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Impact of Cold Shock on the Intrinsic Qualities of a Bioremediated Petroleum Hydrocarbon-Polluted Soil

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ABSTRACT: *The present study investigated the effects of cold shock on the intrinsic qualities of a bioremediated petroleum hydrocarbon-polluted soil. Into already perforated buckets were measured 5kg sun-dried top soil (0-10cm). Waste engine oil (WEO) was added to soil and mixed thoroughly to obtain similar concentrations of 5 % w/w oil in soil. The entire setup was divided into 5 treatment sets, depending on the interval of exposure to a total of 250ml of ice pellets at a time;daily exposure (1PD), twice per week at equal intervals (2PW), once weekly (1PW), fortnightly (2PM), and once monthly (1PM). The control soil did not receive any cold treatment. Results showed significant reductions in heavy metal and polyaromatic hydrocarbon concentrations. Immediately after soil was contaminated with waste engine oil concentration of vanadium 0.08mg/kg, but was beyond detectable limit in all the treatment and control at after exposure to cold shocks 3 months later (MAP). Total PAH at 3 MAP was 142.81mg/kg in 1PD, 96.27mg/kg in 2PW, 196.31 mg/kg in 1PW and 147.39mg/kg in 1PM, compared to 1055.15mg/kg immediately after soil was contaminated with WEO. It is therefore demonstrated that exposing soils to low temperature shocks at most twice in a week offered comparatively better remediative capabilities for the soil.*

Keywords: Bioremediation, cold shock, hydrocarbon, petroleum, natural attenuation, temperature.

Introduction

In any developing countries like Nigeria, it is not an uncommon feature to find dark or oily spots on roads, open vacant plots, walk ways, and even in agricultural lands. These spots are usually evidences of indiscriminate waste engine oil disposal. When engine oil escapes from the car, it has the ability to go very far distances, most used engine oil flows down to water bodies in form of run offs. Oil spills cause an extremely harmful site in the environment it has negative impact or both plants and wildlife while bring suffering to man and economic losses. The effect of oil spills on wildlife have received media interest, oil spill also destroy plant since the oil also damage plants ability to carry out its basic life function such as photosynthesis.

Waste engine oil can be a contaminant in the soil and drinking water. Little amount, 50 to 100ppm, of wasted engine oil can pollute a plant. The productivity of a soil is reduced when it is polluted with wasted engine oil [1]. Oil contaminated soil looks waxy and prevent water movement from top of the soil [2], and floods when wet. Changes in the soil structure and configuration of enzymes are the effect of plant due to oil pollution [3]. Heavy metal content in an oil polluted soil prevents growth of most plant species. Some heavy metals are needed in little quantities in plant species. For example, Copper is very important in plant metabolism. It activates enzymes; it is involved in protein synthesis and carbohydrate, nucleic acid and lipid metabolism. When there is increase of heavy metal in plant, it reduces the growth of that plant, e.g. rice plant [4].

Soil that is polluted with oil gives an unpleasant condition for plant growth [1]. Oil penetration and accumulation to plant brings damage to cell membranes and leakage of cell contents [5]. Leaf chlorosis and plant dehydration was seen in the growth of cereals that was grown in an oil polluted soil [6].Oil spills on soil surface forms a crust. The crust formed may prevent the growth of plant due to insufficient water and nutrient element that cannot enter the soil. Soils that are polluted with oil spills will not be able to take water from the top except from the sides. Isirimah et al., [7] reported that he observed reduction in the presence of nitrogen in the soil with high oil pollution. There was an increase in the presence of phosphorus level, as the level of oil in the soil increased by 2% thereafter there was a reduction [7].

Contamination control ways that involve physical and chemical methods have always increased the problem instead of removing it. Biodegradation method is very cheap and attractive [8]. The major agent in the removal of petroleum hydrocarbon is microorganism [9]. Bacteria, yeast, filamentous fungi and algae are some of the microorganism [10, 11]. There is no microorganism that has been found to be able to totally remove a petroleum hydrocarbon molecule. Facundo et al. [12] however stated that there are different species or strains of the same species which are able to removing different groups of hydrocarbon.

Vidali [13] reported that temperature was one the factors that affected the efficiency of bioremediation. Temperature affects soil microbial composition and processes and as such has a prominent effect on remediative capabilities of oil-polluted soils. Soil temperature is one of the important factors controlling activity and survival of microorganisms as well as the rate of organic matter decomposition among all the ecological factors. Temperature of both soil and air determine the rate of biological degradation processes in the soil, as well as the soil moisture content [14]. Increasing the temperature increases the rate of degradation of organic compounds in soil [15]. The growth of micro-organisms usually doubles for every 10^{o}C rise in temperature [16]. Increasing temperature also decreases adsorption, which makes more organic material available for microorganisms to use for the degradation [14]. The use of hydrocarbon by micro-organisms can take place at temperatures ranging from -2 to 70 ^{o}C [17]. Microbial degradation of hydrocarbons occurs even at 0 ^{o}C. Microorganism can grow at temperature ranging from sub-zero to more than 100 ^{o}C. Microbes are divided into four groups based on the temperature range at which they can grow in. In an effort to improve the bioremediation process in cold regions researchers have used treatability experiment to know the presence or absence of microbial activity for a particular contaminant or group of contaminant to determine the optimum requirement such as nutrients [18].

Several factors determines the bioremediation technology most suitable for a specific site, factors such as site conditions, indigenous microorganism population, and quantity, the type and toxicity of contaminant chemicals present. Making the best or most effective use of the environmental factors that affect the progress of bioremediation activity has a crucial role in its success. This may cause a reduction in maintenance cost; successful running of the system throughout the year, successful mineralization of the contaminants, and returning the site to its normal functional ecosystem. This needs an understanding of the microorganisms and the conditions required for them to cause effective

bioremediation. This informs the basis for the present study, the object being to investigate the effect of cold shock on the remediative capability of an oil-polluted soil.

Materials and Methods

Sample preparation:

Top soil (0-10 cm), of predetermined physicochemical property (Table 1), was collected from a randomly marked area measuring 10 x 10m on a fallow land situated within University of Benin campus, Benin City, Nigeria. Thereafter, 10kg sun-dried soil was each measured into large perforated 45 cm-diameter bowl with 8 random perforations made with 2 mm diameter nails at the bottom of each bowl. Soils in each bowl were thoroughly mixed with waste engine oil (WEO) to obtain a uniform mixture of 5%w/w oil-in-soil concentration. For clarity, the 100 g WEO measured 135.2 ml.

Methodology adopted

Having predetermined the water holding capacity of the soil to be 209 ml/kg soil, each bowl was initially wetted with 1000ml of water initially, before ice treatments were introduced subsequently. The total volume of ice pellets placed on each bowl was 500ml at a time. The pellets were placed in such a way that the entire surface area of the soil was covered equally with ice pellets. The experimental set up consisted of 5 cold treatments and a control. The cold treatments included placements of ice pellets on the total surface area of the oil-polluted soils at 5 different intervals; daily exposure (1PD), twice per week at equal intervals (2PW), once weekly (1PW), fortnightly (2PM), and once monthly (1PM). The control soil did not receive any cold treatment. Treatments were placed in a well ventilated screen house with inherent room temperature (25 - 32^{o}C) for 3 months, after which the soil was analyzed for heavy metal and polycyclic aromatic hydrocarbons.

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 Spectrometer.

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.

Fractionation Procedure:

Preparation of the Fractionation Column

A glass wool fibre was inserted into the base of the column. The column must be a polypropylene type. 10 g of silica gel (60-200 mesh size, Davidson Grade 850 or its equivalent) was weighed, pre-conditioned by baking at 105^{o}C overnight, and the column was packed with it. The base of the column was tapped to pack the silica gel properly. The column was eluted very well with n-hexane, and care was taken not to allow it to dry out during this period.

Fractionation of the Sample Extract

Using a pipette, 1 ml of the sample extract was transferred to the top of the column. It was eluted with 60 ml of n-hexane to the get the aliphatic hydrocarbons and the eluates collected in a conical flask. While the hexane was almost getting dry, 40 ml of DCM was added to elute the PAH components. Also, the eluates were collected in another conical flask. The sample eluates were then concentrated to 1 - 3 ml using the rotary evaporator, before being transferred into sample vials.

GC Analysis

The GC analysis began by first injecting 1l 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)
Results of the analyses were done in triplicates.

Identification of Soil Microorganisms

Isolation and characterization of bacterial and fungal oil degraders was carried out using the methods of Cowan and Steel [19] and Cheesebrough [20].

Phytoassessment

After 3 months of exposing the soils to the various cold treatments, the soils were phytoassessed to ascertain the success of remediation. This was done by sowing cowpea (*Vigna unguiculata* cv. Ife Brown) in remediated soils. Plant parameters used for assessment were those recorded only

during seedling growth stage of the test plant. These included the number of days taken for seedling emergence, percentage emergence, height of emergents, fresh and dry weights of emergents, percentage survival of emergents, as well the duration taken for the appearance of chlorotic and necrotic tissues in the developed seedlings, including the number of days taken to record total death of all seedlings.

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 [21, 22]:

Hazard Quotient (HQ)

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

When HQ > 1: Harmful effects are likely due to contaminant in question
When HQ = 1: Contaminant alone is not likely to cause ecological risk
When HQ < 1: Harmful effects are not likely

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

$TEQ = \sum T_i \times TEF$

Where TEQ = Toxic Equivalency, T_i = PAH concentration in soil, TEF = Toxic Equivalency factor.

Results and Discussion

The present study investigated the impact of cold shock on the intrinsic bioremediation of a waste engine oil-polluted soil. Chemical composition of waste engine oil and topsoil used for the experiment are presented on Table 1. Immediately after soil was contaminated with waste engine oil the total heavy metal acid was 3154.80mg/kg (Table 2) comprising of Copper (10.32mg/kg), Manganese(1.08mg/kg), Nickel (0.68mg/kg), Vanadium (0.08mg/kg), Iron (3005.34mg/kg), Chromium (18.32mg/kg), Cadmium (26.21mg/kg), Lead (8.52mg/kg) and Zinc (65.25mg/kg).

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

Parameters	WEO (mg/kg)	Soil (mg/kg)
Naphthalene	25.95	0
Acenaphthylene	7.62	0
2-bromonaphthalene	28.32	0
Acenaphthene	21.25	0
Fluorene	42.33	0
Phenanthrene	4.2	0.85
Anthracene	19.65	0
Fluoranthene	33.21	0
Pyrene	24.09	0
Benzo(a)anthracene	41.09	0
Chrysene	116.04	0
Benzo(b,j,k)fluoranthene	38.05	0
Benzo(a)pyrene	118.24	40.28
Indeno(1,2,3-cd)pyrene	131.05	5.24
Dibenzo(a,h)anthracene	34.22	12.25
Benzo(g,h,i)perylene	59.66	19.24
Copper, Cu	17.92	0
Manganese, Mn	3.1	0
Nickel, Ni	0.03	0
Vanadium, V	0.93	0
Chromium, Cr	26.85	0.08
Lead, Pb	12.96	0
Iron, Fe	3395.27	298.62
Cadmium, Cd	32.51	0
Lead, Pb	11.65	0
Zinc, Zn	124.42	0

Detectable limit is 0.0001mg/kg

Three months later copper in the control was 3.15mg/kg in 2PW. Vanadium was beyond detectable limit in all the treatment and control but 2PW (0.8mg/kg), Nickel and Manganese were totally remediated at three months after pollution. Comparatively efficiency of heavy metal removal in the soil was highest at 1PW (38.13%) and lowest at 2PM (26.15%)

Table 2: Soil parameters (heavy metals) at 3 months after pollution

Heavy metal components	Immediately after soil contamination	3 MAP						LSD (0.05)
		Control	1PD	2PW	1PW	2PM	1PM	
		Heavy metal contents (mg/kg)						
Cu	10.32	5.15	4.06	6.96	5.42	5.61	4.05	*0.49*
Mn	1.08	BDL	BDL	BDL	BDL	BDL	BDL	*0.65*
Ni	0.68	BDL	BDL	BDL	BDL	BDL	BDL	*0.23*
V	0.08	BDL	BDL	0.08	BDL	BDL	BDL	*0.009*
Fe	3005.34	2069.25	1969.45	1931.239	1865.21	2269.49	2108.25	*203.42*
Cr	18.32	2.88	5.25	3.98	5.21	6.35	8.35	*2.67*
Cd	26.21	11.05	11.20	8.28	6.85	9.62	11.02	*2.14*
Pb	8.52	3.62	1.37	1.48	BDL	0.09	BDL	*1.06*
Zn	65.25	40.34	45.58	54.30	56.67	38.71	37.02	*10.28*
Total heavy metal	3154.80	2134.29	2038.91	2007.33	1939.36	2329.87	2168.69	-
Efficiency (%)	-	32.35	35.37	36.37	38.53	26.15	31.26	-

BDL = below detection limit of 0.0001mg/kg. Efficiency was calculated only from mean of heavy metals obtained. LSD (0.05) = least significant difference among mean values on similar rows at 5% confidence limit.

Hazard quotient to show ecological toxicity of heavy metal components of waste engine oil polluted soil at three months after pollution have been presented in Table 3. Ecological toxicity was not indicated for Copper, Manganese, Nickel, Vanadium and Lead in all treatment and control, however ecotoxicity of heavy metal attributed to oil polluted soil in the present study were attributed to Iron , Chromium and Cadmium which had concentration of ecological concern been higher than permissible limit (Table 3). Hazard quotient of Zinc was greater than one at day one making it at that point a heavy metal of ecological concern. However three months later it was reduced to safer level in 1PD, 2PM, 1PM and control soils. With regards to hazard quotient to determine toxicity heavy metal component of waste engine oil polluted soil against soil microbes and microbial processes at three months after pollution, the Fe concentration in soil were at toxic levels. Although the concentration of Cr and Cd immediately after soil contamination with WEO were beyond safe levels tolerable for microbial activities and processes, levels after 3 months were eventually tolerable; indicating significant remediation.

Remarkably, most decontamination of heavy metals in the present study could be attributed to both biotic (e.g. microbial activity and processes) and abiotic processes (e.g. adsorption onto soil colloids, transformation and evaporation) which play an important role in freshly contaminated soils but are of minor importance in soils with aged pollution. Metals in soil need to be removed from the matrix by solubilization in a liquid phase. Afterwards, they can be concentrated in the desolubilization phase [23]. Microbiological processes can either solubilize metals, thereby increasing their bioavailability and potential toxicity, or immobilize them, and thereby reduce the bioavailability of metals. These biotransformations are important components of biogeochemical cycles of metals and may also be exploited in bioremediation of metal contaminated soils [24, 25, 26].

Soil microorganisms can detoxify metals by valence transformation, extracellular chemical precipitation, or volatilization. They can enzymatically reduce some metals in metabolic processes that are not related to metal assimilation. More so, although the toxic metals remain in the soil, once they are bound to the microorganisms, they become less bioavailable. It is well known that bacteria can bind metals to their cell surfaces, but, unfortunately, their natural binding capacity is generally insufficient to significantly mitigate metal contamination [27].

Total polyaromatic hydrocarbon content of the soil was 1055.15mg/kg immediately after soil was contaminated with waste engine oil. Three months later however total polyaromatic hydrocarbon reduced to 142.81mg/kg in 1PD, 96.27mg/kg in 2PW, 196.31mg/kg in 1PW and 147.39mg/kg in 1PM, compared to 204.44mg/kg in the control, this shows the efficiency of bioremediation was highest in 2PW (90.88) and lowest in 1PW (81.40) compared to the control 85.48mg/kg. Although Acenaphthylene 2-biomonaphthalene, Acenaphthene, Fluoranthene and Pyrene had values ranging from 19.74mg/kg to 42.53mg/kg, immediately after soil was contaminated with waste engine oil, they were entirely remediated in all treatment and control soil at three months after pollution (Table 4).

Table 3: Hazard quotient to determine toxicity of heavy metal components of waste engine oil-polluted soil at 3 months after pollution

Heavy metal components	Immediately after soil contamination	3 MAP					
		Control	1PD	2PW	1PW	2PM	1PM
HQ to determine ecological toxicity							
Cu [40.00]	0.26	0.13	0.10	0.17	0.14	0.14	0.10
Mn [100.00]	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Ni [30.00]	0.02	0.00	0.00	0.00	0.00	0.00	0.00
V [2.00]	0.04	0.00	0.00	0.0008	0.00	0.00	0.00
Fe [200]	15.03*	10.35*	9.85*	9.66*	9.33*	11.35*	10.54*
Cr [1.00]	18.32*	2.88*	5.25*	3.98*	5.21*	6.35*	8.35*
Cd [4.00]	6.56*	2.77*	2.80*	2.07*	1.71*	2.41*	2.76*
Pb[50.00]	0.17	0.07	0.03	0.029	0.00	0.0018	0.00
Zn [50.00]	1.31*	0.81	0.91	1.09*	1.33*	0.77	0.74
HQ to determine toxicity against soil microbes and microbial processes							
Cu [100]	0.10	0.05	0.04	0.06	0.05	0.06	0.04
Mn [10]	0.11	0.00	0.00	0.00	0.00	0.00	0.00
Ni [200]	0.0034	0.00	0.00	0.00	0.00	0.00	0.00
Fe [30]	100.18*	68.98*	65.65*	64.38*	62.17*	75.65*	70.28*
Cr [10]	1.83*	0.29	0.53	0.39	0.52	0.64	0.84
Cd [20]	1.31*	0.55	0.56	0.41	0.34	0.48	0.55
Pb [50]	0.17	0.07	0.03	0.03	0.00	0.0018	0.00

*toxicity indicated. Values in bracket are permissible limits [21]. HQôs were calculated only from the mean values of heavy metals in Table 2.

Although there was comparatively increased PAH remediations in the cold-treated soils compared to the control, Wu *et al.* [28] however reported that low temperatures affect the rates of biodegradation through the modification of the physical nature of the contaminants. For petroleum hydrocarbons, a decrease in temperature results in increased viscosity, decreased volatilization and increased water solubility (resulting in higher toxicity of short-chain alkanes), and decreased bioavailability of some compounds such as long-chain alkanes [29]. Beside of these effects, the environmental temperature influences microbial activity. Due to the Q10 effect, reaction rates are reduced in the cold, however, local environmental conditions select for populations with high activities at low temperatures. These reported effects may not have been significantly recorded in the present study probably because the soil was constantly monitored under room temperatures, but only received ice blocks to immediately induce a drastic shift in temperature. Successful bioremediation at low temperatures has been however described for arctic and antarctic soils [30].

The researchers also noted that the treatments with ice were better than the control probably because of increased soil moisture [31]. As the ice block melted, they enhanced the soil moisture, which incidentally is sine qua non to successful natural attenuation [13, 31]

Hazard quotient to determine ecological toxicity of polyaromatic hydrocarbon component of waste engine oil polluted soil at three months after pollution are presented on (Table 5) Hazard quotient for polyaromatic hydrocarbon values indicated on Table 5 where all greater than one at day one after pollution. These were however significantly reduced to values less than one in both treated and controlled soil in Acenaphthene, Flourene, Fluoranthene and Pyrene, indicating that these pH components did not pose any ecological treat. Contamination factor values of Anthracene (292.4) were reduce to 183.2 in the control and 128.1 in 1PD at three months after pollution. However contamination factor values in 2PW, 1PW, 2PM and 1PM were zero. These indicated that the later treatment where better measures for reducing ecotoxicity of Anthracene in the oil polluted soil.

Table 4: Polyaromatic hydrocarbon content of oil-polluted soil after 3 months oil pollution

PAH components	Immediately after soil contamination	3 MAP						LSD (0.05)
		Control	1PD	2PW	1PW	2PM	1PM	
	PAH contents of soil (mg/kg)							
Naphthalene	31.02	BDL	12.00	12.48	BDL	16.27	BDL	*5.62*
Acenaphthylene	19.74	8.92	BDL	BDL	BDL	1.52	0.99	*5.62*
2-bromonaphthalene	35.21	16.93	BDL	BDL	BDL	BDL	BDL	*9.65*
Acenaphthene	37.41	13.81	1.20	BDL	0.98	6.81	12.96	*6.85*
Fluorene	45.22	BDL	12.32	BDL	BDL	16.51	10.00	*8.63*
Phenanthrene	35.66	17.85	12.86	13.13	22.61	16.96	17.96	*7.09*
Anthracene	29.24	18.32	12.81	BDL	BDL	17.09	BDL	*9.25*
Fluoranthene	42.53	BDL	BDL	BDL	BDL	BDL	BDL	*0*
Pyrene	38.22	11.66	6.88	BDL	2.99	6.55	5.21	*4.28*
benzo(a)anthracene	53.87	21.14	13.82	BDL	28.65	BDL	BDL	*11.54*
Chrysene	123.54	19.76	16.18	14.85	25.23	19.74	19.46	*8.65*
benzo(b,j,k)fluoranthene	59.44	21.19	13.67	15.34	30.13	17.71	21.38	*8.24*
benzo(a)pyrene	198.42	23.57	16.70	18.24	44.87	22.75	22.18	*9.24*
indeno(1,2,3-cd)pyrene	169.54	3.06	2.29	1.29	4.14	4.43	2.47	*1.08*
dibenzo(a,h)anthracene	63.48	3.87	3.82	3.21	3.52	16.31	11.05	*2.68*
benzo(g,h,i)perylene	72.61	24.36	18.26	17.73	33.19	21.96	23.73	*11.07*
Total PAH (mg/kg)	1055.15	204.44	142.81	96.27	196.31	184.61	147.39	-
Efficiency (%)	-	80.62	86.47	90.88	81.40	82.50	86.03	-

BDL = below detection limit of 0.0001mg/kg. Efficiency was calculated only from mean values of PAH fractions obtained. LSD (0.05) = least significant difference among mean values on similar rows at 5% confidence limit.

Short-chain alkanes become less volatile and more water-soluble at low temperatures. This results in slower evaporation and a decreased probability that the microbes will come into contact with the compounds, both of which delay degradation. The researchers suggest that this may be one of the reasons why there were particularly no statistical differences in the bioremediation efficiencies in the cold-treated soils altogether. Cold conditions may cause other alkanes to precipitate from crude oil as waxes, rendering them inaccessible to microbes. Temperature can also affect hydrocarbon utilization: bacteria readily metabolize isoprenoids at 30° C but have difficulty doing so at 4° C. Although many species can withstand freezing and thawing, bacteria cease growth and metabolism altogether at temperatures below -12° C due to the formation of intracellular ice [32]. Low temperatures are, however, not always detrimental. Some hydrocarbons become less water-soluble at lower temperatures. Their lower solubility may reduce the potential toxicity of those particular compounds to members of the microbial community and could explain observations of slower but greater overall biodegradation at low temperatures [32].

Table 5: Hazard quotient to determine ecological toxicity of PAH components of waste engine oil-polluted soil at 3 months after pollution

PAH components	Immediately after soil contamination	3 MAP					
		Control	1PD	2PW	1PW	2PM	1PM
Naphthalene[0.10]	310.20*	0	120.00*	124.80*	0	162.70*	0
Acenaphthene[20.00]	1.87*	0	0	0	0	0	0
Fluorene[30.00]	1.51*	0	0.41	0	0	0.55	0
Phenanthrene[0.10]	356.60*	178.50*	128.60*	131.30*	226.10*	169 .60*	176.60*
Anthracene[0.10]	292.40*	183.20*	128.10*	0	0	0	0
Fluoranthene[0.10]	425.30*	0	0	0	0	0	0
Pyrene[0.10]	382.20*	0	0	0	0	0	0
benzo(a)anthracene[0.10]	538.70*	211.90*	138.20*	0	286.50*	0	0

**Toxicity indicated. Values in bracket are permissible limits [21]. HQ's were calculated from mean PAH values.*

Concentration of toxic equivalency (TEQ) of polyaromatic hydrocarbon components of waste engine oil polluted soil at three months after pollution is presented in Table 6. Total concentration of toxic equivalency was 216.61mg/kg in the oil polluted soil at day one these where reduced to values ranging from 17.09mg/kg to 45.54mg/kg in treated and controlled soil at three months after pollution. The total concentration of toxic equivalency (TEQ) of threes casinogenic polyaromatic hydrocarbon mixtures of the soil sample exceeded the method B cleaner level for Benzo (a) pyrene which was put at 0.13mg/kg [22] and as such the cleanup level for Benzo(a) pyrene was not meet in these particular soil samples.

Table 6: Concentration of toxic equivalency (TEQ) of PAH components of waste engine oil-polluted soil at 3 months after pollution

PAH components (mg/kg)	Immediately after soil contamination	3 MAP					
		Control	1PD	2PW	1PW	2PM	1PM
Chrysene [0.01]	1.24	0.19	0.17	0.15	0.25	0.19	0.19
Benzo(a)pyrene [1.0]	198.42	23.57	16.70	18.24	44.87	22.74	22.1
Indeno(1,2,3-cd)pyrene [0.1]	16.95	0.31	0.23	0.13	0.41	0.44	0.25
Total TEQ (mg/kg)	216.61	24.07	17.09	18.52	45.54	23.38	22.62

Values in bracket are permissible limits [21]. TECôs were calculated from mean PAH values.

Total bacteria count in the control soil was 4.3 x 10^5cfu/g at three months after pollution; comprising of *Micrococcus variants, M. luteus, Clostridium sp, Bacillus pumilis, B. subtilis, Enterobacter aevogenes* (Table 7). Bacteria count in the treated soil were generally less than those in the control; this values ranging from 3.3 to 4.0 x 10^5cfu/g, the lowest heterotrophic bacteria count was obtained in 1PD (3.3 x 10^5cfu/g). Percentage hydrocarbon degrading bacteria was highest in the control (67.44%) followed by 1PD (66.67%) and least in 1PM (30.00%), the implication being that the frequency of each shocks increased the percentage hydrocarbon degraders in the treated soils. commonest among the hydrocarbon degrading bacteria were *Micrococcus Varians* and *Bacillus pumilis.* Comparatively the percentage hydrocarbon degrading fungi among the heterotrophic counts were relatively higher than those of the bacteria. For example percentage degrading fungi in 1PM was 60% where as percentage degrading bacteria in the same treatment was 30%. Similarly percentage degrading fungi and bacteria where both 80.00% and 67.44% respectively. However there where relatively more bacteria count than the fungi count recorded in the present study. Prominent hydrocarbon degrading fungi species where *Aspergillus niger* and *Penicillium sp.*

Comparatively, cold-treated soils had better remediation efficiencies compared to the control soils. The two genera that are typically well represented at cold, petroleum-contaminated sites are *Acinetobacter* and *Pseudomonas*, both of which contain numerous species that can survive solely on hydrocarbon compounds [33]. Colonies of hydrocarbon-degrading species usually exist in very low abundance before the site becomes polluted. Upon exposure to oil, these colonies thrive and can expand to nearly complete dominance of the viable microbial community during the span of contamination [32]. The amount of time between contamination and microbial "bloom" will be greater if the population or species needs to acclimate to the pollutant, which is often the case for populations in previously uncontaminated sites. In the present study, *Pseudomonas* sp was isolated.

There was relatively more bacteria count than the fungi count recorded in the present study. Prominent hydrocarbon degrading fungi species where *Aspergillus niger* and *Penicillium* sp. This is supported by Ikhajiagbe [34]; Ikhajiagbe and Anoliefo [35, 36, 37] (2010, 2011, 2012).

Phytoassessment results showed significant differences ($p<0.05$) in plant parameters between untreated and cold-treated soils (Table 8). Although there was no significant differences among values obtained for plant growth parameters among the cold-treated soils. However, plants in the cold-treated soils, irrespective of the exposure time, had better growth performance compared to those in the oil-polluted soils that were not exposed to cold treatments. For example, dry weights of emergents at 9 days after sowing in cold-treated oil-polluted soils ranged from 0.195 ï 0.205g, compared to 0.112g in the untreated oil-polluted soil. Similarly, percentage survival of cowpea seedling in oil-polluted soil at 2 weeks after sowing was 61.25 ï 63.80% when sown in cold-treated soils, compared to 14.66% when soil was not cold-treated. Within 2 weeks, all seedlings sown in the untreated oil-polluted soil died, where as those in the cold-treated soils remained shortly after 3 weeks.

Conclusion

This study was carried out of a motive to set a cost effective way to remediate contaminated soil. Contamination of the environment by hydrocarbon is mainly due to accidental release, industrial process, waste oil from mechanic shops etc. One of the cost effective, ecofriendly means of achieving this goal is monitored natural attenuation. Attenuation is affected by a myriad of physical factors including temperature changes. The present study thus demonstrated that exposing soils to lower temperature at most twice in a week offered comparatively better remediative capabilities for the soil, and also coupled with the fact that the melted ice provided additional required moisture for improved microbial activity in the oil-polluted soil.

Table 7: Microbial composition of polluted and control soils at 3 months after pollution

Sample identity	Bacterial isolates Identified	Bacteria counts ($x10^5$ cfu/g)	Hydrocarbon Bacteria Degraders Counts	Percentage hydrocarbon degraders (%)	Fungal isolates Identified ($x10^5$ cfu/g)	Fungal counts ($x10^5$ cfu/g)	Hydrocarbon Fungal Degraders Counts	Percentage hydrocarbon degraders (%)
Control	**Mi.va, Mi.lu, Cl.sp* **Ba.pu , *Ba.su, En.ae*	4.3	2.9	67.44	**Fu.so, *As.ni, *Pe.sp*, *Mu.sp*; *Ge.sp*	4.0	3.2	80.00
1PD	**Mi.va, Mi.ro, *Ba.pu*	3.3	2.2	66.67	**As.ni, *Pe.sp*, **As.fv, *Pe.no*	1.8	1.0	55.56
2PW	**Mi.va, Mi.ro, *Ba.pu, En.ae*	3.9	2.3	58.97	**As.ni, *Pe.sp*, **As.fv, *Pe.no* *Ge.sp*, *Tr.sp*	2.9	2.1	72.41
1PW	**Mi.va,*Ba.pu, *Ba.su, En.ae*	3.7	2.1	56.76	**As.ni, *Pe.sp, Ge.sp, Tr.sp*	2.2	0.9	40.91
2PM	**Mi.va,*Ba.pu,Mi.lu, Cl.sp*	3.6	1.8	50.00	**As.ni, *Pe.sp*, *Ge.sp*, *Tr.sp*	1.9	0.8	42.11
1PM	**Ba.su,Mi.lu, Cl.sp*	4.0	1.2	30.00	**As.ni, *Pe.sp, Ge.sp*, *Tr.sp*	1.5	0.9	60.00

**hydrocarbon degraders*; *Mi.va Micrococcus varians; Mi.lu M. luteus; Mi.ro M. roseus ; Cl.sp Clostridium* sp; Ba.pu *Bacillus pumilis; Ba.su B subtilis; En.ae Enterobacter aerogenes; Fu.so Fusarium solani; As.ni Aspergillus niger;As.fv A. Flavus; Pe.sp Penicillium* sp; Pe.no *P. notatum* ; Mu.sp *Mucor* sp; Ge.sp *Geotrichum* sp; Tr.sp *Trichoderma* sp;

Table 8: The effects of soil amendment on some growth Parameters of *Vigna unguiculata* (var. Ife Brown) after 2 months

	Control (unpolluted)	Polluted soil	1PD	2PW	1PW	2PM	1PM
No. of days taken for seedling emergence	3.92^{b}	5.69^{a}	5.13^{a}	6.01^{a}	5.29^{a}	5.13^{a}	6.09^{a}
Percentage emergence at 1 WAS (%)	95.96^{a}	41.23^{c}	58.52^{b}	63.33^{b}	59.62^{b}	53.68^{bc}	62.51^{b}
Height of emergents at 9DAS (cm)	16.06^{a}	7.62^{c}	11.20^{b}	12.20^{b}	11.65^{b}	10.98^{b}	11.52^{b}
Dry wt. of emergents at 9DAS (g)	0.301^{a}	0.112^{c}	0.205^{b}	0.212^{b}	0.199^{b}	0.195^{b}	0.201^{b}
Percentage survival of emergents at 2WAS	91.86^{a}	14.66^{c}	63.22^{b}	64.29^{b}	61.25^{b}	62.14^{b}	63.80^{b}
1^{st} Day of noticed yellowing (DAS)	21.22^{a}	7.28^{c}	11.21^{b}	11.65^{b}	10.52^{b}	11.50^{b}	12.52^{b}
Day of noticed necrosis in plant (DAS)	0^{c}	11.85^{b}	19.32^{a}	18.92^{a}	17.56^{a}	17.92^{a}	19.25^{a}
Day recorded total death of all seedlings (DAS)	0^{c}	15.56^{c}	23.25^{b}	24.03^{b}	22.95^{b}	23.54^{b}	22.68^{b}

Values are means of 10 determinations. Means on the same rows with similar alphabets do not differ significantly ($p>0.05$) from each other. DAP –days after planting; WAP - weeks after planting.

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