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Original Research Article

Toxicological Indices of Crude Oil-Polluted Soil Ecosystem

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Abstract

Assessment of the level of contaminations from possible impact of crude oil on soil ecosystem is imperative for the determination of environmental acceptability. This study investigated this impact *ex-situ* using a culture-dependent approach to evaluate the total microbial counts; physicochemical tools to determine the cation exchange capacity (CEC), metal leachates, exchangeable bases (Mg, Ca, Na and K), pH, total petroleum hydrocarbon (TPH) and the overall effects on plants as indices of toxicity. The experiment demonstrated that at 1.5 – 3.5% contamination across days-zero to -28, there was a significant ($p<0.05$) increase in total petroleum hydrocarbon (TPH) from 0.03 ± 0.00 to 0.07 ± 0.00 with increase in acidity from pH 5.2 ± 0.00 to 4.0 ± 0.00 and a reduction in cation exchange capacity (CEC) from 0.82 ± 0.05 to 0.70 ± 0.11mEq and exchangeable bases with an augmented increase in phytotoxic elements and metal leachates. A reduction in microbial biomass from control, 1.30 x 10^9, to 3.6 x 10^8 cfu on week one as contamination increased and induction of hydrocarbonclastic organisms thereafter across weeks two and four, 3.88 x 10^8 and 4.40 x 10^8 cfu respectively was an indication of a reduction in microbial diversity. Ecosystem dynamics and mineralization were impaired and disrupted and the entire soil biochemistry altered with adverse effects on plant health.

Keywords: Microorganisms, TPH, CEC, Metal leachates, Exchangeable bases, pH.

INTRODUCTION

Environmental pollution as a consequence of impact from crude oil, petroleum-derived products, as well as polycyclic aromatic hydrocarbons (PAHs) and volatile aromatics (benzene, toluene, ethylbenzene, xylene) collectively indicated as BTEX causes soil ecosystem toxicity and degradation. Ecosystem being a community of living organisms in conjunction with non-living components of the environment that are interactive is adversely affected from this impact. These products deteriorate soil, air, and water ecosystems (Wang and Bartha, 1990; Sctompka, 1999). The soil is very essential for human existence for various reasons, in particular agriculture. However, several abuses from anthropogenic means has made the soil to be the first line of recipient of oil pollution such as petroleum (crude oil) and petroleum-by-products, dumping of wastes and other contaminating substances (Ebulue *et al*., 2017, 2020; Osam, 2011; Nwaugo *et al*., 2006, 2009).

The soil is a prime factor in agricultural productivity and socio-economic activities; therefore, any threat or substantial impairment usually affects the people's livelihood and galvanizes into public outcry. These contaminants, though biodegradable are the limiting factors to soil fertility and hence crop productivity, as they create an unsatisfactory condition for life in the soil because they are toxic to soil organisms and to plants (Delille and Pelletiemo, 2002; Wyszkowska *et al*., 2002). They can bio-accumulate in food chains where they disrupt biochemical or physiological activities of many organisms Onwurah *et al*., (2007); due to poor aeration they cause on the soil, nutrient immobilzation and lowering of soil pH (Atuanya, 1987; Achuba and Peretiemo-Clark, 2008). They have the potentials to trigger carcinogenic and mutagenic activities within the soil Krahl *et al*., (2002) Onwurah (2007); alter the succession of microorganisms Kaplan and Kitts (2004), which is directly associated with the induction and activities of soil enzymes Wyszkowska *et al*., (2002) Wyszkowska and Kucharski (2004) and synthesis of soil adenosine triphosphate (ATP).

The most obvious effect of pollutants' exposure to microbial communities is direct toxicity which results to rapid death. The extent of loss in

microbial activity/ biomass and alteration in activities of their exudates and pH can be used to assess the toxicity of that pollutant in the environment. Thus hydrocarbons increase the abundance of hydrocarbon degrading microorganisms, but on the other hand induce a limitation in microbial diversity (Ebulue *et al*., 2017). The consequent loss in mineral elements as they are leached and become bio-unavailable to plants could lead to a reduction in crop yield, thus the response of plants to oil pollution is unambiguously negative (Chaineau *et al*., 1997; Solanito *et al*., 1997). The cation exchange capacity (CEC) which is the ability of the soil particles to hold cations is lost and replaced by phytotoxic elements such as cadmium, aluminum and manganese ions. This effect which altered the entire soil biochemistry could disrupt ecosystem dynamics by slowing soil organic matter mineralization and associated nutrient remineralization (Ebulue *et al*., 2017).

Research Design

This research was designed for a-forty-two-day investigation in consideration of the volatility and biodegradability of hydrocarbons: day- zero, -14, -28, -42; within which, the activities of the aforementioned parameters were determined.

MATERIALS AND METHODS

Crude oil was obtained from Port Harcourt Refinery, Rivers State, Nigeria and the soil sample was obtained from Owerri, Imo State, a neighbouring state to Rivers State with an augar inserted about fifteen centimeters into the soil. Ten grams (10g) of soil contaminated with different concentrations (1.5, 2.5, and 3.5% w/w) of crude oil were used for the determination of the aforementioned parameters.

Determination of pH of crude oil-contaminated soil

Principle: Advanced Bench pH Meters 3510 suitable for easy readout of pH was used.

Procedure: Soil inoculation was carried out by weighing 10g of sieved soil sample into four different test tubes. To the first tube, 0.15g of crude oil in 10g soil sample (corresponding to 1.5%), was added and mixed thoroughly with a steering rod. This procedure was repeated for 1.5, 2.5, and 3.5% with 20ml of deionized water added; and into the 4th tube, the control, 20ml of deionized water was only added to 10g soil and mixed thoroughly by hand. Into the homogenous slurry formed was immersed pH meter probe (Jenway model) and was allowed to stabilize at 25^{o}C. The pH values were then determined after calibration with buffer solution of pH 7.0 and 4.0.

Determination of total petroleum hydrocarbon (TPH)

Total petroleum hydrocarbon was determined gravimetrically by the method of Odu *et al*., (1989), to provide an estimate of the available total hydrocarbon with time.

Leachates from crude oil-polluted soil

This is based on the principle that leaching materials are transferred from a stabilized matrix to liquid medium such as ground water or solutions. The toxicity characteristics leaching procedure (TCLP) extraction is expected to test contaminated soils and sediments, sludges, petroleum contaminated soils, waste oils or fuels.

TCLP extract preparation: Extract the solid sample, separate the liquid extract from the solid sample by filtration. The extracted solution was then analyzed for leachable metal using Inductively Coupled Plasma Emission Spectrometry (ICP-MS).

Procedure: The toxicity characteristics leaching procedure (TCLP) is a leaching test used to determine the content of metals in contaminated materials [19]. The Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-MS) was used for analysis of total metals and TCLP extract.

Determination of cation exchange capacity (CEC)

Principle: The principle of flame photometry was used.

Procedure: 25ml of 1N potassium chloride was added into the 10g soil sample, shaken for 20 minutes, and filtered with Whatman No.1 filter paper.10ml of the filtrate was titrated against 0.1N sodium hydroxide and the cation exchange capacity was evaluated.

$$\text{mEq CEC/100g Soil} = \frac{T \times N \times 100 \times 100}{50 \times 5}$$

Where,
T = Titre value
N = Normality
100 = For total leachate
50 = Aliquots volume taken
5 = Weight of soil sample used

Determination of exchangeable bases

Magnesium:

Principle: Titration based on color changes and the titre value was used to determine the presence of magnesium.

Procdure: Ten (10) ml of the leachate was pipetted into a conical flask and 25ml of distilled water was added and shaken thoroughly. 10ml of 0.1N ammonium buffer 7.4 solution and a pinch of Erichrome black-T indicator were added to the mixture and titrated with 0.01M EDTA till the colour changed from brown to blue. The titre value was then used to calculate the amount of magnesium present in the sample.

$$\text{mEq Mg/100g soil} = \frac{\text{T x N x 100g Soil}}{\text{10 x 5}}$$

Where,
T = Titre value
N = Normality of EDTA.

Calcium:

Ten (10) ml of the leachate was pipette into a conical flask and 25ml of distilled water was added. Then 20ml of 0.1N potassium hydroxide and a pinch of calcium indicator were added to the mixture and titrated with 0.01M EDTA till the greenish pink colour disappears. The titre value obtained was used to calculate the amount of calcium present in the soil sample.

$$\text{mEq Ca/ 100g soil} = \frac{\text{T x N x 100g Soil}}{\text{10 x 5}}$$

Where,
T = Titre value
N = Normality of EDTA

Sodium and Potassium:

Principle: Colorimetric technique is used to determine the concentration of colored compound in solution by measuring its absorbance. Flame photometry measures the emission of radiation by neutral atoms. The neutral atoms are obtained by introducing the sample into flame, hence the name flame photometry.

Procedure: Sodium and potassium were determined colorimetrically by running the 1.0N ammonium acetate filtrate in a flame photometer with standards of sodium and potassium. Using known graph factors (GF) or slopes for the standards of Na (6.644) and K (17.738) at 520 nm and 560nm wavelengths respectively, sodium and potassium levels in the soil samples were determined from the equation:

Readings (value) x GF x 100/5g = Na or K in ppm

Determination of microbial population in the crude oil-impacted soil

Sterilization of materials

The Petri dishes were washed with tap water, dried in a dryer at a temperature of 45^{o}C; then oven-dried at 210^{o}C for 2hr. The test tubes, Erlenmeyer flask, pipette tips, crucible, spatula and beakers were autoclaved at a temperature of 120^{o}C and fifteen pounds pressure for 15 min.

Bacterial Culture

To sterile water, 10g of soil sample was aseptically introduced into test tubes, tightly capped and vortexed for 5min. Thereafter, 1ml was aseptically transferred into 9ml of sterile distilled water, and ten-fold serial dilutions were carried out. 0.1ml of the solution from the fourth dilution was evenly spread on an already prepared nutrient agar plate and the culture was incubated for a period of 24h. After the incubation period, the total viable count was determined by counting the colony forming units (cfu) and distinct colonies were isolated.

Identification of isolates

The isolates were subjected to the routine bacterial identification procedure using Bergey's Manual of Systematic Bacteriology (Baumann and Schubert, 1994).

Statistical Analysis

The results were expressed as mean ± standard deviation (SD). All results were compared with respect to the control. Comparisons between the concentrations and control were made by using Statistical Package for Social Sciences (SPSS) version 20 and One-way Analysis of Variance (ANOVA). Differences at $p < 0.05$ were considered significant.

RESULTS

Table 1: Exchangeable bases and cation exchange capacity (CEC) of crude oil-polluted soil

Parameter	Control	Treatment		
		1.5%	2.5%	3.5%
Na (mEq)	5.0±0.01^{a}	4.81±0.00^{c}	4.60±0.11^{e}	4.2±0.05^{g}
K (mEq)	1.22±0.01^{a}	1.02±0.05^{c}	0.88±0.05^{e}	0.72±0.01^{g}
Ca (mEq)	12.4±0.00^{a}	11.0±0.20^{c}	10.6±0.00^{e}	10.0±0.20^{g}
Mg(mEq)	11.6±0.02^{a}	11±0.57^{c}	10.4±0.05^{e}	9.80±0.20^{g}
CEC(mEq)	0.82±0.05^{a}	0.79±0.05^{c}	0.72±0.00^{e}	0.70±0.11^{g}

The reduction in exchangeable bases and cation exchange capacity (CEC) paralleled increase in concentration of pollution as shown in Table 1.

Table 2: Leachate test analysis at 2.5% pollution, pH 4.2

Parameter	Total (T)	Leached (TLCP) (ppm)
Cd	0.70 ± 0.05	0.60
Ni	0.94 ± 0.00	0.82
Cr	1.01 ± 0.00	0.90
Mn	13.40 ± 0.01	10.06
Fe	5.48 ± 0.00	2.80
Results are expressed as mean ± SD n = 3		

Leachate test analysis at 2.0% pollution, pH 4.2

Table 2 shows the toxicity characteristics leaching procedure (TCLP) which is the leaching test used to determine the metals that leached from crude oil polluted soil.

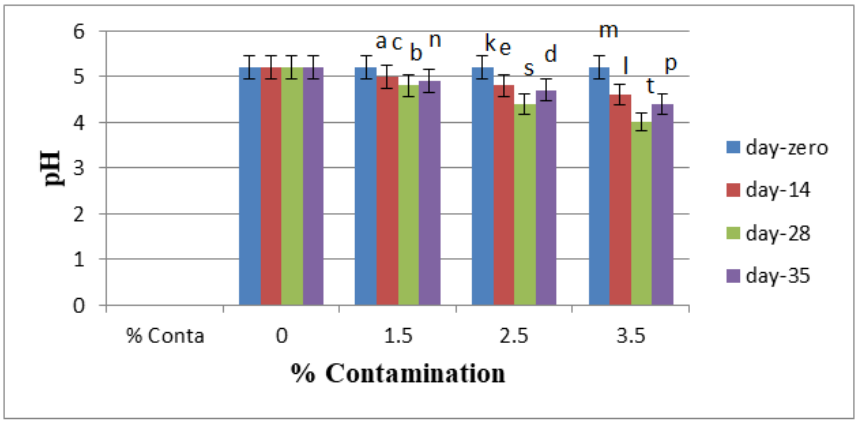

Fig 1: pH of the soil polluted with crude oil Comparison between groups: Bars with different letters differ significantly, (p<0.05)

Effect of spent engine oil on soil pH

Relative to the control, there was a progressive reduction in pH values which was statistically significant (p<0.05) between groups. The oiled soil increased in acidity in a concentration and time dependent manner up to day-28; beyond which, there was a decline in acidity; i.e., pH values began to appreciate.

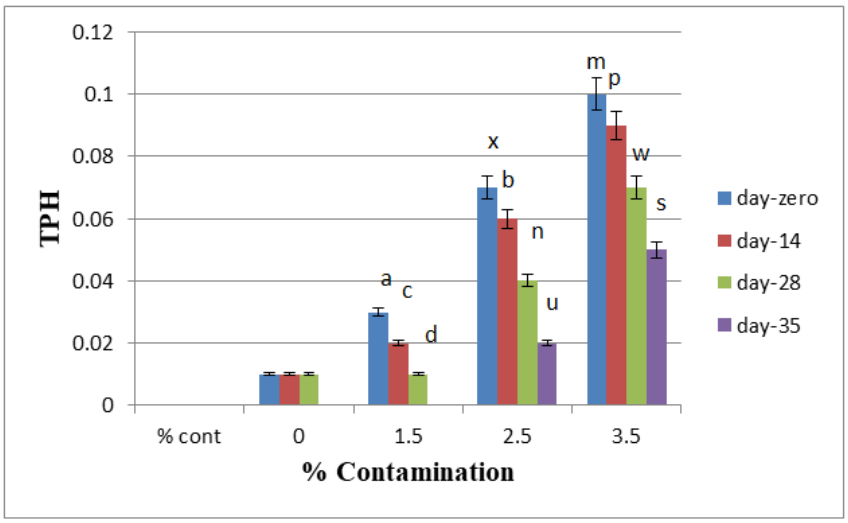

Fig 2: Total petroleum hydrocarbon (TPH) of the crude oil-polluted soil Comparison between groups: bars grouped in different letters differ significantly (p<0.05)

Total petroleum hydrocarbon (TPH) of the crude oil-polluted soil

Following the contamination, there was a synergistic increase in total petroleum hydrocarbon (TPH) which was significantly (p<0.05) different between groups. The increase declined overtime as presented in Figure 2.

Table 3: Total microbial population in the crude oil-impacted soil (x 100)

% Contamination	Week 0ne (cfu/g)	Week Two (cfu/g)	Week Four (cfu/g)	Week six (cfu/g)
Control 1.30×10^{9}				
1.5	$3.60\ x10^{8}$	$3.88\ x10^{8}$	$4.40\ x10^{8}$	$4.01\ x10^{8}$
2.5	$3.26\ x10^{8}$	$3.48\ x10^{8}$	$4.62\ x10^{8}$	$4.40\ x10^{8}$
3.5	$2.02\ x10^{8}$	$2.80\ x10^{8}$	$3.32\ x10^{8}$	$3.52\ x10^{8}$

The prejudicial nature of crude oil reduced the biomass as the concentration increased on week one. Thereafter, there was insurgence of hydrocarbonclastic organisms as presented in Table 3.

Microbial isolates in the crude oil-impacted soil
Bacterial strains: *Pseudomonas aeroginosa*, *Flavobacteria*, *Nocardia*, *Corynebacteria*, *Mycobacteria*, *Micrococcus sp*, *Rhodococcus*, *Streptomyces*, *Bacillus sp*, *Arthrobacter* and *Cyanobacteria*.

Fungal strains: *Fusarium sp*, *Aspergillus niger*, *Candida sp*, and *Penicillium.*

DISCUSSION

Maintenance of ecological equilibrium is a necessity of every natural ecosystem (Ebulue *et al*., 2017, Ebulue, 2022). Any biological disequilibrium as a result of impact of hydrocarbon from crude oil or any xenobiotics will provoke the insurgence of indigenous microbial communities to biodegrade the foreign compounds and bring the ecosystem to a balance and equilibrium. This is the hallmark of the entire ecosystem function (Ebulue *et al*., 2017, Ebulue, 2022). Utilization of chemical contaminants incidented on the soil by different microbial communities as sources of carbon and energy ameliorates a wide range of contaminants from oily waste.

Soil pH which is an important physical property refers to acidity or alkalinity, which is a measure of the concentration of hydrogen ions $[H^{+}]$. It is defined by the equation: $pH = -\text{Log}\ [H^{+}]$. Soil with a large $[H^{+}]$ is acidic (i.e., low pH). This acidity increases the solubility of elements which increases their mobility, lability and probability of leaching into ground water (Ebulue, 2020) while when the $[H^{+}]$ is low, the soil is basic (i.e., high pH), and as a result, cations will be on the particle exchange sites causing lower probability of leachability (Zhang *et al.*, 2007). From this investigation, the positive correlation between the pH of the soil and the amount of crude oil added may be an implication that crude oil pollution led to a reduction in soil pH which was statistically significant. The reduction in pH was in a concentration and time dependent manner from 5.2 ± 0.0 to 4.0± 0.0 which cuts across days-zero to -28 at concentrations 1.5 – 3.5 %. The lowered pH reflected accelerated metabolism and accelerated demand for electron acceptors thus creating a reducing environment. This could be attributable to microbial metabolism of the hydrocarbon present in the crude oil contaminated soil, which consequently gave rise to the production of organic acids that resulted to the increase in the acidity of the affected ecosystem. This is replete with the report of (Osuji and Nwoye, 2007; Osam, 2013; Ebulue, 2020). This increase in acidity would likely affect plant growth, microbial succession and metabolism and leachability of metals.

The results obtained in this study also revealed that crude oil contamination non-significantly ($p > 0.05$) reduced the levels of the soil exchangeable bases (Na, K, Ca and Mg) compared to those in the control. This development portends a serious danger as it may affect the fertility of agricultural soil and eventually the performance of crops that require these mineral elements for growth and crop yield.

The cation exchange capacity (CEC) which is the ability of the soil particles to hold cations was reduced and would likely be replaced by phytotoxic elements such as aluminum and manganese ions, thus leading to the death of crops / plants. The overall effects which altered the entire soil biochemistry could disrupt ecosystem dynamics by slowing soil organic matter mineralization and associated nutrient remineralization (Ebulue *et al*., 2017).

Toxicity characteristics leaching procedure (TCLP) and total metal content were used in this study for crude oil analysis. Increase in acidity (low pH) favours the leachability of heavy metals from contaminated soil into the ground water and also makes the heavy metal fractions to become more labile. The result of this study demonstrated that a pH decrease for instance, from 5.2 – 4.0 enhanced such effects. Thus soil pH is a major factor influencing metal chemistry in the soil (Gambrell, 1994).

The intense infusion of degradable petroleum hydrocarbon from 0.03 ± 0.0 to 0.07 ± 0.00mg/ml that cuts across days-zero to -28 at concentrations 1.5 – 3.5% observed in this study following an exposure of soil ecosystem to crude oil would have likely stimulated aerobic and anaerobic microbial metabolism. So, as oxygen became limiting, utilization of alternate electron acceptors produced an increased reducing environment. This report is in harmony with the findings of (Osuji and Opiah, 2007; Ebulue, 2022). High level of hydrocarbon causes oxygen deprivation and reduction in gaseous diffusion as reported by and these usually have far reaching implications on the flora and fauna of

the affected area, and hence, soil fertility (Osuji *et al*., 2004).

CONCLUSION

Crude oil spill on soil adversely affects ecosystem dynamics. Microbial insurgence as a consequence of crude oil pollution and subsequent mineralization of hydrocarbons lowers the pH. The increase in acidity accelerated leachability and metal mobilization with a loss in cation exchange capacity (CEC) of the soil and consequently leading to plants' absorptive increase in phytotoxic elements and death. All these are notable indices of ecosystem toxicity as a result of crude oil impact.

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