelodun | Osun Central |
| 5 | Ife | 7028’59.9”N & 4033’17.6”E | Ife | Osun East |
| 6 | Ilesa | 7040’52.2”N & 4047’15.7”E | Ilesa | Osun East |

These locations were selected due to their exposure to microplastic pollution from urbanization and waste disposal activities. For each location, five different sampling sites were chosen to obtain a representative sample. A sterile soil auger was used to collect approximately 500 grams of topsoil from a depth of 10 cm. The samples were placed in sterile polyethylene bags, labeled, and transported to the laboratory under controlled temperature conditions for further analysis.

**Pre-treatment of Soil Samples**

The collected soil samples were air-dried at room temperature for 24 hours to remove moisture. The dried samples were then homogenised by passing them through a 2 mm sieve to ensure uniformity. This step was essential for removing larger debris and stones, ensuring that only fine soil particles remained for subsequent microbiological analyses.

**Preparation of media**

Nutrient agar and Bushnell Hass (BH) broth used in this research work were prepared according to the manufacturer’s instructions by dissolving 27 g and 3.27 g of nutrient agar and BH media in 1000 ml of distilled water in a conical flask. The mouth of the conical flask was corked with cotton wool wrapped with foil paper; it was gently shaken, and the agar media were allowed to homogenise in a water bath at 100ºC. After homogenising, the media were sterilised inside an autoclave at 121ºC for 15 minutes. The agar media were allowed to cool before dispensing into Petri dishes and then allowed to solidify before use.

**Serial Dilution and Plating**

Ten-fold serial dilutions were prepared by adding 1 ml of prepared sample into 9 ml of sterile distilled water in a test tube. This process was repeated up to a dilution factor of 105. From each dilution, 0.1 ml was plated on nutrient agar (NA) plates using the spread plate method. The plates were incubated at 28°C for 48 hours. Colonies appearing on the plates were counted to determine the total viable count (TVC) and recorded as colony-forming units per gram of soil (cfu/g).

**Purification of Isolates**

The distinct bacterial colonies observed on the nutrient agar were subcultured on fresh agar plates to obtain pure cultures. Each isolate was streaked onto nutrient agar using an inoculating loop and incubated at 28°C for another 24 hours. Purity was confirmed by observing the morphological characteristics of the colonies, ensuring that only uniform colonies were selected for further analysis.

**Morphological and Biochemical Characterisation**

The isolated bacteria were subjected to morphological and biochemical tests to aid in their identification. Gross colonial morphology includes colour, edges, and elevation through visual observation, while shape and Gram reaction were observed through the aid of a microscope using x100 objective lens.  Other biochemical tests include the catalase test through the use of hydrogen peroxide (H₂O₂) reagent for bubbles observation, the citrate test through the use of Simmons citrate agar slant producing colour changes, and the sugar fermentation test (lactose and sucrose) through the use of respective sugars for the production of gas and colour change.

**Molecular Characterisation**

The isolate was identified using molecular techniques involving DNA extraction, polymerase chain reaction (PCR), and DNA sequencing. For DNA extraction, a Thermo Scientific NanoDrop 2000 was used to quantify DNA purity and concentration. PCR amplification of the 16S rDNA region was performed using a Bio-Rad T100 Thermal Cycler, with universal primers targeting bacterial 16S rRNA. The amplified products were then purified using a QIAquick PCR Purification Kit (Qiagen) and sequenced with a Applied Biosystems 3500 Genetic Analyzer. The resulting 16S rRNA sequences were analyzed and aligned using the BLASTn tool against the NCBI GenBank database for identification, achieving a 99% similarity with the closest published sequences.

**RESULTS**

The physicochemical parameters and microbiological assessment of plastic polluted soil sample from six locations within three (3) geopolitical zones of Osun State were presented in Tables below:

**Table 1: Physicochemical parameters of plastic polluted soil from six (6) locations within three (3) geopolitical zones of Osun State.**

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Samples Cordinates**  **pH** **Temperature (0C)**

**S1** 70’40’14.5”N & 4013’38.1”E 8.8+0.1 250C+0.1

**S2** 7046’22.1”N & 4026’31.6”E 8.6+0.1 29.10C+0.1

**S3** 70’46.25.6”N & 4033’52.9”E 8.2+0.1 280C+0.1

**S4** 7055’11.3”N & 4040’12.2”E8.5+0.1 270C+0.1

**S5** 7028’59.9”N & 4033’17.6”E8.7+0.1 270C+0.1

**S6** 7040’52.2”N & 4047’15.7”E8.3+0.1 270C+0.1

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**KEYS**

**S1:** Iwo **S2:** Ara **S3:** Osogbo **S4:** Ikirun **S5:** Ife **S6:** Ilesa



**Figure 1:** Physicochemical parameters of of Microplastic Polluted Soil

**Table 2: Total Viable Bacterial Count of Plastic Polluted Soil from six (6) locations within three (3) geopolitical zones of Osun State**

**Samples Dilution factors (CFU/g)**

 **10-1 10-5**

 S1 3.5 x 106 1.2 x 106

 S2 2.8 x 106 0.9 x 106

 S3 3.0 x 106 1.0 x 106

 S4 4.0 x 106 1.5 x 106

 S5 2.5 x 106 0.8 x 106

 S6 3.2 x 106 1.1 x 106



**Figure 2:** Total Viable Bacterial Count of Microplastic Polluted Soil

**Table 3: Colonial, morphological and biochemical characterization of isolates**

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**Characteristics Isolate 1 Isolate 2 Isolate 3 Isolate 4**

Colour Cream Cream Cream Cream

Shape Rod Rod Rod Rod

Elevation Flat Slighly raised Raised Flat

Edges Irregular Irregular Smooth Irregular

Gram staining +ve +ve +ve +ve

Catalase ND ND ND ND

Citrate utilizatization +ve +ve -ve +ve

Lactose +ve +ve -ve +ve

Sucrose +ve +ve -ve +ve

Organisms ***Bacillus*  *Bacillus*  *Bacillus Streptococcus***

***subtilis licheniformis amyloliticus spp***



**Figure 3:** Occurrence of Isolates from Sample Sites

**DISCUSSION**

The soil's pH across the zones was alkaline, ranging from 8.2 to 8.8. Alkaline conditions, such as these, can influence microbial activities and plastic degradation rates. Previous studies have indicated that soil pH can directly impact microbial population density and enzyme activities related to plastic degradation (FES, 2021). For example, alkaline pH can promote the growth of certain bacterial species like Bacillus subtilis, which are known to degrade plastics (Yao *et al.*, 2022). Additionally, the soil temperature varied between 25°C and 29.1°C, with warmer temperatures facilitating microbial activity, including the breakdown of plastics (Lv *et al.*, 2024). This observation aligns with the findings of Emmanuel-Akerele *et al.* (2022), who reported that soil temperatures within this range support the enzymatic activities of plastic-degrading microorganisms.

The total viable bacterial count (TVBC) of the soil samples revealed a significant presence of bacteria, with CFUs (colony-forming units) ranging from 2.5 x10⁶ to 4.0 x10⁶ at 10⁻¹ dilution factor and from 0.8 x10⁶ to 1.5 x10⁶ at a 10⁻⁵ dilution factor. The high bacterial load observed, particularly in Ikirun (S4) and Iwo (S1), suggests that these areas may host abundant microbial communities capable of utilizing plastics as a carbon source. This observation is supported by studies documenting that high bacterial counts in microplastic-polluted soils indicate increased microbial activity aimed at plastic degradation (Nadeem *et al.*, 2022).

The identification of the bacterial isolates, including Bacillus subtilis, Bacillus licheniformis, and Streptococcus spp., suggests the presence of microorganisms known for their plastic-degrading capabilities. Bacillus subtilis, for example, has been extensively studied for its ability to produce enzymes like lipases and proteases that can degrade various forms of plastics, including polyethylene and polystyrene (Yao *et al.*, 2022). Similarly, Bacillus licheniformis has been shown to secrete extracellular enzymes that break down plastic polymers. The presence of these bacteria in polluted soils is indicative of potential biodegradation activities, as reported by recent studies focusing on microbial adaptation in plastic-contaminated environments (Lv *et al.*, 2024). The results are consistent with previous reports that microplastic pollution alters the microbial community structure, promoting the growth of plastic-degrading bacteria (Lamela *et al.*, 2023; Jumaah, 2015). The isolated bacterial species, especially from the Bacillus genus, are widely recognized for their role in biodegradation. These findings highlight the potential of these microbial species as key agents in bioremediation efforts, as demonstrated by similar works in plastic-polluted ecosystems (Yao *et al.*, 2022). The ability of these bacteria to utilize citrate and sucrose, as seen in the physiological tests, indicates their metabolic versatility, which is essential for surviving and thriving in harsh, plastic-laden environments. This adaptability is critical for the biodegradation process, as microbial metabolism is often driven by the need to utilize available carbon sources, including plastics, for survival (Lv *et al.*, 2024).

Overall, the findings authenticate Bacillus species' superior degradation efficiency and suggest exploring engineered Bacillus consortia to enhance degradation, as proposed by Emmanuel-Akerele *et al.* (2022). These results reaffirm the critical role of Bacillus species in addressing plastic pollution through bioremediation.

**Conclusion**

This study isolated and identified microplastic-degrading bacteria from plastic-polluted soils in Osun State, Nigeria. Among the identified species, *Bacillus subtilis* and *Bacillus amyloliticu*s demonstrated the highest plastic degradation efficiency, achieving up to 45% weight loss over eight weeks. The findings highlight the potential of Bacillus strains for the bioremediation of plastic waste, offering a sustainable approach to mitigating environmental pollution. The alkaline pH and favourable temperatures of the soils significantly supported bacterial activity and degradation processes. These results underscore the importance of leveraging microbial resources for addressing the global challenge of plastic pollution.

**Recommendation**

To address plastic pollution effectively, large-scale bioremediation efforts should prioritise the application of Bacillus subtilis and Bacillus amyloliticus, given their demonstrated efficiency in degrading plastics. Continuous monitoring of soil conditions, such as pH and temperature, is essential to optimise bacterial activity and degradation rates. Furthermore, future research should focus on engineering microbial consortia and exploring the enzymatic pathways involved to enhance the bioremediation process.

**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. No financial support or sponsorship was received from any commercial entities, and all analyses were conducted objectively and independently. This research was performed solely for academic purposes and to advance understanding in the comparative nutritional assessment of tomato varieties.

**Disclaimer**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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