Comparative Studies of Biostimulation and Phytoremediation in the Mitigation of Crude Oil Toxicity in Tropical Soil

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ABSTRACT: Comparative studies of biostimulation using local materials and phytoremediation in the mitigation of crude toxicity on tropical soil of the Niger-Delta of Nigeria were carried out. 5% pollution level of crude oil were done alongside the control. Remediation treatments were done after one week of pollution using the following materials: Batch A : 20g of NPK 15:15:15; batch B: 50g of poultry dung; batch C: 5 seeds of *Vigna unguiculata* grown on the polluted soil (phytoremediation); batch D: control (pollution but no remediation); and batch E: double control (no pollution and no remediation treatment). Soil analyses result indicated that the different remediation treatments have potentials to ameliorate crude oil toxicity at different degrees because of their ability to increase the nutrient content and decrease the total hydrocarbon content of the soil. There was no statistical difference in the soil pH, Zinc and Copper contents between and within treatments throughout the study period. Highest crude oil biodegradation and improvement in nutrient content of the soil were observed in NPK 15:15:15 and phytoremediation treatments indicating that these are good remedial treatment options in the mitigation of crude oil toxicity. @ JASEM

The Niger Delta ecoregion of Nigeria has been associated with frequent oil spills resulting from oil pipeline vandalization, tanker accidents and accidental rupture of oil pipelines. These mishaps result in the release of crude oil and refined petroleum products into the terrestrial and aquatic environments (Okpokwasili and Amanchukwu, 1988). The intensity of oil damage depends upon a number of abiotic and biotic factors including the season of the spill, type and amount of oil, prevailing weather condition and soil compositions (Alexander & Webb, 1987; Pezeshki *et al.,* 2000). Despite more stringent environmental regulations, the risk of an oil spill affecting these ecosystems is still high and which we must accept as inevitable (Kinako, 1988; Venosa and Zhu, 2002). For possible elimination of these effects, it is imperative to clean up these pollutants from the environment   by applying remedial measures (Ellis *et al*., 1990).

Bioremediation has emerged as a highly promising secondary treatment option for oil removal since it first application after the 1989 Exxon Valdez spill (Bragg *et al*., 1994). Several methods are made to crude oil bioremediation. This include biostimulation in which the growth of indigenous oil degraders are stimulated by the addition of nutrients or other growth limiting substrates; Bioaugmentation, in which known oil degrading bacterial are added to supplement the existing microbial population; and phytoremediation, in which the contaminant is degraded by the growth of higher plants. This research attempts to compare the use of biostimulation and phytoremediation using local materials in the mitigation of crude oil toxicity in

soil. Such will enable one to identify the best option in the treatment of crude oil contaminant.

MATERIALS AND METHODS

The experiment was carried out at the University of Port Harcourt Botanic Garden located about 26km North- west of the city of Port Harcourt along the East-west road between Latitude 4.00^{o}N and 5.00^{o}N and longitude 6.30 and 7.30^{o}E in the tropical rainforest belt of Southern Nigeria. The soil in the area is always low in nutrient content due to the leaching of the nutrient down the earth profile by rainfalls which are always very heavy in the area (NLNG, 1995). Surface soil (loamy- clay) was collected from the garden and thoroughly mixed. It was then filled into 35 black polythene bags of diameter 25cm and height 17cm leaving a space of 6cm from the top to give room for the addition of crude oil, remediation materials and water. The sides and bases of the bags were perforated to increase soil aeration and to avoid water logging. The bags were then arranged into 5 batches of 7 replicates each designated as A, B, C, D and E. Batches A – D were polluted with crude oil obtained from Nigeria National Petroleum Corporation, Port Harcourt while D was not polluted. The crude oil was thoroughly mixed with the soil in the bags. One week after pollution treatment, the different remediation material/ treatments were applied. Batch A was remediated with 20g/bag of NPK 15:15:15 (inorganic fertilizer); B was remediated with 50g/bag of poultry dung (organic fertilizer); C was phytoremediated with *Vigna unguiculata*; D received no remediated treatment (control) while E acted as the double control with no pollution   and no remediation

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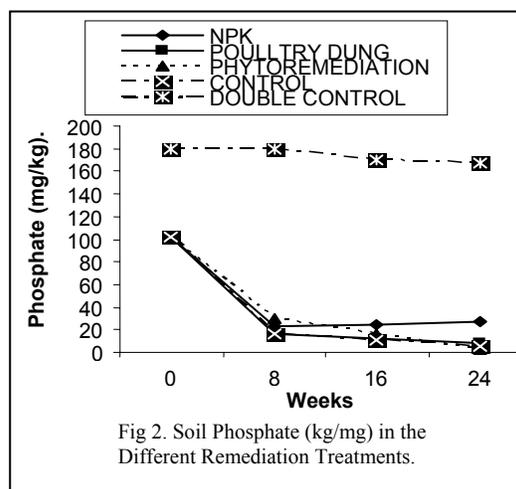treatment. The remediation treatment were repeated after 8 weeks.

The NPK 15:15:15 was obtained from Agricultural Development Programme, Port Harcourt; the poultry dung from a poultry farm at Bori while the *Vigna unguiculata* was bought from Mile 1 market, Port Harcourt. Weeding was done on the plots when the need arose. Composite soil samples were taken from batch for soil analyses. Soil sampling started one week after crude oil pollution. This was taken as the 0 week. Subsequent soil samples were taken at every 8 week to determine the physico-chemical parameters. The following soil parameters were analyzed: Soil pH was analyzed using Jennway 3015 pH Meter. Total organic carbon, phosphorus and Nitrogen were determined by the Oxidation, Ascorbic acid and Kjedahl methods respectively (Stewart *et al.*,1974). Total hydrocarbon content were analyzed by the Spectrophometric method adapted from Stewart *et al.*, (1974). Zinc and copper were first digested for 15 minutes in electro thermal heater before analysis using atomic absorption spectrophometer. The Carbon- Nitrogen ratio was obtained by dividing the total Carbon by the total Nitrogen for each bag. All data collected were subjected to two-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The physico-chemical characteristics of the soils in the treatment options are presented in Figs 1-8. As shown in Fig. 1, soil Nitrogen increased significantly between and within treatments with time (p=0.05). At the end of the study period, highest increment in soil Nitrogen were experienced in batch A (11.20 – 37.41mg/kg) and C (11.20 – 33.89mg/kg). the increase in Nitrogen content in batch A treated with NPK 15:15:15 was as a result of the fact that the fertilizer supplied the Nitrogen (which are always limiting in polluted soil). The increased in the Nitrogen in the phytoremediation treatment (batch C) was as a result of Nitrogen fixation by the *Vigna unguiculata* (a legume) through the symbiotic association with *Rhizobium*. Thus the plant (*Vigna* sp) does not compete with the microorganisms for limited supplies of available Nitrogen at oil contaminated sites rather its increase the soil Nitrogen (Gudin and Syratt, 1975). Phosphate was found to follow a similar pattern as Nitrogen except in the phytoremediation treatment (batch C) which showed a gradual decrease with time as shown in Fig. 2. The reason for the reduction in the phosphate content in this treatment is probably due to the use of the phosphate for growth and development by the introduced plant (*Vigna* sp) in addition to the one used by the oil-degrading microorganisms.

Fig 1: Soil Nitrogen (mg/kg) in different Remediation Treatment

Fig 2. Soil Phosphate (kg/mg) in the Different Remediation Treatments.
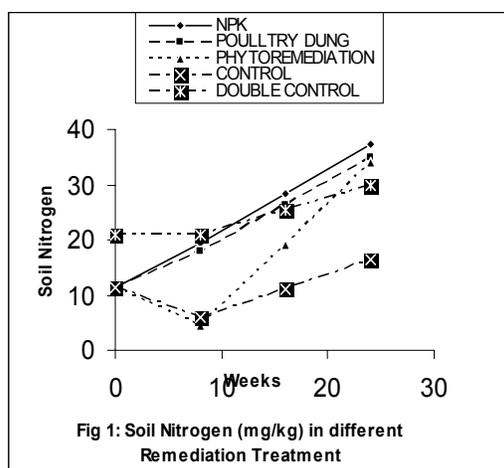
The addition of inorganic fertilizer and the fixation of Nitrogen in the phytoremediation option might have removed the nutrient limitation for microbial activity of biodegradation. It has been reported that addition of Nitrogen and phosphorus enhances biodegradation of polluted soil presumably by removing the Nitrogen and Phosphorus limitation resulting from low natural level (Lee *et al.*, 1993, Odokuma & Ibor, 2002). High nutrient level stimulated increase in microbial population and activities leading to high energy (carbon) demand by the oil-degrading microbes. This

has resulted in the reduction in the total organic carbon (TOC) in the remediation treatments especially in batch A (NPK) and C (phytoremediation) as shown in Fig. 3. This reduction in total organic carbon with time also imply that total hydrocarbon content (THC) loss may have increase with time in these treatments. This is true as shown in Fig. 4 in which THC decreased significantly in the remediated soils than in the control (p=0.05) such that at the end of the study highest loss was evident in the NPK and phytoremediation treatments (675 –

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130mg/kg). The higher hydrocarbon loss in the NPK treatment than the poultry dung treatment is in line with Lee *et al*. (1995) who reported that inorganic fertilizer has greater effect in stimulating crude oil degradation by increasing the total heterotrophic microbial growth and activity. The same applies to the phytoremediation treatment in which in addition to the Nitrogen fixed, the plant (*Vigna* sp) also provide root exudates of carbon, energy, nutrient, enzymes and sometimes oxygen to microbial population (Campbell, 1996; Cunningham *et al*., 1996; Vance, 1996) thereby stimulating the biodegradation activities (Gunther *et al*., 1996; Atlas & Bartha, 1998).
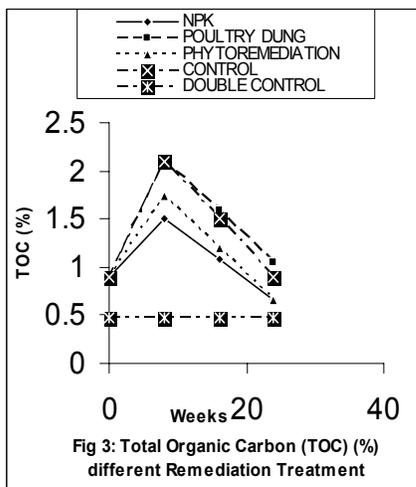

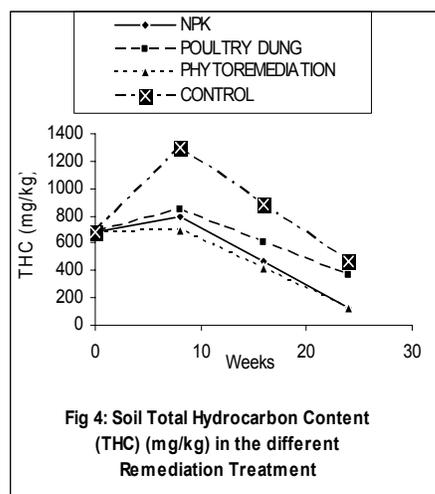Fig 3: Total Organic Carbon (TOC) (%) different Remediation Treatment

Fig 4: Soil Total Hydrocarbon Content (THC) (mg/kg) in the different Remediation Treatment
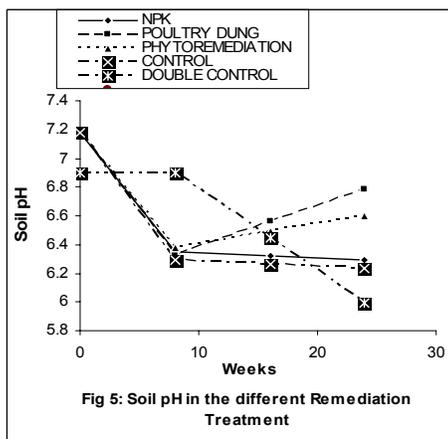

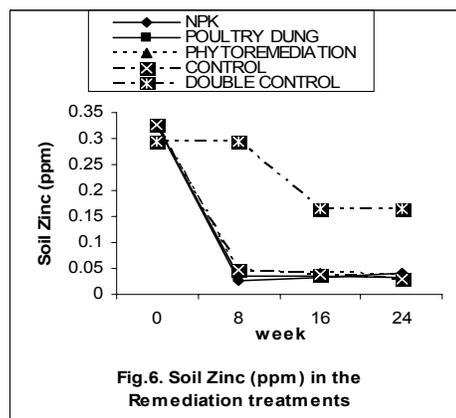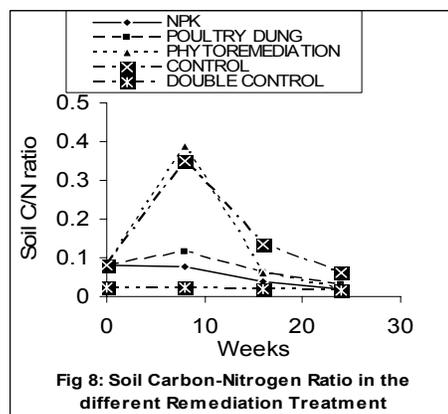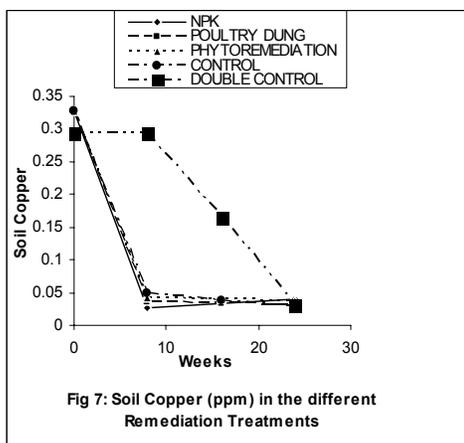Fig 5: Soil pH in the different Remediation Treatment

Fig.6. Soil Zinc (ppm) in the Remediation treatments

There was no significant difference between the various remediation treatments for soil pH, Zinc and Copper (Figures 5, 6 and 7). This range of pH favoured oil degradation by microorganisms as observed on similar studies that pH range of 6-9 provides better conditions for mineralization of hydrocarbons since most bacteria capable of metabolizing hydrocarbons develop best at pH condition close to neutrality (Atlas & Bartha, 1992; Manuel *et al*., 1993). The low and non-significant level of Zinc and Copper is an indication of non-toxicity of the heavy metals in the polluted soils.

Fig. 8 indicated that carbon- Nitrogen (C/N) ratios were significantly reduced in all the treatments. Highest reduction was recorded in the NPK treatment. The fact that lower C/N ratio were recorded for the treatment supplied with inorganic fertilizer indicated that some nutrients were lacking (Odokuma & Dickson, 2003). This meant that hydrocarbon degradation and loss increased with smaller C/N ratios justifying the use of nitrogenous nutrient sources to aid biodegradation.

* Corresponding author: Tanee, F. B. G.

Fig 7: Soil Copper (ppm) in the different Remediation Treatments

Fig 8: Soil Carbon-Nitrogen Ratio in the different Remediation Treatment

Conclusion: In conclusion, biostimulation (inorganic and organic fertilizer) and phytoremediation (*Vigna* sp) had the tendencies in stimulating oil biodegradation process, indicating that they are good remediation treatment option in crude oil polluted soil. The fact that option A (inorganic fertilizer) has the highest hydrocarbon loss and highest nutrient content than the other treatment options indicated that it is a prefer remedial measure followed by phytoremediation while organic manure (poultry dung) proved to be the least effective for crude oil bioremediation.

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