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Bioremediation Potentials of Bacteria Isolates from Hydrocarbon Contaminated Soil of Nakasari area of Sokoto Metropolis, Sokoto State, Nigeria.

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ABSTRACT
Hydrocarbon contamination of soil remains a significant environmental problem in many urban and semi-urban areas due to indiscriminate disposal of petroleum products and continuous mechanical activities. This study investigated the emulsification activity and hydrocarbon utilization potential of bacteria isolated from hydrocarbon-contaminated soil collected from Nakasari, Sokoto metropolis, Sokoto State, Nigeria. Soil samples were collected from mechanic workshop sites and subjected to standard microbiological analyses for isolation, enumeration, and characterization of bacterial species. A total of five bacterial species were identified using morphological and biochemical tests, namely *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus thuringiensis*, *Paenibacillus polymyxa*, and *Bacillus cereus*. The emulsification activity (E24) of the isolates was evaluated using used engine oil as the hydrophobic substrate, while hydrocarbon utilization potential was assessed by monitoring changes in optical density (OD600) in mineral salt medium supplemented with petrol as the sole carbon source over a 12-day incubation period. The results showed varying emulsification indices among the isolates, with *Bacillus cereus* exhibiting the highest emulsification activity (50.0%), while *Paenibacillus polymyxa* showed the lowest (36.7%). All isolates demonstrated the ability to utilize hydrocarbons, as indicated by progressive increases in optical density, with Bacillus cereus and Bacillus subtilis recording the highest growth rates. The findings indicate that hydrocarbon-contaminated soils in Nakasari harbour diverse bacterial species with significant emulsification and hydrocarbon degradation capabilities. These indigenous bacteria possess strong potential for application in the bioremediation of hydrocarbon-polluted environments.

Keywords:
Biosurfactant,
Emulsification activity,
Hydrocarbon utilization,
Bioremediation.

INTRODUCTION

Hydrocarbon contamination of soil is a major environmental concern worldwide due to the persistence, toxicity, and ecological disruption caused by petroleum-derived compounds (Majeed *et al.*, 2025). In urban and semi-urban areas such as Nakasari in the Sokoto metropolis of Sokoto State, Nigeria, soil contamination often arises from activities including automobile repair workshops, fuel storage and distribution, indiscriminate disposal of petroleum products, and minor oil spill incidents. These hydrocarbons, which include aliphatic and aromatic compounds, are hydrophobic in nature and tend to bind strongly to soil particles, reducing their availability for natural degradation processes (Galperin *et al.*, 2022). As a result, contaminated soils may experience reduced fertility, altered microbial diversity, and long-term environmental degradation.

Addressing this challenge requires sustainable and cost-effective remediation strategies that can restore soil quality while minimizing further ecological disturbance (Ghisman *et al.*, 2025).

Bioremediation has emerged as a promising approach for the cleanup of hydrocarbon-polluted environments, relying on the metabolic capabilities of microorganisms to degrade complex organic pollutants into less harmful substances (Alaidaroos, 2023; Egbomuche *et al.*, 2024). Among these microorganisms, bacteria play a central role due to their metabolic diversity, rapid growth rates, and ability to adapt to harsh environmental conditions. Indigenous bacteria isolated from hydrocarbon-contaminated soils are particularly valuable because they are already adapted to the local environment and the specific pollutants present (Chen *et al.*, 2023).

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These bacteria can utilize hydrocarbons as sources of carbon and energy, converting them through enzymatic processes into simpler compounds such Understanding the hydrocarbon utilization potential of such bacteria is therefore essential for evaluating their effectiveness in environmental remediation.as carbon dioxide, water, and biomass (Tiwari *et al.*, 2026).

A critical factor influencing bacterial hydrocarbon degradation is emulsification activity, which is closely linked to the production of biosurfactants (Ibrar *et al.*, 2022). Biosurfactants are surface-active compounds produced by certain bacteria that reduce surface and interfacial tension between water and hydrophobic substances such as oil. By emulsifying hydrocarbons, biosurfactants increase the surface area of oil droplets and enhance their solubility in water, making them more accessible for microbial uptake and metabolism (Sah *et al.*, 2022). This process is particularly important in soil environments, where hydrocarbons are often trapped within soil pores and tightly bound to particles. Bacteria with high emulsification activity are therefore considered more efficient degraders because they can overcome the physical limitations associated with hydrocarbon bioavailability (Pandolfo *et al.*, 2023).

The assessment of both emulsification activity and hydrocarbon utilization potential provides a comprehensive understanding of the bioremediation capacity of bacterial isolates (Hossain *et al.*, 2022). While hydrocarbon utilization reflects the ability of bacteria to metabolize petroleum compounds directly, emulsification activity indicates their capacity to facilitate and enhance this process. Studies have shown that bacterial strains exhibiting strong emulsification properties often demonstrate higher degradation efficiencies, especially when dealing with complex hydrocarbon mixtures (Pandolfo *et al.*, 2023). Evaluating these properties in isolates from contaminated soils enables researchers to identify the most promising candidates for bioremediation applications, either as individual strains or as part of microbial consortia (Zhang and Zhang, 2022).

Investigating the emulsification activity and hydrocarbon utilization potential of bacteria isolated from hydrocarbon-contaminated soils in Nakasari, Sokoto State, is therefore of both scientific and environmental significance (Makut *et al.*, 2022). Such studies contribute to the understanding of local microbial diversity and adaptive mechanisms in polluted environments, while also providing practical solutions for managing soil contamination in the region. The findings can inform the development of environmentally friendly remediation strategies that are tailored to local conditions, reduce reliance on expensive physicochemical methods, and promote sustainable environmental management (Azuazu *et al.*, 2023). Ultimately, harnessing the capabilities of indigenous bacteria represents a viable pathway toward restoring contaminated soils and protecting public and ecological health in Sokoto metropolis and similar settings (Enerijiofi *et al.*, 2025).

MATERIALS AND METHODS

Study Area

The study was conducted using soil samples collected from a selected hydrocarbon-contaminated garage site located in the Nakasari area of Sokoto South, Sokoto State. Two sampling sites were selected within the same auto-mechanic workshop environment characterised by intensive vehicle maintenance activities and petroleum hydrocarbon residues. The study area lies between latitude 13.0447°N and longitude 5.2686°E as shown in Figure 1. below.

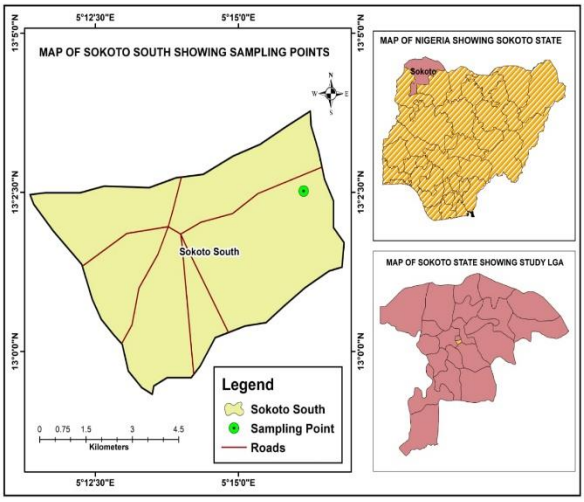

Figure 1: Map of Sokoto South, Sokoto State showing a selected automobile mechanic workshop sampling sites at Nakasari (coordinates: longitude 13.0447°N, latitude 5.2686°E).

Sample Collection

Prior to sample collection, surface debris and other impurities were removed from the soil. Sample of petroleum contaminated soil was collected randomly in duplicate at a depth of 4-5cm into a clean polythene bag from Nakasari garage, Sokoto metropolis. After collecting the samples, they were immediately transported to the laboratory (Huang *et al.*, 2021).

Preparation of Media

The media were prepared according to the manufacturer's instruction.

Nutrient Agar

About 28g of nutrient agar was weighed using an electronic weighing balance and dissolved into 1000ml of

distilled water in a sterile conical flask. It was swirled to mix, the mixture was heated with a water bath to dissolve thoroughly. The mixture was then sterilized in an autoclave at 121ºC for 15mins. The media was allowed to cool at 40ºC, which was then poured into sterile Petri dishes and was allowed to solidify (Kumar[1] *et al.,* 2025).

Mineral Salt Medium

Inorganic Salts (6.0g of NaHPO4, 3.0g of KH2PO4, 1.0g of NH4Cl, 0.5g of NaCl, 0.2g of MgSO4.7H2O, 0.02g of CaCl2 and 0.001g of FeSO4.7H20) was accurately measured and dissolved in 1000ml of distilled water. The pH was checked and adjusted to around the neutral pH (7.0). Then the mixture was poured into flask and was sterilized using the autoclave at 121˚C for 15 minutes. The medium was allowed to cool and 1% petroleum was added as a carbon source and mixed slowly to disperse it evenly (Mahgoub *et al.,* 2023).

Isolation of Bacteria

Exactly 1g of the collected sample was added to 9ml of distilled water in a test tube and it was whirled gently to have a homogenous mixture. Using a pipette, 1ml was drawn from the mixture and was serially diluted by adding 1ml of the sample mixture to 9ml of distilled water in a test-tube making it 10^{-1}, from 10^{-1} up to 10^{-7}. Aliquot (0.1ml) was pipetted from test-tube 10^{-7} and inoculated on the surface of nutrient agar (solid media), and a sterile bent glass rod was used to spread it evenly over the media. The plate was then incubated in an inverted form for 24 hours at 37˚C. After 24 hours of incubation, the visible bacterial colony was subculture onto new media plates to obtain pure culture using streak plate method (Tegegn *et al.,* 2025).

Morphological and Biochemical Classification of Isolates

Gram Staining

The bacterial colony was picked using a sterile wire loop and placed on a clean grease free slide containing a drop of distilled water, and the colony was emulsified in the distilled water to make a smear. The smear was allowed to air dry and was passed through the flame 2 – 3 times in a sweeping manner to heat fix the smear. The heat fixed smear was flooded by crystal violet (Primary stain) for 60 seconds and it was rinsed off with clean water, the slide was flooded again with lugol's iodine which serves as the mordant for 60 seconds and it was rinsed off with clean water, then the decolourizer was added to the slide for 2 – 3 seconds and immediately rinsed off. Lastly, the counter stain (safranin) was added for 60 seconds rinsed with clean water and was placed in a draining rack to dry. After drying, a drop of oil immersion was placed on the stained slide and viewed using ×100 objective lens of the microscope (Das *et al.,* 2025).

Biochemical Tests for Isolate Characterization

Biochemical tests helps identify and classify bacteria based on their metabolic and enzymatic activities.

Catalase Test

A loop full of the bacterial Isolates from the inoculated agar plate was placed on a clean slide and 1-2 drops of 3% hydrogen peroxide was placed onto the colonies. Presence of gas bubbles indicates a positive reaction and absence of the bubble indicates a negative reaction (Rauschenbach *et al.,* 2017).

Indole Test

About 3ml of sterile tryptone water was poured in a test tube and the isolated organism was added. The test tube was incubated at 37°C for up to 24 hours. After incubating, 0.5ml of Kovac's reagent was added and examined. Appearance of a ring-like red colour on the surface layer within 10 minutes indicates indole positive result (Tegegn *et al.,* 2025).

Citrate Utilization Test

Simon citrate agar was inoculated with the isolate and incubated at 37^0C for 48hrs. The presence of a bright blue colour indicates citrate positive while absence of bright blue colour indicates negative (Sheikh, 2018).

Starch Hydrolysis

The Starch-containing agar plate was inoculated with the test organism and incubated at 37ºC for 24hrs. After incubation, the plate was flooded with lugol's iodine solution. A positive result will show a clear zone around the bacterial growth, while negative result will show no clear zone (Stark, 1951).

Methyl Red - Voges-Proskauer Test

The test organism was inoculated in MR-VP broth and incubated for 24hours at 37ºC. Twelve (12) drops of 5% solution of alpha-naphthol and four (4) drops of 40% potassium hydroxide (KOH) was added to the broth after incubation. The mixture was agitated vigorously and left to stand. Positive test leads to production of red or pink color (ETHIOPIA and HAMMAD, 2023).

Urease Test

Colony from the stock culture was sub cultured into nutrient agar to obtain a fresh culture. Heavy inoculums was fetched from the nutrient agar using sterile wire loop and streaked on the slant surface of the urea medium. It was incubated for 24hrs at 37^0C. The development of a pink/red signifies urease positive; if colour remains unchanged (yellow/orange) it signifies negative (Tegegn *et al.,* 2025).

Oxidase Test

An oxidase reagent (1% Tetramethylparaphenylene diamine dihydrochloride) was placed on the Whatman filter paper and a bacterial colony was smeared on the

paper. The presence of the enzyme oxidase was observed by the appearance of a purple colour (Fardami *et al.*, 2022).

Triple Sugar Ion (TSI)

A sterile wire loop was used to pick an isolate and it was used to stab the butt of the TSI agar and the slant surface was streaked and before incubation at 37°C for 24 hours. Observation of the butt becoming yellow indicates glucose fermentation, while yellow coloration at the slant dictates lactose and sucrose fermentation. Appearance of crack or bubbles at the butt indicates gas formation while hydrogen sulphide was determined by observation of a black coloration in the media (Parumasivam *et al.*, 2024).

Preparation of Stock Culture

Nutrient agar was prepared and dispensed into Sterile McCartney bottles with up to half of it. The McCartney bottles containing the agar were autoclaved at 121^0C for 15minutes, the bottles were then removed and and kept in a slant position to cool and solidify. A sterile wire loop was used to pick a single colony of confirmed bacteria colonies and streaked on the surface of the solidified media and then incubated at 37^0C for subsequent use (James and Natalie, 2014).

Emulsification Activity

An aliquot of 2 ml of used engine oil being the sole carbon source was measured and supplemented with the same amount of cell-free supernatant. The mixture was homogenized with a vortex for 2 minutes and was allowed to stand undisturbed for 24 hours. After 24 hours, the height of the stable emulsion layer was measured using ruler (CHIZOBA, 2021). Emulsification index (E_{24}) was calculated using the formula below:

$$\text{Emulsification index (E24)} = \frac{Height\ of\ Emusification\ Layer}{Total\ Net\ Height} \times 100$$

Hydrocarbon Utilization Potentials Using Changes in Optical Density (OD600)

About one (1) ml of the grown inoculums was added into a 250 ml conical flask containing 2% (v/v) petrol oil in 100 ml of mineral salt medium. Another flask was prepared without the inoculum as the Control flask. The flask was incubated in a shaker incubator at 35˚C for 12 days, at 120 rpm. The growth of the isolates was determined using the optical density during petroleum oil degradation for 12 days. 2 ml of the isolate suspension and the control was aseptically dispensed into the cuvette of UV-VIS spectrophotometer, the optical density was measured using UV-VIS spectrophotometer at 3 days interval, that's 3, 6, 9 and 12 days at 600nm (Aina *et al.*, 2025).

RESULTS AND DISCUSSION

Mean Bacterial Colony Count

Table 1 presents the results of bacterial colony count from site A-NMS and B-NMS of Hydrocarbon contaminated soil samples. Sample A-NMS has the highest colony count of 14.1×10^7cfu/g and Sample B-NMS has the lower colony count which is 5.3×10^7cfu/g.

Table 1: ***Bacterial Colony Counts Isolated from Hydrocarbon Contaminated Soil Samples***

Sample	Mean ± Standard (Cfu/g)
A-NMS	14.3 ± 0.2
B-NMS	5.4 ± 0.3

Keys: A-NMS = Nakasari Mechanic Site A, B-NMS = Nakasari Mechanic Site B

Morphological and Biochemical Identification of Bacterial Isolates

Table 2 shows the results of the morphological and biochemical identification of the bacterial isolated from the hydrocarbon contaminated soil samples. *Bacillus subtilis, Bacillus megaterium, Bacillus thuringiensis, Paenibacillus polymyxa* and *Bacillus cereus* were identified after the biochemical tests.

Table 2: Morphological and Biochemical identification of Bacterial Isolates

Samples	G	Shp	Sp	Gl	Su	La	Gs	H_2S	Ca	Ci	In	St	MR	VP	Ox	Ur	Organisms
NMS-1	+	Rod	+	+	-	-	-	+	+	-	+	+	+	-	+	-	*Bacillus subtilis*
NMS-2	+	Rod	+	+	-	-	-	+	+	+	+	+	+	-	+	-	*Bacillus megaterium*
NMS-3	+	Rod	+	+	+	+	-	+	+	-	+	+	+	-	-	-	*Bacillus thuringiensis*
NMS-4	+	Rod	+	+	+	+	-	+	+	+	-	+	+	-	-	-	*Paenibacillus polymyxa*
NMS-5	+	Rod	+	+	-	-	+	+	+	-	-	+	+	-	+	-	*Bacillus cereus*

Keys: G= Gram Reactions, Shp= Shapes, Sp= Spores, Gl=Glucose, Su=Sucrose, La=Lactose, Gs=Gas Production, H_2S= Hydrogen Sulphide, Ca=Catalase, Ci=Citrate, In=Indole, St=Starch, MR=Methyl Red, VP=Voges-proskaurer, Ox=Oxidase, Ur=Urease.

Frequency of Occurrence of the Bacterial Isolates

Table 3 presents the frequency of occurrence of the bacterial isolates. A total of eleven (11) bacterial isolates with varying frequency of occurrence were identified from the hydrocarbon contaminated soil samples. *Bacillus subtilis* had the highest percentage frequency of occurrence (36.36%), and it was found to be the most predominant species among the isolates that were isolated from the hydrocarbon contaminated soil samples. Followed by *Bacillus cereus* (27.27%) and *Bacillus megaterium* (18.18%). while *Bacillus thuringiensis* (9.09%) and *Paenibacillus polymyxa* (9.09%) are the least prevalent.

Table 3: Frequency of Occurrence of the Bacterial Isolates

S/N	Identified Bacteria	Frequency of Occurrence	Percentage Frequency
1	*Bacillus subtilis*	4	36.36%
2	*Bacillus megaterium*	2	18.18%
3	*Bacillus thuringiensis*	1	9.09%
4	*Paenibacillus polymyxa*	1	9.09%
5	*Bacillus cereus*	3	27.27%
	TOTAL	**11**	**100%**

Emulsification Activity (E24) of the Bacterial Isolates

Table 4 presents the results for Emulsification Activity (E_{24}) of the five (5) identified bacterial species. *Bacillus cereus* has the highest emulsification activity (50.0%), followed by *Bacillus thuringiensis* (46.7%) and *Bacillus subtilis* (46.7%). Followed by *Bacillus megaterium* with the emulsification activity (43.3%) and *Paenibacillus polymyxa* (36.7%) have the least emulsification activity.

Table 4: Emulsification Activity (E_{24}) of the Bacterial Isolates

S/N	Organisms	Emulsification Activity (E_{24})
1	*Bacillus subtilis*	46.7%
2	*Bacillus megaterium*	43.3%

3	*Bacillus thuringiensis*	46.7%
4	*Paenibacillus polymyxa*	36.7%
5	*Bacillus cereus*	50.0%

Hydrocarbon Utilization Potentials of the Bacterial Isolates Using Changes in Optical Density (OD600) per 3 Days Interval

Table 5 present the results for the Hydrocarbon utilization potential using changes in optical density (OD_{600}) of the five (5) identified bacterial species. At Day 3, *Bacillus cereus* (0.300) has the highest OD_{600} value followed by *Bacillus subtilis* (0.293), *Bacillus megaterium* (0.182) and *Paenibacillus polymyxa* (0.122). *Paenibacillus polymyxa* (0.122) has the least OD_{600} value. At Day 6 of incubation, *Bacillus subtilis* (0.429) was found to have the highest OD_{600} value followed by *Bacillus thuringiensis* (0.279), *Bacillus cereus* (0.249) and *Paenibacillus polymyxa* (0.205), While *Bacillus megaterium* (0.146) has the least OD_{600} value. At Day 9 of incubation, *Bacillus cereus* (0.861) has the highest OD_{600} value followed by *Bacillus subtilis* (0.810), *Bacillus thuringiensis* (0.643) and *Bacillus megaterium* (0.540) then *Paenibacillus polymyxa* (0.360) being with the least value. After 12 Days of incubation, *Bacillus cereus* (0.889) has the highest OD_{600} value followed by *Bacillus subtilis* (0.845), *Bacillus thuringiensis* (0.701) and *Bacillus megaterium* (0.563) then *Paenibacillus polymyxa* (0.551) having the least OD_{600} value.

Table 5: Hydrocarbon Utilization Potentials of the Bacterial Isolates Using Changes in Optical Density (OD_{600}) per 3 Days Interval

Organisms	**Day 03**	**Day 06**	**Day 09**	**Day 12**
Bacillus subtilis	0.293	0.429	0.810	0.845
Bacillus megaterium	0.182	0.146	0.540	0.563
Bacillus thuringiensis	0.117	0.279	0.643	0.701
Paenibacillus polymyxa	0.122	0.205	0.360	0.551
Bacillus cereus	0.300	0.249	0.861	0.889

In this research, hydrocarbon contaminated soil sample was used to identify bacteria present and these bacteria were screened for their potential to utilize hydrocarbon and their emulsification activity. The soil contaminated by wastes and oil spills from Nakasari garages in Sokoto metropolis do have significant concentration of hydrocarbon. In this study, Emulsification activity and hydrocarbon utilization potential assay were carried out and the potent bacteria were revealed from the five bacteria isolated. According to Chakraborty *et al.* 2016, hydrocarbon pollution creates selective pressures that can favor the growth of certain resistant species, some of which may be harmful to humans.

Table 1 illustrated the results of bacterial mean colony counts. The bacterial counts were found to range from 5.3 and 14.1×10^{7}cfu/g, which is in alignment with the work of Obayori (2008) on a topic titled "Degradation of Some Petroleum Hydrocarbon Pollutants by Micro-Organism from Contaminated Tropical Soils". The high number of colony may be due to adaptation of microbial community to long-term contamination increasing their efficiency in hydrocarbon degradation and growth.

Five distinct bacterial species where identified as shown in Table 2 which are *Bacillus subtilis, Bacillus megaterium, Bacillus thuringiensis, Paenibacillus polymyxa* and *Bacillus cereus*. The occurrence of *Bacillus* spp. in the soil samples from the hydrocarbon contaminated sites might be due to their ability to form endospore, allowing them to survive harsh and toxic environment. The identification of *Bacillus* spp. in this study agrees with that of Al-Dhabaan (2019) carried out at Dhahran, Saudi Arabia. Likewise the identification of *Paenibacillus polymyxa* in this research aligns with the work of Rawayau *et al.,* (2025).

In this study, *Bacillus subtilis* was isolated from hydrocarbon contaminated soil. The Presence of *Bacillus subtilis* is *conspicuous* and aligns with the findings of Tabari and Tabari (2017) in their research titled "Characterization of a biodegrading bacterium, Bacillus subtilis, isolated from oil-contaminated soil", where they highlighted some certain characteristics that encourage its survival despite the contamination by hydrocarbon.

The prescence of *Bacillus megaterium* in this research is in consistent with the findings of Khan and Asthana (2011) showing its adaptability to different ecological changes. They also emphasized that the specie can be a significant agent of bioremediation due to its ability of producing extracellular enzymes that can break down hydrocarbon into simpler, less toxic compounds.

The isolation of *Bacillus thuringiensis* in this research was associated with its spore forming characteristics and

capability of contributing immensely in remediation thus, degrading hydrocarbons with complex structures including phenanthrene as supported by Obayori *et al.*, (2024) in their study carried out at Mechanic village, Dalemo, Alakuko axis of Lagos State, Nigeria.

Paenibacillus polymyxa identified from hydrocarbon contaminated soil sample in this study is supported by Omotayo *et al.* (2013), which shows its ability to engange in symbiotic interactions with other soil microbes, facilitating cooperative degradation of hydrocarbon. This view of isolating this bacteria specie from hydrocarbon contaminated soil is also supported by Rawayau *et al.* (2025)

Finally, the occurrence of *Bacillus cereus* in soil contaminated with hydrocarbon may be due to the bacteria possessing metabolic versatility, including the ability to degrade certain hydrocarbons through production of biosurfactant which enhance the bioavailability of hydrophobic hydrocarbons, enhancing their breakdown. This allows them to thrive in polluted soil as supported by Hussain *et al.* (2024) and Ezeonuegbu *et al.* (2025).

Table 3 presents the frequency of occurrence of the bacterial isolates. A total of twelve (11) bacterial isolates with varying frequency of occurrence were identified from the hydrocarbon contaminated soil samples. The five (5) bacterial species are *Bacillus subtilis, Bacillus megaterium, Bacillus thuringiensis, Paenibacillus polymyxa* and *Bacillus cereus. Bacillus subtilis* had the highest percentage frequency of occurrence (36.36%), and it was found to be the most predominant species among the isolates that were isolated from the hydrocarbon contaminated soil samples. Followed by *Bacillus cereus* (27.27%) and *Bacillus megaterium* (18.18%). while *Bacillus thuringiensis* (9.09%) and *Paenibacillus polymyxa* (9.09%) are the least prevalent. The high prevalence of *Bacillus* species in hydrocarbon contaminated soil samples may be due to their ability to form endospore, which enables them to survive extreme environmental stress including toxic pollutants. This is in concordant with the study of Al-Dhabaan (2019).

Emulsification activity of the five (5) identified bacterial species at 24 hours as depicted in Table 4 shows diverse potential of the isolated organisms to stabilize emulsions between two immiscible liquids, thereby exposing their individual ability to produce biosurfactant. Table 4 highlighted the emulsification activity (E_{24}) of the five (5) identified bacterial species. *Bacillus cereus* shows the highest emulsification activity of up to 50.0% as justified by Durval *et al.* (2019). That may be due to its ability to secrete both glucosamine-based polysaccharide bioemulsifier and a monoglyceride biosurfactant that acted synergistically lower surface tension and stabilize emulsions effectively as strongly supported by the work of Cooper and Goldenberg (1987). Followed by *Bacillus subtilis* and *Bacillus thuringiensis* having a high emulsification activity of (46.7%) both, which is in alignment with Wu *et al.* (2022); Bajelani *et al.* (2023) respectively. Furthermore, *Bacillus megaterium* happens to have the emulsification activity (43.3%) which is at a moderate level in consistent with the findings of Singh *et al.* (2022). *Paenibacillus polymyxa* exhibited the lowest emulsification index (36.7%), which may be due to production of insufficient biosurfactant concentration in the medium and possibly different lipopeptide composition that favors antifungal activity rather than emulsification (Sarwar *et al.*, 2018).

Optical Density at 600nm (OD_{600}) was used to monitor the growth of the five (5) identified bacterial species to unveil their individual hydrocarbon utilization potential as petrol was used as carbon source. Their turbidity was measured using a spectrophotometer for 4 days at 3 days intervals (Day 3, 6, 9 and 12) as shown in Table 5 above. All the five isolates shows a significant value of turbidity elucidating that they are able to utilize and breakdown the provided hydrocarbon as a carbon source for growth and survival. After taking the all readings and observations at Day 12, the bacterial specie with the highest value appears to be *Bacillus cereus* (0.889) which may be due to possession of diverse degradation pathways thereby utilizing alkane, methylcyclohexane, toluene, xylene and benzene degradation routes, showing a broad enzymatic capability for breaking diverse hydrocarbons (Christova *et al.*, 2019), also indicating strong adaptation and metabolic efficiency in utilizing petroleum as its sole carbon source which is in agreement with findings of Hussain *et al.*, (2024) conducted at Lahore, Pakistan. *Paenibacillus polymyxa* (0.551) has the least value, this may be due to its hydrocarbon-degrading enzymes and surfactant production being weaker and condition dependent than other *Bacillus* species, reducing its ability to emulsify and metabolize hydrophobic substrates as stated by Gudiña *et al.* (2015). Consequently, it exhibits lower cell density (OD_{600}) in hydrocarbon media,reflecting minimal biomass formation and poor substrate utilization efficiency especially when hydrocarbon is the only carbon source as justified by a research conducted by Timmusk *et al.* (2021).

CONCLUSION

This study investigated the emulsification activity and hydrocarbon utilization potentials of bacteria isolated from hydrocarbon-contaminated soil in the Nakasari area of Sokoto Metropolis, Sokoto State, Nigeria. The identified bacterial species *Bacillus subtilis*, *Bacillus cereus, Bacillus thuringiensis, Bacillus megaterium*, and *Paenibacillus polymyxa* demonstrated notable adaptability to hydrocarbon-polluted conditions. Variations in their emulsification index (E_{24}) and optical

density (OD_{600}) values reflected differences in biosurfactant production and hydrocarbon degradation efficiency. Among these, *Bacillus cereus* and *Bacillus subtilis* exhibited superior emulsification and growth performance, indicating strong hydrocarbon metabolism. Overall, the presence of these efficient hydrocarbon-degrading and emulsifying bacteria suggests that Nakasari soil harbors diverse and resilient microbial communities with great potential for bioremediation of hydrocarbon-contaminated environments.

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