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# Microbial Isolates in Microplastic-Polluted Soil

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**Abstract**

Microplastic pollution is a growing environmental concern, with plastic debris fragmenting into microscopic particles that contaminate soil ecosystems. Microplastics have become a major environmental concern due to their persistent presence in various ecosystems, including soil. They can act as pollutants and have detrimental effects on the environment and human health. This study aimed at isolating and identifying microbes (Bacteria and Fungi species) present in microplastic-polluted soil. Microplastic-polluted soil samples were collected from three sites (which were Kwararafa University Area Wukari to serve as the site 1, Federal Road Safety Commission Office Area Wukari to be the site 2, while Marmara Area Wukari to be the site 3), from two points; A and B and then packaged in brown envelopes which were then transferred to the Laboratory for further analysis after air-drying them for 24 hours. Then 1gram of each soil sample was weighed and suspended in 9mls of distilled water respectively; Six (6) folds of distilled water were prepared for serial dilution of each soil sample. 1ml of each sample was pour plated on NA, MAC, BA, EMB, CLED and SDA respectively. Then a smear of bacteria was prepared and allow to air dry for 30min and heat fix using flame after which was viewed under the microscope. The results obtained from this study showed that *Staphylococcus spp* are large, medium, small, and pinpoint in size, irregular and spherical in shape, light pink, creamy grey in color, smooth,



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mucoid and dried texture. *Escherichia coli* and *Klebsiella spp* here has similar morphological characteristics, both in size, shape, color, texture, margin and elevation as *Staphylococcus spp*. *Bacillus spp* and *Pseudomonas spp* here has similar morphological identification but differ only in their shape where *Bacillus spp* has a filamentous shape but *Pseudomonas spp* has rhizoid shape respectively. In terms of texture, *Bacillus spp* are Smooth, dried and mucoid but *Pseudomonas spp* are only smooth, dried texture. *Bacillus spp* and *Pseudomonas spp* only differ in their cell morphology where *Bacillus spp* appears to be rods with spores while *Pseudomonas spp* appears as short and long rods in pairs. They react positive to catalase, oxidase and citrate tests while negative to indole test. *Aspergillus Niger* and *Rhizopus stolonifer* were found to be the major fungi isolated from the collected sample. *Aspergillus Niger* and *Rhizopus stolonifer* are spherical in shape and are large in size. *Aspergillus Niger* appears powdery and *Rhizopus stolonifer* appears to fluffy, wood-like. In terms of color and pigmentation *Aspergillus Niger* species are black, black creamy on reverse while *Rhizopus stolonifer* appears grayish in color. The discovery of these microbes in microplastics-polluted soil offers a ray of hope for mitigating plastic pollution. By delving deeper into their capabilities and fostering collaboration between microbiologists and environment. This research will contribute to a broader understanding of the ecological impact of microplastics on soil health and functioning. By identifying microbes associated with microplastics, the specific mechanism of action employed by these bacteria in degrading microplastics can be studied subsequently.

**Keywords:** Microplastics, Microbes, Bacteria, Fungi, Pollutants, Soil

## INTRODUCTION

One of the main problems nowadays that threaten the sustainable development and human health is environmental degradation of pollutants (Rahman *et al.,* 2021). One of the major environmental pollutants in this current dispensation are microplastics, (Microplastics) contamination is widespread and has become a significant global environmental concern because of rising usage (Hasnine *et al.,* 2021). There are still many questions about Microplastics in terrestrial ecosystems, as recent research on Microplastics contamination has mainly focused on aquatic habitats (Marshall *et al.,* 2020). The population of the world increased at an average annual rate of 1.68 % between 1955 and 2015 (Lozano *et al.,* 2021; Rillig *et al.,* 2021). The amount of waste generated by people has increased as a result of this dramatic increase in the world population (Elbasiouny *et al.,* 2021). Recent decades have seen a significant increase in global awareness of the impact that Microplastic soil contamination has on both terrestrial and aquatic ecosystems (Sridharan *et al.,* 2021).

Researchers have reported that plastic waste accumulation puts pressure on the environment. Due to the overuse of plastic products and careless disposal of plastic trash, has become a global problem (Sridharan *et al.,* 2021). These plastics are high molecular weight polymers, long-chain molecules comprised of repeating structural monomers (Hossain *et al.,* 2021). These Long-chain molecules exhibit powerful van der Waals forces, and as a result of prolonged exposure to certain factors, these plastics may undergo degradation, yielding microplastics. There are many different types of discarded plastics in the environment, ranging in size from nanometers to centimeters, including macroplastics, meso-plastics, microplastics, and nanoplastics (Elbehiry *et al.,* 2021). Any plastic component with a diameter between 1 μm and 5 mm is considered a Microplastic (Zhang *et al.,* 2020). Microplastics can be secondary, created during the breakdown and degradation of bigger plastics, or main, resulting directly from the usage of products and materials that contain Microplastics (Yang *et al.,* 2021). Microplastics when released in the environment they have the ability to disperse widely. They can be carried by the wind after being released into the environment, washed off the soil by rain or storm water run-off, and then they can enter the aquatic environment. Through ingestion, inhalation, and translocation, Microplastics can biomagnify and enter the food chain as well as the human body (Anik *et al.,* 2021). Soil microbes are microorganisms that live in the soil and play a crucial role in the soil ecosystem. These microorganisms include bacteria, fungi, archaea, viruses, and protozoa. They contribute to various ecological functions such as nutrient cycling, organic matter decomposition, and disease suppression.

## MATERIALS AND METHODS

### Study Area

This study was conducted in Microbiology laboratory, Federal University Wukari, Taraba State, Nigeria. Wukari a famous city located formerly in Gongola State of Nigeria. Wukari lies between Taraba State and Benue State and it's bounded in the south by Benue state, north by Gassol LGA, east by Donga LGA and Takum, west by Ibi LGA. The relative humidity of Wukari ranges from 14 to 77 %.Wukari covers an area of 4,308 $km^2$ and with a population of about 241,546 at the 2006 census, a traditional state rich with various cultures, norms and value (Otitoju and Lewis, 2021).

**Sample Collection**

Three sampling sites were precisely mapped out for soil samples collection, which were Kwararafa University Area Wukari to serve as the site 1, Federal Road Safety Commission Office Area Wukari to be the site 2, while Marmara Area Wukari to be the site 3.During the collection, the soil samples were collected into an envelope and were labeled as site (S1), site (S2), and site (S3) having point A (PA) and point B (PB) respectively. The point at the sampling sites was 50 m apart. Little quantity of the soil samples was collected from each point of the sampling sites using stainless steel hand trowel at the depth of 0−15 cm into an envelope and then transported to the laboratory. The soil samples from each sub site were introduced into their various containers and labeled as S1, S2 and S3 with their various point PA and PB respectively.

**Sample Preparation**

The soils samples collected from the various sites were sieved then air-dried for at least 24 hours, weighed, then packaged into the various container (bottles) and well labeled, after then the samples were put into the refrigerator until it was time to perform analysis on them.

**Serial Dilution**

1gram of each soil sample was weighed and suspended in 9mls of distilled water respectively; Six (6) folds of distilled water were prepared for serial dilution of each soil sample. 1ml of each sample was pour plated on NA, MAC, BA, EMB, CLED and SDA respectively. 1ml of each sample was spread plated on the respective culture media. The pour plates where allowed to cool and solidify before incubating at 37°C for 18-24hrs

**Gram Staining**

A smear of bacteria was prepared and allow to air dry for 30min and heat fix using flame. It was flood with crystal violet for 1min. then wash off with distilled water. Then Apply lugol's iodine for 1min. And then wash off with distilled water. Later decolorize with acetone or ethyl alcohol. And then wash off with distilled water. Then flood with safranine for 30 seconds. Then wash off with distilled water and allowed to air dry, then place a drop of immersion oil on the stained smear and view under ×100 objective lens

**Catalase test:**

This is a test to ascertain the ability of bacteria to produce catalase that reduces hydrogen peroxide to water and oxygen. Growths of samples were scraped with wired loop. It was then suspended in a drop of 3% $H_2O_2$ on a slide, then examined for bubble formation. If effervescence occurs, it is confirmatory positive test for catalase production, but if it does not occur it is negative test for catalase production.

**Citrate Utilization test:**

Citrate is acted upon by enzyme citrase which produces oxaloacetic acid and acetate. These are enzymatically converted to pyruvate and $CO_2$. During reaction, the medium becomes alkaline as the $CO_2$ combines with Na and $H_2O$ to form sodium carbonate which is alkaline. Simmon's citrate medium slants were prepared. Samples were inoculated into 86 agar slants and incubated for 24-48 hrs at 37°. Positive result is indicated by blue colour slope and no colour change indicates a negative result.

**Indole test:**

Inoculating a tube of tryptophan broth with each bacterial isolate, then incubate the tubes at 37°C for 24-48 hours. After incubation, add a few drops of kovac's reagent to the broth culture and shake the tube gently and observe for a red colored ring at the broth-reagent interface. a red ring indicate the production of indole from tryptophan by the bacteria.

**Oxidase test:**

This test depends on presence of certain oxidases in bacteria that will catalyse the transport of electrons between electron donors in the bacteria and a redox dye- tetraethyl p-phenylene-diamine which is reduced to a deep purple if positive. A strip of filter paper was moistened with freshly prepared 1% solution of the reagent. Immediately, a speck of culture was rubbed on it with a loop. Positive test is indicated with an intense deep purple blue within 1060 seconds. No colour change after 60 seconds indicates a negative result.

**Triple Sugar Iron (TSI) test:**

This test depends on ability of bacteria to ferment lactose, sucrose and glucose and the production of hydrogen sulphide. TSI agar medium was prepared, dispensed in test tubes, sterilized and allowed to set as slopes. Slants were inoculated with samples and

incubated for 18-24 hours at 37°C. Yellow butt, red slant indicates positive glucose fermentation. Yellow butt, yellow slant indicates positive lactose and/or sucrose fermenting. Red butt, red slant indicates neither glucose, lactose, sucrose fermenting. Black precipitate at bottom of slant indicates $H_2S$ production.

## RESULTS AND DISCUSSION

### Morphological identification of bacterial species isolated from microplastic-polluted soil

Table 1 below indicates the presence of microorganisms (microbes) in the soil samples obtained at the three sites. The table also shows the morphological characteristics of these microbes that were identified which are; *Staphylococcus spp, Escherichia coli, Klebsiella spp, Bacillus spp, Pseudomonas spp*. It also explains the cultural morphology of these identified microorganisms present in the soil samples, which is the size, shape, color, texture, margin, elevation of all the bacteria.

The bacterium *Staphylococcus spp* are large, medium, small, and pinpoint in size, irregular and spherical in shape, light pink, creamy grey in color, smooth, mucoid and dried texture, and appears to has an entire, undulate in margin. It elevation is convex, flat and raised. *Escherichia coli* and *Klebsiella spp* here has a similar morphological characteristics, both in size, shape, color, texture, margin and elevation as *Staphylococcus spp*. *Bacillus spp* appears to be large, medium, and small in size, spherical, irregular and filamentous shape, as well as whitish, pink, and grey color. While in terms of texture *Bacillus spp* are Smooth, dried and mucoid but only differ from other isolated bacteria in its elevation appears, where it is slightly elevated, and has a filiform margin. *Pseudomonas spp* here has similar morphological identification but differ only in their shape where *Pseudomonas spp* are large, medium, small and pinpoint in size, irregular, spherical and rhizoid shape respectively. *Pseudomonas spp* has smooth, dried texture, light pink, whitish grey, creamy color. It also has a flat and slightly raised elevation. *Pseudomonas spp* are large, medium, pinpoint and small in size, and has a smooth and dried texture.

**Morphological appearance of the Gram Stained Bacteria from Microplastics-polluted soil**

Table 2 below shows the result of biochemical tests of bacterial species isolated from Microplastics-polluted Soil which describes how these microorganisms are arranged when view under the microscope. And also their reaction to certain biochemical test.

Based on the result obtained from the table below shows that the cell morphology of *Escherichia coli* are rods in pairs, which also test positive to catalase and indole test, and then test negative to oxidase and citrate test.

The *Staphylococcus spp* are cocci cluster in terms of cell morphology, response positive to catalase and citrate test and negative to oxidase and indole test.

*Klebsiella spp* are mostly short rods in their cell morphology and these species of microorganisms test positive to catalase and citrate, but to oxidase and indole, they test negative.

*Bacillus spp* are also rods with spores in terms of their cell morphology, they also test positive to catalase, oxidase and citrate test, while test negative to indole test. *Pseudomonas spp* cell morphology are short and long rods in pairs, where they react positive to catalase, oxidase and citrate tests while negative to indole test.

**Some selected biochemical analysis carried out on the identified bacteria species**

Table 3 below also shows the result of biochemical analysis carried out on isolated bacterial species from microplastic-polluted soil. These tests are glucose test, sucrose test, lactose test, gas test and these tests help to show if these identified microorganisms can utilize glucose, lactose and sucrose which are triple sugar iron. *Staphylococcus spp, Escherichia coli, Klebsiella spp*, *Bacillus spp,* and *Pseudomonas spp* are the microbes used to carry out these tests.

*Staphylococcus spp* are generally non-motile, gram-positive cocci that do not ferment any of the sugars in the triple sugar iron agar. Therefore, the results for *Staphylococcus spp* on a triple sugar iron test would be acid slant (A)/acid butt (A) with no gas production (no slant). *Escherichia coli*is a motile, gram-negative rod-shaped bacterium that ferments all three sugars (glucose, lactose, and sucrose) in the triple sugar iron agar. The result show an alkaline slant (K)/acid butt (A) with gas production (indicated by bubbles in the medium).*Klebsiella spp* are also gram-negative rod-shaped bacteria and most strains can ferment lactose and

sucrose but not glucose. It form and alkaline slant (K)/ acid butt (A) with no gas production. *Bacillus spp* are gram-positive, rod-shaped bacteria. Some bacillus species can ferment various sugars in the triple sugar iron agar, while other cannot. However, most *Bacillus spp* are obligate aerobes and may not grow well under the anaerobic conditions in the triple sugar iron agar butt. Therefore, the results for *Bacillus spp* on a triple sugar iron test can be variable, but they often show no change in the butt (no fermentation) with either an acidic or alkaline slant depending on the specific sugar fermentation pattern. While *Pseudomonas spp* are gram-negative rods that typically do not ferment any of the sugar in the triple sugar iron agar and do not produce gas. Therefore, the results for pseudomonas spp on a triple sugar iron test would be acid slant (A)/acid butt (A) with no gas production (no slant).

**Morphological identification of fungal species isolated from microplastic-polluted soil**

Table 4 below shows the isolated fungi which are; *Aspergillus Niger* and *Rhizopus stolonifer* and their morphological characteristics as seen below

*Aspergillus Niger* and *Rhizopus stolonifer* were found to be the major fungi isolated from the collected sample.

*Aspergillus Niger* appears spherical in shape and large in size, appearance powdery, black color and pigmentation.The black or dark brown color of *Aspergillus Niger* is a key diagnostic feature. Where the black, globose (spherical) conidia produced on phialides (specialized spore-producing structures) arranged in flask-shaped structures called conidial head. Colonies may also have a yellowish or reddish exudate (secreted material) on the surface. *Aspergillus Niger* is slightly elevated in terms of elevation with an entire margin

While *Rhizopusstolonifer* appears to be fluffy, wood-like in terms of appearances and appears grayish in color/pigmentation. It is also highly elevated with entire margin, spherical in shape, large in size. *Rhizopus stolonifer* grow rapidly and has a characteristic cottony appearance. It produces white of slightly off-white sporangia (spore-containing structures) visible on the aerial hyphae.

Both *Aspergillus Niger* and *Rhizopus stolonifer* are known for their ability to degrade various organic

materials, including some types of plastics. Their presence in the soil indicates the possibility that they might be contributing to their degradation. Researchers has made it known that some fungi utilizes these plastics as their carbon source.

Table 1.Morphological identification of bacterial species isolated from microplastic-polluted soil

| S/N | **Bacteria Isolates** | ***Staphylococcus spp*** | ***Escherichia coli*** | ***Klebsiella spp*** | ***Bacillus spp*** | ***Pseudomonas spp*** |
|---|---|---|---|---|---|---|
| 1 | Size | Large, medium, small, pinpoint | Large, medium, small, pinpoint | Large, medium, small, pinpoint | Large, medium, small | Large, medium, small, pinpoint |
| 2 | Shape | Irregular, spherical | Irregular, spherical | Irregular, spherical | Spherical, irregular, filamentous | Irregular, Rhizoid, spherical |
| 3 | Color | Light pink, creamy grey | Light pink, creamy grey | Light pink, creamy grey | Whitish, pink, grey | Light pink, whitish grey, creamy |
| 4 | Texture | Smooth, mucoid, dried. | Smooth, mucoid, dried. | Smooth, mucoid, dried. | Smooth, dried, mucoid | Smooth, dried |
| 5 | Margin | Entire, undulate | Entire, undulate | Entire, undulate | Entire, undulate, filiform | Entire, undulate |
| 6 | Elevation | Convex, flat, raised | Convex, flat, raised | Convex, flat, raised | Flat, slightly, elevated | Flat, slightly raised |

Table 2: Morphological appearance of the Gram Stained Bacteria from Microplastics-polluted soil

| S/N | **Bacteria Isolates** | ***Staphylococcus spp*** | ***Escherichia coli*** | ***Klebsiella spp*** | ***Bacillus spp*** | ***Pseudomonas spp*** |
|---|---|---|---|---|---|---|
| 1 | Cell Morphology | Cocci in cluster | Rods in pairs | Short rods | Rods with spores | Short & long rods in pairs |
| 2 | Catalase | Positive | Positive | Positive | Positive | Positive |
| 3 | Oxidase | Negative | Negative | Negative | Positive | Positive |
| 4 | Citrate | Positive | Negative | Positive | Positive | Positive |
| 5 | Indole | Negative | Positive | Negative | Negative | Negative |

Table 3: Some selected biochemical analysis carried out on the identified bacteria species

| S/N | Biochemical Parameters | ***Staphylococcus spp*** | ***Escherichia coli*** | ***Klebsiella spp*** | ***Bacillus spp*** | ***Pseudomonas spp*** |
|---|---|---|---|---|---|---|
| 1 | Glucose | - | + | - | - | - |
| 2 | Sucrose | - | + | + | + | - |
| 3 | Lactose | - | + | + | + | - |
| 4 | Gas | - | + | - | - | - |

**Key:** + means positive and - means negative

Table 4: Morphological identification of fungal species isolated from microplastic-polluted soil

| S/N | Fungal Isolates | *Aspergillus Niger* | *Rhizopus stolonifer* |
|---|---|---|---|
| 1 | Shape | Spherical | Spherical |
| 2 | Size | Large | Large |
| 3 | Appearance | Powdery | Fluffy, wood-like |
| 4 | Color/pigmentation | Black, black creamy on reverse | Grayish |
| 5 | Elevation | Slightly elevated | Highly elevated raised |
| 6 | Margin | Entire | Entire |

## DISCUSSION

This research was carried out in Federal University Wukari, Biology Laboratory. Our investigation on microbial communities of microplastic-polluted soil has shed light on the fascinating interplay between these novel substrates and the resident soil microbiome. The isolation and characterization of these microbes provide valuable insights into the potential ecological consequences of microplastics pollution.

From the result obtained in table 1 where the cultural morphology of bacteria species were identified, bacteria like *Staphylococcus spp, Escherichia coli, Klebsiella spp, Bacillus spp* and *Pseudomonas spp* were present in the soil samples, we also went further to the identification of the morphological characteristics such as their shape, size, color, texture, margin, and elevation. Where some of these bacteria species differs in their morphology but others are similar to each other in shape or size. *Staphylococcus spp* were having large, medium, small, pinpoint size, irregular, spherical shape, light pink, creamy grey in color, smooth, mucoid, dried texture, and entire, undulate margin, convex, flat, raised elevation. *Escherichia coli*

appears large, medium, small, pinpoint, irregular, spherical shape, light pink, creamy grey color, smooth, mucoid and dried texture, entire, undulate margin. *Klebsiella* spp here appears to be same large, medium, small, pinpoint in size, irregular, spherical shape, with the same color, texture, margin and elevation. *Bacillus spp* filiform as it margin and *Pseudomonas spp* are Large, medium, small, pinpoint in size, Irregular, Rhizoid, spherical in shape, Light pink, whitish grey, creamy in terms of it color appearance, Smooth, dried texture, Entire, undulate margin, Flat, slightly raised elevated.

From table 2 where the morphological appearance of the gram stained bacteria from microplastics-polluted soil was identified for *Staphylococcus spp* to be cocci in cluster, *Escherichia coli* is rods in pairs, *Klebsiella spp* short rods, whereas *Bacillus spp* appears to be rods with spores and *Pseudomonas spp* short and long rods in pairs.

During some selected biochemical analysis carried out on these microbes, *Staphylococcus spp* test positive to citrate, catalase, indole, oxidase, glucose. In term of these glucose test, it indicate that these microbes utilize glucose as carbon source, while some microorganisms like *Escherichia coli* and *Bacillus spp* cannot utilize glucose, so it indicate negative. *Klebsiella spp* and *Pseudomonas spp* here test negative to sucrose test.

While the test for the presence of these Fungi and some cultural morphology of them. *Aspergillus Niger* and *Rhizopus stolonifer* were the fungi species found in the microplastics-polluted soil. *Aspergillus Niger* and *Rhizopus stolonifer* has similar shape, size and margin. But differ in their appearance where *Aspergillus Niger* appears to be Powdery while *Rhizopus stolonifer* appears Fluffy, wood-like.

## CONCLUSION

The aspect of this research is evaluating the potential of isolated microbes to degrade microplastics. Certain microbes may possess enzymatic machinery capable of breaking down specific polymer types. Identifying these “microplastics-degrading" microbes could pave the way for future bioremediation strategies to address microplastics pollution in soil.

Soil is a rich habitat for various microorganisms, both beneficial and harmful. Primary and secondary metabolites released by bacteria in soil are beneficial to increase the nutrient contents in soil, promote plant growth and also play a major role in nutritional chains. While pathogenic strains are commonly less present as compared to non-pathogenic

strains, disturbance of the microfloral habitat by human actions such as littering, defecation by animals, climate change etc may lead to accumulation of infectious microorganisms. *Staphylococcus spp, Escherichia coli, Klebsiella spp, Bacillus spp* and *Pseudomonas spp* have the capability of degrading microplastics as their carbon source.

Microbial interactions with microplastics might not always be beneficial. Some microbes might inadvertently enhance the persistence of microplastics by fragmenting them into even smaller particles, potentially increasing their bioavailability and posing a greater threat to soil organisms. Additionally, microplastics could act as carriers for harmful microbes or pollutants, introducing new threats to soil health.

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