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The interaction of heavy metals and nutrients present in soil and native plants with arbuscular mycorrhizae on the riverside in the Matanza-Riachuelo River Basin (Argentina)

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HIGHLIGHTS

- Metals and nutrients in soils and plants are present in a contamination gradient.
- Plants can grow along the basin from low to very highly contaminated sites.
- Plants concentrate more metals in the roots than in the aboveground tissues.
- AM in *H. bonariensis* roots and spores in soil reflect the contamination gradient.

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ABSTRACT

This study assessed the contamination by heavy metals (Cr, Cu, Pb, Zn), and nutrients (N, P) in soils and native plants, and the effect of the concentration of those elements with the density of arbuscular-mycorrhizal (AM) spores in soil and colonization in roots from the riverside of the Matanza-Riachuelo River Basin (MRRB). The concentration of metals and nutrients in soils and plants (*Eleocharis montana*, *Cyperus eragrostis*, *Hydrocotyle bonariensis*) increased from the upper sites (8 km from headwaters) to the lower sites (6 km from the mouth of the Riachuelo River) of the basin. AM-colonization on the roots of *H. bonariensis* and spore density in soil decreased as the concentrations of metals in soil and plant tissues increased from the upper to lower sites of the basin within a consistent gradient of contamination associated with land use, soil disturbance, population, and chemicals discharged into the streams and rivers along the MRRB. The general trends for all metals in plant tissue were to have highest concentrations in roots, then in rhizomes and lowest in aerial biomass. The translocation (TF) and bioconcentration (BCF) factors decreased in plants which grow from the upper sites to the lower sites of the basin. The plants tolerated a wide range in type and quantity of contamination along the basin by concentrating more metals and nutrients in roots than in aboveground tissue. The AM spore density in soil and colonization in roots of *H. bonariensis* decreased with the increase of the degree of contamination (Dc) in soil.

1. Introduction

Watercourses in the Matanza-Riachuelo River Basin (MRRB) in the Buenos Aires Province (Argentina) are potentially subjected to different types of contaminants from agricultural and urban runoff, industrial effluents, sewage treatment plants and leaching from domestic garbage dumps (Ronco et al., 2008). The lower part of the basin was characterized in 2013 by Green Cross Switzerland as one of the ten most polluted sites in the World (http://www.greencross.ch; http://cyt-ar.com.ar/cyt-ar/index.php/Cuenca_Matanza-Riachuelo).

Heavy metals that represent a potential hazard to humans and animal health, are frequently detected in contaminated environments, including soils and sediments, and they have gradually become one of the major ecological concerns worldwide (Deng et al., 2004; Yoon et al., 2006). Within this context, an evaluation of the ecologic impact produced in the sediments, soils and plants by human activities should be at a high priority in order to find sustainable and economical strategies for improving environmental conditions.

Plants and soil microorganisms can be used as biological tools to assess the level of environmental contamination with an aim at a subsequent cleanup of contaminated areas through bioremediation

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techniques. Phytoremediation is one of the cost-effective ecological technologies to improve, alleviate or stabilize areas contaminated by heavy metals (Shah and Nongkynrih, 2007). The plants for this purpose must tolerate high level of contaminants within the immediate environment of their roots and then to regulate both the absorption of metals from the rhizosphere along with the distribution of the contaminant within the different plant tissues (Mac Farlane et al., 2007).

Arbuscular mycorrhizal (AM) fungi have been demonstrated to alleviate heavy metal stress on plants and thus provide an attractive platform to advance plant-based environmental clean-up (Miransari, 2011; Wang et al., 2012). During its symbiotic interaction the hyphal network functionally extends the root system of their hosts. Thus, plants in symbiosis with AM fungi acquire the capability of taking up heavy metals from an enlarged soil volume and concentrate metals in the root system (Miransari, 2011).

When the climatic and soil conditions change by disturbance or chemical load the plant-community also became altered; and hence, the specific source of the cause-and-effect relationships between plant and soil with respect to plant's absorption of metals from the soil becomes difficult to attribute either to the soil, to the plant alone, or to the two in combination. In order to evaluate the environmental impact, an option to overcome these problems is to collect the same plant species over a wide range of soil conditions and plant communities at different locations contaminated with metals and under same regional climatic conditions so that the later influence remains constant.

In this research we therefore sampled the same native wetland plants on the banks of streams and rivers along the MRRB from close to the headwaters down to the river's mouth in the Río de la Plata. These plants are frequently subjected to flooding and sediment deposition and grow on soils–sediment substrates contaminated with heavy metals (Rendina and Fabrizio de Iorio, 2012). The use of native plants for remediation purposes and the diagnosis of contamination are relevant because native plants often survive, grow, and reproduce better under the environmental stress than plants introduced from other environments.

The primary objective of the present research was to characterize the distribution and pattern of the metal contamination in the MRRB by: a) measuring the concentration of heavy metals (Cr, Cu, Pb, Zn), and nutrients (N, P) present in the soils along with the biomasses of the native plants that grow in the riverside zones of streams and rivers in order to assess the contamination of the environment throughout the basin, b) assessing the ability of the plants to absorb metals and nutrients from the soil solution and then to allocate those inputs in either the aboveground or the belowground biomasses, c) evaluating the capability of the plants to associate with the AM fungi on soils of different levels of contamination and, d) determining the feasibility of using the plants used in this study for phytoremediation purposes. The hypothesis is that the concentration of metals and nutrients in soil and plants increases from the sites of the upper basin to the sites in the lower basin, and these concentrations were inversely related with the AM colonization in roots and the AM spore density in soil. With the increase of the contamination, the plants concentrate more metals and nutrients in the roots than in the aboveground tissues and this decreases the symbiosis with AM fungi.

2. Materials and methods

2.1. Study area

The MRRB comprises approximately 2250 km^2, 90% being located within the Province of Buenos Aires and 10% within the city of Buenos Aires, where nearly 4 million of people live. The climate in the area is subtropical with a maximum average temperature in January of 23.9 °C, a minimum average in July of 10.4 °C, and an annual rainfall of around 1200 mm. The dominant soils near the alluvial plain of the rivers and streams are classified as Arguidolls, Natraquolls and Natraqualfs (De Siervi et al., 2005).

2.2. Selected sites and soil and plant sampling procedure

The sampling area comprised 5 sites on the banks of the Morales stream, the Chacon stream, the Matanza river and the Riachuelo river; with all of those sites lying along the approximately 86 km from the headwaters to the mouth into the Rio de la Plata (Supplementary Fig. 1). Site 1 was situated approximately 8.3 km from the headwaters of the Morales Stream at an altitude of 32 m above sea level, site 2 at 13.5 km from the headwaters and 26 m above sea level in the Las Heras County – along with site 1 – with a population density of approximately 20 habitants per km^2. Site 3 lays on the Chacon stream at 27.9 km and 16 m above sea level in the Marcos Paz County (100 habitants per km^2), site 4 was on the Matanza River at 40.9 km and 11 m above sea level in the Virrey del Pino County (1500 habitants per km^2), and site 5 on the Riachuelo River at 79.5 km from the headwaters at 5.3 m above sea level in the Avellaneda Borough (8500 habitants per km^2). The main economic activity around the first two sites involves bovine feedlots, poultry and porcine productions, and some extensive agriculture (mainly soybean, corn and sunflower). The corresponding activities around site 3 are similar to those in sites 1 and 2, but with more emphasis on extensive agriculture. Sites 4 and 5, located in peri-urban and urban centers respectively, are surrounded by industrial areas, especially by tanning factories in site 5.

Two groups of native plants present in all the sampled sites of the riverside zones were studied. The first was composed by *Eleocharis montana* (Kunth) Roem and *Cyperus eragrostis* Lam., both Cyperaceae (non-mycorrhizal plants); while the second group was composed by *Hydrocotyle bonariensis* Comm. ex Lam., an Araliaceae (mycorrhizal plant). From each site, 5 samples of adult individuals of similar size of *E. montana* and *H. bonariensis* together with their respective belowground biomass and rhizospheric soils were collected at approximately a distance not less than 20 m between samples. The exception was at site 5, where *E. montana* plants were absent and *C. eragrostis* were collected instead.

2.3. Soil and plant analyses

Each soil sample collected was uniformly mixed and separated into two portions, one being used fresh to measure AM spore density in soil, and the other processed to measure the soil's chemical properties, metals and nutrients. The metals measured in soils and plant tissues were: Cr, Cu, Pb and Zn. The Cu and Zn are also nutrients and usually called essential metals. Along the text, we refer to metals for Cr, Cu, Pb and Zn and nutrients for N and P. Total Cr, Cu, Pb and Zn concentrations were determined by digesting a powdered soil sample with $HNO_3 + HClO_4 + HF$ and then were analyzed by atomic absorption spectrophotometer. The extractable metal of soils was determined by the DTPA method (Levei et al., 2010). The agronomical variables: soil pH (1:2.5 soil:water), (EC) electrical conductivity (Jackson, 1964), (OM) organic matter (Richter and Von Wistinghausen, 1981), total N (Bremmer and Mulvaney, 1982), and available P (Kurtz and Bray, 1945) were also measured in soil.

The aboveground biomass of the plants under study had different morphologies. The aerial part of *E. montana* was mainly composed of photosynthetic shoots with small leaves. The aerial biomass of *C. eragrostis* consisted of tall and erect stems with pointed leaves radiating from the top. For both plants, all the aboveground biomass was analyzed together and referred to as shoot. The aerial part of *H. bonariensis* was composed by petioles and leaves, those being analyzed together and referred to as leaves. The belowground biomass of both groups of plants comprised both rhizomes and roots; each of which was analyzed separately; with the exception of *C. montana* at site 5, where the rhizome and root tissues were mixed and analyzed together in a single sample because of insufficient rhizome biomass for analysis.

The belowground biomass was washed with tap and distillated water. The roots of each species were cut in portions of 2-cm long, mixed homogeneously and then separated in two portions; one was used fresh to measure AM colonization and the other portion was oven dried at 70 °C for 48 h for chemical analyses. The rhizome and the aerial biomass were also oven-dried for 48 h, and both, the dried aboveground and belowground tissues were reserved for analyzing Cr, Cu, Pb, Zn, N and P concentrations in the tissue. The aboveground and belowground biomasses were digested and analyzed separately in sulfuric acid to determine N concentration by the Kjeldahl method (Jackson, 1964), and P concentration by a modification of the Murphy Riley method (John, 1970).

2.4. Translocation and bioconcentration factors

The translocation factor (TF) for metals and nutrients within the plants was defined as the ratio of the concentration of a metal or nutrient in the shoot tissue over the concentration of this metal or nutrient in the root tissue. This factor reflects the translocation ability of a species to transfer an element from the roots to the shoots (Stoltz and Greger, 2002). Similarly, the bioconcentration factor (BCF) of a plant with respect to a given soil was expressed as the ratio of the concentration of a metal or nutrient in the root tissue over the concentration of that metal or nutrient in the soil (Ali et al., 2013). The measure of the total metal in soil is commonly used in the definition of soil quality-standards, but that parameter provides no information regarding the nature of the metal or its mobility in soil (Silveira et al., 2006; Walter et al., 2006). For this reason, we used the measure of the extractable metal by the DPTA method to estimate the values of BCF and to associate the concentration of metals in the soils with the concentration of metals in the plant tissue.

2.5. Assessment of AM spore density in soil and AM colonization in roots

AM-fungal spores were extracted and recorded from samples of 100 g of rhizosphere soil of the three species collected. The soil sample was wet-sieved and decanted (Gerdeman and Nicolson, 1963) and the supernatant centrifuged in a sucrose gradient (Walker et al., 1982). Mycorrhizal root colonization was measured in fresh roots cleared and stained in lactic acid–glycerol Trypan blue. Twenty-five root segments per plant sample were examined under a microscope at ×200 magnification. The fraction of root length colonized (MC), and root length containing arbuscules (AC) was determined following Mc Gonigle et al. (1990) and number of entry points (EP) per mm of root colonized, along root fragments at ×200 magnification were measured (Amijee et al., 1989).

2.6. Contamination indexes in soil

For assessing the contamination of the soils by metals along the MRRB we calculated the contamination factor (Cf) and the contamination degree (Dc) used by Kwon and Lee (1998), which are defined as follows:

$$\mathrm{Cf} = \mathrm{Cm}/\mathrm{Cref} \tag{1}$$

where, Cm is the concentration of a particular metal in soil and Cref is the concentration of this metal in a soil used as a reference level; and:

$$\mathrm{Dc} = \sum \mathrm{Cf} \tag{2}$$

where Dc represents the summation of each Cf measured in soil.

The reference soil used for this study was a Typic Natraquoll used for pasture growth and currently grazed by bovines for meat production and characteristic of the study area. The main soil properties are: pH of 8.5, electrical conductivity at 5.1 dS m^{-1}, 2.8% organic C, 0.3% total N and 13.3 mg kg^{-1} soil of available P (Escudero and Mendoza, 2005; García and Mendoza, 2008). Total Cr, Cu, Pb and Zn were measured in this soil, and those values were used as a reference to calculate the Cf values of each metal at each site.

For calculating the reference levels of total N in soil we used an average value of 0.24% N (0.12%–0.47%) which was taken from a series of previous studies of 20 non-fertilized grassland soils sampled within the Province of Buenos Aires and currently used for pasture growth (Escudero and Mendoza, 2005; García and Mendoza, 2008). For calculating the reference value of available P in soil we used the guidelines of the Pampas Region in the Buenos Aires Province given by Sainz Rozas et al. (2012) for crop production, where the soil P levels were categorized from very low (<10.0 mg kg^{-1}) to very high (>25 mg kg^{-1}) level in soil.

2.7. Data analysis

The chemical properties, nutrients, extractable and total metals of the soil from the selected five sites associated to each plant species were analyzed by a one way ANOVA, and the mean separation was performed by the Tukey's test after the normality and homogeneity of variances were verified. Non-normal data were transformed to logarithm, square root and arcsine or no-transformed and analyzed by the Kruskal–Wallis non-parametric test to compare the soil sites and plant species.

Simple linear regressions plotted from the 25 observations for each of the two groups of plants were used to describe: a) the relationship between the concentration of metals or nutrients of the aboveground tissue and the respective concentrations of these metals or nutrients on the root tissue and, b) the relationship between the concentration of each metal or nutrient in the aboveground plus the root tissue and the extractable metals or nutrients in the soil. The relationship between variables was considered significant when the *P* value of the correlation coefficient was <0.05. In addition to the significance of the relationship between the variables, the significance in the deviation of the slope from zero was tested (GraphPad Prism, version 4.0, 2003).

Simple non-linear regressions were used to describe: a) the relationship between the TF and BCF for the metals or nutrients and the concentrations in the root and extractable metals or nutrients in soil respectively, and b) the relationship between the AM spore density in soil and the AM colonization in roots and the concentration of the total and extractable metal in soil. The relationship between variables was considered significant when the *P* value of the correlation coefficient was <0.05.

3. Results

3.1. Soil site chemical characteristics

The rhizospheric soils collected from *E. montana* (sites 1–4), *C. eragrostis* (site 5), and *H. bonariensis* (sites 1–5) were moderately alkaline (Table 1). The EC was high in site 3 and classified as saline, in the other sites the EC values were lower than 4.0 dS m^{-1} and classified as non-saline (Table 1). The OM, N and P from the soils of the studied plants varied among the sites and showed, in general, the lowest values in sites 1 and 2, intermediate in site 3, increased gradually in site 4 and showed a marked increase in site 5 (Table 1). The total N in site 5 was 0.24% and doubled the reference value from non-contaminated soils. Available P in the soils at sites 1 and 2 was either below or at the limit of the reference value of 10 μg g^{-1} while at sites 3–5 the values were very high and 25 mg kg^{-1} of P used as a reference value (Table 1). The total N and OM in the soils of both groups of plants were the agronomical variables that most positively correlated with the concentrations of metals in soils (Pearson's correlation coefficient was always

Table 1
Chemical properties of the soil from *Eleocharis montana–Cyperus eragrostis* (*Eleoch–Cyper*) and *Hydrocotyle bonariensis* individuals collected from sites 1–5 along the MRRB.

Chemical property	Plant species	Site 1	Site 2	Site 3	Site 4	Site 5
pH (1:2.5 w)	*Eleoch–Cyper*	7.47 a	8.29 c	7.39 a	7.46 a	7.65 b
	Hydrocotyle	7.86 b	8.22 c	7.28 a	7.33 a	7.37 a
EC (dS m^{-1})	*Eleoch–Cyper*(1)	2.02 a	2.76 ab	9.50 c	2.95 b	3.04 b
	Hydrocotyle	1.82 a	1.96 a	4.16 b	2.39 a	3.64 b
OM (%)	*Eleoch–Cyper*(1)	3.08 a	3.23 a	3.46 a	2.98 a	7.26 b
	Hydrocotyle	4.31 b	1.53 a	4.08 b	4.64 b	13.07 c
N (%)	*Eleoch–Cyper*(1)	0.24 ab	0.13 a	0.30 c	0.20 a	0.49 d
	Hydrocotyle	0.29 b	0.14 a	0.29 b	0.25 b	0.63 c
P (μg g^{-1})	*Eleoch–Cyper*	5.06 a	7.47 a	77.95 c	94.35 d	66.98 b
	Hydrocotyle	7.30 a	10.83 a	40.42 b	64.31 c	45.94 b
Extractable metal (μg g^{-1})						
Cr	*Eleoch–Cyper*(1)	0.07 a	0.11 b	0.14 c	0.15 c	0.22 d
	Hydrocotyle(3)	0.17 b	0.16 b	0.17 b	0.08 a	0.28 c
Cu	*Eleoch–Cyper*(2)	5.09 ab	4.55 a	8.77 b	20.25 c	64.28 d
	Hydrocotyle(3)	3.83 a	3.22 a	6.01 b	15.92 c	86.72 d
Pb	*Eleoch–Cyper*(1)	2.74 b	0.85 a	2.28 b	4.10 c	19.19 d
	Hydrocotyle	1.01 a	1.03 a	1.78 b	6.25 c	19.02 d
Zn	*Eleoch–Cyper*(1)	1.47 a	2.35 b	18.75 c	26.08 d	54.92 e
	Hydrocotyle(2)	2.33 a	2.35 a	17.54 b	25.18 c	62.98 d
Total metal (μg g^{-1})						
Cr	*Eleoch–Cyper*(3)	21.03 a	22.09 a	26.55 a	32.92 a	891.64 b
	Hydrocotyle(3)	22.08 a	21.22 a	33.50 b	47.96 b	1537.96 c
Cu	*Eleoch–Cyper*(2)	38.83 a	54.56 a	44.30 a	79.10 b	180.69 c
	Hydrocotyle(1)	22.35 a	23.44 a	38.49 b	70.28 c	237.59 d
Pb	*Eleoch–Cyper*(1)	55.96 a	50.04 a	54.96 a	80.09 b	182.83 c
	Hydrocotyle(1)	39.68 a	39.21 a	43.67 a	67.59 b	177.22 c
Zn	*Eleoch–Cyper*(1)	100.58 a	104.31 a	165.82 b	211.93 c	832.12 d
	Hydrocotyle(1)	92.45 a	87.94 a	128.02 b	171.75 c	742.88 d

Different letters for each chemical property and plant species within each row indicate significant differences among sites at a level of $P < 0.05$ by the Tukey's test. (1) log = log transformed data. (2) sqrt = square root transformed data. (3) kw = Kruskal–Wallis non-parametric test.

significant at a level of $P < 0.010$). Both the extractable and the total metals were the lowest in soils at sites 1 and 2 and the highest in the soil at site 5, and for most of the cases, the values from sites 3 and 4 were in between those of sites 1 and 2 and site 5 (Table 1).

3.2. Contamination indexes in soil

The method of assessing contamination in the soils suggests that for all the sites, plant species and metals analyzed, the Cf was always 1 to or greater than 1, and at site 5 markedly so for the two groups of plants. This demonstrates that the soils sampled along the MRRB had metal levels above those of the soil used as reference (Table 2). The table also indicates a consistent increase from site 1 through site 5 in the levels of all the metals and plants, and that most of the soils from sites 1–4 were categorized as moderately contaminated for all four metals, having Cf values ranging from 1 to nearly 3 (Table 2). Moreover, the soils from both plants at site 5 had a very high Cf with values higher than 6 for all four metals in the soil from both *C. eragrostis* and *H. bonariensis* with an exception for Pb in *C. eragrostis* soil where the values were 5.9.

The degree of contamination (Dc) index calculated by Eq. (2) likewise indicated that the soil associated to both groups of plants at site 5 was categorized as being at a very high degree of contamination for all metals together (Table 2). Meanwhile, the soils from both plants at site 1 and from *H. bonariensis* at site 2 had low degrees of contamination, and the soils from both sites 3 and 4 had moderate degrees of contamination (Table 2).

Table 2
Values of contamination factor (Cf)(1) and degree of contamination (Dc)(2) by metals for the reference soil(3) for contamination assessment of the soil sites associated to the plants studied along the Matanza-Riachuelo River Basin (MRRB).

Plant species	Soil site	Contamination factor				Degree contam.
		Cr	Cu	Pb	Zn	
E. montana	1	1.1	1.7	1.8	1.4	6.0 ± 0.3
	2	1.2	2.4	1.6	1.4	6.5 ± 0.7
	3	1.4	1.9	1.8	2.2	7.3 ± 0.1
	4	1.7	3.4	2.6	2.9	10.6 ± 0.1
C. eragrostis	5	46.3	7.9	5.9	11.2	71.3 ± 8.3
H. bonariensis	1	1.2	1.0	1.3	1.3	4.6 ± 0.1
	2	1.1	1.0	1.3	1.9	4.6 ± 0.2
	3	1.7	1.7	1.4	1.7	6.5 ± 0.5
	4	2.5	3.1	2.2	2.3	10.0 ± 0.5
	5	79.9	10.3	5.7	10.0	106.0 ± 7.8

(1)Reference soil (μg g^{-1}): Cr 19.3, Cu 23.0, Pb 31.0, Zn 74.1.
(2)Cf < 1 low contamination factor; Cf 1–3 moderate contamination factor, Cf 3–6 high contamination factor, Cf > 6 very high contamination factor. Dc < 6 low degree of contamination, Dc 6–12 moderate degree of contamination, Dc 12–24 high degree of contamination, Dc > 24 very high degree of contamination.
(3)$Dc = \sum Cf \pm S.E.$

3.3. Relationship between metals and nutrients in plant tissue

Fig. 1 illustrates the relationships between the concentration of each metal in the aboveground or rhizome of the plants and the concentration of the corresponding metal in the root tissue. When the relationship between the aboveground or rhizome and the roots tissue was significant ($P < 0.050$), both the points representing the 25 observations from all the sites taken together and the corresponding fitted line were drawn; but when the variables did not correlate each other, only the points were drawn (Fig. 1a–h).

For both groups of plants (*E. montana* and *C. eragrostis* plus *H. bonariensis*) the concentrations of metals in the aerial tissue did not significantly change with increases in the concentration of those respective metals in the root tissue (Fig. 1a–h). Furthermore, with three exceptions (Cr, Zn in *C. eragrostis* and Cr in *H. bonariensis*; Fig. 1a, g and b respectively), the concentration of metals in the rhizomes also did not change with increases in the concentration in the root tissue. In general, the plants concentrated more metals in root tissue than in the aerial and/or rhizome tissue (Supplementary Table 1).

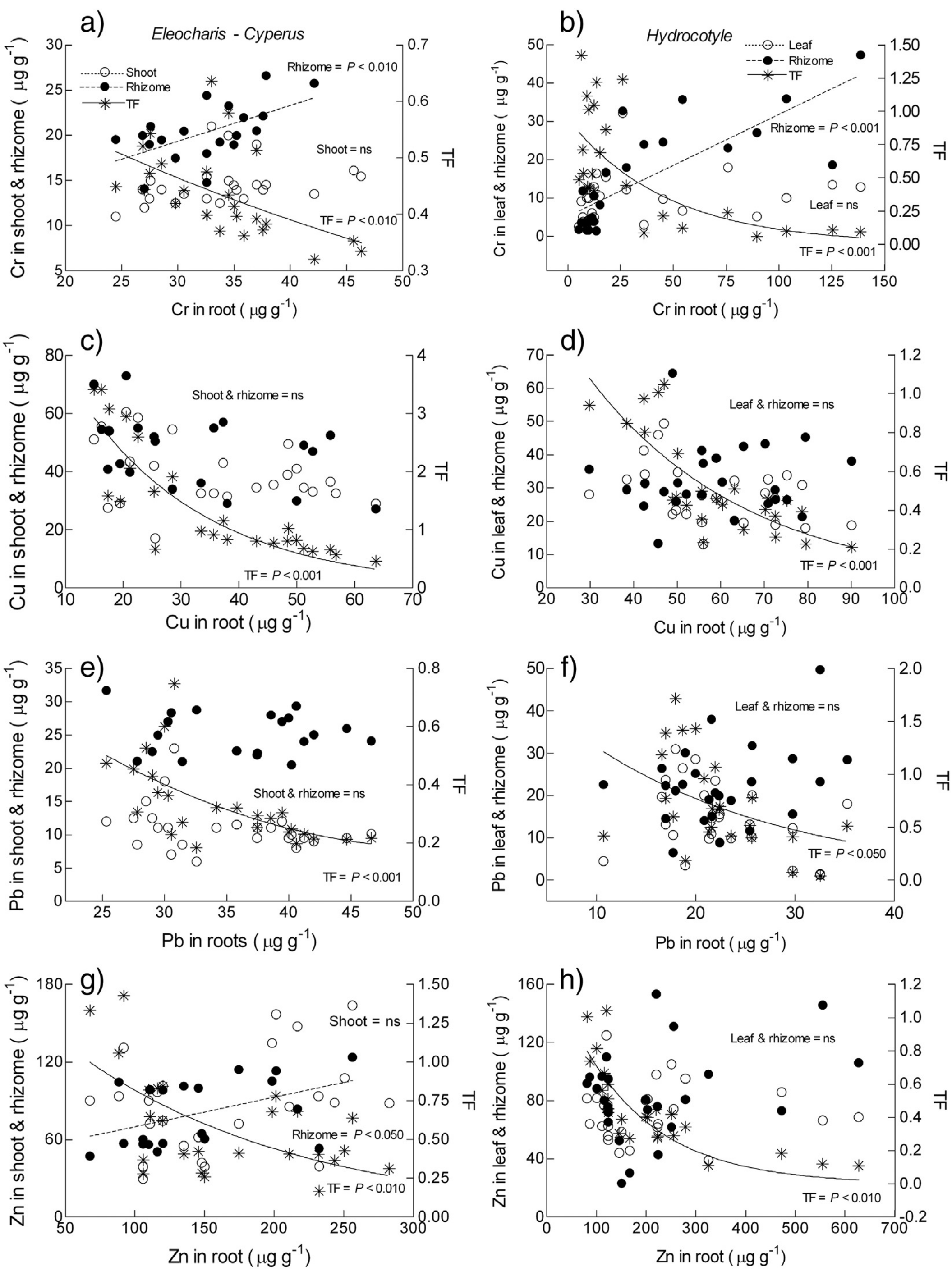

Fig. 1. The observed and fitted values for all observations on the concentration of Cr (a, b), Cu (c, d), Pb (e, f) and Zn (g, h) in the aboveground (shoot or leaves) and rhizome tissues (left ordinate) are graphed as a function of each respective metal concentration in the roots of the plants collected from the sampling sites 1 through 5. The TF (translocation factor) observed and fitted ($y = a \exp(cx)$) values (right ordinate) are also plotted as a function of the concentration of that metal in the root tissue. The fitted lines drawn were significant at the indicated level of *P*. The non-significant fitted lines were indicated as ns.

The concentrations of N in the aboveground and the belowground tissues of both groups of plants showed a different pattern from that described above for the distribution of the metals in plant tissues (Fig. 2a, b). The N in the aboveground tissues of the *E. montana*–*C. eragrostis* plants (0.8–2.6%) and of *H. bonariensis* (1.4–4.1%) plants was always higher than the concentration in their rhizomes for each concentration of N measured in their respective roots (Fig. 2a, b). Nevertheless, the concentrations of P in the aboveground and belowground tissues did not correlate, and were within the same concentrations (0.1–0.8%) of both groups of plants (Fig. 2c, d).

3.4. Relationship between metals and nutrients in plant tissue with extractable metals and nutrients in soil

The concentration of the extractable metals in the soils correlated positively with the metal levels in the aerial, rhizome and root tissues ($P = 0.0236$, $P = 0.0028$ and $P = 0.0009$, respectively) and did so more closely than did the total metal measured in the soils ($P = 0.3878$, $P = 0.1585$, 0.0041, respectively). Because of these correlation patterns, the data on the extractable metals in the soil were preferentially used – than the measures of the total

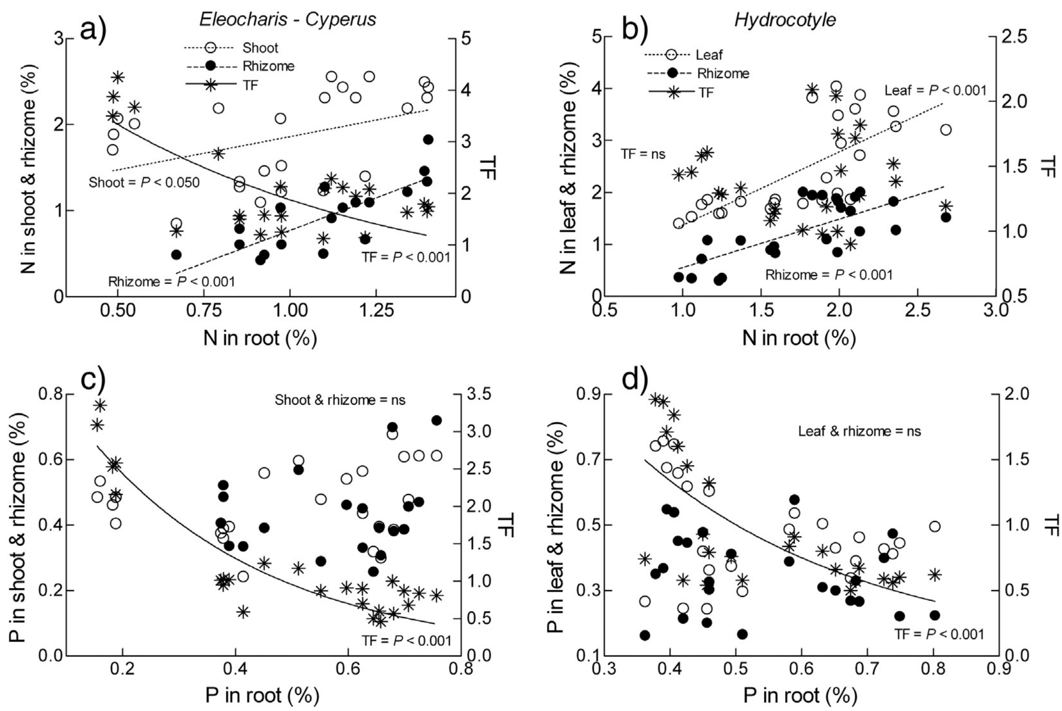

Fig. 2. The observed and fitted values for all observations on the concentration of N (a, b) and P (c, d) in the aboveground (shoot or leaves) and rhizome tissues (left ordinate) are graphed as a function of each respective nutrient concentration in the roots of the plants collected from sampling sites 1 through 5. The TF (translocation factor) observed and fitted ($y = a \exp(cx)$) values (right ordinate) are also plotted as a function of the concentration of that nutrient in the roots. The lines fitted were all significant at the indicated level of *P*. The non-significant fitted lines were indicated as ns.

metals in soils – to study the associations between the metal and nutrients in the soil and those respective concentrations in the plant tissues.

There was a simple lineal relationship between the concentrations of each metal in the roots of both groups of plants and the corresponding extractable metal in soil (Fig. 3), but with two exceptions: one for the extractable Pb in the soil of the *E. montana*–*C. eragrostis* (Fig. 3e); and the second exception was the lack of any correlation between the levels of Cu in the roots and the soil from the *H. bonariensis* plants (Fig. 3d). However, the concentration of metals in the aboveground tissue did not correlate with the corresponding extractable metal in soil (Fig. 3). For the eight combinations between plants and metals, two exceptions were present and both with respect to the *E. montana*–*C. eragrostis* plants (Fig. 3a, e).

The relationship between the concentrations of N and P in the plant tissues and the N and P levels in the soils exhibited a different pattern from that shown by the metals (Fig. 4). For each of these nutrients, the plants concentrated as much or more in the aboveground tissue than in the roots biomass. The N levels in the shoots of the *E. montana*–*C. eragrostis* plants averaged 1.85 ± 0.2%, while in the roots it decreased significantly ($P < 0.050$) from 1.40% to 0.50% when the concentration in the soil increased from 0.10 to 0.70% N (Fig. 4a). The N levels in the leaves and roots of *H. bonariensis* averaged 2.5 ± 0.4% and 1.8 ± 0.2% respectively, and neither concentration changed significantly with increases in N from 0.12% to 0.80% in the soil (Fig. 4b). The dynamics of the P levels between the tissues and the soils differed from that of N. At low levels of available P in soil (5–10 μg P g^{-1}) the concentration of P in the roots was higher than the concentration of P in either shoots or leaves of both plants (Fig. 4c, d), but at soil P concentrations above 50 μg P g^{-1} the concentration in the shoots and roots was similar in the *E. montana*–*C. eragrosits* plants (Fig. 4c), but higher in the leaves than in the roots of *H. bonariensis* plants (Fig. 4d).

3.5. Translocation factor (TF) and bioconcentration factor (BCF) for metals and nutrients

The TF decreased when the concentration of Cr, Cu, Pb and Zn in the roots of both groups of plants increased (Fig. 1). The decline in the TF value was well described by an exponential decay equation ($y = a \exp(cx)$); where y is the concentration of metal in the aerial biomass, a and c are coefficients, and x represents the concentration of metal in the roots. The highest concentrations of the metals in the roots were always found in sites 4 and 5, but especially in site 5, and were associated with the lowest TF values (Fig. 1). Conversely, the lowest concentrations of metals in the roots of the plants from sites 1–2 were associated with the highest TF values (Fig. 1). The changes in the TF values for N and P also decreased with increases in the concentration of N and P in the root tissue (Fig. 2). The sole exception was for the N levels in *H. bonariensis*, where the TF values did not change upon increases in the %N in the root (Fig. 2b).

The BCF decreased with increases in the extractable metals or nutrients in the soil (Figs. 3, 4). These decreases were likewise described by an exponential decay equation having three coefficients ($y = a + b \exp(cx)$). The variables and coefficients have the same meaning as for TF, and b is an extra parameter used to correctly describe the shape of the function. The BCF values decreased gradually from about 500 to 150 when the extractable Cr increased from site 1 through 5 in the soils of the *E. montana*–*C. eragrostis* plants (Fig. 3a), but changed inconsistently for the *H. bonariensis* plants (Fig. 4b). For the other metals, the BCF values decreased rapidly at first with little change of the extractable metal in the soil, and then slowed down at minimal levels with further increases in that extractable metal (Fig. 3c–h). The BCF values of N and P changed in a pattern similar to that of the metals (Fig. 4a–d).

3.6. Relationship between AM spore density in soil and AM colonization in roots with metals and nutrients in soil

The AM-spore densities in soils from the roots of both groups of plants and the MC index of the *H. bonariensis* roots decreased

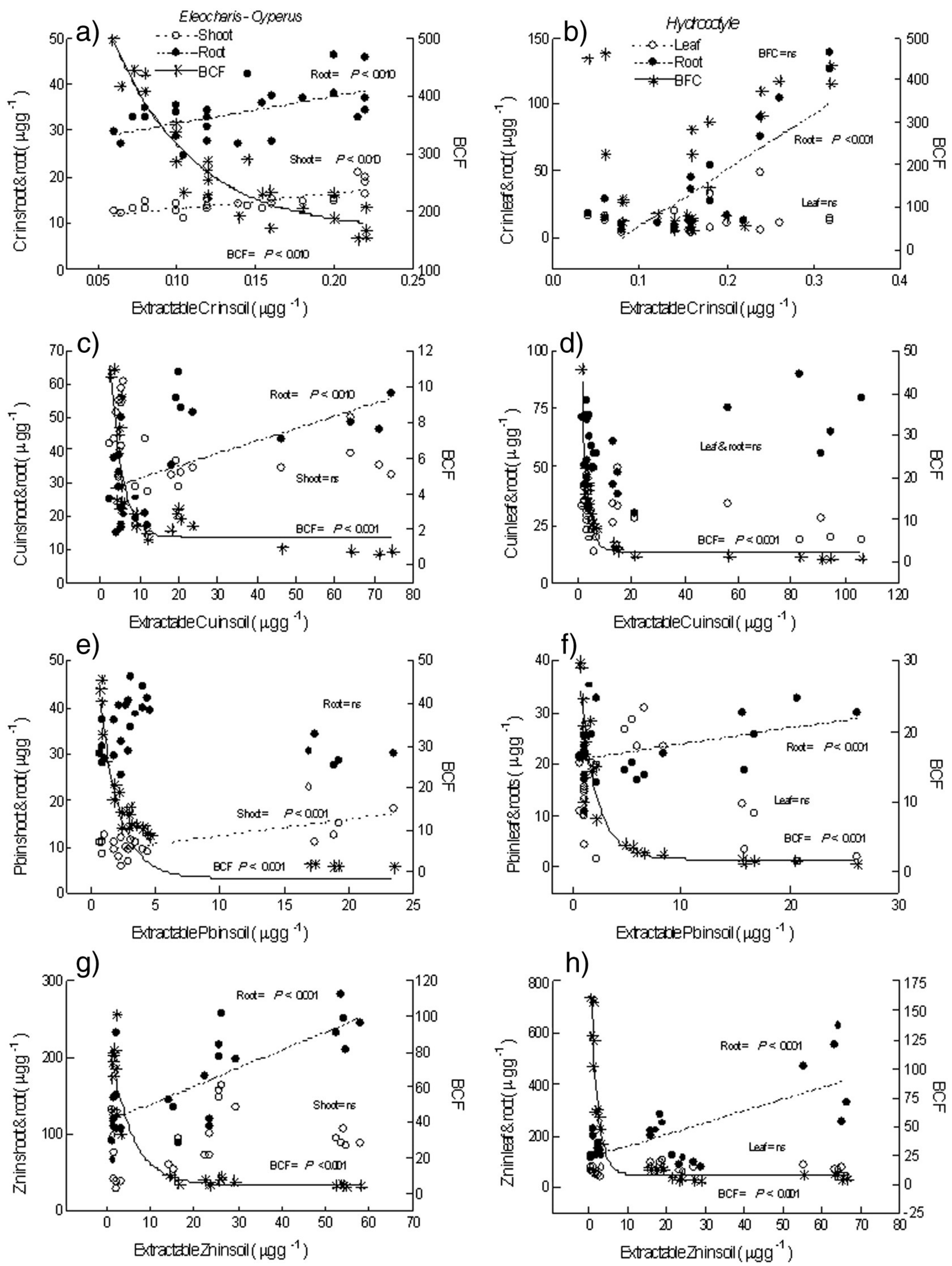

Fig. 3. The observed and fitted values for all observations on the concentration of Cr (a, b), Cu (c, d), Pb (e, f) and Zn (g, h) in the aboveground (shoot or leaves) and rhizome tissues (left ordinate) are graphed as a function of each respective extractable metal in the soils associated with the plants collected from sites 1 through 5. The BCF (bioconcentration factor) observed and fitted ($y = a + b \exp(cx)$) values (right ordinate) are also plotted as a function of the extractable metals in soil. The fitted lines were all significant at the indicated level of *P*. The non-significant fitted lines were indicated as ns.

exponentially ($P < 0.001$) with increases in the total or extractable metals in the soils (Supplementary Fig. 2a–h). Except for the extractable Cr in the soil, where the spore counts and the MC index did not correlate with the concentrations present from *H. bonariensis* soil (Supplementary Fig. 2b), the spore densities decreased markedly at first with increases in the total or extractable metals in the soil, abruptly reaching a constant minimum value near 1–3 spores g^{-1} of soil at the high levels of metals in the soils from sites 4 and 5 (Supplementary Fig. 2). The mean value of the spore densities collected from the rhizospheric soil of *H. bonariensis* decreased from 49 ± 6 (S.E.) in site 1 to 3.4 ± 0.9 spores g^{-1} of dry soil in site 5. Although the *E. montana* and *C. eragrostis* species are not mycorrhizal plants, AM spores were nevertheless recorded in their associated soils; probably arising from other mycorrhizal individuals within the plant community. The spore densities

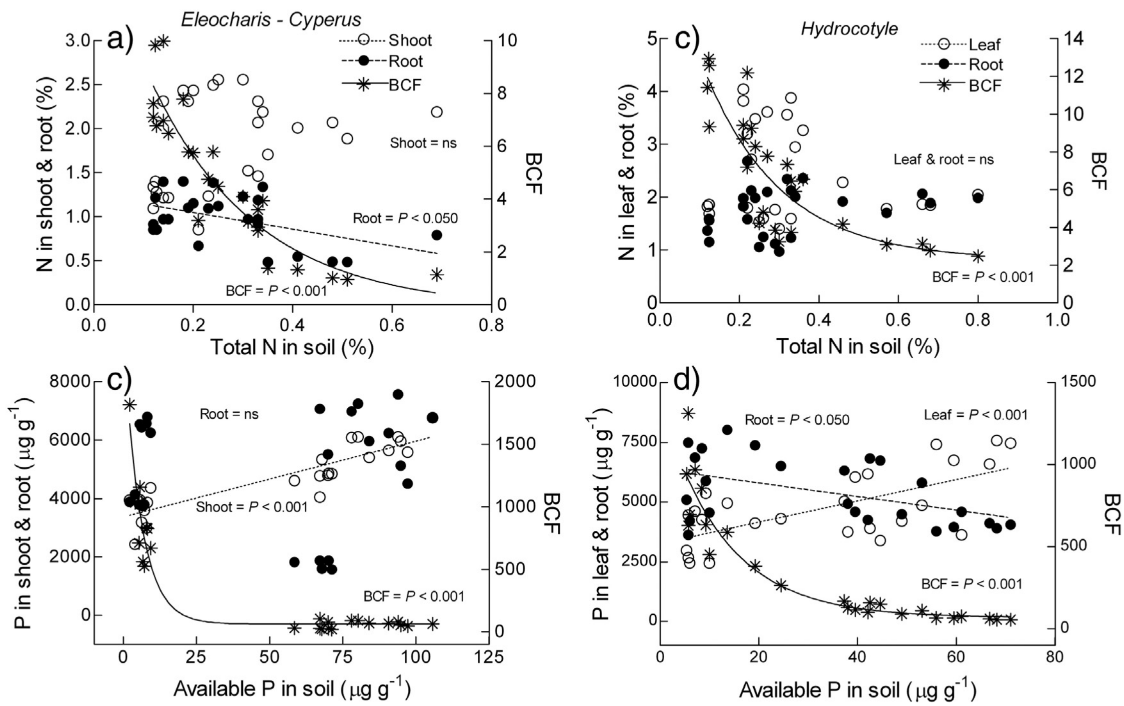

Fig. 4. The observed and fitted values for all observations on the concentration of N (a, b) and P (c, d) in the aboveground (shoot or leaves) and root tissues (left ordinate) are graphed as a function of each nutrient (total N, available P) in the soils associated with the plants collected at the sampling sites 1 through 5. The BCF (bioconcentration factor) observed and fitted ($y = a + b \exp(cx)$) values (right ordinate) are also plotted as a function of N and P in soil. The fitted lines were all significant at the indicated level of *P*. The non-significant fitted lines were indicated as ns.

were lower in the *E. montana*–*C. eragrostis* soils than in the *H. bonariensis* soils, with mean values of 17 ± 1 in site 1 to 0.9 ± 0.1 spores per g of soil in site 5.

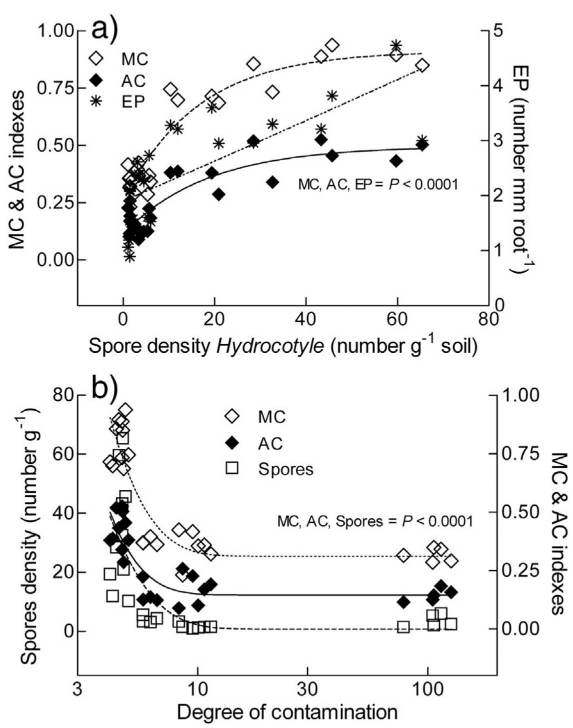

Fig. 5. The MC and AC indexes (left ordinate) and the quantity of the EP (right ordinate) per mm of colonized root of *H. bonariensis* are graphed against the spore density of arbuscular mycorrhizae in the associated soils at sites 1 through 5 (a). The spore density in soil (left ordinate) and the MC and AC indexes (right ordinate) are plotted as a function of the degree of metal contamination (Dc) at the sampling sites 1 through 5 (b). The fitted lines ($y = a + b \exp(cx)$) were all significant at the indicated level of *P*.

The nutrients N and P in the soils exhibited a different association with the AM-fungal variables. The total soil N in did not correlate with those variables in either plant species (Supplementary Fig. 3a), but increases in the available P in the soil resulted in exponential decreases in the spore densities and in the MC indexes ($P < 0.001$) of the *H. bonariensis* plants (Supplementary Fig. 3b).

The MC index was highest at site 1 with a value of 0.88 ± 0.02, but thereafter decreased progressively to 0.31 ± 0.01 in site 5. The AC index and the number of EP also decreased consistently from sites 1 through 5. The AC values were 0.49 ± 0.02 and 0.14 ± 0.01; while the EP (number of EP per mm of colonized root) was 3.5 ± 0.3 to 1.5 ± 0.02, for sites 1 and 5, respectively. The MC and AC indexes together with the EP values in the *H. bonariensis* roots were significantly correlated with the spore density in the different soils (Fig. 5a). The three AM variables measured in *H. bonariensis* plants (spore density, MC, and AC) decreased exponentially with increases in the Dc index in the soils from sites 1 and 2 through site 5 (Fig. 5b). Accordingly, the decrease from sites 1 through 3 was particularly pronounced but then approached constant values between sites 4 and 5 (Fig. 5b).

4. Discussion

The present study showed that the native plants *E. montana*, *C. eragrostis* and *H. bonariensis* can colonize the riverside zones of the streams and rivers of the MRRB over the 86 km from the headwaters to the mouth into the Rio de la Plata through a progressively increasing contamination gradient. The first two soil-sampling sites (1 and 2) located in the upper basin, where the main economical activities are agriculture and livestock had low to moderate values of Cf and low Dc with heavy metals. Site 3, where the principal activity is farm livestock with extensive agriculture, and site 4, a peri-urban center surrounded by scattered industrial areas, tested moderate Cf values and were both characterized by a moderate Dc. Finally site 5, situated in a major urban center with two centuries of industrial activities and a population of approximately 8500 habitants per km^2, showed very high level of Cf values for each metal and very high Dc with values between 71 for the soil of the

C. montana and 106 for the soil of *H. bonariensis*, both widely exceeded the limit of 24 given by Kwon and Lee (1998) as being characterized by a very high Dc. Although N and P are not contaminants, their concentrations in the soil sites formed a gradient similar to the one exhibited by the metals. The metals, nutrients and microorganisms in the soils and plants tissue were associated with the land use, industrial activity and human populations and, reflected a gradient of chemicals discharged into the streams and rivers along the MRRB.

The mobility and availability of metals in the soils are generally low, especially when the soils are high in pH, clay content and organic matter (Rosselli et al., 2003), on which description pertains to the sampling sites along the basin and may explain why the OM content of the soil – together with N – was the agronomical variable that much more closely correlated with the extractable or total metals in the soils.

Despite the wide range of metal contamination among the sites, the studied plants could still grow, and *H. bonariensis* in particular was able to associate with the AM fungi in the soils of the riverside along the MRRB. The three plants studied had become adapted to grow within a wide range of metal concentration in the soil and could regulate both the absorption of the metals by the root from the soil solution and the translocation and distribution of the metals within the plant tissues. The plants of this study have the ability to maintain little changes in the concentration of metals in the aboveground tissue when the concentration of metals in the root tissue and the soil increases to levels characterized as high and very high contamination, as were present at sites 4 and 5. The tolerance mechanism when the metal concentration in soil increased is centered on the control of metal uptake from the soil solution by the roots along with the subsequent distribution of metal throughout the plant tissue to minimize the amount of metal transported to the shoots as a protective mechanism to safeguard the photosynthetic tissue. This mechanism is reflected by the consistent decrease observed in the TF values upon increases in the concentrations of the metals in the roots of the two groups of plants.

The uptake and accumulation of metals by plants is affected by many factors. In general, the plant species, metal's characteristics and concentration, the soil properties and soil disturbance have been accepted as the main factors that control the mechanisms of plant metal processing (Mac Farlane et al., 2007). Previous works in Cyperaceae plants reported that *Cyperus malaccensis* Lam., growing in sites close to metal mines (Deng et al., 2004), concentrated more Cu, Pb and Zn in roots than in shoots and these concentrations were higher than the concentrations reported in the present work (Table S1). Similarly, *Cyperus esculentus* from a site of an industrial area concentrated more Cu, Pb and Zn in roots than in shoots (Yoon et al., 2006), and reported values were higher for Cu and Pb but similar for Zn compared with the values of the present work (Table S1). Furthermore, Yoon et al. (2006) found that *Hydrocotyle americana* from the same site of the industrial area also concentrated more Cu, Pb and Zn in roots that in shoots and the values were higher than the concentrations reported in *H. bonariensis* in our work. These previous results suggest that the Cyperaceae and Araliaceae plants studied in the present work are tolerant with respect to metal contamination in soil and can grow with higher level of contamination than those measured in this work.

Chromium is a highly toxic element — especially as Cr^{6+}, which is more toxic than the Cr^{3+} form, even though the concentration in tissue and the removal efficiency by wheat, rape and buckwheat were higher in Cr^{3+} (Kleiman and Cogliatti, 1998). The two groups of plants of the present work accumulated a relatively higher amount of Cr in the bellow and aboveground tissues, especially in site 5 (Table S1), compared to the 5.2 $\mu g\ g^{-1}$ of Cr in tissue reported as critical for most of the higher plants (Shanker et al., 2005).

The TF values for the metals and nutrients decreased when the concentrations in the roots increased. Moreover, the plants absorbed metals and nutrients from the soil and translocated those elements within themselves in different ways. Whereas for metals the concentration in the aboveground tissues underwent little change when the concentration in the roots increased, the N levels in the aerial tissues increased together with the concentration in the roots; or alternatively, as with P, the levels of both nutrients in both tissues became equivalent. In addition, in most instances the TF values for N and P were generally higher, and the values for the metals lower, than unity. These differing patterns indicated that the plants concentrated a higher proportion of nutrients than those of metals in the aboveground tissues, whereas the metals became more concentrated in the roots than in the aboveground tissues. Because such plants are adapted to the environment and N and P are essential nutrients, the plants are able to concentrate much more of the nutrients in their tissues than is needed for adequate growth. In wetlands, Gopal and Ghosh (2009) found under high nutrient loading that *Cyperus involucratus* may concentrate 1.4–4.3% of the N in the shoots and 1.1–4.5% in the roots as opposed to 0.2–0.5% of the P in the shoots and 0.1–0.7% P in the roots. Similarly, Gopal and Ghosh (2009) observed that *Hydrocotyle umbellate* concentrated 1.5–4.5% of the N and 0.2–1.3% of the P in the leaves, with these concentrations being within the range of N and P in the roots for *H. bonariensis* reported in this study. These results are consistent with our findings.

The extractable metals of soils were positively associated with increases in the concentrations of those metals in the roots for both groups of plants but the concentration in the aboveground tissue did not vary with those increases. This pattern constitutes evidence indicating the ability of the plants to accumulate more metals in the roots than in shoots or leaves in the face of increased concentrations of the metals in the banks from the MRRB. This capacity is also shown by the exponential decay of the BCF for each metal when the extractable value in the soils increased from the low contaminated to high and very highly contaminated sites.

The plants and the chemical methods of assay reflected the status of contamination by metals in the soils in different ways. While the concentrations of metals in the plant tissue changed gradually with the different levels of contamination, the concentration of metals extracted from the soils by the chemical methods changed markedly when the contamination increased from the soils of sites 1 to 5. Although, the metals extracted from the soil at sites 1–4 were within a narrow range of concentrations, the levels extracted in the soil of site 5 increased broadly at about 1 to 11 times the average values for sites 1–4. This difference was even more pronounced in measurements of the total metals in the soil where the concentrations were 2–48 times higher at site 5 than the average values for sites 1–4. These results suggest that the plants are less affected by marked changes in the contamination by metals in the soil than would be predicted by chemical methods used for assessing soil–metal contamination. This difference is highly relevant and constitutes a significant concern when either determining the degree of metal contamination in sites, selecting plants for remediation, or prescribing clean-up protocols because the chemical methods may overestimate the status of effective contamination since the plants may tolerate up to high level of contamination when growing in highly contaminated soils.

The magnitude of the AM fungal variables measured in the *H. bonariensis* plants was inversely related with the contamination status measured by Dc with respect to all four metals in the soils. Nevertheless, it is difficult to attribute the decrease in those AM fungal variables to one or more specific factors because both the concentrations of metals and nutrients in the soils increased from sites 1–5. It is known that increases in the available P and OM in the soil diminish the AM association with plants (Smith and Read, 2008). However, low amounts of AM spores in the rhizosphere of *H. bonariensis* managed to effect a colonization of more than the 30% of the root length colonized by the AM fungal community, especially at sites 4 and 5, which finding suggests a high efficiency of the native AM spore to colonize the root system despite high and even very high levels of contamination in the soil. Arbuscules are the primary sites of nutrients and C exchange between the symbionts (Smith and Read, 2008), and near of 14–19% of the root length was colonized by arbuscules at sites 4–5. It is argued that the native AM fungal community and the *H. bonariensis* plants may establish a functional symbiosis in highly to very highly contaminated sites. Several reports have shown that the AM community is able to alter soil metal availability and to contribute to plant metal tolerance, metal translocation and uptake via diverse pathways (Miransari, 2011). Hence, we concluded that *H. bonariensis* is a species with the ability to maintain and propagate the native AM fungal communities in highly contaminated sites and thus can be used as an inoculum in environmental restoration programs throughout the MRRB. In contrast, the Cyperaceae roots were not colonized by AM fungi at any of the five sites in the MRRB, nevertheless we found AM fungal spores in the rhizopheric soil of the Cyperaceae plants that could be attributed to the contribution of the soil inocula from other mycorrhizal species within the plant community.

The TF and BCF indexes can be used to estimate the potentiality of plants for phytoremediation purposes. Two criteria have been proposed for determining whether a species is metal hyperaccumulator. The first is the finding of a BCF > 1000 and the second the presence of more than 0.5% of a given metal in the dry biomass (Olguín et al., 2002). In our study none of the plants were found to have a sufficient metal concentration in their aboveground tissues to be qualified as hyperaccumulator (Baker and Brooks, 1989). The two Cyperaceae plants and *H. bonariensis* being rather tolerant plants that tend to restrict both soil-root and root-aboveground transfers, have a lower accumulation of metals in their tissues as a result of TF values less than one, with the exception of *E. montana* that in some instances had TF values higher than one for the essential metals Cu and Zn.

5. Conclusions

Within the complex pollution present along the MRRB an elucidation of which metal has the most toxic influence on environmental health is difficult. Native plants such as *E. montana* and *C. eragrostis* and *H. bonariensis* – the last of these being highly symbiotically associated with native AM fungal communities – can cope with and grow within low to very highly contaminated areas. Metals, nutrients and AM fungi in the soils and plants were associated with the land use and extent of the population present and consequently reflected a gradient of chemicals discharges into the streams and rivers of the basin. In addition to *H. bonariensis*, other AM-plants should be identified for confirming the potential use of the AM-plant symbiosis as indicator of the level of the environmental contamination and disturbance in the riverside of the MRRB.

The plants and the chemical methods employed in the study mirrored the contamination status of the soils by metals in different ways. The plants were less affected by marked changes in the levels of contamination than would have been predicted by the chemical analyses of the soil. This difference is a relevant concern when characterizing the level of contamination in sites, cleaning aspects or when selecting plants for remediation purposes.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.09.105.

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