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STUDIES OF FOLIAR BIOCONCENTRATION OF METALS BY *VERNONIA AMYGDALINA* IN A MODEL HEAVY METAL-POLLUTED SOIL

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Abstract: The study was undertaken to investigate the capability of *Vernonia amygdalina* Delile to bioaccumulate heavy metals in a model heavy metal polluted (MHP) soil. Stems cuttings of *V. amygdalina* were planted in soils polluted with 15, 45, and 90 mg/kg concentrations of Cd and Pb and in combination. After 4 months, results showed a significant reduction in height of plants grown in MHP soils compared to the control ($p<0.05$). However, the number of primary root branches per plant significantly increased with exposure to MHP soils (31 – 49) compared to the control (29). Metal accumulation by *V. amygdalina* in MHP soil was significant. Phytoaccumulation efficiency of plant leaves was highest (11.47%) when metal concentration was least, compared to 2.34% efficiency of the test plant in 90 mg(Pb)/kg-polluted soil. The concentration of Cd in the leaves of *V. amygdalina* was 2.51 mg/kg compared to a residual soil concentration of 5.83mg/kg in the 15mg Cd-polluted soil. The amount of Cd lost in the soil was highest with higher soil metal concentration.

Keywords: model heavy metal soil, phytoremediation, phytoaccumulation, bitter leaf, bioconcentration.

INTRODUCTION

The environment suffers incessantly from the influence of industrial and economic development, leading to anthropogenic disturbance of the ecosystem (Musa *et al.*, 2019). Other natural geologic activities also contribute to environmental pollution (Cortez and Ching, 2014). The eventual accumulation of toxic substances emanating from such processes, such as heavy metals, have continued to pose severe dangers to human and animal health. Plants as the primary producers upon which verily environment depends have suffered great damages as a result of heavy metals. Plants have demonstrated a natural propensity to take in heavy metals in their growing substrates and transfer them as the food web advances (Musa *et al.*, 2019). This has led to the death of many plants, changes in morphology and physiology, and bioaccumulation in some plants, posing a more grave risk as they are consumed along with the food web. According to Helmentstine (2014), heavy metals like Pb, Cd, and Zn are toxic to living organisms even at low concentrations, therefore, the removal of these metal contaminants is pointedly necessary.

Physicochemical approaches to heavy metals removal are often high-priced, also necessitating high energy involvement and costly technology (Asubiaro *et al.*, 2018). They are also not all the time effective with the removal of contaminants at low concentrations. Most importantly, these methods are not eco-friendly in the long run; they modify soil structure and reduce soil productivity thereby making the soil unsuitable for plant growth (Atuanya, 1987; Ikhajiagbe, 2010). The biological approach, on the other hand, promotes re-establishment and enhances soil productivity in the run. It is able to reduce heavy metals contents to eco-friendly levels at inexpensive costs, particularly because it achieves this fit by only natural means.

A number of biological processes of soil remediation exist, including soil inoculation with specific soil microorganisms, or bioamendment of contaminated soils to enhance soil recovery periods (Musa, 2019). However, the commonest bioremediation technique involves the use of plant species to clean up denuded lands. The study of plant behavior in heavy metal contaminated soils allows the identification and selection of plant species with remediation capabilities. In most parts of tropical Africa, like Nigeria, *Vernonia amygdalina*, is grown almost everywhere in rural areas for its culinary and medicinal importance. Anoliefo *et al* (2006; 2008) identified *V. amygdalina* among the prevalent species found within and around those plants that were prevalent in and around auto mechanic workshops as well as workshops of welders and artisans in Asaba and Benin City, Nigeria. Ikhajiagbe and Anoliefo (2012), suggested that *V. amygdalina* may be a suitable candidate for phytoremediation because of its hyperaccumulation abilities.

The omnipresent nature of this plant, wide consumption, and prevalence of this plant even in disturbed environments informed the basis for the present study. The present study aims to investigate the capability of *Vernonia amygdalina* to bioaccumulate in its different parts, heavy metals in a model heavy metal-polluted soil.

MATERIALS AND METHODS

Stems cuttings of *Vernonia amygdalina* with a thickness of 1.6 – 1.8 cm and length of 30 cm were planted in soils treated with 3 different concentrations

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(15, 45, and 90 mg/kg of metal in soil) of Cd (as $CdCl_2$) and Pb (as $PbCl_2$). The metals were also used in combined forms of equal proportion (50/50). The experiment was laid out in a screen house for 4 months following a randomized block design experiment in three replicates. Plants were assessed for morphological changes (such as plant height [cm], number of leaves per plant, number of primary and secondary branches, intermodal length [cm], length of the main root [cm], number of chlorotic leaves per plant, and number of necrotic leaves per plant) due to heavy metals. Rhizospheric microorganisms were identified using the methods of Cowan and Steel (1974), Cheesebrough (2001), and Fu *et al.* (1993). Residual HM contents were determined according to APHA (1985). The phytoaccumulation efficiency (PE) was calculated to include the proportion of accumulated contaminant in the plant part compared to the concentration of a contaminant that was applied to the soil. The mean and statistical error of data was calculated. Results were presented as the mean of the 3 replicates and separated using Ducan’s multiple range test at $p<0.05$ (Ogbeibu, 2005).

RESULTS AND DISCUSSION

Morphological Responses of *V. amygdalina*

After four experimental months, results from (table 1) showed a significant reduction in height of plants grown in heavy metal-contaminated soils (24.1-34.4 cm) compared to the control (48.7 cm). This may be that heavy metals have inhibited cytoplasmic enzymes and damaged cell structures due to oxidative stress (Assche and Clijsters, 1990) or have negatively influenced the PGP microorganisms found around the plant rhizosphere leading to declining plant growth (Schaller and Diez, 2011). This result agreed with the work of Kibra (2008) who recorded a significant reduction in height of rice plants growing on soil contaminated with 1 mg Pb/kg. Another study by Ghani (2010) examined the effect of Pb and Cd on the growth of maize and showed a significant reduction in growth and protein content of maize. The result also did not agree with the work of Ikhajiagbe *et al.*, (2016) who observed no significant differences in height of *V. amygdalina* in oil concentrated soil.

Furthermore, there was no significant difference in the length of the main root of plants exposed to metal pollution (38.2 – 49.6 cm) compared to the control (40.8 cm) (Table 1). However, there was an increase in the length of the main root in the polluted soil (38.2-49.6 cm) compared to the control (40.8 cm). Also, the number of primary root branches per plant significantly increased with exposure to metal pollution (31 – 49) compared to the control (29). Increases in the number of secondary branches, primary branches, and the intermodal length were observed in the polluted soil compared with the control. This may be in a bid for the plant to take up more nutrients that would neutralize the heavy metals. This result agreed with the work of Whiting *et al.*, (2000), Gove *et al*., (2002), Liu *et al*., (2015) in a Cd and Pb polluted soil. There were more chlorotic and necrotic leaves in the polluted soil than the control. This result followed Vernay *et al.*, (2008) who suggested that heavy metals such as Pb and Cd induce morphological changes such as necrosis and chlorosis in younger leaves of the *Datura innoxia* plant grown in heavy metals contaminated substrate. According to Kabata-Pendias (2001), most of the reduction in growth parameters of test plants in heavy metals polluted media is a result of reduced photosynthetic activities, plant mineral nutrition, and reduced activity of some enzymes.

Table 1

Morphological response of *V. amygdalina* to metal polluted soil after 4 months

Parameters	15	45	90		15	45	90		15	45	90	Ctrl	LSD(0.05)
	mg (Pb)/kg (soil)				mg(Cd)/kg(soil)				mg(Cd+Pb)/kg(soil)				
Plant height (cm)	24.1	32.1	32.1		28.4	22.8	28.4		34.4	25.4	20.4	48.7	6.8
Total number of leaves/plant	58.6	45.6	57.6		44.1	56.7	58.6		55.1	64.5	52.7	67.1	9.4
Number of secondary branches	4.6	3.4	2.9		3.1	3.8	12.3		3.9	4.4	5.9	4.4	2.0
Number of primary Branches	5.1	5.3	4.1		3.9	7.4	9.3		4.2	3.8	4.4	5.9	2.1
Internodal length of pry. branch (cm)	13.2	12.6	16.4		15.4	13.5	16.4		12.3	16.4	13.7	11.2	3.4
Length of main root (cm)	46.2	41.2	38.6		42.4	51.3	48.4		38.2	47.3	49.6	40.8	10.2
Number of pry root branches	31.6	43.9	31.1		32.9	36.9	49.4		46.8	44.2	42.1	29.4	9.4
Number of chlorotic leaves/plant	8.5	9.5	12.8		4.4	8.5	10.2		6.4	15.4	14.9	3.5	3.2
Number of necrotic leaves/plant	4.5	10.4	12.1		6.2	5.5	8.1		5.9	7.4	5.4	5.4	2.8
Number of Senesced leaves/plant	18.6	11.1	11.6		5.1	12.9	19.9		11.1	16.9	22.9	8.4	5.7

Metal Accumulation by *V. amygdalina*

Results from (table 2) showed that after 4 months experimental period, *V. amygdalina* accumulated a significant amount of Pb and Cd in the heavy metal polluted soil compared to the control. This may be because the control soil has no detectable Pb and Cd compared to the heavy metal polluted soil. This indicates that the test plant has a natural propensity to take up heavy metals found in its growth media. This can also be attributed to the morphology and nature of this plant (Ivano *et al.*, 2007). This also points out the hypothesis by Ikhajiagbe and Anoliefo, (2012) that *V.*

amygdalina may be a candidate for phytoremediation. Phytoaccumulation efficiency of plant leaves was highest (11.47%) when the metal concentration in soil was least, compared to 2.34% efficiency for the test plant in 90 mg (Pb)/kg-polluted soil (Table 2).

The concentration of Cd in the leaves of *V. amygdalina* after 4 months was 2.51 mg/kg compared to a residual soil concentration of 5.83mg/kg in the 15mg/kg Cd-polluted soil. Meanwhile, in the 90mg/kg, the concentration of Cd in leaves of *V. amygdalina* after 4 months was 12.0mg/kg compared to the residual soil concentration of 8.76 mg/kg. The amount of Cd lost in the soil was highest when the soil was polluted with higher concentrations of the metal. This remediation was not due to accumulation in the leaves by the plant, but perhaps to other factors. Moreover, accumulation efficiency, being concentration-dependent, was enhanced at lower concentrations of metal in soil. The control plant in all the experiments showed the least leaves concentration of both Pb and Cd, this may be due to the reduced amount of Pb and Cd in the control soil.

Table 2

Metal accumulation by *V. amygdalina* in Pb-polluted soil after 4 months

Treatment	**Residual Soil conc. (mg/kg)**	**Foliar accumulation (mg/kg)**	**Phytoaccumulation factor**	**Phytoaccumulation efficiency (%)**
	Pb only in soil			
Pb-15mg/kg	11.1	1.72	0.15	11.47
Pb-45mg/kg	20.51	1.46	0.07	3.24
Pb-90mg/kg	42.53	2.11	0.05	2.34
Control soil	0.50	0.81	1.62	-
	Cd only in soil			
Cd-15mg/kg	5.83	2.51	0.43	16.73
Cd-45mg/kg	3.52	9.05	2.52	20.11
Cd-90mg/kg	8.76	12.0	1.37	13.33
Control soil	0.39	0.11	0.28	-
	Both Pb and Cd in soil			
(Pb)Pb/Cd-15mg/kg	5.10	2.53	0.21	33.73
(Pb) Pb/Cd-45mg/kg	11.14	9.05	0.56	40.22
(Pb) Pb/Cd-90mg/kg	28.00	12.03	0.43	26.73
Control soil (Pb)	0.50	0.81	1.62	-
(Cd) Pb/Cd-15mg/kg	2.03	5.46	2.69	72.80
(Cd) Pb/Cd-45mg/kg	2.45	7.11	2.90	31.60
(Cd) Pb/Cd-90mg/kg	9.22	10.22	1.11	22.71
Control soil (Cd)	0.39	0.11	0.28	-

Microbial Composition of Model Heavy Metal-polluted Soil

In the present study *Bacillus* sp. and *Aspegillus niger* were among the predominant species found in the rhizosphere region of soil, and these organisms have been previously reported for remediation of contaminated soils (Okoh *et al.,* 2001; Okoh, 2003; Ojumu *et al.*, 2005; Atagana, 2008; Asubiaro *et al.*, 2018). (Table 3). This shows that the rhizosphere of *V. amygdalina* encourages their growth and may indicate the ability of these microbes to remove heavy metals in polluted environments.

Musa (2019) have also identified *Bacillus sp.* flourishing in arable land, six weeks after pollution with Pb. This result is also consistent with the work of Ugoh and Moneke (2011) who reported that the bacterial isolates from the soil contaminated with petroleum products from different sites showed *Pseudomonas sp*., *Bacillus sp*., and *Klebsiella spp.* However, *Bacillus sp.* was observed to have the lowest growth in petroleum contaminated sites. Alexander (2014) only reported *Mucor* sp., *Penicillium* sp., and *Rhizopus* sp present in a model heavy metal polluted soil in Keffi, Nasarawa State. This result was not consistent with the present study in the case of *Aspergillus niger*.

Table 3

Microbial isolates of heavy metal-polluted soil sown with *V. amygdalina* after 4 months

Parameters	**15**	**45**	**90**		**15**	**45**	**90**		**15**	**45**	**90**	**Ctrl**
	mg(Pb)/kg				**mg(Cd)/kg**				**mg(Cd+Pb)/kg**			
Bacteria												

Micrococcus sp.	+	+	+		+	+	+		+	+	+	+
Pseudomonas aeruginosa.	+	+	-		+	-	-		+	+	+	+
Bacillus sp.	+	+	+		+	+	+		+	+	+	+
Staphylococcus sp.	+	+	-		+	+	-		+	+	+	-
Fungi												
Mucor sp.	+	+	+		+	+	+		+	+	+	+
Penicillium sp.	+	+	+		+	+	+		+	+	+	+
Aspergillus niger	+	+	+		+	+	+		+	+	+	+
Fusarium sp.	+	+	-		+	+	+		+	-	-	+
Rhizopus sp.	+	-	-		+	+	+		-	+	+	+
Trichoderma sp.	+	+	-		+	-	+		+	+	-	+
+ present; - absent.												

CONCLUSIONS

The ability of *Vernonia amygdalina* for uptake and translocation of Cd and Pb was ascertained in this study. The results proved the ability of the test plant to phytoacummulate Pb and Cd. However, a significant reduction in plant height, a significant increase in the number of primary and secondary root branches per plant, and an increase in intermodal length were observed in the polluted soil compared to the control soil. Also, the test plant was observed to encourage activities of some beneficial bacteria and fungi that were previously proven to flourish in Cd and Pb polluted soils.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Beckley Ikhajiagbe, Musa Saheed Ibrahim and James Ogunro designed and executed the study. Musa Saheed Ibrahim and Ephraim Aliu analyzed the data. Beckley Ikhajiagbe and Musa Saheed Ibrahim prepared the drafts. Ephraim Aliu and James Ogunro and Musa Saheed Ibrahim wrote the final manuscript.

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