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Research Article Soil Nutrients Variation During Phytoremediation of Crude oil Polluted Soil Using *Mariscus alternifolius* and *Fimbristylis ferruginea*

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Abstract

Background and Objective: Crude oil pollution affects the soil nutrients availability and the ecological unit of the soil through surface assimilation and adsorption of soil particle. The excess carbon introduced into the soil could result in constraints in soil nutrients. This study was carried out to investigate the alteration of nutrients during phytoremediation of crude oil polluted soil using *Mariscus alternifolius* Vahl. and *Fimbristylis ferruginea*. **Materials and Methods:** The crude oil polluted soil was collected from agricultural farmland located in Ogoniland, Nigeria while the unpolluted soil was collected from the premise of agricultural farmland in the University of Port Harcourt. Mature and viable seeds of *M. alternifolius* and *F. ferruginea* were collected from wild. The effect of *M. alternifolius* and *F. ferruginea* on different soil nutrients such as calcium, magnesium, total nitrogen, potassium, phosphorus, sulphur and moisture content were studied. The pot experimental study was carried out for 12 weeks spanning from September-December, 2017. Standard protocols were adopted for both the field and laboratory procedures. **Results:** After 12 weeks of remediation, significant reductions in calcium, magnesium, total nitrogen and potassium levels were observed in the vegetated soils. However, phosphorus, sulphur and moisture contents of the vegetated soils significantly increased. Such an increase as recorded in the moisture content of the soils was typical for any biodegradation process. **Conclusion:** It is, therefore, worthy to note that certain soil nutrients could diminish following phytoremediation of crude oil polluted soils.

Key words: Polluted soil, crude oil pollution, Fimbristylis ferruginea, soil nutrients, Mariscus alternifolius, phytoremediation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soil is a central portion of the natural environment housing a broad range of organisms and supports the dispensation of plant species¹. It regulates the movement of water and chemical substances between the atmospheric and terrestrial environment and also functions as source and storage for gases such as oxygen and carbon dioxide². Inorganic mineral nutrients in soil are fundamental for the growth and development of vegetation and reproduction tissues. They are significant in enzymatic reaction where they function as cofactors³. In plants, macronutrients are usually found at concentrations greater than 0.1% of dry tissue weight and consist of nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and sulphur (S)⁴. The macronutrients containing carbon, hydrogen and oxygen are regarded as essential for plant growth⁵.

The pollution of the soil resulting from natural or anthropogenic sources can provoke enormous disturbances in the ecological balance and places the health of organisms and other living beings on earth under risk. It inhibits the development and flourishing of crops and could be imbibed by crops which might cause severe health challenges when consumed. Such may result in elevated soil salinity and such soil turns noisome for vegetation and at times barren¹. Soil pollution resulting from crude oil spillage hinders the growth of plant, productivity of the soil and the availability of nutrients⁶. It affects the ecological unit of the soil through surface assimilation and adsorption of soil particle which could emanate when there is an introduction of a surplus carbon which may not be feasible for use by the microbial population thus leading to limitation in soil nutrients⁷. Oil spillage also introduces non-organic compounds, carcinogens and growth inhibiting chemicals obtainable in crude oil into the ecosystem⁸ and protracted exposure of these contaminants may instigation kidney and liver diseases, bone marrow mutilation and intensified risk of cancer⁹.

Abnormal levels of soil essential nutrients resulting from crude oil in the soil have been reported to result in loss of chlorophyll which ultimately leads to dawdling or stunted growth. This may arise due to decline in cell division, protein content of seeds and the vegetative parts and early maturity which may affect yield and quality. It may also give rise to symptoms such as dark to blue-green colouration on older leaves, purpling of leaves, brown spots, chlorosis, twisting, deformation, inhibition and formation of small tiny yellow spots^{3,5,6,10}. The availability of soil nutrients after phytoremediation could depend on the choice of plant. This is because micro-organisms responsible for nutrient recycling thrive specifically in the rhizosphere of certain plants. This study was undertaken to determine the variation of some soil nutrients during phytoremediation of crude oil polluted soil using *Mariscus alternifolius* Vahl. and *Fimbristylis ferruginea*.

MATERIALS AND METHODS

This study was carried out at the University of Port Harcourt Ecological Center for 10 months between May 2017 and February, 2018.

Polluted soil samples were obtained from a crude oil contaminated agricultural farmland located in Bodo community of Ogoniland, Rivers state, Nigeria. Unpolluted soil was obtained from an agricultural site located in the University of Port Harcourt, Nigeria. The soil samples were collected using unused and sterile plastic bags sealed with a rubber band and transported to the Ecological Centre, University of Port Harcourt, where the pot experiment was carried out.

Mariscus alternifolius and *F. ferruginea* seeds identified as per plant species growing in the spill agricultural farmland were sourced from the wild within the premise of the University of Port Harcourt.

Samples for laboratory analysis, with the exception of the samples for the determination of moisture content which was not air dried, was thoroughly mixed, air dried, mixed again and passed through 2 mm sieve to remove gravel and debris.

Study design: Pot experiments were used to achieve this research. Four groups, set up in triplicate were prepared. The groups included a positive control group (unpolluted soil), negative control group (polluted soil) and two treatment groups. Prior to this, mature and viable seeds of M. alternifolius and F. ferruginea were propagated onto soil with no history of pollution. This soil was collected from the premise of an agricultural site of the University of Port Harcourt, Nigeria. Soil sample collected from this site also served as the control for the group marked as the positive control group. Soil samples which served as negative control and as well were used for the treatment groups were collected from agricultural farmland with a crude oil spill. The propagated seeds at the seedling level were transferred to the experimental pots containing 8 kg of soil properly designated for each. The control groups were unvegetated. Treatment was closely monitored for 12 weeks ensuring adequate moistening was attained.

Laboratory analysis: The reagents used for this study were of analytical grade with high purity. The methods as described by Motsara and Roy¹¹ and adopted by Chukwuma *et al.*⁴ were employed for the estimations of total nitrogen (TN), exchangeable calcium (Ca), exchangeable magnesium (Mg), available phosphorus (P) and available sulphur (S). Potassium (K) concentration was determined by microwave digestion method as adopted by Mwegoha and Kihampa¹² and Rashid *et al.*¹³ where finely powdered soil was digested at 95°C for 1 h using aqua regia comprising of HCl and HNO₃ (3:1). Moisture content (MC) was determined by the gravimetric method as described by Cunniff¹⁴, where a known weight of soil samples was dried at 105°C to constant weight and reweighed when cooled in a dessicator.

Total nitrogen: A gram of soil, K_2SO_4 (1.5 g), CuSO₄ (0.7 g) and H_2SO_4 (30 mL) were homogenized in a conical flask. The content was heated until frothing ceased. The solution was boiled briskly until it became clear (sky blue colour appeared) and then digested further for 30 min. To the receiving flask were made available 25 mL and 3 drops of 0.1 M HCl and methyl red indicator, respectively. Thirty millilitres of 35% NaOH was situated in the distilling flask followed by the addition of 20 mL the digest in a manner that the contents did not mix. This was followed by heating the contents for 30 min to distil the ammonia. Thereafter, 0.1 M NaOH of the distillate was then used for the titration of the excess acid in the distillate. Total nitrogen (%) was calculated as follows:

$$N (\%) = \left\{ \frac{1.401 \left[(V_1 M_1 - V_2 M_2) - (V_3 M_1 - V_4 M_2) \right]}{W} \right\} \times df$$

Where:

- V₁ = Millilitres of standard acid put in receiving flask for samples
- V₂ = Millilitres of standard NaOH used in titration
- V₃ = Millilitres of standard acid put in receiving flask for blank
- V₄ = Millilitres of standard NaOH used in titrating blank
- M_1 = Molarity of standard acid
- M_2 = Molarity of standard NaOH
- W = Weight of sample taken (1 g)
- df = Dilution factor of sample (if 1 g was taken for estimation, the dilution factor will be 100)

Exchangeable calcium: Into 5 g soil was dispensed 25 mL of neutral normal ammonium acetate. The conical flask was shaken on rotator mixer for 5 min and filtered. Three crystals of versenate were introduced into the 5 mL

aliquot followed by the addition of 5 mL of 16% NaOH and 40 mg Murexide indicator before titrating with 0.01 N ethylenediaminetetraacetate (EDTA) until colour change was observed. The calcium content of the soil was calculated as follows:

$$N_1 V_1 = N_2 V_2 \frac{N_1 = [N_2 V_2]}{V_1}$$
$$= \frac{\text{Normality of EDTA} \times \text{Volume of EDTA}}{\text{Aliquot taken (mL)}}$$

Hence, N_1 (normality) is the equivalent of Ca^{2+} present in 1 L of aliquot; Therefore:

$$Ca^{2+}$$
 me/L = $\frac{\text{Normality of EDTA} \times \text{Volume of EDTA}}{\text{Aliquot taken (mL)}} \times 1000$

Exchangeable calcium plus magnesium: Five gram air-dried and homogenized soil was placed in a 150 mL flask and 25 mL of neutral normal ammonium acetate solution was added. This was shaken on a rotator mixer for 5 min and filtered. A 5 mL aliquot was taken and 3 crystals of carbamate added followed by the introduction of 5 mL of ammonium chloride-ammonium hydroxide buffer solution. Three drops of Eriochrome Black T (EBT) indicator was further added and solution titrated with 0.01 N EDTA until the colour changed to bright blue or green and no tinge of wine-red colour remained. The calcium plus magnesium content of the soil was calculated as follows:

$$N_1 V_1 = N_2 V_2 \text{ or } N_1 = \frac{\left[N_2 V_2\right]}{V_1}$$
$$= \frac{\text{Normality of EDTA} \times \text{Volume of EDTA}}{\text{Aliquot taken (mL)}}$$

Hence, N_1 (normality) is the equivalent of $Ca^{2+}+Mg^{2+}$ present in 1 L of aliquot; Therefore:

$$Ca^{2+} + Mg^{2+} me/L = \frac{Normality of EDTA \times Volume of EDTA}{Aliquot taken (mL)} \times 1000$$

Milli-equivalent (me) of $Mg^{2+} = me (Ca^{2+}+Mg^{2+})$ -me of Ca^{2+}

Available phosphorus: The standard curve was first prepared by dissolving 0.2195 g of pure dry KH_2PO_4 in 1000 mL of distilled water (dH₂O). This solution, containing 50 µg P mL⁻¹ was preserved as a stock standard solution of phosphate. Ten milliliters of 50 µg P mL⁻¹ solution was diluted to 0.5 L with dH₂O. This solution contained 1 μ g P mL⁻¹ (0.01 mg P mL⁻¹). About 0, 1, 2, 4, 6 and 10 mL from 1 µg P mL⁻¹ solution were delivered in separate 25 mL flasks and to each flask were added 5 mL of Bray's extractant No. 1 (0.03 M NH₄F in 0.025 M HCl) and molybdate reagent (5 mL) and diluted to 20 mL of dH₂O. One millilitre of dilute SnCl₂ solution was added, shaken and composited to 25 mL mark with distilled water after which it was allowed for 10 min for a blue colour to develop. The resultant solution was read spectrophotometrically at 660 nm. The absorbance was thus plotted against "µg P". Phosphorus extraction was initiated by mixing 50 mL of Bray's extractant with 5 g of the soil sample. The solution was shaken briskly for 5 min using a mechanical shaker and thereafter filtered using Whatman No. 1 filter paper. To 5 mL filtrate was added 5 mL molybdate reagent. The solution was diluted to 20 mL with dH₂O, shaken and 1 mL of the dilute SnCl₂ solution added. The final volume was then made up to 25 mL with dH₂O which was shaken thoroughly, allowed standing for 10 min and read at 660 nm. Blank was prepared similarly but without the soil. The soil available phosphorus was calculated as follows:

Phosphorus (P) (kg ha⁻¹) =
$$\frac{A}{1000000} \times \frac{50}{5} \times \frac{2000000}{5} = 4A$$

where, weight of the soil taken is 5 g, volume of the extract is 50 mL, volume of the extract taken for estimation is 5 mL, amount of P observed in the sample on the standard curve is A (μ g) and weight of 1 ha of soil down to a depth of 22 cm is taken as 2 million kg.

Available sulphur: In a conical flask containing 20 g of the soil sample, 100 mL of monocalcium phosphate extracting solution (500 mg P L⁻¹) was added, shaken for 1 h and thereafter filtered. About 10 mL of the clear filtrate was placed in a 25 mL volumetric flask and 2.5 mL 25% HNO₃ and 2 mL of acetic-phosphoric acid added. The solution was diluted to 22 mL with dH₂O, stoppered and shaken. Furthermore, 0.2 g of BaCl₂ crystal and 0.5 mL BaSO₄ seed suspension were added. This was stoppered, inverted thrice and left for 10 min, after which a further inversion for 10, 5 min and one more 10 min was carried out. The solution was left to stand for 15 min and 1 mL of gum acacia-acetic acid solution was added. The volume was made up to 25 mL, inverted 3 times and set aside. After one and half hours, the flask was inverted 10 times and the turbidity measured at 440 nm (blue filter). The blank was prepared similarly but without soil and was read side by side with test samples. To prepare the standard curve, 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mL of working standard solution (10 mg S L⁻¹) were laid into series of 25 mL

volumetric flask to obtain 25, 50, 75, 100, 125 and 150 μ g of S. The turbidity was developed following same method as described above. Standard curve was thereafter prepared by plotting readings obtained from turbidity that developed against S concentration. The soil available sulphur was calculated as follows:

Available sulphur (SO₄⁻-S) in soil (mg kg⁻¹) =
$$\frac{W \times 100}{10 \times 20} = \frac{W}{2}$$

Where:

 $W = Quantity of S(\mu g)$ as obtained on the X-axis against an absorbance reading (Y-axis) on the standard curve

20 = Weight of the soil sample (g)

100 = Volume of the extractant (mL)

10 = Volume of extractant (mL) in which turbidity is developed

Potassium: Two and a half grams of finely powdered soil was transferred to a crucible and mixed with 10 mL of aqua regia comprising of HCl and HNO_3 (3:1) and digested at 95 °C for 1 h. After cooling, the digest was diluted to 50 mL using dH₂O and allowed to settle overnight and thereafter filtered. The concentration of K was determined by atomic absorption spectrometry (SensAA).

Moisture content: A 3 g soil was situated into the empty dish and spread uniformly using a spatula. The dish with soil was further conveyed to the oven, set at 105°C and left for 3 h. After drying, the dish with partially covered lid enclosing the dried sample was conveyed to the dessicator to cool and reweighed. The moisture content was calculated as follows:

Moisture (%) =
$$\frac{W_1 - W_2}{W_1} \times 100$$

Where:

 W_1 = Weight (g) of sample before drying W_2 = Weight (g) of sample after drying

Statistical analysis: Results are expressed as mean ± standard deviation of triplicate determination. To detect a significant difference between the groups, statistical analysis was carried out using one-way analysis of variance (ANOVA) and the Student t-test. Data between groups were analyzed by the Bonferroni test using Statistical Package for the Social Science (SPSS®) Version 20 statistics software at 95% (p<0.05) confidence level while data between the periods were analyzed by the Student t-test.

RESULTS

The results of exchangeable calcium, exchangeable magnesium, available sulphur, total nitrogen, available phosphorus, potassium and moisture content of the remediated soil are presented in Table 1-7.

Exchangeable calcium: The polluted control soil before planting showed significantly (p<0.05) lower values $(3.00\pm1.00 \text{ me } \text{L}^{-1})$ of exchangeable calcium when compared with the unpolluted control soil $(8.00\pm1.00 \text{ me } \text{L}^{-1})$ as shown in Table 1. The exchangeable calcium content of *M. alternifolius* remediated soil and *F. ferruginea* remediated soil, across the remediation period showed no significant difference (p<0.05) when compared with the corresponding baseline values with the exception *M. alternifolius* remediated soil which by 12 WAP showed a significantly (p<0.05) decreased value $(1.43\pm0.40 \text{ me } \text{L}^{-1})$.

Exchangeable magnesium: The exchangeable magnesium of the polluted control soil before planting showed a significantly (p<0.05) lower values $(2.00\pm0.10 \text{ me L}^{-1})$ when compared with the unpolluted control soil $(4.00\pm0.10 \text{ me L}^{-1})$ as shown in Table 2. Compared to the corresponding baseline values, there was a significant (p<0.05) decrease in the exchangeable magnesium contents of *M. alternifolius* remediated soil $(1.07\pm0.12 \text{ me L}^{-1})$ and *F. ferruginea* remediated soil $(1.33\pm0.68 \text{ me L}^{-1})$ 12 WAP.

Available sulphur: The available sulphur of the polluted soil before planting showed a significantly (p<0.05) lower value ($66.25\pm1.00 \text{ mg S L}^{-1}$) when compared with the unpolluted soil ($118.74\pm1.00 \text{ mg S L}^{-1}$) as shown in Table 3. Compared to the corresponding baseline values, no significant difference (p<0.05) was observed in the remediated soils across the period with the exception of *M. alternifolius* remediated soil (95.83±15.88 mg S L⁻¹) at 4 WAP and *F. ferruginea* remediated soil ($103.33\pm13.48 \text{ mg S L}^{-1}$) at 8 WAP, which showed significantly (p<0.05) increased values.

Total nitrogen: The percentage total nitrogen contents of the remediated soils are represented in Table 4. Before planting, the polluted soil recorded significantly (p<0.05) lower value ($0.69\pm0.01\%$) when compared to the unpolluted soil ($0.93\pm0.01\%$). Twelve weeks after planting, a significant (p<0.05) reductions in percentage total nitrogen content of *M. alternifolius* remediated soil ($0.28\pm0.06\%$) and *F. ferruginea* remediated soil ($0.30\pm0.05\%$) were recorded.

Available phosphorus: The soil available phosphorus levels of the remediated soils are represented in Table 5. The polluted soil before planting showed a significantly (p<0.05) lower value (34.59 ± 1.00 kg ha⁻¹) when compared to the unpolluted soil (125.41 ± 1.00 kg ha⁻¹). However, when compared to the corresponding baseline values, no significant difference (p<0.05) was observed in the remediated soils across the period with the exception of *M. alternifolius* remediated soil which by 4 and 8 WAP showed significant (p<0.05) increase and decrease, respectively.

Table 1: Exchangeable calcium content (me L⁻¹) of *M. alternifolius* and *F. ferruginea* remediated soils

Groups	BP	4 WAP	12 WAP
Unpolluted control	8.00 ± 1.00^{a}	7.83±0.29ª	7.27±0.40ª
Polluted control	3.00 ± 1.00^{b}	3.00 ± 0.50^{b}	2.63±0.29 ^b
M. alternifolius	3.00 ± 1.00^{b}	2.93±0.12 ^b	1.43±0.40 ^{c,*}
F. ferruginea	3.00 ± 1.00^{b}	2.93±0.12 ^b	1.83±0.29°

Values are mean \pm standard deviation of triplicate determination. Values in the same column with different letters (a, b, c) are significantly different at p<0.05. *p<0.05 compared to the corresponding values before planting. BP: Before planting, WAP: Week(s) after planting

Table 2: Exchangeable magnesium content (me L^{-1}) of *M. alternifolius* and *F. ferruginea* remediated soils

Groups	BP	4 WAP	12 WAP
Unpolluted control	4.00±0.10 ^a	3.93±0.12ª	3.73±0.12ª,*
Polluted control	2.00 ± 0.10^{b}	2.10 ± 0.10^{b}	1.83 ± 0.06^{b}
M. alternifolius	2.00 ± 0.10^{b}	1.97±0.15 ^b	1.07±0.12 ^{c,*}
F. ferruginea	2.00 ± 0.10^{b}	1.87 ± 0.12^{b}	1.33±0.68 ^{c,*}

Values are mean \pm standard deviation of triplicate determination. Values in the same column with different letters (a, b, c) are significantly different at p<0.05. *p<0.05 compared to the corresponding values before planting. BP: Before planting, WAP: Week(s) after planting

Table 3: Available sulphur (mg S L⁻¹) content of *M. alternifolius* and *F. ferruginea* remediated soils

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Groups	BP	4 WAP	8 WAP	12 WAP	
Unpolluted control	118.74±1.00ª	7.67±2.01 ^{a*}	67.92±31.03 ^{a,b}	12.72±3.61ª,*	
Polluted control	66.25±1.00 ^b	101.58±36.88 ^b	77.08±3.82ª,*	83.75±46.65 ^{a,b}	
M. alternifolius	66.25±1.00 ^b	95.83±15.88 ^{b,*}	86.25±45.48 ^{a,b}	95.00±56.97 ^{a,b}	
F. ferruginea	66.25±1.00 ^b	100.83±26.36 ^b	103.33±13.48 ^{b,*}	62.58±14.03 ^b	

Values are mean ± standard deviation of triplicate determination. Values in the same column with different letters (a, b) are significantly different at p<0.05. *p<0.05 compared to the corresponding values before planting. BP: Before planting, WAP: Week(s) after planting

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Table 4: Total nitrogen con	itent (%) of M. alternifo	<i>lius</i> and <i>F. ferruginea</i> remediated soils	
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Groups	BP	4 WAP	12 WAP
Unpolluted control	0.93±0.01ª	0.76±0.05ª,*	0.34±0.06 ^{a,*}
Polluted control	0.69±0.01 ^b	0.51±0.05 ^{b,*}	0.26±0.06 ^{a,*}
M. alternifolius	0.69±0.01 ^b	0.52±0.09 ^b	0.28±0.06ª,*
F. ferruginea	0.69±0.01 ^b	0.53±0.04 ^{b,*}	$0.30 \pm 0.05^{a,*}$
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Values are mean±standard deviation of triplicate determination. Values in the same column with different letters (a, b) are significantly different at p<0.05. *p<0.05 compared to the corresponding values before planting. BP: Before planting, WAP: Week(s) after planting

Table 5: Available phosphorus content (kg ha⁻¹) of *M. alternifolius* and *F. ferruginea* remediated soils

Groups	BP	4 WAP	8 WAP	12 WAP
Unpolluted control	125.41±1.00 ^a	164.72±5.56ª,*	26.27±7.62 ^{a,b,*}	66.12±14.96ª,*
Polluted control	34.59±1.00 ^b	69.25±28.65 ^b	8.32±0.68 ^{c,*}	46.98±15.53 ^{b,c}
M. alternifolius	34.59±1.00 ^b	87.53±23.19 ^{b,*}	13.73±5.52 ^{a,c,*}	44.00±12.04 ^c
F. ferruginea	34.59±1.00 ^b	96.86±54.14 ^b	27.68±7.61 ^b	21.18±16.29 ^b

Values are mean \pm standard deviation of triplicate determination. Values in the same column with different letters (a, b, c) are significantly different at p<0.05. *p<0.05 compared to the corresponding values before planting. BP: Before planting, WAP: Week(s) after planting

Table 6: Potassium concentration (mg kg⁻¹) of *M. alternifolius* and *F. ferruginea* remediated soils

Groups	BP	12 WAP
Unpolluted control	409.00±1.00 ^a	222.26±110.61ª.*
Polluted control	641.42±10.00 ^b	364.32±58.96 ^{a,b,*}
M. alternifolius	641.42±10.00 ^b	367.59±58.49 ^{b,*}
F. ferruginea	641.42±10.00 ^b	$308.74 \pm 56.08^{a,b,*}$

Values are mean \pm standard deviation of triplicate determination. Values in the same column with different letters (a, b) are significantly different at p<0.05. *p<0.05 compared to the corresponding values before planting, BP: Before planting, WAP: Week(s) after planting

Potassium: The soil potassium concentrations of the remediated soils are presented in Table 6. The polluted soil, before planting showed a significantly (p<0.05) higher value ($641.42\pm10.00 \text{ mg kg}^{-1}$) when compared to the unpolluted soil ($409.00\pm1.00 \text{ mg kg}^{-1}$). There was a significant (p<0.05) decrease in soil potassium concentrations of *M. alternifolius* remediated soil ($367.59\pm58.49 \text{ mg kg}^{-1}$) and *F. ferruginea* remediated soil ($308.74\pm56.08 \text{ mg kg}^{-1}$) 12 WAP.

Moisture content: The soil percentage moisture content of the polluted soil before planting showed a significantly (p<0.05) lower value ($9.67\pm0.01\%$) when compared with the unpolluted soil ($10.33\pm0.10\%$) as shown in Table 7. When compared to the corresponding baseline values, there was a significant (p<0.05) increase in soil percentage moisture content of the remediated soil groups over time.

DISCUSSION

The six mineral elements, calcium, magnesium, sulphur, nitrogen, phosphorus and potassium are required in large amounts and are thus regarded as macronutrients¹⁵.

The significantly lower exchangeable calcium and magnesium recorded in the polluted soil when compared to the unpolluted soil before planting may be due to the crude oil contamination of the soil. This corroborates with Akubugwo *et al.*¹⁶, who opined that the concentrations of exchangeable cations (Ca^{2+} and Mg^{2+}) increase with an increase in pollution. However, the subsequent reduction of the exchangeable cations in the vegetated soils over time may be as a result of the changes in the pH of the soils since pH affects the availability of these exchangeable cations. This may be supported by the findings of Ngobiri *et al.*¹⁷, who associated low pH with loss of exchangeable bases owing to displacement reactions in the soil colloidal complex and excess water that could lead to eluviations and leaching.

The decrease in the sulphur content of the polluted soil when compared to the unpolluted soil before planting may be due to the crude oil pollution. However, environmental factors have contributed to the observed sulphur contents of the soils overtime. This finding corroborated with previous study which opined that oxidation reactions of elemental sulphur are faster in alkaline soils than in acidic soils and of the soil and environmental factors affecting oxidation rate, temperature and soil pH have the greatest effect¹⁸.

The significantly lower nitrogen content of the polluted soils, when compared with the unpolluted soils, may be due to the presence of petroleum hydrocarbons in the soil. This confirmed the previous report that indicated low nitrogen reserve in petroleum hydrocarbons contaminated soil and further corroborated with another report that crude oil pollution leads to a reduction in soil total nitrogen^{19,20}. However, the decrease in the percentage of total nitrogen of the vegetated soils over time may be due to its utilization by the plant species and associated micro-organisms. Since the plant species are not leguminous plants, recycling of this nutrient may be impaired. The reason may be that micro-organisms responsible for fixing nitrogen were either absent or deficient since nitrogen-fixing bacteria are mostly supported by legumes²¹. On the other hand, may have played

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Groups	BP	4 WAP	8 WAP	12 WAP
Unpolluted control	10.33±0.10ª	21.00±3.18ª,*	7.55±2.34ª	19.11±1.95ª,*
Polluted control	9.67±0.01 ^b	5.89±0.38 ^{b,*}	17.11±1.64 ^{b,*}	28.33±0.67 ^{b,*}
M. alternifolius	9.67±0.01 ^b	13.44±1.84 ^{c,*}	17.11±1.17 ^{ь,*}	16.33±2.52 ^{c,*}
F. ferruginea	9.67±0.01 ^b	13.56±2.50 ^{c,*}	15.89±2.27 ^{b,*}	$21.00 \pm 2.02^{a,c,*}$

Table 7: Moisture content (%) of *M. alternifolius* and *F. ferruginea* remediated soils

Values are mean ± standard deviation of triplicate determination. Values in the same column with different letters (a, b, c) are significantly different at p<0.05. *p<0.05 compared to the corresponding values before planting. BP: Before planting, WAP: Week(s) after planting

a significant role in the overall activities of the micro-organism responsible for fixing nitrogen. Earlier reports recognized that nitrogen fixation may be restricted by soil acidity²². The deleterious effect of low pH on nodulation was previously demonstrated by Rice²³ and Penney *et al.*²⁴. Likewise, a field experiment was also conducted on the effects of pH on Rhizobia numbers in soil and on nodulation and nitrogen fixation using alfalfa and red cover. The findings showed that nitrogen fixation was strongly affected by soil pH (<6.0) and thus attributed low nitrogen level to progressively poorer nodulation as the pH decreased²⁵ below 6.0.

The significantly lower available phosphorus obtained in the polluted soil when compared to the unpolluted soil before planting may be due to the crude oil contamination. This corresponds with the previous reports by Okolo *et al.*⁷, Benka-Coker and Ekundayo²⁵ and Wang and Wu²⁶, who stated that crude oil contamination could lead to a decrease in soil available phosphorus. However, the pH of the vegetated soils may have been responsible for the amount of available phosphorus recorded in the vegetated soils over time²⁷. This finding corroborates a study which associated a decrease in soil available phosphorus at the end of the remediation to the inability of the phytoremediation plant to fix phosphorus thus utilizing the soil available phosphorus²¹. According to the United State Department of Agriculture (USDA), soils with inherent pH values of 6-7.5 are ideal for phosphorus availability, while pH values<5.5 and between 7.5 and 8.5 limits phosphorus availability²⁸.

The significantly higher concentration of potassium in the polluted soil when compared with the unpolluted soil before planting may be due to the crude oil pollution of the soil. However, the potassium concentration of the vegetated soils decreased after treatment. This finding corroborates with Ekperusi and Aigbodion²⁹, who reported an increase in the concentration of potassium after crude oil contamination but decreased after the application of treatment. Nonetheless, Ezeaku and Egbemba³⁰ opined that low pH could lead to loss of potassium due to displacement reactions in the soil colloidal complex.

The crude oil content of the polluted soils may be the reason for the lower moisture content recorded in the

polluted soil when compared to the unpolluted soil, before planting. This is in line with the report of Abosede³¹, who opined that crude oil might have negative effects on some soil physical properties such as decreased pore spaces and blockage of soils. Essien and John³² reported significantly low moisture content in polluted soil compared to unpolluted soil and thus concluded that crude oil spillage reduces soil moisture availability or holding capacity or increase moisture deficit in agricultural soils thereby damaging plant growth and yield. It has also been reported that high crude oil concentrations in soil could clog soil pores and reduce water and oxygen penetration^{33,34}. However, the increase in the moisture content of the vegetated soils over time is an indication of the reduction in crude oil content of the soils and corroborates with some reports by Osuji and Onojake³⁶ and Zhang et al.³⁷. Since crude oil can bind soil particles together such can decrease water permeability thus causing artificial flooding of the surface soil³⁵. It was however found that treatment using F. ferruginea restored the polluted soil towards normalcy, with regards to the moisture content. However, treatment using *M. alternifolius* nosedived indicating a failure in restoration.

CONCLUSION

This study concluded that calcium, magnesium, total nitrogen and potassium concentrations were retarded in the vegetated soils while a significant increase was observed in the phosphorus, sulphur and moisture content of the vegetated soils which are typical for any biodegradation process.

SIGNIFICANCE STATEMENT

This study discovered that phytoremediation of crude oil polluted agricultural soils using certain plant species could result in the reduction in some vital soil nutrients after remediation. This study will help researchers to uncover the critical areas of nutrients depletion during post-remediation.

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