

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

## Abstract.

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

**Keywords.** Microflora, Physico-Chemical, Waste Engine Oil (WEO), PAH, Heavy Metal, Contaminated Soils, Owerri, Kumasi.

## Introduction.

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

petroleum hydrocarbons is one of the world's most common environmental problems because they belong to the groups of persistent organic contaminants [1,2].

Generally, the accumulation of contaminants in soils can have destructive effects on the environment and human health; it can enter the food chain and seriously affect animal and human health [3]. Thus, suitable solutions for management or disposal of waste engine oil and indeed all other petroleum wastes must be found.

The aim of this preliminary study was to investigate the effects of waste engine oil on the microflora and physicochemical quality of soil and which of the two sites, namely Owerri in Nigeria and Kumasi in Ghana had the highest level of contamination that could pose a matter of public health concern.

### **Literature Review.**

The availability of oil in any economy is usually a major boost to its development but this does not come without the attendant effects on the immediate environment from which it is explored [4], distributed and its waste products disposed. Environmental pollution with petroleum products has been recognized as one of the most serious current problems [5,6,7]. Pollution phenomena results in inter-woven negative circumstances; it causes significant changes in the zoosphere and phytosphere, as well as to microorganisms, leading to the disappearance of many species; decrease in soil fertility and support for plant and animal life and consequently humans who benefit from these plants and animals (meat, hides, skin, etc) suffer [8].

According to [9][E1], hydrocarbons are considered among the strongest soil pollutants. Any form of hydrocarbon spills on both land and in water can have devastating effects on the biota of an environment because they contain poisonous compounds that constitute potential danger to plants and animals. These poisons can pass through the food web of an area and may eventually be eaten by humans [10,11,12,13]. Contamination of soil with petroleum derivatives like WEO is often more in municipal soils [14] because of the presence of diverse kinds of automobiles and machinery which has resulted in an increase in the use of motor oils and other lubricants. This could result in spillages which can constitute nuisance to the environment due to their persistent nature [15,16]. Furthermore, it could make the soil unsuitable for plant growth for a while or several months or years [17].

69 Waste engine oil is a common environmental contaminant [18], it can cause great damage to  
70 sensitive environments [19] and alter microbial population [6,20]. It may contain components  
71 like Lead, Cadmium and other potentially toxic metals [21]. WEO on soil changes soil colour,  
72 this may have untold health hazard on the technicians/ artisans who mostly live in their  
73 workshops because they cannot afford the cost of good accommodation. This hardens the  
74 soles of their feet most times which may alter their ability to move normally over time [5].  
75

76 The fact that hydrocarbon contamination of soil is of particular environmental concern  
77 because they exhibit carcinogenic and mutagenic properties [22] has increased scientific  
78 interest in examining its distribution, fate and behavior in the environment [11,12,13,23,24].  
79 Engine oils are altered during use by vehicles, motor bikes, generators and other machinery  
80 because of the breakdown of additives, contamination with products of combustion and the  
81 addition of metals from the wear and tear of the engine. It is recognized that the major  
82 components consist of aliphatic and aromatic hydrocarbons such as phenol, naphthalene,  
83 benzo(a)anthracene, benzo(a)pyrene, fluoranthene, lead, cadmium and other potentially toxic  
84 metals [25]. These heavy metals are dangerous because they tend to accumulate and are stored  
85 faster than they are broken down [26]; they cause nutrient imbalance; toxicity of soils and  
86 unfavorable plant growth (if any) [27] due to reduction in the level of available plant nutrients  
87 or a rise in toxic levels of certain elements like such as Iron-iron and Zinezinc.  
88



89  
90 **Image 1. Magazine Mechanic Village in Kumasi, Ghana**  
91 **State**  
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94 **Image 2. Orji Mechanic Village in Owerri, Imo**  
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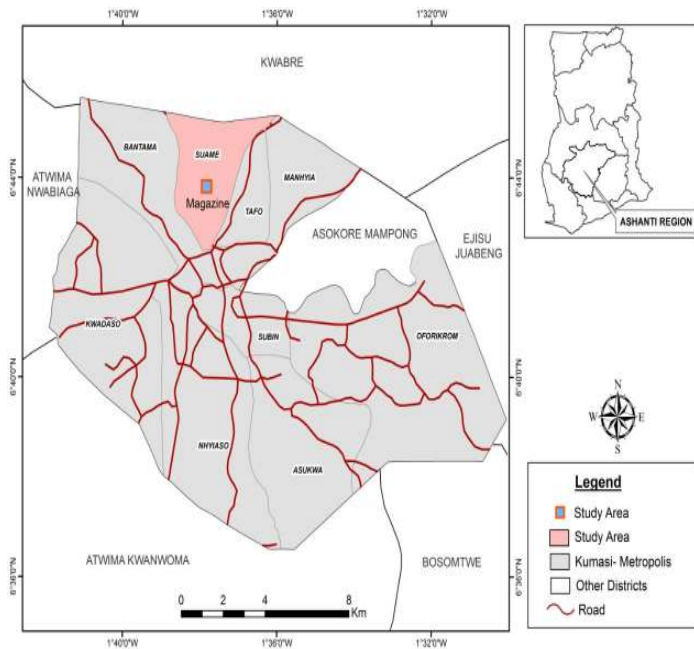


Fig. 1. Map of study area in Suame, Kumasi, Ghana

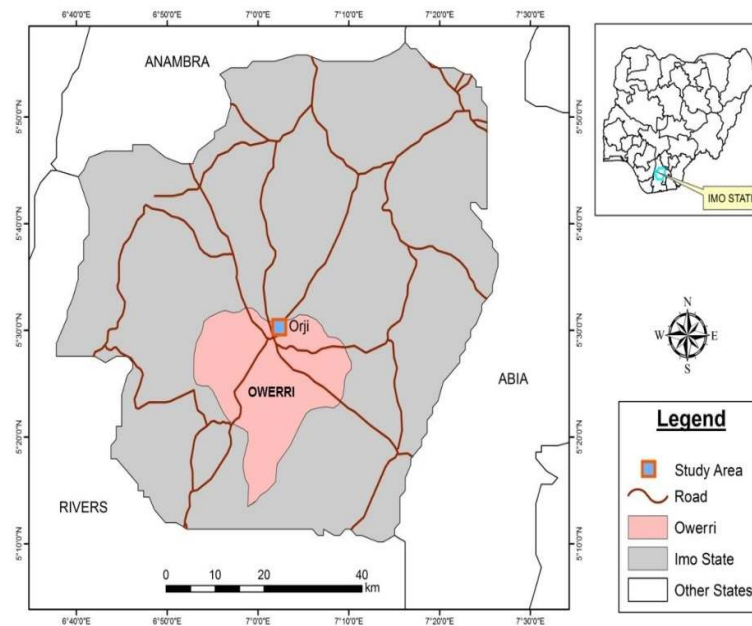


Fig. 2. Map of study area in Owerri, Imo State, Nigeria

## Materials and Methods.

### a) Sample collection

Waste Engine Oil (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 respectively, 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 (Image 1, 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) [28].

Sample two (HCS-O) was collected from the *Orji* Mechanic Village in Owerri, Imo State, Nigeria (Image 2, 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) [29].

### b) Microbiological analyses

The total heterotrophic bacterial and fungal counts (THBC and THFC) of the respective soil samples were taken using pour plate technique while the hydrocarbon utilizing bacterial and fungal counts (HUBC and HUFC) were taken after culture of the respective soil samples using ~~vapour~~vapor phase transfer [30].

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 SubcommissionSub  
commission on *Trichoderma* and *Hypocrea* (ISTH).

#### c) Physico-chemical analyses

The respective soil samples were examined for some physico-chemical parameters such as 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 were carried out using the cold extraction GC-FID method while the heavy metals content was using the AAS:APHA methods 3010-3110 [31,32,33].

#### e) Statistical Analysis

All data from this study were analyzed using the Statistical Package for the Social Sciences (SPSS) (version 20) by analysis of variance on ranks to compare the means of the different results obtained [E2].

### Results and Discussion.

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 bacteria, *Bacillus cereus* occurred in all the control samples (CS-O and CS-K), these organisms are predominant in soil [34] and are known to be facultative anaerobes [35]. The reports of [34,36,37,38] [E3] corroborates this result. These reports also suggest that *Bacillus cereus* can be isolated from uncontaminated soils from 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. [39,40,41,42] [E4] had in

their respective studies corroborated this finding. It can further be inferred that *Bacillus subtilis* is a major isolate from soils contaminated with any kind of hydrocarbon.

Three fungal species namely *Aspergillus carbonarius*, *Cunninghamella* sp. and *Rhizopus* sp. were isolated from the two control samples (CS-O and CS-K). These are fungi species commonly found in uncontaminated soil. In their respective studies [43][E5] and [44][E6] isolated *Aspergillus carbonarius* from uncontaminated soil. Similar observation of *Aspergillus carbonarius* presence in uncontaminated soil was made by [45][E7]. *Cunninghamella* sp was isolated from uncontaminated soil by [46]. The isolation of *Rhizopus* sp. from uncontaminated soil has been presented in several reports [46,47,48,49]. These reports corroborate the results of this study.

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 fungi species of soil, though their presence in the hydrocarbon contaminated soils would suggest that they can utilize hydrocarbon 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 [50]. [51][E8] 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”. These reports corroborate the results of this study.

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  $3.85 \pm 0.01$  and  $3.79 \pm 0.01$  respectively while their controls had mlog of THBC of  $3.89 \pm 0.01$  and  $3.69 \pm 0.01$  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 ( $4.24 \pm 0.02$ ).

The mlog of the Hydrocarbon Utilizing Bacterial Counts (HUBC) for the HCS-K and HCS-O samples were  $4.37 \pm 0.05$  and  $4.35 \pm 0.03$  respectively while their controls had  $4.06 \pm 0.05$  and  $3.98 \pm 0.03$  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  $4.12 \pm 0.02$  and  $4.22 \pm 0.01$  respectively while their controls had  $4.24 \pm 0.01$  and  $4.34 \pm 0.02$  respectively; there was no significant difference ( $P < 0.05$ ) between the HUFC of all the samples.

The total heterotrophic bacterial counts during this 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. These results corroborate the reports of [52,53][E9].

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). [30][E10] 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”, [54][E11] also observed higher total heterotrophic fungal counts in the control samples than in the contaminated soils. These reports agree with the results of this study.

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 [55,56][E12].

The hydrocarbon utilizing fungal (HUF) counts were higher in the controls (CSO and CSK) than in the contaminated samples. It has been reported that hydrocarbon utilizing fungi are ubiquitous and can be isolated from both contaminated and uncontaminated soils [57] and that all sites can harbor hydrocarbon degrading fungi [51]. Due to the fact that these soils have not been impacted with hydrocarbon contaminant, their numbers were higher. This finding corroborates the report of [58][E13].

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

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

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

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

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

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

The mean sulphate content of the respective controls, CS-K and CS-O were  $41.65 \pm 0.01$  and  $41.40 \pm 0.02$  while samples HCS-K and HCS-O had sulphate contents of  $157.675 \pm 0.05$  and  $281.500 \pm 0.04$  respectively.

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 [59]. The contaminated samples were however slightly more acidic than the controls, this is as a result of the contamination. These results corroborate the respective reports of [59,60,61] [E14].

The total hydrocarbon content (THC) of the contaminated samples was more than in the controls. This implies that there has been accumulations of hydrocarbons at the sites the samples were collected. The result obtained from this study corroborates that of [62] [E15].

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 [63,64,65,66,67].

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 [66] and can enhance soil fertility [67]. The increased levels of these macronutrients as recorded from this study agree with the reports of [65,66,67].

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

| Samples | Micro-organisms isolated |                                |
|---------|--------------------------|--------------------------------|
|         | Bacteria                 | Fungi                          |
| HCS-K   | <i>Bacillus subtilis</i> | <i>Rhizopus oryzae</i>         |
|         |                          | <i>Trichoderma spirale</i>     |
| CS-K    | <i>Bacillus cereus</i>   | <i>Aspergillus carbonarius</i> |
|         |                          | <i>Rhizopus sp.</i>            |
|         |                          | <i>Cunninghamella sp.</i>      |
| HCS-O   | <i>Bacillus subtilis</i> | <i>Rhizopus oryzae</i>         |
|         |                          | <i>Rhizopus sp.</i>            |
|         |                          | <i>Trichoderma spirale</i>     |
| CS-O    | <i>Bacillus cereus</i>   | <i>Aspergillus carbonarius</i> |
|         |                          | <i>Rhizopus sp.</i>            |
|         |                          | <i>Cunninghamella sp.</i>      |

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 Owerri.

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

| Sample type | Mean logarithm of microbial counts (cfu/g) |                          |                           |                          |
|-------------|--------------------------------------------|--------------------------|---------------------------|--------------------------|
|             | THBC                                       | THFC                     | HUBC                      | HUFC                     |
| CS-K        | 3.79 ± 0.01 <sup>b</sup>                   | 3.91 ± 0.01 <sup>d</sup> | 4.06 ± 0.01 <sup>b</sup>  | 4.24 ± 0.01 <sup>d</sup> |
| HCS-K       | 3.85 ± 0.01 <sup>c</sup>                   | 3.79 ± 0.07 <sup>c</sup> | 4.37 ± 0.01 <sup>ef</sup> | 4.12 ± 0.02 <sup>c</sup> |
| CS-O        | 3.69 ± 0.01 <sup>a</sup>                   | 4.24 ± 0.02 <sup>f</sup> | 3.98 ± 0.03 <sup>a</sup>  | 4.34 ± 0.02 <sup>e</sup> |
| HCS-O       | 3.89 ± 0.01 <sup>d</sup>                   | 3.98 ± 0.01 <sup>e</sup> | 4.35 ± 0.01 <sup>e</sup>  | 4.22 ± 0.01 <sup>d</sup> |

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

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

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

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

The levels of Bromonaphthalene<sup>[E19]</sup> in samples CS-K and CS-O were both  $0.00 \pm 0.00$  whilst HCS-K and HCS-O had  $5.43 \pm 0.03$  and  $5.85 \pm 0.41$  respectively.

Samples CS-K and CS-O both had Acenaphthene<sup>[E20]</sup> levels below detectable limits ( $0.00 \pm 0.00$  mg/l) while samples HCS-K and HCS-O had  $5.43 \pm 0.10$  and  $5.45 \pm 0.02$  respectively.

The levels of Fluorene<sup>[E21]</sup> in the samples CS-K and HCS-K were  $5.90 \pm 0.02$  and  $6.33 \pm 0.04$  respectively while CS-O and HCS-O had Fluorene<sup>[E22]</sup> levels of  $5.81 \pm 0.03$  and  $6.57 \pm 0.01$  respectively.

The Anthracene<sup>[E23]</sup> levels in samples CS-K, HCS-K, CS-O and HCS-O were  $5.97 \pm 0.02$ ,  $9.43 \pm 0.03$ ,  $5.79 \pm 0.02$  and  $6.55 \pm 0.02$  respectively.

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

Fluoranthene levels in control samples CS-K and CS-O were both  $0.00 \pm 0.00$  whereas HCS-K and HCS-O had  $6.03 \pm 0.02$  and  $5.68 \pm 0.01$  respectively.

The mean levels of ~~Pyrene~~<sup>[E24]</sup> in samples CS-K and CS-O were both  $0.00 \pm 0.00$  while samples HCS-K and HCS-O both had ~~Pyrene~~ level of  $5.83 \pm 0.03$ .

The mean levels of ~~Benzo~~<sup>[E25]</sup>-a-anthracene ~~was~~<sup>[E26]</sup>  $0.00 \pm 0.00$  for samples CS-K and CS-O. Samples HCS-K and HCS-O had ~~Benzo~~<sup>[E27]</sup>-a-anthracene levels of  $6.61 \pm 0.01$  and  $14.65 \pm 2.01$  respectively.

The levels of Chrysene in all the control samples were  $0.00 \pm 0.00$  mg/l while samples HCS-K and HCS-O had ~~Chrysene~~ level of  $6.29 \pm 0.03$  and  $20.15 \pm 0.02$  mg/l respectively.

The levels of ~~Benzo~~~~benzo~~(b,j,k)~~fluoranthene~~<sup>[E28]</sup> in samples HCS-K and HCS-O were  $8.38 \pm 0.05$  and  $16.15 \pm 0.02$  mg/l respectively. The controls, CS-K and CS-O both had ~~0.00  $\pm$  0.00~~ ~~Benzo~~~~benzo~~(b,j,k)fluoranthene levels.

The levels of Benzo(a)pyrene in samples CS-K and CS-O were  $0.00 \pm 0.00$ , samples HCS-K and HCS-O had ~~16.45  $\pm$  1.05~~ and  $15.25 \pm 0.16$  respectively.

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

The mean levels of ~~Dibenzo~~~~dibenzo~~(a,h)anthracene in samples CS-K and CS-O were  $0.00 \pm 0.00$  while samples HCS-K and HCS-O had ~~9.23  $\pm$  0.01~~ and  $15.23 \pm 0.05$  respectively.

The mean levels of ~~Benzo~~~~benzo~~(g,h,i)perylene in the respective samples differ significantly with the controls at  $p < 0.05$ . Samples HCS-K and HCS-O had ~~Benzo~~~~benzo~~(g,h,i)perylene levels at  $8.30 \pm 0.01$  and  $10.28 \pm 0.02$  respectively.

The concentrations of these sixteen (16) EPA target PAHs (EPA-16) assayed showed that in the hydrocarbon contaminated samples (HCS-K and HCS-O), they were detected in varying concentrations (mg/kg). The control samples (CS-K and CS-O) had just three of them namely Fluorene, ~~Anthracene—anthracene~~ and ~~Phenanthrene—phenanthrene~~ in small amounts. Formation of PAH is a resultant effect of incomplete combustion of organic matter through the condensation of ethylenic radicals in the gas phase to form larger polycyclic compounds <sup>[68]</sup>. 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 <sup>[69]</sup>. Benzo(a)anthracene, ~~Chrysene—chrysene~~ and ~~Benzo~~~~benzo~~(b,j,k)fluoranthene are implicated as carcinogens according to US-EPA <sup>[70]</sup>. According to <sup>[71]</sup><sup>[E29]</sup>, PAHs are ubiquitous in both urban and rural environments; it is very common to detect low levels in soils. The presence therefore of ~~Fluoranthene~~~~fluoranthene~~,

~~Anthracene-anthracene~~ and ~~Phenanthrene-phenanthrene~~ in the controls corroborates that report. The trend in values for PAHs in the contaminated samples corroborates the respective reports of <sup>[61,71]</sup>.

The levels of heavy metals namely Iron (Fe), ~~Manganese-manganese~~ (Mn), ~~Nickel-nickel~~ (Ni), ~~Zinc-zinc~~ (Zn), ~~Lead-lead~~ (Pb) and ~~Chromium-chromium~~ (Cr) in WEO contaminated soil samples and their controls are shown in Table 5.

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

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

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

The level of Zn in samples CS-K and CS-O were  $1.96 \pm 0.14$  and  $1.945 \pm 0.02$  respectively. These values do not differ significantly at  $p < 0.05$ . Samples HCS-K and HCS-O had Zn levels of  $8.75 \pm 0.04$  and  $5.100 \pm 0.02$  respectively. These values differ significantly at  $p < 0.05$ .

Samples CS-K and CS-O both had Pb levels of  $0.77 \pm 0.03$ , these do not differ significantly at  $p < 0.05$ . Samples HCS-K and HCS-O had Pb levels of  $6.81 \pm 0.01$  and  $1.55 \pm 0.01$  respectively.

The levels of Cr was detectable in only samples CS-K, HCS-K and CS-O which had  $0.26 \pm 0.01$ ,  $0.29 \pm 0.03$  and  $0.25 \pm 0.03$  respectively, the values differ significantly at  $p < 0.05$ .

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<sup>[E30]</sup> the reports of several researchers <sup>[63,64,72,73]</sup>.

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

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

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

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

382

383 HCS-K – Hydrocarbon contaminated soil from Kumasi; CS-K – Control soil from Kumasi; HCS-O – Hydrocarbon contaminated soil from  
 384 Owerri; CS-O– Control soil from Owerri.

385

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

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

388  
389 **Table 5. Levels of heavy metals in WEO contaminated soil samples and their controls**  
390 **(mg/kg)**

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

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

## Conclusion

Engine oils contain additives which are broken down during the use by vehicles, motor bikes, generators and other machinery, resulting in contamination with the product during combustion and the addition of metals from the wear and tear of the engine. It has been reported that the major components consist of aliphatic and aromatic hydrocarbons such as phenol, naphthalene, benz(a)anthracene, benzo(a)pyrene, fluoranthene, lead, cadmium and other potentially toxic metals <sup>[25]</sup>. These metals result in nutrient imbalance, toxicity of the soils and unfavourable growth (if any) of plants in the ecosystem. Heavy metals can bind to vital cellular components such as structural proteins, enzymes and nucleic acids, and interfere with their functioning <sup>[74]</sup>. Symptoms and effects can vary according to the metal or metal-compound, and the dose involved. So many hazards<sup>[E31]</sup> such as autistic<sup>[E32]</sup> spectrum disorder, depression, headaches, skin problems, thyroid problems, cardiovascular diseases, depletion of immune system and cancer, digestive and hormone problems, autoimmune diseases, low libido, impotence and prostrate problems are associated with humans being exposed to heavy metals <sup>[75,76]</sup>. From my findings, the effects of waste engine oil on the microflora and physico-chemical quality of the soils are deleterious, 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. It is therefore recommended that treatment plants for waste engine oil be constructed in these mechanic villages and that WEO are carefully pooled/ treated so that their negative effects on man and the environment would be thus minimized or eliminated.

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