Some preliminary observations on the zinc and cadmium accumulative capacity of four woody species (Celtis australis,

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SOME PRELIMINARY OBSERVATIONS ON THE ZINC AND CADMIUM ACCUMULATIVE CAPACITY OF FOUR WOODY SPECIES (*CELTIS AUSTRALIS*, *QUERCUS ILEX*, *SYRINGA REFLEXA* AND *VIBURNUM TINUS*)

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SUMMARY - The experiment was conducted in order to observe zinc and cadmium accumulative capacity and to evaluate the potential use of four woody ornamental species (*Celtis australis*, *Quercus ilex*, *Syringa reflexa* and *Viburnum tinus*) in the phytoremediation process. Zinc and cadmium were added together to the substrate as sulphate salts (20 replications per species for each group). At the same time, citric acid was added to two different groups of plants to evaluate its specific role on plant's phytoremediation capacity. The results showed that the four species cannot be considered as metal accumulators but instead as metal tolerant plants. Plant growth was not significantly inhibited by the increasing metals' concentrations in the substrate. In all the four tested species, cadmium and zinc were predominantly accumulated into root system but showed different translocation patterns into aerial parts. Also, the presence of citric acid proved no effect on the translocation of the two metals, but in some cases its addition led to an improved absorption from the substrate. At the end, biological absorption coefficient (BAC) was calculated. *Quercus ilex*, *Syringa reflexa* and *Viburnum tinus* resulted as "strong cadmium absorbers" while *Celtis australis* looked like an "intermediate absorber". More, zinc BAC values were generally lower than 1 (intermediate absorption) for *Quercus ilex*, *Syringa reflexa* and *Viburnum tinus*, while *Celtis australis* showed a weak zinc absorption coefficient.

Key words: *BAC, citric acid, ornamental shrubs, ornamental trees, phytoremediation*.

INTRODUCTION

Soil pollution has recently attracted considerable public attention. Due to many human actions such as the mining and smelting of metalliferous, electroplating, gas exhaust, the energy and fuel production, the application of fertilizers and pesticides, metal pollution has become one of the most serious environmental problems today (Alkorta *et al*., 2004). Phytoremediation is an emerging technology that uses plants to remove, degrade or reduce heavy metals and organics pollutants from contaminated soils (Salt *et al*., 1998). It is considered an effective, low cost, preferred cleanup option for moderately contaminated areas (Weis and Weis, 2004), that only recently started to receive greater attention as a valid alternative to traditional physicochemical remediation methods. Five main subgroups of phytoremediation that use different strategies of remediation have been identified: phytoextraction, in which plants extract metals from soils and concentrate them in the harvestable parts of plants; phytodegradation, in which plants, in conjunction with microbes, are able to degrade organic contaminant through their

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metabolic processes; phytostabilization, that is the bioavailability decrease and mobility of pollutants in soils, reducing the risk of further environmental degradation by leaching into the ground water or by airborne spread; rhyzofiltration, in which plants roots absorb toxic metals from polluted effluents, and phytovolatilisation, that is the use of plants to volatilize contaminants from soil or water into the atmosphere (Pulford and Watson, 2002; Burken and Schnoor, 1997; Dushenkov et al., 1995; Kumar *et al*., 1995; Salt *et al*., 1995; Vangronsveld *et al*., 1995). The idea of using plants to remediate metal polluted soils came from the discovery of "hyperaccumulators", plants that have the ability to accumulate large amounts of contaminants by translocating metals from root to shoot (Brown, 1995; Vasquez *et al*., 1992). The success of phytoremediation depends upon the ability of a plant to uptake and translocate heavy metals (Chen *et al*., 2003). Plants can accumulate chemical elements essential for growth and development such as Fe, Mn, Zn, Cu, Mg and Mo. In addition, some of them have the capacity to accumulate heavy metals with no known biological functions, such as Cd, Cr, Pb, Co, Se and Hg (Baker and Brooks, 1989; Raskin *et al*., 1994). Among these elements, zinc and cadmium are two of the most widely diffused pollutants. Zinc (Zn) is an ubiquitous phytotoxic contaminant. It can reduce crop yields and may represent a potential hazard to the food chain (Luo *et al*., 2000). Most plants have Zn concentrations between 30 and 100 $\mu g\ g^{-1}$ dry weight; concentrations above 300 $\mu g\ g^{-1}$ are considered toxic (Marschner, 1995). Previous works showed that *Thlaspi caerulescens* can accumulate up to 10000 μg Zn g^{-1} dry weight in the shoots without showing any phytotoxicity symptoms or reduction in growth (Shen *et al*., 1997; Brown et al., 1995). Cadmium (Cd) is a heavy metal naturally present in soil at concentrations of slightly more than 1 mg Kg^{-1} (Peterson and Alloway, 1979). It is a highly toxic pollutant to most organisms, having a toxicity 2-20 times higher than many other heavy metals (Vassilev *et al*., 1998). Cd content in soil has been dramatically increased by anthropogenic sources including smelters and agricultural applications of fertilizer and sewage sludge. Cd is neither an essential nor a beneficial element for plants and may even cause numerous physiological toxic responses in plants. A foliar concentration above 100 $\mu g\ g^{-1}$ dry weight is considered exceptional and is used as a threshold value for Cd hyperaccumulator (Baker *et al*., 2000). Unfortunately, most of the metal-accumulating identified species have small sizes and/or are slow growing plants producing low biomass (Kumar *et al*., 1995), and these characteristics severely limit the use of hyperaccumulator plants for environment cleanup. Because of a deeper penetration of woody plants' roots into the soil (several meters), shrubs or trees species, rather than herbaceous plants, could be useful to treat deeper contamination. Besides, in agreement with various authors, trees are potentially the lower-cost plant type to use for phytoremediation (Garbisu and Alkorta, 2001; Stomp *et al*., 1994) and would be attractive plants especially to renew the vegetation of metal-polluted sites (Kopponen *et al*., 2001). Successful application of plants for remediation of contaminated soils depends on the availability of metals. The synthetic chelating agents (such as organic acids) are used in remediation of soil contaminated by heavy metals, in order to improve the transfer of metals to the soil liquid phase (Naidu and Harter, 1998). For example, optimum cadmium mobility in soil is achieved at pH = 4.5 - 5.5 (Bingham *et al*., 1980); therefore, altering soil pH by adding an acid is a viable method for increasing cadmium bioavailability. The aim of this preliminary study is to observe zinc and cadmium accumulative capacity and to evaluate the potential use in the phytoremediation process of four woody ornamental species (*Celtis australis*, *Quercus ilex*, *Syringa reflexa* and *Viburnum tinus*). Also, citric acid addition to soil has been performed in order to evaluate chelating agent performing.

MATERIALS AND METHODS

Experiments were carried out during spring-summer period (May-September) at the Department of Horticulture of the University of Florence. Two-years-old seedlings of four woody ornamental species (*Celtis australis*, *Quercus ilex*, *Syringa reflexa* and *Viburnum tinus*) were grown into Ø 20 cm pots (3 L volume) filled with peat and perlite (1:1, v:v, pH 5) and placed outdoor under a polyethylene sheet to avoid any rainy substrate leaching. Pots were daily irrigated with a modified Hoagland's nutrient solution by a computerized drip irrigation system (pH 6, EC 1.5 mS/cm, NO_3^- 102 ppm, NH_4^+ 5 ppm, PO_4^{3-} 29 ppm, K^+ 153 ppm, Ca^{2+} 88, Mg^{2+} 18 ppm, SO_4^{2-} 37 ppm, Fe^{2+} 1 ppm as EDDHA), without any drain collecting. Plants have been divided into five different treatments related to metal concentrations and citric acid addition (Table 1). Zinc and cadmium have been added together to the substrate as sulphate salts at the beginning of the experiment (20 replications per species for each treatment). Neither metals nor citric acid have been added to the controls.

Plants performances were determined by repeated biomass production measurements (dry weight and relative growth rate) during the experimental period and by chemical analysis to verify metals content into vegetal tissues. Plant material was sampled at the end of June, July, September and November (n=5), and separated into leaves, shoots and roots. Dry weight was obtained after drying the samples in an oven at 70°C for at least 48 h. Relative growth rate (RGR) has been calculated as reported by Hunt (1978). Chemical analysis were carried out by digesting plant tissues (500 mg DW) in a mixture of concentrated HNO_3 and $HClO_4$ (2:1 v.v.) using a digester (VELP Scientifica, Italy). Total zinc and cadmium content in plant tissues was detected using an optical emission spectrometer (ICP-OES, Inductively Coupled Plasma-Optical Emission Spectrometry, PerkinElmer, OPTIMA 2000 DV). Biological Absorption Coefficient (BAC), showing the absorption capacity of the plant, was calculated as the ratio of heavy metal concentration in plant tissues vs. that in the substrate (Nagaraju and Karimulla, 2001). The experiment consisted in a randomized block design, a single block referred to a single treatment. Each treatment was composed by 20 plants, each plant representing a replicate. Data were analysed by one-way ANOVA, and means (n=5) were separated using Tukey's statistical test ($P \leq 0.05$). Statistical analysis were made using GraphPad Prism 4.0 (GraphPad® software).

RESULTS AND DISCUSSION

Plant growth was not inhibited neither by the increase of metal concentrations into the substrate, nor by the addition of citric acid, as no significant differences among treatments have ever been monitored in dry weights during the experimental period (Fig. 1). Relative growth rate was not influenced by zinc and cadmium addition showing no sig-

Tab. 1 - Total amount of metals (as sulphate salts) and citric acid added to the substrate during the experimental period.

Treatment	Total $ZnSO_4$ (mg/l)	Total $CdSO_4$ (mg/l)	Citric acid (mg/l)
control	-	-	-
(A)	300	3.25	-
(B)	400	16.25	-
(A + citrate)	300	3.25	750
(B + citrate)	400	16.25	750

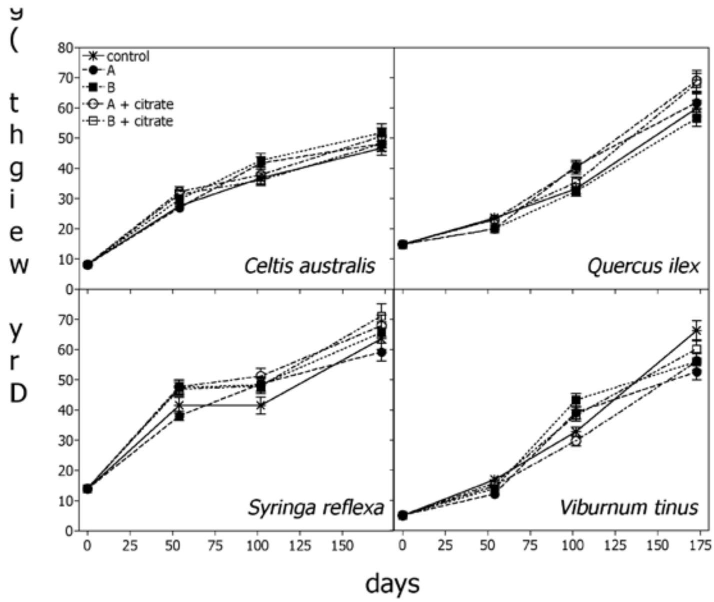

Fig. 1. - Total dry weight in *Celtis australis*, *Quercus ilex*, *Syringa reflexa* and *Viburnum tinus* subjected to different Cd and Zn treatments. Vertical bars show ± SD. Data were analysed by one-way ANOVA and means (n=5) were separated using Tukey's test (P<0.05).

Tab. 2 - Relative Growth Rate (RGR, mg g^{-1} d^{-1}) calculated for each species (see Table 1 for the description of treatments). Data were analysed by one-way ANOVA to evaluate the zinc and cadmium effects. Means inside each row (n=5) were separated by Tukey's test and showed no significant differences for P<0.05.

	control	A	B	A + citrate	B + citrate
Celtis australis	3.35	3.52	3.57	3.82	3.52
Quercus ilex	7.69	8.27	7.76	9.15	8.83
Syringa reflexa	9.30	9.37	9.47	9.95	10.60
Viburnum tinus	14.85	13.52	13.44	13.89	14.92

nificant differences among treatments (Table 2). More, visual assessment showed no toxicity symptoms on treated plants' leaves. It is well established that a plant requires a balance between the uptake of essential metals and the ability to protect sensitive cellular activity and structures from excessive non-essential metals uptake (Garbisu and Alkorta, 2001) in order to maintain a correct and effective growth. Total amounts of absorbed cadmium and zinc as well as the effect of citric acid addition and the metal distribution in plant organs are reported in Fig. 2 and 3 respectively. As expected, according to Whiting *et al*. (2000), all the treatments showed a greater zinc and cadmium content than control. Cadmium content (Fig. 2) in *Celtis australis* plants was higher in (B) treatment, compared to the lower concentration. However, citric acid addition did not influence cadmium accumulation as no significant differences were found among treatments at the same cadmium concentrations. In *Quercus ilex*, (A) plants showed a significant lower cadmium content compared to the other treatment. The addition of citric acid

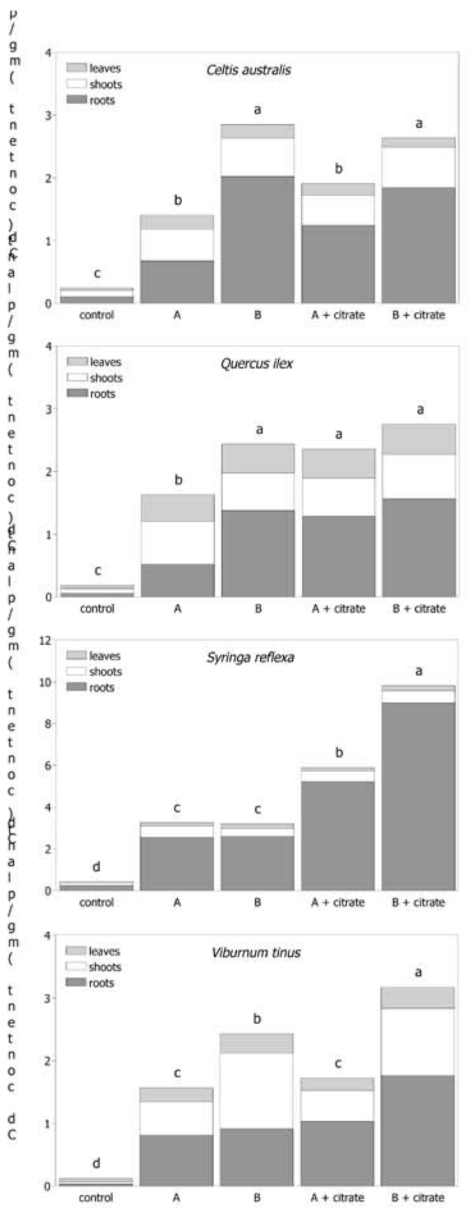

Fig. 2. - Cadmium content in leaves, shoots and roots of *Celtis australis*, *Quercus ilex*, *Syringa reflexa* and *Viburnum tinus* plants grown at different Cd substrate concentrations (3.25 and 16.25 mg*l^{-1}) and the presence of citric acid. Data were analysed by one-way ANOVA and means (n=5) were separated using Tukey's test (P<0.05). Different letters show statistically significant differences in total dry weights among species.

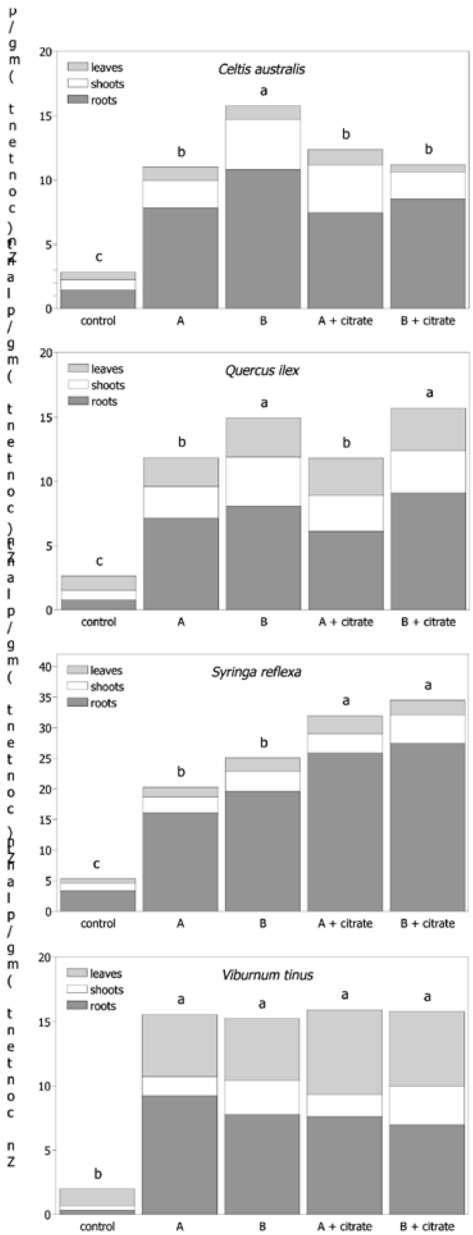

Fig. 3. - Zinc content in leaves, shoots and roots of *Celtis australis*, *Quercus ilex*, *Syringa reflexa* and *Viburnum tinus* plants grown at different Zn substrate concentrations (300 and 400 mg*l^{-1}) and the presence of citric acid. Data were analysed by one-way ANOVA and means (n=5) were separated using Tukey's test (P<0.05). Different letters show statistically significant differences in total dry weights among species.

was effective only at lower cadmium concentrations, as no significant differences were monitored among treatments at the highest concentrations: citric acid was probably able to play a positive role in metal absorption only at a lower cadmium concentration, without any effect at higher concentration. On the contrary, a clear and important role played by citric acid was evident in *Syringa reflexa*. In fact, increased cadmium content into the substrate showed no effect on plant tissues (i.e. (A) and (B) treatments showed no significant differences). However, citric acid addition strongly improved cadmium accumulation, especially at higher cadmium concentration, due to a more pronounced cadmium bioavailability. No differences in cadmium content at any metal level in the substrate were detected in *Vinurnum tinus*, but a strong increment in accumulation followed the addition of citric acid. Moreover, (B+citrate) treated plants showed the highest cadmium content, which was significantly different than the other treatments.

Regarding zinc content, all treatments led to higher values compared to control, but species showed different behaviours. For example, zinc accumulation was positively affected by substrate metal concentration in *Celtis australis* and *Quercus ilex* (Fig. 3), but plants showed a total insensitivity to citric acid, as no significant differences were observed among relative treatments. Also, citric acid addition to B treatment in *Celtis australis* led to a lower zinc accumulation compared to B treatment alone, probably due to a higher but toxic zinc bioavailability, which interfered to metal absorption and/or accumulation. On the contrary, *Syringa reflexa* and *Viburnum tinus* showed no significant differences between any zinc level. Instead a different citric acid effectiveness was observed: citric acid improved zinc accumulation in *Syringa reflexa* but no effect was detected in *Viburnum tinus* .

Concerning organ distribution, cadmium (Fig. 2) and zinc (Fig. 3) were predominantly accumulated into root system with a different translocation pattern into aerial parts among species. Cadmium and zinc contents into *Syringa reflexa* root system varied from 60 to 90% of the total amount of plant metal content. In *Celtis australis* this percentage ranged from 50 to 75% while in *Quercus ilex* and *Viburnum tinus* from 30 to 60%. Other Authors reported different results. For example, *Salix* clones had a higher aboveground heavy metals translocation potential (Greger and Landberg, 1999; Vyslouzilovà et al., 2003), showing that cadmium and zinc were more accumulated in leaves than in twigs and roots, even if clones could present variable metal uptake capacity. In our case, the mechanisms involved in cadmium and zinc translocation from root to shoot were probably the limiting factor for metal transport rate. In fact, plants reacted differently to heavy metals stress, their resistance and tolerance depended on the ability of the single species to activate molecular mechanisms, such as metal sequestration in the cell wall and/or in vacuoles usually by binding with appropriate ligands (Chaney et al., 1997). Plants were able to change soil metal availability in a direct manner (uptake) or indirectly by the use of different mechanisms like exudation of complexing agents or respiration of roots (Hammer and Keller, 2002). Numerous studies showed that by lowering the pH of a soil the absorption of heavy metals (by soil particles) could decrease and thus increase their concentration in soil solution (Turgut *et al*., 2004; Kulli *et al*., 1999; Salt *et al*., 1995; Albasel and Cottenie, 1985; Dijkshoorn *et al*., 1983; Harter, 1983). However, our results indicate that citric acid did not actively stimulate cadmium and zinc translocation from root to aerial parts, in contrast with the study carried out by Chen et al. on *Raphanus sativus* (2002). These Authors proved that leaf heavy metals content increased with the addition of citric acid to contaminated soil. At the end, the Biological Absorption Coefficient (BAC) was calculated in order to investigate the uptake capacity of the tested species. BAC is one of the most important biogeochemical parameters for mineral exploration and is defined as the concentra-

Tab. 3. Values of BAC calculated for each treatment in the four tested species (see Table 1 for the description of treatments).

	Celtis australis		*Quercus ilex*		*Syringa reflexa*		*Viburnum tinus*	
	Cd	Zn	Cd	Zn	Cd	Zn	Cd	Zn
A	0.71	0.07	1.40	0.13	1.98	0.17	1.85	0.17
B	0.27	0.10	0.38	0.17	0.40	0.24	0.56	0.18
A + citrate	0.94	0.08	1.95	0.13	3.06	0.30	2.07	0.19
B + citrate	0.25	0.07	0.42	0.17	0.91	0.30	0.72	0.19

tion of an element in plant ashes *versus* the concentration of the same element into the substrate. Perelman (1966) classified mineral absorption intensity into five groups, related to BAC values: "intensive absorption" (BAC 10-100), "strong absorption" (BAC 1-10), "intermediate absorption" (BAC 0.1-1), "weak absorption" (BAC 0.01-0.1), and "very weak absorption" (BAC 0.001-0.01).

Table 3 reports BAC values for both species and treatments. Cadmium BAC values were normally higher in (A) plants in all species. More, citric acid addition had a positive effect in enhancing BAC values. *Quercus ilex*, *Syringa reflexa* and *Viburnum tinus* appeared as strong cadmium absorbers when grown in lower cadmium substrate concentration, as BAC ranged from 1 to 3 both in normal and citric acid-addicted plants. On the contrary, *Celtis australis* acted like an intermediate absorber (0.71). The increase in cadmium substrate content led to a strong decrease in BAC values, both with or without citric acid. These data seem to agree with Dickinson and Pulford (2005), as tissue concentrations of cadmium tended to be higher in highly contaminated soils, but BAC values were lower, suggesting that translocation of cadmium from soil to plant tissues is less effective in highly contaminated soils. On the contrary, zinc BAC values were generally lower than 1 (intermediate absorption) for all the treated plants of *Quercus ilex*, *Syringa reflexa* and *Viburnum tinus*, while *Celtis australis* showed a weak zinc absorption coefficient.

CONCLUSIONS

Our data underline that the four species cannot be considered as metal accumulators but as metal tolerant plants. *Syringa reflexa* seems to appear the only effective species and it could be successfully used for phytostabilization, probably due to the greater surface area of thin and long roots which contributes to a better absorption of heavy metals (An and Donald, 2003). In fact, species with numerous thin and fine roots can accumulate a higher quantity of metals than species with few thick roots (Das *et al*., 1997). With phytostabilization plants can also physically stabilize the soil against erosion and runoff. In addition, plant activity like water and element uptake can help to keep the heavy metals within the plant/soil system, and, thus, to prevent their leaching to the groundwater. On the contrary, the other three species showed a lower capacity in metal uptake than *Syringa reflexa*. So, their application for phytoremediation purposes appear to be ineffective, despite high rates of root absorbed metal translocated to the aerial organs in *Viburnum tinus*.

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