Taylor & Francis
Taylor & Francis Group

International Journal of Phytoremediation

ISSN: 1522-6514 (Print) 1549-7879 (Online) Journal homepage: https://www.tandfonline.com/loi/bijp20

In vitro lead tolerance and accumulation in three *Chrysanthemum* cultivars for phytoremediation purposes with ornamental plants

Agripina Ramírez, Gregorio García, Olaf Werner & Rosa M. Ros

To cite this article: Agripina Ramírez, Gregorio García, Olaf Werner & Rosa M. Ros (2020): *In vitro* lead tolerance and accumulation in three *Chrysanthemum* cultivars for phytoremediation purposes with ornamental plants, International Journal of Phytoremediation, DOI: 10.1080/15226514.2020.1731730

To link to this article: https://doi.org/10.1080/15226514.2020.1731730

Published online: 28 Feb 2020.

INTERNATIONAL JOURNAL OF PHYTOREMEDIATION
https://doi.org/10.1080/15226514.2020.1731730

In vitro lead tolerance and accumulation in three *Chrysanthemum* cultivars for phytoremediation purposes with ornamental plants

Agripina Ramírez[a], Gregorio García[b], Olaf Werner[c], and Rosa M. Ros[c]

[a]Instituto de Innovación en Biotecnología e Industria, Calle Olof Palme esquina Núñez de Cáceres, Urb. San Gerónimo, Santo Domingo, Dominican Republic; [b]Departamento de Ingeniería Agronómica, ETSIA, Universidad Politécnica de Cartagena, Cartagena, Spain; [c]Departamento de Biología Vegetal, Facultad de Biología, Universidad de Murcia, Murcia, Spain

ABSTRACT

The use of ornamental plants for the phytoremediation of potentially toxic elements in polluted soils is an interesting task. It makes possible to combine environmental restoration, re-use of land, and the production of goods and services of economic interest. In this work, *in vitro* experiments using three cultivars of *Chrysanthemum* (Asteraceae) were carried out with 0, 300, 600, 900, and 1500 mg/kg of lead concentrations for a period of 12 weeks. The objective was to obtain data about their lead tolerance and bioaccumulation capacity in order to know their potential as phytoremediators in a densely populated Caribbean area of the Dominican Republic with a high concentration of lead in soils. The variations in biomass, root growth as well as accumulation of this element in the plants were measured. The results suggest that the three cultivars have a good potential for phytoextraction at moderate pollution levels, as they showed a good bioaccumulation of lead, which had mild effects on their biomass production and root elongation. Additional studies should be carried out to assess their effectiveness as phytoextractors under field conditions, as well as other alternative uses that could generate esthetic, environmental, and/or economic benefits for tropical areas contaminated by Pb.

KEYWORDS
Asteraceae; Caribbean; floriculture; heavy metals; phytoextractors; tropical areas

Introduction

Certain trace elements that are not essential for plant growth and metabolism, such as lead (Pb), can generate environmental pollution problems due to their accumulation in the food chain, which implies a risk to ecosystems and the human population (Wenzel *et al.* 2003). High concentrations of potentially toxic elements in plants tend to inhibit seed germination, growth, and the development of explants, as well as alter many physiological and biochemical processes (Sanita di Toppi and Gabbrielli 1999). They also affect the developing radicle and the growth of the plant (Monni *et al.* 2001). Essential biological functions such as enzymatic and non-enzymatic activities, in addition to photosynthesis may be affected by high concentrations of Pb (Sharma and Dubey 2005; Song *et al.* 2018; Zhou *et al.* 2018). Likewise, according to these authors, this toxic element can cause structural damage as well as affect the balance of nutrients and water in the plant, which may eventually induce intoxication symptoms that can affect the development of plants. The tolerance of a plant species to the availability of high concentrations of metals in the medium may greatly affect its usefulness in the field of phytoremediation (Pilon-Smits 2005).

The use of plants to remove, degrade, or reduce the toxicity of certain chemical elements and substances is known as phytoremediation, a low cost and effective technology under certain circumstances (Organum and Bacon 2006). Phytoremediation comprises a series of phytotechnologies, including the elimination of contaminants by phytoextraction techniques, and their immobilization by phytostabilization techniques (Raskin 1995; Salt *et al.* 1995). Phytoextraction is considered efficient when the edaphic levels of a certain pollutant decrease to reach acceptable levels (LeDuc and Terry 2005). The success of any phytoextraction strategy depends on the identification of suitable plant species which accumulate high concentrations of metals and produce large quantities of biomass; however, few species of plants are adapted to live in soils polluted with metals (Reeves 1992).

Phytoextraction, also called phytoaccumulation, is one of the most interesting techniques due to its ability to completely remove toxic waste from polluted soils; it is based on the fact that the root systems of plants capture and absorb contaminants for storage in the aerial part, mainly in leaves and stems (Cunningham and Berti 2000; Manousaki *et al.* 2008; Nagajyoti *et al.* 2010). Some plants can capture, tolerate, and even hyperaccumulate trace elements and other toxic substances from the environment, soil, and water (Sharma and Dubey 2005). Nevertheless, most of the plants that grow in soils rich in metallic elements are not hyperaccumulators, but heavy metal-excluding species, and therefore

CONTACT Agripina Ramírez agripinars@gmail.com Instituto de Innovación en Biotecnología e Industria, Calle Olof Palme esquina Núñez de Cáceres, Urb. San Gerónimo, Santo Domingo, Dominican Republic.

they can be used in phytostabilization programs. A relevant feature of these excluder plants is that they can transform elements into less active forms, without removing them from the substrate (Chaney *et al.* 1997), using mechanisms of root absorption, adsorption, and accumulation, as well as precipitation of toxic substances in the rhizospheric environment (Wong 2003).

For this reason, only some plant species can be selected for phytoremediation purposes, depending on their ability to tolerate, accumulate, and/or modify polluting elements. Thus, through proper management, environmental restoration can be made compatible with contaminated land use and the production of goods and services of economic interest, as suggested by LeDuc and Terry (2005). In this respect, the dual utility of phytoremediation technology should be noted, since it has a positive economic impact while generating the environmental recovery of the polluted soil. Therefore, it is necessary to redirect the lines of current research toward more applied aspects, which is of particular interest for developing countries.

The ability of some ornamental species for the phytoremediation of potentially toxic elements and the creation of benefits was evaluated by Nakbanpote *et al.* (2016). These authors emphasized the advantages of using this kind of plant, in order to enhance landscape esthetics and enable recovery of the environment, while generating employment and economic benefits. There is also the interest of decreasing the risk of contaminants entering the food chain because many of these plants are not edible. When addressing the study of the tolerance and accumulation level of a metal by plants, and their phytoremediation potential, there are several strategies to follow. Probably the best way is to cultivate them under field conditions (Salt *et al.* 1995), but another good and practical way is to grow them in an *in vitro* system, as it allows a quick characterization of plants for their effectiveness in tolerating and accumulating metals (Kališová-Špirochová *et al.* 2003; Di Lonardo *et al.* 2011; Wiszniewska *et al.* 2015).

The present work focuses on the study of the effect of Pb on the growth of several plants of agronomic interest for the Dominican Republic, where, for example, lead is the most important pollutant of the soils of the municipality of Haina, located in the province of San Cristóbal. This densely populated area was considered as the third most contaminated site on the planet, due to the enormous accumulation of heavy metals, especially Pb (Blacksmith Institute 2006; McCartor and Becker 2010). Three cultivars (cv) of the genus *Chrysanthemum* L. (Asteraceae) were studied in an *in vitro* culture system, as information on the impact of heavy metals on the tissues of these plants remains small and fragmentary. In general, chrysanthemums are very popular cut flowers and ornamental pot plants of high economic value (Miñano *et al.* 2009). The variations in the levels of growth, as well as accumulation of this element in the plants, were measured in five different treatments with increasing Pb concentrations in the substrate in order to examine their mechanisms of tolerance and resistance to Pb and their capacity as hyperaccumulators.

The main goal of this research was to evaluate the lead tolerance and bioaccumulation of three cv of the genus *Chrysanthemum* as a previous step to know their potential use as phytoremediators, for phytoextraction or phytostabilization purposes. Subjecting the plants to the stress of contamination by Pb in the substrate we intended to explore their possible use in contaminated soils of a densely populated Caribbean area of the Dominican Republic while generating economic benefits.

Materials and methods

Plant material

After evaluating the most frequent cultivated plants of commercial interest or thought to be of potential interest in the Haina area, cv belonging to the genus *Chrysanthemum* were selected, according to their availability in the country, economic value, and biological characteristics.

The following commercial cv were studied: ‘Renella,’ ‘Reyellow,’ and ‘Sheena.’ All three cv belong to the *Chrysanthemum indicum* L. group (PlantScope database: https://www.plantscope.nl/pls/pswprd/!psw_main.pagina). The plants were obtained from the work collection of the Institute of Innovation in Biotechnology and Industry (IIBI) of Santo Domingo, Dominican Republic. They were imported into the country as potted ornamental plants for use in gardening and as cut flowers for decoration.

The “International Code of Nomenclature for Cultivated Plants” (Brickell *et al.* 2009) was followed.

A summarized description and breeder information of each taxon are given below:

‘Renella’ is a relatively robust perennial plant, 65–75 cm stem length, with about five capitula each that can have different color. Breeder: Deliflor (obtained from Sierra Flowers Trading Inc.).

‘Reyellow’ is a perennial very compact plant, 60–70 cm stem length, with single, deep yellow capitula with 4 cm in diameter. Breeder: Chrysanthemum Breeder Bv (An) (obtained from The International Union for the Protection of New Varieties of Plants, UPOV).

‘Sheena’ is a tender perennial plant, 100–150 cm stem length with pale capitula and keeled flowers, darker toward the center, produced in late autumn. Breeder: Twyford Plant Lab. Ltd./Southern Glass House Produce L/Golstock Breeding (obtained from UPOV).

In vitro *cultures*

The growth of plants in controlled laboratory conditions using a culture medium containing different Pb concentration allows knowing the response of the plants toward this contaminant. In this way, the behavior of plants is not influenced by the interaction between several toxic elements or other environmental factors, as would be the case under field conditions (Rout and Das 2003).

In order to have genetically uniform plant material, we used vegetative parts of plants and not seeds. The ends of the stems of plants grown in a nursery for six weeks were excised. Each fragment, 1 cm in length and 1 cm in diameter contained the meristematic ends (the apical vegetative point and the nearest young leaves). Prior to inoculation *in vitro*, the plants were cleaned with liquid soap and rinsed with distilled water. They were then placed in a 70% ethanol solution for 20–30 s, rinsed again with sterile deionized water, and placed in a 10% (*w/v*) solution of commercial bleach (1.58% sodium hypochlorite) for about 20–30 min (Salas Salmerón 2007; Felek *et al.* 2015). Subsequently, they were rinsed three more times with sterile deionized water. The disinfected plants were placed in glass flasks of 100 ml capacity, containing 20 ml of the Murashige and Skoog (MS) basal medium (Murashige and Skoog 1962) supplemented with 3% sucrose, at a pH of 5.7 and solidified with 6.3 g/L agar. The flasks were then placed in a dark incubation chamber at a temperature of 25 °C until growth began (1–17 weeks). They were then allowed to grow for five weeks with a photoperiod of 8 h of darkness and 16 h light at 25 ± 3.0 °C, after which treatment with Pb was started. To this end, the seedlings were transplanted into the flasks containing the culture medium to which the Pb was added and allowed to incubate for 12 weeks in order to simulate the actual process of phytoremediation. The stage of plants development analyzed corresponded to their young vegetative system, without flowers.

Five different treatments were applied, consisting of the addition to the basal MS medium of increasing Pb concentrations (0, 300, 600, 900, and 1,500 mg/kg) in the form of Pb nitrate ($Pb(NO_3)_2$), which gave equivalent concentrations in mg/kg in the growth substrate. The 0 mg/kg concentration was used as control; 300 mg/kg as a low-moderate Pb concentration; 600 mg/kg as a high moderate and $\geq$900 mg/kg as a high concentration.

These concentrations corresponded to the range used in similar studies (García *et al.* 2004) and coincided with the range to which these taxa could be exposed in the Haina area soils (Ramírez Sánchez 2017). Three replicates of each treatment were made for each cv, making a total of 15 samples of each cv.

Processing and analysis of plant samples

At harvest, the plants were rinsed with tap water, paying special attention to the cleaning of the root portion. A brush was used in order to remove the traces of culture medium and avoid damage to the roots. Subsequently, they were rinsed twice with distilled water. The length of the main root of the cultivated explants was measured and the fresh weight of the plants was then quantified. Also, the dry weight was obtained after fragmentation and drying at 60 °C for 48 h in an oven. They were then crushed in a mill and approximately 5 g dry weight was taken from each. An Ohaus precision balance, model AR2140, was used for weighing. Subsequently, the samples were digested with a mixture of HNO_3 (65% concentration) and H_2O_2 (30% concentration) in a 4:1 ratio, in a microwave oven (Li *et al.* 2007). The elemental composition was analyzed by Atomic Absorption Spectroscopy (EAA) (Thermo Scientific iCE 3000 Atomic Absorption Spectrometer) at the Laboratory of Mineralogy of IIBI.

Quality control

High-quality grade reagents provided by Fisher-Scientific were used, while solutions and dilutions were performed with purified water obtained from an Elix 3/Milli-Q Element system (Millipore, Billerica, MA, USA). All glassware and polyethylene material used were previously treated for at least 24 h in 10% NHO3 (*v/v*) and rinsed with deionized water before use.

Reagent blanks and reference samples were used to document the bias and precision of the analytical process. A reagent blank refers to an error in test results that comes from the reagents themselves and that should be subtracted from test results. In order to avoid these errors, Pb free reagents were used. As a consequence, all analytical results for these reagent blanks were zero. On the other hand, one reference sample was prepared for every 10 samples to be analyzed by adding a known and variable quantity of the element of interest to a given amount of pure water. These reference samples were sent blindly to the analysis laboratory with each batch of samples. For the analyzed reference samples the mean recovery value, in percentage, was around 92 ± 3.

In addition, replicates were used to improve the accuracy of the calculations and for statistical purposes. In this sense, it is generally better to work, at least, with three replicates, when possible.

Bioaccumulation factor

The phytoextractive or bioaccumulative capacity of Pb from each cv was evaluated from the bioaccumulation factor (BF). This factor indicates the efficiency of plants to accumulate in their tissues the metal present in the substrate in which it grows and is equal to the concentration of this element in the plant tissues, divided by the content of that same element in the substrate (Vyslouzilova *et al.* 2003). For each cv three replicates per treatment were made.

Statistical analyses

The standard descriptive statistics for metal concentrations as mean values and standard deviation of biomass production and root elongation as well as the BF were calculated using program R 3.3.1 (R Development Core Team 2016). The same program was used to determine the regression lines between variables. For the correct interpretation of the data, it was important to estimate in what proportion the observed values fit the regression line. This function is fulfilled by the coefficient of determination R^2 with values ranges between 0 and 1 and is equal to the square of the correlation coefficient. Since a regression line with a slope other than 0 shows that at least one of the concentrations of Pb used differs from the others in terms of the expected

Table 1. Average biomass production (g dry weight ± standard deviation) for each *Chrysanthemum* cultivar studied and treatment with different concentrations of Pb in the culture substrate.

Cultivar	0 mg/kg	300 mg/kg	600 mg/kg	900 mg/kg	1,500 mg/kg
Chrysanthemum 'Renella'	0.12 ± 0.04	0.14 ± 0.03 $p = 0.76908$	0.14 ± 0.03 $p = 0.76908$	0.12 ± 0.02 $p = 0.92707$	0.11 ± 0.03 $p = 0.92077$
Chrysanthemum 'Reyellow'	0.12 ± 0.06	0.12 ± 0.01 $p = 0.78419$	0.13 ± 0.02 $p = 0.62027$	0.06 ± 0.02 $p = 0.34180$	0.05 ± 0.01 $p = 0.22094$
Chrysanthemum 'Sheena'	0.21 ± 0.02	0.16 ± 0.00 $p = 0.31154$	0.17 ± 0.07 $p = 0.46360$	0.05 ± 0.02 **$p = 0.01348$**	0.07 ± 0.01 **$p = 0.02675$**

The p values (obtained by the Conover test and Benjamini & Hochberg correction) indicate the probability that the biomass reached with the Pb treatment is equal to the biomass of the control (0 mg/kg). Significant p values ($p < 0.05$) are marked in bold.

value and does not specify how many differ or identify them, a Conover test (Conover and Iman 1979) was used to identify treatments that resulted in significant deviations from the control samples. This test is non-parametric, so that it does not depend on the assumptions necessary to carry out an ANOVA. The necessary calculations were carried out using the PMCMR package available under R (Pohlert 2014). When carrying out multiple comparisons, the correction of Benjamini and Hochberg (1995) was applied in order to adjust the p values.

Finally, for the visual presentation of the statistical data in the form of diagrams, the ggplot2 package (Wickham 2009) was used (within R 3.1.1; R Development Core Team 2016).

Results and discussion

Studies of *in vitro* grown plants can be considered as a first approach to research the ability of a plant to resist one or more elements under "ideal" conditions. In this work, three variables were evaluated in the three *Chrysanthemum* cv selected when growing in substrates to which increasing amounts of Pb were added. These variables were: the production of plant biomass, the root growth, and the magnitude of the bioaccumulation of the contaminating element by the plants.

Effect of Pb concentration in the substrate on biomass production

The total biomass of the studied cv was examined as a measure of their response to the stress produced by Pb. The average dry weight expressed in g ± standard deviation for each cv used and the different treatments with Pb (Table 1) was obtained.

'Reyellow' and 'Sheena' showed biomass reductions of up to 75% when grown at or above 900 mg/kg Pb concentrations in the substrate; although in the case of 'Reyellow' the Conover test was not significant. In 'Renella', there were no significant effects on biomass production as a function of Pb concentration in the substrate (Figure 1).

It was also verified that many of the plants exposed to the highest concentrations of Pb (900 and 1,500 mg/kg) died or suffered leaf fall and general decay. Only in the 300 mg/kg treatments were 'Renella' and 'Reyellow' able to maintain good growth throughout the experiment, essentially similar to that of the control group. In the 900 and 1,500 mg/kg treatments, plants of 'Reyellow' and 'Sheena' showed difficulties in maintaining normal growth. This suggests a strong impact of Pb on growth in both cv, particularly in 'Sheena', which may be due to the metal-induced toxicity interfering with the normal physiological processes of these plants. Some of the described physiological damages are related to the increase in electrolyte leakage and malondialdehyde content in leaves, the decrease in peroxidase activity, soluble protein level and photosynthetic pigment content in leaves, and the chloroplast structure change (Gallego *et al.* 1996; Shakoor *et al.* 2014; Song *et al.* 2018; Zhou *et al.* 2018). Also, physiological functions can be lost due to cell membrane permeability increase, and decrease in plasticity and elasticity of the cell wall when plants are treated with Pb (Cao *et al.* 2005).

Our findings are in accordance with the results obtained by previous authors. For example, Mishra *et al.* (2006) and Chen *et al.* (2015) pointed in *Ceratophyllum demersum* L. to the interaction between the duration of exposure and the Pb concentration in the substrate as an important factor influencing the biomass production. Also, Song *et al.* (2018) observed the inhibition of biomass accumulation in *Larix olgensis* A. Henry when Pb accumulation in fine roots and leaves took place. With our results, it can be stated that the effects of Pb on plant growth may vary not only between plant species, as Hu *et al.* (2012) observed, but even between cv of the same species.

Effect of Pb concentration in the substrate on root growth

The effects of Pb toxicity on root length of the studied cv, estimated as an indicator of overall plant growth and stress status, are shown in Table 2. The rapid inhibition of root growth is one of the primary effects of Pb toxicity in plants, probably due to the hitch of cell division in the root apex (Eun *et al.* 2000; Fahr *et al.* 2013; Nas and Ali 2018).

All the plants studied presented a negative and strong relationship between the values of the root length and the increasing concentration of Pb in the substrate (Figure 2). 'Renella' and 'Sheena' showed particularly strong reductions in root length (43 and 57%, respectively) at and above 300 mg/kg of Pb in the substrate. At 900 and 1,500 mg/kg, the reductions reached values of more than 75 and 95%, respectively. 'Reyellow' manifested a more moderate effect on root length, with a reduction of 33% for 600 mg/kg of Pb in the substrate, reaching maximum reduction for the highest concentration of Pb considered (1,500 mg/kg). These results show that even the lowest concentration of Pb

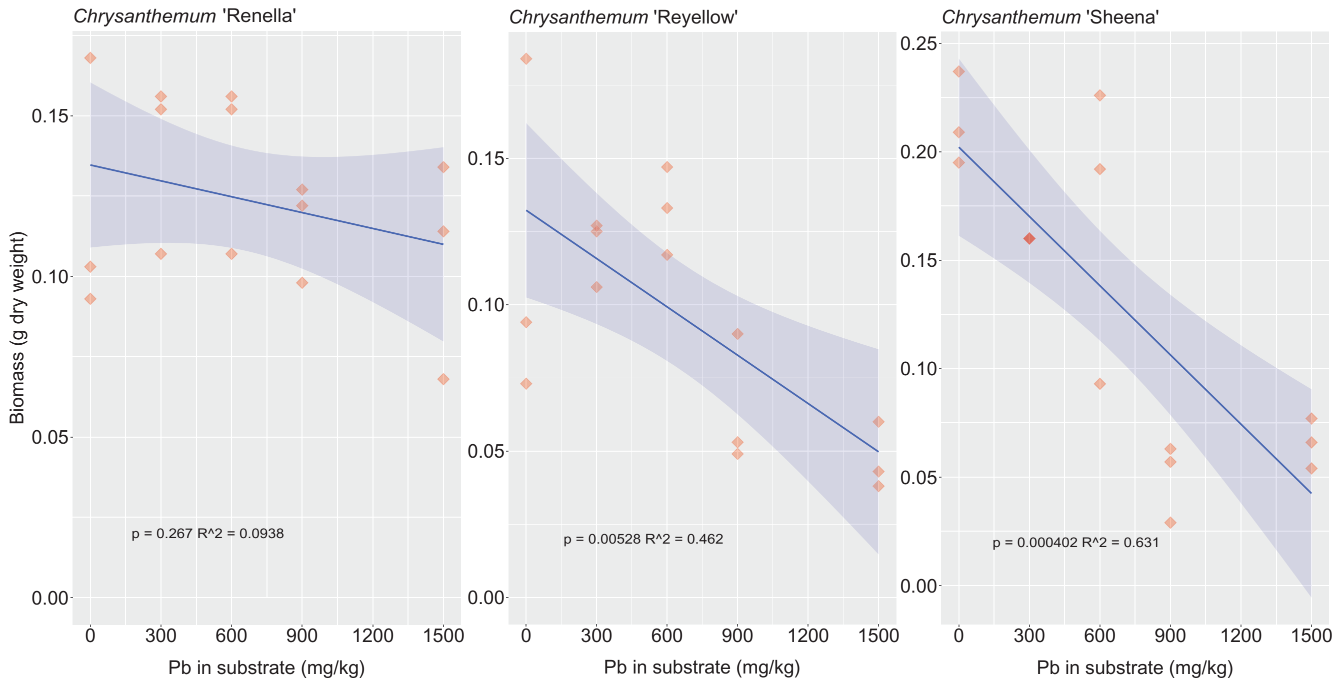

Figure 1. Graphical representation of average biomass production (expressed in g of dry weight) achieved by the *Chrysanthemum* cultivars studied for each of the Pb treatments (expressed in mg/kg) after 12 weeks of *in vitro* culture. The regression line is marked in blue, and the confidence interval (95%) is indicated by the dark blue zone. The *p* value indicates the probability that the concentration of Pb has no significant effect on biomass production and the coefficient of determination R^2 with value ranges between 0 and 1 is equal to the square of the correlation coefficient.

Table 2. Root length (mm ± standard deviation) for each *Chrysanthemum* cultivar studied and each concentration of Pb in the culture substrate.

Cultivar	0 mg/kg	300 mg/kg	600 mg/kg	900 mg/kg	1,500 mg/kg
Chrysanthemum 'Renella'	20.67 ± 8.02	12.47 ± 2.54	11.10 ± 4.71	5.23 ± 0.87	4.60 ± 0.69
		$p = 0.35915$	$p = 0.35915$	**$p = 0.02658$**	**$p = 0.01789$**
Chrysanthemum 'Reyellow'	8.77 ± 3.02	11.97 ± 4.05	5.57 ± 1.60	6.60 ± 2.25	3.47 ± 1.27
		$p = 0.58320$	$p = 0.28682$	$p = 0.48060$	$p = 0.15849$
Chrysanthemum 'Sheena'	22.67 ± 9.29	10.00 ± 6.14	6.93 ± 5.40	1.10 ± 0.85	1.50 ± 0.40
		$p = 0.31530$	$p = 0.13379$	**$p = 0.02467$**	**$p = 0.03524$**

The *p* values (obtained by the Conover test and Benjamini & Hochberg correction) indicate the probability that the root length reached with the Pb treatment is equal to the root length of the control (0 mg/kg). Significant *p* values ($p < 0.05$) are marked in bold.

(300 mg/kg) produced a reduction in root growth of some of the taxa considered, although significant differences with plant control values were generally found for Pb concentrations above 600 mg/kg.

There is, then, clear evidence of a strong impact of Pb on root development in the affected plants even at moderate Pb concentration in substrate, which coincides with the observations of other authors (Mani *et al.* 2015). It should not be forgotten that Pb accumulates mainly in the roots of plants, and its transfer to the aerial parts is difficult (Yoon *et al.* 2006), which increases the risk of deleterious effects in these underground parts of the plants.

The root length clearly shows how Pb affects root growth as the Pb concentrations increase in the culture medium, which corresponds to the description of a nonspecific visual symptom (Sharma and Dubey 2005). These results reflect those described by other authors. For example, Hussain *et al.* (2013) observed that the root–shoot length in *Zea mays* L showed inhibition in seed germination at different lead concentrations. Also in other vascular plant species, a decrease in the length and root dry mass under Pb toxicity was reported in *Triticum aestivum* L. (Dey *et al.* 2007; Kaur *et al.* 2013), *Pisum sativum* L. (Malecka *et al.* 2009), and *Sedum alfredii* Hance (Gupta *et al.* 2010).

A particular case is 'Renella' cv, which seemed not to be affected in its above surface biomass production even at the highest Pb concentration in substrate, although it had an impact on the root's growth. Even though usually root elongation inhibition implies that fresh and dry weights of both roots and shoots are also reduced (Fahr *et al.* 2013), this does not always happen under *in vitro* conditions where mineral salts and water are not limiting factors, particularly in the case of hyperaccumulators and young plants (Seregin and Ivanov 2001).

Therefore, in our experiment, root length of all the studied cultivars was reduced by the different treatments, although the statistical tests were not significant for 'Reyellow'. While the physiological and biochemical effects of Pb in roots in the case of *Chrysanthemum* are not known, authors such as Fahr *et al.* (2013) reported effects of lead on root development and plant physiology, emphasizing damages at cellular level, mineral homeostasis and genotoxicity.

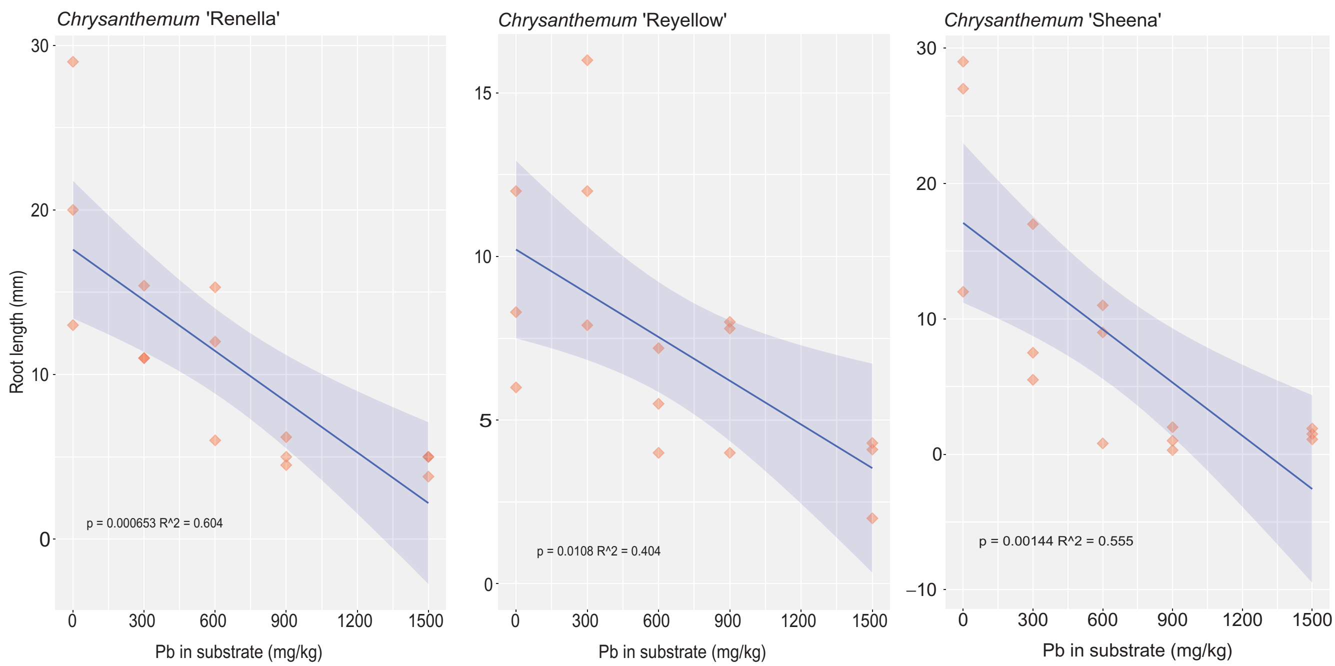

Figure 2. Graphical representation of the average root length (expressed in mm) reached by the *Chrysanthemum* cultivars studied for each of the Pb treatments (expressed in mg/kg) after 12 weeks of *in vitro* culture. Regression line, confidence interval, and R^2 information is the same as in Figure 1. The *p* value indicates the probability that the concentration of Pb has no significant effect on the length of the roots.

Table 3. Average concentration of Pb in the tissues of plants (mg/kg ± standard deviation) for each *Chrysanthemum* cultivar studied and each concentration of Pb in the culture substrate, as well as the average concentration values for all treatments.

Cultivar	0 mg/kg	300 mg/kg	600 mg/kg	900 mg/kg	1,500 mg/kg	Average values all treatments
Chrysanthemum 'Renella'	0.00 ± 0.00	1431.57 ± 173.46 $p = 0.16937$	1464.91 ± 440.22 $p = 0.16937$	1699.98 ± 239.18 $p = 0.07021$	5029.36 ± 1031.69 **$p = 0.00389$**	1925.16
Chrysanthemum 'Reyellow'	0.00 ± 0.00	1321.14 ± 247.25 $p = 0.18985$	1178.46 ± 138.00 $p = 0.19963$	3863.58 ± 2700.26 **$p = 0.02395$**	3224.82 ± 1895.88 **$p = 0.02675$**	1917.60
Chrysanthemum 'Sheena'	22.53 ± 3.54	2289.01 ± 986.20 $p = 0.11044$	2322.66 ± 1945.12 $p = 0.08921$	3884.05 ± 1388.57 **$p = 0.01036$**	1937.87 ± 246.73 $p = 0.14412$	2091.22

The *p* values (obtained by the Conover test and Benjamini & Hochberg correction) indicate the probability that the amount of Pb in the tissues grown under different treatments is equal to that of the control treatment (0 mg/kg). Significant *p* values ($p < 0.05$) are marked in bold.

Pb concentration in plant tissues. BF

In relation to the average concentration of Pb in plant tissues (Table 3), the plants showed visible symptoms of intoxication for the highest concentrations of Pb in the substrate (900 and 1,500 mg/kg). These symptoms consisted in the decrease in root density and the woody and thick appearance of the roots, what agrees with Seregin and Ivanov (2001) who observed that roots can also respond via changes in volume and diameter, with the production or inhibition of lateral roots. In the case of 'Renella', the levels of Pb concentration showed a progressive increase from 300 to 1,500 mg/kg (Figure 3). 'Reyellow' and 'Sheena' showed a similar gradual but significant increase in Pb accumulation up to 900 mg/kg; however, 'Reyellow' showed a slight decrease in Pb concentration (16%), and 'Sheena' a strong decrease (50%) in Pb concentration in the tissues from 900 to 1,500 mg/kg (Table 3). In this respect, Chen *et al.* (2015) also found that the concentration of Pb in some plants fell after reaching a maximum. When plants are developed in substrates affected by toxic trace elements, such as Pb, they can be affected by these elements to a greater or lesser extent (Sridhara Chary *et al.* 2008; Zhuang *et al.* 2009; Zhao *et al.* 2010; Khillare *et al.* 2012; Minkina *et al.* 2012; Chen *et al.* 2014).

Therefore, in general, an increase in the substrate Pb concentration had a positive effect on the bioaccumulation of Pb by all the cv considered. In any case, one aspect that must considered is the effect of the plant biomass on the accumulation rate. To the extent that the BF is a ratio between the concentration of the element studied in the plant tissues and the concentration of this element in the substrate in which the plant lives, the greater or lesser biomass itself has no effect on this accumulation rate. A separate issue is the fact that, especially in woody species (Aman *et al.* 2018) or with a full development (Kaur *et al.* 2018), a greater development of biomass implies the appearance of vegetative or reproductive structures in which accumulation of the elements can be different regarding the considered plant organ, what might entail a dilution of the final concentration bioaccumulated by the plant, mainly if we focus our analysis only in roots and leaves. This aspect should be taken into account when considering an experiment under field conditions and for long periods of time, in which the plant can develop a higher biomass, with vegetative and reproductive structures, so the bioaccumulative behavior of

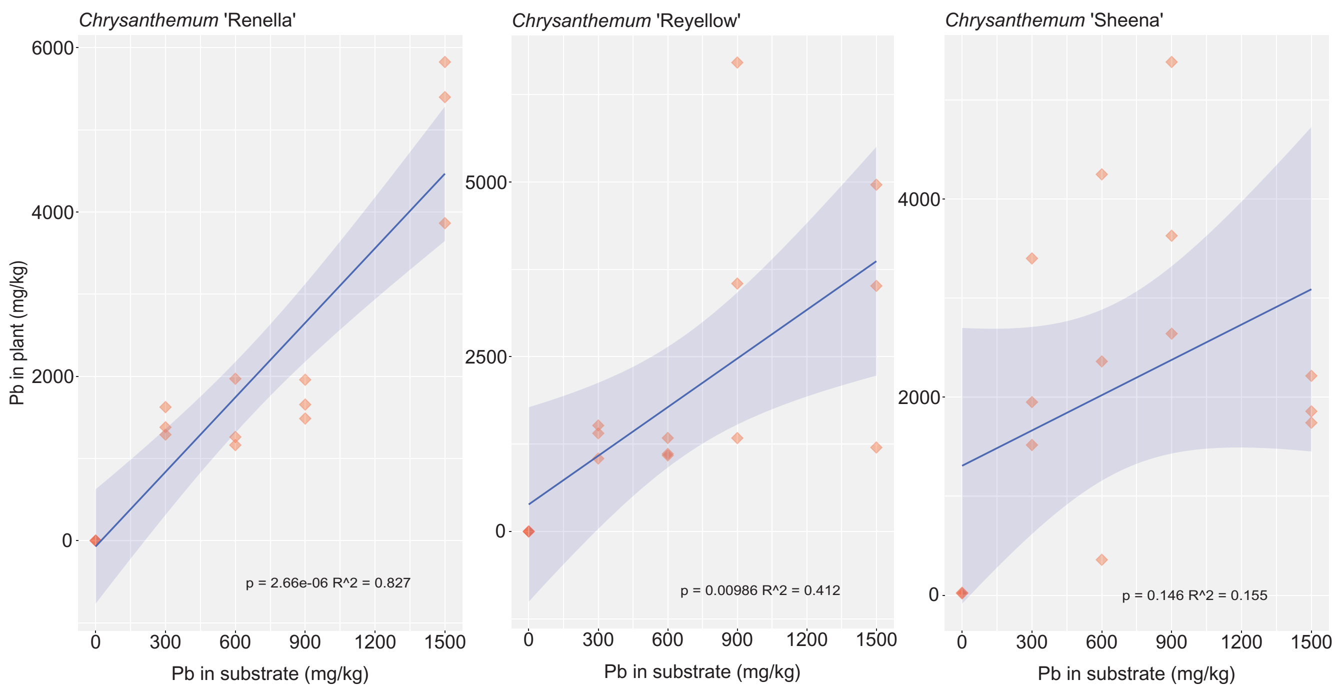

Figure 3. Graphical representation of the average concentration of Pb (expressed in mg/kg) in the tissues of the *Chrysanthemum* cultivars studied for each of the Pb treatments (expressed in mg/kg). Regression line, confidence interval, and R^2 information is the same as in Figure 1. The *p* value indicates the probability that the concentration of Pb in the substrate has no significant effect on the concentration of Pb in plants.

Table 4. Average bioaccumulation factor (BF) for each *Chrysanthemum* cultivar studied and each treatment with different concentrations of Pb in the substrate, as well as the average values for all treatments.

Cultivar	300 mg/kg	600 mg/kg	900 mg/kg	1,500 mg/kg	Average values all treatments
Chrysanthemum 'Renella'	4.77	2.39	1.89	3.35	3.10
Chrysanthemum 'Reyellow'	4.40	1.96	4.29	2.15	3.20
Chrysanthemum 'Sheena'	7.63	3.87	4.32	1.29	4.27

these plants may not be the same as the one observed in this laboratory experiment. In this respect, it has been suggested that high concentrations of Pb can cause alterations in cell membranes that can adversely affect the ability of the soil/plant barrier to control its absorption (Yang *et al.* 2000). However, the tendency observed in our study indicates that the absorption and translocation of Pb toward the aerial part is proportional to the increase in Pb in the medium, at least under the controlled conditions of an *in vitro* assay as developed here.

In relation to the fact that the Conover test in all the cv was not significant at Pb concentrations of 300 and even 600 mg/kg (Table 3), which also showed a high standard deviation, it must be considered that the Benjamini and Hochberg correction was used to correct the effects of multiple comparisons. A comparison of two groups by a Student's *t* test without correction gives much lower *p* values (data not shown).

The three cv studied exhibited different BF values, although in all cases they were clearly >1.0 (Table 4). The average BF value for all treatments was 4.27 for 'Sheena', 3.20 for 'Reyellow' and 3.10 for 'Renella'. It can be seen in Figure 4 how the BF depends on the concentration of Pb in the growing medium. 'Sheena' was the only cv in which there was a correlation between the BF and the Pb values in the substrate.

Interest of the cultivars studied in the field of phytoremediation

The use of selected plant species for metal phytoremediation programs (Vangronsveld *et al.* 2009) or even to eliminate chemical compounds (Wang *et al.* 2018) has become a very frequent and interesting tool. When looking for suitable species for a metal phytoremediation program several factors must be taken into account. First of all, they must be tolerant, but in the case of phytoextraction they must also have good biomass production and high metal accumulation in above ground parts of the plant, or in the case of phytostabilization a good root system and accumulation in the aerial parts (Vangronsveld *et al.* 2009). Therefore, they should also show good levels of accumulation in aerial parts, with BF >1.0 for the case of phytoextraction and quite the opposite for the case of phytostabilization (García Fernández and Angeler 2001; Pilon-Smits 2005; Wei *et al.* 2008; Vangronsveld *et al.* 2009).

Taking into consideration the BF values and also the Pb content of the tissues of the cv studied, the latter greater than the threshold value used by various authors when considering a taxon as hyperaccumulator (1,000 mg/kg) (Baker and Brooks 1989), these three taxa can be considered more efficient than other cited as Pb accumulators (Bech *et al.* 2002; Salas Salmerón 2007; García-Salgado *et al.* 2012). We deduce that the three cv are clearly Pb hyperaccumulators,

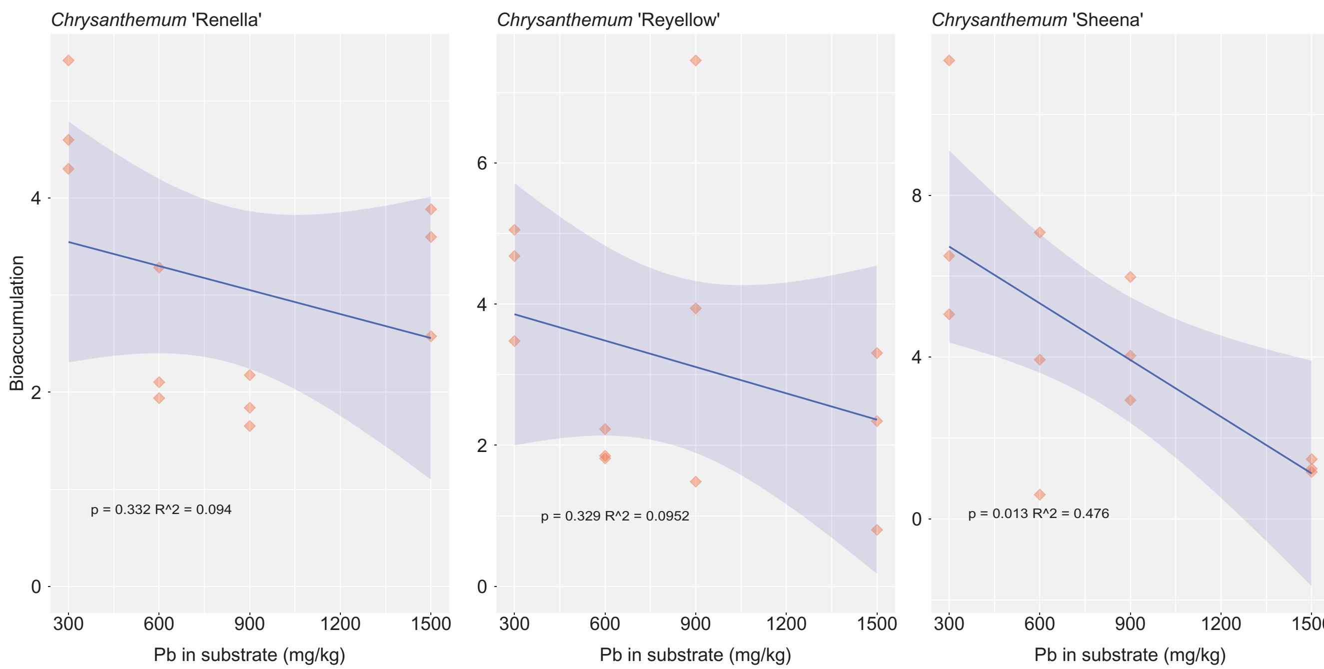

Figure 4. Average bioaccumulation factor (BF) of the *Chrysanthemum* cultivars studied under the different Pb treatments. Regression line, confidence interval, and R^2 information is the same as in Figure 1. The *p* value indicates the probability that the concentration of Pb in the substrate has no significant effect on the BF.

with an absolute increase in the concentration of this element in tissues as the substrate concentration increased, although maximum accumulation in 'Reyellow' and 'Sheena' occurred at 900 mg/kg. However, the regression lines showed a positive slope in all cases, except 'Sheena', which did not show a significant regression.

Tolerance to the presence of Pb was then evaluated to complete the evaluation of this capacity within the field of phytoremediation. This was done by reference to the production of biomass and the degree to which root growth was affected.

In relation to total biomass production, it should be noted that the presence of Pb in the growing environment produced a significant decrease in biomass in most of the treatments in 'Sheena'. However, the opposite was true for 'Renella' and 'Reyellow' cv, which showed no significant effects on biomass production, seemingly reflecting its high tolerance to the action of Pb.

As regards root growth, it should be noted that the most tolerant (lower degree of root growth reduction) toward increasing concentrations of Pb in the substrate was 'Reyellow' cv, followed by 'Renella' cv.

With respect to the Pb concentration in plant tissues, this parameter increased gradually in all three cv up to 900 mg/kg in 'Reyellow' and 'Sheena' and up to 1,500 mg/kg in 'Renella'.

In light of these findings, it can be said that 'Renella' presents great potential for phytoextraction purposes, as it shows a good bioaccumulation of Pb, while its biomass production is little affected and its root elongation moderately so. In either case, all the studied cultivars showed sufficient potential to be considered for phytoextraction purposes, especially in environments with moderate or low levels of Pb contamination.

As regards the genus *Chrysanthemum* in general, it should be noted that, according to Abdullah and Sarem (2010), it has a good capacity to accumulate Pb, and is considered very useful for reducing toxic levels in soils after several months of cultivation. However, metal concentration in the plant tissues was higher in roots and decreased progressively in shoots and flowers, results that are in accordance with Seregin and Ivanov (2001) and Sharma and Dubey (2005) who stated that the content of the heavy metals in plant organs decreases in the order: roots > leaves > stems > inflorescences > seeds. Based on these data, and with a view to its large-scale application, it would be necessary to assess the levels of accumulation in the above ground parts of the plant under real production conditions (Vangronsveld *et al.* 2009).

In the specific case of *C. indicum*, when it was subjected to increasing concentrations of Pb in the substrate, it was observed that above a concentration of 50 mg/kg of Pb in the soil, the plant showed an important reduction in size, as well as in root length and foliar and root biomass (Mani *et al.* 2015). These authors concluded that *C. indicum* showed phytoextraction potential in Pb-contaminated soils, despite the very low threshold from which plants suffer negative effects on their growth. In this respect, data from our experiments under controlled conditions revealed much higher tolerance levels for the studied taxa, with almost normal biomass in substrates with a Pb concentration of up to 600 mg/kg, which is a threshold almost 12 times higher than that described by Mani *et al.* (2015). However, in terms of root length, these thresholds were slightly lower to those observed for biomass production.

The data of Mani *et al.* (2015) are in agreement with the obtained in our *in vitro* study and suggest that the three cv studied here (that belong to the *C. indicum* group) could be used for phytoextraction of areas moderately polluted with lead in the Dominican area of Haina, with Pb concentration values in soil below 600 mg/kg. Nevertheless, there is still a need to make an assessment of the tolerance, accumulation of Pb at each part of the plant and degree of biomass production in the studied cv under field conditions in order to evaluate the real phytoextractor potential of these taxa, as well as the risks associated with the aerial parts of the plants according to their level of Pb.

Studies of phytoremediation in tropical areas have mainly been carried out in Asian areas, such as India, Malaysia, and Thailand (Ang *et al.* 2010; Nakbanpote *et al.* 2016), but far fewer works deal with tropical Caribbean areas. Particular note should be taken of Thailand, where wide scale commercial and technological development has taken place in the cultivation of various ornamental species for the production of flowers and even seeds (Nakbanpote *et al.* 2016).

The use of ornamental plants for phytoremediation such as the studied *Chrysanthemum* cv, has the advantage of preventing accumulated heavy metals from entering the food chain (Nakbanpote *et al.* 2016; Lajayer *et al.* 2019). An important aspect to consider is that the main interest of ornamental plants lies in their aerial parts (flowers, foliage, scent, *etc.*), while their roots are irrelevant in this regard. As an example of this, various authors (Chintakovid *et al.* 2008; Liu *et al.* 2008; Trigueros *et al.* 2012; Cui *et al.* 2013; Chaturvedi *et al.* 2014; Pérez-López *et al.* 2014) showed that several ornamental genera such as *Antirrhinum* L., *Calendula* L., *Erica* L., *Nerium* L., and *Tagetes* L., among others, concentrate heavy metals in roots.

What is for sure, in all events, is that the *Chrysanthemum* cv used in this study could be grown for landscape and decorative purposes at the same time than for phytoremediation of these lands. Even during their growth in the field, they have an esthetic value enriching the landscape, especially in urban areas (Prapagdee and Wankumpha 2017). They would certainly modify the aspect of the affected area, making it an attractive landscape for tourism. But only if the accumulation level in the capitula were low, as some authors seem to remark (Seregin and Ivanov 2001; Sharma and Dubey 2005; Mani *et al.* 2015), these plants could be marketed as cut flowers generating wealth and employment for the local population, and providing a steady source of incomes (Nakbanpote *et al.* 2016; Lajayer *et al.* 2019).

In any case, it would be very important that parts of the plants containing the highest concentration of heavy metals are not dispersedly discarded after death or harvest, but managed and disposed safely; techniques such as composting, compacting, pyrolysis, as well as use of bio sorbents as biochar and the biogas production are under development (Ghosh and Singh 2005; Prasad and Prasad 2012; Godwin *et al.* 2019).

In order to consider this as a model to follow in the Haina area, there are two considerations that should be taken into account. One is the pollution level of the contaminated area and the tolerance of the plants to this level of contamination, and the other one refers to the bioaccumulation of Pb in the aerial parts of the plants. In accordance to our data, the studied taxa could get a good growth rate at pollution levels up to 600 mg/kg, what could allow implementing phytoremediation programs in big areas with a moderate Pb pollution level in the Haina area. Regarding the bioaccumulation of Pb in the above ground parts of these plants, unfortunately, our *in vitro* experiments did not provide information about the metal accumulation in roots and shoots separately. In this regard, it would be necessary to complete the entire cycle of vegetative development and flowering of the studied cv in order to assess the bioaccumulation level in the aerial parts under field conditions.

In light of the above considerations, we consider that proper use of these ornamental plant taxa would add a new aspect to the utilization of plants for the management and restoration of polluted terrestrial ecosystems in tropical areas.

Conclusions

The results obtained from plants grown in laboratory (*in vitro*) lead us to deduce that the three *Chrysanthemum* cv analyzed in this work are good Pb accumulators, even hyperaccumulators. We are dealing, therefore, with taxa that, at least from the physiological point of view and under laboratory conditions, reveal a high degree of tolerance and accumulation even at moderate Pb contamination levels. Therefore, it can be concluded that all three cv present very good potential for the phytoremediation of Pb polluted soils. However, due to reduction of biomass production and root's length with increasing presence of lead, these cv could be used for phytoextraction purposes in the Haina area in soils with a moderate Pb pollution (below 600 mg/kg). Although 'Renella' showed the greatest potential, all the studied taxa have sufficient potential to be considered for phytoextraction work. Prior to its application to real scale, this study should be complemented with cultivation experiences under field conditions for long growth periods, so that the real aptitudes of these taxa and organs such as foliage and flowers could be assessed in the Haina area and the Caribbean in general. In any case, these ornamental taxa would contribute to the natural beautify improving the esthetic values of the Haina landscape and, in the case of low Pb accumulation level in the flowering shoots, these cultivars could also allow the commercial production of inedible products, such as cut flowers, providing incomes for the local population. Nevertheless, the parts of the plants containing high Pb concentrations should be safely managed. Finally, more data are needed on the field yield of the cv studied to quantify the underlying economic and environmental benefits, and therefore the viability of this promising technology in tropical areas.

Acknowledgments

We express our gratitude to Diego Rivera (University of Murcia) for his advice on nomenclatural and taxonomical questions related to the cultivars studied. Also, to Andrea Werner for revising the English text.

Funding

This work was supported by "Fondo Nacional de Innovación y Desarrollo Científico y Tecnológico" (FONDOCYT) of the Dominican Republic under [Grant number 2010-2B1-003].

ORCID

Agripina Ramírez http://orcid.org/0000-0003-0140-152X
Gregorio García http://orcid.org/0000-0001-6686-9903
Olaf Werner http://orcid.org/0000-0003-0601-3964
Rosa M. Ros http://orcid.org/0000-0003-2115-2911

References

Abdullah S, Sarem SM. 2010. The potential of *Chrysanthemum* and *Pelargonium* for phytoextraction of lead-contaminated soils. Jordan J Civ Eng. 4:409–416.

Aman MS, Jafari M, Reihan MK, Motesharezadeh B. 2018. Assessing some shrub species for phytoremediation of soils contaminated with lead and zinc. Environ Earth Sci. 77(3):82. doi:10.1007/s12665-018-7256-2.

Ang LH, Tang LK, Ho WM, Hui TF, Theseira GW. 2010. Phytoremediation of Cd and Pb by four tropical timber species grown on an ex-tin mine in Peninsular Malaysia. Int Sch Sci Res Innov. 4(2):70–74. doi:10.5281/zenodo.1061521.

Baker AJM, Brooks RR. 1989. Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. Biorecovery. 1:811–826.

Bech J, Poschenrieder C, Barceló J, Lansac A. 2002. Plants from mine spoils in the South American area as potential sources of germplasm for phytoremediation technologies. Acta Biotechnol. 22(1–2):5–11. doi:10.1002/1521-3846(200205)22:1/2<5::AID-ABIO5>3.0.CO;2-B.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol. 57:289–300. doi:10.2307/2346101.

Blacksmith Institute. 2006. Project completion report: used lead acid bacteria site remediation. New York (USA): Blacksmith Institute [accessed 2019 Sep 5]. https://web.archive.org/web/20061103005328/http://www.blacksmithinstitute.org/site10c.php.

Brickell CD, Alexande C, David JC, Hetterscheid WLA, Leslie AC, Malecot V, Xiaobai Jin Cubey JJ. 2009. International code of nomenclature for cultivated plants (ICNCP). Eigth Edition. Scri Hortic. 10:1–184. [accessed 2019 Sep 5]. http://www.actahort.org/chronica/pdf/sh_10.pdf.

Cao Y, Huang R, Cao Z. 2005. Effects of Pb stress on the physiological and biochemical traits of maize. J Maize Sci. 13(3):61–64.

Chaney RL, Malik M, Li YM, Brown SL, Brewer EP, Angle JS, Baker AJ. 1997. Phytoremediation of soil metals. Curr Opin Biotechnol. 8(3):279–284. doi:10.1016/S0958-1669(97)80004-3.

Chaturvedi N, Ahmed MJ, Dhal NK. 2014. Effects of iron ore tailings on growth and physiological activities of *Tagetes patula* L. J Soils Sediments. 14(4):721–730. doi:10.1007/s11368-013-0777-0.

Chen L, Luo S, Li X, Wan Y, Chen J, Liu C. 2014. Interaction of Cd-hyperaccumulator *Solanum nigrum* L. and functional endophyte *Pseudomonas* sp. Lk9 on soil heavy metals uptake. Soil Biol Biochem. 68:300–308. doi:10.1016/j.soilbio.2013.10.021.

Chen M, Zhang LL, Li J, He XJ, Cai JC. 2015. Bioaccumulation and tolerance characteristics of a submerged plant (*Ceratophyllum demersum* L.) exposed to toxic metal lead. Ecotoxicol Environ Saf. 122:313–321. doi:10.1016/j.ecoenv.2015.08.007.

Chintakovid W, Visoottiviseth P, Khokiattiwong S, Lauengsuchonkul S. 2008. Potential of the hybrid marigolds for arsenic phytoremediation and income generation of remediators in Ron Phibun District, Thailand. Chemosphere. 70(8):1532–1537. doi:10.1016/j.chemosphere.2007.08.031.

Conover WJ, Iman RL. 1979. On multiple – comparison procedures. Technical report, LA-7677-MS, Los Alamos Scientific Laboratory; [accessed 2016 Nov 18]. https://permalink.lanl.gov/object/tr?what=info:lanl-repo/lareport/LA-07677-MS.

Cui S, Zhang T, Zhao S, Li P, Zhou Q, Zhang Q, Han Q. 2013. Evaluation of three ornamental plants for phytoremediation of Pb-contaminated soil. Int J Phytoremediation. 15(4):299–306. doi:10.1080/15226514.2012.694502.

Cunningham SD, Berti WR. 2000. Phytoextraction and phytostabilization: technical, economic, and regulatory considerations *Chrysanthemum indicum* of the soil–lead issue. In: Terry N, Bañuelos GS, editors. Phytoremediation of contaminated soil and water. Boca Ratón (FL): Lewis Publisher, CRC Press. p. 359–376.

Dey S, Dey J, Patra S, Pothal D. 2007. Changes in the antioxidative enzyme activities and lipid peroxidation in wheat seedlings exposed to cadmium and lead stress. Braz J Plant Physiol. 19(1):53–60. doi:10.1590/S1677-04202007000100006.

Di Lonardo S, Capuana M, Arnetoli M, Gabbrielli R, Gonnelli C. 2011. Exploring the metal phytoremediation potential of three *Populus alba* L. clones using an *in vitro* screening. Environ Sci Pollut Res Int. 18(1):82–90. doi:10.1007/s11356-010-0354-7.

Eun SO, Youn HS, Lee Y. 2000. Lead disturbs microtubule organization in the root meristem of *Zea mays*. Physiol Plant. 110(3):357–365. doi:10.1111/j.1399-3054.2000.1100310.x.

Fahr M, Laplaze L, Bendaou N, Hocher V. E, Mzibri M, Bogusz D, Smouni A. 2013. Effect of lead on root growth. Front Plant Sci. 4:175. doi:10.3389/fpls.2013.00175.

Felek W, Mekibib F, Admassu B. 2015. Optimization of explants surface sterilization condition for field grown peach (*Prunus persica* L. Batsch. Cv. Garnem) intended for *in vitro* culture. Afr J Biotechnol. 14(8):657–660. doi:10.5897/AJB2014.14266.

Gallego SM, Benavides MP, Tomaro ML. 1996. Effect of heavy metal ion excess in sunflower leaves: evidence for involvement of oxidative stress. Plant Sci. 121(2):151–159. doi:10.1016/S0168-9452(96)04528-1.

García Fernández G, Angeler DG. 2001. Plantas restauradoras para recuperar ambientes contaminados. Quercus. 182:24–27.

García G, Faz Á, Cunha M. 2004. Performance of *Piptatherum miliaceum* (Smilo grass) in edaphic Pb and Zn phytoremediation over a short growth period. Int Biodeterior Biodegradation. 54(2):245–250. doi:10.1016/j.ibiod.2004.06.004.

García-Salgado S, García-Casillas D, Quijano-Nieto MA, Bonilla-Simón MM. 2012. Arsenic and heavy metal uptake and accumulation in native plant species from soils polluted by mining activities. Water Air Soil Pollut. 223(2):559–572. doi:10.1007/s11270-011-0882-x.

Ghosh M, Singh SP. 2005. A review on phytoremediation of heavy metals and utilization of its by products. Appl Ecol Env Res. 3(1):1–18. doi:10.15666/aeer/0301_001018.

Godwin PM, Pan Y, Xiao H, Afzal MT. 2019. Progress in the preparation and application of modified biochar for improving heavy metal ion removal from wastewater. J Bioresour Bioprod. 4(1):31–42. doi:10.21967/jbb.v4i1.180.

Gupta DK, Huang HG, Yang XE, Razafindrabe BHN, Inouhe M. 2010. The detoxification of lead in *Sedum alfredii* H. is not related to phytochelatins but the glutathione. J Hazard Mater. 177(1–3):437–444. doi:10.1016/j.jhazmat.2009.12.052.

Hu R, Sun K, Su X, Pan YX, Zhang YF, Wang XP. 2012. Physiological responses and tolerance mechanisms to Pb in two xerophils: *Salsola passerine* Bunge and *Chenopodium album* L. J Hazard Mater. 205–206:131–138. doi:10.1016/j.jhazmat.2011.12.051.

Hussain A, Abbas N, Arshad F. 2013. Effects of diverse doses of lead (Pb) on different growth attributes of *Zea mays* L. Agric Sci. 4(5):262–265. doi:10.4236/as.2013.45037.

Kališová-Špirochová I, Punčochářová J, Kafka Z, Kubal M, Soudek P, Vaněk T. 2003. Accumulation of heavy metals by *in vitro* cultures of plants. Water Air Soil Pollut Focus. 3(3):269–276. doi:10.1023/A:1023933902452.

Kaur R, Bhatti SS, Singh S, Singh J, Singh S. 2018. Phytoremediation of heavy metals using cotton plant: a field analysis. Bull Environ Contam Toxicol. 101(5):637–643. doi:10.1007/s00128-018-2472-8.

Kaur G, Singh HP, Batish DR, Kohli RK. 2013. Lead (Pb)-induced biochemical and ultrastructural changes in wheat (*Triticum aestivum*) roots. Protoplasma. 1:53–62. doi:10.1007/s00709-011-0372-4.

Khillare PS, Jyethi DS, Sarkar S. 2012. Health risk assessment of polycyclic aromatic hydrocarbons and heavy metals via dietary intake of vegetables grown in the vicinity of thermal power plants. Food Chem Toxicol. 50(5):1642–1652. doi:10.1016/j.fct.2012.01.032.

Lajayer BA, Moghadam NK, Maghsoodi MR, Ghorbanpour M, Kariman K. 2019. Phytoextraction of heavy metals from contaminated soil, water and atmosphere using ornamental plants: mechanisms and efficiency improvement strategies. Environ Sci Pollut Res. 26:8468–8484. doi:10.1007/s11356-019-04241-y.

LeDuc DL, Terry N. 2005. Phytoremediation of toxic trace elements in soil and water. J Ind Microbiol Biotechnol. 32(11–12):514–520. doi:10.1007/s10295-005-0227-0.

Li MS, Luo YP, Su ZY. 2007. Heavy metal concentrations in soils and plant accumulation in a restored manganese mineland in Guangxi, South China. Environ Pollut. 147(1):168–175. doi:10.1016/j.envpol.2006.08.006.

Liu JN, Zhou QX, Sun T, Ma LQ, Wang S. 2008. Identification and chemical enhancement of two ornamental plants for phytoremediation. Bull Environ Contam Toxicol. 80(3):260–265. doi:10.1007/s00128-008-9357-1.

Malecka A, Piechalak A, Tomaszewska B. 2009. Reactive oxygen species production and antioxidative defense system in pea root tissues treated with lead ions: the whole roots level. Acta Physiol Plant. 31(5):1053–1063. doi:10.1007/s11738-009-0326-z.

Mani D, Kumar C, Patel NK, Sivakumar D. 2015. Enhanced clean-up of lead-contaminated alluvial soil through *Chrysanthemum indicum* L. Int J Environ Sci Technol. 12(4):1211–1222. doi:10.1007/s13762-013-0488-5.

Manousaki E, Kadukova J, Kalogerakis N. 2008. Phytoextraction and phytoexcretion of Cd and Pb by the salt cedar (*Tamarix smyrensis* Bunge): a new combined phytoremediation process. In: Management Committee Meeting, editor. COST Action 859, Meeting of working group 4, Phytotechnologies in practice – biomass production, agricultural methods, legacy, legal and economic aspects, Book of abstracts, October 15–17. Verneuil-en-Halatte (France): Ineris. p. 22.

McCartor A, Becker D. 2010. Blacksmith Institute's world worst pollution problems report 2010. Top six toxic threats. New York (USA): Blacksmith Institute; [accessed 2019 Sept 5]. http://www.worstpolluted.org/files/FileUpload/files/2010/WWPP-2010-Report-Web.pdf.

Miñano HS, González-Benito ME, Martín C. 2009. Molecular characterization and analysis of somaclonal variation in chrysanthemum cultivars using RAPD markers. Sci Hortic. 122(2):238–243. doi:10.1016/j.scienta.2009.05.001.

Minkina TM, Motuzova GV, Mandzhieva SS, Nazarenko OG. 2012. Ecological resistance of the soil–plant system to contamination by heavy metals. J Geochem Explor. 123:33–40. doi:10.1016/j.gexplo.2012.08.021.

Mishra S, Srivastava S, Tripathi RD, Kumar R, Seth CS, Gupta DK. 2006. Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere. 65(6):1027–1039. doi:10.1016/j.chemosphere.2006.03.033.

Monni S, Uhlig C, Hansen E, Magel E. 2001. Ecophysiological responses of *Empetrum nigrum* to heavy metal pollution. Environ Pollut. 112(2):121–129. doi:10.1016/S0269-7491(00)00125-1.

Murashige T, Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant. 15(3):473–497. doi:10.1111/j.1399-3054.1962.tb08052.x.

Nagajyoti PC, Lee KD, Sreekanth T. 2010. Heavy metals, occurrence and toxicity for plants: a review. Environ Chem Lett. 8(3):199–216. doi:10.1007/s10311-010-0297-8.

Nakbanpote W, Meesungnoen O, Prasad M, 2016. Potential of ornamental plants for phytoremediation of heavy metals and income generation. In: Prasad MNV, editor. Bioremediation and bioeconomy. UK: Elsevier. p. 179–217.

Nas FS, Ali M. 2018. The effect of lead on plants in terms of growing and biochemical parameters: a review. MOJ Eco Environ Sci. 3(4):265–268. doi:10.15406/mojes.2018.03.000.98.

Organum N, Bacon F. 2006. Bioremediation technologies. In: Alvarez PJJ, Illman WA, editors. Bioremediation and natural attenuation. New Jersey (USA): John Wiley & Sons. p. 351–455.

Pérez-López R, Márquez-García B, Abreu MM, Nieto JM, Córdoba F. 2014. *Erica andevalensis* and *Erica australis* growing in the same extreme environments: phytostabilization potential of mining areas. Geoderma. 230–231:194–203. doi:10.1016/j.geoderma.2014.04.004.

Pilon-Smits E. 2005. Phytoremediation. Annu Rev Plant Biol. 56(1):15–39. doi:10.1146/annurev.arplant.56.032604.144214.

Pohlert T. 2014. The pairwise multiple comparison of mean ranks package (PMCMR). R package; [accessed 2019 Sep 5]. https://cran.r-project.org/web/packages/PMCMR/vignettes/PMCMR.pdf.

Prapagdee B, Wankumpha J. 2017. Phytoremediation of cadmium polluted soil by *Chlorophytum laxum* combined with chitosan immobilized cadmium-resistant bacteria. Environ Sci Pollut Res. 24(23):19249–19258. doi:10.1007/s11356-017-9591-3.

Prasad MNV, Prasad R. 2012. Nature's cure for cleanup of contaminated environment – a review of bioremediation strategies. Rev Environ Health. 28:181–189.

R Development Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0; [accessed 2019 Sep 5]. https://www.r-project.org/.

Ramírez Sánchez A. 2017. Evaluación del Potencial Fitorremediativo para el Control de la Exposición al Plomo y Otros Metales y Restauración Ambiental en Haina, República Dominicana [dissertation]. Murcia (Spain): University of Murcia; [accessed 2019 Sep 5]. https://www.tdx.cat/handle/10803/405948#page=1.

Raskin I. 1995. Phytoextraction: the use of plants to remove heavy metals from soils. Environ Sci Technol. 29(5):1232–1238. doi:10.1021/es00005a014.

Reeves RD. 1992. The Hyperaccumulation of nickel by serpentine plants. In: Baker AJM, Proctor J, Reeves RD, editors. The vegetation of ultramafic (serpentine) soils. Andover (UK): Intercept. p. 253–277.

Rout GR, Das P. 2003. Effect of metal toxicity on plant growth and metabolism: I. Zinc. Agronomie. 23(1):3–11. doi:10.1051/agro:2002073.

Salas Salmerón FK. 2007. Selección *in vitro* de plantas tolerantes a plomo para su uso en fitorremediación [Bachelor's Degree in Biotechnology Specialist]. Mexico D.F (Mexico): Universidad Autónoma Metropolitana; [accessed 2019 Sep 5]. http://tesiuami.izt.uam.mx/uam/aspuam/presentatesis.php?recno=14183&docs=UAMI14183.PDF.

Salt DE, Blaylock M, Kumar NP, Dushenkov V, Ensley BD, Chet I, Raskin I. 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Nat Biotechnol. 13(5):468–474. doi:10.1038/nbt0595-468.

Sanita di Toppi LS, Gabbrielli R. 1999. Response to cadmium in higher plants. Environ Exp Bot. 41:105–130. doi:10.1016/S0098-8472(98)00058-6.

Seregin IV, Ivanov VB. 2001. Physiological aspects of cadmium and lead toxic effects on higher plants. Russ J Plant Physiol. 48(4):523–544. doi:10.1023/A:1016719901147.

Shakoor MB, Ali S, Hameed A, Farid M, Hussain S, Yasmeen T, Najeeb U, Bharwana SA, Abbasi GH. 2014. Citric acid improves lead (Pb) phytoextraction in *Brassica napus* L. by mitigating Pb-induced morphological and biochemical damages. Ecotoxicol Environ Safe. 109:38–47. doi:10.1016/j.ecoenv.2014.07.033.

Sharma P, Dubey RS. 2005. Lead toxicity in plants. Braz J Plant Physiol. 17(1):35–52. doi:10.1590/S1677-04202005000100004.

Song J, Zhang H, Duan C, Duan CW, Cui XY. 2018. Exogenous application of succinic acid enhances tolerance of *Larix olgensis* seedling to lead stress. J Res. 29(6):1497–1505. doi:10.1007/s11676-017-0579-0.

Sridhara Chary N, Kamala CT, Samuel S. 2008. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. Ecotoxicol Environ Safe. 69(3):513–524. doi:10.1016/j.ecoenv.2007.04.013.

Trigueros D, Mingorance MD, Rossini Oliva S. 2012. Evaluation of the ability of *Nerium oleander* L. to remediate Pb-contaminated soils. J Geochem Explor. 114:126–133. doi:10.1016/j.gexplo.2012.01.005.

Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnevajova E, et al. 2009. Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ Sci Pollut Res Int. 16(7):765–794. doi:10.1007/s11356-009-0213-6.

Vyslouzilova M, Tlustos O, Szakova J, Pablicova D. 2003. As, Cd, Pb and Zn uptake by different *Salix* spp. grown at soils enriched by high loads of these elements. Plant Soil Environ. 49(5):191–196. doi:10.17221/4112-pse.

Wang J, Fu G, Li W, Shi Y, Pang J, Wang Q, Lü W, Liu C, Liu J. 2018. The effects of two free-floating plants (*Eichhornia crassipes* and *Pistia stratiotes*) on the burrow morphology and water quality characteristics of pond loach (*Misgurnus anguillicaudatus*) habitat. Aquac Fish. 3(1):22–29. doi:10.1016/j.aaf.2017.12.001.

Wei S, Zhou Q, Mathews S. 2008. A newly found cadmium accumulator – *Taraxacum mongolicum*. J Hazard Mater. 159(2–3):544–547. doi:10.1016/j.jhazmat.2008.02.052.

Wenzel WW, Unterbrunner R, Sommer P, Sacco P. 2003. Chelate-assisted phytoextraction using canola (*Brassica napus* L.) in outdoors pot and lysimeter experiments. Plant Soil. 249(1):83–96. doi:10.1023/A:1022516929239.

Wickham H. 2009. ggplot2: elegant Graphics for Data Analysis. New York (NY): Springer-Verlag.

Wiszniewska A, Hanus-Fajerska E, Smoleń S, Muszyńska E. 2015. *In vitro* selection for lead tolerance in shoot culture of *Daphne* species. Acta Sci Pol Hortorum Cultus. 14(1):129–142.

Wong MH. 2003. Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. Chemosphere. 50(6):775–780. doi:10.1016/S0045-6535(02)00232-1.

Yang YY, Jung JY, Song WY, Suh HS, Lee Y. 2000. Identification of rice varieties with high tolerance or sensitivity to lead and characterization of the mechanism of tolerance. Plant Physiol. 124(3): 1019–1026. doi:10.1104/pp.124.3.1019.

Yoon J, Cao X, Zhou Q, Ma LQ. 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Sci Total Environ. 368(2-3):456–464. doi:10.1016/j.scitotenv.2006.01.016.

Zhao K, Liu X, Xu J, Selim HM. 2010. Heavy metal contaminations in a soil–rice system: identification of spatial dependence in relation to soil properties of paddy fields. J Hazard Mater. 181(1–3):778–787. doi:10.1016/j.jhazmat.2010.05.081.

Zhou J, Zhang Z, Zhang Y, Wei Y, Jiang Z. 2018. Effects of lead stress on the growth, physiology, and cellular structure of privet seedlings. PLOS One. 13(3):e0191139. doi:10.1371/journal.pone.0191139.

Zhuang P, McBride MB, Xia H, Li N, Li Z. 2009. Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. Sci Total Environ. 407(5):1551–1561. doi:10.1016/j.scitotenv.2008.10.061.