Periodic Effects of Crude Oil Pollution on Some Nutrient Elements of Soils Treated Over a 90 Day Period Using Schwenkia Americana L. and Spermacoce ocymoides Burm. f.

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ABSTRACT

Crude oil contamination of the environment awfully impede soil ecosystem, through adsorption and surface assimilation of soil particles, contributing to excess carbon which might be unfeasible for use by the microbial populace, thereby bringing about constraints in soil nutrients. This study investigated the effects on some soil nutrient elements brought about by crude oil contamination. Laboratory analyses were carried out using standard methods. When compared to the values before planting, results obtained within the 90 days planting, revealed a significant decrease in the treated soils’ exchangeable calcium, exchangeable magnesium, total nitrogen, phosphorus and potassium contents whereas a significant increase was recorded in the sulfur content. This indicates a deficiency of these nutrients in soils phyto-remediated over a 90 day period, and as such imperative for such soils to be augmented with nutrients before use for agricultural and other related purposes.

Keywords: Nutrient Element, Polluted Soil, Phytoremediation, Schwenkia americana L., Spermacoce ocymoides Burm. f.

1. INTRODUCTION

Crude oil contamination often retards the yield of plants and soil productivity owing to its effect on some soil elements essential for the growth of plants. Inorganic mineral nutrients in soil are fundamental for the blossoming growth and development of plants. Abnormal levels of these essential nutrients induce deleterious effects on the plants.

1.1 Crude Oil Contamination

Crude oil extraction in Nigeria began since the 1960s and has so far resulted to more than 4000 spills, leading to the loss of about 2 million barrels of into the environment [1]. Crude oil contamination of the environment dreadfully affects the ecological unit of the soil via adsorption and surface assimilation of soil particles, causal to excess carbon which might be unfeasible for use by the microbial community thereby bringing about constraints in soil nutrients. Furthermore, it introduces into the environment non-organic compounds, carcinogens, and growth inhibiting chemicals whose prolonged exposure could be harmful to man [2]. The release of petroleum hydrocarbons into the environment is the leading and most reported form of environmental pollution [3] thus giving rise to blockage of air spaces between soil particles which may reduce aeration, hence a condition of aerobiosis is created which leads to root stress in plants and can affect soil growth parameters [4].
1.2. Soil Nutrient Elements and Functions in Plants

Oil spillage commonly retards plant growth, soil productivity [5] and nutrients availability. Inorganic mineral nutrients in soil are vital for the thriving growth and development of both vegetation and reproduction tissues, as well as enzymatic reaction, where they function as cofactors [6]. 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) [7]. Abnormal levels of these essential nutrients have thus been reported to bring about loss of chlorophyll leading to dawdling or stunted growth due to decline in cell division, reduction in protein content of seeds and the vegetative parts, early maturity which would result in a significant reduction in yield and quality, symptoms of dark to blue-green colouration on older leaves of some plants, purpling of leaves, brown spots in older leaves surrounded by chlorotic areas, chlorosis along the edges of leaves, twisting and deforming growing tips, inhibition of root elongation, interveinal chlorosis of older leaves, root growth inhibition, inhibition of protein synthesis, drastic decrease in chlorophyll content of the leaves and formation of small tiny yellow spots [6] - [8].

Nitrogen (N) is acquired from soil in two forms: either as ammonium (NH₄⁺) or nitrate (NO₃⁻) ions, with nitrate being reduced chemically to ammonium before incorporation into organic molecule. Various secondary metabolites are received from amino acids and include low relative molecular mass compounds exploited in osmoregulation (e.g. betaine) and stress responses (e.g. phytosiderophores). Nitrogen is imperative for photosynthesis. It is involved in minerals and perks up protein properties in crops and vegetables. Phosphorus (P) guarantees satisfactory plant growth. It is available in plants as orthophosphate ions (HPO₄²⁻, H₂PO₄⁻) and remains a key in energy transfer in ATP in photosynthesis and respiration. It also plays a major role in root development [6] - [8]. Potassium (K) is vital in plant water relations processes but does not form any vital organic compounds in plants unlike N and P. Nonetheless, its presence is fundamental for plant growth and enzyme activation that promotes metabolism. K is absorbed by plants as monocalcium cation (K⁺). Aside passive uptake, potassium also enters plant root through high- and low-affinity transporters [7] [9]. Calcium (Ca) is obtainable to plants as Ca²⁺. It is a foremost element of cell walls and essential in membrane formation and plasticity. It affects cell division through maintenance of membrane permeability and cell integrity. Besides the roles of stimulus-response coupling and intracellular pools activation, Ca unites with anions including organic acids, sulphates, and phosphates to act as detoxifier by neutralizing organic acids in plants [10]. Magnesium (Mg) content of soils varies between 0.05 % and 0.5 %. It enters soil via easily weatherable ferromagnesium minerals including biotite, serpentine, hornblende and olivine and other secondary clay minerals. Mg is accessible to plants as Mg²⁺, and its transport is passive impelled by ionophores in which Mg²⁺ moves down against the electrochemical gradient. Preponderance of Mg²⁺ absorbed by plants is hoarded in the vacuole and amasses to torgor generation and alters balancing of anions. Beside these roles, Mg functions as a cofactor in numerous enzymatic reactions that activates phosphorylation. It is required to stabilize ribosome particles and helps stabilize nucleic acids structure and sugar movement in plants. In plants, Sulphur (S) is available as sulphate ion, SO₄²⁻ via roots and also absorbed in the form of SO₂ through stomatal openings. Sulphur is indispensable in forming plant proteins because of its presence in certain amino acids. It is prominent in B-vitamins, biotins and thiamines, and co-enzyme A metabolism. It aids seed production, chlorophyll and nodule formation [6] [8]. This study was therefore carried out to ascertain the periodic effects of crude oil pollution on some selected core macronutrients essential for plant growth.

2. MATERIALS AND METHODS

2.1 Study Area

Ogoniland has a terrific account of pollution by crude oil usually a resultant of anthropogenic activities. It covers about 1,000 km² in south-east of Niger Delta and has a populace close to 832,000 comprising largely of the Ogoni people.
2.2 Study Design

After assessing an Ogoniland spill site, crude oil polluted agricultural soil samples were randomly collected and bulked. The unpolluted soil sample was collected from an agricultural farmland of the Department of Agricultural Science, University of Port Harcourt. These were carried out using unused and sterile plastic bags sealed with rubber bands, before being taken to the Ecological Centre of the University of Port Harcourt. Thereafter, the concentrations of some nutrient elements were assessed before the homogenized polluted and unpolluted soil samples were potted and set up in triplicate. Prior to this, some indigenous plants collected from the spill site were identified and assigned herbarium number. Mature seeds of the identified plant species were collected from wild and propagated onto a sterile unpolluted soil after ascertaining their viability. Following the germination of the seeds, the seedlings were transplanted into the experimental pots earmarked for treatment while having unvegetated polluted and unpolluted soil samples set up as controls. The changes that occurred over time on the nutrient elements of the vegetated soil samples were ascertained.

2.3 Laboratory Analyses

Reagents used for this study were of analytical grade with high purity.

The methods as described by Motsara and Roy [12] were adapted for the estimation of total nitrogen (TN), exchangeable calcium (Ca), exchangeable magnesium (Mg), available phosphorus (P), available sulphur (S) and soil pH.

Kjeldahl method was employed for total nitrogen estimation. Into a conical flask containing 1 g of homogenized soil sample was introduced K$_2$SO$_4$ (1.5 g), of CuSO$_4$ (0.7 g) and H$_2$SO$_4$ (30 mL), mixed and heated until frothing ceased. The resultant solution was boiled briskly until it became clear (sky blue colour appeared) and then digested further for 30 minutes. 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 distilling flask in a manner that the contents did not mix and trailed by heating the contents for 30 minutes to distil the ammonia. 0.1 M NaOH of the distillate was then used to titrate the excess acid in the distillate.

The Versenate method was used for calcium and calcium plus magnesium estimations. Twenty five millilitres of neutral normal ammonium acetate was introduced into conical flasks containing 5 g each of air dried and homogenized soil samples. The contents of the flasks were wobbled on a rotator mixer for 5 minutes and filtered through Whatmann No. 1 filter paper. Three crystals of versenate were introduced into each new conical flask containing 5 mL aliquots apiece for calcium estimation, and 40 mg Muroxide indicator powder for calcium estimation, and 5ml of ammonium chloride – ammonium hydroxide buffer solution and 3 drops of EBT indictor for calcium plus magnesium estimation. The solutions were titrated using 0.01 N EDTA solutions until colour changes were observed.

Standard curve was prepared for the determination of available phosphorus by dissolving 0.2195 g of pure dry KH$_2$PO$_4$ in 1000 mL of distilled water. Ten mL of this 50µg P/mL solution was diluted to 0.5 litres with deionized water, and contained 1µg P/mL (0.01mg P/mL) solution. 0, 1, 2, 4, 6 and 10 mL of 1µg P/mL 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) diluted with 20 mL of dilute SnCl₂ solution was added, shaken and composted to 25-mL mark with distilled water, then left for 10 minutes for a blue colour to develop before reading absorbance at 660 nm. The absorbance was thus plotted against “µg P. Phosphorus extraction was initiated by incorporating 50 mL of Bray’s extractant with 5 g of soil sample. The solution was wobbled briskly for 5 minutes 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 H₂O, shaken and 1 mL of the dilute SnCl₂ solution added, and the final volume made up to 25 mL with deionized water which was shaken thoroughly and left at 660 nm using blank prepared similarly but without the soil after allowing to stand for 10 minutes.

For the determination of available sulphur, 100 mL of monocalcium phosphate extracting solution (500mg P/litre) was added into conical flask containing 20 g of soil sample and the flask quivered for 60 minutes. The resultant solution was thereafter filtered using Whatman No 1 filter paper. 10 mL of clear filtrate was laid in 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 distilled water, stoppered and shaken. To 0.2 g of BaCl₂ crystal was added 0.5 mL BaSO₄ seed suspension. This was stoppered, inverted thrice and left for 10 minutes, after which a further inversion for 10 minutes, 5 minutes and another 10 minutes were carried out in tandem. The solution was left to stand for 15 minutes and 1 mL of gum acacia-acetic acid solution added. The volume was made up to 25 mL, inverted 3 times and set aside. After 1 hour, the flask was inverted 10 times and 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/litre) were laid into series of 25-mL volumetric flask to obtain 25, 50, 75, 100, 125 and 150 µg of S. The curve was prepared by plotting readings obtained from the developed turbidity against S concentration.

The pH of the soil samples was determined using a calibrated pH meter, which was ready for use when it indicated pH as per buffers used for the calibration. This was followed by measuring 10 g of soil samples into a 50-mL beaker and 20 mL distilled water added and absorbed, allowed for 30 minutes and stirred. The pH of the soil samples were read afterwards.

2.4 Statistical Analysis

Results of all the studies are expressed as mean ± standard error of triplicate determination. To detect a significant difference between the groups, statistical analysis was carried out using one way analysis of variance (ANOVA). 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 periods were analyzed using Student t-test.

3. RESULTS AND DISCUSSION

The six mineral elements, N, Ca, Mg, S, P and K are requisite in great quantities and consequently regarded as macronutrients [13].

The observed levels of Ca²⁺ and Mg²⁺ in the polluted soils compared to the unpolluted soil before planting (BP) may be due to the crude oil contamination of the soil. While the observed decrease in Ca²⁺ and Mg²⁺ 90 days after planting (DAP) may be an indication of their utilization by the treatment plants for optimal growth, it may, however, result from low pH observed at this period. The latter argument conforms with Ngobiri et al. [14] who associated low pH with loss of exchangeable bases due to displacement reactions in the soil colloidal complex and excess water that could lead to eluviation and leaching loses.

Elemental sulphur is unavailable to the plant for uptake and must be converted by thiobacillus bacteria to sulphate (SO₄²⁻), the accessible form of sulphur for plants, which is an acid-producing process requiring water and oxygen. Oxidation is more rapid in warm, moist soil with high organic matter contents. Oxidation reactions of elemental sulphur proceed slower in acid soils than in alkaline soils, and amid the factors affecting oxidation rate, pH and temperature have the utmost effect [15]. These factors governing the optimum availability of sulphur in the soil may be the reason for the obtained sulphur content values in this study.

The lower total nitrogen content of the polluted soils, when compared with unpolluted soil, confirms the earlier report [15] that indicated low value of nitrogen reserve in petroleum hydrocarbon contaminated soil. This also corroborates Agbogidi et al. [17] who reported that crude oil pollution leads to reduction of total nitrogen. The decrease in total nitrogen over time in all the groups may be due to the utilization of nitrogen by soil microorganisms and treatment plants without fixing it back. This may indicate the absence of nitrogen fixing bacteria which may have resulted from either the type of plants used for remediation since nitrogen fixing bacteria thrive more in legumes or the pH of the soil [18]. If the latter is true, it may be appropriate to state that at pH near neutral (pH 7), the microbial (if present) conversion of NH₄⁺ to nitrate (nitrification) by N fixing bacteria is rapid, and crops generally take up nitrate, while at soil pH < 6, nitrification slows down since N fixing bacteria, such as Rhizobium, declines with increase in acidity, and only plants with the capability to absorb NH₄⁺ may have an advantage. This conforms to earlier reports.
which recognized that nitrogen fixation may be restricted by soil acidity even in the absence of toxic amounts of Al and Mn.

Phosphorus content, BP, was observed to be higher in polluted soils compared to the unpolluted soil. According to Akubugwo et al. [22], crude oil contamination could lead to elevated soil available phosphorus. P was thereafter found to decrease with time in all the groups. This decrease may be associated with its uptake by plants. Even so, P is absorbed by plants in the orthophosphate forms generally as H$_2$PO$_4^-$ or HPO$_4^{2-}$ and the amount of these ions in the soil solution is determined by soil pH. According to the USDA [23], soils with intrinsic pH values of 6-7.5 are idyllic for phosphorus availability, while pH values <5.5 and between 7.5 and 8.5 limits phosphorus availability. However, the inability of the treatment plants to fix phosphorus may also be a limiting factor for the decrease in the remediated soils; hence they utilized the available phosphorus in competition with microorganisms in the rhizosphere for their growth and metabolic activities, thus lessening the soil available phosphorus. A similar finding was reported by Tanee and Kinako [18]. They associated such 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 phosphorus.

Similar to the data obtained from this study, Ekperusi and Aigbodion [24] previously reported an increase in the concentration of potassium (K) after crude oil contamination but decreased after treatment. Likewise, the decrease in K 90 DAP may be due to its utilization by plants and soil microorganisms. However, it has been reported [25] that low pH could lead to loss of potassium due to displacement reactions in the soil colloidal complex, suggesting dislodgment reaction in the cation exchangeable Al$^{3+}$.

Soil pH is a vital index that controls several physicochemical reactions. It is possible that the presence of residual hydrocarbon spills hindered the leaching of basic salts which were responsible for the raised pH recorded in polluted control soil BP. The binding of the oil with soil particulate matter probably became the major resistance to the removal of the basic ions, thereby corresponding with Onuh et al. [26] who reported an increase in pH with increased crude oil contamination. Nonetheless, the pH of all the groups across the 90 days remediation period remained within the optimal pH for maximal plant function of 6.0-7.5 [27] and DPR [28] accepted range of 5.5 to 6.5. Albeit microbial secretions can lead to acidification of the soil, however, excess watering can influence the rate of soil acidification depending on the rate of percolation of water through soil and likewise, organic matter decay to form carbonic acid and other weak organic acids. These effects of these factors may have contributed relatively to the soil acidity [29].

![Figure 3.1. Total nitrogen content of S. americana and S. ocymoides treated soils](image-url)
Figure 3.2. Exchangeable calcium content of *S. americana* and *S. ocymoides* treated soils

Figure 3.3. Exchangeable magnesium content of *S. americana* and *S. ocymoides* treated soils

Figure 3.4. Sulphur concentration of *S. americana* and *S. ocymoides* treated soils
4. CONCLUSION

The awful effect of crude oil contamination brings about constraints in soil nutrients. The soils treated for a 90 day period appeared to have decreased soil essential nutrients. This, therefore, entails that plant-treated polluted soils require nutrient augmentation before being used for farming and other related purposes.
5. REFERENCES

[1] C. C. Chukwuma, J. C. Ikewuchi, C. Ekeke, and M. O. Monanu, Phytoremediation of Crude Oil Polluted Agricultural Soil Using Schwenkia americana L. and Spermacoceocymoides Burm. f., *International Journal of Biochemistry Research & Review*, vol. 23, no 4, pp. 1-12, 2018.

[2] C. C. Chukwuma, M. O. Monanu, J. C. Ikewuchi, and C. Ekeke, Variance in Protease, Dehydrogenase, Phosphatase and Respiratory Activities during Phytoremediation of Crude Oil Polluted Agricultural Soil Using Schwenkia americana L. and Spermacoceocymoides Burm. f. *Annual Research & Review in Biology*, vol. 28, no 6, pp. 1-9, 2018.

[3] K. Singh, and S. Chandra, Treatment of petroleum hydrocarbon polluted environment through bioremediation: A review. *Pakistan Journal of Biological Sciences*, vol. 19, no 1, pp. 1-8, 2014.

[4] W. M. Shukry, G. H. S. Al-Hawas, R. M. S. Al-Moaikal, and M. A. El-Bendary, Effect of petroleum crude oil on mineral nutrient elements, soil properties and bacterial biomass of the rhizosphere of jojoba. *British journal of environment and climate change*, vol. 3, no 1, p. 103, 2013.

[5] M. A. Ekpo and I. L. Nwaankpa, Effect of crude oil on microorganisms and growth of ginger (*Zingiber officinale*) in the tropics. *J. Sustainable Trop Agric Res.*, vol. 16, pp. 67-71, 2005.

[6] J. A. Silva and R. Uchida, Essential Nutrients for plant Growth: Nutrient functions and deficiency symptoms. *Plant nutrient management in Hawaii’s Soils Approaches for Tropical and Subtropical Agriculture*. Chpt 3. pp. 31-55, 2000.

[7] M. A. Grusak, Plant Macro and Micronutrient Minerals, *Encyclopedia of Life Sciences*, Natural Publishing group, 2001.

[8] R. Pandey, Mineral Nutrition of Plant, *Plant Biology and Biotechnology*, New Delhi: Springer, 2015, pp. 499-538.

[9] M. Gierth and P. Mäser, "Potassium Transporters In Plants," *M. Giertha, and P. Maser, Potassium Transporters In Plants, Ulf-Ingo (Ed.),* 2007.

[10] D. S. Bush, "Calcium regulation in plant cells and its role in signaling." *Annual review of plant biology*, vol. 46, no 1, pp. 95-122, 1995.

[11] K. W. Nkpaa, M. O. Wegwu and E. B. Essien, Heavy metals concentration in four selected seafood from crude oil polluted waters of Ogoniland, Rivers State, Nigeria, *Archives of Applied Science Research*, vol. 5, no. 4, pp. 97-104, 2013.

[12] M. R. Motsara and R. N. Roy, *Guide to laboratory establishment for plant nutrient analysis*, Rome: Food and Agriculture Organization of the United Nations, 2008, vol. 19.

[13] P. J. White and P. H. Brown, Plant nutrition for sustainable development and global health. *Annals of botany*, vol. 105, no 7, pp. 1073-1080, 2010.

[14] C. N. Ngobiri, A. A. Ayuk and I. I. Awunuso, Differential degradation of hydrocarbon fractions during bioremediation of crude oil polluted sites in Niger delta area. *J. Chem. Soc. Nig.*, vol. 32, pp. 151-158, 2007

[15] J. Eriksen, M. D. Murphy and E. Schnug, The soil sulphur cycle. In *Sulphur in agroecosystems*, Dordrecht: Springer, pp. 39-73, 1998.

[16] M. Lehtomaker and S. Niemela, Improving microbial degradation of oil in soil. *Am. Boil.*, vol. 4, pp. 126-129, 1975
[17] O. M. Agbogidi, P. G. Eruofor, S. O. Akparobi, S. O. and G. U. Nnaji, Evaluation of Crude Oil Contaminated Soil on the Mineral Nutrient Elements of Maize (Zea mays L.) *Journal of Agronomy*, vol. 6, no 1, pp. 188-193, 2007

[18] F. B. G. Tanee, and P. D. S. Kinako, Comparative studies of biostimulation and phytoremediation in the mitigation of crude oil toxicity in tropical soil. *J. Applied Sci. Environ. Manag.*, vol. 12, pp. 143-147, 2008.

[19] W. A. Rice, Effect of CaC0₃ and inoculums levels on nodulation and growth of alfalfa in an acid soil. *Canadian Journal of Soil Science*, vol. 55, pp. 245-250. 1975.

[20] W. A. Rice, D. C. Panney and M. Nyborg, Effects of acidity on Rhizobia numbers, nodulation and nitrogen fixation by Alfalfa and Red Clover. *Canadian Journal of Soil Science*, vol. 57, pp. 197-203, 1977.

[21] D. C. Panney, M. Nyborg, P. B. Hoyt, W. A. Rice, B. Siemens and D. H. Laverty, An assessment of the soil acidity problem in Alberta and northeastern British Columbia. *Canadian Journal of Soil Science*, vol. 57, pp. 157-164, 1977.

[22] E. I. Akubugwo, G. C. Ogbuji, C. G. Chinyere and E. A. Ugbogu, Physicochemical properties and enzymes activity studies in a refined oil contaminated soil in Isiukwuato, Abia State, Nigeria. *Biokemistri*, vol. 21, no. 2, 2009.

[23] United States Department of Agriculture- Natural Resources (USDA-NRCS), Soil Phosphorus. *Soil Quality Kit-Guides for Educators*. [http://www.nrcs.usda.gov/internetingFSE/pdf](http://www.nrcs.usda.gov/internetingFSE/pdf).

[24] O. A. Ekperusi and F. I. Aigbodion, Bioremediation of petroleum hydrocarbons from crude oil-contaminated soil with the earthworm: *Hyperiodrilus africanus*. *Biotechnology*, vol. 5, no. 6, pp. 957-965, 2015.

[25] P. I. Ezekaku and B. O. Egbemba, Yield of Maize (Manoma spp) affected by automobile oil waste and compost manure. *Academic Journals*, vol. 13, pp. 1250-1256, 2014

[26] M. O Onuh, D. K. Madukwe and G. U. Ohia, Effects of poultry manure and cow dung on the physical and chemical properties of crude oil polluted soil. *Sci.World J.*, vol. 3, no 2, pp. 45-50, 2008.

[27] J. L Smith and J. W. Doran, Measurement and use of pH and electrical conductivity for soil quality analysis., in *Methods for assessing soil quality*. Soil Science Society of America Madison, WI, 1996, vol. 49, pp. 169-182.

[28] Department of Petroleum Resources (DPR), Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (EGASPIN), Ministry of Petroleum and Natural Resources, Abuja, Nigeria, p. 314, 2002.

[29] B. F. Carver and J. D. Ownby, Acid Soil Tolerance in Wheat. *Advances in Agronomy*, Academic Press, 1995, vol. 54, pp. 117-173.