

# Exploring the metal phytoremediation potential of three *Populus alba* L. clones using an in vitro screening

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## Abstract

**Purpose** This work was planned for providing a useful screening tool for the selection of *Populus alba* clones suitable for phytoremediation techniques. To this aim, we investigated variation in arsenic, cadmium, copper, and zinc tolerance, accumulation and translocation in three poplar clones through an in vitro screening. Poplars have been widely proposed for phytoremediation, as they are adaptable to grow on contaminated areas and able to accumulate metals. The investigation of possible differences among poplar clones in metal tolerance and accumulation deserves to be deeply studied and exploited for the selection of the more suitable tool for phytoremediation purposes.

**Methods** In vitro multiplied microshoots of a commercial and two autochthonous *P. alba* clones were subcultured on hormone-free WPM medium for 1 month and then transferred for 2 weeks onto media containing different concentrations of the metals investigated. At the end of the treatments, plantlets were sampled, weighed, and mineralised by wet ashing. Metal concentrations were determined by ICP-OES.

**Results** For the metal concentration used in the experiments, our clones of *P. alba* showed variation in metal tolerance,

metal accumulation and content. The fast-growing commercial clone, even if rarely showing the highest plant metal concentration, displayed the highest metal content, suggesting biomass production as the key factor in evaluating the phytoextraction capacity of *P. alba* clones for the metals studied.

**Conclusions** Data demonstrated that in vitro screening of cuttings represents a valuable way of assessing the ability of different poplar clones to take up, tolerate and survive metal stress.

**Keywords** Poplar · Metals · Tolerance and accumulation variability · Clonal selection

## 1 Introduction

To remove contaminants from polluted sites, unconventional techniques involving biological processes can have strong potentialities. In particular, the so-called phytoextraction technology makes use of plants to extract metals from soil, thus being less expensive and less damaging to the environment than conventional remediation systems consisting mainly in soil excavation and incineration (Vassilev et al. 2004; Van Nevel et al. 2007). On the other hand, phytostabilization technology uses plants for stabilising contamination in place preventing further spreading and transfer into food chains (Vangronsveld et al. 2009).

The efficiency of phytoextraction depends, besides the substrate type, on several characteristics of the plant, such as the ability to accumulate and translocate metals to the aerial parts, a fast growth and a deep and extended root system.

Phytoextraction by woody plants (dendroremediation) presents several advantages. The large biomass yield can be used for economic opportunities, such as bioenergy, solid

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wood production and reconstituted products (Rockwood et al. 2004). The extended and deep root apparatus can explore a large volume of soil; the impact on trophic chains is low, and the adaptability of some tree species to grow in marginal soils is well-established. Metal uptake by trees is reported to be not as high as hyperaccumulators but, due to a greater yield of biomass, the removal of metals from soil could be more effective respect to hyperaccumulating plants (Fischerová et al. 2006; Mench et al. 2009; Vangronsveld et al. 2009). So, these plants may provide a viable alternative to the usual low-yielding hyperaccumulators that, in any case, are not well-represented at temperate latitudes (Dickinson et al. 2009).

Several hardwood species have been proposed for phytoremediation, and poplars are among these. In fact, these Salicaceae are known not only to be adaptable to grow on contaminated areas, but also to be capable of accumulating heavy metals (Pulford and Watson 2003). Moreover, poplar cultural management by means of short rotation coppice cultures is another advantageous feature to be considered in phytoremediation strategies (Rockwood et al. 2004).

Poplars, whose genome has been entirely sequenced (Tuskan et al. 2006), present also the advantage of being generally easy to propagate and, showing fast growth and high biomass production, are therefore useful for short-rotation harvest. In addition, they can be propagated by in vitro techniques and are conveniently exploited in genetic engineering (Confalonieri et al. 2003).

For instance, transgenic white poplar has been obtained expressing a gene for a metallothionein-like protein, thus enhancing tolerance to  $\text{CuCl}_2$  in in vitro culture (Balestrazzi et al. 2009); transgenic yellow poplar overexpressing *mer A9* and *mer A18* genes, showed increased tolerance to high mercuric concentration, developing higher biomass and higher amount of Hg (0), which evaporates through the cell surface, compared with non-transformed plants (Che et al. 2003). Anyhow, that evidence of gene transfer and expression in poplar is just related to sterile culture environment.

Some studies have reported differences among poplar clones in heavy metal accumulation (Laureysens et al. 2004) and in the partitioning of elements within the tree organs (Fischerová et al. 2006; Robinson et al. 2000, 2005; Unterbrunner et al. 2007). So, for improving the possible application of these plant species to phytoextraction techniques, there is the need of investigating variation in metal tolerance, accumulation and translocation among the different poplar clones. To this purpose, we used an in vitro screening as an effective tool to exploit *Populus alba* L. clonal variability. This kind of study can represent a valuable method for the set-up of phyto-recurrent selection cycles, as proposed by Zalesny et al. (2007), from which clones representing the right combination of high levels of

trace element uptake and high productivity can be rapidly individuated and chosen for field tests. An in vitro screening reduces not only the growth period and the treatment time length of the plants but also the space required for the experiments. Moreover, environmental factor variability is also reduced. On the other hand, such screenings suffer from the lack of guarantee that tolerance/accumulation patterns found in that short-term experiments will persist with time and occur also in field. So, data obtained by an in vitro screening always need to be confirmed by field performance trials; anyhow, Watson et al. (2003) and Pulford et al. (2002) pointed out that results obtained in hydroponics and in field experiments have a good agreement.

This study was aimed at evaluating the response of three clones of *P. alba* L. to different heavy metals, in particular cadmium, zinc and copper, and to the metalloid arsenic in terms of tolerance, accumulation and translocation in an in vitro system. Finding a way to quickly characterise poplar clones for their effectiveness in tolerating and accumulating metals is of fundamental importance in an initial screening for the individuation of the most promising clones for field trials on metal-polluted soils.

## 2 Materials and methods

### 2.1 Plant material and in vitro growth conditions

In vitro proliferating microshoots of a commercial and two autochthonous *P. alba* L. clones, Villafranca (Vil), Fiorentini (Frt) and Querce (Qrc), respectively, were subcultured on Woody Plant Medium (WPM, Lloyd and McCown 1980), added with 2% sucrose, 0.72% agar (B&V, Reggio Emilia, Italy) and 0.2 mg l<sup>-1</sup> benzyl adenine, at pH 5.5, in 100-ml baby-food glass jars (Sigma-Aldrich, Milano, Italy) sterilised by autoclaving at 121°C and 108 kPa for 20 min. Culturing microshoots originated from axillary bud proliferation of shoot tips collected from adult trees of the mentioned clones, according to Confalonieri et al. (2003).

The aseptic cultures were incubated in a growth chamber at 23±1°C with a 16-h photoperiod (40 μE m<sup>-2</sup> s<sup>-1</sup>) and routinely subcultured (approximately every 4 weeks). Microshoots were finally transferred to phytohormone-free WPM medium for 4 weeks before metal treatments to remove the cytokinin carry-over effect and enhance shoot elongation.

### 2.2 Metal treatments

Well-developed shoots were cut at uniform size (two internodes; 1.5-cm tall) and transferred to phytohormone-free WPM medium containing a series of Na<sub>2</sub>HAsO<sub>4</sub> (0, 5, 10

and 250  $\mu\text{M}$ ),  $\text{CuSO}_4$  (0, 5, 50 and 250  $\mu\text{M}$ ),  $\text{CdSO}_4$  (0, 5, 50 and 250  $\mu\text{M}$ ) and  $\text{ZnSO}_4$  (0, 250, 1,000 and 2,000  $\mu\text{M}$ ) concentrations; the other medium composition and conditions being the same as previously described. Twenty plants per clone per concentration (four glass jars per treatment per clone containing five shoots each) were used in the experiment. Other 20 rootless shoots per clone were dried at 80°C for 24 h and then weighed to estimate a mean value of the dry biomass at the beginning of the experiment.

### 2.3 Determination of metal toxicity, metal concentration and content

After 15 days of treatment, plantlets were gently removed from the medium and roots were carefully washed. In the case of heavy metal-treated plants, roots were also desorbed with 10 mM  $\text{CaCl}_2$  solution for 10 min to remove the adhering metals from the cell walls.

Plantlets were separated into shoots and roots, dried at 80°C for 24 h and then weighed. The dry biomass production (dry biomass at the end of the experiment minus dry biomass at the beginning of the experiment previously estimated) was used as a measurement of the metal toxic effects (Baker and Walker 1989). To compare clones, we calculated a tolerance index as the ratio of root or shoot dry biomass production on metal medium to root or shoot dry biomass production on metal-free medium, thus obtaining for each metal three series of tolerance indexes for the three clones.

Roots and shoots of plantlets exposed to  $\text{CuSO}_4$ ,  $\text{CdSO}_4$  and  $\text{ZnSO}_4$  were mineralised by wet ashing on hot plate (100–300°C) in plugged beakers with 65%  $\text{HNO}_3$  and 60%  $\text{HClO}_4$  (5:2, v/v). Instead, root and shoots of As-exposed plantlets were digested in 10 ml 65%  $\text{HNO}_3$  in closed glass tubes at room temperature because of metal volatility. Reference material was included in the analysis.

Metal concentrations were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 2000 DV, Perkin–Elmer Instruments). Metal contents were calculated as the product between mean dry biomass of plantlets and mean metal concentration in each plant.

Data were rearranged in graphs using the SigmaPlot 8.0 programme (SPSS, Chicago, IL).

### 2.4 Statistical analysis

All treatments were performed in quintuplicate and repeated in at least four independent experiments.

Statistical analysis was carried out with one-way and two-way analysis of variance (ANOVA) using the statistical programme Statistica 6.0 (StatSoft, 2003). A posterior comparison of individual means was performed using Tukey's test. Clones and elements were considered as

independent variables. Means were considered different at probability values of  $P < 0.05$ .

## 3 Results

### 3.1 Biomass production and tolerance index

Root and shoot dry biomass in *P. alba* clones in presence of increasing metal concentrations is reported in Tables 1 and 2.

In all clones, a significant negative effect of metal treatment on root dry biomass was shown only at the highest concentration for arsenic and zinc treatments.

The reduction in dry biomass due to the presence of cadmium in the culture medium was always significant in the Vil clone, whereas no effect was detected in the other two clones.

The clones displayed a very strong root growth inhibition at the highest copper concentrations, showing roots so reduced to be excluded from the analysis, while the lower concentrations had no significant effect.

The highest values of root dry biomass were always significantly shown by the Vil clone, except in the case of cadmium treatment.

In all clones, roots were significantly more affected by metal toxicity than shoots. In fact, even if a decrease in the weight of shoot dry biomass was shown for some metal treatments, it was never significant in this plant organ.

As in roots, in shoots, the highest values of dry biomass were also shown by the Vil clone, even if only for copper and zinc treatment and significantly only in respect to the Qrc clone.

Expression of the tolerance to metal treatments as tolerance indexes (Fig. 1) enabled a comparison of clones. Factorial ANOVA demonstrated that the three clones of *P. alba* showed significant differences in root dry biomass only in their responses to cadmium treatments. For shoots, no significant difference was detected (data not shown).

### 3.2 Metal accumulation in roots and shoots

Although in all plants, the metal concentration in the tissues increased with the external metal concentration, and metal concentrations were generally higher in roots than in shoots, the three clones differed significantly in root and shoot metal accumulation (Fig. 2).

Root arsenic concentrations were significantly higher in Qrc clone as compared with the other ones at the highest concentration used ( $P < 0.05$ ). Arsenic accumulation in shoots was not significantly different in the three clones.

As for cadmium accumulation, among the clones Frt showed the lowest cadmium values in both roots ( $P < 0.05$ ) and shoots ( $P < 0.05$ ).

**Table 1** Root dry biomass (milligrammes) of *P. alba* clones after metal treatment (micromolars)

	CdSO <sub>4</sub>			CuSO <sub>4</sub>			ZnSO <sub>4</sub>		
	Vil	Qrc	Frt	Vil	Qrc	Frt	Vil	Qrc	Frt
Control	3.75±0.38	1.21±0.22	1.49±0.38	3.75±0.38	1.21±0.22	1.49±0.38	3.75±0.38	1.21±0.22	1.49±0.38
5 μM	3.19±0.35	1.36±0.27	1.66±0.22	1.87±0.34	1.41±0.27	1.58±0.30	2.98±0.51	1.19±0.27	1.00±0.23
50 μM	3.28±0.47	1.51±0.19	1.45±0.31	2.09±0.32	1.72±0.16	1.48±0.29	2.63±0.46	1.47±0.29	1.13±0.30
250 μM	1.36±0.42	0.17±0.09	0.32±0.27	0.66±0.27	1.54±0.17	1.58±0.34	-	-	-

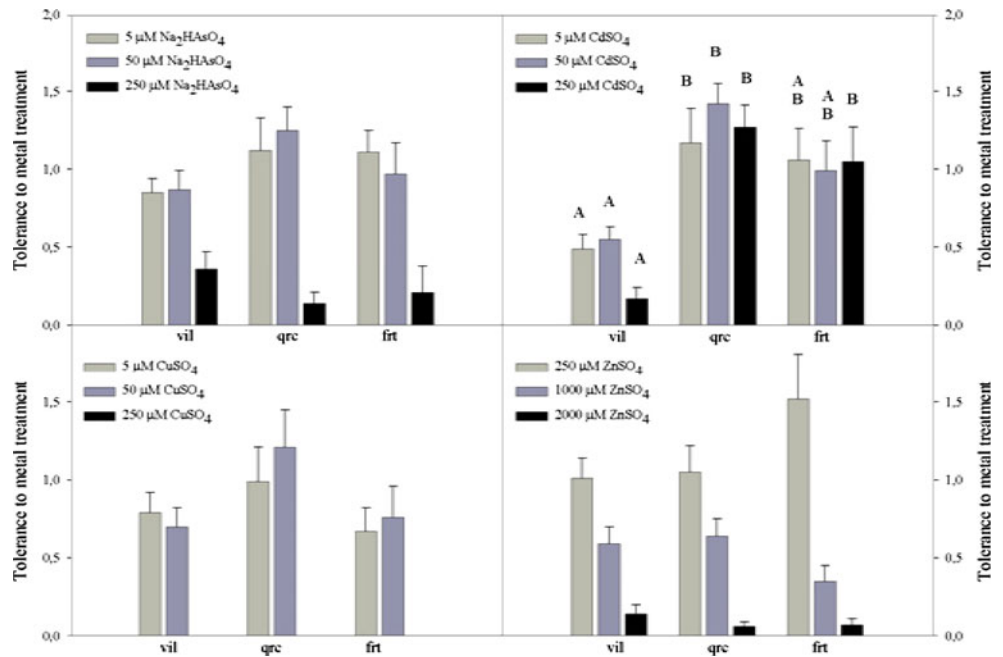
*Vil* Villafranca, *Frt* Fiorentini, *Qrc* Querce. Values are means ± standard error; significant differences between the means (at least  $P < 0.05$ , according to ANOVA) appear with different letters, capital for interclonal and lower case for intracolonial differences

**Table 2** Shoot dry biomass (milligrammes) of *P. alba* clones after metal treatment (micromolars)

	CdSO <sub>4</sub>			CuSO <sub>4</sub>			ZnSO <sub>4</sub>		
	Vil	Qrc	Frt	Vil	Qrc	Frt	Vil	Qrc	Frt
Control	8.03±1.11	2.95±0.64	6.11±1.06	8.03±1.11	2.95±0.64	6.11±1.06	8.03±1.11	2.95±0.64	6.11±1.06
5 μM	8.63±0.92	2.98±0.43	5.92±1.36	7.51±1.89	2.85±0.59	6.93±0.78	8.20±0.99	3.56±0.78	4.86±0.65
50 μM	7.05±0.84	3.07±0.59	6.34±1.02	5.96±0.90	3.45±0.42	6.23±0.58	9.45±0.79	2.98±0.62	4.81±1.28
250 μM	5.70±0.96	3.37±0.59	5.45±1.22	6.30±0.82	3.54±0.49	4.73±1.18	9.86±0.58	3.38±0.59	5.14±1.20

Values are means ± standard error; significant differences between the means (at least  $P < 0.05$ , according to ANOVA) appear with different letters, capital for interclonal and lower case for intracolonial differences

**Fig. 1** Metal tolerance indexes of the three *P. alba* clones. Letters above the histograms, when present, indicate the significant differences among clones according to the Tukey's test



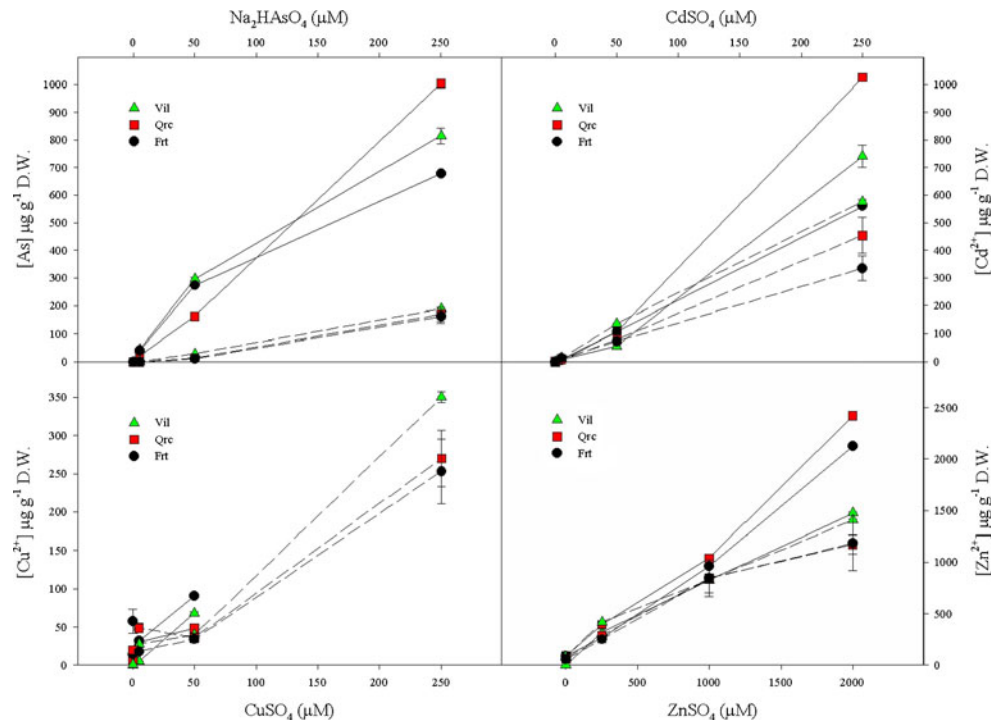
Copper concentrations in roots were not measurable at the highest concentration used for the absence of rooting. At the other concentrations, copper accumulation was generally higher in the Frt clones ( $P < 0.05$ ). In shoots, for the copper concentrations that allowed the root to grow, copper concentrations were not significantly different among the clones. At 250  $\mu\text{M}$   $\text{CuSO}_4$ , the Vil clone showed statistically significant higher values of copper accumulation in shoot, but this datum will be excluded

from the discussion due to the too much higher toxicity of this concentration.

Zinc concentrations were more homogeneous among the clones; the only statistically significant difference was that the Vil clone accumulated this metal to a lesser extent in the roots in respect to the other clones ( $P < 0.01$ ).

Within each clone, linear correlation between metal concentration in root and metal concentration in shoot was highly significant ( $0.946 < r < 0.999$ , at least  $P < 0.05$ , Table 3).

**Fig. 2** Root (solid line) and shoot (dashed line) metal accumulation (microgrammes per gramme dry weight) of the three clones of *P. alba* (mean  $\pm$  standard error) after exposure to increasing metal concentrations



**Table 3** Shoot/root ratio in metal concentration of *P. alba* clones, calculated as the angular coefficient of the linear regression between metal shoot concentration and metal root concentration

	Vil	Qrc	Frt
As	0.23±0.03 aA 0.978*	0.17±0.01 aA 0.997**	0.24±0.06 aA 0.946*
Cd	0.74±0.08 bB 0.987*	0.43±0.02 bA 0.997**	0.59±0.01 bA 0.999***
Zn	0.89 ±0.02 cB 0.999***	0.47±0.11 bA 0.950*	0.54±0.11 bA 0.961*

First row indicates the ratio, second row the regression coefficient  
 Values are mean ± standard error; significant differences between the means appear with different letters, capital for interclonal and intertreatment and lower case for intraclonal differences. *r* values are reported, \**P*<0.05, \*\**P*<0.01, \*\*\**P*<0.001

This correlation was not calculated for Cu as it prevented sufficient root growth at the higher concentration used. As the shoot/root metal concentration ratio never changed in an exposure-dependent way for any of the clones at the metal concentrations used (data not shown), the angular coefficient of this regression line was used to estimate the shoot/root metal concentration ratio itself. This coefficient showed significant differences among the clones for the different metals, even though its range of variation was only from 0.17 (Qrc for arsenic) to 0.89 (Vil for zinc; Table 3). The highest coefficients were shown for zinc and cadmium, the Vil clone displaying the highest, while the lower coefficient was for arsenic, without any significant difference among the clones. Within each clone, the angular coefficient for arsenic was always significantly lower than the other (*P*<0.05); the one for zinc was significantly higher only in Vil clone (*P*<0.05).

### 3.3 Metal content

Root and shoot metal content in *P. alba* clones in the presence of metal treatments is reported in Tables 4 and 5. In all the clones, metal content was always higher in the shoots than in the roots, except for the lowest arsenic concentrations used.

The presence of the metal in the culture medium significantly resulted in an increased root metal content for all the metals, but this increase was never proportional to metal treatment. Among the clones, the Vil clone showed the significantly highest contents for arsenic, copper and zinc, whereas in the case of cadmium the Qrc clone displayed a significantly higher root content for the highest concentration used.

Shoot metal content varied significantly both intra and inter clones. For all the metals tested, shoot content increased proportionally to metal treatment. The significantly

**Table 4** Root total content per plant (microgrammes per plant) in *P. alba* clones after metal treatment (micromolars)

	CdSO <sub>4</sub>			CuSO <sub>4</sub>			ZnSO <sub>4</sub>		
	Vil	Qrc	Frt	Vil	Qrc	Frt	Vil	Qrc	Frt
Control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	< 0.01	< 0.01	< 0.01
5 µM	0.14±0.01 aA	0.03±0.01 aA	0.07±0.01 aA	0.02±0.01 aA	0.02±0.01 aA	< 0.01	0.04±0.01 aA	0.02±0.01 aA	0.02±0.01 aA
50 µM	0.97±0.14 bB	0.25±0.03 bA	0.39±0.08 aAB	0.12±0.02 aA	0.19±0.02 aA	0.16±0.03 aA	0.07±0.01 cB	0.10±0.03 bA	0.10±0.03 bA
250 µM	1.10±0.34 bB	0.17±0.09 abA	0.21±0.18 aA	0.49±0.20 bA	1.58±0.17 bB	0.88±0.19 bA	-	2000 µM 1.80±0.25 cB	2000 µM 0.18±0.11 abA

Values are means ± standard error; significant differences between the means (at least *P*<0.05, according to ANOVA) appear with different letters, capital for interclonal and lower case for intraclonal differences

Values in the range of nanograms were not reported (indicated as <0.01) even if included in the statistical analysis



**Table 5** Shoot total content per plant (microgrammes per plant) in *P. alba* clones after metal treatment (micromolars)

	CdSO <sub>4</sub>				CuSO <sub>4</sub>				ZnSO <sub>4</sub>						
	Vil	Qrc	Frt	Control	Vil	Qrc	Frt	Control	Vil	Qrc	Frt	Control	Vil	Qrc	Frt
Na <sub>2</sub> HAsO <sub>4</sub>	n.d.	n.d.	n.d.	Control	n.d.	n.d.	n.d.	Control	< 0.01	0.10±0.01	0.66±0.06	Control	1.15±0.09	0.47±0.04	0.67±0.06
5 μM	0.02±0.01	n.d.	n.d.	5 μM	0.18±0.03	0.06±0.01	0.15±0.01	5 μM	aA	0.39±0.03	0.39±0.04	0.18±0.01	aA	aA	aA
50 μM	0.39±0.02	0.11±0.01	0.13±0.01	50 μM	1.62±0.12	0.63±0.03	0.85±0.04	50 μM	aA	bA	aA	0.35±0.04	bB	bA	bAB
250 μM	2.21±0.18	1.33±0.10	1.75±0.20	250 μM	7.10±0.47	3.64±0.22	3.57±0.38	250 μM	bA	aA	aA	1000 μM	cB	cA	cA
	cB	bA	bA		cB	cA	cA		cB	bA	bA	2000 μM	dB	dA	dA

Values are means ± standard error; significant differences between the means (at least  $P < 0.05$ , according to ANOVA) appear with different letters, capital for interclonal and lower case for intracolonial differences. Values in the range of nanograms were not reported (indicated as <0.01) even if included in the statistical analysis

highest values of metal content were shown by Vil clone for all the metals.

#### 4 Discussion

The success of phytoremediation techniques is indeed based on the availability of properly selected plant material. Among the species indicated as potential candidates for the remediation of contaminated soil, poplars play an important role, due to their growth, genetic and cultural characteristics (McGrath et al. 2001; Pulford and Watson 2003; Vassilev et al. 2004). In this work, three clones of *P. alba* were studied to evaluate their patterns of variation in metal tolerance, accumulation and translocation, as these fundamental features should form the criteria to be followed in screening plants for the recovery of polluted soils.

A plant, to be utilised in phytoextraction, must have the essential requisite of metal tolerance, to guarantee the defence 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 (Krämer 2005; Vangronsveld et al. 2009). 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 (Zacchini et al. 2009).

In the present work, the root system of poplar clones, analysed by mean of dry biomass production per plant, showed some differences in metal tolerance. Generally, all the metals, except cadmium for Qrc and Frt clones, showed a significant toxic effect on root dry biomass production at some or all the concentrations used. Comparing the clones among them through the calculation of the tolerance index, significant variation was shown only in respect to cadmium, the Qrc and Frt clones being the most tolerant for the concentrations used. Our findings are in accordance with previous investigations and confirm poplar variability in metal tolerance (see for example Zacchini et al. 2009; and Dos Santos Utmazian et al. 2007).

In *P. alba* clones, the root responded more sensitively than the shoot to toxic metal concentrations, and the reduction in shoot biomass production was never significant for all the clones and all the metals for the concentration used.

The highest biomass values were generally showed by the Vil clone, confirming the well-known ability of fast growth of this commercial clone. This feature was present either with or without metal treatment, suggesting that even if this clone was generally the most sensitive, the reduction in its growth led to a biomass production that was anyway higher than the autochthonous, and more tolerant, clones.

Acclimation of trees to metal stress has been studied using a variety of indices, such as cell suspension cultures

and callus cultures, but seedling growth is the most frequently used index, regardless of their greater sensitivity to unfavourable conditions than mature trees (Pulford and Watson 2003). In fact, also in the present work, this index was able to find out variation in metal tolerance in the studied clones.

The accumulated metal concentration in roots and shoots was affected by the external concentration. In shoots, metal concentration widely exceeded the leaf approximate metal concentrations thought to be toxic (Kabata-Pendias and Pendias 2001). Considering the non-significant effect of metal treatments on shoot growth for the concentrations used, data showed that poplars can accumulate high levels of these metals with no adverse effect on their biomass production and matched results already obtained in field (Laureysens et al. 2004).

Metal concentration in the roots was always higher than in the shoots, suggesting for our *P. alba* clones and the studied elements a metal exclusion strategy from stems and reproductive tissue by retaining the metal in the roots, thus avoiding its toxicity. Similar results on metal partitioning in hydroponics were found by Zacchini et al. (2009) and Dos Santos Utmazian et al. (2007). Trees can differ greatly in their metal accumulation pattern (Pulford and Watson 2003) but a preferential root allocation of metals remains a widespread behaviour to face metal toxicity in the majority of the plants.

Metal accumulation was variable among clones in root, showing the Qrc clone the highest values, except in the case of copper. In shoot, variation was detected, but it was significant only for cadmium, with Frt clone displaying the lowest concentrations. So, clones with contrasting phenotypes for accumulation-related traits were found, thus suggesting that such screenings deserve to be deepened as they can provide real opportunities for improving both phytostabilization and phytoextraction techniques.

Only in the case of cadmium did the clones with the highest root concentration also show the highest shoot concentration and a clearly correlated pattern for root and shoot metal accumulation.

*P. alba* clones did not show any tendency to lower the metal shoot/root concentration ratio as the metal concentration in the medium increased. We can speculate that, in *P. alba*, the lack of this behaviour, generally thought to represent a defence strategy against the toxic effect of the metal, could be due to the fact that, with the external metal concentrations selected for this study, shoot metal concentrations did not reach levels toxic enough to trigger such a response. Among the populations, significant variation was found in this ratio, identified as a fundamental trait for the plant suitable for phytoextraction (Vangronsveld et al. 2009), showing the Vil clone significantly higher values for cadmium and zinc although the concentrations of heavy

metals used in this study were different. So, data suggested that each clone can be characterised by a different efficiency in root-to-shoot transport that deserve to be investigated, as variability in shoot/root concentration ratio could contribute to the generation of the differences found in the levels of metal accumulation in the shoot. Anyway, the lack of any relation between arsenic and zinc shoot concentration and root concentration suggests that, among *P. alba* clones, the higher shoot concentrations are due to other feature than higher shoot/root concentration ratios for these metals.

Despite the higher root metal concentration, metal content was generally higher in the shoots than in the roots in all the clones. This result depends on the huge difference in both biomass production and metal sensitivity between the two organs and is one of the reasons why not only metal hyperaccumulators but also high biomass-producing excluder plants can be effectively used in phytoextraction techniques (Vangronsveld et al. 2009).

Also, metal content showed significant variation among the clones. In root, metal content did not increase proportionally to metal medium concentration probably because of the severe effect on root growth imposed by the metal themselves, whereas, in shoot, a proportional increase was found and, in fact, this organ showed a higher metal tolerance, probably due to the lower metal concentration accumulated. Generally, and always in the shoot, the Vil clones showed the highest values of content for all the metals tested. So, even if the plant metal concentration was rarely the highest, this clone showed the highest metal content, suggesting the biomass production as the key factor in evaluating the phytoextraction capacity of *P. alba* clones for the metals studied.

Finally, variation was present at a more significant degree in metal accumulation and content than in tolerance, so this is another reason to carry on in vitro screening even if different clones seem to respond in the same way to metal stress in the terms of biomass production.

## 5 Conclusions

The tree long generation time acts to prevent a fast selection of metal-tolerant genotypes, the generation of which is random or induced by the pollutant (Dickinson et al. 1991). Nonetheless, their basal tolerance mechanisms may allow them to cope with higher metal concentrations than agricultural crops (Riddell-Black 1993) and to survive in contaminated soil, although usually with a reduced growth rate (Dickinson et al. 1992). In this context, the present data demonstrated that in vitro cultures of cuttings really represent a valuable way of assessing the ability of different poplar clones, to take up, tolerate and survive metal stress,



thus representing an initial effective screening for the individuation of the most promising clones for field trials on metal-polluted soils. In fact, for the metal concentration used in the experiments, our clones of *P. alba* showed variation in metal tolerance, metal accumulation and content that deserve to be studied and exploited for the selection of the more suitable tool for phytoremediation purposes. Nevertheless, studies on the effect of the synergistic presence of different metals on clone behaviour and studies proving if cuttings accurately reflect the responses of older trees are still needed.

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