



PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
<https://www.ajol.info/index.php/jasem>
<https://www.bioline.org.br/ja>

J. Appl. Sci. Environ. Manage.
Vol. 28 (10B Supplementary) 3265-3271 October, 2024

Physicochemical Properties Before and After Bioremediation of Crude Oil Polluted Soils from Ilaje, Ondo State, Nigeria

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ABSTRACTS: Bioremediation helps in the reduction environmental pollutants from air, water, soil, flue gasses, Industrial effluents etc., in natural or artificial settings. Hence, the objective of this paper was to investigate the physicochemical properties before and after bioremediation of crude oil polluted soils collected from Ilaje, Ondo State, Nigeria using appropriate standard techniques. Data obtained show that the pH values before and after bioremediation in sampling stations were A: (5.16 ± 0.00^a ; 5.25 ± 0.003^a); B: (5.27 ± 0.00^b ; $5.40 \pm 0.06^{a,b}$) and C: (5.46 ± 0.02^c ; 5.50 ± 0.06^b) respectively. The results before treatment indicates lower values of pH obtained for soils from contaminated sites. The results showed that there is an increase in the pH of the soils after the treatment, reduction in the amount of available phosphorus in the polluted soil in compares with the control. It also reveals decrease in the amount of organic carbon, organic matter, nitrogen, and potassium and sodium contents of the soil after the treatment. This shows that the bioremediation treatment is effective in the removal of some contaminants in the soil and in the improvement of the physicochemical properties of the soil.

DOI: <https://dx.doi.org/10.4314/jasem.v28i10.38>

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Cite this Article as: EKUNDAYO, F. O; ADEGUNLOYE, D. V; OYEWUMI, O. O. (2024). Physicochemical Properties Before and After Bioremediation of Crude Oil Polluted Soils from Ilaje, Ondo State, Nigeria. *J. Appl. Sci. Environ. Manage.* 28 (10B Supplementary) 3265-3271

Dates: Received: 21 August 2024; Revised: 29 September 2024; Accepted: 08 October 2024 Published: 31 October 2024

Keywords: Physicochemical; Soils; polluted; unpolluted; Bioremediation

Soils are particulate materials of the outer crust of the earth surface formed from the continuous weathering of the underlying parental rocks. Therefore, the type of soil is a function of the nature of the underlying rocks. Soil formation has been reported to be combination of various interrelated factors of parental materials, climate, organisms, topography and time (Arotupin and Akinyosoye, 2008). Soil is important to everyone directly or indirectly. Soil is a complex ecosystem where living organisms play a key role in the maintenance of its properties. Soil is a highly complex medium influenced by environmental and physicochemical parameters, creating a varied habitat for a diverse range of soil microorganisms. Soil quality

can be assessed by analyzing different physicochemical parameters with the analysis of microbial diversity. These are the various indicators which provide the actual condition, nature and quality of the soil. It is a known fact that soil microorganisms are fundamental for terrestrial processes as they play an important role in various biogeochemical cycles by contributing to plant nutrition and soil health (Mocali and Benedetti, 2010). This hidden biodiversity could be a great resource of natural products for agricultural and biotechnological applications (Steele and Streit, 2005). Assessing and preserving the diversity of soil microorganisms is thus crucial. Soil is considered to be the skin of the earth and interfaces with its

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lithosphere, hydrosphere, atmosphere and biosphere. Soil consists of a solid phase (minerals and organic matter) as well as a porous phase that holds gases and water (Chukwuemeka *et al.*, 2017). The most critical biotransformations at stake (degradation of pollutants, synthesis of biofuels and production of novel drugs) require a whole microbial community to be performed. For instance, no single microbe is capable of converting ammonia to nitrate but consortium of microbes can do this very efficiently. These communities are likely to explain the farming mystery of "suppressive soil" in which a pathogen is known to persist but causes little damage to the crops. The activities of suppressive soil communities are quite beneficial to agriculture ensuring the quality and provision of ecosystem services (NRCC, 2007). Soil is the key component of natural ecosystem and environmental sustainability depends largely on sustainable ecosystem (Adenipekun, 2008; Onuh *et al.*, 2008a; Adedokun and Ataga, 2007). Crude oil pollution adversely affects the soil ecosystem through adsorption to soil particles, provision of an excess carbon that might be unavailable for microbial use and an induction of a limitation in soil nitrogen and phosphorus (Baker and Herson, 1994; Atlas, 1981). These processes which affect drastically soil enzymatic activities result in a very slow biodegradation of crude oil polluted soils (Ijah *et al.*, 2008; Okolo *et al.*, 2005). Consequently, various soil amendments have been used in bioremediation strategies to hasten the process for the actualization of sustainable ecosystem. The effects of crude oil pollution on the properties of soil have been the subject of many studies. Okolo *et al.* (2005) reported that oil pollution increase carbon and reduces soil nitrates and phosphorus. Similarly, Adedokun and Ataga (2007) reported that any contact of soil with crude oil results in damage to the soil microorganisms and plants while Onuha *et al.* (2003) among others have shown that crude oil pollution prevents oxygen exchange between soil and the atmosphere due to hydrophobic properties of oil. In Nigeria, most of the terrestrial ecosystem and shore-lines in oil producing communities are important agricultural land under continuous cultivation. The adverse effects of crude oil pollution on these arable agricultural lands have given rise to various soil treatment options such as the use of surfactants, alternate carbon substrates, organic and inorganic manures and bioremediation plants as bioremediation strategies (Ijah *et al.*, 2008; Onuh *et al.*, 2008a, b; Okolo *et al.*, 2005; Burd *et al.*, 2000; Raskin *et al.*, 1997; Obasi *et al.*, 2013). The population and kinds of microorganisms present in soil depend on many environmental factors; nutrients availability, available moisture, degree of aeration, pH, temperature etc. Soil bacteria and fungi play pivotal

roles in various biochemical cycles and are responsible for the recycling of organic compounds (Ogunmwony *et al.*, 2008). Therefore, results obtained from physicochemical analysis give information about soil health (Ogunmwony *et al.*, 2008). Therefore, the objective of this paper is to investigate the physicochemical properties before and after bioremediation of crude oil polluted soils collected from Ilaje, Ondo State, Nigeria.

MATERIALS AND METHODS

Sample Collection: The crude oil polluted soil samples were collected in Ojumole of Ugbonla, Igbokoda, within the coastal area of Ondo State (Ilaje Local Government Area) Nigeria. The sample was collected from 10 cm depth using soil auger into sterile bags and transported aseptically to the laboratory at the Federal University of Technology, Akure, Nigeria, for chemical and microbiological analyses.

Physicochemical analysis of soil samples: The physicochemical properties of the above soil samples were determined. The parameters measured included pH, calcium, magnesium, sodium, cation exchange capacity, organic carbon, organic matter, potassium, particle size, sodium, phosphorus and nitrogen in the Department of Crop Soil and Pest Management, The Federal University of Technology, Akure, (FUTA), Ondo State, Nigeria.

pH Determination: Soil samples collected from the crude oil polluted site was used for physicochemical analysis. Twenty grams of air-dried soil was weighed into a 100mL glass beaker. Then, 50 mL water was mixed with it with a glass rod, and allowed to stand for 30 minutes. The suspension was stirred every 10 minutes during this period. After 1 hour, the suspension was stirred again. A combined pH meter electrode was put into the suspension (about 3-cm deep). The reading was taken after 30 seconds. The pH meter combined electrode was removed from the suspension and rinsed thoroughly with distilled water into a separate beaker and the excess water was carefully dried with a tissue. Twenty (20) grams of each soil samples were weighed and put in a 100 ml beaker. Twenty millimeters of distilled water were added to the sample. The suspension was left for 2 minutes, with occasional stirring using glass rod in order to enable it reach equilibrium. The pH of the suspension was determined using a pH meter (AOAC, 2012).

Determination of Soil Moisture content: Two (2) grams of the sample was weighed into a previously weighed crucible. The crucible plus sample taken was then transferred into the oven set at 100°C for 24 hours

$$\text{Organic Carbon} = \frac{(B - T) * M * 0.003 * 1.33}{W_t} * 100 \quad (5)$$

Where B = Blank titre value; T= Sample titre value; M= Molarity of ferus sulphate; 0.003= 1 mL of 0.167 M K₂Cr₂O₇= 3 mg carbon =0.003 g; 1.33= Walkey constant from assumptions of 75% Organic Carbon attached; W_t= weight of sample

Determination of sodium, potassium, calcium, magnesium and Phosphorus: Determination of mineral contents was done according to the methods described by AOAC (2012). The following minerals: sodium, potassium, calcium, magnesium and phosphorus were assayed for. All analyses were carried out in triplicates. The mineral composition of samples used was determined by wet-hatching method followed by reading of the level of mineral. Sample (soil) of one gram in triplicate each were weighed into porcelain crucible and placed in muffle furnace. The temperature was raised gradually to 450°C. The sample was hatched at 550°C for 56 hours. After cooling to room temperature (28°C), the hatch was dissolved in 1mL 0.5% (v/v) HNO₃. The sample volume was made up to 100 mL and the level of mineral present was analyzed with atomic absorption spectrophotometer Buck 201 VGP. The mineral content was calculated using equation 6 (AOAC, 2012).

$$\text{Mineral (mg/g)} = \frac{R * V * D}{W_t} \quad (6)$$

Where R= solution concentration; V= volume of sample digest; D= dilution factor and W_t. = weight of sample.

Determination of Calcium and Magnesium: Soil sample (10g) of the sieved sample was weighed and poured into a Lab cup, filtered into a sample bottle. 10 mL of the extract was poured into a 250 mL conical flask, 5 mL of NH₃ (Ammonia solution) was added, 3 and 2 drops of KCN and Erichrome black T were respectively added, then titrated with 0.01M EDTA. Colour change was noticed from wine to deep blue. Reading was then taken at the deep blue point (AOAC, 2012).

Determination of Phosphorus Content: Two grams (2g) of soil was weighed and passed through 2 mm sieve into a crucible. The soil was ignited at 50°C for one hour in the muffle furnace. It was removed after one hour and allowed to cool to room temperature. Thirty millilitres (30 mL) of 0.1 M H₂SO₄ was added to the ignited sample and to the same weight of unignited sample. It was stirred and filtered into 10 mL

standard flask and the solution was brought to mark with distilled water. Phosphorus content was determined in ignited and nonignited extracts using spectrophotometer at wavelength of 830 nm (AOAC, 2012).

Determination of sodium, potassium concentrations: Soil sample (10g) of the sieved sample were weighed and poured into lab cup, then filtered into a sample bottle. Flame photometer was used to read the level of Na and K after standardizing it with respective minerals. The percentage individual element was carried out using equation 7 (AOAC, 2012).

$$\text{ppm (sample)} = \frac{R * V * D}{W_t} \quad (7)$$

Where D= Dilution factors; V=Final volume of digest or extract; R= ppm obtained from graph; W_t= Weight of sample used.

Determination of Soil Nitrogen: Soil sample (1g) was weighed into 500 mL Kjeldahl flask, and 10 mL concentrated H₂SO₄ with half kjeldahl catalyst tablet was added. The flask with content on the digestion stand as heat until the solution becomes clear and soil residue remaining is white, it was heated further for few minutes to ensure complete digestion. The digestion was allowed to cool, and distilled water was added to it and transferred into 50 mL volumetric flask and made up to mark with distilled water (AOAC, 2012).

Kjeldhal distillation apparatus was used 5mL of 2% Boric acid was pipetted into a 100mL conical flask, 3 drops of mixed indicator added, the condenser tip was dropped inside the boric acid such that the condenser tube was below the surface of the boric acid solution. 10mL of digest was pipetted into the reaction chamber and 10 mL of 40% of NaOH added. The joints were closed and distillation commenced immediately. 50 mL of distillate was collected inside the receiving flask. The distillate was titrated with 0.01m HCL and the titre value was noted and the nitrogen level calculated using equation 8 (AOAC, 2012).

$$\text{Nitrogen (\%)} = \frac{M * T * 0.014 * V_1}{V_2} * 100 \quad (8)$$

Where T= Control titre value; M= Molarity of acid; V₂= volume of digest used in the distilled; V₁= final volume of digestion

RESULTS AND DISCUSSION

The physicochemical properties of the soil samples before bioremediation treatment are presented in the

table 1 above. On the basis the soil content of sand (47.00 ± 0.00), clay (37.00 ± 0.00) and silt (16.00 ± 0.00) for samples A and B and sand (51.00 ± 0.00), clay 33.00 ± 0.00 and (16.00 ± 0.00); the soils can be classified as clayey-sand. Sample A has the highest values of percentage of clay, organic matter and nitrogen, this may be due to presence of crude oil contaminants while sample C has the highest percentage of sand and phosphorous. The result of pH showed that unpolluted soil has higher pH values (5.46 ± 0.02) than polluted soil (5.16 ± 0.00). Also, phosphorous of unpolluted soil (8.94 ± 0.01) was higher than polluted soil (4.336 ± 0.01).

Soil texture is a measure of the physical properties of the soil. These properties include plasticity of the soil, water retention capacity, soil productivity, soil permeability and ease or toughness of tillage of the soil (Amos- Tautua *et al.*, 2014). The soil texture showed it to be a sandy-loamy soil (sand>clay>silt). This soil therefore has the potential to hold more water within the particles due to the presence of a relatively high percentage of clay (Brady, 1996). Sandy soils retain little water and therefore percolation of water through it is high and so promotes ground water contamination while clayey texture prevents water.

Table 1: Physicochemical properties of crude oil polluted soil and unpolluted soil before and after Bioremediation

Parameters	Samples					
	Before		After		Before	
	A	After	B	After	C	After
pH	5.16 ± 0.00^a	5.25 ± 0.003^a	5.27 ± 0.00^b	$5.40 \pm 0.06^{a,b}$	5.46 ± 0.02^c	5.50 ± 0.06^b
OC (%)	2.48 ± 0.01^c	0.86 ± 0.01^c	1.85 ± 0.01^b	1.24 ± 0.01^b	1.34 ± 0.00^a	1.82 ± 0.02^a
OM (%)	4.27 ± 0.00^c	1.49 ± 0.00^a	3.17 ± 0.00^b	2.15 ± 0.03^b	2.31 ± 0.01^a	3.14 ± 0.01^c
N (%)	0.48 ± 0.01^c	0.20 ± 0.00^a	0.20 ± 0.00^a	0.28 ± 0.01^b	0.26 ± 0.01^b	0.32 ± 0.01^c
Sand (%)	47.00 ± 0.00^a	N.D	47.00 ± 0.00^a	N.D	51.00 ± 0.00^a	N.D
Clay (%)	37.00 ± 0.00^a	N.D	37.00 ± 0.00^a	N.D	33.00 ± 0.00^a	N.D
Silt (%)	16.00 ± 0.00^a	N.D	16.00 ± 0.00^a	N.D	16.00 ± 0.00^a	N.D
P (mg/kg)	5.06 ± 0.02^b	5.91 ± 0.00^b	4.36 ± 0.01^a	8.40 ± 0.17^c	8.94 ± 0.00^c	4.02 ± 0.01^a
Ca (cmol/kg)	2.09 ± 0.03^a	2.00 ± 0.01^a	1.90 ± 0.00^b	2.10 ± 0.05^a	2.40 ± 0.01^c	2.60 ± 0.02^b
Mg (cmol/kg)	$1.00 \pm 0.00^{a,b}$	1.00 ± 0.01^a	0.90 ± 0.02^a	1.00 ± 0.02^a	1.10 ± 0.15^c	1.30 ± 0.06^b
K (cmol/kg)	0.91 ± 0.00^c	0.33 ± 0.01^a	0.47 ± 0.01^b	0.40 ± 0.01^b	0.23 ± 0.00^a	0.82 ± 0.01^c
Na (cmol/kg)	1.09 ± 0.01^c	0.46 ± 0.00^a	0.57 ± 0.01^b	0.56 ± 0.01^b	0.30 ± 0.02^a	0.96 ± 0.02^c
Zn (cmol/kg)	0.67 ± 0.02^b	N.D	0.81 ± 0.00^b	N.D	0.45 ± 0.39^a	N.D
Ni (cmol/kg)	N.D	N.D	N.D	N.D	N.D	N.D
Pb (cmol/kg)	$0.15^b \pm 0.00$	N.D	0.20 ± 0.00^c	N.D	0.07 ± 0.00^a	N.D
Co (cmol/kg)	0.09 ± 0.01^c	N.D	0.07 ± 0.00^b	N.D	0.01 ± 0.00^a	N.D
Cr (cmol/kg)	6.33 ± 0.01^c	N.D	8.54 ± 0.01^b	N.D	1.40 ± 0.02^a	N.D

Values are presented as mean \pm S.D. mean values are significantly different at $P \geq 0.05$. Mean values with similar superscript are not significantly different at $P \geq 0.05$.

Keys: A - Crude oil polluted soil, N. D - Not determined, P - Phosphorous, K - Potassium, Ni- Nickel, B - Crude oil polluted soil, OC - Organic carbon, Ca - Calcium, Na - Sodium, Pb- Lead, Co- Cobalt C - Unpolluted soil, OM - Organic matter

The table 1 reveals the pH, percentage organic carbon, organic matter, nitrogen, sand, clay, silt, amount of phosphorous, calcium, magnesium, potassium, sodium, zinc, nickel, lead, cobalt and chromium in the soil samples. The soil samples have low nitrogen and organic matter content. This may be as a result of the detrimental effects of oil pollution (Ogbonnal *et al.*, 2009). The lower values of pH obtained for soils before bioremediation from contaminated sites and for samples A and B respectively are lower than that of the control. Findings after bioremediation revealed that there is an increase in the pH of the soils after the treatment. Therefore, it could be inferred that the presence of oil impacts acidity on the soil. The increase in pH after bioremediation as observed in this study contradicts the reports of Onuh *et al.* (2008a) who observed an increase in pH values. The result of the pH obtained from this study is lower to the values obtained by Chikere *et al.* (2019) who reported slight alkaline pH (7.8) for crude oil polluted soil while

pristine soil had slightly acidic pH (6.5). However, the values of the pH obtained from this study is within the acceptable standards of 5.5 to 6.5 (DPR, 2002). The pH which is the degree of acidity or alkalinity of soil affects not only the physicochemical properties but also the flora and fauna of soil. Thus, it determines the availability of many nutrients for plant growth and maintenance (Obasi *et al.*, 2013). Strong acidic soils (pH 4 to 5) have been reported to have high concentration of soluble aluminum and manganese salts, which are toxic to plants. Consequently, the lowered pH values observed in the polluted soils can be raised by liming through appropriate application of calcium and magnesium compounds. Also, it is known that carbon mineralization and organic matter breakdown are rapid in neutral-to-slightly alkaline soils (Hunt, 1996; Obasi *et al.*, 2013). The pH of the soils according to the United States Department of Agriculture, soil pH range classification is represented in tabular format. From the table, it was obtained that

the nature of the soil is slightly acidic. pH value is a measure of the hydrogen or hydroxyl ion activity of the soil water system which indicates whether the soil is acidic, neutral or alkaline in reaction. Crop growth suffers much both under very low as well as high pH. Soil pH (acidity and alkalinity) play the greatest influence on availability of nutrients to plants and the type of organism found in the soil. The pH also affects the solubility of metal and therefore its availability to plants is made more accessible to plants at acidic pH. The pH is defined as the hydrogen ion concentration. It is the measure of the acidic property of matter. In areas with high rainfall, soils tend to be more acidic in nature. This is because the basic cations are forced off the soil colloids by the mass action of hydrogen ions from the rain as those attach to the colloids (Edoris *et al.*, 2017). It also revealed decrease in the amount of organic carbon, organic matter, nitrogen, potassium and sodium content of the soil after the treatment. This shows that the bioremediation treatment is effective in the removal of some contaminants in the soil and in the improvement of the physicochemical properties of the soil. The values for the physicochemical analysis of these oil polluted samples were higher than those obtained by Perez *et al.* (2017). This decrease observed contradicts the findings of other researchers who reported increase in values of percentage organic matter and organic carbon (Obasi *et al.*, 2013; Onuh *et al.* (2008a); Ogboghodo *et al.*, 2005). Sample A has the highest values of percentage of clay, organic matter and nitrogen while sample C has the highest percentage of sand and phosphorous. The decrease in the available nitrogen and phosphorus with increased levels of crude oil pollution may be attributed to the limitation induced by the introduction of excess carbon to the soil since crude oil is a rich source of hydrocarbon (Atlas, 1981). It is evidenced from this study that bioremediation treatment modify the physical, chemical and biological properties of crude oil polluted soils and this will improve their nutritional status for enhanced agronomic performances.

Conclusion: The study revealed that crude oil pollution adversely affect soil physicochemical properties, then the use of nanoparticles and biosurfactants was effective in treatments of the polluted soil as observed findings after treatment. Thus, leading to the improvement of the physicochemical properties of the soil which is an important factors that farmers always consider that lead to fertile soil, thereby increasing crop productivity and reduced food scarcity in the nation. Hence, this can be employed in oil polluted environments.

Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability Statement: Data are available upon request from the corresponding author

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