CLASSIFICATION OF PLANTS ACCORDING TO THEIR HEAVY METAL CONTENT AROUND NORTH MARA GOLD MINE, TANZANIA: IMPLICATION FOR PHYTOREMEDIATION

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ABSTRACT

Plants like other living organisms respond differently under different environmental conditions. An elevated level of heavy metals is one of the stresses which results into three classes of plants depending on their heavy metal content. The classes of plant species according to their accumulated heavy metals around North Mara Gold Mine were not known. To study such classes, a line transect of 700m long was established opposite the gold mine wastes. A total of eight sampling points were systematically established each after every 100m in that transect. Fifteen plant species were sampled; at least one species per sampling point. Approximately 5g of the root and shoot portions of the plants were separately collected from each plant. Three soil samples were also collected at each sampling point where vegetations were previously sampled. The soils and vegetations were analyzed for heavy metals (copper, lead, chromium, zinc, cadmium and nickel) by AAS. Of 15 plant species; 10, 6, 6 were hyperaccumulators, excluders and indicators for heavy metals respectively. Detailed studies on the agronomical requirements, optimizations, growth rates and the incidence of pests and diseases are required on the identified heavy metal hyperaccumulator plants for possible future remediation of the study area.

INTRODUCTION

In their natural environment plants survival, growth and reproduction depend on the soil physical and chemical characteristics changes. To survive such changes, plants must adapt and those that fail to are eliminated. One area that stresses plants is the pressure of heavy metals. Baker and Walker (1990), categorize plants into three groups according to their strategies for growing on metal-contaminated soils; metal excluders, indicators and accumulators or hyperaccumulators. Metal accumulators (hyperaccumulators) are plant species that concentrate metals in their above-ground tissues to levels far exceeding those present in the soil or in the non-accumulating species growing nearby. These plants are capable of extracting heavy metals from soils and concentrate them in their shoots, and they are widely used in phytoremediation. Accumulated heavy metals have been reported to play physiological and ecological functions, for example in prevention of bacterial and fungal diseases (Cutraro 2005). Some species can hyperaccumulate one particular each, while others hyperaccumulate more than one metal each. Metal excluders are plants which effectively limit the levels of heavy metal translocation within them and maintain relatively low levels in their shoot over a wide range of soil levels; however, they can still contain large amounts of metals in their roots (Baker and Walker 1990). Phytoremediation technology uses metal accumulation and exclusion abilities of plants to cleanup heavy metal polluted areas (Baker and Walker 1990, Schnoor 2002). Metal indicators are plants that accumulate metals in their above-ground tissues and the metal levels in the tissues of these plants generally reflect metal levels in the soil (Baker and Walker 1990). However, under continued uptake of heavy metals these plant species

die-off. Heavy metal indicator plants render biological and ecological functions in that they are possible indicators of pollution and useful in absorption of pollutants (Kvesitadze *et al.* 2006).

Determination of hyperaccumulator and excluder plant species is based on strict criteria. A plant is classified as a hyperaccumulator for heavy metal (s) when it meets four criteria; (a) shoot/root quotient (level of heavy metal in the shoot divide by level of heavy metal in the root) > 1, (b) extraction coefficient (level of heavy metal in the shoot divide by total level of heavy metal in the soil) > 1; extraction coefficient gives the proportion of total heavy metal in the soil which is taken up by the plant shoot/aerial part of the plant (Harrison and Chirgawi 1989, Rotkittikhun et al. 2006), (c) higher levels of heavy metals of 10 - 500 times the levels in normal plants (uncontaminated plants) according to Allen (1989) and Fifield and Haines (2000), also, (d) more than 1000µg/g of copper, lead, nickel, chromium; or more than 100µg/g of cadmium or more than 10000µg/g of zinc (Shen and Liu 1998, Ginocchio and Baker 2004, Yangun et al. 2004, Yangun et al. 2004, Boularbah et al. 2006, Rotkittikhun et al. 2006). Furthermore, a plant which has high levels of heavy metals in the roots but with shoot/root quotients less than 1 is classified as a heavy metal excluder (Boularbah et al. 2006). According to Baker and Walker (1990) an indicator plant species is the one of which the levels of heavy metals in the tissues are similar to those in the surrounding environment; soil.

According to Baker and Brooks (1989), metal hyperaccumulation is generally restricted to species growing at a given locality due to a great variation in physical, chemical and biological factors which exist among contaminated areas. Therefore, the present study aimed at classifying plants growing around North Mara Gold Mine for the possibility of getting hyperaccumulator

plants which could be used to extract heavy metals, some of which are already in elevated levels (Almås *et al.* 2009).

MATERIALS AND METHODS The study area

The current study was carried out in the vicinity of North Mara Gold Mine. The mine is about 100 kilometers east of Lake Victoria and 20 kilometers south of the Kenya-Tanzania border. Sampling was done at Nyabirama open cast of North Mara Gold Mine, located between latitudes 1°28' S and 1°27' S and along longitudes 34°29' E and 34°28' E.

The global positioning system (GPS) was used in recording the coordinates and geographical Information System (GIS) was used to locate the map of the study area (Figure 1).

Sampling

Sampling was done during commencement of the rainy season and cessation of the dry season. A line transect of 700m long was established opposite the gold mine wastes. Eight sampling points were systematically established after every 100m. A total of 15 plant species as shown in Table 1 (list of plants studied) were collected for analysis of their heavy metal content; at least one species per sampling point. Approximately 5g of the root and shoot portions were taken from the rhizosphere of the collected plants. Identification of the collected plant species was done based on keys or comparison with authentic specimens. These samples were labelled, air dried, placed in paper bags and transported to the University of Dar-es-Salaam for laboratory analysis.

Three soil samples were also collected at each sampling point where vegetations were previously sampled; the samples were then mixed to constitute composite samples per sampling point.

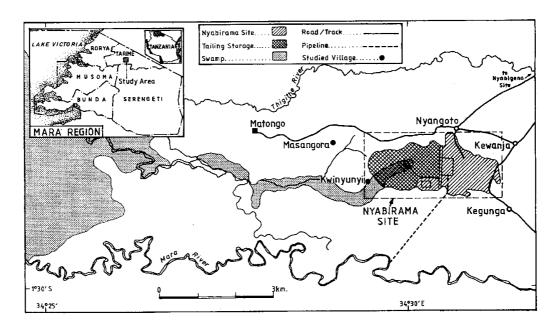


Figure 1: Location of the Sampling Area around North Mara Gold Mine

Soil

In order to determine total levels of heavy metals, the composite soil samples were airdried to constant weights. They were then ground into fine powder using pestle and mortar and sieved through 2mm plastic mesh to avoid metal contamination. A subsample of 2g was taken from each composite sample and put in a test tube. To each sample a 6ml 5:1 Nitric acid and Perchloric acid mixture was added for digestion following procedures by Allen (1989). To achieve digestion, mixtures were then heated in a Kjeldahl Thermo apparatus at 200°C until complete digestion was achieved. During heating, explosion of the contents was prevented by adding porcelain chips to the digestion tubes. After digestion, samples were left to cool to room temperature. Then, samples were prepared for heavy metal analysis by adding distilled water to 50ml. Analysis of the levels of heavy metals was done at the University of Dar es Salaam, College of Engineering and Technology; using a Perkin-Elmer 3100 Atomic Absorption Spectrophotometer.

Plants

Before the analyses root and shoot samples were thoroughly washed by distilled water to remove all adhering soil particles. Samples were then oven dried to constant weights at 105°C. Each dried sample was ground to powder using a wearing blender (Model type A 10 Janke and Kunkel GBH a Co. KG) according to Allen (1989). One gram of each sample was used for analysis. These samples were then digested using 5ml of 5:1 of concentrated Nitric (HNO₃) and Perchloric acid (HClO₄) mixture according to Allen (1989), then heated in a Kjeldahl vessel at 120° C until complete digestion was achieved. Subsequent procedures were similar to those for soil samples (above). Digestion and analytical efficiency of AAS was validated using a standard reference material of tomato leaf (SRM 1573a, National Institute of Standards and Technology, NIST). The percentage recoveries from the analysis of the standard reference material by the procedures that were currently used were 80% Zn, 85% Cu, 90% Ni, 83% Pb, 85% Cd and 70% Cr.

Table 1: Levels of Cu, Pb, Cr, Zn, Cd and Ni in Plant Roots and Shoots, and Soils around North Mara Gold Mine

DI .	Copper (µg/g)			Lead (µ	g/g)		Chromium (µg/g)		
Plant species	Soil	Root	Shoot	Soil	Root	Shoot	Soil	Root	Shoot
Ludwigia stolonifera (Ghill. & Pers) Raven.	9.9	ND	ND	546.56	506	459	ND	ND	ND
Sphaeranthus gomphrenoides O. Hoffm.	9.9	ND	ND	546.56	2080	1522.5	ND	ND	ND
Leersia hexandra SW.	9.9	ND	ND	546.56	422	832.5	ND	ND	ND
Commelina benghalensis L.	9.9	ND	ND	546.56	127	299	ND	ND	ND
Sphaeranthus kirkii Oliv.	8.25	ND	ND	278.44	237	261	ND	ND	ND
Typha capensis Pers.	8.25	ND	ND	278.44	543	379	ND	ND	ND
Cyperus articulatus L.	13.61	1	ND	527.59	355	297	66.83	ND	ND
Fuirena umbellata Rottb.	25.17	ND	ND	298.24	207	52	ND	42.00	36.00
Agave sisalana Perr.	25.17	ND	ND	298.24	338	28	ND	30.00	26.00
Cyperus exaltatus L.	13.61	ND	ND	527.59	227	92.5	66.83	ND	ND
Crinum papilosum L.	11.0	1	4	260.0	382	231	ND	29.00	39.00
Hoslundia opposita Vahl.	11.55	12	1	263.18	315	475	ND	52.00	44.00
Pluchea dioscoridis (L) DC.	11.55	ND	1	263.18	300	128	ND	47.50	24.00
Hygrophylla auriculata (Schumach) Heine.	45.38	ND	ND	304.43	2600	549	ND	7.00	17.00
Ipomoea batata (L) Lam.	6.19	NA	ND	231.83	NA	775	ND	NA	ND

Table 1 Cont.

DI (Zinc (μg/g)			Cadmium (μg/g)			Nickel (μg/g)		
Plant species	Soil	Root	Shoot	Soil	Root	Shoot	Soil	Root	Shoot
Ludwigia stolonifera (Ghill. & Pers) Raven.	115.09	65	66	13.2	14	21	1165.73	757	558
Sphaeranthus gomphrenoides O.	115.09			13.2			1165.73		
Hoffm.		155	142.5		155	47.5		3485	1885
Leersia hexandra SW.	115.09	39	90	13.2	55	37.5	1165.73	452	1962.5
Commelina benghalensis L.	115.09	33	81	13.2	19	30	1165.73	791	673
Sphaeranthus kirkii Oliv.	95.7	27	28	13.61	77	64	669.9	334	511
Typha capensis Pers.	95.7	36	71	13.61	12	39	669.9	806	498
Cyperus articulatus L.	94.05	72	42	9.08	51	30	1105.91	842	915
Fuirena umbellata Rottb.	64.35	56	58	15.26	41	47	879.04	595	365
Agave sisalana Perr.	64.35	50	34	15.26	40	34	879.04	915	517
Cyperus exaltatus L.	94.05	30	145	9.08	20	47.5	1105.91	851	1930
Crinum papilosum L.	81.0	29	32	11.0	37	52	833.0	825	781
Hoslundia opposita Vahl.	82.09	42	41	11.55	37	42	834.9	903	890
Pluchea dioscoridis (L) DC.	82.09	82.5	39	11.55	170	71	834.9	2110	919
Hygrophylla auriculata (Schumach) Heine.	89.51	35	47	7.84	30	63	731.78	721	1031
Ipomoea batata (L) Lam.	45.38	NA	112.5	9.9	NA	185	548.63	NA	2742.5

RESULTS Levels of Heavy Metals in the Roots and Shoots of Plant Species and Soils

Table 1 presents the levels of the selected heavy metals in soils, shoots and roots. The

extent of accumulation of heavy metals by the plants studied differed with the type of metal. The root of *Hoslundia opposita* had the highest level of copper of 12µg/g while, in all the plant species except *Cyperus* articulatus, Crinum papilosum and Pluchea dioscoridis the levels of copper were below detection limits. The highest level of lead of 2600µg/g was found in the root of Hygrophylla auriculata while, the lowest level of lead of 28µg/g was found in the shoot of Agave sisalana. The highest level of chromium was 52µg/g which was found in the root of *Hoslundia opposita*. However, the lowest levels of chromium in most plant species were below detection limit. In addition, the results in Table 1 indicate that, the highest level of zinc of 155µg/g was in the root of Sphaeranthus gomphrenoides; while the lowest level of zinc of 27µg/g was found in the root of Sphaeranthus kirkii. Furthermore, the highest level of cadmium of 185µg/g was found in the shoot of Ipomoea batata, while the lowest level of cadmium of 12µg/g was found in the root of Typha capensis. The results in the same table indicate that the highest level of nickel of 3485µg/g was found in the root of Sphaeranthus gomphrenoides while the lowest level of nickel of 334µg/g was found in the root of Sphaeranthus kirkii.

Furthermore, the results in Table 1 show that the level of total copper in the soil ranged from 6.19 to $45.38\mu g/g$. Total lead in the soil ranged from 231 to $546.56\mu g/g$, while, total chromium ranged from below detection limit to $66.83\mu g/g$. Furthermore, the level of total zinc ranged from 45.38 to $115.09\mu g/g$, total cadmium from 7.84 to $15.26\mu g/g$ and total nickel ranged from 548.63 to $1165.73\mu g/g$.

Shoot/Root Quotients and Extraction Coefficients for Plant Species

Table 2 summarizes the results which indicate that *Crinum papilosum* is the only species which had a shoot/root quotient > 1 for copper.

Both Crinum papilosum and Hygrophylla auriculata had shoot/root quotient > 1 for chromium. The results also show that Leersia hexandra, Commelina benghalensis, Sphaeranthus kirkii and Hoslundia opposita

had shoot/root quotients > 1 for lead. Furthermore, Ludwigia stolonifera, Leersia hexandra, Commelina benghalensis, Sphaeranthus kirkii, Typha capensis, Fuirena umbellata, Cyperus exaltatus, Crinum papilosum and Hygrophylla auriculata had shoot/root quotients > 1 for zinc.

In the case of nickel, the species which had shoot/root quotients > 1 were Leersia hexandra, Sphaeranthus kirkii, Cyperus articulatus, Cyperus exaltatus, Hoslundia opposita and Hygrophylla auriculata.

The same results also show that Ludwigia stolonifera, Commelina benghalensis, Typha capensis, Fuirena umbellata, Cyperus exaltatus, Crinum papilosum, Hoslundia opposita and Hygrophylla auriculata had shoot/root quotients > 1 for cadmium.

The same table indicates that, in the case of copper, the extraction coefficients were 0.34 for *Crinum papilosum* and 0.8 each for *Hoslundia opposita* and *Pluchea dioscoridis* while for the case of chromium, the extraction coefficients were not workable because the levels of chromium were not detectable.

The extraction coefficients for Sphaeranthus gomphrenoides, Leersia hexandra, Typha capensis, Hoslundia opposita and Hygrophylla auriculata were > 1 for lead. The results of Table 2 also show that Sphaeranthus gomphrenoides and Cyperus exaltatus had extraction coefficients > 1 for zinc.

Table 2 also shows that, the extraction coefficients for Sphaeranthus gomphrenoides, Leersia hexandra, Cyperus exaltatus, Hoslundia opposita, Pluchea dioscoridis and Hygrophylla auriculata were > 1 for nickel.

 Table 2:
 Extraction Coefficients and Shoot/Root Quotients for 14 Plant Species Growing around North Mara Gold Mine

Plant species	Copper		Lead		Chromium		Zinc		Cadmium		Nickel	
	EXT.C	S/R.Q	EXT.C	S/R.Q	EXT.C	S/R.Q	XTE.C	S/R.Q	EXT.C	S/R.Q	EXT.C	S/R.Q
Ludwigia stolonifera (Ghill. & Pers) Raven.	NA	NA	0.84	0.90	NA	NA	0.57	1.01	1.59	1.5	0.47	0.74
Sphaeranthus gomphrenoides O. Hoffm.	NA	NA	2.78	0.73	NA	NA	1.23	0.91	3.59	0.30	1.62	0.54
Leersia hexandra SW.	NA	NA	1.523	1.973	NA	NA	0.782	2.308	2.84	0.68	1.68	4.34
Commelina benghalensis L.	NA	NA	0.54	2.35	NA	NA	0.70	2.45	2.27	1.57	0.57	0.85
Sphaeranthus kirkii Oliv.	NA	NA	0.93	1.10	NA	NA	0.29	1.03	4.70	0.83	0.76	1.53
Typha capensis Pers.	NA	NA	1.36	0.69	NA	NA	0.74	1.97	2.86	3.25	0.74	0.62
Cyperus articulatus L.	NA	NA	0.56	0.83	NA	NA	0.44	0.58	3.30	0.58	0.83	1.09
Fuirena umbellata Rottb.	NA	NA	0.17	0.25	NA	0.85	0.90	1.03	3.08	1.14	0.42	0.61
Agave sisalana Perr.	NA	NA	0.09	0.08	NA	0.86	0.52	0.68	2.22	0.85	0.59	0.57
Cyperus exaltatus L.	NA	NA	0.17	0.40	NA	NA	1.54	4.83	5.23	2.37	1.75	2.27
Crinum papilosum L.	0.34	4	0.87	0.60	NA	1.34	0.39	1.10	4.50	1.40	0.94	0.95
Hoslundia opposita Vahl.	0.08	0.08	1.81	1.50	NA	0.84	0.49	0.97	3.63	1.13	1.07	0.99
Pluchea dioscoridis (L) DC.	0.08	NA	0.48	0.42	NA	0.50	0.47	0.47	6.14	0.41	1.10	0.44
Hygrophylla auriculata (Schumach) Heine.	NA	NA	1.80	0.21	NA	2.42	0.52	1.34	8.036	2.1	1.41	1.43

DISCUSSION

Generally, plant species or even populations react differently when exposed to elevated levels of heavy metals. Some plants accumulate heavy metals; others exclude them while other plant species are sensitive. To be classified in either category plants must pass certain criteria as explained in the introductory part.

In this study, we evaluated fifteen plant species against a number of heavy metals, *viz.*, copper, lead, chromium, zinc, cadmium and nickel based on the criteria stated. So, on the basis of the results presented in Tables 1 and 2; table 3 summarizes the plant species in their respective classes.

 Table 3:
 Classes of 14 Plant Species around North Mara Gold Mine

Plant species	Hyperaccumulator	Excluder	Indicator
Ludwigia stolonifera (Ghill. & Pers) Raven			Cd and Pb
Sphaeranthus gomphrenoides O. Hoffm.		Ni and Cd	
Leersia hexandra SW.	Pb and Ni		
Commelina benghalensis L.	Cd		
Sphaeranthus kirkii Oliv.			Pb
Typha capensis Pers	Cd	Ni	
Cyperus articulatus L.		Cd	Zn
Fuirena umbellata Rottb	Cd and Cr		
Agave sisalana Perr.	Cr		Zn, Pb and Ni
Cyperus exaltatus L.	Cd, Zn and Ni	Pb	
Crinum papilosum L.	Cd and Cr		Ni
Hoslundia opposita Vahl	Cr, Cd and Pb		
Pluchea dioscoridis (L) DC.	Cr	Ni and Cd	Pb and Zn
Hygrophylla auriculata (Schumach) Heine	Cr, Cd and Ni	Pb	

NOTE: Some of the plant species met only some of the requirements for hyperaccumulation

In this study, *Leersia hexandra* and *Hoslundia opposita* were classified as lead hyperaccumulators. These plant species met only three criteria for lead hyperaccumulation; thus, they have an implication of carrying out

phytoremediation around North Mara Gold. *Hygrophylla auriculata* and *Cyperus exaltatus* were classified as lead excluders in the present study. *Ludwigia stolonifera*, *Sphaeranthus kirkii*, *Agave sisalana* and

Pluchea dioscoridis were classified as lead indicators.

The current study also classified Leersia hexandra. Cyperus exaltatus and Hygrophylla auriculata as nickel hyperaccumulators after meeting all the four criteria for nickel hyperaccumulation. These plant species implied to be used to extract nickel from the study area. Cyperus digitalis has also been reported by Ogundiran and Osibanjo (2008) in Nigeria as one of the plant species capable of accumulating higher levels of nickel in shoots.

Sphaeranthus gomphrenoides, Typha capensis and Pluchea dioscoridis were classified as nickel excluders. Indicator plant species for nickel were Agave sisalana and Crinum papilosum. Cyperus exaltatus met three conditions for onlv hyperaccumulation. The present study identified indicator plant species for zinc toxicity as Pluchea dioscoridis, Agave sisalana and Cyperus articulatus.

The present study also, classified Commelina benghalensis, Typha capensis, Fuirena umbellata, Cyperus exaltatus, Crinum papilosum, Hoslundia opposita and Hygrophylla auriculata as cadmium hyperaccumulators. These species met only three criteria for cadmium hyperaccumulation. It follows therefore that such species have implications of extracting cadmium from the contaminated soil around North Mara Gold Mine. Some of the above findings are similar to those reported by Ellis et al. (1994) who identified Typha latifolia as a cadmium hyperaccumulator which was previously used in sludge and waste substrate sanitation in London, United Kingdom. Also, Anoliefo et al. (2008) identified Cyperus rotundus as cadmium hyperaccumulator in Nigeria.

The cadmium excluders identified in the present study were Sphaeranthus gomphrenoides, Pluchea dioscoridis and Cyperus articulatus. Cadmium indicator

was Ludwigia stolonifera. Elifantz and Tel-Or (2002) reported on the efficiency of Ludwigia stolonifera as a biofilter for cadmium-contaminated water in Israel.

papilosum. In this study Crinum Hygrophylla auriculata, Fuirena umbellata, Agave sisalana, Hoslundia opposita and Pluchea dioscoridis were classified as chromium hyperaccumulators. Crinum papilosum and Hygrophylla auriculata were so classified on the basis of only two criteria chromium hyperaccumulation. for Furthermore, Fuirena umbellata, Agave sisalana, Hoslundia opposita and Pluchea dioscoridis met only one criterion for chromium hyperaccumulation. Probably more criteria for chromium hyperaccumulation could have been met by these plant species if there were higher levels of the metal in the study area.

The levels of copper were very limited in both soils and plant tissues, to a large extent being below detection limits. Thus, it was not possible to classify plants on the basis of their relations to copper levels in the soils. Unfortunately, leaf ages were not considered in the present study during collection of plant samples; because sampling was carried out during the offset of the dry season hence it was not easy to demarcate older and younger leaves. This is because, Boyd et al. (1999) reported that older leaves tend to have higher levels of nickel and chromium and less level of copper than younger leaves that tend to have lower levels of nickel and chromium and higher levels of copper. Furthermore, zinc and lead contents are not affected by leaf ages. The limited levels of copper at the study area was probably not greatly attributed to plant content rather soil content, as in some of the plant materials collected there were higher levels of both nickel and chromium. Probably, the sources of copper in the shoots depended upon foliar absorption and not greatly from the soils. Sweet potatoes had more than 10 times

levels of shoot zinc, lead, cadmium and

nickel than normal sweet potato shoots in the present study. It was not possible to classify the species because its root samples were consumed by rodents during the process of drying. However, other studies have classified it as a hyperaccumulator being effective in removing toxic metals from contaminated soils in Poland (Por_bska and Ostrowska 1999). Although sweet potatoes have accumulation potential may be, can not be selected as an accumulator because both its tuber and shoot are edible.

All in all, classification of indicator plant species requires careful consideration. This is because, some of the plant species classified as indicators in the current study were possibly hyperaccumulators or excluders during their early stages of metal uptake.

Plant species or even populations react differently when exposed to elevated levels of heavy metals. Some plants accumulate heavy metals, others exclude them while other plant species are sensitive (indicators). In relation to heavy metals, many of the plant species identified by other researchers as heavy metal hyperaccumulators vary depending on the climatic conditions, soil characteristics and other factors (Pivertz 2001).

Though the present study has identified species of plants that can probably be used in phytoremediation and detailed studies on agronomical requirements and optimization, influence of watering regimes and the incidence of pests and diseases are required on the identified heavy metal hyperaccumulators for possible future remediation of areas around North Mara Gold Mine.

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