Trace Metals Bioaccumulation Potentials of Three Indigenous Grasses Grown on Polluted Soils Collected Around Mining Areas in Pretoria, South Africa

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Abstract

The rapid increase in the number of industries may have increased the levels of trace metals in the soil. Phytoremediation of these polluted soils using indigenous grasses is now considered an alternative method in remediating these polluted soils. The present study investigated and compared the ability of three indigenous grasses as bioaccumulators of trace metals from polluted soils. Seeds of these grasses were introduced into pots containing polluted soil samples after the addition of organic manure. The seeds of the grasses were allowed to germinate and grow to maturity before harvesting. The harvested grasses were later separated into shoots and roots and the trace metal contents were determined using ICP –MS. From all the grasses, the concentrations of trace metals in the roots were more than those recorded in the shoot with a significant difference ($P < 0.05$). The transfer factor (TF) showed that Zn was the most bioaccumulated trace metals by all the grasses followed by Pb, Mn, and Cu respectively. Chromium concentration from the shoot of the grasses was in the order *Urochlora moasambicensis* > *Themeda trianda* > *Cynodon dactylon*. The study concluded that the three grasses used were all able to bioaccumulate trace metals in a similar proportion from the polluted soils. However, since livestock feed on these grasses, they should not be allowed to feed on the grasses used in this study especially when harvested from a polluted soil due to their bioaccumulative potentials.

Introduction

Trace metals are mostly introduced into the environment through the emission of gases from factories (the fossil fuel combustion is the burning of coal, oil, or natural gas. Trace metals could also arise from natural sources and anthropogenic sources such as mining, agriculture and industrial activities (Scragg, 2006). Many parts of South Africa are generally affected by trace metals pollution, especially in areas around the mining industries (Olowoyo *et al.*, 2013). Mining activities encourage the disturbance of the plant cover, lack of organic matter, great relief depression and the degradation of soil structure which in turn results in an increase in the erosion risk (Izquierdo *et al*., 2005). During mining, tailings (heavier and larger particles established at the bottom of the flotation cell during mining) are directly discharged into natural depressions, including onsite wetlands resulting in high concentrations of trace metals (De Volder *et al*., 2003).

Trace metals cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another (Garbisu & Alkorta, 2001). However, it is possible for plants to absorb these trace metals in their tissues hence, reducing their levels in the soil (Olajire & Ayodele, 2002). From the soil, metal passes the root surface first before it moves from the soil solution to the plant (Jadia & Fulekar, 2008). Special plant membrane proteins recognize the chemical structure of essential

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metals; these proteins bind the metals and are then ready for uptake and transportation (Axelsen & Palmgren, 2001). Since the metal is complex within a chelate it can be translocated upwards in the xylem without being adsorbed by the high cation exchange capacity of the xylem (von Wiren *et al*., 1999). After harvesting the plants, the shoots may be taken out, incinerated to reduce volume and disposed as dangerous waste in order to reduce the level of trace metals in the soil.

Several studies have pointed to the use of indigenous grasses as phytoremediator; however, the ability of grasses to bioaccumulate trace metals from the soil seems to be plant and metal specific. The study of Mulungisi *et al.* (2009) reported that *Cynodon dactylon* have a potential of being classified as hyperaccumulator of trace metals and *Hyparrhenia hirta* has the ability to accumulate manganese which have severe health hazard to mammals and humans. Also in the study conducted by Nethengwe (2012) *Berkhenya coddii* plant was used to de-contaminate copper (Cu) and nickel (Ni) from the contaminated metallurgical waste residue known as Rustenburg Base Mine Refineries waste. A study was performed in a green house, where *B. coddii* was reported to have the ability to absorb nickel and copper into their tissues especially in their leaves (Nethengwe, 2012).

In South Africa, it is often common to see *Themeda triandra*, a perennial grass that grows mostly in an undisturbed soil especially in areas of average to high rainfall. The grass may also grow in any type of soil; it thrives well in a clay soil with a support of high organic matter content (Leistner, 2000). On the other hand, *Urochlora mosambicensis* also a perennial grass and a drought enduring grass, is also widely distributed in Southern Africa. The plant can tolerate both acidic and alkaline soils (Leistner, 2000). However, these two grasses ability as regards to phytoremediation potentials has not been investigated.

Apart from the aforementioned, there is also a growing concern on rehabilitation of previously polluted areas and identification of different indigenous grasses and the metals they can bioaccumulate from the soil. The present study therefore compared the bioaccumulation potentials of *Themeda trianda* and *Urochlora mosambicensis* with *Cynodon dactylon* that was previously reported in literature as good hyperaccumulator (Mulungisi *et al.,* 2009; Soleiman *et al*., 2009).

Materials and method

The experiment was conducted in a greenhouse in order to check and limit the environmental influence. Soil samples were collected from two sites which were previously reported to have polluted soils (Olowoyo *et al*., 2015) and were used to plant the grasses**.** The soil samples were placed in twenty – four pots and treated with cow dung and chicken droppings in order to provide needed nutrient for the grasses (Detpiratmongkol *et al*., 2014). The pots were divided into two groups, with each group containing soils collected from sites 1 and 2 respectively (Site 1: Eland Platinum Mine and Site 2: Production Unit, Sefako Makgatho Health Sciences University). Each group comprised 12 pots with each grass having 4 pots in each case representing 4 replicates.

Soil samples were analysed for trace metal content before the commencement of the experiment. The analysis of trace metals from the soil samples was carried out using three acid digestion methods. The soil samples were air dried for 8–10 days in the laboratory. The dried soil samples were ground using mortar and pestle and sieved through 2 mm steel sieve to obtain homogenized fine particles and stored in plastic bags. Thereafter, 1 g of soil sample was mixed with 9 ml of 65% Nitric acid and 2 ml hydrogen peroxide, 3 ml of perchloric acid and then placed in higher performance microwave unit 12000 mega digester at 180 °C for ~2 hours under 250 W. After digestion, the samples were left to cool and then transferred to 100ml volumetric flasks where they were filled with deionised water till the mark of 100 ml volumetric flask. Then the samples were left for 24 hours to settle down, and the resulting solution was analysed using ULTIMA Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and soil *p*H was determined in deionised water using a *p*H meter.

Soil samples were then filled into 10 kg pots and used for the cultivation of indigenous pasture grasses (*Cynodon dactylon*, *Themeda trianda* and *Urochlora mosambicensis*). Seeds of the grasses were collected from the inflorescences of the matured grass plants and introduced to the pot plants, watered daily and allowed to grow to maturity before harvesting for a period of 16 weeks to reach full maturity.

The grass samples after harvesting (16 weeks after planting) was separated into two different parts namely the shoot and the root. The grasses were then oven dried at 70° C for 48 hrs, ground and digested for analysis using 2 ml of nitric acid (HNO_3) and 3 ml of perchloric ($HClO_4$) (Olowoyo *et al.*, 2015) and analysed using ULTIMA Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

The ability of the plants to bio accumulate trace metals were determined using the formula described by Yangun *et al.* (2004).

i. $\frac{\text{Shoot}}{\text{Root}} > 1$ is metal accumulator

ii. $\frac{\text{Shoot}}{\text{Root}} < 1$ is metal excluder

However, bioconcentration factor (BCF) was calculated using the formula

$$(\text{BCF}) = \frac{\text{metal concentration in aerial part plants}}{\text{soil metal concentration}}$$

(Rotkittikhum *et al.*, 2006)

Statistical analysis

Analysis of Variance (ANOVA) was used to determine the significant differences between the growth rates and uptake of trace metals from the three grasses using SPSS version 21.0.

Results and discussion

The soil *p*H from all the pots ranged from 6.66 ± 1.28 – 8.32 ± 1.23. Trace metals mobility is enhanced mostly in acidic soils (Lion & Olowoyo, 2012). The present study added organic manure to the collected soil samples in order to facilitate the growth of the plants. This amendment might have restricted the mobility of trace metals in the soil. The organic manure can provide sorption sites for trace metals thus affecting their mobility in soil (Ross, 2011). The rapid growth noticed might be connected to the addition of the organic manure as reported by Detpiratmongkol *et al.* (2014) in a separate study using Kalmegh plant. However, despite the introduction of the organic amendment, the grasses used were still able to uptake most of the trace metals from the soil especially when the transfer factor is considered (Tables 5 and 6). In the case of Zn, it was reported in literature that organic matter can favour bioavailability of Zn due to the fact that Zn is associated with organic matter and this may result in high decomposition and oxidation of organic matter with time (Ogunkunle *et al*., 2013). This phenomenon of Zn bioaccumulation by all the grasses in our study could then be traced to the high affinity of Zn with organic matter.

The mean concentration of Pb from the harvested grasses from all the pots ranged from 0.18 ± 0.10 µg/g – 8.34 ± 0.33 µg/g. From all the grasses, the mean concentrations recorded from the roots were more than those recorded from the shoots and the differences were significant ($P <$ 0.05) (Tables 2 and 3). There was no significant difference in the value of Pb recorded for all the grasses ($P >$ 0.05).

The concentration of Mn was in the range 54.00 ± 0.47 µg/g – 444.00 ± 7.69 µg/g for all the grasses from all the pots. The highest mean value was recorded from the roots of *Themeda trianda* harvested from soil collected from Site 1. The lowest mean value was recorded from the shoots of *Cynodon dactylon* harvested from soil collected from Site 2. Similar observation noted for the accumulation of Pb in shoots and roots was also noted with Mn. The differences obtained in shoots and roots of all the plants were significant ($P <$ 0.05). However, there was no significant variation in the values of Mn obtained from all the grasses ($P >$ 0.05).

With Cr, the mean concentrations ranged from 3.90 ± 0.01 µg/g – 69.9 ± 1.93 µg/g. The highest mean value was recorded from the roots of *Cynodon dactylon* harvested from soil collected from Site 1. The lowest mean value was recorded from the shoots of *Urochlora mosambicensis* harvested from pots containing soil collected from Site 2. Cr concentrations in the shoot of the plants from soil collected from Site 1 was in the order *Urochlora moasambicensis* > *Themeda trianda* > *Cynodon dactylon* while from the plants harvested from soil collected from Site 2 was in the order *Themeda trianda* > *Cynodon dactylon* > *Urochlora mosambicensis*. There was no significant difference in the concentration of Cr from the shoots of the grasses ($P >$ 0.05).

The concentration of Cu for all the different parts from the grasses in all the pots ranged from 11.40 ± 0.28 µg/g – 44.90 ± 0.29 µg/g. The highest mean levels for these metals was recorded from the roots of *Cynodon dactylon*, however, there was no significant difference in the concentration of this metal from all the grasses either from the roots or shoot ($P >$ 0.05).

Zn concentration ranged from 492.00 ± 30.7 µg/g – 1300.00 ± 24.5 µg/g. The highest mean value was recorded from the roots of *Themeda trianda* harvested from soils collected from Site 1 mine. The lowest mean value was recorded from the shoots of *Urochlora mosambicensi* harvested from soil Site 2 with a value of 492 ± 30.7 µg/g. Just as in the case of Cu, there was no significant difference in the values obtained for Zn either from the shoot or the root ($P >$ 0.05).

The transfer factor (TF) showed that elements such as Zn, Mn and to some extent Pb were translocated from the roots to the shoots of the grasses (Table 4). The values of TF were > 1 and in some cases closer to 1 suggesting that all the grasses can act as bioaccumulator (Sekabira *et al*., 2011). Most of the values for element such as Cu were close to 1 from all the plants harvested

Table 1

Mean concentrations of trace metals in soils collected from different sites

Soil	*Trace Metals (mg/kg)*				
	Cu	*Zn*	*Mn*	*Pb*	*Cr*
S1	108.67 ± 36.45	143 ± 65.28	282.77 ± 73.86	32.35 ± 10.69	317.97 ± 35.40
S2	91.94 ± 36.48	66.05 ± 4.85	315.53 ± 84.82	34.93 ±1 1.80	269.77 ± 30.74

S1 (Crocodile River mine); S2 (Control SMU)

Table 2

Trace metal concentration of plant parts (shoots and roots) harvested from soils collected from Eland Platinum mine (S1)

Plants	*Plant Parts*	*Trace Metals (mg/kg)*				
		Cu^{b}	*Zn*^{b}	*Mn*^{ab}	*Pb*^{a}	*Cr*^{b}
Themeda trianda	Shoots	17.8 ± 0.37	932 ± 9.3	99 ± 1.68	3.09 ± 0.06	13.4 ± 0.35
	Roots	21.2 ± 0.75	1300 ± 24.5	444 ± 7.69	3.82 ± 0.012	61.4 ± 0.77
Cynodon dactylon	Shoots	14.0 ± 0.40	535 ± 7.3	59.0.66	1.88 ± 0.04	7.6 ± 0.25
	Roots	18.9 ± 0.62	691 ± 10.5	181 ± 2.57	2.06 ± 0.025	31.2 ± 0.30
Urochlora mosambicensis	Shoots	18.2 ± 0.30	652 ± 9.8	121 ± 1.48	0.18 ± 0.10	4.4 ± 0.16
	Roots	27.4 ± 0.24	812 ± 25.7	478 ± 6.90	0.19 ± 0.015	69.9 ± 1.93

a: Significant differences in the values obtained for roots and shoots from all the plants ($P < 0.05$)
b: No significant differences in the values obtained from all the plants ($P > 0.05$)

Table 3

Trace metal concentration of plant parts (shoots and roots) harvested from soils collected from Sefako Makgatho Health Sciences University (S2)

Plants	*Plant Parts*	*Trace Metals (mg/kg)*				
		Cu^{ab}	*Zn*^{ab}	*Mn*^{ab}	*Pb*^{ab}	*Cr*^{ab}
Themeda trianda	Shoots	13.6 ± 0.34	584 ± 16.00	71 ± 2.62	1.97 ± 0.05	6.2 ± 0.24
	Roots	49.5 ± 0.24	550 ± 20.2	83 ± 1.49	8.34 ± 0.33	21.8 ± 0.88
Cynodon dactylon	Shoots	11.4 ± 0.28	518 ± 7.2	54 ± 0.47	1.74 ± 0.08	3.9 ± 0.05
	Roots	44.9 ± 0.23	497 ± 5.5	73 ± 0.75	7.22 ± 0.099	19.6 ± 0.06
Urochlora mosambicensis	Shoots	11.7 ± 0.83	492 ± 30.7	58 ± 4.21	1.78 ± 0.10	5.6 ± 0.34
	Roots	40.5 ± 0.58	565 ± 3.0	58 ± 1.10	6.05 ± 0.445	17.7 ± 0.25

a: No significant differences in the values obtained for the elements from the shoots of the plants ($P > 0.05$)
b: No significant differences in the values obtained for the elements from the shoots of the plants ($P > 0.05$)

TABLE 4

Translocation Factor of Themeda trianda, Cynodon dactylon and Urochloa mosambicensis from different sites

Plants	*Site*	*Trace Metals (mg/kg)*				
		Cu	*Zn*	*Mn*	*Pb*	*Cr*
Themeda trianda	S1	0.83	0.71	0.22	0.81	0.22
	S2	0.25	1.06	0.85	0.23	0.28
Cynodon dactylon	S1	0.74	0.77	0.32	0.91	0.24
	S2	0.25	1.04	0.73	0.24	0.19
Urochlora mosambicensis	S1	0.66	0.80	0.25	0.94	0.06
	S2	0.29	0.87	1.00	0.29	0.32

TABLE 5

Bio-accumulation factor (BCF) for plant parts harvested from Eland Platinum mine (S1)

Plants	*Plant Parts*		*Trace Metals (mg/kg)*			
		Cu	*Zn*	*Mn*	*Pb*	*Cr*
Themeda trianda	Shoots	0.16	6.51	0.35	0.09	0.04
	Roots	0.20	9.09	1.57	0.12	0.19
Cynodon dactylon	Shoots	0.13	3.74	0.21	0.05	0.02
	Roots	0.18	4.83	0.64	0.06	0.09
Urochlora mosambicensis	Shoots	0.17	4.60	0.42	0.01	0.01
	Roots	0.25	5.68	1.70	0.06	0.21

TABLE 6

Bio-accumulation factor (BCF) for plant parts harvested from Sefako Makgatho Health Sciences University (S2)

Plants	*Plant Parts*		*Trace Metals (mg/kg)*			
		Cu	*Zn*	*Mn*	*Pb*	*Cr*
Themeda trianda	Shoots	0.15	8.84	0.22	0.05	0.02
	Roots	0.53	8.32	0.26	0.24	0.08
Cynodon dactylon	Shoots	0.12	7.84	0.17	0.05	0.01
	Roots	0.50	7.52	0.23	0.21	0.07
Urochlora mosambicensis	Shoots	0.12	7.50	0.18	0.05	0.02
	Roots	0.44	8.55	0.18	0.17	0.07

from Site 1 which may also indicate the possibility of Cu translocation from the roots of the plants to the shoot. The behavioural pattern as regards translocation for all the plants were similar with the exception of Mn from plants harvested from soil collected from Site 2.

All the plants accumulated more of the trace metals in their roots than the shoot from the present study. The ability of plants to bioaccumulate more trace metals in their roots than the shoot has been reported in literature. The study of Hazrat *et al*. (2012) showed that the order of concentrations of trace metals was the roots greater than the shoot. Similar observation was also made by Peralta *et al.* (2001) where alfalfa plants accumulated more of the trace metals in the roots than any other part.

Generally from the study, *Urochlora mosambicensis* and *Themeda trianda* seem to have competed favourably as a good biomonitor like *Cynodon dactylon* owing to the transfer factor (TF) depicted in Tables 5 and 6. There is no evidence from our study that suggests that the two other plants cannot be used as bioaccumulators. The findings from our study are in agreement with the study of Sekabira *et al.* (2011) where *Cynodon dactylon* and *Commelina benghalensis* were both used as phytoremediator and phytostabilsers. The result showed that both plants bioaccumulated Cu, Pb, Cd and Zn effectively from the soil. The choice of *Cynodon dactylon* in most cases by researchers may be due to its ability to cover soil quickly and the ability to hold soil together in order to reduce erosion. Similar observation was also noticed for *Urochlora mosambicensis* and *Themeda trainda* when introduced in this study and the ability of these plants to grow quickly and cover the soil rapidly was previously reported by (Leistner, 2000).

In conclusion, the three grasses used for the study were all able to bioaccumulate trace metals in a similar proportion from the polluted soils used in the study. The transfer factor (TF) showed that the grasses can translocate Zn, Mn and mostly Zn from their roots to their shoot while the bioconcentration factor (BCF) showed that three grasses can bioaccumulate Zn effectively from the soil despite the amendment used. The author thus, recommended that since most livestoock feed on these grasses, animals should not be allowed to feed on the grasses used in this study especially when harvested from a polluted soil due to the bioaccumulative potentials.

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