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Original Citation:

Zinc and cadmium tolerance of Hyssopus officinalis L. and Satureja montana L. plants / S.Mugnai; E.Azzarello; C.Pandolfi; S.Mancuso. - In: ACTA HORTICULTURAE. - ISSN 0567-7572. - STAMPA. - 723(2006), pp. 361-366.

Zinc and Cadmium Accumulation in *Hyssopus officinalis* L. and *Satureja montana* L.

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Keywords: aromatic plants, citric acid, heavy metals, phytoremediation, toxicity

Abstract

Accumulation of cadmium and zinc in two Mediterranean plants, *Hyssopus officinalis* L. and *Satureja montana* L., is described here. Two concentrations for each metal have been used in an artificially contaminated substrate: 200 and 1400 ppm zinc, and 21 and 108 ppm cadmium. Plant growth has been monitored for three months after metal addition. Total metal uptake has been determined by ICP-OES measurements on samples harvested at 30, 60 and 90 days from the beginning of the experiment. *Hyssopus officinalis* L. and *Satureja montana* L. seemed to be tolerant to both cadmium and zinc, as progressive metal accumulation vs. time has been observed with no toxicity symptoms. Zinc and cadmium concentrations have also been measured in the different plant organs: a significant metal translocation to the upper parts (stem and leaves) was detected. Specifically, *Hyssopus* seemed to be more efficient in translocating Zn than Cd; by contrast, Satureja accumulated higher quantities of cadmium in the aerial parts compared to *Hyssopus*. The addition of an organic chelator (citric acid) to substrate was also considered, as a means to improve metal bioavailability and explore the possible use of these two species for phytoremediation. Citric acid enhanced zinc and cadmium accumulation, mostly at root level.

INTRODUCTION

Heavy metal contamination of agricultural soil may represent a relevant environmental problem, since it can reduce crop yield and negatively affect the safety of plant products as food and feeds (Zheljazkov et al., 2005). The ingestion of heavy metals with medicines and foods can cause accumulation in organisms, producing serious health hazards connected to acute or chronic toxicity (Goodman and Gilman, 1990; Abou-Arab et al., 1999).

Heavy metals can be present in spices and medicinal plants, sometimes at concentrations exceeding the tolerance limits. Recently, some studies have been published, reporting high heavy metal content in medicinal and aromatic plants collected from commercial supply areas (Šovljanski et al., 1992; Dwivedi and Dey, 2002). This could be mainly attributed to the contamination of soil and water, the addition of certain fertilizers and herbicides, and also to traffic pollution.

In addition, there is increasing evidence that some aromatic and medicinal species show good tolerance to metal contaminated soil, suggesting the possibility of their use in phytoremediation (Jeliazkova and Craker, 2002; Scora and Chang, 1997; Zheljazkov and Warman, 2003; Zheljazkov et al., 2005).

Starting from these considerations, the present research was developed to study the accumulation of zinc and cadmium, two of the most common soil contaminants, in *Hyssopus officinalis* L. and *Satureja montana* L., with the main aim of detecting the risk of attaining critical concentrations, from a safety perspective. Moreover, we also started to preliminary explore the possible use of these two Mediterranean species for phytoremediation, by the use of an organic chelator, such as citric acid.

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Acta Hort 723, ISHS 2006

MATERIALS AND METHODS

Seedlings of *Hyssopus officinalis* L. and *Satureja montana* L. were grown in 1.5 l pots, filled with peat and perlite (1:1, v:v) and placed outdoors. Eight experimental treatments were obtained by the combinations of zinc and cadmium concentrations, with or without citric acid, reported in Table 1. 20 plants were randomly assigned to each treatment. The adopted heavy metal concentrations were in the range of those occurring in polluted soil.

The plants were irrigated twice a day with a Hoagland-half strength nutrient solution, by a computerized drip irrigation system (pH 6, EC 1,5 mS/cm). Contaminants and citric acid were supplied to the substrate as salts (zinc sulphate, cadmium sulphate, citrate, Sigma-Aldrich).

Plant material was sampled 30, 60 and 90 days after the beginning of the experiment, for biomass determination and chemical analyses. Each plant was separated into leaves, shoots and roots. Before measurements, roots were gently washed. Dry weights were obtained after oven drying the samples at 70°C for 48 h.

Chemical analyses 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 digestor (VELP Scientifica, Italy). Total Zn and Cd concentrations were analyzed using an optical emission spectrometer (ICP-OES, Inductively Coupled Plasma-Optical Emission Spectrometry, PerkinElmer, OPTIMA 2000 DV).

The experiment was arranged in a completely randomised design, with individual plants acting as replications; five plants per treatment were randomly collected and used for the biometric and chemical determinations. The data have been analyzed by one-way ANOVA; differences among means were detected using Tukey's test ($P \leq 0.05$). The GraphPad Prism 4.0 (GraphPad Software, Inc.) package was used for statistical analyses.

RESULTS AND DISCUSSION

Plant Growth and Biomass Production

Plant biomass was not affected by any of the experimental treatments, for both species under consideration (Table 2) suggesting a lack of toxicity at the used concentrations.

Metal Uptake

The pattern of metal accumulation during the experimental period is shown in Figure 1. Both species were able to extract and accumulate large amounts of the two metals, compared to the control. The process was relatively slow and, as previously stated, the progressive accumulation of metals occurred without any evident toxicity symptoms; only for the 1400 ppm zinc treatment, added with citric acid, plants rapidly died (data not reported). A compartimentation of the two toxicants within the plant perhaps occurred, but further analyses are needed and planned to clarify this process.

At the end of the experiment, total cadmium content was 5-6 times higher at the highest cadmium concentration compared to the 21 ppm treatment both in *Hyssopus* and *Satureja*. For zinc, the ratio between the highest concentration treatment and the 200 ppm treatment was 4 times for *Satureja* and 7 times for *Hyssopus*. The addition of citric acid to the substrate strongly increased metal uptake, mainly at the highest cadmium concentration, as reported by Marchiol et al. (2004) in *Brassica napus* and *Raphanus sativus*. On the contrary, Chen et al. (2003) found that the addition of citric acid decreased the total amount of lead and cadmium absorbed by radish (*Raphanus sativus*).

Kim et al. (1994) reported an average Cd and Zn content of 0.39 and 27.78 ppm respectively, in 291 samples of several medicinal plants grown in unpolluted sites. A comparison with our results, in which hyssop and savoury were able to accumulate from 16 to 96 ppm of Cd and 84 to 645 ppm of Zn, clearly indicates the risk of potential health hazards if these species are grown on polluted soil.

Concentrations of toxic metals exceeding tolerance limits were reported also by Al-Saleh and Chudasoma (1994), Scora and Chang (1997), Zheljazkov et al. (2005) and Mamani et al. (2005) for plants cultivated or spontaneously growing in polluted areas.

Metal Distribution within the Plants at Final Harvest

The partitioning of zinc and cadmium at final harvest in the different organs is shown in Figure 2.

At low concentration (200 ppm), zinc was distributed quite uniformly among organs in both species. In *Hyssopus*, zinc concentration increased almost exponentially, as a function of treatments, in all organs, with higher rates in roots; in fact, at 1400 ppm, zinc content was 12-fold higher in roots and 4.5-fold higher in leaves than in the 200 ppm treatment. The addition of citric acid to the 200 ppm treatment caused a significant increase in total zinc accumulation (two-fold higher) both in roots and leaves (Fig. 2a). In *Satureja*, the raise of zinc concentration was compatively lower in leaves and shoots, with respect to *Hyssopus* (Fig. 2b). The addition of citric acid to the 200 ppm treatment caused a significant increase of the zinc content of roots (2 fold) shoots (1.2 fold) and leaves (1.5fold). As a whole, *Hyssopus* was able to store higher amount of zinc than *Satureja* at 1400 ppm, in both the roots and leaves.

Cadmium was mainly stored in the roots, with significant increases from the lower to the higher substrate concentration (202 ppm in *Hyssopus* and 125 ppm in *Satureja,* at 108 ppm in the substrate). *Satureja* was able to accumulate higher cadmium concentration in shoots than *Hyssopus* (Figs. 2c and 2d). The addition of citric acid to the 21 ppm treatment had no effect in *Hyssopus*, but resulted in a significant increase of cadmium content, at 108 ppm (Fig. 2c). In *Satureja*, the addition of citric acid determined an increase of cadmium concentration in roots, for both the two concentrations in the substrate, and in leaves, for the 108 ppm treatment (Fig. 2d). Previous studies on the use of organic acids to increase the mobility of heavy metals in the soil report controversial results. Kayser et al. (2000), adding amendments as NTA (nitrilotriacetate) to the soil, demonstrated a 58-fold increase of cadmium solubility, but only a two-fold increase of its plant accumulation. Recently, Turgut et al. (2004) showed that control plants of *Helianthus annus* were more efficient in the uptake and translocation of cadmium than citric acid treated plants. However, in our conditions, citric acid significantly increased zinc and cadmium accumulation at root level, and the subsequent translocation to the shoot and leaves.

CONCLUSIONS

Satureja and *Hyssopus* plants contained high levels of cadmium and zinc when grown in an artificial polluted substrate, indicating potential health hazard for consumers, especially since no visible symptoms of heavy metals toxicity on plant growth were observed. Therefore, monitoring toxic metal levels in aromatic and medicinal plants is important mainly for assuring safety and quality to the consumers.

Besides this fact, the high level of heavy metal accumulation may suggest the potential of these species for phytoremediation programs in Mediterranean areas, although the small size of *Satureja montana* may be a limit for its practical use.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Susanna Pucci, Microanalysis Lab. of Department of Organic Chemistry for her kind help and support in ICP-OES analysis on plant materials.

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Tables

Table 1. Total amount of metals and citric acid supplied for each treatment. Metal concentrations are referred to soil dry weight.

Treatments
1. Control
2. Zinc 200 ppm
3. Zinc 200 ppm + citric acid 5 mg g^{-1}
4. Zinc 1400 ppm
5. Cadmium 21 ppm
6. Cadmium 21 ppm + citric acid 5 mg g^{-1}
7. Cadmium 108 ppm
8. Cadmium 108 ppm + citric acid 5 mg g^{-1}

Table 2. *Hyssopus officinalis* L. and *Satureja montana* L. total dry mass (g) in the different zinc and cadmium treatments after 90 days of treatment.. Results are reported as means ± SD. All treatments are not significantly different ($P \leq 0.05$).

Treatments	*Hyssopus officinalis*	*Satureja montana*
1. Control	23.90 ± 6.38	20.61 ± 3.88
2. Zinc 200 ppm	22.62 ± 2.30	19.84 ± 1.78
3. Zinc 200 ppm + citric acid	21.15 ± 5.62	19.20 ± 1.70
4. Zinc 1400 ppm	19.12 ± 4.06	18.67 ± 1.92
5. Cadmium 21 ppm	25.97 ± 1.63	22.34 ± 3.53
6. Cadmium 21 ppm + citric acid	20.86 ± 3.32	21.71 ± 2.58
7. Cadmium 108 ppm	27.05 ± 2.00	19.79 ± 1.45
8. Cadmium 108 ppm + citric acid	25.94 ± 4.06	21.98 ± 1.50

Figures

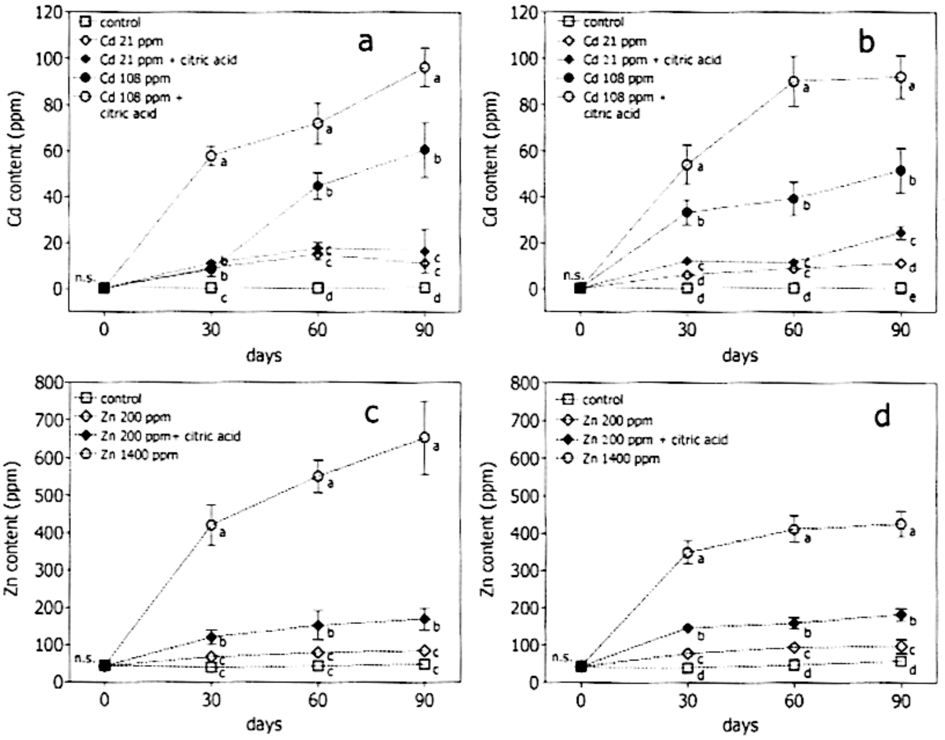

Fig. 1. Cadmium and zinc content (ppm total plant dry weight) determined after 30, 60 and 90 days in a) and c) *Hyssopus officinalis*, b) and d) *Satureja montana*. Vertical bars indicate ± SD (n=5). Different letters at each harvest date refer to significant differences for $P \leq 0.05$, when means were separated by Tukey's test.

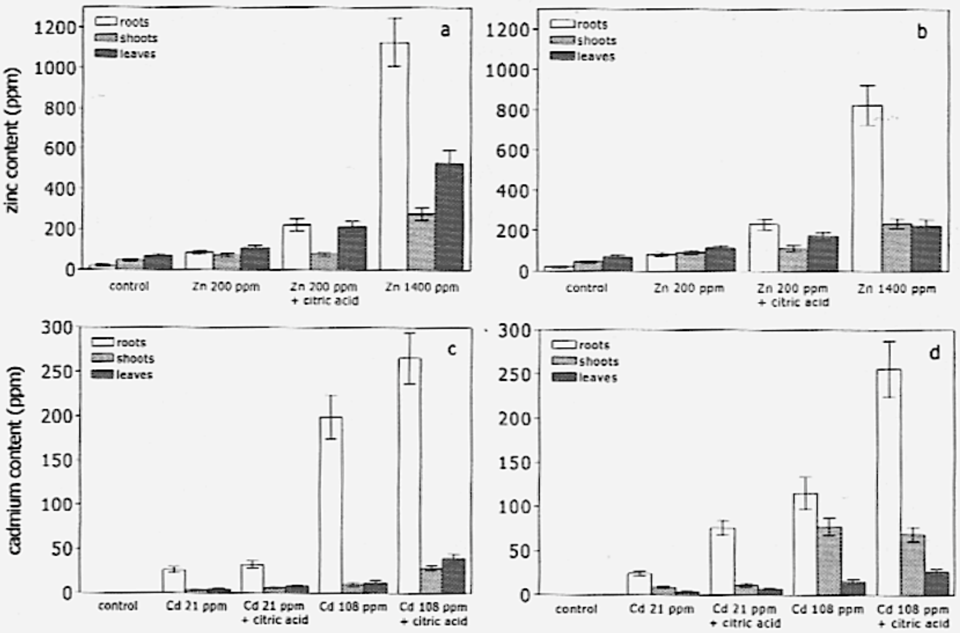

Fig. 2. Zinc and cadmium content (ppm referred to root, shoot and leaf dry weight) in a) and c) *Hyssopus officinalis* and b) and d) *Satureja Montana* at final harvest. Vertical bars indicate ± SD (n=5).