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Distribution of Cu, Fe, Mn, and Zn in Two Mangroves of Southern Brazil

Ana Paula Lang Martins Madi[1], Maria Regina Torres Boeger[2] and Carlos Bruno Reissmann[3*]
[1]Programa de Pós-Graduação em Ecologia e Conservação; Setor de Ciências Biológicas; Universidade Federal do Paraná; Curitiba - PR - Brasil. [2]Departamento de Botânica; Setor de Ciências Biológicas; Universidade Federal do Paraná; Curitiba - PR - Brasil. [3]Departamento de Solos e Engenharia Agrícola; Setor de Ciências Agrárias; Universidade Federal do Paraná; Curitiba - PR - Brasil

ABSTRACT

This study assessed the relation between Cu, Fe, Mn, and Zn in the soils of the mangroves of Antonina and Guaratuba, on the coastline of the State of Paraná, and in the leaf tissues of A. shaueriana, R. mangle, *and* L. racemosa *through the analysis of correlation levels between these two compartments. Leaf samples were collected for ten individuals of each species in a 1000 m^2 area delimited in each mangrove. Soil samples from 0-10 cm depth were taken from under the crown projection area of the selected trees to be submitted to chemical analysis. In the soils, metallic micronutrients presented the following order: Fe > Mn > Zn > Cu. In the leaves, concentrations were species dependent. In* A. shaueriana *and* R. mangle, *the profile was Mn > Fe > Zn > Cu, while in* L. racemosa, *the sequence was: Fe > Mn > Zn > Cu. Correlation analyses revealed only four significant correlations for Mn, Zn, and Cu in the soil and plant compartments. These results suggested that significant correlations depended on abiotic factors, inhibition between the elements, and immobilization and/or adsorptions of these metals by the soil.*

Key words: *Avicennia schaueriana, Laguncularia racemosa, Rhizophora mangle,* mangrove, micronutrients

INTRODUCTION

Mangrove environments are influenced by a complex interaction of biotic and abiotic factors, which control the metallic and non-metallic nutrients available to plants species (Reef et al. 2010). Their soil receives and retains metals coming from different sources such as freshwater, saltwater, as well as water runoff (Saenger and McConchie 2004; Kannappan et al. 2012), be they due to natural processes (Kabata-Pendias and Pendias 2001), or anthropogenic activities (Tam and Wong 2000). In terms of ecosystem, metals can be classified either as nutrients (Broadley et al. 2012) or, depending on their density, trace (d < 1 g kg^{-1}) (Broadley et al. 2012), or heavy metals (d > 5g cm^3) (Epstein and Bloom 2004). When they act as micronutrients, heavy metals supply plants metabolic necessities and their lack may impair the whole enzymatic system (Gupta 2001). Nonetheless, since their excess can alter cell membrane permeability, they can inhibit enzyme activity and interfere with photosynthesis (MacFarlane and Burchett 1999). Therefore, plants usually react differently in terms of use, storage and tolerance of metals in their different parts (MacFarlane et al. 2003).

The analysis of metals concentration in different organs of mangrove plants has proved to be a more accurate instrument than mere soil analysis.

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[*] Author for correspondence: reissman@ufpr.br

In fact, soils retain metals in different fractions and are submitted to the hydrodynamic variations typical of the ecosystem (Saenger and McConchie 2004; Cuzzuol and Rocha 2012), while tissues act as bioindicators (MacFarlane et al. 2003). Evidence of low correlation between the metals in the soil and leaf tissues (Bhosale 1979; Peterson et al. 1979; Sadiq and Zaidi 1994; Cuzzuol and Rocha 2012) may indicate a low bioavailability, and/or a high selectivity of the plants (MacFarlane et al. 2003). The analytical procedures do not consistently reveal the complexity of bioavailability, distribution, and assimilation processes of the elements affecting the plants (Parker et al. 2001). Despite this limitation, altogether, these procedures suggest that mangroves act as an efficient biogeochemical barrier to metal transport (Cuzzuol and Rocha 2012).

The dual role of Cu, Fe, Mn, and Zn, which can be either nutrients, or toxic elements in mangrove ecosystems, has been addressed by several studies (MacFarlane et al. 2003; Andrade et al. 2005; Kannappan et al. 2012). They also have been investigated for their physiological functions together with the mechanisms that control their absorption or exclusion in the plants that grow in this ecosystem (Machado et al. 2002; Cuzzuol and Rocha 2012).

Given this context, this study tested the relation between the Cu, Fe, Mn, and Zn content of the soils and leaf tissues of plants from two mangroves located on the Parana coastline. Since both have different forest structure and soil classification (Histosol and Gleysoil), we also analyzed the level of correlation between them.

MATERIAL AND METHODS

Study Area

This study was performed in two mangrove areas located in the cities of Antonina and Guaratuba, State of Parana, Brazil. Antonina, with an area of 460 km^2, is located west of the Paranagua Bay. Guaratuba is situated on the Guaratuba Bay. It represents the second largest estuary ecosystem of the State of Parana coastline, with 48.72 km^2).

The geographical position, edaphic, and climatic characteristics of these two mangrove areas are presented in Table 1. Rainfall and temperature data referred to 2010 and were provided by the Paranagua station of the Sistema Meteorológico do Paraná- Paraná State Meteorological System (Simepar).

Table 1 – Geographic position, climatic and edaphic characteristics from Antonina and Guaratuba mangroves.

	Antonina	**Guaratuba**
Geographic Position	25°29'S 48°42'W	25°50'S 48°34'W
Average Temp. (°C) (Min./Max.)	20.5ºC (16.7ºC/26.4ºC)	20.8ºC (18,0ºC/24,2ºC)
Climate*	Cfa	Cfa
Annual rainfall *	2733 mm	3183 mm
Soil type**	Histosol tiomorphic saline sodic	Gley soil tiomorphic saline sodic
Interstitial water salinity (‰)**	16.3‰	24.4‰
Redox Potential (mV)**	-327.2mV	-316.9mV

Simepar; ** Boeger et al. (2011).

Material Collection

Ten dominant individuals of each species were sampled in a 1000 m^2 area delimited in each mangrove in order to survey the entire narrow wetland strip parallel to the body of water. In Antonina, individuals were marked in the middle region of the Rio Nhundiaquara estuary, and in Guaratuba in the middle region of the Rio dos Pinheiros estuary. Fully expanded mature leaves (Römheld 2012) of *Rhizophora mangle* L., *Avicennia racemosa* (L.) Gaertn and *Avicennia Avicennia* Stapf & Leachman were collected with a trimmer in July 2010 from the middle region of their crown foliage exposed to the north (Reissmann et al. 1999). Sprouting and senescent leaves were excluded. Collected material was washed and dried at 60°C to constant weight and then ground to powder and submitted to nitric-perchloric digestion (Jones and Case 1990). Copper (Cu), Iron (Fe), Manganese (Mn) and Zinc (Zn) contents were determined through Inductively Coupled Argon Plasma Optical Emission Spectrometry (ICP-OES).

In order to determine the correlations between the soil and plants, 0-10 cm soil samples were collected (Nielsen and Andersen 2003) from four points in the crown projection of the selected trees (June 2010), using a 10 cm ϕ PVC tube. Samples were dried in open air before they were crushed and sieved to obtain air dried thin fraction samples (ϕ < 2mm). Analyses to determine Cu, Fe, Mn, and Zn were performed according to the methods of soil analyses manual (Embrapa 1997).

Data Analysis

Univariate analyses were used to verify the leaf response and pedological variables within each mangrove and between them. Differences between the variables were determined through two variance analyses (*one-way* ANOVA), considering the species as a factor and the mangroves successively. Dependent variables were the micronutrients in the leaves and soil. The Fisher LSD test using a 5% significance level was performed following the ANOVA tests. Conditions were tested through the Bartlett test at 5% (homogeneity of the variability) and the Kolmogorov-Smirnov test, also at 5% (gaussianity) (Zar 1999). Pearson correlations verified the relations between the measured variables. All the analyses were performed using Statistica software. When significant correlations were found, ($p<0.05$), regression equations were established.

RESULTS

The chemical analyses of the soil of both the mangroves presented high values of micronutrients content as compared to the standards of chemical fertility in Brazil. As regards micronutrients concentration profile in the soil, a Fe > Mn > Zn > Cu decreasing pattern was found in all the species studied. Soil in Antonina presented higher Mn and Fe contents, statistically differing from that of Guaratuba mangroves for all the species. In the Antonina mangroves, only Mn in the soil under *R. Mangle* differed from the two other species. *A. shaueriana* and *L. racemosa* statistically differed from the soil in Guaratuba for the content of Zn. The content of Cu in the soil under *L. racemosa* was the only to statistically differ between both the mangroves (Table 2).

Leaf concentration presented inter and intraspecific variations. The nutritional profile of the micronutrients in the leaves followed a Mn > Fe > Zn > Cu decreasing pattern in *A. shaueriana* and *R. mangle* in the two studied areas. In *L. racemosa*, Fe and Mn were inverted in the two mangroves: Fe > Mn > Zn > Cu (Table 3). Values of the leaf Cu in the Guaratuba mangrove were approximately two to three times higher than in Antonina. In both mangroves, *L. racemosa* presented higher concentrations of Fe. *R. mangle* showed higher concentrations of Mn (approximately two times as high as that of *A. shaueriana* and up to five times higher than that of *L. racemosa*) and lower levels of Zn and Cu. As in Antonina mangrove, the leaf levels of Mn were different in all the species in Guaratuba.

Table 2 - Soil micronutrients contents under *A. shaueriana*, *L. racemosa* and *R. mangle* trees, from Antonina and Guaratuba mangroves, at 0-10 cm depth.

	Antonina			**Guaratuba**		
	A. schaueriana	*L. racemosa*	*R. mangle*	*A. schaueriana*	*L. racemosa*	*R. mangle*
Fe soil (mg dm^{-3})	319±6.43 aA	320± 4.69aA	310±5.12 aA	265±7.65 aB	261±9.96 aB	268±4.41 aB
Cu soil (mg dm^{-3})	1.6±2.30 aA	0.9± 0.25aB	1,4±0.27 aA	2.0±0.18 aA	1.9±0.25aA	1.8±0.21 aA
Mn soil (mg dm^{-3})	58± 15.04aA	52±9.43aA	40±8.73bA	22±10.55aB	21±8.94aB	29±6.40 aB
Zn soil (mg dm^{-3})	8.9±1.66 aA	8.8±1.81aA	8.7±2.50aA	5.3±2.09 bB	6.1±1.81abB	7.2±2.30aA

Fisher LSD test ($p<0.05$). Values with different lower case letter, in the same line and the same area, are statistically different. Values with different upper case letter, in the same line, between areas for the same species are statistically different. Values following the averages represent the standard deviation.

Table 3 - Average values of leaf metal concentrations of *A. shaueriana*, *L. racemosa* e *R. mangle* from Antonina and Guaratuba mangroves.

	Antonina			**Guaratuba**		
	A. schaueriana	*L. racemosa*	*R. mangle*	*A.schaueriana*	*L. racemosa*	*R. mangle*
Cu(mg kg^{-1})	1.4±0.60aB	0.8±0.35abB	0.7±0.60bB	2.7±0.57aA	2.3±0.48aA	1.5±0.90bA
Fe(mg kg^{-1})	108±22.81bA	330±136.5aA	57±12.4bA	98±15.7bA	179±41.8aB	70±13.9bA
Mn(mg kg^{-1})	138±29.7bA	71±19.71cA	339±38.42aA	185±13.85bA	70±7.89cA	345±73.23aA
Zn(mg kg^{-1})	12.7±2.48aB	14.3±1.95aB	5.1±1.96bA	16.9±2.71aA	16.4±1.19aA	5.7±0.77bA

Fischer LSD test ($p<0.05$) Values with different lower case letter, in the same line and same area, are statistically different. Values with different upper case letter, in the same line, between areas for the same species, are statistically different. Values following the averages represent the standard deviation.

Four significant Pearson correlations ($p< 0.05$) were observed between the studied elements in the plant and soil compartments: Mn soil x Mn plant for *A. avicennia* in Antonina ($r = 0.64$) and in Guaratuba ($r=0.74$), Zn soil x Zn plant ($r= 0.65$) and Cu soil x Cu plant ($r= -0.85$), both for *L. racemosa* in Guaratuba. The relation expressed in regression form dimensioned the behavior between the leaf concentrations and soil contents (Figs. 1A, 2B, 2C, 2D).

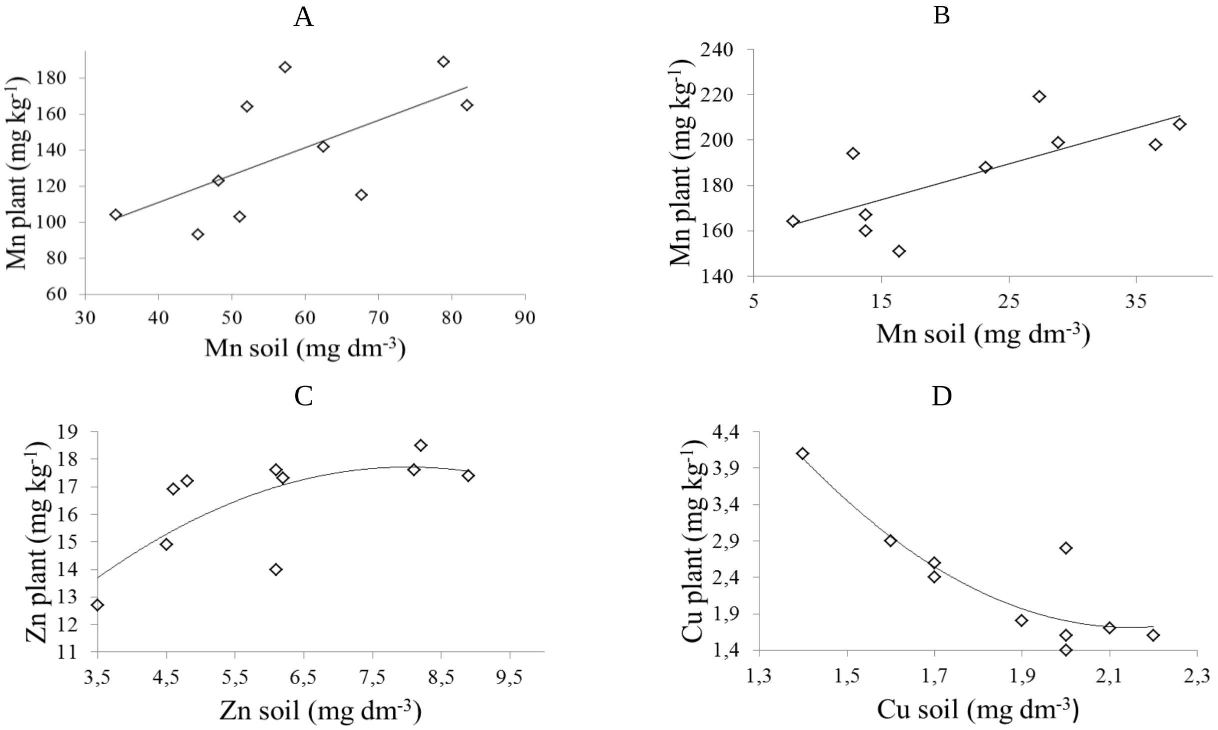

Figure 1 - Regression curves between Mn soil and Mn plant in *A. schaueriana* - Antonina mangrove (A), Mn soil and Mn plant in *A. schaueriana* - Guaratuba mangrove (B), Zn soil and Zn plant in *L. racemosa* - Guaratuba mangrove (C), and Cu soil and Cu plant in *L. racemosa* - Guaratuba mangrove (D). Mn: manganese; Zn: zync; Cu: copper.

DISCUSSION

In the studied areas, the differences in Fe, Mn, Cu, and Zn concentrations in the soils, both between the species and mangroves could be related to temporal (Cuzzuol and Rocha 2012) and spatial variations, which were linked to the chemical and physical characteristics of the soil (Bernini et al. 2006; 2010). Tides variation also interfered on the availability of chemical elements (Lacerda et al. 1986), resulting in a concentration/dilution effect on the nutrients of this ecosystem (Ong Che 1999). In the two studied mangroves, the micronutrient concentrations in the soil were between two (Cu) and up to a hundred times higher (in the case of Mn) than the highest reference concentrations of soil fertility standards (SBCS 2004). In this case, such high values did not seem to represent a negative factor for the plants, since they were minimized by a series of biotic and abiotic factors (Lacerda et al. 1993; Machado et al. 2005; Jiang et al. 2009).

The anoxic conditions of soil promote sulfide formation, which prevents absorption of the elements by the plants (Lacerda et al. 1993). In the soil/roots interface, the oxidation of Fe and Mn forms Fe plaques, creating a further barrier to the absorption of these elements (Lacerda et al. 1993, Machado et al. 2005, Jiang et al. 2009). Fe plaques are originated from the oxidation in the rhizosphere through roots aerenchyma, which diffuses oxygen into the rhizospheric soil (Ong Che 1999). They are composed of a mixture of iron hydroxides (Wang and Peverly 1996). These plaques can also adsorb Zn and Cu, which are eventually desorbed and used by plants (Otte

1989). Since, they depend on the induction processes occurring in the rhizosphere (Hinssinger 1998), these mechanisms do not take place in an even form among the species. In the case of Cu, the strong complexation with organic matter in the soil (Stevenson 1986) and retention in the root cell walls (MacFarlane et al. 2003) represent an ascending inhibition factor preventing this element to reach the plants (Amberger 1988). In anoxic environments, Mn tends to undergo reduction, thus being more available (Barber 1984). Differently from Cu, Mn tends to accumulate more in the aerial parts of the plant than in the roots (Cicad 2004).

The composition of the original material and the micronutrient forms of occurrence in soil has an influence on their availability for plants, and also to the interaction between these plants and soil.

However, soil nutritional profile did not reflect entirely in the three species. Only in *L. racemosa*, there was a correspondence between the leaf profile and soil. Average values of leaf concentrations were consistent with the values described in the literature on Brazilian mangroves (Bernini et al. 2010; Bernini and Rezende 2010; Cuzzuol and Rocha 2012). The variation in the leaf concentrations observed among the studied species (Table 3) was partly due to the salt excluding condition of the *Rhizophora* gender species, or to the salt including condition of the *Avicennia* and *Avicennia* gender species (Lacerda et al. 1985; Bernini et al. 2006). Specifically, the low leaf levels of Fe, Zn and Cu in *R mangle* could be due to the salt exclusion mechanism, which affected the absorption of these elements (Lacerda et al. 1985).

In their study on southeastern Brazilian mangroves, Machado et al. (2005) concluded that *A. Avicennia*, *L. racemosa*, and *R. mangle* developed efficient inhibitory mechanisms against Fe, Mn, and Zn through the formation of Fe plaques. Accordingly, the ascending inhibition of Fe and Zn access to leaves took place within root tissues, while that of Mn was due to the immobilization of Mn in the rhizosphere soil. Higher concentrations of Mn in *R. mangle* and of Fe in *L. racemosa* have also been observed in other Brazilian mangroves (Bernini et al. 2006). In the case of Fe, in particular, beyond the above descriptions, the genetic efficiency of plants in the mechanisms involving morphological and anatomical alterations of the roots is also to be considered (Römheld and Marschner 1986, Mengel and Kirkby 1987). These alterations impact the development of cell structures able to absorb Fe in the reduced form (White 2012).

The soil-plant (leaf) correlations observed for Mn, Zn, and Cu are diverging from other studies (Defew et al. 2005; Bernini et al. 2006). MacFarlane et al. (2003) observed a positive correlation for Zn ($r=0.62$, $p<0.05$) for *A. marina* and Ong Che (1999) showed a positive correlation for Cu ($r=0.89$, $p<0.05$). However, this was observed only between the root tissues and mangrove soil. This controversy was due to various (edaphic and metal speciation) factors interfering in these relations (Ong Che 1999), including ion acquisition mechanisms and root absorption (Hissinger 1998), analytic procedures used for the samples, mainly in the case of Mn (Barber 1984), and the tissues studied (MacFarlane et al. 2003; Defew et al. 2005).

For *A. avicennia*, correlations between Mn in the soil and plants in the two studied mangroves could be attributed to a higher spatial variability of this element within the areas, which generated the concentration gradient observed in the leaves (Tam and Wong 2000). Correlation between the soil and plant Zn for *L. racemosa* in the Guaratuba mangrove could also be attributed to the observed availability of soil Zn, associated to a spatial variability, allowing a proportional absorption by the plants (Tam and Wong 1995; 2000). The negative correlation between the soil and plant Cu, for *L. racemosa* in the Guaratuba mangrove was not due only to the strong retention of Cu in the organic matter and in the roots. The correlation between the soil-plant for Cu has already been reported with low soil Cu concentrations (MacFarlane et al. 2003). Studies on *A. marina* have demonstrated that from a given concentration in soil (200 ppm), Cu accumulated in the roots without transferring to the aerial parts (MacFarlane and Burchett 2002), showing that it was blocked in the roots. The retention strength of Cu in the soil matrix (sediment and/or interstitial water) may depend on the nature of the ligands (Cao et al. 2004), pH and interaction with other ions (Stevenson 1986). In this sense, it has been stated that in seawater, 99% of Cu is complexed to organic ligands (Leal and Van Den Berg 1998).

The regression curves for Mn showed an even growing tendency at 80 and 40 mg g^{-1} levels of the soil Mn in the Antonina and Guaratuba mangroves, respectively (Figs. 1A and 1B). The curve for Zn showed a leaf saturation starting from approximately 7.0 mg g^{-1} of soil Zn (Fig. 1C). The distinct tendencies for Mn and Zn were referred to the inherent characteristics of these elements, interactions of soil with the rhizosphere, and specific characteristics of the plant (Lombi et al. 2001).

Beyond the already known factors affecting Cu, Fe, Mn, and Zn leaf concentration, such as tide variation and interstitial water salinity, the immobilization and/or adsorption of these metals by the soil was very strong in this ecosystem. This contributed to the low number of significant correlations encountered between the nutrients in soil and in the plants. Despite the high correlation observed for Mn and Zn, the studied areas seemed to be very promising to study metallic elements in mangroves. Given the understanding of the soil-plant relations on the low number of significant correlations between the soil and plant compartments, different plant organs must be considered and the analytic methods should be adjusted for this type of environment. Data collected confirmed that, as already reported in other studies, mangrove vegetation acted as a biochemical barrier against the transportation and exportation of metals between the mangrove and the nearby coastal ecosystem.

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