

1 EFFECTS OF WASTE ENGINE OIL ON THE MICROFLORA AND PHYSICO- 2 CHEMICAL QUALITY OF SOILS FROM TWO WEST AFRICAN COUNTRIES.

3 Abstract.

4 A study of the bioremediation of diverse levels of simulated contamination of waste engine
5 oil (WEO) in soil using natural attenuation, bioaugmentation, ~~Myc~~  mediation and
6 phytoremediation was carried out. This required a preliminary study of the microbiological
7 and physico-chemical quality of waste engine oil contaminated soils from as it were, a
8 “naturally” contaminated site, as a check that the characteristics of the “natural” and
9 simulated samples match. Thus, WEO contaminated soil samples were randomly collected
10 with controls and pooled together respectively from Owerri, Imo State, in the Eastern part of
11 Nigeria and Kumasi in the Ashanti region of Ghana.  The most occurring microorganism
12 isolated from all the samples is *Bacillus* sp. The ~~mu~~  of the total heterotrophic bacterial
13 counts (THBC), total heterotrophic fungal counts (THFC), hydrocarbon utilizing bacterial
14 count (HUBC) and hydrocarbon utilizing fungal counts (HUFC) for the WEO contaminated
15 samples from Kumasi and Owerri were 3.85 ± 0.01 and 3.89 ± 0.01 ; 3.79 ± 0.07 and 3.98 ± 0.01 ;
16 4.37 ± 0.01 and 4.35 ± 0.01 and 4.12 ± 0.02 and 4.22 ± 0.01 respectively. Some Physico-chemical
17 parameters like pH, total hydrocarbon, total organic carbon, phosphate, nitrate and sulphate
18 content; polycyclic aromatic hydrocarbon (PAH) and heavy metals content in the respective
19 samples were analyzed.

20
21 **Keywords.** Microflora, Physico-Chemical, Waste Engine  ~~Oil~~, ~~PAH~~  Heavy Metal,
22 Contaminated Soils, Owerri, Kumasi.

23 Introduction.

24 Millions of gallons of waste engine oil are disposed in trash, on land and into sewers.
25 Pollution with waste engine oil is in fact an endemic problem in most African countries. This
26 is because of its discharge into the environment without adequate treatment as a result of non
27 availability of waste engine oil recycling facilities. Furthermore, most open plots of land are
28 usually leased out by their owners to vehicle mechanics and other artisans who utilize them
29 as their workshops. These mechanics thus dispose of these substances in and around these
30 places. Some even dispose of WEO into run-off, gutters, water drainages, etc, thus easing the
31 access of WEO into nearby agricultural lands and/or water bodies. Soils being a component
32 of natural ecosystems are usually worst hit. The ecosystem is altered; agricultural activities
33 are affected as the soil receives water soluble fractions of the WEO. Some are recycled but it
34 has the potential of contaminating soil, groundwater and surface water. Soil contamination by

35 petroleum hydrocarbons is one of the world's most common environmental problems because
36 they belong to the groups of persistent organic contaminants (Huang et al, 2005; US EPA
37 2000).

38

39 Generally, the accumulation of contaminants in soils can have destructive effects on the
40 environment and human health; it can enter the food chain and seriously affect animal and
41 human health (Khan, 2005). Thus, suitable solutions for management or disposal of waste
42 engine oil and indeed all other petroleum wastes must be found.

43

44 The aim of this study, apart from it being a preliminary study, is to check between Owerri in
45 Nigeria and Kumasi in Ghana, the effects of waste engine oil on the microflora and physico-
46 chemical quality of soil, which level of contamination is higher and/or has become a matter
47 of public health concern.

48

Literature Review.

49 The availability of oil in any economy is usually a major boost to its development but this
50 does not come without the attendant effects on the immediate environment from which it is
51 explored (Ikhajiagbe et al, 2014), distributed and its waste products disposed. Environmental
52 pollution with petroleum products has been recognized as one of the most serious current
53 problems (Udeani et al, 2009; Hamzah et al, 2010; Berte-Corti et al, 2007). Pollution
54 phenomena results in inter-woven negative circumstances; it causes significant changes in the
55 zoosphere and phytosphere, as well as to microorganisms, leading to the disappearance of
56 many species; decrease in soil fertility and support for plant and animal life and consequently
57 humans who benefit suffer (Engelking, 2000).

58

59 According to Ayotamuno et al, 2009, hydrocarbons are considered among the strongest soil
60 pollutants. Any form of hydrocarbon spills on both land and in water can have devastating
61 effects on the biota of an environment because they contain poisonous compounds that
62 constitute potential danger to plants and animals. These poisons can pass through the food
63 web of an area and may eventually be eaten by humans (Gibson and Parales, 2000; Semple et
64 al, 2001; Stroud et al, 2007, 2009). Contamination of il with petroleum derivatives like
65 WEO is often more in municipal soils (~~Adam et al, 2002~~; Clark, 2003) because of the
66 presence of diverse kinds of automobiles and machinery which has resulted in an increase in
67 the use of motor oils and other lubricants. This could result in spillages which can constitute

68 nuisance to the environment due to their persistent nature (Husaini et al, 2008; Plohl et al,
69 2002). Furthermore, it could make the soil unsuitable for plant growth for awhile or several
70 months or years (~~Obire and Nwaubeta, 2002~~).
71

72 Waste engine oil is a common environmental contaminant (US EPA, 2001), it can cause great
73 damage to sensitive environments (Hans, 1993) and alter microbial population (Toledo et al,
74 2000; Hamzah et al, 2010). It may contain components like Lead, Cadmium and other
75 potentially toxic metals (Joanne et al, 2008). WEO on soil changes soil colour, this may have
76 untold health hazard on the technicians/ artisans who mostly live in their workshops because
77 they cannot afford the cost of good accommodation (Pic. 1); this hardens the soles of their
78 feet most times which may alter their ability to move normally over time (Udeani et al, 2009).
79

80 The fact that hydrocarbon contamination of soil is of particular environmental concern
81 because they exhibit carcinogenic and mutagenic properties (~~Mozuk~~ and Piotrowska-Seget,
82 ~~2010~~) has increased scientific interest in examining its distribution, fate and behavior in the
83 environment (Alexander, 1995, 2000; Semple et al, 2001, 2003; Stroud et al, 2007, 2009).
84 Engine oils are altered during use by vehicles, motor bikes, generators and other machinery
85 because of the breakdown of additives, contamination with products of combustion and the
86 addition of metals from the wear and tear of the engine. It is recognized that the major
87 components consist of aliphatic and aromatic hydrocarbons such as phenol, naphthalene,
88 benzo(a)anthracene, benzo(a)pyrene, fluoranthene, lead, cadmium and other potentially toxic
89 metals (Jain et al, 2009). These heavy metals are dangerous because they tend to accumulate
90 and are stored faster than they are broken down (Abioye, 2011); they cause nutrient
91 imbalance; toxicity of soils and unfavorable plant growth (if any) (Odu, 2000) due to
92 reduction in the level of available plant nutrients or a rise in toxic levels of certain elements
93 like Iron and Zinc.



Pic. 1: A mechanic resident at the Orji, Owerri, Imo State, Nigeria mechanic village having a meal.



Pic. 2. Magazine Mechanic Village in Kumasi, Ghana



Pic. 3. Orji Mechanic Village in Owerri, Imo State

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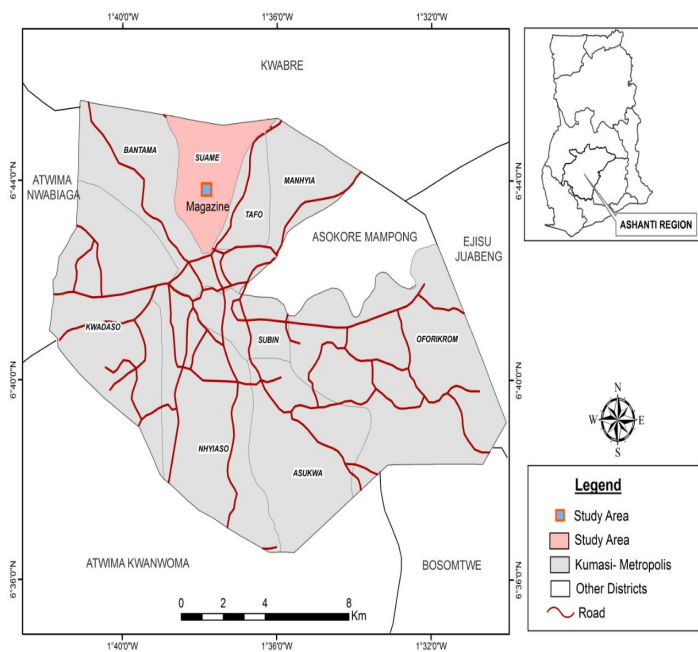


Fig. 1. Map of study area in Suame, Kumasi, Ghana

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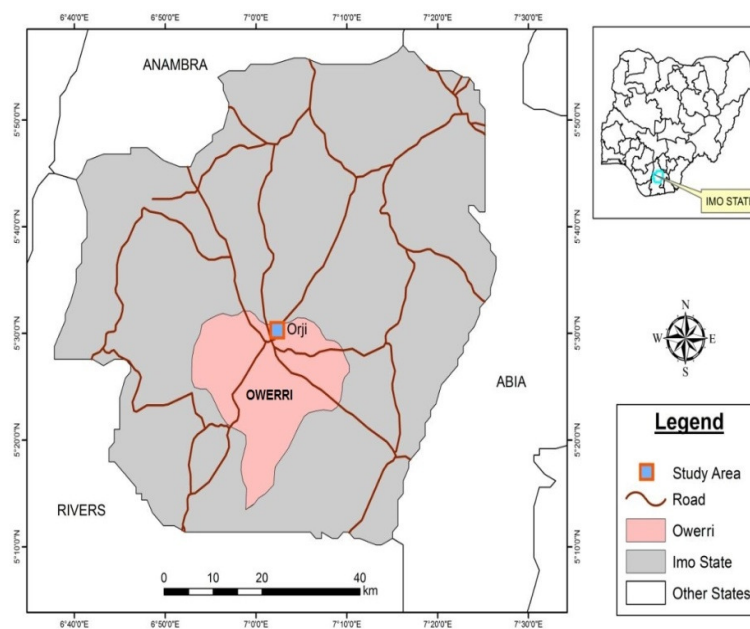


Fig. 2. Map of study area in Owerri, Imo State, Nigeria

Materials and Methods.



a) Sample collection

~~WEO~~ contaminated soil samples with their respective controls were collected randomly at a depth of 0 – 15cm from four locations each in Kumasi, Ghana and Owerri, Nigeria, for investigation. These were pooled together and designated HCS-K and HCS-O respectively and their controls C-K and C-O respectively.

Sample One (HCS-K) was collected from the *Magazine* Mechanic Village in Kumasi, Ghana (Pic. 2, Fig. 1). Kumasi is the capital of the Ashanti Region of Ghana, approximately 300 miles (480 km) north of the Equator and 100 miles (160 km) north of the Gulf of Guinea. It is the largest city in Ghana with a population size of two million and thirty five thousand and sixty four (2,035,064) (GSS, 2012).

Sample two (HCS-O) was collected from the *Orji* Mechanic Village in *Owerri*, Imo State, Nigeria (Pic. 3, Fig. 2). Owerri is the capital of Imo State, in the south-east region of Nigeria. It consists of twenty seven (27) local government areas and is approximately 5,530 square kilometres in area. It is bordered to the east by the Otamiri River and the south by the Nworie River with a population size of three million, nine hundred and thirty four thousand, eight hundred and ninety nine (3,934,899) (NPC, 2006).

b) Microbiological analyses

The total heterotrophic bacterial and fungal counts (THBC and THFC) and hydrocarbon utilizing bacterial and fungal counts (HUBC and HUFC) were taken after culture of the respective soil samples using vapour phase transfer (Chikere and Chijioke-Osuji, 2006).

For the characterization and identification of the bacterial isolates, molecular assays were carried out on the samples using nucleic acid as a template. Following DNA extraction, Polymerase Chain reaction (PCR) (TP 63) was employed to amplify copies of the partial 16S fragment of rDNA in vitro. Sequencing reactions (TP 66) were undertaken and identification was done by comparing the sequence with those available in EMBL via the EBI.

Characterization and identification of fungal isolates was done using ITS rDNA sequencing analysis. Sequencing reactions were undertaken by comparing the sequence obtained with those available in the European Molecular Biology Laboratory (EMBL) through the

European Bioinformatics Institute (EBI) and the International Subcommittee on *Trichoderma* and *Hypocrea* (ISTH).

c) Physico-chemical analyses

The respective soil samples were examined for some physico-chemical parameters like pH (using distilled water method); total hydrocarbon content (using APHA colorimetry method); total organic carbon (using refluxing method); phosphate content (using colorimetry method); nitrate content (using brucine method) and sulphate content (using turbidimetric method).

d) Analyses of polycyclic aromatic hydrocarbons and heavy metals

Polycyclic aromatic hydrocarbon (PAH) content of the respective samples was carried out using the cold extraction GC-FID method while a check on the heavy metals content was done using the AAS:APHA methods 3010-3110 (APHA, 1985; ASTM, 1982; ERI, 1999).

Results.

Identified microorganisms isolated from controls and hydrocarbon contaminated soils obtained from Kumasi and Owerri are presented in Table 1. The microorganisms that occurred in the hydrocarbon contaminated soil sample from Kumasi are *Bacillus subtilis*, *Rhizopus oryzae* and *Trichoderma spirale* while its control soil had *Bacillus cereus*, *Aspergillus carbonarius*, *Cunninghamella* and *Rhizopus* sp. The microorganisms that were identified from the hydrocarbon contaminated soil from Owerri are *Bacillus subtilis*, *Rhizopus oryzae*, *Rhizopus* sp. and *Trichoderma spirale*; its control had *Bacillus cereus*, *Aspergillus carbonarius*, *Cunninghamella* and *Rhizopus* sp.

The mlog of the total heterotrophic and hydrocarbon utilizing bacterial and fungal counts are shown in Table 2. The mlog of the THBC for the HCS-K and HCS-O samples were 3.85 ± 0.01 and 3.79 ± 0.01 respectively while their controls had mlog of THBC of 3.89 ± 0.01 and 3.69 ± 0.01 cfu/g respectively. There was a significant difference ($p < 0.05$) between the mean THBC of all the samples.

The mlog of the Total Heterotrophic Fungal Counts (THFC) was highest in CS-O (4.24 ± 0.02).

The mlog of the Hydrocarbon Utilizing Bacterial Counts (HUBC) for the HCS-K and HCS-O samples were 4.37 ± 0.05 and 4.35 ± 0.03 respectively while their controls had 4.06 ± 0.05 and 3.98 ± 0.03 respectively. There was a significant difference between mlog of the HUBC of all the samples.

The mlog of the Hydrocarbon Utilizing Fungal Counts (HUFC) for the HCS-K and HCS-O samples were 4.12 ± 0.02 and 4.22 ± 0.01 respectively while their controls had 4.24 ± 0.01 and 4.34 ± 0.02 respectively; there was no significant difference ($P < 0.05$) between the HUFC of all the samples.

The physicochemical parameters of hydrocarbon contaminated soil samples and their controls are presented in Table 3.

The pH values of the respective samples range between 5.18 ± 0.57 and 5.40 ± 0.29 with all values having no significant difference at $p < 0.05$.

The total hydrocarbon contents (THC) have the lowest values the controls, with sample CS-O having the least THC followed by CS-K which has a value of 675.50 ± 8.03 . The THC of HCS-O is 4305.25 ± 0.86 while that of HCS-K is 5835.25 ± 0.08 .

The percentage total organic carbon (TOC) contents of the control samples CS-K and CS-O were 6.11 ± 0.05 and 6.12 ± 0.09 respectively, both values do not differ significantly ($p < 0.05$) whereas samples HCS-K and HCS-O have TOC contents of 9.09 ± 0.06 and 9.89 ± 0.01 respectively, these values differ significantly at $p < 0.05$.

The phosphate content of the control samples CS-K and CS-O were 9.65 ± 0.08 and 9.925 ± 0.01 respectively. Both values are not significantly different at $p < 0.05$. HCS-K and HCS-O samples have phosphate contents of 127.7 ± 0.57 and 97.200 ± 0.08 mg/kg respectively. They differ significantly at $p < 0.05$.

The mean nitrate contents of samples CS-K and CS-O are 0.27 ± 0.02 and 0.29 ± 0.01 mg/kg respectively. The values have no significant difference at $p < 0.05$ while Samples HCS-K and HCS-O have nitrate contents of 31.25 ± 0.02 and 15.78 ± 0.002 mg/kg respectively.

The mean Sulphate content of the respective controls, CS-K and CS-O were 41.65 ± 0.01 and 41.40 ± 0.02 while samples HCS-K and HCS-O have Sulphate contents of 157.675 ± 0.05 and 281.500 ± 0.04 respectively.

201 **Table 1. Microorganisms isolated from hydrocarbon contaminated soils obtained from Kumasi, Owerri and their controls.**

Sample	Species	Family	Order	Class	Shape	Chromogenesis
HCS-K	<i>Bacillus subtilis</i> (Bacteria)	Bacillaceae	Bacillales	Bacilli	Circular & Undulating, motile, tree-like and spreading	Creamy White
	<i>Rhizopus oryzae</i> (Fungi)	Mucoraceae	Mucorales	Mucormycotina	Irregular and Spreading	White and fluffy with dark and white dense spores
	<i>Trichoderma spirale</i> (Fungi)	Hypocreaceae	Hypocreales	Sordariomycetes	Circular	Pink coloured and pasty
CS-K	<i>Bacillus cereus</i> (Bacteria)	Bacillaceae	Bacillales	Bacilli	Circular & Undulating, motile, tree-like and spreading	Creamy White
	<i>Aspergillus carbonarius</i> (Fungi)	Trichocomaceae	Eurotiales	Eurotiomycetes	Circular	White and fluffy with dark spores
	<i>Rhizopus</i> sp. (Fungi)	Mucoraceae	Mucorales	Mucormycotina	Circular	White and fluffy base with dense white spores
	<i>Cunninghamella</i> sp. (Fungi)	Cunninghamellaceae	Mucorales		Circular with rough edges	White and pasty
HCS-O	<i>Bacillus subtilis</i> (Bacteria)	Bacillaceae	Bacillales	Bacilli	Circular & Undulating, motile,	Creamy White

					tree-like spreading and	
	<i>Rhizopus oryzae</i> (Fungi)	Mucoraceae	Mucorales	Mucormycotina	Irregular Spreading and	White and fluffy with dark and white dense spores
	<i>Rhizopus</i> sp. (Fungi)	Mucoraceae	Mucorales	Mucormycotina	Circular	White and fluffy base with dense white spores
	<i>Trichoderma spirale</i> (Fungi)	Hypocreaceae	Hypocreales	Sordariomycetes	Circular	Pink coloured and pasty
CS-O	<i>Bacillus cereus</i> (Bacteria)	Bacillaceae	Bacillales	Bacilli	Circular & Undulating, motile, tree-like and spreading	Creamy White
	<i>Aspergillus carbonarius</i> (Fungi)	Trichocomaceae	Eurotiales	Eurotiomycetes	Circular	White and fluffy with dark spores
	<i>Rhizopus</i> sp. (Fungi)	Mucoraceae	Mucorales	Mucormycotina	Circular	White and fluffy base with dense white spores
	<i>Cunninghamella</i> sp. (Fungi)				Circular with rough edges	White and pasty

202

203 HCS-K – Hydrocarbon contaminated soil from Kumasi; CS-K – Control soil from Kumasi; HCS-O – Hydrocarbon contaminated soil from Owerri; CS-O – Control soil from
 204 Owerri.

205 **Table 2. Mean logarithm of microbial counts in contaminated and control soil samples**

Sample type	Mean logarithm of microbial counts			
	THBC	THFC	HUBC	HUFC
CS-K	3.79 ± 0.01^b	3.91 ± 0.01^d	4.06 ± 0.01^b	4.24 ± 0.01^d
HCS-K	3.85 ± 0.01^c	3.79 ± 0.07^c	4.37 ± 0.01^{ef}	4.12 ± 0.02^c
CS-O	3.69 ± 0.01^a	4.24 ± 0.02^f	3.98 ± 0.03^a	4.34 ± 0.02^e
HCS-O	3.89 ± 0.01^d	3.98 ± 0.01^e	4.35 ± 0.01^e	4.22 ± 0.01^d

206 Means $\pm$ SD (in same column) with different letters in superscripts differ significantly ($p <$
 207 0.05

208

209 The Polycyclic Aromatic Hydrocarbon (PAH) contents of the respective samples are shown
 210 in Table 4.

211 The mean levels of naphthalene in the control samples, CS-K and CS-O were below
 212 detectable limits (0.00 ± 0.00) while sample HCS-K has 5.40 ± 0.07 and HCS-O has $5.40 \pm$
 213 0.33 . The PAH contents for all the samples are significantly different from the controls but do
 214 not differ significantly between themselves at $p < 0.05$.

215 Acenaphthalene in the CS-K and CS-O samples were 0.00 ± 0.00 each, HCS-K has $5.68 \pm$
 216 0.07 while HCS-O has 5.63 ± 0.24 .

217 The levels of Bromonaphthalene in samples CS-K and CS-O were both 0.00 ± 0.00 whilst
 218 HCS-K and HCS-O have 5.43 ± 0.03 and 5.85 ± 0.41 respectively.

219 Samples CS-K and CS-O both have Acenaphthene levels below detectable limits (0.00 ± 0.00
 220 mg/l) while samples HCS-K and HCS-O have 5.43 ± 0.10 and 5.45 ± 0.02 respectively.

221

222 The levels of Fluorene in the samples CS-K and HCS-K were 5.90 ± 0.02 and 6.33 ± 0.04
 223 respectively while CS-O and HCS-O have Fluorene levels of 5.81 ± 0.03 and 6.57 ± 0.01
 224 respectively.

225 The Anthracene levels in samples CS-K, HCS-K, CS-O and HCS-O were 5.97 ± 0.02 , $9.43 \pm$
 226 0.03 , 5.79 ± 0.02 and 6.55 ± 0.02 respectively.

227 Phenanthrene level in samples CS-K, HCS-K, CS-O and HCS-O were 6.02 ± 0.03 , $5.55 \pm$
 228 0.01 , 6.02 ± 0.04 and 8.38 ± 0.05 respectively.

229 Fluoranthene levels in control samples CS-K and CS-O were both 0.00 ± 0.00 whereas HCS-
 230 K and HCS-O have 6.03 ± 0.02 and 5.68 ± 0.01 respectively.

231 The mean levels of Pyrene in samples CS-K and CS-O were both 0.00 ± 0.00 while samples
 232 HCS-K and HCS-O both have Pyrene level of 5.83 ± 0.03 .

233 The mean levels of Benzo-a-anthrace was 0.00 ± 0.00 for samples CS-K and CS-O. Samples
 234 HCS-K and HCS-O have Benzo-a-anthrace levels of 6.61 ± 0.01 and 14.65 ± 2.01
 235 respectively.

236 The levels of Chrysene in all the control samples were 0.00 ± 0.00 mg/l while samples HCS-
 237 K and HCS-O have Chrysene level of 6.29 ± 0.03 and 20.15 ± 0.02 mg/l respectively.

238 The levels of Benzo(b,j,k)fluoranthene in samples HCS-K and HCS-O were 8.38 ± 0.05 and
 239 16.15 ± 0.02 mg/l respectively. The controls, CS-K and CS-O both have 0.00 ± 0.00
 240 Benzo(b,j,k)fluoranthene levels.

241 The levels of Benzo(a)pyrene in samples CS-K and CS-O were 0.00 ± 0.00 , samples HCS-K
 242 and HCS-O have 16.45 ± 1.05 and 15.25 ± 0.16 respectively.

243 The mean levels of Indeno(1,2,3cd)pyrene in samples CS-K and CS-O were 0.00 ± 0.00 .
 244 Samples HCS-K and HCS-O have Indeno(1,2,3cd)pyrene levels at 6.55 ± 0.08 and $26.95 \pm$
 245 2.02 respectively.

246 The mean levels of Dibenzo(a,h)anthracene in samples CS-K and CS-O were 0.00 ± 0.00
 247 while samples HCS-K and HCS-O have 9.23 ± 0.01 and 15.23 ± 0.05 respectively.

248 The mean levels of Benzo(g,h,i)perylene in the respective samples differ significantly with
 249 the controls at $p < 0.05$. Samples HCS-K and HCS-O have their Benzo(g,h,i)perylene levels at
 250 8.30 ± 0.01 and 10.28 ± 0.02 respectively.

251 The levels of heavy metals namely Iron (Fe), Manganese (Mn), Nickel (Ni), Zinc (Zn), Lead
 252 (Pb) and Chromium (Cr) in WEO contaminated soil samples and their controls are shown in
 253 Table 5.

254 Fe levels in the samples differ significantly with the controls at $p < 0.05$. Samples CS-K and
 255 CS-O have Fe levels of 63.43 ± 0.25 and 63.18 ± 0.01 respectively, both values have no
 256 significant difference at $p < 0.05$ while samples HCS-K and HCS-O have Fe levels of $93.75 \pm$
 257 0.02 and 55.37 ± 0.25 respectively.

258 The levels of Mn in samples CS-K and CS-O were 1.05 ± 0.05 and 1.04 ± 0.01 respectively;
 259 these values do not differ significantly but differ significantly from the rest of the samples at
 260 $p < 0.05$. Samples HCS-K and HCS-O have Mn levels of 0.90 ± 0.03 and 0.77 ± 0.01
 261 respectively.

262 Ni levels in all the samples were below detectable limits but for sample HCS-K which has
 263 0.11 ± 0.01 mg/l Ni level and differs significantly with the level of Ni in the rest of the
 264 samples at $p < 0.05$.

265 The level of Zn in samples CS-K and CS-O were 1.96 ± 0.14 and 1.945 ± 0.02 respectively.
 266 These values do not differ significantly at $p < 0.05$. Samples HCS-K and HCS-O have Zn
 267 levels of 8.75 ± 0.04 and 5.100 ± 0.02 respectively. These values differ significantly at
 268 $p < 0.05$.
 269 Samples CS-K and CS-O both have Pb levels of 0.77 ± 0.03 , these do not differ significantly
 270 at $p < 0.05$. Samples HCS-K and HCS-O have Pb levels of 6.81 ± 0.01 and 1.55 ± 0.01
 271 respectively.
 272 The levels of Cr was detectable in only samples CS-K, HCS-K and CS-O which have $0.26 \pm$
 273 0.01 , 0.29 ± 0.03 and 0.25 ± 0.03 respectively, the values differ significantly at $p < 0.05$.

274 **Table 3. Physicochemical parameters of WEO contaminated soil samples and their controls**

	pH	THC	TOC	P ⁺	N ³⁺	S ⁺
CS-K	5.40 ± 0.29 ^a	675.50 ± 8.03 ^a	6.11 ± 0.05 ^a	9.65 ± 0.08 ^a	0.27 ± 0.02 ^a	41.65 ± 0.01 ^a
HCS-K	5.18 ± 0.57 ^a	5835.25 ± 0.08 ^d	9.09 ± 0.06 ^b	127.70 ± 0.57 ^c	31.25 ± 0.02 ^f	157.675 ± 0.05 ^e
CS-O	5.40 ± 0.29 ^a	670.25 ± 0.22 ^a	6.12 ± 0.09 ^a	9.25 ± 0.01 ^a	0.29 ± 0.01 ^a	41.40 ± 0.02 ^b
HCS-O	5.20 ± 0.26 ^a	4305.25 ± 0.86 ^c	9.89 ± 0.01 ^d	97.200 ± 0.08 ^c	15.78 ± 0.002 ^d	281.500 ± 0.04 ^f

Means ± SD (in same column) with different letters in superscripts differ significantly (p < 0.05)

- All values apart from pH are in mg/kg.

275
 276 HCS-K – Hydrocarbon contaminated soil from Kumasi; CS-K – Control soil from Kumasi; HCS-O – Hydrocarbon contaminated soil from
 277 Owerri; CS-O– Control soil from Owerri.

278

279 **Table 4. PAH content of WEO contaminated soil samples and their controls (mg/kg)**

PAH	Samples			
	CS-K	HCS-K	CS-O	HCS-O
Naphthalene	0.00 ± 0.00 ^a	5.40 ± 0.24 ^b	0.00 ± 0.00 ^a	5.40 ± 0.33 ^b
Acenaphthylene	0.00 ± 0.00 ^a	5.68 ± 0.07 ^c	0.00 ± 0.00 ^a	5.63 ± 0.02 ^c
Bromonaphthalene	0.00 ± 0.00 ^a	5.43 ± 0.03 ^b	0.00 ± 0.00 ^a	5.85 ± 0.41 ^c
Acenaphthene	0.00 ± 0.00 ^a	5.43 ± 0.10 ^b	0.00 ± 0.00 ^a	5.45 ± 0.02 ^b
Fluorene	5.90 ± 0.02 ^b	6.33 ± 0.04 ^f	5.81 ± 0.03 ^a	6.57 ± 0.01 ^g
Anthracene	5.97 ± 0.02 ^b	9.43 ± 0.03 ^g	5.79 ± 0.02 ^a	6.55 ± 0.02 ^d
Phenanthrene	6.02 ± 0.03 ^c	5.55 ± 0.01 ^b	6.02 ± 0.04 ^c	8.38 ± 0.05 ^d
Fluoranthene	0.00 ± 0.00 ^a	6.03 ± 0.02 ^d	0.00 ± 0.00 ^a	5.68 ± 0.01 ^c
Pyrene	0.00 ± 0.00 ^a	5.83 ± 0.03 ^c	0.00 ± 0.00 ^a	5.83 ± 0.03 ^c
Benzo-a-anthrace	0.00 ± 0.00 ^a	6.61 ± 0.01 ^b	0.00 ± 0.00 ^a	14.65 ± 2.01 ^d
Chrysene	0.00 ± 0.00 ^a	6.29 ± 0.03 ^b	0.00 ± 0.00 ^a	20.15 ± 0.02 ^c
Benzo(b,j,k)fluoranthene	0.00 ± 0.00 ^a	8.38 ± 0.05 ^b	0.00 ± 0.00 ^a	16.15 ± 0.02 ^d
Benzo(a) pyrene	0.00 ± 0.00 ^a	16.45 ± 1.05 ^d	0.00 ± 0.00 ^a	15.25 ± 0.16 ^c
Indeno(1,2,3cd) pyrene	0.00 ± 0.00 ^a	6.55 ± 0.08 ^b	0.00 ± 0.00 ^a	26.95 ± 2.02 ^c
Dibenzo(a,h)anythracene	0.00 ± 0.00 ^a	9.23 ± 0.01 ^c	0.00 ± 0.00 ^a	15.23 ± 0.05 ^d
Benzo(g,h,i) perylene	0.00 ± 0.00 ^a	8.30 ± 0.01 ^b	0.00 ± 0.00 ^a	10.28 ± 0.02 ^c

280 Means ± SD (in same **row**) with different letters in superscripts differ significantly (p < 0.05)

281
282 **Table 5. Levels of heavy metals in WEO contaminated soil samples and their controls**
283 **(mg/kg)**

Samples	Heavy metals					
	Fe	Mn	Ni	Zn	Pb	Cr
CS-K	63.43 ± 0.25 ^e	1.05 ± 0.05 ^c	0.00 ± 0.00 ^a	1.96 ± 0.14 ^b	0.77 ± 0.03 ^c	0.26 ± 0.01 ^{bc}
HCS-K	93.75 ± 0.02 ^f	0.90 ± 0.03 ^b	0.11 ± 0.01 ^b	8.75 ± 0.04 ^d	6.81 ± 0.01 ^e	0.29 ± 0.03 ^c
CS-O	63.18 ± 0.01 ^e	1.04 ± 0.01 ^c	0.00 ± 0.00 ^a	1.945 ± 0.02 ^b	0.77 ± 0.03 ^c	0.25 ± 0.03 ^b
HCS-O	55.37 ± 0.25 ^c	0.77 ± 0.01 ^a	0.00 ± 0.00 ^a	5.100 ± 0.02 ^c	1.55 ± 0.01 ^d	0.00 ± 0.00 ^a

284 Means ± SD (in same column) with different letters in superscripts differ significantly (p < 0.05)

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Discussion.

The discharge of waste engine oil into the environment is common in most developing countries. Taking Nigeria for example, about 20 million gallons of WEO is generated annually from mechanic workshops and discharged into the environment (Faboya, 1997; Adegoroye, 1997). Following the report of USEPA (1996), just one litre of used (waste) engine oil is enough to contaminate one million gallons of freshwater. Used engine oil is also capable of rendering the environment unsightly and constitutes a potential threat to humans, animals and vegetation (Adelowo et al, 2006). The quality of life on earth is highly related to its environment. For life to sustain, it is very important that the environment remains clean and healthy. In recent times, due to the increase in industrialization and utilisation of petroleum products, not to forget negligence using them, the environment is getting damaged. To clean up especially the subsurface hydrocarbon concentration is a worldwide problem whose solution is bioremediation which has potentials to provide a low cost, non intrusive, natural method to render toxic substances in soil less harmful or even harmless over time.

i) Microorganisms isolated from WEO contaminated soils and their controls

The bacteria, *Bacillus cereus* occurred in all the control samples (CS-O and CS-K), these organisms are predominant in soil (Amin et al., 2015) and are known to be facultative anaerobes (Wikipedia, 2014). Amin et al. (2015) had in their study on “identification of *Bacillus* species from soil and evaluation of their antimicrobial properties” isolated *B. cereus* from normal soils in Iran. Yilmaz et al. (2006) had in their study about the antimicrobial activities of some *Bacillus* spp. strains isolated *Bacillus cereus* from soils in Turkey. Fen et al. (2012) in their study on the isolation, identification and characterization of a glyphosate degrading bacterium, *Bacillus cereus* CB4 also isolated this bacterium from soil in China. Villain et al. (2006) in their study isolated *B. cereus* from soils in America. These reports therefore suggest that *Bacillus cereus* can be isolated from normal soils in any geographical region of the world.

Bacillus subtilis was isolated from the WEO contaminated soil samples (HCS-K and HCS-O). This suggests that the species *subtilis* has affinity for hydrocarbons. Al-Sharidah et al. (2000) had in their study on the isolation and characterization of two hydrocarbon-degrading *Bacillus subtilis* strains from oil contaminated soil of Kuwait corroborated this finding. In their report, the hydrocarbon sources were diesel fuel, n-decane or naphthalene. In the report of Khalid and

Shaimaa (2016), conducted in Baghdad-Iran, there was a 100% appearance rate of *Bacillus subtilis* in soils contaminated with diesel oil and scorched oil. Nwaogu et al. (2008) reported that *Bacillus subtilis* is a major bacterial isolate from diesel oil contaminated soil. Working on petrol contaminated soil selected randomly from oil refineries in India; Shekhar et al. (2015) isolated and identified five indigenous bacterial strains, including *Bacillus subtilis*, with hydrocarbon bioremediation efficiency. Jalilzadeh et al. (2014) in their study, report that *Bacillus subtilis* was isolated from crude oil contaminated soils. It can therefore be inferred that *Bacillus subtilis* is a major isolate from soils contaminated with any kind of hydrocarbon.

From the two control samples (CS-O and CS-K) three fungal species, *Aspergillus carbonarius*, *Cunninghamella* sp. and *Rhizopus* sp. were isolated. These are normal fungi species commonly found in soil. In their respective studies Barberis et al. (2014) and Leong et al. (2007) isolated *Aspergillus carbonarius* from normal soil and used it in the production of ochratoxin A. Similar observation of *Aspergillus carbonarius* presence in normal soil was made by Afini et al. (2016). *Cunninghamella* sp was isolated from normal soil by Ziaee et al. (2016). The isolation of *Rhizopus* sp. from normal soil has been presented in several reports (Mehedi et al., 2013; Ziaee et al., 2016; Nakuleshwar et al., 2013; Rakesh and Kavita, 2014). These reports corroborate my findings and indicate that these are common fungal species present in normal soils.

In the contaminated soils (HCS-O and HC-K) three fungal species, *Rhizopus* sp., *Rhizopus oryzae* and *Trichoderma spirale* were isolated. *Rhizopus oryzae* and *Trichoderma spirale* were isolated from the hydrocarbon contaminated soil from Kumasi while *Rhizopus* sp., *Rhizopus oryzae* and *Trichoderma spirale* were isolated from the hydrocarbon contaminated soil from Owerri. These are also normal fungi species of soil, though their presence in the hydrocarbon contaminated soils would suggest that they can utilise hydrocarbons as a source of energy. It has been reported that *Rhizopus oryzae* was isolated from an age long (10 years) hydrocarbon contaminated soil in Iran (Arani and Sonakebriaeezade, 2015). Allamin et al. (2014) report that *Rhizopus oryzae* was one of the fungal isolates in their study on the “distribution of hydrocarbon degrading fungi in soil in Kukawa, Borno State, Nigeria”. The source of their samples was sites covering petroleum exploration stations in Kukawa, Borno State, Nigeria.

ii) Total heterotrophic bacterial and fungal counts from WEO contaminated soils and their controls

The total heterotrophic bacterial counts during preliminary study were in the order HCSO>HSK>CSK>CSO (3.89, 3.85, 3.79, 3.69). Hydrocarbons in soil provide a source of carbon for microbial growth and this explains the higher total heterotrophic bacterial counts in the WEO contaminated soils compared to the control soils. Alrumman et al. (2015) report that there are higher heterotrophic bacteria counts in diesel contaminated soil whilst Bundy et al. (2002) indicates that heterotrophic bacteria counts increased with the addition of diesel and kerosene to soils.

The total heterotrophic fungal (THF) counts in the control soil from Owerri (CS-O) are higher than those of the control soil from Kumasi (CS-K) and the counts in the controls are also higher than in the respective contaminated samples (HCS-O and HCS-K). Chikere and Chijioke-Osuji (2006), observed that crude oil contaminated soil from Uzere Flow Station, recorded higher THF counts in the control than in the contaminated soil. Working on “sludge contaminated soil of petroleum refinery”, Chaudry et al. (2012), also observed higher total heterotrophic fungal counts in the control samples than in the contaminated soils. They attribute this to the toxicity of the hydrocarbons. These studies indicate that the high total heterotrophic fungal count is a marker of soil health. This suggests that the control soils from Nigeria and Ghana are of a higher quality than contaminated soils.

iii) **Hydrocarbon utilizing bacterial and fungal counts from WEO contaminated soils and their controls**

The hydrocarbon utilizing bacteria (HUB) counts in the hydrocarbon (WEO) contaminated soil from Kumasi (HCS-K) was more than in the hydrocarbon contaminated soil from Owerri (HCS-O). This implies that the extent of contamination at the points of sample collection at Magazine is higher than that at the Orji Mechanic Village because isolation of high numbers of hydrocarbon degrading bacteria from polluted environments is commonly taken as evidence that these microorganisms are the active microflora of that environment. This finding corroborates the reports of Okoh (2003), who recorded that hydrocarbon degrading bacteria are present in higher numbers in hydrocarbon contaminated soils than in uncontaminated environments and Lawson et al. (2012), who reported higher counts in hydrocarbon utilising bacteria in diesel contaminated soils than in the control.

The hydrocarbon utilizing fungal (HUF) counts were higher in the controls (CSO and CSK) than in the contaminated and simulated samples. It has been reported that hydrocarbon utilizing fungi are ubiquitous and can be isolated from both contaminated and uncontaminated soils (Obire and Anyanwu, 2009) and that all sites can harbor hydrocarbon degrading fungi (Allamin et al., 2014). Due to the fact that these soils have not been impacted with hydrocarbon contaminant, their numbers were higher. It has been reported by Chikere et al. (2011) that hydrocarbon contamination is lethal to hydrocarbon utilizing fungi at the initial stages till they adapt to the contaminant (unlike bacteria which doesn't require time to adapt) thus, the lower counts in the contaminated samples. This finding corroborates that of Dawoodi *et al.* (2015) working on heterotrophic and oil-utilizing fungi in soils, reported that the control sample has higher counts of oil-utilizing fungi than those of the contaminated soils.

iv) Physico-chemical parameters of WEO contaminated samples and their controls

The pH of the contaminated and control soil samples did not have any significant difference at $p < 0.05$. The reason that the pH of the samples are in the acidic range can be attributed to the time of collection which was during the rainy season. It has been recorded that soils tend to be acidic during the rainy seasons due to the leaching effect on the exchange sites in cation exchange (Oyem and Oyem, 2013). The contaminated samples were however slightly more acidic than the controls, this may be as a result of the contamination. Achuba and Peretiemo-Clarke (2008) report that spent engine oil in soil causes poor soil aeration, immobilization of soil nutrients and lowering of soil pH, as observed in the samples. The low pH values as observed in this research confirms the reports of Oyem and Oyem (2013) who recorded similar pH values (5.3 and 5.7 for two crude oil contaminated soil samples from different locations) on some soil samples in a study on series of tests to evaluate the physicochemical effect of crude oil spill on soil. Acidic pH values (5.46 to 5.58 over 14 months) were also reported by Ikhajiagbe and Anoliefo (2011) in their study of the natural attenuation of a-14-month-old waste engine oil polluted soil. Iwegbue et al. (2008) in their study on soil profiles of automobile mechanic waste dumps also recorded a pH value of 5.40 for the control soil and a contaminated soil.

The total hydrocarbon content (THC) of the contaminated samples was more than in the controls. The concentrations of hydrocarbon in the HC-K and HC-O are relatively high compared to their controls; this implies that there has been accumulations of hydrocarbons at the sites the samples were collected. The results obtained from this study corroborates that of Iwegbue et al. (2008)

who reported higher total hydrocarbon contents in a study of soil profiles of automobile mechanic waste dumps compared to the controls.

The total organic carbon (TOC) contents of the contaminated samples are expectedly higher than in the controls. This is as a result of the hydrocarbon in those samples which has increased the bulk of carbon and organic matter in them. Similar findings of high TOC in hydrocarbon contaminated soil has been reported by Benka-Coker and Ekundayo (1995) in their study on the effects of an oil spill on soil physico-chemical properties; Ekundayo and Obuekwe (1997) in their study on the effects of an oil spill in a Typic Paleudult of Midwestern Nigeria; Ekperusi and Aigbodion (2015) in their study on bioremediation of crude oil contaminated soil and Agbogidi et al. (2007) in their study on the evaluation of crude oil contaminated soil on the mineral nutrient elements of maize (*Zea mays* L.).

The soil macronutrients Nitrate, Phosphate and Sulphate in the contaminated samples were higher than in the controls. Studies have shown that hydrocarbons in soil at low to moderate levels can improve soil content of some nutrient elements (Agbogidi et al., 2007) and can enhance soil fertility (Mengel and Kirkby, 1987). The increased levels of the macronutrients as recorded from this study agrees with the reports of Ekperusi and Aigbolion (2005) who recorded increased levels of these nutrients in crude oil contaminated soil in their study on bioremediation of petroleum hydrocarbons using earthworms and Agbogidi et al. (2007) in their study on effect crude oil contamination on the mineral nutrient elements of maize.

v) **Polycyclic Aromatic Hydrocarbon (PAH) contents of WEO contaminated soils and their controls**

The concentrations of the sixteen (16) EPA target PAHs (EPA-16) assayed showed that all the hydrocarbon contaminated samples (HCS-K and HCS-O) were detected in varying concentrations (mg/kg). The control samples (CS-K and CS-O) had just three of them namely Fluorene, Anthracene and Phenanthrene in small amounts. Formation of PAH is a resultant effect on incomplete combustion of organic matter through the condensation of ethylenic radicals in the gas phase to form larger polycyclic compounds (Lane, 1989). The PAH results from the study show that the heavy PAHs (from pyrene down) constituted the largest group of compounds with high concentrations in the contaminated samples. These PAHs fall within the category of PAHs with the highest health risk especially at prolonged exposure (ATSDR, 1999a). Benzo(a)anthracene, Chrysene and Benzo(b,j,k)fluoranthene are implicated as carcinogens

according to US-EPA (California Environmental Protection Agency, 1994). According to Obini et al. (2013), PAHs are ubiquitous in both urban and rural environments; it is very common to detect low levels in soils. The presence therefore of Fluoranthene, Anthracene and Phenanthrene in the controls corroborates that report. The trend in values for PAHs in the contaminated samples corroborates the reports of Obini et al. (2013) who determined the levels of PAH in soils contaminated with spent motor oil in Abakaliki Auto-mechanic village, Nigeria and Ikhajiagbe and Anoliefo (2011) in their report on the natural attenuation of a-14-month-old waste engine oil polluted soil.

vi) Heavy metals content of WEO contaminated soils and their controls

There was a significant build up of heavy metals in the contaminated samples (HC-K and HC-O) compared to the controls (CS-K and CS-O). These results corroborates the reports of several authors namely, Benka-Coker and Ekundayo (1995) who reported such build up in zones of heavy impact at an oil spill site in the Niger Delta area on Nigeria; Ekundayo and Obuekwe (1997) in a similar study at an oil spill site in a Typic Palenduit of Midwestern Nigeria, Ikhajiagbe and Chijioke-Osuji (2015) who reported an increased heavy metals concentration in soil upon introduction of waste engine oil and Dike et al. (2013) who recorded increased heavy metals contents in waste engine oil contaminated soils compared to the control in their study on the remediation of used motor engine oil contaminated soil using soil washing treatment approach.

Conclusion

Pollution by hydrocarbon and hydrocarbon products has been recognised as the most significant contamination problem (Snape et al, 2001). This results in attendant negative consequences. From my findings, the effects of waste engine oil on the microflora and physico-chemical quality of the soils are negative, affecting the physico-chemical quality and microbial population of the impacted soils. The concentrations of some PAH components especially the high molecular weight PAHs were more in the hydrocarbon contaminated sample from Owerri and the heavy metals (Iron, Manganese, Zinc, Lead, Nickel and Chromium) content were more in the hydrocarbon contaminated sample from Kumasi. These pose a risk to human life especially those of the poor artisans who live in these mechanic villages and are more exposed to these chemicals/elements.

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