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Physicochemical Properties and Enzymes Activity Studies in a Refined Oil Contaminated Soil in Isiukwuato, Abia State, Nigeria

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Abstract

Soil Physicochemical properties and enzyme concentration were evaluated in soil from a refined-oil contaminated community in Isiukwuato, Abia State three years after the spill. The soil enzymes examined were urease, lipase, oxidase, alkaline and acid phosphatases. Results show a significant (P< 0.05) decrease in the activities of these enzymes in the contaminated soil relative to the control. Soil temperature, Organic Carbon(OC), Organic Matter (OM), Exchangeable Acidity (EA), Saturation Base (SB) and Effective Cation Exchangeable Capacity (ECEC) were significantly higher (p<0.05) in the polluted soil. Soil Nitrogen (N), Phosphorus (P), Calcium (Ca) and Magnesium (Mg) were also elevated in contaminated soil. These results suggest that the soil is not yet suitable for agricultural activity

Keywords: Refined-Oil Spill, Soil Enzymes, Physicochemical Properties, Eluama

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INTRODUCTION

Petroleum oil pollution results from human activities such as drilling, manufacturing, storing, transporting, waste management of oil and vandalization of oil pipe lines[1]. The massive and extensive pollution of the environments by petroleum industries constitute socioeconomic and public health hazards[2]. Petroleum oil pollution exerts adverse effects on plants indirectly by making toxic minerals in the soil more available to plants[3].

Crude oil pollution also leads to deterioration of soil structure, loss of organic matter contents, loss of soil mineral nutrients such as Potassium, Sodium, Calcium, Magnesium, Nitrogen, sulphate, Phosphate and Nitrate etc. It also exposes soil to leaching and erosion[4]. The activities of soil enzymes such as dehydrogenase, oxidase, lipases, urease and alkaline phosphatases are reduced or inhibited[5]. Literature is replete with reports on the effects of oil pollution on soil, aquatic life, ground and surface water[6]. Information concerning enzymes activity studies in oil contaminated soils are still scanty, to this direction. This work therefore aimed at increasing available information concerning the effect of refined-oil contaminated soil physicochemical properties and enzymes activity on farmland.

MATERIALS AND METHODS

Area of Study

Eluama in Isiukwuato Local Government Area of Abia State, Nigeria. It is located along the Nigeria National Petroleum Corporation (NNPC) high pressure oil pipelines used for distribution of petroleum products from the refinery in Port Harcourt to parts of the country. The main occupation of the inhabitants is farming.

Collection of Soil Samples

Immediately after this spill field, reconnaissance surveys were conducted to identify and access extent of soil pollution. Three year after the spill the findings of these survey, were to divide into three and classified as either heavy, medium or light polluted. Land within 50 meters of vandalized pipe represented heavy polluted, Land within 50 meters and up to 100m medium polluted, and up to 150m light polluted. An unpolluted farmland in the community serves as control.

A sampling area 50m x 50m was mapped out in each of the classified zones denotes heavy, medium and light polluted and also for the control site. Each zone in the study area and control was divided into ten quadrants. Soil samples at a depth of 10 – 15 cm (top soil) and 15 – 30 cm (subsurface soil) were collected at random and pooled each quadrant by using a plastic auger. The samples were taken and kept in plastic bags. The samples were transported down to Abia State University, Uturu immediately for analysis in refrigerated coolers to arrest microbial growth.

Preparation of soil samples for analysis

Soil samples were air-dried and sieved with 2mm mesh according to Allen *et al.*[7]. The temperature of each sample was measured at the site by immersing the bulb of the thermometer in soil and taking the reading in ^{0}C. Soil pH was determined by the method of Bates [9] using air-dried samples. To 20g of air-dried soil (2mm sieve) in 50ml beaker, 20ml distilled water was added, the contents stirred occasionally with a glass rod and then allowed to stand for 30 minutes.

The electrodes of pH meter were inserted into the suspension and pH reading recorded. Organic carbon was determined using the method of Walkey and Black[10]. 1.0g of sieved soil was weighed into 300ml beaker and treated with 10ml of potassium dichromate ($K_2Cr_2O_7$) to wet the sample. 20ml of concentrated sulphuric acid was immediately added to the wet mixture. The beaker and its contents were swirled for 20 minutes. 200ml of distilled water was then added to the contents of the beaker. 25ml of 0.5N ferrous ammonium sulphate was added and titrated using 0.4N potassium permanganate.

Exchangeable cations Ca^{2+}, k^{+}, Na^{+} Mg^{2+}) were determined using method of Jackson[11]. 2.5g soil sample was transferred into a conical flask followed by an addition of 25ml of IN ammonium acetate CH_3COONH_4). The mixture was shaken for 45 minutes and the extract was filtered into glass beakers. Aliquots of the filertate was used to determine the Ca^{2+}, K^{+}, Na^{+} using flame photometer.

Magnesium concentration was determined by Versanate EDTA titration method.

To determine Exchangeable acidity, 2.5g of soil sample was transferred into a conical flask, followed by addition of 25ml of IN potassium chloride. The mixture was shaked for 20 minutes and filtered. 10ml of filterate was titirated using 0.10N sodium hydroxide. The Effective Cation Exchange Capacity and Base Saturation was estimated by the formula;

$$\% BS = \frac{TEC \times 100}{ECEC}$$

Where BS = Base Saturation; TEC = Total Exchangeable Base Cation and ECEC = Effective Cation Exchange Capacity.

Heavy metals were analyzed using atomic absorption spectrophotometer (ASS) Unican 939/959 model using the method described by AOAC[12].

Enzyme Analysis

Concentration of soil dehydrogenase was estimated as described by Casida *et al.*[13]. 5.0g of different soil samples were mixed each with 10ml of 0.25% aqueous triphenyl tetrazolium chloride in specimen bottles. 0.1 tribuffer solution was added; the bottle were sealed and incubated at 37^0C for 6 hours. The reduced triphenyl formazan formed was extracted with 10ml of methanol. The resultants were centrifuged for 10 minutes and the supernatants were taken, the absorbances of the supernatant were read at 485nm using methanol as blank.

The soil lipase concentration was estimated by the method described by Saiuburumaruign *et al.*[14]. Urease and Hydrogen Peroxidase concentration was determined by method of Nannipieri *et al*[15].

The activities of both acid and alkaline phosphatases were determined using the methods of Tabatabai (24h which involved the use of Nitrophenylphosphate with $CaCl_2$ and NaOH added to step the reactions and reading the result at 410nm).

Data Analysis

Results were analyzed using Analysis of Variance (ANOVA) and means were compared for significant at $p<0.05$ using Duncan's multiple range analysis.

RESULTS

The results obtained in the physicochemical parameter are shown on Table 1. The highest temperature values of 32.33 and 33.00 were observed in heavily impacted soil. The pH values showed a unique trend as the top soil samples were slightly more acidic than bottom soil samples. There was decreased in moisture content. The control sites had the highest value 34.26 Phosphorus ranged from 16.80 – 15.94 in heavily polluted, 16.50 – 12.22 in medium polluted and 8.80 – 8.40 in light polluted site. Organic carbon and organic matter were less than 3.5 in both polluted and control sites. The values of nitrogen increase with increase in pollution. No significant variation was observed in Na^+ and K^+ in both polluted and control sites. There was significant decrease in EA and ECEC from polluted sites to control sites.

Table 1: Physiocochemical Properities Of Soil In Eluama Affected By Refined Petroluem Oil Spillage

	HP1	HP2	MP1	MP2	LP1	LP2	C1	C2
Temp. (^{0}C)	32.33±2.38^{bc}	33.00±1.73^{bc}	29.67±1.02^{ac}	30.33±1.28^{ac}	29.00±1.73^{a}	29.33±0.79^{a}	27.00±1.00^{a}	27.33±0.78^{a}
pH	5.10±1.65^{a}	5.12±1.01^{a}	5.12±0.01^{a}	5.13±1.62^{a}	5.15±0.10^{a}	5.32±0.84^{a}	5.98±1.00^{a}	6.00±1.73^{a}
MC(%)	18.00±2.00^{a}	20.12±2.00^{a}	20.26±0.01^{a}	21.22±0.99^{a}	25.26±1.49^{ac}	26.40±2.11^{bc}	29.40±2.10^{bc}	34.26±0.98^{ac}
OC(%)	1.82±0.01^{c}	1.56±0.01^{cd}	1.23±0.01^{c}	1.20±0.01^{c}	1.06±0.01^{b}	1.05±0.01^{b}	0.98±0.02^{b}	0.51±0.01^{a}
OM(%)	3.13±0.13^{b}	2.07±0.01^{b}	2.08±0.06^{bc}	1.88±0.53^{ac}	2.07±0.11^{bc}	1.82±0.58^{a}	0.88±0.32^{a}	0.80±0.01^{a}
N(%)	0.21±0.01^{c}	0.13±0.01^{ab}	0.15±0.01^{bd}	0.13±0.02^{ab}	0.13±0.01^{ab}	0.10±0.01^{a}	0.05±0.01^{a}	0.05±0.02^{a}
P(mg/g)	16.00±0.01	15.94±0.02^{c}	15.80±0.02^{c}	12.22±0.01^{b}	8.80±0.01^{a}	8.40±0.01^{a}	8.50±0.01^{a}	0.05±0.02^{a}
Ca(mg/g)	2.40±0.01^{b}	1.80±0.02^{b}	2.40±0.02^{b}	1.60±0.10^{a}	2.40±0.01^{a}	1.20±0.01^{a}	1.10±0.01^{a}	0.80±0.01^{a}
Na(mg/g)	0.82±0.01^{a}	0.42±0.01^{a}	0.44±0.01^{a}	0.42±0.02^{a}	0.44±0.01^{a}	0.04±0.01^{a}	0.42±0.01^{a}	0.04±0.01^{a}
K(mg/g)	0.06±0.01^{a}	0.05±0.01^{a}	0.06±0.01^{a}	0.04±0.01^{a}	0.05±0.01^{a}	0.04±0.01^{a}	0.05±0.01^{a}	0.04±0.01^{a}
Mg(mg/g)	1.60±0.01^{b}	0.80±0.01^{a}	1.20±0.01^{ab}	0.40±0.01^{a}	0.80±0.01^{a}	0.40±0.01^{a}	0.80±0.01^{a}	0.40±0.01^{a}
EA(mg/g)	1.21±0.01^{b}	0.96±0.01^{a}	1.04±0.01^{b}	0.96±0.02^{b}	0.88±0.01^{a}	0.80±0.01^{a}	0.64±0.01^{a}	0.56±0.02^{a}
ECEC(mg/g)	5.62±0.01^{bc}	4.25±0.01^{ac}	5.22±0.02^{b}	4.03±0.01^{ac}	3.90±0.01^{ac}	3.22±0.01^{a}	2.95±0.01^{a}	2.56±0.01^{a}
BS(%)	80.70±0.01^{bc}	78.23±0.01^{ac}	80.80±0.01^{bc}	78.13±0.01^{ac}	77.41±0.01^{a}	76.62±0.01^{a}	77.44±0.02^{a}	78.23±0.01^{ac}

Figures are mean ± SD. Figures in row bearing different alphabets differs significantly P<0.05 (n = 3)

HP1 = Heavy polluted 0 – 15 *HP2 = Heavy polluted 15 – 30*
MP1 = Medium polluted 0 – 15 *MP2 = Medium polluted 15 - 30*
LP1 = Light polluted 0 -15 *LP2 = Light polluted 15 – 30*
C1 = control 0 – 15 *C2 = Control 0 – 15*

Table 2: Heavy Metals Concentrations Of Soil Samples In Eluama Affected By Refined Petroleum Oil Spillage

Metals	HP1	HP2	MP1	MP2	LP1	LP2	C1	C2
Cr(mg/g)	44.68±0.11[b]	49.23±0.05[c]	43.92±0.0.68[b]	48.55±0.51[b]	36.15±0..03[a]	40.56±0.40[b]	36.11±0.03[a]	36.15±0.03[a]
Cu(mg/g)	17.28±0.02[bc]	17.54±0.56[bc]	12.29±0.02[bc]	13.72±0.12[ab]	8.31±0.12[a]	9.30±0.44[a]	7.81±0.22[a]	8.02±0.22[a]
Mn(mg/g)	17.58±0.44[bc]	17.60±0.14[bc]	14.53±0.20[ac]	14.76±0.59[ac]	12.90±0.19[a]	14.40±0.10[a]	12.03±0.28[a]	12.42±0.11[a]
Pb(mg/g)	15.03±0.08[bc]	15.16±0.19[bc]	14.99±0.11[bc]	15.01±0.16[bc]	11.01±0.12[a]	13.38±0.14[ac]	8.17±0.08[a]	8.89±0.17[a]
Fe(mg/g)	52.20±2.82[a]	54.90±2.56[a]	51.03±2.00[ac]	52.02±2.00[b]	51.03±1.13[ac]	51.71±3.13[ac]	56.10±4.90[bc]	48.19±3.00[b]

Figures are mean ± SD. Figures in row bearing different alphabets differs significantly p<0.05 (n = 3)

HP1 = Heavy polluted 0 – 15 *HP2 = Heavy polluted 15 – 30*

MP1 = Medium polluted 0 – 15 *MP2 = Medium polluted 15 - 30*

LP1 = Light polluted 0 -15 *LP2 = Light polluted 15 – 30*

C1 = control 0 – 15 *C2 = Control 0 – 15*

Table 3: Enzyme activity of soil in Eluama affected by refined petroleum oil sample

	Heavy		Medium		Light		control	
	HP1	HP2	MP1	MP2	LP1	LP2	C1	C2
Dehydrogenase (mg/g/6h)	10.22±0.01[bc]	8.72±0.02[bc]	37.68±0.01[a]	15.78±0.01[ac]	43.70±0.20[a]	28.97±0.01[ac]	30.31±0.01[ac]	27.10±0.01[ac]
Oxidase (mg/g/3h)	1.21±0.01[bc]	0.72±0.01[b]	2.41±0.01[ac]	1.25±0.01[bc]	3.47±0.01[a]	2.71±0.01[ac]	3.41±0.01[a]	2.97±0.01[a]
Alkaline phosphatise (µmol)	0.97±0.01[a]	0.62±0.01[b]	1.24±0.01[a]	0.84±0.01[b]	1.73±0.01[a]	1.36±0.01[a]	1.87±0.01[a]	1.31±0.01[a]
Acid phosphatise (µmol)	1.11±0.01[a]	0.62±0.01[b]	1.54±0.02[a]	0.92±0.02[a]	1.97±0.01[a]	1.21±0.01[a]	1.92±0.01[a]	1.17±0.01[a]
Lipase(mg/g/30nm)	1.30±0.01[bc]	1.09±0.01[bc]	4.30±0.01[a]	2.71±0.01[ac]	3.90±0.01[a]	3.30±0.01[a]	3.24±0.01[ac]	3.10±0.01[ac]
Urease (mg/g/24h)	1.02±0.01[bc]	0.92±0.01[b]	1.34±0.01[bc]	1.67±0.01[bc]	3.12±0.01[a]	2.02±0.01[ac]	3.17±0.01[a]	2.21±0.01[ac]

Figures are mean ± SD. Figures in row bearing different alphabets differs significantly p<0.05 (n = 3)

HP1 = Heavy polluted 0 – 15 *HP2 = Heavy polluted 15 – 30*

MP1 = Medium polluted 0 – 15 *MP2 = Medium polluted 15 - 30*

LP1 = Light polluted 0 -15 *LP2 = Light polluted 15 – 30*

C1 = control 0 – 15 *C2 = Control 0 – 15*

Table 2 shows the heavy metal concentrations of soil samples in Eluama affected by refined-petroleum oil spillage concentration of Cr, Cu, Mn, Pb followed the same pattern and were quite significant at (p<0.05) as compared to control site. The results of enzymatic activity of soil in Eluama are shown in Table 3. Dehydrogenases were the most affected group of enzymes but heavily polluted site had the least concentration. Lipases and urease values for both the control polluted sites were less than 5, while Alkaline and Acid Phosohatases were less than 2 in both control and polluted sites.

DISCUSSION

Results of the physiochemical properties of soil in Eluama affected by refined petroleum oil spillage are shown in Table 1. The data indicated that available phosphorus and total nitrogen levels were elevated in the impacted soil compared to their controls. The observed increases were greatest in the heavy polluted sites. The temperature of the impacted soil was higher compared with control Akubugwo *et al.*[6] reported similar increase in temperature. This increase in temperature may be due to biochemical reactions following oil pollution. The pH increased non-significantly (p<0.05). Concentrations of Exchangeable cations (K^+, Na^+, Ca^{2+}, Mg^{2+}) increased with increased in pollutions. Onyeike *et al.*[16] reported such increase in Exchangeable cation of soil from crude oil polluted soil in Ogoni land. Potassium (K^+) values in polluted and control soil samples were within the range for the low fertility class of Nigeria soils[17]. Unpolluted land areas contained cations which may be due to anthropogenic origin increase in the metal contents in the polluted soil must definitely have arisen from oil[18]. Kakulu *et al.*[19] observed that crude petroleum had contributed to some extent to the metal pollution in the Nigeria Delta area. The moisture content of the polluted soil samples reduced. This may be due to coating of the soil surface by hydrophobic hydrocarbon that reduces the water holding capacity of the soil and reduction in the binding property of clay soil[20]. There were significant increases at (p<0.05) in organic carbon and organic matter, these may be attributed to the metabolic processes following oil spillage that facilities agronomical addition of organic carbon from petroleum hydrocarbon by reducing the carbon mineralizing capacity of the microflora[21].

It is discernible from Table 2 that petroleum oil pollution generally increased the concentrations of heavy metals (Cr, Cu, Mn, Pb, Fe) in the polluted soil samples were significantly (p<0.05) higher than those in the unpolluted soils, the increase in the concentrations of heavy metals in the polluted soil may be due to hydrocarbon pollution[18,19]. The observed high level of Fe^{2+} in the soils under the influence of crude oil spillage may be attributable to the reduction of Fe^{3+} to Fe^{2+} due to favourable reducing condition provided by oil pollution. The high concentration of Fe^{2+} in the soil renders the soil toxic for plant growth, hence an overall pollution[16].

The decreases were more in heavily polluted soils than medium, light and control soils (Table 3). This decrease affects mostly dehydrogenases, followed by oxidases and lipases. Alkaline and acid phosphatases were least affected. Reduction in these enzymes agreed with Wyzskowska and Kucharisk[22]. Low dehydrogenases in the soil polluted by petroleum spillage were also noticed by Malachowoskajutsz *et al.*[23.]

Galas *et al.*[24] and Zhang *et al.*[25] reported undesirable depressions in the concentration of these enzymes which indicates low activities of microorganisms in the polluted soil.

This study has shown that petroleum oil spillage affected the concentration of some inorganic cation, and enzymes activity in the soils of the studied area. The concentration of some ions and enzymes analyzed were on the high side, indicating their high pollution potential, especially if treatment measures are not taken. The results here therefore indicate that the farmlands in Eluama are not yet suitable for agricultural activity.

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