Pb, Zn AND Cd TOLERANCE AND ACCUMULATION IN POPULATIONS OF *THLASPI CAERULESCENS*

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ABSTRACT: Industrial soils near Zn-Pb mines of Mibladen and Zaida (Northern West Morocco) are exposed to high environmental stress related to heavy metal pollution (Zn, Pb, Cd and Cu) from waste disposal sites.

The use of plant species to stabilize or remove pollutants from soils, generally defined as phytoremediation. Moreover, the revegetation of metal-polluted areas allows to establish a vegetal cover that will limit the further dispersion of metal-contained soil particles through the water and the wind erosion.

This study tackles screen plants growing on contaminated soil to determine their potential for metal accumulation. A non-metallicolous (NM) ecotype of *Thlaspi caerulescens* and a metallicolous (M) ecotype are compared for growth and Pb, Cd and Zn accumulation in shoot and root in six metal contaminated soils and one normal soil. The growth of individuals from normal soil was greater than that of individuals from metal-contaminated soils. Both ecotypes had similar growth. The NM populations had markedly higher root: shoot ratio compared to M populations. The results thus indicate both ecotypes of *T. caerulescens* are highly tolerant of zinc and Cd. Ecotype NM has constitutively higher Zn uptake capacity than M ecotype and grew less well than ecotype M. *T. caerulescens* species accumulate higher amount of Zn and Cd in their tissues in polluted soil and both of the two ecotypes the root Pb concentrations were much greater than those of the shoot Pb contents.

The results obtained indicate that *Thlaspi caerulescens* from both normal and metal-contaminated soils are interesting material for phytoremediation of zinc and cadmium.

INTRODUCTION

Mining and industrial activities, waste accumulation, are recognised as the main sources of metal pollution of soils (Clemente *et al.* 2007; Del Rio *et al.* 2006; Freitas *et al.* 2004; Wong 2003). Mine tailings are known to have the highest environmental effects (Dudka and Adriano 1997) due to the high concentration of heavy metals (Wong *et al.* 1998; Norland and Veith 1995), and the high incidence of erosion agents (wind and water run-off) (Conesa *et al.* 2007). The threat that heavy metals pose to human and animal health is aggravated by their long-term persistence in the environment (Yoon 2006). Most conventional remediation approaches do not provide acceptable solution to toxicity caused by metal pollution (Wang *et al.* 2007). The development of phytoremediation technology for site decontamination has spurred recent interest in the mechanisms by which metals are accumulated in plants, Pollard *et al.* (2002). Phytoremediation is an emerging technique that offers the benefits of working in situ, being low cost (McGrath *et al.* 2002; Salt *et al.* 1998), and can provide long-lasting and

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aesthetic solution for remediation of contaminated sites (Ma *et al.* 2001). One of the strategies of phytoremediation of metal-contaminated soil is phytoextraction, i.e. through uptake and accumulation of metals into plant shoots, which can then be harvested and removed from the site. Another application of phytoremediation is phytostabilization where plants are used to minimize metal mobility in contaminated soils (Yoon *et al.* 2006). A few species, *c.* 400, (Baker *et al.* 2000), are classified as heavy metal hyperaccumulator plants. However, the physiological and molecular mechanisms underlying the hyperaccumulation trait are still largely obscure (Assunção *et al.* 2003). *Thlaspi caerulescens* is relatively well studied because it's known to hyperaccumulate zinc. It can be classified as a facultative métallophytes growing on metallicolous as well as non-metallicolous soil (Dechamps *et al.* 2005).

Recent work has pointed out the existence of variation in metal accumulation capacity, both within and between populations (Roosens *et al.* 2003; Assunção *et al.* 2001; Escaré *et al.* 2000; Meerts and Van Isacker 1997; Pollard and Baker 1996; Lloyd-Thomas 1995).

A lot of studies have been conducted about variation of accumulation in populations situated on contaminated or non contaminated soils (Dechamps *et al.* 2005). These were found to accumulate Zn to much higher concentrations than metallicolous populations when growing in their natural, Zn poor soils, non metallicolous populations also their concentration factor (concentration ratio of Zn in shoots to Zn in soil) is higher compared to metallicolous populations on metal rich soils (Reeves *et al.* 2001; Lloyd-Thomas 1995).

In this paper, we assess Zn, Cd tolerance and accumulation in a metallicolous (M) ecotype of *T. careulescens* and non metallicolous (NM) ecotype., we test whether growth Zn, Cd and Pb accumulation of two ecotypes.

METHODS

Seed and Soil Sampling

Seed Sampling

Seeds of *T. caerulescens* metallicolous were collected in the vicinity of F.T. Laurent le Minier in the Pb-Zn mining district of les Malines (Northe of Montpellier, South of France). Seeds of *T. caerulescens* non-metallicolous were sampled on the Southern Border of the Larzac plateau (North of Montpellier, Southern France). Therefore, the two populations used in this study are assumed to be representative of there respective ecotype.

Soil Sampling

Soil substrates were collected from a soil mining of Mibladen and Zaida (North West, Morroco). In The lead layer of Zaïda, two representative of soil industrial waste were collected, Z1 corresponds to Tailings around old lead mine workings and Z2 corresponds to the residues of treatments.

In the mine of Mibladen, Three sites of samplings were collected, M1 correspond to the residues of treatment and S1 and S2 to the residues of foundry.
The unpolluted soil sample originated from an agricultural site of Kenitra (East Morocco).

Tests of Germination

Seeds of *Thlaspi caerulescens* were collected manually on the site, they are preserved at the laboratory under the environmental conditions (20°C approximately) safe from the light. The tests of germination are carried out of Plastic Petri dishes. The collected seeds are posed on cotton soaked with distilled water. The Petri dishes were placed safe from the light. Twenty seeds are sown on pots containing the various substrates. The latter are humidified by distilled water.

Plants Sample Analysis

Seeds were germinated in Petri dishes. Twenty seedlings were transplanted into each pot. There were three replicates of either ecotype for each soil. The 84 pots were watered with distilled water as necessary.

The plants were growing for three months. The aerial biomass shoots and roots of plants samples were then harvested, rinsed in distilled water, dried 48 h at 60°C and weighted. Subsamples were digested with a mixture of nitric with hydrochloric acid. The concentration of Zn, Cd, and Pb in the digest was determined using ICP-MS (Inductively Coupled Plasma Mass Spectrometry).

Soils Analysis

500 mg soil subsamples were digested with 10 mL of a 3:1 HCl/HNO_3 mixture. The digested was evaporated to dryness filtered and finally made up to 25 ml with deionised water, 1 mL of the filtered extract was then analysed for Cd, Zn and Pb by ICP-MS.

Statistical Analysis

Variation of heavy metals composition of cultivated plants were analysed by spss 11.5, correlation between biomass, Zn, Cd and Pb were examined. All analyses were performed with spss 11 and origin 6.

RESULTS

Soil metal concentration

The metals contents were determined in soil samples from (T), Zaïda (Z1), (Z2); Mibladen (Mib1), S1 and S2. S1 and S2 which are a mine spoil (Table 1) showed extremely high concentrations of Zn, Pb and Cd in agreement with data obtained from calamine sites in Lelligen (LC) (Assunção *et al.* 2003) (Table 2). The soil from the polluted site of the high Moulouya was highly enriched in Zn, Cd and Pb with maximal concentrations of up to 160200 mg/kg Zn (S2), 368 mg/kg Cd (S2) and 1211 mg/kg Pb (S1) (Table 2).

Table 1. Location and characteristics of the sites of soils collection

Stations	Site location	Sustratum
T	Kenitra	Normal soil
M 1	Mibladen	Tailings around old lead mine workings
S1	Mibladen	Mine spoil and waste from fondery
S2	Mibladen	Mine spoil and waste from fondery
Z1	Zaida	Tailings around old lead mine workings
Z2	Zaida	Lead mine spoil

Metal Tolerance

Metal tolerance was assessed in plants from the populations of M1, S1, S2, Z1 and Z2. Plants from S1and S2 were highly tolerant to zinc. Z1 and T maintained normal growth. The results confirm that both ecotypes of *T.caerulescens* are highly tolerant of Zn and Cd. Ecotype NM grew less well than ecotype M (Figure. 1).

Metal Concentrations in Plants

A non-metallicolous (NM) and a metallicolous (M) ecotype of *Thlaspi caerulescens* are compared for growth and Pb, Cd and Zn accumulation in shoot and root in a pot experiment in six soil, the levels of the traces elements analyzed of non metallicolous ecotype were slightly less greater than metallicolous ones.

Table 2. Soils analyses from the sites of: Mibladen: (M 1), (S1) and (S2); Zaïda: (Z1), (Z2) and La Calamine: (LC). Soils samples were taken at dept of 0-15 cm. Concentrations of metals is expressed as mg/kg; n, number of subsamples of soils analysed

		T (n=3)	Mib1 (n=3)	S1 (n=3)	S2 (n=3)	Z1 (n=3)	Z2 (n=3)	LC[1] (n=3)
Soil metal content	Zn	38	7582	89142	160200	1099	867	101563
	Cd	0.1	2	207	368	0.5	0.2	217
	Pb	11	817	1212	318	214	32	8998

[1] Assunção *et al.* (2003).

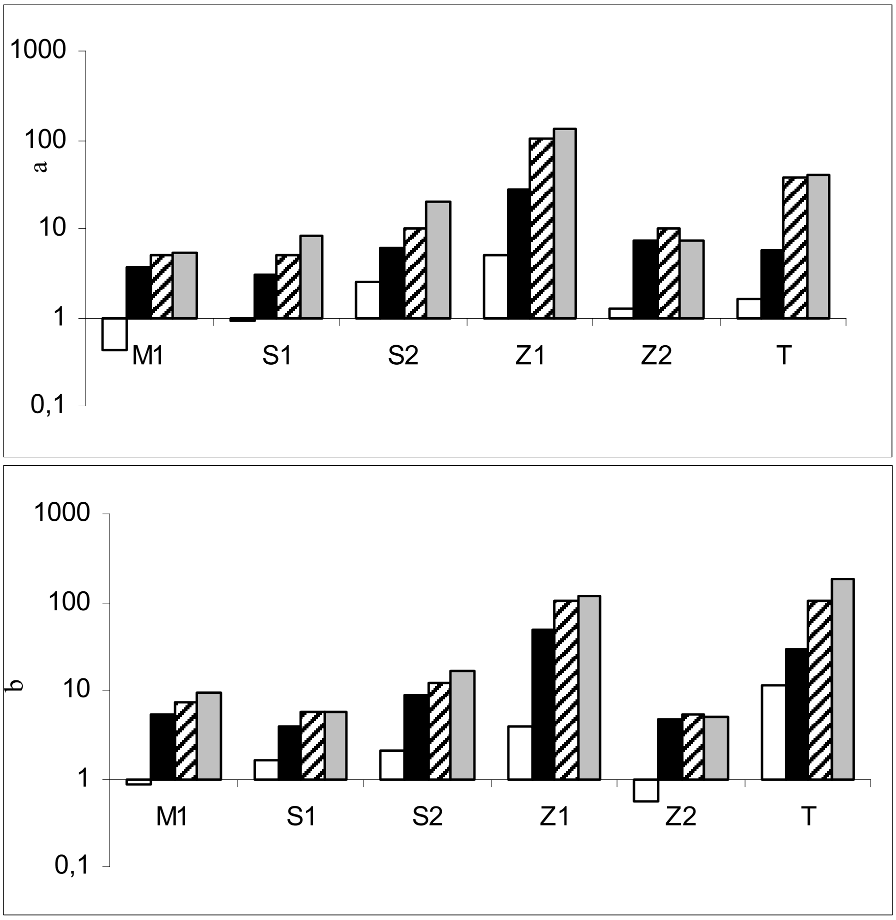

Figure 1. Logarithmic values of tolerance of metallicolous (a) and non metallicolous (b) populations in the *Thlaspi caerulescens* cultivated in six types of soil; a black bars refers a first month of growth, second month is showed by the white bars, gray and barred bars represent the third and fourth month respectively

Figures 2 and 3 compares metal concentrations in roots and shoots between the two populations of *T.caerulescens* growing in unpolluted and polluted soil. Both of the two populations, it shows that Cd and Zn are more concentrated in shoots, however Pb concentrations in roots were much greater than those of the shoot Pb contents, indicating low mobility of Pb from the roots to the shoots and immobilization of heavy metals in roots, so plant uptake of heavy metals from soil occurs either passively with the mass flow of water into the roots, or through active transport crosses the plasma membrane of root epidermal cells (Yoon *et al.* 2006). Perhaps the high accumulation of Pb in soils, and particularly so in roots, may possibly slow down, or almost completely stop the transport to the aerial tissues since Pb may accumulate in the cell wall (Marmiroli *et al.* 2005). It has recently been reported that

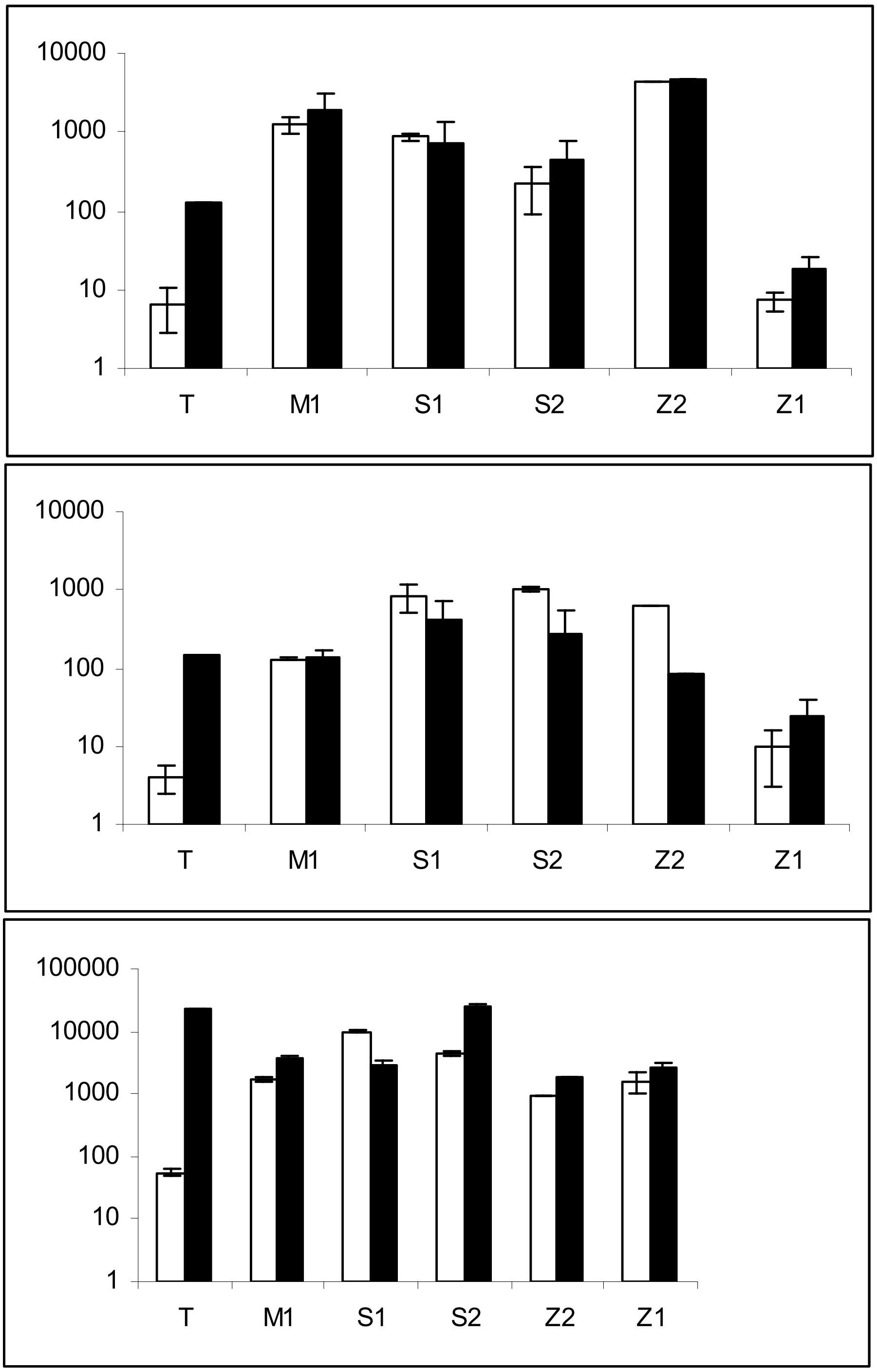

Figure 2. Logarithmic values of Pb , Cd and Zn accumulation (means±SE) in the shoot of *Thlaspi caerulescens* metallicolous (white bars) and non metallicolous (black bars) populations cultivated in six types of soil

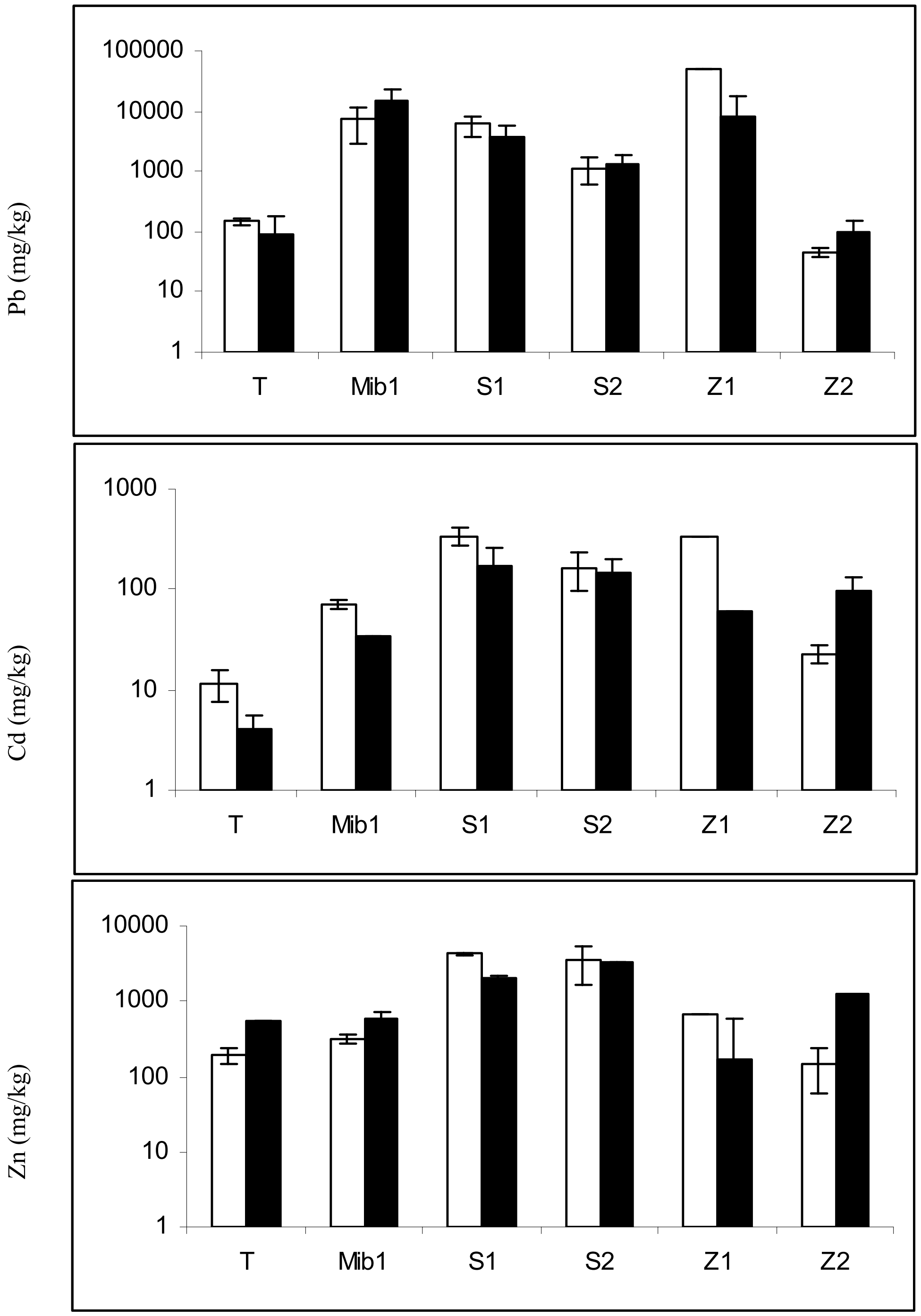

Figure 3. Logarithmic values of Pb , Cd and Zn accumulation (means±SE) in the roots of *Thlaspi caerulescens* metallicolous (white bars) and non metallicolous (black bars) populations cultivated in six types of soil

despite the adaptation of this ecotype of *T. caerulescens* to soils containing Cu, Zn and Pb, Cu strongly inhibits growth unlike Pb does (Walker and Bernal 2004). It is possible that perhaps Pb compete to bind the membrane transporters (Martinez *et al.* 2006).

However, the differences between root and shoot concentrations indicated a restriction of the internal transport of metals from the roots towards stems and green leaves (Del Rio *et al.* 2006). Such metal immobilisation in root cells, as emphasized by the MS/MR (shoot/root metal concentration quotient) quotient < 1 (Table 4), is related to an exclusion strategy (Baker 1981).

On the other hand, the results in Table 4 revealed that *T. caerulescens* species accumulate higher amount of Zn and Cd in their tissues in polluted soil, similar findings were reported by Al-Garni, (2006) in cowpea plants. Mechanism of transport to aerial parts for Zn was more efficient that for Pb (Del Rio *et al.* 2006). Trends of Zn concentrations in plant species parts differed from those of Pb. Dahmani-Muller *et al.* (2000) reported in *Cardaminopsis halleri* (L.) that Zn and Cd were actively transported from roots to stems and leaves. According to the definition of Zn hyperaccumulators (plant species whose shoots contain > 10,000 mg/kg (Baker and Brooks 1989), our results showed that *Thlaspi* plant can be considered as a hyperaccumulator for Zn (Table 2). Same finding has also been observed in four species *C. album*, *C. intybus*, *C. dactylon* and *M. nicaeensis* (Del Rio *et al.* 2006).

Bioaccumulation and Translocation Factors

The definition of metal hyperaccumulation has to take in consideration not only the metal concentration in the above ground biomass, but also the metal concentration in the soil. Both bioaccumulation factor (BF) and translocation factor (TF) have to be considered while evaluating whether a particular plant is a metal hyperaccumulator (Gonzaga *et al.* 2006; Ma *et al.* 2001).

For each metal, Bioaccumulation factors (BF) were calculated for heavy metal content of plant parts (roots or shoots) (mg/kg) to heavy metal content of soil (mg/kg), (Table 3).

Table 3 shows BF comparisons between the different organs of *T. careulescens* metallicolous and non metallicolous. The distribution of observed polluants (Pb, Cd and Zn) was very different according to ecotypes species and parts. Pb concentration of root was 23 times higher than those of shoot. However, Cd and Zn concentration of plant parts was somewhat different, moreover trend of plant parts' metal concentration slightly reversed, shoot was 37 and 43 times higher than root respectively. The average values of Pb BF in roots of *T. caerulescens* non metallicolous are higher than in shoots but Cd and Zn BF in roots are lower than those calculated in the shoots. The translocation factor for metals within a plant was expressed by the ratio of metal (shoot) to metal (root) to show metal translocation properties from roots to shoots (Saleh Al-Garni 2006; Stoltz and Greger 2002; Tu and Ma 2002). Table 4 shows that Zn TF in both ecotypes of *T. caerulescens* is higher than Cd and Pb.

Comparison of Bioaccumulation among Origins

In our study, populations from different origins did not accumulate metals to the same extent in their shoots: M accumulated significantly less zinc and lead than NM. This difference in accumulation capacity has also been observed in hyperaccumulator species such as *A. maritima ssp halleri, A. halleri*, Bert *et al.* (2000) or *A. elatius,* Deram *et al.* (2006).

Concerning these well studied species, Escarré and *al.* (2000) showed that the NM accumulated twice to three times more zinc in aboveground parts than M.

Forward to explain such observations two non-exclusive hypotheses could be put:

(1) the NM plants could develop heavy metal active cellular mechanisms, such as chelation of metals in the cytosol by phytochelatins that are involved in the detoxification of heavy metals and, thus, increase tolerance to metal stress, Deram *et al.* (2006a), Hall and Williams, (2003) and Hall (2002) for review; or

(2) M could restrict zinc and cadmium accumulation in aerial parts. Restriction in the transport or delay in metal translocation from roots to shoots, often referred to as a strategy for heavy metal tolerance, is well-documented (Deram et *al.* 2006a; Brekken and Steinnes 2004; Dahmani- Muller *et al.* 2000; Ernst et *al.* 1992; Baker and Walker 1990).

Table 3. Intraspecies comparisons of bioaccumulation factors in exposed shoots: (a) and roots; (b) of *T. caerulescens* metallicolous (M) and non metallicolous (NM)

(a)	Pb		Cd		Zn	
	M (n=3)	NM (n=3)	M (n=3)	NM (n=3)	M (n=3)	NM (n=3)
T	1.03±2.2	9.7±nm	17.09±5.16	56.6*±nm	0.15 + 0.13	75.7 + nm
Mib1	0.82±0.83	1.3±2.32	59.37±8.44	81.7±119.2	0.13±0.08	2.4±2.02
S1	0.96±2.1	0.7±11.12	3.68±68.30	2.8±16.87	0.11±0.02	0.1±0.15
S2	0.75±5.4	1.4±14.1	2.77±14.05	0.7±31.89	0.03±0.03	0.2±0.29
Z1	0.14±0.14	0.4±0.43	14.63±8.59	30.2±15.61	0.77±3.67	2.2±2
Z2	84.09±nm	20.2±nm	98*±nm	23.1*±nm	0.98±nm	1.7±nm

(b)	Pb		Cd		Zn	
	M (n=3)	NM (n=3)	M (n=3)	NM (n=3)	M (n=3)	NM (n=3)
T	2.3*±4.8*	6.7±20.7	49.7*± 11.16	15.3±5.5	0.5 + 1	1.7 + 8.6
Mib1	4.84±11.07	10.2±16.3	2.85*±2.4*	7.2*±30.1*	0.02±0.02	0.4±0.9
S1	6.71±4.8*	3.8±37.5	1.52±15.60	1.1±4.5	0.05±0.01	0.04±0.01
S2	3.86±17.19	4.1±21.1	0.46±10.51	0.4±5.1	0.03±0.15	0.03±0.1
Z1	0.93±0.57	1.9±3.5	33.9±6.01	12.1*±4.4*	0.1±0.55	1±0.6
Z2	97.6*±nm	6.7±0.6	53.7*±nm	12.8*±23.7*	0.71±nm	0.1±31.1**

Bioaccumulation factors (BF) are expressed by the means±standard errors of 3 replicates. nm: not measured. *: values recorded were multiplied by 10; **: values were multiplied by 10^6.

Table 4. Ratio of root to shoot metal concentration of *T. caerulescens* metallicolous and non metallicolous

	Pb		Cd		Zn	
	M (n=3)	NM (n=3)	M (n=3)	NM (n=3)	M (n=3)	NM (n=3)
T	0.04±0.05	1.4±nm	0.3± 0.5	37±nm	0.30+0.13	43.4+nm
M1	0.17±0.07	0.13±0.14	2.1±0.3	1.1±0.4	5.5±4.33	6.3±2
S1	0.14±0.04	0.19±0.3	2.43±4.4	2.43±3.7	2.35±1.45	1.5±10
S2	0.19±0.31	0.3±0.7	6.04±1.3	1.9±6.2	1.27±0.18	7.7±2.8
Z2	0.16±0.24	0.2±0.1	0.43±1.4	0.25±0.4	7.52±6.7	2.2±3.3
Z1	0.9±nm	2.1±nm	1.8±nm	1.38±nm	1.83±nm	2.12±nm

Translocation factors (TF) are expressed by the means±standard errors of 3 replicates, nm: not measured.

T.carelescens, like *A. elatius*, has been experimentally demonstrated that this species, originating from contaminated soils, seems to have evolved a complex metal homeostasis network system which regulates its uptake and distribution within the plant effectively protecting the metabolic processes (Clemens *et al.* 2002). Regarding M, plants appear to restrict translocation of metal in order to better tolerate soil extremely contaminated with zinc and cadmium (Deram *et al.* 2006b).

CONCLUSIONS

This study was conducted to screen plants growing on a contaminated site to determine their potential for metal accumulation. To conclude, the two ecotypes of plant species were able to form a vegetal cover on Cd, Pb, and Zn-polluted soils. The main findings of this work were that: (i) It is increasingly clear that hyper-tolerance is fundamental to hyperaccumulation, and high rates of uptake and translocation are observed in *T.caerulescens*; and (ii) Pb is less accumulated in the shoots than roots. iii) For all the polluted soils, both of Cd and Zn TF greater than one. Our results suggest that non-metallicolous populations might be better candidates to phytoremediation.

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