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ScienceDirect

Science of the Total Environment 372 (2007) 372 – 381

Science of the Total Environment

An International Journal for Scientific Research into the Environment and its Relationship with Humankind

www.elsevier.com/locate/scitotenv

Zinc and cadmium accumulation among and within populations of the pseudometalophytic species *Arrhenatherum elatius*: Implications for phytoextraction

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Received 2 June 2006; received in revised form 20 September 2006; accepted 20 September 2006
Available online 8 December 2006

Abstract

The purpose of this study was to investigate, under standard conditions, the bioaccumulation of zinc and cadmium in *Arrhenatherum elatius*, a perennial grass with a high biomass production. Nine populations of three different origins were tested: three metallicolous populations (mpop); three non-metallicolous populations (nmpop) and three populations developing on soils moderately metal polluted (medpop). We have found that bioaccumulation differs among these populations, with nmpop accumulating significantly more zinc ($p<0.0001$) and cadmium ($p<0.0001$) than mpop. Indeed, we have observed a concentration of 325 mg kg^{-1} of zinc and 52 mg kg^{-1} of cadmium in *A. elatius* shoots from mpop, whereas in nmpop, the concentration reached on average 524 mg kg^{-1} zinc and 83 mg kg^{-1} cadmium. In the same way, medpop accumulated as much zinc but more cadmium than nmpop. Moreover, the standard deviation of medpop was larger than the one for mpop and nmpop. Indeed, some *A. elatius* samples from medpop presented a high metal content whereas, others presented low concentrations in their shoots (ranging from 60 to 210 mg kg^{-1} cadmium). Hence, these medpop exhibited a large variability among and within populations in accumulating zinc and cadmium in their shoots. Based on these results, the possibility of selecting *A. elatius* plants with the best accumulating capacity from medpop was proposed. We concluded that if the accumulation capacity is genetically controlled in *A. elatius*, this species fulfils this necessary condition for efficiently increasing species bioaccumulation by crossbreeding *A. elatius* plants with the higher accumulation capacity.

Keywords: *Arrhenatherum elatius*; Bioaccumulation; Cadmium; Metallicolous; Phytoextraction; Zinc

1. Introduction

Phytoextraction technology, defined as the use of plants to remove toxic metals from soils into the

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0048-9697/$ - see front matter
doi:10.1016/j.scitotenv.2006.09.026

harvestable of their portion biomass (Cunningham et al., 1995), has become firmly established in the literature. Heavy metal uptake and accumulation by higher plants depends on: (1) the nature and the speciation of the metals; (2) soil factors, and; (3) the characteristics of the plant itself (see Bargagli, 1998; Brooks, 1998). Thus, in addition to optimizing agronomic practice, one of the key steps in phytoextraction remains the selection of plant species.

Until recently, phytoextraction has relied on the use of plants known as hyperaccumulators, defined as plants which accumulate about 10 times more metal than non-accumulator plants growing in the same environment (Chaney, 1983; Brooks, 1998). However, hyperaccumulators are species which often have a low biomass and a slow growth rate, leading to a slow time frame for metal uptake and soil decontamination. These plants are also limited to the range of metals that can be hyperaccumulated (Brooks, 1998; Anderson et al., 2001). Moreover, there are many metals for which few hyperaccumulators exist, for example lead, which remains a problematic contaminant in soils from industrial areas.

As a consequence, there has been considerable focus on the potential for using non-hyperaccumulator species with a higher biomass or growth rate. The goal with these species is to optimize metal bioaccumulation potential. Aside from genetic modifications, increasing species bioaccumulation performance is mainly achieved using artificial selection by crossbreeding, assuming that the species represents a significant bioaccumulation improvement and that this characteristic is genetically controlled.

Primary studies concerning the variability of bioaccumulation in non-hyperaccumulator species were based on agricultural or market plants for human consumption (Vangronsveld, 1998a,b; Hough et al., 2004). Their aims were to select cultivars to minimize the introduction of heavy metals into food chains and so limit the consequences of soil pollution on human health. Ouzounidou et al. (1997) observed, for instance, that the variability between wheat cultivars for cadmium accumulation in leaves could reach 250%. In the phytoextraction context, studies are carried out with the opposite purpose and species are selected for their ability to accumulate and extract metals from polluted soil. Huang and Cunningham (1996) showed that *Brassica juncea* (variety 211000) and *B. juncea* (variety 531268) extract about twice the amount of lead than *B. juncea* (L.) Czern, with EDTA added. Ligneous species have also been studied. Seventy genotypes of *Salix viminalis* were tested for their capacity to accumulate cadmium (Greger, 1999). Results showed that the best genotype of *Salix* was able to extract 216 g ha^{-1} $year^{-1}$ of cadmium, compared with 35 g ha^{-1} $year^{-1}$ extracted by the hyperaccumulator *Thlaspi caerulescens* and with 43 g ha^{-1} $year^{-1}$ extracted by *Alyssum murale*, both growing in similar conditions.

Given the fact that variations in accumulation capacity are genetically controlled, most published studies have so far dealt with hyperaccumulator species. The genetic variations between populations, in their ability to accumulate zinc and cadmium, have been investigated. Those studies compared zinc hyperaccumulation capacity between metallicolous and non-metallicolous populations in the species *Armeria maritima* (Kohl, 1997); zinc and cadmium accumulation in *Arabidopsis halleri* (Bert et al., 2002); zinc, lead and cadmium accumulation in *T. caerulescens* populations (Baker et al., 1994; Meerts and Van Isacker, 1997; Escarré et al., 2000; Frérot et al., 2003; Assunçao et al., 2003) and zinc and nickel accumulation in *T. caerulescens* and *Thlaspi pindicum* (Taylor and Macnair, 2006). Considering non-hyperaccumulator species, studies are very few. They mainly concern dicotyledenous species such as *Silene vulgaris*, considering cadmium-tolerant and cadmium-sensitive ecotypes (Ernst et al., 1992; Chardonnens et al., 1998). For monocotyledons, comparisons between populations from polluted and non-polluted origins do not directly focus on accumulation capacity but on tolerance, as studied by Coughtrey and Martin (1979) in *Holcus lanatus* for zinc, lead and cadmium or by Karataglis (1980) in *Anthoxanthum odoratum* for zinc.

In this study, *Arrhenatherum elatius*, a perennial grass with a high biomass, was selected as a study species for its ability to tolerate high concentrations of heavy metals in its shoots. In Northern France, this species grows equally well on unpolluted sites (fallow land, meadow, colliery slag heaps) and on polluted sites such as metalliferous areas near smelters (Petit, 1980; Ducousso et al., 1990; Deram et al., 2000). This ability defines a pseudometallophyte species (Lambinon and Auquier, 1963) and prompted us to explore the potential for *A. elatius* to be exploited for its ability to remove heavy metals from the soil. Through experimentation, it has been shown that *A. elatius* could be effective for phytoremediation operations when growing either on cobalt/copper/nickel ore or on base-metal tailings rich in lead (Deram et al., 2000). *A. elatius* could also be exploited to remove cadmium in the vicinity of smelters in Northern France (Deram et al., 2006). In this area, the cadmium concentration in soils can exceed by a factor of more than 300 the regional background average of 0.40 mg kg^{-1} (Sterckeman et al., 2002).

In the context of using non-hyperaccumulator species in phytoextraction, it is essential to improve our knowledge regarding the extent and partitioning of variation in accumulation of heavy metals by natural populations (Pollard et al., 2002). Thus, we aimed to investigate differences between and within populations of *A. elatius* for zinc and cadmium accumulation in order to evaluate the potential for increasing *A. elatius* bioaccumulation capacity. Metallicolous and non-metallicolous populations were used. In addition, populations from moderately contaminated soils were studied. This third origin was investigated in order to describe better the genetic variations of metal bioaccumulation parameters.

Thus, the purposes of this work were: (1) to study the metal bioaccumulation capacity of non-metallicolous populations of *A. elatius*, grown on soil highly contaminated with zinc and cadmium; (2) to compare bioaccumulation levels in *A. elatius* originating from metallicolous, non-metallicolous and medium populations, and; (3) to discuss the genetic origin of the potential bioaccumulation variability. This approach enabled us to evaluate the possibility of artificial selection in an attempt to increase the bioaccumulation capacity of *A. elatius* for zinc and cadmium.

2. Materials and methods

2.1. Selected populations

The study was carried out in northern France. Nine populations of *A. elatius* were tested for their metal bioaccumulation capacity in leaves. Three metallicolous populations (mpop) were selected on account of the high levels of metal pollution (Zn, Pb, Cd) in the soils they were growing on. These three sites were all old slag heaps where residues resulting from the processing of lead and zinc ores were dumped.

The first one, the Auby site, (mpopA) is located in the immediate vicinity of the Umicore plant which has been processing zinc ore since the end of the 19th century. In the 1970s, the industrial process of Umicore changed. No atmospheric emissions in this zone have occurred since then. The study site used to be an area where process waste was dumped but it has since been transformed into fallow land. The second site, Mortagne-du-Nord (mpopB) is situated near a zinc smelter. The activity of this factory ceased in 1963. This site, a calamine grassland, is situated several hundred meters from the plant, which has been destroyed. The third site (mpopC) is located in Courcelles-les-Lens, near the Pb–Zn smelter of Metaleurop Nord, which was in production from 1870 until early 2003. On this site, there is still an enormous slag heap influencing atmospheric emissions, whereas slag deposits have been taken away on the two other sites. Within a radius of 4–5 km around the Umicore and Metaleurop Nord sites, the metal contents of the soil plow layer, suitable for phytoextraction can reach 350, 8000 and 40,000 mg kg^{-1} of soil dry matter, of cadmium, lead and zinc respectively (Petit, 1980; Deram, 2003). These three sites are colonized by a particular type of vegetation known as "trace element grassland" which consists of obligate metallophytes such as *A. maritima* subsp. *halleri* (Wallr.) Rothm (present at Auby and Mortagne) and *A. halleri* (L.) O'Kane and Al-Shehbaz (present at all three sites).

Three non-metallicolous populations (nmpop) were selected on account of the metal contents of their soils being close to the regional level established by Sterckeman et al. (2002). The population of Sainghin-en-Mélantois (nmpopA) was from fallow land and the population of Rouvroy (nmpopB) from fallow land adjacent to a colliery heap. The population nmpopC was sampled from the Université des Sciences et Technologies de Lille (USTL) campus. These three sites were colonized predominantly by *A. elatius*.

In addition, three populations growing on moderately polluted soils (medpop) were also selected: Auby (medpopA), Evin Malmaison (medpopB) and Leforest (medpopC). Both nmpop and medpop grow on agricultural soils and present monospecific populations of *A. elatius*. Fig. 1 shows the locations of the populations studied. Table 1 summarizes the main physico-chemical characteristics of the topsoil (0–30 cm) at the studied sites.

2.2. Experiment design

At each location and for each population, 30 plants were randomly selected, isolated and marked. All the seeds from every plant were collected and considered as a family. Among each family, three seeds were randomly selected and sown in artificially polluted growth media (500 g/pot; diameter: 12.5 cm), the composition of which was as follows: compost (80% blond peat, 20% perlite 3–6 mm, NPK 14–16–18, pH (water) 5.3–5.8, salinity 0.5 g L^{-1}, with negligible concentrations of heavy metals) was polluted with 1000 mg kg^{-1} of zinc and 300 mg kg^{-1} of cadmium. Both zinc and cadmium were added as nitrate salts to the total amount of compost and mixed with a cement mixer. Samples of the growth medium were then analyzed to verify zinc and cadmium concentrations and contamination homogeneity. Zinc and cadmium concentrations were based upon preliminary studies dealing with the tolerance of *A. elatius* (Languereau-Leman, 1999), and interactions of zinc and cadmium in compost influencing bioaccumulation (Deram, 2003). More

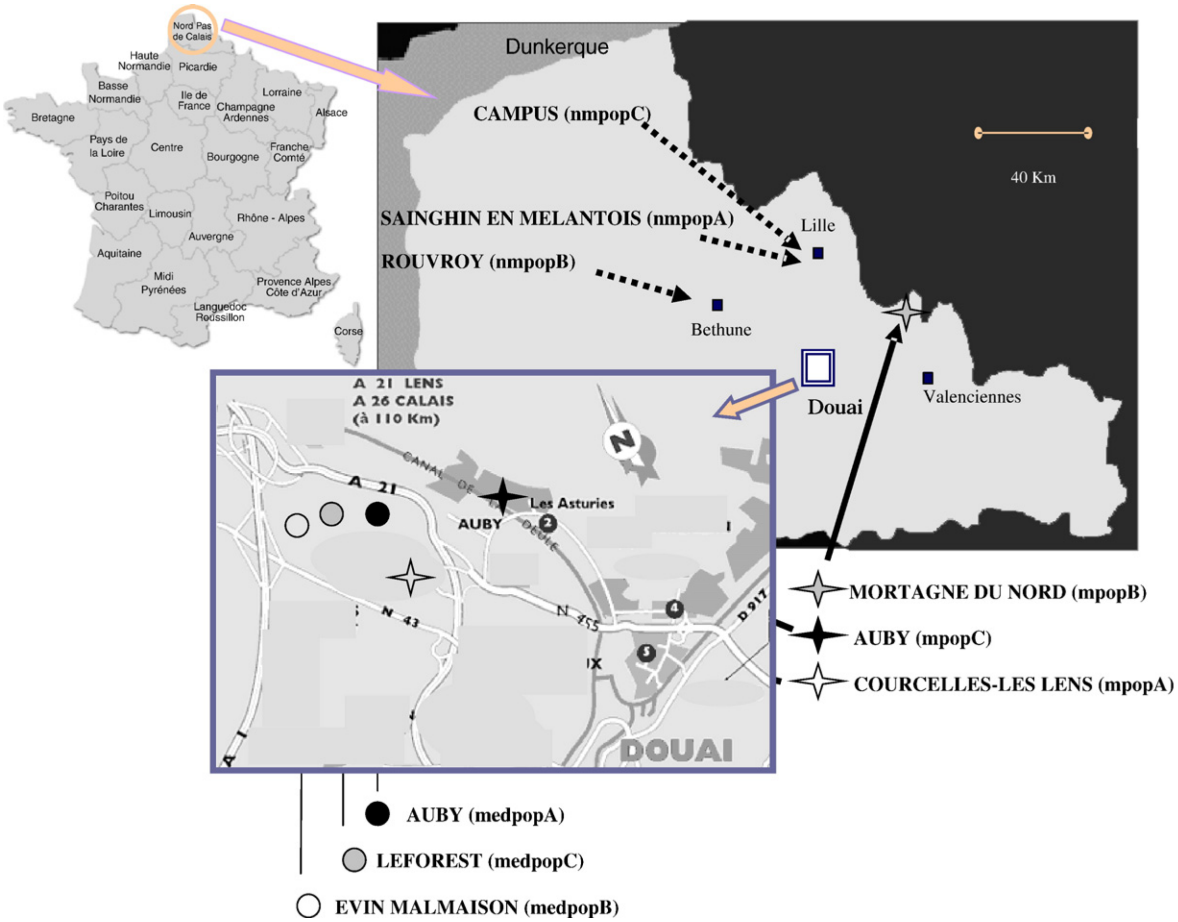

Fig. 1. Location of the nine populations from which 30 *Arrhenatherum elatius* plants were collected (30 × 9 populations).

precisely, zinc and cadmium interactions were studied considering the same compost, pot preparation and greenhouse conditions. Sixteen composts, artificially polluted, with both zinc and cadmium nitrates were prepared, combining the following concentrations of zinc: 0; 300; 1000 and 3000 mg kg^{-1} with the following concentrations of cadmium 0; 2; 20 and 200 mg kg^{-1}. Three pots of each treatment were prepared and three seeds were sown in each pot. Twelve weeks after sowing, the three seedlings were harvested and considered as one

Table 1
Chemical and physical parameters of topsoils (0–30 cm) at the nine study sites

	mpopA	mpopB	mpopC	medpopA	medpopB	medpopC	nmpopA	nmpopB	nmpopC
pH	6.8	6.2	8.2	7.9	7.1	8.2	7.8	7.8	7.8
O.M.	460.8	104.3	59	82.6	32.6	54.5	68.5	105.1	71
CEC	39.3	18.1	nd	14.3	13.4	16.4	14.4	15	14.3
Exchangable Ca	3.43	1.76	nd	4	3	18	4.24	6.6	4.46
Exchangable Mg	0.233	0.151	nd	0.196	0.130	0.126	0.098	0.150	0.103
P_2O_2	0.023	0.066	nd	0.133	0.128	0.158	0.191	0.120	0.126
Cd tot.	278.5	50.6	33.2	28.5	25.4	32.9	5.4	4.3	5.7
Zn tot.	31,230	6,438	5,127	3,630	2,700	3,860	122	189	117
$Cd_{Ac\text{-}EDTA}$.	138.5	32.6	23.7	15.4	14.5	15.7	2.5	1.7	2.7
$Zn_{Ac\text{-}EDTA}$	8,316	2,815	1,006	1,740	1,240	1,910	46.6	58.9	35.9

OM: organic matter (g kg^{-1}); CEC: cation exchange capacity ($cmol^{+}$ kg^{-1}); Zn tot. and Cd tot.: total zinc and cadmium concentrations in soils (mg kg^{-1}); $Zn_{Ac\text{-}EDTA}$ and $Cd_{Ac\text{-}EDTA}$: zinc and cadmium concentrations in soils (mg kg^{-1}) extracted with NH_4OAc-EDTA 1 M; nd: missing values.

Table 2
Zinc and cadmium concentrations in *Arrhenatherum elatius* shoots versus populations and origins

	Zinc, mg kg^{-1}	Cadmium, mg kg^{-1}
mpopA	332.1±70.7	46.1±15.3
mpopB	309.4±89.3	61.6±27
mpopC	333.2±72	45.3±12.4
mpop	**324.6±77.7**	**51.6±21.1**
medpopA	461.2±81.7	119.7±29
medpopB	411±199.1	106.4±42.7
medpopC	485.8±265.6	147.3±40.2
medpop	**481.7±353**	**123±40.6**
nmpopA	568.7±119	75.1±35.8
nmpopB	492.6±104.7	90.1±54.3
nmpopC	444.5±142.9	83.9±114.7
nmpop	**524.4±275**	**82.9±75.3**

(Geometric means±standard deviation).

sample. The samples were analyzed as described in Section 2.3. No significant interaction between zinc and cadmium was observed. In other words, zinc concentrations in shoots were not significantly different whatever the concentration of cadmium in soil, and vice versa. Data analyses and previous experiments have provided evidence that this might be due, at least in part, to experimental conditions, and in particular to the high organic matter contents of the composts. Consequently, we are able in the present study to simultaneously add zinc and cadmium to the compost.

These soils were prepared 1 week before sowing. All pot trials were carried out in a greenhouse at USTL. Temperatures were maintained in the range 18–24 °C and an 8–16 h (dark–light) photoperiod was used. There was no humidity control. Pot trials were regularly watered with tap water and randomized twice a week. Each pot was in an individual dish limiting leaching and avoiding contamination of other pots.

2.3. Shoot samples collection and analysis

Six weeks after sowing, the three seedlings of a family were harvested. Their aerial parts were considered as one sample (*n*=30, for each population). Samples were not washed since they were not exposed to atmospheric deposition.

In order to determine metal concentrations in their aerial parts, *A. elatius* shoot samples were acid digested and tested for their zinc and cadmium concentrations at the Laboratoire de Génétique et Evolutions des Populations Végétales (GEPV) on the USTL campus. Shoots were dried in an oven at 40 °C to constant weight. The dried shoot samples were weighed and digested by a hot mixture of $H_2SO_4/HNO_3/H_2O_2$ in a ratio of 1:3:3 (v/v/v) at 130 °C for 2 h (Hoenig's methods, 1981). Samples were stored in polyethylene bottles for one night (4 °C). Concentrations of zinc and cadmium were determined by flame (air/acetylene) atomic absorption spectrometry (Varian Spectr. AA-10), following the main steps of QA/QC Requirements and Performance Standards for SW-846 Method 7000 Series, Atomic Absorption Methods for metals (Muldoon, 2004).

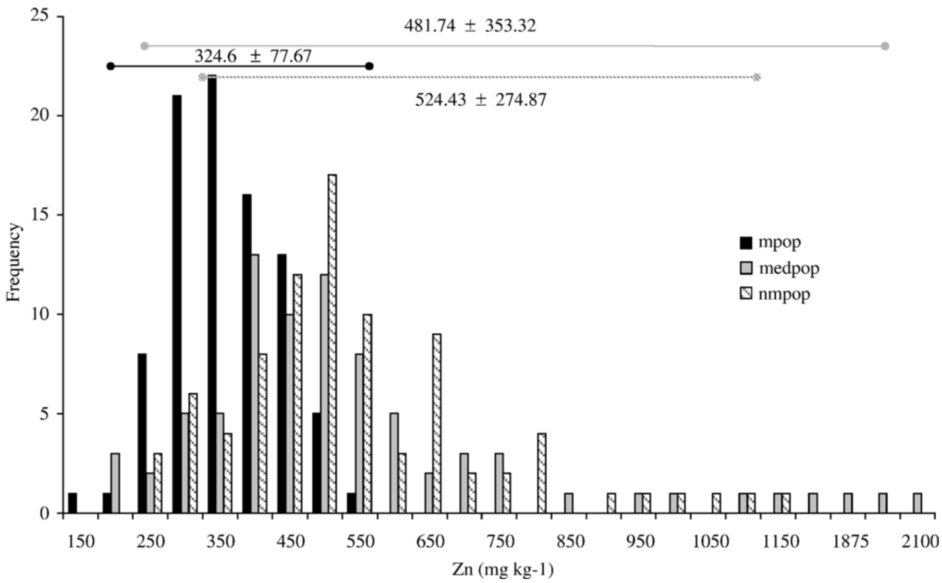

Fig. 2. Distribution of zinc concentrations in *Arrhenatherum elatius* shoots versus origins. Numbers near the horizontal lines are the geometric means± standard deviation (mg kg^{-1} d.m.; *n*=90 per origin).

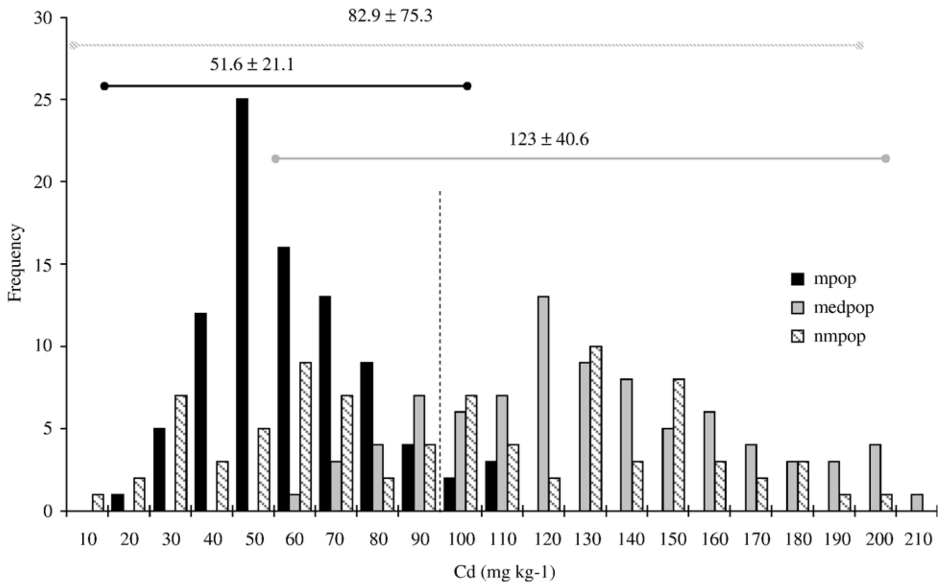

Fig. 3. Distribution of cadmium concentrations in *Arrhenatherum elatius* shoots versus origins. Numbers near the horizontal lines are the geometric means ± standard deviation (mg kg^{-1} d.m.; $n = 90$ per origin).

2.4. Statistical analysis

General Linear Model analyses of variance (ANOVAs) were conducted on the dataset to compare accumulation of zinc and cadmium in shoots between and within *A. elatius* populations. For each ANOVA, the normality of the distribution (Kolmogorov-Smirnov) and the homogeneity of variance (Bartlett's test) were tested. ANOVAs, Student's tests and all statistical analysis were conducted with *Statistica* software (version 5.1, 1997).

3. Results

Seedlings of *A. elatius* grew well. No phytotoxicity signs, such as chlorosis, necrosis or low biomass, were observed, compared with seedlings growing on compost without heavy metal, and in similar conditions. Zinc and cadmium concentrations in shoots of *A. elatius* are summarized in Table 2.

3.1. Zinc bioaccumulation

Zinc accumulation in shoots was statistically different depending on the origins of the populations (mpop/medpop: $p < 0.0001$; mpop/nmpop: $p < 0.0001$; medpop/nmpop: n.s.). Nmpop and medpop accumulated 524 ± 275 mg kg^{-1} and 482 ± 353 mg kg^{-1}, respectively, whereas mpop accumulated significantly less (324 ± 78 mg kg^{-1}). The samples with the highest accumulating capacity belonged to medpop, which was not the best bioaccumulating origin population on average. Those samples contributed to a wider distribution, slightly asymmetric, and skewed in favor of high accumulating seedlings (< 1000 mg kg^{-1}). In contrast, the standard deviation of mpop was smaller compared to nmpop and medpop (Fig. 2).

The mpop and the nmpop did not show significant intra-origins differences in their zinc bioaccumulation ($p < 0.608$ and $p < 0.336$, respectively). On the other hand, all medpop populations did not accumulate zinc to the same extent ($p < 0.002$), medpopB accumulating significantly less zinc than medpopC (Student, $p < 0.038$). Considering the nine populations, medpopC did not show the best accumulating capacity of zinc on average but seedlings of this population constituted the highest range of cadmium accumulation, ranging from 200 to 2000 mg kg^{-1}.

3.2. Cadmium bioaccumulation

Depending on their origins, populations accumulated cadmium differently ($p < 0.0001$) with medpop > nmpop > mpop. Nmpop and medpop accumulated 83 ± 75 mg kg^{-1} and 123 ± 41 mg kg^{-1}, respectively. Medpop accumulated about twice the amount of cadmium as mpop.

As for zinc, mpop standard deviation was restricted compared with medpop and nmpop populations. There were no significant intra-origins differences among nmpop populations ($p < 0.197$). High variability among seedling accumulation capacities characterized these populations. No dominating mode stands out (Fig. 3). However, there were significant intra-origins differences among medpop populations ($p<0.001$). MedpopC was statistically the population with the highest cadmium accumulation capacity among the nine tested populations ($p<0.0001$).

4. Discussion

4.1. Bioaccumulation capacity of A. elatius

Considering all the samples, the zinc accumulation was very low in shoots, ranging from 150 to 2100 mg kg^{-1} whereas the typical mean zinc concentrations in hyperaccumulator species is >10,000 mg kg^{-1} (Brooks, 1998). The fact that *A. elatius* could not be considered as a good zinc accumulator is in agreement with the results obtained under natural conditions. Schwartz et al. (2001) analyzed aerial parts of *A. elatius* growing on a soil heavily polluted with zinc at Mortagne-du-Nord (2290 mg kg^{-1} zinc, in litter). They reported an average bioaccumulation of close to 498 mg kg^{-1}. In the same manner, Deram et al. (2006) observed at Courcelles-les Lens that NH_4OAc-EDTA extractable zinc in soil was 1006 ± 790 mg kg^{-1}, and bioaccumulation was about 1171 ± 897 mg kg^{-1}in shoots of *A. elatius* (Geometric mean; $n=24$).

In contrast, *A. elatius* originating from nmpop bioaccumulated relatively large amounts of cadmium. In our experimental conditions, cadmium concentrations in shoots were close to 100 mg kg^{-1} for this metal. In natural conditions, *A. elatius* individuals from Courcelles-les-Lens were able to accumulate up to 102 mg kg^{-1} cadmium in shoots (Deram et al., 2006). Such high concentrations may allow us to consider this plant as a cadmium hyperaccumulator according to Brooks (1998) (here defined as >100 mg kg^{-1} in dry plant material). Until now, only three plant species have been describe as cadmium hyperaccumulators, namely the Brassicaceous *T. caerulescens* (Brooks, 1998), *Thlaspi praecox* (Vogel-Mikus et al., 2005) and *A. halleri* (Bert et al., 2000). These species are able to accumulate large amount of cadmium in their shoots even if the concentration in soil is low. Moreover, their shoots/roots ratios are always greater than one. *A. elatius* did not behave like these cadmium hyperaccumulators species. Regarding mpop, and as an example, the shoot/root ratio of *A. elatius* never exceeded 0.886 under natural conditions (Deram et al., 2006). Thus, based only on the result of the present study, *A. elatius* does not appear to be a cadmium hyperaccumulator. This is in accordance with the results from other studies demonstrating that monocotyledonous plants take up less cadmium than dicotyledonous plants (Matthews and Thornton, 1982). However, as a result, such concentrations in shoots, observed in natural conditions and in the present experiment, make it possible to consider *A. elatius* as one of the best graminaceous cadmium accumulators compared to data in the literature (Ebbs and Kochian, 1997; Brekken and Steinnes, 2004).

4.2. Comparison of bioaccumulation among origins

In our experimental conditions, populations from different origins did not accumulate metals to the same extent in their shoots: mpop accumulated significantly less zinc and cadmium than nmpop. 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 *T. caerulescens* (Meerts and Van Isacker, 1997). Concerning these well-studied species, Escarré et al. (2000) showed that the nmpop accumulated twice to three times more zinc in aboveground parts than mpop. Non-hyperaccumulator species, such as *H. lanatus* (Coughtrey and Martin, 1979) or *S. vulgaris* (Chardonnens et al., 1998) were also studied and showed that more metals are accumulated in aerial parts in ecotypes growing in uncontaminated soils.

Two non-exclusive hypotheses could be put forward to explain such observations: (1) the nmpop 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 (Hall, 2002 for review; Hall and Williams, 2003), or; (2) mpop 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 (Baker and Walker, 1990; Ernst et al., 1992; Dahmani-Muller et al., 2000; Brekken and Steinnes, 2004). Regarding *A. elatius*, it has been experimentally demonstrated that this species, originating from contaminated soils, restricted and even stopped accumulation in roots and translocation in shoots during biomass growth (Deram et al., 2006). So, *A. elatius*, like other species, 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).

To conclude on this point, mpop plants appear to restrict translocation of metal in order to better tolerate soil extremely contaminated with zinc and cadmium, corroborating Ernst's observations. Indeed, this author suggested that, as soon as the contamination exceeds the phenotypic flexibility of plants, survival on contaminated soils is only possible if tolerant individuals are selected from the non-tolerant populations (Ernst, 1999). As a consequence, selection leads to a decrease of the metal bioaccumulation capacity and its variability within tolerant populations. Considering the accumulation capacity of medpop in this study, *A. elatius* seems to support this hypothesis since pronounced variability and a high accumulation capacity were observed in plants from this origin.

4.3. Selection to increase bioaccumulation?

Considering within population differences in this study (i.e. within populations of the same origin), we found no evidence of a significant difference between mpopA, mpopB and mpopC. This result is different from observations in *T. pindicum* (Taylor and Macnair, 2006) and *T. caerulescens* (Pollard and Baker, 1996; Meerts and Van Isacker, 1997; Escarré et al., 2000; Assunçao et al., 2003), where between-population variations in metal accumulation were observed. However, small amounts of genetic variation in metallicolous populations of these hyperaccumulator species have been shown.

By contrast, a larger variation of zinc and cadmium accumulation in shoots was noted in nmpop. In the same way, medpop accumulated large amounts of cadmium in their shoots and a pronounced variation in the shoot concentrations were observed between and within populations. Indeed, the medpopC population accumulated the largest amount of zinc and cadmium compared with the medpopA and medpopB populations.

This significant component of variance at family level suggests that genetic variation for metal bioaccumulation exists within medpoppop and is available for breeding purpose (Escarré et al., 2000), so we can assume that medpop are more suitable populations for selection.

Hence, these results highlight the potential of artificial selection intended to increase extraction capacity of *A. elatius*. This should be done at population level, taking into account the origins of populations or at plant level using intra-population variability. This could be achieved by a cross-breeding program between the populations originating from moderately polluted soils.

4.4. Application to phytoextraction

In this study, the best accumulator plants of cadmium were generally not the best zinc accumulators. Selection strategies must be designed for each individual metal. For zinc, even if selection of medpopC plants allows us to increase zinc bioaccumulation, it would not induce a sufficient improvement to consider this species for zinc phytoextraction.

On the other hand, variability between origins, populations and even plants is important for cadmium. Medpop had a higher average accumulation capacity than mpop. The best plants in medpop accumulated twice as much cadmium as the hyperaccumulation threshold. This result underlines the observation that artificial selection could be very efficient for cadmium phytoextraction. To this end, medpopA, medpopC, nmpopA and nmpopB are the most effective. As an example, selecting the medpopC, which accumulated on average 147 mg kg^{-1} cadmium, would decrease phytoextraction time by a factor of about three compared to the mpopC (45 mg kg^{-1} cadmium) under our experimental conditions.

Attempting to estimate the period needed to reduce cadmium contents of the nmpopB site down to the regional background level (0.4 mg kg^{-1}), the following assessment was obtained. If we consider a biomass of 10 t ha^{-1}, all the plants containing on average 147 mg kg^{-1} cadmium would provide about 1.47 kg of cadmium. Assuming that the soil contained 4.3 mg kg^{-1} cadmium to be removed to the depth of 30 cm, and assuming a soil density of 1.3, a hectare of soil would contain 7.8 kg of cadmium. Assuming that the plant-available fraction of cadmium remained constant at each growing season, six sequential crops of *A. elatius* should be sufficient to remove most of the cadmium from this soil, in theory.

5. Conclusion

Bioaccumulation results, obtained under the controlled conditions described in this study did not seem to overestimate those obtained under natural conditions. Both suggested that *A. elatius* is a good candidate for cadmium phytoextraction due to its biomass and its capacity to bioaccumulate cadmium. Moreover, our results seem to demonstrate that it is possible to increase the accumulation capacity of *A. elatius* by selecting populations originating from moderately contaminated soils. As a consequence, a future study of great interest would be to consider *A. elatius* for its capacity to accumulate cadmium under natural conditions, with phytoextraction as a goal. However, this process is not applicable in every situation and should be addressed to slightly contaminated sites.

Finally, non-metallicolous populations and populations developing on moderately polluted soils revealed several original characteristics suggesting that they may not have received sufficient attention up to now. Thus, the possibility that populations from low or moderately metal contaminated soils would actually contain greater reserves of genetic variation, and hence perhaps greater potential for bioaccumulation, is of great practical significance and deserves further field investigation.

Acknowledgements

We gratefully thank the Nord- Pas-de-Calais Regional Council and the French Government for their financial support for this research program which has also been subsidised by the European Regional Development Fund (ERDF). We express our sincere thanks to Mike Howsam, Jérôme Lanoy and Peter Denimal for significant revision of the English.

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