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Full Length Research Paper

Performance and risk assessment of Bambara beans grown on petroleum contaminated soil and the biostimulation implications

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The potential of *Vigna subterranean* commonly called Bambara beans for phytoextraction of copper in a crude oil contaminated soil was investigated. The contamination levels of crude oil in the soil for this pot experiment were 2, 4, 6, 8 and 10% (w/v). Amendments were added to increase the uptake and translocation of metal to aerial biomass. A range of amendments was tested for copper uptake enhancement with *V. subterranean* commonly called Bambara beans. Safe levels of exposure to contaminants were further investigated using albino Wistar rats for 28 feeding trial days to trace inherent biomagnifications. Bambara beans extracted up to 88.88 and 43.38 mg/kg into its shoot and root respectively at 10% contaminant dose while achieving 63.17% Cu removal unamended. Poultry manure was the most effective amendment for enhancing copper uptake and translocation into the shoots of Bambara beans with shoot tissue copper levels of 118.13 mg/kg compared to 98.25 and 93 mg/kg obtained for NPK and UREA amendments. 79.28, 70.07 and 70.99% Cu removal was achieved with poultry manure, NPK and UREA amendment respectively. An assay was carried out with animals on inherent biomagnifications. The results showed that the performance of rats fed diets formulated from contaminated soil differed significantly (P > 0.05) from those of the controls used. Hazard characterization revealed negative effects of potentially toxic copper 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.

Key words: Bambarra beans, copper uptake, amendments, diets, performance.

INTRODUCTION

Heavy metal contamination of soils is a major environmental problem worldwide and phytoextraction has emerged as a potential cost-effective and environmentally sustainable technique for removing toxic metals from soils (McGrath and Zhao, 2003). Copper, an essential trace element for plants, is a co-factor for some enzymes involved in photosynthesis, such as plastocyanin, in the detoxification of superoxide radicals, such as dismutase, in respiration such as cytochrome C oxidase, and in the ethylene receptors for the apoplastic oxidases (Pilon et al., 2006). Copper deficiency can lead to a very severe reduction of crop yields. Also, copper is potentially toxic at high concentration, and it has been reported to be eight times more toxic than lead (Fargašovă, 2001). Toxic symptoms or even death are sometimes reported when excessive Cu is found in living tissues (Jamers et al., 2006). Although soil and environmental conditions influence the plant availability of trace metals, Cu accumulation in plants depends to varying extents on the bioavailable fraction of the total Cu concentrations in soils (Ke et al., 2001). Sources of anthropogenic metal contamination include smelting of metalliferous ore, electroplating, gas exhaust, energy and fuel production, application of fertilizers and municipal sludges to land and industrial manufacturing (Blaylock and Huang, 2000; Vasiliadou and Dordas, 2009). Previous work has demonstrated that plants that are effective for phytoextraction display features such as a

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Table 1. Selected physicochemical properties of the soil collected for the pot experiment.

S/N	Parameter	Amount
1	pH	7.04
2	NO_3 (mg/kg)	4055.00
3	SO_4 (mg/kg)	1725.00
4	PO_4 (mg/kg)	13.90
5	% Total Nitrogen	0.34
6	Cl^- (mg/kg)	70.00
7	% TOM	6.00
8	% TOC	3.49
9	Mg (mg/kg)	215.00
10	Na (mg/kg)	112.00
11	Cd (mg/kg)	103.00
12	Cu (mg/kg)	45.00
13	Fe (mg/kg)	18690.00
14	Conductivity(µs)	12.00
15	K (mg/kg)	5.30
16	Ca	516.00

high tolerance to elevated metal concentrations, an ability to accumulate high levels of metal in harvestable parts, a rapid growth rate, a high biomass production in field conditions and a prolific root system (Alkorta et al., 2004). Bambara groundnut (*Vigna subterranean (L.) Verdc*) is a legume grown mainly in the Middle Belt and Enugu State of Nigeria (Doku and Karikari, 1971) and is characterized by a bunch growth among other features. Bambara seeds have been reported to contain 24% crude protein, 6.60 and 1.30% lysine and methionine, respectively (Poulter, 1981; Temple and Aliyu, 1994). The seeds are normally milled and sieved several times to obtain the flour locally called okpa, which is a nutritive human food found in Enugu state of Nigeria.

To date, studies on the exposures implications of diets formulated from phytoremediation exercise are limited. The objective of this study was therefore to determine the suitability of Bambara beans for Cu removal and phytoextraction, the enhancement potential of tested amendments on Cu removal and the toxicity of Bambara beans diet harvested from a contaminated soil on test animal.

MATERIALS AND METHODS

Soil samples were collected from the top layer (20 cm) from an agricultural farm (18°15N) along Eastwest road at Choba community, Rivers state, Nigeria. Freshly collected soil samples were air dried and filtered through a nylon sieve (2 mm) before use in an experimental pot. Selected soil properties were determined using standard methods (Allen et al., 1984) and are shown in Table 1. The experimental pots each had dimension of 45 × 45 cm and were at the Botanical garden of the University of Port Harcourt, Nigeria. Each pot was either crude oil contaminated (2, 4, 6, 8 and 10% (w/v) with no amendments or crude oil contaminated (2, 4, 6, 8 and 10% (w/v) with nutrient amendments (NPK, UREA or poultry manure fertilizers). The amendments were compared on the basis of their toxicity to plants, influence on metal uptake by roots, and ability to manipulate the translocation of metals from roots to shoot tissue. It is desirable to select an amendment that facilitates a high rate of root and shoot growth, with low toxicity, while increasing the rate of metal translocation to the shoots in order to extract metals rapidly from the soil. Pot with no crude oil spiking served as a control in this experiment. There were 3 replicates of each treatment, providing 63 pots in a completely randomized design. Topdressing crude oil spiking was done following the above concentrations derived from a toxicity range test. A fertilizer topdressing of 0.8 g per pot was applied 2 weeks as basal after spiking with crude oil. 10 formazan tested and confirmed viable seeds (scarified to improve germination efficiencies) were planted in each pot containing 2 kg soil two weeks after fertilizer applications. Soil moisture was maintained at about 70% of the water–holding capacity by weighing and watering with deionized water everyday. Growth indices like plant height and leaf area were measured biweekly. Visual observation of plant materials were done at first harvest (6 weeks after germination). The plants and soil were collected at harvest after 3 months of growth. Soil samples were dried, crushed and passed through 2 mm nylon sieves. Cu concentration was determined by atomic absorption spectrophotometry (Varian SpectrAA 220 FS, Varian, Palo Alto, CA, USA) using appropriate lamp. After their heights and root lengths were measured, plants were separated into shoots and roots. The plant samples were gently and thoroughly rinsed with tap and deionized water in succession. Roots were immersed in a solution of 20 mmol L^{-1} Na_2 – EDTA for 30 min to remove extracellular metals before washing with tap water and deionised water. The washed root and shoot samples were dried at 80°C for 48 h, and dry weights determined with analytical balance (PM 480002618). The samples were thereafter ground and digested with aqua-regia (HCl and HNO_3 in the ratio of 3:1). Cu was determined by AAS (Varian SpectrAA 220 FS, Varian, Palo Alto, CA, USA). Blanks and plant standard reference materials were included in each batch for quality control.

Rat feeding study

This study was conducted at the Animal House of the Biochemistry Department of the University of Port Harcourt, Rivers State, Nigeria. A total of 13-diet groups of 3 disease-free female, weaned and mature albino rats, weighing between 100 and 126 g and of the Wistar strain were employed 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 (I). The experimental diets were formulated in such a way that 6:1 industrial feed to Bambara beans flour from the individual cultivated groups (Moses, 2008). Control 1 diet was wholly industrial feed while Control 2 diet was basically Bambara beans flour from the control experiment. Bambara bean seeds were roasted to improve palatability (Moses, 2008) and reduce significantly the effects of the trypsin inhibitors, haemaglutinin, tannic acid, phytic acid and oxalate present in the raw. Onwudike and Eguakun (1994) had reported that raw Bambara groundnut seeds did not support weight gain of broiler chicks but gave a negative protein efficiency value. 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 adopted. Topfeeds grower mash for chicks was used as feed for the animals. 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 and the beddings were kept clean and changed at very regular and frequent

intervals. One of the cleanest, environmentally-friendly and easiest to use beddings, shredded cardboard paper (Du Commun, 2002) was employed and 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 (final weight designated as F) before sacrifice which was done 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).These organs 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℃ for 48 h, weighed, crushed and stored in a dessicator for further nitrogen determination. Parameters measured were body weight change (BWC), feed intake, protein efficiency ratio, (PER), weight of dry carcass (WDC), feed conversion ratio (FCR) and (RGR). Initial live weights of the pullets were determined by weighing them in groups at day-old and individually after 4 weeks of age. Weight gain was determined by subtracting initial live weight from final live weight. Daily feed intake was determined by subtracting the quantity leftover from the quantity given each day. The weighing of rats and feed was done using a top loading (2 kg capacity, Goat Brand^{R}) weighing scale. Weighing of animals took place in the morning hours (7.00 - 8.00 am) each week. Feed conversion ratio was determined as feed intake divided by weight gain. Relative growth rate was calculated as 2.303log (increase in mass/time interval in days) (Onwudike and Eguakun, 1994).

Statistical analysis

The performance of Bambara beans and the exposures implications were analyzed using the statistical package for Social Sciences for Windows version 10.0 (SPSS Inc., Chicago, IL) to perform 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

The potential toxic effects of the contaminant and amendments were assessed by visual observations of the root and tissue during the growth period, in addition to measurements of the root and shoot biomass production after exposure. Observations were made of toxicity symptoms such as wilting, discoloration and leaf necrosis. From visual inspections, Bambara beans were less sensitive to treatment below 10% contaminant concentration. At 10% contaminant concentration, leaves and stems grew tinier. Root growth was also best with poultry manure amendment in comparison with others employed in this study (Figure 9). In line with the findings of Johnson et al. (2009), root growth (data not shown) was more significantly affected than shoot growth due to the addition of chemical amendments for UREA–amended treatment. There was no statistical significant differences in average plant height among the contaminated, un-amended plants, contaminated, NPK–amended plants and Poultry manure–amended plants. Urea–amended, contaminated plants gave stunted growth and less bunch growth above 2% concentration of pollution (Figures 5 to 8). This could be due to an imbalance of nutrients in the soil. Tang et al. (2009) reported similar observations. NPK–amended soil produced no fruits under the conditions of this experiment. Shoot biomass (Figure 4) of poultry manure treatment, PM was approximately 2 times larger than those of UREA and NPK at 12 weeks after germination, WAG and shoot biomass formed a more reliable indicator of plant growth performance than plant height. Markedly increased was plant height and shoot biomass compared with UREA (Figure 4). Therefore, in the pot experiment, PM treatment had the largest effect on plant height (Figure 8) and shoot biomass of Bambara beans. Figure 5 also confirmed the strength in Bambara beans in this technique of interest without soil biomodifications and could go on for a longer study time. Although UREA fertilizer was the least in favour of translocation to the shoot, its efficiency at improving Cu phytoavailability at the root level could be explored.

Figures 2 and 3 show the accumulated concentrations of Cu in the plant tissues at the twelfth week after planting. The root concentrations (mg/kg) were 37 ± 16 to 41.3 ± 7.5; 42.5 ± 11 to 51.75 ± 9; 42.88 ± 9 to 54 ± 12; and 40.9 ± 9 to 45 ± 11 for plants from un-amended (CON); NPK–amended (NPK); UREA–amended (UREA) and poultry manure–amended (PM) contaminated soils respectively. The shoot concentrations (mg/kg) were 86.88 ± 32 to 92 ± 33; 87.63 ± 27 to 98.25 ± 17; 87.5 ± 35 to 93 ± 30 and 87.5 ± 39 to 118.13 ± 41 for plants from un-amended (CON); NPK–amended (NPK); UREA–amended (UREA) and poultry manure–amended (PM) contaminated soils respectively. These confirm tolerance above critical Cu content for toxicity (20 – 30 mg/kg) in most plant species as reported by Marschner (1995) and Tang et al. (2009). As evident in Figure 1, Cu removal was in the other PM > UREA > NPK > CON achieving excellent degradation amounts of 79.28, 70.99, 70.07 and 63.17%, respectively. Copper was detected in the control leaves of *V. subterranean* at low concentrations (7.08 mg/kg in roots and 14.5 mg/kg in shoots) that could be due to cross contamination in the study area, rain or other extraneous factors. This value falls within a narrow normal range of 3 - 20 mg/kg reported by Marschner (1995), below which indicates Cu deficiency. Cu shoot levels across all treatments exceeded those of the root by over 50%. The translocation coefficient is expressed as the ratio of the content of copper in the shoots to that in the roots on either a concentration or total mass basis. The coefficient is a useful indication of the ability of amendments to effect the translocation of metals to shoot tissue. Copper translocation from root to shoot in Bambara beans differed widely with treatments. The addition of UREA fertilizer caused the most noticeable reduction of copper translocation to shoots. Although poultry manure significantly increased copper translocation from root to shoot, un-amended treatment was the next option in this regard.

From the phytoextraction coefficient observed,

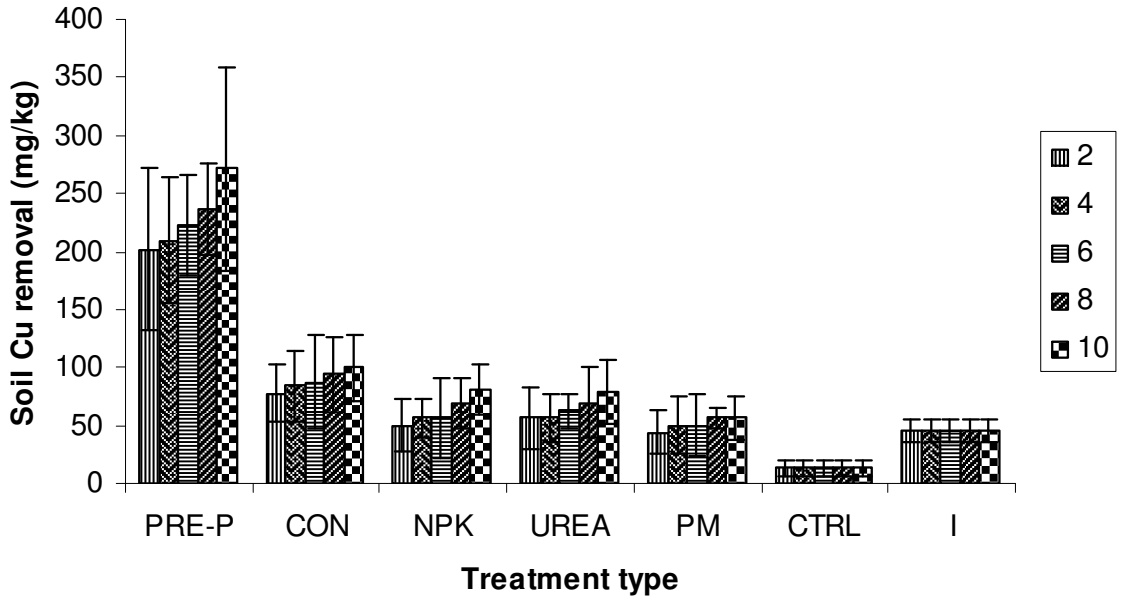

Figure 1. Cu concentrations of soil before and after 12WAG. Values denote mean ± SEM (n = 3).

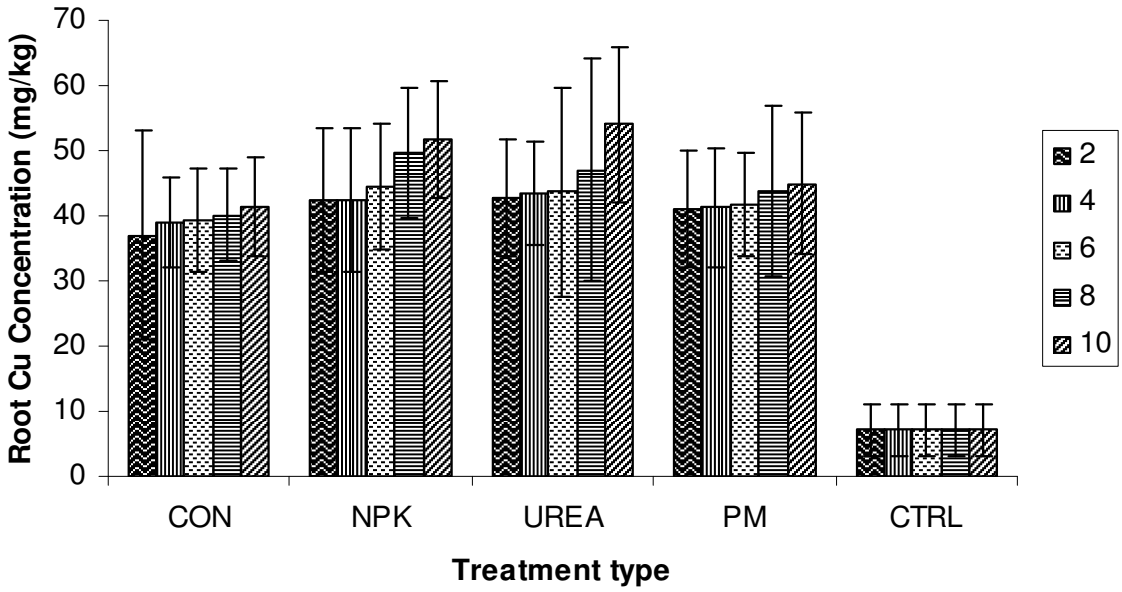

Figure 2. Root Cu concentrations at 12WAG. Values denote mean ± SEM (n = 3).

composting Bambara bean plants grown in Cu–contaminated soil and then applying the compost to a Cu–deficient soil could be an effective technique for remediation of contaminated soils and redistribution of the copper as a plant nutrient for copper–deficient soils. Findings may have been exaggerated in the pot experiment due to the limited soil volume explored by the plant roots in pots compared with field conditions hence the need for a field trial. The Bambara bean grains (Table 3) did contain a Cu concentration sufficiently high to pose a risk for the food chain at 6% and above of contaminant concentration. It exceeded the narrow range (5 – 8 mg/kg) of grain Cu concentrations reported by Tang et al. (2009) and also falls above the Chinese maximum permissible concentration limit for copper in foods 10 mg/kg; GB 15199 – 94, 1994.

Rats fed un-amended contaminated diet at higher spill concentrations suffered enlarged stomach wall after 10 days of feeding trials. Inhibition of trpsin by trpsin inhibitors in raw soya flour had been thought to result in a

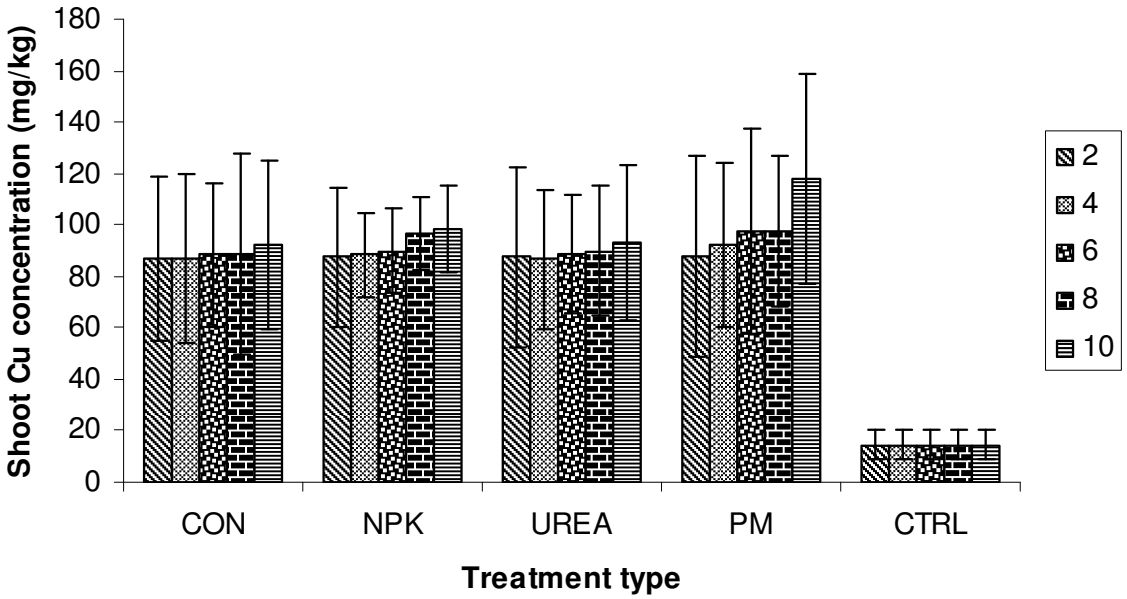

Figure 3. Shoot Cu concentrations at 12WAG. Values denote mean ± SEM (n = 3).

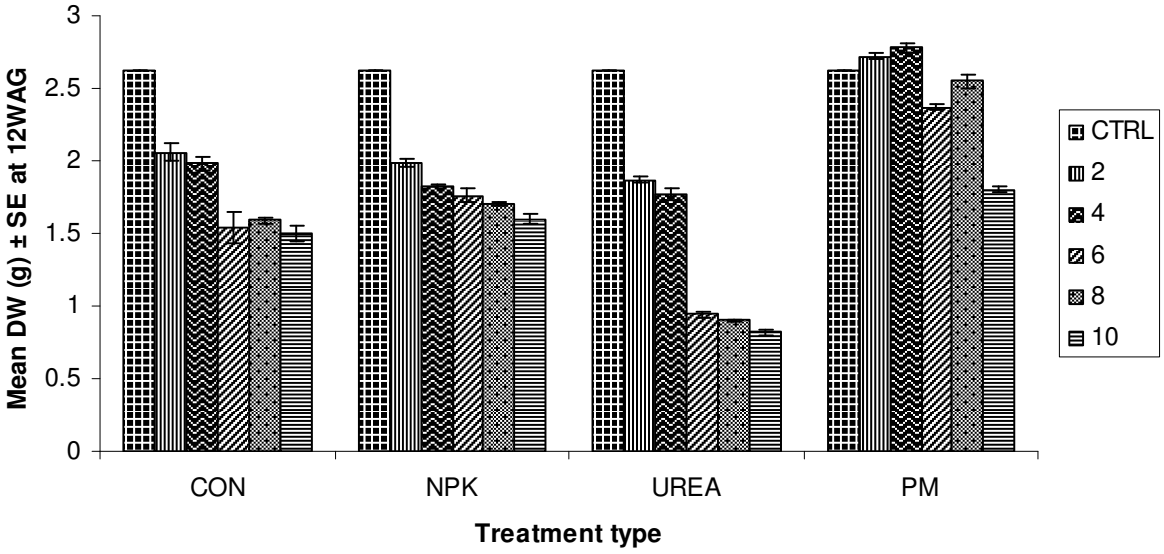

Figure 4. Mean Shoot biomass (g) ± SEM for plants grown for 12 weeks.

Table 2. Mean levels of Cu (mg/kg) in vigna fruits harvested.

TRMT	CTRL	CON	NPK	UREA	PM
2	1.2 ± 0.11	NA	NA	5.03 ± 0.2j	4.44 ± 0.14cd
4	1.2 ± 0.11	5.89 ± 0.32c	NA	5.6 ± 0.41jb	4.7 ± 0.22d
6	1.2 ± 0.11	8.4 ± 0.6d	NA	6.5 ± 0.52jc	4.84 ± 0.3bc
8	1.2 ± 0.11	10.3 ± 0.83ef	NA	NA	5.5 ± 0.17a
10	1.2 ± 0.11	10.97 ± 0.7ef	NA	NA	NA

Values denote mean ± SEM (n = 3). Different letters within each column indicate difference by least significant digit ($p < 0.05$). NA = Not applicable/No fruits.

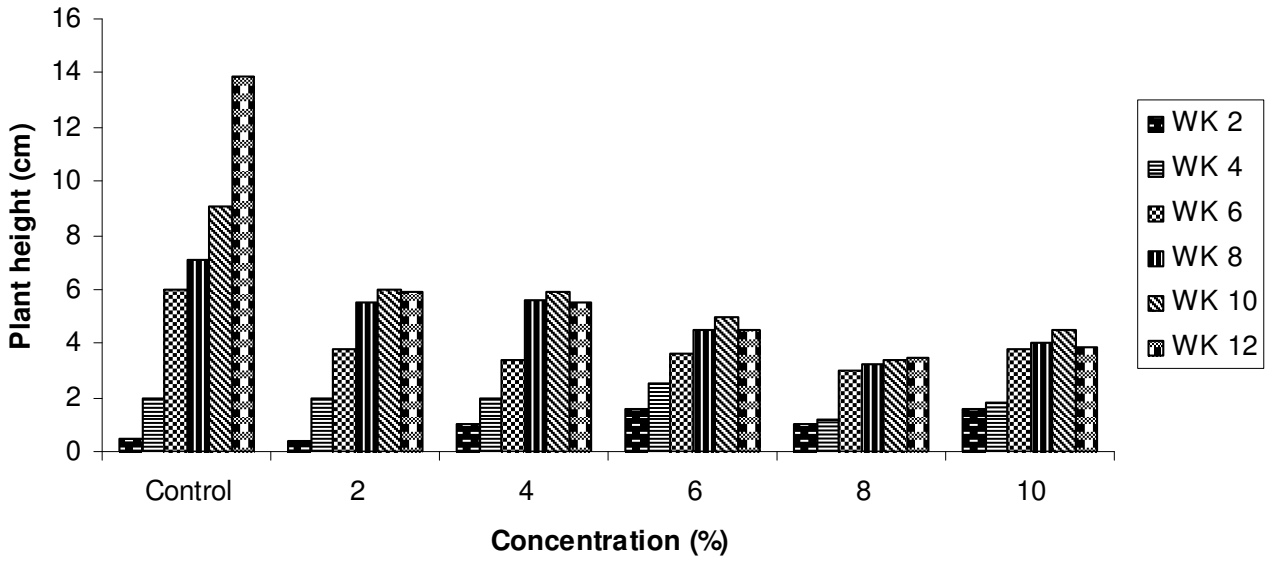

Figure 5. Plant height (cm) for plants grown in contaminated, un-amended soil at 12WAG.

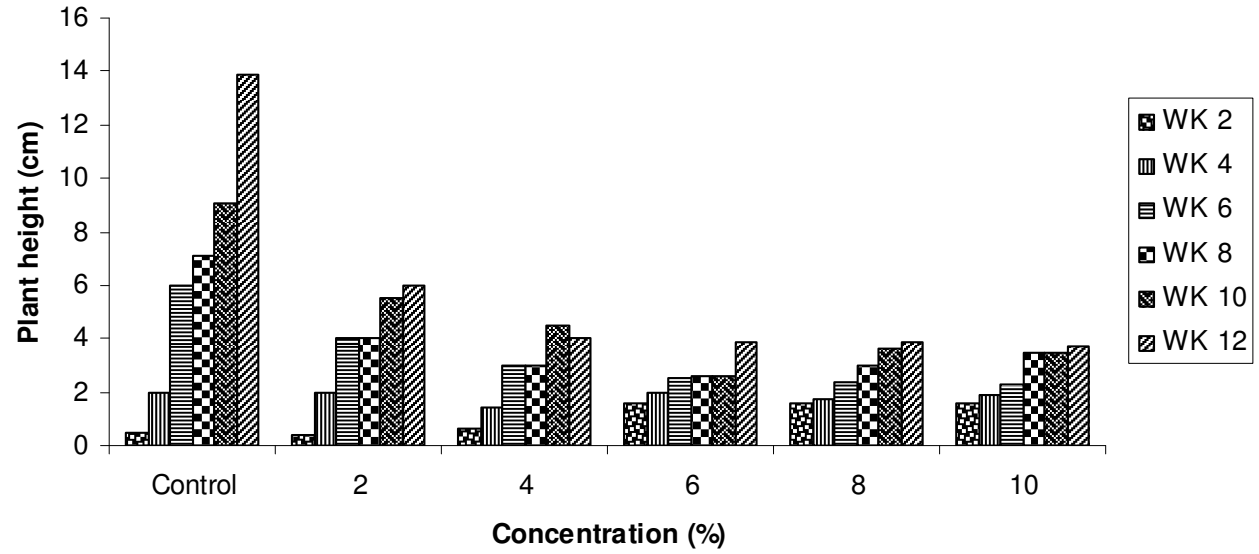

Figure 6. Plant height (cm) for plants grown in contaminated, UREA-amended soil at 12WAG.

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 increment in most organ weight (Table 4) with increased contaminant concentration could be due to increased hepatic enzyme activity, though improvement was recorded with contaminant removal. Amendment ameliorated the effect of pollution. 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 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. The rats fed diet CON gave least relative growth rate. Carcass crude protein (Table 5) decreased with increased contaminant dose while fecal crude protein increased with decreased contaminant dose. Although, the diets met the lysine, methionine and other nutrient requirements of these animals (National Research Council, 1994), increasing levels of contaminants may have adversely affected nutrient utilization probably due to the presence of some antinutritional substances (Onwudike and Eguakun, 1992; Apata and Ologhobo, 1997) and potentially toxic metals contained therein. The poorer live weight gain and final live

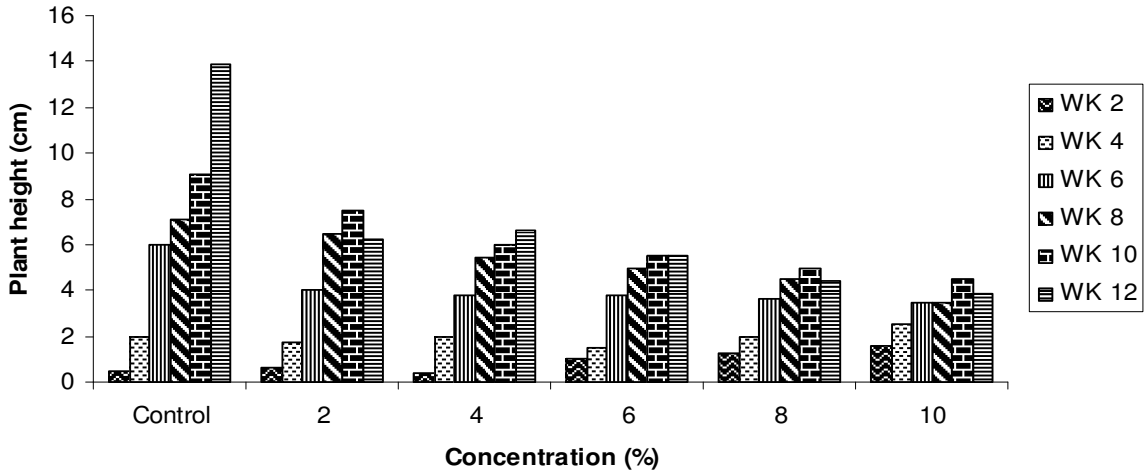

Figure 7. Plant height (cm) for plants grown in contaminated, NPK-amended soil at 12WAG.

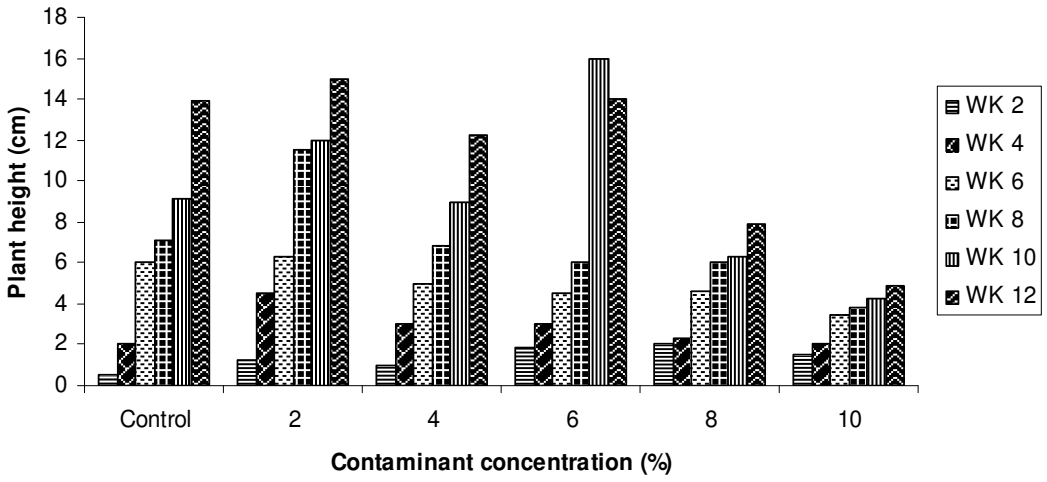

Figure 8. Plant height (cm) for plants grown in contaminated, Poultry manure-amended soil at 12WAG.

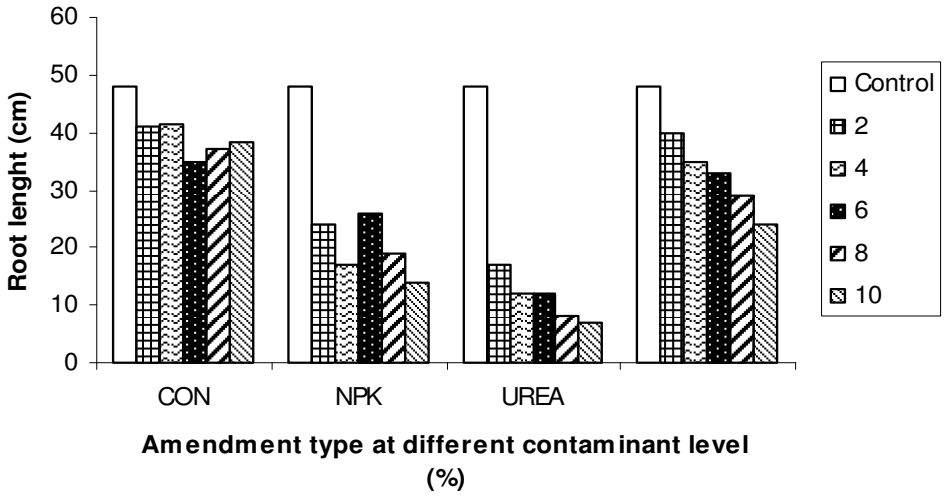

Figure 9. Root length (cm) for plants at different contaminant levels and amendments.

Table 3. 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	$100^{a} \pm 1.1$	$170.7^{i} \pm 7$	$70.7^{a} \pm 8.0$	$1.67^{e} \pm 0.1$	0.98a ± 0.02	$38.22^{h} \pm 6.7$	$0.93^{a} \pm 0.1$
CTRL2	$100^{a} \pm 0.4$	$197^{j} \pm 7.2$	$82^{a} \pm 7.01$	$1.94^{e} \pm 0.1$	$1.03^{a} \pm 0.05$	$45.77^{h} \pm 6.9$	$1.07^{b} \pm 0.2$
4 CON	$118^{b} \pm 0.5$	$173^{k} \pm 8.0$	$55^{b} \pm 8.2$	$1.30^{f} \pm 0.2$	$0.75^{c} \pm 0.04$	$32.08^{i} \pm 7.1$	$0.68^{c} \pm 0.08$
6 CON	$125^{b} \pm 0.8$	$180^{l} \pm 8.0$	$55^{b} \pm 5.7$	$1.30^{f} \pm 0.2$	$0.75^{d} \pm 0.04$	$32.17^{i} \pm 6.9$	$0.68^{c} \pm 0.09$
8 CON	$126^{c} \pm 0.8$	$181^{l} \pm 9.1$	$55^{b} \pm 5.74$	$1.30^{f} \pm 0.2$	$0.76^{dc} \pm 0.03$	$32.05^{i} \pm 7.4$	$0.68^{c} \pm 0.09$
10 CON	$125^{c} \pm 0.6$	$175^{m} \pm 4.9$	$50^{c} \pm 6.0$	$1.18^{g} \pm 0.1$	$0.69^{dc} \pm 0.05$	$31.50^{i} \pm 7.1$	$0.58^{d} \pm 0.05$
2 PM	$115^{a} \pm 0.8$	$188^{j} \pm 4.9$	$73^{a} \pm 6.1$	$1.73^{e} \pm 0.11$	$1.00^{a} \pm 0.09$	$44.26^{j} \pm 6.8$	$0.96^{eb} \pm 0.09$
4 PM	$100^{ab} \pm 0.9$	$170^{n} \pm 5.6$	$70^{a} \pm 6.6$	$1.66^{h} \pm 0.12$	$0.95^{a} \pm 0.07$	$43.34^{j} \pm 6.7$	$0.92^{e} \pm 0.09$
6 PM	$100^{d} \pm 0.4$	$170^{n} \pm 4.8$	$70^{a} \pm 6.1$	$1.66^{h} \pm 0.12$	$0.95^{a} \pm 0.08$	$42.96^{j} \pm 8.1$	$0.92^{e} \pm 0.1$
8 PM	$110^{d} \pm 0.7$	$180^{n} \pm 6.8$	$70^{a} \pm 9.02$	$1.66^{h} \pm 0.1$	$0.95^{a} \pm 0.09$	$37.56^{k} \pm 7.1$	$0.92^{e} \pm 0.1$
2 UREA	$100^{e} \pm 0.7$	$167^{o} \pm 7.2$	$67^{d} \pm 9.4$	$1.59^{i} \pm 0.09$	$0.85^{f} \pm 0.06$	$42.19^{l} \pm 7.7$	$0.87^{f} \pm 0.07$
4 UREA	$125^{e} \pm 1.0$	$190^{p} \pm 4.4$	$65^{d} \pm 7.9$	$1.54^{i} \pm 0.13$	$0.83^{f} \pm 0.06$	$41.27^{l} \pm 8.6$	$0.84^{g} \pm 0.09$
6 UREA	$125^{e} \pm 1.2$	$189^{p} \pm 4.5$	$64^{d} \pm 6.3$	$1.59^{i} \pm 0.2$	$0.86^{g} \pm 0.05$	$41.05^{l} \pm 8.8$	$0.83^{g} \pm 0.06$

Values denote mean ± SEM (n = 3). Different letters within each column indicate difference by least significant digit ($p < 0.05$). CTRL 1 = 1st control group of rats fed commercial feed only; CTRL 2 = 2nd control group of rats fed commercial feed + beans from control experiment, that is, beans harvested from plants grown on un-spiked soil and un-amended soil; PM = rat group fed commercial feed + beans harvested from Poultry manure - amended treatment; UREA = rat group fed commercial feed + beans harvested from UREA fertilizer - amended treatment; CON = rat group fed commercial feed + beans harvested from spiked, un-amended treatment; 2, 4, 6, 8 and 10 placed by CON, UREA and PM represent different oil spill concentrations.

Table 4. Organ weight (g) of animal.

Diet group	Liver	Heart	Kidney	Lungs	Spleen	Pancreas
CTRL 1	$4.15^{a} \pm 0.02$	$0.60^{h} \pm 0.08$	$0.57^{b} \pm 0.04$	$0.60^{i} \pm 0.11$	$0.56^{c} \pm 0.05$	$0.22^{g} \pm 0.09$
CTRL 2	$5.70^{b} \pm 0.04$	$0.35^{i} \pm 0.08$	$0.54^{b} \pm 0.06$	$0.62^{i} \pm 0.08$	$0.57^{c} \pm 0.04$	$0.22^{gi} \pm 0.05$
40 CON	$5.81^{c} \pm 0.04$	$0.40^{j} \pm 0.04$	$0.80^{c} \pm 0.04$	$0.50^{j} \pm 0.09$	$0.58^{d} \pm 0.05$	$0.49^{h} \pm 0.10$
60 CON	$5.98^{c} \pm 0.03$	$0.50^{j} \pm 0.09$	$0.81^{c} \pm 0.04$	$0.51^{j} \pm 0.15$	$0.59^{d} \pm 0.02$	$0.50^{h} \pm 0.10$
80 CON	$6.02^{c} \pm 0.01$	$0.51^{j} \pm 0.04$	$0.81^{c} \pm 0.05$	$0.51^{j} \pm 0.07$	$0.61^{d} \pm 0.04$	$0.52^{h} \pm 0.13$
100 CON	$8.11d^{c} \pm 0.01$	$0.47^{k} \pm 0.05$	$0.55^{d} \pm 0.05$	$0.53^{j} \pm 0.07$	$0.83^{e} \pm 0.06$	$0.53^{h} \pm 0.16$
20 PM	$5.34^{e} \pm 0.02$	$0.38^{ij} \pm 0.09$	$0.81^{e} \pm 0.07$	$0.70^{k} \pm 0.07$	$0.49^{c} \pm 0.06$	$0.35^{i} \pm 0.07$
40 PM	$7.11^{f} \pm 0.05$	$0.44^{ij} \pm 0.09$	$0.82^{e} \pm 0.01$	$0.85^{k} \pm 0.11$	$0.48^{c} \pm 0.06$	$0.35^{i} \pm 0.05$
60 PM	$6.02^{f} \pm 0.03$	$0.50^{l} \pm 0.06$	$1.00^{e} \pm 0.01$	$0.74^{k} \pm 0.11$	$0.60^{e} \pm 0.09$	$0.35^{i} \pm 0.06$
80 PM	$6.30^{g} \pm 0.03$	$0.32^{l} \pm 0.07$	$0.65^{e} \pm 0.04$	$0.75^{k} \pm 0.14$	$0.57^{e} \pm 0.05$	$0.30^{i} \pm 0.14$
20 UREA	$6.31^{g} \pm 0.03$	$0.32^{l} \pm 0.07$	$0.66^{e} \pm 0.04$	$0.57^{l} \pm 0.13$	$0.47^{f} \pm 0.04$	$0.30^{i} \pm 0.13$
40 UREA	$6.34^{g} \pm 0.04$	$0.47^{m} \pm 0.07$	$0.93^{f} \pm 0.02$	$0.94^{m} \pm 0.11$	$0.72^{f} \pm 0.07$	$0.30^{i} \pm 0.09$
60 UREA	$6.34^{g} \pm 0.04$	$0.50^{m} \pm 0.09$	$0.94^{f} \pm 0.04$	$0.96^{m} \pm 0.09$	$0.74^{f} \pm 0.07$	$0.31^{i} \pm 0.11$

Values denote mean ± standard error (n = 3). Means in the same column having the same letters are not significantly different at $p \leq 0.05$.

weight of rats fed diets formulated from 6, 8, and 10% contaminated soils, when compared to those fed control diets and diets formulated from 2% contaminated soil, could also be attributed to imbalance between the first limiting amino acid and the background level of other amino acids (Amaefule and Osuagwu, 2005), considering the amino acid profile of Bambara bean seeds. The feed conversion ratio (FCR) of rats fed diets formulated from 2% contaminated soil did not significantly differ ($P > 0.05$) from each other, irrespective of amendment applied during cultivation but were significantly higher ($P < 0.05$) than those of rats fed diet formulated from 4, 6, 8, and 10% contaminated soil with the exception of those of the UREA amended produce. There was no marked variation among rats fed poultry amended produce at all pollution concentrations considering the FCR (Table 3). Body weight change followed the same pattern as FCR for the rats fed poultry amended produce at all pollution concentrations, while pattern was typical of WDC for rats fed poultry amended produce at all pollution concentrations. PER, FCR, and RGR increased with increasing live weight as shown in Table 3. The toxicity effects were

Table 5. Mean %crude protein in animal.

Diet group	Carcass	Fecal
CTRL 1	42.00a±5.66	23.63b±6.78
CTRL2	54.73b±5.90	16.00c±5.96
40 CON	21.88c±6.00	16.25c±6.73
60 CON	19.38c±5.92	17.25d±6.89
80 CON	13.88c±6.77	18.75d±6.50
100 CON	10.94c±6.55	25.81e±4.99
20 PM	35.00d±7.01	12.25f±7.21
40 PM	34.38d±5.44	13.81f±4.70
60 PM	31.50d±7.12	17.50d±6.72
80 PM	30.25d±7.50	18.69d±6.34
20 UREA	29.75d±6.44	18.50d±6.68
40 UREA	31.00e±6.52	14.88g±5.06
60 UREA	27.56e±6.77	13.56g±5.89

Values denote mean ± Standard Error (n = 3). Means in the same column having the same letters are not significantly different at $p \leq 0.05$.

attributed to the increased phytoavailability of the heavy metals.

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REFERENCES

Alkorta I, Hernandez – Allica J, Becerril JM, Amezaga I, Albizu I, Onaindia M, Garbisu C (2004). Chelate – enhanced phytoremediation of soils polluted with heavy metals. Rev. Environ. Sci. Biotechnol. 3: 55-70.

Allen SE, Grimshaw HM, Parkinson JA, Quarmby C (1984). Chemical Analysis of Ecological Materials. Blackwell Scientific Publications. Oxford London, Edinburgh Melbourne pp. 137-148.

Amaefule KU, Osuagwu FM (2005). Performance of Pullet chicks fed graded levels of Raw Bambarra groundnut (*Vigna subterranean* (L.) Verdc) offal diets as replacement for Soybean meal and Maize. Livestock Res. Rural Dev. 17(5).

Apata DF, Ologhobo AD (1997). Trypsin inhibitor and other anti-nutritional factors in tropical legume seeds. Trop. Sci. 37: 52-59.

Blaylock MJ, Huang JW (2000). Phytoextraction of metals. In: I. Raskin and B.D. Ensley eds. Phytoremediation of toxic metals: using plants to clean-up the environment. New York, John Wiley and Sons, Inc., pp. 53-70.

Doku EV, Karikari SK (1971). Bambarra groundnut. Econ. Bot. 25: 225-262.

Fargašovă A (2001). Phytotoxic effects of Cd, Zn, Pb, Cu and Fe on *Sinapsis alba* L. seedlings and their accumulation in roots and shoots. *Biologia Plantarum* 44: 471-473.

GB 15199 – 94 (1994). National Standard of the People's Republic of China. Tolerance limit of Copper in foods.

Jamers A, Van der VK, Moens L, Robbens J, Potters G, Guisez Y, Blust R, De Coen W (2006). Effect of copper exposure on gene expression profiles in Chlamydomonas reinhardtii based on microarray analysis. Aquat. Toxicol. 80: 249-260.

Johnson A, Gunawardana B, Singhal N (2009). Amendments for Enhancing Copper Uptake by *Brassica juncea* and *Lolium perenne* from Solution. Int. J. Phytoremediation 11: 215-234.

Ke WS, Xi HA, Yang Y, Wang WX, Chen SJ (2001). Analysis on characteristics of phytogeochemistry of *Elsholtzia haichowensis* in Daye Tonglushan copper mine. Acta Ecol. Sinica (In Chinese) 21: 907-912.

Marschner H (1995). Mineral Nutrition of Higher Plants. Lond., UK, Acad.

McGrath SP, Zhao FJ (2003). Phytoextraction of metals and metalloids from contaminated soils. Curr. Opi. Biotechnol. 14: 277-282.

Moses OO (2008). Common uses of Vigna subterranean in Bendel Abia State of Nigeria. Personal communication 2/2/08.

National Research Council (1994). Nutrient Requirements of Poultry, 9th Revised Ed. National Acad. Press, Wash. DC, USA.

Onwudike OC, Eguakun A (1992). Effect of heat treatment on the composition, trypsin inhibitory activity, metabolizable energy level and mineral bioavailability of Bambara groundnut meal with Poultry. In Proceedings XIX World's Poultry Congress, Amsterdam, the Netherlands 20-24 Sept., 1992 pp. 508-513.

Onwudike OC, Eguakun A (1994). Nutritional Evaluation of Raw and Heat-Treated Bambara groundnut meals for Starter Broilers birds. J. Agric. Technol. 2: 38-46.

Pilon M, Abdel – Ghany SE, Cohu CM, Gogolin KA, Ye H (2006). Copper cofactor delivery in plant cells. Cur. Opin. Plant Biol. 9: 256-263.

Poulter NH (1981). Properties of some protein fractions from Bambara groundnut (*Voandzeia subterranea* (L) Thouras). J. Sci. Food Agric. 32: 44-50.

Tang M, Hu F, Wu L, Luo Y, Jiang Y, Tan C, Li N, Li Z, Zhang L (2009). Effects of Copper – Enriched Composts applied to Copper – Deficient Soil on the Yield and Copper and Zinc Uptake of Wheat. Int. J. Phytoremediation 11: 81-93.

Temple VJ, Aliyu R (1994). Proximate Composition of the Cream Coloured decorticated Seeds of Bambara groundnut (*Voandzeia subterranea* (L) Thouras). BioSci. Res. Communication. 6: 51-54.

Vasiliadou S, Dordas C (2009). Increased concentration of soil cadmium affects on plant growth, dry matter accumulation, Cd, and Zn uptake of different Tobacco cultivars (*Nicotiana tabacum* L). Int. J. Phytoremediation 11: 115-130.