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Heavy metal accumulation in *Pelargonium hortorum*: Effects on growth and development

Acumulación de metales pesados en *Pelargonium hortorum*: Efectos sobre el crecimiento y el desarrollo

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Abstract. Ornamental plants have been proposed for growing in heavy metal (HM) contaminated soils, and also for phytoremediation. We evaluated (1) biomass production and (2) HM accumulations in *Pelargonium hortorum*. Plants were grown for 16 weeks on HM (cadmium, chromium, copper, lead, nickel and zinc) enriched soils. Treatments were i) control, non-enriched soil, ii) medium concentration treatment and iii) high concentration treatment. Four destructive harvests were carried out, and roots, stems, leaves, and flowers were analyzed each time. Concentrations of HM were determined using ICP. Significant reductions in biomass production were observed in HM-enriched soils compared with the control. Different indexes confirmed that *P. hortorum* was affected by HM. Heavy metals concentrations were higher in roots than shoots. Plant uptake rates of HM in roots and shoots showed different patterns for each element. Flowering was highly sensitive to HM soil concentrations.

Key words: Heavy metals, Ornamental plants, Tolerance index, Enriched soils, Plant heavy metal accumulation.

Resumen. Las plantas ornamentales han sido propuestas para crecimiento en suelos contaminados con metales pesados, y también para su uso en fitoremediación. Se evaluó la producción de biomasa y la acumulación de metales pesados en plantas de *Pelargonium hortorum*. Las plantas crecieron durantes 16 semanas en suelos enriquecidos con cadmio, cromo, cobre, plomo, níquel y cinc. Los tratamientos fueron i) control, suelo no enriquecido, ii) tratamiento concentración media y iii) tratamiento concentración alta. Se realizaron cuatro cosechas destructivas y se analizaron raíces, tallos, hojas y flores en cada cosecha. Las concentraciones de metales pesados se determinaron mediante espectrometría de emisión óptica con plasma acoplado inductivamente. La producción de biomasa fue reducida significativamente en los suelos enriquecidos con metales pesados comparado con las plantas en suelo control. Diferentes índices confirmaron que *P. hortorum* fue afectada negativamente por la aplicación de metales pesados. Las concentraciones de metales pesados fueron mayores en las raíces que en la parte aérea de las plantas. La tasa de absorción radical y la producción de biomasa mostraron diferentes patrones dependiendo de los metales pesados estudiados. La floración fue un parámetro muy sensible a la concentración de metales pesados en el suelo.

Palabras clave: Metales pesados, Plantas ornamentales, Índice de tolerancia, Suelos enriquecidos, Acumulación de metales pesados en planta.

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INTRODUCTION

Several ornamental plants have been proposed suitable for growing in heavy metal (HM) contaminated soils, and also phytoremediation. KrishnaRaj et al. (2000) assessed the capacity of scented geranium (*Pelargonium* sp. 'Frensham') to tolerate and accumulate Cr, Ni, and Pb in a hydroponics system. These data provided quantitative evidence for HM tolerance and accumulation potential in the genus *Pelargonium*. Orroño et al. (2008) found that *Pelargonium hortorum* showed better tolerance to HM than other *Pelargonium* species. Occurrence of heavy metals in soils is a consequence of industrialization, particularly in soils of urban areas which show higher levels of HM than those in rural regions (Kelly et al., 1996; Lavado et al., 1998).

Heavy metal accumulation in plants has received much attention, and those above-cited studies can be viewed from three different points of view. Firstly, the effect of HM on plant biomass production and HM accumulation. When toxic elements are absorbed by plants, toxicity problems (at a biochemical level) usually result in reduced biomass production either at a plant organ or whole plant scale (Hagemeyer, 1999). Decreases in plant biomass are generally correlated with an increase in absorbed elements concentration (Marschner, 1995). For instance, high metal concentrations were measured on shoots following growth inhibition of plants subjected to metal toxicity (Hajiboland, 2005). Therefore, any plant growing in HM contaminated soil usually increases their HM tissue concentration. Heavy metal uptake rates (UR) were developed to quantify HM transport from soil to plants (Singh & Agrawal, 2007).

Secondly, the timing when plants accumulate HM. Most research has developed short-term studies. For example, Lutts et al. (2004) studied the accumulation of cadmium and zinc during a few days after germination of *Atriplex halimus*. Few studies have addressed how HM concentrations vary at different plant growth stages. Perronnet et al. (2003) reported that cadmium and zinc accumulation varied with plant organs and age during growth of the hyperaccumulator *Thlaspi caerulescens*. Stem zinc concentration decreased with time, while cadmium concentration remained constant, despite an increase in biomass. Young leaves exhibited higher cadmium concentration than older leaves, but the reverse was true for zinc. Dinelli & Lombini (1996) found that metal concentrations in plant organs were higher at early vegetative growth stages due to relatively higher nutrient uptake than plant growth rate on soils derived from copper mine spoils. At flowering time, minimal copper and other heavy metal concentrations were detected. Other works have shown that metals may influence resource allocation during sexual reproduction (Saikkonen et al., 1998), and delay flowering (Brun et al., 2003).

Lastly, plant organs differ in HM accumulation. In most plants, roots, stems, leaves, fruits and seeds exhibit different HM concentrations, with roots containing the highest and seeds the lowest HM levels (Kloke et al., 1994). Different indexes have been proposed to evaluate the capacity of a plant for metal accumulation in harvested organs. This is useful for phytoremediation. Among those indexes are the shoot:root ratio and the tolerance index (TIN) (Antosiewicz, 1995). The relative growth rate (RGR) is also used to determine the ability of a plant to accumulate metals (Lutts et al., 2004). The first two indexes, are usually applied at final harvest, but the latter needs to be applied at different harvesting times during the growing season.

We studied the ability of the ornamental plant species *Pelargonium hortorum* (Geraniaceae), 'Common Geranium', for growing on HM contaminated soils. Our objectives were i) to determine the accumulation and its uptake rate in roots, stems, leaves and flowers of HM, throughout different harvesting times; ii) to evaluate biomass production by applying HM accumulation indexes and iii) to analyze the effect of HM on flowering development.

MATERIALS AND METHODS

We conducted a pot study using a Typic Argiudoll soil. Physical and chemical properties of this soil were measured using standard techniques (Klute, 1986; Sparks et al., 1996); major values for these properties were: sand 17.1%; silt 57.1%; clay 25.8%; 1: 2.5 (w/v) pH 6.1; organic matter 3.90%; total nitrogen (Kjeldahl) 0.22%; extractable phosphorus (Bray & Kurtz) 12.5 mg/kg; and exchangeable potassium 1.35 meq/100g. A completely randomized design with three treatments and eight replications per treatment were performed. Treatments were as follows: i) Control, not contaminated soil (T0), ii) Medium HM concentration (T1), and iii) High HM concentration (T2). Treatments T1 and T2 received cadmium (Cd) as cadmium nitrate; chromium (Cr) as chromic acid; copper (Cu) as copper chloride; lead (Pb) as lead nitrate; nickel (Ni) as nickel sulfate, and zinc (Zn) as zinc sulfate. The T1 treatment contained the following HM concentrations: 10, 250, 250, 80, 500 and 300 mg/kg Cd, Cu, Cr, Ni, Pb and Zn, respectively. The T2 treatment included the same metals, but twice as much the T1 concentration treatment. Salts were carefully mixed with the soil, and subjected to wet/dry cycles during a three month period prior to the onset of the experiment. This procedure reduced any overestimation of metal bioavailability (Basta et al., 2005). It was because after mixing, metals interacted with the soil matrix reaching a new equilibrium (Martinez y Motto, 2000).

Uniform plantlets were selected, and three were transplanted to plastic pots of 2000 cm^3 volume. Subsequently, only one plant per pot was maintained, and the other two were removed. Approximately every four weeks, over a period of 16 weeks, two plants were harvested from each treatment. Plant material was oven-dried at 60°C after washing to determine

dry weight and heavy metal content in roots, stems, leaves, and flowers. Plant material was first digested with a nitric-perchloric acid mixture (Jones & Case, 1990), and HM content was then determined by ICP-AES (Sparks et al., 1996), and expressed on a dry weight basis.

Several indexes were calculated: (1) Shoot/root ratios; (2) TIN, which represents the biomass ratio for plants grown in HM enriched-soils relative to those grown in HM non enriched, control-soils (Antosiewicz, 1995); (3) the RGR for each harvesting interval, based on the following equation:

$$RGR = (\ln W2 - \ln W1) / (t2 - t1),$$

where W2 and W1 represent total plant dry weights at times t1 and t2, respectively (Lutts et al., 2004).

Finally, UR ($mg.plant^{-1}.d^{-1}$) were calculated as:

$$\text{Heavy metal uptake rates (UR)} = \frac{M2*W2 - M1*W1}{t2 - t1}$$

where M1 and M2 are metal concentrations in plant tissue, and W1 and W2 are plant biomasses at times t1 and t2 (Singh & Agrawal, 2007).

Dry matter yields, HM concentrations in different organs, shoot/root ratios, and RGR were analyzed using Statistics 8.0. Data were subjected to a two-way analysis of variance (ANOVA) for each sampling time to examine significant differences between treatments (T0, T1, and T2), harvesting times, and HM concentrations in different plant organs. Differences between individual means were tested using Tukey's Test at the 0.05 significance level.

RESULTS

Heavy metal stress had no effect on plant survival, and all plants were alive at the end of the experiment. However, plant biomass was affected by increases in soil HM concentrations (Table 1). Aerial biomass production was significantly reduced ($p<0.05$) at 3rd and 4th harvests in plants from the T1 and T2 treatments compared to T0 plants. At the last sampling date, biomass was lower ($p<0.05$) (1) in the T2 treatment for stems, and (2) in T1 treatment for leaves, compared to T0 treatments. The interaction of *treatment x harvest* was significant ($p<0.05$) (Table 1). In T1 treatments some replications did not produce flowers, while in T2 treatments there was not flower production at all. Root biomass decreased significantly ($p<0.05$) in T2 treatments compared with the T0 and T1, but there were no differences ($p>0.05$) among harvesting times within each treatment (Table 1).

A change in biomass distribution between roots and shoots, and the inhibition of shoot growth on HM contaminated soils was confirmed by the shoot/root ratio. In T0 treatment this ratio increased significantly from 7 to 16 from the first to the fourth harvest. Meanwhile, the shoot/root ratio in both the T1 and T2 treatments remained between 4 to 8, and it was significantly lower ($p<0.005$) in T2 treatment (data not shown). The tolerance index showed significant differences between harvesting times ($p<0.05$) (Fig. 1), and T1 treatment exhibited TIN values greater than 100% for the 1st and 2nd harvest, indicating a net increase in biomass relative to the control. TIN values for T1 and T2 treatments were lower than 100% for the 3rd and 4th harvests, showing a net decrease in biomass, and growth inhibition. Relative growth rates increased throughout the experiment (Fig. 2). However, they were lower ($p<0.05$) in HM contaminated soils (T2) than in the control, showing a delay in plant growth rates.

In general, the increase in HM content in plants was consistent with the metal concentration in soil. Heavy metal accumulation in roots (Table 2) was higher than that in the other organs (roots > stems > leaves > flowers). Only Cu and Zn were detectable in all treatments, but they showed no significant accumulation differences among the T0, T1 and T2 throughout the study. The other HM´s were not detectable in the Control. Heavy metal accumulation in T1 treatment, at different harvesting times (Table 2) showed a similar pattern to T2 treatment. Root Cd, Ni, and Zn accumulations increased significantly over time, and continued to accumulate throughout the experiment. Chromium, Cu and Pb, on the other hand, exhibited no consistent trend throughout time. Accumulation of HM in stems and leaves (Tables 3

Table 1. Biomass accumulation in *Pelargonium hortorum* per treatment and harvesting time.
Tabla 1. Acumulación de biomasa en *Pelargonium hortorum* por tratamiento y época de cosecha.

		Biomass (g)			
Harvest	Treat.	Roots	Stems	Leaves	Flowers
1st	T0	0.6 ± 0.1 a	2.1 ± 0.3 b	2.3 ± 0.2 abc	0.8 ± 0.9
2nd		0.4 ± 0.1 a	1.7 ± 1.0 b	0.6 ± 0.2 c	0.6 ± 0.1
3rd		0.6 ± 0.3 a	3.5 ± 0.5 ab	4.2 ± 0.7 ab	1.5 ± 0.4
4th		0.8 ± 0.1 a	4.2 ± 0.3 a	5.4 ± 1.22 a	1.3 ± 0.5
1st	T1	0.5 ± 0.2 b	2.1 ± 0.1 b	1.9 ± 0.9 bc	0.3*
2nd		0.6 ± 0.3 a	2.2 ± 0.4 ab	1.4 ± 1.0 bc	0.4*
3rd		0.4 ± 0.1 a	1.8 ± 0.4 b	1.2 ± 1.3 bc	0.5*
4th		0.9 ± 0.1 a	2.3 ± 0.3 ab	0.6 ± 0.0 c	0.2*
1st	T2	0.5 ± 0.0 b	2.1 ± 0.3 b	0.7 ± 0.1 bc	nfp
2nd		0.4 ± 0.1 b	1.5 ± 0.6 b	0.4 ± 0.0 abc	nfp
3rd		0.4 ± 0.2 b	2.5 ± 0.7 ab	0.1 ± 0.1 ab	nfp
4th		0.3 ± 0.1 b	1.9 ± 0.2 b	0.2 ± 0.2 bc	nfp

*no replicates
nfp: no flower production
*falta de réplicas
nfp: no producción de flores
Means followed by the same letter do not differ statistically at p≤0.05.
Promedios seguidos por la misma letra no difieren significativamente a p≤0,05.

Fig. 1. Tolerance index of *Pelargonium hortorum* plants for Medium (T1) and High HM concentration (T2) treatments for different harvesting dates. Means followed by the same letter do no differ statistically (p≤0.05; Tukey´s test).
Fig 1. Índice de tolerancia de las plantas de *Pelargonium hortorum* en los tratamientos concentración media (T1) y alta (T2) en diferentes momentos. Las medias seguidas por la misma letra no difieren estadísticamente (p≤0,05; Tukey´s test).

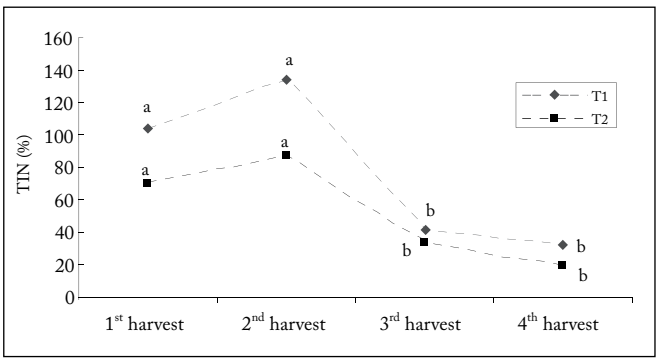
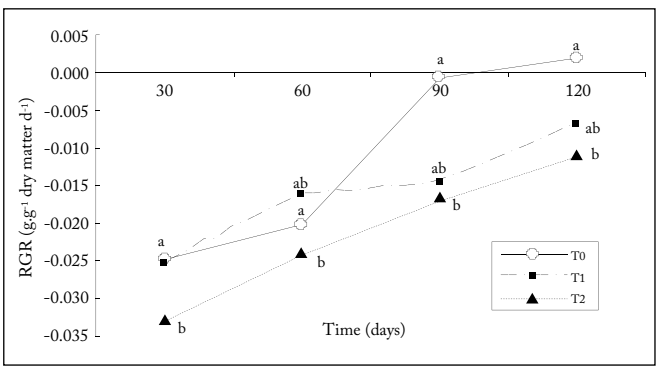


Fig. 2. Relative growth rate (RGR) of *Pelargonium hortorum* plants during the experimental period. Means followed by the same letter do no differ statistically (p≤0.05; Tukey´s test).
Fig 2. Tasa relativa de crecimiento (TRC) de la plantas de *Pelargonium hortorum* durante el periodo experimental. Las medias seguidas por la misma letra no difieren estadísticamente (p≤0,05; Tukey´s test).



and 4) showed no significant differences ($p<0.05$); although a subtle pattern was inferred as plant growth proceeded, leaves at the 4th harvesting time exhibited lower HM levels than leaves at the 1st harvesting time. There was a significant treatment effect ($p<0.05$), but differences in HM concentrations for harvesting times were not significant ($p>0.05$) in stems and leaves. The exceptions were HM concentrations of Cd, Ni and Pb in plant roots (Table 2). This result was likely due to a consistently higher concentration of HM in roots. A significant *treatment x harvest* interaction ($p<0.05$) was detected for root Cd and Ni, but in general the accumulation of HM did not increase considerably over the growing season.

Table 5 summarizes flower HM concentrations among the three treatments. Only Cu and Zn were measured in the Control. Although T1 treatment did not have an adequate number of flowers for statistical analysis, it appears that HM exhibited an increase in concentrations with respect to T0 treatment. The high HM concentrations in T2 resulted in the absence of flowering.

Figure 3 shows Cu and Zn uptake rates (mg plant^{-1} d^{-1}) for roots and aerial biomass in T0 treatment. Rates of Cu uptake by plants were very stable for roots and aerial biomass. Zn showed variable uptake rates, especially in roots, but there was not a clear tendency across the duration of the study. Zinc uptake rates by roots in T1 treatment (Fig. 4) were positive at the beginning and end of the growth period, and the lower uptake rates occurred at 90 days from study initiation. Cadmium, Cu and Pb, and to a lesser extent Ni, uptake rates were basically constant throughout harvests. Rates of Zn uptake in aerial biomass were positive in T1 treatment (Fig. 4). Zinc, and to a lesser extent Ni, tended to decrease across time. The remaining HM showed more stable uptake rates. Uptake rates of HM by roots and the aerial part were less clear at T2 treatment (Fig. 5). These plant organs showed either positive Zn and Ni uptake rates at the beginning or negative uptake rates for these HM at the end of the growth period. Rate of uptake progressively decreased with plant development. Cadmium, Cr, Cu and Pb were more stable.

Fig. 3 Heavy metal uptake rate (mg plant^{-1} d^{-1}) for roots (a) and aerial biomass (b) of *Pelargonium hortorum* in T0 treatment as a function of harvesting date.
Fig. 3. Tasa de absorción de metales pesados (mg plant^{-1} d^{-1}) de raíces (a) y biomasa aérea (b) en plantas de *Pelargonium hortorum* en el tratamiento control en función de la fecha de cosecha.

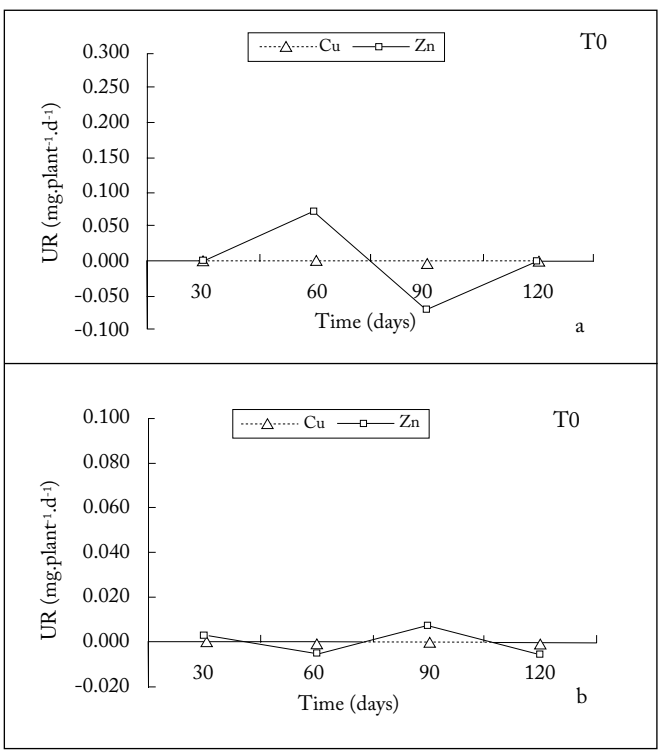


Table 2. HM accumulation in roots of *Pelargonium hortorum* per treatment and harvesting date.
Tabla 2. Acumulación de metales pesados en raíces de *Pelargonium hortorum* por tratamiento y fecha de cosecha.

Harvest	Treatm	Metal in roots (ppm)					
		Cu	Zn	Cd	Ni	Cr	Pb
1st	T0	12.9 ± 2.9	50.5 ± 145.5	bdl	bdl	bdl	bdl
2nd		84.3 ± 60.1	77.8 ± 17.1	bdl	bdl	bdl	bdl
3rd		11.4 ± 1.5	53.8 ± 4.2	bdl	bdl	bdl	bdl
4th		6.3 ± 0.4	26.6 ± 0.9	bdl	bdl	bdl	bdl
1st	T1	187.0 ± 58.0	2397.9 ± 283.7	24.6 ± 3.2	323.3 ± 14.4	100.7 ± 37.3	164.3 ± 59.3
2nd		323.7 ± 36.5	3596.3 ± 4.9	40.8 ± 3.2	473.3 ± 64.4	123.8 ± 24.1	222.1 ± 22.6
3rd		122.4 ± 43.3	2753.7 ± 17.5	25.8 ± 0.6	359.8 ± 14.2	89.6 ± 14.8	99.5 ± 44.6
4th		118.7 ± 8.0	3815.9 ± 22.4	40.2 ± 1.4	721.5 ± 90.7	90.5 ± 22.5	111.7 ± 12.7
1st	T2	646.8 ± 65.8	5003.2 ± 2127.1	73.2 ± 10.4	815.6 ± 169.9	225.8 ± 34.6	202.4 ± 46.4
2nd		748.2 ± 398.2	7271.7 ± 932.9	94.4 ± 13.9	1009.1 ± 3.2	347.5 ± 89.2	340.5 ± 52.8
3rd		466.1 ± 125.3	9501.9 ± 966.0	129.0 ± 10.7	1245.3 ± 16.9	194.8 ± 19.4	152.2 ± 34.0
4th		941.8 ± 80.6	9417.1 ± 699.1	120.5 ± 12.8	1550.5 ± 228.0	353.0 ± 11.5	270.1 ± 45.6
ANOVA	Treatm.	***	***	***	**	***	***
	Harvest	ns	ns	*	**	ns	*
	Treat x harv.	ns	ns	*	*	ns	ns

Significance levels for treatments, harvesting times and the interaction *treatment* × *harvesting times* are shown: *p<0.05; **p<0.01; ***p<0.001; bdl: Below detection limit.
Niveles de significancia para tratamientos, épocas de cosecha, y la interacción *tratamiento x época de cosecha*: *p<0,05; **p<0,01; ***p<0,001; bdl: Por debajo del límite de detección.

Table 3. HM accumulation in stems of *Pelargonium hortorum* per treatment and harvesting time.
Tabla 3. Acumulación de metales pesados en tallos de *Pelargonium hortorum* por tratamiento y fecha de cosecha.

Harvest	Treatm	Metal in stems (ppm)					
		Cu	Zn	Cd	Ni	Cr	Pb
1st	T0	3.25 ± 2.7	43.0 ± 8.1	bdl	bdl	bdl	bdl
2nd		3.5 ± 1.5	27.9 ± 8.0	bdl	bdl	bdl	bdl
3rd		4.4 ± 0.2	41.7 ± 12.0	bdl	bdl	bdl	bdl
4th		1.9 ± 1.9	14.9 ± 12.9	bdl	bdl	bdl	bdl
1st	T1	26.2 ± 0.6	449.2 ± 42.3	7.8 ± 0.8	83.0 ± 1.6	20.8 ± 5.1	39.1 ± 5.7
2nd		24.8 ± 4.9	672.2 ± 195.9	12.6 ± 4.3	118.6 ± 39.2	18.6 ± 3.1	43.6 ± 5.8
3rd		37.4 ± 9.5	1010.6 ± 66.6	14.1 ± 2.2	149.9 ± 19.7	30.4 ± 8.5	70.3 ± 18.4
4th		33.2 ± 4.4	1110.7 ± 101.1	12.7 ± 2.8	166.6 ± 16.1	26.3 ± 0.6	79.9 ± 4.7
1st	T2	104.7 ± 47.6	3633.9 ± 1587.3	55.3 ± 29.8	571.8 ± 243.1	61.2 ± 33.7	113.6 ± 60.5
2nd		103.7 ± 23.9	5614.5 ± 964.7	96.7 ± 28.9	1017.2 ± 179.0	63.8 ± 20.0	152.6 ± 48.9
3rd		57.2 ± 47.5	3260.7 ± 3155.9	38.0 ± 33.1	830.5 ± 817.2	42.9 ± 33.1	82.8 ± 73.2
4th		51.8 ± 3.2	3333.2 ± 417.9	37.4 ± 0.1	545.1 ± 15.7	31.6 ± 0.2	74.7 ± 3.7
ANOVA	Treatm.	***	***	*	**	***	***
	Harvest	ns	ns	ns	ns	ns	ns
	Treat x harv.	ns	ns	ns	ns	ns	ns

Significance levels for treatments, harvesting times and the interaction *treatment* × *harvesting times* are shown: *p<0.05; **p<0.01; ***p<0.001; bdl: Below detection limit.
Niveles de significancia para tratamientos, épocas de cosecha, y la interacción *tratamiento x época de cosecha*: *p<0,05; **p<0,01; ***p<0,001; bdl: Por debajo del límite de detección.

Table 4. HM accumulation in leaves of *Pelargonium hortorum* per treatment and harvesting time.
Tabla 4. Acumulación de metales pesados en hojas de *Pelargonium hortorum* por tratamiento y fecha de cosecha.

Harvest	Treatm	Metal in leaves (ppm)					
		Cu	Zn	Cd	Ni	Cr	Pb
1st	T0	10.0 ± 0.8	50.1 ± 9.4	bdl	bdl	bdl	bdl
2nd		6.6 ± 0.7	38.8 ± 0.2	bdl	bdl	bdl	bdl
3rd		6.9 ± 1.3	36.9 ± 2.4	bdl	bdl	bdl	bdl
4th		4.1 ± 0.1	26.8 ± 1.1	bdl	bdl	bdl	bdl
1st	T1	21.0 ± 5.0	307.9 ± 92.3	5.0 ± 2.0	77.0 ± 19.1	38.0 ± 18.1	59.0 ± 29.1
2nd		15.9 ± 9.9	300.1 ± 176.0	8.1 ± 0.0	75.9 ± 45.1	33.3 ± 26.3	43.2 ± 36.3
3rd		18.9 ± 2.9	642.5 ± 16.0	9.7 ± 0.7	148.2 ± 11.4	35.8 ± 3.9	46.7 ± 36.3
4th		7.9 ± 0.1	342.6 ± 2.6	5.9 ± 0.1	137.4 ± 15.5	7.9 ± 0.1	17.9 ± 2.2
1st	T2	32.9 ± 15.0	1426.9 ± 689.3	26.0 ± 14.0	328.0 ± 151.2	46.9 ± 14.9	74.9 ± 28.9
2nd		48.0 ± 24.1	1409.5 ± 711.9	24.0 ± 10.0	349.9 ± 130.3	97.0 ± 53.1	135.0 ± 79.2
3rd		13.9 ± 0.1	890.4 ± 15.0	17.8 ± 1.9	160.5 ± 30.6	15.8 ± 0.1	21.8 ± 0.2
4th		20.1 ± 1.9	643.5 ± 326.3	10.8 ± 1.7	109.4 ± 9.4	13.8 ± 4.4	21.3 ± 2.5
ANOVA	Treatm.	*	***	***	*	*	*
	Harvest	ns	ns	ns	ns	ns	ns
	Treat x harv..	ns	ns	ns	ns	ns	ns

Significance levels for treatments, harvesting times and the interaction *treatment* × *harvesting times* are shown: *p<0.05; **p<0.01; ***p<0.001; bdl: Below detection limit.
Niveles de significancia para tratamientos, épocas de cosecha, y la interacción *tratamiento x época de cosecha*: *p<0,05; **p<0,01; ***p<0,001; bdl: Por debajo del límite de detección.

Fig. 4. Heavy metal uptake rate (mg plant^{-1} d^{-1}) for roots (a) and aerial biomass (b) of *Pelargonium hortorum* in the Medium HM concentration treatment (T1) as a function of harvesting date.
Fig. 4. Tasa de absorción de metales pesados (mg plant^{-1} d^{-1}) de raíces (a) y biomasa aérea (b) en plantas de *Pelargonium hortorum* en el tratamiento concentración media (T1) en función de la fecha de cosecha.

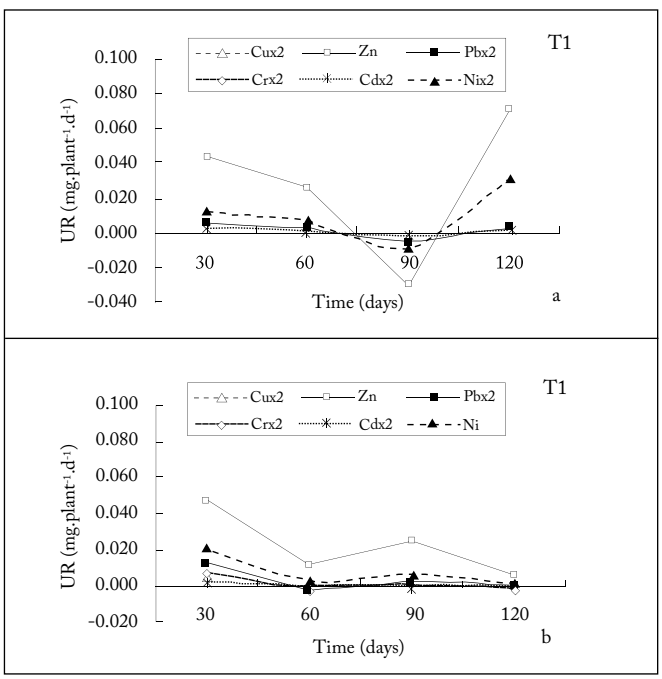
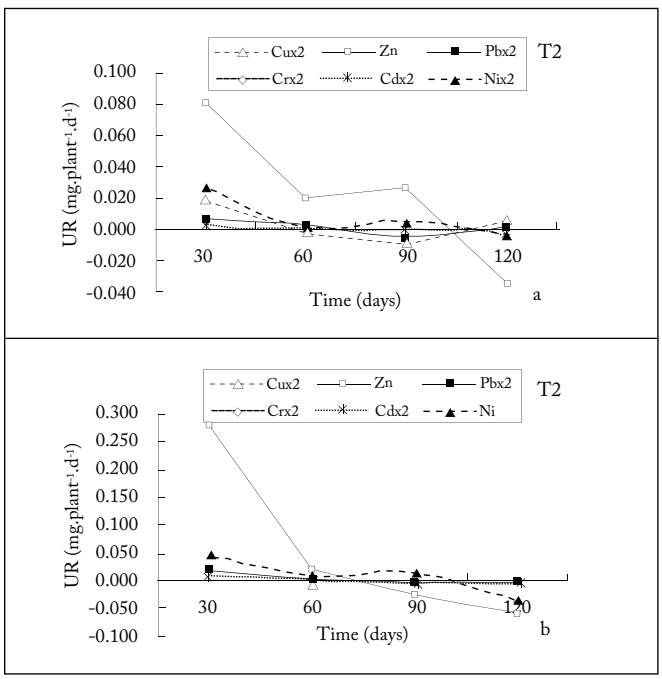


Fig. 5. Heavy metal uptake rate (mg plant^{-1} d^{-1}) for roots (a) and aerial biomass (b) of *Pelargonium hortorum* in the High HM concentration treatment (T2) as a function of harvesting date.
Fig. 5. Tasa de absorción de metales pesados (mg plant^{-1} d^{-1}) de raíces (a) y biomasa aérea (b) en plantas de *Pelargonium hortorum* en el tratamiento concentración alta (T2) en función de la fecha de cosecha.



DISCUSSION

Aerial growth was affected by HM toxicity more than root growth, and roots of *Pelargonium* plants accumulated a significantly greater metal concentration than aerial organs. Other authors have shown that growth of the upper plant parts is more sensitive to heavy metals, in spite of their low metal content compared with roots. It was hypothesized that roots could play an important role in metal retention by preventing an excessive and toxic accumulation in shoots (Mazhoudi et al., 1997). Initial leaf mortality and leaf turnover at harvesting time were higher at T2 than at the T1 treatment. The accelerated senescence caused by HM comes from oxidative damage and increased membrane permeability (Luna et al., 1994).

The lower shoot/root ratios found in plants of T1 and T2 treatments, compared with T0 treatment, clearly show that biomass allocation changes under heavy metal stress conditions between roots and shoots in *Pelargonium* plants. Tolerance indexes have been useful to characterize plant tolerance. TIN values lower than 100% indicate a net decrease in biomass and suggest that plants are HM-stressed. At the same time, TIN values equal to 100% indicate no differences relative to non-HM control treatments. Also, TIN values greater than 100% indicate a net increase in biomass, and suggest that plants express a growth dilution effect (Audet & Charest, 2007). Our TIN values decreased as soil HM concentrations increased (Fig. 1). RGR values progressively increased for all treatments; this response in T1 and T2 treatments suggest that these plants recovered after an initial shock, and that growth was delay under HM stress. Negative values in T1 and T2 treatments (Fig. 2) can be explained by increases in senesced leaf number (Davidson & Campbell 1984); the lower RGR value in T2 treatment may be a consequence of decreases in shoot biomass (Table 1).

Low transport of HM to shoots may be due to saturation of root metal uptake, when internal metal concentrations are high (Zhao et al, 2003). Although we did not find significant effects of harvesting times for leaves, new leaves tended to have lower HM levels than older ones (Table 4). Metal concentrations, therefore, may rise as leaves age simply due to the continued passive metal transport into leaf tissues. Movement of metals into older leaves is a way that some plants have to eliminate some of their metal excess (Verkleij & Schat, 1990). This result disagrees with that of Marschner (1995), who attributed a decline in dry matter mineral content as plants age to an increase in the proportion of structural material (cell wall and lignin) and storage compounds.

By the time of the final harvest, very few plants were flowering on the HM, soil contaminated treatments (Table 5). A delay and inhibition of flowering can be attributed to a disruption in biological processes due to HM stress (Van Assce & Clijsters, 1990). Our results suggest that flowering was very sensitive to HM concentrations (Saikkonen et al., 1998).

Table 5. HM in flowers at different harvesting times. Lack of standard deviations indicate lack of replicates.
Tabla 5. Acumulación de metales pesados en flores en distintas fechas de cosecha. La ausencia de desviación estándar indica ausencia de réplicas.

Harvest	Treatm	Metal in flowers (ppm)					
		Cu	Zn	Cd	Ni	Cr	Pb
1st	T0	21.2 ± 16.0	33.0 ± 11.0	bdl	bdl	bdl	bdl
2nd		10.0 ± 0.0	41.5 ± 12.0	bdl	bdl	bdl	bdl
3rd		9.9 ± 0.0	43.6 ± 19.7	bdl	bdl	bdl	bdl
4th		2.9 ± 4.0	24.9 ± 1.2	bdl	bdl	bdl	bdl
1st	T1	16.5	99.3	16.6	15.6	16.6	15.5
2nd		96.8 ± 116.0	139.2 ± 84.7	8.4 ± 8.8	45.3 ± 43.3	11.1 ± 5.0	17.1 ± 3.4
3rd		13.7	87.6	2.4	87.6	5.7	8.8
4th		17.8	333.3	4.4	88.9	8.9	17.8
1st	T2	nfp	nfp	nfp	nfp	nfp	nfp
2nd		nfp	nfp	nfp	nfp	nfp	nfp
3rd		13.2	153.7	44.1	125.2	5.9	8.3
4th		nfp	nfp	nfp	nfp	nfp	nfp

bdl: Below detection limit.
nfp: No flower production.

bdl: Por debajo del límite de detección.
nfp: No hubo producción de flores.

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