

HEAVY METAL CONTENT IN ACACIA SALIGNA AND ACACIA POLYACANTHA ON SLIME DAMS: IMPLICATIONS FOR PHYTOREMEDIATION

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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.

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

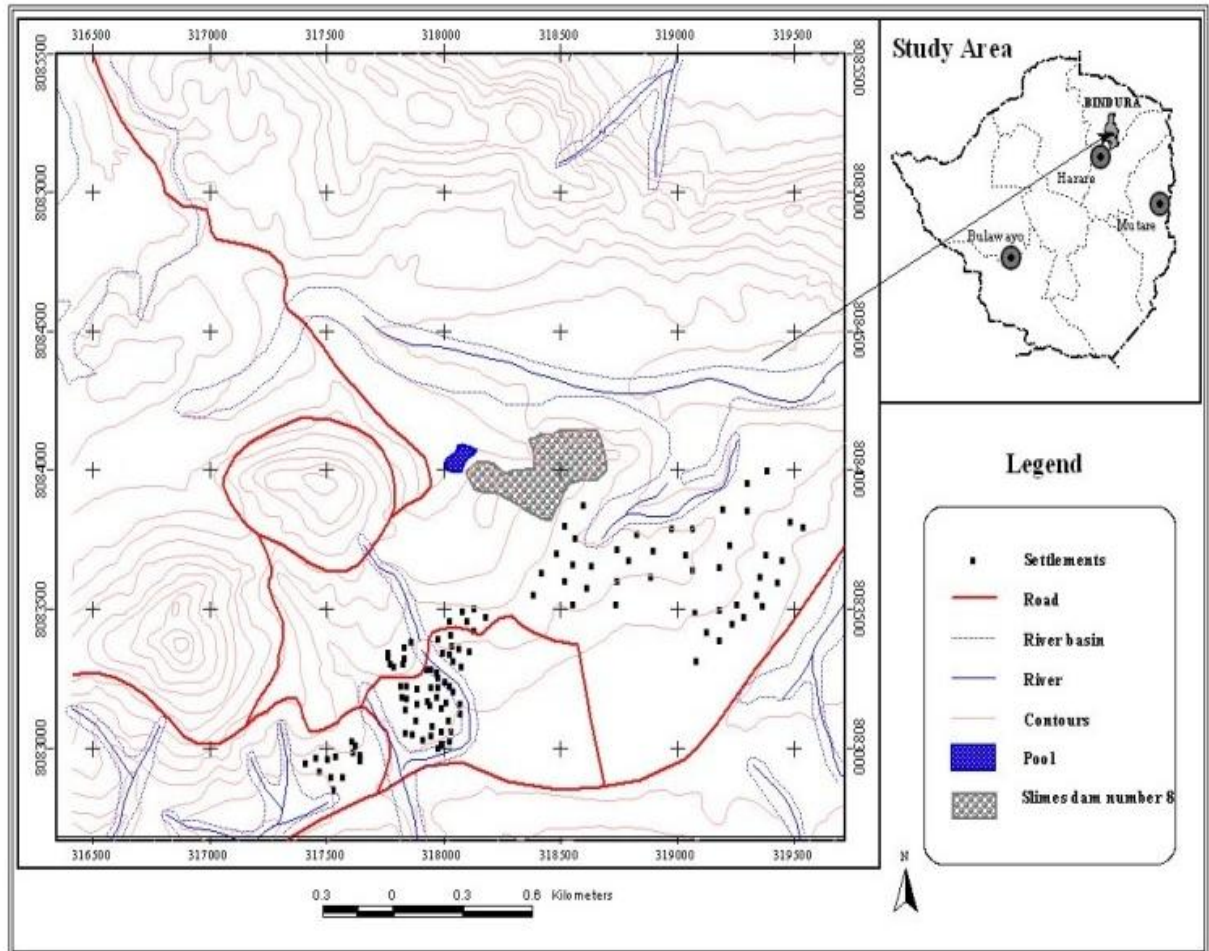
contaminants may be easily and safely processed by drying, ashing or composting. Metals can then be reclaimed from the ashes. This generates recycling avenues and reduces the generation of hazardous waste [6]. Major sources of heavy metal pollution in the environment are mostly anthropogenic, including 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.

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2. MATERIALS AND METHODS

2.1 Study Area



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

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

96 The control site is located 10km outside the slime site. It is characterized by miombo woodlands
97 growing on sandy loam soils enriched with leafy organic matter from the trees and the area is
98 mainly a grazing area. Other species observed besides the acacias are *Brachystegia spiciformis*,
99 *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 cm³ of *aqua regia* (mixture of HCl and HNO₃, ratio 3:1) and 10 cm³ of 30 % H₂O₂ [2]. The H₂O₂ 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°C 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 cm³ 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 80°C 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 500°C.

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 HNO₃ 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 nickel, copper, zinc, iron, arsenic, and lead 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].

$$BAF = \frac{C_{plant}}{C_{soil}}$$

where C_{plant} and C_{soil} 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. It was computed as follows:

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

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].

194 **3. RESULTS AND DISCUSSION**

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196 **3.1 Soil pH**

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

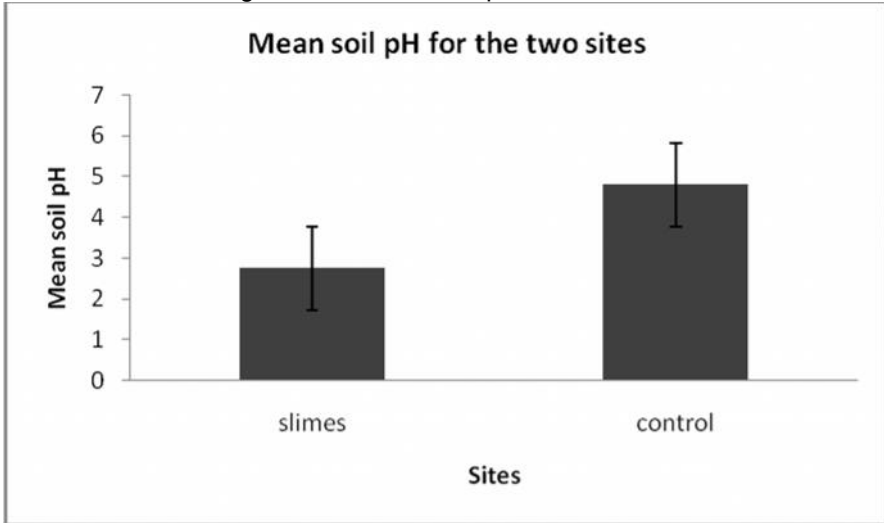


Fig 2. A graph for the mean soil pH at the sites

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204 **3.2 Soil heavy metal contents**

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

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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 copper, zinc and lead ($P < 0.001$) and iron ($P < 0.05$) 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 Nickel and Arsenic 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 nickel, copper, iron, zinc and arsenic 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.

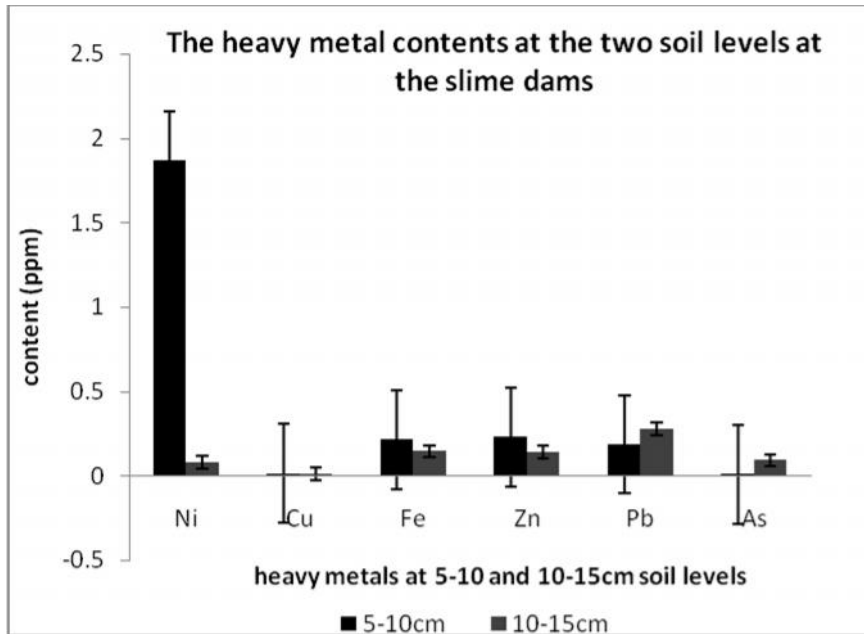


Fig 3. Graph of heavy metal distribution in the two soil levels at the slime dams.
 5-10cm – The top soil level at a depth of five to ten centimeters, 10-15cm bottom soil level at a depth of ten to fifteen centimeters

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, copper, zinc and iron are most abundant at the control sites at different levels as shown in fig 4.

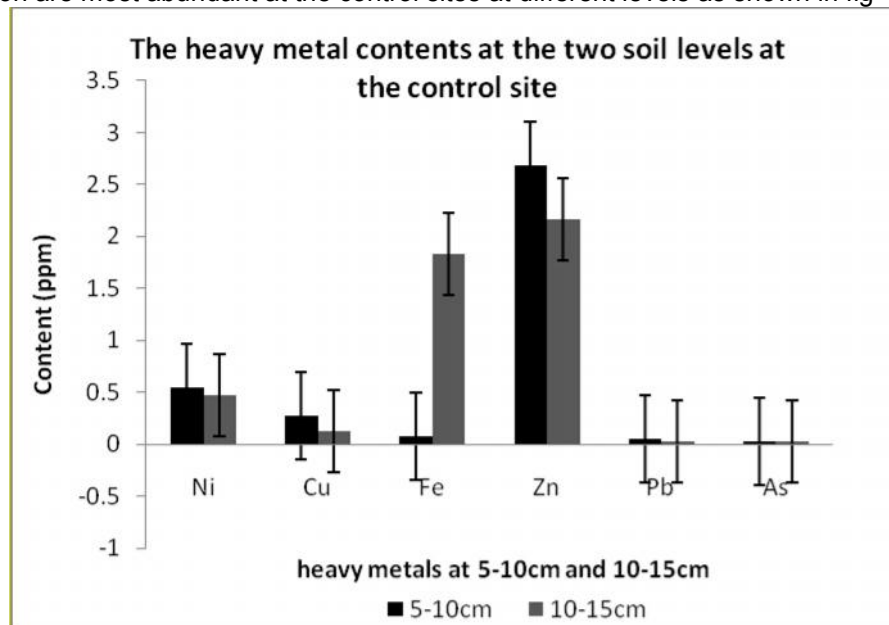


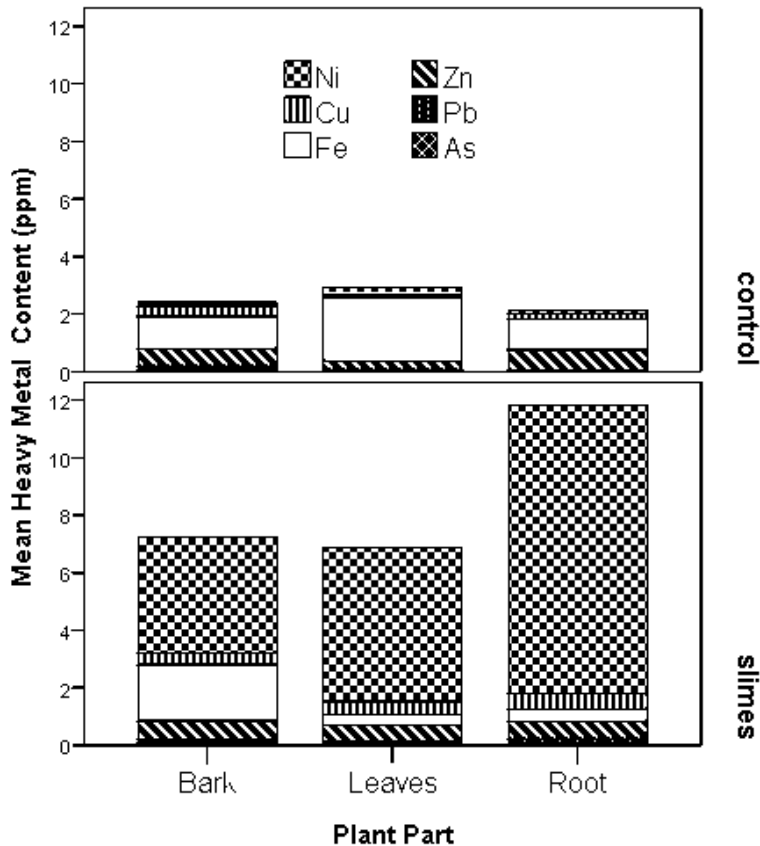
Fig 4. Graph of heavy metal distribution in the two soil levels at the control site
 5-10cm – The top soil level at a depth of five to ten centimeters, 10-15cm bottom soil level at a depth of ten to fifteen centimeters

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

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

268 **3.3 Plant heavy metal contents**

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



276 **Fig 5. Heavy metal contents in the plant parts.**
277 *Bark – heavy metal contents in the bark, leaves – heavy metal contents in the leaves, root –*
278 *heavy metal contents in the roots.*
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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 nickel, iron and copper 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 nickel, copper, iron, lead and zinc. However because the soil contents were not significantly different for nickel and arsenic, 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 zinc, lead and arsenic.

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 zinc whose upward mobility is comparable for the two species. The metals nickel, copper and iron are more upwardly mobile in the two species.

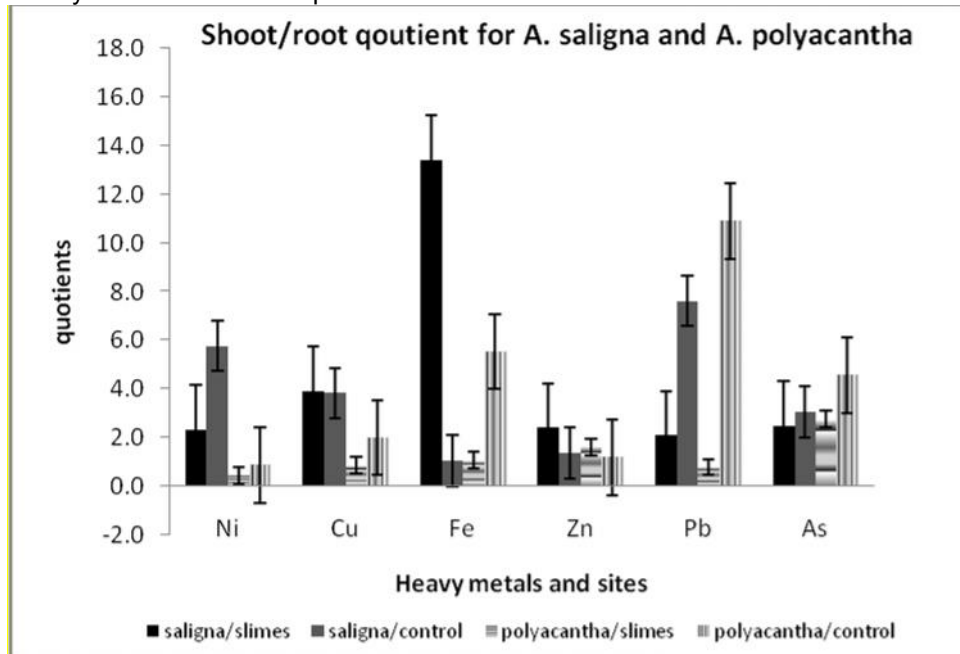


Fig 6. Translocation factors for the two species at the two sites.

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 shows a graph of 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 [10]. The ability of a species to tolerate high metal concentration makes it ideal as an accumulator. The values obtained in their study though on a different species, *Dyera costulata*, on three metals, lead, nickel and zinc also studied in this work showed maximum values of 0.62 for lead 4.00 for nickel and 3.1 for zinc, which are comparable to values obtained for a *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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AUTHORS' CONTRIBUTIONS

Mr. Dzomba, Mr Mhandu and Ms Masvodza worked on formulating the project proposal. Mr. Mhandu and Mr. Dzomba carried out the sampling and laboratory analysis. Mr. Dzomba and Ms Masvodza wrote the manuscript. Mr Dzomba and Mr Masamha assisted in statistical data analysis.

REFERENCES

1. P. Dzomba, S. Nyoni and N. Mudavanhu. Heavy metal contamination risk through consumption of traditional food plants growing around Bindura town, Zimbabwe. *Journal of Toxicology and Environmental Health Sciences*. 2012 ; Vol. 4(5): 92-95
2. Raskin I, Smith RD and Salt DE. Phytoremediation of metals: Using plants to remove pollutants from the environment. *Current Opinion in Biotechnol*.1997; 8(2):221
3. Antonious GF, Kochhar TS and Coolong T. Yield, quality, and concentration of seven heavy metals in cabbage and broccoli grown in sewage sludge and chicken manure amended soil. *Journal of Environmental Science and Health Part A Toxic/Hazardous Substances and Environmental Engineering*. 2012; 47(13):1955-65
4. Gupta S, Nayek S, Saha RN and Satpati S. Assessment of heavy metal accumulation in macrophyte, agricultural soil and crop plants adjacent to discharge zone of sponge iron factory. *Environmental Geology*. 2008; 55:731-73
5. Griffin A R, Midgley SJ, Bush D, Cunningham PJ, Rinaudo AT. Global uses of Australian acacias – recent trends and future prospects. *Diversity and Distributions*. 2011; 17(5):837 - 847.
6. Ghosh M and Singh SP. A review on phytoremediation of heavy metals and utilization of its by products. *Applied and ecology and environmental research* 31:1-18.
7. Akan JC, Abdulraham FI, Sodipo OA and Lange AG. 2010. Physicochemical parameters of soil and vegetable samples from Gongulon Agricultural site, Maiduguri state Nigeria. *Journal Am. Science*. 2005; 6:12.
8. Mupangwa JF, The growth performance of exotic and indigenous tree species in rehabilitating active gold mine tailings Dump at Shamva mine in Zimbabwe. *Journal of Applied Sciences and Environmental Management*. 2004; (9):2
9. Bhargavi NVL and Sudha PN. Effect of Salinity and pH on the Accumulation of Heavy Metals in Sunflower (*Helianthus Annus*) Plant. 2011; Available at: <http://www.eco-web.com/edi/110706.html>

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10. Chaney RL, Mielke HW and Sterrett SB. Speciation, mobility and bioavailability of soil lead. *environmental geochemistry and health* 9:105-129.
11. Coates W. 2005. Tree species selection for a mine tailings bioremediation project in Peru, short communication in *biomass and bioenergy*. 1994; 28 (4): 418–423
12. Compton H. Using Phytoremediation to clean up sites. 1999; www.epa.gov/superfund/accomp/news/phyto.htm
13. Prica M, Dalmacija, B, Roncevic S, Krcmar, D and Becelic M. A comparison of sediment quality results with acid volatile sulfide (AVS) and simultaneously extracted metals (SEM) ratio in Vojvodina (Serbia) sediments. *Science of the Total Environment*. 2007; 389:235-44.
14. Peplow D. Environmental impacts of mining in eastern Washington. Center for Water and Watershed Studies Fact Sheet. Seattle. 1999; 1-9.
15. Raskin I and Ensley BD. Phytoremediation of toxic metals. eds. John Wiley and Sons. New York; 2000.
16. Erakhrumen A and Agbontalor A. Phytoremediation an environmentally sound technology for pollution prevention, control and remediation in developing countries. *Educational Research and Review*. 2007; 2(7): 15-156.
17. Erdei L, Mezsi LG, Mcs I, Vass I, Fglein F and Bulik L. Phytoremediation as a program for decontamination of heavy-metal polluted environments. *Proceedings of the 8th Hungarian Congress on Plant Physiology and the 6th Hungarian Conference on Photosynthesis*; 2005..
18. Friberg L, Nordberg GF and Vouk V. *Handbook on the toxicology of metals*. Elsevier. Amsterdam; 1986.
19. Ghafoori M, Majid NM, Islam MM and Luhath S. Bioaccumulation of heavy metals by *Dyera costulata* cultivated in sewage sludge contaminated soil. *African Journal of Biotechnology* 10(52) 10674-10682
20. Maharia RS, Dutta RK, Acharya R and Reddy AVR. Heavy metal bioaccumulation in selected medicinal plants collected from Khetri copper mines and comparison with those collected from fertile soil in Haridwar, India. *Journal of Environmental Science and Health Part B Pesticides Food Contaminants and Agricultural Wastes*. 2010; 45(2):174-81
21. Raskin I, Smith RD and Salt DE. Phytoremediation of metals: Using plants to remove pollutants from the environment. *Current Opinion in Biotechnol*. 1997; 8(2):221
22. Sastre J, Sahuquillo A, Vidal M, and Rauret G. Determination of Cd, Cu, Pb and Zn in environmental samples: microwave assisted total digestion versus aqua regia and nitric acid extraction. *Anal. Chim. Acta*. 2002; 462: 59–72.
23. Thompson GD, Dirk U Bellstedt, Margaret Byrne, Melissa A Millar, David M Richardson. Cultivation shapes genetic novelty in a globally important invader. *Molecular Ecology*. 2012; 21(13):3187-99.

- 449 24. Trueby P. Impact of heavy metals on forest trees from mining areas. Proceedings of the
450 International Conference on Mining and the Environment III. Sudbury. Ontario. Canada,
451 2003.
- 452 25. Kindu M , Tadesse Y Glatzel G , Amha Y. Performance of eight tree species in the
453 highland Vertisols of central Ethiopia: growth, foliage nutrient concentration and effect on
454 soil chemical properties New Forests. 2012; 32(3):285-298
- 455 26. Post JA, Kleinjan CA, Hoffmann JH and Impson FAC. Biological control of *Acacia cyclops*
456 in South Africa: The fundamental and realized host range of *Dasineura dielsi* (Diptera:
457 Cecidomyiidae). Biological Control. 2009. 53(1):68-75.
- 458
459 27. Tadros JM. The physiology and biochemistry of the multipurpose forest trees species:
460 *Leucaena leucocephala*, *Prosopis juliflora*, *Acacia saligna*, and *Eucalyptus citriodora* as
461 influenced by water stress. Purdue University publication. Available at:
462 <http://docs.lib.purdue.edu/dissertations,2003>.