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Article

Hydrocarbon Load and The Occurrence of Diverse Species of Pseudomonads in Ibeno Wetland Soil, Southern Nigeria

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Abstract: The degradation of hydrocarbon by indigenous biosurfactant-producing species of pseudomonas stands out as a natural remediation phenomenon in the Ibeno coastal soils. Standard microbiological and biotechnological methods were employed to investigate the bioremediation of hydrocarbon in Ibeno coastal soil using an indigenous strain of biosurfactant-producing Pseudomonas aeruginosa. The Physicochemical analysis of the soil included the determination of pH, total organic carbon available phosphorus, total organic nitrogen exchangeable cations, exchangeable acidity, particle size, total petroleum hydrocarbon petroleum aromatic hydrocarbon load, availability of heavy metals, density of heterotrophic bacteria and hydrocarbon utilizing bacteria. The study revealed a slightly acidic soil that is predominantly sandy (with a mean sand fraction of 87.47%, followed by 9.81% clay and 2.2% silt) but, poor in nitrogen and phosphorus contents. However, the results of the microbial analysis showed that the Ibeno coastal soil has a high heterotrophic status with a mean total heterotrophic bacterial (THB) count of 7.9 x104 CFU/g with only 1.9 x102CFU/g (0.024 %) of the heterotrophs (HUB) endowed with the ability to utilize hydrocarbon. Pseudomonads were also detected and their densities in the coastal soil ranged from 5.6 x 103CFU/g to 6.0 x 103 CFU/g. These included; Pseudomonas cepacia, P. putida, S. maltophila, P. aeruginosa, P. flourescens, P. diminuta and P. stutzeni. Amongst the isolates, P. aeruginosa and P. flourescens with percentage occurrence rates of 100% and 75% respectively, were the major occurring pseudomonas species isolated from Ibeno coastal soil. The available Pseudomonads augment the Ibeno coastal soil by the production of biosurfactants.

Keywords: Hydrocarbon load, diverse species, pseudomonads, Ibeno Wetland soil, Southern Nigeria

Introduction

The rate of environmental degradation in the global south is alarming, especially, arising from increase in population and the production and use of fossil fuels [1][2]. In the Niger Delta region and some other African States, oil exploration and use threaten the health of the environment and affects creatures including humans[3][4]. Ogoniland is one of the many communities in Nigeria that is heavily polluted by petroleum exploration[5][6]. The environmental assessment of Ogoniland by the United Nations Environment Programme (UNEP) in August 2011 provides a picture of the exploration impact and the urgent need for cleanup and restoration of the polluted environment[7]. Issues like oil spill is commonplace and its involves the release of petroleum hydrocarbon to the environment; through various ways including drilling rigs, offshore platforms, and pipeline vandalization[8][9].

Crude oil is observed as the most important source of energy worldwide[10]. Ibanichuka[11] and Onuoha[12] reported that the routine extraction and drilling of fossil energy resources cause serious environmental problems in Nigeria. Crude oil and refined fuel spills have been observed to damage natural ecosystems in France, Alaska, the Galapagos Islands, the Gulf of Mexico, the Niger Delta region in Nigeria, and many other places worldwide[13][14]. The quantity of oil spilled during accidental spills ranges from a few hundred tons to several hundred thousand tons [e.g., Deepwater Horizon Oil Spill, Atlantic Empress, and Amoco Cadiz][15][16]. Because of the Niger Delta Region's remoteness, which prevents emergent environmental response, Spills have been shown to have a significant influence on ecosystems[17]. The effects of oil contamination are many; including damage to plant and animal lives. A variety of chemicals found in crude oil is highly hazardous to both human health and the environment due to their cytotoxic, mutagenic, and carcinogenic properties[18].

Materials and Method

Collection of Soil Sample

The soil samples for the study were obtained from Ukpenekang, within the Ibeno wetland of Nigeria. Like other coastal areas located in the region, the Ibeno wetlands soil is under the constant influence of petroleum exploration and exploitative activities. Qua Iboe Light crude oil is produced from numerous offshore fields in the Bight of Bonny, which is brought to the shore through a seabed pipeline system to the Qua Iboe Terminal. The terminal (4°20′N, 7°59′E) is located on the eastern side of the wetland soil and contains nine crude oil storage tanks, with a total capacity of 4.5 million barrels (m bbls)[19]. A large quantity of the wetland soil was transferred to the Experimental Garden of the Department of Microbiology, University of Uyo for investigation.

Determination of Physicochemical Properties

Samples were analyzed for pH, total organic carbon (TOC), available phosphorous, total nitrogen (TON), total salinity, nutritive salts, particle sizes, and exchangeable cations.

Determination of Total Petroleum Hydrocarbon (TPH) in Test Samples

The sample, immediately after collection was treated with 5.0ml of 50% of H_2SO_4 to stop hydrocarbon breakdown. Petroleum hydrocarbon was extracted with dichloromethane (5 x 10ml) by liquid-liquid extraction in duplicate samples. Before extraction, a-androstane was added to each flask as an internal standard. The extracts were dried over Na_2SO_4, concentrated in a vacuum rotary evaporator, dried under a gentle nitrogen stream, and further treated as follows:

(a) Separation Procedure

All extracts were fractioned accordingly; each extract was re-suspended in 1ml of dichloromethane and loaded into a glass column (30 x 1 cm i.d) and filled with 10g each of 50% water-deactivated alumina (70-230 mesh, merck). Total petroleum hydrocarbon fraction (TPH) was obtained by eluting with 100ml of dichloromethane.

(b) GC – FID Analysis

Saturated compounds of TPH fractions were verified by Gas chromatography equipped with a flame ionization detector (GC-FID). A Gas Chromatography Termoquest Trace 2000 was used. Compounds were separated on a capillary column DB5 (25m by 0.32mm [i.d.] 0.25-um film thickness). The column temperature was held at 350C for 2 minutes and then programmed up to 310C at a rate of 40C min-1. This final temperature was held at 350C for 2 min. The detector and inlet temperature were

set at 3200C and 2900C respectively. The Helium flow was 1.1 ml min-1 and the injection volume was 1μL.

Determination of PAH Load in Test Soil Samples

The PAHs in the samples were extracted and fractioned as described. Fifty (50ml) of the sample was measured and spiked with a pre-deuterated PAH cocktail as an internal standard (naphthalene-d8, acenaphthylene-d8, anthracene-d10, acenaphthene-d10, fluoranthene-d10, phenanthrene-d10, florine-d10, pyrene-d10, chrysene-d12, benzo [a] anthracene-d12, beno [ghi] perylene-d12 dibenzo[a,h] anthracened14, and indenol [1,2,3-ed] pyrene-d12, (ES2528, promo chem, Wesel, Germany) and extracted with dichloromethane using temperature programmed Soxhlet extractor at 65°C for 24 hours. The extracts were column-packed with 30g of alumina deactivated with 4.5% water. PAHs were eluted with 50ml of hexane/dichloromethane (95/5%, v/v) and the polar fractions were eluted dichloromethane. The PAH fractions were concentrated by rotary evaporation. Before GC/MS analysis, fractions were dried under nitrogen and re-dissolved in dichloromethane.

GC/MS Analysis and Qualification of PAH

A gas chromatograph (GC, Hewlett-Packard HP 6890 series) coupled to a mass spectrometer (MS, model 5971, Hewlett-Packard) was used to quantify extractable organic PAHs. Aliquots of each sample were injected using a 30:1 split ratio onto 30m, 0.25mm inner diameter, Hp-MS 5% phenyl methyl siloxane capillary column. The operational conditions were as follows: 40-2800C at 60C/min; injector temperature of 3000C, scan range 40-500 amu; scan rate, 1.53 scans/s; and source temperature of 3200C. Helium was used as the carrier gas (at 1.5mL/min). The PAHs were determined in selective ion-monitoring mode with an ionization energy of 70ev. The m/z peaks corresponding to the molecular masses of individual PAH were used for identification and qualification. Concentrations of PAHs were calculated relative to the pre-deuterated internal standard.

Microbiological Analysis of Test Soil

Determination of Count of Density of Pseudomonads

Use of Pseudomonad selective Agar Base: A solid culture medium for selective isolation of Pseudomonas aeruginosa and others was carried out according to the Pharmacopecal Harmonized method and 15022717 Standards. Glycerol Additive, Cetremide – Nalidixic (CP) selective Agar (01-609) selective solid medium for the detection of Pseudomonas aeruginosa and others according to the EN12780 and 15016266 Standards.

Determination of Counts of Heterotrophic Bacteria in Test Soil

Microbial load in soil samples was determined after the classical ten-fold decimal dilutions method[20], using the pour plate and spread plate techniques. The Bacto nutrient agar was employed for the enumeration of heterotrophic bacteria.

Determination of Density of Hydrocarbon Utilizing Bacteria in the Test Soil and Screening for Degradability of the Isolates

The vapor phase transfer method described by Okpokwasili, Amanchukwu[21] was adopted. The growth medium was an un-supplemented minimal Medium (MM) containing Agar as the solidifying agent and the carbon and energy sources were severed from sterile Whatman filter paper soaked in crude petroleum, supplied in the lid of the inoculated plate on a yeast plate containing streptomycin and Chloramphenicol at 50gl-1. The plates were seeded with inoculum from the enrichment culture using the spread plate technique. The culture plates were incubated at room temperature for 14 days. Colonies that formed on the plates were picked as crude-oil-degrading bacteria, and purified before characterization.

Screening of Crude and PAH Degrading Potentials of Bacterial Isolates

Crude oil utilizing the potentials of the bacteria isolates was determined using the hydrocarbon overlay method. Fifteen (15) g of agar-agar was added to Mineral Salt Medium (same composition as enrichment medium), sterilized, and allowed to set. The solidified plates were overlaid with 1% (v/v) sterile crude oil, and allowed to set for about 15 to 30 minutes. Then , the test isolates were streaked on the surface of the plates.

Screening for bacteria with PAH utilizing potentials was carried out using a modified overlay method adopted for isolating crude oil utilizing bacteria. After sterilizing the medium, it was dispensed

into Petri dishes (15 ml) and allowed to set. Naphthalene crystals (0.5g) were dissolved in 10 ml acetone and used to coat the surface of the solidified agar plates. The same procedure was carried out for Anthracene. The coated plates were subsequently kept for 24 hours for the carrier solvent to volatilize. The test isolates were then inoculated onto the plate using the streaking method. All inoculated plates were incubated at room temperature for 5-15 days with periodic observation. Colonies that eventually developed showed areas of clearing; and were selected and rated. The utilization was rated based on the diameter and luxurious nature of the developed colonies, i.e., '+', '++', or '+++' indicating the magnitude of potentials.

Maintenance of Pure Bacterial Isolates

Discrete colonies from incubated plates were picked with the aid of a sterile wire loop and subcultured on freshly prepared NA using the streak plate technique. The process of subculturing ensures that pure cultures are obtained. Pure bacterial colonies were stocked on Nutrient Agar slants and preserved in a refrigerator at 40C for characterization and identification.

Characterization and Identification of Bacterial Isolates

Stock cultures were aseptically picked and subcultured on freshly prepared NA plates. Bacterial isolates were characterized and identified presumptively based on their morphological, cultural, physiological attribute, microscopic examination, and biochemical characteristics using the method described by Kurnianto[22]. The tests adopted included Gram's staining, spore stain, flagella stain, coagulase, oxidase, catalase, urease, indole, citrate, methyl red, Voges-Proskauer, starch hydrolysis, sugar fermentation, nitrate reduction, and hydrogen sulphide production tests.

Hydrocarbon Remediation Studies

Precisely 4 kg of the coastal soil was carefully and separately placed in 2ft x 2ft twelve wooden boxes. The soil samples were simulated (contaminated) with 20.34 mg/kg, 24.23 mg/kg, and 32.85 mg/kg of hydrocarbon derived from 5%, 10%, and 20 % of Bonny Light crude oil respectively, and allowed to mimic natural crude oil degradation for 48 hours. Thereafter the activities of indigenous soil microorganisms in the contaminated soils were augmented with graded doses; 3.9 x 104 CFU/15ml, 5.2 x 104 CFU/20ml, and 6.5 x 104 CFU/25ml of 24-hour-old batch culture of biosurfactant producing strain of Pseudomonas aeruginosa representing a viable cells count of 2.6 x 103 CFU/ml and exposed for remediation within 12 weeks (84 days).

Heterotrophic Status and Hydrocarbon Attenuating Potential of Microbes in Bio-augmented Soil

The effects of bioaugmentation with the biosurfactant-producing strain of Pseudomonas aeruginosa on the heterotrophic status and crude oil degradability of the indigenous microbial population were determined. The total heterotrophic bacteria (THB) and hydrocarbon utilizing bacterial (HUB) counts were determined by the pour plate and vapor phase transfer methods respectively as described by Chikere[23].

Result and Discussion

Diverse Species of Pseudomonads Isolated from Ibeno Coastal Soil

The morphological and biochemical characteristics of Pseudomonads isolated from Ibeno coastal soil (Table 2). The isolates included: *P. cepacia, P. putida, S. maltophila, P. aeruginosa, P. flourescens, P. diminuta* and *P. stutzeni.* Amongst the isolates *P. aeruginosa* and *P. flourescens* with percentage occurrence rates of 100% and 75% respectively; were the most common pseudomonads encountered in the coastal soil (Table 2).

Table 1: Occurrence rate of the Pseudomonads in Ibeno coastal soil

Isolate	Location 1	Location 2	Location 3	Location 4	Occurrence Rate (%)
P. cepacia	-	-	-	+	25
P. putida	+	-	+	-	50
S. maltophila	-	+	-	-	25
P. aeruginosa	+	+	+	+	100
P. flourescens	+	+		+	75

P.diminuta	-	+	-	+	50
P.stutzeni	-	+	-	-	25

Table 2: Morphological and biochemical characteristics of Pseudomonads isolated
Key: R – Rod, Ao – Present, Oo – Absent, MM – Multitrichous, Monotrichous, P - Polar

ISO. NO	CELL SHAPE	GRAM STAIN	CATALASE	COAGULASE	MR TEST	MOTILITY	URASE TEST	OXIDASE TEST	PHOSPHOTASE	ARGINASE	SPORE TEST	GLUCOSE	LACTOSE	MALTOSE	MALITOLE	ZYLOSE	H_2S test	Citrate test	Voges Pros test	ORGANISMS
1	R	-	+	-	-	M M, P	+	+	-	-	-	A	A	A	A	A				*Pseudo monas cepacia*
2	R	-	+	-	-	M M,	+	+	-	+	-	A	O	O	O	A				*Pseudo monas*
3	R	-	+	-	-	M M, P	-	+	+	-	-	A	A	A	O	O				*S. maltophi la*
4	R	-	+	-	-	M, P	+	+	+	+	-	A	O	O	A	A				*Pseudo monas aerugino sa*
5	R	-	+	-		M, P	-	+	-	-	-	O	O	O	O	O				*P.dimin uta*
6	R	-	-	-	-	M, P	+	+	+	+	-	A	O	A	A	O				*P. flouresce ns*
7	R	-	-	-	-	M, P	-	+	+	-	-	A	O	A	A	A				*P.stutze ni*

Table 3: Physicochemical properties, TPH, and PAH Load of the Ibeno coastal soil

Parameter	Unit	Sample 1	Sample 2	Sample 3	Mean
pH		6.5	6.7	6.6	6.6
Total Org. C	%	3.5	2.6	3.05	3.05
Total N	%	0.09	0.06	0.075	0.075
Avail. P	mg/kg	2.9	1.4	2.15	2.15
Particle Size:					
Sand	%	89.00	85.94	87.47	87.47
Silt	%	2.20	2.20	2.2	2.2
Clay	%	8.80	10.82	9.81	9.81
Exchangebale Base:					
Exch. Ca	cmol/kg	5.1	5.0	5.05	5.05
Exch. Mg	cmol/kg	2.1	2.0	2.05	2.05
Exch. Na	cmol/kg	0.06	0.06	0.06	0.06

Exch. K	cmol/kg	0.11	0.11	0.11	0.11
Exch. Acidity	cmol/kg	2.8	2.8	2.8	2.28
ECEC	cmol/kg	10.1	10.0	10.05	10.05
Hydrocarbon Load:					
TPH		*Composite*	*Sample*		4.142
PAHs		*Composite*	*Sample*		0.8959
Microbiological Properties					
THB	CFU/g	8.1×10^5	7.9×10^5	7.7×10^5	7.9×10^5
TP	CFU/g	6.0×10^3	5.8×10^3	5.6×10^3	5.8×10^3
HUB	CFU/g	2.1×10^2	1.9×10^2	1.7×10^2	1.9×10^2

Key: THB = Total heterotrophic bacterial count, HUB = Hydrocarbon utilizing bacteria count, TP = Pseudomonads count

Microbial Properties of Test Soil during Enhanced Remediation with *Pseudomonas aeruginosa*

The un-contaminated soil was characterized by heterotrophic bacteria which ranged from 5.6 $x10^4$ – 8.8 $x10^5$ CFU/ml and hydrocarbon-utilizing bacteria (2.8 $x10^1$ – 6.2 $x10^2$) CFU/ml. The results recorded that hydrocarbon contamination readily increased the density of hydrocarbon-utilizing bacteria in the coastal soil. This was concentration-dependent and occurred mostly at the beginning of the process (1-14 days). The densities of the indigenous population of heterotrophs in crude oil polluted soil were retarded by immediate exposure and the effect was also concentration dependent. The hydrocarbon reduced the doubling value of diminutive microbes (index of growth over time) in treated soil and the cell's recovery rate.

Bioaugmentation with biosurfactant-producing *Pseudomonas aeruginosa* raised heterotrophic bacteria over time leading to a general reduction in the activities of hydrocarbon-utilizing bacteria (HUB) in the contaminat soil. The effect of bioaugmentation on soil heterotrophism was determined by both the level of hydrocarbon contamination and loads of bio-surfactant-producing *Pseudomonas aeruginosa* added to the soil. At all levels of contamination, bio-augmentation with 6.5 $x10^4$ CFU/ml (25 ml of broth culture) of bio-surfactant-producing *Pseudomonas aeruginosa* gave the best recovery rates.

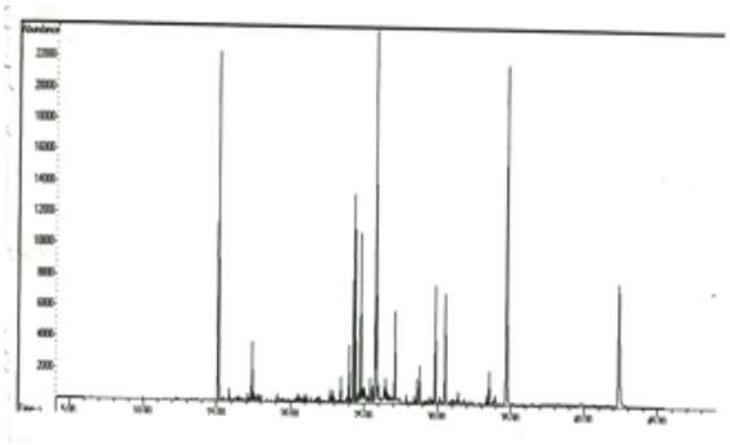

Figure 1: PAH (0 Day) Control

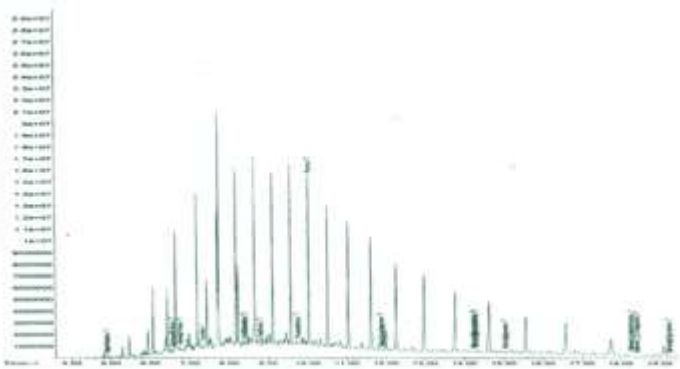

Figure 2: PAH (0 Day) 5% Contamination Level

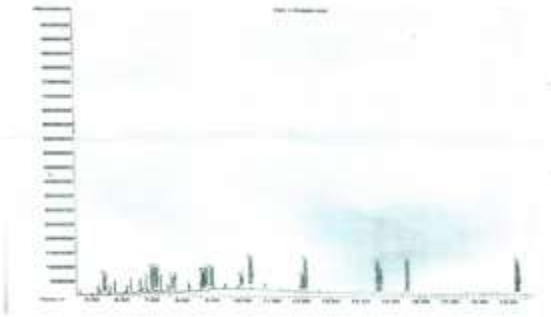

Figure 3: PAH (12th Week) 5% Contamination Level

Figure 4: PAH (0 Day) 10% Contamination Level

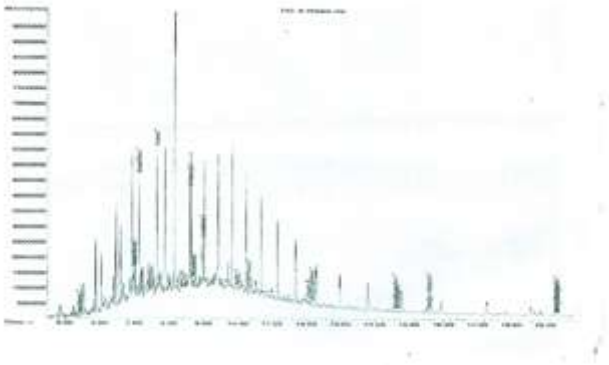

Figure 5: PAH (6th Week) 10% Contamination Level

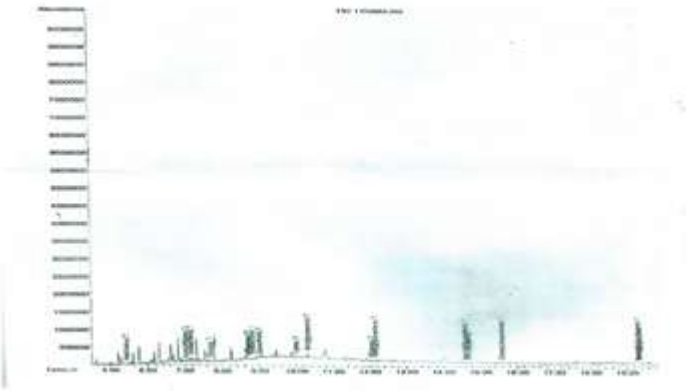

Figure 6: PAH (12th Week) 10% Contamination Level

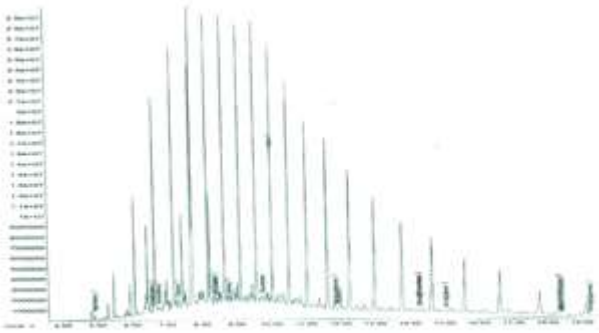

Figure 7: PAH (0 Day) 20% Contamination Level

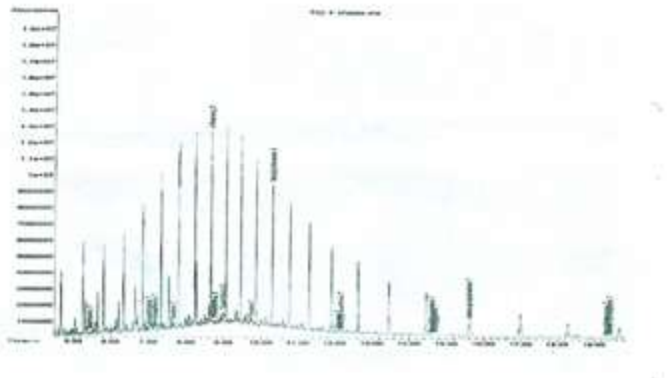

Figure 8: PAH (6th Week) 20% Contamination Level

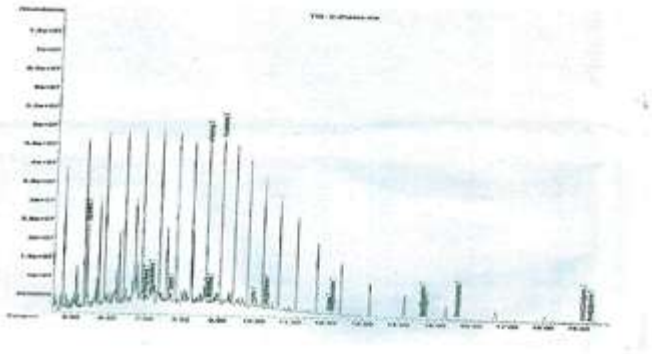

Figure 9: PAH (12th Week) 20% Contamination Level

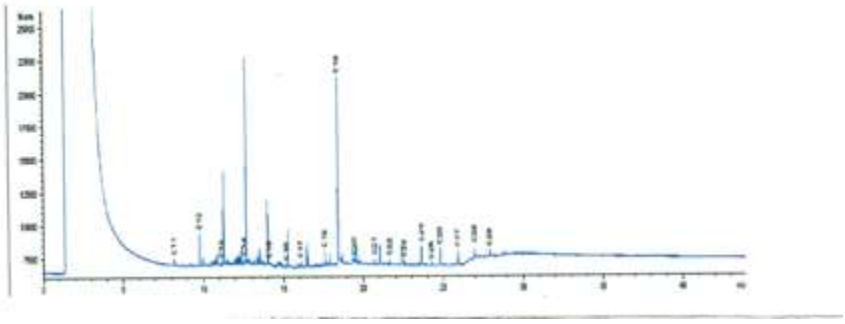

Figure 10: TPH (0 Day) Control

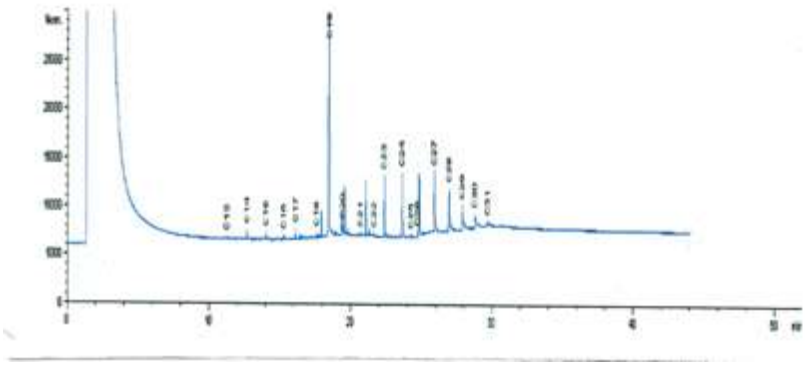

Figure 11: TPH (0 Day) 5% Contamination Level

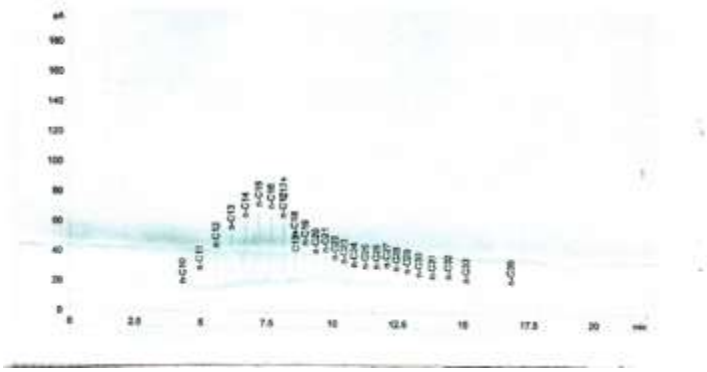

Figure 12: TPH (6th Week) 5% Contamination Level

Figure 13: TPH (12th Week) 5% Contamination Level

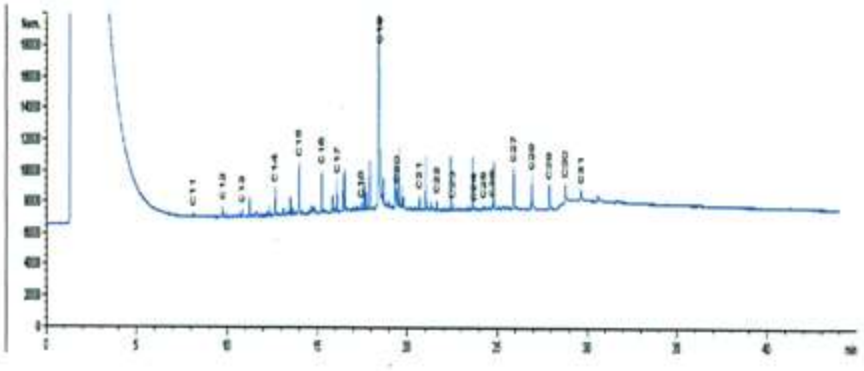

Figure 14: TPH (0 Day) 10% Contamination Level

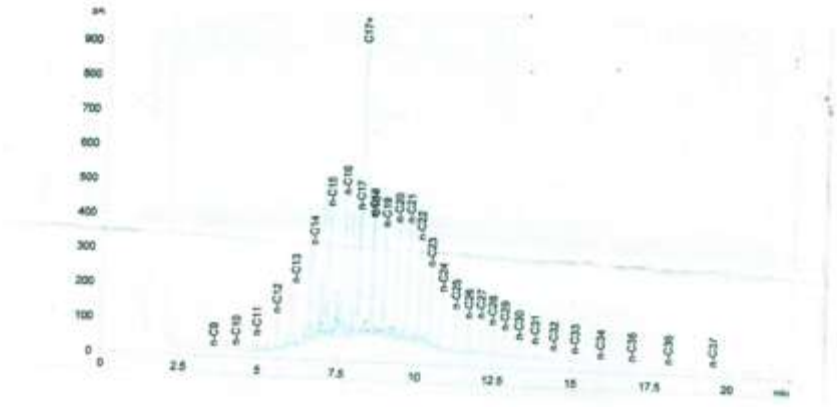

Figure 15: TPH (6th Week) 10% Contamination Level

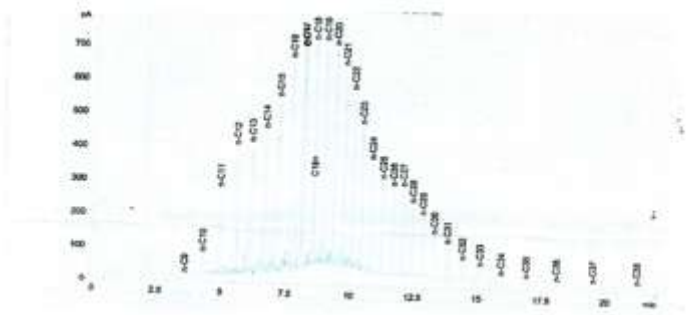

Figure 16: TPH (12th Week) 10% Contamination Level

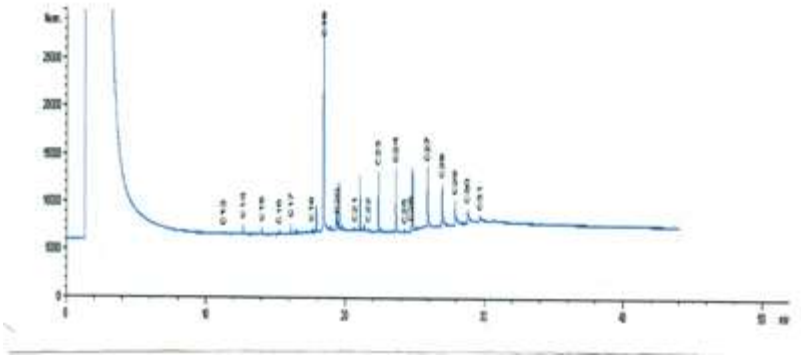

Figure 17: TPH (0 Day) 20% Contamination Level

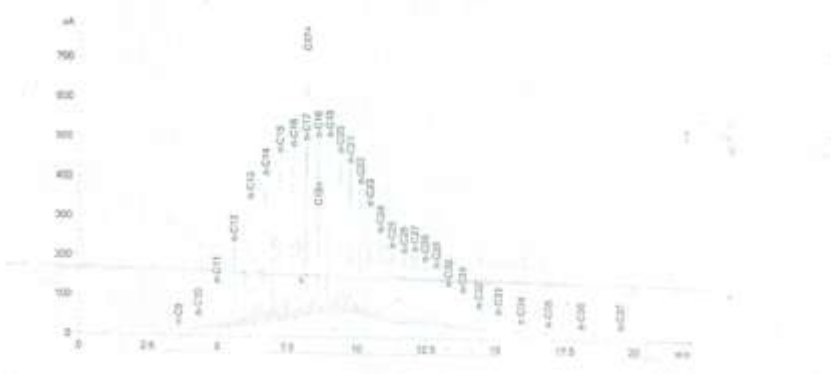

Figure 18: TPH (6th Week) 20% Contamination Level

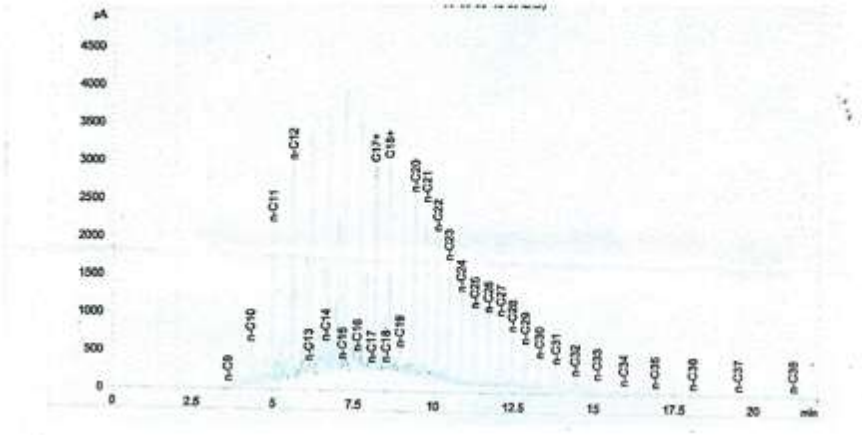

Figure 19: TPH (12th Week) 20% Contamination Level

Table 4: Remediation of Total Petroleum Hydrocarbon (mg/kg) in coastal soil augmented with 2.6 x 10^3 CFU/25ml of 24-hour-old batch culture of biosurfactant-producing strain of *Pseudomonas aeruginosa*

S/N	Parameter	Control	0-week			6-weeks			12 – weeks		
			5% cont.	10% cont.	20% cont.	5% cont.	10% cont.	20% cont.	5% cont.	10% cont.	20% cont.
1	$C_8 - C_{11}$	0	0	0	0	0	0	0	0	0	0
2	C_{12}	0	0	0	2.8635	0	0	1.0425	0	0	1.7845
3	C_{13}	0.1245	0	1.1245	1.1245	0	1.4525	1.7456	0	1.0657	1.0436
4	C_{14}	0.0531	0	0.8568	1.6531	0	0.0342	2.0032	0	1.1786	1.6032
5	C_{15}	0	0	0.7211	2.2305	0	1.4292	1.9832	0	0.6672	1.3098
6	C_{16}	0	0	0.2541	1.5644	0	0	1.6001	0	0	1.0301
7	C_{17}	0	0.8568	0.3564	2.2058	1.2068	1.6312	0.4332	0.7680	0.8590	0.7621
8	C_{18}	0.0101	0.5688	0.1245	1.5607	1.1088	1.3230	1.2490	1.2358	1.0589	0.5840
9	C_{19}	0	3.1225	4.0287	4.0248	1.4525	1.3478	1.3298	1.0035	0.9886	0.6908
10	C_{20}	0.2977	1.2562	1.1014	1.8977	0	0.3498	1.0485	0	0.5685	0.9546
11	C_{21}	0.3122	1.1265	1.1145	0.3122	1.1065	1.2294	1.4054	0	1.0067	1.0640
12	C_{22}	0.0427	0.0568	0.0247	1.3427	0.0068	0.0348	1.4587	0.0348	0.3458	1.0027
13	C_{23}	1.1048	1.5638	1.1244	1.1048	0.5638	0.9638	1.0583	0.2008	0.7885	0.8003
14	C_{24}	0.3407	1.4522	1.2501	2.3407	0	1.0200	1.3420	0	0.2330	0.7076
15	C_{25}	0.0254	1.9856	0.1425	0.0254	1.0856	1.2346	0.3565	0.9230	0.0907	0
16	C_{26}	0.5122	0.2587	1.0245	1.5122	1.2587	1.3511	0.4321	0.3897	0.0031	0.0678
17	C_{27}	0.0717	2.0145	1.2047	1.6917	1.0105	1.2315	0.0945	0.0095	0.8734	0.0087
18	C_{28}	0.5922	1.0258	1.0657	2.5928	1.0208	1.1245	0.0045	1.2808	0.6225	0
19	C_{29}	0.3627	1.0699	1.0255	1.3687	1.0699	1.0922	1.3022	0.0809	0.4802	0.8262
20	C_{30}	0.1956	0.9856	0.8524	1.2987	0.3056	0.2034	0	0	0.3004	0
21	C_{31}	0.0965	0.6225	2.2385	0.0965	0.0025	0.1234	0	0	0.0141	0
22	C_{32}	0	0.5210	0	0	0.3210	0.8930	0	0.4020	0.0740	0
23	C_{33}	0	0.4552	1.1355	0	0.0552	0.5692	0.3400	0.0072	0.0532	0
24	C_{34}	0	0.5114	2.4355	0	0	0	0.0034	0	0	0.0672
25	C_{35}	0	0.4129	0	0	0	0	0	0	0	0
26	C_{36}	0	0.3245	0	0	0	0	0	0	0	0
27	C_{37}	0	0	0	0	0	1.3422	1.0212	0	0	0
28	C_{38}	0	0.1524	0	0	0	0	0	0.9770	0.7842	0.8390
29	$C_{39} - C_{40}$	0	0	0	0	0	0	0	0	0	0
	Total	**4.1421**	**20.3467**	**24.2305**	**32.8546**	**11.5751**	**19.9808**	**21.2539**	**6.3130**	**12.0463**	**15.1462**

Table 5: Remediation of Polycyclic Aromatic Hydrocarbon (mg/kg) in coastal soil augmented with 2.6 x 10^3 CFU/25 ml of 24-hour-old batch culture of biosurfactant-producing strain of *Pseudomonas aeruginosa*

Summary of levels of PAH components during the 12 weeks biodegradation Period

S/N	Parameter	Control	0-week			6-weeks			12 - weeks		
			5% cont.	10% cont.	20% cont.	5% cont.	10% cont.	20% cont.	5% cont.	10% cont.	20% cont.
1	Naphthalene	0.0000	0.3598	1.3671	1.6971	0.2700	0.0701	0.5001	0.0500	0.0171	0.0671
2	2-methylnapthalene	0.0005	0.2395	0.3381	1.0781	0.2300	1.9401	0.4801	0.0150	0.0781	0.0501
3	Acenapthene	0.0000	0.2517	0.6592	0.9892	0.3300	0.9902	2.7612	0.6800	0.0412	0.3602
4	Acenapthylene	0.0000	0.1758	0.5428	0.9576	0.5800	0.2303	0.7428	0.0300	0.5016	0.8403

5	Fluorene	0.0000	0.4398	0.5678	0.8918	0.4398	0.5710	1.3103	0.2000	0.0218	0.0503
6	Phenanthrene	0.0000	0.2090	1.1201	1.3911	0.2090	0.2912	0.9801	0.1600	0.4011	0.6701
7	Anthracene	0.0000	0.5876	1.0002	0.8452	1.1706	1.1204	0.1002	0.0400	0.8102	0.5702
8	Fluoranthene	0.0070	0.3211	0.4012	0.9467	0.1311	0.0611	0.1012	0.0700	0.6051	1.3201
9	Pyrene	0.0030	0.4020	0.5122	0.8690	0.1320	0.5207	0.0812	0.0900	0.0370	0.2205
10	Benz(a)anthracene	0.0091	0.1391	0.6391	0.8631	0.1391	0.0501	0.1201	0.0500	0.0301	0.1301
11	Benzo(b)fluoranthene	0.1101	1.0101	0.0304	0.6384	0.0101	0.0911	0.0404	0.0700	0.1104	0.0704
12	Chrysene	0.0852	0.0632	0.2352	0.9676	0.1632	0.0426	0.1032	0.0402	0.0516	0.0716
13	Benzo(k)fluoranthene	0.0011	0.2401	0.4703	0.6907	0.0601	0.0505	0.1203	0.0620	0.0465	0.0905
14	Benzo(a)pyrene	0.0106	0.3478	0.4778	0.8576	0.3418	0.0911	0.0928	0.0806	0.0576	0.0606
15	Dibenz(a,h)anthracene	0.3006	0.2763	0.6763	0.7993	1.2213	0.0393	0.0410	0.0306	0.0993	0.0533
16	Benzo(g,h,i)perylene	0.2283	0.1683	0.3079	0.9068	0.1683	0.1610	0.1609	0.0713	0.0968	0.1080
17	Indeno(1,2,3-cd)pyrene	0.1203	0.3412	0.5702	0.9534	0.3412	0.0803	0.0702	0.0603	0.0614	0.0124
	Total	**0.8958**	**5.5789**	**9.9159**	**16.3427**	**5.9776**	**6.4011**	**8.7161**	**1.8000**	**3.0687**	**5.3501**

The Niger Delta wetland environments (aquatic, marine, and land) have been affected by oil spill and these events have greatly influenced the fertility status of the soil. The present study has revealed a slightly acidic soil that is predominantly sandy (with a mean sand fraction of 87.47%, followed by 9.81% clay and 2.2% silt), poor in nitrogen and phosphorus levels. The low status of nitrogen and phosphorus in the soil is an index of less fertility and a weak nutrient mineralization potential. Oil spills would readily increase soil acidity, retard organic waste decomposition, and the general fertility of the soil. An increase in the acidity level of soil conversely leads to a decrease in the presence of Ca, Mg, Na, K, NH_3, N, and phosphorus but an increase in nutritive salts contents of soil[24][25].

Heavy metals such as copper (17.8mg/kg), zinc (42.1mg/kg), nickel (10.8mg/kg), chromium (3.6mg/kg), cadmium (1.5mg/kg) and iron (618mg/kg) were detected in the Ibeno coastal soil. Ijah[26] reported a high level of micronutrients, and heavy metals in some selected wetland soil profiles in Akwa Ibom State. Akpan reported a moderate value of nickel and vanadium in some samples of the shoreline soil. Studies have shown that trace elements e.g. cadmium, copper, chromium, nickel, and lead are among a wide variety of contaminants that have an affinity for soil sediments[27][28] while Devatha[29] also revealed the availability of heavy metals in the crude oil contaminated soils.

However, the results of the microbial analysis showed that the Ibeno coastal soil has a high heterotrophic status with a mean total heterotrophic bacterial (THB) count of 7.9 $x10^4$CFU/g with only 1.9 $x10^2$CFU/g (0.024 %) of the heterotrophs (HUB) endowed with the ability to utilize hydrocarbon. Pseudomonads were also detected and their densities in the coastal soil ranged from 5.610^3CFU/g to $6.0x10^3$CFU/g.

Conclusion

In this study, some species of pseudomonads were encountered and these included *Pseudomonas cepacia, P. putida, S. maltophila, P. aeruginosa, P. flourescens, P. diminuta,* and *P. stutzeni. Amongst the isolates, P. aeruginosa* and *P. flourescens* with percentage occurrence rates of 100% and 75% respectively, were the most common pseudomonad species isolated from Ibeno coastal soil. *Pseudomonas aeruginosa* is generally described as ubiquitous in natural settings of such soil and water ecosystems. Similarly, the meta-analysis revealed that samples obtained from environments with intense human contact had a higher prevalence of *P. aeruginosa* than in environments with less human impact. While high in coastal soil, its low occurrence in amended agricultural soils has been reported. Using direct viable count-fluorescent antibody (DVC-FA) technique. Also explained that cells reactive to an antibody against *P. aeruginosa* were widely present in Tokyo Bay. It is indicative that *P. aeruginosa* is widely present in coastal marine environments.

REFERENCES

[1] J. Wang and W. Azam, "Natural resource scarcity, fossil fuel energy consumption, and total greenhouse gas emissions in top emitting countries," Geoscience Frontiers, vol. 15, no. 2, Art. no. 101757, 2024.

[2] U. S. Yousaf, F. Ali, B. Aziz, and S. Sarwar, "What causes environmental degradation in Pakistan? Embossing the role of fossil fuel energy consumption in the view of ecological footprint," Environmental Science and Pollution Research, vol. 29, pp. 33106–33116, 2022, doi: 10.1007/s11356-021-17895-4.

[3] A. A. Kadafa, "Environmental impacts of oil exploration and exploitation in the Niger Delta of Nigeria," Global Journal of Science Frontier Research: Environment & Earth Science, vol. 12, no. 3, pp. 19–28, 2012.

[4] O. Adekola and G. Mitchell, "The Niger Delta wetlands: Threats to ecosystem services, their importance to dependent communities and possible management measures," International Journal of Biodiversity Science, Ecosystem Services & Management, vol. 7, no. 1, pp. 50–68, 2011.

[5] P. A. Onuh, T. J. Omenma, C. J. Onyishi, C. U. Udeogu, N. C. Nkalu, and V. O. Iwuoha, "Artisanal refining of crude oil in the Niger Delta: A challenge to clean-up and remediation in Ogoniland," Local Economy, vol. 36, no. 6, pp. 468–486, 2021.

[6] A. Nwozor, "Depoliticizing environmental degradation: Revisiting the UNEP environmental assessment of Ogoniland in Nigeria's Niger Delta region," GeoJournal, vol. 85, no. 3, pp. 883–900, 2020.

[7] Shell Nigeria, *The UNEP Environmental Assessment of Ogoniland*, 2016. [Online]. Available: https://www.shell.com.ng

[8] P. Itaa, L. F. Kale, F. Itaa, and C. Itaa, "Effects of oil spills on aquatic lives: A study of Kporghor Community in Tai Local Government Area, Rivers State," 2023.

[9] S. Kuppusamy, N. R. Maddela, M. Megharaj, and K. Venkateswarlu, "The fate of total petroleum hydrocarbons in the environment," in *Total Petroleum Hydrocarbons: Environmental Fate, Toxicity, and Remediation*, Cham, Switzerland: Springer, 2020, pp. 57–77.

[10] N. A. Abraham, L. O. Odokuma, and G. C. Okpokwasili, "Oily sludge degrading potentials of single and consortium of autochthonous bacterial species," GSC Advanced Research and Reviews, vol. 8, no. 3, pp. 93–101, 2021.

[11] G. Ibanichuka, "Environmental pollution and degradation in Nigeria: A legal approach," AJIEEL, vol. 8, no. 1, pp. 1–9, 2023.

[12] C. A. Onuoha, N. C. Ngobiri, E. B. Ochekwu, and P. Onuoha, "Environmental challenges awareness in Nigeria: A review," African Journal of Environment and Natural Science Research, vol. 5, no. 2, pp. 1–14, 2022.

[13] A. A. Ibrahim, U. A. Khalifa, A. Sani, A. M. Gado, G. Ismail, M. A. Ibrahim, and U. D. Adam, "Bioremediation: A biological tool for environmental mitigation," International Research Journal of Modern Engineering and Technology Science, vol. 1, pp. 649–655, 2021.

[14] C. C. Peter, J. S. Nworu, L. Arisabor, and O. K. Berezi, "Environmental phytoremediation study of oil spill site using common vegetables: A review," Green Reports, vol. 3, no. 7, pp. 16–24, 2022.

[15] L. Omene, "Assessing the socioeconomic vulnerability to oil spill from the Deepwater Horizon oil spill: A case study on coastal counties in the Gulf of Mexico," Ph.D. dissertation, Texas Southern Univ., Houston, TX, USA, 2019.

[16] D. A. D'Silva, "Impact of global oil spills: Wasting energy, jobs, and the environment—A detailed study," Ph.D. dissertation, College of Management and Economics Studies, UPES, Dehradun, India, 2020.

[17] S. Mohammadiun, G. Hu, A. A. Gharahbagh, J. Li, K. Hewage, and R. Sadiq, "Evaluation of machine learning techniques to select marine oil spill response methods under small-sized dataset conditions," Journal of Hazardous Materials, vol. 436, Art. no. 129282, 2022.

[18] M. Gana, C. S. Olusanya, A. D. Shaba, B. B. Adamu, and A. D. Idris, "Biodegradation of diesel and crude oil using Corynebacterium sp. and Lysinobacillus fusiformis 5b stimulated with

biosurfactant, biochar, and iron oxide nanoparticles," Covenant Journal of Physical and Life Sciences, vol. 9, no. 2, 2021.

[19] K. E. Ukhurebor, H. Athar, C. O. Adetunji, U. O. Aigbe, R. B. Onyancha, and O. Abifarin, "Environmental implications of petroleum spillages in the Niger Delta region of Nigeria: A review," Journal of Environmental Management, vol. 293, Art. no. 112872, 2021.

[20] I. Franciosa, "Autochthonous starter culture selection for PGI Salama Piemonte production through the microbiota analysis," Ph.D. dissertation, Univ. of Brest, Brest, France, 2021.

[21] G. C. Okpokwasili and S. C. Amanchukwu, "Petroleum hydrocarbon degradation by Candida species," Environment International, vol. 14, pp. 243–247, 1998.

[22] M. A. Kurnianto, H. D. Kusumaningrum, and H. N. Lioe, "Characterization of Streptomyces isolates associated with estuarine fish *Chanos chanos* and profiling of their antibacterial metabolites crude extract," International Journal of Microbiology, 2020.

[23] C. B. Chikere, C. C. Obieze, and B. O. Chikere, "Biodegradation of artisanally refined diesel and the influence of organic wastes on oil-polluted soil remediation," Scientific African, vol. 8, Art. no. e00385, 2020.

[24] G. Agegnehu, C. Yirga, and T. Erkossa, *Soil Acidity Management*. Addis Ababa, Ethiopia: Ethiopian Institute of Agricultural Research (EIAR), 2019.

[25] U. R. Obot and A. E. Gobo, "The physicochemical properties of Ibeno wetland soil, Akwa Ibom State, Nigeria," International Journal of Bioscience, vol. 1, no. 1, pp. 76–84, 2009.

[26] C. A. Ijah, F. O. Umoh, O. A. Essien, I. Gu, and V. I. Moses, "Profile distribution of iron and zinc in soils formed from three different parent materials in Akwa Ibom State, Nigeria," 2023.

[27] V. L. B. Souza, S. D. Barbosa, and L. C. D. da Silva, "Soil metals and sediments: A review," in *Proc. International Nuclear Atlantic Conference (INAC 2021): Nuclear Technology—Reducing Our Carbon Footprint and Increasing Quality of Life*, 2021.

[28] Y. Hamid, L. Tang, B. Hussain, M. Usman, Q. Lin, M. S. Rashid, and X. Yang, "Organic soil additives for the remediation of cadmium contaminated soils and their impact on the soil–plant system: A review," Science of the Total Environment, vol. 707, Art. no. 136121, 2020.

[29] C. P. Devatha, A. Vishnu Vishal, and J. Purna Chandra Rao, "Investigation of physical and chemical characteristics of soil due to crude oil contamination and its remediation," Applied Water Science, vol. 9, pp. 1–10, 2019.