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Impact of Organic Mulching on the Enhanced Natural Attenuation of a Petroleum Hydrocarbon Polluted Soil

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ABSTRACT: *The remediation of oil polluted soil has been a major problem in oil producing countries and investigations on the use of mulch and various soil amendments has been on. In order to identify the organic material that would enhance remediation of crude oil-contaminated soil, the present study was undertaken. Waste engine oil-polluted soils (5% w/w) were made into beds of 120cm × 60cm × 15cm dimension, and then mulched with saw dust, dried cow dung, wood ash, dried ruderal weeds, and dried crushed Chromolaena odorata plants. The set up was left for 3 months on an open field. The result revealed that there were over 88% reductions of polyaromatic hydrocarbon contents in soil 3 months after mulching, from 833.62mg/kg to 103.88mg/kg in the oil-polluted soil. Significant reductions from the original concentrations of Fe, Mn, Zn, Cu, Cr, Cd, Pb, Ni and V were also achieved. Achromobacter spp., Bacillus pumilis, Sarcina spp. and Micrococcus spp. were prevalent bacteria species found in the polluted soils, while prevalent fungi species included Aspergillus niger, Penicillium, spp. and Fusarium spp. Contamination factor, probable efficient concentration and hazard quotient were generally higher in unmulched soil, compared to the mulched ones. Results of phytoassessment, using seedling development at 3 weeks for yardstick for adjudging the success of remediations, showed improved seedling development in the sawdust-mulched soil (87.62% survival), compared to unmulched oil-polluted soils (25.98% survival).*

Keywords: Attenuation**,** *Chromolaena*, mulching, intrinsic remediation, petroleum hydrocarbon, soil amendment

Introduction

Petroleum pollution due to crude oil has been on the forefront of most discourse on soil pollution, but crude oil or its products are obviously not the only causes of petroleum pollution. Waste engine oil has been adjudged a more serious threat than crude oil alone [1, 2]. Motor mechanics use crude oil products extensively in servicing of vehicles, such services involves emptying of used engine oil, filling of new ones and cleaning of motor parts with fuel and these used motor oils contaminate natural environment with hydrocarbon which spread horizontally on the groundwater surface thereby causing serious groundwater contamination [3]. The effects of oil in soil are very devastating and as such the need to clean up those sites using various methods of remediation has become very imperative. There are different remediation processes and some of these processes are controversial, especially when they involve low temperature. Some of these remediation processes are various physical chemical, thermal processes and biological techniques involved in the cleaning up of oil contaminated site [4].

Biological processes of remediation are a much more friendly method of clean up. However, Ikhajiagbe [2] noted that for effective bioremediation particularly that hinged on improved microbial activity and processes the use of soil amendments is highly recommended. Apart from positive effects on soil physiochemical properties, it also enhances the inherent soil microbial community. Since the use of organic mulching in some agricultural processes and practices sometimes involves decaying of the organic materials, the implication for soil include enhanced soil fertility, improved soil moisture, regulation of soil temperature, to mention a few; and these properties are implicated in enhanced soil microbial activity. This would eventually enhance intrinsic bioremediation in the affected soil. Enhanced intrinsic bioremediation (natural attenuation) as an active in-situ remediation method, enhances natural biodegradation within the saturated zone by the introduction of factors like oxygen [5], moisture [6], or organic materials [7, 8] to stimulate natural processes by changing the sub-soil condition [5]. Microbiological activity also helps to reduce slowly severe contaminant concentration.

Natural attenuation processes under favourable conditions, reduce the mass, toxicity, mobility, volume, and/or concentration of contaminants in soil and groundwater. The researchers therefore hoped to investigate the suitability for organic mulching as a bioremediation method, as well as preference of organic mulching material for the venture. The main idea below the use of organic mulch in bioremediation is that hydrocarbon-degrading microorganisms would prefer to utilize added organic material, rather than hydrocarbons, intensively proliferate, and eventually decompose the hydrocarbons following the depletion of sub-stance added [9].

Mulch is usually but not exclusively organic material, permanently or temporarily applied to bare soil or around existing plants to increase soil organic matter, and to improve fertility by establishing patterns of nutrient cycling [10, 11]; and these soil factors are as important in bioremediation of oil-contaminated soils. Commonly available organic mulches include leaves, grass clippings, peat moss, saw dust, animal dung, dried leaves, wood and bark chips.

Since organic mulches are composed of plant materials, they add small amounts of nutrients to the soil through decomposition. When organic mulches such as fresh leaves, wood chips, and straw, are used, a considerable amount of nitrogen is taken from the soil by the micro-organisms decomposing the organic matter. Plant materials used in the present study was a combination of local weeds available in the study area. These included a combination of *Asystsia gangetia, Croton lobatus, Digitaria horizontalis, Euphorbia heterophyllia, Euphorbia hirta, Eleucine indica, Paspalum serobiculatum, Scleria naumanniana,* and *Solanum nigrum.* Anoliefo *et al*. [12] had earlier identified some of these weeds as probable candidates for phytoremediation based on certain tolerance indices. *Chromolaena odorata* was also selected based on a study by Nweke and Okpowasili [13], who showed that *Chromolaena odorata* contained some active compounds that when they are decayed, could serve as nutrient for micro organisms present in the soil which enable them to degrade long chain hydrocarbons.

Soil microbes act both as a source and sink of available nitrogen through opposing processes of mineralization and immobilization (sequestration of inorganic nitrogen in microbial biomass), and subsequent remineralization of nitrogen as soil microbes die and are decomposed [14, 15]. Consequently, cow dung was also been selected as a mulching material in the present study. Apart from investigating the suitability of these mulching materials for enhanced natural attenuation of the oil-polluted soil, phytoassessment of the remediated soils would also be carried out, using seedling development responses as yardstick for measuring remediation success.

Materials and Methods

Site Location

The site was located beside Department of Plant Biology and Biotechnology Botanic garden and directly opposite the Animal and Environmental Biology animal husbandry building. The study started on the 14th day of December 2012, and lasted for 3 months.

Soil collection

Top soil (0-10cm) was gathered at random spots beside the Botanic Garden and pooled together to obtain composite sample. Physiochemical composition of the soil was determined before use.

Mulch material collection

Whole plants of *Chromolena odorata* were collected from a fallow land on UNIBEN Campus. They were crushed and dried before use. Wood ash was obtained from a kitchen at NASU Canteen/restaurant. Sawdust was obtained from a saw mill at Isihor, opposite Army Barracks. Cow dung was obtained from an abattoir at Oluku. The dung was sun-dried and later crushed into near powdery form by using a mortar and pestle.

Research Methods

Forty (40) kg of sun-dried soil was thoroughly mixed with WEO to obtain a constant concentration of 5% w/w oil-in-soil. The contaminated soils were then poured into bare ground and made into beds of the following dimension; 120cm long, 60cm wide and 15cm high. In order to ensure that oil or heavy metal fractions did not percolate to ground water, polythene material was spread and buried 15cm below soil surface, just before the contaminated soils were poured on the bare ground and made into beds (Fig. 1). The idea was to excavate all polluted soil materials at the end of the experiment. A schematic diagram is shown below.

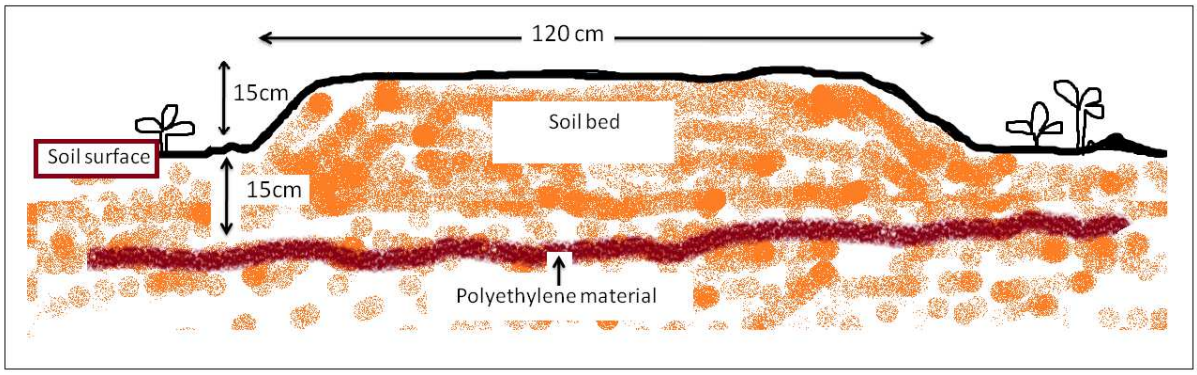

Fig. 1: Schematic cross section of the bed.

A total of 18 beds were made, as there were 5 different mulching materials to be used per bed and then a control (unmulched bed). These were replicated 3 times.

Parameters Studied

At the beginning and at 3 months after pollution (MAP), various parameters were assayed, including presence of weed(s) on soil surface, soil physiochemical parameters, total poly aromatic hydrocarbon content of soil, as well as soil microbial composition (fungi and bacteria).

Soil Physicochemical 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 selected soil physicochemical properties and the heavy metal fractions as well as PAH.

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.

Determination of Polyaromatic Hydrocarbon Contents of Polluted Soil by Gas Chromatography (GC)

A 10 g sample was extracted with methylene chloride (DCM). The extract was filtered through anhydrous sodium sulphate to remove any trapped water molecule. This was followed by a clean- up/ fractionation of the sample extract into aliphatic and aromatic (PAH) components. Finally, the components were concentrated using a rotary evaporator for GC analysis, using FID as detector. Model of GC used was AGILENT 6890.

The GC analysis began by first injecting 1 µL of the sample extract into the GC, and the results calculated as follows:

$$\text{Sample (mg/kg)} = \frac{\text{Area x F.vol x 1000}}{\text{Rf x Wt}}$$

Where,

Rf = Response factor = Total Area / Total Concentration, obtained from instrument calibration with standards.

Area is obtained from the chromatogram output.

F.vol is the final volume of the concentrated extract (in ml)

Wt is the initial weight of the homogenized sample (in grams)

Soil analyses were done in triplicates.

Identification of Soil Microorganisms

The soil samples were air-dried and sieved through a 2 mm mesh to remove undesirable material. The dilution series for the soil sample was done by transferring 1 gram of the soil to nine (9 ml) millimetres of sterile distilled water in sterile glass containers as blank. The glass containers were shaken for 5 minutes and was taken as 10^{-1} dilution factor, 10 ml were then transferred from the 10^{-1} dilution into another 9 ml blank to obtain a 10^{-2} dilution and same process of transfer was repeated twice to obtain a dilution factor of 10^{-4}.

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 bacteria 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, was used as the enrichment medium with 8 % (v/v) filter sterilized oil as the sole carbon source. The medium was dispensed into in 100 ml Erlenmeyer flasks and autoc1aved at 121 ^{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· 33 ifunctional shaker (B. Bran Scientific and Instrument Company, England). After 10 days, 1 ml of enriched media was transferred eshly 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 and fungal counts respectively using the methods described by Cowan and Steel [16] and Cheesebrough [17]. These were carried out in triplicates.

Computation of Contamination Factor (CF)

CF expresses the ratio between the eventual concentrations of pollutant against its pre-contamination reference.

$$CF = \frac{\text{Concentration of pollutant}}{\text{Pre-contamination Concentration}}$$

When CF > 1, inherent concentration element in soil was due to exogenous application of contaminant (i.e. WEO), in which case the metal concentration was higher than values obtained in the original unpolluted soil.

Computation of Hazard Quotient (HQ)

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

$$HQ = \frac{\text{Measured concentration}}{\text{Toxicity reference value or selected screening benchmark.}}$$

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

Screening benchmarks were available at Efroymson *et al.* [18].

Computation of Concentration of Toxic Equivalency (TEQ) for Polycyclic Aromatic Hydrocarbons (PAH).

Toxic Equivalency factors (TEF) are toxicity potency factors used as a consistent method to evaluate the toxicities of variable mixtures of organic compounds.

$TEQ = \Sigma Ti \times PEF$

Where TEQ = Toxic equivalency
T*i* = PAH concentration in soil
PEF= Potency equivalency factor [19]

Bioremediation Efficiency

This is regarded as the proportion (%) of contaminant that was bioremediated compared to a measured concentration at the start point. In the present study, the reference start point was at 1 week after pollution (1WAP). This was calculated as;

$$\text{Efficiency}(\%) = \frac{\text{Measured concentration at 3MAP}}{\text{Concentration at 1WAP}} \times \frac{100}{1}$$

Statistics

Statistical analysis of data was done using the *SPSS-15* statistical software, and means were separated by using the Least Significant Difference. Other forms of statistics were those of ecological significance that required comparison with standard benchmark [18, 19].

Results and Discussion

The results showed that weeds that eventually emerged after 3 months in the soil with the sawdust mulch included *Asystsia gangetia, Solanum nigrum, Eleusine indica,*and *Paspalum serobiculatum;* while those in the dried weeds-mulched soils included *Asystasia* gangetica and *Eleusine indica* respectively. Anoliefo *et al*., [12] suggested earlier that some of these weeds were oil-tolerant and as such, where candidates for phytoremediation. It was also observed that no weed grew on the bed containing *C. odorata*. This was basically due to the fact that *C. odorata* contains some allepathic substances [13]. Also it has been recorded that some organic mulching such as *C. odorata* directly provides organic carbon inputs to soil, which suppresses weeds and used to reduce soil erosion in organic farming systems [20].

Table 1: Identified weed species on each soil bed at 3 months after pollution.

S/N	Mulch	Weeds identified	Family
1	Saw dust	*Asystsia gangetia*	Acanthaceae
		Solanum nigrum	Solanaceae
		Eleusine indica	Poaceae
		Paspalum serobiculatum	Poaceae
2	Wood ash	*Paspalum serobiculatum*	Poaceae
		Eleusine indica	Poaceae
3	Cow dung	*Asystasia gangetica*	Acanthaceae
		Eleusine indica	Poaceae
4	*Chromolaena odorata*	No weed	Not available
5	Dried weeds	*Asystasia gangetica*	Acanthaceae
		Eleusine indica	Poaceae
6	Control	*Digitaria horizontalis*	Poaceae
		Eleusine indica	Poaceae
		Croton lobatus	Euphobiaceae
		Scleria naumanniana	Cyperaceae

Total PAH was highest in unmulched soil (Table 2). However, remediation of these compounds was better in the mulched soils but more effective in the saw dust mulched soil. PAH content of the soil 1 WAP was 833.62mg/kg. Total PAHs (and bioremediation efficiencies) were 160.10 mg/kg (80.79%) in wood ash-mulched soil, 240.48 mg/kg (71.15%) in dried weeds-mulched soil, 212.01mg/kg (74.56%) in *C.*

Odorata-mulched soil and 215.92mg/kg (74.09%) in cow dung-mulched soil. There was total (100%) remediation of acenaphthene at all levels. The total PAH reduction discussed may have resulted from volatilization, diffusion and microbial degradation in a dissolved state [21, 22].

The physical properties of petroleum hydrocarbons have an effect on their biodegradation. At very low concentrations, hydrocarbons are soluble in water, but most oil spill incidents release petroleum hydrocarbons in concentrations far excess of the solubility limits [23, 24]. The degree of spreading of oil in the surface area of soil is important for microbial colonization by hydrocarbon-degrading microorganisms [25]. Also, soil moisture is a major control on microbial and microfaunal community structure and activity in the soil [26], and this may have been significantly affected by the mulches.

Hazard quotients (HQ) for PAH in the polluted soils obtained are presented in Table 3. Collectively, HQ's for PAH at 3 MAP were lowered with the use of mulch, indicating a reduction in toxicity potential of the PAH after 3 months. At 1 WAP, HQ was very high in napthalene (6.1), acenaphthene (0.11), flourene (0.39), phenanthrene (747.90), anthracene (65.20), Fluoranthene (433.80), pyrene (180.10) and benzo(a)pyrene (621.00). However, HQ was greater than unity (HQ>1) in all except acenaphthene and flourene, which indicated a toxic situation. But at 3 MAP, the HQ concentration of acenaphthene and flourene was also lowered to zero (0). HQ values of some other PAHs in all mulched soils, ranged from 4.20 to 21.48 for phenanthrene, 139.2 to 182.4 for anthracene, 275.80 to 576.30 for benzo(a)pyrene. All of these presented a HQ>1 situation. However, for Fluoranthene and pyrene, HQ<1 for saw dust, wood ash and cow dung mulched soils, signifying no toxicity.

The use of mulch in the present study was significant in reduction of the PAH. This is because organic mulch have been suggested to conserve soil moisture, suppress weeds, enhance aesthetics [27], help keep the soil temperature constant so that the activity of the microorganisms can continue at an even rate [28] and raise the pH slightly, making the soil reaction more alkaline [29]. However, from the results earlier presented, saw dust mulch was the most efficient in the reduction of PAH. Sutherland *et al*, [30] had reported that microorganisms growing in sawdust substrate produce enzymes used in metabolizing the hydrocarbons. Sawdust has been reported to produce enzymes which enable *Pseudomonas ostreatus* to bind with flouranthrene metabolite [31]. The result obtained was also in agreement with earlier findings of Ikhajiagbe and Anoliefo [8].

Significant reductions were observed when the toxicity equivalents of the PAH were calculated in Table 4. At 1 WAP, toxicity equivalents (TEQ's) was 40.58 mg/kg in benzo[a]pyrene, but zero values were obtained for both benzo[a]anthracene and chrysene respectively. TEQ's of PAH in saw dust-mulched soil were 0 mg/kg in benzo(a)anthracene and chrysene, 27.58 mg/kg in benzo[a]pyrene and 1.58 in indeno(1,2,3-cd)pyrene). In the *C. odorata*-mulched soil were 1.74 mg/kg in benzo(a)anthracene, 0.09 mg/kg in chrysene, 52.33 mg/kg in benzo[a]pyrene, and 1.20 mg/kg in indo(1,2,3-cd)pyrene. The benzo[a]pyrene values, were higher than benchmark TEF values of the PAH of 0.1 mg/kg. All other values exceeded the benchmark clean-up level [19]. The implication is that the cleanup level for benzo[a]pyrene according to Cal-EPA [19] was met not for amended soil samples. But the TEF values of benzo(a)anthracene and chrysene where zero (0) in sawdust- and wood ash-mulched soils which did not exceed the required benchmark values. This implied that clean up of these PAHs were met in these soils.

Table 5 shows physiochemical parameters of the soil in the present study. The pH value for polluted and unpolluted soil samples generally were within acidic range (5.58 - 5.75). On application of mulches, a slight increase towards neutrality was observed (6.02 - 6.89). Soil pH as earlier discussed influences the mobility of nutrients and metals, impact microbial activity and alter the community compositions. According to Gianfreda *et al*., [29] heavy metals are bio-available between pH 3.5 - 6.0 below which they will become less bio-available under alkaline condition. This may be one of the reasons why there was negatively correlation between the heavy metals and the pH, as the pH recorded in this present study was above 6 in the mulched soil. The slight increases in pH could be as a result of high metabolic activities possibly resulting from the production of intermediate metabolites in the mulched soils. Ano and Ubochi [32], reported that cow dung and wood ash contains a pH of (6.7 - 11.9). However, Dibble and Bartha [33] reported a pH range of 6.5-8.0, for optimum mineralization of hydrocarbons with organic materials.

Electrical conductivity (EC) which was 349µs/cm 1 WAP, was significantly lowered with the use of mulch 3MAP, with dried weeds having the lowest concentration of 230µs/cm. This shows that dried weeds are capable of reducing the conductivity of a crude oil polluted soil. This confirms the previous work of Osuji and Nkoye [34]. It is however possible that mulching materials were not directly responsible for the observed changes in EC since organic compounds like crude oil cannot conduct electrical current very well. However, it may be due to the fact that anoxic biodegradation mechanism through direct dehydrogenation allowed the anaerobic metabolism of hydrocarbons in the presence of an electron acceptor such as nitrate ion, which may be responsible for the observed differences in EC.

Lehtomake and Niemela [35] reported a low value of N, K, Ca, Mg and P reserve in petroleum hydrocarbon contaminated soil as recorded in the present study. However, at 3 MAP, the application of mulch increased the soil concentrations of total organic carbon, total nitrogen, Na, Mg, P, Ca and Cl. The changes observed in contents of nitrogen, potassium, calcium, magnesium and phosphorus could be attributed to the organic mulches added to the soil which on decomposition and mineralization release the different nutrients in the soil.

Although results showed that organic mulching had no effects on soil texture, it however was quite effective in reducing the concentrations of heavy metals. At 1 WAP the concentration of Fe was (1539.24 mg/kg), Mn (32.62mg/kg), Zn (91.54mg/kg) and Cu (54.36 mg/kg). However, on application of mulch, significant reductions were recorded. At 3 MAP, concentration of Fe was 987.62 mg/kg in the unmulched soil, but reduced to 821.65 mg/kg and 837.14 mg/kg in the soils mulched with sawdust and dried weeds respectively. It was observed that there were slight increases in some metal concentration in the mulched soils, compared to the ones that were not mulched. For example, concentration of Cd in the unmulched soil at 3 MAP was 4.22 mg/kg, but slightly increased to a range of 4.50 – 4.70 mg/kg in the polluted soils mulched with sawdust, wood ash, dried weeds, and cow dung. This may have been contributed by the mulching materials. The values for contamination factor presented on Table 6 showed that only Mn, Cu, Pb, and Ni were heavy metals with a less-than-one contamination factor (CF<1); the implication being that these heavy metals had been remediated to values below their original concentrations before soil was contaminated with waste engine oil, thus indicating significant remediation.

Similarly, there were significantly lower hazard quotients (HQ) upon mulching of oil-polluted soil (Table 7). HQ for determination of toxicity of resident pollutant heavy metals to ecological processes were above normal with regards to Fe, Zn Cr, Cd, and V. This implied that for the plant ecologist, the remediation of heavy metals by mulching after 3 months may not have been in the ecologist's favour. However, heavy metal levels were well within statutory ranges for microbial activities and processes (Table 7). Similar quotients were used in the studies by Ikhajiagbe [2].

The reductions recorded in the concentrations of heavy metals in the present study were as a result of the added mulches which were able to increase the degradative ability of microorganism by acting on microbial enzymatic activities to transform or degrade the contaminants from the environment [36]. The mechanisms by which these microorganisms act on heavy metals includes biosorption (metal sorption to cell surface by physiochemical mechanisms), bioleaching (heavy metal mobilization through the excretion of organic acids or methylation reactions), biomineralization (heavy metal immobilization through the formation of insoluble sulfides or polymeric complexes) intracellular accumulation, and enzyme-catalyzed transformation (redox reactions) [37]. The mulches also provided a permanent solution as a result of complete mineralization of these contaminants in the environment [38].The result obtained in this study is in agreement with the work of Ray and Ray, [39] who recorded a reduction in Cr and other heavy metal concentrations with the application of mulch.

Table 2: Polyaromatic hydrocarbon contents of the soil in the present study

PAH (mg/kg)	Original soil used for the study	1 WAP	3 MAP						LSD (0.05)
			Soil mulched with						
			No mulching	Sawdust	Wood Ash	Dried weeds	*C. odorata*	Cow dung	
Naphthalene	BDL	0.61	BDL	17.16	14.52	13.83	11.02	16.20	*5.21*
Acenaphthylene	BDL	1.16	1.32	BDL	14.36	BDL	BDL	16.22	*4.08*
2-bromonaphthalene	BDL	3.91	1.53	BDL	15.09	14.98	13.64	17.22	*2.96*
Acenaphthene	BDL	2.31	BDL	BDL	BDL	BDL	BDL	BDL	*1.98*
Fluorene	BDL	11.57	2.59	BDL	14.85	14.11	3.09	BDL	*3.95*
Phenanthrene	0.85	74.79	44.84	0.42	0.72	1.25	2.14	0.89	*5.01*
Anthracene	BDL	6.52	BDL	18.24	15.36	14.72	13.92	17.28	*8.65*
Fluoranthene	BDL	43.38	17.34	BDL	BDL	14.04	6.93	BDL	*9.25*
Pyrene	BDL	18.01	BDL	BDL	BDL	14.45	18.37	BDL	*6.32*
benzo(a)anthracene	BDL	33.06	BDL	BDL	16.71	20.50	17.54	18.46	*4.08*
Chrysene	BDL	17.39	BDL	BDL	BDL	6.17	9.03	BDL	*3.21*
benzo(b,j,k)fluoranthene	BDL	124.13	87.58	BDL	4.48	15.33	13.24	2.20	*11.51*
benzo(a)pyrene	40.28	62.10	40.58	27.58	38.07	51.65	52.33	59.63	*8.80*
indeno(1,2,3-cd)pyrene	5.24	29.46	BDL	15.31	2.76	20.40	12.05	18.52	*4.25*
dibenzo(a,h)anthracene	12.25	336.17	77.90	1.82	2.40	14.59	19.46	25.78	*9.93*
benzo(g,h,i)perylene	19.24	69.05	32.65	23.35	20.78	24.46	19.25	23.52	*11.55*
Total PAH	77.86	833.62	306.33	103.88	160.10	240.48	212.01	215.92	-
*Efficiency (%)	-	-	63.25	87.54	80.79	71.15	74.56	74.09	-

** Efficiency (%) was calculated as percentage changes in TPAH with respect to 1 WAP. BDL below detectable limit (0.0001 mg/kg), WAP. Weeks after pollution, MAP. Months after pollution. N/A. Not available. Efficiency was calculated only from mean value of PAH obtained. LSD (0.05) = least significant difference among mean values on similar rows at 5% confidence level.*

Table 3: Hazard quotient for ecotoxicity of polyaromatic hydrocarbon contents of the soil in the present study

PAH (mg/kg)	Original soil used for the study	1 WAP	3 MAP					
				Mulched soil				
			No mulching	Sawdust	Wood Ash	Dried weeds	*C. odorata*	Cow dung
Naphthalene [0.1]	0	6.1	0	171.6	145.2	138.3	110.2	162
Acenaphthene [20.0]	0	0.11	0	0	0	0	0	0
Fluorene [30.0]	0	0.39	0.09	0	0.50	0.47	0.10	0
Phenanthrene [0.1]	0.85	747.90	448.40	4.20	7.20	12.51	21.40	8.90
Anthracene [0.1]	0	65.20	0	182.40	153.60	147.20	139.20	172.80
Fluoranthene [0.1]	0	433.80	173.40	0	0	140.40	69.30	0
Pyrene [0.1]	0	180.10	0	0	0	144.50	183.70	0
benzo(a)pyrene [0.1]	40.28	621.00	405.80	275.80	380.70	516.50	523.30	596.30
Total PAH [0.1]	77.86	2054.60	1027.69	634.00	687.20	1099.88	1047.20	940.00

Values in bracket means HQ benchmark values [18]. HQ's were calculated only from the mean values obtained for PAH in Table 2.

Table 4: Toxicity equivalency of polyaromatic hydrocarbon contents of the soil in the present study

PAH (mg/kg)	Original soil used for the study	1 WAP	3 MAP					
				Mulched soil				
			No mulching	Sawdust	Wood Ash	Dried weeds	*C. odorata*	Cow dung
Benzo(a)anthracene [0.1]	0	3.30	0	0	0	2.05	1.75	1.84
Chrysene [0.01]	0	0.17	0	0	0	0.06	0.09	0
Benzo(a)pyrenez [1.0]	40.28	62.1	40.58	27.58	38.07	51.65	52.33	59.63
Indeno(1,2,3-cd)pyrene [0.1]	0.52	2.94	N/A	1.53	0.27	2.04	1.20	1.85
Total TEC	40.80	68.51	40.58	29.11	38.34	55.80	55.37	63.32

Values in parenthesis means TEF benchmark values [19].TEC were calculated from mean values of PAH in Table 2.

Table 5: Physiochemical parameters of the soil in the present study from 1 WAP to 3 MAP

Parameters	Original soil used for the study	1 WAP	3 MAP						LSD (0.05)
				Mulched soil					
			No mulching	Sawdust	Wood Ash	Dried weeds	*C. odorata*	Cow dung	
pH	5.58	5.75	5.63	6.02	6.89	6.25	6.85	6.84	*1.05*
EC (µs/cm)	300	349	333	329	305	230	321	470	*13*
TOC (%)	0.41	5.81	4.68	5.68	5.98.	5.51	5.48	5.45	*0.29*
Total Nitrogen (%)	0.62	0.52	0.49	0.48	0.47	0.41	0.43	0.50	*0.31*
EA (meq/100 g soil)	0.20	0.52	0.44	0.49	0.52	0.43	0.39	0.54	*0.11*
Na (meq/100 g soil)	10.90	1.63	1.09	1.62	2.09	1.30	2.09	2.10	*0.09*
K (meq/100 g soil)	1.65	0.04	0.03	0.10	0.09	0.07	0.10	0.12	*0.01*
Ca (meq/100 g soil)	15.60	3.68	3.50	4.29	4.28	3.25	3.67	5.24	*0.56*
Mg (meq/100 g soil)	11.30	3.09	2.89	2.39	3.05	2.44	2.08	3.93	*1.01*
P (mg/kg)	153.00	19.5	20.65	16.54	13.08	13.52	12.09	15.62	*2.65*
NH_4N (mg/kg)	25.40	20.21	20.32	18.62	20.61	20.21	18.25	23.93	*3.22*
NO_2 (mg/kg)	15.01	13.36	11.09	10.65	11.03	10.36	11.11	11.29	*2.98*
NO_3 (mg/kg)	30.75	18.62	16.52	14.68	15.03	15.01	14.93	16.36	*2.28*
SO_4 (mg/kg)	14.63	48.21	36.58	42.65	49.33	50.66	50.34	54.92	*6.21*
Clay (%)	4.43	4.43	4.43	4.43	4.43	4.43	4.43	4.43	*1.08*
Silt (%)	7.82	7.82	7.82	7.82	7.82	7.82	7.82	7.82	*1.50*
Sand (%)	87.82	87.82	87.82	87.82	87.82	87.82	87.82	87.82	*3.22*
Fe (mg/kg)	1009.21	1539.24	987.62	821.65	798.68	837.14	931.83	970.82	*54.21*
Mn (mg/kg)	15.29	32.65	28.41	18.04	20.55	20.21	21.22	23.56	*6.32*
Zn (mg/kg)	12.03	91.54	81.65	78.65	79.65	78.30	75.37	62.95	*8.24*
Cu (mg/kg)	BDL	54.36	35.25	30.65	32.06	29.12	24.46	33.69	*4.22*
Cr (mg/kg)	BDL	9.35	5.09	4.98	4.98	5.56	3.03	5.80	*0.84*
Cd (mg/kg)	BDL	5.26	4.22	4.63	4.69	4.50	3.56	4.70	*0.71*
Pb (mg/kg)	BDL	6.58	4.68	4.19	4.96	4.38	5.01	5.11	*1.03*
Ni (mg/kg)	BDL	3.64	2.60	1.98	2.33	2.56	2.13	3.78	*0.09*
V (mg/kg)	BDL	3.95	2.19	1.68	2.49	2.30	1.84	2.91	*0.18*

BDL below detectable limit (< 0.0001 mg/kg or 10^{-4}mg/kg). LSD (0.05) = least significant difference among mean values on similar rows at 5% confidence level.

Table 6: Contamination factor of mean heavy metal contents of the soil in the present study

Parameters	Original soil used for the study	1 WAP	3 MAP					
				Mulched soil				
			No mulching	Sawdust	Wood Ash	Dried weeds	*C. odorata*	Cow dung
Fe	1009.21	1.52	0.97	0.81	0.79	0.82	0.92	0.96
Mn	15.29	2.13	1.85	1.17	1.34	1.32	1.38	1.54
Zn	12.03	7.60	6.77	6.53	6.62	7.33	6.26	7.72
Cu	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$
Cr	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$
Cd	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$
Pb	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$
Ni	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$
V	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$	$>10^4$

Table 7: Hazard quotient for determining of toxicity of mean heavy metal contents of the soil in the present study

Parameters	1 WAP	3 MAP					
			Mulched soil				
		No mulching	Sawdust	Wood Ash	Dried weeds	*C. odorata*	Cow dung
		Hazard quotient to determine ecological toxicity					
Fe	7.69	4.93	4.10	3.99	4.18	4.65	4.85
Mn	0.32	0.28	0.18	0.20	0.20	0.21	0.23
Zn	1.83	1.63	1.57	1.59	1.57	1.50	1.25
Cu	1.35	0.88	0.76	0.80	0.72	0.61	0.84
Cr	9.35	5.09	4.98	4.98	5.56	3.03	5.80
Cd	1.31	1.05	1.15	1.17	1.12	0.89	1.17
Pb	0.13	0.09	0.08	0.09	0.08	0.10	0.10
Ni	0.12	0.08	0.06	0.07	0.08	0.07	0.12
V	1.97	1.09	0.84	1.24	1.15	0.92	1.45
		Hazard quotient to determine toxicity to microbial activities and processes					
Fe	7.69	4.93	4.10	3.99	4.18	4.65	4.85
Mn	0.32	0.28	0.18	0.20	0.20	0.21	0.23
Zn	0.91	0.81	0.78	0.79	0.78	0.75	0.63
Cu	0.54	0.35	0.30	0.32	0.29	0.24	0.33
Cr	0.93	0.50	0.49	0.49	0.55	0.30	0.58
Cd	0.26	0.21	0.23	0.23	0.22	0.17	0.23
Pb	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Ni	0.04	0.02	0.02	0.02	0.02	0.02	0.04
V	0.19	0.10	0.08	0.12	0.11	0.09	0.14

Table 8: Microbial composition in treated and control soils at 3 months after pollution

	No mulching	Sawdust	Wood Ash	Dried weeds	*C. odorata*	Cow dung
			Bacterial species			
Achromobacter sp	+	+	-	+	+	+
Bacillus pumilis	+	+	+	+	+	+
B. subtilis	+	-	+	+	+	+
Sarcina sp	+	+	-	-	-	+
Micrococcus varians	-	+	+	+	+	+
M. luteus	-	-	-	-	-	+
Proteus vulgaris	-	+	-	+	+	+
Pseudomonas aeruginosa	+	-	+	+	+	-
Het. bacteria (x 10^5 cfu/g)	2.3^{b}	2.8^{ab}	2.5^{ab}	2.6^{ab}	2.8^{a}	3.2^{a}
Hyd. Deg. bacteria (x 10^5 cfu/g)	1.5^{ab}	1.0^{b}	2.0^{a}	1.8^{a}	2.0^{a}	2.0^{a}
% Hyd	65.22	35.7	80.0	69.2	71.4	62.5
			Fungal species			
Aspergillus niger	+	+	+	+	+	+
A. fumigatus	-	+	-	+	+	+
Penicillium sp	-	-	-	-	-	-
P. notatum	+	+	+	+	-	-
Fusarium sp	-	-	-	-	-	+
F. solani	+	-	-	+	+	-
Rhizopus sp	+	+	+	-	+	+
Het. Fungi (x 10^5 cfu/g)	2.9^{a}	2.3^{ab}	1.8^{b}	2.2^{b}	1.8^{b}	2.0^{b}
Hyd. deg. Fungi (x 10^5 cfu/g)	1.8^{a}	1.5^{ab}	0.7^{c}	1.0^{bc}	1.1^{bc}	1.1^{bc}
% Hyd	62.0	65.2	38.8	45.4	61.1	55.0

(+)= present, (-)=absent, %Hyd=Percentage hydrocarbon degraders, Het= Heterotrophic. Means of values on the same rows carrying the same superscript alphabets do not differ significantly (p>0.05) from each other.

Table 8 presents microbial composition in the mulched and unmulched soils at 3 months after pollution. Both bacteria and fungi species were present in the soil. *Achromobacter* sp, *Bacillus pumilis B. subtilis, Sarcina* sp, *Micrococcus varians, M. luteus, Proteus vulgaris, Pseudomonas aeruginosa* were dominant bacteria species in the present study while *Aspergillus niger, A. fumigatus, Penicillium* sp, *P. notatum, Fusarium* sp, *F. solani, Rhizopus* sp were dominant fungi species in the present study and it was observed that *M. luteus* and *Fusarium* sp were absent in all soils except cow dung-mulched soil. Percentage hydrocarbon-degrading bacteria (80.0%) was highest in the wood ash-mulched soil, where as it was least in the sawdust-mulched soil. similarly, percentage hydrocarbon-degrading fungi ranged from 38.8% in the wood ash-mulched soil to 65.2% in sawdust-mulched soil

WEO pollutants usually inhibit soil microbial development. These effects of microorganisms on them have been reported to depend on the concentrations of the pollutant [40]. The presence of these organisms in pollutants must have been as a result of their tolerance to the pollutants. However, different species of microbes are capable of degrading different groups of hydrocarbons, found in oil [41]. Therefore, the microorganism in the present study may have been involved in the remediation process, considering the fact that they were prevalent, even in high concentrations of pollution.

Soil organic mulches have an enormous potential of sustaining diverse populations of microorganisms, as they are capable for bioremediation. They can act as a soil ameliorant capable of changing pH, moisture content, salinity, temperature, soil structure and acting as a nutrient source, thereby improving the contaminated soil environment for indigenous microbial degradative activity [8]. Such organisms including bacilli, pseudomonads, mesophilic, thermophilic and lignin-degrading fungi, all with the potential to degrade a variety of aromatic pollutants. In the present study, it was observed that the saw dust mulched soil had the greatest percentage of hydrocarbon degrading fungi. This may be due to the fact that the saw dust substrate provided more conducive environment that supported their growth and probably produced substrate matrix during its growth that were used in metabolizing the hydrocarbons.

Effects of mulching on seedling development of test crop (*Zea mays*) for up to 3 weeks after sowing showed that percentage seedling emergence at 1 week after sowing (WAS) in the control (unpolluted soil) was 100%, compared to 36.81% in the unmulched oil-polluted soil (Table 9). Percentage emergence in the mulched soils ranged from 55.56% in the dried weeds-mulched soil to 88.88% in the saw dust-mulched soil. At 1 WAS, seedling height was 12.65 cm, being the highest in all the mulched treatments, and lowest in cow dung-mulched soil (8.95 cm). Seedling height in the control, however, was 18.62 cm (Table 9). It took 10.52 days for seedlings in the non-mulched oil-polluted soils to experience leaf chlorosis, compared to 19.21 days in the seedlings in the sawdust-mulched soils. Percentage survival in the mulched soils ranged from 48.65 – 87.62%, compared to 25.98% in the unmulched oil-polluted soil. Percentage survival in the control was 100%. Oil-polluted soil could also become unsuitable for plant growth due to a reduction in the level of available plant nutrients or a rise to a toxic level of elements such as manganese [42]. This heavy metal content of oil-contaminated soil imposes metabolic disorders and growth inhibition on most of the plant species

One major hindrance to germination and organism survival in the soil is the hydrophobic nature of the soil which can be caused by PAH presence. Cerniglia [43] reported that PAHs are hydrophobic compounds and their persistence in the soil is chiefly due to their low water solubility condition which can lead to plant experiencing water loss. Oil-polluted soil hampered growth and development of *Zea mays* used as biotest. However, this condition was alleviated by various mulching material such as sawdust, wood ash, dried *C. odorata*, cow dung used due to their various physical, chemical and biological properties [44] and some already established facts which was earlier discussed. Also, the enhanced nitrogen mineralization may substantially stem from the turnover of microbial biomass as Bonde *et al.* [45] estimated that microbial biomass contributed to 55–89% of total mineralized nitrogen during a 40 week incubation period.

Table 9: Effects of mulching on seedling development of test crop (*Zea mays*) for up to 3 weeks after sowing.

	Percentage emergence (%) @ 1 WAS	Height of emergent in 1 WAS (cm)	1st Day of noticed yellowing (DAS)	Day of noticed leaf necrosis in plant (DAS)	Percentage survival of emergents at 3WAS
Control	100.00^{a}	18.62^{a}	0^{d}	0^{f}	100.00^{a}
No mulch	36.81^{d}	7.36^{d}	10.52^{c}	15.95^{e}	25.98
Sawdust	88.88^{b}	12.65^{b}	19.21^{a}	31.66^{a}	87.62^{b}
Wood ash	77.78^{b}	10.68^{bc}	13.25^{b}	21.62^{c}	65.39^{c}
Dried weeds	55.56^{c}	8.98^{cd}	14.21^{b}	21.05^{c}	56.98^{cd}
C. odorata	77.78^{b}	9.98^{c}	13.65^{b}	19.25^{c}	48.65^{d}
Cow dung	66.67^{bc}	8.95^{cd}	18.65^{a}	26.95^{b}	63.88^{c}
Mean (mulched)	67.25	9.77	14.92	22.75	58.08

Means on the same column with similar alphabetic superscripts do not differ from each other significantly (p.>0.05)

Enhancement of microbial biomass and activities and potential nitrogen availability may reflect the plant productivity. Osubor and Anoliefo [46] have extensively studied the effects of oil pollution on seed germination of crop plants, and all agree that oil pollution adversely affected crop germination. Udo and Fayemi [42] reported that maize germination was adversely affected by the pollution of the soil and effect being proportional to the level of crude oil pollution and oil contaminated soil, generally causing delayed seed emergence. But according to the present study *Zea mays* showed quick and proper growth 3 months after mulching has been carried out on the polluted soil which therefore means that remediation was effective.

Conclusion

According to the present study, the addition of soil amendments enhanced the soil's remediative capabilities. The use of sawdust was preferable for the intrinsic bioremediation polyaromatic hydrocarbons.

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