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Safety evaluation of the impact of metals contaminants on ecological receptors

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Abstract

The toxic effects on *Vigna subterranea* plants grown for 12 weeks in a simulated petroleum (up to 100ml/2kg soil) contaminated soil was investigated. The soil samples in which the plants were established were either un-amended, or amended with NPK, or Urea, or Poultry manure. The rate of metal uptake, plant growth and fruition, which was best with poultry manure amendment were measured. Phytoextraction of metals was effective. Harvested fruits were fed whister albino rats for 28 days to trace the inherent biomagnifications. Hazard characterization revealed negative effects of potentially toxic metals on organ weight, Protein Efficiency Ratio and weight change.

Key Words: Phytochemistry; Food safety; Metals contaminants; Exposure.

Introduction

Petroleum is a complex mixture of hydrocarbons that form from the partial decomposition of biogenic materials [1]. Elemental analysis revealed spectra due to trace elements such as Ca, Fe, Mg, Cu, Zn, Na, Ni, K and Mo in crude oil were recorded using Laser induced breakdown spectroscopy (LIBS) technique [2]. The environmental impacts associated with the exploration and exploitation of crude oil have been a popular area of experimental research in Nigeria in the last three decades, therefore, literature treating this subject is quite prolific. 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 [3]. These methodologies, and their plant-assisted variants, phytoremediation usually considered environment-friendly treatments, 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. Grain legumes of Bambara groundnuts serve as a cheap source of protein to a large proportion of the population in poor countries of the tropics and are called 'Okpa' in Igboland in Nigeria. Bambara groundnut (*Vigna* *subterranea* (L.) Verdc), an indigenous African legume, plays an important socio-economic role in the semi-arid regions of Africa [4]. The dry seeds may be roasted and eaten with palm kernel as snacks. Protein functionality tests on the ground seed indicate that it can compete with or replace other conventional flours in a range of processed products [5]. The seed makes a complete food, as it contains sufficient quantities of proteins, carbohydrates and lipid [6] [5]. Bambara groundnut is a drought resistant crop grown in marginal, low-input, environments. At this time, there is insufficient information on impacted field produce, physiology and behavior of the flora and exposure implications for a reasoned assessment. Research on rats is to validate preliminary findings and points towards potential health risks.

Materials and Methods

Cultivation: The surface (0-15cm) 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 < 2mm, and used for basic characterization analysis using the method of Allen et al. [7]. The zero treatment (control) included in the design was exposed to same condition as other treatment groups but was not spiked with crude oil. Fresh poultry manure, PM used was characterized with composition as follows: Total Nitrogen, 1.09; Phosphorus as P_2O_5, 1.01; Potassium K_2O, 0.55; and Calcium, 3.62. Spiking with Bonny light crude oil in the concentrations of 2, 4, 6, 8, and 10% (v/w) in a 45 x 45cm polyethylene bag after conducting toxicity trials to arrive at these concentrations were done. All amendments were added by top-dressing 2 weeks after contamination and before planting. Bambara beans were seeded into soil groups after 2 weeks of soil preparation while watering. Up to 3 – 4 holes 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. Dried plants materials and harvested seeds (roasted) of Bambara beans, finely ground, acid – digested using HNO3 and HCl in the ratio of 3:1 respectively and the concentrations of Cd, Fe, Cu, Mg, and Na in the seeds and leaves were determined by graphite furnace atomic absorption spectrophotometry (GTA – 110/SpectrAA50, Varian, Inc., Palo Alto, CA, USA). The limit of detection for this equipment is 0.001. Every sample was taken, labeled and preserved appropriately. Transportation to the laboratory was made using ice chest coolers. Analysis was conducted using scientifically accepted methodology (APHA and ASTM) and high quality standard non – expired reagents. There was no deviation whatsoever from the quality standard prescribed for this experiment.

Rat feeding study: A total of 13-diet groups of 3 disease-free female, weaned, mature albino rats, weight range between 100 and 126g of the wistar strain were designed in this 28-day feeding study in three replicates. The rats were weighed and acclimatized for 7 days and reweighed. The weight after 7 days acclimatization formed the initial weight. Apart from CTRL 1 diet group, which was wholly commercial feed only, other groups, had their diets formed from 6:1 industrial feed to Beans flour from the various diet groups. Unsuitable animals were culled and daily heath monitoring (posture, vitality, consumption, elimination, hydration), use of PPEs, minimal noise, vibration, stress, disturbance, pest management program and general cleanliness of the cage and environment were some QA/QC strategies. Topfeeds Grower mash of composition: Crude Protein, 16%; Fats/oil, 5%; Crude fibre (kcal/kg), 7%; Calcium, 1%; Methionine, 0.36, Salt(min), 0.3; Metabolisable Energy(min), 11.1MJ; Available Phosphorus, 0.70%; Lysine, 0.75% ; and Net weight/kg, 25Kg) 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 [8] [9], 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 after a careful exposition to Anesthesia Induction Chamber (Havard Apparatus). All national and local regulations on handling of animals were adhered to. 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^oC for 48 hours, weighed, crushed and stored in desiccators for further nitrogen determination.

Statistical analysis: 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

Brown patches (Fig I) on the leaves in contaminated and unamended treatment showed a toxicity symptom.

Fig I: Brown/burnt patches on leaves seen on plants in CON treatment

Studies showed decreasing phytomass with increasing spill concentration though Bambara beans basically were crude oil spill - induced drought tolerant as rare death cases were recorded. Application of amendments to contaminated soil here significantly increased the soil pH, and promoted better growth and yields in bans. However, Bambara beans showed little response to applications of Urea which stuntedness could be due to Biuret in Urea fertilizer. The concentration of cadmium in the soil solution fell significantly when poultry manure was applied to contaminated soil whereas comparable levels where seen in the produced fruits (Tables I and

II) and these constitute safety concerns [10]. Fruition was not supported within the 12 weeks of study for NPK group though bunch and radiant growth was evident (Tables I and II).

Table I: Mean levels of Cd (mg/kg) in Bambara beans harvested

TRMT	CNTRL	CON	NPK	UREA	PM
V20		NA	NA	14.38d±4.11	4.38f±6.78
V40		11.88b±5.44	NA	16.25e±4.33	13.75e±9.04
V60		13.13b±6.01	NA	18.13e±4.51	14.38e±5.99
V80		16.88c±6.22	NA	NA	16.25ge±7.11
V100	0.63a±5.21	16.25cd±6.50	NA	NA	NA

Values are means ±SE (n = 3). Means in the same column having the same letters are not significantly different at p ≤ 0.005. NA = Not applicable as fruits were not produced with these regimes. CTRL = Control experiment; NPK = NPK-amended treatment; PM = Poultry manure- amended treatment; UREA = Urea- amended treatment; 20, 40, 60, 80, and 100 represent different spill volumes/ 2kg soil simulated.

Table II: Mean levels of Cu (mg/kg) in Bambara beans harvested

TRMT	CTRL	CON	NPK	UREA	PM
V20		NA	NA	<0.001	<0.001
V40		<0.001	NA	<0.001	<0.001
V60		<0.001	NA	<0.001	<0.001
V80	<0.001	<0.001	NA	NA	<0.001

Observed low magnesium and potassium levels (data not shown) for NPK could account for non-fruit yield in that regime. The contaminant placed the plant/microbe community under stress as evident in above ground- level-fruition seen for un-amended group at the highest spill concentration adopted (Fig II).

Fig II: Stress induced early fruition at 100ml/2kg soil contamination above soil as opposed to below soil fruition taken by plant normally at eleven weeks after germination, 11WAG.

Nutrient elements analysed were affected by spill. AAS analysis revealed Urea- 30.9%, NPK- 30.71%, PM- 49.03%, Un-amended- 33% for Cd; Urea- 34.78%, NPK- 19.13%, PM-37.39%, Un-amended- 17.17% for Mg (data not shown); Urea- 31.26%, NPK- 20.9%, PM- 32.84%, Un-amended- 19.34% for Na; Urea- 20.77%, NPK- 18.87%, PM- 28.32, Un-amended- 24.54% for Cu; and Urea- 34.23%, NPK- 41.53%, PM- 47.69%, Un-amended- 18.46% for Fe (data not shown) at the highest spill concentration (Table III).

Table III % Shoot Performance (extraction) of Bambara bean plant at the end of experiment at the highest contaminant dose

Metals	CON	NPK	UREA	PM
Cd	33.00	30.71	30.90	49.03
Mg	17.17	19.13	34.78	37.39
Na	19.34	20.90	31.28	32.84
Cu	24.54	18.87	20.77	28.32
Fe	18.46	41.53	34.23	47.69

Soil mineral availability and plant mineral uptake was affected by crude oil and effects were ameliorated by biostimulants. Increased Cd levels in grain for UREA (Table I) treatment group could be due to fertilizer components. Cu was not detected in all grains analyzed. Soil characteristic geology and manure components contributed to high Fe content recorded for all samples. Animal body weight gain was highest in CTRL 2 diet group followed by rats fed CTRL 1, then PM diet group in which a systematic body weight decrease was observed with increased spill concentration. Increase in body weight was significantly ($p \leq 0.05$) higher in CTRL 2 followed by CTRL 1 and PM.

The interaction between the rhizosphere microbial community and the contaminant left some contaminants in the fruits which were formed underground. 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. 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. Inhibition of trypsin by trypsin 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 [11].

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 (Table 1). PER for rats fed Nutrend was 1.67 [12]. This compared well with PER found for control and PM diet groups. The rats fed diet CON gave least relative growth rate (Table IV).

Table IV Performance characteristics of albino rats fed different diets for 28 days

Diet group	I(g)	F(g)	BWC (g)	PER	FCR	WDC	RGR
CTRL 1	100	170.7	70.7	1.67	0.98	38.22	0.93
CTRL2	100	197	82	1.94	1.03	45.77	1.07
40 CON	118	173	55	1.30	0.75	32.08	0.68
60 CON	125	180	55	1.30	0.75	32.17	0.68
80 CON	126	181	55	1.30	0.76	32.05	0.68
100 CON	125	175	50	1.18	0.69	31.50	0.58
20 PM	115	188	73	1.73	1.00	44.26	0.96
40 PM	100	170	70	1.66	0.95	43.34	0.92
60 PM	100	170	70	1.66	0.95	42.96	0.92
80 PM	110	180	70	1.66	0.95	37.56	0.92
20 UREA	100	167	67	1.59	0.85	42.19	0.87
40 UREA	125	190	65	1.54	0.83	41.27	0.84
60 UREA	125	189	64	1.59	0.86	41.05	0.83

Values are means ± SE for 3 rats per dietary group (n = 3). Means in the same column having the same letters are not significantly different at p ≤ 0.005. BWC = Body weight change; CTRL 1 = Group fed commercial feed only, CTRL 2 = Group Fed commercial feed + control seeds; F = Final weight; I = Initial weight; CON = Diet group fed seeds harvested from contaminated and un-amended treatment; NPK = Diet group fed seeds harvested from NPK-amended treatment; PM = Diet group fed seeds harvested from Poultry manure- amended treatment; UREA = Diet group fed seeds harvested from Urea- amended treatment; WDC = Weight of dry carcass; Protein Efficiency Ratio, PER = Body weight change at the end of the feeding period/Protein consumed; Feed Conversion Ratio, FCR = Body weight change at the end of the feeding period / Quantity of feed consumed; Relative Growth Rate, RGR = 2.303log (increase in mass/time interval in days); 20, 40, 60, 80, and 100 represent different spill volumes/ 2kg soil simulated.

Carcass crude protein decreased with increased contaminant dose while fecal crude protein increased with decreased contaminant dose (data not shown). Also, there was no marked variation between carcass crude protein between PM and UREA diet groups. Low FCR in CON diet group could be related to their high fecal crude protein.

The effect of feeding these diets on the organ weights of rats is shown in table V.

The increment in most organ weight with increased contaminant concentration could be due to increased hepatic enzyme activity [13], though improvement was recorded with contaminant removal. Pancreatic enlargement was evident with animals-fed with significant contaminated formulations and is in conformity with the findings of Tudor and Dayan [11]. Visual inspection of their lungs at autopsy revealed gross pathological changes. This could be due to some physiological variations. The significant difference in pancreas weight obtained for rats fed CON diets over those of other groups was indicative of pancreatic enlargement. This was also visible at autopsy.

Table V Organ weight (g) of Animal

Diet group	Liver	Heart	Kidney	Lungs	Spleen	Pancreas
CTRL 1	4.15c±0.02	0.60a±0.08	0.57a±0.04	0.60a±0.11	0.56a±0.05	0.22a±0.09
CTRL2	5.70d±0.04	0.35b±0.08	0.54a±0.06	0.62a±0.08	0.57a±0.04	0.22a±0.05
40 CON	5.81e±0.04	0.40c±0.04	0.80b±0.04	0.50b±0.09	0.58a±0.05	0.49b±0.10
60 CON	5.98e±0.03	0.50d±0.09	0.81b±0.04	0.51b±0.15	0.59a±0.02	0.50b±0.10
80 CON	6.02e±0.01	0.51d±0.04	0.81b±0.05	0.51b±0.07	0.61a±0.04	0.52b±0.13
100 CON	8.11f±0.01	0.47d±0.05	0.55c±0.05	0.53b±0.07	0.83b±0.06	0.53b±0.16
20 PM	5.34c±0.02	0.38b±0.09	0.81b±0.07	0.70c±0.07	0.49a±0.06	0.35c±0.07
40 PM	7.11g±0.05	0.44b±0.09	0.82b±0.01	0.85c±0.11	0.48a±0.06	0.35c±0.05
60 PM	6.02g±0.03	0.50d±0.06	1.00b±0.01	0.74c±0.11	0.60a±0.09	0.35c±0.06
80 PM	6.30g±0.03	0.52d±0.07	0.65b±0.04	0.75c±0.14	0.57a±0.05	0.30c±0.14
20 UREA	6.31g±0.03	0.32e±0.07	0.66b±0.04	0.57d±0.13	0.47a±0.04	0.30c±0.13
40 UREA	6.34g±0.04	0.47d±0.07	0.93b±0.02	0.94c±0.11	0.72a±0.07	0.30c±0.09
60 UREA	6.34g±0.04	0.50d±0.09	0.94b±0.04	0.96c±0.09	0.74a±0.07	0.31c±0.11

Values are means ±SE for 3 rats per dietary group (n = 3). Means in the same column having the same letters are not significantly different at $p \leq 0.005$.

The implications for the biotechnology industry, already suffering from a generic backlash against food grown in Niger Delta – could be severe. This implies that the industries need do rigorous hazard assessment, do it repeatedly and monitor it. Further sensory evaluation on humans, shell life and microbial studies should be carried out if natives or industries must make use of such produce. Also, Bambara beans could be useful at phytoextraction of Cd, Cu and Fe into its harvestable portion and could be explored in the area of phytoremediation.

Conclusion

The ability of the amendment in reducing the phytotoxicity and the measurable plant uptake of contaminants were key findings. Also, the early fruition with high (10% v/w) dose of contaminant is indicative of stress-response. Poultry manure compared well with control experiment especially at lower dose of the contaminants and is therefore recommended for such management practices. Rats fed un-amended contaminated diet at higher spill concentrations suffered enlarged stomach wall after 10 days of feeding trials. 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. Pancreatic enlargement was evident with animals-fed with significant contaminated formulations due to some physiological variations. Poultry manure amendments however, reduced the inherent phytotoxicity.

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