Changes in Heavy Metal Contents of a Waste Engine Oil Polluted Soil Exposed to Soil pH Adjustments

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Authors' contributions

Author BI, in collaboration with authors GOA, EOO and NA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors EOO and BI managed the analyses of the study. Authors BI and NA managed the literature searches. All authors read and approved the final manuscript.

Research Article

ABSTRACT

The present study investigated the role of soil pH adjustments in heavy metals concentrations in waste engine oil (WEO) - polluted soils. Sun-dried top soil (0-10cm) was measured into buckets. WEO was added to soil and mixed thoroughly to obtain similar concentrations of 2.5% w/w oil in soil. The polluted soil was thereafter amended with NPK (15:15:15) fertilizer to enhance microbial activity. The buckets were transferred into a well ventilated screen house with inherent constant room temperature (27ºC). The entire setup was divided into 5 sets. Each set was wetted daily with 200ml of different pH solutions (pH 3, 5, 7, 9, and 11) for a period of 3 months. There were significant reductions in heavy metal concentrations particularly at pH of 5. There were significant reductions in total hydrocarbons contents (THC) of polluted soils at 2 months after pollution from 1882.32 mg/kg at pH 3 to 325 mg/kg at pH 5, compared to THC of soil at 1 week after pollution at pH 7 (3425.63mg/kg).

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1. INTRODUCTION

The petroleum industry has created economic boom for many countries and at the same time led to environmental and socio-economic problems. The environmental impacts associated with exploration and exploitation of crude oil has been a major area of experimental research in the last three decades. Pollution caused by the refined products such as engine oil and diesel have not been given the proper attention it deserves. There are several components of the oil, including solvents and detergents added during the blending process, aliphatic hydrocarbons and PAHs distilled from crude oil, and heavy metals from engine wear, and these are either toxic in themselves or can combine with products of combustion to generate carcinogens and endocrine disrupters [1].

Oil-contaminated soils are of environmental concern because they are unsuitable for agricultural and recreational uses and are potential sources for surface and ground water contamination. The PAHs components of the oil have very low water solubility and often tightly bound to soil particles. Oil-polluted soil could also become unsuitable due to a reduction in the level of available plant nutrients or a rise to a toxic level of elements such as manganese [2]. This heavy metal content of oil-contaminated soil imposes metabolic disorders and growth inhibition on most of the plant species. Some heavy metals are essential micronutrients for plant species. For instance, copper plays an important role in several plant metabolic processes. However, at excess of specific limits, these metals adversely affect plant growth [3,4,5]. Whisman et al. [6] reported presence of heavy metals such as vanadium, lead, aluminum, nickel and iron in unused oil products, with high values in used ones. It is therefore important that various means by which these pollutants are removed from the environment be carefully considered. This is even more important considering the fact that there are several remediation technologies, some of which are controversial, particularly when they involve physical and chemical methods. These methods which are the most widely used procedures for clean-up are not entirely simple or environmentally favourable. This therefore underscores the need for environmental friendly approaches to remediation. One of such approaches relies on the soils inherent abilities to remediate contaminants [4, 7].

Successful natural attenuation of oil polluted soils are a combination of several forces in synergism – plant action, microbial degradation and sequestration, percolation of water soluble fractions, volatilization of light weight hydrocarbon fractions, and a host of other possible mechanisms. However, a number of factors, physical, chemical or biological, however affect this inherent soil ability [4]. It is therefore the aim of the present study to investigate the effects of various levels of soil pH on the remediation of heavy metals constituent of waste engine oil (WEO) - polluted soil.

2. MATERIALS AND METHODS

Top soil (0-10cm), of known physicochemical properties (Table 1), was collected randomly from an area measuring 50 x 50m on a fallow land situated near the Department of Plant Biology and Biotechnology Screen House, University of Benin, Benin City, Nigeria. Thereafter, 5kg sun-dried soil was each placed into large perforated bowls with 5 random perforations made with 2 mm diameter nails at the bottom of each bowl. WEO was obtained randomly from petrol engine vehicles in Benin City, Nigeria, and pooled together to obtain a composite sample. Oil was added to soil in the bowls and mixed thoroughly to obtain similar concentrations of 2.5% w/w oil in soil. The polluted soil was thereafter amended with 4g NPK

(15:15:15) fertilizer [8] to enhance microbial activity. The buckets were transferred into a well ventilated screen house with inherent constant room temperature of 27ºC.

Five different pH solutions were prepared. A 1M NaOH solution was carefully added to distilled water (pH 7) to obtain pH 9 and pH 11 solutions. While orthophosphoric acid was added to distilled water to obtain pH of 3 and 5. The pH was read on a pH meter. These solutions were prepared and stored in clean jerry cans. The entire setup was divided into 6 sets. Each set was wetted daily with 200 ml of specific pH solutions. Care was taken to ensure that soils were equally wetted, with no leakages from the perforations. This was necessary to check that water-soluble fractions of WEO did not escape through the leakages. Each bucket was wetted every morning for a period of 3 months. The control soil was wetted with distilled water (pH 7) at the same rate. The soil was slowly and carefully turned every week to ensure that contents settling at the bottom of the bowls due to percolation and downward movement were equally mixed.

2.1 Soil Physicochemical Analyses

Soils were dried at ambient temperature (22-25ºC), 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 heavy metal fractions by atomic absorption spectrophotometry [9].

2.2 Identification of Soil Microorganisms

Isolation and characterization of bacterial and fungal oil degraders was carried out using the methods of Sabba [10].

2.3 Computation of Contamination Factor (CF)

CF expresses the ratio between the eventual concentrations of pollutant against its pre-industrial concentration.

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

In the present study, background/pre-contamination concentration is referred to as concentration of the heavy metal just before exogenous application of the WEO pollutant (Table 1).

2.4 Computation of Hazard Quotient (HQ)

HQ expresses the possibility of the contaminant being an ecological risk or a contaminant of potential ecological concern (COPEC). 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 are available at Efroymson *et al.* [11].

2.5 Computation of Environmental Risk Factor (ERF)

The environmental risk factor (ERF) is expressed by the following equation,

$$ERF = QV - \frac{Ci}{QV}$$

Where
QV= Quality Value (background/pre-contamination concentration).
C_i =Heavy metal concentration in the soil fractions.
ERF < O = Potential ecological threat.
ERF > O = No threat.

Table 1. Physical and chemical properties of soil before waste engine oil contamination

Parameters	Units	Soil
pH	-	6.11
Electrical conductivity	µs/cm	301.00
Total Org. matter	%	0.61
Total nitrogen	%	0.12
Exchangeable acidity	meq/100 g soil	0.22
Water-holding capacity	ml(water)/kg(soil)	83.00
K	meq/100 g soil	1.43
Ca	meq/100 g soil	15.26
Mg	meq/100 g soil	10.97
P	mg/l	153.00
Clay	%	7.90
Silt	%	13.90
Sand	%	78.20
Fe	mg/kg	998.80
Mn	mg/kg	16.71
Zn	mg/kg	12.12
Cu	mg/kg	4.98
Cr	mg/kg	2.08
Cd	mg/kg	ND
Pb	mg/kg	ND
Ni	mg/kg	3.60
V	mg/kg	0.76
Total hydrocarbon content	mg/kg	224.06

ND: Not detected ($\leq$0.001 mg/kg)

3. RESULTS AND DISCUSSION

Heavy metal contents of soil at 3 months after exposure to waste engine oil pollution and soil pH adjustment are presented on Table 2. At One week after pollution (1 WAP), Fe in soil was 1097.34 mg/kg at pH of 7. At 3 months after pollution (MAP), Fe content in the same soil pH decreased to 870.42 mg/kg. At pH 3 Fe content was 726.42 mg/kg and this further increased to 981.84 mg/kg. At pH 11, Mn in soil at 1 WAP was 18.4 mg/kg; 3 months later Mn in soil ranged from 9.2 mg/kg at pH 3 to 12.3 mg/kg in pH 11. Similarly, Zn at 3 MAP ranged from 13.7mg/kg - 15.8 mg/kg within pH ranges of 3 - 11, as against 16.4 mg/kg at 1 WAP. There was reduction in total Cd content of soil from 1.42 mg/kg at 1 WAP – 0.92 mg/kg at 3 months later at pH 7 (Table 2). Obviously, heavy metal content of soil at pH values near neutrality was lower than neutral (pH 7). Similarly, at pH 3 and 5 metal contents were lower, compared to neutrality. There were decreases in Ni and V contents at 3 MAP, compared to values obtained at 1 WAP. Total hydrocarbon content (THC) of soil at 1 WAP (pH 7) was 3425.63mg/kg, compared to 963.32mg/kg at pH7. At pH 3, THC was 1882.32mg/kg and 1204.43mg/kg at pH 11. However at pH values closed to neutrality (5 and 9) THCs were 325.63mg/kg and 433.98mg/kg respectively.

Although soil microorganisms can degrade organic contaminants, metals need immobilization or physical removal. In immobilization of metals, they are biotransformed by microbial activity to organic compounds, thereby ensuring their bio-unavailability. Microbial transformation of metals serves various functions. Generally, this occurs either by redox conversions of inorganic forms or conversions from inorganic to organic forms and vice versa [12]. Reduction of metals can occur through dissimilatory reduction where microorganisms utilize metals as a terminal electron acceptor for anaerobic respiration. For example, oxyanions of chromium [13, 14] can be used in microbial anaerobic respiration as terminal electron acceptors. In addition, microorganisms may possess reduction mechanisms that are not coupled to respiration, but instead are thought to impart metal resistance. Another mechanism of metal reduction is methylation. Microbial methylation plays an important role in the biogeochemical cycle of metals, because methylated compounds are often volatile. For example, Pb can be biomethylated to dimethyl lead [15]. Microbes may possess reduction mechanisms that are not coupled to respiration, but instead are thought to impart metal resistance. Methylation is another possible mechanism of metal reduction by microbial action. A number of different bacterial species including *Pseudomonas* sp., *Escherichia* sp., *Bacillus* sp., and *Clostridium* sp. have been implicated in the biomethylation of heavy metals [15]. The present study thus isolated *Pseudomonas* sp., *Bacillus* sp., and *Clostridium* sp from polluted soils, and these organisms may have been involved in the methylation of Fe, Cr and Mn [16]. Although many soil microbes carry out a number of transformations of metals, soil microbial activity and functioning can be affected by high concentrations of metals. Significant reductions in microbial biomass [16] and soil respiration [17] have been found in metal contaminated soils, compared to uncontaminated soils. On the other hand, microorganisms have evolved different resistance mechanisms to avoid toxic effects of metals. Adaptation is thus an important mechanism behind the responses of microbes to the presence of soil contaminants [18] and may therefore result in the compensation of an adverse effect by the increased activity of the remaining microbiota.

Table 3 shows contamination factor (CF) of soil at 3 MAP under varying soil pH adjustment. CF of Fe at pH 3 was 0.73, whereas at pH 11 it increased to 0.98. CF of Mn in soil ranged from 0.47 – 0.74 at 3 MAP. THC at 3 MAP was 1.45 at pH 5, compared to 4.30 at pH 7. This is a significant reduction from values obtained (15.29) at 1 WAP (pH 7). The CF explains the

possibility for the inherent concentrations of contaminants in the soil to be primarily due to exogenous application of oil treatments (i.e. CF > 1), compared to pre-contamination levels [4].

Hazard Quotient (HQ) expresses the possibility of the contaminant being an ecological risk or a contaminant of potential ecological concern (Table 4). At 3 MAP, there was reduction in HQ of Fe at 1 WAP from 5.4867 to a range of 3.2727 – 4.9092 three months later (Table 4). For these values, HQ>1. Hence harmful effects were likely due to Fe concentration in soil. HQ of Mn at both 1 WAP and 3 MAP (0.092 – 0.184) were generally less tan unity. Therefore this implies the harmful effect imposed by heavy metals were not likely due to Mn. It should be noted however that HQ values at pH 5 were lowest irrespective of the heavy metals considered, compared to other pH values.

The environmental risk Factor (ERF) as a pollution index determines environmental risk in other to establish potential threat to resident organisms [4, 18]. ERF values were presented as greater than zero, except for V, the implication being that potential ecological threat was implicated for V (Table 5).

At 1 WAP, total bacteria count was 3.4 x 10^5 cfu/g (Table 6). However, at 3 MAP, total bacteria count was 3.9 x 10^5 cfu/g. Total hydrocarbon degraders increased from 1.7 x 10^5 cfu/g at 1 WAP (pH 7), to 3.4 x 10^5 cfu/g at pH 5 during 3 MAP as against 2.8 x 10^5 cfu/g at pH 3 and 2.9 x 10^5 cfu/g at pH 9. Total heterotrophic fungal count was 5.2 x 10^5 cfu/g at 1 WAP (pH 7), and then increased to 6.0 x 10^5 cfu/g 3 months later at the same pH. Fungi species present included *Aspergillus niger, Trichoderma* sp*., Penicillium* sp*., Rhizopus* sp*.* at pH 9 (Table 6). *Aspergillus niger* and *Penicillium* sp were the hydrocarbon degraders present. Predominant hydrocarbon-degrading bacterial species was *B. subtilis,* followed by *M. varians.* Soil pH is important because most microbial species can survive only within a certain pH range. The majority of soil microbes thrive in neutral pH (6-7) due to the high availability of most nutrients in this pH range, but there are examples of microbes (especially fungi) that can tolerate pH of 1 to 13 [19]. Some organisms that can tolerate extreme pH include bacteria in *Halomonas* and Archea *Archaeoglobus*. Alterations in pH can render essential microbe enzymes inactive and/or denature proteins within the cells and prevent microbial activity from occurring. pH changes can also effect microbes in their access to metals and organics that react differently under varied pH régimes [19]. Majority of bacteria exhibit growth optima at or near neutral pH values. Increase of pH causes deprotonation of metal ions binding sites exposed to cellular surfaces [20]. Decreasing pH causes competition between protons and positively charged metal ions. However these rules concern only cations [21]. In general, an increase in soil pH tends to decrease the availability of Calcium, Magnesium, Sodium, Potassium, Ammonia, Nitrogen and Phosphorus, whereas decrease in soil pH results in decreasing availability of nitrate and chloride [22]. Soil nutrient availability has also been previously linked with increased microbial activity [4, 23].

Table 2. Heavy metals of soil 3 months after soil exposure to waste engine oil pollution and soil pH adjustments

		Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V	THC
		(mg/kg)									
1 WAP	pH7	1097.34^a	18.4^a	16.4^a	5.63^a	2.83^a	1.42^a	1.03^a	2.95^a	3.55^a	3425.63^a
3 MAP	pH3	726.42^c	9.2^{bc}	13.9^b	4.04^{bc}	1.78^b	0.65^b	0.67^c	1.83^b	3.08^b	1882.32^b
	pH5	763.21^{bc}	7.9^c	13.7^b	3.34^c	1.65^b	0.79^b	0.53^c	1.67^{bc}	2.56^d	325.63^d
	pH7	870.34^b	10.3^{bc}	14.3^{ab}	4.63^{ab}	2.09^b	0.92^b	0.98^{ab}	1.87^b	2.87^{bc}	963.32^c
	pH9	654.54^c	11.9^b	15.8^{ab}	3.96^{bc}	2.11^{ab}	0.98^b	0.79^{abc}	1.38^c	2.48^d	433.98^d
	pH11	981.84^{ab}	12.3^b	14.6^{ab}	4.07^{bc}	1.97^b	1.06^{ab}	1.03^a	1.98^b	2.93^b	1204.43^c
LSD (0.05)		116.34	3.1	2.3	1.26	0.73	0.41	0.28	0.39	0.34	282.34

WAP Weeks after pollution, MAP months after pollution. Means along the same column with similar alphabetic superscripts do not differ significantly (p<0.05) from the other.

Table 3. Contamination factor (CF) of soil 3 months after soil exposure to waste engine oil pollution and soil pH adjustments

		Fe	Mn	Zn	Cu	Cr	$^+$Cd	$^+$Pb	Ni	V	THC
1 WAP	pH7	1.19	1.10	1.36	1.13	1.36	$\geq 10^4$	$\geq 10^4$	0.82	4.55	15.29
3 MAP	pH3	0.73	0.56	1.15	0.81	0.86	$\geq 10^4$	$\geq 10^4$	0.51	3.95	8.40
	pH5	0.76	0.47	1.13	0.67	0.79	$\geq 10^4$	$\geq 10^4$	0.46	3.28	1.45
	pH7	0.87	0.62	1.18	0.93	1.00	$\geq 10^4$	$\geq 10^4$	0.52	3.68	4.30
	pH9	0.66	0.71	1.31	0.80	1.01	$\geq 10^4$	$\geq 10^4$	0.38	3.18	1.94
	pH11	0.98	0.74	1.21	0.82	0.95	$\geq 10^4$	$\geq 10^4$	0.55	3.76	5.38

$^+$pre-contamination values for Cd and Pb were $\leq 10^{-4}$ mg/kg. MAP months after pollution

Table 4. Hazard Quotient (HQ) of soil 3 months after soil exposure to waste engine oil pollution and soil pH adjustments

		Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V
1 WAP	pH7	*5.4867	0.184	0.164	0.563	0.283	0.071	0.0206	0.0378	0.0394
3 MAP	pH3	*3.6321	0.092	0.139	0.040	0.178	0.033	0.0134	0.0203	0.0342
	pH5	*3.8161	0.072	0.137	0.033	0.165	0.039	0.0106	0.0186	0.0284
	pH7	*4.3517	0.103	0.143	0.046	0.209	0.046	0.0196	0.0208	0.0319
	pH9	*3.2727	0.119	0.158	0.039	0.211	0.053	0.0158	0.0153	0.0276
	pH11	*4.9092	0.123	0.146	0.041	0197	0.053	0.0206	0.0220	0.0326

HQ>1, toxicity is indicated. WAP Weeks after pollution, MAP months after pollution

Table 5. Environmental Risk Factor (ERF) of soil 3 months after soil exposure to waste engine oil pollution and soil pH adjustments

		Fe	Mn	Zn	Cu	Cr	$^+$Cd	$^+$Pb	Ni	V	THC
1 WAP	pH7	998.7	15.6	10.8	3.85	0.64	$\geq 10^4$ $\geq 10^4$	$\geq 10^4$ $\geq 10^4$	2.78	-3.91*	208.7
3 MAP	pH3	998.07	16.1	10.9	4.17	1.20	$\geq 10^4$	$\geq 10^4$	3.01	-3.29*	215.5
	pH5	998.04	16.2	10.9	4.31	1.22	$\geq 10^4$	$\geq 10^4$	3.14	-2.61*	222.6
	pH7	997.92	16.1	10.9	4.05	1.28	$\geq 10^4$	$\geq 10^4$	3.08	-3.02*	219.8
	pH9	998.14	15.9	10.8	4.18	1.08	$\geq 10^4$	$\geq 10^4$	3.17	-2.50*	222.1
	pH11	997.82	15.9	10.9	4.16	1.07	$\geq 10^4$	$\geq 10^4$	3.15	-3.09*	218.7

ERF<1, toxicity is indicated. MAP months after pollution
$^+$*pre-contamination values for Cd and Pb were $\leq 10^{-4}$ mg/kg*

Table 6. Total colony counts of bacteria and fungi obtained from waste engine oil polluted soil exposed to 3 months of soil pH adjustment

	Soil pH	Bacteria counts (x10^5 cfu/g)	Hydrocarbon bacteria Degraders Counts(x10^5 cfu/g)	Bacteria isolates	Fungal counts (x10^5 cfu/g)	Hydrocarbon Fungal Degraders Counts(x10^5 cfu/g)	Fungi isolates
1WAP	pH 7	3.4	1.7	*Micrococcus varians* *Bacillus subtilis* Clostridium sp. *Pseudomonas sp.	5.2	2.8	*Aspergillus niger* *Penicillium sp
3 MAP	pH 3	6.0	2.8	*Micrococcus varians* *Bacillus subtilis* Clostridium sp.	3.6	1.9	*Aspergillus niger* *Penicillium sp
	pH 5	7.3	3.4	*Micrococcus varians* *Bacillus subtilis* Clostridium sp. *Pseudomonas sp.	8.9	4.6	*Aspergillus niger* Trichoderma sp. *Penicillium sp
	pH 7	3.9	1.7	*Bacillus subtilis* Clostridium sp. *Pseudomonas sp	6.0	2.8	*Aspergillus niger* *Penicillium sp Rhizopus sp.
	pH 9	5.1	2.9	*Micrococcus varians* *Bacillus subtilis* Clostridium sp.	6.8	3.4	*Aspergillus niger* Trichoderma sp. *Penicillium sp Rhizopus sp.
	pH 11	4.0	1.9	*Micrococcus varians* *Bacillus subtilis*	3.0	1.4	*Aspergillus niger* *Penicillium sp

*hydrocarbon degraders

4. CONCLUSION

Improved microbial activity is tied to pH changes. Therefore, it is possible that the changes observed in the heavy metal contents of the soil in the present study may be invariably due to changes in nutrient contents of soil which affected microbial action on the heavy metals. Therefore, for enhanced remediation of soil heavy metal components, a pH range most favourable to enhance nutrient availability, and have concomitant effect on increased soil microbial activity, is key to successful remediation. The present study suggests that biodegradation of petroleum hydrocarbons could be optimal when soils were constantly irrigated with a solution at pH 5.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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