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Phytoextraction of Cadmium from Petroleum Contaminated Soil by *Vigna subterranean*

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Abstract

Pot experiments were carried out to investigate cadmium (Cd) uptake and accumulation by Vigna subterranean from soil artificially spiked with Nigerian Bonny light crude oil (up to 100mL/2kg soil). Effects of amendments such as poultry manure, NPK and UREA fertilizers on phytoextraction were also investigated. Cd uptake levels of 108.97mg kg^{-1} and 56.25mg kg^{-1} for the shoots and roots, respectively within 12 weeks of study, giving a shoot: root quotient of 1.94 at the highest contaminant dose, were observed. Shoot and root Cd concentrations increased linearly with increasing contaminant dose with or without amendments except for UREA that peaked at 8% contamination. Cadmium uptake by shoots were 101.48, 101.68, 101.75, 108.83 and 108.97 mg kg^{-1} at 2, 4, 6, 8 and 10% (w/v) contamination, respectively suggesting that additional cadmium removal could be achieved by successive revegetation over a growing period. Amendments generally improved Cd phytoavailabilty, thus the levels found in tissues. There was no significant difference between the plant's performance under stress in the nutrient un-amended and amended treatments except at the highest dose of contaminant in the poultry manure amended regime. The results indicate that V. subterranean is a Cd hyperaccumulator with a high capacity to accumulate Cd in the shoots.

Keywords: *Vigna subterranean*; Contamination; cadmium extraction; shoot: root quotient (SRQ); Amendments.

INTRODUCTION

Heavy metal contamination of soils is a major environmental problem worldwide [1-2] and phytoextraction has emerged as a potential cost-effective and environmentally sustainable technique for removing toxic metals from soils [3]. Phytoextraction and phytoremediation have been applied successfully for cleaning – up soils contaminated with metals from tannery sludge [4]. Cadmium, Cd is a heavy metal that normally occurs in low concentrations in soils. However, up to 100-120 mg/kg dry weight was reported by Lombi et al. [4]. Sources of anthropogenic metal contamination include smelting of metalliferous ore, electroplating, gas exhaust, energy and fuel production, the application of fertilizers and municipal sludge to land, and industrial manufacturing [6-8]. Ingestion of cadmium contaminated foods and drinks gives rise to local gastro intestinal symptoms including vomiting, diarrhea and in severe cases shock and kidney failure [9].

The main route of Cd absorption for humans is via the food chain and is accumulated mainly in the plant leaves and, to a smaller extent, in other parts of the plant such as fruits and grains. Its uptake is affected by soil factors, agronomic practices, plant species and genotype [10].

Bambara beans/groundnut (*Vigna subterranean* (L.) Verdc), an indigenous African legume, is a rich source of protein and along with other local sources of protein and may be roasted and eaten with palm kernel as snacks [11]. It is one of the under-utilized legumes in the poor countries of the tropics. Bambara groundnut could be used to remediate oil-contaminated soils owing to its unique characteristics viz: toughness to stress, good root system, and bunch growth. The yield however, has to be disposed of properly.

The present study investigated: 1) the uptake of Cd by Bambara beans, 2) the effects of amendments on the phytoextraction efficiency, and 3) the growth response of Bambara beans in different oil spill concentrations.

MATERIALS AND METHODS

17 cores of surface soil (sandy loam) samples (0-15cm depth) from agricultural grassland located at Choba, Eastwest Rivers State of Nigeria with no history of pollution, were collected using a clipped quadrat technique in a stratified random sampling design for optimal sample representation and composited prior to use. Activities in the neighborhood of study site include palmwine tapping, thatch making, firewood fetching and the likes. The air-dried, gently crushed soils were sieved (2mm screen), chemical and physical properties of the soils were also determined prior to experimentation as described by Stewart et al. Crude oil sourced from Nigerian National Petroleum Corporation (NNPC PHRC) was characterized as shown in Table 1 below. [12]. 2kg soil was bagged in sixty-three (63) 45 × 45cm cellophane pots each to represent three replicates of four treatment groups outside the control experiment that consisted of no spiking, nutrient un-amended soil. The four treatments included crude oil contaminated, nutrient un-amended CON; crude oil contaminated, NPK amended NPK; crude oil contaminated, Urea amended UREA and crude oil contaminated, poultry manure amended PM soil groups. Unplanted replicates were also provided for further comparison. The replicates were spiked with crude oil to 2, 4, 6, 8 and 10 % (v/w) derived from toxicity range tests and allowed natural settlement while watering for two weeks. The spiked soil was characterized at this juncture and labeled (PRE-P). % composition of dried fresh poultry manure sourced from a De people's poultry farm in Akwaibom state of Nigeria is as follows - Nitrogen, 1.98; Phosphorus, 1.74; Calcium, 5.25; Potassium, 2; and Magnesium, 4.79. UREA and NPK fertilizers were sourced

from Agricultural Development Programme (ADP) in Port Harcourt, Nigeria. 0.8g of amendments were added according to the method of Akobundu [13] and allowed for two weeks while watering moderately with distilled water sourced from Shell Petroleum Development Company, Port Harcourt of Nigeria.

Table 1: Character of Crude oil used in the study

S/N	Parameter	Amount	S/N	Parameter	Amount
1	API gravity at 60F	38.1	11	Viscosity @ 100F Deg fsu	37.8
2	Specific gravity	0.84	12	Sediment and Water (bsw) v/v%	Trace
3	Characterization factor	11.75	13	Organic Chlorides	8.0
4	Color	Brownish-green	14	Copper strip corrosion	IB
5	Acid number	0.39	15	Carbon residue w/w%	0.92
6	Pour point deg F.	35	16	Iron wt (ppm)	1.0
7	Salt Content Lbs/1000bbl	77.9	17	Vanadium wt (ppm)	2.0
8	Reid water pressure	4.9	18	Nickel wt (ppm)	4.0
9	Sulphur w/w%	0.18	19	Crude volume v/v%	32.7
10	Viscosity @ 60F Deg fsu	54.7	20	Density @ 15^{o}C	0.93

Bambara bean seeds were sourced from Enugu state of Nigeria and were subjected to formazan test, scarification and imbibition for viability and germination enhancement. Average of 10 seeds/ pot were planted and thinned to three plants/ pot after 10 days of germination/ seedling emergence. The pots were irrigated with distilled water to keep the moisture at about 70% of the water – holding capacity. Measurement of growth parameters (leaf area, plant height, and root length) commenced two weeks after germination, WAG at 2-weeks intervals of growth period. Available crop residues/remains, including straw and organic wastes were put back to maintain the humus content of their soils by incorporation. At the end of the 12 weeks study period, harvested plants were separated into shoots and roots. The plants were washed first with tap water and then with deionized water. Roots were immersed in a solution of 20mmol 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^{o}C for 48h, and then their dry weights were recorded and were ground and digested with aqua-regia (HCl and HNO_3 in the ratio of 3:1). Cd was determined by atomic absorption spectrophotometry (Varian SpectrAA 220 FS, Varian, Palo Alto, CA, USA). Blanks and plant standard reference materials were included in each batch for quality control.

Statistical Analysis

Analysis of variance was performed on each measured variable and means and standard errors (SE) were calculated. The least significant difference ($p < 0.05$) was used for multiple comparisons among treatment means. Descriptive statistics were calculated using the SPSS v. 13.0 software package and Microsoft Excel.

RESULTS AND DISCUSSION

The specific root surface area, phytomass, leaf size and shoot size were reduced under the effects of petroleum. However, *Vigna subterranean* exhibited good adaptation and tolerance in stress conditions simulated. Plant roots were observed to be most elongated in un-amended crude oil contaminated soil in search of water and nutrients. A simple index of plant vigor, measured as the ratio of the plant weight to plant height was relatively high and thus recommendable for this technique of interest. Jeopardized fruit yield seen with NPK amended treatment could be linked with observed low magnesium level (data not shown). Leaf area decreased with time for all treatments after 6 weeks of growth (data not shown). Approximately 28% and 19% Cd removal was achieved in unplanted, unamended soil at 2 and 10% respectively (Table 2a). These levels were improved to 34% and 27% using UREA amendments (Table 2b).

Table 2a: Soil Cadmium removal (mg/kg) achieved at plant harvest

TRMT	CON	NPK	UREA	PM	PRE-P
IXTER	11.60a±5.55	11.60a±5.55	11.60a±5.55	11.60a±5.55	NA
CTRL	8.76 q±4.33	8.76 q±4.33	8.76 q±4.33	8.76 q±4.33	NA
2	136.25d±6.22	125.50m±6.12	124.62k±5.22	104.12d±5.43	304.50o±6.13
4	137.70de±4.55	127.95m±6.12	126.37de±6.07	105.75d±5.23	304.87o±6.34
6	148.25e±4.56	137.00me±6.34	126.65ef±9.02	116.05ed±6.00	314.88op±6.23
8	148.25e±4.62	147.45e±7.44	127.20ef±11.02	116.17e±6.20	318.12pq±9.12
10	169.15fg±5.32	149.00f±4.53	139.25n±7.56	128.85fg±7.07	329.75r±7.77

Values are means ±SE, n = 3, values followed by different letters in the same column are significantly different at p < 0.05. NA = Not applicable.

Table 2b: Soil Cadmium removal (mg/kg) achieved at the 12th Week in unplanted soil

TMT	CON	NPK	UREA	PM	PRE-P
2	221.43h±5.85	203.15h±10.02	203.08h±6.44	205.22hi±6.05	304.50o±6.13
4	222.06h±4.44	204.08hi±7.23	203.99hi±4.32	205.27hi±6.22	304.87o±6.34
6	234.38hi±7.04	210.38j±8.57	208.36hij±4.11	221.43h±6.12	314.88opq±6.23
8	255.51jkl±8.32	238.23jk±8.24	232.51ij±7.92	241.60ij±5.76	318.12pq±9.12
10	268.25lmj±7.76	239.19jkl±9.01	239.13ijk±9.01	247.05jkl±4.66	329.75r±7.77

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

On the other hand, phytoremediation further improved the removal efficiencies to 55% and 49%; 59% and 55%; 59% and 58%; 66% and 61% for unamended; NPK amended; UREA amended and poultry amended types (Table 2a). Cd, a potentially toxic heavy metal has its observed levels exceed the intervention value of 12mg/kg proposed by EGASPIN ([14], but was not very significant when compared with the values obtained during baseline studies. These values compare favorably with values reported for Osuji et al. [15] and Chen et al. [16] that described Cd levels ranging from 175 - 378 mg/kg as high. The high concentration of heavy metals in the polluted soils could be attributed to the presence of these metallic ions and trace elements in the crude oil. Cadmium hyperaccumulators are defined as plants that are capable of accumulating more than 100mg Cd/kg in the shoots [17]. Until now, only a small number of Cd hyperaccumulators have bee identified, including A. halleri, T. caerulescens and S. alfredii Hance [18]. In the present study, Cd concentrations in *V. subterranean* shoots varied from 101.48 to 139.38mg/kg. Biomass production showed no statistically significant differences, even at contaminant concentrations up to 10% (w/v) (Fig 6). This indicates that Bambara beans can tolerate and accumulate high concentrations of Cd. However, the plants grew slowly and remained radiant as reported for *S. jinianum* [19]. The present results indicate that Bambara beans accumulated more Cd in the shoot than in the roots (tables 3 and 4).

Table 3: Mean Cadmium uptake (mg/kg) by root

TMT	CON	NPK	UREA	PM
CTRL	0.89c±0.01	0.89c±0.01	0.89c±0.01	0.89c±0.01
2	30.13h±0.85	33.15h±1.00	34.98h±1.44	33.22h±6.05
4	30.21h±1.44	40.08h±1.23	35.99h±1.32	33.27h±6.22
6	36.38ijh±1.00	40.38h±1.57	51.13ih±2.11	40.43hg±6.12
8	41.10ij±1.32	50.23hk±1.24	51.51hg±1.90	50.60hi±5.76
10	56.25j±1.76	66.00lm±2.00	90.13jh±2.00	71.05jk±4.66

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

Table 4: Mean Cadmium uptake (mg/kg) by Shoot

TMT	CON	NPK	UREA	PM
CTRL	9.73b±0.22	9.73b±0.22	9.73b±0.22	9.73b±0.22
2	101.48f±2.22	104.33n±1.33	103.50f±0.33	111.50f±0.56
4	101.68f±1.23	105.99f±0.34	103.65f±1.05	116.68fg±0.60
6	101.75g±1.89	108.65f±1.09	106.88fg±1.05	118.79g±0.78
8	108.83ij±3.22	110.75gf±1.07	108.83gh±0.88	138.83hi±1.34
10	108.97ijkg±2.58	110.98i±1.05	94.97i±0.88	139.38hij±1.78

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

The biggest reduction in dry matter did not happen in low-biomass cultivars, but in cultivars with toxic contaminant dose. Cadmium toxicity was shown to affect plant growth in the present study only at higher concentrations. Furthermore, this was influenced significantly by nutrient level (especially with poultry manure amendment); water content and agronomic practices (tillage). Also, significant increases in Cd concentrations resulted in marked decrease in plant height and could be due to some genetic mechanisms of Cd tolerance in Bambara beans.

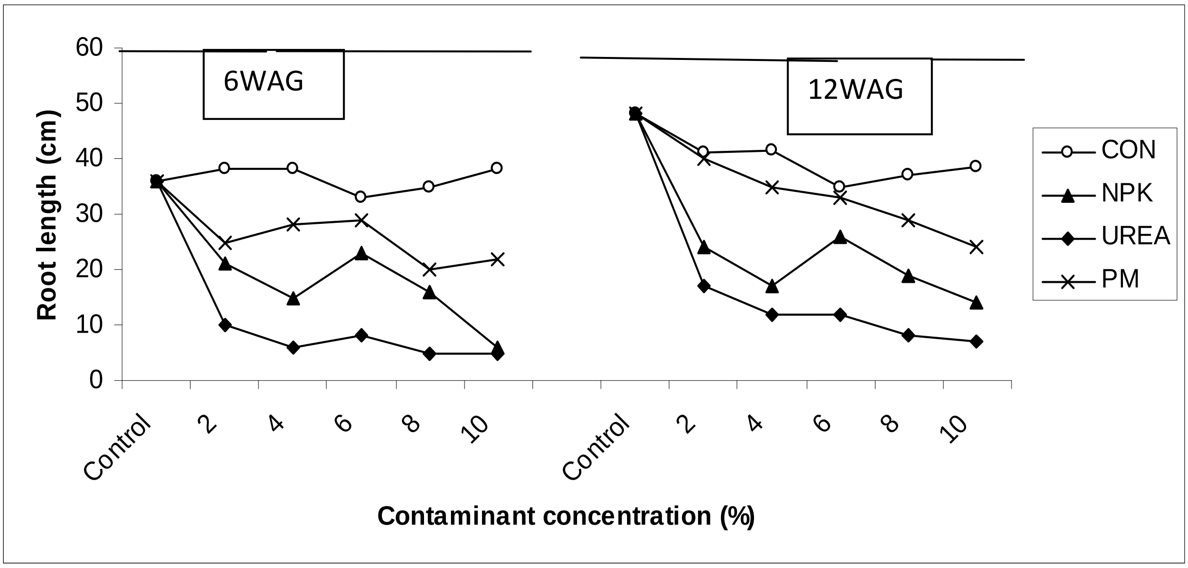
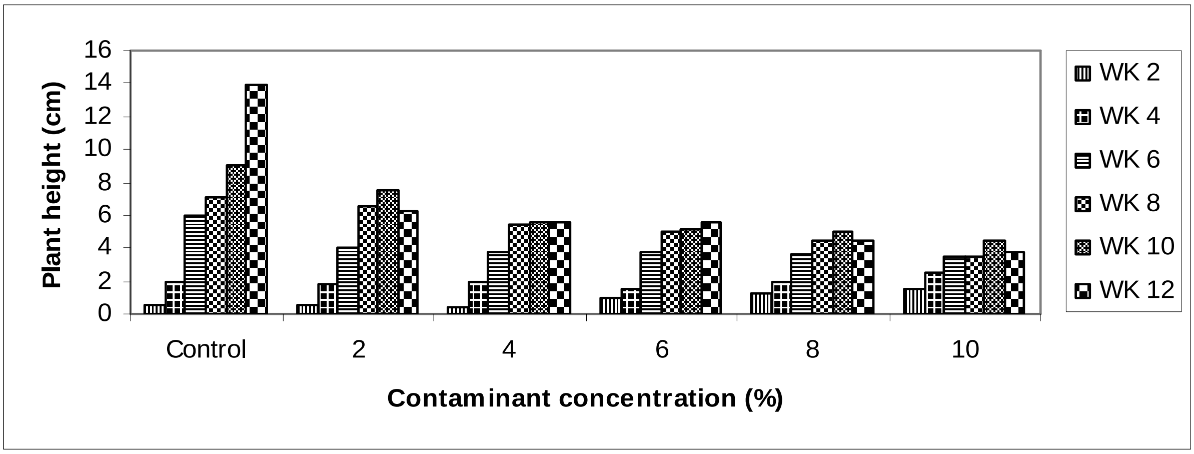


Fig 1 Mean Root length (cm) by Bambara beans at 6 and 12 WAG

Comparison of trace metal levels from post impact assessment, PIA and post remediation assessment, PRA showed that the amounts after remediation activities were lower than those of PIA. The mean Cd level of 329.75 mg/kg during PIA was over 59% higher than its corresponding PRA values. Heavy metal, Cd concentrations in vegetative samples were greater than maximum permissible levels, MPL of 0.3µg/g for plants grown in contaminated soil and showed significant variation ($p \geq 0.05$) compared to control and were considered potentially unsafe for human consumption. Generally, Cd extraction from a petroleum contaminated soil was efficient with *Vigna subterranean*, un-amended or amended with biostimulants and could be improved by successive revegetation and/ or extended time as growth indicators were still active as at the end of experiment. Root length is supportive of this suggestion as it increases were recorded going from 6WAG to 12WAG (Fig 1).

Fig 2 Plant height (cm) observed trend with time during the study period for CON



Compared to other amendments, poultry manured plants developed longer roots in search of nutrients as it is not readily available as NPK and UREA fertilizers. UREA amendment not only stunted the growth of plants as that in contaminated and un-amended treatment (Fig 2), but did not support effective translocation to the leaves. The acidity generated when ammonium get converted to nitrate could be a factor.

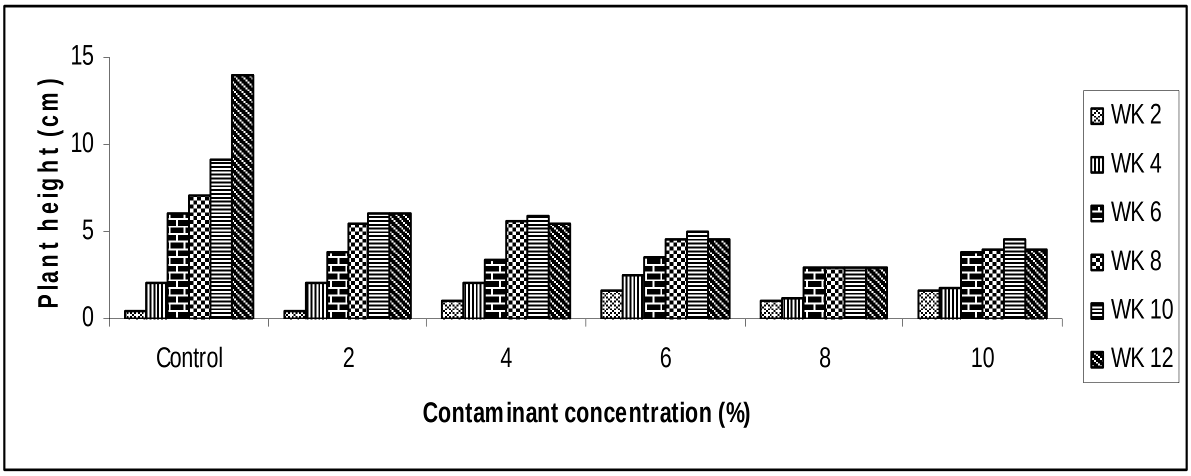


Fig 3 Plant height (cm) observed trend with time during the study period for NPK

Also, NPK treatment as well as POULTRY MANURE enhanced plant growth significantly in contrast to that observed with UREA (Figs 3, 4 and 5). Shoot biomass produced (Fig 6) is also indicative of best amendment option in this specie - specific technique using POULTRY MANURE. The effect of the contaminant concentration was significantly different especially without POULTRY amendment usin more reliable growth index called produced biomass.

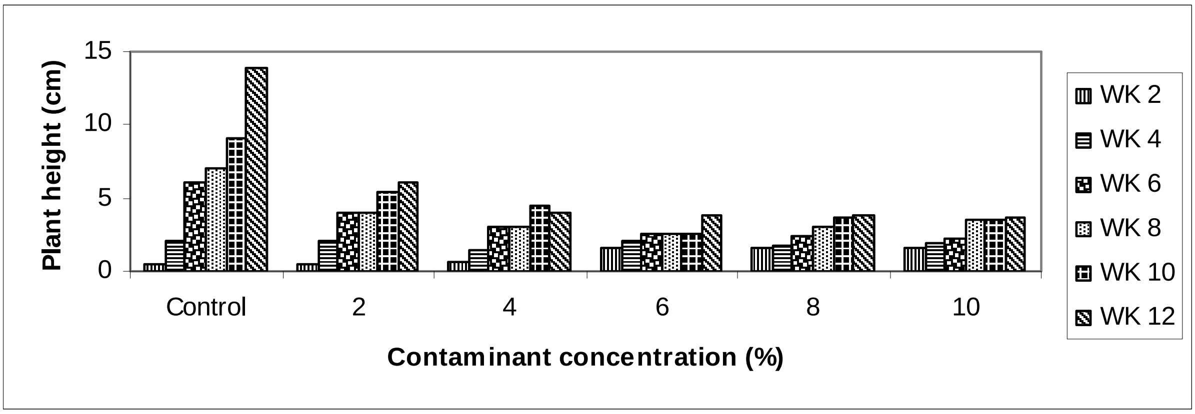


Fig 4 Plant height (cm) observed trend with time during the study period for UREA

Translocation coefficient expressed as the ratio of the Cd concentration in the shoots to that of the roots, was best promoted with poultry amendments. This is a useful indication of the ability of amendments to improve contaminant concentration to the shoots. This may equally mean that *V. subterranean* has a naturally high rate of solute translocation owing to the uptake mechanism inherent in dicotyledons. Also, Bambara beans provided a vegetative cover that did not endanger local ecosystems as well as exposing soil to inherent drought.

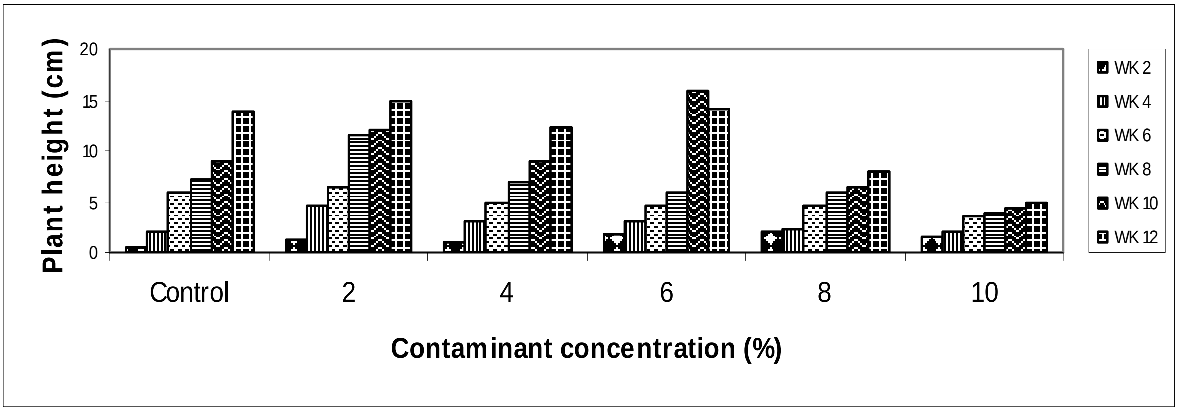


Fig 5 Plant height (cm) observed trend with time during the study period for PM

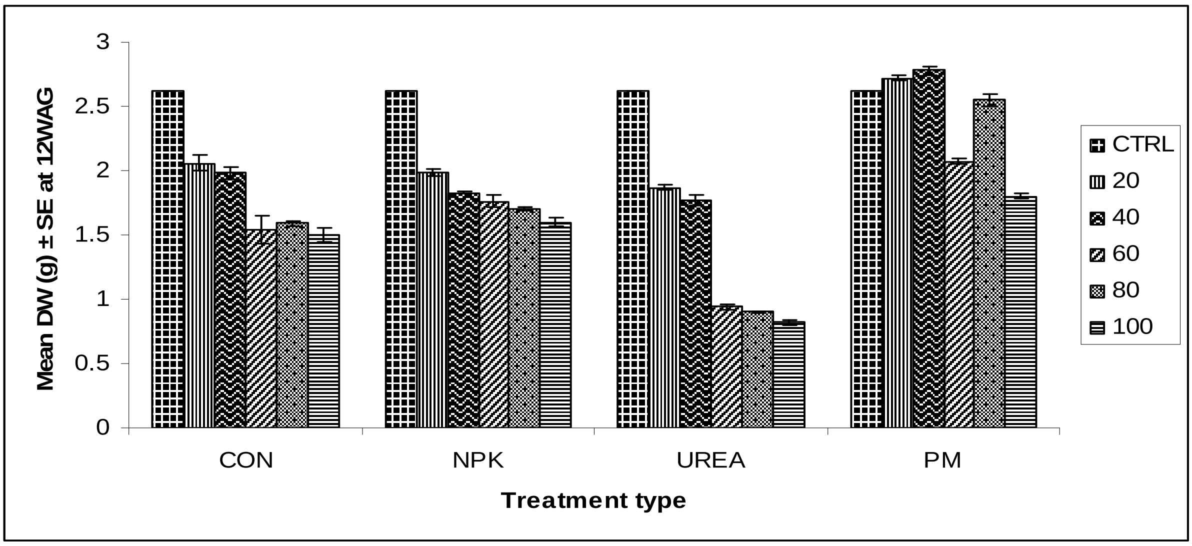


Fig 6 Produced dry biomass (g) at 12 Weeks after germination, WAG

This present study indicates that Bambara beans can tolerate Cd concentrations up to 329.75mg/kg without a statistically significant reduction in dry matter yield, which is similar to published results for *S. jinianum* ([18] and S. alfredii Hance [19]. The Cd concentrations in the shoots and roots increased linearly with increasing Cd concentration in the soil as was observed for *A. halleri* [20]. The bioaccumulation factors for Cd were more than 1 when the plants were grown in Oil contaminated soil, indicating that *V. subterranean* is a hyperaccumulator that also has a high capacity to accumulate Cd in the shoots. As far as literature can take us to date, this is the first report of Cd hyperaccumulation by Bambara beans. Therefore, this plant species can be enlisted to the Cd hyperaccumulators that may prove useful for future studies on the mechanisms of Cd hyperaccumulation by higher plants and on the phytoremediation of soils contaminated with Cd.

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