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Bio-Removal Potential of Halo-Tolerant *Micrococcus luteus* in Crude Oil-Polluted Water Remediation

Jumbo, E. I[1]., Robinson, V. K[2]., Ikechukwu, C[3] and Ikor, P. U[4].
[1,3,4] Federal Polytechnic of Oil and Gas, Bonny, Rivers State.
[2]Department of Microbiology, Rivers State University, Npkolu, Oroworukwo, Port Harcourt
***Corresponding Author:** *lizzyibjumbo@gmail.com*

ABSTRACT

This study assessed the bioremediation efficiency of halotolerant *Micrococcus luteus* in crude oil-polluted water from Bonny, Rivers State, Nigeria. Eleven bacterial genera were isolated; *Micrococcus* sp., *Pseudomonas* sp., and *Bacillus* sp. showed strong halotolerance (growth at 10–40% NaCl) and hydrocarbon utilisation. *Micrococcus luteus* was selected for further study. A 90-day microcosm experiment was conducted using 3% (v/v) crude oil-contaminated water under four treatments: sterilised polluted water (control), unsterilised polluted water (natural attenuation), sterilised polluted water + *M. luteus*, and sterilised polluted water + *M. luteus* + NPK fertiliser. TPH was monitored by gas chromatography; microbial counts and physicochemical parameters were analysed periodically. The highest TPH degradation (77.0%) was recorded in the *M. luteus* + NPK treatment (TPH reduced from 8.992 mg/L to 2.065 mg/L), followed by *M. luteus* alone (39.3%), natural attenuation (25.0%), and sterile control (<5%). Nutrient amendment significantly increased microbial populations and accelerated hydrocarbon removal while reducing BOD, COD, TOC, and heavy metals. Halotolerant *Micrococcus luteus*, when augmented with NPK, offers a highly effective, eco-friendly approach for bioremediation of crude oil-contaminated saline environments.

Keywords: Crude-Oil Polluted Water, Bio-Removal, Bioremediation Efficiency, *Micrococcus luteus*, Halo-Tolerant.

Introduction

Crude oil consists of four primary fractions: saturated hydrocarbons, aromatics, resins, and asphaltenes (Wu *et al.,* 2014). Additionally, the complex mixture of paraffinic, aromatic hydrocarbons, as well as nitrogen, oxygen, and sulphur-containing compounds, along with a variety of metal-containing organic and inorganic compounds, is the main constituent of crude oil (Haritash *et al.,* 2009; Bachmann et al., 2014). Crude oil compounds are potentially toxic to various eukaryotic and prokaryotic organisms (Abbasian *et al.,* 2016). Different chemical, physical, and biological methods have been applied for the removal of crude oil residues in marine environments (Bacosa *et al.,* 2013; Seddighi *et al.,* 2015; Nwadiogbu *et al.,* 2016). In comparison with the traditional methods, the microbial bioremediation of crude oil is a more efficient way; this is because of its economy and no secondary contaminations (Kuyukina *et al.,* 2013; Ferradji *et al.,* 2014; Roy *et al.,* 2014).

Bioremediation is an effective, cost-saving, and eco-friendly approach to re-establishing deteriorated environments (Kumar *et al.,* 2019). There are several types of bioremediations, including microbial remediation (microbes), phytoremediation (plants), and myco-remediation (fungi) (Arora *et al.,* 2017; Afzal *et al.,* 2019; Kulshreshtha *et al.,* 2014).

In crude oil bioremediation, crude oil-degrading bacteria are used to eliminate pollutants from contaminated waters. Halophilic or salt-loving bacteria are very divergent. They are also those that can be found in water bodies with five times greater salt concentration than the ocean water. There are about 70 genera with over 150 species of them reported. They are classified into different groups based on the salt component requirements (De Lourdes *et al.,* 2013). The groups have slight halophiles with 2–5% NaCl, moderate halophiles with 5–20% NaCl, extreme halophiles with 20–30% NaCl, and halotolerant microorganisms, which are the strains that grow in between 0–5% salinity (Mohammadipanah *et al.,* 2015).

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Additionally, they offer potential applications in various fields of biotechnology. The degradation or transformation of a range of organic and inorganic pollutants is among the application fields of these groups of halophiles. High salinity and nutrient availability (nitrogen and phosphorus) act as the factors limiting the biodegradation process by microorganisms in polluted areas. The present study therefore investigated the bio-removal potential of halotolerant *Micrococcus luteus* in crude oil polluted water.

Materials and Methods

Description of the Study Area

The study area was the Bonny coal beach shoreline in Bonny Local Government Area of Rivers State, Nigeria. In southern Nigeria's Rivers State, on the Bight of Bonny, is the traditional seaside town of Bonny (formerly Ibani). Additionally, it serves as the capital of the Kingdom of Bonny. Today, Bonny Island is a key location for oil exports (Frynas, 2000).

Collection of Water Samples

A water sample was collected from the Bonny River in the Bonny Local Government Area of Rivers State, using sterile bottles by submerging the bottles in the water. The bottles containing the water samples were properly labelled for identification and transported in an ice box to the Microbiology laboratory, Department of Microbiology, Rivers State University, for microbiological analyses.

Nitrogen, phosphate and potassium (NPK) fertilizer was obtained from the Agriculture Development programme at Rumuodumaya, Rivers State, Nigeria.

Enumeration and Isolation of Total Heterotrophic Bacteria

The total heterotrophic bacterial load of the sample was enumerated using the spread plate method (Ibiene *et al.,* 2024). In this method, ten-fold serial dilutions of the water samples were carried out with the aid of a sterile 1ml pipette to obtain a dilution of 10^{-6}. Aliquots from the 10^{-4} dilution were transferred into the centre of a freshly prepared nutrient agar (NA) plate in duplicates.

Inoculated plates were later spread evenly using a sterile bent glass rod before incubating at 37 °C for 24-48 hours. After incubation, plates were observed for growth. The colonies in the respective plates for the different samples were counted and recorded. This was used in enumerating the bacterial population while distinct colonies on plates were subcultured and purified by carefully streaking on freshly prepared NA plates.

The total Heterotrophic Bacteria Count (THBC) Counts of the sample was calculated using the formulae below;

$$\text{Colony Forming Unit Per Millilitre } \left(\frac{\text{CFU}}{\text{ml}}\right) = \frac{\text{Number of Colonies}}{\text{Dilution x Volume plated (0.1ml)}} \quad \text{Equation 1}$$

Identification of Bacterial Isolates

The bacterial isolates were identified using morphological and biochemical methods. The morphological and biochemical tests as described by Prescott et al. (2011) were used for identification.

The morphological characteristics involved the texture of colony, colour, shape, Gram reaction and motility, while the biochemical characteristics involved sugar fermentation (maltose, sucrose, glucose, lactose and mannitol), citrate, oxidase, methyl red, Voges-Proskauer, catalase, and indole tests.

Enumeration and Isolation of Hydrocarbon Utilizing Bacteria (HUB)

The Hydrocarbon Utilizing Bacteria (HUB) in the water samples was enumerated using the vapour phase transfer method (Owhonka and Obire, 2019) on prepared Bushnell Hass agar.

In the vapour phase transfer method, sterile filter paper discs that were soaked in filter-sterilized crude oil (which served as the carbon source) were aseptically transferred to the inside cover of the inoculated Petri dishes and incubated for 7 days at 37 °C.

The agar was fortified with 50mg/ml ketoconazole to inhibit the growth of fungi. The colonies after incubation were counted and distinct colonies were purified and stored as described earlier.

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Preliminary Screening Test

Salt Tolerance Test

The bacterial isolates were screened for their ability to utilize higher concentrations of salt despite being isolated from the marine water. The salt tolerance test was carried out as described by Cheesbrough (2006). In this method, two concentrations of salt (10 and 40%) were prepared by dissolving 10 and 40 grams of NaCl separately into 100 mL of distilled water. The brine was swirled homogeneously for easy mixing, and 9ml each was transferred into well-labelled test tubes (test tubes were labelled according to the salt concentrations for the isolates tested). These were later sterilized at 12ºC for 15 minutes at 15 Psi. On cooling, 1ml of 24 hours cultures of bacteria were transferred into the labelled brine concentrations. Incubation followed at 37 ºC for 24 hours. After incubation, cultures were plated out on nutrient agar. Isolates showing higher colony-forming units were selected as the best halo-tolerant for bioremediation. Thus, + represents weak tolerance, ++ indicated high tolerance while the negative sign (-) showed no tolerance (Odokuma and Akponah, 2010)

Bioremediation Experiments of Crude-Oil Polluted Water

Enhanced bioremediation technology influenced by microbes and nutrients was employed to test the impact of nitrogen, phosphorus, potassium (NPK) and *Micrococcus luteus* on the components of crude-oil biodegradation. Before the biodegradation of the crude oil, the crude oil-polluted water was sterilized at 121 °C for 15 mins at 15 Psi.

This was done to ensure the sterility of the water so that only inoculated organisms can be investigated on their remediation ability (King et al., 2024). Another control set-up was not sterilized, in order to monitor natural attenuation. Sterilized crude-oil-polluted water samples (1500 mL of 3%) were transferred into twenty-five glass containers and were labelled accordingly. The addition of the bacterial culture followed. The experimental setup is presented in Table 1. The experiment was for duration of three (3) Calendar months.

Percentage Biodegradation

Percentage biodegradation was calculated as follows:

Step 1: Amount of pollutant remediated equals to Initial pollutant concentration (Day 1) minus Final pollutant concentration at the end of experiment (Last day).

Step2: Percentage (%) Bioremediation equals to Amount of pollutant remediated divided by Initial pollutant concentration (Day 1) multiplied by 100.

BC =IC – FC Eqn. 1 (Douglas et al., 2024)

Where;
BC = Amount of pollutant remediated
IC = Initial concentration of pollutant (Day 0 or 1)
FC = Final concentration of pollutant at end of experiment (Last day)

$$\textbf{\% Bioremediation} = \frac{\textbf{BC x100}}{\textbf{IC}} \quad \text{Eqn. 2}$$

Table 1: Experimental Set-Up of Bioremediation

Experimental Set-Up	Volume of Water	Nutrient Supplement	Volume of Isolate	Final volume
Crude oil Polluted water (UPW)	1500mL	None	-	1500ml
Sterilized Polluted water (SPW)	1500mL	None	-	1500ml
PW + *M. luteus*	1500mL	None	15mL	1515ml
PW + *M. luteus* + NPK	1500mL	5.0g	15mL	1515ml

Keys: PW= Polluted water; *M. luteus* = *Micrococcus luteus*; NPK= Nitrogen, Phosphorus, potassium (inorganic fertilizer).

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Physicochemical Parameters of Water Samples

The physicochemistry of the water samples carried out included pH, electrical conductivity, salinity, total petroleum hydrocarbon (TPH), total organic carbon, heavy metals, turbidity, biochemical oxygen demand (BOD), total dissolve solid (TDS), and total suspended solids (TSS). The method of the various parameters was done as described by (APHA, 2012). Analysis of Heavy metals (Lead (Pb), Cadmium (Cd), Chromium (Cr) and Nickel (Ni) of the water samples was done by using atomic absorption spectroscopy (Sriadibhatla, 2013).

Statistical Analysis

The microbial counts were presented in log on Microsoft Excel (v21). The mean and standard deviations of microbial counts were analysed using SPSS (v 27). The means were compared using ANOVA. Means showing significant differences were separated using the Duncan Multiple range test at a significant level of 0.05. The percentage occurrence of isolates as well as graphical presentations were carried out on Microsoft Excel (v16)

Results

Results of the Total Heterotrophic bactaria and hydrocarbon-utilizing bacterial loads of the water sample was 3.5×10^{6} and $3.0\times10^{2,}$ respectively. The baseline results showing the physicochemical, heavy metal parameter and TPH showed that the pH, salinity, turbidity, nitrate, phosphate, BOD, COD, total organic carbon (TOC), chromium (Cr), Iron (Fe), Lead (Pb),

Total petroleum hydrocarbon (TPH) and electrical conductivity (EC) were 6.4, 26.16 mg/l, 91.5 NTU, 0.6 mg/l, 20.5 mg/l, 510.13 mg/l, 5550 mg/l, 0.01 mg/l, 0.07 mg/l, 0.01 mg/l, 7.166 mg/l, 5200 mg/l and 4502µs-1 , respectively.

Results of the phenotypic and biochemical characterization the bacteria isolated from the water samples showed that eleven bacterial genera belonging to *Staphylococcus* sp, *Serratia* sp, *Pseudomonas* sp, *Shigella* sp, *Vibrio* sp, *Bacillus* sp, *Micrococcus* sp, *Alcaligenes* sp, *Enterobacter* sp, *Proteus* sp, and *Providencia* sp were isolated.

Amongst the eleven bacterial isolates, only *Pseudomonas, Alcaligenes, Micrococcus* and *Bacillus* sp were isolated from crude oil because they showed crude oil utilization potential, while all eleven isolates were isolated from the water samples.

Results of the bacterial isolates that were screened for the ability to tolerate sodium chloride at higher concentrations (halo-tolerant bacteria) are presented in Table 2. Results showed that out of the eleven bacterial isolates screened for 10% and 40% sodium chloride, only three (3) isolates, such as *Micrococcus* sp, *Pseudomonas* sp, and *Bacillus* sp, were able to grow at all concentrations. Thus, they were selected for further study.

Results showing the trend of the total heterotrophic bacterial counts during the period of bioremediation are presented in Fig. 1. Results showed that the total bacterial load of the treatments for Day 1 ranged from 0.0 to 2.0 $\times10^{7}$ CFU/mL.

Table 2: Screening Result of Bacteria for Halo-tolerance

Isolate	Salt Concentration	
	10%	40%
Staphylococcus sp	+	-
Serratia sp	+	-
Pseudomonas sp	+	+
Shigella sp	+	-
Vibrio sp	+	-
Bacillus sp	+	+
Micrococcus sp	+	+
Alcaligenes sp	+	-
Enterobacter sp	+	-
Proteus sp	+	-
Providencia sp	+	-

Keys: + = Growth, - = no growth

Results in Figure 1 also showed that there were no significant differences ($P < 0.05$) in the total heterotrophic bacteria load in Day 1 across the treatments, with the set-up containing *M. luteus* supplemented with NPK fertilizer having the highest growth.

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In Day 15, the total heterotrophic bacterial load of the treatments ranged from 0.0 to 2.5×10^7 CFU/mL. High bacteria counts were observed, and even though there was no significant difference ($P > 0.05$) in the THB of PW +*M. luteus* +NPK and PW +*M. luteus* , the THB of PW+*M. luteus* was significantly higher ($P < 0.05$) than the THB of PW and SPW.

Similarly, the high HUB of PW+*M. luteus* +NPK, despite not being significantly different from PW+*M. luteus*, was significantly ($P < 0.05$) higher and different from SPW and PW, respectively. On Day 30, the total heterotrophic bacterial load of the treatments ranged from 0.0 to 7.4×10^7 CFU/ml.

The results further showed that the peak of bacterial growth was on Day 30. The set-up PW+*M. luteus* had the highest THB, and it was significantly different ($P < 0.05$) from THB recorded in SPW and Polluted water (PW).

For Day 45, the total heterotrophic bacterial load of the treatments ranged from 0.0 to 3.1×10^7 CFU/ml.

There was a decline in the THB for day 45 as compared to the previous days (Days 1, 15, and 30), which increased exponentially.

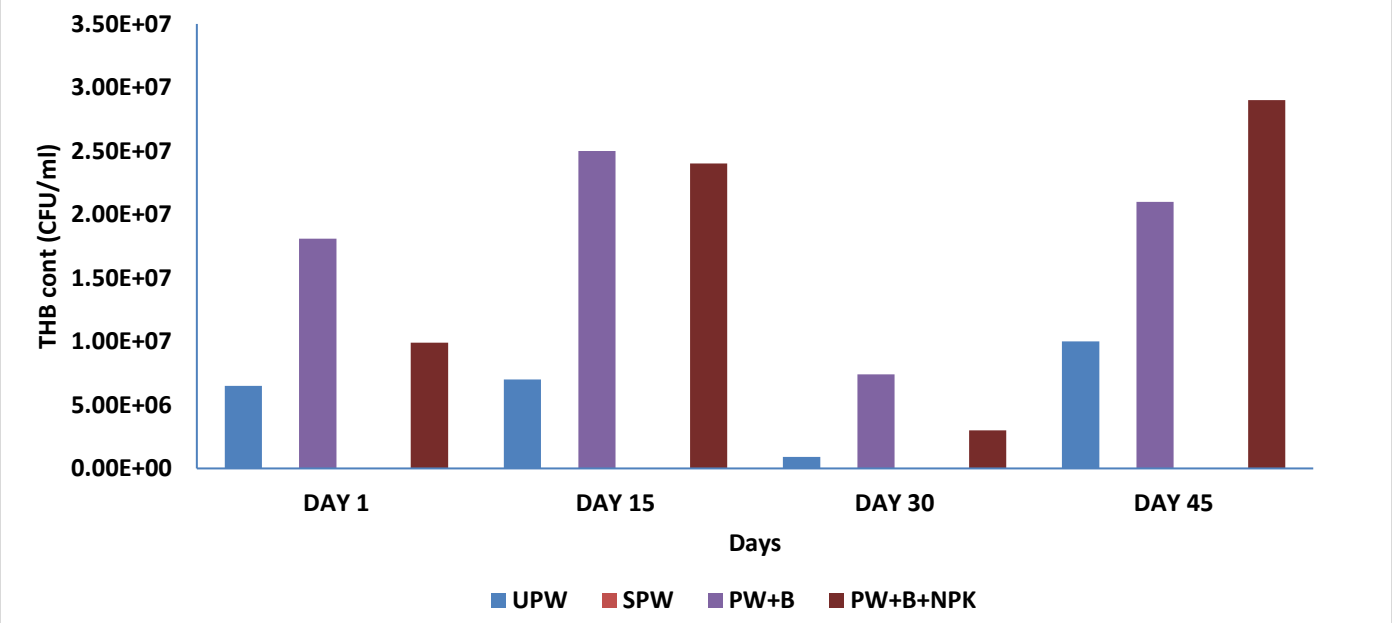

Fig.1: Trend of the total heterotrophic bacterial counts during the bioremediation period

Keys: SPW= sterilized water, B= *M. luteus*; NPK= inorganic fertilizer; UPW = polluted water

Results showing the trend of hydrocarbon-utilizing bacteria during the remediation period are presented in Fig. 2. Results showed that the mean range of the hydrocarbon-utilizing bacterial load for Day 1 was 0.0 to 8.0×10^4 CFU/mL. Results also showed that there were significant differences ($P < 0.05$) in the hydrocarbon-utilizing bacteria.

Results further showed that the hydrocarbon-utilizing bacterial load of the set-up PW+*M. luteus* +NPK was significantly ($P < 0.05$) higher than counts recorded in other treatments.

On Day 15, the mean range of the hydrocarbon-utilizing bacterial load was 0.0 to 1.6×10^5 CFU/mL. While on Day 30, the mean range of the hydrocarbon-utilizing bacterial load was 0.0 to 1.04×10^5 CFU/ml. For Day 45, the mean range of the hydrocarbon-utilizing bacterial load was 0.0 to 2.3×10^5 CFU/ml. There were significant differences in the HUB counts amongst the treatment options.

The chromatogram of the breakdown of TPH components is presented in Figure 3 to Figure 8.

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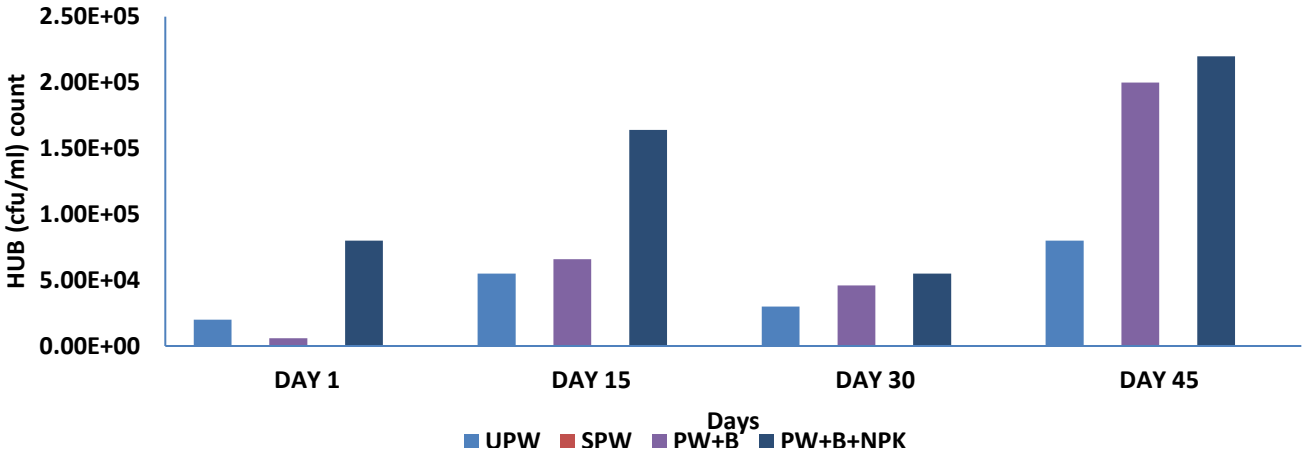

Fig. 2: Trend of the Hydrocarbon Utilizing bacterial counts during the bioremediation period
Keys: SPW= sterilized water, B= *M. luteus*; NPK= inorganic fertilizer; UPW = Polluted water

Fig. 3: Total Petroleum Hydrocarbon of Contaminated water with *M. luteus* amended with NPK (Day 15)

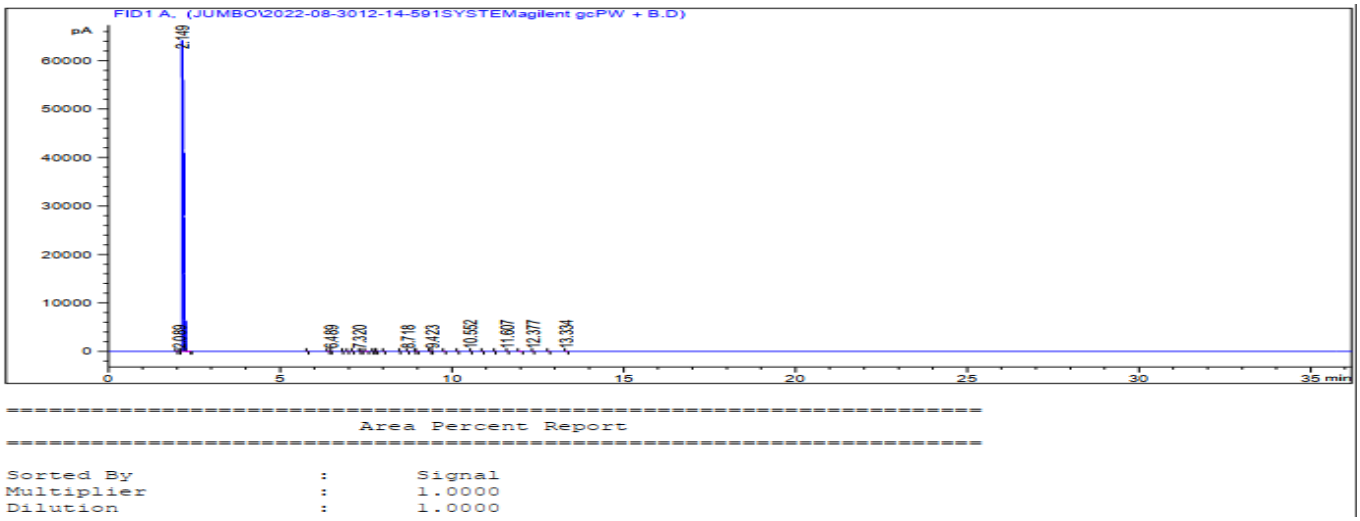

Fig. 4: Total Petroleum Hydrocarbon of Contaminated water with *Micrococcus luteus* (Day 15)

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Fig. 5: Total Petroleum Hydrocarbon of Sterilized Crude oil Contaminated water (Day 15)

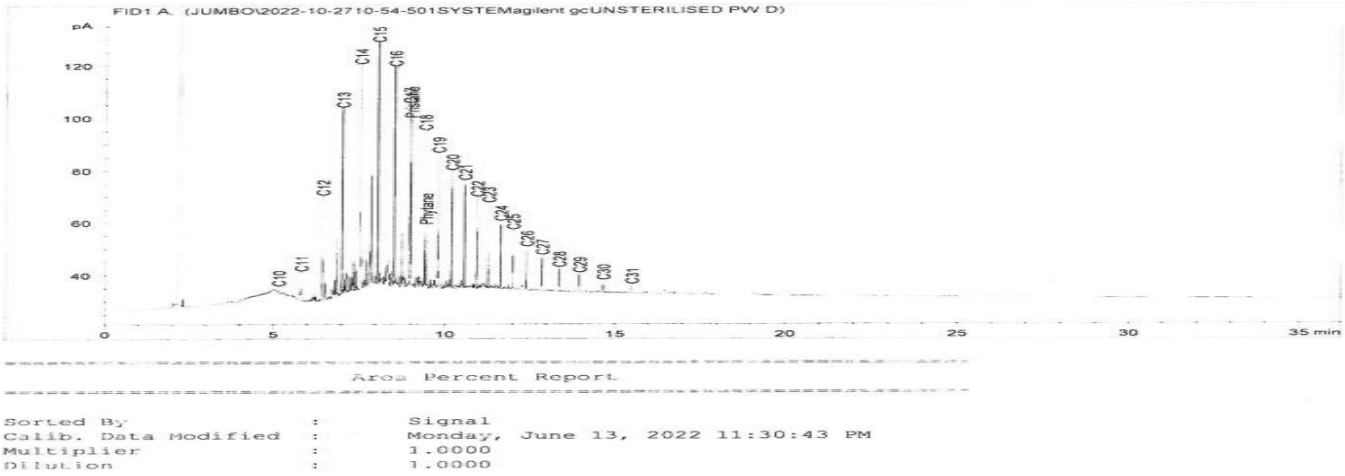

Fig. 6: Total Petroleum Hydrocarbon of Crude oil Contaminated water (Day 45)

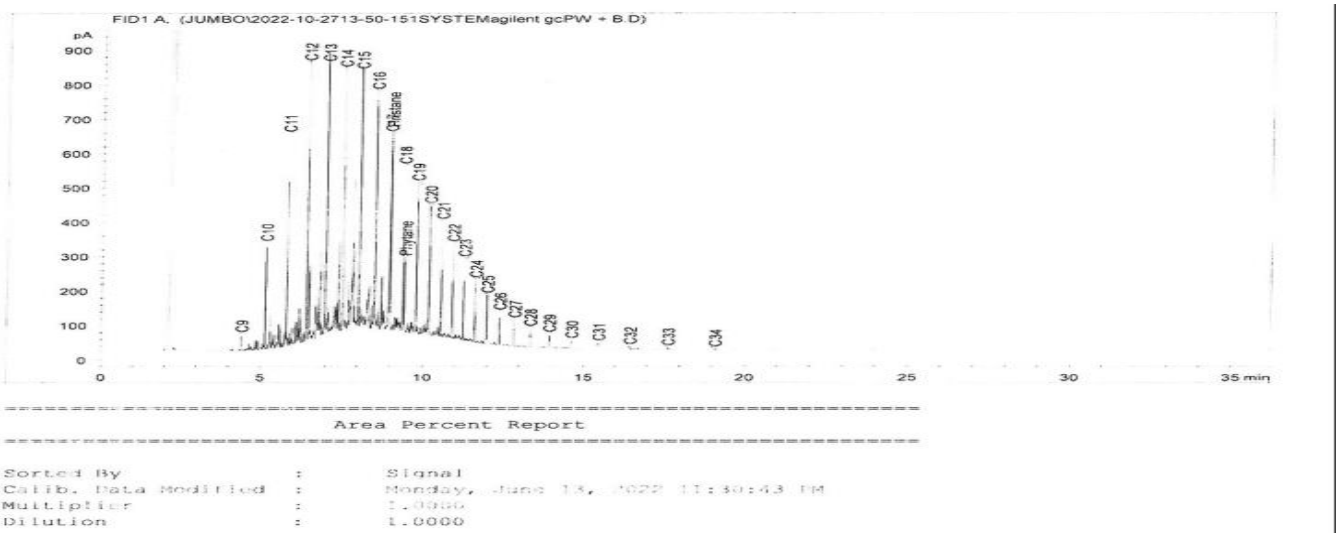

Fig 7: Total Petroleum Hydrocarbon of Contaminated water with *Micrococcus luteus* (Day 45)

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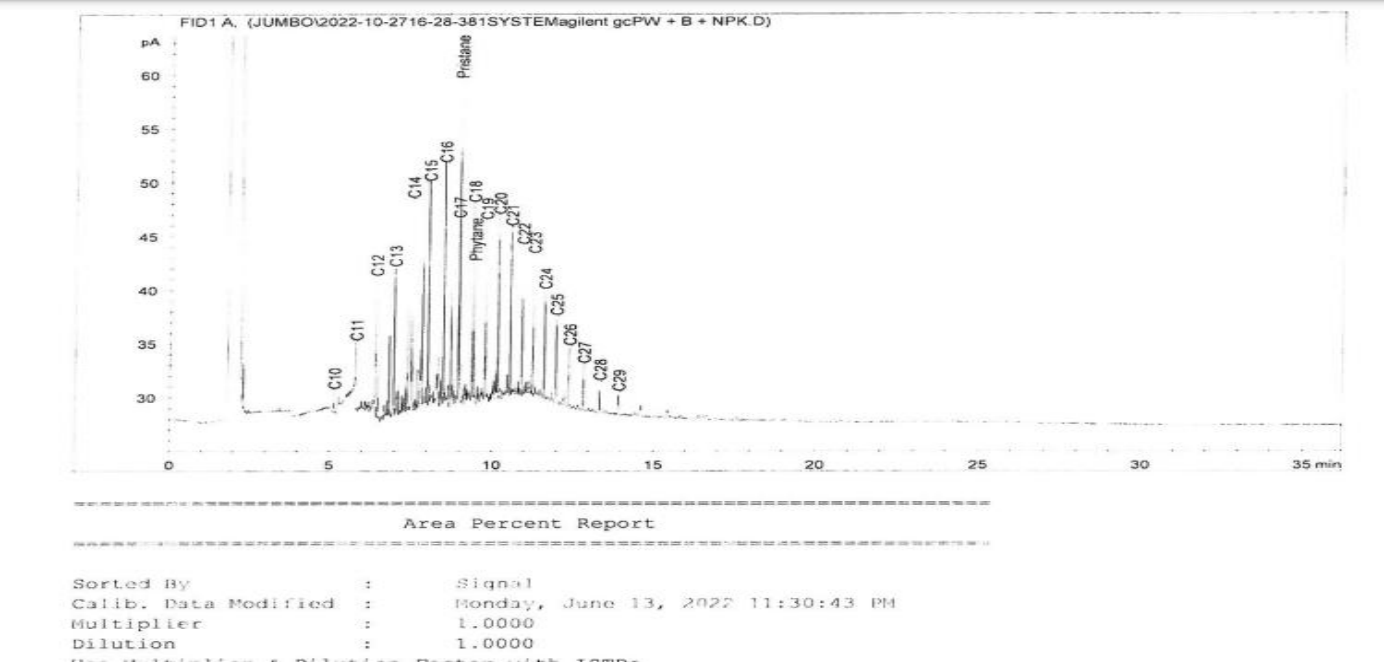

Fig. 8: Total Petroleum Hydrocarbon of Contaminated water with *M. luteus* amended with NPK (Day 45)

Results of the physicochemical parameters such as changes in the pH, biological oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), phosphate, nitrate and total petroleum hydrocarbon (TPH) is presented in Figs. 9-15, respectively. Results showed slight fluctuations in all the parameters as initial values were different from the final values.

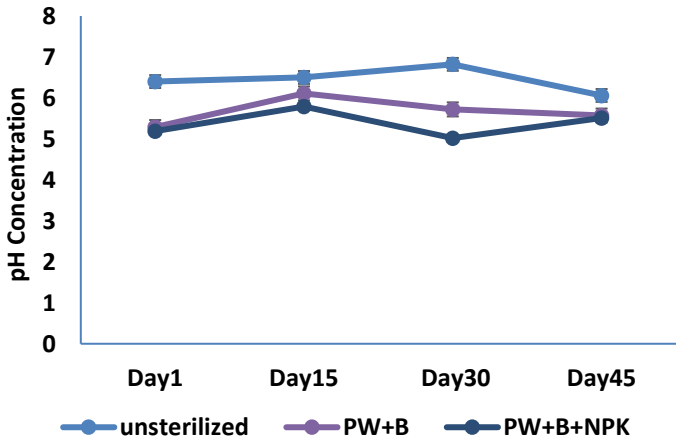

Fig. 9: Change in pH during Bioremediation

Keys: SPW= sterilized water, B = *M. luteus*; NPK= inorganic fertilizer.

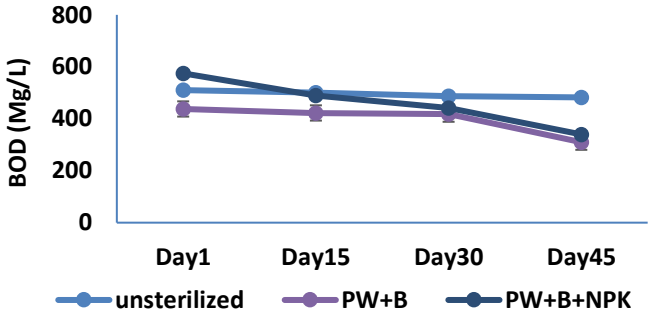

Fig. 10: Change in BOD during Bioremediation

Keys: SPW= sterilized water, B= *M. luteus*; NPK= inorganic fertilizer

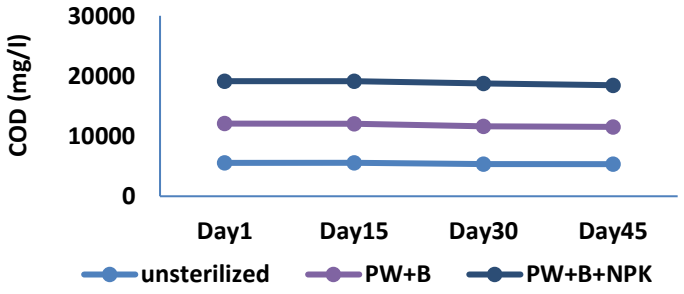

Fig. 11: Change in COD during Bioremediation

Keys: SPW= sterilized water, B=*M. luteus*; NPK= inorganic fertilizer

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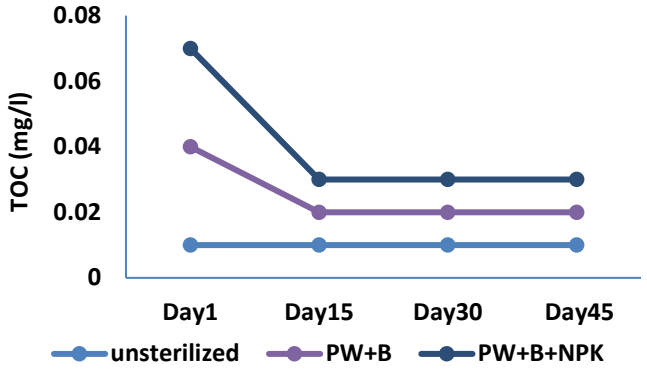

Fig. 12: Change in TOC during Bioremediation
Keys: SPW= sterilized water, B= *M. luteus*; NPK= inorganic fertilizer

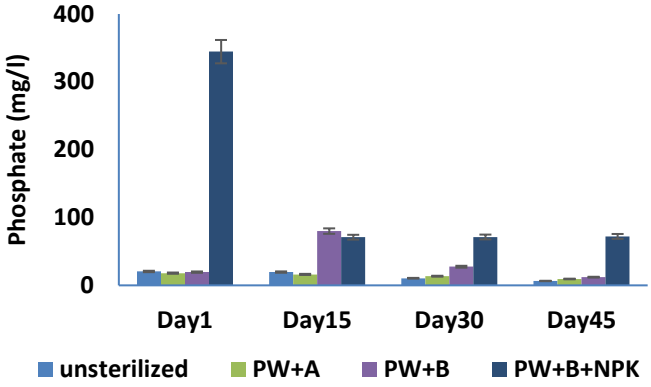

Fig. 13: Change in Phosphate concentration during Bioremediation
Keys: SPW= sterilized water, B= *M. luteus*; NPK= inorganic fertilizer

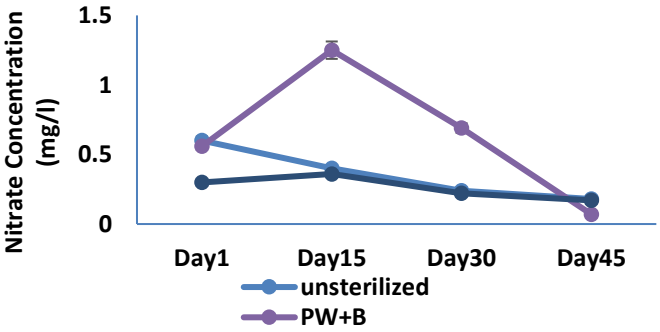

Fig. 14: Change in Nitrate concentration During Bioremediation
Keys: SPW= sterilized water, B= *M. luteus*; NPK= inorganic fertilizer

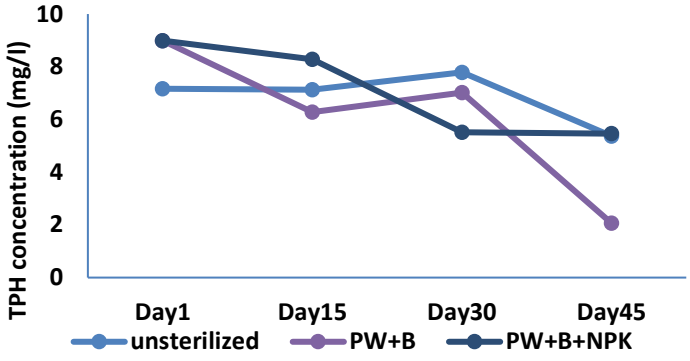

Fig. 15: Change in TPH concentration during Bioremediation
Keys: SPW= sterilized water, B= *M. luteus*; NPK= inorganic fertilizer.

Results of the heavy metals and percentage biodegradation of the crude oil (TPH) are presented in Figs. 15 & 16. Results showed that the percentage remediation of sterilized crude oil contaminated water, unsterilized crude oil contaminated water, PW+B, and PW+B+NPK was 25.0, 77.0, and 39.3%, respectively (fig. 17).

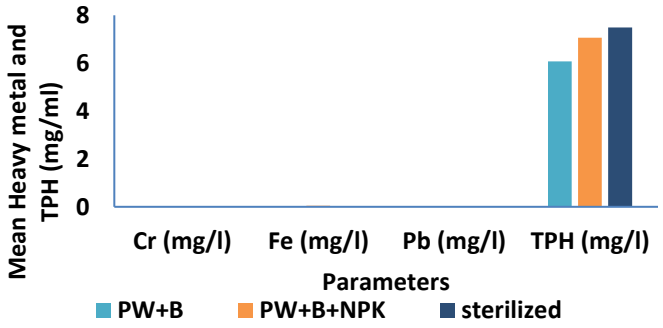

Fig. 16: Mean Heavy metals with regard to TPH
Keys: SPW= sterilized water, B= *M. luteus*; NPK= inorganic fertilizer; Cr: chromium; Fe: Iron; Pb: Lead; TPH: Total Petroleum Hydrocarbon

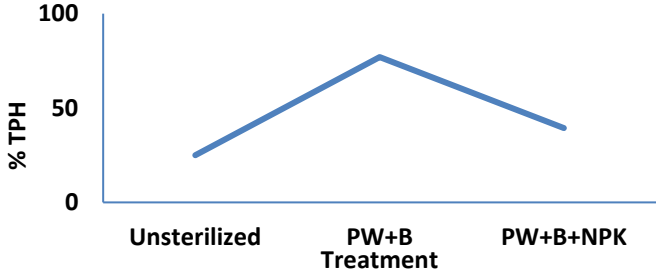

Fig. 17: Percentage change in TPH Keys: SPW= sterilized water, B= *M. luteus*; NPK= inorganic fertilizer.

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Results of the mean physicochemical properties of the treatments are presented in Table 3.

Table 3: Mean Physicochemical Parameters during Bioremediation

Treatments	pH	EC	Salinity	Turbidity	Nitrate (NO^{3-})	Phosphate (PO_4)	BOD	COD	TOC
sterilized	7.04±0.542 ^{d}	4199.75±10.24 ^{b}	26.33±0.19 ^{ab}	51.20±16.49 ^{a}	0.66±0.24 ^{a}	50.92±39.40 ^{a}	445.19±4.96 ^{bc}	5862.75±574.50 ^{a}	0.01±0.00 ^{a}
Polluted	6.45±0.31 ^{cd}	4449.50±35.00 ^{c}	26.07±0.18 ^{a}	43.76±40.76 ^{a}	0.35±0.18 ^{a}	14.26±6.89 ^{a}	494.89±12.66 ^{c}	5444.0±121.25 ^{a}	0.01±0.000 ^{a}
PW+B	5.67±0.34 ^{ab}	4025.50±1.0 ^{ab}	26.53±0.01 ^{b}	40.90±6.28 ^{a}	0.64±0.48 ^{a}	34.77±30.80 ^{a}	396.83±58.64 ^{b}	6383.00±173.17 ^{a}	0.015±0.01 ^{a}
PW+B+NPK	5.378±0.34 ^{a}	5268.0±0.00 ^{d}	33.34±0.406 ^{d}	445.0±30.71 ^{b}	0.26±0.08 ^{a}	139.74±136.5 ^{a}	461.39±98.09 ^{bc}	7041.50±90.349 ^{a}	0.01±0.01 ^{a}

Note :*Means with similar superscript down the group share no significant difference (*P > 0.05*)

keys: BOD: Biological Oxygen Demand; COD: chemical oxygen demand, TOC: total organic carbon; PW: sterilized water; NPK: inorganic fertilizer, EC: electrical conductivity, SPW= sterilized water, B= *M. luteus*; NPK= inorganic fertilizer.

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Discussion

This study evaluated the bioremoval potential of halotolerant *Micrococcus luteus* in crude oil polluted water. The high total heterotrophic bacterial count over the Hydrocarbon utilizing bacteria observed in the water sample could be attributed to the medium (Nutrient agar) which contains varying nutrients in their respective quantities that support different microbial types. Crude oil despite being a very important mineral resources has been reported to be one of the major pollutants of the water and soil environment especially when it spills into these environments as it could cause selection of microbial types in that environment while inhibiting those not adapted to it (Tangahu *et al.,* 2017). Douglas and Tamunonegiyeofori (2019) reported that higher concentrations of crude oil deposit could lead to a uniform reduction in species diversity and population of microorganisms. Thus, this statement also corroborates with the bacterial distribution across the samples in the present study which showed that the water samples had aerobic bacteria of eleven genera. Furthermore, the presence of light carbon chain petroleum hydrocarbons in some of the treatments may have contributed to the modest overall heterotrophic bacteria. This demonstrated that some THB were also able to use and degrade TPH (Alrumman, 2015).

Halophiles are great sources of enzymes that can not only endure and carry out reactions effectively in harsh environments but are also salt-stable (Kumar *et al.,* 2012). Thus, the bacterial isolates were screened for their ability to utilize higher salt concentrations despite being isolated from the estuarine water body. Amongst these isolates, *Bacillus* sp and *Micrococcus* sp were the most halophilic bacterial isolates. The dominance of *Bacillus* sp could be attributed to its wide distribution in the environment as well as its ability to survive and withstand harsh environmental conditions due to the presence of endospores (Prescott *et al.,* 2011). *Bacillus* sp was among the bacterial isolates reported by Kumar *et al* (2012) as halotolerant bacterial isolate. More so, *Bacillus* sp, *Micrococcus* sp and *Pseudomonas* sp have been reported to be hydrocarbon utilizers. Douglas and Tamunonegiyeofori (2019) isolated *Pseudomonas* sp, *Micrococcus* sp, *Bacillus* sp, *Serratia* sp and *Acinetobacter* sp as hydrocarbon utilizing bacteria which agreed with the present study.

However, *Acinetobacter* sp reported in their study was not isolated in this present study. Similarly, *Santhini et al* (2009) isolated seven bacterial isolates which is comprised of *Bacillus* sp, *Serratia* sp, *Pseudomonas* sp and *Micrococcus* sp as hydrocarbon utilizing bacteria which agreed with the present study. The only difference in the hydrocarbon utilizing bacterial isolates is that while they isolated seven, the present study only isolated five bacterial genera as hydrocarbon utilizers. This difference in bacterial diversity could be attributed to the prevailing nutrient and other environmental factors, which might have selectively influenced the presence of these bacterial types, thereby limiting other isolates that could not withstand or utilize the available nutrients (Das and Chandran, 2011; Douglas and Tamunonegiyeofori, 2019). It could also mean that these five bacterial isolates are the main crude oil degraders in this environment, thus, their ability to be isolated. According to Santhini *et al*. (2009), the ability to isolate high numbers of certain oil degrading microorganisms from oil oil-polluted environment is commonly taken as evidence that these microorganisms are the active degraders of that environment. Although *Bacillus* sp is very diverse in different environments, *Micrococcus* sp is a genus of bacteria that occurs in a wide range of environments, including dust, water, and soil, with the ability to grow in environments with little water of high salt concentration. Some of its strains have been reported to be useful in hydrocarbon degradation (Santhini *et al.*, 2009).

The disparity in the microbial load of the total heterotrophic bacteria and the hydrocarbon-utilizing bacteria could be attributed to their potential in utilizing the crude oil components, time as well as the available nutrients or level of exposure to hydrocarbon content. This agreed with Kawo and Bacha (2016) who had a similar view and attributed the low proportion of crude oil utilizers to the high total heterotrophic populations to the fact that the environment from which the samples were obtained might not have been previously exposed to heavy and consistent crude oil pollution. Generally, the microbial load of the bacteria (*Micrococcus luteus*) used for the bioremediation gradually increased from the initial population on day 1 to higher populations on day 45 during the period of bioremediation, and this corroborates with the degradation rate of the crude oil.

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The highest bacterial population was observed on Day 45 and this could be due to the utilization of hydrocarbon and organic nutrients (nitrate and phosphate). Contrary to the findings of the present study, Sang-Hwan *et al*. (2007) reported that during the first 30 days of the 105-day testing period, the population of bacteria that broke down hydrocarbons rose quickly. They suggested that this discovery could be used as a gauge for whether bioremediation of oil-polluted soils is feasible. However, over time, as a result of oil-resistant components with high chains and in the presence of fewer nutrients, bacterial growth and oil degradation declined (Schaefer and Juliane, 2007). This corroborates the present study which also showed a decline in the bacterial population on Day 30. Furthermore, Ramsay *et al.* (2000) found that continuous ventilation and fertiliser addition had a significant impact on the proliferation of hydrocarbon degrading microorganisms when they studied the effects of bioremediation on the microbial population in oil deposits. According to Van Gestel *et al.* (2001), the population of bacteria in an oil-polluted environment has increased significantly.

The degradation of total petroleum hydrocarbon (TPH) by the bacteria and in the water in the treatment samples as observed, showed a significant difference ($P< 0.05$) with the control from the statistical point of view. Thus, crude oil degradation in set-ups without NPK fertiliser was less while set-ups with NPK fertiliser had higher crude oil degradation rate. This corroborates the study of Chorom *et al.* (2010) who also made similar observation that oil degradation was lower in the samples without treatment but faster in the samples treated with 2 tons/ha of fertiliser than it was in the samples treated with 1 ton/ha of fertiliser. There was a significant ($P< 0.05$) reduction in the TPH content from the initial stage of the remediation process to the final. The *Micrococcus luteus* supplemented with NPK reduced the TPH values from 8.992 to 2.065mg/l while the *Micrococcus lutus* without supplements reduced TPH values from 8.992 to 5.461mg/l. Similar findings were reported by Sang-Hwan *et al.* (2007) who found that while only 18% of the hydrocarbon was removed from the non-fertilized treatment, the initial amount of oil-polluted soil (9320344 mg/kg) was reduced by 42-51% in the fertilized treatment. The ability of *Micrococcus* sp to utilize hydrocarbons is well documented.

In a previous study, *Micrococcus* sp was reported to possess a higher hydrocarbon degradative potential with a percentage remediation of 93.7% than *Bacillus* sp (Kawo and Bacha, 2016). This also could mean that these set-up had better attachment to the hydrocarbon molecules, especially since it has been reported that petroleum degradation, amongst other factors, is mediated by attachment of the microbial cells to the substrates (Das and Chandran, 2011). Despite this observation, addition of NPK was a key in the degradation of the hydrocarbon compound especially as the set up having the best percentage remediation were observed from that supplemented with NPK. This agrees with Chorom *et al*. (2010), who also reported that the lack of organic feeding matters, will limit the oil degradation.

Conclusion

In conclusion, this study highlights the significance of halotolerant *Micrococcus* sp. in the bioremediation of crude oil-polluted water. The microbacterial dual ability to tolerate salinity and degrade hydrocarbons shows its potential as a bioresource for sustainable water treatment strategies. Leveraging the bioremediation capacities of halotolerant *Micrococus* sp. contributes to addressing the global challenge of water pollution and underscores the importance of harnessing natural organisms for environmental restoration.

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