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Full Length Research Paper

Heavy metal contents and microbial diversity of waste engine oil-polluted soil in some public and commercial centres in Benin City metropolis, Nigeria.

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Abstract
This study investigated the heavy metal contents and microbial diversity of waste engine oil-polluted soil in some public and commercial centres in Benin City metropolis, Nigeria. The study was carried out in four Local Government Areas (Ikpoba Okha, Ovia North East, Egor and Oredo) within Benin Metropolis; the administrative headquarters of Edo State of Nigeria. Certain plots within the study area were selected based on the presence of waste engine oil spill on the ground, either due to activities of mechanics, parked vehicles, stationary diesel engines, or oil dump sites. However, in the present study only mechanic workshops (MW), bakeries (BK), generating plant houses (GN), as well as spare part dealer shops (SP) located within the City were selected. Field reconnaissance studies were carried to estimate the extent of pollution on surrounding soil, using the presence and absence of some flora and fauna, and developmental defects on some flora. Top soil (0 - 10 cm) was randomly collected from about 30 cm from the periphery of the spill sites and then pooled together to obtain composite samples. These were labelled appropriately, and immediately transported in polythene bags, covered with aluminium foil papers, to the laboratory for heavy metal and microbial assay.
Keywords: Hydrocarbons, heavy metal, waste engine oil, bioremediation,

Introduction
Environmental pollution with petroleum products such as crude oil has been recognized as one of the most serious current problems especially when associated with accidental spills on large-scale (Mandri and Lin, 2007). In Nigeria, oil spills at auto mechanic workshops have been left uncared for over the years and its continuous accumulation is of serious environmental concern, because of the hazard associated with it. For instance, the spent motor oil disposed of improperly contains potentially toxic substances such as benzene (carcinogens), lead, arsenic, zinc and cadmium, which can seep into the water tables and contaminate ground water (Igwe e*t al.*, 2008). It results in loss of soil quality, accumulation of toxic compounds by plants and animals that are directly hit, and consequently, serious health hazards such as anemia and tremor, which can cause death.

Although soil contamination lead to a significant change in soil microbial diversity, an important factor for mineralization of soils (Ikhajiagbe and Chijioke-Osuji, 2012); it is however noted that petroleum hydrocarbons in soil also stimulate the growth of indigenous microbial populations, which are capable of utilizing the petroleum hydrocarbons as their carbon and energy source thereby degrading the contaminants. The ability to degrade hydrocarbon substrates is exhibited by a wide v*ariety* of bacteria genera *(*Dally *et al.*, 1997;

Bogan *et al*., 2003; Malakootian *et al*., 2009; Abdulsalam and Omale, 2009; Abdulsalam *et al.*, 2011). The main object of the study is to investigate the diversity of soil microorganisms in waste engine oil-polluted soils in auto mechanic workshops, bakeries, generating plant houses, as well as spare part dealer shops in Benin City, Nigeria. This is very important because for most soils, the restoration of soil health upon contamination by petroleum hydrocarbons and heavy metals is imperative. Most soils are restored by natural processes, called attenuation or intrinsic bioremediation (Ikhajiagbe and Anoliefo, 2011, 2012a, 2012b; Ikhajiagbe et al. 2013); and this is mainly achieved by a consortium of microorganisms inherent in such soils (Anoliefo and Ikhajiagbe, 2011).

The bacteria most commonly encountered are the Gram-negative species of the alpha proteobacteria group, such as species of *Pseudomonas*, *Sphingomonas*, *Moraxella*, *Acinetobacter*, *Alcaligenes*, and *Proteus*. Other important groups are the low G+C Gram-positives, such as *Bacillus* and *Micrococcus*, and the high G+C Gram-positives, particularly the actinomycetes (Amund, 2000; Wackett and Hershberger, 2001; Parales *et al*., 2003). *Pseudomonas* species are often isolated from hydrocarbon-contaminated sites and hydrocarbon-degrading cultures. Members of this genus have a broad affinity for hydrocarbons and can degrade selected alkanes, alycyclics, thiophenes and aromatics (Vankateswaran *et al*., 1995; Allen *et al*., 1997). Polycyclic aromatic hydrocarbons (PAHs) are among the most recalcitrant components of crude oil (Kanaly and Harayama, 2000). The isolated crude oil degraders belong to the genera *Micrococcus*, *Corynebacterium*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Moraxella*, *Aeromonas*, *Acinetobacter* and *Vibrio*. The flora reflects the normal heterotrophic bacteria present in soil, and native genera seem to be crude oil utilizers. Several other workers also reported on the above genera as hydrocarbon-degrading microorganisms (Atlas, 1981; Leahy and Colwell, 1990; Banat *et al*., 2000). In general, a bacterial consortium shows the maximum percentage (78%) of degradation of crude oil after 20 days of incubation.

Although microorganisms are present in contaminated soil, they cannot necessarily be there in the numbers required for bioremediation of the site. Their growth and activity must be stimulated. Carbon is the most basic form of nutrient required by living organism. In addition to this, the bacteria also need macronutrients like nitrogen and phosphorous to ensure effective degradation of the oil. The optimum nutrient balance required for hydrocarbon remediation is Carbon: Nitrogen: Phosphorus in the ratio of 100:10:4. In general, at least 1 ppm of ammonium nitrogen and 0.4 ppm of orthophosphate needs to be present. Pathways can be influenced by further adjusting volumes of bio-nutrients (U S Congress, 1991).

Materials and methods

Study Area

This study was carried out in four Local Government Areas (Ikpoba Okha, Ovia North East, Egor and Oredo) within Benin Metropolis, the administrative headquarters of Edo State of Nigeria. Benin City is located within longitude 5^{o} 24' and 6^{o} 35' E and latitude 5^{o} 36' and 5^{o}47' of the equator. The area has a hot humid climate with two distinct seasons - the wet season (May to November) and dry season (November to April). Mean annual rainfall is about 2,500mm, which displays a bi-modal pattern characteristic of the Southern parts of West Africa.

Experimental procedures

Field Reconnaissance

Certain plots within the study area were selected based on the presence of waste engine oil spill on the ground, either due to activities of mechanics, parked vehicles, stationary diesel engines, or oil dump sites. However, in

the present study only mechanic workshops (MW), bakeries (BK), generating plant houses (GN), as well as spare part dealer shops (SP) located within the City were selected.

Field reconnaissance studies were carried out to identify the extent of pollution on the surrounding soil, using the presence and absence of some flora and fauna, and developmental defects on some flora. A reconnaissance survey was also important in order to obtain a visual assessment of the extent of oil cover on the soil surface. During this visit the specific sampling points, based on the aforementioned facilities, where pinned down for selection. These included Generator plant house at Benzito Fast Food, Uwasota (Egor GN), Obey God Bakery, off Nova Road (Egor BK), Mechanic workshop at Eyeye Street, off Uwasota (Egor MW), and Spare parts dealer shop at Uwelu (Egor SP) all in Egor Local Government Area. There was also Generator plant house at Winner Chapel, Sapele Road (Ikpoba GN), Bakery off old Agbor Road, Agbor park, Ikpoba Hill (Ikpoba BK), Mechanic workshop at Aduwawa (Ikpoba MW), and Spare parts dealer shop by Alohan Filling Station, off Sapele Road (Ikpoba SP) all located within Ikpoba Okha Local Government Area. Four areas in Oredo Local Government Area were also selected including a Generator plant house at Stella Obasanjo Hospital, off Sapele Road (Oredo GN), Bakery near Oba Market, Ring Road (Oredo BK), Mechanic workshop along Country Home Road, off Sapele Road (Oredo MW) and Spare parts dealer shop along Country Home Road, off Sapele Road (Oredo SP). Finally, at Ovia North East Local Government Area, the sites selected were Generator plant house at the Faculty of Life Sciences, UNIBEN (Ovia GN), Efe Bakery, Isiohor (Ovia BK), Mechanic workshop at Oluku (Ovia MW) and Spare parts dealer shop at Oluku (Ovia SP).

Collection of Soil Samples

Top soil (0 - 10 cm) was randomly collected from about 30 cm from the periphery of the spill sites and then pooled together to obtain composite samples. These were labelled appropriately, and immediately transported in polythene bags, covered with aluminium foil papers, to the laboratory for heavy metal and microbial assay. Soil collection was usually in the early morning (between 6.00 - 7. 00 am).

Soil Heavy Metal Analyses

Soils were dried at ambient temperature (22-25^{o}C), crushed in a porcelain mortar and sieved through a 2-mm (10 meshes) stainless sieve. Air-dried <2 mm samples were stored in polythene bags for subsequent analysis. The <2 mm fraction was used for the determination of heavy metal fractions as well as total hydrocarbon content (THC).

Extraction of Micronutrients in Soils by Hydrochloric Acid Method

Ten (10) g of soil was weighed into a 250 ml plastic bottle. 100 ml of 0.1 m HCI was added, stoppered, and then shaken for 30 minutes. The mixture was filtered through Whitman filter paper No.42. and then Fe, Cu, Mn, Zn, Cd, Cr, Pb, Ni, and V were determined in the filtrate by Atomic Absorption Spectrometry.

Computation of Hazard Quotient (HQ) for Heavy Metal Fractions

Calculation of the HQ expresses the possibility of the contaminant being an ecological risk or a contaminant of potential ecological concern (Ikhajiagbe, 2010). The hazards Quotient is expressed by the following equation:

$$HQ = \frac{\text{Measured concentration}}{\text{Selected screening benchmark.}}$$

When HQ > 1: Harmful effects are likely due to contaminant in question

When HQ = 1: Contaminant alone is not likely to cause ecological risk

When HQ < 1: Harmful effects are not likely

The selected screening benchmark is available in Efroymson *et al.* (1997).

HQ was calculated for both ecotoxicity of the various heavy metal fractions in the soil and their toxicity to soil microbial activities and processes.

Heterotrophic Bacterial and Fungal Counts

The spread plate method was employed in taking the heterotrophic bacteria counts. One (1) ml of the serially diluted portion of 10^{-4} of each soil sample was inoculated onto nutrient agar plates for bacterial count and Potato dextrose agar plates for fungal counts. The plates were inoculated at room temperature for 24 hours and 72 hours respectively, for bacteria and fungi growth. After incubation colonies were then counted and the colony forming unit (cfu/g) of the soil samples determined.

Isolation of Bacterial and Fungal Oil Degraders

Bushnell- Haas (BH) medium ($MgSO_4$, 0.20 g/I; $CaCl_2$, 0.02 g/l; $K_2H,P0_4$, 1 g/l; NH_4NO_3, 1 g/l; $FeCl_3$, 0.05 g/l; KH_2PO_4, 1 g/l; pH 7.0 (Atlas, 1994) was used as the enrichment medium with 8 % (v/v) filter – sterilized oil as the sole carbon source. The medium was dispensed into 100 ml Erlenmeyer flasks and autoc1aved at 12l ^{0}C for 15 minutes. Thereafter, 5 g of each soil sample was inoculated into each flask of the medium and incubated at 130 rpm at room temperature in a HY-4 multifunctional shaker (B. Bran Scientific & Instrument Company, England). After 10 days, 1 ml of enriched media was transferred into freshly prepared enrichment media and incubated under the same conditions as described above. Serial dilutions from the third enrichment process were inoculated onto nutrient agar plates and potato dextrose agar plates for oil-degrading bacterial counts and funga1 counts respectively. To prevent bacterial growth antibiotics were added to the Potato dextrose agar plates and fungicide to the nutrient agar to prevent fungal growth. Distinct colonies were counted and sub-cultured to obtain pure colonies which were then stored on slants for further studies.

Characterization and Identification of Bacterial Oil-degrading Isolates

The bacterial isolates that were predominantly isolated were identified to their species level using conventional microbiological and biochemical tests as described by Cowan and Steel (1973), Cheesebrough (2000) and Aneja (2003).

Computation of Microbial Biodiversity Studies

Biodiversity of microorganisms (bacteria and fungi only) was computed using the formulae below. Only weeds that were >3 cm high were counted.

Given that,

S= total number of species

N= total number of individuals

n*i*= number of individuals in the i^{th} species

Species Richness Indices

Margalef's index, $d = \frac{S-1}{\ln(N)}$

Menhinick's index, $D = \frac{S}{\sqrt{N}}$

Diversity Indices

Shannon Index, $H^{I} = -\sum_{i=1}^{s} pi \ln pi$

Where $pi = ni / N$

Shannon-Weiner's index, $H = \frac{N \log N - \sum_{i=1}^{s} fi \log fi}{N}$

This index gives the level for which a plant population consists of several species in cohabitation.

Evenness Indices

Evenness, $E = \frac{H}{\log S}$, while $E^{I} = \frac{H^{I}}{\ln S}$

The index varies between 0 and 1, where E=1 gives the situation when all species are equally abundant.

Simpson's Dominance Indices

Simpson's index, $C = \sum_{i=1}^{s} pi^2$

$$D = \sum_{i=1}^{s} \left[\frac{ni(ni-1)}{N(N-1)} \right]$$

The index varies between 0 and 1, and gives the probability that two individuals drawn at random from a population belong to the same species.

Results

The soil content of chromium, around generator plants (GN) in Egor was 1.08 mg/kg compared to 0.09 mg/kg in Oredo (Table 1). Zinc in soil collected around Ikpoba Okha GN was 65.22 mg/kg, compared to 211.08 mg/kg in Ovia North East. For soil collected within auto mechanic workshop, soil concentration of chromium ranged from 0.98 to 2.19 mg/kg, whereas manganese ranged from 1.09 to 9.65 mg/kg. The highest value for these metals was obtained in Egor. Similarly higher values of zinc, iron and cadmium were obtained in Egor, compared to other sites.

At auto spare parts stores, soil content of chromium at Egor was 1.09 mg/kg, compared to that of Ovia North East and Ikpoba Okha which were 0.98 mg/kg respectively. Soil content of manganese was 6.90 mg/kg in Egor and 2.56 mg/kg in Ovia respectively. For soil collected around Bakery, chromium ranged from 0.28 mg/kg at Ikpoba Okha to 0.68 mg/kg at Egor while zinc ranged from 29.67 to 16.22 mg/kg; and obviously both values were the highest of soil content within bakery in the four L.G.As.

Table 1: Heavy metal contents of waste oil-polluted soil collected from designated sites within Benin metropolis

	Cr	Mn	Zn	Fe	Cd	Total
			(mg/kg)			
			Soil around Generator Plant			
Egor GN	1.08	3.52	92.62	1652.22	0.006	1749.45
Ikpoba GN	1.02	0.56	65.22	1601.22	0.003	1668.02
Oredo GN	0.09	0.69	33.74	1362.42	*Bd*	1396.94
Ovia GN	1.32	6.33	211.08	1632.25	0.006	1850.99
			Soil within auto mechanic workshop			
Egor MW	2.19	9.65	205.54	1864.03	0.062	2081.47
Ikpoba MW	1.82	1.59	155.86	1647.64	0.022	1806.93
Oredo MW	0.98	1.09	58.57	1258.19	*Bd*	1318.83
Ovia MW	2.01	9.34	165.57	1365.23	0.054	1542.20
			Soil within auto spare parts stores			
Egor SP	1.09	6.98	185.51	1720.65	0.020	1914.25
Ikpoba SP	0.98	0.96	59.82	1563.63	*Bd*	1625.39
Oredo GN	0.42	0.38	26.95	1305.21	0.019	1332.98
Ovia SP	0.98	2.56	62.48	1493.25	*Bd*	1559.27
			Soil around bakery			
Egor BK	0.68	1.05	6.25	1630.41	*Bd*	1638.39
Ikpoba BK	0.28	0.19	3.95	1598.64	*Bd*	1603.06
Oredo BK	0.49	0.33	16.22	1298.21	*Bd*	1315.25
Ovia BK	0.39	0.28	29.67	1482.21	*Bd*	1512.55
Mean	0.99	2.84	86.19	1529.71	0.012	1619.75

Bd - below detectable limit (0.0001 mg/kg); GN generator plant house, BK bakery, MW mechanic workshop, SP spare parts shop

Hazard quotient (HQ) for toxicity of heavy metals contents of waste oil – polluted soil to microbial activities and processes was less than unity for heavy metals Cr, Mn, and some Cd in soil (Table 2). this indicated that toxicity to microbial activities or processes may not be necessarily due to concentration levels of these metals, but Fe. HQ values for Fe were greater than unity ($HQ_{Fe} > 1$). Table 3 shows microbial species composition of waste oil – polluted soil collected from designated sites within Benin metropolis. Around generating plant house bacteria species present at Egor were *Bacillus subtilis*, *Sarcina* sp., *Clostridium* sp. and *Micrococcus luteus*; while fungal species where *Aspergillus niger, A. flavus, Rhizopus stolonifer, Penicillum notatum* and *Fusarium solani*. At Oredo L.G. A., bacterial and fungal species present at Auto mechanic workshop are as follows *Bacillus* sp., *Clostridium* sp., and *Micrococcus* sp. while fungi species where *Aspergillus niger, Rhizopus stolonifer, Penicillium* sp., *Geotrichum* sp. and *Mucor* sp. Generally, however, the most prevalent bacterial and fungal species in all 16 sites around Benin was *Bacillus subtilis* with frequency of occurrence at about 93.75% and fungal species was *Aspergillus niger* with frequency of occurrence of 100.0% which showed that this species occurred the most.

Table 2: Hazard quotient to show toxicity of heavy metal contents of waste oil-polluted soil collected from designated sites within Benin metropolis to microbial activities and processes

	*Cr [10]	Mn [10]	Zn [NA]	Fe [30]	Cd [20]
			Soil around Generator Plant		
Egor GN	0.108	0.352	NA	55.074*	0.0003
Ikpoba GN	0.102	0.056	NA	53.374*	0.0015
Oredo GN	0.009	0.069	NA	45.414*	$>10^4$ *
Ovia GN	0.132	0.633	NA	54.408*	0.0003
			Soil within auto mechanic workshop		
Egor MW	0.219	0.965	NA	62.134*	0.0031
Ikpoba MW	0.182	0.159	NA	54.921*	0.0011
Oredo MW	0.098	0.109	NA	41.939*	$>10^4$ *
Ovia MW	0.201	0.934	NA	45.507*	0.0027
			Soil within auto spare parts stores		
Egor SP	0.109	0.698	NA	57.356*	0.0010
Ikpoba SP	0.098	0.096	NA	52.121*	$>10^4$ *
Oredo SP	0.042	0.038	NA	43.507*	0.0009
Ovia SP	0.098	0.256	NA	49.775*	$>10^4$ *
			Soil around bakery		
Egor BK	0.068	0.105	NA	54.347*	$>10^4$ *
Ikpoba BK	0.028	0.019	NA	53.288*	$>10^4$ *
Oredo BK	0.049	0.033	NA	43.274*	$>10^4$ *
Ovia BK	0.039	0.028	NA	49.407*	$>10^4$ *

**Toxicity indicated. Values in bracket are permissible limits (Efroymson* et al., *1997); NA not available; GN generator plant house, BK bakery, MW mechanic workshop, SP spare parts shop*

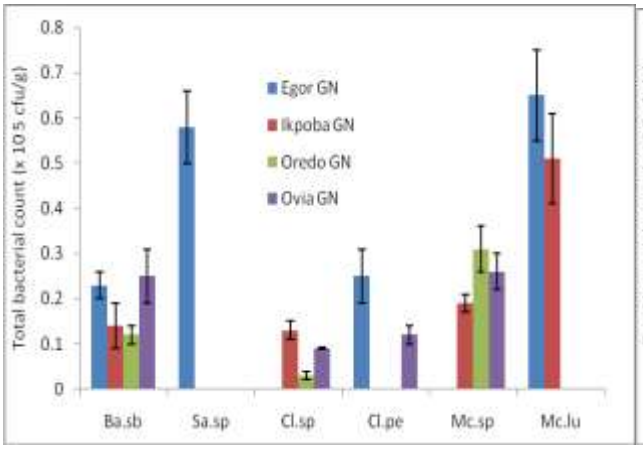
Figs. 1(a-b) show the microbial composition of the bacteria species associated with the waste oil – polluted soil collected from designated sites within Benin metropolis. In Egor L.G., around generator plant, total specific bacterial counts included 0.23 x 10^5cfu/g for *Bacillus subtilis*, 0.58 x 10^5cfu/g for *Salmonella*, 0.42 x 10^5cfu/g for *Micrococcus* sp. However, within spare parts shops in Oredo L.G., total bacteria counts included *Bacillus subtilis* (0.18 x 10^5cfu/g), *Salmonella* sp (0.63 x 10^5cfu/g) and *Clostridium* sp (0.19 x 10^5cfu/g). Figs. 2 (a-d) show fungal composition of species associated with the oil-polluted soil collected from the designated sites. Around generator plant house, in Oredo L.G., the total number of fungal count included *Aspergillus niger* (0.19 x 10^5cfu/g), *Rhizopus stolonifer* (0.06 x 10^5cfu/g),

Penicillium sp (0.31 x 10^5cfu/g), *Geotricum* sp (0.12 x 10^5cfu/g) and *Mucor* sp (0.25 x 10^5cfu/g). In Ovia L.G. around spare parts shop the total number of fungal count was 1.37 x 10^5cfu/g, with the presence of *Aspergillus niger* (0.24 x 10^5cfu/g), *Aspergilus flavus* (0.31 x 10^5cfu/g), Penicillium sp (0.16 x 10^5cfu/g), *Penicillium notatum* (0.14 x 10^5cfu/g), *Geotricum* sp. (0.52 x 10^5cfu/g).

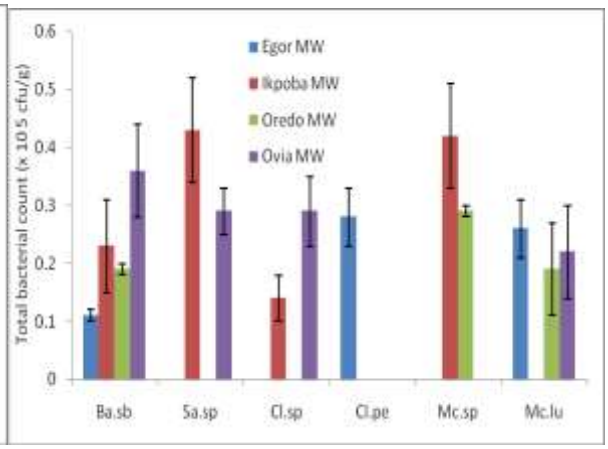
Table 3: Microbial species composition of waste oil-polluted soil collected from designated sites within Benin metropolis to microbial activities and processes

	Bacterial species						Fungal species								
	Ba.sb	*Sa.sp*	*Cl.sp*	*Cl.pe*	*Mc.sp*	*Mc.lu*	*As.ng*	*As.fv*	*Rh.st*	*Pe.sp*	*Pe.no*	*Ge.sp*	*Mu.sp*	*Tr.sp*	*Fu.so*
Soil around Generator Plant															
Egor GN	+	+	-	+	-	+	+	+	+	-	+	-	-	-	+
Ikpoba GN	+	-	+	-	+	+	+	+	-	-	-	+	-	+	-
Oredo GN	+	-	+	-	+	-	+	-	+	+	-	+	+	-	-
Ovia GN	+	-	+	+	+	-	+	+	-	+	-	-	-	-	+
Soil within auto mechanic workshop															
Egor MW	+	-	-	+	-	+	+	-	-	+	-	+	-	-	+
Ikpoba MW	+	+	+	-	+	-	+	-	+	-	-	-	-	+	-
Oredo MW	+	-	-	-	+	+	+	-	+	+	+	-	+	-	+
Ovia MW	+	+	+	-	-	+	+	+	-	-	-	+	-	+	-
Soil within auto spare parts stores															
Egor SP	-	-	+	+	+	+	+	-	+	-	-	-	-	+	+
Ikpoba SP	+	-	+	-	+	-	+	+	-	+	-	-	+	-	+
Oredo SP	+	+	+	-	-	-	+	+	+	+	-	+	-	-	-
Ovia SP	+	+	-	+	-	+	+	+	-	+	+	+	-	-	-
Soil around bakery															
Egor BK	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-
Ikpoba BK	+	-	+	-	+	-	+	+	-	+	-	+	+	-	+
Oredo BK	+	+	-	-	-	+	+	-	-	+	+	-	+	+	-
Ovia BK	+	-	-	+	-	+	+	+	+	-	+	+	-	-	+
Freq. Occ. (%)	93.75	37.50	56.25	43.75	50.00	62.50	100.00	56.25	62.50	62.50	31.25	56.25	31.25	37.50	50.00

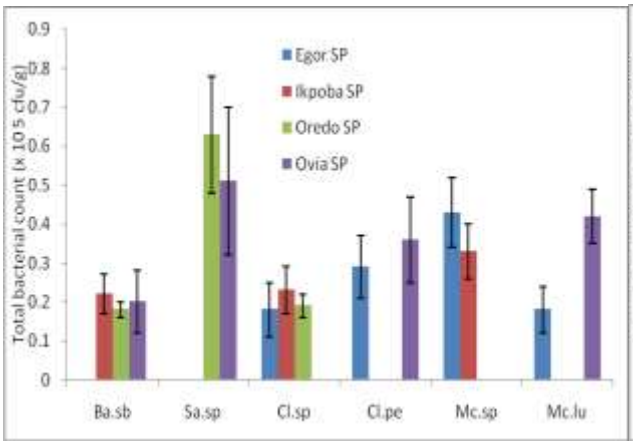
Freq. Occ. (%) frequency of occurrence of microorganism; - Absent,+ present; Ba.sb-Bacillus substilis, Sa.sp- Sarcina sp, Cl.sp- Clostridium sp, Cl.pe- C. perfringens, Mc.sp- Micrococcus sp, Mc.lu- M. luteus, As.ng- Aspergillus niger, As.fv- A. flavus, Rh.st- Rhizopus stolonifer, Pe.sp- Penicillium sp, Pe.no- P. notatum, Ge.sp- Geotrichum sp, Mu.sp- Mucor sp, Tr.sp- Trichoderma sp, Fu.so-Fusarium solani; GN generator plant house, BK bakery, MW mechanic workshop, SP spare parts shop


Total bacterial count (x 10 5 cfu/g)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Egor GN
Ikpoba GN
Oredo GN
Ovia GN
Ba.sb
Sa.sp
Cl.sp
Cl.pe
Mc.sp
Mc.lu

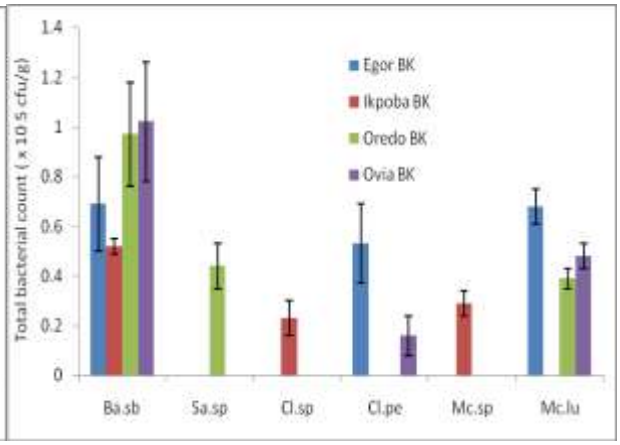
(1a)


Total bacterial count (x 10 5 cfu/g)
0.6
0.5
0.4
0.3
0.2
0.1
0
Egor MW
Ikpoba MW
Oredo MW
Ovia MW
Ba.sb
Sa.sp
Cl.sp
Cl.pe
Mc.sp
Mc.lu

(1b)


Total bacterial count (x 10 5 cfu/g)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Egor SP
Ikpoba SP
Oredo SP
Ovia SP
Ba.sb
Sa.sp
Cl.sp
Cl.pe
Mc.sp
Mc.lu

(1c)


Total bacterial count (x 10 5 cfu/g)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Egor BK
Ikpoba BK
Oredo BK
Ovia BK
Ba.sb
Sa.sp
Cl.sp
Cl.pe
Mc.sp
Mc.lu

(1d)

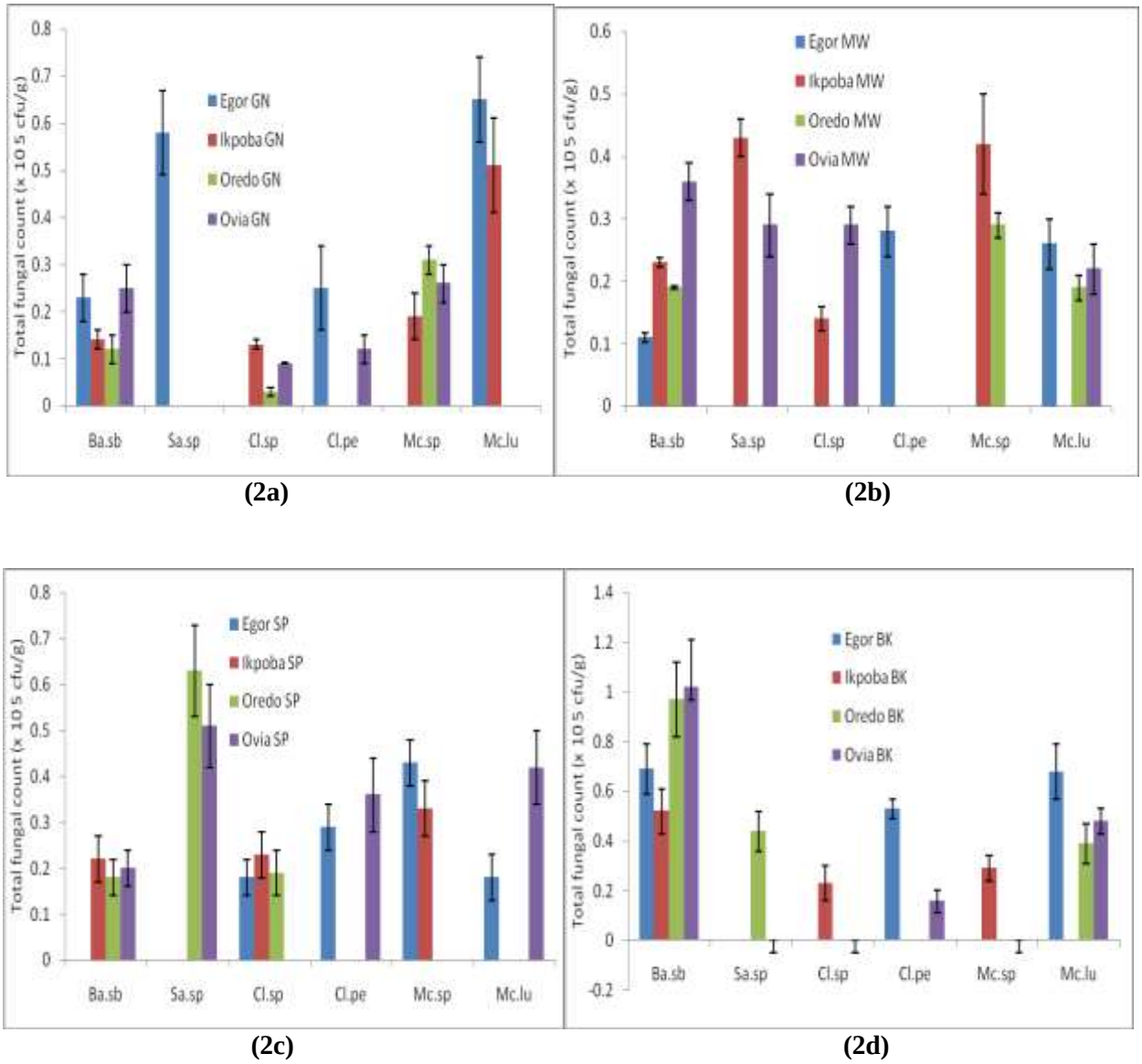


Fig. 2 (a-d): Microbial composition of fungal species associated with the waste oil-polluted soil collected from around generator plants within Benin metropolis. *As.ng- Aspergillus niger, As.fv- A. flavus, Rh.st- Rhizopus stolonifer, Pe.sp- Penicillium sp, Pe.no- P. notatum, Ge.sp- Geotrichum sp, Mu.sp- Mucor sp, Tr.sp- Trichoderma sp, Fu.so-Fusarium solani; GN generator plant house, BK bakery, MW mechanic workshop, SP spare parts shop.*

Table 4 shows diversity and dominance indices for bacteria species associated with the experimental sites. Species richness index for bacteria species associated with waste oil polluted soil collected from Egor generator plant house (GN) was 0.3996 compared to 0.1683 in Ovia generator plant house. The implication is that the oil – polluted soil collected from generator plant in Egor was richest in bacteria species , compared to the other three local government areas. Around the mechanic workshop (MW) the bacteria species with the highest species richness was found at Ikpoba Okha which was 0.2851 and the lowest at Egor (0.519). Diversity index for

bacteria species associated with oil – polluted soil around generator plant house ranged from 0.045 to 0.3424 and the highest index been found in Egor.

Soils collected from around motor spare part in Ikpoba Okha were more diverse in bacteria species (0.8331). By comparing dominance indices for bacteria species in all the 16 sites sampled altogether, dominance index was highest in Ovia BK (0.003864) and lowest in Oredo GN (0.0000499).

Egor L.G. showed the lowest fungi species richness associated with oil – polluted soil which was around spare parts with richness of about 0.1551 compared to the highest species richness at Ovia of about 0.3373 (Table 5). Generally around bakery, the highest fungi species richness was again at Ovia which was 0.3988. Diversity index for fungal species associated with oil – polluted soil around generator plant house ranged from 0.246 to 0.325 and the highest index been found in Ikpoba Okha. Soils collected around mechanic shops (MW) were more diverse at Oredo (0.299) compared to MW's in the study area.

Around spare parts shops, oil – polluted soil sample ranged from 0.17 to 0.331 while the lowest index was at Egor and the highest index at Ovia (Table 5). Evenness index varied between 0 and 1, but for fungi species, the highest value index was at Ovia and Ikpo Okha around the bakeries (0.031) respectively. By comparison, dominance index for fungal species in all the 16 sites sample as highest in Ikpoba GN (0.002637) and lowest in Ikpoba MW (0.000046).

Results (Table 6) also showed that in Egor L.G. around generating plant houses, the presence of certain plant species was recorded*; Acanthospermum hispidum, Cyperus esculentus, Eleusine indica* and *Paspalum scrobiculatum* which were all more than 20 in this location but the likes of *Achryranthes aspera, Acroceras zizanioides, Ageratum conyzoides, Alternanthera repens, Axonopus compressus, Cyperus rotundus, Echinochloa obtusifolia, Kylling erecta, Leptochloa caerulescens, Mariscus alternifolios, Oldenlandia herbacea, Panicum maximum, Peperomia pellucidia, Phyllanthus amarus, Sida acuta* and *Tridax procumbens* were less than 10. At auto – mechanic workshop, in Ikpoba Okha L.G. *Ageratum conhyzoides, Cyperus esculentus, Echinochloa ontusifolia, Leptochloa caerulenscens, Mariscus alternifolios, Oldenlandia herbacea, Panicum maximum, Paspalum scrobiculatum, Phyllanthus amarus, Tridax procumbens* were all less or fewer than 10 each in these locations, while *Alternathera repens* was more than 10 but less than 20, and *Eleusine indica* was more than 20. All plant species found around Bakery in Ovia L.G were less or fewer than 10 each. *Eleusine indicia* was the most predominant plant species, found in all the sites sampled.

Table 4: Diversity and dominance indices for bacteria species associated with the waste oil-polluted soil collected from designated sites within Benin metropolis

Sources	Species richness (Menhinick's) index	Shannon index (H^1)	Evenness index (E^I)	Dominance index (C)	Margalef index
Egor GN	0.3996	0.3414	0.028	0.002611	0.244
Ikpoba GN	0.2267	0.218	0.019	0.002611	-0.0103
Oredo GN	0.1075	0.054	0.005	0.0000499	-0.1857
Ovia GN	0.1683	0.178	0.016	0.000458	-0.0963
Egor MW	0.1519	0.155	0.014	0.000472	-0.1204
Ikpoba MW	0.2851	0.266	0.023	0.001293	0.0757
Oredo MW	0.1566	0.16	0.014	0.00061	-0.1135
Ovia MW	0.2711	0.262	0.022	0.00103	0.0550
Egor SP	0.2524	0.244	0.021	0.000994	0.0275
Ikpoba SP	0.1823	0.831	0.074	0.000627	-0.0757
Oredo SP	0.2337	0.208	0.018	0.001459	0
Ovia SP	9.3482	0.311	0.026	0.001809	0.1685
Egor BK	0.440	0.348	0.029	0.03632	0.3096
Ikpoba BK	0.2430	0.222	0.019	0.001216	0.0138
Oredo BK	0.4207	0.326	0.027	0.003828	0.2752
Ovia BK	0.3879	0.298	0.025	0.003864	0.2270
Mean	0.829688	0.2764	0.02375	0.003703	0.04965

GN generator plant house, BK bakery, MW mechanic workshop, SP spare parts shop

Table 5: Diversity and dominance indices for fungal species associated with the waste oil-polluted soil collected from designated sites within Benin metropolis

Sources	Species richness (Menhinick's) index	Shannon index (H^1)	Evenness index (E^I)	Dominance index (C)	Margalef index
Egor GN	0.26	0.281	0.024	0.000924	0.029
Ikpoba GN	0.3644	0.325	0.027	0.02637	0.171
Oredo GN	0.589	0.246	0.022	0.000782	-0.025
Ovia GN	0.2585	0.253	0.022	0.001425	0.018
Egor MW	0.2733	0.18	0.015	0.001036	0.039
Ikpoba MW	0.443	0.06	0.006	0.00046	-0.293
Oredo MW	0.2856	0.299	0.026	0.0011	0.057
Ovia MW	0.3028	0.276	0.024	0.001975	0.082
Egor SP	0.1551	0.17	0.015	0.000513	-0.132
Ikpoba SP	0.2831	0.29	0.025	0.001144	0.054
Oredo SP	0.1649	0.182	0.016	0.0005074	-0.118
Ovia SP	0.3373	0.331	0.028	0.001721	0.132
Egor BK	0.1600	0.177	0.016	0.000457	-0.125
Ikpoba BK	0.2733	0.362	0.031	0.00171	0.039
Oredo BK	0.2659	0.277	0.024	0.001042	0.029
Ovia BK	0.3988	0.375	0.031	0.002788	0.221
Mean	0.300938	0.25525	0.022	0.002747	0.011125

GN generator plant house, BK bakery, MW mechanic workshop, SP spare parts shop

Table 6: Plant species composition of waste oil-polluted soil collected from designated sites within Benin metropolis to microbial activities and processes

	AH	AA	AZ	AC	AR	AT	AX	CE	CR	EO	EI	EH	KR	LC	MA	OH	PM	PS	PP	PA	SA	TP
Soil around Generator Plant																						
Egor GN	+++	+	+	+	+	-	+	+++	+	+	+++	-	+	+	+	+	+	+++	+	+	+	-
Ikpoba GN	++	++	-	+	-	-	-	+	+	+	++	-	+++	+	+	-	-	++	-	-	+	+
Oredo GN	-	+	+	+	++	-	+	-	-	-	+++	+	+	-	+	+	+	+	+	+	+	-
Ovia GN	+	+	+	+	+++	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Soil within auto mechanic workshop																						
Egor MW	+	+	-	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	-
Ikpoba MW	-	-	-	+	++	-	-	+	-	+	+++	-	-	+	+	+	+	+	-	+	-	+
Oredo MW	-	-	-	+	+	-	-	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+
Ovia MW	-	+	+	+	+	-	+	+	-	+	++	-	-	+	+	+	+	++	+	+	+	+
Soil within auto spare parts stores																						
Egor SP	-	-	+	+	-	+	-	++	+	+	+	-	-	-	+	+	+	-	-	+	+	-
Ikpoba SP	-	-	++	+	-	+	-	++	+	+	+	-	+	-	+	+	+	+	-	+	+	-
Oredo SP	-	-	-	+	+	-	++	+	+	+	+	-	-	-	+	+	+	-	+	+	+	-
Ovia SP	+	-	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Soil around bakery																						
Egor BK	-	+	++	+	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
Ikpoba BK	-	-	+	-	-	-	-	-	+	+	+	++	+	-	+	+	+	++	+	+	+	-
Oredo BK	-	-	+	-	+	-	-	+	+	+	+	-	+	-	+	+	+	+	-	+	-	+
Ovia BK	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+

+++=more than 20 plants per unit area, ++=10 - 20 plants, + = less than 10 plants, - =No plant presence. GN generator plant house, BK bakery, MW mechanic workshop, SP spare parts shop. AH = Acanthospermum hispidum, AA = Achyranthes aspera, AZ = Acroceras zizanioides, AC = Ageratum conyzoides, AR = Alternanthera repens, AT = Andropogon tectorum, AX = Axonopus compressus, CE = Cyperus esculentus, CR = Cyperus rotundus, EO = Echinochloa obtusifolia, EI = Eleusine indica, EH = Euphorbia hyssopifolia, KR = Kyllinga erecta, LC = Leptochloa caerulescens, MA = Mariscus alternifolios, OH = Oldenlandia herbacea, PM = Panicum maximum, PS = Paspalum scrobiculatum, PP = Peperomia pellucid, PA = Phyllantus amarus, SA = Sida acuta, TP = Tridax procumbens.

Discussion

The present study provides information on heavy metal contents and microbial diversity of waste engine oil-polluted soil in some public and commercial centres in Benin city metropolis. Heavy metals present in the soil samples include chromium, manganese, zinc, iron and cadmium. It is not clear exactly what levels of heavy metals in soil are safe or unsafe but microbes has proven to be effective in the bioremediation of these heavy metal so as to enhance crops/plant growth (Robinson *et al.,* 2001; Turgut, 2003). Whisman *et al*. (1974) observed that most heavy metals like V, Pb, Ni and Fe that are below detection in unused lubricants oil gave high concentration values in used oil. However, after exposure of soil to experimental conditions and treatments, some of these heavy metals were below detectable limit.

A number of factors contribute to heavy metal reduction in polluted soils, including soil physicochemistry as well as biological action, most importantly the activities of local resident plant species (Ikhajiagbe and Chijioke-Osuji, 2012). Cataldo and Wildung (1978) reported that one of the factors governing metal availability to plants in soils is the solubility of the metal associated with the solid phase, since in order for root uptake to occur, a soluble species must exist adjacent to the root membrane for some finite period. The rate of release and form of this soluble species will have a strong influence on the rate and extent of uptake and, perhaps, mobility and toxicity in the plant and consuming animals.

The mobility of most heavy metals in the soil and subsoil depends on the physico-chemical properties of the solid and liquid phases. Many chemical changes may occur during the movement of water through the soil including dissolution/precipitation, adsorption/desorption, degradation, filtration and a variety of transport processes (Fic and Schroter, 1989). Trace metals can be found in different physical phases including particulate, colloidal and truly dissolved phases. The fate and transport of metals in the environment depends on these phases and the interaction between them (Wilhelmy *et al.*, 1996).

In this research, it was noticed that there were more heavy metals at mechanic workshop compared to other locations used. This is could be attributed to frequent discard of waste engine oil (WEO) and petroleum by automobile engineers. These WEO and petroleum have been reported to contain relatively large amounts of hydrocarbons, including the highly toxic polycyclic aromatic hydrocarbon (PAH) ((Wang *et al*., 2000) and toxic heavy metals (Edeberi and Nwanokwale, 1981).

In this study, microorganisms isolated from the soil samples include six (6) bacteria which are *Bacillus substilis, Sarcina sp, Clostridium sp, C. perfringens, Micrococcus sp, M. luteus* and nine (9) fungal species which include *Aspergillus niger, A. flavus, Rhizopus stolonifer, Penicillium sp, P. notatum, Geotrichum* sp., *Mucor* sp., *Trichoderma sp., Fusarium solani.*

The most prevalent microorganisms in soil around generator plant and soil within auto mechanic workshop are *Bacillus subtilis* and *Aspergillus niger* while the most prevalent microorganisms in soil within auto spare parts stores are *Clostridium sp*. and *Aspergillus niger. Aspergillus niger* was the most prevalent microorganism in all the soil samples. Microorganisms were more prevalent in soil samples within auto spare parts stores, this may be because, lesser oil pollution and heavy metals were present in the soil samples from auto spare parts stores compared to soil within auto mechanic workshop and soil around generator plant. These microorganisms may have been involved in the remediation process, considering the fact that their prevalence,

even in higher concentrations of pollution, may signify tolerance to these pollutants. These identified as active members of bioremediation microbial consortia by Ekundayo and Obuekwe (1997); Yogambal and Karegoudar (1997); Remero *et al.* (2001); April *et al*. (2000). Irwin (1998) reported that if site assessments reveal that species of indigenous microorganisms are unable to degrade target contaminants, exogenous microorganisms with the required biochemical capabilities can be introduced to successfully degrade specific waste compounds. Bacteria in bio-remediation are prolific. Certain bacteria belonging to the *Bacillus* and *Pseudomonas* species have these desirable characteristics: they consume organic waste thousands of times faster than the types of bacteria that are naturally present in the waste; they grow and reproduce easily, are non-pathogenic, and do not produce foul odours or gas (Efeovbokhan *et al*., 2012). Fungi have been used in the treatment of waste and waste waters and the role of fungi in the bioremediation of various hazardous and toxic compounds in soils and sediments have been established. They have also shown the removal of metals and degradation and mineralization of phenols and other phenolic compound, petroleum hydrocarbons, polycyclic aromatic hydrocarbons, poly chlorinated biphenyls, chlorinated insecticides and pesticides and other substances in various matrices. Saprophytic fungi degrade organic matter to release carbon, nitrogen, and other elements locked up in complexes (Atlas, 1995)

Twenty-two (22) plant species such as *Acanthosperum hispidum, Achyranthes aspera, Acroceras zizanioides, Ageratum conyzoides, Alternanthera repens, Andropogon tectorum, Axonopus compressus, Cyperus esculentus, Cyperus rotundus, Echinochloa obtusifolia, Eleusine indica, Euphorbia hyssopifolia, Kyllinga erecta, Leptochloa caerulescens, Mariscus alternifolios, Oldenlandia herbacea, Panicum maximum, Paspalum scrobiculatum, Peperomia pellucida, Phyllantus amarus, Sida acuta* and *Tridax precumbens* which accumulated heavy metal were found in the waste engine oil soil samples. Soil samples around generator plant had the highest predominance of plant species growth followed by soil samples around bakery and within auto mechanic workshop while soil within auto spare parts stores had the least prevalence of plant species growth. The most predominant plant species in the all the soil samples was *Eleusine indica.* The predominance of *Eleusine indica* in soil samples in all the sites visited indicates that they are the most tolerant to spent engine oil and its heavy metal contents. This phenomenon demonstrates that the family has the highest genetic potential to clean up spent engine oil-contaminated soil.

Conclusion

The present study thus indicates that soil samples used in this study contain high level of heavy metals and present microorganisms that can likely remediate and manage spent engine oil – contaminated soils. Plant species and their families that are tolerant to spent engine oil – polluted soils were also identified.

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