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Characterization and Safety Evaluation of the Impact of Hydrocarbon Contaminants on Ecological Receptors

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Abstract Hydrocarbon-contaminant removal efficiency of Bambara groundnuts and biomagnification was investigated. The crude oil contaminated soil samples in which the plants were established were either un-amended, or amended with NPK, or Urea, or Poultry manure. Amendments improved phytoextraction rates as follows: Urea – 63.37%, NPK – 65.99%, Poultry – manure – 70.04%, for PAH; Urea – 78.80%, NPK – 79.80%, Poultry manure – 87.90%, for BTEX. Hazard characterization from 28-day feeding study revealed negative effects of potentially toxic BTEX and PAH on organ weight, optimum digestibility and animal growth rate. Sleep time decreased with increasing hydrocarbon concentrations probably due to increased liver enzyme activity.

Keywords Phytoremediation · Exposure · Hydrocarbon contamination · Food safety

As Crude oil comes from the well, it contains a mixture of hydrocarbon compounds and relatively small quantities of other materials such as oxygen, nitrogen, sulphur, salt and water. In the refinery, most of these non-hydrocarbon substances are removed and the oil is broken down into its various components, and is blended into useful products. The environmental impacts associated with the exploration and exploitation of crude oil has been a popular area of experimental research in Nigeria especially in the Niger Delta in the last three decades. Over the last decades, there has been an increasing interest in biological methodologies, collectively indicated as bioremediation that may help reduce the risk of organic pollutants in soil and effectively restore polluted sites (Vincenza and Liliana 2007). These methodologies, and their plant-assisted variants, phytoremediation (Al-Awadhi et al. 2009) are usually considered environment-friendly treatments. They constitute essentially a managed or spontaneous process mediated by living organisms (mainly microorganisms) or plant-associated types, which degrade or transform contaminants to less toxic or nontoxic products, with mitigation or elimination of environmental contamination. The pollution of the environment by two classes of aromatic pollutants namely, polycyclic aromatic hydrocarbons (PAHs) and volatile aromatics collectively indicated as BTEX (benzene, toluene, ethylbenzene, xylene) are of major concern (Blaylock and Huang 2000). The biodegradation techniques primarily consist of using degrading microorganisms, which are sometimes supported by added nutrients or aeration (Rezek et al. 2009) and successful phytoremediation in fertilized soil has been reported by Parrish et al. (2004).

Bambara groundnut (*Vigna subterranea* (L.) Verdc), an indigenous African legume plays an important socio-economic role in the semi-arid regions of Africa (Massawe 2005). The seed makes a complete food, as it contains sufficient quantities of proteins, carbohydrates and lipid (Brough 1993). Bambara groundnut is a drought resistant crop that can grow in marginal, low-input, environments, and occurs in the local (Nigerian) environment hence the rationale for choice in this study. Phytoremediation of petroleum contaminated soil using Bambara groundnuts, ecological assessment of the risks associated with soil contamination vis-à-vis exposures to produce from such site and the safe level of plant products used for green remediation form the major aim of this study. Research on

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rats is to validate preliminary findings and points towards potential health risks.

Materials and Methods

The surface (0–20 cm) soil used in this study was collected from an agricultural grassland lying fallow (with no history of pollution) in Choba, a typical Niger Delta Region, Rivers State of Nigeria using a stainless steel hand auger in a randomized sampling quadrat design. The soil samples were air-dried, gently crushed, sieved to <2 mm, and used for basic characterization analysis using the method of Allen et al. (1984). The zero treatment (control) included in the design was exposed to same condition as other treatment groups but was not spiked with crude oil. Soil (2 kg) pots in a 45 × 45 cm polyethylene bag were watered and allowed to equilibrate for 2 weeks. Bonny light Crude oil (sourced from Nigerian National Petroleum Corporation (NNPC), Port Harcourt Refinery) treated soil (topdressing). in the concentrations of 2, 4, 6, 8, 10% (v/w) for ease of statistical analysis, were left for 1 week and moistened for another 1 week to enable it to settle. Thereafter, 0.8 g topdressed Urea, NPK and Poultry manure amendments were applied to selected groups and allowed for 2 weeks. Amendments such as the organic manure were selected for their similarity to naturally produced plant root exudates, while others were chosen for their high complex stability. Amount of amendments added was calculated from the method of Akobundu (1987), $C = (R \times A)/Q$ (where C = amount of amendment, $R = 2$ = a constant, A = weight of soil, and Q = product weight of substance as it is bagged). The % composition of poultry manure used was characterized as Total Nitrogen, 1.09; Phosphorus as P_2O_5, 1.01; Potassium K_2O, 0.55; and Calcium, 3.62. Ten seeds were germinated in the actual treatment soils and thinned down to three after a week of growth. Holes (3–4 per bag) were made to allow for free drain and adequate aeration. Three replicates per treatment were prepared. Growth indices were measured biweekly and plants were harvested at 6 and 12 weeks for comparison, washed thoroughly with deionized water, and sun-dried to constant weight and the shoot dry weight was recorded. Seeds of crop were finely ground and the concentrations of hydrocarbons in the seeds, shoots, leaves and soil were determined using Gas chromatography FID and Horiba Oil content analyzer OCMA-350. Analysis was strictly conducted using scientifically accepted methodology and analar grade and non – expired reagents.

PAHs and BTEX are determined to pose the most significant potential threat to human health due to their known or suspected toxicity and potential for human exposure at these contaminated sites hence this study. A total of 13-diet groups of 3 disease-free female, weaned, mature albino rats, weight range between 100 and 126 g of the wistar strain were designed in this 28-day feeding study. The rats were weighed and acclimatized for 7 days and reweighed. The weight after 7 days acclimatization formed the initial weight. But for CTRL 1 diet group, which was wholly industrial feed only, other groups, had their diets formed from 6:1 industrial feed to Bambara groundnuts flour from the diet groups. Unsuitable animals were culled and daily heath monitoring (posture, vitality, consumption, elimination, hydration), use of personal protective equipment, minimal noise, vibration, stress, disturbance, pest management program and general cleanliness of the cage and environment were some QA/QC strategies. In addition, code and contemporary guidelines for the care and use of animals adopted in Biochemistry Animal house was adhered to. Topfeeds Grower mash of N 1.98; P 1.74 Ca 5.25; K 2; Mg 4.79% composition was employed for rats feeding. Animals had continuous free access to clean tap water and feed. Perforated wire-meshed cages with facilities for feed, water and fecal collection were used. The beddings are kept clean and changed at very regular and frequent intervals. One of the cleanest, environmentally – friendly and easiest to use beddings, shredded cardboard paper was employed. Hay was put underneath to support rats with a semblance of environmental enrichment and a means of following natural instincts. The rats were weighed at the end of the feeding experiment and sacrificed by a careful dislocation of the neck. The carcasses were weighed, labeled and incisions made into the skull, thoracic and body cavities to expose the organs (liver, kidney, heart, spleen, pancreas and lungs), which were excised, trimmed of fat tissues, weighed, inspected for any gross pathological changes and returned into individual carcasses. The carcasses were finally dried in an oven at 80°C for 48 h, weighed, crushed and stored in a dessicator for further nitrogen determination.

Statistical Package for Social Sciences for Windows version 10.0 (SPSS Inc., Chicago, IL) was used to perform one – or two – way analyses of variance and the pearson correlation. Pairs of treatment means were compared for significant differences using least significant difference (LSD) at the 5% level.

Results and Discussion

Results obtained in this investigation are shown in Tables 1, 2 and 3 and Figs. 1, 2 and 3. Studies revealed decreasing phytomass (Fig. 1) with increasing oil spill concentration. Better health and sufficient biomass production within 3 months was evident below 10% contamination. After pollination and fertilization, the developing

Table 1 PAH concentration (mg/kg) achieved at plant harvest

TMT	CON	NPK	UREA	PM	PRE-P
S2	14.58b ± 0.02	9.56c ± 0.63	10.11c ± 0.45	7.01d ± 0.34	30.73e ± 6.94
S4	15.31bc ± 0.01	9.61c ± 0.71	10.44c ± 0.56	7.52d ± 0.80	34.23f ± 6.59
S6	17.21bd ± 0.03	11.45c ± 0.31	12.01c ± 0.51	10.13c ± 1.04	38.08f ± 4.58
S8	17.64d ± 0.68	13.31bc ± 0.80	14.17b ± 0.74	11.83c ± 1.01	42.45h ± 7.07
S10	20.00e ± 0.02	14.67b ± 0.91	15.80b ± 0.96	12.92cd ± 2.01	43.13hi ± 5.01
R2	1.0fi ± 0.00	0.88d ± 0.07	0.82i ± 0.01	0.94k ± 0.01	
R4	1.42fg ± 0.01	0.91d ± 0.01	0.92i ± 0.07	0.99k ± 0.01	
R6	1.60fgh ± 0.01	0.92d ± 0.05	0.98i ± 0.06	1.01k ± 0.03	
R8	1.63gh ± 0.04	1.03de ± 0.09	0.98i ± 0.06	1.05k ± 0.07	
R10	2.03i ± 0.10	1.10de ± 0.08	0.99i ± 0.06	1.06kl ± 0.10	
SH2	13.04j ± 0.89	11.74j ± 1.18	11.90j ± 1.01	12.67j ± 1.00	
SH4	14.60j ± 1.05	12.28j ± 1.02	12.00j ± 1.03	13.22j ± 1.02	
SH6	14.80j ± 1.24	13.17j ± 1.05	12.33j ± 1.01	14.18jl ± 0.95	
SH8	15.62j ± 1.30	13.75jk ± 2.01	13.09j ± 1.01	15.08l ± 1.08	
SH10	21.86kl ± 2.03	13.84jk ± 1.90	13.42jk ± 2.06	16.91lm ± 2.01	NA

CTRLS 0.21a ± 0.02, *CTRLR* 0.002i ± 0.00, *CTRLSH* 0.41i ± 0.07

Values are means ± SE, n = 3, values followed by different letters in the same column for each sample type (soil, root or shoot) are significantly different at $p < 0.05$. *S* Soil, *R* root and *SH* shoot samples. 2, 4, 6, 8 and 10 besides these abbreviations represents spill concentrations. *TMT* Treatment type; CTRL, CON, NPK, UREA, PM and PREP – P groups represents control, crude oil contaminated without nutrient amendment at 12WAG (weeks after germination), crude oil contaminated without nutrient amendment at 12WAG with NPK fertilizer amendment, crude oil contaminated without nutrient amendment at 12WAG with Urea fertilizer amendment, crude oil contaminated without nutrient amendment at 12WAG with poultry manure amendment and pre-plant soil i.e. 2 weeks after contamination/post impact values respectively; *S* Soil, *R* root and *SH* shoot samples. 2, 4, 6, 8 and 10 besides these abbreviations represents %spill concentrations (v/w)

Table 2 BTEX concentration (mg/kg) at plant harvest

TMT	CON	NPK	UREA	PM	PRE-P
S2	2.08a ± 0.09	1.90b ± 0.05	2.02b ± 0.03	0.96c ± 0.08	4.50d ± 0.23
S4	2.21a ± 0.05	1.91b ± 0.05	2.03b ± 0.05	0.99c ± 0.09	5.34e ± 0.61
S6	2.40a ± 0.05	2.01b ± 0.06	2.05b ± 0.05	1.01c ± 0.09	6.00e ± 0.85
S8	3.50ab ± 0.12	2.01a ± 0.08	2.10a ± 0.04	1.14c ± 0.10	8.80f ± 1.01
S10	4.48b ± 0.34	2.02a ± 0.21	2.12a ± 0.09	1.21c ± 0.20	10.00f ± 3.00
R2	0.04c ± 0.01	0.02g ± 0.01	0.02g ± 0.01	0.03g ± 0.01	
R4	0.16c ± 0.01	0.03g ± 0.01	0.02g ± 0.01	0.05g ± 0.01	
R6	0.27cd ± 0.01	0.08g ± 0.01	0.09g ± 0.01	0.11h ± 0.01	
R8	0.59de ± 0.01	0.09g ± 0.01	0.12g ± 0.01	0.22h ± 0.01	
R10	0.81de ± 0.04	0.22g ± 0.01	0.33gh ± 0.03	0.51h ± 0.01	
SH2	2.31f ± 0.21	2.13h ± 0.11	2.03i ± 0.09	2.17i ± 0.23	
SH4	2.83g ± 0.51	2.54hi ± 0.16	2.04i ± 0.09	2.57i ± 0.11	
SH6	3.14g ± 0.82	2.84h ± 0.34	2.65ij ± 0.15	2.89i ± 0.41	
SH8	3.84g ± 1.03	3.05i ± 0.37	2.76ij ± 0.18	3.23i ± 0.22	
SH10	4.50gh ± 1.10	4.05j ± 0.81	3.99k ± 0.09	4.42ij ± 0.32	

CTRLS 0.001 ± 0.00, *CTRLR* <0.001, *CTRLSH* <0.001

Values are means ± SE, n = 3, values followed by different letters in the same column for each sample type (soil, root or shoot) are significantly different at $p < 0.05$. *S* Soil, *R* root and *SH* shoot samples. 2, 4, 6, 8 and 10 besides these abbreviations represents spill concentrations (w/v)

pods were buried under the soil surface except those grown in 10% (v/w) spiked soil and nutrient un-amended that produced their fruits above ground – the contaminant probably placed the plant/microbe community under stress. NPK- amended group produced no fruits at the end of the study period. This could be due to observed low magnesium levels (1 mg/kg as compared with 3.65 mg/kg in control shoot, fresh weight) and potassium levels (33.1 mg/kg as

Table 3 Mean levels of PAH (mg/kg) of harvested fruits

TMT	CTRL	CON	NPK	UREA	PM
2		NA	NA	0.01g ± 0.001	0.02b ± 0.001
4		0.03a ± 0.01	NA	0.01g ± 0.001	0.02b ± 0.001
6		0.03a ± 0.01	NA	0.01g ± 0.001	0.02b ± 0.002
8		0.07c ± 0.01	NA	NA	0.04b ± 0.003
10	<0.001	0.09c ± 0.01	NA	NA	NA

Values are means ± SE, n = 3, values followed by different letters in the same column are significantly different at $p < 0.05$

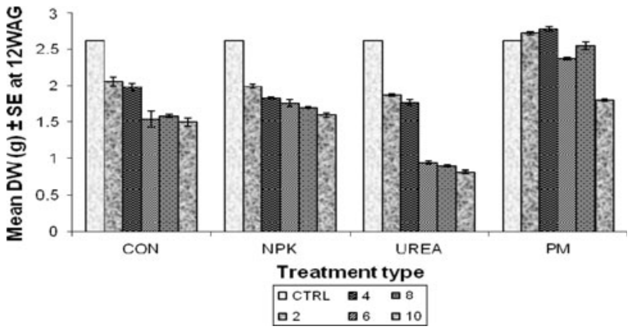

Fig. 1 Produced biomass, DW ± SE of *V. subterranean* at 12WAG for different treatments

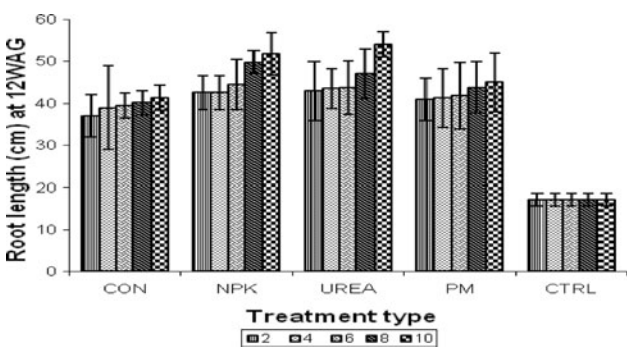

Fig. 2 Observed root length ± SE of *V. subterranean* at 12WAG for different treatments

compared with 56 mg/kg in control shoot, fresh weight) at the end of the experiment and at the highest contaminant dose. Oil toxicity for Bambara groundnuts was also evident in the narrow, scaly, chlorotic and less robust leaves seen throughout the study. BTEX levels in harvested fruits fell below the detection limit of the analytical instrument used while PAH (Table 3) filtered in it. Marked root elongation in response to contamination in search of nutrients and water, was evident in all treatment types (Fig. 2). There was no statistically significant variation among different spill concentrations simulated with respect to hydrocarbons uptake by plants un-amended (Tables 1, 2). Marked increased was observed in exhibited plant height (data not shown) and shoot biomass at mature stage compared with those of the tender age. The stunted growth, along with bluish green coloration exhibited by UREA treated group affected their residual dry weight (Fig. 1). NPK treated group showed similar trend to PM group in its response to hydrocarbon stress on produced biomass and gave negative growth with time. Poultry manure application in particular increased plant height, biomass, and grain yield. Gas chromatographic analysis revealed 53.62% and 55.20% contaminant removal for PAH and BTEX respectively, (Table 1) without nutrient amendments. However, biostimulation significantly enhanced performance to 65.99%, 63.37% and 70.04 PAH and 79.80%, 78.80% and 87.90% BTEX for NPK, Urea and Poultry manure, respectively at 10% (w/v) pollution (Table 2). This is in agreement with the findings of Johnson et al. (2009) and Tang et al. (2009) both which demonstrated the increased degradation with amendments and places *Vigna subterranean* over ryegrass (Rezek et al. 2009) that gave 54.37% and 52.2% PAH removal in fertilized and non-fertilized PAH – contaminated soil, respectively. Successful phytoremediation in fertilized soil has been demonstrated (Parrish et al. 2004). The team went further to state that chemically enhanced phytoextraction (Gupta and Sinha 2007) has been identified as a promising technology to remediate contaminated soil. Residual concentrations of these compounds were low enough to interpret them as biodegraded. Interestingly, desirable uptake potential of 45% BTEX and 50.68% PAH were achieved (Tables 1, 2) within 3 months of study period. Also, the uptake levels seen with the shoot were much higher than those of the roots and such is recommended in phtotoremediation. This process is affected by many factors, including soil properties (water balance, availability of nutrition, etc.), contaminant properties, the extent of contamination, and the bioavailability of contaminants to the appropriate plant species and microorganisms. From the results obtained, Bambara groundnuts could be used as a biomonitor owing to its vast tissue distribution.

Sleep time decreased among study animals with increasing hydrocarbon concentrations probably due to increased liver enzyme activity during the feeding study. Animal body weight gain was highest in rat fed control – produce followed by those fed pure commercial feed, then poultry manure amended diet group in which a systematic body weight decrease was observed with increased spill concentration. Body weight gain was least with contaminated and unamended diet group (not shown). Generally, adequate feed consumption rate recorded revealed that roasting exercise carried out on the Bambara beans before administration; not only inactivated toxic factors especially trypsin inhibitors and hemagglutinins but also improved the taste of the seeds. Rats fed un-amended contaminated diet at higher spill concentrations suffered enlarged stomach wall after 10 days of feeding trials. Inhibition of trpsin

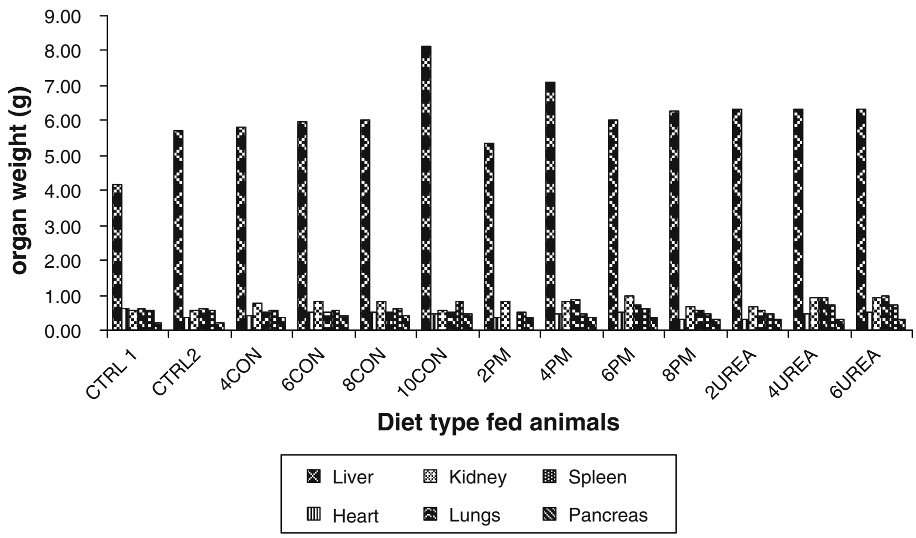

Fig. 3 Observed organ weight (g) of animals fed different diets after 28 days. CTRL 1 = Group fed commercial feed only, CTRL 2 = Group Fed commercial feed + beans from control experiment i.e. beans harvested from plants grown on unspiked soil; PM = Group Fed commercial feed + beans harvested from Poultry manure-amended treatment; UREA = Group Fed commercial feed + beans harvested from Urea- amended treatment; CON = Group Fed commercial feed + beans harvested from spiked un-amended treatment; 2, 4, 6, 8, and 10 added to CON, UREA and PM represent different %spill concentrations (v/w) simulated

by trpsin inhibitors in raw soya flour had been thought to result in a continuous release of the stimulant hormone cholecystokinin from the mucosa of the small intestine bringing about an adaptive response in the pancreas (Tudor and Dayan 1987). The un-amended contaminated diet group recorded the poorest relative growth rate, body weight gain as well as feed conversion ratio and protein efficiency ratio, PER. PER was highest for rats fed Control >PM > UREA > CON diets. There was significant difference ($p \leq 0.05$) among the weights of dry carcass for amended and un-amended diet groups. Carcass crude protein decreased with increased contaminant dose while fecal crude protein increased with decreased contaminant dose. Nutrient amendments ameliorated the effect of pollution on organs (Fig. 3). Visual inspection of their lungs at autopsy revealed gross pathological changes with contamination. This could be due to some physiological variations. The marked varied pancreas weight gain obtained for rats fed CON diets over those of other groups was indicative of pancreatic enlargement and was also visible at autopsy. This implies that the industries need to do rigorous hazard assessment, do it repeatedly and monitor it before usage of such food both industrially and by natives receives approval.

Livestock had a high tolerance to hydrocarbon exposure via feed before clinical symptoms of toxicity manifested. The statistically significant increment in most organ weight with increased contaminant concentration could be due to increased hepatic enzyme activity, though improvement was recorded with contaminant removal. Generally, poultry manure compared well with control experiment especially at lower dose of the contaminants. *V. subterranean* significantly achieved potentially toxic hydrocarbons removal in Petroleum contaminated soils to an acceptable level in comparison to those of ryegrass and others. Hazard characterization revealed negative effects of BTEX and PAH on organ weight, optimum digestibility and animal growth rate. Weight of pancreas showed pancreatic hypertrophy in rats adapted to diets, which contain higher levels of contaminants compared to controls.

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