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TOLERANCE OF SOME MEDITERRANEAN CROPS TO COPPER AND ZINC: IMPLICATIONS IN TOXIC METAL CLEAN UP

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A screening of ten different Mediterranean crops was carried out to test their tolerance to soils polluted with copper and zinc. The methods for copper and zinc determination in plants have been improved, avoiding the time consuming acid digestion. Results showed that zinc toxicity was higher than copper for the assayed plants. In copper-polluted soils, *Lupinus luteus* (lupin) 549 $\mu g\, g^{-1}$ and *Zea mays* (corn) 213 $\mu g\, g^{-1}$ showed the highest amounts dried weight of accumulated metal, mainly detected associated to the root system without significant reduction of biometrical parameters of the plants. In zinc-contaminated soils, corn (1304 $\mu g\, g^{-1}$), *Triticum durum* (wheat) (933 $\mu g\, g^{-1}$) and lupin (654 $\mu g\, g^{-1}$) showed the highest amounts of metal accumulated mainly in the roots, and in all cases, without biometrical reduction.

Keywords: Copper; zinc; phytoremediation; heavy metal accumulation; Mediterranean crop species

INTRODUCTION

Many elements get into the food chain through plants and, among them, are heavy metals. Excessive metal concentration is a potential health risk and in soils it can result in decreased soil microbial activity, soil fertility and therefore yield losses (McGrath *et al.*, 1993). Most

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heavy metal content in plants comes through the root uptake from soil. Nemeck *et al.* (1994) found a relative trend of increasing heavy metal content in agricultural crops in the later years, being metals of anthropogenic origin, considered as more available than those from the parent rock (Grupe and Kuntz, 1988).

For various reasons, there is an increasing concern about copper toxicity in agriculture (Tiller and Merry, 1981). These include the high copper contents in soil caused by the long-term use of copper-containing fungicides (*e.g.*, in Spanish vine-yards), industrial and urban activities (air pollution, city waste and sewage sludge) as well as the application of pig and poultry slurries also high in copper.

Zinc contamination has also become a topic of interest in agriculture and crop physiology, since zinc is the heavy metal most commonly found to occur in highest concentrations in most wastes arising from modern, industrialized countries (Boardman and McGuire, 1990).

Removal of these heavy metals from soils is difficult. The existing physical or chemical methods for soil clean-up are expensive. The use of specialised plant species as bioaccumulators, named phyto-remediation, may provide an effective and *in situ* way to remove heavy metal from contaminated soils (Baker *et al.*, 1994).

On the other hand, phyto-remediation development involves developing new methodologies for plant and soil analysis to facilitate the screening of hyper-accumulator species. The existing procedures for heavy metals measurement in plants usually include dry ashing at 450°C and the combustion residue is then leached with dilute acid or digested in strong acid to bring analytes into solution (Cavero *et al.*, 1992). All of these methods are time consuming, losses are difficult to control, and generally sample size required is too large to determine concentrations in the different organs of the plants.

To improve phyto-remediation, the plant species with high metal tolerance must be known and quick and easy methods to measure these metals in plants and soils must be developed. Accordingly, the objectives of the present study were (i) a screening of ten different Mediterranean crops to test their tolerance to copper and zinc separately, and (ii) the development of an easier and reliable sample pretreatment to extract heavy metals from plants with probe sonication followed by atomic absoprtion measurement. This method has been validated for copper and zinc by application in a certified standard reference material (SRM) prior to use in this study.

MATERIAL AND METHODS

Plant Species

The following plant species were selected considering three criteria: (i) plant species that were cited in literature as phyto-accumulators (*i.e.*, Brassicaceae – Knight *et al.*, 1997 as *Raphanus* or *Brassica*), (ii) plant species that grew on soils under continental Mediterranean climate (*Triticum*, *Scolymus*, *Helianthus*), and (iii) considering species of different plant families used as crops (Poaceae, Fabaceae, Solanaceae, Asteraceae). The selected species were: *Triticum durum* (wheat), *Zea mays* (corn), *Lupinus luteus* (Lupin), *Vicia villosa* (vetch), *Helianthus annus* (sunflower), *Cynara sp.* (artichoke), *Raphanus sativus* (radish), *Brassica napus* (rape), *Lycopersicum sculentus* (tomato) and *Cucurbita pepo* (cucumber).

Soil

A common soil from the Iberian peninsular was used for the experiment. Soil was sampled at Montepri\ncipe (Madrid district, coordinates 40°25'N, 3°51'W), from a site dominated by 20 – 40 year old Spanish oak trees (*Quercus ilex* ssp. *ballota*). Soil was taken with a spade from the A horizon (approximately 0 – 10 cm) after careful removal of overlying layers in early February of 1997. Soil was carried to our laboratory and, previous to its use for experiments was dried at room temperature (20°C) and sieved through 2 mm. Physico-chemical characteristics of soil were: pH 6.36, total nitrogen 1 mg g^{-1}, organic carbon 0.84%, N-ammonium 4.05 µg g^{-1}, N-nitrate µg g^{-1}, phosphate 2.5 µg g^{-1}, texture; 29% sand, 30% silt and 41% clay and a cation exchange capacity of 3.31 meq 100 g^{-1} soil.

Development of the Experiment

1 kg of soil was placed in 1.5 l plastic pots. Soil was artificially contaminated with a 100 or 1000 µg g^{-1} copper nitrate solution and at

40% of field capacity. The same procedure was followed for zinc with a zinc nitrate solution. After addition of heavy metal solutions the soil was thoroughly mixed. An untreated control was also used and for all cases, three replicates were prepared.

Seeds of each plant species were kept in darkness at 4°C for one week, in order to optimize germination. After this period, seeds were placed on plastic trays containing moist vermiculite. The 14 days old seedlings were transferred to pots (four per pot).

Plants were grown for three months at 20°C under controlled photoperiod (14 h day). During all the experiment soil moisture was kept at 70% of field capacity gravimetrically, watering daily with Milli-Q water. Pots were closed at the bottom to avoid losses of heavy metals by leaching. Plants were harvested, and used for biometrical measurements: aerial surface (AS), root surface (RS), aerial length (AL), root length (RL) and dry weight (DW). Biometrical parameter study (AS, RS, AL, and RL) was carried out with a Delta-*T* image analysis system with the DIAS software. After biometrical determination plants were pulverized for heavy metal content analysis.

Analytical Methods

Standards

Spinach leaves were SRM 1570-a from NIST. Nitric acid (HNO_3) and pure standards for spectrometry were from Carlo Erba. Solvents were purchased from Scharlau and other reagents from Panreac.

Plant Treatment

For zinc determination: 10 ml of 1% nitric acid were added to 0.1 g of dried plant weighed in 50 ml centrifuge tubes. Samples were probe sonicated (DR. HIELSCHER UP.200) for 5 min. and centrifuged (HERAEUS, MEGAFUGE 1.0) at 2500 cursive for 10 min. Measurements were performed by atomic absorption spectrometry (PERKIN ELMER 3100). For copper determination: 10 ml of nitric acid 1:9 (v/v) were added to 0.5 g of dried plant weighed exactly in 50 ml centrifuge tubes and the rest was as above.

Validation

Six different samples of the SRM spinachs were subjected to the complete treatment described above and the results were compared statistically with the certified values for zinc and copper by using the Student's *t* test; and there were no significant differences in the variances.

Soils

Sequential extraction of plant-available metal species, or at least of those species that are correlated with plant content or plant uptake, was the selected procedure, because despite the initial treatment of soils with soluble salts, changes could occur during growth. The procedure of Tessier *et al.* (1979) (Fig. 1) the most widely used

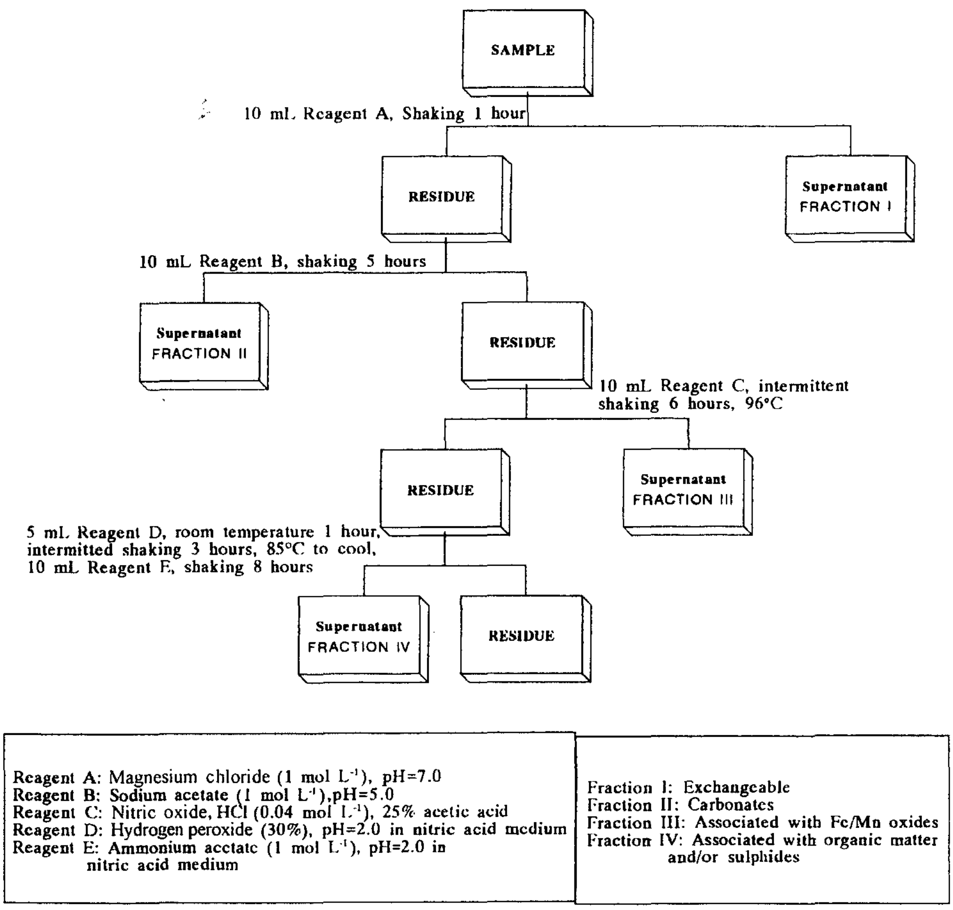

FIGURE 1 Tessier scheme for sequential fractionation of metals in soils.

speciation scheme in the literature (Ramos *et al.*, 1994) was followed, but avoiding the last step using hydrofluoride/hydrochloric acid for silicate bound measurement, since only the plant-available fractions matter. For testing recoveries in the sequential extractions in soil, 100 and 1000 $\mu g\ g^{-1}$ of copper or zinc were added and assayed.

Statistics

For biometrical parameters and plants heavy metal content, one-way ANOVA were used to assess differences between treatments and between plant species. Since homogeneity of variances in the plant, heavy metal contents were not found; Friedman analysis of variance were carried out in all comparistions.

RESULTS

Tolerances varied depending on the heavy metal and plant species. Results showed that zinc toxicity was higher than copper in the assayed conditions. These differences were detected both in the number of tested species that were able to survive in a soil contaminated with each metal, and in the variations of the biometrical parameters considered.

Four of the ten plant species tested were able to survive with 100 $\mu g\,g^{-1}$ of copper and 100 $\mu g\,g^{-1}$ of zinc: vetch, wheat, corn, and lupin but only wheat did it at 1000 $\mu g\,g^{-1}$. Sunflower and tomato succeeded to survive with 100 $\mu g\,g^{-1}$ copper, but the number of replicates was too small to consider it in the following study. Biometrical parameters showed a clear difference between the two heavy metal effects.

In the copper-treated plant species, 3 out of the 4 surviving species showed higher values in all the biometrical parameters than the control plants. Vetch plants grown in 100 $\mu g\,g^{-1}$ copper-polluted soil showed a significant ($p < 0.05$) increase in all biometrical parameters except in root length and aerial root length (Fig. 2). Only wheat plants were able to grow in a 1000 $\mu g\,g^{-1}$ copper-polluted soil. In fact, biometrical parameters showed higher values in 100 $\mu g\,g^{-1}$ than in controls and differences were statistically significant ($p < 0.05$). On the other hand, plants grown in 1000 $\mu g\,g^{-1}$ copper-polluted soil showed lower biometrical parameters than in 100 $\mu g\,g^{-1}$ copper (Fig. 3). Corn

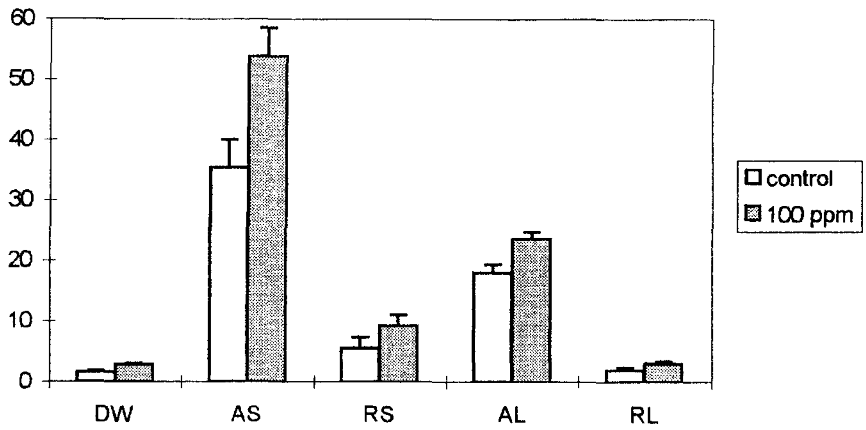

FIGURE 2 Biometrical parameters of vetch plants grown in 100 $\mu g\,g^{-1}$ copper polluted soil and control plants. AS: aerial surface (cm^2); RS: root surface (cm^2); AL: aerial length (cm); RL: root length (cm); DW: dry weight (g). DW and RS values appear multiplied by ten. Error bars represent SE; $n = 4$.

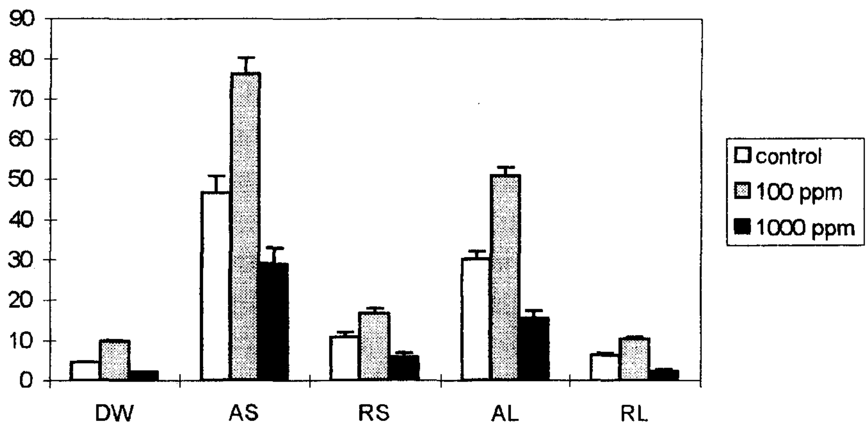

FIGURE 3 Biometrical parameters of wheat plants grown in 1000 and 100 $\mu g\,g^{-1}$ copper polluted soil and control plants. AS: aerial surface (cm^2); RS: root surface (cm^2); AL: aerial length (cm); RL: root length (cm); DW: dry weight (g). DW values appear multiplied by ten. Error bars represent SE; $n = 4$.

plants grown in 100 $\mu g\,g^{-1}$ copper soil, showed higher values in all the biometrical parameters considered than control plants, but differences were not statistically significant on aerial length and root length. No differences were detected when the ratios AS/RS and AL/RL were compared between control plants and in contaminated soil plants (Fig. 4). Moreover, lupins were not affected by the copper soil treatment as the biological parameters show (no statistical differences were detected). Comparing AS/RS and AL/RL ratios, non-significant differences were

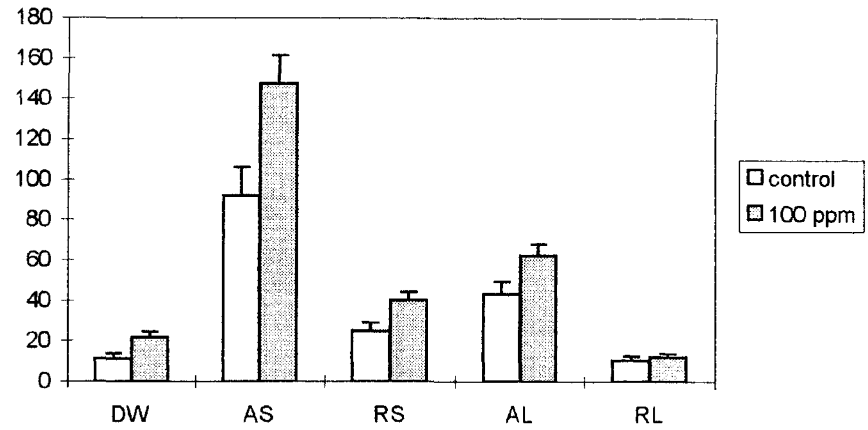

FIGURE 4 Biometrical parameters of corn plants grown in 100 μg g^{-1} copper polluted soil and control plants. AS: aerial surface (cm^2); RS: root surface (cm^2); AL: aerial length (cm); RL: root length (cm); DW; dry weight (g). DW values appear multiplied by ten. Error bars represent SE; $n = 4$.

detected between control plants and those grown in contaminated soils (Fig. 5).

Zinc toxicity seemed to be higher than copper. Only 4 of the 10 plant species tested were able to grow in 100 μg g^{-1} zinc-polluted soils, and all biometrical parameters of that species were lower than those in unpolluted soil. Vetch plants (Fig. 6) grown in 100 μg g^{-1} zinc-polluted soil showed statistical differences in biometrical parameters with controls, except for root length ($p < 0.05$). As far as wheat plants are concerned (Fig. 7) a significant decrease ($p < 0.05$) was detected in all biometrical parameters as compared to control plants except for dry weight. That inhibition was especially marked in the root system length, as AL/RL ratios showed: 4.76 in controls and 6.03 in 100 μg g^{-1} zinc-polluted soils. Corn plants grown in 100 μg g^{-1} zinc-polluted soil (Fig. 8) showed smaller values in biometrical parameters (except for root length) than untreated plants ($p < 0.05$) and those grown in 100 μg g^{-1} copper-polluted soil. The corn plants growing in 100 μg g^{-1} zinc soil showed a reduction in the root system length (AL/RL in controls 4.3, AL/RL in 100 μg g^{-1} zinc added 5) and an increase in the root system surface as compared to control plants (SA/SR in controls 3.7, SA/SR in 100μg g^{-1} zinc added 2.6). Lupin plants grown in 100 μg g^{-1} zinc- or in control soil did not show statistical differences in any biometrical parameter (Fig. 9).

As regards to analytical methods (Tab. I) there was no statistical significant differences ($p < 0.05$) between certified and found copper

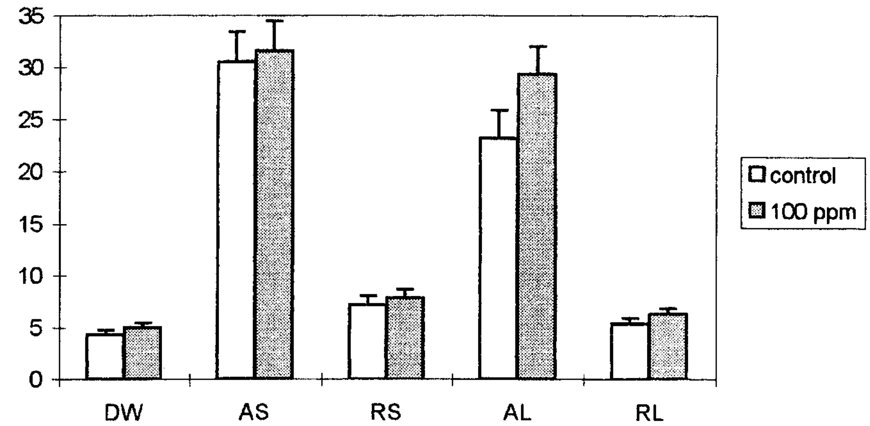

FIGURE 5 Biometrical parameters of lupin plants grown in $100\,\mu g\,g^{-1}$ copper polluted soil and control plants. AS: aerial surface (cm^2); RS: root surface (cm^2); AL: aerial length (cm); RL: root length (cm); DW: dry weight (g). DW values appear multiplied by ten. Error bars represent SE; $n = 4$.

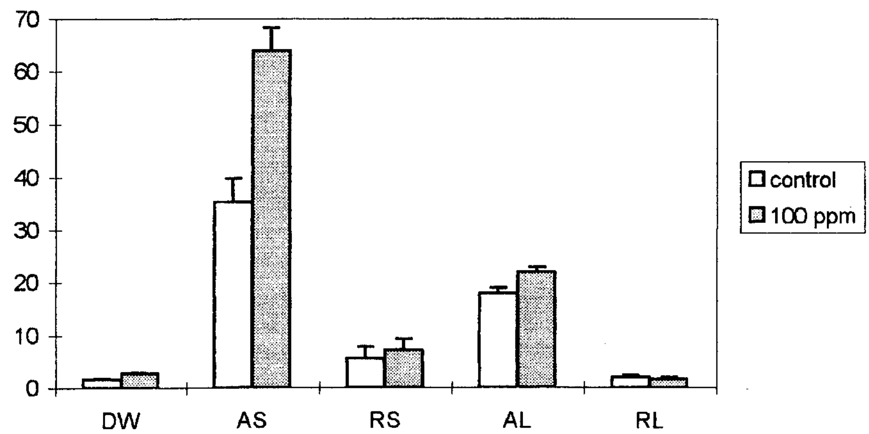

FIGURE 6 Biometrical parameters of vetch plants grown on $100\,\mu g\,g^{-1}$ zinc polluted soil and control plants. AS: aerial surface (cm^2); RS: root surface (cm^2); AL: aerial length (cm); RL: root length (cm); DW: dry weight (g). DW and RS values appear multiplied by ten. Error bars represent SE; $n = 4$.

content in the spinach reference material employing the sonication method instead of calcination. Although for zinc, the statistical study with the Student t-test, showed a significant difference ($p < 0.05$) between the result and 100%, and values can be considered as very good recoveries for this method. So, the proposed method for copper and zinc analysis in plants can be considered reliable. Moreover, Table II shows copper and zinc ions total recovery from the sequential

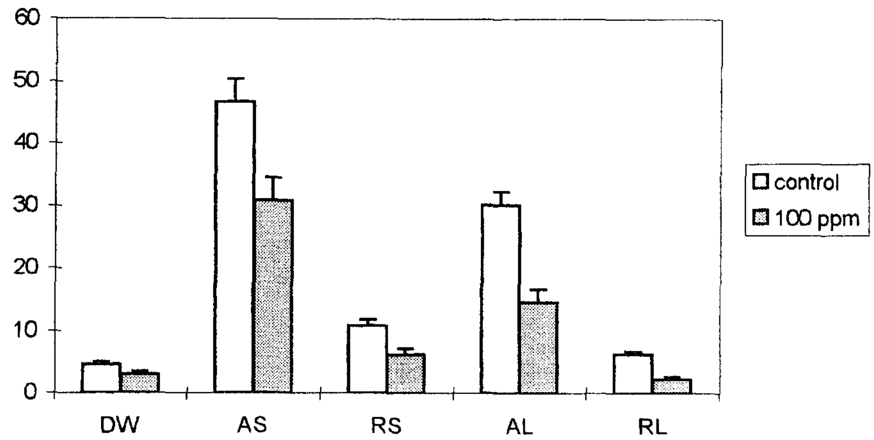

FIGURE 7 Biometrical parameters of wheat plants grown in $100\,\mu g\,g^{-1}$ zinc polluted soil and control plants. AS: aerial surface (cm^2); RS: root surface (cm^2); AL: aerial length (cm); RL: root length (cm); DW: dry weight (g). DW values appear multiplied by ten. Error bars represent SE; $n = 4$.

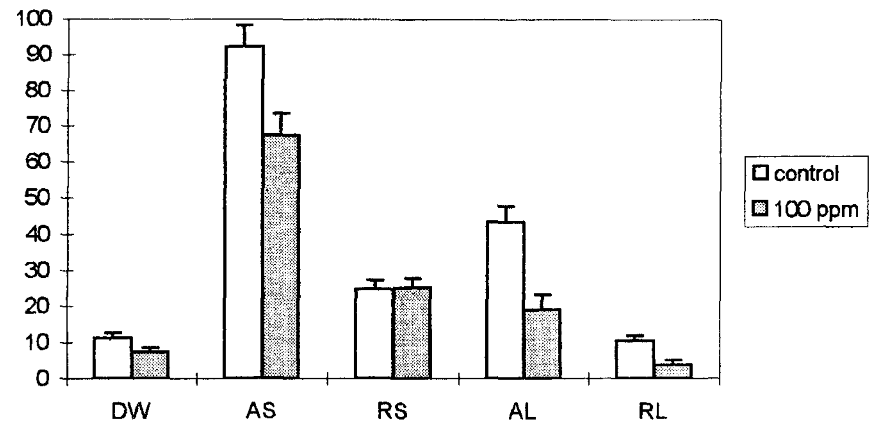

FIGURE 8 Biometrical parameters of corn plants grown in $100\,\mu g\,g^{-1}$ zinc polluted soil and control plants. AS: aerial surface (cm^2); RS: root surface (cm^2); AL: aerial length (cm); RL: root length (cm); DW: dry weight (g). DW values appear multiplied by ten. Error bars represent SE; $n = 4$.

extraction method in soils. As could be expected, the total ion added was recovered in both cases and in the two contamination levels, but distributed in different fractions.

The heavy metal content in the plant species considered was quite different depending on the heavy metal considered. Copper contents on tested plants appear on Table III. Statistical differences ($p < 0.05$) were found in all the plant species considered between plants grown in

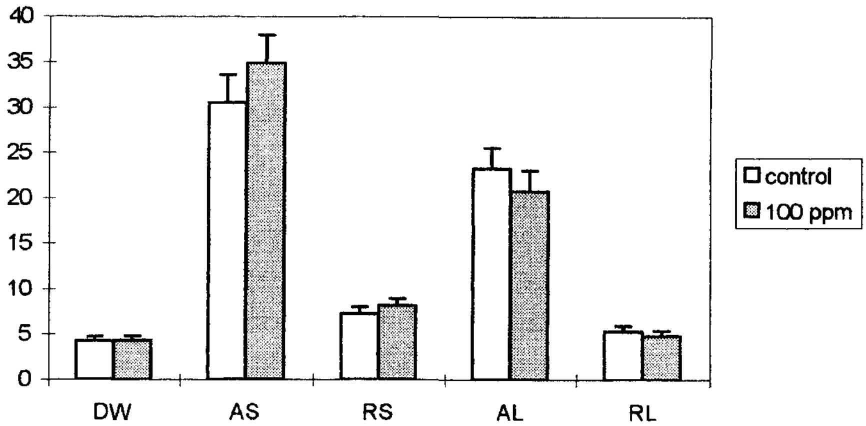

FIGURE 9 Biometrical parameters of lupin plants grown in 100 $\mu g\,g^{-1}$ zinc polluted soil and control plants. AS: aerial surface (cm^2); RS: root surface (cm^2); AL: aerial length (cm); RL: root length (cm); DW: dry weight (g). DW values appear multiplied by ten. Error bars represent SE; $n = 4$.

TABLE I Recoveries of copper and zinc with the sonication method in spinach leaves ($n = 6$)

Concentration of Cu	($\mu g\,g^{-1}$)	*Concentration of Zn*	($\mu g\,g^{-1}$)
Certified material	Found	Certified material	Found
12.2 ± 0.6	11.1 ± 0.6	82.0 ± 3.0	80.1 ± 0.9

TABLE II Recoveries of copper and zinc from soils with the sum of fractions in the sequential extraction method ($n = 3$). Concentration in $\mu g\,g^{-1}$

	Concentration assayed	*Concentration found*	*Recovery* (%)
Zinc	100	98 ± 9	98 ± 9
	1000	957 ± 27	96 ± 3
Copper	100	97 ± 3	97 ± 3
	1000	952 ± 43	95 ± 4

polluted and control soils. Lupin plants showed higher concentrations of copper: 207 times over untreated lupin plants and no reduction in biometrical parameters as pointed out previously. Vetch plants showed lower levels of copper than lupin (10.95 times over untreated plants) while wheat plants grown in 100 $\mu g\,g^{-1}$ copper-polluted soil showed 22.6 times more copper than control ones with a low percentage of heavy metal content on aerial part of the plants (3.6%). Corn plants had 42.6 times more copper than their controls.

TABLE III Copper content of plants ($n = 4$)

	Control			*100 $\mu g\,g^{-1}$ Cu*					
	AP[a]	*RS*[a]	*Total*[a]	*AP*[a]	*RS*[a]	*Total*[a]	[*P*]/[*C*][b]	%*AP*[c]	%*RS*[d]
Wheat	6.6 ± 0.6	9.5 ± 0.3	7.2 ± 0.7	18 ± 3	489 ± 17	163 ± 19	22.63	3.64	96.36
Corn	5.6 ± 0.7	3.6 ± 0.7	5.0 ± 0.6	15 ± 3	395 ± 25	213 ± 12	42.60	3.65	96.35
Lupin	na	na	2.6 ± 0.1	na	na	540 ± 37	207.69	na	na
Vetch	na	na	6.7 ± 0.5	na	na	73 ± 5	10.95	na	na

AP: aerial part; RS: root system.

[a] units $\mu g\,g^{-1}$;

[b] ratio total copper in polluted samples [P] and copper in controls [C];

[c] percentage of total copper in aerial part;

[d] percentage of total copper associated to root system.

na: data not available.

These plants did not differ statistically in biometrical parameters and the larger percentage of heavy metal is associated to the root system (96.35%), as is the case of wheat.

Zinc content in plants appear in Table IV. As happened with copper, plants showed statistically significant highest amounts of heavy metal when growing in a polluted soil than when growing in control soil. The highest amounts of zinc were found in corn plants (28.3 times more in plants grown on polluted soil) and lupin (12.3 times). In case of corn, the percentage of heavy metal content in the aerial part in relation to that associated to the root system was 33.1: 66.9, whereas it was 11.4: 88.5 in lupin. Moreover, it is important to remark that corn plants were bigger, which means a higher total amount of heavy metal. Vetch plants showed a concentration quite similar to wheat (2.3 and 3.8 times more than plants of each respective species grown in control soil). Wheat plants accumulated heavy metals quite differently than the other plant species tested: 11.4% in the aerial part and 88.5% on the root system.

Regarding the total content of zinc and copper in the treated soils (Tab. V) main trends in the results indicate that the number, size of the plants and time of plantation have not been enough to show a decrease of the metals, since the weight of the plants in relation to that of the soil was very small. It is necessary to highlight the effect of lupin and corn, those plant species that had shown better remediation capacity. Lupin produced a significant decrease for copper in polluted soils and corn did not reach different statistical methods with controls, but mainly due to the error size. These values are in agreement with higher copper content in lupin and corn plants growing in polluted soils in relation with controls than in other plants. These preliminary results would guide later works allowing a bigger growth of the plants for more notorious effects.

Considering the different studied fractions (Tab. V), it was possible to see that in polluted soils where the above cited plant species were grown, heavy metal content (exchangeable) in fraction I decreased from the start of the experiment to harvest time. It must be noted that although both metals were added as soluble salts, copper was extracted mainly in fractions II and IV, associated to carbonates and to organic matter, while zinc remained mainly in fraction I (exchangeable) in control soils, but it increased in fractions II and III in soils with plants growing in it. As a general trend, fraction III for both metals (associated with iron or manganese oxides) increased in soils with plants growing in them, in

TABLE IV Zinc content of plants ($n = 4$)

	Control			100 $\mu g\, g^{-1}$ *Zn*					
	AP[a]	*RS*[a]	*Total*[a]	*AP*[a]	*RS*[a]	*Total*[a]	[*P*]/[*C*][b]	%*AP*[c]	%*RS*[d]
Wheat	189 ± 36	311 ± 33	242 ± 13	763 ± 90	1860 ± 101	933 ± 63	3.87	29.08	70.92
Corn	55 ± 1	35 ± 5	46 ± 3	938 ± 76	1892 ± 117	1304 ± 106	28.34	33.14	66.86
Lupin	43 ± 5	86 ± 6	53 ± 5	313 ± 81	2419 ± 134	654 ± 58	12.33	11.45	88.55
Vetch	na	na	146 ± 15	na	na	393 ± 25	2.32	na	na

AP: aerial part; RS: root system.
[a] units $\mu g\, g^{-1}$;
[b] ratio total zinc in polluted samples [P] and zinc in controls [C];
[c] percentage of total zinc in aerial part;
[d] percentage of total zinc associated to root system.
na: data not available.

TABLE V Zinc and copper content ($\mu g\ g^{-1}$) in different fractions and total in soils ($n = 4$)

Copper	*Fraction I*	*Fraction II*	*Fraction III*	*Fraction IV*	*Total*	[a]
Control Soil	< 0.1	46.1 ± 0.6	5.8 ± 0.4	44 ± 4	**96 ± 4**	ns
Lupin Soil	< 0.1	26 ± 6	6 ± 4	21 ± 15	**53 ± 25**	*
Corn Soil	1.14 ± 0.06	34 ± 2	18.1 ± 0.8	41 ± 8	**95 ± 10**	ns
Wheat Soil	< 0.1	8 ± 7	15 ± 6	44 ± 5	**68 ± 12**	ns
Vetch Soil	< 0.1	16 ± 6	16 ± 6	57 ± 11	**92 ± 9**	ns
Zinc						
Control Soil	85 ± 9	11 ± 2	3.1 ± 0.2	< 0.05	**99 ± 8**	ns
Lupin Soil	52 ± 4	26 ± 4	8.4 ± 0.5	< 0.05	**86 ± 6**	ns
Corn Soil	67 ± 3	28 ± 3	2.9 ± 0.2	< 0.05	**98 ± 7**	ns
Wheat Soil	64 ± 3	25 ± 2	6 ± 1	< 0.05	**95 ± 5**	ns
Vetch Soil	50.8 ± 0.6	33.8 ± 0.7	19 ± 4	< 0.05	**104 ± 5**	ns

[a]Statistical significance:
*$p < 0.05$;
ns = non significant;
Fraction I: Exchangeable;
Fraction II: Carbonates;
Fraction III: Associated with Fe/Mn;
Fraction IV: Associated with organic matter and /or sulphides.

relation with control soils. Fraction IV (complexed with organic matter and/or sulphides) showed amounts of both heavy metal were almost constant in the two sampling times.

DISCUSSION

Studies on phyto-remediation in Mediterranean soils are scant, mainly using crop plant species instead of hyper-accumulators (in the way proposed by Baker and Brooks, 1989) such as *Thlaspi caerulescens* (Reeves and Brooks, 1983). Information about this topic is extremely important in order to design strategies to remove heavy metal from contaminated soils in this area of the world, or to switch the way of soil exploitation focusing on this problem. It should be stressed that the interest of the impact nowadays, suffering problems as the effects of an uncontrolled leak of 5000 tons of sludge containing metalliferous mining residues, occurred recently in the south of Spain, close to a Natural Reserve (Parque Nacional de Doñana).

The proposed analytical method for copper and zinc extraction in plants has proved to be accurate and simpler than calcination, while the fractionation of metal contents in soils gave good total recoveries and permitted more information on the metal evolution than a simpler extraction.

Our results showed the well-known fact that zinc is more toxic to plants than copper (Schat *et al.*, 1997). In that sense, plant species that did not survive on either polluted soil showed the characteristic chlorosis on leaves due to an iron deficiency (Bergman, 1988; Woolhouse, 1983) and in case of copper, the high metal concentration could also result in higher lipid peroxidation and thus, a destruction of thylakoid membranes (Matoo *et al.*, 1986).

The amounts of copper found in plants ranged from $73\,\mu g\,g^{-1}$ in vetch to $540\,\mu g\,g^{-1}$ in lupin. These values are larger than those found by other authors in plants grown under a multiple heavy metal pollution, where copper is present. Daniel *et al.* (1997) found levels varying between 2.9 to $27\,\mu g\,g^{-1}$ studying 28 different wild plant species. That difference with our results could be due to: (i) the way that those plant species use to take heavy metals, (ii) the possible interaction between metals on the multiple contamination, and (iii) the complexity of a mono-specific system. It is striking that these same authors found the highest amounts of copper in plants belonging to the Compositae family (*e.g.*, *Cinchorium inthybus*, *Crepis biennis*) while in the present study precisely the plants of this family (*Helianthus annus*, *Cynara sp.*) could not survive in copper-polluted soils. These contradictory results may be explained as above.

As biometrical parameters show, lupin was the plant species least affected by the maximum copper content. It is interesting to point out that lupins showed root nodules, that were not present in vetch. Thus, nitrogen nutrition cannot be ruled out as the reason of differences between growth and copper content in these plant species, since lupin certainly had a nitrogen supply. Nevertheless, it may be possible that lupin plants have metal sequestration mechanisms that change rhizosphere pH, as McGrath *et al.* (1997) reported recently. These authors suppose that shifts on rhizosphere pH increase mobile heavy metal forms. In this sense, Lucas *et al.* (1998) and Cieslinski *et al.* (1998) found organic acids exuded by four different lupin species and wheat that could be related with this effect. Nevertheless the mechanisms that involve changes on rhizosphere pH and metal sequestration are not actually clear, and will probably depend on the plant species and the type of heavy metal, since no pH fluctuations have been detected in the rhizosphere of some hyper-accumulators such as *Alyssum murale* or *Thlaspi sp.* (McGrath *et al.*, 1997).

Corn plants also showed high levels of copper ($213\,\mu g\,g^{-1}$) without statistical significant decreases on biometrical parameters, specially root growth, which is a clear indicator of copper toxicity (Lexmond and Vorm, 1981). It has been reported that in maize, the synthesis of cystine-rich phytochelants is induced by supplying high concentrations of cadmium and also to a less extent by copper (Tukendorf and Rauser, 1990; Keltjens and Beusichem, 1998); considering our results, corn plants must not have any mechanism to translocate copper to aerial parts of the plant, since 96.3% of total metal content was found in the root system.

In the case of zinc, the amounts of that this metal found in plants ranged from $339\,\mu g\,g^{-1}$ in vetch to $1304\,\mu g\,g^{-1}$ in corn. These amounts are smaller than those found on hyper-accumulators such as *Thlaspi caerulescens* (McGrath *et al.*, 1997) in which levels ranging between 3100 to $8100\,\mu g\,g^{-1}$ have been reported. Considering the results reported by Daniel *et al.* (1997), the same considerations as for copper may be pointed out. These authors found, in a study on a multiple heavy metal contaminated soil, zinc concentrations range from 43 to 68 $\mu g\,g^{-1}$ in wild plant species.

As discussed previously, the Poaceae family plant species assayed in the present study (corn and wheat), showed the highest levels of zinc. Boruvka *et al.* (1997) found that *Poa sp.* and *Festuca sp.* plants exposed to cadmium, lead and zinc pollution, showed higher amounts of these metals in the root system (as in the present work) than in the shoot. Values found by these authors were 147 $\mu g\,g^{-1}$ in the aerial part and around 1700 $\mu g\,g^{-1}$ in the root system.

As explained above, corn plants showed the highest amounts of zinc, mainly found in roots (around 66%). However, an increase in the root system was detected compared to control plants which is the opposite response to zinc toxicity (Godbold *et al.* 1993). On the contrary, and consistent with the cited authors, wheat plants showed a larger root system. The response found in corn could be explained as a strategy of these species consisting of a greater development of the root system to be able to explore more soil to find "clean portions".

CONCLUSIONS

Plants respond to elevated concentrations of heavy metals in soils with two basic strategies: (i) exclusion mechanisms, where plants avoid excessive uptake and transport of metals, and (ii) accumulation and sequestration mechanisms, where more or less large amounts of metals are taken and translocated to plant shoots (Baker, 1981). In both mechanisms, root exudates seem to play an important role, since they could change chemical species of the heavy metals in soil, thus faciliting their mobility (McGrath *et al.*, 1997; Knight *et al.*, 1997; Lorenz *et al.*, 1997). This is one of the reasons that makes it of interest of deeper knowledge of the exudation process.

Another interesting point related to plant mechanisms involved in the response to elevated levels of heavy metals and strategies related to phyto-remediation is how metals distribute in the plant. The classical strategies for remediation by plants are based in those plant species able to translocate heavy metals mainly to aerial parts, in order to pick up metals in harvesting. In this way, excellent accumulators (hyper-accumulators) such as *Thlaspi caerulescens* have the size problem: those plants are too small for a good harvest. An alternative could be to use plant species that accumulate heavy metals in roots. Plants able to survive in polluted soils, without a decrease in biometrical parameters (tolerant plants) will also fit this goal. With those plants, the strategy to follow for soil recuperation would be different: production of plants free of heavy metals (at least to sub-toxic levels), that could be used for consumption. It could be the case of corn, wheat or lupin in Mediterranean soils polluted with zinc or copper.

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