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# HEAVY METAL AVAILABILITY IN *PELARGONIUM HORTORUM* RHIZOSPHERE: INTERACTIONS, UPTAKE AND PLANT ACCUMULATION

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□ *The rhizosphere is a key area for the plant metal uptake. We studied heavy metal availability in the rhizosphere and the bulk soil, the interactions between metal ions, and their effects on heavy metal uptake and accumulation by* Pelargonium hortorum*s (geranium). A pot experiment with plants of geranium was conducted in a soil spiked with cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn) singly or in combinations. Bulk soils showed higher concentrations of extractable metals than rhizosphere soils, and metals accumulated preferentially in roots relative to aerial biomass. Regression analysis showed that soil extractable Cr, Ni and Pb were related ($R^2 = 0.90$) to their concentration in plants, but there was no correlation between soil and plants for Cd, Cu, and Zn. Larger concentrations of metals were found when they were added in combinations rather than individually, and availability and uptake were directly related to the level of metals applied.*

**Keywords:** heavy metal availability, heavy metal interactions, bulk soil, rhizosphere soil, *Pelargonium hortorum*

## INTRODUCTION

The importance of the rhizosphere in plant nutrition has been known since the beginning of the 20th century (Hinsinger et al., 2003). The rhizosphere is defined as the volume of soil distinct from the bulk soil, which is influenced by root activity (Hinsinger, 1998). Acidification and release of root exudates are two common mechanisms by which plants modify the rhizosphere. The rhizosphere has an important influence on the availability or solubility of nutrients such as phosphorus, as well as on the availability of heavy metals (Marschner et al., 1987).

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Some studies have indicated that there is a higher concentration of available heavy metal forms in the rhizosphere than in the bulk soil (Wang et al., 2002). One of the most important factors controlling the availability and plant uptake of heavy metals is soil pH. Roots are responsible for substantial changes in the pH of the rhizosphere as a result of the differential uptake rates of cations and anions (Haynes, 1990; Hinsinger, 2001). In order to maintain their charge balance, roots release protons whenever they take up more cations than anions, and take up protons when the opposite occurs (Hinsinger et al., 2003).

On the other hand, the levels of metals found in plants are often correlated with the levels present in the environment (Vesk and Allaway, 1997). Accordingly, the concentration of heavy metals in the soil solution is another very important factor that regulates the absorption of nutrients and heavy metals by plants (Cancès et al., 2003). The determination of bioavailable metals is probably more significant than the analysis of total concentration, that is why the former allows a general better prediction of the risk of metal uptake by plants and its mobility in the system (Pueyo et al., 2004). The uptake and accumulation of heavy metals have been studied in several plant species. Ornamental plants have even been proposed as suitable for phytoremediation in urban areas. KrishnaRaj et al. (2000) and Arshad et al. (2008), for instance, assessed the capacity of scented geraniums (*Pelargonium* sp.) to tolerate and accumulate heavy metals, whereas Orroño et al. (2009) found that *Pelargonium hortorum* showed better tolerance to heavy metals than other *Pelargonium* species.

The main source of heavy metals in soils is the anthropogenic pollution, which is usually not limited to a single pollutant. Several metals may be present together in potentially toxic concentrations in the soil. For example, Yoon et al. (2006) and Kabala and Singh (2001) found areas with high concentrations of several metals [mainly copper (Cu), lead (Pb) and zinc (Zn) together]. In other locations, cadmium (Cd), chromium (Cr) and nickel (Ni) have been reported together in contaminated urban soils (Lavado et al., 1998).

When a plant is exposed to more than one heavy metal, interactions between them may occur both at the root surface, affecting uptake, and within the plant, affecting translocation and toxicity (Pahlsson, 1989). Basically, when two or more elements coexist in the soil, plant uptake of heavy metals is subjected to the antagonistic, additive and synergetic effects that they exert on one another (Ernst and Nelissen, 2000). For example, Cd, Cu, Pb and Zn show a wide range of interactions. Cadmium interacts with Cu and Zn at the root surface, whereas Pb interacts with Zn and Cd within the plant. Some of these interactions are antagonistic (Zn with Cu and Cd), whereas others are synergistic (Cd with Zn at the root surface, Pb with Cd within the plant) (Kabata-Pendias and Pendias, 2000). Although interactions between metals are commonly observed, they are often complex and contradictory, and have been rarely reported in the rhizosphere. Thus, the aims of this work were to study heavy metal availability in the rhizosphere as compared to that in the bulk soil, the interactions between those metal ions, and heavy metal uptake and accumulation by *Pelargonium hortorum* plants.

## MATERIALS AND METHODS

A pot experiment was conducted in a greenhouse using the A horizon of a Typic Argiudoll as a substrate, spiked with Cd, Cr, Cu, Pb, Ni, and Zn. The heavy metals were applied to the soils at two concentration levels called hereafter as the medium level (ML) and the high level (HL) treatments. A completely randomized design was performed with 13 treatments and two replications per treatment (in the case of single metals) and three replications per treatment (in the case of multiple heavy metal application). Treatments were: i) T0, a Control, non-spiked soil; ii) T1, soil spiked with the six heavy metals together at the ML concentration; iii) T2, soil spiked with the six heavy metals together at the HL concentration; iv) T3, three heavy metals together (Cu, Pb, Zn) at the ML concentration; v) T4, three heavy metals together (Cu, Pb, Zn) at the HL concentration; vi) T5, three heavy metals together (Cr, Cd, Ni) at the ML concentration; vii) T6, three heavy metals together (Cr, Cd, Ni) at the HL concentration; viii) T7, soil spiked only with Cd at the HL concentration; ix) T8, soil spiked only with Cr at the HL concentration; x) T9, soil spiked only with Cu at the HL concentration; xi) T10, soil spiked only with Pb at the HL concentration; xii) T11, soil spiked only with Ni at the HL concentration; and xiii) T12, soil spiked only with Zn at the HL concentration. The concentrations used were based on the results of previous experiments (Orroño and Lavado, 2009b; Orroño et al., 2009). The quantity of the salts applied to the soil in the ML treatment to reach the final concentrations found in Table 1 were 0.027 g cadmium nitrate $kg^{-1}$ soil, 0.57 g chromic acid $kg^{-1}$ soil, 0.54 g copper chloride $kg^{-1}$ soil, 0.32 g nickel sulfate $kg^{-1}$ soil, 0.80 g lead nitrate $kg^{-1}$ soil and 1.33 g zinc sulfate $kg^{-1}$ soil, whereas the HL treatments received twice the quantity of the same salts. The bioavailability of metals added to the soil as salts usually differs from the bioavailability of native metals (Basta et al., 2005). Then, spiked soils were subjected to wet/dry cycles for three months prior to the onset of the experiment to allow the metals to react with the soil components and become incorporated into the soil matrix.

A rhizopot of 16 cm in height and 14.5 cm in diameter as that designed by Silva Gonzaga et al. (2006) was used to grow the plants. A central compartment (10 cm in height and 7.5 cm in diameter) of nylon cloth (mesh size 45 um) was used to separate the rhizosphere from the bulk soil. Root

**TABLE 1** Heavy metal application rates of the experimental treatments

| | HM Application Rate (mg kg$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| Treatment | Cd | Cr | Cu | Pb | Ni | Zn |
| T0 | 3.8 | 5.4 | 18.0 | 26.9 | 14.7 | 35.9 |
| T1 | 10 | 250 | 200 | 500 | 80 | 300 |
| T2 | 20 | 500 | 400 | 1000 | 160 | 600 |
| T3 | | | 200 | 500 | | 300 |
| T4 | | | 400 | 1000 | | 600 |
| T5 | 10 | 250 | | | 80 | |
| T6 | 20 | 500 | | | 160 | |
| T7 | 20 | | | | | |
| T8 | | 500 | | | | |
| T9 | | | 400 | | | |
| T10 | | | | 1000 | | |
| T11 | | | | | 160 | |
| T12 | | | | | | 600 |

growth was limited to the central compartment (500 g soil) within the nylon cloth.

One healthy plant of similar size was carefully transplanted into each pot, at the beginning of the experiment. The pots were then arranged in a completely randomized design. Plants were watered every two or three days throughout the study to keep the water content near to field capacity. After 16 weeks all plants were harvested, washed with deionized water, and dried at 70°C for 24 h. Plant biomass was divided into stems, green leaves, dead leaves, flowers and roots. Rhizosphere soil (i.e., that in the plastic net, filled with fine roots), and bulk soil (i.e. that outside the central compartment) were collected (Figure 1). Both soils were analyzed for plant-available exchangeable heavy metal forms, by shaking 3 g of air-dried soil with 20 mL of 0.1 M calcium chloride ($CaCl_2$) for 16 h. The pH value was determined in a soil:water suspension (1:2.5) after shaking, and electrical conductivity (EC) was measured on saturation paste extracts.

Statistical analyses in the rhizosphere and bulk soils were conducted using SAS version 8.2 (SAS Institute, Cary, NC, USA). When data were not normally distributed, log transformations were used and the PROC MIXED procedure was used for two-way ANOVA to determine the significance of the main factors (treatments and rhizosphere versus bulk soil) and their interactions. Data on plant heavy metal concentrations were subjected to one-way ANOVA and comparisons between means were made using Tukey's test at a 0.05 level of significance. Data were log-transformed to achieve normality. Single correlation analysis was performed and Pearson's correlation coefficient was undertaken in order to assess the relationship between metals in the soil solution and metal uptake by plants.

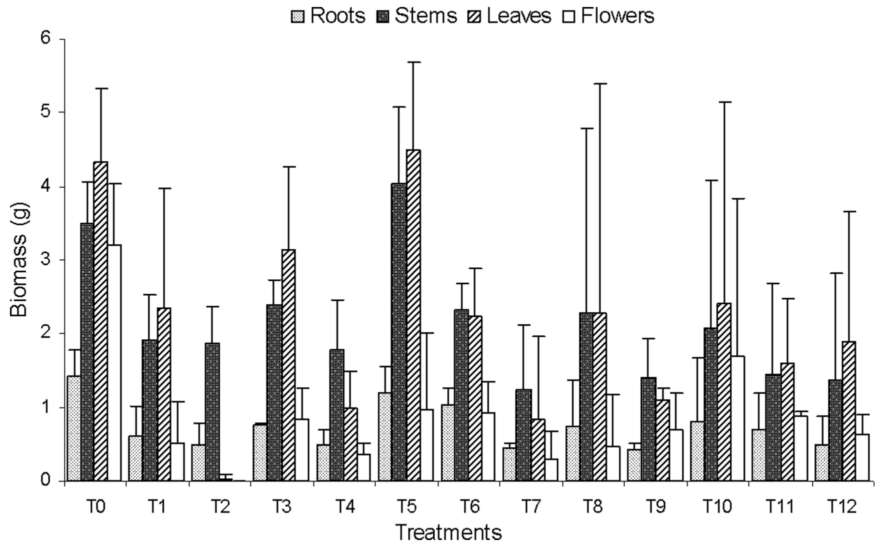


**FIGURE 1** Roots, stems, leaves and flower biomass of *Pelargonium* plants at harvest. Values are means ± standard deviations.

## RESULTS

Total plant biomass at harvest (Figure 1), was significantly affected by treatments and was highly affected with increase in heavy metal concentration. Exposure to heavy metals caused a significant decrease in aerial dry mass, especially leaves and flower dry matter production, but generally had no significant effect on root mass.

Figure 2 shows the concentrations of the $CaCl_2$-exchangeable metals in the rhizosphere and bulk soils. In some treatments (T2 and T4), the extractable heavy metals content in the rhizosphere after harvest differed significantly from that in the bulk soil. Treatment and soil interactions were significant for Cd, Ni and Zn (Figures 2A, 2D and 2F), which indicated that treatments had a different behavior in the rhizosphere and bulk soils. Consequently, it was not possible to evaluate the factors "treatment" and "soil" separately. In contrast, Cr, Cu and Pb showed significant effects only in their extractable forms, which implies that these metals had the same availability in the rhizosphere and bulk soils (Figures 2 B, 2C, and 2E).

Table 2 shows the variations in soil pH and EC between the different treatments after the growth and harvest of *P. hortorum* plants. The rhizosphere pH globally varied between 4.50 and 7.00, whereas that of the bulk soil varied between 4.60 and 6.70, with differences of 0.1–0.5 pH units. In treatments T1, T2, T5 and T12, there were significant differences in soil pH, but only in T5 the pH was lower in rhizosphere soil. After plant growth, the EC of spiked treatments was higher than that of the control soil. The values ranged globally from 0.2 to 1.1 dS $m^{-1}$ for rhizosphere soils and from 0.4

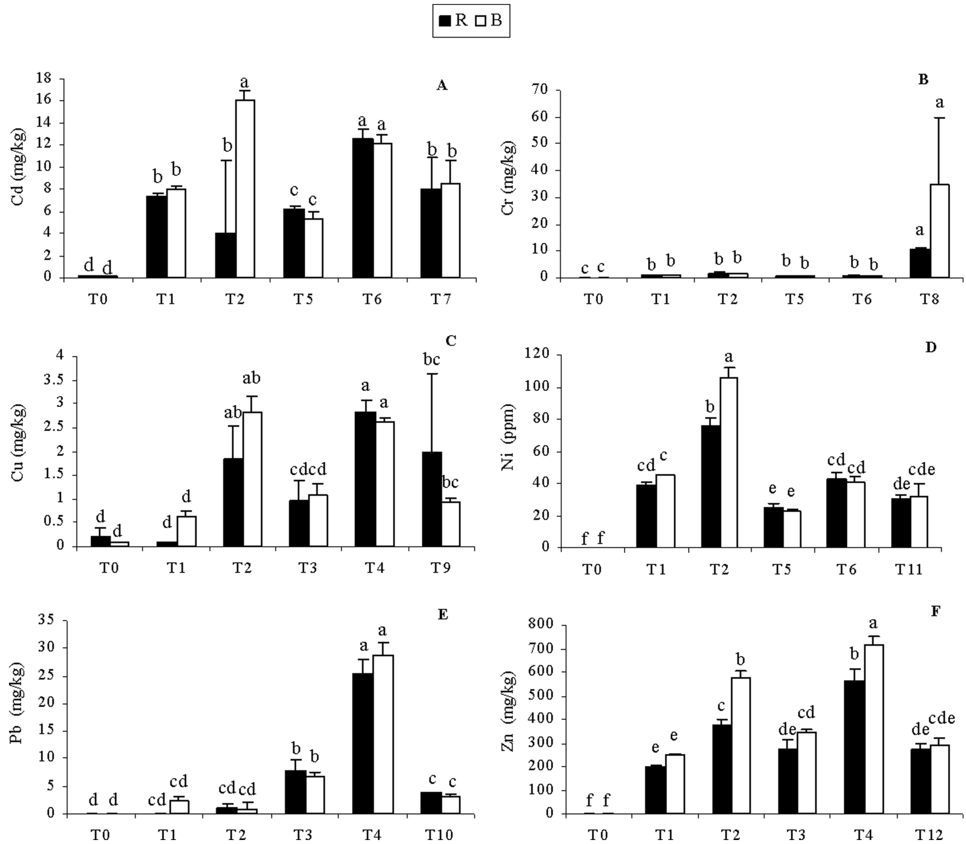


**FIGURE 2** Concentrations of $CaCl_2$-exchangeable metals in rhizosphere (R) and bulk (B) soils of *P. hortorum* plants grown in contaminated soils. Values are means ± standard deviations with n = 3. Treatment means were compared using Tukey's test at $P < 0.05$.

to 3.2 for bulk soils (Table 2). The differences in soil EC between the treatments was not significant in most cases, with the exception of treatments T1, T2 and T4, in which, significantly lower values were found in rhizosphere soils as compared with bulk soils.

Figure 3 shows the distribution of heavy metals in *P. hortorum* tissues. Concentrations of Cd, Ni and Pb (Figures 3A, 3D and 3E) in T0 treatment were far below the detection limit in all plants tissues. Tissue concentrations of heavy metals were higher in all HL treatments as compared with controls and ML treatments, especially for roots and stems. Metal content at harvest showed large differences between different organs and treatments; in general, higher HM contents were observed in roots, intermediate in stems, lower in leaves (Figure 3) and even lower in flowers. Flower production was very sensitive to heavy metals, therefore, couldn't be performed the statistical analysis of these data. Applications of single heavy metals (at the HL concentration) showed less plant accumulation. The exception was Cr,

**TABLE 2** pH of rhizosphere soils (R) and bulk soils (B) after growth of *Pelargonium hortorum* plants (mean value ± SD)

| | pH | | CE | |
|---|---|---|---|---|
| Treatment | R | B | R | B |
| T0 | 5.4 ± 0.14 | 5.8 ± 0.19 | 0.2 ± 0.02 | 0.4 ± 0.06 |
| T1 | 5.2* ± 0.01 | 4.7* ± 0.04 | 0.3* ± 0.00 | 1.9* ± 0.45 |
| T2 | 5.1* ± 0.35 | 4.6* ± 0.07 | 0.4* ± 0.10 | 3.2* ± 1.06 |
| T3 | 4.5 ± 0.06 | 4.6 ± 0.10 | 1.1 ± 0.03 | 1.7 ± 0.36 |
| T4 | 4.6 ± 0.17 | 4.6v0.14 | 0.7* ± 0.18 | 3.2* ± 0.49 |
| T5 | 5.5* ± 0.18 | 5.8* ± 0.12 | 0.4 ± 0.15 | 0.3 ± 0.13 |
| T6 | 5.7 ± 0.15 | 5.6 ± 0.37 | 0.4 ± 0.12 | 0.8 ± 0.58 |
| T7 | 6.6 ± 0.06 | 6.5 ± 0.01 | 0.3 ± 0.03 | 0.4 ± 0.05 |
| T8 | 7.0 ± 0.15 | 6.7 ± 0.04 | 0.2 ± .09 | 0.8 ± 0.41 |
| T9 | 6.5 ± 0.02 | 6.3 ± 0.08 | 0.9 ± 0.22 | 0.9 ± 0.01 |
| T10 | 6.3 ± 0.08 | 6.4 ± 0.08 | 0.9 ± 0.39 | 1.3 ± 0.14 |
| T11 | 6.6 ± 0.04 | 6.6 ± 0.04 | 0.7 ± 0.30 | 0.6 ± 0.10 |
| T12 | 6.6 ± 0.02 | 6.2* ± 0.01 | 0.5 ± 0.14 | 1.1 ± 0.34 |

Data are the means of duplicates. Rows means followed by asterisk are significantly different according to Tukey's test at $P < 0.05$.

since its plant concentration was greater when it was added alone than in combinations with other metals. Dead leaves showed great variability within the same treatments, and generally showed greater heavy metal contents as compared to the leaves collected at the end of the experiment (data not shown).

Table 3 shows the correlation between plant uptake and $CaCl_2$-extractable heavy metals. In general, the results showed significant correlations and a close relationship between metal content in plant tissues and metal content in both soils. The exceptions were Cd, Cu and Zn in some plant organs.

## DISCUSSION

In the present study, both synergistic and antagonistic effects were observed on plant growth. It is known that excessive heavy metal in the media can adversely affect plant growth, development, and reproduction (Brun et al., 2003; Ryser and Sauder, 2006). Treatments 1, 2, 3 and 4 at HL exerted similar effects on plant biomass, although treatment 2 at HL had a significant detrimental effect on leaves and flower biomass; this is a synergistic response in which 6 metals in mixture have a greater inhibitory effect on growth than 3 metals. In contrast, treatments 9 and 10, at both concentrations, showed less inhibition of growth. The former fact suggests that the toxic effect of more phytotoxic metal (Cr) was attenuated in the presence of Cd and Ni; so there is an antagonistic effect in which the toxicity is reduced for some mixtures. Except in the case of Cr, the combined toxic effect of all heavy metals

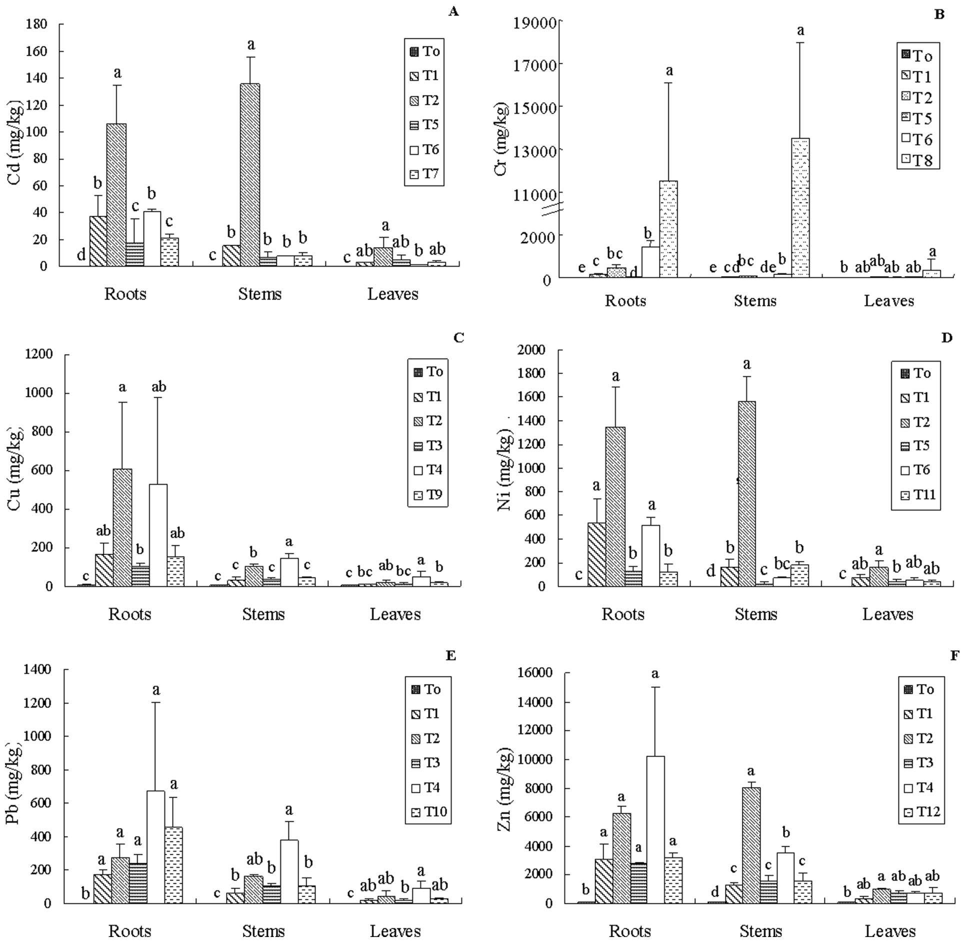


**FIGURE 3** Concentration of heavy metals in different plant organs. Values are means ± standard deviations with n = 3 for treatments in combinations and n = 2 for single metals. Treatment means were compared using Tukey's test at $P \leq 0.05$.

at HL was more pronounced compared to their single treatments, showing again a synergistic effect. This last finding agrees with Gladkov (2007) who found a considerable inhibitory effect on whole plants of *Agrostis stolonifera* for combined application of Pb, Cd and Zn.

*Pelargonium hortorum* plants were able to reduce the labile fraction of Cd, Ni in T2 and Zn in T2 and T4 in the rhizosphere soil compared to that observed in the bulk soil. Hinsinger (1998) explained the depletion or enrichment of the rhizosphere by the capacity of the soil to replenish the more soluble forms of metals in that zone. Hence, it is reasonable to assume that the uptake of these metals at HL was greater than the supply through the diffusion from the bulk soil. This agrees with results of Lorenz et al. (1997) who also found that concentrations of Cd and Zn decreased in soil solution during growth of radish plants. On the other hand, the Cd, Ni and Zn solubility in ternary and multiple applications were always lower in ML treatments compared to HL treatments. This is consistent with previous findings (Orroño and Lavado, 2009a). In contrast, Cr, Cu, and Pb showed different situations: Cr was more available at T8, Cu at T2 and T4, and Pb at T3 and T4. Chromium availability increased when supplied singly and decreased when applied with other metals (antagonist type effect). In our experiment, the increased Cr availability could be explained by the neutral pH of the soil; in fact, hexavalent chromium is an anion, and its sorption decreases with increasing soil pH (Guertin et al., 2004).

Unlike Cr, Cu availability tended to increase when supplied at HL either singly, in ternary combination or in combination with other five heavy metals. It is possible that in the present experimental conditions Cu availability was related to the level of Cu in the soil. In general, since Cu had a higher affinity for soil organic matter (OM), it seems possible that at higher concentrations of Cu in the soil (HL treatments) organic matter binding sites may be exhausted, resulting in a higher concentration of free metal ions (the bioavailable fraction). This explanation agrees with previous findings (Orroño and Lavado, 2009b). In contrast, Pb availability was reduced when supplied with the other five heavy metals (antagonist type effect), but when supplied alone or in ternary combination, availability increased (synergistic type effect). Lead is notorious for the lack of soil mobility, primarily due to metal precipitation as insoluble phosphates, carbonates and hydroxides (Blaylock and Huang, 1999). The increased availability of Pb in ternary combination may be related to the antagonistic interaction between Cu and Zn (Adriano, 1986; Kabata-Pendias and Pendias, 2000), which would allow Pb to be more available for plant uptake.

Acidity is known to influence heavy metal removal since H+ can be considered as competitive cations in ion exchange processes (Inglezakis et al., 2003). The pH of the rhizosphere can be up to two units different from the bulk soil, depending on plant and soil factors (Marschner, 2003). However, the bulk soils of the present experiment were more acidic than the rhizosphere soils, which suggests that acidification of the rhizosphere was not operative on metal availability. Electrical conductivity, which is a measure of the dissolved salts, was significantly lower in treatments T1, T2 and T4 in rhizosphere soils than in bulk soils. This result could be accredited to an intense absorption of soluble heavy metal salts by *P. hortorum* roots.

The bioaccumulation of a metal is known to be influenced by the occurrence of other metals, resulting in an inhibited or enhanced bioaccumulation of one metal in the mixture (An et al. 2004). Treatments with higher concentrations of the available heavy metal showed more metal accumulation in plant tissues, especially in roots, with the exception of Pb. This suggests that absorption of Pb is probably not concentration-dependent.

Zinc was the only metal showing accumulation in the leaves, since this is a micronutrient and moves relatively easily from the root to plant tops (Greger, 2004).

Some synergistic effects have been observed for selected pairs of elements that can modify mobility/availability of the metal (Kabata-Pendias and Pendias, 2000). In our case, Zn had a synergistic impact on Cd accumulation of T2, which resulted in an increase Cd content in roots, stems and leaves of *P. hortorum* plants. It is known that Zn induces dissociation of Cd sorbed onto the binding sites as a result of competition for these sites and increased Cd in soil solution (Angelova et al., 2007). These results differ from those reported by Roosens et al. (2003), who found that high total soil Zn concentration diminishes Cd uptake in *Thlaspi cearulescens* Prayon.

The lower uptake of Ni by plants in treatments T5 and T11 is difficult to explain because Cd and Ni have the same charge and hence have a similar behavior in soils. Perhaps they compete for the same trans-membrane carriers when they are together in triple combination with Cr. Kinetic data from Crowley et al. (1991) have demonstrated that essential Cu2+, Zn2+ and Ni2+ and nonessential Cd2+ compete for the same trans-membrane carrier. Similarly, Nakazawa et al. (2001) found that Ni uptake was inhibited by the coexistence of Cd in the nutrient solution. This antagonism could be due to the competition for available absorption sites at the root surface or to the formation of non-absorbable complexes in the soil solution.

According to MacFarlane and Burchett (2002), exposure to metals in combinations may result in increased cellular uptake and toxicity. In the present work, the degree to which *Pelargonium hortorum* absorbed and accumulated heavy metals on a single-element application basis was much lower than that with combined applications, except in the case of Cr. Our results differ from those by Smilde (1981), who worked with sewage sludge spiked with metal salts and found higher tissue concentrations for metals added separately rather than in combination. The toxic effects of Cr are primarily dependent on the metal speciation, which determines its uptake, translocation and accumulation. The increased Cr uptake in T8 might be due to disruption of plasma membranes at high concentrations of this element in the soil and the subsequent reduction of the soil-plant barrier (Hall et al., 2002). On the other hand, the pathway of Cr (VI) transport is an active mechanism involving carriers of essential anions such as sulfate (Shanker et al., 2005). Perhaps, when Cr is alone, it competes for those trans-membrane carriers more effectively. Lower Cr uptake in treatments T0, T1, T2, and T5 could be explained by the interactions between the metal ions in the multi-contaminated soils studied.

The present results suggest that Pb uptake was depressed in the presence of Cu and Zn. There are reports of antagonistic interaction effects on Pb uptake for the Pb-Zn combination. According to Kabata-Pendias and Pendias (2000), the Pb-Zn antagonism adversely affects the translocation of each element from roots to tops. Likewise, Clarkson and Lutge (1989) reported that $Cd^{2+}$, $Pb^{2+}$ and $Zn^{2+}$ interact competitively for uptake in higher plants. Finally, the lower metal concentrations in leaves and flowers indicate an important restriction of internal metal transport and may be related to an exclusion strategy (Baker et al., 1981).

Salim et al. (1993) showed that the concentrations of Cd, Cu and Pb in radish plants increase as the concentration of these metals increase. The Pearson's correlations matrix showed in most cases that a strong relation exists between plant metal uptake and metal availability in both soils. The exceptions were Cd, Cu and Zn in some plant tissues either in rhizosphere or bulk soils. The lack of correlation in these cases only implies that extractable heavy metals alone are not dominant in determining Cd, Cu and Zn uptake by the plant; other factors, which could interact in different ways, may influence these results. For example, metal absorption by the plants can be affected not only by the soil heavy metal content but also by factors such as the crop and the age of the plants and by soil properties like pH, the cation exchange capacity and the organic matter content (Adriano, 1986).

## CONCLUSIONS

Our data indicate that even the lowest metals levels applied in this study influence the growth and reproduction of *P. hortorum*. Shoots and roots react differently to heavy metal stress; aerial biomass was severely affected in a number of treatments, becoming particularly inhibited the formation of new leaves and flowers. Metal availability did not appear to be affected by the pH of rhizosphere soil and HM distribution inside the plant varied with the organ and specific metal under study. In general, the higher HM accumulation was found in roots. Thus, our results suggest that competition between metal ions at root surface can play a major role in metal uptake and accumulation by *Pelargonium hortorum* plants.

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