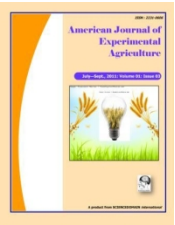

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Heavy Metal Content in *Acacia saligna* and *Acacia polyacantha* on Slime Dams: Implications for Phytoremediation

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Authors' contributions

Authors PD, FM DRM worked on formulating the project proposal. Authors FM and PD carried out the sampling and laboratory analysis. Authors PD and BM wrote the manuscript. Author PD and BM assisted in statistical data analysis.

Research Article

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ABSTRACT

Aims: To determine heavy metal content in *A. saligna* and *A. polyacantha* so as to ascertain their possible use in phytoremediation
Place and Duration of Study: Bindura University of Science Education, Chemistry and Biological Sciences Dept, P. Bag 1020, Bindura, Zimbabwe. The study was carried out between December 2011 and January 2013.
Methodology: Two sites which are the slime dams at a local gold mine in Bindura and a control site 10km outside the slimes were used in the study. A total of four sampling points each in the form of 5x5m quadrants were established after every 100m in transects, 700m long separately established on the control and slime dams. Soil samples at 5-10cm and 10-15cm levels as well as roots, leaves and bark from five sampled plants were collected at each sampling point. The metals content was analyzed using Atomic Absorption Spectrometry and Inductively Coupled Plasma. The bioaccumulation factor and the shoot/root quotient were computed in Microsoft excel. Analysis of Variance was carried out using SPSS and Genstat Version 16.

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Results: The present study shows that *A. saligna* and *A. polyacantha* accumulate heavy metals with biological accumulation factor (BAF) value results indicating significant differences between the slimes and control sites. Both species had BAF values for nickel, copper and iron greater than one except for zinc, lead and arsenic. The shoot/root quotients showed that nickel, copper and iron are translocated to the shoots in the species as compared to zinc, lead and arsenic.
Conclusion: *A. saligna* and *A. polyacantha* showed evidence of accumulation of nickel, copper and iron and therefore may be used for phytoremediation and restoration purposes at mine slime dams.

Keywords: Heavy metals; phytoremediation; bioaccumulation factor; translocation factor; Acacia polyacantha; Acacia salina.

1. INTRODUCTION

Contamination of soil, water and food plants with toxic heavy metals due to mining activities in mining towns is still a major environmental and human health problem [1]. While methods such as excavation and burial of contaminated soil at designated waste sites have been suggested such methods are not popular due to huge costs. They cost over a million United States dollars per acre [2]. There is still a need for researches in effective and affordable methods of counteracting this challenge [3,4]. Phytoremediation offers attractive options. It takes advantage of the fact that living plants can act as solar driven pumps that can extract and concentrate particular elements from the environment [5]. Harvested plant tissue that would have accumulated heavy metal contaminants may be easily and safely processed by drying, ashing or composting. Metals can then be reclaimed from the ashes. This generates recycling avenues and reduces the generation of hazardous waste [6]. Major sources of heavy metal pollution in the environment are mostly anthropogenic, including mining activities, effluent discharges and waste disposal [7]. In trace concentrations, many metals are essential to life and have several vital functions in biological processes but in excess the same metals can be toxic. It has been observed that even long after mining activities have ceased heavy metals continue to persist in the environment [8]. They can enter the food chain when taken up by plants during farming and eventually affect human health [9]. Heavy metals poison animals and humans by disrupting cellular enzymes, which use nutritional minerals such as magnesium, zinc and selenium for their function. Toxic metals replace these nutrients and bind their receptor sites, causing diffuse symptoms by affecting nerves, hormones, digestion and immune function [10].

A survey of most mine slime dumps in Bindura town shows that *Acacia polyacantha* and *Acacia saligna* grows very well in these areas. The plants depicted no stress or any stunted growth. Thus the proliferation of *Acacia polyacantha* and *Acacia saligna* on slime dams makes them an important object of research. While *A. polyacantha* is an indigenous species in Zimbabwe *A. saligna* is exotic and native to Australia and falls within a family of acacias commonly known as Australian wattles. The species spread to many parts of the world and is now considered the most widely planted non-timber species with around 600,000 ha established worldwide [11]. Both species have been mentioned in other studies as ideal for phytoremediation [12,13]. *A. saligna* was reported to have a high biomass and fast growth rate [11]. *A. polyacantha* out-performed indigenous species like *Bauhinia thonningii* in biomass production and growth rates [13,14]. Coates [11] indicated in his studies that the fast growth rate of *A. Saligna* makes it ideal for reforestation of mine dumps [5]. There is still

need for more work on the capacities of both species as accumulators as well as the physiology involved [15]. Therefore this research sought to assess heavy metal content of *Acacia polyacantha* and *Acacia saligna* with an aim of finding out if they can probably be used in heavy metal decontamination of the slime mine dumps.

2. MATERIALS AND METHODS

2.1 Study Area

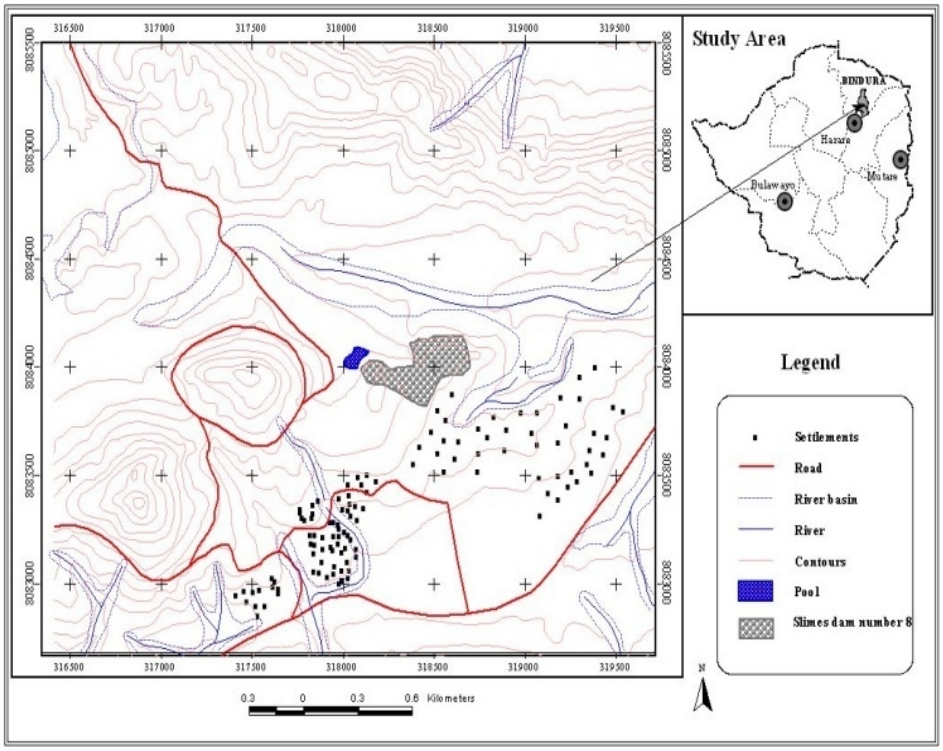

Fig. 1. A map of Bindura town where the study area is located

The slimes site is located 2km north-west of a gold mine concentrator plant at the gold mine in Bindura Fig. 1, a town located 88 km North-East of Harare. The dump site lies at an altitude of 1070m above sea level and it has a capacity to hold about 37mega tones of tailings. The weather is characterized by a tropical climate with distinct wet and dry seasons. The area is generally rich in gold, nickel, copper and cobalt. Geologically the area is typically a greenstone configuration composed mainly of basaltic rocks banded with iron formations and volcanic tuffs. As a reforestation measure, the mine initiated planting of *Acacia* species in the area. The area is also dominated by *Brachystegia spiciformis, Brachystegia spiciformis* and *Julbernadia globiflora*.

The control site is located 10km outside the slime site. It is characterized by miombo woodlands growing on sandy loam soils enriched with leafy organic matter from the trees and the area is mainly a grazing area. Other species observed besides the acacias are

Brachystegia spiciformis, Brachystegia spiciformis, Albizia antunesiana, Faurea rochetiana and *Bauhinia thonningii.*

2.2 Sampling Protocol

Sampling was carried out at the slime dams alongside a control site 50km outside, with a presumption that it was well separated from the mine. A line transect of 700 m long was established on the sites and a total of 4 sampling sites were systematically established after every 100 m in line transects. Four 5x5 m quadrants were established on the sites one at each established sampling point in the line transects.

2.3 Plant Sample Collection

Leaf, bark and root samples were collected from plants of *Acacia polyacantha* and *Acacia saligna* species at the slime dams and the control site. Five individual plants were sampled in each quadrant using simple random sampling. The plants of heights between 40 and 50cm were selected as this was an estimate for similar ages. The samples were separately collected from each plant and were wrapped in aluminum foil and labelled. All the samples were kept in a cooler box with ice during transportation and brought to the laboratory according to [16]. Heavy metal contents were determined in the plant parts.

2.4 Soil Collection

Three soil samples were also collected at each sampling point where plant samples were previously taken. The samples were then mixed to constitute composite samples per sampling point. In each plot, soil samples were collected at two depths, 5-10 and 10-15 cm using a soil auger [2]. In all cases, soil samples were placed in clean plastic bags, sealed and transported to the laboratory. Soil samples were air-dried, ground into fine powder using pestle and mortar and passed through 2mm mesh sieve [2]. Soil samples were analyzed for pH and heavy metal content.

2.5 Sample Analysis

2.5.1 Quality assessment and control

This was achieved by analyzing results in triplicates and use of certified reference standards to ascertain reproducibility and accuracy as shown in Table 1. Distilled water was used throughout the study. Glassware was properly cleaned and the reagents used were of analytical grade. Reagent blank determinations were used to correct the instrument readings.

Table 1. Plant certified reference material concentration (ppm) mean ± SE

Metal	Certified value	Measured value	Recovery
Copper	120±0.4	120±0.5	100
Zinc	260±0.5	260±0.5	100
Nickel	1.2±0.01	1.1±0.05	92
Lead	73±0.5	73±0.5	100
Arsenic	1.6±0.5	1.5±0.01	94

2.5.2 Soil pH

The soil pH was measured in a suspension at a soil-to-water ratio of 1:2 using a pH meter.

2.5.3 Soil heavy metal analysis

Two grams of the soil samples were weighed into nitric acid-washed glass beakers. Soil samples were digested by the addition of 20 cm^3 of *aqua ragia* (mixture of HCl and HNO_3, ratio 3:1) and 10 cm^3 of 30 % H_2O_2 [2]. The H_2O_2 was added in small portions to avoid possible overflow leading to loss of material from the beaker. The beakers were covered with a watch glass, and heated at 90^oC for two hours. The beaker wall and watch glass were washed with distilled water and the samples filtered out to separate the insoluble solid from the supernatant liquid and the filtrate was collected. The volumes were adjusted to 100 cm^3 with distilled water. All the samples and blanks were stored in plastic containers.

2.5.4 Plant heavy metal content analysis

Root and shoot samples were thoroughly washed with distilled water to remove all adhering soil particles. The plant samples were weighed to determine the fresh weight and dried in an oven at 80ºC for 72 hours to determine their dry weight [2]. The dry samples were crushed in a mortar and the resulting powder digested by weighing 0.5 g of oven-dried ground and sieved soil (<1 mm) into a nitric acid-washed porcelain crucible and placed in a muffle furnace for four hours at 500ºC.

The crucibles were removed from the furnace and cooled. Ten mL of 6M HCl was added, covered and heated on a steam bath for 15 minutes. Another 1 mL of HNO_3 was added and evaporated to dryness by continuous heating for one hour to dehydrate silica and completely digest organic compounds. Finally, 5 mL of 6 M HCl and 10 mL of water were added and the mixture heated on a steam bath to complete dissolution. The mixture was cooled and filtered through a Whatman no.1 filter paper into a 50 mL volumetric flask and made up to the mark with distilled water.

Determination of Ni, Cu, Zn, Fe, As, and Pb in soil and plant samples was made in triplicates directly on each of the final solution using Atomic Absorption Spectroscopy (AAS). Determination of arsenic was also made in triplicates directly on the final solution using Inductively coupled plasma (ICP).

2.5.5 The bioaccumulation factor (BAF)

The index of the plants' ability to accumulate metals from soils was calculated as follows [11].

$$\mathbf{BAF} = \frac{\mathbf{cplant}}{\mathbf{csoil}}$$

where Cplant and Csoil represent the heavy metal concentration in plant parts and soils respectively.

2.5.6 The shoot/root quotient (SRQ)

This may also be referred to as translocation factor (Ti) and it gives the leaf/root metal concentration and it depicts the ability of the plant to translocate the metal species from roots to leaves at different concentrations [17,18,19,20]. It was computed as follows:

$$\text{SRQ} = \frac{S}{R}$$

where S and R represent the level of heavy metals in the plant parts (leaves and bark) and R the level of heavy metals in the roots respectively [13,21].

3. RESULTS AND DISCUSSION

3.1 Soil pH

The pH values for the soils on the mine dump samples ranged from 2.0 to 4.7 and can be classified as acidic. The pH values at the control site ranged between 4.7 and 6.8 and can be classified as acidic to neutral. Fig. 2 shows the mean pH for the two sites.

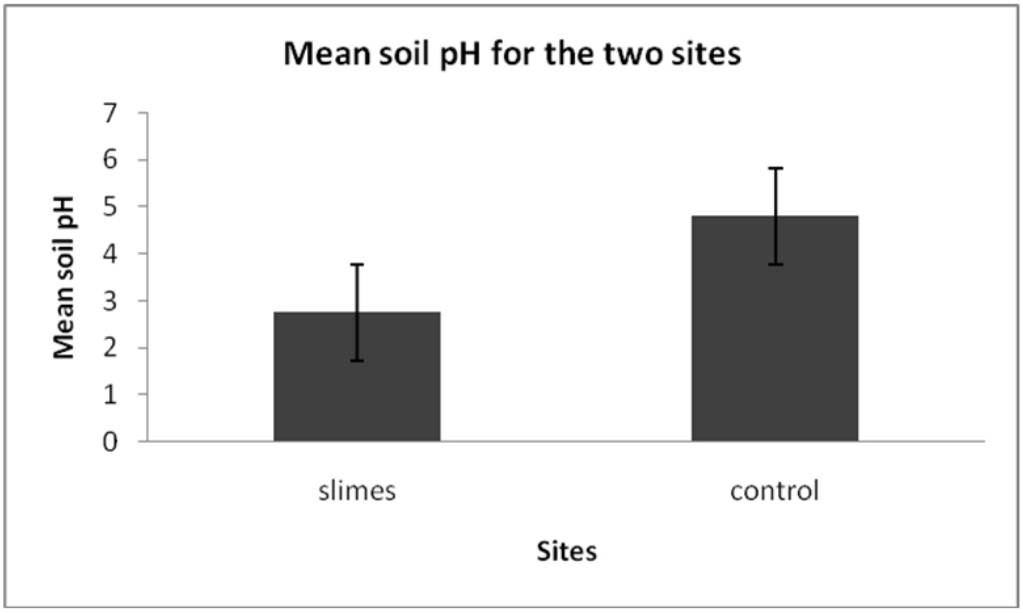

Fig. 2. A graph for the mean soil pH at the sites

3.2 Soil Heavy Metal Contents

The sampling points within sites had non-significant variation (P>0.05) for all the heavy metals, an indication of homogeneity among the sampling points. This means that the sampling strategy was effective and there was no bias in the strategy as all points within sites were uniform as shown in Table 2.

Table 2. Mean heavy metal concentration (ppm) for the soils at the control and slime dams

		Ni	As	Cu	Fe	Pb	Zn
SITE	**SLIMES**	0.97ns	0.307ns	0.013***	0.18*	0.234***	2.45***
	CONTROL	0.51ns	0.057ns	0.201***	0.96*	0.043***	0.184***
	S.E	0.192	0.0949	0.0171	0.205	0.0169	0.0904
LEVEL	**5-10cm**	1.21**	0.0197*	0.146**	0.14*	0.121ns	1.454**
	10-15cm	0.28**	0.0616*	0.069**	0.99*	0.155ns	1.155**
	S.E	0.192	0.00949	0.0171ns	0.205ns	0.0169	0.0904
SAMPLING POINT	**1**	0.76ns	0.00949ns	0.103ns	0.55ns	0.155ns	1.17ns
	2	0.72ns	0.0398ns	0.0103ns	0.55ns	0.123ns	1.43ns
	3	0.74ns	0.0405ns	0.109ns	0.56ns	0.137ns	1.24ns
	4	0.75ns	0.0424ns	0.115ns	0.6ns	0.138ns	1.377ns
	S.E	0.271	0.0401	0.02041	0.29	0.0239	0.1279

*S.E.= standard error . ns= no significant difference (p > 0.05). *p <0.05 **P<0.01 ***p<0.001 ppm = parts per million 5-10cm – The top soil level at a depth of five to ten centimeters, 10-15cm bottom soil level at a depth of ten to fifteen centimeters, sampling pt: any one of four sampling points established at the two sites in the form of 5x5 quadrabts*

Significant differences were observed between the sites for Cu, Zn and Pb (P<0.001) and Fe (P<0.05) and non-significant differences for nickel and arsenic in the soil samples P>0.05. Table 2 shows the means and standard errors. The analysis shows an indication that there was heavy metal accumulation at the slimes for four of the six metals considered. The non-significant differences for Ni and As might be an indication that the control site was not ideal for these metals as it was only 10km from the mine dumps as substantive quantities of the metals were at both sites. The mine being a Nickel Mine would result in an abundance of the metal in the Bindura locality; hence a more far removed control site would have been better.

The soil depths, 5-10 and 10-15 cm are significantly different (P<0.01) for Ni, Cu, Fe, Zn and As and non-significantly different for lead. This shows a sign of movement of the metals between the levels as well as availability of the heavy metals in the soil layers. Fig. 3 shows the heavy metal concentrations in the two levels.

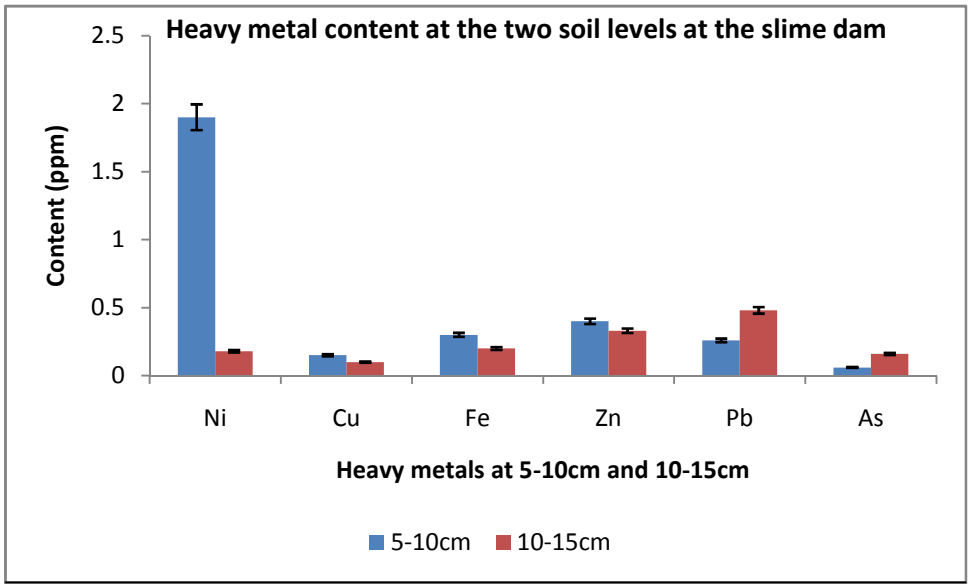

Fig. 3. Heavy metal distribution in the two soil levels at the slime dams
5-10cm –The top soil level at a depth of five to ten centimeters, 10-15cm bottom soil level at a depth of ten to fifteen centimeters

It was observed that nickel is the most abundant heavy metal in the slimes at the 5-10 cm level, a confirmation that deposits of the metal were made at the slimes and on the other hand, Cu, Zn and Fe are most abundant at the control sites at different levels as shown in Fig. 4.

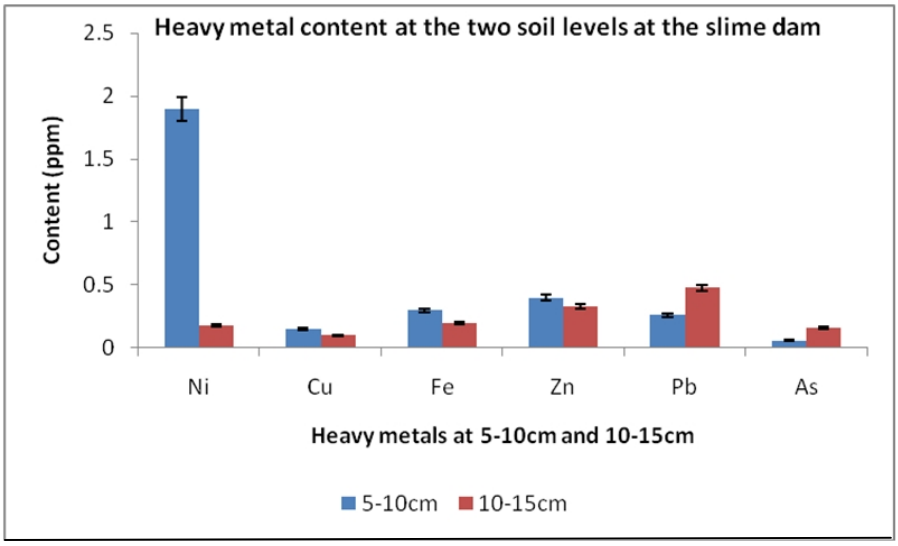

Fig. 4. Heavy metal distribution in the two soil levels at the control site
5-10 cm – the top soil level at a depth of five to ten centimeters, 10-15cm bottom soil level at a depth of ten to fifteen centimeters

The control site had a much less tree species density than the slimes which underwent reforestation for land reclamation by the mine. This may have resulted in the lower levels of some of the heavy metals observed at the slime dams. Zinc is abundant at the control site in both levels.

The low pH at the slimes may have contributed to accumulation of heavy metals at the slimes [4,22,23]. Soil acidity dramatically affects the cation exchange capacity (CEC) of soil by limiting the available exchange sites. H^+ bind to soil particles tighter than other cations, thus, any metal bound to a soil particle will get knocked off in the presence of excess H^+ [3,24,25]. At high pH (>7), cations are less bioavailable because they have less competition from H^+ for available binding sites. Many cations bind to free hydroxyl groups (OH^-) and form insoluble hydrous metal oxides, which are unavailable for uptake, such as $CdCO_3$. This could explain why there was an accumulation of some of the heavy metals at the slimes.

3.3 Plant Heavy Metal Contents

Generally the plants at the dumps contain more metals than plants at the control a reflection of the nature of the different sites. The roots at the slimes contain the highest quantity of heavy metals especially nickel, most likely as roots are the first point of contact compared to the bark and leaves, whereas the leaves at the control contain the most iron a reflection of abundance of this metal at the site. Fig. 5 summarizes mean heavy metal contents for different plant parts for the two species.

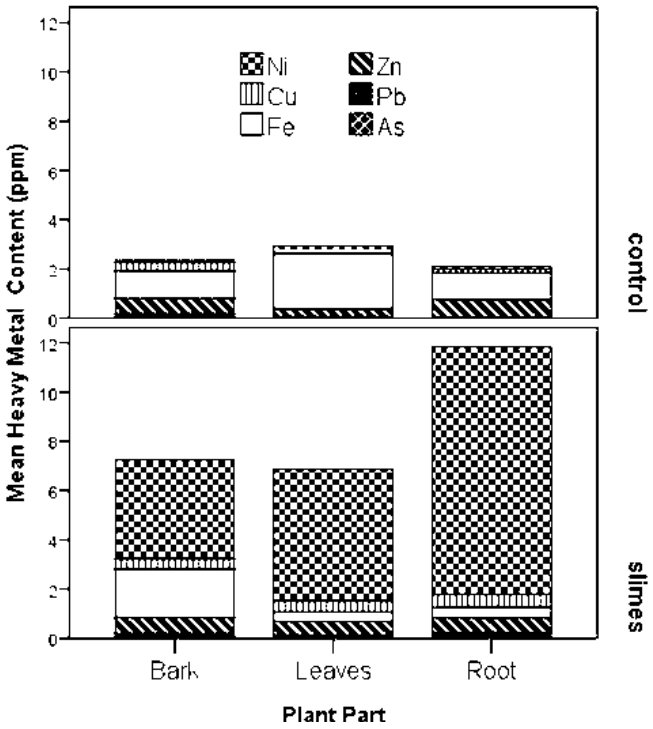

Fig. 5. Heavy metal contents in the plant parts

Bark – heavy metal contents in the bark, leaves – heavy metal contents in the leaves, root – heavy metal contents in the roots.

The contents become meaningful when expressed relative to soil contents, as the bioaccumulation factor.

3.4 Biological Accumulation Factor (BAF)

When BAF values were computed, the mean values shown in Table 3 were observed. Values greater than 1 indicate a net accumulation by the plant whereas values below 1 show net accumulation in the soil. Generally, therefore, the species are hyperaccumulators for Ni, Fe and Cu as the BAFS are greater than 1. However they are comparable as the ANOVA shows they are the same.

The results indicated significant differences between the slimes and control sites for BAFs, which suggests the species are hyper accumulators for Ni, Cu, Fe, Fe and Zn. However because the soil contents were not significantly different for Ni and As, a better control site will be ideal in future studies with no abundance of this metal, so that precise conclusions are made for this metal.

The plant parts are not statistically different with respect to BAF values in other words no particular plant part leaves, bark or roots specifically accumulate the metals more than the other. The species are also not significantly different with respect to BAFs but they are both on the high side as BAFS are greater than one except for Zn, Pb and As.

Table 3. Mean bioaccumulation factor for the species and plant parts at the sites

		Ni	As	Cu	Fe	Pb	Zn
Site	**SLIMES**	3.32*	0.38ns	17.86***	2.57**	0.76**	1.66***
	Control	0.17*	0.55ns	0.50***	0.77**	0.31**	0.11***
	S.E	0.762	0.078	2.72	1.195	0.168	0.111
Species	***A. polyacantha***	1.89ns	0.55ns	9.47ns	1.09ns	0.46ns	0.80ns
	A.SALIGNA	1.59ns	0.38ns	8.89ns	2.25ns	0.61ns	0.97ns
	S.E	0.933	0.078	2.72	1.195	0.168	0.111
Plant part	**Leaves**	2.64ns	0.33ns	10.29ns	0.88ns	0.32ns	0.90ns
	Root	1.50ns	0.23ns	8.80ns	1.11ns	0.39ns	0.81ns
	Bark	1.09ns	0.84ns	8.45ns	3.02ns	0.89ns	0.95ns
	S.E	0.933	0.095	3.332	1.464	0.206	0.136

*S.E=standard error, ns=no significant difference P>0.05 * p<0.05 ** P<0.01. ***p<0.001*

From similar work on cabbage and broccoli grown on amended soils the overall bioaccumulation factor (BAF) of seven heavy metals in cabbage leaves and broccoli heads revealed that cabbage and broccoli were poor accumulators of Cr, Ni, Cu, Cd, and Pb (BAF <1), while BAF values were >1 for Zn and Mo [1]. According to work by Maharia et al., [20] the bioaccumulation factors (BAF) significantly lower BAF<1 values of Cu and Cr were found in the medicinal plants *Ocimum sanctum, Cassia fistula, Withania somnifera and Azadirachta Indica* Only *Withania somnifera* showed very high metal bioaccumulation BAF >1 [14].

3.5 Translocation Factor

The results show that the metals are translocated from roots to shoots more in *A. Saligna* than *A. Polyacantha* as this species shows higher translocation factor across the metals,

except for Zn whose upward mobility is comparable for the two species. The metals Ni, Cu and Fe are more upwardly mobile in the two species.

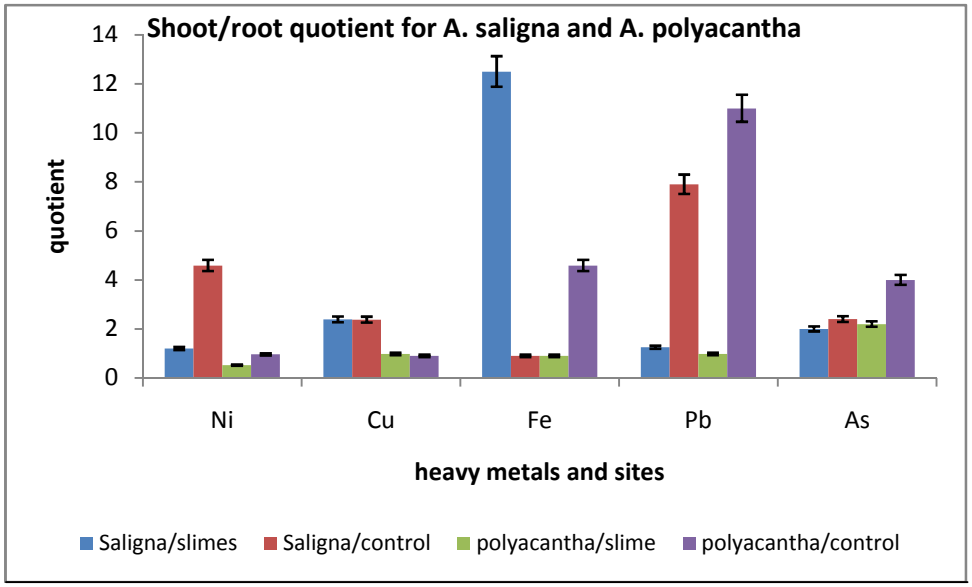

Fig. 6. Translocation factors for the two species at the two sites

Saligna/slimes – is the shoot/root quotient for *A. saligna* at the slimes Saligna/control – is the shoot/root quotient for *A. saligna* at the control site, polyacantha/slimes – is the shoot/root quotient for *A. polyacantha* at the slimes polyacantha/control – is the shoot/root quotient for *A. polyacantha* at the control site.

Fig. 6 depicted the translocation factors for the metals and the species. This means that destruction of the upper plant will be effective at removing these metals from the environment. The fast growth rate reported in literature for *A. saligna* may contribute to the high translocation factor observed for the species. Both the ability of a species to accumulate high quantities of elements per biomass unit and the possibility of high biomass production over a given time and area are important.

Work by Ghafoori et al., [19] confirms that the translocation factor increases with increased concentration of heavy metals. The ability of a species to tolerate high metal concentration makes it ideal as an accumulator [10,26,27]. The values obtained in their study though on a different species, *Dyera costulata,* on three metals, Pb, Ni and Zn also studied in this work showed maximum values of 0.62 for Pb 4.00 for Ni and 3.1 for Zn, which are comparable to values obtained for *A. saligna* and *A. polyacantha*.

4. CONCLUSION

There was evidence of heavy metal accumulation by *A. saligna* and *A. polyacantha* according to data gathered in this study. These two species, *A. saligna* and *A. polyacantha* are two of the available options for phytoremediation at the dumps and the mine should continue to propagate them to remedy heavy metal toxicity in the environment. Harvesting and incinerating the plant will potentially facilitate remediation of the slime dams.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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