Environ Earth Sci (2016) 75:742
DOI 10.1007/s12665-016-5513-9

THEMATIC ISSUE

Lead and zinc determinations in *Festuca arundinacea* and *Cynodon dactylon* collected from contaminated soils in Tandil (Buenos Aires Province, Argentina)

Carolina B. Albornoz[1,2,3] · **Karen Larsen**[1,3] · **Roberto Landa**[2] · **Miguel A. Quiroga**[3] · **Roberto Najle**[1] · **Jorge Marcovecchio**[4]

Received: 29 May 2015 / Accepted: 25 February 2016 / Published online: 25 April 2016

Abstract Heavy metal soil contamination is one of the most serious environmental problems, considering both their persistence and progressive accumulation which makes possible the transference to other systems, and could potentially affect human health and ecosystems functioning. The total concentration of lead (Pb) and zinc (Zn) in soil and plant samples from *Festuca arundinacea* and *Cynodon dactylon* naturally developed (in situ) and within experimentally contaminated soils (ex situ) was measured. Pb and Zn obtained results showed that the average values in industrial soils were significantly higher ($p < 0.0001$) than those corresponding to controls values (472 % more for Pb and 288 % more for Zn). There was a positive significant correlation between Pb levels in soil and roots ($r = 0.99$) and leaves ($r = 0.98$) of *C. dactylon*, and between Zn levels in soil and roots ($r = 0.94$) and leaves ($r = 0.91$) of *C. dactylon*. *Festuca arundinacea* plants were experimentally exposed to Pb-contaminated soil during different times (0, 5 and 10 days). Two indicators were calculated: the bioaccumulation factor (BCF) and translocation factor (TF). Five day BCF was 0.25, while for 10 days one was 0.72. This showed that the value of BCF varied according to the exposure time, and *F. arundinacea* showed to be highly tolerant to Pb species. TF was low (T_1: 0.18 and T_2: 0.09) because the higher accumulation occurred in roots. Considering that roots of *F. arundinacea* and *C. dactylon* were their largest metals reservoir, they could be used as indicators of metal contamination within soils.

Keywords Contaminated soil · Lead · Zinc · *Cynodon dactylon* · *Festuca arundinacea* · Phytoremediation

This article is part of a Topical Collection in Environmental Earth Sciences on "3RAGSU", guest edited by Daniel Emilio Martinez.

✉ Carolina B. Albornoz
carolinaalbornoz24@gmail.com

[1] Lab. de Ecotoxicología y Biología Celular, Tandil, Argentina

[2] Lab. de Análisis Bioquímicos y Minerales, Tandil, Argentina

[3] CIVETAN, U.N.C.P.B.A, Tandil, Argentina

[4] Instituto Argentino de Oceanografía. IADO-CONICET/UNS, Bahía Blanca, Argentina

Introduction

Urban ecosystem has been characterized as a complex of both natural and anthropic factors (Karim et al. 2014). According to Xiao et al. (2013), trace metals occur within nature as sediments, rocks and soil components. Anthropic activities have produced a significant increase in trace metal concentration within soils and waters up to levels of toxicity or very close to (Bai et al. 2009, 2011). So, mining exploitation (Lv et al. 2014; Odumo et al. 2014), energy production (Rodríguez Martin et al. 2013), other industrial activities (Kaitantzian et al. 2013), burning of fossil fuels within vehicular traffic (Argyraki and Kelepertzis 2014), pesticides and fertilizers application (Koch and Rotard 2001), or soils and waters pollution due to e-waste recycled (Wu et al. 2015) are good examples of such activities.

Trace metals are considered to be toxic pollutants due to their persistence within the environment, their bioaccumulation ability (Bai et al. 2012) and their potentiality to produce teratogenic, mutagenic and carcinogenic effects on the biota (Vithanage et al. 2012), affecting both human and animal health (Ok et al. 2004). Heavy metals, such as zinc, lead, cadmium, copper or nickel, are environmental pollutants of great concern (United States Environmental Protection Agency 1997). The European Commission Directive (Directive 86/278/EEC 1986) declares that these substances cause hazard to the life of plants or animals when their concentrations surpass certain values. By the way, levels of 375 μg g^{-1} DM for lead (Pb) and 600 μg g^{-1} DM for zinc (Zn) corresponding to soils with agricultural use and 1000 μg g^{-1} DM for Pb and 1500 μg g^{-1} DM for Zn for industrial soils have been proposed in Argentina (Decreto No. 831/1993).

On the other hand, increasing interest in using biological indicators such as plants for monitoring soil, air and water pollution has been observed in recent years (Bonanno and Lo Giudice 2010; Peng et al. 2008). Plants absorb trace metals (i.e., cadmium, lead, chromium or zinc) which do not have known biological functions and could be toxic at low concentrations. Plants capture soil minerals in response to concentration gradients and induced by ion-selective uptake via roots or by diffusion of elements in the soil. Moreover, accumulation level differs between and within the different species (Huang and Cunningham 1996; McGrath et al. 2002). Within this framework, it is important to highlight that phytoremediation—especially phytoextraction—is a decontamination of soils methodology, which uses plants to remove inorganic contaminants, primarily heavy metals (Garbisu and Alkorta 2001; Yang et al. 2005).

Tandil is a town located in the southwest of Buenos Aires Province, in Argentina. Metallurgical production (including smelting) is the main industrial activity within this city. Foundry processes present different methods, applied technologies and type of metal such as ferrous (steel and Fe) and non-ferrous (Pb, Zn, Cu, Ni). Within these processes, the sands are used to make molds and replicate a metal piece. Molding sands are the main waste generated by the mentioned foundries, which have been used for many years for filling surfaces. Moreover, waste foundry sands are recovered and reused for the mentioned productive process. However, a significant volume of sands used in the molding process is not recovered; instead, these sands are abandoned in diggings, quarries and brick factories or are alternatively used to level the soil surface.

The aims of the present study were as follows: (a) to determine Pb and Zb concentrations within soil and plant root samples from different areas of Tandil (in situ study); (b) to assess Pb uptake by roots and leaves of two plant species planted in soils with trace metal levels (ex situ study). Heavy metal concentrations were analyzed in all samples in order to evaluate the quality of the terrestrial environment within the study area; in addition, the usefulness of the two plant species as indicators of metal contamination within the study area was also verified.

Materials and Methods

Sampling procedure

In situ experiments

Soils samples were collected in different sites close to Tandil city, Buenos Aires Province, Argentina (Fig. 1).

The study area includes as follows: (A) the Industrial Park of Tandil (IPT); (B) the Urban Area (UA); y (C) places far from both A and B (C).

1. The IPT is located near the urban area, about 4 km from the city center. The site is bounded by strategic access routes and occupies an area of ~1 km^2. The samples collected in areas from IPT were considered I_1, I_2 and I_3.
2. Soil samples (UA_1, UA_2 and UA_3) of land whose surfaces were covered with the remains of foundry waste (molding sand) were obtained within the urban area. Moreover, soil samples were taken from a distant land (200 m of the place that had remains of foundry waste) to perform the analysis of control soil (UAC) within this area.
3. Samples collected in three sites further from IPT, UA and cultivated land were considered control ones (C_1, C_2 and C_3).

All samplings were performed in triplicate. Samples were obtained at 0–20 cm depth, were mixed to obtain a homogeneous sample (1 kg) and were preserved in plastic bags. Forage samples (*Cynodon dactylon*) were collected from urban area and preserved in plastic bags. Roots and leaves were separated for the study of the plants.

Ex situ experiments

Soil Soil from the control zone (C) was obtained to build up the ex situ experiments, and it was dried, homogenized and passed through a 2-mm sieve. This soil was considered control sample and attained a final concentration of 600 μg Pb g^{-1} DM. Control and contaminated soils were placed in plastic pots (1 kg/pot). Both pots (with control and contaminated soil) were analyzed for triplicate to determine their metal content.

Forage Seeds of *Festuca arundinacea* were germinated in a germination chamber alternating temperatures of 16 h in the dark at 20 °C and 8 h at light at 30 °C during 14 days (ISTA 2012). After this procedure, germinating seeds were transplanted into pots with control soils during 14 days.

In addition, seedlings of *F. arundinacea* were transplanted into pots exposed experimentally to Pb-contaminated soil to

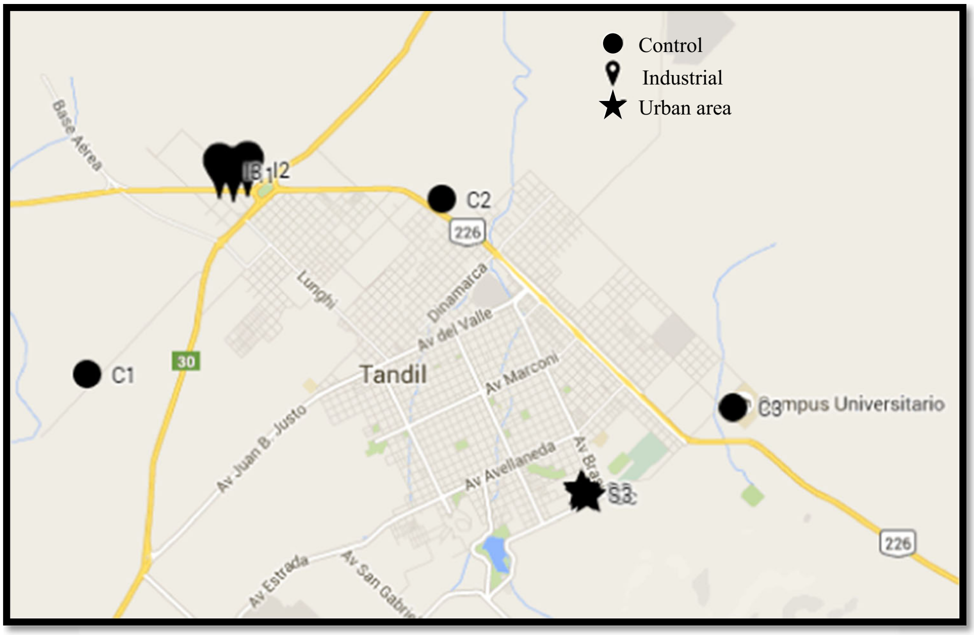

Fig. 1 Location map of sampling sites in soils and plant samples collected in Tandil (Bs. As, Argentina). Control soils (*C*), industrial soils (*IPT*) and urban area (*UA*)

start the treatment during different times (T_0: 0 day; T_1: 5 days and T_2: 10 days). Pots of day 0 were considered control treatment. Roots and leaves were separately analyzed during forage study.

Samples preparation and analysis

Heavy metal determination

Plant (previously washed with distilled water) and soil samples were dried in a heater (70 °C) up to constant weight, and subsequently ground and homogenized. 0.5 g of homogeneous sample was mineralized through acid digestion with 0.5 ml HNO_3 and $HClO_4$ (3:1) during 6 h at room temperature, and 12 h at 95 °C to destroy the whole organic matter content in order to determine the trace metals concentrations. Samples were centrifuged at 1200 g during 15 min. After the extracted supernatant, samples were diluted to measure metals with the analytical instruments. Pb and Zn content in soil and forage was determined by atomic absorption spectroscopy (AAS, GBC 906, Australia) (Dean and Rains 1975; Price 1979).

Bioaccumulation factor (BCF), translocation factor (TF) and removal percentage

Bioaccumulation factor (BCF) and translocation factor (TF) are widely used in phytoremediation studies (Tu et al. 2003; Zhuang et al. 2007) to evaluate the ability of different plant tissues assimilating trace elements from soil as well as their ability to translocate these elements from roots to aboveground tissues, respectively.

Translocation factor

Plants were analyzed to determine the translocation factor (TF) of metals from roots to aerial parts, which has demonstrated to be extremely useful in phytoremediation studies (Jadia and Fulekar 2009).

The BCF and TF formulas are given as follows (Rauf et al. 2011; Ghosh and Singh 2005):

$$\mathrm{BCF} = \frac{C_{\text{plant tissue}}}{C_{\text{soil}}}$$

where $C_{\text{plant tissue}}$ (mg/Kg) is Pb concentration in plant tissue (i.e., leaves and roots) and C_{soil} (mg/kg) is Pb concentration in soil.

$$\mathrm{TF} = \frac{C_{\text{above ground}}}{C_{\text{root}}}$$

Where $C_{\text{above ground}}$ (mg/Kg) and C_{root} (mg/kg) are Pb concentrations in the aboveground plant tissues and plant roots, respectively.

Removal percentage

The removal percentage was used to calculate the efficiency of phytoremediation on Pb-contaminated soils. It was calculated following the algorithm:

$$\%\text{Removal} = \frac{[(C_i - C_f) \cdot 100]}{C_i}$$

where: C_i is the starting metal concentration in the soil and C_f is the final metal concentration in the soil (Prasad and Frietas 2002).

Statistical analysis

Statistical analyses were performed using the program GraphPad InStat 3.0. The results were assessed through parametric tests: t test and analysis of variance (ANOVA). The variance homogeneity was checked by Bartlett's test. Logarithmic transformations performed by the post hoc Tukey or nonparametric tests were applied in cases where the data were not normally distributed. The significance level was $p < 0.05$. Pearson's correlation coefficient (1986) was applied to perform a correlation analysis of Pb and Zn concentrations within soil and forage (root and leaves).

Results

In situ experiments

IPT and Control

Pb average values within industrial soils (I) have showed to be significantly higher ($p < 0.0001$) than those corresponding to control (C) ones. A similar situation has been observed within Zn concentrations in control and industrial soils samples, where (I)-Zn average values were significantly higher ($p < 0.0001$) than those recorded in (C). The increasing percentages of values above described were 472 % within Pb concentration, and 288 % in Zn concentrations, being in both cases major in industrial soils than in control ones (Fig. 2).

The same kind of analysis has been performed within the study of forage (*Cynodon dactylon*), considering separately the roots and leaves. Values of Pb and Zn in roots of *C. dactylon* corresponding to contaminated soils (I) were higher than roots from control soils (C). Roots showed higher Pb values ($p < 0.001$) than Zn ones ($p < 0.05$). Roots of grasses, corresponding to industrial soil, surpassed 551 % Pb concentration, and 258 % Zn concentration, in relation to the control samples (Fig. 3). Significant differences were statistically greater for Pb ($p < 0.001$) than those for Zn ($p < 0.05$).

Urban area

The results of Pb and Zn concentrations as obtained were quite high because metal values in soil and remains of molding sand ($S_{1,2,3}$) that belongs to an urban area were higher than those from control soils (S_c). This remarks the higher differences in concentration of Pb and Zn in soil in comparison with control ones (***$p < 0.001$).Waste molding sands showed higher differences in the concentration of Pb (***$p < 0.001$) than Zn (*$p < 0.05$). The levels of Pb in soil samples from the affected area exceeded 742 % those from control one, and waste molding sand did by 766 %. In relation to Zn concentrations, the levels in soils were 509 %, and waste molding sand 258 % higher, respectively, than the reference soil (Fig. 4).

Another test was conducted in an urban area directed to study the correlation of metals (Pb and Zn), between the roots (R) and the leaves (L) of the forages from both the control site (S_c) and contaminated ones (S_1, S_2 and S_3). Average concentrations of Pb (µg Pb g^{-1}DM) in soils and roots of *C. dactylon* samples from the same sites (Fig. 5) showed a correlation coefficient of $r = 0.99$ in relation to

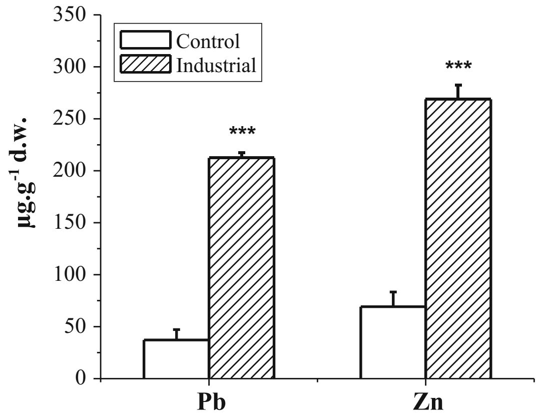

Fig. 2 Concentration of Pb and Zn in Controls and Industrial soils. ***$p < 0.001$

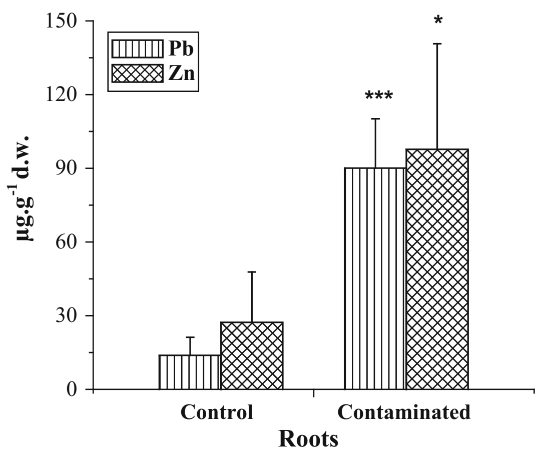

Fig. 3 Concentrations of Pb and Zn (µg g^{-1} D.M.) in roots of *Cynodon dactylon* from control and contaminated soils. Pb ***$p < 0.001$; Zn *$p < 0.05$

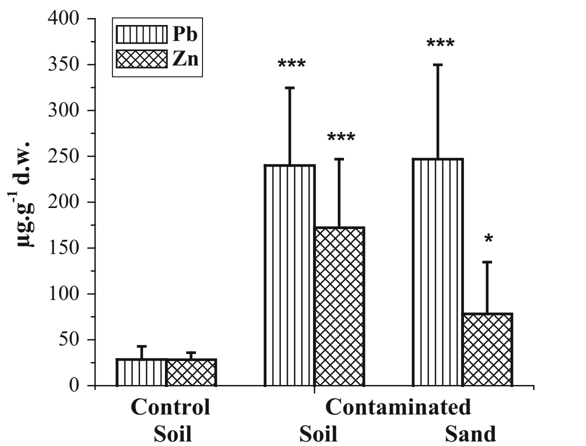

Fig. 4 Concentrations of Pb and Zn (μg g^{-1} D.M.) in control soil (S_c), contaminated soil and remains of molding sand from urban area (S). * $p < 0.05$; *** $p < 0.001$

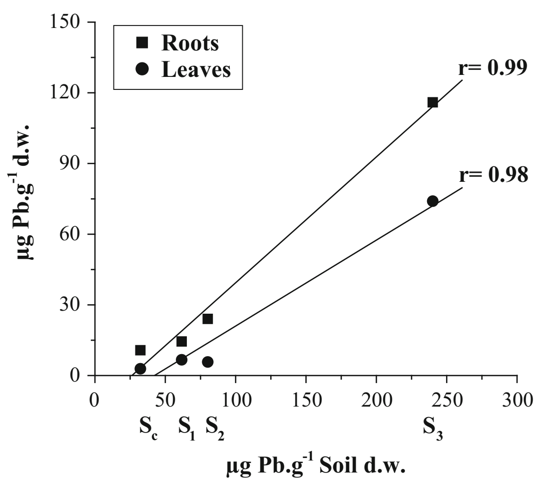

Fig. 5 Correlation analysis of Pb (μg g^{-1} D.M.) between the roots and the leaves of *Cynodon dactylon* from different sampling sites (S_c, S_1, S_2 and S_3) from urban area. Correlation analysis positive, root $r = 0.99$; leaves $r = 0.98$

the concentration of soil, while an $r = 0.98$ has been obtained for leaves of *C. dactylon*. In addition, a positive correlation analysis between Pb concentrations from different soil sampling sites and parts of the plants has also been recorded.

Besides, bioaccumulation factor was estimated considering the concentration of Pb in roots and soils of each sampling site. The results were as follows: S_c: 0.33; S_1: 0.23; S_2: 0.30 and S_3: 0.48. Greater metal accumulation was observed in plant roots than in leaves. Average concentrations of Zn (μg Zn g^{-1}DM) in samples of soils (S_c; S_1; S_2 and S_3) and roots of *C. dactylon* from these same sites (Fig. 6) had an $r = 0.94$ in relation to soil concentration, whereas that leaves of *C. dactylon* had an $r = 0.91$

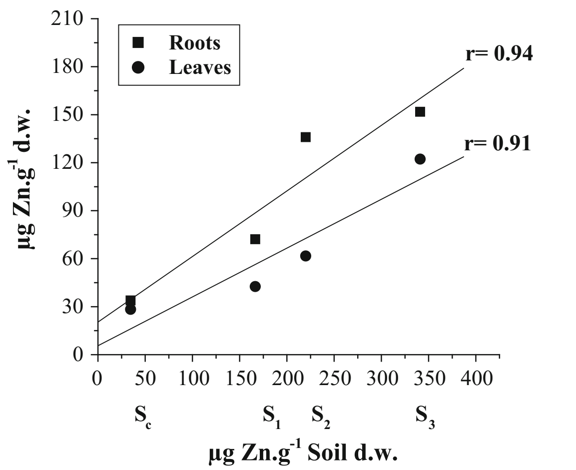

Fig. 6 Correlation analysis of Zn (μg g^{-1} D.M.) between the roots and the leaves of *Cynodon dactylon* from different sampling sites (S_c, S_1, S_2 and S_3) from urban area. Correlation analysis positive, root $r = 0.94$; leaves $r = 0.91$

in relation to soil concentration. Bioaccumulation factor calculated with concentrations of Zn in root of the plants and soils was S_c: 0.98; S_1: 0.43; S_2: 0.62; and S_3: 0.45. A positive correlation between the concentrations of Zn obtained from different soil sampling sites and in different parts of the plants has also been observed.

Ex situ experiments

Plants *F. arundinacea* were experimentally exposed to Pb-contaminated soil during different times (T_0: 0 day; T_1: 5 days; and T_2: 10 days). The study started with a Pb initial concentration within soil T_0: 611.23 ± 5.99 μg Pb g^{-1} DM. At the same time, roots and leaves presented lower metal concentrations (i.e., 7.67 ± 0.35 and 0.69 ± 0.23 μg Pb g^{-1} DM, respectively). After five days, soils concentration was reduced to 595.17 ± 21.64 μg Pb g^{-1} DM, while in roots and leaves was increased to 150.06 ± 0.83 and 27.4 ± 2.16 μg Pb g^{-1} DM, respectively. After 10 days, soil concentration showed 437.35 ± 50.07 μg Pb g^{-1} DM; meanwhile, root levels notoriously increase up to 314.2 ± 19.18 and leave levels were similar (28.66 ± 1.18 μg Pb g^{-1} DM) than the time before (5 days before) Pb concentration in soil decreased during the process ($r = 0.90$), while in plants Pb concentration increased. The roots accumulate higher concentrations of metal in relation to the time exposure and had a $r = 0.99$, while that increase in the leaves means an $r = 0.87$. The reduction in Pb concentrations in soil throughout the days is expressed by a removal percentage of the metal of 28 % (Fig. 7). Two indicators were calculated to evaluate the efficiency of phytoremediation of *F. arundinacea*: the bioaccumulation factor (BCF) and the translocation factor

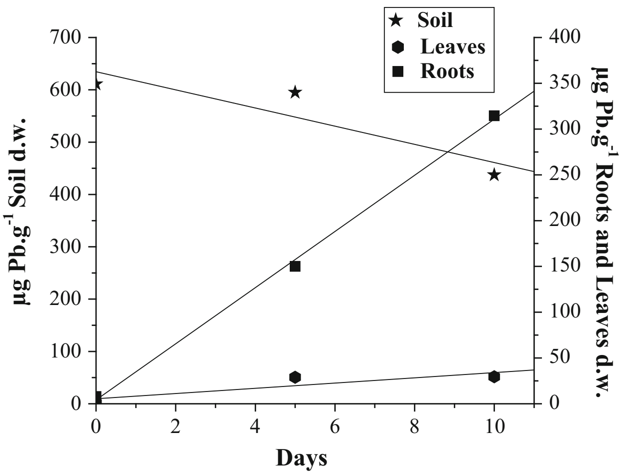

Fig. 7 Concentrations of Pb (µg g^{-1} D.M.) in control soil (S_c), contaminated soil (611.23 µg Pb g^{-1} D.M.) and in roots and leaves of *Festuca arundinacea* exposed during distinct time intervals to experimental soils. Pb concentrations in soil showed a negative tendency ($r = 0.90$) while in the roots and leaves were positive ($r = 0.99$ and $r = 0.87$, respectively)

(TF). BCF was 0.25 to five, and 0.72 to ten days. This fact showed that the value of BCF varied according to the time, and *F. arundinacea* is a species that tolerates high Pb concentration. TF was low (T_1: 0.18 and T_2:0.09) because the much higher accumulation occurred in roots.

Discussion

Total contents of Pb and Zn within samples from both industrial and urban zones have exceeded the corresponding soil background values. Similar results have been reported by other authors within soils, wetlands and urban sediments from different environments (i.e., Xiao et al. 2012, 2013; Bai et al. 2015; Mahar et al. 2015). In addition, reference values of 375 µg g^{-1} DM for lead (Pb) and 600 µg g^{-1} DM for zinc (Zn) within soils with agricultural use, and 1000 µg g^{-1} DM of Pb and 1500 µg g^{-1} DM of Zn within industrial soils have been proposed in Argentina. Within this framework, it could be observed that the values of Pb (212.4 ± 4.95 µg Pb g^{-1} DM) and Zn (268.77 ± 13.59 µg Zn g^{-1} DM) as recorded in soils nearby to industries within the study area did not exceed the admitted values of Argentina's legislation. Nevertheless, the obtained results within present study have showed to be higher than acceptable values from other countries; i.e., the Netherlands legislation establishes maximum values up to 85 µg Pb g^{-1} DM and 140 µg Zn g^{-1} DM (NMHPPE 1991), while Chile's one does at 75 µg Pb g^{-1} DM and 175 µg Zn g^{-1} DM (INN 2004).

It had been accepted that plants can be effectively used as biomonitors of heavy metal environmental pollution (Rossini and Mingorance 2006). The content of lead and zinc in the root of *Cynodon dactylon* growing in soils nearby to industries was superior to the values determined in roots of specimens growing in control soil (Fig. 3). These results are indicative of the ability of the plant *C. dactylon* to take up metal ions from soils contaminated with heavy metals. Similar results have been reported by Kim et al. (2003) and Halasz et al. (2012) who have studied Pb and Zn contents in *Polygonum thunbergii*. In addition, Pederson et al. (2002) have presented the levels of these metals in numerous adventive plants (*Urtica* spp., *Artemisia* spp., *Stenactis annua* (L), *Nul polygonum sachalinense Fr. Scidt*, etc.) as well as in samples of sixteen different forages, which have showed similar levels than those here reported.

Concentrations of Pb and Zn in samples of soils and in roots and leaves of *Cynodon dactylon* obtained from urban areas are shown in Figs. 5 y 6. The bioaccumulation of Pb and Zn has decreased according to the following order: soil > roots > leaves. Positive linear correlations were found when Pb concentrations obtained from samples of different soil sites and roots of *C. Dactylon* ($r = 0.99$) or leaves ($r = 0.98$) were plotted. Similar positive correlations were also reported by Madejon et al. (2002).

The bioaccumulation factor demonstrated that Zn has greater power of translocation from the roots to the leaves in comparison with Pb. There was also a significant positive correlation between Zn levels in soil and roots or leaves of *C. dactylon* ($r = 0.94$ and $r = 0.91$, respectively). Madejón et al. (2002) have demonstrated that there was a significant positive correlation between Pb and Zn levels in samples of soil (EDTA values) and *C. dactylon* ($r = 0.63$ and $r = 0.845$). *C. dactylon* had higher tolerance to Pb, Zn and Cu accumulation when the metal concentrations in soils were increased (Shu et al. 2002; Leung 2013).

Furthermore, it has been demonstrated that Pb is bioaccumulated by *F. arundinacea* within the study conditions (Fig. 7). During the 10-day period, the plant absorbed Pb from the soil. Thus, Pb concentration in the soil was decreased during the same time. Two indicators were calculated to evaluate the efficiency of phytoremediation of *F. arundinacea*: the bioaccumulation factor (BCF) and translocation factor (TF). BCF was 0.25 to five and 0.72 to ten days. This showed that the value of BCF varied according to the exposure time, as well as that *F. arundinacea* is species which tolerates high Pb concentration. Liu et al. (2008) in a comparable study have demonstrated that in all samples they have reviewed, the BCF has varied between 0.002 and 0.9, but always showed to be <1. On the other hand, the Pb BCF as obtained in the study of Yoon et al. (2006) was lower than that found by Kim et al. (2003), which varied between 5 and 58, and simultaneously was higher than those reported by Stoltz and Greger (2002) (BCF = 0.004–0.45). BCF is a significant factor to be taking into account when considering the phytoremediation potential of plant species (Zhao et al. 2003).

TF values were low (T_1: 0.18 and T_2:0.09) because the higher accumulation occurred in roots. Liu et al. (2008) have reported Pb translocation factors ranging from 0.02 to 0.93. The international literature largely reported that translocation of Pb from roots to leaves tends to be generally very low, due to its strong binding at root surfaces and cell walls (Jarvis and Leung 2002; Pourrut et al. 2013).

Both bioconcentration factors (BCF) and translocation factors (TF) can be used to estimate a plant's potential for phytoremediation purpose.

Conclusions

Based on current studies carried out in Tandil city, in nearer areas to the industries, it was shown that potentially toxic trace metal (Pb, Zn) values as recorded in soils and plants from the area intensified the need to propose short-term remediation tools, which could allow the improvement of the environmental condition, avoiding effects which nowadays could impact on local society.

Obtained results within this study have allowed the proposal of phytoremediation as a possible tool to amend trace metals soil contents, considering those previously described within in situ and ex situ experiments, as well as field observations on metals of concern.

This study pointed out that *C. dactylon* presented a linear behavior between concentrations of Pb and Zn and soil ones at urban areas. Although both metals were absorbed by the plants, the assimilation of Zn was greater in their tissues. In relation to the species *F. arundinacea*, it can be concluded that they have a great potential for Pb accumulation, even considering that the study was carried out during 10 days of exposure. The main reservoirs were the roots. Accordingly, a significant decrease in the Pb concentration in soil was observed.

This study pointed out that *F. arundinacea* and *C. dactylon* can be used as indicators of metal contamination within soil, and the roots have demonstrated to be the largest reservoirs of metals.

Acknowledgments The authors are grateful to the Universidad Nacional del Centro de la Provincia de Buenos Aires, particularly Veterinary Research Center Tandil (CIVETAN-CONICET) for their permanent support.

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