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Cadmium phytoextraction potential of different *Alyssum* species

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ABSTRACT

This work was planned for providing useful information about the possibility of using serpentine adapted plants for phytoextraction of cadmium, element scarcely represented in such metalliferous environment. To this aim, we investigated variation in cadmium tolerance, accumulation and translocation in three *Alyssum* plants with different phenotypes: *Alyssum bertolonii*, that is a serpentine endemic nickel hyperaccumulator, and two populations of *Alyssum montanum*, one adapted and one not adapted to serpentine soils.

Plants were hydroponically cultivated in presence of increasing concentrations of $CdSO_4$ for two weeks. For the metal concentration used in the experiments, the three different *Alyssum* populations showed variation in cadmium tolerance, accumulation and content. The serpentine adapted population of *A. montanum* showed statistically higher cadmium tolerance and accumulation than *A. bertolonii* and the population of *A. montanum* not adapted to serpentine soil thus deserving to be investigated for phytoextraction purposes. Furthermore, as for the kinetic parameters of the cadmium uptake system, *A. montanum* serpentine population presented a low apparent K_m value, suggesting a high affinity for this metal of its uptake system, whereas the V_{max} values were not significantly different among the plants.

Present data revealed metallicolous plants are also suitable for the phytoremediation of metals underrepresented in the environment of their initial origin. Nonetheless, field trials on real contaminated soils are essential.

1. Introduction

Phytoextraction, together with phytostabilization, phytovolatilization, phytodegradation, and other plant-based technologies, is a subset of phytoremediation used to remediate or stabilize contaminants in the environment [1]. In particular phytoextraction is the technology that uses plants to extract elements from polluted or mineralized soils, and accumulate them in harvestable organs and tissues in order to remove the pollutants/contaminants from the field [2]. Plants suitable for phytoremediation purposes have to show tolerance to trace element/s, fast growing rate on polluted soil, and metal accumulation on harvestable organs [3].

Trace element phytoextraction, as any other technology, has both advantages and limitations. The main advantages are: it has reasonable costs, due to plant's ability to work as a solar-driven pump, extracting and concentrating particular elements from the environment; there is the possibility of trace element recycling, as the ash of some hyperaccumulators can consist of significant quantities of trace elements and there is no need to pay for safe disposal; it works without disturbing further the site, which is of very great importance for its public acceptance [2]. The main limitations of this technology are: it can only be used for low to moderately contaminated soils; its applicability is limited to surface soils at rooting depth; it is limited to the plant available fraction of the trace elements and it is time-requiring [3].

Hyperaccumulators are effective in phytoextracting and/or phytomining metals or other pollutants from contaminated or mineralized soils [4,5]. For these reasons, metal hyperaccumulators can be used as solar-driven ion-pumps, capable of removing and concentrating metals from the substrate [6] thus extracting the metal from polluted soils through the so-called "green" technologies [7].

From the very beginning of the introduction of the trace element phytoextraction concept, the key question about the preferable use of trace element hyperaccumulator plants or of high biomass producing crop species, is still in debate. Chaney et al. [8] reported that trace element hyperaccumulation is a more important trait than dry biomass yield. In support to this statement, they theoretically calculated that zinc removal by hyperaccumulator and high biomass plants and drew that in any case the use of hyperaccumulators resulted in higher trace element extraction. However, the opposite view also exists [3].

Today, approximately 500 trace element accumulating taxa, belonging to at least 100 plant families, have been identified [9].

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Most of the hyperaccumulator plant species are able to accumulate just one trace element, but there are also multitrace element accumulators. For example, some populations of *T. caerulescens* are found to have not only high levels of zinc, but also of cadmium, cobalt, and some other trace elements [10], whereas others do not display this capability [11].

One of the aspects that can be improved to increase phytoextraction yield is certainly the selection of the most suitable plant species. In this context, it is important the selection of ecotypes with high biomass production and the optimization of their cultural condition. At the same time the possibility of these plants to also extract metals not present in their natural environment can offer an opportunity that merit to be studied. In this study we evaluated the potential of the serpentine endemic nickel hyperaccumulating plant *A. bertolonii* Desv. [12] to tolerate and accumulate cadmium, an important environmental contaminant, extremely toxic to living organisms and human health. This metal is scarcely present in serpentine soils, known for having low Ca to Mg ratio and high content of heavy metals, especially nickel, chromium and cobalt [13]. Furthermore, we compared the response to Cd of *A. bertolonii* to that of two populations of *Alyssum montanum* L., harvested from serpentine and normal soil in the same region. In this way, we could compare three distinct phenotypes; serpentine adapted nickel hyperaccumulator, serpentine adapted non-hyperaccumulator and non-serpentine adapted, in relation to cadmium tolerance and accumulation. Beyond giving information on the possible use of these plants in phytoextraction techniques, this model system could also shed light on the still debated existence in nature of the co-tolerance phenomenon. Co-tolerance occurs when metal-tolerant plants can also tolerate high and detrimental concentrations of those metals present at low, non-toxic levels in their environment [14]. Studying cadmium response in serpentine adapted plants could provide useful hints on such intriguing topic.

2. Materials and methods

2.1. Plant material and experimental conditions

Plants and seeds of *Alyssum bertolonii* Desv. were collected in a serpentine outcrop at Pieve Santo Stefano, Arezzo, Italy (PSS). Plants and seeds of *A. montanum* L were harvested in the serpentine outcrop of Monterufoli (MR) and from a normal soil in Monte Prata (MP) respectively. Concentration of nickel in the soil has been measured after nitric and perchloric acid digestion [15] and was respectively of $1228 \pm 46\ \mu g\ g^{-1}$ (PSS), $1480 \pm 47\ \mu g\ g^{-1}$ (MR) and $37 \pm 0.19\ \mu g\ g^{-1}$ (MP).

The soil pH, measured following the method of Sparks [16], was around 7.1, 7.5 and 7.3 for PSS, MR and MP, respectively. The soils showed traceable amounts of cadmium, ranging from 1 to $3\ \mu g\ g^{-1}$ [15].

Ten adult plants, of the same size for each species and population, were randomly collected over the population entire spatial distribution. Roots and shoots were washed with milliQ-water three times, dried at 70 °C for 24 h and then weighed and mineralised by wet ashing with a mixture of concentrated HNO_3 and $HClO_4$ (5:2 v/v) on an electric thermostatic plate (300 °C). Certified materials (grade BCR, Fluka Analitycal, Sigma–Aldrich) were the reference samples used to verify the accuracy of the method. The certified reference materials were digested as described above. Nickel and cadmium concentrations in the digests were determined using ICP-OES. Standard solutions were prepared by using available commercial stock solutions (Fluka Analytical, Sigma–Aldrich).

Seeds were germinated for 3 days in the dark on floating trays held in 1 l vessels containing 400 ml of continuously aerated Arnon solution [17] diluted 1:10, pH 5.5 ± 0.1 with the following composition: KNO_3 0.06 mmol L^{-1}, $Ca(NO_3)_2 4H_2O$ 0.03 mmol L^{-1}, $NH_4H_2PO_4$ 0.01 mmol L^{-1}, $MgSO_4 7H_2O$ 0.02 mmol L^{-1}, $FeSO_4 7H_2O$ 0.18 μmol L^{-1}, tartaric acid 0.9 μmol L^{-1}, H_3BO_3 4.6 μmol L^{-1}, $MnCl_2 4H_2O$ 0.92 μmol L^{-1}, $CuSO_4 5H_2O$ 0.03 μmol L^{-1}, $ZnSO_4 7H_2O$ 0.077 μmol L^{-1}, and H_2MoO_4 0.06 μmol L^{-1}. The culture conditions were a 12 h (day) photoperiod, provided by Philips TDL 58W/33 fluorescent tubes (160 μmol $m^{-2}s^{-1}$), at 23 ± 1 °C and a relative humidity of 60–65%.

2.2. Determination of cadmium tolerance

After germination, the floating trays with seedlings were placed in the same Arnon fresh hydroponic solutions containing 0, 0.25, 0.5, 1, 2, 5 and 10 μM $CdSO_4$. After 11 days of growth, root and shoot lengths of 30 plantlets for each treatment were measured and chosen as a measurement of metal toxic effects on plants [18]. To compare populations among them, we calculated a tolerance index as the ratio of root or shoot elongation on $CdSO_4$ containing solutions to root or shoot elongation on $CdSO_4$ free solutions.

For a quantitative estimation of root cadmium tolerance, experimental data points were fitted to a sigmoid curve to obtain four-parameter logistic function and tolerance parameters were calculated.

2.3. Cadmium accumulation

The plantlets, grown in the above-mentioned conditions, were rinsed with milliQ-water and the roots were carefully washed with $Pb(NO_3)_2$ 10 mM at 4 °C for 10 min to de-absorb metals adhering to the root cell wall. The plantlets were then divided into shoots and roots and processed as described above for cadmium determination.

2.4. Cadmium accumulation in excised roots

Roots of two-weeks-old plantlets were excised and incubated in solutions containing different $CdSO_4$ concentrations ranging between 0 and 5 μM. After 1 h, samples were washed with milliQ-water and desorbed in ice-cold (4 °C) $Pb(NO_3)_2$ 10 mM for 10 min. Cadmium was determined as described above.

2.5. Statistical analysis

All treatments were performed in triplicate and the significance of differences was analysed by one-way and factorial ANOVA followed by a HSD-Tukey test for post-hoc comparisons between unequal samples performed with Statistica 6 (StatSoft, 2003).

For the analysis of toxicity data, the curve fitting and the estimate of kinetic parameters, custom-made worksheets and program files for SigmaPlot 8.0 (SPSS Inc., Chicago, IL) were used.

3. Results

3.1. Metal concentration in field collected plants

Table 1 shows nickel and cadmium concentrations in roots and shoots of the field collected plants of *A. bertolonii* and the two populations of *A. montanum*.

The nickel concentrations were statistically different between the three plants ($p < 0.001$). The highest values were shown by *A. bertolonii* and the metal amount was far higher in shoots than in roots. The lowest nickel concentrations were shown by *A. montanum* Monte Prata (MP) and no significant differences were found between roots and shoots, whereas, *A. montanum* Monterufoli

Table 1
Metal concentration (mean ± standard error) in field collected plants of *A. bertolonii* and *A. montanum*.

Metal ($\mu g\,g^{-1}$ d.w.)	Population					
	A. bertolonii Pieve S. Stefano (PSS)		*A. montanum* Monterufoli (MR)		*A. montanum* Monte Prata (MP)	
	Roots	Shoots	Roots	Shoots	Roots	Shoots
Nickel	396 ± 38	7040 ± 1036	120 ± 15	47 ± 16	3.68 ± 1.69	2.40 ± 0.17
Cadmium	0.30 ± 0.04	n.d.	0.49 ± 0.01	0.32 ± 0.06	0.19 ± 0.04	0.19 ± 0.05

(MR) showed higher nickel concentrations in roots than in shoots ($p < 0.001$).

Regarding cadmium concentrations, only small amounts of the metal were detected in roots and shoots of the three plants.

3.2. Effects of cadmium on growth

Root and shoot length variation in *A. bertolonii* and in the two populations of *A. montanum* in the presence of increasing cadmium ($CdSO_4$) concentrations are reported in Fig. 1. Plants from all the three populations showed a decrease in root and shoot growth with increasing cadmium concentration in the nutrient solution. At the lowest exposure level used in the experimentation, all plants already showed a significant reduction in root and shoot growth in respect to plants grown in absence of cadmium ($p < 0.001$).

Tolerance to cadmium treatments is given as index of tolerance (Fig. 2) which is given by the ratio of root or shoot elongation on $CdSO_4$ containing solutions to root or shoot elongation on $CdSO_4$ free solutions. Root tolerance index did not show any significant difference at the lowest and at the two highest cadmium concentration used, whereas, *A. bertolonii* Pieve Santo Stefano (PSS) showed a significantly lower tolerance than *A. montanum* MR at 0.5 μM ($p < 0.05$) and than *A. montanum* MR and MP at 1 and 2 μM ($p < 0.001$). Also for shoot tolerance index, no significantly differences between the plants were observed at the lowest and at the two highest cadmium concentrations used. *A. bertolonii* PSS showed to be less tolerant than *A. montanum* MR and MP at 0.5 and 1 μM cadmium concentration, while at 2 μM PSS was less tolerant than MP but no significantly different than MR.

The negative effect of cadmium treatment on root growth was analysed with a regression model and in all the three plants, data fitted significantly to a logistic dose-response relationship (Table 2). EC_{50} was calculated as a tolerance parameter; the highest value was shown by *A. montanum* MR, followed by *A. montanum* MP and at last *A. bertolonii* PSS (Table 2).

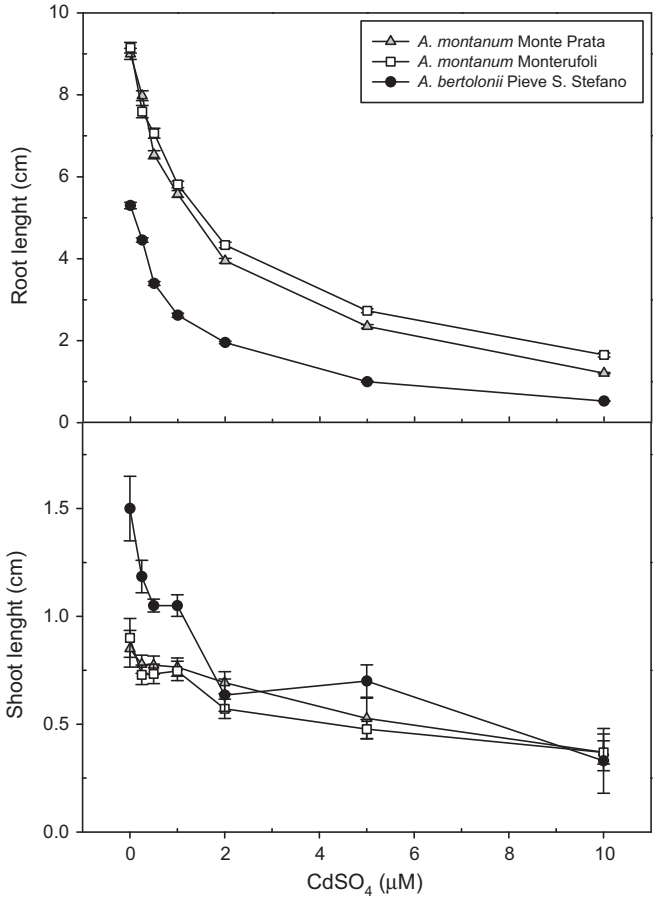

Fig. 1. Root and shoot length (mean ± standard error) of *A. bertolonii* and *A. montanum* two week old-plantlets treated with 0, 0.25, 0.5, 1, 2, 5, 10 μM $CdSO_4$ for eleven days.

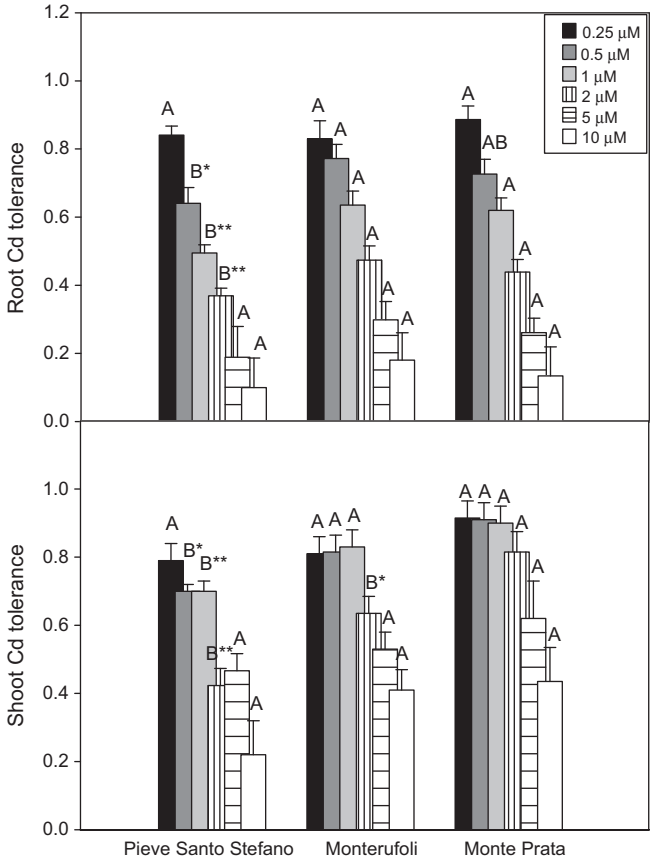

Fig. 2. Root and shoot cadmium tolerance indexes (mean ± standard error) of *A. bertolonii* and *A. montanum* two week old-plantlets treated with 0, 0.25, 0.5, 1, 2, 5, 10 μM $CdSO_4$ for eleven days. Letters above the histograms indicate significant differences among plants from the three different populations (* $p < 0.05$, ** $p < 0.001$).

Table 2
Cadmium tolerance of *A. bertolonii* and *A. montanum* plantlets expressed as EC_{50} (mean ± standard error). *r* and *p* values are reported. Significant differences between the means appear with different letters (* $p < 0.05$).

	Population		
	A. bertolonii Pieve S. Stefano (PSS)[a]	*A. montanum* Monterufoli (MR)[b]	*A. montanum* Monte Prata (MP)[c]
EC_{50} (μM $CdSO_4$)	0.97 ± 0.19 B*	1.86 ± 0.36 A	1.57 ± 0.33 AB

[a] $r = 0.99$, $p = 0.048$.
[b] $r = 0.99$, $p = 0.014$.
[c] $r = 0.99$, $p = 0.018$.

3.3. Cadmium accumulation

The cadmium concentrations in roots and shoots of all three plants increased with increasing cadmium exposure (Fig. 3) and the metal concentrations showed a significant raise ($p < 0.05$ for roots and shoots of PSS and roots of MR; $p < 0.001$ for shoots of MR and roots and shoots of MP) already at the first exposure level (0.25 μM). Moreover, values showed a saturating trend in all populations both in roots and in shoots.

Regarding cadmium concentrations in roots, significant differences ($p < 0.05$) between the three plants were found and, except for the lowest concentration used, the highest values were shown by *A. bertolonii* PSS, followed by *A. montanum* MP and at last MR.

Shoot cadmium accumulation was not significantly different between the three populations, except for the two highest exposure levels (2 and 5 μM), where *A. bertolonii* PSS exhibited the lowest concentrations ($p < 0.001$) and *A. montanum* MR the highest ones.

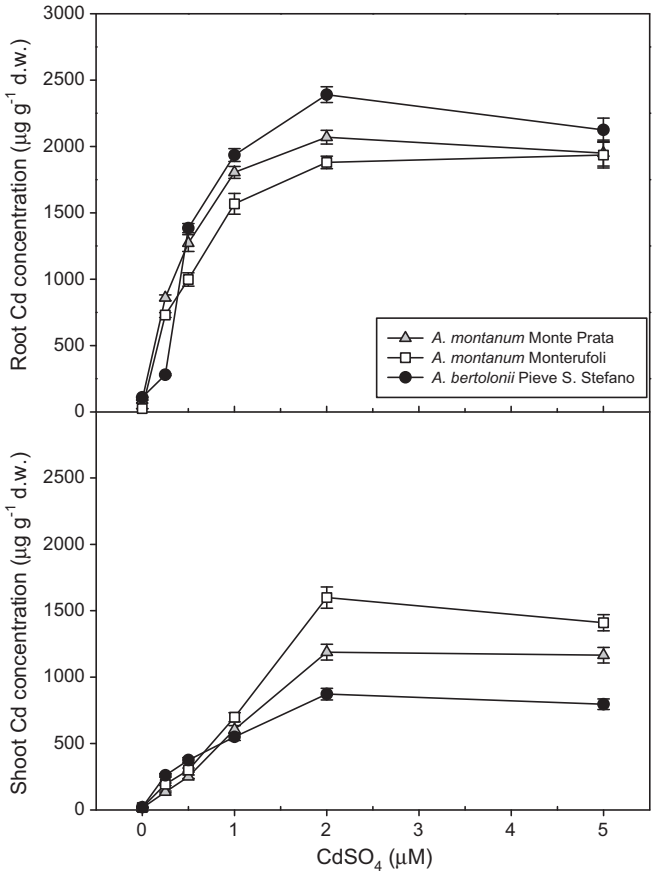

Fig. 3. Root and shoot cadmium concentration (mean ± standard error) of *A. bertolonii* and *A. montanum* two week old-plantlets treated with 0, 0.25, 0.5, 1, 2, 5, 10 μM $CdSO_4$ for eleven days.

Within each plant, a significant linear correlation between cadmium concentration in root and cadmium concentration in shoot was found. As the shoot:root metal concentration ratio never changed in a exposure-dependent way for any of the three populations at the cadmium concentrations used, the angular coefficient of this regression line was used to estimate the shoot:root metal concentration ratio itself (Table 3). The lowest mean value of this coefficient was found in *A. bertolonii* PSS, followed by *A. montanum* MP and at last *A. montanum* MR. Significant difference ($p < 0.05$) was found between *A. bertolonii* PSS and *A. montanum* MR.

3.4. Cadmium content per plant

Total cadmium contents per plant, calculated as the product between mean cadmium concentrations found in tissues of each plant and mean dry weight of plantlets, are reported in Table 4. The three plants showed a significant increase in total cadmium amount already at the first exposure level (0.25 μM) compared to the control ($p < 0.001$), both in roots and in shoots, except for roots of *A. bertolonii* PSS that showed a significant increase at 0.5 μM.

The variation of cadmium concentration in roots and shoots of all the plants followed a saturating trend similar to that observed in Fig. 3, although in this case *A. bertolonii* PSS showed the lowest concentration of metal in roots at 1 and 5 μM ($p < 0.05$). At the other exposure levels there were not significant differences between the populations for root cadmium concentrations.

Regarding the total cadmium concentration in shoots, *A. bertolonii* PSS exhibited the lowest values at 1, 2 and 5 μM ($p < 0.001$), while significant differences between the two populations of *A. montanum* were at 2 and 5 μM where MR showed higher values than MP ($p < 0.05$).

3.5. Cadmium accumulation in excised roots

Fig. 4 reports cadmium accumulation in excised roots of the three plants treated for 1 h with increasing concentrations of cadmium ($CdSO_4$). In all cases, it was observed a significant increase of cadmium concentration in excised roots was observed already at the first exposure level in respect to the control ($p < 0.05$). For most of the concentrations used, *A. bertolonii* showed the lowest root cadmium concentrations ($p < 0.05$).

Excised roots accumulation data were elaborated in order to compare uptake kinetics of cadmium by roots of the three plants of *Alyssum* in the different experimental conditions used. Results showed that the kinetic for cadmium influx into roots followed a hyperbolic pattern that fitted very well to a Michaelis–Menten function (*R* ranging from 0.92 to 0.98).

Table 5 shows the kinetic parameters of the cadmium uptake system, where K_m is an approximate measure of the affinity of the substrate for the uptake system and V_{max} is the maximum velocity at which the uptake system transports the ion. The V_{max} values for roots treated with cadmium were not significantly different between the three plants, whereas for K_m a significant difference

Table 3
Shoot:root ratio in cadmium concentrations of *A. bertolonii* and *A. montanum* plantlets calculated as the angular coefficient of the linear regression between cadmium shoot concentration and cadmium root concentration. r and p values are reported. Values are mean ± standard error; significant differences between the means appear with different letters (* $p < 0.05$).

	Population		
	A. bertolonii Pieve S. Stefano (PSS)[a]	*A. montanum* Monterufoli (MR)[b]	*A. montanum* Monte Prata (MP)[c]
Shoot:root ratio	0.32 ± 0.05 B*	0.81 ± 0.18 A	0.60 ± 0.15 AB

[a] $r = 0.95$, $p = 0.004$.
[b] $r = 0.91$, $p = 0.011$.
[c] $r = 0.88$, $p = 0.018$.

Table 4
Total cadmium concentrations (ng) in whole roots and shoots of *A. bertolonii* and *A. montanum* plantlets treated with $CdSO_4$ (μM). Values are mean ± standard error. Roots or shoots intra-populations statistical differences are reported with lowercase letters (at least $p < 0.05$), roots or shoots inter-population statistical differences are showed with uppercase letters (at least $p < 0.05$).

Treatment ($CdSO_4$ μM)	Population					
	A. bertolonii Pieve S. Stefano (PSS)		*A. montanum* Monterufoli (MR)		*A. montanum* Monte Prata (MP)	
	Roots	Shoots	Roots	Shoots	Roots	Shoots
Control	18.97 ± 3.07 aB	9.00 ± 1.96 aB	6.94 ± 1.26 aA	0.32 ± 0.10 aA	14.14 ± 6.47 aAB	14.54 ± 3.18 aB
0.25	42.00 ± 5 bA	134.4 ± 11.20 bA	199.19 ± 23.98 bB	181.69 ± 20.66 bB	174.93 ± 17.68 bB	142.43 ± 29 bAB
0.5	243.41 ± 29 cA	197.44 ± 27.15 cA	287.47 ± 34.60 cA	275.21 ± 36.37 cA	238.63 ± 43.24 bcA	229.03 ± 35.63 bA
1	252.22 ± 37.06 cA	240.88 ± 27.75 cdA	357.74 ± 77.27 cdA	636.82 ± 64.90 dB	349.02 ± 40.52 cA	540.9 ± 94.72 cB
2	217.37 ± 24.92 cA	310.25 ± 52.7 dA	441.50 ± 35.94 dB	1508.53 ± 258.0 eB	346.26 ± 61.70 cAB	1022.69 ± 183.0 dB
5	162.94 ± 31.73 cA	285.76 ± 30.7 dA	314.39 ± 53.93 cdA	1324.25 ± 116.0 eB	279.37 ± 58.88 cA	996.64 ± 123.0 dB

were found between *A. montanum* MR and *A. bertolonii* PSS, the latter showing a higher value than the other one ($p < 0.001$).

4. Discussion

Cadmium concentrations in plants harvested from the field revealed the presence of small amount of this element in roots and shoots of the three plants. Furthermore, in aerial parts cadmium concentration was well under the toxic limits for shoots [19], thus excluding any possible plant pre-adaptation to this element. The low cadmium concentration found in plants is likely to depend not only on the low cadmium concentration showed by these soils, but also on their alkaline pH that presumes a very low mobility of cadmium in such environments [19]. Mineral analysis also showed a high nickel concentration in roots and especially in shoots of the nickel hyperaccumulating plant *A. bertolonii*, whereas, far lower values were detected in the two populations of *A. montanum*. Between these two latter, the serpentine population showed a nickel concentration higher than the non-serpentine one and the limits of toxicity [19] and underlined its non-hyperaccumulating phenotype, being its nickel concentrations higher in roots than in shoots.

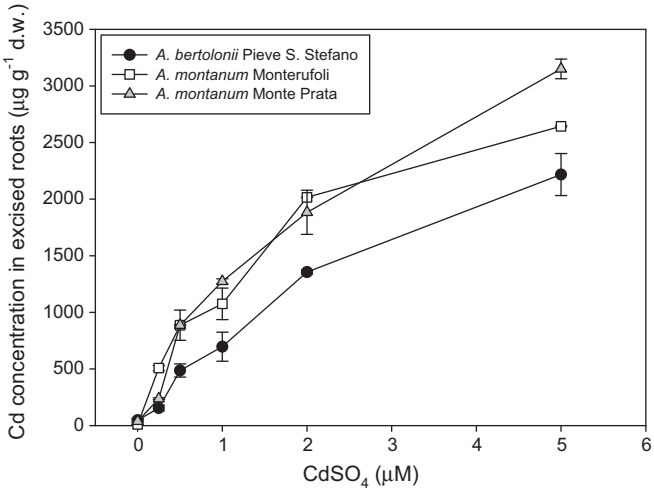

Fig. 4. Cadmium concentration (mean ± standard error) in excised roots of *A. bertolonii* and *A. montanum* two week old-plantlets treated with 0, 0.25, 0.5, 1, 2, 5, 10 μM $CdSO_4$ for 1 h.

Table 5
Kinetic parameters for cadmium influx into roots of *A. bertolonii* and *A. montanum* plantlets. Values are mean ± standard error, r values are reported in the table. Significant differences between the means appear with different letters (** $p < 0.001$).

	Population		
	A. bertolonii Pieve S. Stefano (PSS)[a]	*A. montanum* Monterufoli (MR)[b]	*A. montanum* Monte Prata (MP)[c]
V_{max}	185.33 ± 39.09 A	151.34 ± 11.11 A	202.08 ± 26.66 A
K_m	4.77 ± 0.86 A	1.84 ± 0.29 B**	2.87 ± 0.71 AB

[a] $r = 0.95$.
[b] $r = 0.98$.
[c] $r = 0.92$.

We have seen that roots were more susceptible than shoots to cadmium toxicity in all the *Alyssum* species under evaluation, and, in fact, the reduction in elongation was less significant in shoots than in roots. Although *A. bertolonii* showed the highest shoot length values, either with or without metal treatment, this plant always displayed the lowest dry biomass values for both roots and shoots in respect to *A. montanum* plants (data not showed). Many hyperaccumulator species have a decreased growth rate because of the energetic costs for hyperaccumulation mechanisms [20], and unfortunately this feature represents a further disadvantage in their use in phytoextraction techniques.

In *Alyssum* plants, the presence of cadmium in the culture medium decreased root growth in a population dependent way. In fact, analyzing the tolerance indexes, *A. bertolonii* showed a higher cadmium sensitivity, significant as compared to the serpentine *A. montanum*, whereas the two *A. montanum* populations did not show any difference in cadmium tolerance. Neither the serpentine adapted hyperaccumulating phenotype nor the serpentine adapted non-hyperaccumulating one seemed to have any relationship with Cd tolerance thus excluding the occurrence of any kind of Cd co-tolerance for these species from a nickel, cobalt, and chromium rich environment. Differently, in some cases there is evidence for cadmium co-tolerance supported by tolerance to other metals. For example, Arnetoli et al. [21] found a very high level of hyper-tolerance to cadmium in a population of *Silene paradoxa* from a polimetallic sulphide deposit mainly enriched in copper, arsenic, zinc and lead [22] and with low cadmium levels. Moreover, high cadmium co-tolerance was found in a chromium-tolerant strain in comparison with the wild type one in *Scenedesmus acutus* [23].

Data on root growth were modeled to calculate the EC_{50} values. Results confirmed what suggested by tolerance index analysis, showing the serpentine *A. montanum* as the most tolerant plants, significantly in respect to *A. bertolonii*. Moreover, interestingly, the calculation of the EC_{50} values gave useful hints about a remarkable cadmium tolerance of these plants. In fact, considering the μM range of the EC_{50} values found for the three *Alyssum*, they are far higher than the maximum value of Cd concentration in the solution of non-contaminated soil (about 0.05 μM [19]) and about the half of this value for heavy contaminated soil (about 3 μM [19]). These plants, especially the *A. montanum* species, are likely to have a good potential to grow on cadmium-contaminated soil. In fact, a plant, to be utilized in phytoextraction, must have the essential requisite of metal tolerance, to guarantee the defense of the major physiological and metabolic processes. Nonetheless, this feature should be the result of a combination of metal uptake and reduction of harmful effects and not be simply due to metal exclusion [3,24]. Root tolerance means the preservation of the selective property of the cell membrane and so represents the first step in metal uptake and loading into the xylem vessels [25]. Furthermore, the fact that, in our experiments, shoot growth was less affected than root growth, reinforced the previous consideration.

The accumulated cadmium concentration in roots and shoots was influenced by the external concentration and metal concentration in the roots was always higher than in the shoots in all the three plants. A preferential root allocation of metals remains a widespread behavior to face metal toxicity in the majority of the plants.

As for root cadmium accumulation, *A. bertolonii* showed the higher concentrations, up to 2500 $\mu g\,g^{-1}$, followed by the other *Alyssum* species up to 1900–2000 $\mu g\,g^{-1}$. In shoots, significant differences were found only for the two highest medium concentrations used. The pattern of the differences was diverse from roots and interestingly, inverted. In fact, *A. montanum* plants showed the highest concentrations, up to 1600 and 1200 $\mu g\,g^{-1}$ for MR and MP respectively, whereas *A. bertolonii* showed the lowest, up to only to 900 $\mu g\,g^{-1}$. When the effect of medium cadmium concentration began to be stringent, the most tolerant species displayed the lowest root cadmium concentrations and the highest shoot cadmium concentrations, whereas the most sensitive species, *A. bertolonii*, showed the opposite condition. Probably, the higher cadmium toxicity suffered by the roots of this plant, possibly due to the higher internal cadmium concentration present, impaired all the metabolic processes, cadmium translocation included.

In shoots at the lowest concentration used, all the plants showed tissue concentrations higher than 100 $\mu g\,g^{-1}$, value that is considered the limit for cadmium hyperaccumulation [9]. Considering that for that concentration the toxic effect of cadmium was very low on root growth and not present in shoots, or minimum as in the case of *A. bertolonii*, these plants could be proposed as potential cadmium hyperaccumulators that merit to be studied. After all, also the nickel hyperaccumulator *Thlaspi goesingense* was demonstrated to accumulate cadmium at very high concentrations in its above ground tissue [11]. Values found in shoots were also higher than those found in some hydroponically cultivated *Brassica* plants, generally studied for their remarkable and promising cadmium phytoremediation potential (see for example Qadir et al. [26] and Grispen et al. [27]). The very high concentrations found in the shoots of these *Alyssum* plants may suggest that it is worth studying the behavior of plants also to metals not present in their environment.

However, for really generating an hyperaccumulator phenotype, also a high rate of root-to-shoot translocation has to be present and this was not the case for these *Alyssum* plants for the concentrations used, as root always showed higher cadmium concentrations than shoots.

In any case, the translocation coefficients, identified as a fundamental trait for the plant suitable for phytoextraction [3], were very different in the three plants and the serpentine *A. montanum* showed a value approaching to 1, whereas, the nickel hyperaccumulator showed the lowest. The most cadmium tolerant plants were also those with the highest shoot concentration and the highest translocation coefficient, really suggesting that metal adapted non-hyperaccumulating plants can be useful in exploring the possibility for phytoextraction, rather than too specialized hyperaccumulators. Furthermore, our data also suggest that even non metal adapted plants may be more useful than hyperaccumulators in the extraction of the non hyperaccumulated metals, as is the case of the non-serpentine *A. montanum* and *A. bertolonii*.

As for cadmium content per plant, this incremented with increasing cadmium concentration in the medium and was higher in shoots than in roots despite the higher root metal concentration. This result depends on the huge difference in both biomass production and metal sensitivity between the two organs. The plantlets showed significant differences in cadmium content. In the case of roots, even if *A. bertolonii* showed the highest metal concentrations and the lowest biomass production, it had the lowest metal content; in the case of root the low biomass was the factor that determined the differences in metal content. As for shoot cadmium content, the serpentine *A. montanum* showed the highest concentration and *A. bertolonii* the lowest. This result was generated by both the lowest cadmium concentration and the lowest dry biomass (data not showed) displayed by *A. bertolonii*. Therefore, whereas the serpentine adapted nickel hyperaccumulating phenotype did not seem a useful material for cadmium phytoextraction, the serpentine adapted nickel non-hyperaccumulating phenotype presented the advantage of an higher cadmium phytoextraction capability, confirming the idea that studying metal non present in the environment of metal adapted plants can offer the possibility of a useful selection of plants for phytoextraction and phytostabilisation aim.

To evaluate if differences in metal concentration may depend on differences in root cadmium uptake systems, short-term uptake

studies with excised roots were of fundamental importance to exclude the effect of translocation and evaluate the real ability of the root to take the metal up. Our results showed a significantly different cadmium accumulation in the excised roots of the three plants. Experiments showed that the nickel hyperaccumulating plants accumulated cadmium at a significantly lower level than the other ones for most of the cadmium concentrations used. In fact, the calculation of the kinetic parameters of cadmium uptake presented a significant difference in the apparent K_m values. *A. bertolonii* presented the highest apparent K_m value, suggesting a lower affinity for this metal of its uptake system, whereas the V_{max} values were not significantly different. If there could be a connection between this very low affinity and nickel hyperaccumulation is a question that deserves to be investigated. This low affinity for cadmium is not in contrast with the highest cadmium root accumulation showed by intact plants, as the latter is also the result of shoot translocation that in *A. bertolonii* was the lowest. This fact, combined also with the lowest dry biomass of this plant, could explain the very low amount of cadmium per plant showed by the nickel hyperaccumulating phenotype. As for *A. montanum*, the two species did not present any difference in the cadmium kinetic parameters, but, notwithstanding this result, it is interesting to note that the cadmium content per plant was higher in the serpentine adapted plants, probably because of its higher, even not significant, tolerance to this metal in respect to the non-serpentine adapted population.

5. Conclusion

The selection of the most efficient plant is indeed a key factor in every phytotechnology for soil reclamation, especially for metal phytoextraction that is still far from reality. In fact, gaining information on the intra-specific and inter-specific variability in plant contaminant accumulation and selecting suitable plants remains a challenge [28]. In this context, the present data demonstrated that evaluating metallicolous plant behavior, even toward metals present at low level in the origin environment, could be a good research field for finding plants suitable for phytoextraction. In fact, in respect to the non-serpentine adapted *A. montanum* and the serpentine adapted nickel hyperaccumulator *A. bertolonii*, the serpentine adapted population of *A. montanum* showed remarkable cadmium tolerance and accumulation that merit to be studied and exploited for the selection of more suitable tools for phytoremediation purposes. Nevertheless, studies on plant cultivation on real contaminated soils are needed.

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