

Heavy metal accumulation in geranium (*Pelargonium hortorum*) and effects on growth and quality of plants

D.I. ORROÑO, R.S. LAVADO*

INBA (CONICET/FAUBA) and Facultad de Agronomía, Universidad de Buenos Aires. Av. San Martín 4453 C1417DSE Buenos Aires, Argentina.

Received 18 November 2010 – Received in revised form 11 March 2011 – Accepted 24 March 2011

Keywords: heavy metals; hyperaccumulator; ornamental plants; plant quality parameters.

INTRODUCTION. – Heavy metals (HM) are an increasing environmental problem worldwide and metals, such as Cd, Cr, Cu, Pb, Ni and Zn, are among the most important environmental pollutants. In contaminated areas, i.e. industrial or mining areas and their surroundings, several HM usually co-occur in high concentration. The same happens in urban areas. Chromium, Cd and Ni were selected to represent those metals used widely in industry (ALLOWAY, 1995), whereas Pb, Zn and Cu were selected to represent those commonly found to be elevated in urban environments (CHANEY, 1993). The bioaccumulation of a metal is known to be influenced by the occurrence of other metals and plants show a considerable inhibitory effect when HM act in combination (KABATA PENDIAS and PENDIAS, 2000). To cope with this situation, phytoremediation arose as a suitable technology to remove HM from the soils (PRASAD and FREITAS, 2003; REEVES and BAKER, 2000). The success of phytoextraction depends upon several plant characteristics. The two most important characters are the ability to accumulate large quantities of biomass rapidly and the ability to accumulate large quantities of metals in shoot tissue (BAKER, 1981). It is the combination of high metal accumulation and high biomass production that results in the most metal removal. However, growth changes are the visible response of plants under stress. Heavy metals are known to have adverse effects on both shoot and root growth (ASHMORE, 1997). Furthermore, metals may also influence allocation to sexual reproduction (SAIKKONEN *et al.*, 1998) and delay flowering (BRUN *et al.*, 2003). Reductions in total leaf area per plant were reported by SINGH (2001), among others.

* Corresponding author: lavado@agro.uba.ar

One focus on phytoremediation research is towards the identification of new plant species for such purposes (PRASAD and FREITAS, 2003). Several studies suggest that ornamental plants are able to deal with heavy metals (SHARMA *et al.*, 2004). The special advantage of using ornamental plants is that they may provide an aesthetic value to landscape of contaminated soils and also resolve HM pollution at the same time. Species of the genus *Pelargonium* commonly known as geraniums have been mentioned, in particular, for this purpose (PRASAD and FREITAS, 2003). Some experiments indicated that these species showed tolerance to different levels of Cd, Ni and Pb in the growing media. For example, scented geranium *Pelargonium* sp. "Frensham" studied in nutritive solutions showed hypertolerance to different levels of Cd, Ni (DAN *et al.*, 2002) and Pb (KRISHNARAJ *et al.*, 2000). ARSHAD *et al.* (2008) found that 3 cultivars of scented *Pelargonium* were Pb-hyperaccumulators plants, with high biomass production. In a previous research we found that *Pelargonium hortorum* showed greater tolerance to HM as compared with other *Pelargonium* species (ORROÑO *et al.*, 2009). From those results, we infer that this plant species would have a potential as a crop for phytoremediation purposes.

In a second experiment, however, using also 6 heavy metals together *P. hortorum* failed to act as good phytoremediation plant (ORROÑO and LAVADO, 2009). The last finding might be caused by the simultaneous presence of several metals in soil. Therefore, we decided to study in a first experiment the effects of Cu, Pb plus Zn, and in another experiment the effects of Cd, Cr plus Ni. The first experiment mimics the group of HM found together by KABALA and SINGH (2001) and YOON *et al.* (2006). The second experiment mimics the group of HM found together in other contaminated areas (LAVADO *et al.*, 1998). The overall objective of the present work was to evaluate the potential of *Pelargonium hortorum* plants to accumulate two sets of HM as well as the effects on growth and quality of plants.

MATERIALS AND METHODS. – Greenhouse Experiments. – Two separated experiments were carried out on potted plants in a greenhouse under controlled conditions between autumn to spring 2006. First experiment was carried out between April to July. The second experiment was carried out between August to November. The two experiments were carried out in different periods within the crop cycle because no mayor differences were expected. The greenhouse was vented when the day temperature reached 27°C by opening the side vents and the plants were fogged with water. The greenhouse was under natural light conditions (with no supplemental lighting). Treatments were i) control, a non-contaminated Typic Argiudoll (C), ii) medium level treatment (ML treat-

ment) and iii) high level treatment (HL treatment). Heavy metals were applied as salts, and mixed thoroughly with the soil. Soils were subjected to wet/dry cycles for three months in order to reduce the possibility of overestimating metal bioavailability

Experiment A: in this experiment we studied the combined effect of Cu, Pb and Zn. The levels of salts applied to the soil in ML treatment were 0.54 g copper chloride kg⁻¹ soil, 0.80 g lead nitrate kg⁻¹ soil and 1.33 g zinc sulfate kg⁻¹ soil. The HL group received twice the quantity of the same salts as the soil in the ML treatment.

Experiment B: in this experiment we studied the combined effect of Cd, Cr and Ni. The levels of salts applied to the soil in ML treatment were 0.57 g chromic acid kg⁻¹ soil, 0.027 g cadmium nitrate kg⁻¹ soil, 0.32 g nickel sulfate kg⁻¹ soil. The HL group also received twice the quantity of the same salts as the soil in the ML treatment.

The ML treatment represents the HM content found in some contaminated soils (IMPERATO *et al.*, 2003; LAVADO *et al.*, 1998). The HL treatment represents a high soil contamination status.

Pot Experimental design. – The experimental design for both experiments was at random with three treatments and five replications (pots) per treatment, respectively. Rooted *Pelargonium hortorum* plantlets from a greenhouse commercial stock were transplanted to pots containing 2.5 kg of soil. Average fresh weight of plants at the beginning of Experiment A was 81.6 g while average fresh weight of plants at the beginning of Experiment B was 71.4 g. Plants were watered approximately 80% of field capacity but to prevent leaching of nutrients or HM from soil, plastic trays were placed under each pot. The leachate was added to the pots. At the start of the experiments, and every 15 days after transplanting nitrogen, phosphorous and potassium (N: P: K = 26:6:15) were added to the pots as base fertilizers.

Determination of plant quality parameters and relative growth rate (RGR). – To assess plant height, diameter, and leaf and flower area we used non-destructive determinations. Plants from each experiment were sampled two (40 and 120 days) and three times (40, 80 and 120 days) after transplant. Plant height was measured from the border of the container to the top of the main plant stem with a stainless steel ruler. Plant diameter was measured at 1 cm from the base of the main plant stem. Leaf area was estimated by tracing on a graph paper the outline of leaves and counting the squares of 1 cm². The same method employed for leaf area was used for flower area.

Plants were harvested after 16 weeks and divided into roots, stems, leaves and flowers. Plants were dried at 60°C until they reached constant dry mass and then dry weights were recorded. Mean relative growth rate (RGR) of whole plant dry mass (HUNT, 1978) was estimated according to the following formula:

$$RGR = (\ln(DW2) - \ln(DW1)) / \Delta t$$

where, DW1 and DW2 are the mean dry weights at the beginning and at the end of the time interval, respectively and Δt is the time interval. The RGR indicates the accumulation of biomass present per unit of biomass produced per unit of time.

Chemical analysis of soil and plants. – The main physical and chemical properties of control soil analyzed using standard techniques (SPARKS, 1996) were: sand 17.1%; silt 57.1%; clay 25.8%; 1:2.5 pH 6.1 in water; organic matter 3.9%. Soil samples were collected from each treatment before planting and were extracted with DTPA and with "aqua regia", hydrochloric, perchloric and nitric acid in order to assess the bioavailable

and total HM concentration, respectively (SPARKS, 1996). Plant samples were wet-digested with a mixture 3:1 ratio (v/v) of $\text{HNO}_3/\text{HClO}_4$ concentrate (JONES and CASE, 1990). Cadmium, Cr, Cu, Ni, Pb and Zn concentrations were determined in plant organs and in soil extracts by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using a Baird 20/70 equipment.

Statistical analyses. – The data were analyzed with the Statistix 8.0 program. Analysis of variance (ANOVA) was applied to test significant differences between treatments and HM effects on growth. When data were not normally distributed, log transformations were used. In cases where the F-test results were significant, the means were compared using the Tukey test, with $p < 0.05$ considered to be statistically significant.

RESULTS. – *Heavy metal concentrations in soils and effects on plant growth and RGR.* – Total and available (DTPA) metal concentration in soil and the ratio DTPA/Total (%) are shown in Table 1. Both experiments showed lower total metal levels in control soil, and there was a linear increase of total HM levels with increasing application rate. Values of DTPA extractable HM and the ratio DTPA/Total (%) were lower in control soil and there was an increase in these parameters in ML treatment. However, at HL treatment these values generally decreased or remained constant. The exceptions were Cd and Ni in Experiment B that showed a net increase in these parameters at both levels of metals in soil.

Shoot (stems, leaves and flowers) and root dry weights are shown in Figure 1. Experiment A shoot dry weight significantly decreased with an increasing metal concentration in soil, while root dry weight was affected only at HL treatment. Experiment B shoot and root dry weights were similar at both HM treatments.

Figure 2 shows the RGR of Experiments A and B. In both experiments, RGR of HM treatments were significantly lower than those found in the control treatments. Experiment A at ML and HL treatments had a RGR close to zero. In contrast, RGR in Experiment B was negative at both HM treatments. This experiment suffered a great loss of leaves, which were not accounted for in the measurement of final plant biomass.

Heavy metal effects on plant quality parameters. – Leaf area, flower area, diameter and plant height were significantly influenced by the two levels of HM in the soil (Figs. 3 and 4). Plant height increased over time and the control plants showed significantly higher values than those grown under HM treatments. Plant diameters were similar in the control and ML treatments in Experiment A, and significantly larger in control plants as compared with ML and HL treatments in Experiment B. Leaf

TABLE 1. — Total and available (DTPA) metal concentration in soil and the ratio DTPA/Total (%). Experiment A: Cu, Pb and Zn; Experiment B: Cd, Cr and Ni. Values represent the average of 3 replicates with the standard deviations in parentheses.

Treatment	Fraction extracted	Heavy metal concentration (mg/kg)					
		Experiment A			Experiment B		
		Cu	Pb	Zn	Cd	Cr	Ni
Control	DTPA	5.8 ± 0.0	1.4 ± 0.10	4.6 ± 0.13	0.6 ± 0.0	ND	1.2 ± 0.0
	Total	27.3 ± 1.0	22.5 ± 19.2	37.7 ± 19.4	2.6 ± 2.0	24.7 ± 4.7	14.2 ± 1.0
	DTPA/Total (%)	21.2	6.2	12.2	23		8.5
ML	DTPA	98.4 ± 1.6	137.8 ± 11.2	261.0 ± 6.2	7.4 ± 0.4	49.4 ± 3.3	87.0 ± 0.7
	Total	198.2 ± 22.7	650.1 ± 108.4	414.2 ± 14.2	8.6 ± 1.2	428.1 ± 23.7	107.5 ± 4.5
	DTPA/Total (%)	49.6	26.7	63	86	11.5	80.9
HL	DTPA	212.6 ± 5.9	27.8 ± 6.2	249.6 ± 9.4	17.4 ± 1.3	50.6 ± 5.0	214.2 ± 12.7
	Total	509.3 ± 21.5	1467 ± 264.6	728.4 ± 229.3	17.6 ± 2.2	852.6 ± 29.8	238.5 ± 8.1
	DTPA/Total (%)	41.7	1.89	34.2	98.8	5.9	89.8

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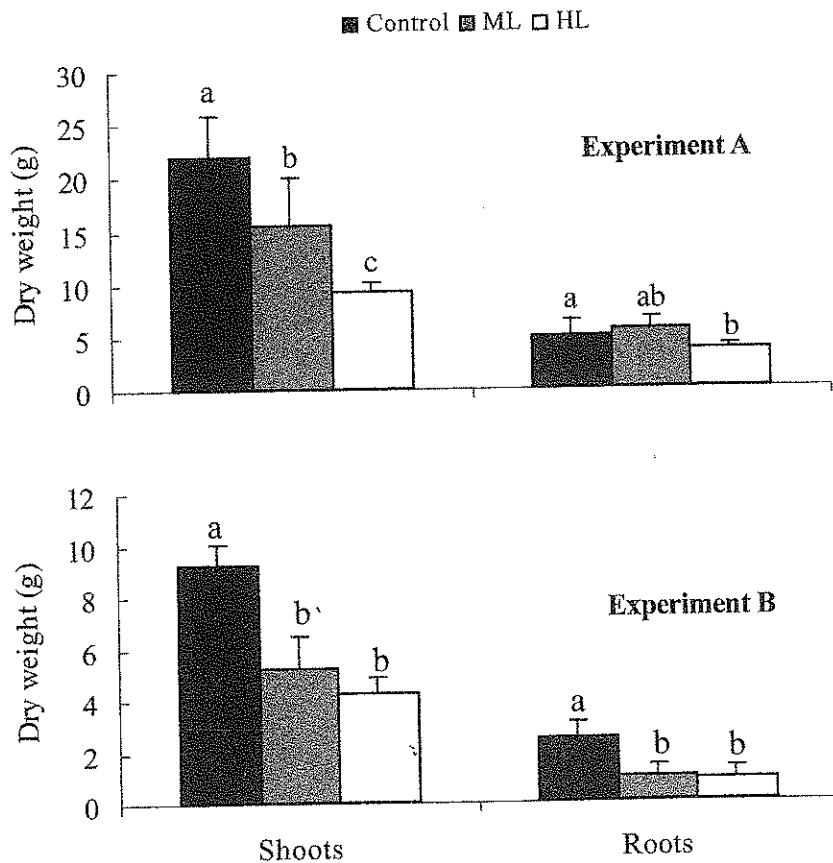


FIG. 1. — Effect of heavy metal treatments on shoot and root dry weights of *P. hortorum*. Experiment A: Cu, Pb and Zn; Experiment B: Cd, Cr and Ni.

area was similar in both experiments: significantly higher in the control intermediate in HL and lower in ML; this parameter tends to diminish due to the growth of new leaves and the senescence of old ones. Flower area was always significantly larger in control plants, and was reduced to a similar extent in plants growing in ML and HL treatments. In Experiment A (Fig. 3), treatments ranked from high to low flower area in the following order control > ML > HL from the beginning of measurements. Experiment B showed very poor flower production during the course of the experiment and for some dates there was no flower production (Fig. 4).

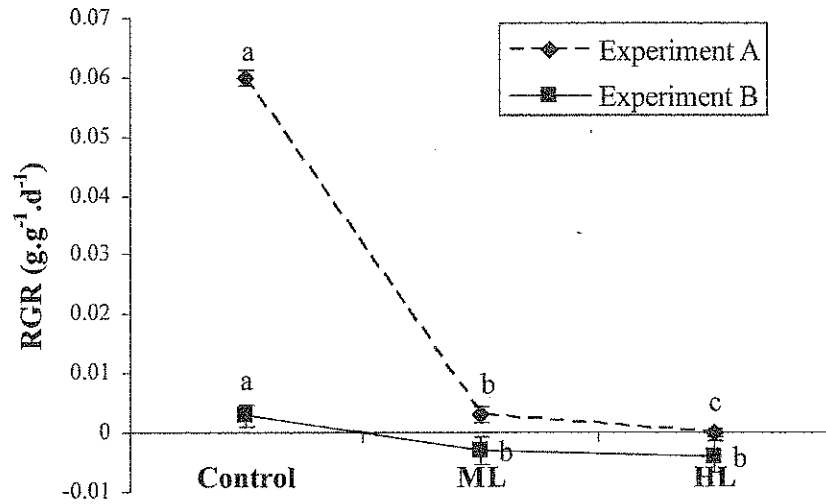


FIG. 2. - Relative growth rate (RGR) of whole plants of *Pelargonium hortorum* in Experiments A and B. Experiment A: Cu, Pb and Zn; Experiment B: Cd, Cr and Ni. Values followed by the same letter do not differ statistically according to Tukey test at $p \leq 0.05$. Values represent the average of 4 replicates with the standard deviations.

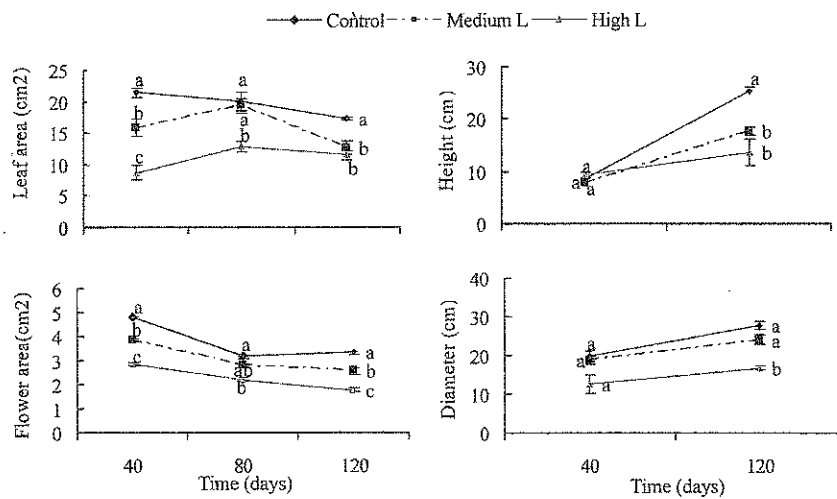


FIG. 3. - Leaf, flower area, plant height and diameter at different time intervals of Experiment A (Cu, Pb and Zn). Date 1: 40 days after transplant; date 2: 80 days after transplant; date 3: 120 days after transplant. Values followed by the same letter do not differ statistically according to Tukey test at $p \leq 0.05$.

Heavy metal concentration in roots, stems, leaves and flowers of P. hortorum. – Heavy metal concentration in roots, stems, leaves and flowers are shown in Figure 5. Metal contents at harvest showed large differences between organs. Concentrations of Cd, Cr, Ni and Pb in control plants were below detection limits in all plants organs. In contrast, Cu and Zn concentrations were fairly similar among plant organs. Although in both experiments HM accumulation was higher in roots, a significant amount of these metals was accumulated in aerial parts. The lowest metal contents were found always in flowers. In both experiments, concentrations of metals were higher in HL treatments as compared with control and ML treatments, especially for roots and stems. Copper, Pb and Zn accumulation in leaves and flowers of Experiment A was higher than the accumulation of Cd, Cr and Ni in Experiment B.

DISCUSSION. – The DTPA extractable/total ratio is a measure of HM “bioavailability” (ULRICH *et al.*, 1999). The relative bioavailability for Cd and Ni was high in both HM treatments (near to 100%), indicating greater availability of the metals. Among the toxic HM, Cd is one of the most mobile elements (KABATA PENDIAS and PENDIAS, 2000).

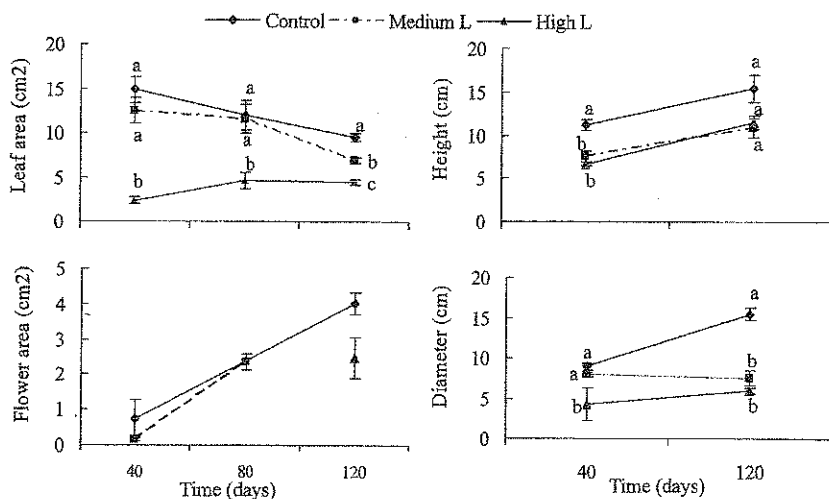


FIG. 4. – Leaf, flower area, plant height and diameter at different time intervals of Experiment B (Cd, Cr and Ni). Date 1: 40 days after transplant; date 2: 80 days after transplant; date 3: 120 days after transplant. Flower area was really poor and for some dates and treatments there were no flowers. Values followed by the same letter do not differ statistically according to Tukey test at $p \leq 0.05$.

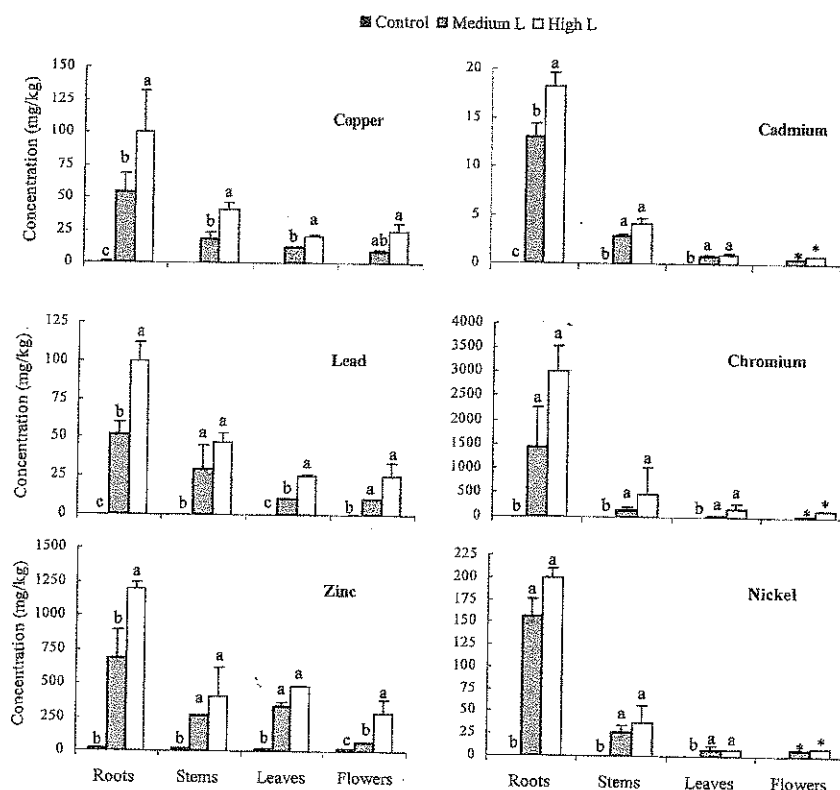


FIG. 5. - Heavy metal content (mg kg^{-1}) in roots, stems, leaves and flowers of *Pelargonium hortorum*, grown in control, ML and HL treatments. Values followed by the same letter do not differ statistically according to Tukey test at $p \leq 0.05$. Values represent the average of 2 replicates with the standard deviations.

In both experiments shoot dry weight was more affected than root dry weight. However, it is well known that root tissues are more sensitive to heavy metal toxicity than aerial tissues; this is a well documented effect of HM (ASHMORE, 1997). It was hypothesized that roots could play an important role in the retention of metals by preventing an excess of toxic accumulation in the shoots (MAZHOUDI *et al.*, 1997). However, plants showed phytotoxic symptoms, such as early senescence of mature leaves. These changes in the growth pattern are related to geranium commercial quality. The reduction in RGR is consistent with results found by other authors (HAAG-KERWER *et al.*, 1999). In the Experiment B, for both heavy metal treatments, the proportion of biomass decreased due to increased senescence and death of leaves recorded throughout the

experiment. In this sense, it is known that morphological changes can help to reduce the growth rates of plants exposed to HM. According to ANTÚNEZ *et al.*, (2001) a high proportion of leaf biomass favors high growth rates, while a high proportion of root biomass correlates negatively with RGR.

The delay in growth is a response commonly observed in a wide range of plants that grow in soils enriched with metals. In both experiments HM treatments negatively influenced height and diameter of the plants. Several authors have reported that metals inhibit root, shoot and stem elongation, and reduce the number and diameter of vascular bundles (BARCELÓ and POSCHENRIEDER, 1990). The inhibitory effect of metals on plant growth was evident by the decrease observed in leaf and flower area, especially in Experiment B. The reduction in leaf area may be associated with increased leaf senescence in HM treatments. In this regard, BARCELÓ and POSCHENRIEDER (1990) reported that HM stress in sensitive plants can induce a series of events that lead to the reduction of water loss, for example by a reduction in the number and size of leaves. One of the parameters most affected was flower production, especially in Experiment B. It should be noted that in this experiment the different photoperiod might have played a relevant role in plant response. According to TOOKE *et al.* (2005) HM stress causes delay in flowering as flower formation is directly related to environmental conditions.

Copper and Zn concentrations in control plants of Experiment B were within normal values (KABATA PENDIAS and PENDIAS, 2000). Our study shows that HM treatments at higher concentrations results in a linear increase in metal accumulation in roots followed by stems, leaves and flowers. This is in accordance with previous findings (ORROÑO and LAVADO, 2009). It is considered that Pb accumulates mainly in the roots of plants, with a very low fraction being transported to the shoot (BLAYLOCK *et al.*, 1997). However, our data suggest that at high concentrations of Pb in soil, transport to the shoot was high. The availability of Pb increased in ternary combination, which may be related to the antagonistic interaction between Cu and Zn (KABATA-PENDIAS and PENDIAS, 2000), which would allow Pb to be more available for plant uptake. The higher DTPA-extractable Cd and Ni in Experiment B and Zn in Experiment A caused more accumulation of these elements in roots and shoots of *P. hortorum*. 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, 1981). According to

REEVES and BAKER (2000) hyperaccumulator species are those able to accumulate in aerial parts $> 100 \text{ mg.kg}^{-1}$ Cd, $> 1.000 \text{ mg.kg}^{-1}$ Ni, Pb, Cu and Cr, or $> 10.000 \text{ mg.kg}^{-1}$ of Zn when grown in soils rich in metals. Although HM reached toxic concentrations in plant tissues (KABATA PENDIAS and PENDIAS, 2000), our data indicate the concentrations of HM in *P. hortorum* were lower than these values. This findings contrast with that of KRISHNARAJ *et al.* (2000) and DAN *et al.* (2002) who found that *Pelargonium* sp. "Frensham" was able to accumulate in roots and shoots Pb, Cd and Ni levels equivalent to metal hyperaccumulator plants. Also the measured Pb concentration do not compare with that of the scented geranium cultivars studied by ARSHAD *et al.* (2008), which also exceed the limit of 0.1% dry weight.

CONCLUSIONS. – Growth of *Pelargonium hortorum* plants was influenced by both sets of metals in soil. The higher the concentration of heavy metal in the soil, the greater was the toxic effect on the plant. Exposure to both sets of HM induced a decrease in shoot and root dry weight. In addition, RGR in metal treatments were significantly lower than those found in the control treatments. Experiment B was more affected than Experiment A probably due to the different sets of HM used. Furthermore, plants ornamental quality was severely affected, mainly leaf and flower production especially at HL treatments. The significantly reduction on plants biomass in addition to the storage of metals mainly in roots and restricted transfer to the aerial parts in both experiments suggested that in contrast to other members of the genus, *P. hortorum* is not hyperaccumulator. Thus, *P. hortorum* can't be considered to be a species with potential for phytoextraction purposes.

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SUMMARY – Two separate pot experiments were carried out for 16 weeks in order to evaluate heavy metal (HM) accumulation on the popular ornamental plant *Pelargonium hortorum* as well as the effects on growth and quality of plants. Experiment A studied the effects of copper, lead plus zinc, and Experiment B the effects of cadmium, chromium plus nickel. Treatments consist of control soil (non-contaminated); medium and high concentrations of copper, lead plus zinc; and medium and high concentrations of

cadmium, chromium plus nickel. Growth parameters such as dry mass, relative growth rate (RGR), leaf and flower area, plant height and diameter were determined. The results showed that exposure to HM induced a decrease in several plant growth parameters. Experiment B was more affected than Experiment A. Quality of plants was also affected, especially leaf and flower area of Experiment B. The significant reduction on plants biomass in addition to the storage of metals mainly in roots and restricted transfer to the aerial parts in both experiments suggested that in contrast to other members of the genus, *P. hortorum* is not hyperaccumulator. Values represent the average of 3 replicates with the standard deviations in parentheses.