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ORIGINAL ARTICLE

Assessment and Biodegradation of Polycyclic Aromatic Hydrocarbons in Soil and Water Around Petroleum Products Depot Suleja, Nigeria

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Abstract

Petroleum contamination constitutes a frequent incidence in various petroleum depots in Nigeria. In this study, the polycyclic aromatic hydrocarbons (PAHs) present in soil and water in communities around Petroleum Products Marketing Company (PPMC) Suleja, Nigeria, were evaluated and degraded using indigenous microorganisms. The samples sites were divided into 7 plots from where samples of water and soil were obtained: one within the PPMC depot, five from communities surrounding the depot, and the control 93,000 km from the depot. The microbial counts were determined using spread plate inoculation technique on minimal salt media. The microbial isolates were characterized and identified based on their cultural, biochemical, and molecular characteristics. The potential of the microbial isolates to utilize 0.05 mL of diesel, kerosene, engine oil, and crude oil was determined in a Bushnell Haas Broth, and the biodegradation was determined by total viable cell counts and spectrophotometry. The ability of the isolates to mineralize PAHs was also evaluated in a minimum salt media. The bacterial isolates were species of *Streptococcus, Pseudomonas, Staphylococcus, Proteus, Escherichia,* and *Bacillus,* while species of *Penicillium, Aspergillus, Mucor*, and *Rhizopus* were isolated among the fungi. *Aspergillus niger* strain ATCC 1015 and *Bacillus thuringiensis* strain M43 showed high capacity to utilize the 16 priority PAHs. The pahE1 gene was used by *Bacillus thuringiensis, Pseudomonas aeruginosa* and *A. niger,* while *Penicillium notatum* used *pahE2* gene for the degradation of the PAH. The current study identified microbial isolates that can utilize priority PAHs, making them beneficial for oil spill bioremediation in tropical environments.

Keywords PAH · Petroleum · Pollution · Degradation · Environment · Bacteria · Fungi

Highlights
- Microorganisms were isolated from the soil and water contaminated by petroleum hydrocarbons
- The isolates were tested for their ability to mineralize PAH
- *A. niger* and *B. thuringiensis* showed high utilization capacity on 16 priority PAHs
- The *pahE1* and *pahE2* genes were used for the degradation of the PAH

Extended author information available on the last page of the article

Introduction

Petroleum is an intricate blend of hydrocarbons and non-hydrocarbon compounds [1]. Petroleum products can pollute soils and contaminate surface and underground water that are used for domestic purposes [2]. Petroleum products are primarily composed of different types of hydrocarbons with traces of some potentially toxic elements. Oil production, transportation, and distribution activities inevitably result in environmental pollution from petroleum products [3].

The polycyclic aromatic hydrocarbons (PAHs) have been recognized as persistent and recalcitrant organic pollutants with a range of toxicities [4]. Based on their chemical structure, they are aromatic in nature and are made up of more than one benzene rings that is only composed of C and H atoms and organized in a straight, angular, or organized form [5]. They are by-products of petroleum refinery, combustion, and other high-temperature industrial methods [4]. PHAs can be found in natural environments including air, soil, water, and wastewater [4–6]. There are hundreds of these compounds in nature, and each one's aromatic ring number and position are different [6], but only 16 of them have been found to pose a serious risk to human health and the environment: These are acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a) anthracene, chrysene, benzo(b)fluoranthene, benzo(a)pyrene, benzo(k)fluoranthene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene, and naphthalene [7]. Reduced immunity, glaucoma, liver and kidney damage, difficulty in breathing, abnormal lung function, and inflammation of the skin can all result from chronic or long-term exposure to PAHs [8].

According to Patel et al. [4], Stading et al. [9], and Imam et al. [10], PAHs also exhibit genotoxicity, teratogenicity, carcinogenicity, and immunotoxicity. Enzymes first chemically alter them into metabolites, which then interact with DNA to cause mutations; poor prenatal development and cardiovascular disease have also been connected to exposure to PAHs [9].

Petroleum products are produced, refined, and transported to depots and other final destinations more often in response to rising consumer demand [11]. These petroleum-related operations frequently cause significant environmental damage [8, 12–16] including leakages from depot reservoir [4]. Communities have had unfavorable interactions with adjacent water and soil [6]. In Petroleum Products Marketing Company Limited (PPMC) depots, long lines of loaded and unloaded trailer tankers are frequently seen; these are possible sources of spillage of petroleum hydrocarbons [17]. The hydrocarbon molecules that make up crude oil and petroleum derivatives are extremely poisonous especially in large concentrations [18]. The negative impact on soil microflora, which is central to the maintenance of a healthy ecosystem and soil fertility, is one of the many environmental effects of soil contamination by petroleum products. Microorganisms that play a significant part in the nutrient cycle may experience gene mutation as a result of petroleum contamination of soil and water [4].

Much of the groundwater used by the communities near the Suleja PPMC facility serves household purposes. Through spills, leaks, disposal of effluent water, and other sources, the PPMC's activities have contaminated these water sources. Due to the large number of people living in communities around the Suleja PPMC depots, as well as unplanned settlement patterns, there has been significant environmental contamination. These PPMC depot populations frequently witness indiscriminate defecation and urination, which are also significant sources of microbiological contamination. This is because there is a lack of

appropriate sanitary structures in the area. As a result, runoff or leachate from open defecation can contaminate the soil, surface water, and possibly subsurface water as well. Even in cases of relatively mild contamination, soil contaminated with petroleum products may result in a reduction in microbial density and activity [4]. The interactions between macro and microorganisms in the rhizosphere can have a significant impact on crop yield and soil fertility [19, 20]. Poor agricultural yields may arise from soil contaminated with petroleum products since it slows down the activities of nitrogen-fixing bacteria [20, 21].

It is usual for depot residents to use available land close to PPMC depots for farming, and the contaminated soil, water, and farm produce could further contribute to the hazards from PAHs. The presence of priority PAHs indicates contamination and gives a general indication of human exposure to their toxicity, and the persistent petroleum product discharges in this environment also pose a serious threat to the microbial population [22]. The aim of this study therefore was to assess and biodegrade PAH in soil and water around the petroleum products depot Suleja, Nigeria.

Materials and Methods

The Study Area

This study was conducted in communities around PPMC depot Suleja, Niger State, Nigeria. Niger State is located in Central North Nigeria and is positioned between Lat. 80 20° N and 110 30° N and Lat. 3030° E and 70 20° E. The Suleja depot is situated along the Maje-Dikko bypass. The PPMC depot began its operation in 1995. The depot has 12 storage tanks each with a holding capacity of 11.2 million liters of petroleum. Communities around the depot are Tunga Shanu, Dikko, Dagwe, Tunga Koro, Maje. It is worth noting that around the depot, there are a lot of commercial activities. Temporary and unauthorized mechanic shops exist in the neighborhoods surrounding the PPMC depot to fix broken-down automobiles. These operations pollute the environment with leaks and spent engine oils [23].

Experimental Design and Sample Collection

Complete Randomized Design (CRD) approach was used for the collection of samples (Table 1; Fig. 1). Soil and water samples were collected in the five communities as well

Table 1 Distance of samplings plots to petroleum products depot and their coordinates

PlotS	Distance (meters)	Community	Coordinates
1	1500	Tunga Shanu	9.250958° N, 7.160060° E
2	500	Dikko	9.253754° N, 7.164931° E
3	1500-(farthest communities)	Petroleum products depot premises	9.254262° N, 7.1668195° E
4	1000	Dagwe	9.2512229° N, 7.1687292° E
5	1500	Tunga Koro	9.2559345° N, 7.1786521° E
6	1000	Maje	9.255818° N, 7.1788452° E
Control	93,000	FUT Minna (Bosso campus)	9.655311° N, 6.5267310° E

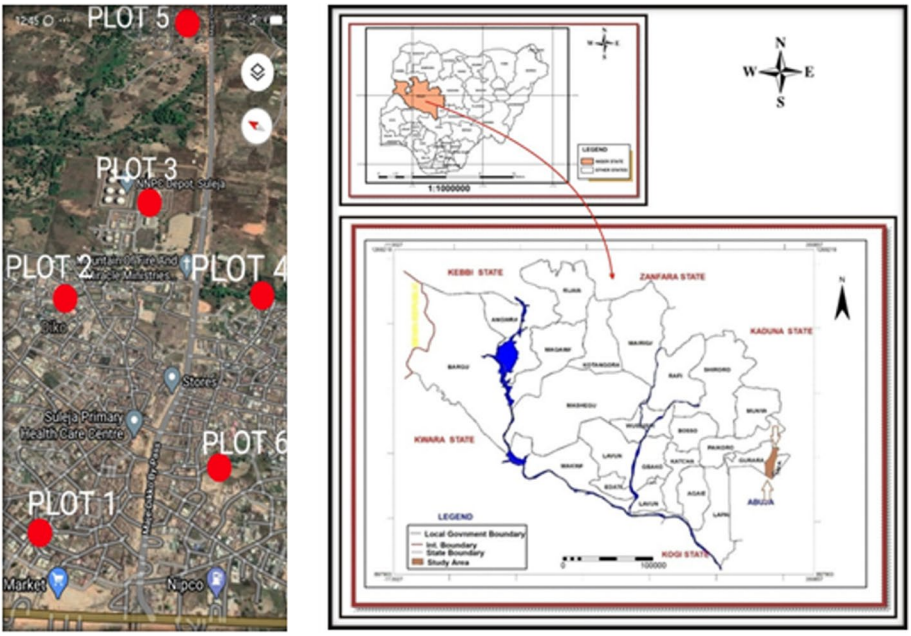


Fig. 1 Study site showing various communities (Plots). Source: Niger State Ministry of Land and Surveys, Minna (2015). Legend- Plot 1: Tunga Shanu, Plot 2: Dikko, Plot 3: Petroleum products depot premises, Plot 4: Dagwe, Plot 5: Tunga Koro, Plot 6: Maje

as within the Depot premises into nitric acid sterilized dark bottles [24]. Samples were also collected 93,000 km from the sample site (control). Samples collection was done for a year period during rainy and dry seasons. Soil samples were collected at 0–15 cm deep and placed in Whirlpak bags while the water samples (30 underground wells, boreholes, and stream) were transported in an ice pack. Both samples were taken to Microbiology laboratory of Federal University of Technology, Minna, Nigeria, for analysis.

Isolation of Microorganism

The bacteria and fungi present in the soil samples were enumerated using 10 g of serially diluted soil samples, and 0.1 mL of the diluent was placed over Nutrient agar (NA) and Sabouraud dextrose agar (SDA) for isolation of bacteria and fungi respectively. While the SDA plates were incubated at room temperature (28 ± 2 ºC) for 5 days, the NA plates were incubated at 30 ºC for 48 h [25]. The organisms that developed after incubation were counted and expressed as colony-forming units per gram of soil (cfu/g).

Isolation and Identification of Bacteria and Fungi that Utilize Hydrocarbons

The total hydrocarbon utilizing bacterial (THUB) and total hydrocarbon utilizing fungal (THUF) counts were determined by plating 0.1 mL of diluent on sterile Bushnell Haas agar (Sigma-Aldrich, USA) [26]. The delivery of the petroleum hydrocarbon was conveyed *via* sterilized (No. 1) Whatman filter papers impregnated with 5 mL of each hydrocarbon

(engine oil, crude oil, kerosine or diesel) on the lids of the inverted plates. The THUB plates were incubated in a shaker (New Brunswick Scientific) at 150 rev/min for 21 days at 30 °C while the THUF were incubated for 5 days at 28 °C. Bacterial isolates were characterized based on their reaction to Gram's staining and biochemical tests [27, 28] while the fungi isolates were identified based on their macroscopic and microscopic characteristics [29]. Molecular identification of the bacterial and fungal isolates was determined by extracting the DNA using CTAB method [30]. Extracted DNA was resuspended in 50 µL sterile water and stored at -20 °C till further analysis.

PCR Analysis Using ITS1 and ITS4 Primers

PCR was carried out with universal 1TS1 and ITS4 using methods described by Farber [31] and Wang et al. [32]. The PCR mix comprised 1 µL of 10X buffer, 0.4 µL of 50 mM $MgCl_2$, 0.5 µL of 2.5 mMdNTPs, 0.5 µL 5 mM ITS1 primer, 0.5 µL of 5 mM ITS4 primer, 0.05 µL of 5 units/ µLTaq with 2 µL of template DNA and 5.05 µL of distilled water to make-up 10 µL reaction mix. The PCR profile used was an initial denaturation temperature of 94°C for 3 min, followed by 30 cycles of 94°C for 60 s, 56°C for 60 s, 72°C for 120 s, the final extension temperature of 72°C for 5 min and held at 10°C.

PCR Reaction mix for PAH Primers

Each of the two PAH gene primers was used in a PCR analysis (Table 2). The PCR mixture consisted of 10X buffer (1 µL), 50 mM $MgCl_2$ (0.4 µL), 2.5 mM dNTPs (0.5 µL), and 5 mM (0.5 µL) forward primer, 5 mM reverse primer (0.5 µL) and 5 units/l Taq (0.05 µL), template DNA (2 µL), and 5.05 µL of distilled water made up to 10 µL reaction mix. The PCR profile started denaturation at 94 ºC for 3 min. This was followed by 30 cycles of 94 ºC for 60 s, 56 ºC for 60 s, 72 ºC for 120 s, and the final extension temperature of 72 ºC for 5 min. The cycle was finally terminated at 72 °C for 10 min and cooled at 4 °C.

Purification of PCR Products

The amplicon was further purified before the sequencing using ethanol purification protocol. To 10 µL of the PCR product, was added 1 µL 2 M NaAct pH 5.2, followed by 20 µL absolute ethanol, kept at -20°C for 1 h, spun at 10,000 rpm for 10 min, washed with 70% ethanol and air-dried. It was then resuspended in 5 µL sterile distilled water and kept at 4°C for sequencing.

Table 2 Primer sets for identifyin PAH-degrading genes

Primer name	Gene sequences
pahE1R	CCTGAGGAATCTCGGACATYT-STGCCCARAA
pahE2R	TTTGGCGGTVACVACYTG
pahE2F	AGCATGGGAACKYTKGGNGA
pahE1F	TGCGGCGGGTGTNAAYGGNAT

DNA Electrophoresis

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0% agarose gels. Agarose gels were prepared by dissolving and boiling 1.0 g agarose in 100 mL 0.5X TBE buffer solution. The gels were allowed to cool down to about 45°C and 10 µL of 5 mg/mL ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, 3 µL of the DNA with 5 µL sterile distilled water and 2 µL of 6X loading dye was mixed together and loaded in the well created. Electrophoresis was done at 80 V for 2 h. The integrity of the DNA was visualized and photographed on UV light source.

PCR for Sequencing

The primers used for the reaction were ITS1 and ITS4. The PCR mix used were 0.5 µL of BigDye Terminator Mix,1 µL of 5X sequencing buffer, 1 µL of M13 forward primer with 6.5 µL distilled water and 1 µL of the PCR product making a total of 10 µL. The PCR profile for sequencing was a rapid profile, the initial rapid thermal ramp was to 96°C for 1 min followed by 25 cycles of rapid thermal ramp to 96°C for 10 s. Rapid thermal ramp to 50°C for 5 s and rapid thermal ramp to 60°C for 4 min, then followed by rapid thermal ramp to 4°C.

Purification of PCR Sequencing Products

The PCR sequence product was also purified before the sequencing running using 2 M Sodium Acetate wash techniques. To 10 µL of the PCR product was added 1 µL 2 M NaAct pH 5.2, then 20 µL absolute ethanol, kept at -20°C for 1 h, spun at 10,000 rpm for 10 min, then washed with 70% Ethanol and air-dried. The mix was re-suspended in 5 µL sterile distilled water and kept at 4°C for sequencing running.

The cocktail mix was a combination of 9 µL of Hi di formamide with 1 µL of purified sequence making a total of 10 µL. The samples will be loaded on the gene sequencer (ABI 3130xl) machine and the data in forms A, C, T, and G were released.

Biodegradation of PAHs by the Bacterial Isolates

The hydrocarbon-utilizing isolates were checked for their ability to utilise PAH as the only carbon and energy substrate in a minimal salt medium containing per liter; Na_2HPO_4 (2.13 g), KH_2PO_4 (1.3 g), NH_4Cl (0.5 g), and $MgSO_4$ (0.2 g). The medium was autoclaved for 15 min at 121 °C. The agar surface of dried plates was evenly coated with acetone solution (0.2 mL) having 0.1% (w/v) of each PAH using an aseptic pipette [33]. Before administering pure cultures of the isolates, the acetone was allowed to evaporate in sterile conditions. The inoculated plates were incubated at room temperature (28 ± 2 ºC) in the dark for 21 days. Fresh PAH-coated agar plates were used to duplicate the colonies with clear zones on the coated agar plates, and the colonies were cultured for 21 days. The isolates that were obtained were selected as PAH degraders.

Determination of PAHs

Gas chromatography - Mass Spectrometry (GC-MS) was used to determine the PAHs in the samples. A sonicator (Ultrasonic bath-Elmsonic S40H) was used to extract hydrocarbons from the samples in line with US SW-846 Method 3550 [34]. A 50:50 (v/v) mixture of acetone and methylene chloride was used to extract 10 g of each soil sample. Before being deposited in an ultrasonic bath, this mixture was appropriately mixed and spiked with 1 mL of a PAH that is the internal standard, a GC coupled with FID-ECD dual detector, TriPlus AS auto-sampler with He as a carrier gas, dual column, and a quadrupole MS (Agilent 5975 MSD), based on USEPA method 8100 [35].

A 2 µL of extracts was introduced into the GC port with the following column settings: HP-5 crosslinked PH-ME siloxane, 30 m in length, 0.25 mm in diameter, and 1 µm in thickness. The helium carrier gas was set in spitless, constant flow mode and a flow rate of 1.2 mL/min. The individual PAH was identified and quantified using an internal calibration standard (EPA-16) that contained known concentrations of the 16 priority PAHs [36].

Determination of Gene for Degradation of PAH

Two bacterial and two fungal isolates were found to be highly effective PAH degraders out of all the microorganisms that were found. The isolates were cultured in ZoBell broth medium (peptone (5 g), yeast extract (1 g), $FePO_4$ 0.01 g in 1 L at pH 7.6) for 36 and 72 h for bacteria and fungi respectively. The microbial cells were spun at 2000 rpm for 1 h, washed 2x with MSM in 10% MCD (mixed-cyclodextrin), in 1 L of distilled water, pH 7.2. In the fume hood at ambient temperature for 36 h, the cyclohexane was left to vaporize. Following this, 100 mL of MSM containing 5 ppm of phenanthrene and the bacterial and fungal inocula were added, resulting in a final optical density of 0.3 measured at 600 nm. According to Hasham et al. [37]., the cultures were shaken at 150 rpm for 24 and 72 h at 30 °C in the dark.

Results

Rate of Petroleum Hydrocarbons Utilized by Bacterial and Fungal Isolates

The bacterial isolates used petroleum products at varied degrees after 21 days (Fig. 2a, c and e, and ST1). The absorbance (600 nm) was the lowest (15.81%) in engine oil, whereas crude oil had the highest (36.04%). Kerosene and diesel had 28% and 19.60%, respectively. The highest absorbance values on crude oil were recorded by *Pseudomonas* and *Bacillus*, at 0.663 and 0.601, respectively. *Proteus mirabilis* has the lowest absorbance on crude oil (0.081–0.41 nm).

The hydrocarbon-using bacteria *Streptococcus faecalis* and *Proteus mirabilis* were the least effective at using petroleum products. Within 21 days of incubation, the fungi utilized the petroleum products in varying degrees (Fig. 2b, d and f, and ST2). Crude oil had the highest percentage of use (43.16%), while engine oil had the lowest (12.64%). The percentages of diesel and kerosene used by the fungal isolates were 18.71% and 25.66%

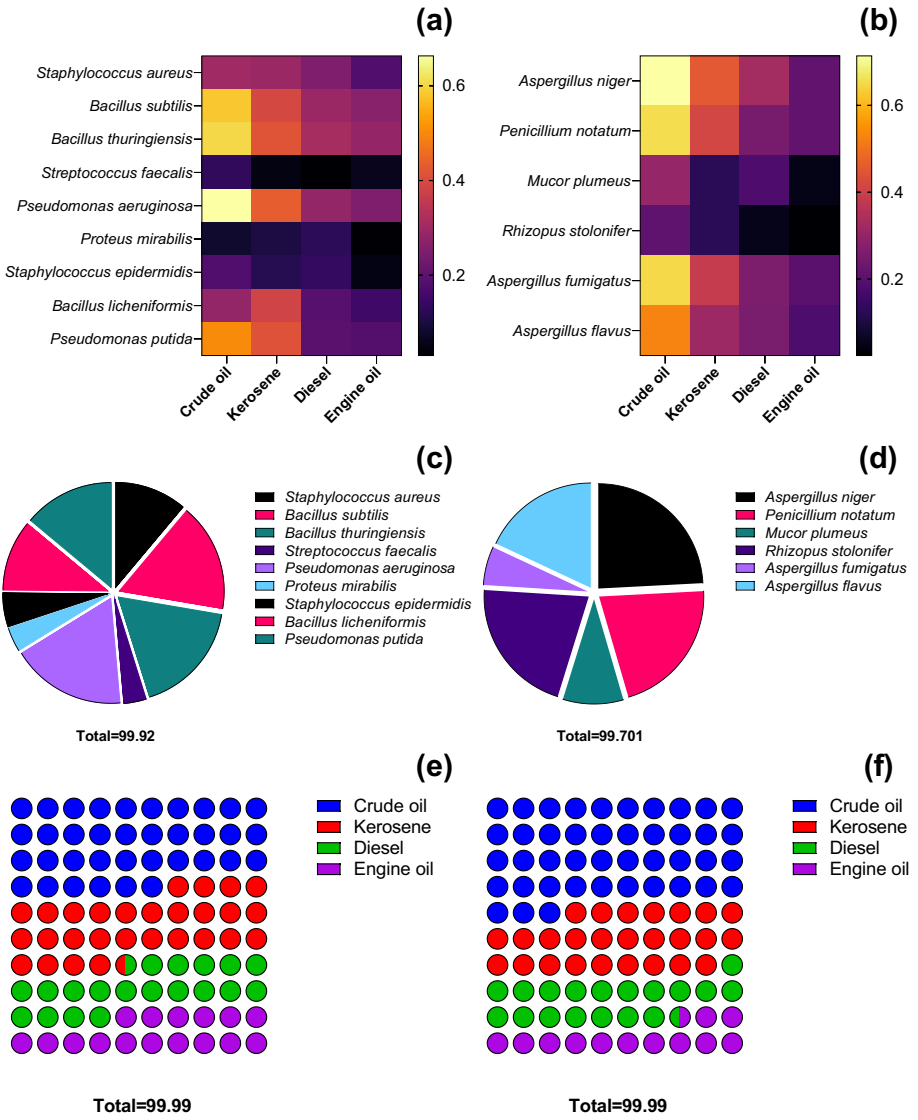


Fig. 2 Response of bacterial species in medium containing different fuels. Utilization efficiencies (A_{600} nm) of petroleum products by bacterial (**a**) and fungal (**b**) isolates after 21 days of incubation. Total % utilization of fuels by different bacterial (**c**) and fungal (**d**) species. Percent of different fuels utilized by different bacterial (**e**) and fungal (**f**) species

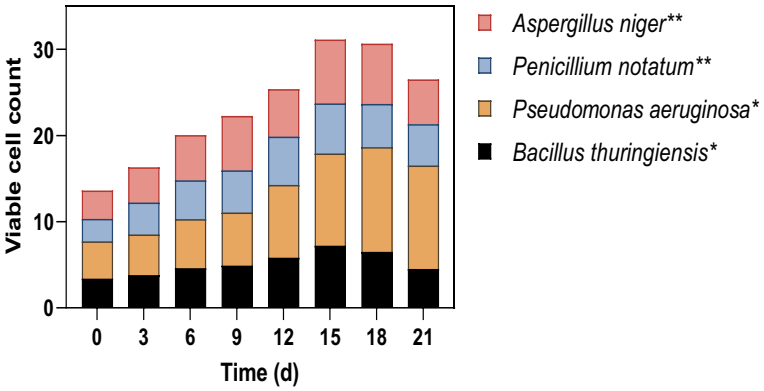


Fig. 3 Mean viable cell counts of hydrocarbon-utilising bacteria and fungi in MSM with crude oil (* $\times 10^6$; ** $\times 10^4$)

respectively. When compared to other fungal isolates, *Aspergillus niger* and *Penicillium notatum* showed a better ability to use petroleum products.

Counts of Petroleum hydrocarbon-Utilising Microorganisms in MSM

After 21 days, substantial differences in viable counts of both hydrocarbon-utilizing bacteria (HUB) and hydrocarbon-utilizing fungi (HUF) were recorded in the MSM amended with crude oil (Fig. 3 and ST3). The crude oil was increasingly emulsified in the medium as the time of degradation proceeded. For *B. thuringiensis* and *P. aeruginosa*, the viable counts increased up to the 15th and 18th day. When compared to other isolates, *P. aeruginosa* had the highest viable count (Fig. 3 and ST3). On day 21, the count dropped to 12.00×10^6 cfu/mL after rising from 4.32 to 12.15×10^6. On days 15 and 18, respectively, the count of *P. notatum* and *A. niger* increased to 5.80×10^4 and 7.60×10^4.

The Molecular Identity of Screened Isolates

After the 16S ribosomal RNA gene of the isolates was characterized, the bacterial isolates (B7 and B4) had 99.0% and 98.8% similarity to *P. aeruginosa* PA14 and *B. thuringiensis* strain M43 respectively while Fs2 and Fs3 (fungal isolates) were 98.5% and 98.9% similar to *A. niger* strain ATCC 1015 and *P. notatum* strain P72-30 respectively. The DNA size of the bacterial isolates was 1600 bp while the fungal isolates had a size of 500 bp.

The Concentrations of Priority PAHs in Soil Samples

The concentrations (mg/kg) of USEPA 16 priority PAHs designated in the soil of various Plots are presented in Table 3. Plots 1–5 had 6 of the PAHs while Plot 6 had 5. No PAH was found in the control Plot. Benz(a)anthracene, fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene were not found in all the 5 Plots while 2-methylnaphthalene, phenanthrene, benzo(b)fluoranthene were found present in all 5 experimental Plots. Pyrene and chrysene were both found in Plot 2 while 1-Methylnaphthalene was only found in Plot 3.

Table 3 The concentrations of Priority PAHs in soil samples in the various plots

PAHs (mg/kg)	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Ctr
1-Methylnaphthalene (NAP) LMW	ND	ND	16.00	ND	ND	ND	ND
2-Methylnaphthalene (NAP) LMW	20.03	8.00	8.00	36.00	8.00	8.05	ND
Acenaphthylene (ACY) LWW	ND	84.00	84.09	152.00	64.00	172.00	ND
Acenaphthene Acenaphthene (ACE) LWW	4.00	ND	ND	4.00	4.00	28.00	ND
Fluorene (FLU) LWW	ND	ND	ND	ND	116.00	ND	ND
Phenanthrene (PHEN) LWW	428.00	584.03	324.00	452.00	376.00	388.00	ND
Anthracene (ANT) LWW	16.00	112.00	ND	32.00	ND	ND	ND
Fluoranthene (FLTH) HWW	ND	ND	ND	ND	ND	ND	ND
Pyrene (PYR) HWW	ND	12.00	ND	ND	ND	ND	ND
Benzo(a)anthracene (B[a]A) HWW	ND	ND	ND	ND	ND	ND	ND
Chrysene (CHRY) HWW	ND	4.11	ND	ND	ND	ND	ND
Benzo(b)fluoranthene (B[b]F) HWW	52.00	244.00	40.00	32.00	4.00	172.00	ND
Benzo(a)pyrene (B[a]P) HWW	ND	ND	ND	ND	ND	ND	ND
Benzo(k)fluoranthene (B[k]F) HWW	ND	ND	ND	ND	ND	ND	ND
Indeno(1,2,3-cd)pyrene (INP) HWW	4.00	32.00	28.00	ND	4.00	ND	ND
Dibenz(a,h)anthracene (DBA) HWW	144.00	ND	44.00	32.00	ND	184.10	ND
Benzo(g,h,i)perylene (B[ghi]P) HWW	20.00	ND	8.00	ND	ND	ND	ND

ND – not detected, mg/kg – milligram per kilogram, Ctr – control., LMW : Low molecular weight ; HMW : High molecular weight

Table 4 Concentrations of USEPA priority PAHs present in water samples in the various plots

PAHs (μg/mL)	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Ctr
1-Methylnaphthalene (NAP) LMW	0.1371	0.2092	0.1576	0.1809	0.1024	0.1298	ND
2-Methylnaphthalene (NAP) LMW	0.1242	0.3312	0.1426	0.1636	0.0929	0.1176	ND
Acenaphthylene (ACY) LWW	0.004	0.0031	ND	0.0028	0.0061	0.0028	ND
Acenaphthene Acenaphthene (ACE) LWW	0.0004	0.0008	0.0001	0.0001	0.0002	0.0002	ND
Fluorene (FLU) LWW	0.0024	0.0048	0.0017	0.0036	ND	0.0029	ND
Phenanthrene (PHEN) LWW	0.0005	0.0016	0.004	ND	0.0012	0.0006	ND
Anthracene (ANT) LWW	0.0003	0.0211	0.0024	ND	0.0007	0.0004	ND
Fluoranthene (FLTH) HWW	ND	ND	ND	ND	ND	ND	ND
Pyrene (PYR) HWW	ND	ND	ND	ND	ND	ND	ND
Benzo(a)anthracene (B[a]A) HWW	0.002	ND	0.0067	ND	0.001	ND	ND
Chrysene (CHRY) HWW	ND	ND	0.0001	ND	0.0001	ND	ND
Benzo(b)fluoranthene (B[b]F) HWW	ND	0.0033	0.0022	ND	ND	ND	ND
Benzo(a)pyrene (B[a]P) HWW	ND	ND	ND	ND	0	ND	ND
Benzo(k)fluoranthene (B[k]F) HWW	ND	0.0008	ND	0.0011	ND	0.0001	ND
Indeno(1,2,3-cd)pyrene (INP) HWW	ND	ND	0.0004	ND	ND	0.0008	ND
Dibenz(a,h)anthracene (DBA) HWW	0.0008	0.003	ND	0.0019	ND	0.0028	ND
Benzo(g,h,i)perylene (B[ghi]P) HWW	ND	ND	ND	ND	ND	ND	ND

μg/mL- microgram per kilogram, ND- not detected, Ctr- control, LMW : Low molecular weight ; HMW : High molecular weight

The highest concentration of the PHA was phenanthrene and its most abundant in Plot 2 (584.03 $mgkg^{-1}$) while Plot 6 had the highest concentration of acenaphthylene (172 mg/m) and Benzo(b)fluoranthene (172 $mgkg^{-1}$) and dibenz(a,h)anthracene (184 $mgkg^{-1}$).

The Concentrations of Priority PAHs in Water Samples

The concentrations (µg/mL) of the priority PAHs in water samples from the Plots are presented in Table 4. Acenaphthene 1-methylnaphthalene, 2-methylnaphthalene, were present in all the Plots. Plot 2 had the highest concentration of 2-Methylnaphthalene 0.3312 µg/mL Benzo(a)pyrene, pyrene, benzo(g,h,i) and perylene, and fluoranthene were not found in all the experimental Plots.

PAH Degradation by*Bacillus thuringiensis* M43

Figure 4d and ST7 show the outcomes of biodegradation of priority PAHs by *B. thuringiensis* M43. The low molecular weight (LMW) PAHs were utilized by *B. thuringiensis* more than the high molecular weight (HMW) PAHs. BgP, DBA, NAP and InP were completely mineralized. The PAHs that *B. thuringiensis* used the least in the medium were CHR (2.50%), BbF (0.50%), and BkF (43.00%). On the PAHs priority pollutants, *B. thuringiensis* had 65.75%.utulization. The chromatograms are shown in ST 9.

PAH Degradation by*Pseudomonas aeruginosa* PA14

The 16 priority PAHs pollutants were well used by *P. aeruginosa* strain PA14 (Fig. 4c, ST6). The isolate mineralized 4 of the PHAs (FLA, BgP, InP, and DBA). The least PAH utilized were NAP (70.50%) and CHR (71.00%) for LMW and HMW PAHs. *P. aeruginosa* demonstrated reasonably considerable attraction to both LMW and HMW PAHs. For *P. aeruginosa* strain PA14, the overall degradation rate was 84.80%. The chromatograms are shown in ST 10.

PAH Degradation by*Aspergillus niger* ATCC 1015

Four of the 16 priority PAHs, including benzo(g,h,I,)perylene, dibenz(a,h)anthracene, naphthalene and Indeno(1,2,3,-cd)pyrene were completely metabolized by the *A. niger* strain ATCC 1015, according to the results of the GC-MS analysis (Fig. 4a, ST4). Fluorene was the least utilized LMW PAH (68%) while Chrysene was the least utilized HMW PAH (49.50%). After 21 days, *A. niger* had broken down 72.11% of the PAHs The chromatograms are presented in ST 11.

PAH Degradation by*Penicillium notatum* P72-30

Figure 4b and ST5 provide the GC-MS findings on the mineralization of the priority PAHs by *P. notatum* P72-30. LMW PAHs were used by the organism more than HMW PAHs. FLA, DBA, and InP were completely mineralized in the medium. NAP was least utilized among LMW PAHs (39.00%), whereas BaP was least utilized among HMW PAHs (22.00%). *P. notatum* used all of the PAHs in the medium to varied degrees and exhibited a 63.65% overall degradation rate. The chromatograms are shown in ST 12.

Fig. 4 Retention time (min), residual concentration (µg/ml) and degradation percent of USEPA priority PAHs by by *Aspergillus niger* strain ATCC 1015 (**a**), *Penicillium notatum* strain P72-30 (**b**), *Pseudomonas aeruginosa* strain PA14 (**c**), and *Bacillus thuringienis* strain M43 (**d**). Strains and 100% degrded PAHs fractions were as follows – ATCC 1015 (NAP, DAHA, IND, BENGHIP), P72-30 (FLUA, DAHA, IND, BENGHIP), PA14 (BTM, BENAA, BENK, DAHA, IND, BENGHIP), and M43 (BTM, NAP, DAHA, IND, BENGHIP). [BTM (Benzene,1,2,3trimethyl); NAP (Naphthalene); 2MN (2-Methylnaphthalene); ACNY (Acenaphthylene); ACNE (Acenaphthene); FLU (Fluorene); ANT (Anthracene); PHEN (Phenanthrene); FLUA (Fluoranthene); PYR (Pyrene); BENAA (Benz(a)anthracene); CHR (Chrysene); BENB (Benzo(b) Fluoranthene); BENK (Benzo(k) Fluoranthene); BENAP (Benzo(a) Pyrene); DAHA (Dibenz(a,h) Anthracene); IND (Indeno (1,2,3-cd) pyrene); BENGHIP (Benzo (g, h, i) perylene)]

Bacterial and Fungal Genes for PAH Degradation

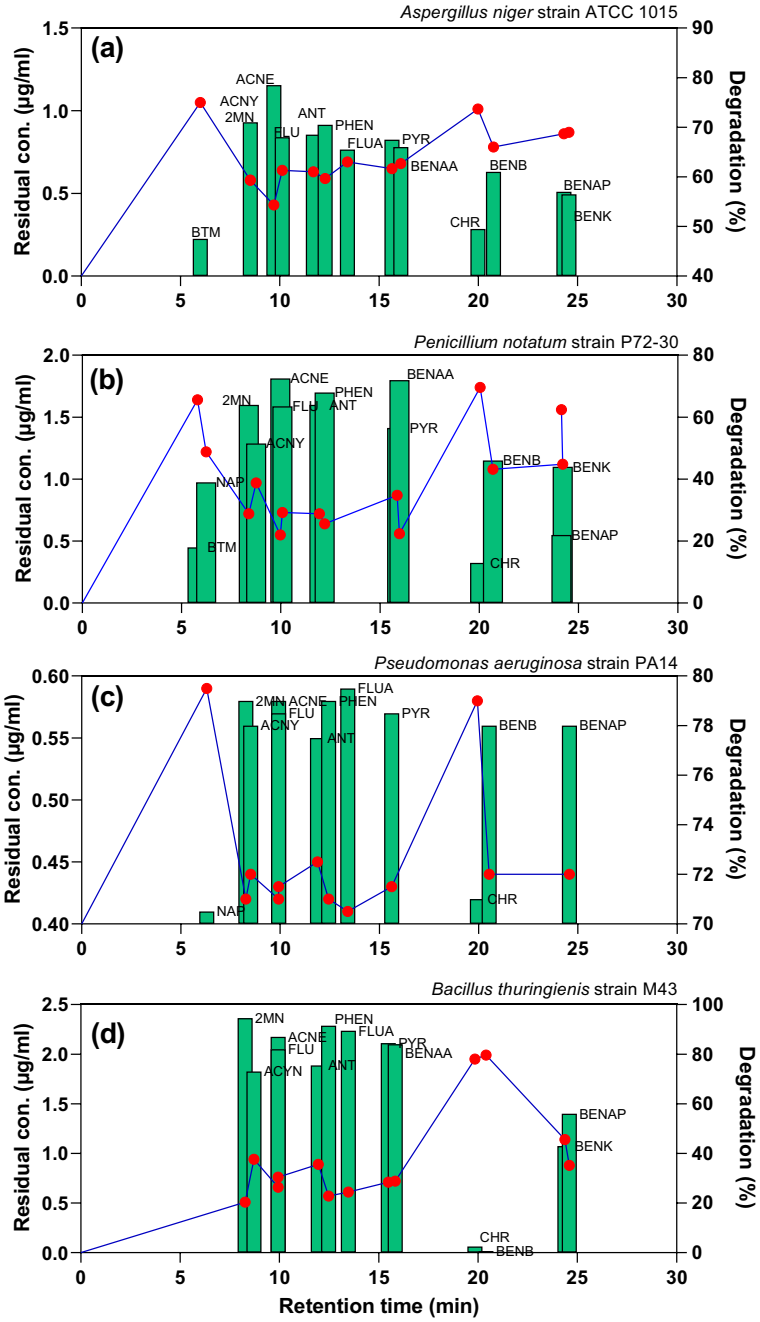
The gene for PAH degradation detected in the bacterial and fungal isolates and their accession numbers are presented in Table 5. Only one of the two primers used was able to amplify each of the two bacterial isolates. The gene sequence of *P. aeruginosa* PA14 was amplified by pahE2, whereas a portion of the gene sequence of *B. thuringiensis* strain M43 was amplified at 580 bp by pahE1. The gene sequences of both fungal isolates (*P. notatum* strain P72-30 and *A. niger* ATCC 1015) were amplified using only the pahE1. At 580 bp, all of the isolates were amplified.

Discussion

Microorganisms were isolated from petroleum hydrocarbon-contaminated soil and water in and around Suleja PPMC depots and screened for their ability to mineralise PAHs. The highest microbial count was found from the genus *Bacillus*, which also contains the species *B. subtilis, B. thuringiensis*, and *B. licheniformis*. Additionally, Bacilli and *P. aeruginosa* were found in all the experimental Plots. The findings by Osarumwense et al. [38] that *Bacillus* and *Pseudomonas* effectively break down hydrocarbons by creating surfactants and lipase and other enzymes are supported by the dominance of these bacteria in the present study. Agu et al. [39]. also reported that *Bacillus* sp. has the capacity to produce spores, which enable microorganisms to tolerate the hazardous effects of petroleum pollutants. The findings of the investigation into the 16 prioritized PAH in the soil from the Plots revealed that the Plots were contaminated and polluted by PAH pollutants at varying quantities. The presence of LMW PAH, HMW PAH, and carcinogenic PAHs in the Plots is of grave public health concern.

All the samples contained phenanthrene and 2-methylnaphthalene (both are LMW PAH) in concentrations ranging from 324 g/kg to 584 g/kg and 8 g/kg to 36 g/kg, respectively. According to National Toxicology Program [40], naphtha is a potential human carcinogen and a respiratory toxicant. Naphthalene and its isomers are known to generate olfactory tumours in rats [40], and to be extremely toxic to pulmonary tissues in a number of different organisms [41]. Petroleum product spills and incomplete combustion could be the cause of the high concentration of NAP and PHE in the Plots [42]. Maje - Plot 2, a neighborhood well known for its long lines of fuel trucks, mechanical shops, and black market (illegal) gas stations, has the greatest concentration of PAH.

Both carcinogenic and non-carcinogenic PAHs became apparent when the HMW PAHs (PYR, BbF, CHR, InP, DBA, and BgP) were identified. The largest concentrations of HMW PAHs were found in DBA and BbF. Ellanhom et al. [43] assert that BbF and DBA are dangerous pollutants connected to human cancer. Despite the fact that the total



PAH concentrations in this study fell below the 1000 g/kg, 1500 g/kg, and 5 mg/kg recommended levels from guidelines for cleanups in Denmark, the Netherlands, and Australia, respectively [44, 45], the values obtained showed a slight to moderate level of pollution of the Plots by these pollutants and demand for continual monitoring of the study area.

Table 5 PAH-degrading gene detected in bacterial and fungal isolates

Isolate name	Strain	PAH degrading gene		GenBank accession No
		pahE1	*pahE2*	
Bacillus thuringiensis	M43	+	–	KY307913.1
Pseudomonas aeruginosa	PA14	–	+	KY963575.1
Penicillium notatum	P72-30	+	–	ATCC16834
Aspergillus niger	ATCC 1015	+	–	KJ439163.1

+ sign indicates the PCR product was detected, – sign indicates PCR product was not detected

While Wang et al. [32]. reported a very high PAH concentration > 13,000 g/kg in the petroleum-contaminated area of Loess Plateau, China, Muze et al. [46] indicated a lower PAH concentration in auto-mechanic workshops. Studies by Ogoko et al. [47] and Adeyi and Oyeleke [48] also noted soil PAH concentrations exceeding allowable limits.

According to this study, 12 of the USEPA's 16 prioritized PAH contaminants were found in the water samples, whereas 4 of the contaminants were not. The health of the local community is seriously at risk due to the presence of HMW PAH (BaA, CHR, BbF, DBA BkF, and InP) in the water samples. These HMW PAHs have a number of hazardous qualities that can be toxic to humans and other organisms, including the ability to cause cancer and mutation. Some of the inhabitants of these Plots use PAH-contaminated water for agricultural and domestic purposes. In the body fat, PAHs have the potential to deposit in a number of tissues where they exert their effects. According to Ibe et al. [49]., anthropogenic activities in the study Plots were mostly responsible for the occurrence of PAHs. These Plots contain a number of petroleum product sales stations, which have been a constant source of oil spills onto the Plots.

The PAH having the highest concentration was Benz(a)anthracene, which is known to be highly hazardous, mutagenic, and/or carcinogenic and rapidly absorbs from the GIT [50]. According to Bjorseth [51], the LMW PAHs discovered in this investigation are likely human carcinogens. They have significant harmful effects on humans and microbes, including genotoxicity, inflammation of the skin, teratogenic characteristics, kidney and liver dysfunctioning, as well as gastrointestinal distress [50].

The PAH concentrations in the water obtained for the current study is up to 0.3312 g/L in the Plots. Methyl naphthalene concentrations ranged from 0.1209 to 0.209 g/L, with an average level of 0.1520 g/L, which is below the permissible limit of 0.2 g/L [36]. The concentration found in Plot 2 (0.2092 g/L), however, was above the acceptable boundaries, indicating that the water is contaminated and unfit for human consumption. The average level of 2-methylnaphthalene (0.3091 g/L) was much higher than the allowable limit and hence important for public health.

Naphthalene (LMW PAH, C10H8) exhibits substantial acute toxicity to microorganisms despite not being a human carcinogen [52, 53]. Although the mean concentrations of the PAHs under investigation were below the allowable limits, their occurrence in the Plots remains a public health concern. Similar PAH values to those found in the current study were found in Betem subterranean water by Ideriah and Nwinaa-ie [54(2015), however Edokpayi et al. [54] found greater PAH concentrations in rivers and effluents (13.174–26.382 mg/L). Additionally, according to Adeniyi et al. [6], the PAH concentration in South Africa's Buffalo River Estuary ranged from 14.91 to 206.00 g/L.

The microbes that were investigated in this study were able to use hydrocarbons at different rates. This concurs with the findings of Ikuesan [55]. In this investigation, *Penicillium* and *Aspergillus* showed a high capacity for degradation. By producing hydrocarbon-degrading enzymes, tolerating the toxic effects of hydrocarbons, and having an adaptive physiology that can tolerate harsh environments, microorganisms may be able to use hydrocarbons.

This outcome is consistent with reports from Chukwura et al. [56], which also noted some fungal isolates from mechanics shops had substantial degrading capacities. According to Ikuesan [55], the differences in the rates of degradation relate to the inherent capabilities of the isolates. It might also be because the organisms have biosurfactants or enzymes that can be used for degradation or the presence of certain petroleum compounds that the microbes can use as energy and carbon sources [57, 58].

The presence of PAHs in the environment is a major cause for concern, especially when their concentrations are above the permitted limits. White rot fungus, *Ganoderma lucidum* strain CCG1, *Alcaligenes denitrificans* and species of *Rhodococcus*, *Mycobacterium*, and *Pseudomonas*, are only a few of the bacteria and fungi that have been identified as using PAHs [59]. Numerous bacterial and fungal species have the ability to completely mineralize specific PAHs into intermediate products of metabolism like CO_2 and water [60]. However, the findings from this current study that some microbial species can break down these resistant and deadly pollutants (PAHs) are worthy of noting and further research. In the current study, *P. aeruginosa* PA14 had the highest total PAHs degradation (84.80%) potential, followed by *A. niger* ATCC 1015 (72.11%), *B. thuringiensis* M43 (65.75%), and *P. notatum* P72-30 (63.77%). These isolated organisms demonstrated that they can be developed for the degradation of PAH-contaminated environments.

Naphthalene was the PAH mostly degraded by *B. thuringiensis* M43, *A. niger* ATCC 1015, *P. aeruginosa* PA14 and *P. notatum* strain P72-30 in descending order. Ijah and Ukpe [61], Ijah and Ndana [62] and Ijah and Antai [63] have also reported the biodegradation efficiency of *Bacillus* species in petroleum hydrocarbon polluted environment. Pathak et al. [64]. stated that *Pseudomonas* sp. HOB1 is a potent isolate for degrading elevated concentrations of naphthalene.

P. aeruginosa PA14 had the highest percentage of anthracene (ANT) utilization among the four isolates (77.5%), whereas *P. notatum* P72-30 had the lowest (64.0%). The capacity of the isolates to use ANT may be due to their ability to create an enzyme that can convert ANT to anthraquinone, which can then be converted to dihydroxy-anthraquinone, which is less toxic than the parent molecule [65]. In a liquid culture of *Phanerochaete chrysosporium* isolated from an oil-contaminated soil, Brodkorb and Legge [66] found a reduced percentage (37.7%) of ANT after mineralization.

InP and DBA were totally mineralized by each and every microbial strain in the media. All the 4 isolates, with the exception of *B. thuringiensis* M43, metabolized BgP. The least amount (56.5%) of perylene was degraded by *P. notatum* strain P72-30. This might have something to do with the tolerability of the PAH toxicity in the medium by the isolates. The microbial isolates least degraded CHR (34%), BbF (46.37%), and BaP (53.12%). Kotterman et al. [67]. reported that white rot fungi completely degraded benzo(a)pyrene. PAHs, CHR, BbF, and BaP have strong resistance to biodegradation by the 4 isolates.

The PahE1 and PahE2 genes have been reported to be involved in PAH degradation [68, 69]. The PahE1 primer was able to amplify the two fungal isolates, but not the PahE2 primer. This revealed that the PahE2 was missing from the organisms. When compared to PahE1 primer, which amplified only the *B. thuringiensis* M43 gene, PahE2 primer

amplified only the *P. aeruginosa* PA14 gene, demonstrating that the sequence coding for the PAH degrading gene, PAHE1, can be found in the organism.

Conclusion

The study found that samples of soil and water in the community around PPMC depot, Suleja Nigeria were contaminated with PAHs at varied concentrations, with a higher concentration of LMW-PAH, indicating recent contamination from primarily human sources. According to the findings of this study, the four isolates *B. thuringiensis* M43, *P. aeruginosa* PA14, *P. notatum* P72-30, and *A. niger* ATCC 1015 mineralized PAH as their only carbon or energy source and each had either of the PAH-degrading genes (*pahE1* and *pahE2*) in their genomes.

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Data Availability All the data pertaining to this manuscript has been included in the manuscript.

Declarations

Ethical Approval This article does not contain any studies with human participants or animals.

Consent to Participate All authors agree mutually with the participation and publication of this work and declare that this is original research.

Consent to Publish All authors agree mutually to publication of this work.

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