J. curcas and *Manihot esculenta* are potential super plants for phytoremediation in multi-contaminated mine spoils.

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Abstract. Phytoremediation approaches have increasingly been applied in environmental remediation projects. In this study, the potentials of *Manihot esculenta (M. esculenta), Vigna unguiculata (V. unguiculata)* and *J. curcas curcas (J. curcas*) in remediating multi-contaminated mine spoils was evaluated. The target potentially toxic elements (PTEs) are Cd, As, Zn, Pb, and Hg. The test plants were grown and monitored under growth stress conditions for 270 days. Using inductively Coupled Plasma- Mass Spectrometry and EPA method 200.8, the total elemental contents in the shoot and root parts of the plants were determined. Significant differences ($p< 0.01$) were observed in the uptake performance of the test plants. For example, *M. esculenta* and *J. curcas*, bioaccumulated 50- 80 % of the various baseline PTE contents in their root parts at 270 days after planting. In contrast, < 27 % of PTEs were found in the root parts of *V. unguiculata*, except Zn at 70 %. Growth stressors and soil PTEs were factors that reduced biomass production in respective plants by 25 %. Cummulatively, the performance order *M. esculenta*> *J. curcas*> *V. unguiculata* was observed for multi-contaminant removal in soils. Techniques for enhancing the easy cultivation of the test plants are recommended to enhance their applicabilities in phytoremediation projects.

1 Introduction

Global attempts to satisfy the wants of mankind drove the era of industrialisation several decades ago. As a results, an increased competition between industrial activities and arable lands have given rise to the degradations of the ecosystem. Characteristically, mining, and other industrial processes have continually and significantly supplied varying amounts of potentially toxic elements (PTEs) in the environment [1–3]. Potentially toxic elements maybe either metals or metalloids established to be detrimental to living organisms if

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present in soil at levels beyond tolerable thresholds [4, 5]. Apart from few metalloids like arsenic (As) and non-metals (Sb), most PTEs such as Cd, Mn, Pb, Zn, Fe, Co are metals. Potentially toxic elements in soils may either be geogenic or manmade source [2, 4]. However, the anthropogenic sources accounts for greater portions of soil contamination owing to increasing industrialisation (pharmaceutical, agrochemical) and resource extractions (mining and processing) [2, 3, 6, 7]. Despite their harmful properties, most PTEs can be appraised as vital plant nutrients (Zn, Mo, Ni, Fe) and plant physiological enhancers (Co, Se, V and Cr) at the right concentrations in soil [1, 4, 8].

The presence and persistence in the environment often aggravate their health risk levels to humans [3, 9–12]. This is due to the possibility of eating contaminated foods and the associated food chain magnification [13]. Notable human health issues resulting from exposures to PTEs are well documented and may include, silicosis, skin defects, pnuemonocosis, respiratory and heart related diseases [3, 14, 15]. Others involve reduced fertility in humans, mental detarations, malfunctioning of vital organs, and birth defects [16, 17]. PTEs are also able to reduce the normal functioning of plant's physiological and metabolical activities. Thus, resulting in undesirable growth, diseases, and low yields of arable and tree crops.

To this end, environmental media contaminated with high PTEs require clean up to avert threats to sound human health in society. Popular conventional techniques used in soil remediation may include; isolation and containment, mechanical separation, ex-situ and in-situ soil washing with chemicals, and bioremediation (plants and microbe use) [2, 18]. All these remediation techniques have certain merits and drawbacks, as they leave secondary residual effects in soils. Nonetheless, phytoremediation (use of accumulator plants in soil cleaning) presents the least harmful effects, cost-effective amongst other techniques and suits the increasing quest for environmental sustainability [4, 18–21]. Phytoremediation may be grouped based on their operating mode and they include; phytoextraction, phytostabilisations, phytofiltration, phytodegradation, phytovolatilisation, and rhizodegradedation [22]. Their applicability is however dependent on the goal of the planned soil remediation programme, as various plants have special attributes.

Nonetheless, the deployment of well-adaptive indigenous hyperaccumulators is central for enhanced success rates during phytoremediation [3, 4]. Thus, selected plants must among other properties should be able to grow, withstand and absorb high PTEs contents in soils conveniently. In this study, we assessed how three native African plants could successfully remediate Cd, As, Hg, Zn and Pb from a gold mine spoils. In our view, this was the maiden study of exploring M. esculenta in soil cleaning experiment under growth stress conditions.

2 Materials and Methods

Figures and tables, as originals of good quality and well contrasted, are to be in their final form, ready for reproduction, pasted in the appropriate place in the text. Try to ensure that the size of the text in your figures is approximately the same size as the main text (10 point). Try to ensure that lines are no thinner than 0.25 point.

2.1 Material Source and Experimental Setup

Pre-analysed bulk soil samples (Table 1) from an alluvial gold mine spoils were used in a pot experiment. For control purposes, topsoil from forest areas >2 km away from known human disturbances were used. Each pot was filled with 12 kg of treated soil, watered, and made to stand for 7 days before planting. The experiment which laid in a randomized complete block design with 4 replication lasted for 270 days after planting (DAP). All specimen used were procured from certified plant growers. Before the actual experiment, *J. curcas* seeds were nursed and transplanted for use after 5 weeks of germination and averaged 7cm in plant height. For *M. esculenta* they were pre-sprouted for 14 days using its stem cuttings whilst V. unguiculata was directly sown by seeds. Plants were watered weekly at 70 % field capacity. No pest control was done throughout the study. Weed controls were done only at 80, 170 and 260 DAP, respectively. However, for the control pots, weeds were monthly controlled. The entire experiment was setup outdoor to mimic natural field conditions. No temperature control was done throughout the study.

2.2 Material Source and Experimental Setup

Plant biomass (shoot and root parts) were sampled at 90, 180 and 270 DAP respectively. Soil samples were collected only at 270 DAP. Plants were carefully harvested to cause fewer or no injuries to the roots. Where roots were lost to the soil, their debris were sufficiently reclaimed. Harvested specimens were washed under running water to remove all soil particles and rinsed with deionised water. Initial parameters (fresh weight and length) were measured, air-dried, oven-dried at 75 °C for 48 hours, cooled, weighed, and milled accordingly with ZM1000, Retsch, Germany. Sample weight of 100 mg each was microwave digested with Ethos plus 2, MLS as recommended by Krachler et al., [23] and Okoroafor et al., [24]. The digestion was aided by 3 mL nitric acid and 0.1 mL hydrofluoric acid. The total concentration of the obtained extracts was measured using ICP-MS (Xseries 2, Thermo Scientific). The elemental value obtained per sample was multiplied by the corresponding dry biomass weight per plant to obtain the actual total contents in each plant.

For the total elemental contents in soils, 0.5 g of pulverised sample, and 2 g of even portions of Na_2CO_3 and K_2CO_3 were homogenised for the melting digestion technique as employed by Okoroafor et al., [24]. Thus, heated the mixture at 900 degrees for half an hour in a muffle furnace, cooled and liquified with 50 mL of a solution of 2 mol/L nitric acid and 0.5 mol/L citric acid. The resultant solutions were also measured for their target elements using ICP-MS (X series 2, Thermo). We used 10 ug/L of rhodium and rhenium for quality control measurement purposes.

2.3 Estimation of phytoremediation potentials of test plants

The movement of PTEs from soil to plant indices was used to ascertain the suitability of the selected test plants for remediation after the experiment. This involved estimating how much either the shoots or roots had stored PTE from the soil through their bio-accumulation coefficient (BAF) in equation 1 or bio-concentration factor (BCF) in equation 2, relative to the post remediation soil PTE content [1, 4].

$$BCF = PTE\ in\ Root/soil\ \text{(mg/kg)} \tag{1}$$

$$BAF = PTE\ in\ Shoot/soil\ \text{(mg/kg)} \tag{2}$$

Only estimated BCF or BAF values not less than 1 (< 1) were considered suitable accumulators. But to understand the test plants' capacity to move target PTEs from their root zones into above ground structures we calculated the translocation factor (TF) using equation 3.

$$TF = PTE\ in\ Shoot/root\ \text{(mg/kg)} \tag{3}$$

TF values ≥ 1 are preferable for phytoextraction projects. Whereas plants having BCF $> 1 <$ TF were appraised as potential phytostabilisers by this study [4, 25].

2.4 Data Analysis

Using IBM SPSS statistic 28, the Welch's Analysis of Variance (ANOVA) and Games-Howell's post-hoc tests were deployed to compare the mean values for estimated/ measured parameters (total elemental contents in plant parts, dry biomass weight, root lengths) at 95 % confidence level. Graphs were generated using Microsoft excel 2020.

3 Results and discussion

Background Values
The initial background soil information for the contaminated mine spoil (CMS) and topsoil (TS) for control before the planting are presented in Table 1.0 below. Generally, the soil textures were silty-clay for the contaminated mine spoil (CMS) and loamy-clay for the control soil used (CS). Plant available nutrients and trace element concentrations varied sharply between the two soil types. Whereas CMS had higher concentrations of PTEs and very low soil nutrients, the TS had the reverse. Varying temperatures and humidity were experienced throughout the experiment and averaged 35.2 ℃ and 65 %, respectively.

Table 1. Chemical characteristics of background soils for experiment.

Parameter	CMS	TS
Cadmium (Cd) mg/kg	30.78	0.74
Arsenic (As) mg/kg	368.17	6.20
Lead (Pb) mg/kg	37.10	0.00
Mercury (Hg) mg/kg	6.43	0.30
Zinc (Zn) mg/kg	288.21	34.80
Soil Carbon	0.90	4.5
Total N (%)	0.08	0.50
Available P (mg/kg)	3.82	5.04
pH	4.33	5.85
EC (is/cm)	1970.00	1200.00
Mg (Cmol/ kg)	0.40	0.60
Ca (Cmol/kg)	2.20	1.80
K (Cmol/ kg)	0.09	0.20

After our assessments, all three test plants were efficient in removing total soil Hg, Cd and Pb signicantly ($p < 0.01$) in the mine spoil. More specifically, for Zn removal the order *J. curcas*> *M. esculenta*> *V. unguiculata* was observed at 152.36, 128.59 and 71.06 mg/ kg, respectively. For As removal the order *M. esculenta*> *J. curcas*> *V. unguiculata* occurred at 206.92, 189.54 and 79.82 mg/ kg, respectively. For Hg removal, *V. unguiculata*> *M. esculenta*> *J. curcas* at 05.11, 04.52 and 3.97 mg/ kg, respectively was attained. Average Cd uptake potentials was as follows *J. curcas*> *M. esculenta*> *V. unguiculata* at 22.90, 20.05 and 19.90 mg/ kg, respectively. Lastly, the order *M. esculenta*> *V. unguiculata*> *J. curcas* at 24.30, 22.04 and 20.45 mg/ kg, was respectively, ascertained for Pb (Figure 1 and 2).

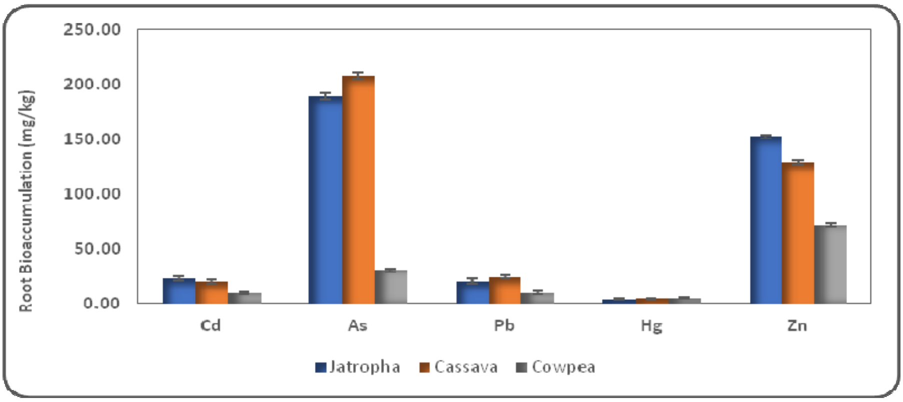

Fig. 1. PTEs bioaccumulation in the roots of test plants.

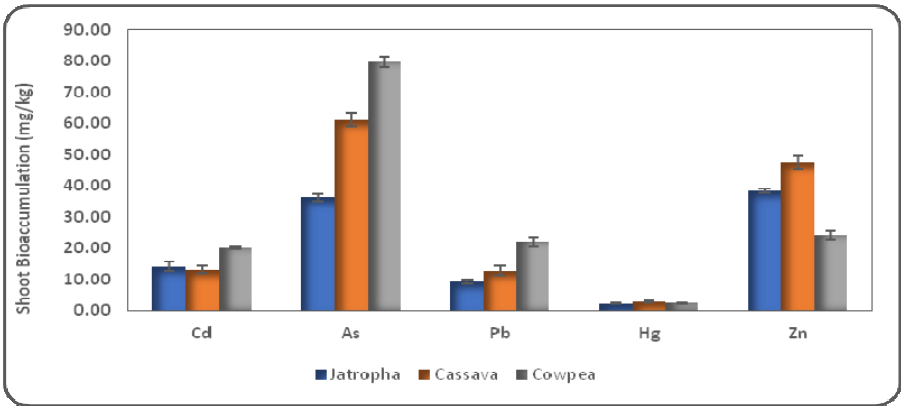

Fig. 2. PTEs bioaccumulation in the shoot of test plants.

Statistically, we found a strong level of significance ($p< 0.01$) in root uptake between all treatments throughout the study. Even though *J. curcas* and *M. esculenta's* uptake of Cd and Pb were comparable at $p= 0.29$ and $p= 0.35$, respectively (post hoc test). J. curcas further mimicked *M. esculenta* and *V. unguiculata* bioaccumulation of soil Hg at $p= 0.55$ and $p= 0.15$, respectively. This indicated a possibility for a common transport mechanism of soil Cd, Pb and Hg in both plants. At 270 DAP the different plants shoot bioaccumulation of $p< 0.01$ was observed for of all target PTEs. The shoots of *J. curcas* and *M. esculenta* had similar uptake capacities for Hg and Cd at $p> 0.86$ and $p> 0.19$ respectively. Also, *J. curcas* and *V. unguiculata* had comparable soil Hg uptake at $p> 0.99$.

A deeper insight on the bioaccumulation factor (BAF), bioconcentration factor (BAC), translocation factor (TF), and the remaining total soil PTE contents after the 270 DAP experiment are presented in Table 2. Calculations showed how both *J. curcas* and *M. esculenta* obtained very high BCF values due to their strong abilities to retain high contents of PTEs in their roots than shoots. Thus, leading to poor (< 1) TF value for all target PTEs. *For V. unguiculata*, it obtained high (>1) TF and BAF values due to its capacities to bioaccumulate more PTEs in its above ground biomass. The differences in biomass output between all treatments are presented in Table 3. Except for *V. unguiculata*, there were no significant differences ($p> 0.05$) between biomass production of *M. esculenta* and *J. curcas* grown in the contaminated soils and controls.

Table 2. Basic phytoremediation indices of test plants.

Parameters (mg/kg)	*J. curcas*					*M. esculenta*					*V. unguiculata*				
	Cd	**As**	**Pb**	**Hg**	**Zn**	**Cd**	**As**	**Pb**	**Hg**	**Zn**	**Cd**	**As**	**Pb**	**Hg**	**Zn**
Soil PTE	5.08	134.56	8.36	2.04	106.06	6.88	115.04	5.02	2.16	129.92	6.98	244.02	6.62	1.04	187.22
BAF	2.77	0.27*	1.11	1.12	0.36*	1.91	0.53*	2.55	1.31	0.3*	2.87	0.33*	3.33*	2.25	0.13*
BCF	4.51	1.41	2.46	1.95	1.44	2.89	1.81	4.84	2.09	1.00	1.35	0.12*	1.50	4	0.38*
TF	0.61*	0.19*	0.45*	0.57*	0.25*	0.6*	0.29*	0.53*	0.63*	0.3*	2.13	2.64	2.21	0.46*	0.34*

X = < 1 mg/kg*

Table 3. Average biomass production of test plants in different growth media.

Plant type	**Treatments**	**Dry biomass weight (g) mean ± SD for DAP**		
		90	180	270
M. esculenta	Control soil	141.6 ± 11.1	229.0 ± 09.9	314.7 ± 23.4
	Contaminated soil	134.0 ± 06.3	183.4 ± 07.6	282.2± 15.7
J. curcas	Control soil	137.4 ± 08.6	217.7 ± 07.2	284.1 ± 20.6
	Contaminated soil	121.5 ± 06.3	188.7 ± 11.0	261.7± 9.0
V. unguiculata	Control soil	159.8 ± 06.4	258.6 ± 09.0	332.5± 11.1
	Contaminated soil	136.2 ± 07.3	233.7 ± 08.1	249.4 ± 14.6

4 Discussions

Influence of soil and plant characteristics on trace element bioaccumulation

Plants have varying inherent abilities to tolerate and/ or bioaccumulate soil contaminants [26]. Bioaccumulation is generally aided by metal dissolving acidic substances associated with plant roots. Despite this, the geo-chemical behaviour and interaction of elements in soil can strongly influence how much PTEs can be mobilised for absorption, translocation and/ or storage by plants. Soil contaminants mostly associate with organic matter in soils and/ or as carbonates [24, 26]. This makes the bioavailability of toxic elements a single but crucial factor to determine the success of any phytoremediation [24].

In this study, we found highly competitive capacities between *J. curcas* and *M. esculenta* in bioaccumulating more soil PTEs than *V. unguiculata* plants. This might be driven by a common transport mechanism in taking up soil Cd, Pb and Hg in both plants owing to similarities in both plants' physiology (same family Euphobiaceace) [24, 27].

This was aided by the secretion of more phytosiderophores in their roots and the relatively longer roots for deeper reach in soils [26, 28]. The less transferability (TF< 1) of bioaccumulated PTEs into the above ground parts of both *M. esculenta* and *J. curcas* plants were adaptive strategies for survival in metal-rich soils and growth stress conditions [13].

For instance, the root tubers of *M. esculenta* are prominent storage organ. They extended much deeper in soils and hold high amount of water than the remaining plants tested. This means, inadequate water for warm temperatures over short durations are insufficient to significantly impede growth. This is confirmed by the variation in total biomass output between plants grown in CMS and TS (Table 3). As only 8, 10 and 25 % biomass loss were observed for *M. esculenta, J. curcas,* and *V. unguiculata,* respectively, by 270 DAP under growth stress conditions.

The observed transfer coefficient (TC) for *M. esculenta* and *J. curcas* (<1) for all PTEs did not satisfy contemporary selection criteria for hyperaccumulators (*e.i, they should be able to store higher amounts of soil contaminants in the above root parts*) as the best plants for phytoremediation. However, we argue that root storage plants hold sufficient potentials for remediation as their uptake indices (calculated BCF for all PTEs was between 1.00-4.84 and 1.41- 4.51mg/ kg for *M. esculenta* and *J. curcas*, respectively) outperformed some recommended hyperaccumulators in total plant uptake [29, 30]. Using root efficient cleaning plants rather offer advantages of limiting potential toxicities to primary consumers in the food web. Parisa et al.,[31] recommended the use of *J. curcas* in phytoremediation after its significant performance in sewage sludge cleaning trials. In Germany, Plant species like *Populus tremula, Tanacetum vulgare, Lotus corniculatus* and *Agrostis capillaris* have also proven effective in removing concentrations of Cd, Zn, As, Pb from former mine sites [12].

It is believed in this study that *V. unguiculata* plants were sensitive mainly to high As and Zn in soils. This led to lower adaptive capacities to prevailing growth stresses. This was manifested through the yellowing of leaves in most growth periods than the remaining plants. Nevertheless, it remained the plant in this study with the shortest lifespan. Therefore, possible stoppage of active crop metabolism are blamed for the relatively poor PTE bioaccumulation efficiency. However, instances where plants as young as 2 weeks have been established to have high phytoextraction potentials than older ones exist. Even though, older plants may have more biomass to compensate same [32].

5 Conclusion and recommendation

Under low nutrient, less water, high temperature and competitive weeds growing regime, *Manihot esculenta* and *J. curcas curcas* were able to remove averagely, 152.36 and 128.59 of zinc, 206.92 and 189.54 mg/kg of As, 20.05 and 22.90 mg/kg for Cd from contaminated gold mine spoils. No significant differences existed amongst the test plants in soil Hg removal. Even though numerically, *Vigna unguiculata* was only efficient in bioaccumulating about 05.11 out of the 6.43 mg/kg in soils. Cumulatively, the efficiency of the test plants after 270 DAP was in the descending order *Manihot esculenta*> *J. curcas curcas*> *Vigna unguiculata.* This study therefore categories *Manihot esculenta* and *J. curcas curcas* as potential phytostabilisers. It was established that growth stress conditions and potentially toxic elements reduced biomass output of plants up to 25 %. Techniques to optimize easy planting and harvesting of these phytostabilisers like *Manihot esculenta* and *J. curcas curcas* are encouraged to fast track future phytoremediation projects. Generated biomass produced from these plants can be used for biomass production. The seeds from the *J. curcas* plant can processed into biodiesel and lubricants for the automobile industry.

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