International Journal of Science and Technology Research Archive

ISSN: 0799-6632 (Online)

Journal homepage: https://sciresjournals.com/ijstra/

(RESEARCH ARTICLE)

Comparative study of microbial tolerance and degradation potentials to herbicides in crude oil-polluted soil

Chika Christiana NWANKWO [1,*], Margaret GBARALE [1] and Oluchi RoseColette NLEMOLISA [2]

[1] *Department of Microbiology Technology, School of Science Laboratory Technology (SSLT), University of Port Harcourt, Port Harcourt, Rivers State Nigeria.*
[2] *Department of Microbiology, Federal University of Technology, Owerri, Imo State PMB 1526, Nigeria.*

International Journal of Science and Technology Research Archive, 2026, 10(02), 010-021

Publication history: Received on 09 March 2026; revised on 19 April 2026; accepted on 22 April 2026

Article DOI: https://doi.org/10.53771/ijstra.2026.10.2.0017

Abstract

This study isolated bacteria and fungi from crude oil-polluted soil and tested their tolerance and degradation potentials in the presence of glyphosate, paraquat and chlorpyrifos. Bacterial isolates and their percentage occurrence were *Pseudomonas putida* (29%), *Bacillus cereus* (18%), *Bacillus subtilis* (13%), *Chromobacterium violaceum* (11%), *Clostridium* sp. (8%), *Serratia marcescens* (8%), and *Corynebacterium* sp. (8%), while fungal isolates and their percentage occurrence were *Fusarium* sp. (13%), *Aspergillus niger* (34%), *Rhizopus* sp. (22%), *Aspergillus terreus* (13%), and *Penicillium* sp. (19%). The total heterotrophic bacteria and total fungal count from oil-polluted soil ranged between 1.15 - 9.75±0.22 × 10^4 cfu/g and between 0.5 - 4.4±0.35 × 10^3 sfu/g, respectively. *Chromobacterium violaceum* and *A. niger* were the most tolerant isolates. Both had a growth count of 4.7 and 2.9, respectively, in the presence of 1000 µg/l of glyphosate and a tolerance index of 1.35 and 0.83, followed by *Bacillus subtilis* and *Penicillium* sp. The degradation potential of *Chromobacterium violaceum* and *A. niger* was ascertained at an optical density of 540nm; *Chromobacterium violaceum* ranged between 0.1-0.5 (glyphosate), 0.2-1.0 (paraquat) and 0.2-1.0 (chlorpyrifos) while that of *A. niger* ranged between 1.0-3.1 × 10^2 sfu/mL (glyphosate), 1.8-2.2 × 10^2 sfu/mL (chlorpyrifos) and no growth was observed in paraquat. Bioremediation of glyphosate, paraquat and chlorpyrifos is a feasible technique using appropriate biodegraders. Bacteria and fungi show specificities in the degradation of herbicides and also at varying volumes of herbicides. Therefore, a consortium of microorganisms can be considered while seeking eco-friendly bioremediation solutions.

Keywords Agrochemical; Biodegradation; Microorganism; Mitigation; Pollution

1 Introduction

Agriculture is constantly trying to increase productivity; one strategy to achieve this goal is the use of herbicides (Pileggi *et al.*, 2020). These chemical agents act by blocking the biosynthesis of amino acids, carotenoids or lipids, or by interrupting the flow of electrons in the process of photosynthesis. Nevertheless, massive use of herbicides and other pesticides leads to contamination of agricultural soils, river systems, and nearby groundwater, changing the structure and function of soil microbial communities. Herbicides directly or indirectly impact organisms other than their primary targets, including humans (Zaller & Bruhl, 2021). Herbicide use and misuse also cause selection pressure on microbes in soil and water, possibly resulting in changes to microbial processes, especially if there are genes encoding enzymes related to herbicide degradation (Pileggi *et al.*, 2020). Xenobiotic compounds may increase the production of reactive oxygen species (ROS) (Silvestre, 2020). These compounds affect the survival of microorganisms that subsequently need to develop strategies to adapt to these conditions to maintain their ecological functionality. Without adaptation, specific populations of microorganisms will likely disappear (Pileggi *et al.*, 2020). Researchers have tested the use of several

* Corresponding author: Chika C. NWANKWO, Corresponding email chika.nwankwo@uniport.edu.ng , Tel: +(234)8033384882

bioremediation technologies, aiming to render environmental herbicide contaminants non-harmful or less harmful (Osman *et al.,* 2022; Umar *et al.,* 2024). These promising environmental technologies are based on microbial metabolic activities (via enzymes) to transform toxic components into harmless molecules. While some microorganisms able to transform recalcitrant compounds have indeed been isolated, trials of their use in environmental applications have been disappointing. Therefore, before degradation is achieved, microbial communities must develop survival strategies against the stresses induced by herbicides (Arora, 2020; Eththiligoda & Aththanayake, 2023). Adaptation of bacteria to stressful environments is achieved by the interaction of several systems in a complex manner. This study, therefore, isolated herbicide-tolerant soil bacteria and fungi to degrade glyphosate, paraquat and chlorpyrifos herbicides. Glyphosate, paraquat and chlorpyrifos are commonly used by farmers to control weeds; by their application in agriculture, they leave behind residual toxic chemicals that are harmful to humans and other animals. Therefore, complete degradation to non-toxic or less toxic compounds using bio-degraders is a sustainable and eco-friendly technique.

2 Materials and Methods

2.1 Soil sample collection

Farmyard top soil, subsoil and oil-polluted soil samples were obtained at a depth of 10cm from three different farmland locations of Port Harcourt, Rivers State, Nigeria. These locations had different climatic and ecological conditions. Using a sterile spatula, 500g of the soil samples were collected and put in sterile polythene bags, homogenised properly and transported to the laboratory for physicochemical and preliminary microbiological analyses. Herbicides were purchased from local retailers in Port Harcourt, Rivers State. The choice of herbicides was based on their popular use in farming and flower lawns in the Niger Delta region. Herbicides used in the experimental stages were prepared in 250mL conical flasks at the concentrations recommended for use by farmers: 250, 500, 750 and 1000 ppm.

2.2 Physicochemical characterisation of soil and herbicides

Physical and chemical properties (pH, particle size, soil organic matter, biological oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), soil organic carbon, total nitrogen, phosphorus, electrical conductivity, total exchangeable bases, calcium, magnesium, sodium and potassium) of freshly homogenized soil sample was characterized before the addition of herbicides, following the standard methods.

2.3 Soil pH measurement

The pH of the soil samples was determined using a pH meter (Metler Toledo Seven compact series). Soil particle size was determined according to the modified hydrometer method of Andres *et al.* (2014) and Beretta *et al.* (2014).

2.4 Soil moisture content measurement

Soil moisture content was measured by the dry oven method (Ataikiru & Ajuzieogu, 2023). Five grams (5g) of the sample was measured into an already weighed Petri dish (a). The sample was transferred to a Petri dish into the oven and allowed to stand for 1h at 105℃. After this, the sample was allowed to cool in a desiccator and weighed (b). Percentage moisture is given as:

$$\% \text{ Moisture} = \frac{a - b}{\text{Sample weight}}$$

2.5 Soil electrical conductivity (EC) measurement

Soil Electrical conductivity (EC) was ascertained by weighing 5 g of each sample into a clean beaker, adding 50 ml of distilled water and homogenising properly using a stirrer or glass rod. The calibrated conductivity meter was submerged in the samples to determine the conductivity.

2.6 Soil organic carbon (SOC) measurement

Soil percentage carbon and organic matter were determined by Ataikiru & Ajuzieogu (2023). Soil organic carbon (SOC) was estimated by oxidation with potassium dichromate and titration with ferrous sulphate using diphenylamine as an indicator. At the end point of the titration, the colour of the indicator will change from violet to green. Soil organic matter (SOM) was calculated from SOC using the equation:

$$\% \text{ SOM} = \% \text{ OC} \times 1.724$$

2.7 Total Nitrogen Content Measurement

The total nitrogen content was measured by the Kjeldahl method (AOAC, 2005). One gram (1 g) of the soil sample was weighed and introduced into the Kjeldahl flask. A selenium catalyst was added, and 20 ml of concentrated H_2SO_4 was also added to the mixture. The digestion flask with the mixture was heated in the DK20 heating digester block, beginning with a temperature of 80 °C and later 350 °C. The contents of the digestion vials were heated until the volume was reduced to 3–4mL. The contents of the digestion flask were cooled, and the volume was made up to 100 mL in a volumetric flask. Ten (10) millilitres of each sample extract was pipetted into a Kjeldahl flask, to which 20mL of 40% NaOH was added and distilled. The distillate was collected (in a 250 ml conical flask) over 10 ml of 4% boric acid, and three drops of indicator were added and allowed to stand for 5 min. The presence of nitrogen gave a light blue colour.

Two hundred millilitres (200 ml) of the distillate were titrated with 0.1 N HCl until the colour changed from light blue to grey and suddenly turned pink. A blank titration was conducted on the sample solution. The weight of nitrogen was calculated as follows: 14g of nitrogen contained in a milliequivalent weight of ammonia. The percentage of nitrogen in the sample was calculated as follows:

$$\text{Total N \%} = \frac{14(\text{A} - \text{B}) \times \text{acid conc} \times 100}{1000 \times l}$$

Where A = volume of standard HCl used in the titration of the sample
B = volume of standard HCl used in the blank titration
14 = atomic weight of nitrogen
l = mass of the sample in grams

2.8 Phosphorus measurement

Available phosphorus was determined by weighing a soil sample (5g) into the plastic bottle and adding 40mL of the extracting solution. This was homogenised for 1 min and filtered with Whatman filter paper No. 42. Three to five drops of conc. H_2SO_4 were added and left to stand for about 2h. The clear supernatant was decanted. The filtrate or supernatant was then measured for available phosphorus.

2.9 Organic Carbon Content Measurement

The organic carbon content was measured using the wet dichromate oxidation method. A portion of soil (0.05 g) was measured in an Erlenmeyer flask. Ten millilitres of concentrated sulfuric acid, 10 mL of 0.1667 M $K_2Cr_20_7$ and 10 mL of orthophosphoric acid were added. After the addition of 200 ml of distilled water, the solution was left to stand for 30 min on an asbestos sheet and titrated again with solutions of FeSO 0.333 M with the diphenylamine indicator (Devasia & Madhu, 2011).

2.10 Total exchangeable bases measurement

For total exchangeable bases, 5 g of air-dried soil was weighed into a plastic bottle, and 100 mL of neutral 1 M ammonium acetate was added. The mixture was shaken mechanically for 30 min and filtered into a 100 ml volumetric flask using a No 42 Whatman filter paper. This was made up with the acetate to the mark. Na (589-nm wavelength) and K (766.5 nm wavelength) were determined with a Flame Photometer, while Ca and Mg by Atomic Absorption Spectrophotometer. Ten to 20mL of soil saturation extract was pipetted into a 250-ml Erlenmeyer flask. This was diluted to 20–30mL with distilled water. 2–3mL 2 N NaOH solution and about 50 mg ammonium purpurate indicator were added. This was titrated with 0.01 N EDTA. The colour change went from red to purple. A drop of EDTA was added every 10 s. For Magnesium measurement, 10–20mL soil saturation extract was pipetted into a 250-mL flask, and diluted to 20–30mL with distilled water. Then, 5 mL buffer solution was added with a few drops of Eriochrome Black Indicator. This was titrated with 0.01 N EDTA until the colour changed from red to blue. For measurement of calcium, 50mL of the sample filtrate was taken, and 0.1 N HCI was added to decompose bicarbonates. It was then boiled to expel CO_2, after which it was cooled, and 2 ml of NaOH was added to produce a pH of 12–13. Murexide indicator was added, and the solution was slowly titrated with EDTA, and continuous stirring ensued until the proper endpoint. The solution changed from pink to purple. The concentration of calcium is calculated.

2.11 Measurement of heavy metals

For heavy metals analysis, soil samples were oven-dried at 70–80°C for 24h to remove all moisture. Dried samples were milled into a fine powder of 80µm. Following this, 1.0g of the dried sample was measured into a digestion tube and 10mL of 98% nitric acid was added. This was then placed in a water bath and allowed to boil for about 72h, after which it was allowed to cool and the contents transferred into a 100cm^3 volumetric flask and made up to the volume mark with water. The solution was used for the determination of mineral elements. The solution was also analysed for arsenic, cadmium, lead, and mercury using the Atomic Absorption Spectrophotometer (AAS, PerkinElmer model 2130) (Ataikiru & Ajuzieogu, 2023).

2.12 Characterisation of herbicides

The herbicides were characterised using a gas chromatography-mass spectroscopy (GC-MS) (Ataikiru & Ajuzieogu, 2023). The different compounds were identified after preparing and digesting samples using the gas chromatography-mass spectrophotometer Model 7820 (Agilent instruments, USA) based on mass to charge ratio.

2.13 Microbiological analysis of soil samples

Isolation of bacteria and fungi in the soil samples was carried out. Serial dilution was done using one (1) gram of soil sample suspended in 9mL of sterile physiological saline. Aliquots (0.1mL) of the dilutions were plated out using appropriate media for the enumeration of microorganisms. Potato dextrose agar (PDA) (Sigma Aldrich) fortified with chloramphenicol agar (0.1g/L) was used for the enumeration of fungi (Lone *et al.*, 2014). Plate count agar (PCA) (Sigma Aldrich, Steinheim, Germany) fortified with griseofulvin (50µl/mL) was used for the enumeration of heterotrophic bacteria (Ataikiru *et al.*, 2019).

2.14 Characterisation and identification of bacterial and fungal isolates

The bacterial isolates were subcultured onto plate count agar to obtain a pure culture. The isolates were characterised and identified by microscopic, Gram reaction and biochemical (indole, coagulase, citrate utilisation, sugar fermentation, methyl red and Voges-Proskauer) characteristics. The Microgen Kit was used to identify the isolates to the species level. The bacterial isolates were identified by standard microbiological methods. This includes the identification of Gram-negative bacteria using the API® 20E identification kit (Ajayi & Abiola, 2018; Hauwa *et al.*, 2023). The fungal isolates were sub-cultured onto potato dextrose agar (PDA) to obtain a pure culture and used for various evaluations and identification. Microscopic observations were done on mounted cultures using lactic acid. The morphological traits of each fungal colony were observed and recorded as described in fungal atlases (Klich, 1990).

2.15 Performance of the potential bioremediation activities of the tolerant organisms on herbicides

Bioremediation activities of the herbicide-tolerant bacteria and fungi were investigated on the basis of COD and TOC. Firstly, the chemical oxygen demand (COD) and total organic carbon (TOC) values in the enriched environment in which the microorganisms were found were measured. All of the experimental studies were performed in triplicate, and the average value of these replicates was used. While the open reflux method specified in standard method 5220B was used for COD analysis in the study, in TOC analyses, the standard method high temperature combustion method 5310A was preferred with the Shimadzu TOC-V device. For COD assays, a DR860 HACH spectrophotometer was used in the closed-reflux colourimetric method to calculate the COD concentration. The test values are the average of three readings, and the measurement discrepancies were found to be less than 0.02. The effectiveness of COD and TOC elimination was discovered using Equation (1) and Equation (2) (Erguven *et al.*, 2023).

$$COD\ removal\ (\%) = \frac{COD_{initial} - COD_{final}}{COD_{initial}} \times 100 \quad (1)$$

$$TOC\ removal\ (\%) = \frac{TOC_{initial} - TOC_{final}}{TOC_{initial}} \times 100 \quad ((2)$$

$COD_{initial}$ and COD_{final} (mg/L) are the values of initial COD and COD after the bioremediation process at time t, respectively. $TOC_{initial}$ and TOC_{final} (mg/L) are the values of initial TOC and TOC after the bioremediation process at time t, respectively. 1ml of 24h bacterial culture and 1ml of the spores of the fungal isolates (each ml of fungi contains approximately 10^6 colony-forming units) were added to the media containing various concentrations of the pesticides (500 and 1000 ppm) and control (without pesticides) for bioremediation activities. Sampling for bacterial isolates was done after 24 h, 48 h and 168 h, while sampling for fungal isolates was done after 7 days, 21 days and 28 days. The samples were filtered through 0.45µm filter paper, and bioremediation studies were followed by COD and TOC analyses

on these filtrates. To inhibit the activity of fungal colonies during this process, one drop of freshly prepared 1N H_2SO_4 was added to the filtrates. The assays were performed in triplicate.

2.16 Effect of herbicides on bacterial and fungal growth

The effect of herbicide degradation was determined by optical density (bacteria) and wet weight (fungi). After bioremediation, the effect of pesticide degradation on bacteria was monitored by measuring the optical density of the media turbidity (as an indicator of bacterial growth) (Yadav *et al.*, 2015). After fungal incubation, the mycelia of fungi were harvested, weighed using an electronic balance, and the wet weight of the fungal mycelium. Wet biomass of the fungal mycelium is accounted for as the growth parameter of the fungi. From the duplicate values, the mean values were derived and recorded. Growth of the fungal isolates was calculated with the following formula (Sangeetha *et al.*, 2018):

$$\frac{W1}{W2} \times 1000$$

Whereas, W2 = Wet weight of mycelium in control; W1= Wet weight of mycelium in pesticide utilisation trials.

3 Results and Discussion

3.1 Physicochemical characteristics of oil-polluted soil

The result, as presented in Table 1, reveals the physicochemical parameters of the polluted soil sample. The result shows physical parameters such as pH, temperature and chemical parameters such as total nitrogen, phosphate, manganese, etc.

Table 1 Physicochemical characteristics of oil-polluted soil

Parameters	**Mean ± SE**
pH	8.8± 0.20
Temperature (^oC)	28.6± 0.33
Electrical conductivity (µS/cm)	1213± 2.05
Total nitrogen %	14.60± 0.16
Total organic carbon (%)	9.22±0.32
Sulphate (mg/ L)	31.2±0.22
Nitrate (mg/ L)	15.42± 0.51
Phosphate (mg/L)	8.20± 0.02
Chloride (mg/ L)	13.31±0.68
Copper (mg/ L)	10.29± 0.49
Zinc (mg/ L)	8.80± 0.17
Manganese (mg/ L)	2.92± 0.56

Values are means of duplicates: ± Standard error of the mean

3.2 Total viable counts of bacterial and fungal isolates from oil-polluted soil samples

The result, as presented in Table 2, reveals the total heterotrophic bacteria counts (THBCs) (cfu/g) and total fungal counts (TFCs) (sfu/g) of the soil samples.

Table 2 Total viable counts of bacterial and fungal isolates from agricultural and oil-polluted soil

Soil Sample codes	Total heterotrophic bacterial count (cfu/g×10^4)	Fungi (sfu/g× 10^3)
CS_{1a}	9.75±0.22	0.95±0.32
CS_{1b}	4.5±0.33	NG
CS_2	6.05±0.00	4.4±0.35
CS_2	30.0±0.43	0.5±0.02
FS_1	1.15±0.00	2.2±0.15
FS_2	9.50±0.10	3. 0±0.00

Counts represent means of triplicate samples ± standard error

Key: cfu/g= Colony forming units per gram; sfu = spore forming units per gram; SE = Standard error; NG = No growth

The result shows that sample CS_2 had the highest THBC of 30.0±0.43 ×10^4 cfu/g and sample FS_1 had the least THBC of 1.15 ×10^4 cfu/g, while CS_2 had the highest TFC of 4.4±0.35 ×10^3 sfu/g and CS_{1b} had no fungal growth.

3.3 Morphological and biochemical characterisation and identification of isolates

The morphological and biochemical characterisation test results of the bacterial isolates are represented in Table 3, while the macroscopic and microscopic characterisation of fungal isolates are represented in Table 4. A total of 10 bacteria and 7 fungi were isolated from both samples. The bacterial isolates were identified as *Chromobacterium violaceum*, *Pseudomonas putida*, *Nocardia* sp., *Corynebacterium* sp., *Bacillus* spp., *Serratia marcescens* and *Clostridium* sp., and the fungi are *Penicillium* sp., *Aspergillus terreus, Aspergillus niger, Rhizopus* sp., and *Fusarium* sp.

Table 3 Biochemical characteristics of the bacterial isolates

Isolate ID	Catalase	Citrate	Glucose	Lactose	Indole	MR	VP	Motility	Starch	TSI	H_2S	Gram stain	Possible genera
FM1	+	+	+	-	-	-	-	+	+	A A+	-	-	*Chromobacterium violaceum*
FM2	+	-	-	-	-	-	-	+	+	A A -	-	-	*Pseudomonas putida*
FM3	+	+	+	-	-	+	-	-	-	A A+	+	+	*Nocardia* sp.
FM4	+	+	+	-	-	+	+	+	+	A B -	+	+	*Bacillus cereus*
FM5	+	-	+	-	-	-	+	+	+	A B -	+	+	*Bacillus subtilis*
FM6	+	-	+	-	-	+	+	-	-	A B -	+	+	*Corynebacterium* sp.
FM7	+	+	+	-	-	-	+	+	+	A B -	-	-	*Serratia marcescens*
FM8	+	-	-	-	-	-	-	+	+	A A -	-	-	*Pseudomonas putida*
FM9	-	+	+	+	-	-	-	-	+	A B -	+	+	*Clostridium* sp.
FM10	+	-	-	-	-	-	-	+	+	A A -	-	-	*Pseudomonas putida*

'+' = Positive, '–' = Negative

Table 4 Morphology and microscopic characteristics identification of fungal isolates

Isolate code	Macroscopy	Microscopy	Probable fungi
FMF1	Cream non-cracked reverse, green powdery mycelia, white margin.	Long hyaline hyphae with brush-like conidiophores	*Penicillium* sp.
FMF2	Golden Brown non-cracked reverse, white margin, Brown mycelia with shiny crystals.	Presence of globes spores	*Aspergillus terreus*
FMF3	Cream cracked reverse, black powdery mycelia, cream margin	Thick-walled hyphae, unbranched, bearing conidia. Presence of scattered black globose spores	*Aspergillus niger*
FMF4	Cream non-cracked reverse, white woolly mycelia with black spores	Presence of hyphae, sporangium and spore	*Rhizopus* sp.
FMF5	Pink non-cracked reverse, white woolly mycelia	Presence of septate hyphae bearing conidia	*Fusarium* sp.
FMF6	Cream cracked reverse, white margin, greenish dense mycelia.	Presence of thin, long, branched septate conidiophores bearing conidia	*Penicillium* sp.
FMF7	Yellowish non-cracked reverse, white margin, greenish mycelia with shiny crystals.	Presence of bluish round spore, thin hyphae	*Penicillium* sp.

3.4 Percentage occurrence of bacterial isolates from oil-polluted soil

The result, as presented in Figure 1, reveals the percentage occurrence of bacterial isolates from the oil-polluted soil samples. The result shows that *Pseudomonas putida* had the highest occurrence.

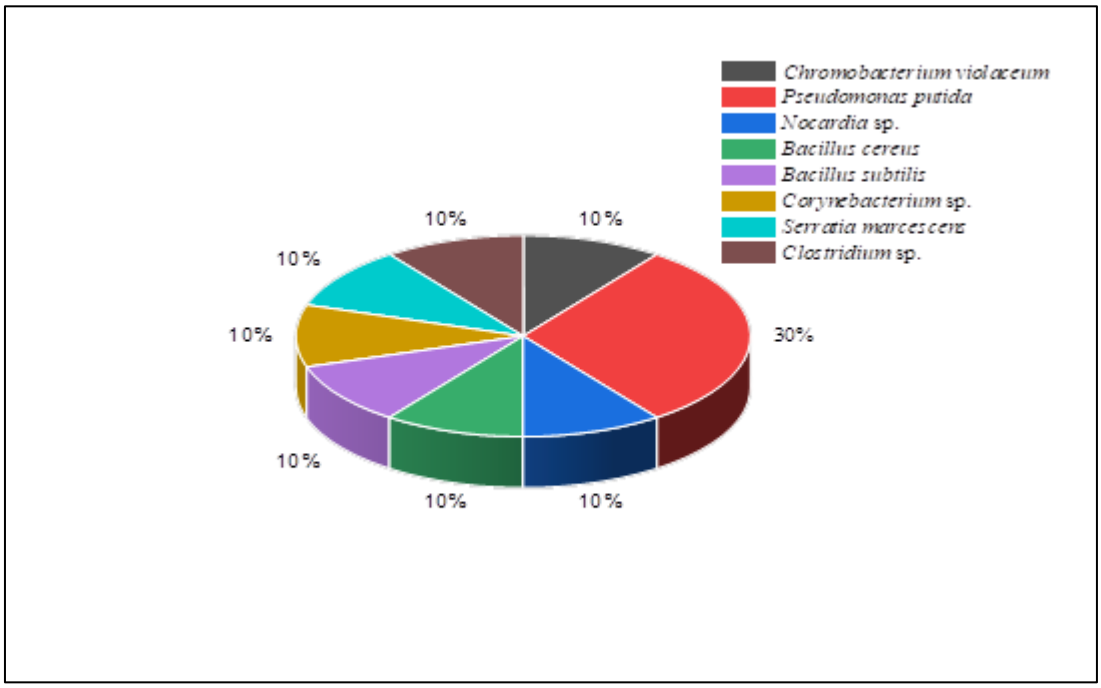

Figure 1 Percentage occurrence of bacterial isolates from oil-polluted soil

3.5 Percentage occurrence of fungal isolates from oil-polluted soil

The result, as presented in Figure 2, reveals the percentage occurrence of fungal isolates from oil-polluted soil. The result shows that the highest occurring isolate was *Penicillium* sp. at 42.9%.

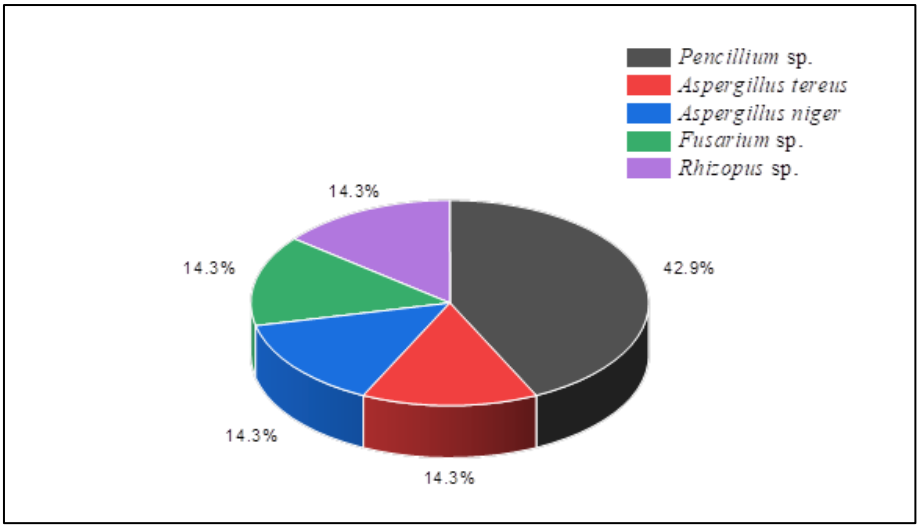

Figure 2 Percentage occurrence of fungal isolates from oil-polluted soil

3.6 Screening of Microbial Isolates for growth in the presence of test herbicides

The result, as presented in Table 5, reveals the growth of screened microbial isolates in the presence of glyphosate, paraquat and chlorpyrifos. The result shows that *Chromobacterium violaceum* had the highest growth in the presence of herbicides among the bacterial isolates, while *Chlostridium* sp. and *Corynebacterium* sp. had the least growth. Also, the result shows that *Aspergillus niger* had the highest growth in the presence of the herbicides among the fungal isolates, while *Rhizopus* sp. and *Fusarium* sp. had the least growth.

Table 5 Screening of microbial isolates for growth in the presence of test herbicides

Code		**Control**	**Growth in the presence of 1000 µg/l of herbicides**		
			Glyphosate	**Paraquat**	**Chlorpyrifos**
	Bacterial Isolates				
FM1	*Chromobacterium violaceum*	3.5	4.7	3.68	2.86
FM2	*Pseudomonas putida*	3.72	2.42	1.8	2.09
FM3	*Nocardia* sp.	3.3	1.89	1.01	1.55
FM4	*Bacillus cereus*	2.7	1.81	1.05	1.11
FM5	*Bacillus subtilis*	2.21	1.65	1.36	0.32
FM6	*Corynebacterium* sp.	2.86	1.12	0.86	NG
FM7	*Serratia marcescens*	3.35	2.34	1.09	1.21
FM9	*Clostridium* sp.	2.48	1.3	0.46	NG
	Fungal Isolates				
FMF1	*Penicillium* sp.	2.5	1.2	1.8	1
FMF2	*Aspergillus terreus*	2.9	1.3	1.5	1.1
FMF3	*Aspergillus niger*	2.7	2.2	1.9	1.6
FMF4	*Rhizopus* sp.	2.2	0.1	0.1	0.1
FMF5	*Fusarium* sp.	2	0.4	0.2	0.9

NG = No growth

3.7 Herbicide tolerance index of bacterial isolates

The result, as presented in Figure 3, reveals the bacteria isolates tolerance index to the herbicides. The result shows that *Chromobacterium violaceum* was the most tolerant, while *Clostridium* sp. was the least tolerant to the herbicides.

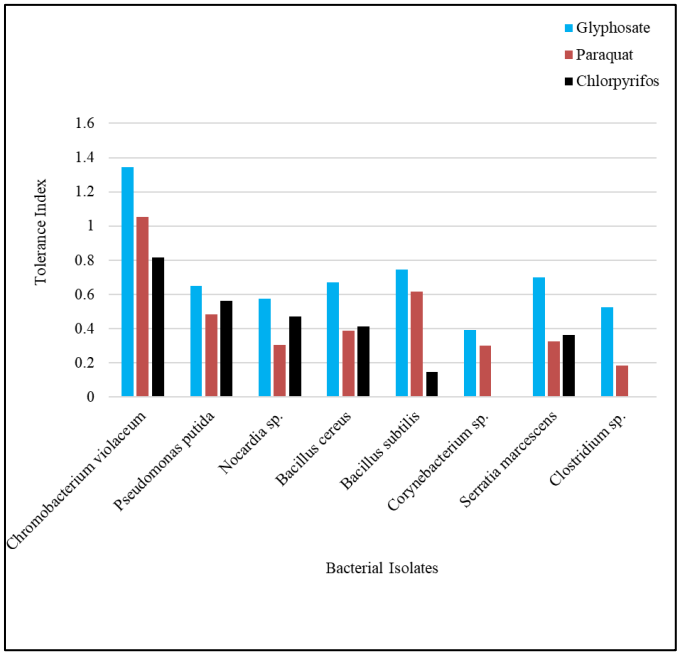

Figure 3 Herbicide tolerance index of bacterial isolates

3.8 Herbicide tolerance index of Fungal isolates

The result, as presented in Figure 4, shows the herbicide tolerance index of fungal isolates. The results shows that *Aspergillus niger* was the most tolerant to herbicides, while *Rhizopus sp.* was the least tolerant.

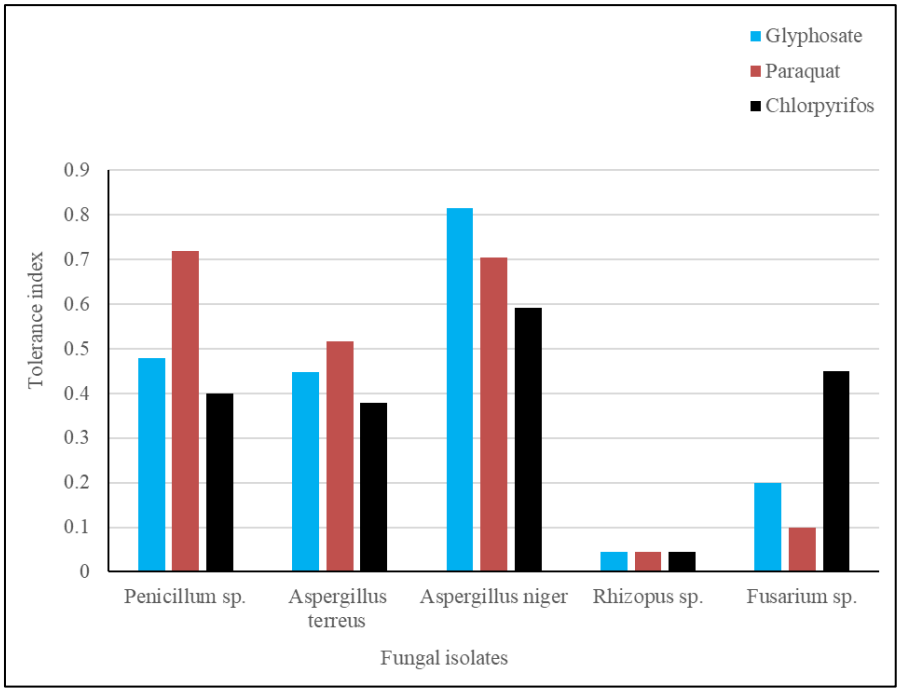

Figure 4 Herbicide tolerance index of Fungal isolates

3.9 Biodegradation of test herbicides by *Bacillus subtilis* and *Aspergillus niger*

The result, as presented in Figure 5, shows the biodegradation of the herbicides by *Chromobacterium violaceum*. The results show that *C. violaceum* degraded paraquat and chlorpyrifos better than it did glyphosate after 168 hr incubation time. Figure 6 shows the biodegradation of the herbicides by *Aspergillus niger*. The result shows that *A. niger* degraded glyphosate very well, followed by chlorpyrifos, but could not degrade paraquat.

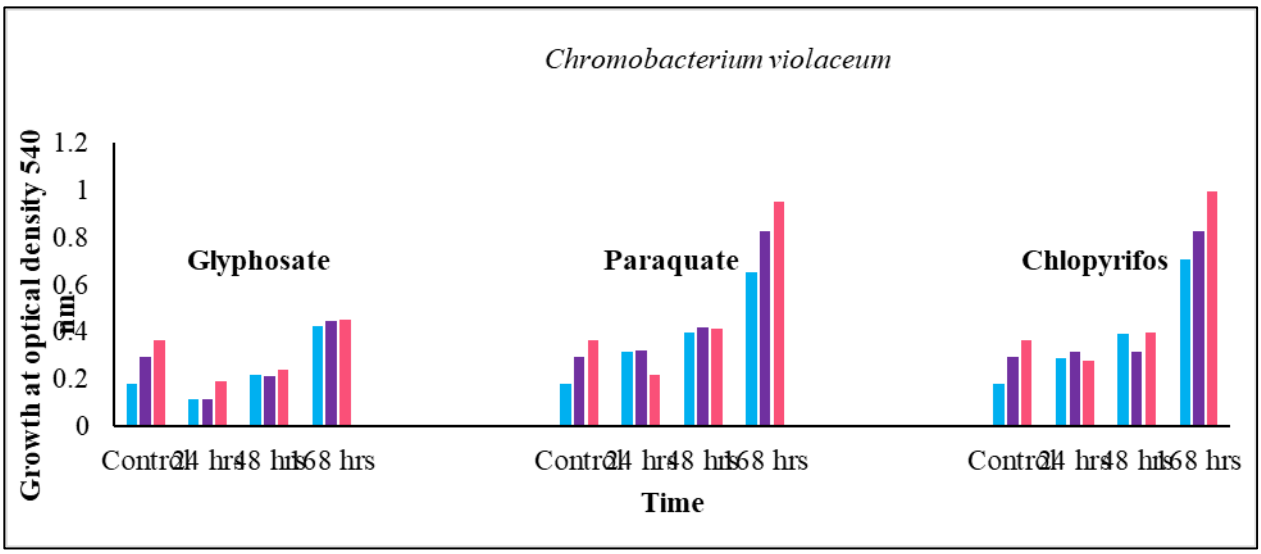

Figure 5 Biodegradation of test herbicides by *Chromobacterium violaceum* at 24hr, 48hr and 168hr

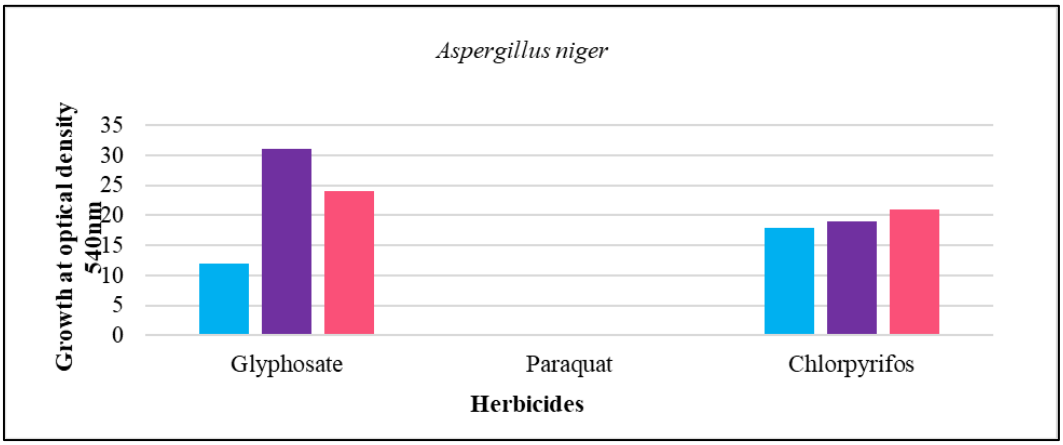

Figure 6 Biodegradation of test herbicides by *Aspergillus niger* at 168 hours

4 Discussion

All the isolates showed some degree of utilisation of the three herbicides glyphosate, paraquat and chlorpyrifos, except *Corynebacterium* spp., which could not grow in the presence of chlorpyrifos (Makut & Bello, 2018). Although fungi and bacteria both grew in the presence of the herbicides, their growth varied, as seen in their individual tolerance indices. Among the bacterial isolates, *Chromobacterium violaceum* showed the highest tolerance index, followed by *Bacillus subtilis*. These two bacteria are good candidates for the remediation of sites contaminated by glyphosate, paraquat and chlorpyrifos (Ni *et al.,* 2016, 2025).

Biodegradation of the three herbicides by *Chromobacterium violaceum* shows that the bacterium degraded paraquat and chlorpyrifos more effectively than glyphosate. *Chromobacterium violaceum* has been reported by other authors as an herbicide degrader (Abbas *et al.,* 2019; Almeida *et al.,* 2017; Lei *et al.*, 2023).

Biodegradation test of the three herbicides by *Aspergillus niger* showed that the fungal isolate could only degrade glyphosate and chlorpyrifos, but showed zero tolerance to paraquat. There are reports of *A. niger* degradation of herbicides by some researchers (Magnoli *et al.*, 2022; Yang *et al.*, 2022).

5. Conclusions

Bioremediation of glyphosate, paraquat and chlorpyrifos is a feasible technique based on the results obtained from this study. However, bacteria and fungi show specificities in the degradation of herbicides and also at varying rates per genus of the bacteria and fungi. Therefore, a consortium of microorganisms, both fungal and bacteria should be considered when considering bioremediation of herbicides such as glyphosate, paraquat and chlorpyrifos. The synergistic collaboration of other bio-degrading microorganisms as a consortium could increase the degradation rate.

Compliance with ethical standards

Acknowledgments

The authors are very grateful to the University of Port Harcourt, Nigeria, for the research funds provided and technical support.

Disclosure of conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

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