EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 1 Number 5 (December-2014) pp. 16-27

www.ijcrbp.com

Original Research Article

Heavy Metals Concentration in *Adenodolichos rhomboideus* (O. Hoffm.) Harms. Forage Growing on Mining Tailings in South East of Democratic Republic of Congo: Influence of Washing, pH and Soil Concentrations

M. I. Tshibangu[1]*, V. I. Nsahlai[2], M. H. Kiatoko[3] and J. L. Hornick[4]

[1]University of Lubumbashi, Faculty of Agriculture, Department of Animal Production, Democratic Republic of Congo

[2]University of KwaZulu-Natal, Animal and Poultry Science, School of Agricultural Sciences & Agribusiness, South Africa

[3]University of Kinshasa, Faculty of Agriculture, Department of Animal Production, Democratic Republic of Congo

[4]University of Liège, Faculty of Veterinary Medicine, Department of Animal Production, Belgium

Corresponding author.

A b s t r a c t

A study was carried out in the mining zone, south east of the DRC, characterized by high soil concentrations of Cu, Co, Zn and Pb. This study aimed to (1) evaluate the contamination of leaves of *Adenodolichos rhomboideus* by some trace metals (Cu, Co, Zn and Pb) as a function of pH and total concentrations in the soil; and (2) determine the phytoremediation and phytostabilisation potential of this species. Samples of leaves were either washed or left unwashed. Transfer and accumulation of metals were evaluated as a biological concentration factor (BCF), translocation factor (TF) and bio-accumulation coefficient (BAC). Soil pH values varied from 6.2 to 6.5. Soil heavy metals concentrations varied highly. Those in washed leaves minerals concentrations were largely reduced. All soil values differed ($p<0.05$) except Cu concentration. For unwashed leaves concentrations, sites differed ($p<0.05$) and Zn concentration differed ($p<0.05$) between topographies. For washed leaves, only Zn concentration was affected by sites. *A. rhomboideus* supported soil contaminated with Cu, Co, Zn and Pb; fodder contained potentially toxic Cu concentrations for sheep. It can be used in phytoremediation, in phytostabilisation and in phytoextraction of soil contaminated by Zn, Pb and Co and Zn.

K e y w o r d s

Adenodolichos rhomboideus

Contamination

Forage

Ruminant

Trace elements

Introduction

The south-east of Democratic Republic of Congo (DRC) is characterized by the presence of extractive industries and ores treatment; primarily copper, cobalt, zinc, nickel, and incidentally uranium (Banza et al., 2009). The natural concentration of metals in metalliferous soils can reach thousands of mg/kg, but would decrease rapidly when the soil deviates from a deposit (Ernst, 1974). However, for a century, the development of extractive industries and treatment of ores has contributed to the anthropogenic dispersion of metals and contamination of surfaces much faster than those occupied by natural outcrops. New types of metalliferous habitats have thus appeared (Ginocchio et al., 2002). They constitute a very heterogeneous unit (Remon et al., 2005) since their origin vary according to the nature of the ground, ores exploited and the industrial processes. Sectors affected by contaminations are both urban and rural. Mining in Katanga is in transition. Although artisanal mining is still widespread, industrial mining - often involving multinational companies - is on the increase (Amnesty International, 2013; Lydall and Auchterlonie, 2011).

Flora of the area is strongly influenced by a six months dry season (Malaisse et al., 1999) and consecutively by high metals concentration in the soil and many forage species have dried and disappeared, especially grasses. Some forage plants look green all the time.These conditions favour the development of metallophytes and metallo-resistant plants, some of which can be utilized to initiate or promote the restoration of degraded ecosystems. Native plant species adapted to high levels of heavy metals can be doubly exploited; first to re-colonize damaged areas and produce forages for ruminants in the dry season.

Among herbaceous leguminous plant species, *Adenodolichos rhomboideus* (O. Hoffm.) Harms. has the property to remain green all year round. This species, used as forage and in traditional medicine, grows in various places in the south-east of DR. Congo: at Likasi, Kambove, Kundelungu, Lusinga, Masombwe, Kipushi, Kinsevere, Luiswishi, Kasombo, Lubumbashi, and Tshinsenda (INEAC, 1954; Meerts, 2008). It grows in grassy savannas, in shrubby savannas, in the clear forest and also on periodically wet sands (INEAC, 1954), and its roots spread out up to two meters (Tshibangu, 2010).

A. rhomboideus is a metallo-resistant species, encountered on copper hills, on normal and moderate to heavily contaminated soils (Faucon et al., 2011; Malaisse et al., 1999; Meerts, 2008). In a mining environment, *A. rhomboideus* forage can contain high levels of heavy metals by absorption from the soil or from atmospheric contamination. Some metals are macro and micro elements essential for organisms, but these minerals can become toxic at relatively low concentrations in the soil (Apostoli et al., 1998; Larry, 2008). *A. rhomboideus* forage is used as feed for ruminants in dry season (Tshibangu et al., 2014). Animals consuming these feeds do not live long enough to develop diseases because they are slaughtered and consumed by humans. Certain trace elements, are contaminants in the entire food chain from upstream (soil, water, atmosphere), to humans, via animal and vegetable production. Some studies have shown that river water, fish, faeces and urine of people and animals living in this areas contained high levels of heavy metals such as Cu, Co, Pb and Zn (Banza et al., 2009; Katemo et al., 2010; Mobinzo, 2010).

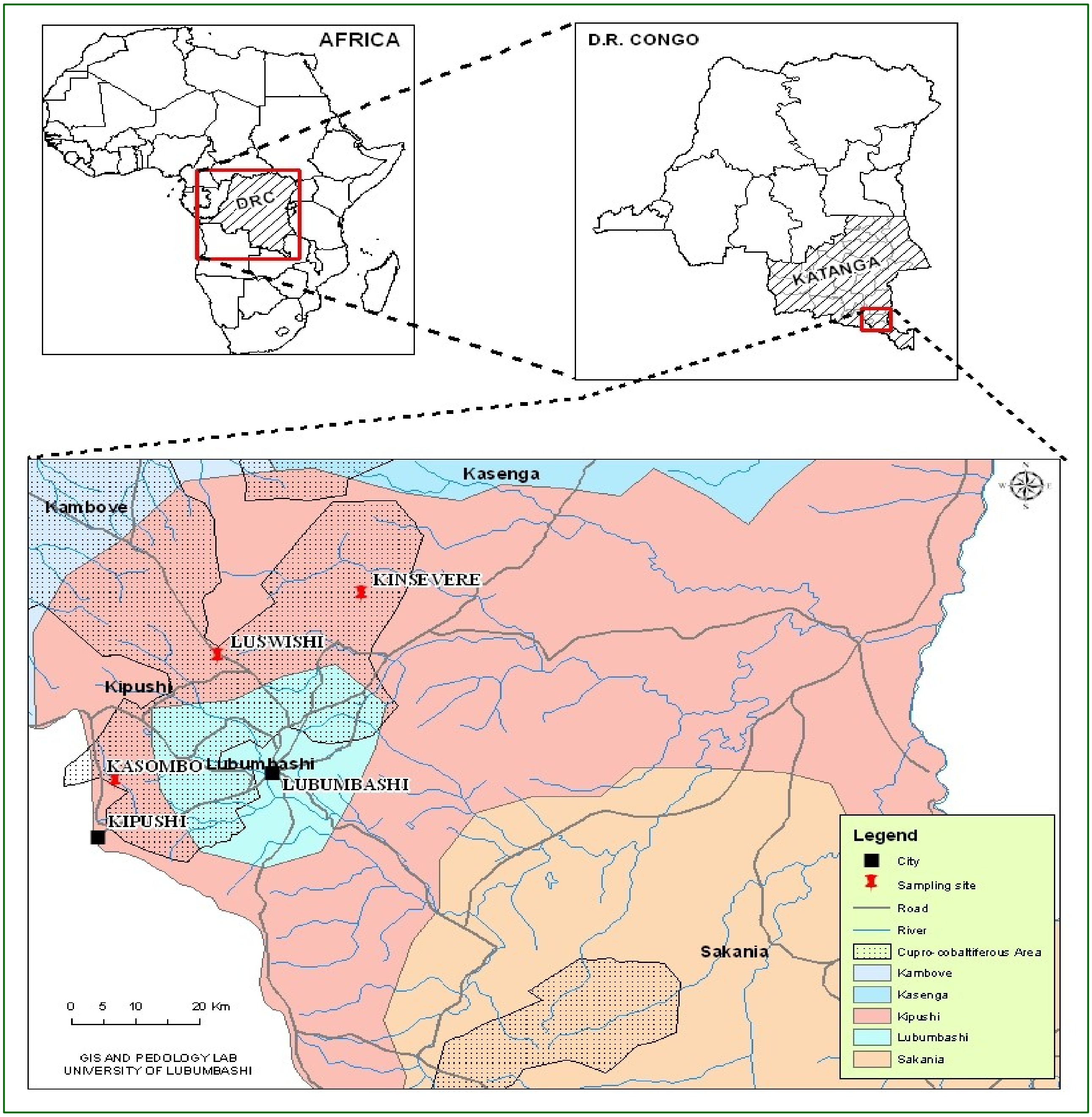
Thus, to better appreciate the dangers associated with the use of forage at Lubumbashi and its surroundings, a preliminary study was conducted to assess the state of contamination of *A. rhomboideus* in dry season. Specifically, this study aims to assess the first the toxicological quality (level of heavy metal contamination Cu, Co, Zn and Pb) of *A. rhomboideus* leaves as a function of pH and total concentrations of these nutrients in the soil of mining areas around Lubumbashi. And soil sites mowing and study the adaptability of this species on contaminated soil or not by heavy metals characteristics of the region to determine its phytoremediation and phytostabilisation potential.

Materials and methods

Sites of the study

The study was conducted in three mining sites around Lubumbashi (Luiswishi, Kinsevere and Kasombo) chosen for their proximity with dwellings. The climate is subtropical and the altitude is about 1300 m. The rainy season is unique, lasting from November until early April. Rainfall is about 1300 mm per year and the average annual temperature about 20°C (Faucon, 2009; Malaisse et al., 1999). Luiswishi mine is located at 1320m altitude, 26°33' 45” E, 11°03' 25” S, 25 km North-West of Lubumbashi. Kinsevere mine is located at 1240m altitude, 027°34' 58.0” E, 11°22' 01.5” S, 37 km North of Lubumbashi and Kasombo II mine is located at 1320m altitude, 027°18' 21” E, 11°41' 17” S, 35 km in the South-West of Lubumbashi (Fig. 1).

Fig. 1: Maps of Africa (left above), the Democratic Republic of Congo (right side above) and part of the Katanga province (below). The study took place in southern Katanga in different samplings areas of Lubumbashi (capital), Kasombo, Kinsevere and Luiswishi.



Sampling

Samples of the plant, *A. rhomboideus* (Fig. 2) were collected from the shallows, slopes and trayson each of the three sites. Whole plants of were harvested at the same places where soil profiles were dug for soil sampling. Roots (Fig. 3) were separated directly from the aerial parts of plants, washed and tops sampled into two parts. Fractions were packaged and labelled. In the laboratory, one of two aliquot of each aerial part was washed with deionized water containing Alconox (Faucon et al., 2007). Leaf samples and roots were dried for 72 h at 60°C, ground through a mill (IKA WEKKE M20), and placed in flasks for laboratory analysis. Regarding soil profiles, two layers were considered according to the colour of soil and samples were collected from the bottom up within 30 to 50 cm following most plant roots distribution. Soil samples of different layers were put in bags and labelled. After drying at room temperature, sampled soils were crushed, sieved (2 mm mesh) according to the AFNOR X31-151 (Alsac, 2007) and stored in flasks pending analyses.

Fig. 2: *Adenodolichos rhomboideus*-habit; local name Kitutula (Kitabwa); Pande (Luiswishi); Kinkampe (Tshiluba), Kimamba (Bemba) (INEAC, 1954, Tshibangu, 2010).

Fig. 3: *Adenodolichos rhomboideus* roots

Chemical analyses

Dry matter concentration was determined after drying leaves and root in an oven at 105°C for 24 h. Soil total concentrations of copper, cobalt, zinc and lead were measured in duplicate, and results reported in mg/kg dry soil. The pH was determined using a pH-meter glass electrode in a soil to distilled water ratio of 1: 2.5.The mineral content of the soil was determined according to the method described by Alsac (2007). Digestion was carried out on 0.5 g soil with 6 ml of hydrochloric acid and 2 ml of nitric acid (aqua regia) at 95°C for 75 min on a heating block. The digest was then adjusted to 50 ml. Mineral content of roots and leaves were done using atomic absorption spectrometry, according to the NF EN ISO 17294-1 and 17294-2 French standard method (Hébrard-Labit and Meffray, 2004; Sebei et al., 2005). The minimum detection limit for each of these metals in leaves and roots samples were Cu: 3 ppb, Co: 5 ppb, Pb: 10 ppb and Zn: 1ppb.

Calculations and statistical analyses

Data were analysed following the generalized linear model (Proc GLM, SAS, 1999). The model accounted for the mains effects of sites of samplings, topography, depth of horizon, washing, and their interactions. Averages were compared between them using Student t-test. Concentration, transfer and accumulation of metals from soil to roots, from roots to leaves and from soil to leaves were evaluated in terms of Biological Concentration Factor (BCF), Translocation Factor (TF) and Bio-accumulation Coefficient (BAC) according to the formulas: BCF= [metal] root/ [metal] soil (Yoon et al., 2006); TF = [metal] leaves/ [metal] root (Cui et al., 2007; Li et al., 2007); BAC = [metal] leaves/ [metal] soil (Cui et al., 2007; Li et al., 2007).

Results

Soil pH

Values of pH were highest at Kinsevere and lowest at Kasombo. Values of pH were higher at the lowlands than at other topographies. Surface layers had higher ($p<0.001$) pH values than deep layer. Soil pH values normally range from 3.0 to 8.4 (Table 1), which encompasses the range in this study. Kaya (2008) observed soil pH ranging from 5.1 to 6.1 at Kinsevere. These values are lower than those found in this work and could be due to samples being exclusively taken from soils colonized by *A. rhomboideus*.

Heavy metals concentration in soil

Trace metals concentrations of soil colonized by *A. rhomboideus* are given in Table 1. Copper concentrations were not significantly different at Kasombo and Luiswishi ($p<0.01$) but lower at Kinsevere. Copper concentrations were not affected by topography, but superficial soil layers had higher ($p<0.001$) copper levels than deep layers. Cobalt concentrations at Kasombo and Kinsevere were similar but lower ($p<0.001$) than at Luiswishi and the lowlands had lower cobalt concentration than other topographies ($p<0.05$).

Table 1. Heavy metals concentration and pH in soils colonized by *Adenodolichos rhomboideus* according to site, topography (Top) and depth horizon at Lubumbashi.

Parameters	Site (1)			Topography (2)			Depth (3)		SEM	Effects (P>F)					
	KAS	KINS	LUI	S	T	Sl	SL	DL		Site	Top.	Dep.	Site*Top.	Site*Dep	Top.*Dep
Cu	1029^a	1763^b	1058^a	1298^a	1246^a	1306^a	1672^b	894^a	188	**	NS	***	*	NS	NS
Co	108^a	150^a	801^b	220^a	390^b	449^b	463^a	243^b	82	***	*	***	***	***	*
Zn	146.0^a	70^b	73^b	51^a	160^b	78^a	131^a	61^b	36	*	**	*	*	**	NS
Pb	89^a	61^b	58^b	55^a	90^b	64^a	81^a	58^b	10	*	**	**	NS	**	NS
pH	6.2^a	6.5^b	6.3^c	6.4^a	6.3^b	6.3^b	6.4^a	6.2^b	0.2	***	***	***	***	***	***

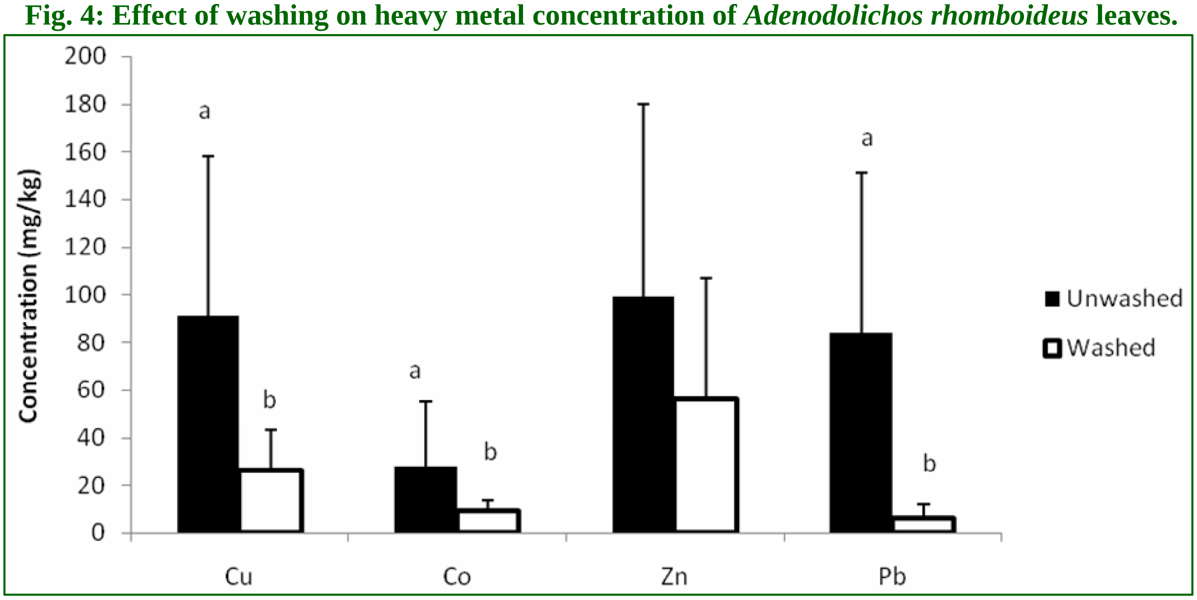
Values with different superscript letters in a row are significantly different ($p<0.05$).
1: KAS – Kasombo; KINS – Kinsevere; LUI – Luiswishi;
2: S – shallows; T – Trays; Sl – Slopes;
3: SL - superficial layers; DL - deep layers; Dep – Depth;
Top: topography; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; NS: not significant; SEM: standard error of the mean

Surface layers were much richer in these heavy minerals than deep layers ($p<0.001$). Some interactions were significant. Levels of zinc at Kinsevere and Luiswishi were similar but lower ($p<0.05$) than at Kasombo, and those on trays were higher ($p<0.01$) than in the other two topographies, both being similar. The level of zinc was higher ($p<0.05$) in surface than in deep soil. The interactions between sites and topographies ($p<0.05$) and between sites and depths ($p<0.01$) affected zinc concentration. Higher concentrations of lead occurred at Kasombo site, and on trays; and values were higher ($p<0.001$) for surface than deep layers. The interaction of sites and depths affected ($p<0.01$) lead concentration. Some soils presented elevated levels of trace metals while others had levels at or below those of soils considered as normal in the literature (Kabata and Pendias, 2001), especially for cobalt.

Generally, heavy metal concentrations were highest on trays, except copper and cobalt which were highest along the slopes. Trays in this region are elevated and have lower plant cover than shallows and slopes. It is therefore possible that they receive atmospheric dust which has high concentrations of heavy metal. This would also explain why trays tended to have high heavy metals concentration. Higher concentrations of heavy metal occurred in surface than deep layers of soil, irrespective of topography considered (excepted cobalt). Kabata and Pendias (2001) noted that atmospheric inputs of zinc and copper were higher than exports in surface horizons of different ecosystems. Copper concentration in soil is 100 mg/kg of dry soil (Downs et al., 2000; Kabata and Pendias, 2001). In south-eastern soils of DR of Congo, they vary between 100 and 100,000 mg/kg dry soil (Faucon, 2009).

All soils studied had very high copper concentrations relative to that found in soils. Cobalt concentration in soils is in the order of 0.1 to 70 mg/kg of dry soil with an average of 7.9 mg/kg (Kabata and Pendias, 2001), and 10 to 100,000 mg/kg of dry soil in south-eastern DRC. Concentrations found in these study sites are also higher than in soils because the study area is located in the Copper and Cobalt belt (Fig. 1). Zinc concentration in soil range from 64 to 220 mg/kg dry soil (Cabrera et al., 1999; Kabata and Pendias, 2001). Zinc concentrations at Kasombo site were higher than this range. Soils of south Katanga are poor in zinc with the exception of Kipushi soil (Malaisse et al., 1999) otherwise named Kasombo (in this study). Lead concentration in common soils vary between 15 and 200 mg/kg soil (Davies, 1997; Kabata and Pendias, 2001). All soils had values within this range. According to Kabata and Pendias (2001), lead contamination of surface soils can be the result of its high concentration in atmospheric air which is typical of industrial areas, major communication routes and certain agricultural or urban areas. All study sites were characterised by strong mining activity which could explain the high levels observed in soils.

Fig. 4: Effect of washing on heavy metal concentration of *Adenodolichos rhomboideus* leaves.



(a, b: for each metal, side by side bars with different letters refer to means significantly different at $p<0.05$).

Mineral concentrations in leaves, roots and breeding implications

Levels of trace metals in leaves of *A. rhomboideus* are presented in Table 2. For all elements, site affected concentrations of unwashed samples, Kinsevere having the lowest values. For washed samples, sites had similar concentrations of copper, cobalt, and lead; and zinc concentrations were higher (p<0.05) at Kasombo than at Kinsevere and Luiswishi. Topography effects were not significant, except in the case of zinc concentration in unwashed leaves where shallows had the lowest (p<0.05) average value. No interaction was significant. Element concentrations were consistently lower in washed than in unwashed samples. These effects were generally significant, except in the case of zinc (Fig. 4).

Table 2. Heavy metal concentration in *Adenodolichos rhomboideus* leaves according to site, topography and washing, at Lubumbashi.

Parameter	Washing status	Site (1)			Topography (2)				Effect (P >F)		
		KAS	KINS	LUI	S	T	Sl	SEM	Site	Top	Site*top
Cu	Unwashed	63^{Aa}	42^{Ab}	168^{Aa}	85^{Aa}	98^{Aa}	90^{Aa}	27.8	**	NS	NS
	Washed	20^{Ba}	12^{Aa}	45^{Ba}	18^{Ba}	30^{Ba}	29^{Ba}	9.5	NS	NS	NS
Co	Unwashed	14^{Aa}	10^{Aa}	60^{Ab}	29^{Aa}	25^{Aa}	30^{Aa}	2.7	*	NS	NS
	Washed	10^{Aa}	3^{Aa}	13^{Ba}	8^{Ba}	11^{Ba}	7^{Ba}	1.3	NS	NS	NS
Zn	Unwashed	185^{Aa}	24^{Ab}	87^{Ab}	38^{Ab}	107^{Aab}	151^{Aa}	33	*	*	NS
	Washed	103^{Aa}	26^{Ab}	39^{Aab}	37^{Aa}	58^{Aa}	73^{Aa}	12.3	*	NS	NS
Pb	Unwashed	107^{Aa}	8^{Ab}	136^{Aa}	78^{Aa}	65^{Aa}	78^{Aa}	21.7	**	NS	NS
	Washed	7^{Ba}	3^{Aa}	7^{Ba}	4^{Ba}	9^{Ba}	4^{Ba}	1.4	NS	NS	NS

1 : KAS – Kasombo; KINS – Kinsevere; LUI – Luiswishi.
2: S – shallows; T – Trays; Sl – Slopes;
Values with different uppercase letters in a row are significantly different at p<0.05.
Values with different uppercase capital letters in one column are significantly different at p<0.05.
*: p<0.05; **: p<0.01; ***: p<0.001; NS: not significant
SEM: standard error of the mean

Heavy metal concentration in *A. rhomboideus* roots is presented in Table 3. Site affected (p<0.05) the concentration of cobalt and lead with highest level observed at Luiswishi and the lowest at Kasombo. The effect of topography was not significant. Several factors control the selective accumulation of metals in plant and their bioavailability: soil and climatic conditions, plant genotype and agronomic techniques (Chunilall et al., 2005).

Metal solubility depends primarily on pH (Tremel-Schaub and Feix, 2005). Low pH increases generally element phytoavailability because protons have a high affinity for negative charges present on colloids, and then come into competition with metal ions towards these sites, which induces a relaxation of trace elements in soil pore water (Tremel-Schaub and Feix, 2005). In contrast, precipitation reactions and adsorption of metals on colloidal particles increases with pH. These particles are driven by leaching away from roots. This phenomenon restricts the absorbability of the element from the soil solution and its translocation in plant tissues (Liu et al., 2005).

In particular, the bioavailability of zinc, and therefore its transfer, is mainly influenced by pH and total concentration in soil, and the exchangeable form decreases with increasing pH (De Haan and Visser-Reyneveld, 1996). Acidic soil thus facilitates the transfer of Cu, Co, Zn and Pb into the plant, but the removal of these elements increases significantly only below pH 6 (Tremel-Schaub and Feix, 2005). This phenomenon can partly explain these low levels of metals found in leaves of *A. rhomboideus* because the latter has grown in highly contaminated soils, but with pH ranging from 6.2 to 6.4. However, higher heavy metals level in forage from Luiswishi is due to the fact that this site is in full operation. *Adenodolichos* green leaves are eaten by herbivores mainly during the dry season (Omokanye et al., 2001; Dung et al., 2000). High levels of heavy metals in these leaves thus are a source of contamination for domestic herbivores.

Table 3. Heavy metal concentration in *Adenodolichos rhomboideus* roots according to site and topography at Lubumbashi.

Parameters	Site (1)			Topographies (2)			Effects		SEM
	KAS.	KINS.	LUI.	S	T	Sl	Site	Topo.	
Cu	52.3^{a}	67.3^{a}	70.3^{a}	56.3^{a}	80.0^{a}	53.7^{a}	NS	NS	8.12
Co	5.3^{a}	11.7^{ab}	31.3^{b}	16.0^{a}	17.0^{a}	15.3^{a}	*	NS	3.8
Zn	62.0^{a}	23.0^{a}	38.3^{a}	41.3^{a}	41.0^{a}	41.0^{a}	NS	NS	7.1
Pb	92.0^{a}	113.3^{ab}	130.7^{b}	115.0^{a}	115.3^{a}	105.7^{a}	*	NS	4.0

1: KAS – Kasombo; KINS – Kinsevere; LUI – Luiswishi;
2: S – shallows; T – Trays; Sl – Slopes;
Values with different superscript letters in a row are significantly different at $p<0.05$.
*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; NS: not significant; SEM: standard error of the mean.

Cu is a metal whose solubility is less affected by pH but which complexes with soluble organic matter at about pH 6.5 (Barber, 1995). Levels recommended for domestic ruminants are of the order of 4 to 15 mg Cu/kg DM (NRC, 2007; Riviere, 1991). Malaisse et al. (1999) found a value of 13 mg/kg DM in the leaves of *A. rhomboideus* at Luiswishi. Levels of copper in unwashed and washed leaves were beyond the recommendation for ruminants, except washed leaves at Kinsevere. Cattle are relatively resistant to excess copper and can tolerate up to 10 times the recommendations, but sheep can develop acute forms of poisoning (nephrotoxicity, icterus) when levels reach 20 mg of Cu/kg DM (Rivière, 1991). Goats can tolerate 60 mg/kg DM without any sign of intoxication (NRC, 2007); furthermore, no signs of acute toxicity occurred within 35 days of offering Cu at 100 to 300 mg/kg DM (NRC 2007). Copper concentrations in A. *rhomboideus* forage are thus considered as toxic to sheep but can support cattle and goats.

Cobalt is a necessary element for rumen flora function, and for the synthesis of vitamin B12 for which 11 mg/kg DM has been found (Malaisse et al., 1999); washed leaves of *A. rhomboideus* at Luiswishi had a similar concentration. Cobalt requirements for ruminants range from 0.07 to 1 mg/kg DM (TCORN, 1999). NRC (2000) recommended the maximum Co level ruminants can tolerate in their diet is 10 mg/kg DM. At this level, the functioning of the animal's body is not altered and meat from such animals is safe. Co levels in washed leaves of *A. rhomboideus* around Lubumbashi are potentially toxic.

Malaisse et al. (1999) found a value of 29 mg Zn/kg DM in shoots of *A. rhomboideus* at Luiswishi. This value is comparable to values observed at Kinsevere, Luiswishi, and in shallows, but much lower than at Kasombo, which is an area where zinc is being mined. In a collection of 383 samples of plant leaves harvested in the area of Katanga copper-cobalt belt, the average zinc concentration was 85 mg/kg DM (Brooks et al., 1987), a value within our observation. Zinc concentration required to cause toxicity is much greater than the recommended. Ruminants' requirement for Zn is 50 mg/kg DM (Rivière, 1991) and the maximum tolerable for cattle is 500 mg/kg (NRC 2000). Zinc in leaves of *A. rhomboideus* would therefore not pose a toxic challenge to ruminants.

With regard to lead, environmental contaminations were all very high, except forage from Kinsevere that was less contaminated. Lead is currently being heavily mined at Kasombo and Luiswishi and sites for quarrying and mining of this metal are close to study sites. Lead concentration in plants under natural conditions without contamination range from 0.1 to 10 mg/kg DM, with an average of 2 mg/kg DM (Kabata and Pendias, 2001). This average values is below levels found in unwashed forages but is comparable to washed forages. According to Kabata and Pendias (2001) atmospheric deposition are a significant source of contamination of plants by this metal. NRC (2000) has recommended a value of 30 mg/kg DM as an upper limit cattle can tolerate, which is lower than 45 mg/kg DM proposed by Douay and Sterckeman (2002). These concentrations are more than those in all washed and unwashed forages from Kinsevere, but lead concentration in unwashed forages from Kasombo and Luiswishi would be toxic to ruminants.

Accumulation characteristics

BAC values were generally weak, excepted for zinc, at Kasombo. BCF values were low for copper, cobalt and zinc (excepted at Kasombo for zinc) but >1 for lead (Table 4). TF values were generally low, except for cobalt at Kasombo and zinc at Kasombo and Kinsevere. There was no trend highlighting the effect of topography or depth on accumulation. Normal levels of lead, copper and zinc in aerial parts of plants (Zu et al., 2004) are respectively 5, 10 and 100 mg/kg DM. These levels of heavy metals in cupro-cobalticultural plants vary from 10 to 10,000 mg/kg DM for copper and 4 to 6200 mg/kg DM for cobalt (Faucon, 2009). There are several ways to define the hyper accumulation properties of heavy metal in plants. Plant species are considered as hyperaccumulator for copper, cobalt, or lead when they have concentrations exceeding 1000 mg/kg DM (Faucon et al., 2007). The corresponding value for Zn is 10,000 mg/kg DM (Baker and Brooks, 1989).

Table 4. Bioaccumulation coefficient (BAC), concentration factor (BCF) and translocation factor (TF) of Cu, Co, Zn and Pb in *Adenodolichos rhomboideus* according to site, topography and depth of horizon at Lubumbashi.

Site	Topography	Depth	BAC				BCF				TF			
			Cu	Co	Zn	Pb	Cu	Co	Zn	Pb	Cu	Co	Zn	Pb
KASOMBO	Shallows	P1	0.01	0.06	1.53	0.14	0.014	0.04	2.22	1.68	0.74	1.83	0.69	0.09
	Shallows	P2	0.07	0.12	1.2	0.14	0.091	0.07	1.74	1.68				
	Slopes	P1	0.02	0.09	1.17	0.04	0.078	0.05	0.39	0.88	0.23	1.8	3.0	0.05
	Slopes	P2	0.03	0.1	3.34	0.08	0.129	0.06	1.11	1.72				
	Trays	P1	0.01	0.08	0.18	0.04	0.043	0.04	0.1	0.45	0.3	1.8	1.88	0.09
	Trays	P2	0.06	0.12	2.55	0.15	0.188	0.06	1.36	1.6				
KINSEVERE	Shallows	P1	0.01	0.01	0.53	0.01	0.03	0.06	0.47	2.17	0.24	0.11	1.11	0.01
	Shallows	P2	0.01	0.01	0.3	0.02	0.031	0.06	0.27	2.42				
	Slopes	P1	0.01	0	0.37	0.01	0.031	0.03	0.32	1.68	0.19	0.13	1.15	0.01
	Slopes	P2	0.01	0	0.45	0.01	0.03	0.03	0.39	1.93				
	Trays	P1	0	0.04	0.88	0.15	0.035	0.12	0.78	2.39	0.14	0.36	1.12	0.06
	Trays	P2	0.02	0.07	0.2	0.07	0.125	0.2	0.18	1.15				
LUISWISHI	Shallows	P1	0.02	0.03	0.45	0.03	0.108	0.08	0.32	2.02	0.15	0.34	1.42	0.02
	Shallows	P2	0.02	0.03	0.97	0.05	0.133	0.09	0.68	2.84				
	Slopes	P1	0.03	0.01	0.39	0.11	0.023	0.02	0.45	1.76	1.42	0.29	0.85	0.06
	Slopes	P2	0.08	0.02	0.93	0.17	0.057	0.07	1.09	2.76				
	Trays	P1	0.05	0.01	0.41	0.19	0.072	0.02	0.42	2.19	0.69	0.71	0.98	0.09
	Trays	P2	0.05	0.03	0.59	0.19	0.077	0.04	0.6	2.26				

Plant species can also be characterized by ratios of trace metal concentrations in soil, roots and shoots DM. Baker and Whiting (2002) suggested that the accumulating species is characterized by a ratio of concentrations in the shoots to the soil concentration greater than 1 (BAC> 1); species known as "excluder" having a ratio below 1. This agrees with Rotkittikhum et al. (2006) who suggested accumulation occurs when the concentration of an element in the aerial part of a plant is higher than in the soil or the concentration ratio of leaf/soil should be greater than 1 (BAC). This test reveals the ability of a species to accumulate metals (Chen et al., 2004). Secondly, the concentration of an element in the aerial parts of a plant should be 10 to 500 times higher than normal levels, and thirdly, the concentration ratio leaves/roots must be greater than 1 (TF> 1). Normal copper concentration in plants is within the range 2 and 50 mg/kg (Faucon et al., 2007). The leaf copper concentration of *A. rhomboideus* was not ten times the normal levels and did not exceed 1000 mg/kg DM. On the other hand, none of these three coefficients (BAC, BCF and TF) was > 1. Thus, the species can be considered as neither accumulator nor hyperaccumulator of copper according to the defined criteria.

The normal levels for cobalt in plants are in the order of 1 mg/kg (Faucon et al., 2007). These results show that *A. rhomboideus* is not a hyperaccumulator of cobalt. However, aTF> 1 shows that this element has a capacity of being translocated from root to leaves of *A. rhomboideus* species. Zinc values obtained in the leaves remained below 10,000 mg/kg. BAC values were>1 for zinc at Kasombo site. Consequently, *A. rhomboideus* has the ability to accumulate zinc from soils to the leaves, and is excluder for other three trace elements. With BCF>1, lead is easily absorbed into roots from soil, but a TF <1 suggests that lead is not translocated from roots to leaves. Several authors have shown that lead stops at plant roots where it accumulates without being fed into the aerial parts, probably because of the low capacity of translocation (Cunningham et al., 1995; Huang and Cunningham, 1996). It should be noted that the high atomic weight of lead than other metals studied makes it an element to be classified separately, and probably more difficult to translocate.

Conclusions

Adenodolichos rhomboideus growing on soils of the region of Lubumbashi is contaminated by some trace metals. However, the plant does not accumulate copper, cobalt, zinc or lead. It is suitable for phytostabilisation of soils contaminated with lead and zinc and, to some extent, for the phytoextraction of soils contaminated by cobalt and zinc. This species is more appropriate for phytoremediation of soils contaminated by zinc because of its BAC, BCF and TF coefficients. Unwashed forage of *A. rhomboideus* contains excessive amounts of copper for sheep feeding, and excess cobalt and lead for domestic ruminants. Even washed leaves still contain excessive levels of copper for sheep. Further research should be conducted to study the effect of this forage on performance of ruminants and heavy metal accumulation in serum, bone, kidney, and liver of animals.

Acknowledgements

This work was financed by the 'Coopération Universitaire au Développement' (CUD, Belgium) in the framework of the project P3 (troisième programme). We thank all the partners of this project. We also thank Mrs. Lubalega Tolerant, Kisimba Kibuye, Tshibungu Nkulu and Mukadi Panya for their invaluable help for plant and soil preparation and assistance on samples collection.

References

Alsac, N., 2007. Analysis of heavy metals (As, Cd, Cr, Cu, Ni,Pb, Zn et Hg) in soils by ICP-MS. Ann. Toxicol. Anal. 19, 37–41.

Amnesty International, 2013. Profits and loss: Mining and human rights in Katanga, Democratic Republic of the Congo-Report.

Apostoli, P., Kiss, P., Porru, S., Bonde, J.P., Vanhoorne, M., 1998. Male reproductive toxicity of lead in animals and humans. Occup. Environ. Med. 55, 364-374.

Baker, A.J.M., Brooks, R.R., 1989. Terrestrial higher plants which hyperaccumulate metallic elements: A review of their distribution, ecology and phytochemistry. Biorecover. 1, 81–126.

Baker, A.J.M., Whiting, S.N., 2002. In search of the holy grail-a further step in understanding metal hyperaccumulation. New Phytol. 155, 1–7.

Banza, L.N.C., Tim, S.N., Haufroid, V., Decrée, S., De Putter, T., Smolders, E., Ilunga, K.B., Luboya, N.O., Ilunga, N.A., Mutombo, M.A., Nemery, B., 2009. High human exposure to cobalt and other metals in Katanga, a mining area of the Democratic Republic of Congo. Environ. Resvol. 109(6), 745-752.

Barber, S.A., 1995. Soil Nutrient Bioavailability: A Mechanistic Approach. John Wiley and Sons, Inc., New York, NY. pp. 180-201.

Brooks, R.R., Naidu, S.M., Malaisse, F., Lee, J., 1987. The elemental content of metallophytes from the copper/cobalt deposits of Central Africa. Bull. Soc. R.Bot. Belg. 119, 179–191.

Cabrera, F., Clemente, L., Diaz, B.E., Lopez, R., Murillo, J.M., 1999. Heavy metal pollution of soil affected by the Guadiamar toxic flood. Sci. Total Environ. 242, 117-129.

Chen, Y., Shen, Z., Li, X., 2004. The use of vetiver grass (*Vetiveria zizanioides*) in the phytoremediation of soils contaminated with heavy metals. Appl. Geochem. 19, 1553–1565.

Chunilall, V., Kindness, A., Jonnalagadda, S.B., 2005. Heavy metal uptake by two edible *Amaranthus* herbs grown on soils contaminated with lead, mercury, cadmium and nickel. J. Environ. Sci. Heal. A 40, 375-384.

Cui, S., Zhou, Q., Chao, L., 2007. Potential hyper-accumulation of Pb, Zn, Cu and Cd in endurant plants distributed in an old smeltery, northeast China. Environ. Geol. 51, 1043-1048.

Cunningham, S.D., Berti, W.R., Huang, J.W., 1995. Phytoremediation of contaminated soils. Trends Biotechnol. 13(9), 393-397.

Davies, B.E., 1997. Heavy metal contaminated soils in an old industrial area of Wales, Great Britain: Source identification through statistical data interpretation. Water Air Soil Pollut. 94, 85-98.

De Haan, F.A.M., Visser-Reyneveld, M.I., 1996. Soil Pollution and Soil Protection. International Training Centre (PHLO), Agricultural University, Wageningen. pp. 81-116.

Douay, F., Sterckeman, T., 2002. Teneur en Pb, Cd et Zn dans les végétaux cultivés aux lentours d'usines métallurgiques des éléments traces métalliques dans les sols. Approches fonctionnelles et spaciales. INRA, France.

Downs, T.J., Cifuentes, E., Ruth, E., Suffet, I., 2000. Effectiveness of natural treatment in a wastewater irrigation district of the Mexico City Region: a synoptic field survey. Water Environ. Res. 72, 4–21.

Dung, D.D., Omokanye, A.T., Lamidi, O.S., 2000. Short-terme intake and "in sacco" degradability of mixture of tropical legumes. Tropicult. 18(4), 164-166.

Ernst, W., 1974. Heavy-metal Vegetation of the Earth. Fischer Verlag, Stuttgart (in German).

Faucon, M.P., 2009. Ecology and biology of the conservation of the métallophytes. The case of *Crepidorhopalon perennis* and *C. tenuis* (Scrophulariaceae) of the cupro-cobaltiferous grounds of Katanga. Dissertation, Free University of Brussels.

Faucon, M.P., Ingrid, P., Collinet, G., Mahy, G., Ngongo, L.M., Meerts, P., 2011. May rare metallophytes benefit from disturbed soils following mining activity? The case of the *Crepidorhopalon tenuis* in Katanga (D. R. Congo). Restor. Ecol. 19(3), 333-343.

Faucon, M.P., Shutcha, M., Meerts, P., 2007. Revisiting copper and cobalt concentrations in supposed hyperaccumulators from SC Africa: influence of washing and metal concentrations in soil. Plant Soil 301, 29–36.

Ginocchio, R., Toro, I., Schnepf, D., 2002. Copper tolerance in populations of *Mimulus luteus* var. *variegatus* exposed and non exposed to copper pollution. Geochem. Explor. Environ. Anal. 2, 151-156.

Hébrard-Labit, C., Meffray, L., 2004. Comparison of Methods for Analysis of Trace Metals ETM and Polycyclic Aromatic Hydrocarbons PAHs in Soils and Plants (Study reports), Transport, Urban Planning and Public Buildings Studies Centre Networks (CERTU), Engineering Design Centre of the Equipment, Lyon, France.

Huang, J.W., Cunningham, S.D., 1996. Lead phytoextraction: Species variation in lead uptake and translocation. New Phytol. 134, 75-84.

INEAC (National Institute for Agronomic Research in Belgian Congo), 1954. Flora of the Belgian Congo and Rwanda-Urundi: Spermatophyta. Executive Committee of the Flora of the Belgian Congo and the Botanical Garden of the State, Vol. VI, Brussels.

Kabata, P.A., Pendias, H., 2001. Trace elements in soils and plants. CRC, 3rd Edn. USA.

Katemo, M.B., Colinet, G., André, L., Chocha, M.A., Marquet, J.P., Micha, J.C., 2010. Evaluation of contamination of the food chain by trace elements (Cu, Co, Zn, Pb, Cd, U, V and As) in the basin of the upper Lufira (Katanga/DR Congo). Tropicult. 4, 246-252.

Kaya, M. D., 2008. Contribution to the study of the factors of the spatial distribution of metal elements traces content in soil and sediments of Gécamines area. M.Sc. thesis in Vegetable Biology and Environment (BVE), Faculty of agronomic.University of Lubumbashi, Lubumbashi.

Larry, E.J., 2008. Nutritional Disorders: Mineral Deficiency and Toxicity. 18th Edn. Merk Manual for Healthcare Professionals. Merk & Co., Inc., Whitehouse Station, NJ, USA.

Li, M.S., Luo, Y.P., Su, Z.Y., 2007. Heavy metal concentrations in soils and plant accumulation in a restored manganese mineland in Guangxi, South China. Environ. Pollut., 147, 168-175.

Liu, Y., Zhu, Y.G., Chen, B.D., Christie, P., Li, X.L., 2005. Yield and arsenate uptake of arbuscular mycorrhizal tomato colonized by *Glomus mosseae* BEG167 in As spiked soil under glasshouse conditions. Environ. Int. 31, 867–873.

Lydall, M.I., Auchterlonie, A. M., 2011. The Democratic Republic of Congo and Zambia: A Growing Global “Hotspot” for Copper-Cobalt Mineral Investment and Exploitation. The Southern African Institute of Mining and Metallurgy. 6th Southern Africa Base Metals Conference.

Malaisse, F., Baker, A.J.M., Sophie, R., 1999. Diversity of plant communities and leaf heavy metal content at Luiswishi copper/cobalt mineralization, Upper Katanga, Dem. Rep. Congo. Biotechnol. Agron. Soc. Environ. 3(2), 104–114.

Meerts, P., 2008. Flora of Copper SCI. Numerical Image Library. Free University of Brussels. ttp://bib18.ulb.ac.be/cdm4/item_viewer.php?CISOROOT=/bst003&CISOPTR=4&REC=1. Accessed 28 Jully 2012.

Mobinzo, K. S., 2010. Space and temporal distribution of the contamination to the copper of the town of Lubumbashi established by the study of faeces of the goats as bioindicatory.MSc. Thesis.Faculty of veterinary medicine.University of Lubumbashi (in French).

NRC (National Research Council), 2000. Nutrient Requirements of Beef Cattle. 20th Edn. National Academy Press, Washington DC.

NRC (National Research Council), 2007. Nutrient Requirements of Small Ruminants Sheep, Goats, Cervids and New World Camelids. National Academy Press, Washington DC.

Omokanye, A.T., Balogun, R.O., Onifade, O.S., Afolayan, R.A., Olayeni, M.E., 2001. Assessment of preference and intake of browse species by Yankasa sheep at Shika, Nigeria. Small Ruminant Res. 42, 203-210.

Remon, E., Bouchardon, J.L., Cornier, B., Guy, B., Leclerc, J.C., Faure, O., 2005. Soil characteristics, heavy metal availability and vegetation recovery at a former metallurgical landfill: Implications in risk assessment and site restoration. Environ. Pollut. 137, 316-323.

Rivière, R., 1991. Handbook of Feed of Domestic Ruminants in Tropical Area. Institute of Breeding and Veterinary Medicine of the Tropical Countries (IEMVT), Paris (in French).

Rotkittikhun, P., Kruatrachue, M., Chaiyarat, R., Ngernsansaruay, C., Pokethitiyook, P., Paijitprapaporn, A., Baker, A.J.M., 2006. Uptake and accumulation of lead by plants from the Bo Ngam lead mine area in Thailand. Environ. Pollut. 144, 681–688.

SAS Institute, 1999. SAS® Statistics Users Guide, Statistical Analysis System. 5th Edn, 8.2 version, Carry, NC, SAS Institute Inc..

Sebei, A., Chaabani, F., Ouerfelli, M. K., 2005. Impacts of mining wastes on the soil and plants in the Boujaber area (NW Tunisia): Chemical fractionation of heavy metals in soils. Geo. Eco. Trop. 29, 37-50.

TCORN, 1999. A reappraisal of the calcium and phosphorus requirements of sheep and cattle. Nutr. Abstr. Rev. Ser. B 61, 573–612.

Tremel-Schaub, A., Feix, I., 2005. Soil Contamination: Soil Transfers to the Plant. 2nd Edn. ADEME/EDP Sciences, Cedex. p. 422.

Tshibangu, M. I., Nsahlai, V. I., Kiatoko, M. H., Hornick, J.L., (2014). Nutritive value of *Adenodolichos rhomboideus* leaves compared with *Leucaena leucocephala* and *Stylosanthes guianensis* forages in indigenous goats in Lubumbashi (DRCongo). Biotechnol. Agron. Soc. Environ. 18(2), 165-173.

Tshibangu, M.I., 2010. Bromatologic composition of fodder, pH variation and the contents of heavy metals of soil colonized by *Adenodolichos rhomboideus* (case of the mining sites of Lubumbashi). M.Sc., thesis, University of Lubumbashi (in French).

Yoon, J., Cao, X., Zhou, Q., Ma, L.Q., 2006. Accumulation of Pb, Cu and Zn in native plants growing on a contaminated Florida site. Sci. Total Environ. 368, 456-464.

Zu, Y.Q., Li, Y., Christian, S., Laurent, L., Lin, F., 2004. Accumulation of Pb, Cd, Cu and Zn in plants and hyperaccumulator choice in Lanping lead-zinc mine area, China. Environ. Int. 30, 567-576.