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Intrinsic Qualities of a Naturally Attenuated Petroleum Hydrocarbon-polluted Seed Bed Exposed to Different Kinds of Mulching Materials

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Authors' contributions

This work was carried out in collaboration between both authors. Author BI designed the study, wrote the protocol and wrote the first draft of the manuscript. Author CCCO managed the literature searches, analyses of the study performed the spectroscopy analysis and author BI managed the experimental process and identified the species of plant. Both authors read and approved the final manuscript.

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ABSTRACT

As materials that help in the conservation of soil moisture, improvement of soil health and fertility, mulches also improve microbial activity, which is *sin qua non* to successful intrinsic bioremediation. The study investigated the effects of mulching with selected materials on the natural attenuation of waste engine oil (WEO)-polluted soil. Top soil was mixed with WEO to obtain 5% w/w oil- in-soil concentration. The soil was made into beds and covered separately with 5 different mulching materials [aluminum foil, polyethylene materials (transparent and black), granite and tarpaulin] for 3 months. Results showed that efficiency of heavy metal reductions was decreased by application of the various mulches. Percentage reduction in total heavy metal content (Cu, Mn, Ni, V, Cr and Pb) was 18.77% in the transparent polyethylene-covered bed and 62.61% in the aluminum foil-mulched soil, compared to 83.65% in the control. Total PAH content in the uncovered soil was 336.80mg/kg,

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indicating a 67.14% bioremediation efficiency, compared to 79.08% in the black polyethylene-covered seed bed and 74.75% in the aluminum foil-mulched bed. Total bacterial count in both treatment and control soils was highest in the tarpaulin-mulched soil (6.8 x 10^5 cfu/g), compared to 1.8 x 10^5 cfu/g in the control (uncovered). Prominent bacteria species were *Bacillus subtilis* and *Micrococcus luteus* whereas predominant fungus species were *Aspergillus niger* and *Penicillium* sp*.* Phytoassessment of the treatment and control soils using transplants of *Amaranthus hybridus* showed highest yield and biomass accumulation in the tarpaulin-mulched soils.

Keywords: Amaranthus; intrinsic bioremediation; mulching; natural attenuation; petroleum hydrocarbon; solarization.

1. INTRODUCTION

Oil pollution has been a major problem in the world at large and most counties has tried to prevent oil spillage or ways to control the spillage so that the general effect of this pollution will not be high. Accidental spills, illegal dumping and careless handlings of spent lube oil in mechanic workshops have been a significant source of environmental pollution, because of the predominantly unstructured practice of automobile vehicle repair services. As it is inevitable for the efficient and effective functioning of the automobile engines, soil contamination with used engine oil is also becoming one of the major environmental problems, mainly due to uncontrollable disposal, particularly in developing economies. Although engine oil is a complex mixture of hydrocarbons and other organic compounds including some organo-metabolic constituents that is used to lubricate the parts of an automobile engine, spent or waste engine oil contains metals and heavy polycyclic aromatic hydrocarbons (PAHs), and these could contribute to chronic hazards including mutagenicity and carcinogenicity [1].

When indiscriminately released into the environment, they increase incidence of oil contamination of agricultural soils. Petroleum can create unsatisfactory conditions for plant growth through a number of processes such as; (a) oil could displace air from soil pore spaces, (b) an increase in the demand for oxygen brought about by activity of oil-decomposing microorganisms, and (c) petroleum hydrocarbon creates hydrophobic environment, which limits water absorption to plant roots [2,3].

Although remediation of polluted soils is important to achieve good soil health and the attendant benefits, soil remediation through physicochemical methods have been reported to be non-ecofriendly. Biological methods on the other hand have been reported to be greatly enhanced by application of soil mulches, which when permanently or temporarily applied to bare soil or around existing plants increases soil organic matter, and also improve fertility. Mulches are used widely to suppress weeds, conserve soil moisture, and enhance aesthetics [4]. The potential of mulch to increase organic matter and establish patterns of nutrient cycling more similar to natural ecosystems has also been recognized. In the present study, the use of inorganic mulches has been employed, including polyethylene materials, textile, and granite.

The use of granite as well as polyethylene materials on soils is very likely to increase the surface temperature of the soil, in a process called solarization. In very many cases, solarization has been used to reduce or decrease the effect of toxicity in the soil caused by oil pollution on plants, a method used to decontaminate the soil. The principle of solarization involves the use of solar heating as a lethal agent for tarps for capturing solar energy by using transparent polyethylene soil mulches. This allow for maximum transmission of solar radiation through the moist soil to reduce the moisture loss from the soil to the environment by mean of evaporation. This process will increase the temperature of the soil through improve heat conduction within the soil owing to its higher moisture level.

In most part of the word, particularly in most urban areas, it is usually not easy gathering organic materials for mulching for the sake of remediative or other agricultural proposes. The most available materials are polyethylene, textile, and aluminium foil from kitchens as well as granite. What can these materials offer as mulches in the attempt to remediate oil-polluted soils?

Solarization is not entirely beneficial with regards to preservation of biodiversity. The rise in temperature achieved during solarization has a direct effect on soil ecology. Many soil-inhabiting organisms are inactivated when exposed to the high temperatures achieved during this process. Nevertheless, research suggests that the heat shock response in most organisms subjected to high temperatures is sustained until death occurs. Tolerance to high temperatures can be influenced by agents that affect the synthesis of heat shock proteins, such as cycloheximide (inhibitor), ethanol, sodium arsenite and anoxic conditions (elicitors). [5] reported significant reductions in heavy metal and PAH contents after the exposure to different periods of heat exposure, with significantly higher remediations with less frequency of exposure to heat shock (monthly).

2. MATERIALS AND METHODS

2.1 Location of Experimental Site

The site chosen for the present study, which lasted for 3 months beginning from December 10, 2012, was the Botanic Graden of the Department of Plant Biology and Biotechnology, University of Benin, Ugbowo Campus.

2.2 Collection of Soil

Arbitrary collection of topmost soil from different locations at the Botanic Garden was carried out. Soils collected were pooled to achieve a combination sample, and then analysed for physiochemical composition.

2.3 Research Methods

A measured quantity of sundried soil (40 kg) was contaminated with waste engine oil (WEO to achieve a uniform concentration of 5% w/w oil-in-soil. Thereafter, the oil-polluted soils were transferred into bare ground with already-buried polyethylene material (see Fig. 1). The polyethylene underlay was provided 15 cm beneath the soil bed to ensure that fractions of oil or heavy metal did not seep into to the ground water. The polluted soils were respectively made into 120 cm x 60 cm x 15 cm beds [6]. At the end of the experiment, the entire oil-polluted soils would be adequately excavated and disposed of.

Each bed was covered completely with the required mulching material completely. These materials included tarpaulin (1.17 mm thick), black nylon (0.98 mm thick), as well as transparent nylon (0.82 mm thick),; aluminum foil, as well as gravel (3 head pan). The control was uncovered. Treatments were replicated 3 times, so that there were a total of 18 beds.

2.4 Soil Physicochemical Analyses

Soils were obtained at random spots in each soil bed and at depth of 7.5 cm below top of soil bed, and then dried at ambient temperature (22 – 25℃). Air-dried soils were then crushed in a porcelain mortar, sieved through a 2-mm mesh stainless sieve, and stored in polythene bags for successive heavy metal and PAH analyses.

Metal analyses were conducted by atomic absorption spectrophotometry according to the methods of [7] and [8]. PAH contents of soil were determined according to [9] and [10]. Isolation and characterization of bacterial and fungal isolates was carried out using the methods of [11] and [12].

3. RESULTS

PAH as well as heavy metal contents of soil and WEO used for the experiment have been presented on Table 1. Soil concentration of Naphthalene was 31.02 mg/kg just before application of mulch, but 3 months after mulch was placed over polluted soil, naphthalene was below detection (>0.0001 mg/kg) (Table 2).

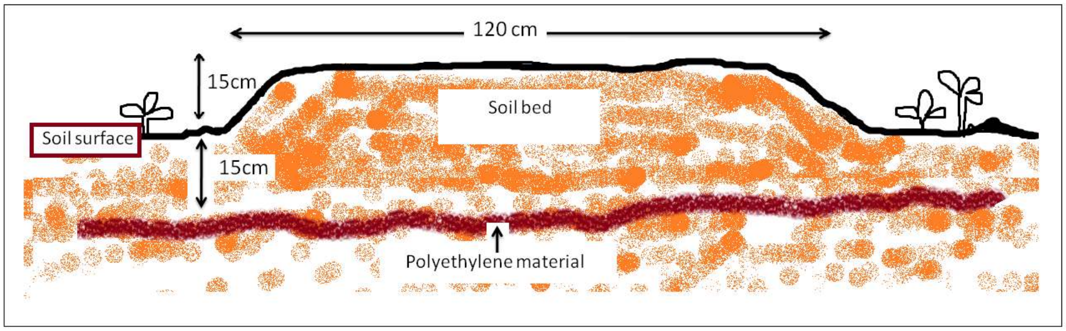

Fig. 1. Schematic cross section of the bed [6]

Total PAH before mulching was 1025.15 mg/kg. Aluminum foil- covered soil bed was 289.56 mg/kg, indicating a 71.75% remediation efficiency. Bioremediation efficiency for PAH components were 78.08% and 71.76% in both black and transparent polyethylene materials respectively. Results also showed that mulching did not favour heavy metal remediation. Although there was total remediation of Mn, Ni and V in all treatments, heavy metal remediation efficiency was 83.62% in the control compared to 18.77% in the transparent material-mulched soil.

Table 1. Chemical composition of waste engine oil and top soil used for the experiment

Parameters	WEO (mg/kg)	Soil (mg/kg)
Naphthalene	26.51	0
Acenaphthylene	8.06	0
2-bromonaphthalene	29.54	0
Acenaphthene	28.21	0
Fluorene	43.25	0
Phenanthrene	3.68	0.85
Anthracene	22.05	0
Fluoranthene	32.52	0
Pyrene	23.05	0
Benzo(a)anthracene	39.51	0
Chrysene	112.51	0
Benzo(b,j,k)fluoranthene	48.06	0
Benzo(a)pyrene	121.98	40.28
Indeno(1,2,3-cd)pyrene	109.54	5.24
Dibenzo(a,h)anthracene	36.04	12.25
Benzo(g,h,i)perylene	64.00	19.24
Copper, Cu	16.58	0
Manganese, Mn	12.05	0
Nickel, Ni	1.65	0
Vanadium, V	0.65	0
Chromium, Cr	16.85	0.08
Lead, Pb	39.25	0

Sensitivity of analytical equipment used 0.0001 mg/kg

Bacterial isolates in the control were *Micrococcus luteus, Clostridium perfringens,* and *Sarcina* sp., whereas fungal isolates were *Penicillium* and *Rhizopus* sp. total bacterial count in the aluminum foil-mulched soil was 3.3 x10^5 cfu/g, whereas hydrocarbon degrading bacteria was 1.1 x10^5 cfu/g (Table 3). Total hydrocarbon bacterial degrader was 3.0 x 10^5 cfu/g in the tarpaulin-mulched soil. Mulching enhanced bacterial presence in the soil. That may have also enhanced the remediation of PAH in the soil.

4. DISCUSSION

Polyaromatic hydrocarbons (PAHs) and heavy metals are toxic environmental pollutants that have accumulated in the environment due to a variety of anthropogenic activities. Remediation of these pollution can be done by various physical, biological and chemical methods. The present study tried to investigate the impact of organic mulching on the natural attenuation of these hydrocarbon polluted soils so as to prevent its detrimental effects.

The total PAH reduction discussed may have resulted from volatilization, diffusion and microbial degradation in a dissolved state [13,14]. Some of such factors that affects the rate and extent of biodegradation of PAHs in soil are microbial population characteristics, physical and chemical properties of PAHs and environmental factors (temperature, moisture, pH, degree of contamination).

According to [15], biodegradation of petroleum hydrocarbons is significantly affected by the physical properties of the latter. For example, the solubility of hydrocarbons in water is enhanced at significantly lower concentrations; the degree, therefore, of oil spreading over an enormous surface area of the soil is key for active and effective colonization by soil microorganisms that effectively utilize hydrocarbons [16]. Also, higher temperatures increase the rates of hydrocarbon metabolism to a maximum. Typically, in the range of 30 to 40℃, thermophilic alkane-utilizing bacteria do exist, decreasing hydrocarbon toxicity. Soil moisture is a major control on microbial and microfaunal community structure and activity in the soil. Physiological stress, such as drought, tends to reduce microbial diversity, favoring those microbes best adapted to coping with the stress [17] and also, soil pH also influences the mobility of nutrients and metals, it also impacts microbial activity and can alter the community compositions. However, extremes in soil pH negatively affect soil microbial activity, particularly their capability for hydrocarbon utilization. Optimal pH therefore is within the region of neutrality [18,19].

Table 2. Polyaromatic hydrocarbon and heavy metal contents of oil-polluted soil after 3 months oil pollution and subsequent mulching

Heavy metal	Oil-polluted soil just before mulching	3 months after pollution					
		Colour of polyethylene mulch					
		Control (Uncovered)	Aluminum	Black	Transparent	Tarpaulin	Granite
		Polyaromatic hydrocarbons					
Naphthalene	31.02	0	0	0	0	0	0
Acenaphthylene	19.74	16.11	0	18.21	16.24	17.18	13.67
2-bromonaphthalene	35.21	16.57	14.58	0	16.63	17.82	14.26
Acenaphthene	37.41	0	0	0	0	17.09	0
Fluorene	45.22	16.56	14.11	18.83	0	17.73	14.13
Phenanthrene	5.66	0.19	0.31	0.04	0.22	0.73	0.88
Anthracene	29.24	19.62	14.99	20.66	20.61	19.48	15.91
Fluoranthene	42.53	18.31	14.05	19.11	16.59	17.52	14.21
Pyrene	38.22	17.95	0	19.73	17.78	17.41	15.22
Benzo(a)anthracene	53.87	26.33	26.07	23.08	21.85	46.01	28.05
Chrysene	123.54	12.66	0	0.38	2.05	13.49	22.21
Benzo(b,j,k)fluoranthene	59.44	6.49	7.55	7.13	21.36	11.31	16.86
Benzo(a)pyrene	198.42	53.85	88.55	55.08	39.57	48.45	68.92
Indeno(1,2,3-cd)pyrene	169.54	43.41	18.62	1.44	22.32	68.04	29.70
Dibenzo(a,h)anthracene	63.48	39.67	46.10	1.21	47.25	64.97	50.81
Benzo(g,h,i)perylene	72.61	49.08	44.63	29.56	47.07	42.80	77.17
Total PAH	1025.15	336.8	289.56	214.46	289.54	420.03	382.00
PAH Rem. Efficiency (%)	-	67.15	71.75	79.08	71.76	59.02	62.74
		Heavy metals					
Copper, Cu	10.64	1.77	6.39	8.32	8.84	9.98	8.91
Manganese, Mn	9.68	BDL	BDL	BDL	BDL	BDL	BDL
Nickel, Ni	0.93	BDL	BDL	BDL	BDL	BDL	BDL
Vanadium, V	0.18	BDL	BDL	BDL	BDL	BDL	BDL
Chromium, Cr	10.09	BDL	BDL	5.24	11.83	10.53	9.90
Lead, Pb	29.21	8.16	16.32	14.18	28.66	14.66	17.08
Total heavy metal	60.73	9.93	22.71	27.74	49.33	35.17	35.89
Metal Rem. Efficiency (%)	-	83.64	62.60	54.32	18.77	42.09	40.90

BDL, below detection limit, 0.0001 mg/kg

Table 3. Microbial composition of mulched oil-polluted soil at 3 months after exposure to treatments

Sample identity	**Bacterial isolates Identified (x10^{4} cfu/g)**	**Bacteria counts (x10^{5} cfu/g)**	**Hydrocarbon bacteria degraders counts (x10^{5} cfu/g)**	**Percentage hydrocarbon-degrading bacteria (%)**	**Fungal isolates identified (x10^{5} cfu/g)**	**Fungal counts (x10^{5} cfu/g)**	**Hydrocarbon fungal degraders counts (x10^{5} cfu/g)**	**Percentage hydrocarbon-degrading fungi (%)**
Control (Uncovered)	_*Micrococcus luteus_ _Clostridium perfringens_ _Sarcina_ sp	1.8	1.1	61.11	_*Penicillium_ _Rhizopus sp_	1.0	0.9	90.00
Aluminum	_*Micrococcus luteus_ _C. perfringens_ _Sarcina_ sp	3.3	1.1	33.33	_*Aspergillus niger_ _*Fusarium solani_ _Rhiszopus stolonifer_	2.7	1.5	55.55
Black	_*Bacillus substilis_ _*M. luteus_ _M. roseus_	4.1	2.4	58.54	_*Aspergillus niger_ _Mucor_ sp _Geotrichum_ sp	1.0	0.2	20.00
Transparent	_*Bacillus pumilus_ _C. perfringens_ _Sarcina_ sp	6.5	3.2	49.23	_*Aspergillus niger_ _Mucor_ sp _Geotrichum_ sp	0.9	0.1	11.11
Tarpaulin	_*Micrococcus varians_ _*Bacillus substilis_ _Achromobacter_ sp	6.8	3.0	44.12	_*Penicillium sp_ _Mucor_ sp _Geotrichum_ sp	0.9	0.2	22.22
Granite	_*Bacillus substilis_ _Pseudomonas_ sp	4.5	2.0	44.44	_*Aspergillus niger_ _*Penicillium sp_ _Mucor_ sp _Geotrichum_ sp	2.1	1.2	57.14

*Hydrocarbon degraders

The present study recorded a number of bacteria and fungi species. *Micrococcus luteus, C. perfringens, Sarcina* sp, *Bacillus substilis, M. luteus, M. roseus, Aspergillus niger, Mucor* sp., and *Geotrichum* sp were present. Waste oil pollutants impede the growth and development of soil microbial populations [20]. However, their presence in oil-polluted soils suggests tolerance patterns. However, diverse soil microbial species have been reported to be capable of degrading or utilizing numerous dissimilar hydrocarbon groups [21].

5. CONCLUSION

In this study which was to investigate the impact of organic mulching on the natural attenuation of hydrocarbon polluted soils so as to prevent its detrimental effects, results show the polyaromatic hydrocarbon and heavy metal contents were decreased by application of the various mulches.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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