Isolation and Identification of Microorganisms from Microplastic-Polluted Soil from Geopolitical Zones in Osun State, Nigeria

Authors

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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 locations in three zones, and their physicochemical properties were analyzed. Microbial isolation and identification were carried out using morphological and biochemical tests standards. The pH ranged from 8.2 to 8.8. and the temperature ranged from 25°C to 29.1°C. Microbiological assessments revealed high bacteria counts, with total viable bacterial counts ranging from 2.5 x 10^6 to 4.0 x 10^6 CFU/g. Four bacterial strains were isolated: *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliticus*, and *Streptococcus spp* while fungal include *Aspergillus niger* and *Aspergillus terreus*. 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: Bacteria, Microplastic, Pollution, Soil.

1.0 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.

1.1 Fate of plastics in Osun Communities

Plastic pollution poses a significant environmental challenge in Osun communities, particularly in the Osun River. Studies have shown the river contains up to 22,079 microplastic particles per liter, one of the highest concentrations globally (Og Research Conservation, 2023). This contamination is largely due to the widespread use and improper disposal of single-use plastics such as sachet water bags, food packaging, and plastic bottles (Wikipedia, 2023). In response, the Osun State Government launched an action plan in 2023 to tackle plastic pollution, aiming to achieve measurable progress by 2024 through policy alignment with international environmental standards (EnviroNews Nigeria, 2023). Despite this, challenges remain, including poor waste management infrastructure and limited public awareness. In areas like Ede, plastics are often burned or dumped into rivers, worsening pollution levels (Bjelica, 2023). Addressing this issue requires improved waste systems, recycling programs, and educational campaigns to reduce plastic waste and its harmful effects.

Figure 1: Dumpsite view from Author Field Work

1.2 Objectives

This study aims to:

- To analyse the physicochemical parameters of the dumpsite soil
- To estimate the total heterotrophic bacteria and fungal count

- To isolate and identify microorganisms from dumpsite soil

2.0 Materials and Methods

2.1 Study Area

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 shown in Table 1. 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.

Table 1: Coordinates of sample location in Osun State

S/N	Location	Gps Location	LGA	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

2.2 Pre-treatment of Soil Samples

The collected soil samples were 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.

2.3 Isolation of Microplastic-degrading Microorganisms

Five-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 10^5. From each dilution, 0.1 ml was placed on prepared sterile nutrient agar (NA) plates and Potato Dextrose Agar (PDA) using the spread plate method. The plates were incubated at 28°C for 48 hours and 72 hours respectively. Colonies appearing on the plates were counted to determine the total heterotrophic bacteria count (TVC) and recorded as colony-forming units per gram of soil (cfu/g).

The distinct bacterial and fungal colonies observed on the nutrient agar and potato dextrose agar were subcultured on fresh agar plates to obtain pure cultures. Each isolate was streaked onto nutrient agar and potato dextrose 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.

2.4 Morphological and Biochemical Characterization

The isolated bacteria were subjected to morphological and biochemical tests to aid in their identification using Fawole and Oso, (2004) method. 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 included the catalase test through the use of hydrogen peroxide (H_2O_2) 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. Fungi isolates were identified with front appearance and reverse appearance with microscopic examination using lactophenol blue.

3.0 Results

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

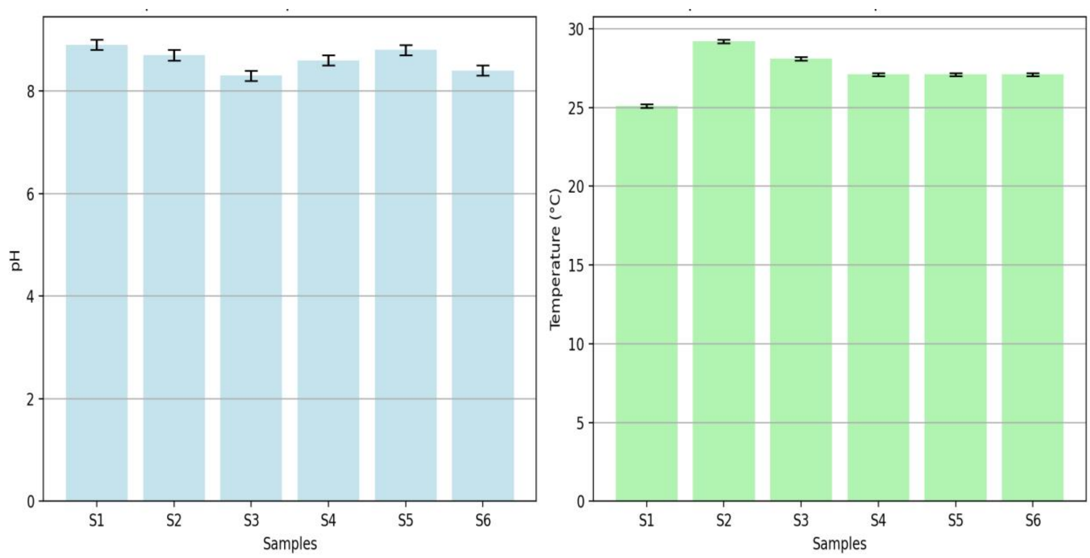

Figure 2: Physicochemical parameters of Microplastic Polluted Soil

KEYS

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

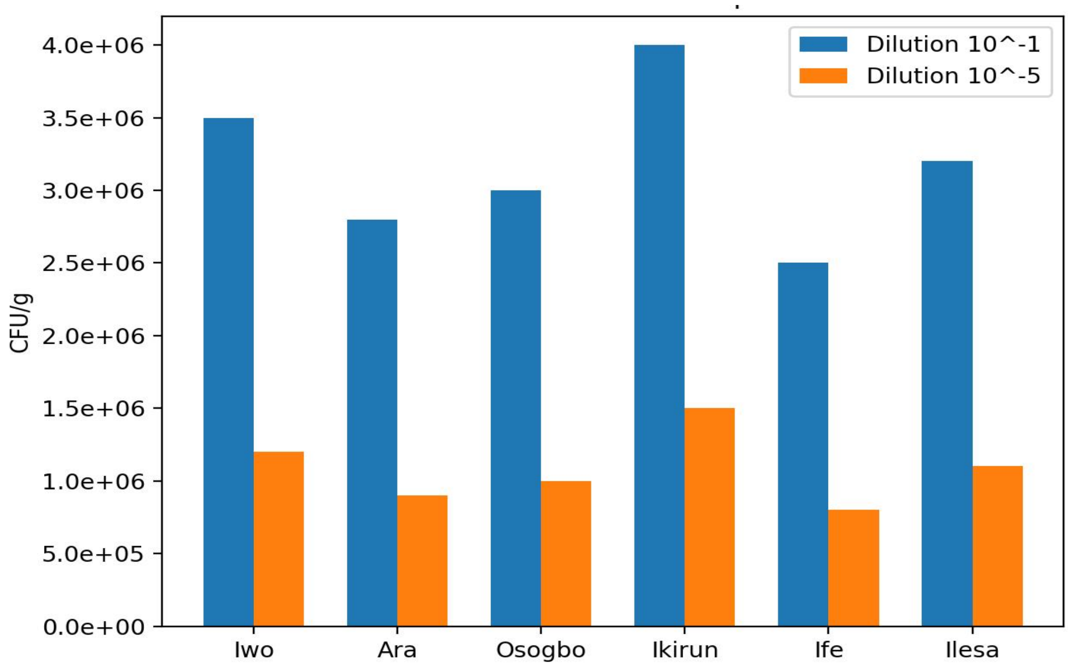

Figure 3: Total heterotrophic Bacteria Count of Microplastic Polluted Soil

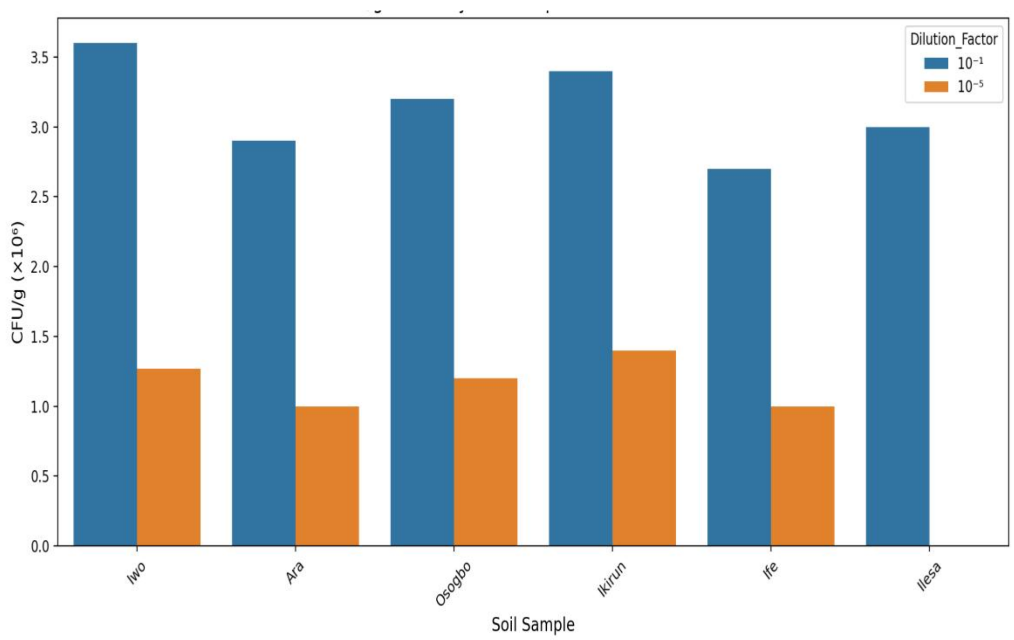

Figure 4: Total Fungal Count of Microplastic Polluted Soil

Table 2: Colonial, morphological and biochemical characterization of bacterial isolates obtained from six locations within geopolitical zones of Osun State.

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
Presume Organisms	***Bacillus subtilis***	***Bacillus licheniformis***	***Bacillus amyloliticus***	***Streptococcus spp***

Table 3: Colonial, morphological and biochemical characterization of Fungal isolates obtained from six locations within three (3) geopolitical zones of Osun State.

Isolate ID	**Colony Appearance**	**Reverse Colony Color**	**Hyphae**	**Conidiophores**	**Spores Size**	**Presume organism**
Isolate 1	Black, powdery, radial colonies with white margins	Pale to yellowish	Septate hyphae	Long,	3.5–5 µm	*Aspergillus niger*
Isolate 2	Cinnamon to light brown,	Yellow to orange	Septate hyphae	Short	2–3.5 µm	*Aspergillus terreus*

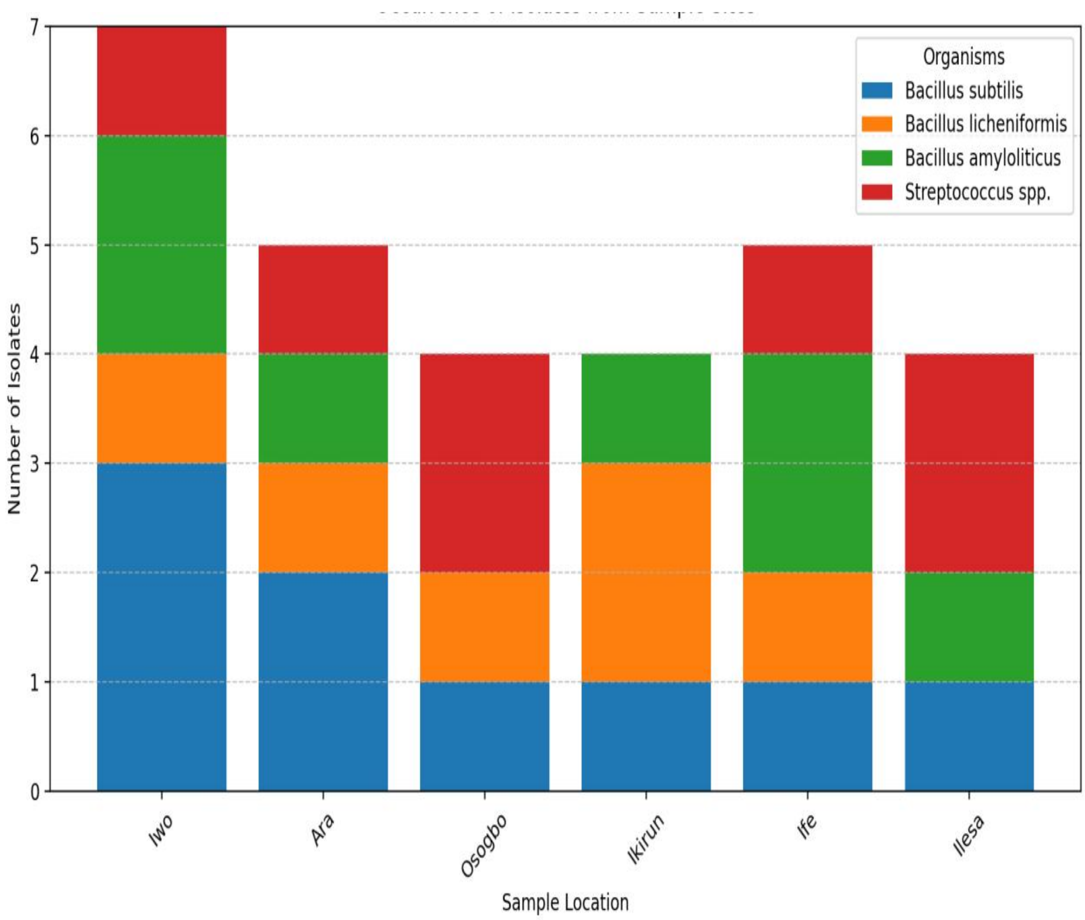

Figure 5: Occurrence of Isolates from Sample Sites

4.0 Discussion of Findings

Due to their potent adsorption and capacity to continuously pollute the environment with compounds, microplastics, a term used to describe plastic trash and particles smaller than 5 mm, have garnered significant attention. Microplastics have recently been found in the ocean, the atmosphere, soil, food, surface rivers and lakes, and even in the seldom visited polar areas and deserts (Gupta *et al.,* 2024).

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 x10^6 to 4.0 x10^6 at 10^{-1} dilution factor and from 0.8 x10^6 to 1.5 x10^6 at a 10^{-5} dilution factor while fungal high count was obtained at 3.60 x10^6 and lowest at 1.0 x10^6 . The high bacterial load observed, particularly in Ikirun (S4) and Iwo (S1) for bacterial while Funagi had lowest at Ara and Ife and highest at Iwo. This 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 isolates, including *Bacillus subtilis*, *Bacillus licheniformis*, *Streptococcus* spp., *Aspergillus niger* and *Aspergillus terreus* 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. The isolated fungi have been reported to have the ability to degrade most especially Aspergillus genera thereby conform with previous finding as earluer stated by Lamela *et al.,* (2003) and they are know to produce degrading enzymes like cutinase. 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* and Fungi specesi' 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.

5.0 Conclusion and Recommendation

This study isolated and identified microplastic-degrading bacteria from plastic-polluted soils in Osun State, Nigeria. 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. 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.

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References

Bjelica, A. (2023). Assessment of plastic pollution in Osun State. Geo-Science Panoramic.

Da Silva, M. R. F., Souza, K. S., Motteran, F., de Araújo, L. C. A., Singh, R., Bhadouria, R., & de Oliveira, M. B. M. (2024). Exploring biodegradative efficiency: A systematic review on the main microplastic-degrading bacteria. *Frontiers in Microbiology, 15*, 1360844.

Emmanuel-Akerele, H. A., Akinyemi, P. O., & Igbogbo-Ekpunobi, O. E. (2022). Isolation and identification of plastic-degrading bacteria from dumpsites Lagos. *Advances in Environmental Technology, 1*, 59-71.

EnviroNews Nigeria. (2023). Osun institutes action against plastic pollution. https://www.environewsnigeria.com/world-environment-day-osun-institutes-action-against-plastic-pollution-environmentalists-seek-alternatives/

Fawole, M.O. and Oso, B.A, (2004). Characterization of bacteria: Laboratory manual of microbiology, Spectrum Publishers, Ibadan.

Frontiers in Environmental Science. (2021). Microplastics increase soil pH and decrease microbial activity. *Frontiers in Environmental Science*.

Gupta, V., Trivedi, A,, Nandeha, N., Monya, D., Dujeshwer, K., Pandey, A.K., and Singh, A. (2024). Micro Plastic Pollution in Soil Environment: A Comprehensive Review. J. Sci. Res. 30(6):412-9.

Jumaah, O. S. (2015). Screening of plastic-degrading bacteria from dumped soil area. *IOSR Journal of Environmental Science, Toxicology and Food Technology, 11*(5), 93-98.

Lamela, P. A. M., Diputado, Y. G., Lumantas, J. M., Gatpatan, I. G., & Cabulong, R. B. (2023). Isolation, characterization, and identification of plastic-degrading bacteria from Bago City landfill, Negros Occidental, Philippines. *Philippine Journal of Natural Sciences, 28*, 24-25.

Lv, S., Li, Y., Zhao, S., & Shao, Z. (2024). Biodegradation of typical plastics: From microbial diversity to mechanisms. *International Journal of Molecular Sciences, 25*(1), 593.

Nadeem, H., Alia, K. B., Muneer, F., Rasul, I., Siddique, M. H., Azeem, F., & Zubair, M. (2022). Isolation and identification of low-density polyethylene degrading novel bacterial strains. *Archives of Microbiology, 203*, 5417–5423.

Og Research Conservation. (2023). Gideon's advocacy for plastic ban. https://ogresearchconservation.org/gideon-plastics-ban/

Ololade, I. A., Apata, A., Oladoja, N. A., Alabi, B. A., & Ololade, O. O. (2023). Microplastic particles in river sediments and water of southwestern Nigeria. *Environmental Science and Pollution Research, 30*, 12345–12356.

Onyekachi, N. S., & Chukwuemeka, I. S. (2022). A review: State of plastic pollution in Nigeria and measures to tackle them. *Journal of Analytical Toxicology and Environmental Studies, 2*(1), 101.

Yalwaji, B. (2022). Plastic pollution in the environment in Nigeria: A rapid systematic review of the sources, distribution, research gaps, and policy needs. *Scientific African, 7*, e00242.

Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., ... & Oda, K. (2016). A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science, 351*(6278), 1196–1199. https://en.wikipedia.org/wiki/Ideonella_sakaiensis

Yao, Z., Seong, H. J., & Jang, Y. S. (2022). Degradation of low-density polyethylene by *Bacillus* species. *Applied Biological Chemistry, 65*(2), 97-104.

Wikipedia. (2023). Osun River. https://en.wikipedia.org/wiki/Osun_River