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Microbial processes in the rhizosphere soil of a heavy metals-contaminated Mediterranean salt marsh: A facilitating role of AM fungi

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Abstract

We investigated the relationship of the zonal pattern followed by the vegetation in a polluted Mediterranean salt marsh, in semiarid south-eastern Spain, with the microbiological and biochemical properties (labile C fractions, oxidoreductases and hydrolases) of the rhizosphere soil of two halophyte species, *Arthrocnemum macrostachyum* and *Sarcocornia fruticosa*, and with the degree of arbuscular mycorrhizal (AM) colonisation in their rhizospheres. Levels of plant biomass and cover were inversely related to heavy metal contents and salinity. The concentrations of Fe, Cu, Mn and Pb extracted with DTPA hardly varied among the different zones of the salt marsh. The dehydrogenase and phosphatase activities, the soluble C and water-soluble carbohydrates concentrations and the extent of root colonisation were greater in the salt marsh zones of lower soil salinity and lower metal concentration. Urease and β-glucosidase activities were not detected in the salt marsh. Plant biomass and cover showed positive relationships with mycorrhizal colonisation ($R = 0.773$, $P < 0.001$; $R = 0.874$, $P < 0.001$, respectively). Mycorrhizal colonisation was negatively correlated with the contents of Pb and Zn in plant tissues. This work supports the view that reduced plant uptake of toxic metals, particularly lead, could be involved in the beneficial effects of AM fungi on plant development in Mediterranean salt marshes contaminated with mining wastes.

Keywords: Enzyme activities; Heavy metal; Arbuscular mycorrhizal fungi; Mining waste; Salt marsh

1. Introduction

The salt marshes along the Mar Menor lagoon, one of the largest coastal lagoons on the Mediterranean Sea, tend to be heavily contaminated with Pb, Cu and Zn, as a consequence of mine wastes from the La Unión-Sierra of Cartagena hills in south-eastern Spain (Ramos et al., 2002; Álvarez-Rogel et al., 2004). The situation of the mine tailings, generally in the Sierra just in the headwater of its water streams, means that some materials from the mine tailings can be readily washed away by the torrential rainfalls typical of the Mediterranean semiarid climate. Recent studies have confirmed the presence of important concentrations of heavy metals in some salt marshes of the Mar Menor; Lo Poyo salt marsh is the one most affected (Álvarez-Rogel et al., 2004). The Mar Menor and associated salt marshes are included in the Ramsar Convention on Wetlands.

An excess of heavy metals in soils has a direct toxic effect on plants, but reductions in below-ground microbial diversity and activities governing the biogeochemical cycles of the major plant nutrients are known to occur concomitantly (Konopka et al., 1999). Microbial populations and activities are fundamental for maintaining soil quality, by

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mediating the processes of organic matter turnover and nutrient cycling (Jeffries et al., 2003). Soil microbial activity has been assessed frequently through biological and biochemical parameters such as biomass C and enzyme activities. Enzymes are potential indicators of the extent to which soil disturbance by a given activity may affect the immediate environment (Pascual et al., 2000). Water-soluble C, as a component of the labile C pool, may also be sensitive to perturbation and stress in soil-plant ecosystems (Ghani et al., 2003) and, therefore, it could be used as a sensitive indicator of soil quality. However, there are relatively few studies regarding the use of such properties as indicators of soil quality in salt marshes and their relationship with the capacity of these ecosystems for maintaining the diversity of plant cover.

The arbuscular mycorrhizal (AM) fungi are a major component of the soil microbial biomass and they are associated symbiotically with plant roots (Smith and Read, 1997). Mycorrhizas have been reported in plants growing on heavy metal-contaminated sites (Chaudhry et al., 1999) and metal-tolerant fungal strains have been isolated (Hildebrandt et al., 1999). However, the study of the effect of AM colonisation on plants growing in metal-contaminated substrates yields contradictory results, because this effect might differ with the host, fungal species and ecotype and phytoavailable metal concentration (Liao et al., 2003). Furthermore, no previous assessment of AM status has been made in salt marshes that have been degraded by mining.

Numerous studies have determined relationships between spatio-temporal gradients of salinity and edaphic moisture and plant zonation in salt marshes of semiarid SE Spain (Álvarez-Rogel et al., 2001), but none of them have examined salt marshes contaminated by heavy metals. We hypothesise that the biomass and activity of rhizosphere microbial communities could have a significant effect on patterns of vegetation in a polluted salt marsh soil, because soil microbes respond quickly to stress factors such as heavy metals. The aim of this study was to assess whether the zonal pattern followed by the vegetation in a polluted Mediterranean salt marsh, from semiarid southeastern Spain, is related to the microbiological and biochemical properties (labile C fractions, oxidoreductases and hydrolases) of the rhizosphere soil of two halophyte species, *Arthrocnemum macrostachyum* and *Sarcocornia fruticosa*, and to the ecological role of AM fungi in their rhizospheres.

2. Materials and methods

2.1. Study sites

The study area is located in Lo Poyo salt marsh (210.6 ha), sited in the coastal area of the Mar Menor. The climate is typically Mediterranean and it is characterized by irregular and intense rainfall events and a harsh dry summer period. The salt marsh was a small saline lagoon which was used for salt extraction in former times, but it was abandoned in the first years of the 20th century. The *Rambla del Beal* crosses the zone and flows into the Mar Menor what is determined as the main source of mine wastes going into Lo Poyo salt marsh (the waterbed of this *Rambla* is strongly affected by the wastes in all of its length). As a consequence, the old saline pools and part of the soils were buried under several millions of tons of wastes. Nowadays, most of the soils of the salt marsh can be classified as Spolic Anthropic Regosols (World Reference Base for Soil Resources, 1998). The vegetation is mainly dominated by halophytic plant species, such as *A. macrostachyum* (Moric.) Moris and *S. fruticosa* (L.) A.J. Scott (Álvarez-Rogel et al., 2004) distributed clearly into bands or zones.

The salt marsh is surrounded by agricultural lands, and the beach between the salt marsh and the Mar Menor is used for recreational activities.

2.2. Sampling procedures

One transect of 105-m length perpendicular to the coastline was drawn from the Lo Poyo salt marsh' zone with the least plant cover (zone more contaminated) to the zones with the highest plant cover (zones less contaminated), in an area of 1000 m^2. Four sampling plots (6×6 m^2) were established in the transect according to the plant zonation: plot I, a short form of *A. macrostachyum* growing in disperse patches (10% cover); plot II, mono-specific stand of a short form of *A. macrostachyum* growing in dense stands (85% cover); plot III, mono-specific stand of a high form of *S. fruticosa* growing in dense stands (100%); and plot IV, almost mono-specific stand of a tall form of *A. macrostachyum* growing in dense stands (97% cover). The slope is nearly flat (less than 1%) and only some small depressions appear. In September 2004, five soil samples from each plot were collected randomly (20 soil samples in total). Each sample consisting of four bulked sub-samples (200 cm^3 soil cores) randomly collected at a fixed depth of 0–20 cm in the rhizospheres of individual plants of similar size and external appearance. Soil strongly adhering to roots and collected at 0–4 mm from the root surface was defined as rhizosphere soil. Rhizosphere soil was allowed to dry at room temperature, weighted and passed through a 2-mm sieve. An aliquot was stored at 2 °C for biological and biochemical analysis and another aliquot was used directly for physico-chemical and chemical analysis.

2.3. Soil physical–chemical and chemical analyses

Soil pH and electrical conductivity were measured in a 1:5 (w/v) aqueous solution. In soil aqueous extracts, water-soluble carbon was determined with an automatic Carbon Analyser for liquid samples (Shimadzu TOC-5050A). Water-soluble carbohydrates were determined by the method of Brink et al. (1960). Total organic carbon was measured by pretreatment with (1:1) HCl to remove

carbonates, followed by combustion at 1020 °C in a Carlo Erba NA 1500 analyser. Particle size distribution was determined using the pipette method after oxidation of the organic matter with H_2O_2 and stirring in a sodium hexametaphosphate solution.

In each sampling plot, the soil redox potential was measured ($n = 3$) with a portable Eh/pH meter at the sampling time when the soil was moist enough. Measures of Eh were corrected (Vepraskas and Faulkner, 2001) by adding 200 mV to the field voltage (value of the standard Ag/AgCl reference electrode).

Total metal contents were determined by nitric–perchloric digestion: 1 g of crushed sample was placed in a Kjeldahl flask, and 10 ml of concentrated HNO_3 plus 10 ml of concentrated $HClO_4$ were added. The mixture was heated at 210 °C for 90 min. When cool, the content of the tubes was filtered through an Albert® 145 ashless filter paper, and the volume completed at 50 ml by washing the Kjeldahl flasks with HCl 0.5 N several times and filtering. DTPA-extractable Fe, Cu, Mn, Zn and Pb were obtained with a 1:5 soil:DTPA relation, as indicated by Soon and Abboud (1993) for metal-contaminated soils. After shaking the suspension for 2 h, it was immediately filtered through Albert® 145 ashless filter paper, diluted when necessary and Fe, Cu, Mn, Zn and Pb measured. All the measures of metal contents were made with an UNICAM 969 Atomic Absorption Spectrometer.

2.4. Soil biochemical analyses

Dehydrogenase activity was determined according to García et al. (1997). For this, 1 g of soil at 60% of its field capacity was exposed to 0.2 ml of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22 °C in the dark. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtering through Whatman No. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.

Urease and *N*-α-benzoyl-L-argininamide (BAA) hydrolyzing protease activities were determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M BAA were used as substrates, respectively. Two milliliters of buffer and 0.5 ml of substrate were added to 0.5 g of sample, which was incubated at 30 °C (for urease) or 39 °C (for protease) for 90 min. Both activities were measured using colorimetric determination of the NH_4^+ released in the hydrolysis reaction (Kandeler and Gerber, 1988).

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. Two milliliters of 0.5 M sodium acetate buffer at pH 5.5 using acetic acid (Naseby and Lynch, 1997) and 0.5 ml of substrate were added to 0.5 g of soil and incubated at 37 °C for 90 min. The reaction was stopped by cooling at 2 °C for 15 min. Then, 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added, and the mixture was centrifuged at 4000 rpm for 5 min. The *p*-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm. Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.

β-Glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrate. This assay is based on the release and detection of PNP. Two milliliters of 0.1 M maleate buffer pH 6.5 and 0.5 ml of substrate was added to 0.5 g of sample and incubated at 37 °C for 90 min. The reaction was stopped with tris-hydroxymethyl aminomethano (THAM) according to Tabatabai (1994). The amount of PNP was determined by spectrophotometry at 398 nm.

2.5. Plant sampling and analyses

Three 0.25 m × 0.25 m sub-plots were sited at random in each of the sampling plots, and all the above-ground biomass was cut with clippers at the surface level. Shoots were carefully washed and weighted in fresh. The plant tissue was dried at 105 °C for 5 h and ground before chemical analysis. Sub-samples were oven-dried at 480 °C, then the ashes were dissolved in concentrated nitric acid and analyzed for Zn and Pb with an UNICAM 969 Atomic Absorption Spectrometer and for P by colorimetry (Murphy and Riley, 1962).

Roots were sub-sampled in three 2-cm cross-sections of the upper, middle, and lower root system. To assess colonisation, roots were cleared with 10% KOH and stained with 0.05% trypan blue (Phillips and Hayman, 1970). The percentage of root length colonized by AM fungi was calculated by the gridline intersect method (Giovannetti and Mosse, 1980). Positive counts for AM colonisation included the presence of vesicles or arbuscules or typical mycelium within the roots.

2.6. Statistical analysis

Data were log transformed to achieve for normality. All data were subjected to an one-way analysis of variance and comparisons among means were made using the least significant difference (LSD) test calculated at $P < 0.05$. Pearson's correlation coefficients between all the soil parameters were assessed. Statistical procedures were carried out with the software package SPSS 10.0 for Windows.

3. Results and discussion

3.1. Physical–chemical parameters and metals in soil

The total metal contents in the soils decreased from plot I to plot IV (Table 1). In general, there was a good correlation between the different total metal contents, indicating a common origin for Fe, Cu, Zn and Pb ($P < 0.001$). The variability and the relations among the metals can be due to several reasons. One factor could be the existence of different materials deposited in the tailings of the mines from which the wastes were transported, and the existence

Table 1
Changes in soil total metals contents (in g kg^{-1} soil) along a spatial vegetation gradient in the Lo Poyo salt marsh contaminated by mine wastes

Plots	Fe	Cu	Mn	Zn	Pb
I	176.6c	0.16b	1.99a	6.55c	10.7b
II	107.0b	0.13b	4.48b	14.51d	9.6b
III	78.9a	0.05a	1.99a	2.31b	5.0a
IV	74.9a	0.04a	1.53a	1.25a	2.8a

Values in columns followed by the same letter are not significant difference at the $P < 0.05$ probability level.

Table 3
Changes in soil physical–chemical properties along a spatial vegetation gradient in the Lo Poyo salt marsh contaminated by mine wastes

Plots	pH (H_2O)	EC (dS m^{-1})	2–50 μm (%)	50 μm–2 mm (%)	Eh (mV)	TOC (%)
I	6.86a	3.49d	80.4c	19.6a	221.9a	0.5a
II	8.15b	3.04c	70.1b	29.9b	228.7a	0.9b
III	8.15b	2.55b	67.9b	32.1bc	220.4a	1.2bc
IV	8.10b	2.26a	62.4a	37.6c	177.0a	1.3c

EC: electrical conductivity; TOC: total organic C.
Values in columns followed by the same letter are not significant difference at the $P < 0.05$ probability level.

of different events of deposition of these wastes. However, we cannot reject the possibility that, after deposition of the wastes, other factors, such as plant and microbial activity, induced metal mobility (Khan et al., 2000) or that flooding (Otero et al., 2000) influenced metal distribution.

The reference values proposed by the Environment Ministry of Spain for Pb in soils for housing estates (300 mg kg^{-1}) and for industrial use (1000 mg kg^{-1}) were surpassed. The level of Zn above which the soil can be considered as polluted in non-industrial zones according to the Environment Ministry of Spain (450 mg kg^{-1}) was also surpassed.

Except for DTPA-Zn, the values of metals extracted with DTPA hardly varied among the different zones of the salt marsh (Table 2). This could be due to the fact that the quantity of metals extracted with DTPA can be influenced by several factors, such as organic matter, content of oxides and hydroxides of metals, pH, and others (Sims and Johnson, 1991). The quantities of Pb extracted with DTPA were still higher than the values proposed by the Environment Ministry of Spain for housing estates.

The lowest pH value of the soil was observed in plot I, corresponding to the zone with the least plant cover (most-contaminated zone) (Table 3). The increase in pH of soil in plot II and successive plots with respect to plot I can reduce metal mobility and decrease its plant uptake in the study zone. This agrees with the decrease in the concentrations of Zn extracted with DTPA found in the plots III and IV. The electrical conductivity decreased from plot I (3.49 dS m^{-1}) to plot IV (2.26 dS m^{-1}). It can be expected that the zone with the highest electrical conductivity value would have a reduced biological activity, resulting from the greater salt content (García et al., 1997). There were no significant differences in soil redox potential along the transect.

The texture of soil in the plots is silty (Table 3). The absence of the clay fraction (<2 μm) suggests that the heavy metals are not interacting with the mineral fraction of soil. In the zone most affected by the polluted sediments (plot I) the content in particles <50 μm was more than 70%. This result was excepted due to the fact that these wastes were originated after grinding and differential floating in the ore processing.

As it was expected, the soil total organic C increased with plant cover (Table 3). The highest TOC concentrations were found in the soil of the plots III and IV. The main input of organic matter to soil is plant litter, decaying aerial parts and roots and rhizomes. Decomposing plant remains may provide humified organic matter to which metals readily bind, influencing the solubility and mobility of these metals. However, the high soil moisture and the high temperatures of the zone favour the rapid mineralization of plant litter and limit the formation of humic substances (Bedford, 2005). In this case, the contribution of soil organic matter to retention of metals by means of metal-humic complexes could be insignificant.

Table 2
Changes in soil metals contents (in mg kg^{-1} soil) extractable with DTPA along a spatial vegetation gradient in the Lo Poyo salt marsh contaminated by mine wastes

Plots	Fe	Cu	Mn	Zn	Pb
I	10.3a	7.8a	8.6a	599.9c	864.9a
II	8.0a	9.5a	10.2a	1082.8d	847.5a
III	9.4a	6.8a	9.3a	223.8b	894.5a
IV	9.6a	7.0a	8.0a	136.2a	770.6a

Values in columns followed by the same letter are not significant difference at the $P < 0.05$ probability level.

3.2. Mycorrhizal infection, soil microbial properties and biomass of both halophytes

Plant biomass increased from plot I to plot IV (Table 4), and was correlated positively with the plant cover of the salt marsh ($R = 0.661$, $P < 0.01$). Increased heavy metals and salt contents affected negatively the biomass and cover of the plant species in the salt marsh (Table 5). It is worth noting that the biomass and cover of the plant species showed positive relationships with mycorrhizal colonisation. Alleviation of heavy metal phytotoxicity by AM fungi has been indicated in several studies (Chen et al., 2003). The AM fungi may act indirectly, for example by enhancing plant P nutrition and increasing plant growth with a resulting dilution effect of the metal in the host plant, or directly, by binding of the metal to the fungal mycelium and immobilisation in the rhizosphere or the roots (Chen et al., 2001). In some experiments, colonisation both reduced host tissue metal concentration and increased biomass production (Chen et al., 2003), but, in others, host

Table 4
Plant biomass, mycorrhizal colonisation, foliar phosphorus and metals and their biological absorption coefficient (BAC, the ratio of metal concentration in shoot to the metal concentration in soil) of *A. macrostachyum* and *S. fruticosa* in the Lo Poyo salt marsh contaminated by mine wastes

Plots	Shoot (g dw)	Colonisation (%)	Foliar P (g kg^{-1})	Foliar Zn (mg kg^{-1})	Foliar Pb (mg kg^{-1})	BAC Zn (mg kg^{-1} plant)/(mg kg^{-1} soil)	BAC Pb (mg kg^{-1} plant)/(mg kg^{-1} soil)
I	2069.8a	2.4a	7.78a	419.1b	248.5b	0.064	0.023
II	1609.1a	9.0b	7.87a	258.1b	138.4b	0.018	0.014
III	5808.6b	21.7c	8.72ab	84.2a	25.8a	0.036	0.005
IV	7541.1b	18.8c	12.02b	60.5a	16.4a	0.048	0.006

Values in columns followed by the same letter are not significant difference at the $P < 0.05$ probability level.

Table 5
Correlation coefficients between chemical, biochemical and growth parameters and mycorrhizal colonisation ($n = 20$)

	Colonisation	Shoot	Plant cover
Total Fe	−0.886***	−0.674***	−0.926***
Total Cu	−0.802***	−0.771***	−0.731***
Total Zn	−0.671***	−0.827***	−0.511*
Total Pb	−0.725***	−0.703***	−0.651**
Foliar P	NS	0.465*	NS
Foliar Zn	−0.697***	−0.859***	−0.775***
Foliar Pb	−0.843***	−0.857***	−0.784***
DTPA Cu	NS	0.686***	NS
DTPA Mn	NS	0.692***	NS
DTPA Zn	−0.620**	−0.837***	−0.511*
DTPA Pb	NS	0.666***	NS
Dehydrogenase	0.636**	0.471*	0.711***
Protease	NS	NS	0.728***
Phosphatase	NS	NS	NS
Water-soluble C	NS	0.510*	NS
Water-soluble CH	0.463*	NS	NS
TOC	0.763***	0.695***	0.816***
pH	0.646**	NS	0.857***
EC	−0.728***	−0.820***	−0.782***
Eh	NS	NS	NS
Colonisation	–	0.773***	0.874***

*, **, ***: Significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. NS: not significant. TOC: total organic C.

growth was enhanced without any apparent reduction in metal uptake (Davies et al., 2001) or host metal concentrations were reduced without any detectable growth benefits (Li and Christie, 2000). The percentage of root length colonised by AM fungi decreased significantly with increasing concentrations of total heavy metals and of Zn extracted with DTPA (Tables 1, 2 and 4). Various heavy metals are fungitoxic, reducing spore germination, mycelial growth and, consequently, mycorrhizal colonisation. An excess of Zn and Cu inhibits spore germination, while colonisation can be reduced in the presence of high levels of Zn, Cu, Ni and Cd (Silva et al., 2005). The inhibition of AM colonisation observed in this study could suggest that these fungi did not contribute to host metal tolerance in the salt marsh. However, mycorrhizal colonisation was correlated negatively with the contents of Pb and Zn in plant tissues. This would be justified in the case of Zn, because the concentration of Zn extracted with DTPA, and available for the plants, decreased along the transect as did AM colonisation. In contrast, the concentration of Pb extracted with DTPA did not vary among the different plots of the transect. These results could indicate that, in the salt-marsh zone with higher colonisation, the fungi might reduce the plant uptake of this toxic metal, which would increase biomass production.

Arbuscular mycorrhizal fungi may improve plant tolerance and growth in sites affected by salinity, by enhancing the supply of nutrients, such as P, and increasing photosynthetic activity and water-use efficiency (Ruíz-Lozano et al., 1996). However, the mycorrhizal colonisation was not related to the concentration of foliar P of the plants grown in this salt marsh. The percentage colonisation by AM fungi showed negative relationships with electrical conductivity (Table 5). The reduced colonisation of both halophytes in the salt-marsh zones with higher concentrations of salt seems to indicate that AM fungi do not contribute greatly to the ecological adaptation of these halophytes to the zones of the salt-marsh environment studied. We also found that AM colonisation of *Inula crithmoides* L. decreased with salinity in an uncontaminated Mediterranean salt marsh (Caravaca et al., 2005).

The interaction of arbuscular mycorrhizal fungi with other rhizosphere microorganisms can affect rhizodeposition and thus the quantity and quality of labile organic C delivered to the soil via fungal hyphae (Marschner et al., 1997). The labile organic matter fraction consists of a heterogeneous mixture of components of varying molecular weight, such as mono- and polysaccharides, polyphenols, proteins and low-molecular-weight organic acids. This fraction can be used as carbon and energy sources by soil microflora and may also have a structural function (Roldán et al., 1994). The study of these fractions is important since they determine soil microbial activity (Ghani et al., 2003). The soil under *A. macrostachyum* showed the highest soluble C and water-soluble carbohydrates values in the salt-marsh zone with the highest plant cover and highest mycorrhizal colonisation of this species (plot IV) (Table 6). A more direct measurement of microbial activity is the dehydrogenase activity, which expresses the biological oxidation processes of soil microorganisms (Nannipieri, 1994). As a respiratory measurement, dehydrogenase activity should be strongly representative of the size and activity of the viable microbial community. In fact, dehydrogenase has been proposed as a measure of global microbial activity in soil under aerobic conditions

Table 6
Changes in soil labile C fractions and biochemical properties along a spatial vegetation gradient in the Lo Poyo salt marsh contaminated by mine wastes

Plots	Water-soluble C (μg g^{-1})	Water-soluble CH (μg g^{-1})	Dehydrogenase (μg INTF 20 h^{-1} g^{-1} soil)	Protease (μmol NH_3 g^{-1} h^{-1})	Phosphatase (μmol PNP g^{-1} h^{-1})
I	88a	15ab	0.022a	0.13a	2.89a
II	85a	13a	0.036b	0.82c	2.73a
III	89a	18ab	0.036b	0.43b	2.85a
IV	115b	19b	0.041b	0.56bc	3.97b

CH = carbohydrates.
Values in columns followed by the same letter are not significant difference at the $P < 0.05$ probability level.

(García et al., 1997). This enzyme is affected by numerous environmental factors, such as soil redox potential and oxygen level (Alef and Nannipieri, 1995). The highest values of dehydrogenase activity were recorded for the salt-marsh zones with *A. macrostachyum* and *S. fruticosa* growing in dense stands (plots II–IV) (Table 6). The decreased dehydrogenase activity in the marsh zone with higher salt and metals concentrations could indicate that the salinity (mainly plot I) and metal contamination inhibit soil microbial activity. In fact, soil dehydrogenase activity was negatively correlated with soil electrical conductivity ($R = -0.597$, $P < 0.01$) and with the concentrations of Fe ($R = -0.733$, $P < 0.001$), Cu ($R = -0.499$, $P < 0.05$), Pb ($R = -0.575$, $P < 0.01$) and Zn ($R = -0.445$, $P < 0.05$) extracted with DTPA. Likewise, the plot with the highest metal contamination had significantly less plant biomass and, therefore, the lack of organic matter input could have reduced the microbial populations and activities. The low level of mycorrhizal colonisation of the plants grown in the plot I could also have promoted a less microbial activity in the soil. In fact, the percentage of root colonised by AM fungi was related positively ($p < 0.01$) with soil dehydrogenase activity (Table 5).

The changes in microbiological activity along the spatial vegetation gradient were also revealed by the variations in protease-BAA, phosphatase, urease and β-glucosidase activities. The measurement of these hydrolase activities can provide an early indication of changes in soil fertility, since they are related to the mineralization of such important nutrient elements as N, P and C (Ceccanti and Garcia, 1994). Protease activity is involved in the hydrolysis of N compounds to NH_4^+, using low-molecular-weight protein substrates. The values of protease-BAA were significantly less in plot I, i.e., in the rhizosphere soil of plants growing in the most-contaminated zone of the salt marsh (Table 6). This was not necessarily a direct effect of metal toxicity because there were no significant correlations between protease activity and concentrations of toxic metals. The decline in protease activity found is probably an effect of a decreased synthesis of this enzyme associated with inhibited microbial growth rather than direct enzyme inhibition by the metals. As for dehydrogenase activity, N-cycle activity was inversely related to salinity ($R = -0.523$, $P < 0.01$). The high protease activity in plots II–IV may be ascribed to the root exudation. Root exudates can be a source of easily-degradable N-compounds, such as amino acids and small peptides, able to induce protease synthesis (García-Gil et al., 2004).

Phosphatases are enzymes with relatively broad specificity, capable of hydrolysing various organic and inorganic phosphate esters, and are involved in the P cycle. Similar to protease-BAA activity, the highest values of phosphatase were in the salt-marsh zone with less salt content and less metal contamination (Table 6). The phosphatase activity was negatively correlated with the concentration of Zn in soil ($R = -0.457$, $P < 0.05$) and the concentration of Mn extracted with DTPA ($R = -0.535$, $P = 0.05$). Deng and Tabatabai (1995) demonstrated that metal ions may inhibit enzyme reactions by complexing with the substrate, by reacting with the protein-active groups of enzymes or by reacting with the enzyme-substrate complex. Phosphatase activity in the rhizosphere may be secreted predominantly by plant roots, associated mycorrhizae and other fungi (Tarafdar and Marschner, 1994). However, the phosphatase activity was not correlated with the colonisation by AM fungi (Table 5).

Urease and β-glucosidase activities were not detected in the rhizosphere soil of the halophytes in the salt marsh. Urease is responsible for breaking down urea into ammonium and β-glucosidase catalyses the hydrolysis of the ends unreduced chains of β-D-glucoside to form β-D-glucose, and indicates the potential for soil organic matter decomposition. Urease and β-glucosidase appear to be more sensitive to heavy metal pollution than protease and phosphatase activities. The data suggest that a heavy metal-contaminated salt marsh loses very common biochemical properties which are necessary for the functioning of the ecosystem. Kandeler et al. (1996) demonstrated that microbial biomass and enzyme activities decreased with increasing heavy metal pollution, under laboratory conditions, but the amount of decrease differed among the enzymes. They also observed that heavy metal pollution severely decreased the functional diversity of the soil microbial community.

Based on the results obtained, it can be concluded that the soil salinity and heavy metal contents negatively affected the degree of colonisation by AM fungi and some parameters indicating soil microbial activity, such as dehydrogenase activity and some hydrolases (urease, protease,

phosphatase, and β-glucosidase), which, in turn, largely determined the plant distribution in the salt marsh. Some evidence from this study suggests that the reduction in plant uptake of toxic metals, particularly lead, could be partially related to the presence of AM colonisation in salt marsh species, although this beneficial effect can be suppressed by high soil salinity.

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