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RESPONSES OF FIVE LOCAL PLANT SPECIES TO METAL EXPOSURE UNDER CONTROLLED CONDITIONS

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ABSTRACT

This work focused on the determination of the accumulator potential of five local plant species for use in phytoremediation. In plants, trace elements such as Zn, Fe, Cu, Co, Mn, Mo and Ni are necessary for biological processes, but their excessive accumulation can be toxic. Other non-essential elements, such as Cd and Pb, can also be absorbed by plants, thus constituting a potential hazard since they enter the food chain. Phytoremediation is the use of plants and their associated microbes to clean up the environment. A controlled trial was conducted with five species (*Vetiveria nigritana* (Benth.), *Oxytenanthera abyssinica* (A. Rich), *Barleria repens* (Ness), *Cymbopogon citratus* (DC.) Stapf and *Lantana camara* (Linn.) on soils contaminated by municipal solid waste at a rate of 15 t/ ha or 5.1 kg of waste. Bioaccumulation of heavy metals, bioconcentration factors, tolerance, and correlations between accumulated metal element content were determined. In this study, it was demonstrated that *Vetiveria nigritana* and *Oxytenanthera abyssinica* could accumulate relatively high concentrations of metals (Cd, Cu, Pb and Zn) compared to the other three species, eg 15-20 mg/ kg of Foliar dry matter Cd, 15 times more than other species. The plant species studied have high bioconcentration factor values, for example 24.7 with Cd in lixisols. All species exhibited high tolerance for metals in terms of *Vetiveria nigritana* and *Oxytenanthera abyssinica* 359.63% and 382.75% respectively at leaf level. Positive and strong correlations are noted between Cd-Zn, Cu-Zn, Cu-Cd, Pb-Cd and Pb-Cu.

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INTRODUCTION

The toxicity caused by heavy metals, found in many soils contaminated by industrial activities, constitutes a significant environmental risk for humans. In order to overcome the main limitations of the methods currently available in pollution abatement strategies, research has been focusing for a few years on the use of plants. It has long been known that the presence of a vegetative cover induces or stimulates the biodegradation of a large variety of organic contaminants (Reilley et al., 1996). In addition, certain so-called metallophyte plants are able to develop normally on sites heavily contaminated by various metals and some of these plants, called hyperaccumulators (Brooks, 1998), are capable of massively storing metals in their aerial parts. . Thus, studies concerning the ecophysiology and metabolism of higher plants in a contaminated environment have gradually made it possible

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to insert the concept of phytoremediation alongside that of bio-remediation of polluted sites. Phytoremediation uses plants capable of collecting toxic metals and accumulating them in their aerial parts (phytoextraction), cleaning polluted waters (phytofiltration), transforming soil pollutants to make them less harmful (phytodegrading), or even to immobilize the metals in the root system of the plant to prevent its leaching into groundwater (phytostabilization) (Lasat 2002, Pilon-Smits 2005). It is an emerging technology that is effective and applicable to a number of pollutants and sites of different characteristics (Lasat 2002, Pilon-Smits and Pilon 2002). The phenomenon of tolerance towards heavy metals in plants has focused the interest of scientists, ecologists, physiologists and biologists but also engineers and chemists for a very long time.

The genetic basis of tolerance has been widely studied (Antonovics et al., 1971, Baker 1987, Macnair 1990, Macnair 1993), but there is little certainty and much more work is needed. However, it is now widely recognized that a significant number of common plant species are able to rapidly colonize newly contaminated environments. This observation supports the hypothesis that an adapted ecotype (or population) can emerge rapidly after a few years, or even in a single generation (Wu et al., 1975). This phenomenon is classically presented as a microevolution model (Macnair, 1993); it is based on the inducibility of tolerance, in populations that usually develop on non-contaminated sites and have a lower average tolerance. In this article, we have studied the responses of five plant species to metal exposure under controlled conditions. Our objectives were to evaluate the accumulative capacity of heavy metals of these plant species by analyzing the levels in the plant organs of each plant and calculate the tolerance of each species to this exposure to metals. It was also to study the correlation between the metal contents in the different tissues of the plant.

MATERIALS AND METHODS

Study sites

The soil samples were taken from the villages of Boni (11 ° 35'N, 3 ° 26'W) and Dossi (3 ° 17'-3 ° 30'West, 11 ° 22'-11 ° 30'North) in Burkina Faso. Soils were then transported to the Polytechnic University of Bobo Dioulasso (4 ° 10'-4 ° 30'West, 11 ° -12 ° North) in Burkina Faso where the experiment was conducted under controlled conditions in a greenhouse. The climate of the experimental sampling sites is of the South Sudanian type with an annual rainfall of between 900 and 1200 mm.

Biological materials

Five plant species were used as test plants: *Vetiveria nigritana* (Benth.), *Oxytenanthera abyssinica* (A. Rich), *Barleria repens* (Ness), *Cymbopogon citratus* (DC) Stapf and *Lantana camara* (Linn.). The plants in species having the same age and having the same morphological development were selected. So:

- *Vetiveria nigritana* plants had a height of 40 cm and the length of the roots reduced to 8 cm,
- *Oxytenanthera abyssinica* plants had 2 cm of circumference of the stem and 30 cm of height; the length of their roots has been reduced to 6 cm,
- *Barleria repens* plants were 1.5 cm in circumference and 40 cm in height; root length reduced to 6 cm,
- *Cymbopogon citratus* plants were 45 cm high and 8 cm long for roots length,
- *Lantana camara* plants were 2.5 cm in circumference of the stem, 50 cm in height and the root length reduced to 6 cm.

Soils

Soil samples were collected in November 2010 at a depth of 0-20 cm in fallow lands (uncultivated). The sampled soils collected at Boni and Dossi are respectively lixisols and vertisols. These two types of soil have different physical and chemical characteristics. The lixisols are characterized mainly by their low content of clays, organic matter, nitrogen and exchangeable bases, their low cation exchange capacity, their strongly to weakly acidic pH. As for the vertisols, they mainly have high contents of swelling clays, a good water retention capacity, a high mineral richness (BU.NA.SOLs, 1985).

Experimentation

The experimental set up is a split-plot factorial device completely randomized with two factors (soil and plant) and four repetitions. The sorted urban waste was used in our experimentation as a source of heavy metal pollution; they were brought into the buckets and thoroughly mixed with the ground. The plants were then transplanted into the plastic pots (inside diameter 30 cm and depth 28 cm) containing 10 kg of soil and placed in the greenhouse. The sorted urban waste was used in our experimentation as a source of heavy metal pollution; they were brought into the buckets and thoroughly mixed with the soil at the rate of 15 t / ha or 5.1 kg of waste.

Conduct of culture

In each pot is planted a plant. The pots are protected by a dried herb roof covered with plastic to prevent rainwater. During the three (03) months of the expe riment, the water content of the soil in the buckets was kept constant at 60% of the maximum soil holding capacity. To keep the soil moisture constant, the buckets were weighed daily, and the amount of distilled water needed was added to keep the weight constant. During the experiment, the average minimum and maximum temperatures were 21 and 36 ° C. The maximum temperature in the greenhouse was 34.5 ° C.

Data collection

At the end of the experiment, the plants are dug out; their roots are rinsed for 15 minutes in a solution containing 10 mM EDTA in order to eliminate the metals present on the surface of the plant organs. Then the fresh masses of leaves, stems and roots are measured. The plants are dried for 48 h in the shade and then at 60 ° C in an oven to constant weight before measuring the dry masses. Subsequently, the leaves, stems and roots are crushed in order to use 100 to 200 mg for the quantification of metals by ICP-MS. For soils, samples of both soils were previously collected and analyzed to measure their initial metal content. At the end of the experiment, it is removed and sieved (2 mm) after being dried. The bioconcentration factor and the tolerant index of heavy metals (Cd, Cu, Pb and Zn) by plant species were evaluated using the following formulas:

$$\text{Bioconcentration factor} = \frac{\text{Concentration in leaves}}{\text{Concentration in soil}} \quad (1)$$

$$\text{Tolerant index (\%)} = \frac{\text{Dry biomass with heavy metals}}{\text{Dry biomass (control)}} \text{ x } 100 \qquad (2)$$

Analysis of metals contents in plant tissues and soils by ICP-MS (Inductively Coupled Plasma - Mass Spectrometry)

An analysis requires 100 to 200 mg of dry matter, whether for samples of leaves, stems, roots or soil. Samples of 100 to 200 mg dry matter are mineralized with 5 ml of 70% HNO3 in a microwave. After dilution to a final concentration of 3.5% HNO3, the content of metal elements is determined by inductively coupled plasma atomic emission spectrometry (Vista MPX, Varian). This device allows fast and accurate multi-element analysis of samples in solution. This technique has very low detection limits (0.01 - 0.1 µg/ l) depending on the elements and matrices.

Soil chemical parameters analysis

Soils are then dried in the shade, and then sieved to 2 mm for determinations of chemical parameters. N-total and P-total contents were determined in the mineralizates using a SKALAR automatic calorimeter (Segmented flow analyzer model SANplus 4000-02, Skalar Holland). K-total was determined using a flame photometer (JENCONS, PFP 7, Jenway LTD, Felsted, England). The assimilable P was determined according to the Bray I method (Bray and Kurtz, 1945. Soil carbon was determined by the Walkley-Black method (Walkley and Black, 1934).) Soil pH was determined after shaking for 1 hour of 20 g of soil sample in 50 ml of distilled water The cation exchange capacity (CEC) was determined by the Metson ammonium chloride method.

Statistical analysis

The variations in metal concentrations between species, between soils or between organs are tested with an analysis of variance (ANOVA) using the Genstat version 10.3 software. The separation of the averages is performed by the Tukey test at the 5% threshold. The Pearson coefficient is used to evaluate the linear correlation between two metal levels in the roots, leaves and stems of different plant species. Indeed, this type of coefficient makes it possible to establish the existence of a link between 2 variables X and Y in order to measure the strength or the intensity of this link. A positive sign indicates that the relationship is proportional, a negative sign that it is inversely proportional. By convention we say that the relation between X and Y is perfect if r = 1; very strong if r> 0.8; strong if r is between 0.5 and 0.8; average if r is between 0.2 and 0.5; low if r is between 0 and 0.2 and zero if r = 0. This was calculated using SPSS version 20 statistical software.

RESULTS

Initial contents of heavy metals

The initial contents of the heavy metals of the waste and the different substrates (soils + waste) are recorded in Table 1. The different substrates have higher zinc contents than the other heavy metals. Cd is the metal with the lowest content.

Soil chemical characteristics

The chemical characteristics of the soils of the different treatments before transplanting (0 months) of the plants and at harvest (3 months) are given in Table 2. The period 0 months corresponds to transplanting.

Table 1. Initial heavy metals content (mg/ kg) of waste and substrates

Samples	heavy metal contents (mg/kg)			
	Cd	Cu	Pb	Zn
Pure waste	1	48	28	313
Lixisols without waste	0.5	28	2	58
Lixisols + 5.1 kg of waste	2	38	26	126
Vertisols without waste	0.1	87	16	59
Vertisols + 5.1 kg of waste	1	70	22	160

The lixisols used have a moderately acidic pH (6.5) while the vertical soils have a moderately basic pH (7.1). The contribution of urban solid waste increased the water level by 2 units and 1 unit respectively for lixisols and vertisols. Irrespective of the treatment and the type of soil, pH water increased with the contribution of waste and the duration of the cultivation. The contribution of urban waste has increased nutrient levels. However, the C / N ratio decreased with the application of waste. In lixisol soils, before transplanting the plants, CEC increased with the addition of waste, while in vertisol, there was a decrease. At the end of cultivation, CEC increased strongly regardless of soil type and larger in vertisol. The CEC of vertisols is 2.5 times higher than that of lixisols in soils before cultivation. On the other hand, in soils after cultivation, it is higher in lixisols compared to vertisols. This difference is 2.3 times; 244 Cmol / kg; 147 Cmol / kg; 46 Cmol / kg and 113 Cmol / kg respectively in soils in the presence of *Vetiveria nigratana*, *Oxytenanthera abyssinica*, *Barleria repens*, *Cymbopogon citraus* and *Lantana camara*.

Heavy metal content in plant organs

The results of the analysis of the contents of heavy metals in plant organs are given in Tables 3, 4 and 5 respectively at the roots, stems and leaves. The accumulation of heavy metals (Cd, Cu, Pb and Zn) in roots, leaves and stems depends on the species (Tables 3, 4 and 5). In lixisols, accumulation of heavy metals at the root level gives Cd contents ranging from 10 to 37 mg / kg, from 2 to 70 mg / kg for Cu, from 0.1 to 120 mg / kg for Pb and between 138 and 510 mg / kg for Zn. In vertisols, 12 to 22 mg / kg of Cd, 3 to 72 mg / kg of Cu, 0.3 to 100 mg / kg of Pb and 129 to 309 mg / kg of Zn were recorded.

In *Barleria repens* stems and in lixisols, the levels are in the range of 11 to 33 mg / kg of Cd, 10 to 64 mg / kg of Cu, 0.3 to 90 mg / kg of Pb at 45 to 480 mg / kg Zn. In vertisols, Cd contents ranging between 8 to 19 mg / kg, 6 to 46 mg / kg for Cu, 0.001 to 86 mg / kg for Pb and 41 to 300 mg / kg are noted. the Zn. In the leaves of *Barleria repens*, the recorded contents are 1 to 19 mg / kg of Cd, 7 to 37 mg / kg of Cu, 0.01 to 109 mg / kg of Pb, 200 to 354 mg / kg of Zn in tropical ferruginous soils. In upland soils, there are Cd contents ranging from 0.01 to 20 mg / kg, 9 to 36 mg / kg for Cu, 0.1 to 168 mg / kg for Pb and 88 to 355 mg / kg for Zn. The highest Cd contents in the leaves were found for *Oxytenanthera abyssinica* (20 mg / kg) and *Vetiveria nigritana* (19 mg / kg) respectively in lixisols and vertisols. The Cd contents in the leaves of the other plants range from 0.01 to 10 mg / kg. Cu accumulation is higher at the roots of *Oxytenanthera abyssinica*. It is 70 mg / kg and 56 mg / kg respectively in lixisols and vertisols. However, *Oxytenanthera abyssinica* accumulates 3 to 32 times more Zn in roots and stems than other species. High Pb levels are recorded with *Oxytenanthera abyssinica* and *Vetiveria nigritana* irrespective of soil type.

Table 2. Chemical characteristics of soils

		Measured parameters							
Treatments	Periods	pHeau	Carbon (g/kg)	N (g/kg)	C/N	P-total (mg/kg)	P-ass (mg/kg)	CEC (C mol/kg)	K-Total (mg/kg)
Lixisol pur	0 month	6.47	0.081	0.006	13	135.7	1.05	4.55	2245
Vertisol pur	0 month	7.14	0.122	0.009	14	152.0	1.13	15.45	1293
Lixisol +DU	0 month	8.34	0.187	0.0168	11	693.2	32.17	5.85	2134
Vertisol +DU	0 month	8.16	0.217	0.0191	11	752.5	67.28	13.35	1824
Lixisol +DU+Vn	3 month	8.64	0.165	0.0168	10	672	64.97	5.00	1935
Vertisol+DU+Vn	3 month	8.47	0.189	0.019	10	790	31.21	11.50	1559
Lixisol +DU+Oa	3 month	8.61	1.55	0.174	9	702	64.13	2024.00	4.55
Vertisol+DU+Oa	3 month	8.47	1.96	0.218	9	805	28.39	1780.00	12.6
Lixisol +DU+Br	3 month	8.21	0.19	0.1	9	720	89.13	2167	4.35
Vertisol+DU+Br	3 month	8.34	0.14	0.007	9	882	17.1	2020	11.65
Lixisol +DU+Lc	3 month	8.61	0.17	0.13	9	722	78.3	2133	4.95
Vertisol+DU+Lc	3 month	8.47	0.16	0.21	9	689	19.39	2087	10.85
Lixisol +DU+Cc	3 month	8.41	0.19	01	11	762	96.32	2200	4.45
Vertisol+DU+Cc	3 month	8.47	0.16	0.21	9	689	19.39	2087	10.85

DU = Urban waste Vn= *Vetiveria nigritana* Oa = *Oxytenanthera abyssinica*
Br = *Barleria repen* Lc = *Lantana camara* Cc = *Cymbopogon citratus*

Table 3. Metal content in the species roots (in mg/ kg). The concentrations are expressed as the averages of 4 replicates for each species

Roots	Cd		Cu		Pb		Zn	
	Lixisols	Vertisols	Lixisols	Vertisols	Lixisols	Vertisols	Lixisols	Vertisols
V. nigritana	17	15	2*	21*	120	100	138	240
O. abyssinica	37	12	70	56	67	0.01*	510	213
B. repens	10	22	34	72	0.1*	5*	224	342
C. citratus	20	22	18*	16*	0.3*	0.5*	204	309
L. camara	16	17	21*	3*	0.3*	0.3*	331	129

*** :** These values are within the range of normal concentrations for plants (Larcher, 2003).

Table 4 . Metal content in the stems of the species (in mg/ kg). The concentrations are expressed as the averages of 4 replicates for each species.

Stems	Cd		Cu		Pb		Zn	
	Lixisols	Vertisols	Lixisols	Vertisols	Lixisols	Vertisols	Lixisols	Vertisols
V. nigritana	11	9	10*	14*	90	86	130	234
O. abyssinica	33	8	64	46	53	0.001*	480*	190
B. repens	21	19	10*	6*	0.3*	0.08*	45*	41*
C. citratus	18	17	19*	14*	1.1*	0.3*	194	300
L. camara	17	16	13*	12*	0.4*	0.4*	60*	102

* : These values are within the range of normal concentrations for plants (Larcher, 2003).

Table 5 : Metal content in the leaves of the species (in mg/ kg). The concentrations are expressed as the averages of 4 replicates for each species

Leaves	Cd		Cu		Pb		Zn	
	Lixisols	Vertisols	Lixisols	Vertisols	Lixisols	Vertisols	Lixisols	Vertisols
V. nigritana	18	20	37	21*	99	168	201	236
O. abyssinica	19	15	30	27*	109	28	200	155
B. repens	3	0,3	21*	33	0.1*	0.17*	354	240
C. citratus	10	2	7*	9*	0.2*	0.1*	220	88*
L. camara	1	0.01*	9*	36	0.8*	0.2*	354	355

*** :** These values are within the range of normal concentrations for plants (Larcher, 2003).

Table 6. ANOVA results for species and soil effects on metal concentrations in roots, leaves and stems of plants

Effects (roots)	Cd	Cu	Pb	Zn
Species	***	***	***	***
Soil	***	***	**	***
Species x soil	***	***	ns	***
Effects (stems)	Cd	Cu	Pb	Zn
Species	***	***	***	***
Soil	ns	**	ns	***
Species x soil	ns	***	ns	***
Effects (leaves)	Cd	Cu	Pb	Zn
Species	***	*	***	**
Soil	***	**	***	***
Species x soil	***	*	***	**

* = $P < 0.05$, ** = $P < 0.005$, *** = $P < 0.001$, ns = not significant.

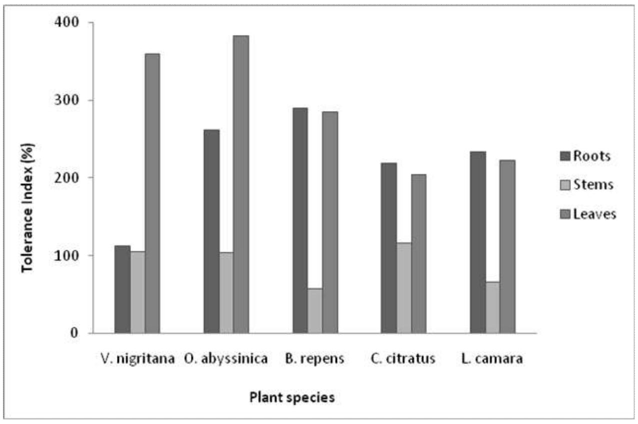

Figure 1. Tolerance index of plant species in lixisols

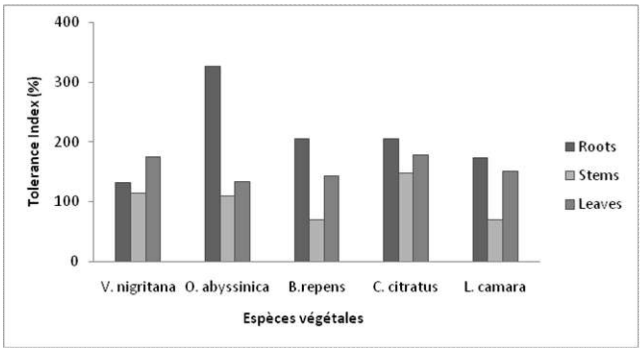

Figure 2. Tolerance index of plant species in vertisols

However, there is a preferential accumulation in the roots and leaves relative to the stems. *Barleria repens*, *Cymbopogon citraus* and *Lantana camara* show very low Cd accumulation between 0.08 and 5 mg / kg. The highest Zn accumulation in the leaves is recorded with *Lantana camara* followed by *Braleria repen* whatever the soil type.

Bioconcentration factors are more important for Cd in lixisols. The only exception is *Lantana camara*, which has a lower Cd content in lixisols than other species (1 mg / kg). Regardless of the type of soil, the lowest values for bioconcentration factors (<0.01 to 0.14) are obtained with Pb in the presence of *Barleria repens*, *Cymbopogon citratus* and *Lantana camara* (Table 7).

Heavy metal content in plant organs

The results of the analysis of the contents of heavy metals in plant organs are given in Tables 3, 4 and 5 respectively at the roots, stems and leaves. The accumulation of heavy metals (Cd, Cu, Pb and Zn) in roots, leaves and stems depends on the species (Tables 3, 4 and 5). In lixisols, accumulation of heavy metals at the root level gives Cd contents ranging from 10 to 37 mg / kg, from 2 to 70 mg / kg for Cu, from 0.1 to 120 mg / kg for Pb and between 138 and 510 mg / kg for Zn. In vertisols, 12 to 22 mg / kg of Cd, 3 to 72 mg / kg of Cu, 0.3 to 100 mg / kg of Pb and 129 to 309 mg / kg of Zn were recorded. In *Barleria repens* stems and in lixisols, the levels are in the range of 11 to 33 mg / kg of Cd, 10 to 64 mg / kg of Cu, 0.3 to 90 mg / kg of Pb at 45 to 480 mg / kg Zn. In vertisols, Cd contents ranging between 8 to 19 mg / kg, 6 to 46 mg / kg for Cu, 0.001 to 86 mg / kg for Pb and 41 to 300 mg / kg are noted. the Zn. In the leaves of *Barleria repens*, the recorded contents are 1 to 19 mg / kg of Cd, 7 to 37 mg / kg of Cu, 0.01 to 109 mg / kg of Pb, 200 to 354 mg / kg of Zn in tropical ferruginous soils. In upland soils, there are Cd contents ranging from 0.01 to 20 mg / kg, 9 to 36 mg / kg for Cu, 0.1 to 168 mg / kg for Pb and 88 to 355 mg / kg for Zn. The highest Cd contents in the leaves were found for *Oxytenanthera abyssinica* (20 mg / kg) and *Vetiveria nigritana* (19 mg / kg) respectively in lixisols and vertisols. The Cd contents in the leaves of the other plants range from 0.01 to 10 mg / kg. Cu accumulation is higher at the roots of *Oxytenanthera abyssinica*. It is 70 mg / kg and 56 mg / kg respectively in lixisols and vertisols.

Table 7. Bioconcentration factors of plants

Plant species	Cd		Cu		Pb		Zn	
	Lixisol	Vertisol	Lixisol	Vertisol	Lixisol	Vertisol	Lixisol	Vertisol
V. nigritana	18	11.11	1.423	0.38	5.21	4.66	1.40	1.25
O. abyssinica	24.70	15	0.75	0.45	6.05	0.96	1.34	0.91
B. repens	3	0.27	0.84	0.97	0.01	0.01	3.40	1.96
C. citratus	8.33	2.5	0.27	0.17	0.01	< 0.01	2.04	0.53
L. camara	0.83	< 0.01	0.27	0.52	0.04	< 0.01	2.18	1.90

In the roots and stems, *Oytenanthera abyssinica* records the highest levels of Zn with 480 mg / kg and 190 mg / kg in the stems and 510 mg / kg and 213 mg / kg in the roots respectively in the lixisols and vertisols. With some exceptions, plant species have accumulated high levels of heavy metals. Quantities are based on species and soil type. ANOVA analysis of variance confirms these results. They clearly indicate that metal concentrations have a highly significant ($p < 0.001$) and significant ($p < 0.05$) correlation with species and soil factors and their interaction (soil x treatment) (Table 6).

Bioconcentration factors of plants

The bioconcentration factors for plant species are given in Table 7. Except for the values of 0.38 and 0.45 of Cu obtained in vertisols, *Vetiveria nigritana* and *Oxytenanthera abyssinica* have a factor of 0.7 to 24.7; which suggests that these two species are accumulators of heavy metals (Cd, Cu, Pb and Zn).

However, *Oxytenanthera abyssinica* accumulates 3 to 32 times more Zn in roots and stems than other species. High Pb levels are recorded with *Oxytenanthera abyssinica* and *Vetiveria nigritana* irrespective of soil type. However, there is a preferential accumulation in the roots and leaves relative to the stems. *Barleria repens*, *Cymbopogon citraus* and *Lantana camara* show very low Cd accumulation between 0.08 and 5 mg / kg. The highest Zn accumulation in the leaves is recorded with *Lantana camara* followed by *Braleria repen* whatever the soil type.

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Tolerance index of the plant species tested

Another way of representing biomass is the use of the tolerance index (Figure 1 and 2). In fact, if the biomass is larger, this means that the cultivar tolerates the treatment well and believes it better than in the absence of metallic contamination. Few tolerance indexes are less than 100%. Regardless of soil type and plant species, root and leaf tolerance indices are greater than 100%. Only *Barleria repens* and *Lantana camara* stems have tolerance indices less than 100%. The highest tolerance indexes are recorded in the leaves of *Vetiveria nigritana* and *Oxytenanthera abyssinica* 359.63% and 382.75% respectively.

Correlations between metal contents in different plant tissues

Correlation coefficients (Pearson correlation) were calculated for each metal combination in the roots, leave and stems (Tables 8 and 9).

Table 8. Pearson correlations of metal content in roots, stems and leaves of plant species in lixisols (correlation values r with significant differences marked by a star)

Cd	Cu	Pb	Roots
0.73**	0.90**	-0.06	Zn
	0.73**	0.35	Cd
		-0.09	Cu
Cd	Cu	Pb	Stems
0.79**	0.97**	0.38	Zn
	0.90**	-0.09	Cd
		0.26	Cu
Cd	Cu	Pb	Leaves
-0.94	-0.49	-0.73	Zn
	0.74**	0.91**	Cd
		0.88**	Cu

**The correlation is significant at P < 0.01.

Lixisols: In the roots, the correlation coefficients for the Cd-Zn and Cu-Cd pairs are strong ($0.5 < r < 0.8$), Cu-Zn is very strong ($r > 0.8$). In the stems, the correlation coefficient Cd-Zn is strong, those of the Cu-Zn and Cu-Cd pairs are very strong ($r > 0.8$). The correlation coefficients of the pairs Pb-Zn and Pb-Cu are average ($0.2 < r < 0.5$). In the leaves, the correlation coefficients between Pb-Cd and between Pb-Cu are very strong, that of Cu-Cd is strong.

Vertisols: In the roots, the coefficients of correlation between Cd-Zn and between Cu-Zn are strong, the other relations are inversely proportional. In the stems, the relationships are inversely proportional regardless of the metal. In the sheets, the correlation coefficients for the Cu-Zn and Pb-Cd pairs are very strong. Other relationships are inversely proportional.

Table 9: Pearson correlations of metal content in roots, stems and leaves of plant species in vertisols (correlation values r with significant differences marked by a star)

Cd	Cu	Pb	Roots
0.66**	0.56*	-0.014	Zn
	0.03	-0.304	Cd
		-0.211	Cu
Cd	Cu	Pb	Stems
-0.40	0.27	0.33	Zn
	-0,74	-0.54	Cd
		-0.16	Cu
Cd	Cu	Pb	Leaves
-0.19	0.83**	0.06	Zn
	-0.22	0.85**	Cd
		-0.21	Cu

*The correlation is significant at P <0,05

**The correlation is significant at P < 0,01.

DISCUSSION

The initial analysis on the two soils of different chemical compositions made it possible to highlight completely different contents of heavy metals (Cd, Cu, Pb and Zn). The highest grades are recorded with Zn followed by Cu and Pb. Cd is at the lowest grade These values are also comparable to those present in the literature (Benton 1998, Larcher 2003, Kabata-Pendias 1992). These levels are higher in vertisols compared to lixisols. The different plant species studied do not behave in the same way in the face of metallic pollution. However, for the same type of soil with metal concentrations considered identical for all species, differences are observed between levels accumulated by plants (Tables 3, 4 and 5). Thus, *Vetiveria nigritana* and *Oxytenanthera abyssinica* generally have higher levels of metals in their plant organs (roots, stems and leaves) than other species. This is especially true for Cu, Pb and Zn in roots and leaves. In general, the heavy metal contents obtained in the dry biomass of the organs are greater than or equal to the values obtained with the critical concentrations above which the effects of toxicity are possible (5-30 mg / kg for the Cd, 20 -100 mg / kg for Cu, 30-300 mg / kg for Pb and 100-400 mg / kg for Zn) according to Larcher (2003). On the other hand, the case of Cd is quite different because the contents are higher in the stems of the three other species.

In addition, the Cd contents vary slightly between soils whatever the plant organ. In general, metal contents are higher in the roots followed by leaves and stems. Several studies have also demonstrated the ability of *Vetiveria nigritana* to accumulate heavy metals in its aerial parts (Truong and Baker 1996, Chen et al. 2000) in aerial parts after phytoremediation on contaminated soil. The work of Truong and Baker (1998) conducted in Thailand concluded that planting rows of hedgerows of *Vetiveria nigritana* would capture agrochemicals, including pesticides, and prevent them from spreading in the environment. Surrounding in South Africa, *Vetiveria nigritana* is used to drain heavy metal toxic tributaries (Al, As, Cd, Cu, Cr, Pb, Zn, etc.) (Truong, 1999). *Oxytenanthera abyssinica* has previously been shown to be useful for the removal of certain toxins from the soil in phyto-remediation (Van et al., 2006).

Results are reminiscent of Thy (2009) with *Lantana camara* on Pb-contaminated soils. Comparable results with other species have been observed with Adhikari et al. (2010). Indeed, this work has shown that *Typha augustifolia* L. is able to accumulate high concentrations of Pb in the roots (1200 mg / kg) and in the leaves (275 mg / kg) while *Ipomoea carnea* L. could also accumulate 1500 and 475 mg / kg respectively in the roots and in the leaves. For Zn, it has been shown that a concentration of 100 mg / kg in feed is considered to be chronically toxic to animals (Dudka et al., 1995). The level of zinc found in the leaves and roots in our study is well above the toxic concentration in the forage. This result is similar to that found in Gipuzkoa (Spain) in *Lolium* sp, a grass, where the Zn concentration far exceeds the critical level (Maiz et al., 2000). If we consider the bioconcentration factors, all the species studied can be considered as Zn accumulators and *Vetiveria nigritana* accumulates all the metals (Cd, Cu, Pb and Zn) (Table 7).

A species is known as an accumulator if its bioconcentration factor is greater than 1, which means that the concentrations measured in the plant are higher than those in the soil. The strongest factors are measured with *Oxytenanthera abyssinica* and are respectively 24.70 and 6.05 for Cd and Pb. Next, *Barleria repens* was also well studied with a factor of 3.40 for Zn in lixisols. These results corroborate those of Klang-Westin and Eriksson (2003) who found bioconcentration factors of Cd (27.9) measured on *S. viminalis* on a soil containing 0.45 mg / kg of Cd. For Zn, the most important bioconcentration factor (1.71) was observed on *S. viminalis* on soil containing 1160 mg / kg Zn (Hammer et al., 2003). Only *Barleria repens* and *Lantana camara* stems have tolerance indices of less than 100% regardless of soil. This means that the species studied can grow on soils contaminated with heavy metals and are therefore interesting in the accumulation of Cd, Cu, Pb and Zn. Positive correlations between the concentrations of two metals (Cd-Zn, Cu-Zn, Cu-Cd, Pb-Cd and Pb-Cu) in the leaves and stems have been demonstrated (Table 8 and 9) suggesting a common transport of the two metals. Cd is able to use Zn transporters, which are present in the plant to absorb the essential element Zn. It has been known for many years that Zn is a competitor ion of Cd (Hawf and Schmid, 1967) and that it decreases Cd uptake (Mengel and Kirkby, 2001). Cd uptake depends on the amount of Zn in the soil and plants generally absorb more Cd if the soil Zn content is low (Kirkham, 2006).

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Conclusion

The results of this present study indicate the accumulative potential of metals by the plant species tested. However, the level of accumulation depends on the plant species, the plant organ and the soil type. Bioconcentration factors are higher with *Vetiveria nigritana* and *Oxytenanthera abyssinica* compared to the other three species; which allows us to say that these two species are more accumulative than the others. In addition, the registered tolerance indexes are all greater than 100; only *Barleria repens* and *Lantana camara* stems have tolerance indices less than 100%. These results also revealed strong correlations (r> 0.5) in the accumulation of heavy metals in the leaves and stems.

These results show that the plant species studied can be grown on soils contaminated with heavy metals. They can therefore be interesting in the accumulation of Cd, Cu, Pb and Zn.

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