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# EFFECTS OF BIOREMEDIATION OF PETROLEUM POLLUTION ON SOIL PHYSICOCHEMICAL PROPERTIES AND METALS BIOAVAILABILITY IN SOUTH-SOUTH NIGERIA

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**Abstract**

The effects of bioremediation on selected soil physicochemical properties and heavy metals bioavailability in South South geopolitical region of Nigeria, was studied. The level of petroleum pollution and remediation the physicochemical and metals bioavailability in bioremediated soils were examined in 20 composite soil polluted and remediated soils each with a composite control samples. The soils were collected, processed and chemically extracted and analyzed according to standard methods and procedures. The results showed adequate remediation levels ranging from about 95 to 98% such that the residual total petroleum hydrocarbons (TPH) were within the Nigeria acceptable levels and below the intervention values. However, a general drop in the soils' pH with average reduction in pH values of 2.32 in the bioremediated sites was observed. Also observed, was the increase in the bioavailability of selected heavy metals with average increase values of 11.91, 13.70, 16.23 and 14.84% for Cr, Pb, Cd and As respectively. Similarly, the percentage increase in bioavailability for Ba, Cu, Ni and Zn were 13.23, 10.90, 10.63 and 11.03 % respectively. The $R^2$ values for the scattered graph plot of pH drop on metal bioavailability ranged from 0.7091 – 0.8988. The increment in the metal bioavailability could be attributed to drop in the pH of the sites during the remediation field work.The study revealed the need for re-conditioning petroleum polluted soils after their bioremediation.

**Key Words**: Bioremediation, Soil pH, Petroleum pollution, Physicochemical properties, Metals bioavailability

## INTRODUCTION

The South South geopolitical region of Nigeria is a host to many oil prospecting, exploration and producing companies due to its rich oil and natural gas reserves. The over six decades of oil and natural gas production in the region has led to several reported and non-documented oil spills either due to faulty installation, accident or sabotage (Daniel and Braide, 2002; Iwegbue and Egobueze, 2002; Intl., 2006). It has been reported that in the recent decades, over 300, 000,000 barrels of petroleum crude and refined products (Okoh, 2003; Virgo, 2010) were released into the region due to various kinds of oil spills (Okoh, 2003; Virgo, 2010). In Nigeria, several government regulations have been enacted to monitor and control environmental pollution and degradation due to the activities of oil and gas exploration and exploitation industries (DPR, 1991; FEPA, 1991). Such regulations required oil companies to clean-up and remediate polluted sites to recommended standards of 50 and 500 mg/kg soil of TPH in residential/agricultural and industrial soils respectively (DPR, 1991; FEPA, 1991). Sometimes, these standards are relatively met and the soils would be left for further natural attenuation to reduce the concentration of the oil contaminants to the acceptable levels. For such natural remediation to be effective, the physicochemical environment of the soil must be favorable to the soil indigenous microbes particularly the oil pollutants degraders (Mmom and Deekor, 2010).

The clean-up of spilled petroleum products and soil remediation processes could lead to unfavorable microbial environment in soils which would slow down the expected further post remediation by natural attenuation. Most companies focus on the reduction of the oil pollutants in the polluted soils to meet regulatory laws and guidelines with no or little attention on the effects of the clean-up and remediation exercises on the soil physicochemical conditions. It has been well documented that degradation of petroleum and its products could decrease soil pH (Adekunle, 2011). This is because final biodegradation processes of Petroleum and its products lead to production of water and carbon dioxide capable of increasing soil acidity (Duarte da Cunha and Leite, 2000).

Excessive acidic pH is detrimental to soil microorganisms (Dibble and Bartha, 1979; Atlas, 1981). Also, metals bioavailability is soil pH dependent (Richards, *et al*. 2000; Antoniadis and Alloway 2002; Clemente, *et al*. 2003; Houben, *et al*. 2013). Furthermore, low crop yields have been reported from petroleum hydrocarbon polluted-bioremediated soils (Odokuma and Ibor, 2002; Agamuthu, *et al*., 2010; Osuji, *et al*. 2010), which may not be attributed to level of residual petroleum pollutants alone. There is need to investigate other factors that could hinder optimal crops yield from Petroleum hydrocarbon polluted-bioremediated soils. Hence, it is necessary to document to what extent remediation of oil polluted soil could affect its physicochemical properties and metal bioavailability, with the view to improving the soils' conditions, particularly for agricultural purposes.

Several works on the effectiveness and efficacy of landfarming or bioremediation of petroleum polluted soils have been reported (Oghoje, S. U., *et al.*, 2021, Oghoje, S. U., *et al.*, 2020 a & b, Okieimen and Okieimen, 2005; Chorom, Sharifi *etal*. 2010; Mmom and Deekor 2010). However, reports on the post remediation effects on soils physicochemical properties, nutrient status particularly as it relate to heavy metals bioavailability have been scanty. Therefore, the aim of the work is to examine the effects of clean-up and remediation on previously oil polluted soils' physicochemical properties including soil pH, electroconductivity, Total Organic Matter (TOM), Total Organic Carbon (TOC), nutrients status, as well as heavy metal bioavailability content with view to recommending steps for enhanced post remediation/ degradation of residual oil pollutants as well as soil conditioning. Therefore, this study determine the physicochemical properties of oil polluted soils before, and after landfarming processes and evaluate the eco-toxicity or metal bioavailability of bioremediated oil polluted soils.The data generated from the study would reveal vital information on the status of bioremediated Petroleum hydrocarbon polluted soils.

## MATERIALS AND METHODS

### 2.1 Soils Sampling

A total of 40 (n =3) composite soil samples comprising of 20 from crude oil polluted sites and 20 controls (A composite control to each site) within five oil producing States of Bayelsa, Cross River, Delta and Edo and Rivers were studied (Figure 1) prior to bioremediation. The same volume of samples was collected from the sites including the controls after the remediation exercise. Therefore, the gross total of samples studied was 80. The samples were collected using soil auger at level of 0 – 30 cm. For each of the polluted and remediation site, the composite control soil samples were randomly taken from about 500 meters (From the site) depending on the geophysical features of the polluted sites. The samples for soil physicochemical analyses were collected into polyethylene bag while the samples for the evaluation of total petroleum hydrocarbon contents were placed in well cleaned 1 litre glass jar with Teflon lined screw cap, cooled to about $4^0$ C in ice jackets for further processing and analyses at EarthQuest Laboratory International, Warri, Delta State, Nigeria. At the laboratory, the samples for the determination of selected physicochemical properties and heavy metals content were air dried at ambient temperature grinded in clean porcelain mortars and sieved through 2 mm sieve and kept in polymer containers for physicochemical analyses.

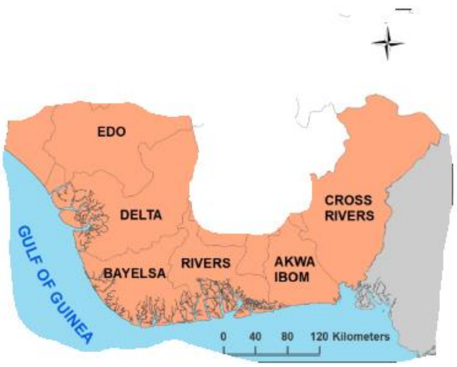


**Figure 1**: A typical map of South South geo-political region of Nigeria

**Physicochemical analyses**

**Particle size analysis**

Particle size analysis of the soil samples were carried out according to Bouyoucos hydrometer method as described by Okalebo, *et al.,* (2002).

**The soils pH analyses**

The pH analyses were carried out at 1:2.5 (soil: water) stirred for about 15 minutes using magnetic stirrers and readings were taken using Orion model 420A pH meter, United Kingdom UK), which was calibrated with buffer solution of pH 4, 7 and 9 prior to usage (Okalebo, *et al*., 2002).

**Electroconductivity**

Electroconductivity was carried out at same soil to water ratio but stirred for about 30 minutes using magnetic stirrers and reading were taking as recommended by Okalebo, *et al.,* (2002).

**Hydrocarbons analyses**

Hydrocarbons analyses were done according to USEPA method 8015B using GC-FID (HP6890,Hewlett Packard,United State of America (USEPA, 1996).

**Heavy metals and cations analyses**

The analyses for sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) were carried out as recommended by ASTM (1982.) as described by Okalebo (2002) methods for acid digestions using flame photometer (Model 410 Sherwood, UK) for K and Na and AAS (GBC Australia for Ca, Mg and the selected heavy metals.

**Total Organic Carbon (TOC) analysis**

Total Organic Carbon (TOC) in the soil was analyzed by rapid wet oxidation method based on Walkey and Black (1954) procedure for the determination of total organic carbon (TOC) as described by OKalebo, *et al.*, (2002).

**Chloride and Sulphate analysis**

Chloride and Sulphate content was evaluated using ASTMD 1411 – 82 test method (APHA, 1989.)

**Nitrate and Phosphate analyses**

The United States Environmental Protection Agency (USEPA) 352.1 in combination with the Chemical Analysis of Ecological Materials (second edition) test methods were used to determine the nitrate content of soil samples. While phosphate in soil samples was determined using UV/Visible (spectrophotometer. The persulfate digestion/ascorbic acid otherwise called Olsen Method, based on the method described in the Chemical Analyses of Ecological Materials (2nd edition), was applied (APHA, 1989, Okalebo, *et al.*, 2002, Murphy and Riley, 1962.)

**2.4 Statistical analysis**

Statistical analyses were carried out by averaging the values from the triplicates of samples from a particular location and the controls. Differences between average controls and samples values were noted for both physicochemical parametersand heavy metals bioavailability. The correlation between the soil pHs change and levels of bioremediation as well as heavy metals bioavailability were determined using regression (scattered lines. All graphical works were done using Microsoft Excel version 2010 software and SigmaPlot®.

**RESULTS AND DISCUSSION**

**The soils' pH**

The effects of bioremediation on some physicochemical properties of the soils are presented in table 1. The results showed that there was a general decrease in the pH (i.e increase in acidity) at the end of the remediation process. A pH range of 3.22 – 6.00 was observed across the bioremediated sites after the remediation as against a pH range of 5.97 – 7.27 for the polluted sites prior to the bioremediation. But there was no much different of in the average pHs of the composite control sites (CCS) before and after the remediation. The observed drop in the pHs of the remediated sites may be attributed to the release of carbon dioxide during the remediation since this gas is one of the by-products of bio-oxidation of Petroleum and its refined products (Duarte da Cunha and Leite, 2000). Previous reports have also implicated Petroleum pollution for the decrease in soil pH (Antoniadis and Alloway, 2002; Clemente, *et al.*, 2003; Houben, *et al.*, 2013).

**Table 1**: Percentage remediation and some chemical properties of the soil after remediation

| Parameters Assayed | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sites | pH BR | pH AR | % Reme | EC (µS/cm) | TOC (%) | $CL^-$ (mg/kg) | $SO_4^{2-}$ (mg/kg) | $NO_3^-$ (mg/kg) | $PO_4^{2-}$ (mg/kg) |
| CCS | 6.47 | 6. 25 | - | 47.000 | 0.270 | 7.998 | 8.85 | 0.15 | 0.38 |
| 1 | 6.47 | 4.20 | 97.14 | 84.00 | 0.550 | 8.497 | 7.00 | 0.01 | 0.31 |
| 2 | 6.97 | 3.90 | 97.57 | 117.00 | 0.51 | 8.497 | 6.17 | 0.01 | 0.10 |
| 3 | 6.71 | 5.84 | 95.44 | 183.20 | 1.10 | 25.99 | 12.90 | 0.06 | 0.23 |
| 4 | 6.82 | 4.70 | 94.56 | 231.80 | 0.38 | 35.49 | 3.59 | 0.08 | 0.05 |
| 5 | 6.58 | 3.22 | 96.58 | 324.5 | 2.54 | 63.44 | 20.24 | 0.06 | 0.40 |
| 6 | 7.17 | 5.40 | 94.80 | 118.70 | 2.83 | 14.23 | 3.49 | 0.07 | 0.21 |
| 7 | 7.17 | 5.20 | 95.00 | 157.40 | 2.40 | 21.00 | 1.04 | 0.05 | 0.09 |
| 8 | 7.27 | 5.10 | 95.10 | 123.00 | 1.79 | 10.55 | 4.46 | 0.07 | 0.22 |
| 9 | 6.97 | 5.10 | 95.40 | 115.40 | 2.91 | 15.22 | 5.50 | 0.05 | 0.23 |
| 10 | 6.97 | 5.50 | 94.70 | 108.20 | 1.33 | 47.15 | 3.42 | 0.12 | 0.01 |
| 11 | 7.17 | 5.10 | 95.60 | 146.60 | 1.15 | 19.89 | 4.49 | 0.08 | 0.11 |
| 12 | 6.67 | 3.60 | 96.60 | 124.20 | 2.87 | 10.99 | 2.74 | 0.04 | 0.15 |
| 13 | 7.27 | 6.00 | 94.80 | 141.80 | 2.79 | 13.72 | 3.50 | 0.07 | 0.09 |
| 14 | 6.57 | 4.20 | 95.90 | 172.50 | 1.13 | 16.88 | 3.92 | 0.08 | 0.18 |
| 15 | 7.07 | 4.20 | 96.40 | 253.00 | 0.11 | 41.692 | 4.03 | 0.01 | 0.02 |
| 16 | 6.37 | 3.80 | 96.10 | 160.00 | 0.14 | 16.322 | 5.84 | 0.04 | 0.08 |
| 17 | 6.47 | 3.70 | 96.92 | 196.00 | 0.13 | 16.048 | 7.64 | 0.13 | 0.02 |
| 18 | 6.47 | 3.90 | 96.10 | 165.00 | 0.11 | 15.774 | 5.38 | 0.03 | 0.01 |
| 19 | 6.57 | 4.10 | 96.00 | 222.00 | 0.102 | 33.741 | 4.48 | 0.02 | 0.03 |
| 20 | 5.97 | 4.30 | 95.20 | 199.00 | 0.098 | 22.498 | 5.84 | 0.02 | 0.06 |

**The Soil Electroconductivity**

The electrical conductivity (EC) values of the soils were relatively high and ranged from 84 – 325 µS/cm compared to the average EC of the control soils which was 47 µS/cm. The observed increase in the EC may be attributed to the acidic conditions of the soils. This is because, as soil solution acidity increases, most metallic salts become more soluble to form ions. Consequently, the soil EC increases. The increase in solubility of metallic compounds could lead to increase in bioavailability of metals that could contaminate plants and animals.

**Total organic carbons**

In this study, we observe relative increase in the TOC of the remediate sites when compared to the control sites. The observed values may be attributed to the presence of petroleum hydrocarbon in the soils even after their remediation exercise. Though over 90% of total hydrocarbons were removed from the soils by the remediation exercise, the observed concentrations in the soils were significant enough to increase the soils TOC.

**The Soil Chloride**

The chloride values ranged from about 8 – 63 mg/kg and were generally high (post-remediation) when compared to the value obtained for the control soils (Table 1). This observed value may be attributed to the drop in the soil pHs during the remediation exercise. Acidic soil enhances the solubility of most metallic chloride and the concentration of soluble salts particularly metallic chlorides determines the salinity of soils. Consequently, the high values of chloride observed in the remediated soils samples could be an indication of high salinity. Soil salinity has negative effects on the ecological life in soil and could results to plasmolysis in plants cells. However moderate concentration of chloride in soils is required to maintain osmotic process between plant roots and inhabiting soil fauna and Annelida

**The soil sulphate**

The sulphate concentration of the soils after the remediation exercise ranged from 1.04 – 20.24 mg/kg including the value of 8.85 mg/kg for the average control soils (ACA, Table1). Except in few cases, the sulphate concentration in the control soils was generally higher than those of the remediated soils. This is an indication that some amount of sulphate would have been used up during the remediation. It has been previously asserted that, sulphate could be reduced to hydrogen sulphide (HS) during remediation of Petroleum polluted soils particularly in an anaerobic condition. HS is an acidic gas which is capable of dropping the soil pH on its oxidation to sulphuric or sulphurus acid.

**The nutrients**

Nitrogen in the form of nitrate or ammonium and available phosphate are among the primary plant nutrients in soils. Nitrogen and phosphorus are particularly needed for bioremediation of Petroleum polluted soils. The results showed value ranges of 0.01 – 0.13 and 0.01- 0.40mg/kg for the nitrogen and available phosphorus respectively. Except in very few samples, the values of the nutrients were generally far lower than the values obtained in the ACS. This is an indication of high depletion in the nutrient levels during the remediation process. This observation was expected since micro-organisms need these nutrients for their metabolisms and in bio-oxidation of petroleum pollutants. Some forms of biostimulation would have be done during the process. But the levels and rate of biostimulation was not evaluated in this study.

**Changes in soil pHs during bioremediation**

**Table 2**: Changes in soil pHs and percentage (%) remediation (Rem.)

| Samples sites | pHs before Rem, | pHs after Rem, | Difference (changes) in pHs | % of TPH removal (% Rem.) |
|---|---|---|---|---|
| ACS (Control) | 6.47 | 6. 25 | 0.22 | NA |
| 1 | 6.47 | 4.10 | 2.47 | 97.14 |
| 2 | 6.97 | 3.90 | 3.07 | 97.57 |
| 3 | 6.71 | 5.84 | 1.91 | 95.44 |
| 4 | 6.82 | 4.70 | 2.12 | 94.56 |
| 5 | 6.58 | 3.22 | 3.36 | 98.06 |
| 6 | 7.17 | 5.40 | 1.77 | 94.80 |
| 7 | 7.17 | 5.20 | 1.97 | 95.00 |
| 8 | 7.27 | 5.10 | 2.17 | 95.10 |
| 9 | 6.97 | 5.10 | 1.87 | 95.40 |
| 10 | 6.97 | 5.50 | 1.47 | 94.70 |
| 11 | 7.17 | 5.10 | 2.07 | 95.60 |
| 12 | 6.67 | 3.20 | 3.47 | 96.60 |
| 13 | 7.27 | 6.00 | 1.27 | 94.80 |
| 14 | 6.57 | 4.20 | 2.37 | 95.90 |
| 15 | 7.07 | 4.20 | 2.87 | 96.40 |
| 16 | 6.37 | 3.80 | 2.67 | 96.10 |
| 17 | 6.47 | 3.70 | 2.77 | 96.92 |
| 18 | 6.47 | 3.90 | 2.57 | 96.10 |
| 19 | 6.57 | 4.10 | 2.47 | 96.00 |
| 20 | 5.97 | 4.30 | 1.67 | 95.20 |

The differences between the soils pHs prior to remediation and post-remediation exercises in relation to the level (%) of remediation is presented table 2. On the average, a pH drop of 2.32 was observed. A drop in soil pH indicated increase in soil acidity. As mention earlier, soil pH is one of the major parameters that could affect soil chemistry and biochemistry as well as the ecological life equilibrium of the environment. Increase in soil acidity could increase the ionization and solubility of many metallic compounds thereby making metals (Including toxic metals) more bioavailable. High soil acidity could be toxic to plants roots, fauna and flora particularly soil microorganisms (Including biomass and soil pollutants' decomposers).

**Soil acidity has direct and indirect** implication on the acidity of the aquifer and surrounding water bodies. This study has shown that bioremediation of Petroleum polluted soil could have devastating effects on the soil physicochemical properties and the entire ecosystem. Therefore, there should be the need for soil reconditioning including liming to follow bioremediation of Petroleum or organic polluted soils.

Figure 2 showed that there is an arithmetical relationship between the levels of bioremediation of organic pollutants (Including Petroleum products). There is a positive correlation with $R^2$ value as high as 0.7847 on a scattered graph plot. The statistical implication of this relationship is that, there would be drop in soils pHs values as the levels of remediation of organic soil pollutants increases provided other environmental factors that could cause increase in soil pH are constant. However, the observed relationship was not perfect and proportional hence the statistical correlation ($R^2$) value is less than 1. This observation could be attributed to the fact, that there are several environmental factors (Including acid rains, aerial acidic gas deposition as well as organic and inorganic anaerobic bio-reactions in soils) that could increase soil acidity. Besides, the actual concentration of organic pollutants bio-decomposed (Remediated) could be a determinant of soil acidity. Suffice to say that that bioremediation of Petroleum polluted soil has the tendency of increasing the soil acidity as demonstrated by this study.

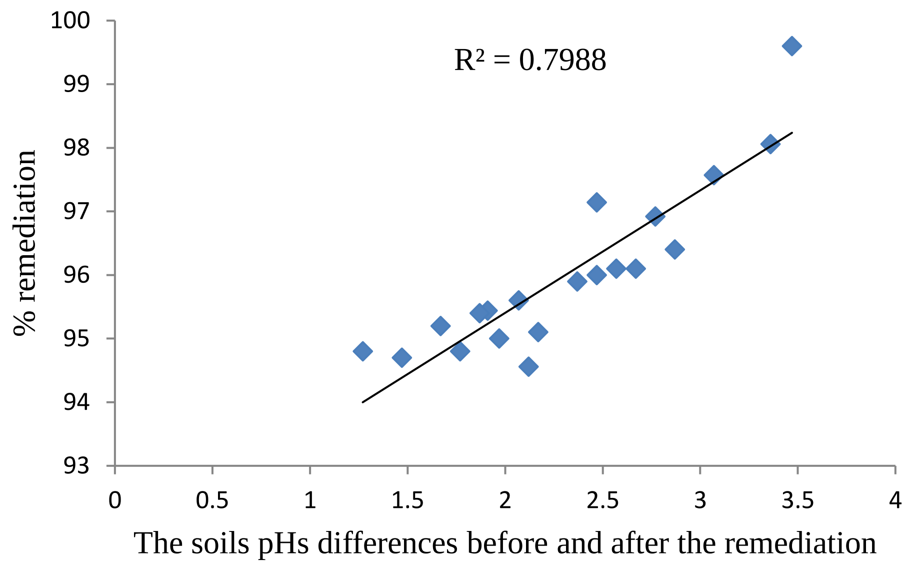


**Figure 2**: Relationship of % Petroleum remediation to soil pH changes

**Metals bioavailability during Soil remediation**

Metal bioavailability is the quantity of metals that could be available or accessible to plants and sometimes referred to as plant available form in the classification of forms of metals in soils. The bioavailable metals include the soluble and exchangeable forms. Soil pH is a master factor in soil chemistry and bio-chemistry. To a large extent, it determines the availability of the various soil nutrients as well as bioavailability of metals in soils (Houben, *et al*., 2013). In this study, eight (8) selected heavy metals including lead (Pb), cadmium (Cd),Arsenic (As), chromium (Cr), barium (Ba), Copper (Cu), Nickel (Ni) and Zinc (Zn) during bioremediation of Petroleum polluted soils was investigated.

**Table 3a**: pH changes and Cr, Pb, Cd and As bioavailability

| | | Heavy metals | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sites | Changes in pHs | Cr (mg/lg) | | | Pb (mg/kg) | | | Cd (mg/kg) | | | As (mg/kg) | | |
| | | Total | BR | AR | Total | BR | AR | Total | BR | AR | Total | BR | AR |
| ACS | 0.22 | 1.12 | - | - | 0.01 | - | - | 8.06 | -- | - | 1.05 | - | - |
| 1 | 2.47 | 10.10 | 1.37 | 1.49 | 11.05 | 1.32 | 1.53 | 11.53 | 1.80 | 2.19 | 11.53 | 1.41 | 1.67 |
| 2 | 3.07 | 12.40 | 2.32 | 2.59 | 10.51 | 1.94 | 2.24 | 10.07 | 1.63 | 1.98 | 10.07 | 2.10 | 2.5 |
| 3 | 1.91 | 9.40 | 1.37 | 1.58 | 6.44 | 1.08 | 1.26 | 21.14 | 1.16 | 1.37 | 21.14 | 1.07 | 1.23 |
| 4 | 2.12 | 13.39 | 1.44 | 1.69 | 6.92 | 1.19 | 1.39 | 19.54 | 1.44 | 1.71 | 19.54 | 1.38 | 1.61 |
| 5 | 3.36 | 10.66 | 2.40 | 2.72 | 9.36 | 1.85 | 2.15 | 13.98 | 2.14 | 2.58 | 13.98 | 2.43 | 2.88 |
| 6 | 1.27 | 6.16 | 0.80 | 0.93 | 10.32 | 0.63 | 0.73 | 12.77 | 0.63 | 0.75 | 12.77 | 0.54 | 0.63 |
| 7 | 1.47 | 14.54 | 1.21 | 1.29 | 8.65 | 1.20 | 1.39 | 12.10 | 0.77 | 0.92 | 12.10 | 0.67 | 0.79 |
| 8 | 1.57 | 8.46 | 1.30 | 1.47 | 9.76 | 0.87 | 0.99 | 14.44 | 0.71 | 0.84 | 14.44 | 0.48 | 0.54 |
| 9 | 1.87 | 12.69 | 1.33 | 1.53 | 17.05 | 1.02 | 1.17 | 12.44 | 0.79 | 0.94 | 12.44 | 0.97 | 1.14 |
| 10 | 1.17 | 4.85 | 0.72 | 0.84 | 13.18 | 0.68 | 0.78 | 14.10 | 0.60 | 0.72 | 14.10 | 0.70 | 0.82 |
| 11 | 2.07 | 16.11 | 1.74 | 2.00 | 9.94 | 1.17 | 1.36 | 15.44 | 1.28 | 1.54 | 15.44 | 1.27 | 1.49 |
| 12 | 3.07 | 12.76 | 2.17 | 2.42 | 13.73 | 1.96 | 2.27 | 9.44 | 2.00 | 2.26 | 9.44 | 2.47 | 2.96 |
| 13 | 1.27 | 4.95 | 0.74 | 0.87 | 8.48 | 0.70 | 0.81 | 13.10 | 0.70 | 0.84 | 13.10 | 0.63 | 0.74 |
| 14 | 2.37 | 12.11 | 1.54 | 1.76 | 10.55 | 1.14 | 1.32 | 10.44 | 1.41 | 1.71 | 10.44 | 1.52 | 1.81 |
| 15 | 2.87 | 12.19 | 2.03 | 2.26 | 11.01 | 1.62 | 1.88 | 21.70 | 1.44 | 1.67 | 21.70 | 1.95 | 2.27 |
| 16 | 2.57 | 10.01 | 1.50 | 1.74 | 5.51 | 1.27 | 1.48 | 18.53 | 1.55 | 1.84 | 18.53 | 1.58 | 1.84 |
| 17 | 2.77 | 11.88 | 2.15 | 2.35 | 7.79 | 1.52 | 1.77 | 7.26 | 1.37 | 1.67 | 7.26 | 1.66 | 1.99 |
| 18 | 2.57 | 20.13 | 2.09 | 2.28 | 8.03 | 1.24 | 1.45 | 11.91 | 1.26 | 1.51 | 11.91 | 1.61 | 1.91 |
| 19 | 2.47 | 12.05 | 1.67 | 1.94 | 11.13 | 1.60 | 1.86 | 8.70 | 1.33 | 1.62 | 8.70 | 1.03 | 1.22 |
| 20 | 1.67 | 4.99 | 0.71 | 0.83 | 9.84 | 0.64 | 0.74 | 14.77 | 0.66 | 0.78 | 14.77 | 0.76 | 0.88 |

Table 3a showed the total, bioavailable fraction (BAF) before remediation (BR) and after remediation (AR) of Cr, Pb, and Cd and As in the 20 Petroleum polluted bio-remediated sites. The BAF for Cr, Pb, Cd and As before the remediation exercise ranged from 0.71 – 2.40, 0.63 – 1.96, 0.60 – 2.14 and 0.48 – 2.47 mg/kg respectively as against their corresponding values of 0.83 – 2.72, 0.73 – 2.27, 0.72 – 2.58 and 0.54 – 2.96 mg/kg after the remediation routine. Similarly, the BAF values for Ba, Cu, Ni, and Zn ranged from, 0.76 – 2.34, 0.67 - 2.46, 1.02 – 2.43, and 1.15 – 2.55 mg/kg respectively before the bioremediation exercise as against their corresponding values of 0.87 – 2.72, 0.76 – 2.77, 1.15 – 2.72 and 1.26 – 2.93 mg/kg. (Table 3b)

**Table 3b**: pH changes and Ba, CU, Ni and Zn bioavailability

| | | Heavy metals | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sites | Changes in pHs | Ba (mg/lg) | | | Cu (mg/kg) | | | Ni (mg/kg) | | | Zn (mg/kg) | | |
| | | Total | BR | AR | Total | BR | AR | Total | BR | AR | Total | BR | AR |
| ACS | 0.22 | 1.12 | - | - | 0.01 | - | - | 8.06 | -- | - | 1.05 | - | - |
| 1 | 2.47 | 11.23 | 1.64 | 1.89 | 11.05 | 1.51 | 1.73 | 11.53 | 1.71 | 1.89 | 8.10 | 2.01 | 2.19 |
| 2 | 3.07 | 15.1 | 2.25 | 2.59 | 12.51 | 1.86 | 2.04 | 10.07 | 2.21 | 2.48 | 15.40 | 2.23 | 2.53 |
| 3 | 1.91 | 15.4 | 1.40 | 1.58 | 6.44 | 1.32 | 1.46 | 21.14 | 1.21 | 1.37 | 19.40 | 1.36 | 1.47 |
| 4 | 2.12 | 19.4 | 1.36 | 1.59 | 6.92 | 1.36 | 1.49 | 19.54 | 1.74 | 1.88 | 13.39 | 1.78 | 1.97 |
| 5 | 3.36 | 13.39 | 2.34 | 2.72 | 11.36 | 2.46 | 2.77 | 13.98 | 2.38 | 2.72 | 10.66 | 2.55 | 2.93 |
| 6 | 1.27 | 10.66 | 0.93 | 0.93 | 10.32 | 0.72 | 0.83 | 12.77 | 1.02 | 1.15 | 16.16 | 1.17 | 1.26 |
| 7 | 1.47 | 16.16 | 1.11 | 1.29 | 18.65 | 1.21 | 1.39 | 12.1 | 1.24 | 1.42 | 8.54 | 1.15 | 1.31 |
| 8 | 1.57 | 14.54 | 1.15 | 1.37 | 19.76 | 1.16 | 1.29 | 14.44 | 1.36 | 1.48 | 12.46 | 1.44 | 1.57 |
| 9 | 1.87 | 7.46 | 1.30 | 1.53 | 17.05 | 1.25 | 1.37 | 12.44 | 1.38 | 1.54 | 9.69 | 1.50 | 1.67 |
| 10 | 1.17 | 8.69 | 0.81 | 0.94 | 13.18 | 0.77 | 0.88 | 14.1 | 1.14 | 1.32 | 14.85 | 1.23 | 1.43 |
| 11 | 2.07 | 9.85 | 1.38 | 1.60 | 9.94 | 1.30 | 1.46 | 15.44 | 1.57 | 1.74 | 26.11 | 1.58 | 1.85 |
| 12 | 3.07 | 26.11 | 2.14 | 2.52 | 13.73 | 2.22 | 2.43 | 9.44 | 2.43 | 2.66 | 12.76 | 2.25 | 2.64 |
| 13 | 1.27 | 12.76 | 0.76 | 0.87 | 12.48 | 0.87 | 0.99 | 13.1 | 1.17 | 1.34 | 14.95 | 1.23 | 1.43 |
| 14 | 2.37 | 14.95 | 1.55 | 1.76 | 10.55 | 1.47 | 1.63 | 10.44 | 1.38 | 1.51 | 26.11 | 1.47 | 1.62 |
| 15 | 2.87 | 18.11 | 1.57 | 1.86 | 11.01 | 1.82 | 1.98 | 21.7 | 1.69 | 1.91 | 12.19 | 1.76 | 2.00 |
| 16 | 2.57 | 12.19 | 1.33 | 1.54 | 13.51 | 1.40 | 1.6 | 18.53 | 1.66 | 1.84 | 12.01 | 1.73 | 1.93 |
| 17 | 2.77 | 12.01 | 1.50 | 1.75 | 9.79 | 1.59 | 1.77 | 7.26 | 1.49 | 1.67 | 12.88 | 1.69 | 1.91 |
| 18 | 2.57 | 2.88 | 1.53 | 1.8 | 10.03 | 1.43 | 1.64 | 11.91 | 1.49 | 1.71 | 20.13 | 1.57 | 1.8 |
| 19 | 2.47 | 20.13 | 1.24 | 1.44 | 11.13 | 1.34 | 1.53 | 8.70 | 1.44 | 1.62 | 12.05 | 1.51 | 1.7 |
| 20 | 1.67 | 12.05 | 1.05 | 1.23 | 9.84 | 0.67 | 0.76 | 14.77 | 1.07 | 1.18 | 10.99 | 1.16 | 1.27 |

The result showed a general increase in the metals bioavailability at the end of the remediation exercise with average percentage increase ranging from 10.23 – 16.23 % (Table 4)

**Table 4**: Percentage increase of metals bioavailability after the bioremediation exercise

| | Heavy metals | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Cr | Pb | Cd | As | Ba | Cu | Ni | Zn |
| | Percentage (%) increase in bioavailability after the remediation | | | | | | | |
| 1 | 8.36 | 13.63 | 17.90 | 15.52 | 13.33 | 12.49 | 9.52 | 8.22 |
| 2 | 10.42 | 13.39 | 17.47 | 16.20 | 13.00 | 8.82 | 10.89 | 11.74 |
| 3 | 13.48 | 14.29 | 15.18 | 13.17 | 11.39 | 9.86 | 11.82 | 7.35 |
| 4 | 14.73 | 14.14 | 16.02 | 14.20 | 14.72 | 8.46 | 7.47 | 9.69 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5 | 11.91 | 14.09 | 16.98 | 15.56 | 14.10 | 11.18 | 12.57 | 12.96 |
| 6 | 13.55 | 13.56 | 16.53 | 14.29 | 0.00 | 13.01 | 10.96 | 7.29 |
| 7 | 6.51 | 13.49 | 16.74 | 14.58 | 13.95 | 12.95 | 12.68 | 12.23 |
| 8 | 11.73 | 12.58 | 15.48 | 11.67 | 15.77 | 9.77 | 7.78 | 8.37 |
| 9 | 12.84 | 12.82 | 16.38 | 15.00 | 15.29 | 8.80 | 10.52 | 10.02 |
| 10 | 14.64 | 13.46 | 16.11 | 14.93 | 13.40 | 12.27 | 13.64 | 13.97 |
| 11 | 13.20 | 13.79 | 16.62 | 14.86 | 13.95 | 11.10 | 10.03 | 14.69 |
| 12 | 10.41 | 13.55 | 11.68 | 16.54 | 15.00 | 8.59 | 8.53 | 14.86 |
| 13 | 14.48 | 13.52 | 16.67 | 14.59 | 12.41 | 12.36 | 12.76 | 13.72 |
| 14 | 12.70 | 13.52 | 17.66 | 16.01 | 11.86 | 10.05 | 8.34 | 9.00 |
| 15 | 10.15 | 13.72 | 13.89 | 13.96 | 15.48 | 8.18 | 11.69 | 11.97 |
| 16 | 13.79 | 14.49 | 15.76 | 14.18 | 13.32 | 12.60 | 9.78 | 10.17 |
| 17 | 8.36 | 14.41 | 18.08 | 16.55 | 14.40 | 9.97 | 10.78 | 11.69 |
| 18 | 8.29 | 14.59 | 16.69 | 15.64 | 15.20 | 12.62 | 12.63 | 12.90 |
| 19 | 13.99 | 13.87 | 17.78 | 15.34 | 13.75 | 12.59 | 11.11 | 11.44 |
| 20 | 14.64 | 13.18 | 14.87 | 13.91 | 14.63 | 12.32 | 9.15 | 8.36 |
| Averages | 11.91 | 13.70 | 16.23 | 14.84 | 13.25 | 10.90 | 10.63 | 11.03 |

This observed outcome may be attributed to the increase in soil acidity due to the bioremediation exercise of the Petroleum polluted soils. Figures 3 (a - d) showed that, there was good positive statistical correlation values rangingfrom 0.7091 (For Cr) – 0.8988 (For As) which buttressed the fact that metals bio-availability is soil acidity dependent (Richards, *et al.*, 2000; Antoniadis and Alloway, 2002; Clemente, *et al.* 2003; Houben, *et al.* 2013).

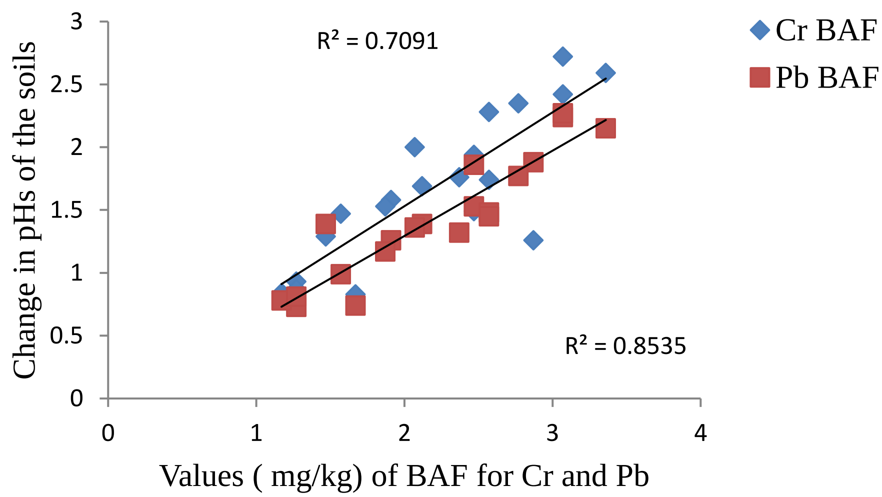


**Figure 3a**: Correlation for changes in soil pHs and Cr and Pb bioavailability

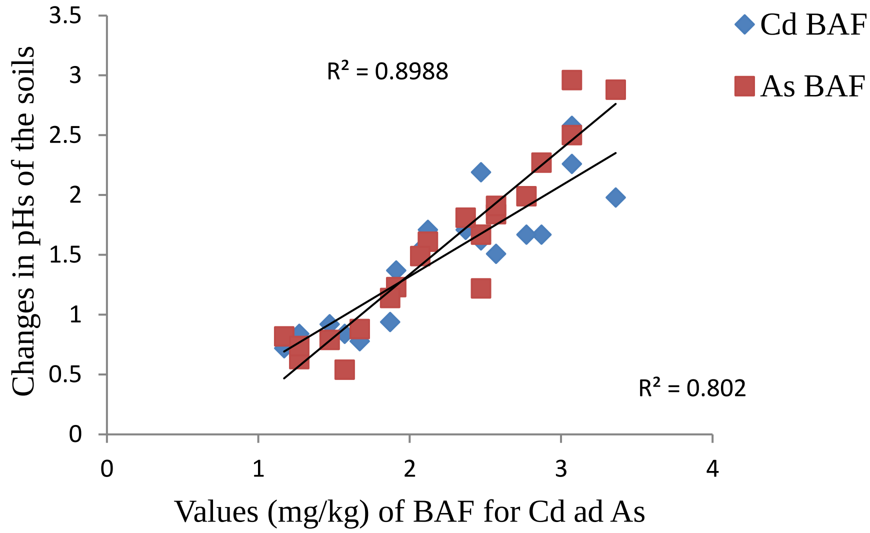


**Figure 3b**: Correlation for changes in soil pHs and Cd and As bioavailability

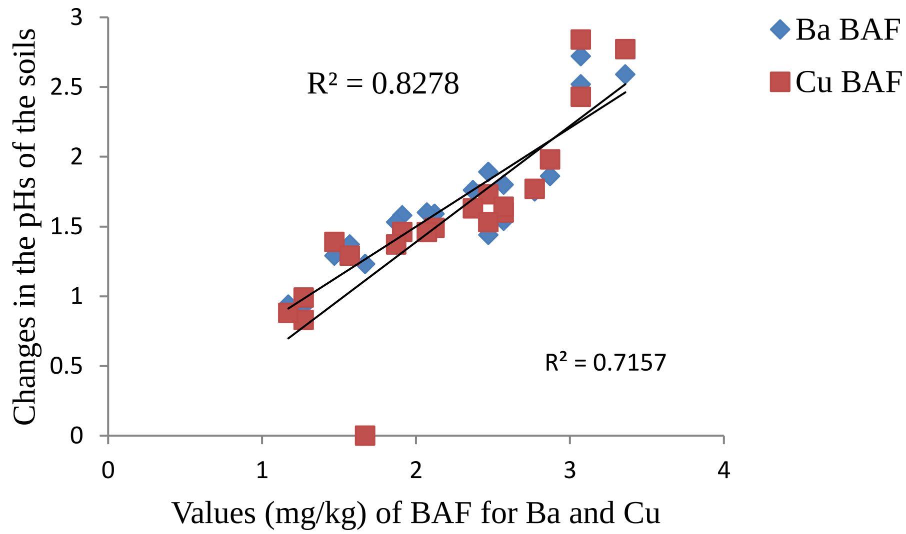


**Figure 3c**: Correlation for changes in soil pHs and Ba and Cu bioavailability

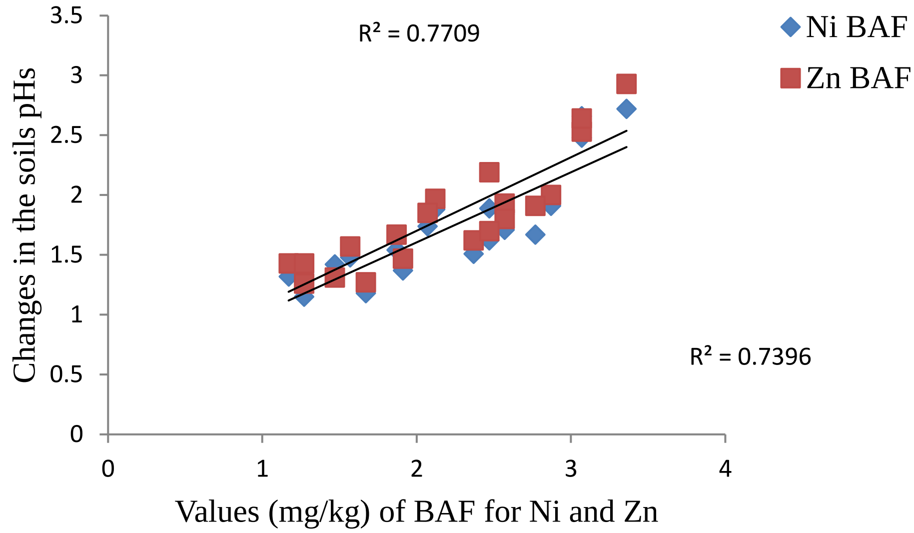


**Figure 3d**: Correlation for changes in soil pHs and Ni and Zn bioavailability

The study has revealed that the relation of metals bioavailability to soil acidity is arithmetical (Figure 3 a – d) and not always proportional. This may be attributed to the several factors that could affect metals bio-availability in the soil environment. For instance, soil type such as clay could make metals less soluble that sandy soil at the same soil pH. Other factors may include the nature and quantity of soil organic matter (SOM). Also, the levels and forms of the metallic compound or complex as well as the presence of similar cations is the soil could contribute significantly to their bioavailability. In the report of Liu *et al.*,(1997), the concentrations of cadmium and lead found in the roots and grains of rice were related to the total concentration of these metals in the soil on which the rice were cultivated. In a similar study in Northern Taiwan, there was a significant correlation between the concentration of cadmium in wheat grains and the concentration in the farm soil (Lee, 1999). However, in this study, the effects of the soil acidity were more significant and correlated to the metal bio-availability in comparison to the total concentration of the metals in the various remediated soils.

Metal bioavailability is of very important health concern. It is one of the routes through which, toxic metals could find theirs ways into human food chain vide vegetables and other plant products. According to Dudka and Market, (1992), only the bioavailable heavy metals are significant to plants and animal. In other words, special attentions and concerns should be focused on this form of metals particularly the toxic heavy metals in soils. This has called for several works on the various factors that could increase the solubility and bioavailability of nutrients and metals in soils (Basta, *et al.,* 2005; Bradham, *et al*., 2006; Wang, *et al*., 2015; Xu, *et al*., 2016; Adamczyk-Szabela and Wolf, 2022). The findings in this study buttress the previous documentations on the relationship of soil acidity to metals bioavailability in a better statistical presentation.

**CONCLUSION**

This study investigated the impact of bioremediation on the physicochemical properties of Petroleum polluted soils with emphasis on metal bioavailability in the remediated soils. It revealed that: bioremediation significantly led to pH drop (i.e. increase in soilacidity) during remediation of Petroleum hydrocarbons polluted soils. Also, there was positive correlation between values of changes in pHs and the percentage of bioremediation of Petroleum polluted soils. Furthermore, bioremediation of Petroleum polluted soil led to significant increase in bioavailability of metals which was attributed to the increase in the soil acidity. Finally, the need to re-condition Petroleum hydrocarbon polluted soils after clean-up and bioremediation particularly for agricultural purposes is established.

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