"Effect of Giant Rat's Tail Grass (*Sporobolus pyramidalis p.beauv*) on Total Petroleum Hydrocarbon (TPH) and Heavy Metals content of Crude Oil Polluted Soils"

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ABSTRACT: Soil contamination by crude oil has been an issue within the oil producing areas of the Niger Delta Nigeria and so, many remediation methods; including phytoremediation, the use of plants, have been adopted for the remediation of the affected soils. Such plants are expected to be safe and effective in the clean-up of hydrocarbons and heavy metals from oil polluted sites. Giant rat's tail grass (*Sporobolus pyramidalis p.beauv*) is among the plants being proposed. This study therefore investigated in eight-weeks, the effects of giant rat's tail grass (*Sporobolus pyramidalis p.beauv*) on the total petroleum hydrocarbon (TPH) and heavy metals content of contaminated soils. Residual TPH and heavy metals (chromium, Cr and lead, Pb) were quantified as an index for assessing the post-phytoremediated crude oil contaminated soils. Crude oil pollution levels of 25ml/kg, 50ml/kg and 100ml/kg on soils were respectively done alongside the unpolluted control. Remediation treatments of the test soils and the control with the plant were done after two weeks of pollution using three young seedlings of giant rat's tail grass. Results from eight weeks remediation period showed that TPH (mg/kg) reduced from 496.55 to 257.90, 578.09 to 241.37, 602.61 to 198.80 and 21.27 to 6.22 on the test soils and the control respectively; Cr (mg/kg) from 5.86 to 1.23, 7.96 to 1.38, 9.76 to 1.65 and 4.26 to 0.63 on the test soils and the control respectively; and Pb (mg/kg) from 4.25 to 1.21, 5.26 to 2.31, 5.12 to 3.93 and 1.96 to 0.43 on the test soils and the control respectively. Soil analysis results from the study indicated that giant rat's tail grass (*Sporobolus pyramidalis p.beauv*) has the potential to ameliorate crude oil toxicity at different crude oil contamination levels because of its ability to significantly ($p > 0.05$) decrease the TPH and heavy metals content of the soil. © JASEM

Keywords: Total Petroleum Hydrocarbon, Heavy Metals, Phytoremediation, giant rat's tail, crude oil contamination

The Niger-Delta region of Nigeria face serious soil contamination problem. As a result of human economic activity, large quantities of soil have been contaminated with petroleum products (Kaimi*et al*., 2006). Petroleum contaminated soil causes pollution of local ground water by organics, threatens the safety of potable water, limits the use of ground water, causes enormous economic loss and ecological disaster, and destroys agricultural production (Wang et al., 2007; Xu*et a1*., 2006). Trace metals in crude oils and other bituminous substances have been recognized worldwide (Nwadinigwe and Nworgu, 1999, Nduka*et al*., 2006). The presence of heavy metals in some environments has therefore been attributed to petroleum prospecting and mining as well as oil spills (Osuji and Onojake, 2004). It is estimated that more than four thousand incidents of crude oil spills have occurred in the Niger Delta region of Nigeria since 1960, releasing several million barrels of crude oil (sometimes containing heavy metals) into the surrounding areas (Nduka *et al*., 2006). The list of elements commonly considered as pollutants is rather short and includes Al, As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Zn and some radionuclides (Shtangeeva, 2006).

Phytoremediation is regarded as a cost-effective method for removal of petroleum from soil and has a great potential in remediation of soil contaminated with petroleum (Joner*et al*., 2002; Ryan et al., 2001).

In addition, this method is not destructive and could remedy the soil structure and recover the biological environment (Maria et al., 2002). Phytoremediation of polluted soil has been applied successfully. Plants enhance the remediation of petroleum-containing soils by various processes, including elimination, destruction or sequestering hazardous substances from the environment (Maila and Cloete, 2002; Vervaeke*et al*., 2003). Phytoremediation field trials showed that the reduction of petroleum hydrocarbons in the rhizosphere was accelerated (Chen *et al*., 2003; Glick, 2003). Yateem*et al*, (2006), investigated the degradation of total petroleum hydrocarbon (TPH) in rhizosphere and non-rhizosphere soil of three domestic plants: Alfalfa (*Medicagosativa*), Broad bean (*Viciasativa*) and Ryegrass (*Loliumperente*). The three plants exhibited normal growth in the presence of 1% TPH; the degradation was more profound in the case of leguminous plant. They reported that the soil cultivated with broad bean and alfalfa has 36.6 and 35.8% respectively, compared with 24% degradation in the case of ryegrass. The key issue for successful phytoremediation was the application of plant species that have the ability to proliferate in highly contaminated soil.

The aim of the present study was to investigate the effects of giant rat's tail (*Sporobolus pyramidalis p.beauv*) on the total petroleum hydrocarbon (TPH) and heavy metals content of polluted soils. Sandy

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loam soils were obtained from the University of Port Harcourt Port Harcourt Nigeria Botanic garden and polluted with crude oil from Nigerian Agip Oil Company (NAOC). The change in TPH, Cr and Pb contents were checked before and after the experiment. The possible factors for these changes are discussed in this paper. The influence on the degradation of hydrocarbons in petroleum polluted soil is presented, providing a theoretical and technical support for phytoremediation of crude oil polluted soil.

MATERIALS AND METHODS

Soil for experiment: Sandy loam soil was collected from the University of Port Harcourt, Port Harcourt Nigeria Botanic Garden, from 0-15cm soil layers and mixed thoroughly to a depth of 15cm. About 5kg of the soil were placed in 20 – cylindrical bucket. The buckets were perforated at the sides and bottom to avoid water logging and also to increase soil aeration. The buckets were arranged in four (4) rows designated as A1, A2, A3 and C of five replicates each.

Soil pollution for experiment: Crude oil obtained from Nigeria Agip Oil Company (NAOC), Omoku, Rivers Nigeria was used as pollutant. Crude oil was added to the 5kg of soil to achieve the following crude oil-contamination levels: 125ml of crude oil for 25ml/kg (A1), 250ml of crude oil for 50ml/kg (A2) and 500ml of crude oil for 100ml/kg (A3). Sample C was unpolluted and served as the control.

Plant for experiment:

Fig1*: Giant rat's tail (Sporobolus pyramidalisp.beauv)*

Young seedlings of giant rat's tail (*Sporobolus pyramidalisp.beauv)* were removed from their growing medium at University of Port Harcourt Port Harcourt Nigeria Botanical Garden and placed in each of the buckets.

Experimental procedures: The crude oil polluted soil was mixed thoroughly by hand in the various buckets. Each bucket contained 5kg soil. At the beginning of the experiment, the soil in the bucket was irrigated until it was saturated. The experiment was conducted over an 8 weeks period. Three young seedlings were transplanted to each bucket, from their growth medium at the University of Port Harcourt. During the experiment period, the irrigation frequency and volume of irrigation water were controlled to prevent crude oil in the soil from washing out. Sample A1, A2, A3 and C were all planted with equal numbers of giant rat's tail (S*porobolus pyramidalis p.beauv).*

Chemical analysis: This chemical process was based on the principle that the analysis of total nutrients requires complete oxidation of organic matter. Digestion can alternatively be achieved through the use of alkaline (instead of acid) or through dry ashing of the sample.

Procedure**:** Ground soil samples of 0.3 ± 0.001g each of oven dried (70ºC) was weighed into a labelled dry and clean digestion tube. A 4.4 ml aliquot of the digestion mixture was added to each tube and also to two reagent blanks. Digestion was allowed to proceed for 2 hours at 360 ºC. The resulting colourless solution was allowed to cool. Twenty five ml distilled water was added to each tube and the solutions were mixed until no more sediment dissolution was observed. The mixtures were allowed to stand for some minutes after which the clear top liquid from the top of the tube was taken out for metal determination (Okalebo*et al,* 1993).

Determination of heavy metal concentrations: Metals were determined for the soil samples which had undergone the earlier described digestion process. The heavy metal test tablet was added to each corresponding digestion extract. The analytes ions in the solutions were determined with AAS where the absorbance were read off with UNICAM 939AA Spectrometer, and their various concentrations in the samples were extrapolated from the standard graphs plotted with concentrations against absorbance. Computations were then done with the various digestion factors.

Determination of Total Petroleum Hydrocarbon: Two gram of soil sample was weighed into a clean extraction container. Ten ml of extraction solvent (dichloromethane, DCM) was added into the sample, mixed thoroughly and allowed to settle. The mixture was carefully filtered into a clean solvent rinsed extraction bottle, using filter paper. The extracts were concentrated to 2ml and then transferred for clean-up/separation.

Moderately packed glass wool (1cm) was placed at the bottom of 10mm ID X 250mm loup chromatographic column. Slurry of 2g of activated silica gel in 10ml methylene chloride was prepared and placed in the chromatographic column. To the top of the column was added 0.5cm of sodium sulphate. The column was rinsed with additional 10ml of methylene chloride. The column was pre-eluted with 20ml of dichloromethane which flowed through the column at a rate of about 2 minutes until

the liquid in the column was just above the sulphate layer. Immediately 1ml of the extracted sample was transferred into the column. The extraction bottle was rinsed with 1ml of dichloromethane and added to the column as well.

The stop-cork of the column was opened and the eluent was collected with graduated cylinder. Just prior to exposure of the sodium sulphate layer to air, dichloromethane was added to the column in 1-2ml increments. Accurately measured volume of 8-10ml of the eluent was collected and was labelled aliphatics.

The concentrated aliphatic fractions were transferred into labelled glass vials with leflons rubber crimp caps for G C analysis.

One micro litre of the concentrated sample was injected by means of hypodermic syringe through a rubber septum into the column. Separation occurs as the vapour constituent partition between the gas and liquid phases. The Sample was automatically detected as it emerges from the column (at a constant flow rate) by the FID detector whose response is dependent upon the composition of the vapour.

Soil was collected for analysis at 0 and 60 days. The sample soil was taken from a depth of 6cm under ground near root, according to the plum blossom method (He, 2001). Soil samples were analyzed for TPH, Chromium, and Lead content.

RESULTS AND DISCUSSION

Degradation of total petroleum hydrocarbons*: In the plant rhizosphere, the soil environment was improved, so that the growth and activity of microorganisms were promoted. Simultaneously, with the growth of the plant roots, the soil became loose. And the oxygen needed in oxidation of pollutant was transported into deeper levels of soil along the root. In addition, the plants root itself secreted organic compounds and enzymes. All of these factors were favourable to the degradation of petroleum hydrocarbons.

Result of an investigation by Reilley*et al,* (1996) indicates that grasses and legumes enhanced the removal of PAHs from contaminated soils. The plant (investigated independently) included the legume alfalfa and three grasses: tall fescue, Sudan grass and switch grass. Pyrene and anthrecene were used as PAH contaminants. Planted soils had significantly lower concentration of PAHs than the unplanted soils, with 30 to 40% more degradation in the planted soils and this, as been noted could be due to enhanced rhizosphere effect.

Furthermore, in a six-month laboratory study, Pradhan *et al*, (1998) have identified that alfalfa, switch grass, and little bluestem were each capable of reducing the concentration of PAHs in soil contaminated at a manufactured gas plant. The initial soil concentration of total PAHs for the three plant treatments and an unplanted control was 184.50±14.00mg total PAHs per kg of soil. After six months, the concentration in unplanted control soil was 135.90±25.50 mgkg^{-1}, while the concentrations in planted treatment were (switch grass = 79.50±3.70mgkg^{-1}, alfalfa = 80.20±8.90mgkg^{-1}, little bluestem = 97.10 ± 18.70 mgkg^{-1}). It was concluded that the degradation of total petroleum hydrocarbons in the planted soil was stimulated by plant roots. Fig 2 illustrated that. Moreover, the degradation of petroleum hydrocarbons in different plant rhizosphere environments was mainly related to the diversity of plant roots and their secretion, i.e. the enzymes. The reason why the degradation rate of petroleum hydrocarbons was enhanced in the rhizosphere was that the secretion of plant roots promoted microbial populations and their activity. The increased microbial population induced by the rhizosphere could improve the degradation of petroleum hydrocarbons. So this relationship between plant and microbes was favourable to degradation of petroleum hydrocarbons in the planted soil.

*Reduction of Chromium***:** Current conventional methods to remediate heavy metal-contaminated soil and water, such as *ex situ* excavation, landfill of the top contaminated soils (Zhou and Song, 2004), detoxification (Ghosh and Singh, 2005), and physico-chemical remediation, are expensive (Danh et al., 2009), time consuming, labour exhaustive and increase the mobilization of contaminants, and destroy the biotic and structure of the soil. Therefore, these remediation techniques are not technically or financially suitable for large contaminated areas (Baccio et al., 2003). Bioremediation was developed as a technology to degrade pollutants into a low toxic level by using microorganisms. However, the use of this technology to remediate contaminated areas by applying living organism was less successful for extensive metal and organic pollutants. Plants are able to metabolize substances produced in natural ecosystems (Vidali, 2001). Phytoremediation is an approach in which plants are applied to detoxify contaminated areas (Garbisu and Alkorta, 2001; Mangkoedihardjo and Surahmaida, 2008).

The post pollution and post phytoremediation mean concentrations of the heavy metal (Cr) in the various samples were further illustrated in fig 3 using a bar chart. From the results sample

Degradation of Lead: The post pollution and post phytoremediation mean concentrations of the heavy metal (Pb) in the various samples were further illustrated in fig 4 using the bar chart.

Results obtained revealed that pollution with crude oil increased the heavy metal concentration of the soil. Chromium and lead in polluted soil samples were significantly (p>0.05) higher than in unpolluted (control) samples. The mean concentrations of the heavy metals increased as the level of pollution increased. The results also showed that *Sporobolus pyramidalis* was effective in reducing the concentrations of the heavy metals. These trends were also observed in past works. Bada and Olarinre (2012) studied the characteristics of soils and heavy metal content of vegetation in oil spill impacted land in Nigeria. Higher heavy metals contents were observed in the soil very close to the source of pollution and this decreased with soil depth. The higher metal content might be due to proximity to the source of pollution. The soils heavy metal concentrations ranged from 0.10 - 0.18 mg Cd/kg, 0.34 - 0.46 mg Zn/kg and 0.28 - 0.44 mg Pb/kg. Nigerian crude oil contains heavy metals which are not completely removed during refining processes (Nwachukwu*et al.*, 1995).
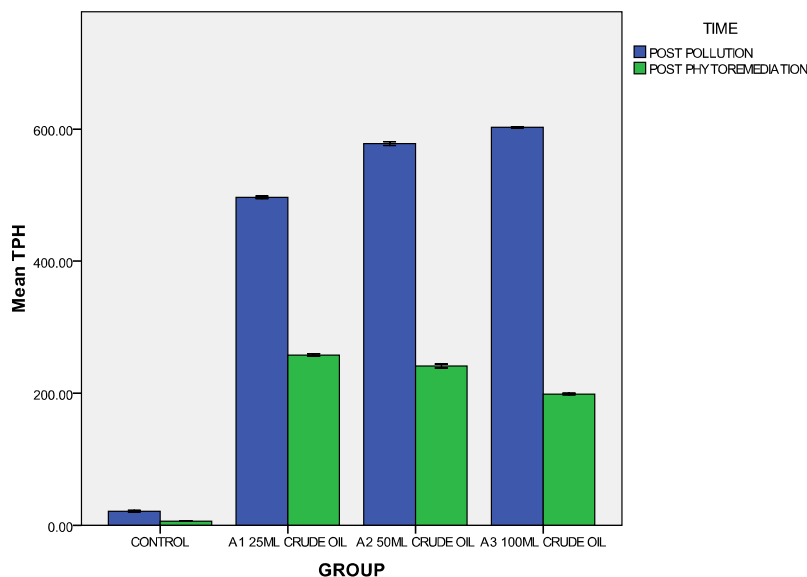

Fig 2: Illustrates the post pollution and post phytoremediation mean total petroleum hydrocarbons concentration of triplicate determinations.
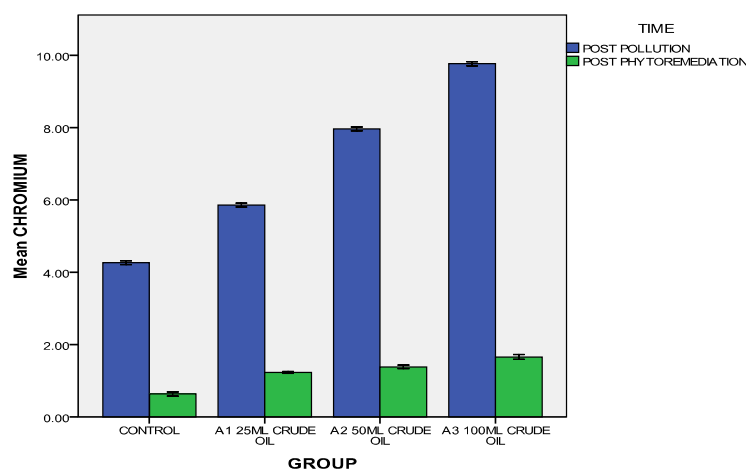

Fig 3: illustrates the post pollution and post phytoremediation mean Crconcentration for triplicate determinations of various samples.
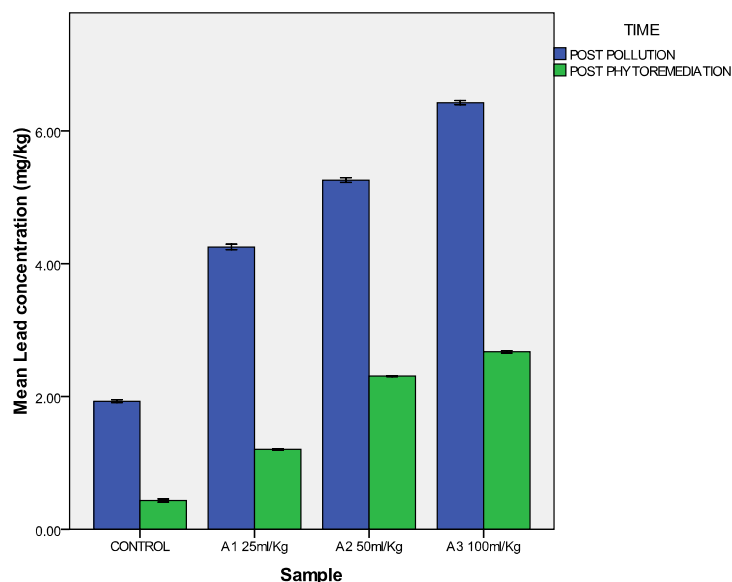
Fig 4: Illustrates the post pollution and post phytoremediation mean Pbconcentration for triplicate determinations of various samples.

REFERENCES

Bada BS, Olarinre TA. (2012). "Characteristics of Soils and Heavy Metal Content of Vegetation in Oil Spill Impacted Land in Nigeria,"*Proceedings of the Annual International Conference on Soils, Sediments, Water and Energy*: 17, Article 2.

Baccio D, Tognetti R, Sebastiani L, VitaglianoC. (2003). Responses of *PopulusdeltoidesPopulusnigra*(*Populuseuramericana*) clone I-214 to high zinc concentrations. *New Phytologist*159(2), 443-452.

Chen YC, Katherine BM, Paul SA. (2003). Pyrene degradation in the rhizosphere of Tall Fescue (*Festucaarundinacea*) and Switchgrass (*Panicumvirgatum L*). *Environmental Science and Technology*37(24), 5778-5782

Danh LT, Truong P, Mammucari R, Tran T, Foster N. (2009). Vétiver grass, *Vetiveriazizanioides*: A choice plant for phytoremediation of heavy metals and organic wastes. *International Journal of Phytoremediation*11(8), 664-691.

Garbisu C, Alkorta I. (2001).Phytoextraction: A cost-effective plant-based technology for the removal of metals from the environment. *Bioresources Technology*77(3), 229-236.

GhoshM, Singh SP.(2005). A review on phytoremediation of heavy metals and utilization of it's by products. *Applied Ecology and Environmental Resources*3(1), 1-18.

Glick BR. (2003). Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnology Advances*21(5), 383-393

He SY. (2001). The analysis and monitor of environment pollutant. 77- 80. Beijing: Chemica1 Industry Press (in Chinese).

Joner EJ, Corgie SC, Amella N. (2002). Nutritiona1 contributions to degradation of polycyclic aromatic hydrocarbons in a simulated rhizosphere.*Soil Biology and Biochemistry*34, 859-864

Kaimi E, Mukaidani T, Miyoshi S.(2006). Ryegrass enhancement of biodegradation in diesel-contaminated soil.*Environmental and Experimental Botany*55(1-2), 110-119

Mangkoedihardjo S, Surahmaida A. (2008). *Jatrophacurcas*L. for phytoremediation of lead and cadmium polluted soil. *World Applied Science Journal*4(4), 519-522.

Maila MP, Cloete TE. 2002. Germination of Lepidiumsativum as a method to evaluate polycyclic aromatic hvdrocarbons (PAHs) removal from contaminated soil. *1nternational Biodeterioration and Biodegradation*50(2), 107-113

Maria T, Thomas GR, Angela S. (2002). Bacterial rhizosphere populations of black poplar and herbal plants to be used for phytoremediation of diesel fuel.*Soil Biology and Biochemistry*34(12), 1883-l892

Nduka JKC, Constance E, Obiakor E. (2006). Selective bioaccumulation of metals by different parts of some fish species from crude oil polluted water. *Bulletin of Environmental Contamination and Toxicology*77, 846-553

Nwachukwu JJ, Oluwole AF, Asubiojo OI, Filby RH, Grimm CA, Fitzgerald S. (1995). A Geochemical evaluation of Niger Delta crude oils.In Oti, M.N. and Postma, G. (Eds.), *Geology of Deltas.*Brookfield VT, USA, 287-300.

Nwadinigwe CA, Nworgu ON. (1999). Metal contaminants in some Nigerian well head crudes comparative analysis. *Journal of Chemical Society Nigeria*24,118-121.

Osuji LC, Onajake CM.(2004). Trace heavy metals associated with crude oil: A case study of Ebocha-8 Oil-spill-pollluted site in Niger Delta, Nigeria. *Chemistry Biodiversity*1, 1708-1715

Ryan KM, Mary K, Friestone M. (2001). Enhanced Phenanthrene biodegradation in soil by slender oat root exudates and root debris. Environ Oual. 30, 19ll-1918

Shtangeeva I. (2006). Phytoremediation of Trace Element contaminated soil with cereal crops: Role of Fertilizers and Bacteria on Bioavailability In: Trace Elements in the Environment. Biogeochemistry, Biotechnology and Bioremediation, Prasad, M.N.V., K.S. Sajwan and R. Naidu (Eds). CRC Press, Taylor and Francis Group, Boca Raton, pp: 549-581

Vervaeke LS, Mertens J. (2003). Phytoremediation prospects of willow stands on contaminated sediment: A field trial. *Environmental Pollution*126(2), 275-282.

Vidali M. 2001. Bioremediation.An overview.*Pure and Applied Chemistry*73(7), 1163-1172.

Wang J, Xu HK, Gup SH.(2007). Isolation and characteristics of a microbia1 consortium for effectively degrading phenanthrene.*Petroleum Science*4(3), 68-75

Xu S, Chen YX, Wu WX. (2006). Enhanced dissipation of phenathrene and pyrene in spiked soils by combined plants cultivation. *Science of Total Environment* 363, 206-215

Yateem A, Balba MT, El-Nawanwy AS, Al-Awadhi N.(2006). Plants-associated microflora and the remediation of oil contaminated soil. International *journal phytoremediation* 2, 183-191.

Zhou QX, Song YF. (2004). Principles and methods of contaminated soil remediation: Science Press, Beijing p.568.