Zinc and cadmium hyperaccumulation by Thlaspi caerulescens from metalliferous and nonmetalliferous sites in the Mediterranean area: implications for phytoremediation

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Received 28 July 1999 : accepted 2 November 1999

SUMMARY

Growth, tolerance and zinc and cadmium hyperaccumulation of Thlaspi caerulescens populations from three metal contaminated soils and three normal soils were compared under controlled conditions. Individuals of six populations were cultivated on five soils with increasing concentrations of zinc (50–25000 µg g⁻¹) and cadmium (1–170 µg g⁻¹). There was no mortality of normal soil populations in the four metal-contaminated soils, but plant growth was reduced to half that of populations from metal-contaminated soils. However, in noncontaminated soil, the growth of individuals from normal soils was greater than that of individuals from metal-contaminated soils. Individuals from normal soils concentrated three times more zinc in the aboveground biomass than those from metal-contaminated soils, but the latter accumulated twice as much cadmium. We conclude that populations of T. caerulescens from both normal and metal-contaminated soils are interesting material for phytoextraction of zinc and cadmium, but to optimize the process of phytoextraction it is necessary to combine the extraction potentials of both type of populations.

Key words: cadmium, growth, metal-contaminated soils, phytoremediation, Thlaspi caerulescens, zinc.

INTRODUCTION

Excessive concentrations of toxic heavy metals such as Zn, Pb, Cd, Cu, Ni and Tl in soils in mining areas and around smelters are sources of serious environmental and health hazards. Consequently, soil-depolluting technics methods are today an important concern. Several authors have recently pointed out that, the use of plant species that hyperaccumulate heavy metals in their aerial parts could be an economically efficient ecological phytoextraction method for cleaning the soil (Raskin et al., 1994; Salt et al., 1995, 1998; Robinson et al., 1998; Leblanc et al., 1999). The concept of hyperaccumulation was originally introduced to define plants containing > 0.1% (1000 µg g⁻¹) of Ni in dried plant tissues (Brooks et al., 1977). However for other metals such as zinc and manganese the threshold is 10000 µg g⁻¹ (1%) of metal in aerial dry matter (Baker & Walker, 1990). Among the northwestern European temperate flora, Thlaspi caerulescens, a herbaceous annual or short-lived perennial, has recently received much attention (Baker et al., 1994; Brown et al., 1995; McGrath et al., 1997; Meerts & Van Isacker, 1997; Shen et al., 1997), because it has been known for a long time to hyperaccumulate zinc (up to 2.5% d.m. (Risse, 1865, cited in Ernst, 1974; Baumann, 1885).

Except for the work of Robinson et al. (1998), no study of T. caerulescens has been devoted to Mediterranean plant populations. Moreover, Meerts & Van Isacker (1997) is the only study comparing the ability to hyperaccumulate zinc and lead of metalliferous and nonmetalliferous populations.
The severe environmental conditions prevailing on heavy-metal-enriched spoil heaps (metal toxicity, lack of nutrients and limited water supply due to porous substrate), are enhanced in the Mediterranean climatic conditions because of prolonged drought. Therefore, if *T. caerulescens* is to be promoted for phytoextraction in this region, it is necessary to use ecotypes adapted to Mediterranean climatic conditions. We therefore studied Zn and Cd accumulation by Mediterranean populations of *T. caerulescens* from heavy-metal-contaminated soils and from normal soils in an area near Montpellier, France. The principal aim of this study was to explore the possibility of phytoremediation of Zn- and Cd-contaminated soils, using populations of *Thlaspi caerulescens* and selecting the more efficient families. The following questions were addressed:

- Do the metalliferous and nonmetalliferous populations differ in Zn and Cd tolerance and accumulation when growing on metal-contaminated substrates?
- Is there any trade-off between growth, accumulation and tolerance?
- Are Zn and Cd accumulation correlated?
- Is there any genetic component for Zn and Cd accumulation in populations and in individuals within populations?

**Materials and Methods**

**Populations**

Populations of *T. caerulescens* J. et C. Presl (Tutin et al., 1993) growing on heavy-metal-polluted soil and on normal soil are distinguished taxonomically in floras of northwestern Europe as subsp. *calaminare* (Lej. et Court.) Dvor. and subsp. *caerulescens*, respectively, mainly on the basis of tenuous characteristics of the silicula (Dvorakova, 1968; Lambinon et al., 1992). However, recent studies based on isozyme polymorphism do not confirm the taxonomic treatment (Koch et al., 1998). Therefore, populations from Montpellier are referred to as metalliferous (from metal-contaminated soils, MCS) and nonmetalliferous (from normal soils, NOR).

The MCS populations were collected in the vicinity of St. Laurent le Minier in the Pb-Zn mining district of Les Malines (40 km north of Montpellier, Fig. 1), from three mine sites abandoned at times ranging from the Middle Ages (MG), to 20 (Pommiers, PO) and 5 (Les Malines, MA) yr ago.

The three NOR populations were sampled on the southern border of the Larzac Plateau, which comprises mainly Mg-carbonate bedrock, at Les Infruts (IN), Saint-Michel (SM) and near the Cirque de Navacelles (CN). Site characteristics are listed in Table 1.

**Seed sampling and germination**

In each population 20–30 flowering plants were collected and grown in pots of their original soil until seeding. Offspring from each individual plant was labelled as a family.

Seeds belonging to 10 families per population were germinated in Petri dishes in a glasshouse with natural light supplemented with three fluorescent
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Table 1. Soil characteristics of collection sites. Concentrations are expressed as μg g⁻¹ ammonium-acetate-EDTA extractable element

<table>
<thead>
<tr>
<th>Locality</th>
<th>pH</th>
<th>Ca (μg g⁻¹)</th>
<th>Zn (μg g⁻¹)</th>
<th>Pb (μg g⁻¹)</th>
<th>Cd (μg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal-contaminated soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pommiers (PO)</td>
<td>7.31</td>
<td>500</td>
<td>6000</td>
<td>4350</td>
<td>17</td>
</tr>
<tr>
<td>Malines (MA)</td>
<td>8</td>
<td>37000</td>
<td>3500</td>
<td>253</td>
<td>16.5</td>
</tr>
<tr>
<td>Middle Age Mine (MG)</td>
<td>6.5</td>
<td>1500</td>
<td>950</td>
<td>1550</td>
<td>13.1</td>
</tr>
<tr>
<td>Normal soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saint Michel (SM)</td>
<td>7.3</td>
<td>7500</td>
<td>50</td>
<td>28</td>
<td>1.4</td>
</tr>
<tr>
<td>Cirque de Navacelles (CN)</td>
<td>7.5</td>
<td>8000</td>
<td>18</td>
<td>38</td>
<td>1.2</td>
</tr>
<tr>
<td>Les Infruts (IN)</td>
<td>7.2</td>
<td>6000</td>
<td>9</td>
<td>17</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of the five substrates used in the experimental treatments; concentrations are expressed as μg g⁻¹ ammonium acetate-EDTA extractable element

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Contaminated soil (%)</th>
<th>Compost (%)</th>
<th>Sand (%)</th>
<th>pH</th>
<th>Ca (μg g⁻¹)</th>
<th>Zn (μg g⁻¹)</th>
<th>Pb (μg g⁻¹)</th>
<th>Cd (μg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>7.67</td>
<td>21000</td>
<td>50</td>
<td>175</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>40</td>
<td>40</td>
<td>7.73</td>
<td>9000</td>
<td>10500</td>
<td>4500</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>30</td>
<td>20</td>
<td>7.83</td>
<td>5500</td>
<td>20100</td>
<td>8000</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td>7.65</td>
<td>6500</td>
<td>20700</td>
<td>9500</td>
<td>120</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>20</td>
<td>0</td>
<td>7.58</td>
<td>4500</td>
<td>25200</td>
<td>14000</td>
<td>170</td>
</tr>
</tbody>
</table>

lamps and under 12:12 h light: dark at 25:10°C, respectively. Seed germination was 90%.

Soil treatments

Germinated seeds were planted in 1-l pots filled with different proportions (by volume of dry soil) of garden compost, sand and a heavily Zn- and Pb-contaminated soil from the Avinieres mine, at Saint Laurent le Minier. The soil was collected from an old retention pond filled with tailings generated during the Zn–Pb ore oxidation process. The mildly alkaline pH of the tailings (7.5–7.9) results from the presence of carbonate material associated with the Zn–Pb hydroxides. The soils have a high metal content, mainly Zn and Cd, which are soluble in these neutral–slightly alkaline pH conditions. The physical and chemical characteristics of the five treatments are shown in Table 2. Treatment 5 was the most toxic, with 80% contaminated soil. Treatments 3 and 4, both comprised 50% toxic soil, but differed in the amount of added compost, resulting in two different levels of fertility. Treatment 1, the control, was a mixture of commercial garden compost and soil from the CEFE experimental field station with a very low organic C content (3.8%) and a low C:N ratio (16). The cationic exchange capacity was high (25 C mol kg⁻¹).

The differing proportions of contaminated soil in the mixtures were used to create a gradient of concentrations of extractable Zn and Cd. However, this was of little importance to our conclusions because most of the analyses were of treatment 5 only. The addition of compost did not change metal concentrations because in treatments 3 and 4, with the same proportion of contaminated substrata but different proportions of compost, the concentration of extractable Zn was the same.

Experimental design

For each treatment and for each population, 10 families of 15 individuals were distributed among three blocks each with one individual per treatment in a split-plot design. The whole plot included the five treatments, and the split plot the two origins (MCS vs NOR). There were thus 6 populations × 10 families × 3 blocks (or repeats) × 5 treatments representing a total of 900 pots. However, in some families the number of germinated seeds was low and therefore only 830 individuals were used in the experiment. Pots were placed in a glasshouse with a natural day:night regime, at temperatures > 4–5°C (to avoid freezing) and watered daily with distilled water. Plants that died during the 2 wk after transplanting were replaced by individuals from the same mother plant, mortality being assumed to be the result of handling.

Measurements

Soil and plant analysis. To analyse the extractable metal concentration of metals of the soil of the collection sites, a bulked sample of 5 subsamples of
soil was taken from each site at a depth of 5–15 cm from around the roots of field plants. The cation exchange capacity of the soil was measured by the ammonium method. Air-dried soil (20 g) was shaken for 30 min with 100 ml of extracting solution (0.5 M ammonium acetate + 0.5 M acetic acid + 0.02 M EDTA at pH 4.65). The extracts were filtered on folded Schleicher & Schull 595 1/2 filters (125 mm diameter) and analysed by flame atomic absorption spectroscopy (AAS). Soils from the different treatments were analysed in the same way (Table 1).

After 365 d of cultivation, the green aerial biomass was collected and dried at 60°C for 3 d. All plants in treatment 5 (80% contaminated soil) and one plant per family in treatment 4 (50% contaminated soil) were mineralized in a mixture of nitric and perchloric acid and their Zn and Cd contents determined by flame AAS. Plants that died before the end of experiment (i.e. after flowering) were collected and processed as the other plants. Mortality before the end of experiment in treatment 5 was very low (7 individuals) and evenly distributed among populations (only SM had two dead individuals); therefore the inclusion of dead plants did not change the results. The plants analysed in treatment 4 were all living.

**Tolerance to contamination level.** The tolerance to toxic soils of the families and populations was estimated as the ratio of the biomass production on metal contaminated treatments (treatment numbers 2 to 5, see Table 2) to the biomass production on the normal soil treatment (treatment number 1).

**Statistical analysis.** Plant biomass and Zn and Cd content were analysed by a split-plot ANOVA, performed with SAS. (SAS, 1996). Populations were nested within the origin factor and families within populations. All the factors were considered as fixed except the factor family, which was random. Because of the occurrence of missing values, Type III sums of squares and the Satterthwaite approximation (the ‘Test’ option in the ‘Random’ statement of the GLM procedure) were used.

**RESULTS**

**Biomass production**

Biomass production was significantly higher in MCS than in NOR populations in contaminated treatments (treatments 2–5) (Fig. 2). Aerial biomass of MCS populations was smallest on noncontaminated soil and greatest at a high level of contamination (treatments 4 and 5). Conversely, aerial biomass of two NOR populations, CN and SM, decreased in contaminated treatments compared with treatment 1 (noncontaminated soil). The third NOR population (IN) had the smallest biomass and was almost insensitive to the different treatments. These results explain the significant effects of ANOVA for population within origins and for the interactions between treatment × origin and treatment × population (Table 3). Moreover, a significant effect of family was found, indicating that within the populations, the response of the families to the five treatments was different (Table 3).

**Tolerance to the toxic soils**

From Table 4 and Fig. 3 it can be seen that MCS populations were nearly twice as tolerant in each
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Table 4. Analysis of variance on the tolerance to the toxic soils of populations of Thlaspi caerulescens from metalliferous and nonmetalliferous origins grown in four treatments

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>2</td>
<td>1.08(1)</td>
<td>1.18 ns</td>
</tr>
<tr>
<td>Tr</td>
<td>4</td>
<td>10.11(1)</td>
<td>10.98*</td>
</tr>
<tr>
<td>Block × Tr</td>
<td>6</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Ori</td>
<td>2</td>
<td>284.93(2)</td>
<td>27.82***</td>
</tr>
<tr>
<td>Pop [Ori]</td>
<td>4</td>
<td>9.63(2)</td>
<td>0.93 ns</td>
</tr>
<tr>
<td>Fam [Pop [Ori]]</td>
<td>52</td>
<td>10.65(2)</td>
<td>12.19***</td>
</tr>
<tr>
<td>Tr × Ori</td>
<td>3</td>
<td>3.34(2)</td>
<td>3.46*</td>
</tr>
<tr>
<td>Tr × Pop [Ori]</td>
<td>12</td>
<td>1.83(2)</td>
<td>1.98*</td>
</tr>
<tr>
<td>Tr × Fam [Pop [Ori]]</td>
<td>156</td>
<td>0.86(4)</td>
<td>0.32 ns</td>
</tr>
<tr>
<td>Residual</td>
<td>379</td>
<td>2.67</td>
<td></td>
</tr>
</tbody>
</table>

Tr, treatment; Ori, origin; Pop, population; Fam, family.
(1) Block × Tr is the error term.
(2) Fam [Pop [Ori]] is the error term.
(3) Tr × Fam [Pop [Ori]] is the error term.
(4) Residual is the error term.
*, P < 0.05; **, P < 0.01; ***, P < 0.001; ns, non-significant.
MS, mean squared.

Fig. 3. Tolerance to the toxic soils (mean ± SE) of three metalliferous (MCS) and three nonmetalliferous (NOR) populations of Thlaspi caerulescens from the Montpellier region (southern France) grown on four toxic substrates with increasing Zn and Cd concentrations (Table 2). CN, Cirque de Navacelles; IN, Les Infruts; MA, Les Malines; MG, mine abandoned in the Middle Ages; PO, Pommiers; SM, Saint-Michel.

contaminated treatment as NOR populations. Within the two origins (i.e. among the three MCS populations or among the three NOR populations) no significant differences emerged between populations when all treatments were combined. Nevertheless there was a significant population effect among MCS populations: Population PO was almost twice as tolerant as MA on the most toxic substrate (treatment 5), but had the same level of tolerance as the two other MCS populations in treatment 4 (50% contamination level). The family effect within populations was also highly significant.

Zinc and cadmium content in aerial parts

The average Zn content of MCS populations ranged from 4500 to 9500 µg g⁻¹, whereas that of NOR populations ranged from 21000 to 30000 µg g⁻¹ (Fig. 4a). This results explains the significant effect of origin in the ANOVA (Table 5). There were also significant differences within NOR populations of which CH was the lowest performer (Fig. 4). There was a clear overall negative correlation between Zn content and biomass production. This relation was verified between populations, both within the NOR (r = −0.40; P < 0.001; n = 84) and MCS (r = −0.30; P < 0.005; n = 85) origins.

There was a significant difference between origins in Cd content (Table 5, Fig. 4a) but, in contrast with what was observed for Zn, the MCS populations showed a higher Cd content (average range 950–1300 µg g⁻¹) than NOR populations (average range 500–700 µg g⁻¹). Significant differences were also found between populations but restricted to MCS, with MA and MG exceeding PO.

Biomass was significantly negatively correlated with Cd content in MCS (r = −0.542; P < 0.001; n = 85) but no such correlation was found in NOR populations.

Uptake of Zn and Cd were positively correlated in both MCS (r = 0.63; P < 0.005; n = 84) and NOR
Table 5. Analysis of variance of Zn and Cd concentrations in aerial parts of Thlaspi caerulescens from metalliferous and nonmetalliferous populations cultivated in treatment 5 (80% contaminated soil + 20% compost)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Zn</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MS</td>
<td>F</td>
</tr>
<tr>
<td>Block</td>
<td>2</td>
<td>1.05(1)</td>
<td>1.95 ns</td>
</tr>
<tr>
<td>Ori</td>
<td>1</td>
<td>174.52(2)</td>
<td>192.20***</td>
</tr>
<tr>
<td>Pop [Ori]</td>
<td>4</td>
<td>4.64(2)</td>
<td>5.11**</td>
</tr>
<tr>
<td>Fam [Pop [Ori]]</td>
<td>52</td>
<td>0.92 (3)</td>
<td>1.71**</td>
</tr>
<tr>
<td>Residual</td>
<td>147</td>
<td>0.54</td>
<td></td>
</tr>
</tbody>
</table>

Tr, treatment; Ori, origin; Pop, population; Fam, family.

(1) Residual is the error term.
(2) Fam [Pop [Ori]] is the error term.
*, P < 0.05; **, P < 0.01; ***, P < 0.001; ns, not significant.

Table 6. Analysis of variance of Zn and Cd aerial shoot uptake of Thlaspi caerulescens from metalliferous and nonmetalliferous populations cultivated in treatment 5 (80% contaminated soil + 20% compost)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Zn</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MS</td>
<td>F</td>
</tr>
<tr>
<td>Block</td>
<td>2</td>
<td>3.47(1)</td>
<td>7.19***</td>
</tr>
<tr>
<td>Ori</td>
<td>1</td>
<td>50.52(2)</td>
<td>80.50***</td>
</tr>
<tr>
<td>Pop [Ori]</td>
<td>4</td>
<td>1.41(2)</td>
<td>2.25 ns</td>
</tr>
<tr>
<td>Fam [Pop [Ori]]</td>
<td>52</td>
<td>0.63(1)</td>
<td>1.31 ns</td>
</tr>
<tr>
<td>Residual</td>
<td>147</td>
<td>0.48</td>
<td></td>
</tr>
</tbody>
</table>

Ori, origin; Pop, population; Fam, family.

(1) Residual is the error term.
(2) Fam [Pop [Ori]] is the error term.
*, P < 0.05; **, P < 0.01; ***, P < 0.001; ns, not significant.

Shoot uptake of zinc and cadmium

The shoot uptake per individual was calculated by multiplying the aboveground biomass by the concentration of Zn or Cd.

Shoot uptake of Zn was twice as high in NOR (average for origin: 19000 µg per plant) as in MCS populations and this was highly significant (Table 6, Fig. 4b). Significant differences were also detected within NOR populations (Fig. 4b).

For Cd shoot uptake the results were the reverse; MCS populations accumulated significantly more (2–3 times) than NOR populations. This result was expected, as MCS grew better and accumulated more Cd on contaminated soils. Significant differences were found between the MCS populations, MA being the best accumulator. There were no significant differences among families within populations (Table 6).

For treatment 4 (1:1 per weight mixture of contaminated soil and compost soil), Zn and Cd contents were similar to those obtained in treatment 5 (data not shown). However, the shoot uptake of Zn and Cd (Fig. 5), was higher in treatment 4 than in the more severe treatment 5 (t-test of comparison of
means, with the two origins and populations pooled, was significant $t = 3.53; P < 0.01$. This result was due to higher biomass production in treatment 4 (Fig. 2). The differences between the two population origins were not as clear as in treatment 5, although shoot uptake of Cd by MA, a MCS population, remained higher than that of the other 5 populations as for treatment 5 (Fig. 5b).

**DISCUSSION**

**Growth and tolerance to metal-contaminated soils**

Data on biomass production during one year's growth on toxic substrata (treatments 2, 3, 4 and 5) indicate a clear ecotypical differentiation between metalliferous and nonmetalliferous populations, the latter attaining only 60% of the growth of the former. The difference was enhanced for the tolerance to the toxic soils as a result of the combined effect of higher growth in toxic treatments and lower growth on the compost soil of the metalliferous populations. On the other hand, the higher performance of nonmetalliferous populations than metalliferous populations in normal soils suggests that the latter have a 'need' for heavy metals. This effect is in agreement with Mathys (1977), who found that Zn had a stimulating effect on growth only in metalliferous populations. This response which might be related to the greater ability of metalliferous individuals relative to that of nonmetalliferous plants to reduce the uptake of the metal (Ernst *et al.*, 1990) and to the mechanisms of sequestration in shoot tissues (Lasat *et al.*, 1996), resulting in a deficiency in availability of Zn for metabolic functions in soils with normal concentrations of Zn (Shen *et al.*, 1997).

Another point of interest is a significant component of variance at family level, suggesting that genetic variation for biomass production is available for breeding purposes. Although the heavy-metal tolerance of metalliferous populations was higher, it appears that nonmetalliferous populations were still capable of growing on toxic soils, demonstrating a constitutive background tolerance, as shown by Baker *et al.* (1994) for several species of *Thlaspi*. This would suggest that metalliferous and nonmetalliferous populations share a common constitutive heavy-metal tolerance, as in metalliferous populations of *Armeria maritima* (Kohl, 1997).

**Zinc and cadmium content**

One of the major findings of our study is the difference between Zn and Cd concentrations in the aerial parts of metalliferous and nonmetalliferous populations. The nonmetalliferous populations accumulated 2–3 times more Zn in aboveground parts than metalliferous populations, whereas the opposite was found for Cd, although the divergence was more limited. The effect of ecological origin, associated with population effect within origin, as already shown by Pollard & Baker (1996), underscores the fact that to optimize the phytoremediation with *T. caerulescens*, it is necessary to work with diverse populations, both metalliferous and nonmetalliferous. Moreover, the significant family effect within populations might allow selection for enhanced Zn and Cd uptake.

Overall, no results similar to ours have previously been documented, although Meerts & Van Isacker (1997) showed a subspecies and family differences in Zn content in material from Belgium (Zn-contaminated soils) and Luxemburg (normal soils). One striking difference between these results and those of the present study is that the nonmetalliferous Luxembourg populations showed a higher mortality than metalliferous Belgian populations in the toxic treatments, whereas in our case there were no significant differences between the two origins and the mortality in toxic treatments was very low (7% for the four toxic treatments).

That nonmetalliferous *T. caerulescens* accumulates more Zn than metalliferous plants has not been found in other species of *Thlaspi* (Reeves & Baker, 1984; Boyd & Martens, 1998), indicating that this peculiarity might be restricted to *T. caerulescens*. Nevertheless, it must be mentioned that in some species of other genera (Baker, 1981; Baker & Walker, 1990; Ernst *et al.*, 1992; Chardonens *et al.*, 1998) more metal is accumulated in aerial parts in ecotypes growing in normal soils. Therefore, that the the behaviour of *T. caerulescens* in relation to Zn is not entirely surprising’. However the opposing patterns of uptake of Zn and Cd between the two origins is somewhat unusual.

Our data for Zn accumulation, reaching 10000 μg g⁻¹ in metalliferous populations, are consistent with those from experiments on contaminated soil by Knight *et al.* (1997), Meerts & Van Isacker (1997), McGrath *et al.* (1997) and Robinson *et al.* (1998). Nevertheless, concentrations of almost 30000 μg g⁻¹ have also been found in natural populations in different sites in northwestern Europe (Baker & Brooks, 1989). In our experiments, the Zn concentrations of 15 individual plants from nonmetalliferous populations were >40000 μg g⁻¹, and those of three individuals were >50000 μg g⁻¹.

In terms of the hyperaccumulation threshold defined by Baker *et al.* (1994), our metalliferous populations were low-level Zn hyperaccumulators (<10000 μg g⁻¹), whereas the nonmetalliferous populations, mainly IN and SM, correspond fairly well to high-level Zn hyperaccumulators (>30000 μg g⁻¹). Thus, metalliferous populations tolerant to Zn behave as excluders (Baker & Walker, 1990) compared with nonmetalliferous populations. Meerts & Van Isacker (1997) reached the same conclusion. Since, according to Baker *et al.* (1994),
100 µg g⁻¹ is the lower limit, the six populations are efficient hyperaccumulators, with average concentrations of Cd of 500−1100 µg g⁻¹. In SM and MA, aerial Cd content was almost eight times higher than that of the Cd available in the soil.

Zinc and cadmium uptake per individual

The ‘agronomical’ value for phytoextraction is shown by the shoot uptake of Zn and Cd by an individual plant. On the whole, uptake of Cd by individuals of metalliferous populations was approximately twice, but of Zn only half, that of nonmetalliferous populations. An important point of this study is that no differentiation occurred between families within populations, leaving the only significant variations for origin (Zn and Cd) and populations (Cd). This is the result of the negative correlation between growth and heavy metal concentration, a common trend encountered by plant breeders which can be overcome by multiple crossing trials.

Another important point concerns the comparisons of shoot uptake between treatments 4 and 5. Plants in the NOR populations had a higher Zn shoot uptake in treatment 4 than in treatment 5, despite higher Zn availability in the latter treatment (+4500 µg g⁻¹). This result shows that phyto-remediation by T. caerulescens can be improved by improving growing conditions (i.e. fertilization), because plants from treatment 4 had higher biomass than those from treatment 5.

To conclude, T. caerulescens populations are able to accumulate such large amounts of Zn and Cd that they are interesting organisms for phytoextraction in the Mediterranean region. Nevertheless there is much variation in potential for accumulation and phytoextraction potential, both between and within populations, whether the plants come from metalliferous or nonmetalliferous sites. An ideal phytoextractor should be able to combine the extraction potentiality scattered among the two groups of populations (i.e. the nonmetalliferous populations for Zn extraction and the metalliferous for Cd extraction). This can be achieved by a cross-breeding programme between the populations of the two origins.

REFERENCES


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