UNIVERSIDAD POLITÉCNICA DE CARTAGENA
DEPARTAMENTO DE CIENCIA Y TECNOLOGÍA AGRARIA

Dynamics of nitrogen, phosphorus and metals in eutrophic wetlands affected by mine wastes. Effects of liming on plant growth and metals mobility

María Nazaret González Alcaraz

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Que la Tesis Doctoral titulada, "Dynamics of nitrogen, phosphorus and metals in eutrophic wetlands affected by mine wastes. Effects of liming on plant growth and metals mobility", ha sido realizada por Dª. María Nazaret González Alcaraz, bajo la dirección y supervisión del Dr. José Álvarez Rogel y del Dr. Héctor Miguel Conesa Alcaraz, dando su conformidad a la misma la Comisión Académica, con la finalidad de que sea presentada ante la Comisión de Doctorado.

La Rama de conocimiento por la que esta tesis ha sido desarrollada es:

☑ Ciencias
☐ Ciencias Sociales y Jurídicas
☐ Ingeniería y Arquitectura

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Fdo.: [redacted]

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D. José Álvarez Rogel y D. Héctor Miguel Conesa Alcaraz, Directores de la Tesis Doctoral "Dynamics of nitrogen, phosphorus and metals in eutrophic wetlands affected by mine wastes. Effects of liming on plant growth and metals mobility".

INFORMA:

Que la referida Tesis Doctoral, ha sido realizada por Dª. María Nazaret González Alcaraz, dando mi conformidad para que sea presentada ante la Comisión de Doctorado.

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LOS DIRECTORES DE LA TESIS

Fdo.: José Álvarez Rogel [redacted]

Héctor Miguel Conesa Alcaraz [redacted]

COMISIÓN DE DOCTORADO

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Summary

The Mar Menor lagoon (SE Spain) is one of the largest coastal lagoons of the Mediterranean basin (135 km^2 surface). The lagoon and its associated wetlands are affected by eutrophic water of agricultural and urban origin, with high loads of nitrogen and phosphorus, and by metal mine wastes carried by the surface watercourses coming from the former mining area of Sierra de Cartagena-La Unión.

The objectives of this work were: 1) To study the biogeochemical processes related to the dynamics of nitrogen and phosphorus in the soil-water-plant system of eutrophic wetlands polluted by metal mine wastes, in order to identify their possible role as sinks or sources of both nutrients, and 2) To assay the effect of liming, presence of plants, and hydric/flooding conditions on metals mobility in the soil-water-plant system of eutrophic wetlands polluted by metal mine wastes, in order to identify the advantages and drawbacks of these conventional remediation treatments.

To achieve these objectives, three experimental studies were performed under greenhouse conditions. In the experiments, soils polluted by metal mine wastes from the Marina del Carmolí and the Lo Poyo salt marshes, and plant species typical of these environments (*Sarcocornia fruticosa* and *Phragmites australis*) were used. In addition, eutrophic water from local surface watercourses and eutrophic water synthetically prepared were employed.

In the first experiment, loam soils from the Marina del Carmolí (pH~7.8) and sandy-loam soils from the Lo Poyo salt marsh (pH~6.2) were used. Three pot treatments were assayed: *S. fruticosa*, *P. australis*, and bare soil. The pots were flooded for 15 weeks with eutrophic water, and pH, Eh, and the concentrations of NO_3^-, PO_4^{3-}, and water-soluble organic carbon in the soil solution were regularly monitored. Before and after the flooding period, a soil P-fractionation was performed.

- In relation to N dynamics, the NO_3^- concentrations in the soil solution decreased between 70 and 90% by the second day of flooding, except in the unvegetated pots with the soil of pH~6.2. Denitrification was the main mechanism associated to the removal of NO_3^-. The role of vegetation in improving the rhizospheric environment was relevant in the soil of pH~6.2 because higher sand content,

lower pH, and higher soluble metal concentrations might strongly hinder microbial activity.

- In relation to P dynamics, the PO_4^{3-} concentrations in the soil solution decreased between 80 and 90% after three hours of flooding, with and without vegetation. The Fe/Mn/Al oxides and the Ca/Mg compounds played an important role in soil P retention. In the pots with *S. fruticosa*, the reductive conditions induced P release from metal oxides and P retention to Ca/Mg compounds. In turn, *P. australis* may have favoured the release of P from carbonates, which was transferred to Fe/Mn/Al compounds.

In the second experiment, soils with fine texture from the Marina del Carmolí (in this case of pH~6.4) and sandy soils from the Lo Poyo salt marsh (in this case of pH~3.1) were used. Each type of polluted soil was mixed with a lime amendment (dose of 20 g kg^{-1}), assaying two treatments: non-limed and limed soil. Cuttings of *S. fruticosa* were planted in pots prepared with the soil treatments. The pots were irrigated for 10 months with eutrophic water and soluble metal concentrations (Al, Cd, Mn, Pb, and Zn) and plant survival, plant biomass, and plant metal content were determined. The lime amendment decreased the concentrations of soluble metals and favoured the growth of *S. fruticosa*, enhancing the capacity of the plant to phytostabilise metals in roots.

In the third experiment, soils with fine texture from the Marina del Carmolí (in this case of pH~6.4) and sandy soils from the Lo Poyo salt marsh (in this case of pH~3.1) were used. Each type of polluted soil was mixed with a lime amendment (dose of 20 g kg^{-1}). Simulated soil profiles (60 cm depth) were constructed and four treatments were assayed: without liming + without plant, without liming + with plant, with liming + without plant, and with liming + with plant. The plant species employed was *S. fruticosa*. Three horizons were differentiated in the soil profiles: A (never under water), C1 (alternating flooding-drying conditions), and C2 (always under water). The pH, Eh, and soluble metal concentrations (Cd, Cu, Fe, Mn, Pb, and Zn) were measured regularly at each depth for 18 weeks. At the end of the experiment a soil metal-fractionation was applied. The lime amendment favoured the growth of *S. fruticosa*, an increase in pH, and a drop in Eh.

- In relation to Fe dynamics, liming decreased Fe solubility, mainly in the soil of pH~3.1, but also facilitated a drop in Eh, favouring the dissolution of amorphous

Fe oxides and hence increasing the concentration of soluble Fe. The plant rhizosphere contributed to the decrease of Eh and actively re-distributed the dissolved Fe along the soil profiles.

- In relation to Cd, Cu, Mn, Pb, and Zn dynamics in the soil of pH~6.4, the lime amendment was effective for immobilising Mn, Zn, and Cd, but not Cu and Pb. The growth of *S. fruticosa* counteracted the effect of liming, strongly increasing the concentrations of soluble metals and distributing them through the soil profiles.
- In relation to Cd, Cu, Mn, Pb, and Zn dynamics in the soil of pH~3.1, liming was effective for immobilising Zn, Cu, and Pb, but not Mn and Cd. The amendment increased the contents of Zn, Mn, and Cd bound to potentially-mobilisable soil fractions at the expense of the most-environmentally-inert fractions.

In conclusion, the choice of the most appropriate phytomanagement techniques for recovering eutrophic wetlands polluted by metal mine wastes depends on the particular characteristics of the soil-water-plant system, the hydric/flooding regime, and the type of pollutant (nitrogen, phosphorus, and metals).

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Chapter 1

Introduction

1.1. Definition and role of wetlands

There are many definitions of wetlands. The Ramsar Convention (1971) defines wetlands as *areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceeded six meters*. Wetlands are often located in the transition lands between terrestrial and aquatic systems, where the water table is usually at or near the surface or the land is covered by shallow water (Cowardin et al., 1979) at a frequency and duration sufficient to support the prevalence of vegetation typically adapted to saturated soil conditions (US EPA, 2010). Reddy and DeLaune (2008) provide a broader definition of wetlands: *biologically active soil or sediment in which the content of water in or the overlying floodwater is great enough to inhibit oxygen diffusion into the soil/sediment and stimulate anaerobic (oxygen-free) biogeochemical processes and support hydrophytic vegetation*. Definitely, three major components constitute a wetland:

- Hydrology (presence of water at or near the surface for a period of time).
- Hydrophytic vegetation (wetland plants adapted to saturated soil conditions).
- Hydric soils (saturated soil conditions with temporary/permanent anaerobiosis).

Wetlands support many environmental functions and values for humans (Ramsar, 2001; US EPA, 2001):

- Flood control
- Coastal protection
- Groundwater recharge
- Sediment trap
- Water quality improvement
- Climate change mitigation
- Biogeochemical cycling
- Sinks for pollutants
- Biological productivity
- Reservoirs of biodiversity
- Fish and wildlife habitat
- Nursery areas
- Recreation, aesthetics, education
- Cultural value
- Source of products for humans

Wetland systems can be very diverse, with high internal spatial and temporal heterogeneity with respect to vegetation, soils, and hydrology. The latter hinders, in many cases, the process of delineating the jurisdictional/legal boundaries of wetlands and of deciding what wetlands are subject to what policies. Thus, regulatory definitions not always coincide with scientific definitions, and national and regional governments develop specific definitions of wetlands or adapt general definitions for their objectives.

The government of Spain, under the Real Decreto 606/2003 of May 23, modifying the Real Decreto 849/1986 of April 11, includes in the definition of wetlands: 1) marsh, peatland, or shallow waters, whether permanent or temporary, with water that is static or flowing, fresh, brackish, or salt, natural or artificial and 2) margins of these shallow waters and adjacent lands, when necessary to prevent the damage to fauna and flora.

1.2. Wetland soils

Depending on their hydrology, wetland soils can be characterized as it follows (Reddy and DeLaune, 2008): 1) flooded with defined water depth above soil surface, 2) saturated soil conditions with no excess of floodwater, and 3) water table below the soil surface at a certain depth depending on soil characteristics. Under the first two conditions, wetland soils can be classified as hydric soils, whereas the third one mimics the characteristics of both wetland and upland soils, depending on soil type and hydrology. Mausbach and Blake-Parker (2001) define hydric soil as *a soil that is formed under conditions of saturation, flooding, or ponding long enough during the growing season to develop anaerobic conditions in the upper part.*

In upland soils, the oxidised forms of chemical species dominate the system, while in wetland soils the reduced forms are more abundant (Reddy and DeLaune, 2008). During flooding or in the absence of oxygen, the oxidised forms are converted into reduced forms through microbial mediated processes. Redox potential (Eh) reflects the intensity of reduction (gain of electrons) (Eq. 1) or oxidation (loss of electrons) (Eq. 2) and is determined by the concentration of oxidants (electron acceptors) and reductants (electron donors).

$\text{Oxidant} + e^- \rightarrow \text{Reductant}$ (Eq. 1) (Reddy and DeLaune, 2008)

$\text{Reductant} \rightarrow \text{Oxidant} + e^-$ (Eq. 2)

Organic matter is the major source of electrons. When it is oxidised, the electrons released are used for reducing reactions. Oxygen is the most preferred electron acceptor for microorganisms. Once the oxygen is depleted in the soil, specialized microorganisms have the capacity to use other electron acceptors, derived from both organic and inorganic sources, for respiration. The sequence of acceptors is NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-}, and CO_2 (Ponnamperuma, 1972) and is dependent on the electron affinity of the electron acceptor and the energy yield:

$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$ (Eq. 3) (Vepraskas and Faulkner, 2001)

$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O$ (Eq. 4)

$MnO_2 + 2e^- + 4H^+ \rightarrow Mn^{2+} + H_2O$ (Eq. 5)

$Fe(OH)_3 + e^- + 3H^+ \rightarrow Fe^{2+} + 3H_2O$ (Eq. 6)

$SO_4^{2-} + 8e^- + 10H^+ \rightarrow H_2S + 4H_2O$ (Eq. 7)

$CO_2 + 8e^- + 8H^+ \rightarrow CH_4 + 2H_2O$ (Eq. 8)

According to Vepraskas and Faulkner (2001) and Otero and Macías (2003), at neutral pH, soils can have three different states in relation to Eh: 1) oxic (Eh > +300 mV, where O_2 is reduced), 2) suboxic (+300 mV > Eh > +100 mV, which corresponds to the reduction of NO_3^-, Mn^{4+}, and Fe^{3+}), and 3) anoxic (Eh < +100 mV, which corresponds to the reduction of SO_4^{2-} and CO_2).

Saturated soil conditions not always lead to anaerobiosis. The factors that may hinder anaerobiosis are the following (Vepraskas and Faulkner, 2001): 1) low temperatures that prevent microorganism's activity, 2) absence of organic matter, 3) water movement that allows soil oxygenation, and 4) elevated pH that displaces oxidation-reduction reactions towards oxidation. Hence, the following conditions are needed for a soil to become anaerobic and to support the reducing reactions (Vepraskas and Faulkner, 2001):

- The soil must be saturated or inundated to exclude O_2.
- The soil must contain organic matter that can be oxidised or decomposed.
- A microbial community must be respiring and oxidizing the organic matter.
- The water must be stagnant or moving very low.

Redox reactions produce characteristic signs in the soil called redoximorphic features (Table 1.1). These indicators of hydric soils are formed, predominantly, by the accumulation or loss of Fe, Mn, S, or C compounds and include specific colour patterns, odours, colour changes that occur on exposure to air, or specific kind of organic material (Vepraskas, 2001).

Table 1.1. Redoximorphic features and related soil processes (modified from FAO, 2006).

Redoximorphic features	Processes
No redoximorphic characteristics	Strongly aerated
Black Mn concretions	Mn^{2+} formation
Reddish Fe mottles and/or brown concretions	Fe^{2+} formation
Blue-green to grey colours, Fe^{2+} always present	Formation of Fe^{2+}/Fe^{3+} oxides (green rust)*
Black colours due to metal sulphides, flammable methane present	Sulphide formation, methane formation

* green rust: Fe-mix compounds

1.3. Wetland vegetation

Cronk and Fennesy (2001) define wetland plants as *those species that are normally found growing in wetlands, or on the water, or where soils are flooded or saturated long enough for anaerobic conditions to develop in the root zone, and that have evolved some specialized adaptations to an anaerobic environment*. Based on their growth form, they difference the following groups of wetland plants:

- Emergent plants: plants that are rooted in the soil, with basal portions below the surface of water, but whose leaves, stems, and reproductive organs are aerial (e.g. *Juncus*, *Rhizophora*, *Spartina*, *Typha*).
- Submerged plants: plants rooted in the soil or rootless, which spend their entire life cycle below the surface of water, with the possible exception of flowering (e.g. *Ceratophyllum*, *Elodea*, *Myriophyllum*, *Utricularia*).
- Floating-leaved plants: plants whose leaves float on the surface of water while their roots are fixed in the soil (e.g. *Nelumbo*, *Nymphaea*).
- Floating plants: plants with leaves and stems floating on the surface of water and roots, if they are present, hanging free in the water (e.g. *Lemna*, *Pistia*, *Spirodela*, *Wolffia*).

The capacity of wetland plants to survive under oxygen-deficient conditions of saturated soils largely depends on the rate of plant respiration and the rate of oxygen supply from photosynthetic tissues to belowground roots (Reddy and DeLaune, 2008). Wetland plants are adapted to reduced oxygen levels through physiological adaptations (such as anaerobic root respiration) and morphological/anatomical adaptations (such as adventitious roots and intercellular airspaces/aerenchyma tissues development). Oxygen provided by aerenchyma tissues is consumed by root respiration or diffused to the rhizosphere via radial oxygen loss (ROL) (Reddy and Delaune, 2008), creating an oxidizing soil layer around the living roots of wetland plants (Figure 1.1). ROL is an important defence mechanism against toxic reduced substances such as Mn^{2+}, Fe^{2+}, and S^{2-} (Jackson and Armstrong, 1999; Tiner, 1999) that are oxidised, and thus immobilized, in the rhizosphere. However, organic exudates produced in the rhizosphere may stimulate the activity of microorganisms and therefore favour anoxic conditions (Otero and Macías, 2002).

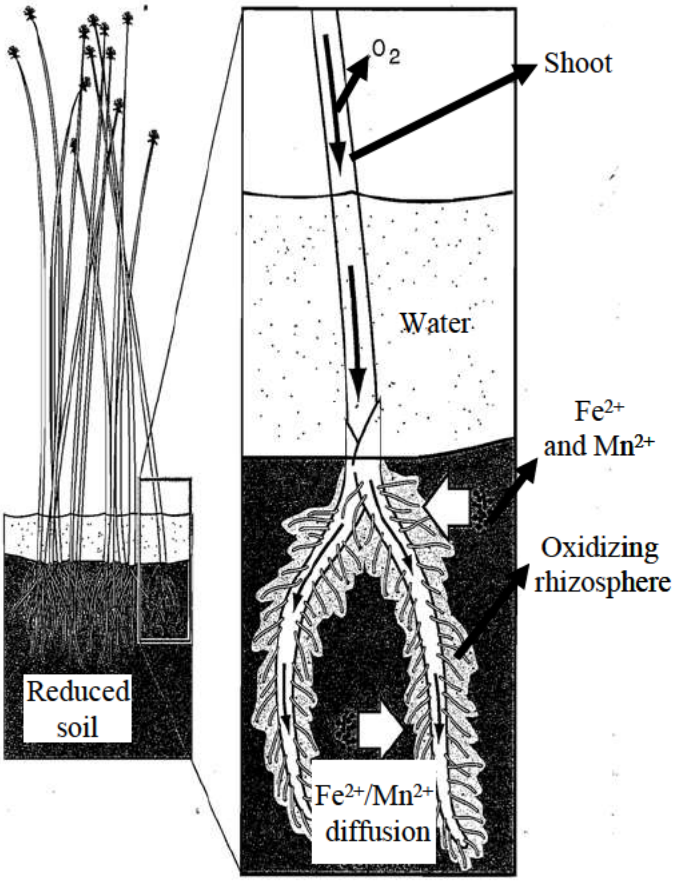

Figure 1.1. Oxidising rhizosphere in wetland plants (modified from Mitsch and Gosselink, 2000).

Many wetland plants are halophytes (plants adapted to high levels of salinity). To successfully grow, halophytes must be able to uptake water and prevent accumulation of excess salt (Cronk and Fennesy, 2001). They have unique adaptations for living in saline environments, e.g.: changes in leaf morphology and orientation to minimize evapotranspiration rate and salt exposure; increases in stomatal and cuticular resistance; changes in tissues elasticity; salt-secreting glands; osmotic adjustment involving inorganic ions, carbohydrates, and organic acids; compartmentalization of metabolites in cell vacuoles and avoidance of ion toxicity and nutrient imbalance (Jones and Corlett, 1992; Tiner, 1999; Touchette et al., 2007; Flowers and Colmer, 2008; Munns and Tester, 2008). Halophytes can be classified in a variety of ways (Waisel, 1972):

- Internal salt content (Ashraf et al., 2006)
    - Excluders: halophytes that exclude salts, e.g. *Atriplex*, *Limonium*.
    - Includers: halophytes that accumulate salts, e.g. *Arthrocnemum*, *Sarcocornia*, *Salicornia*.
- Morphology
    - Excretives: halophytes capable of excrete excess salts from the plant body (Marschner, 1995), e.g. *Atriplex*, *Limonium*, *Tamarix*.
    - Succulents: halophytes capable of store large amount of water within their body to minimize salt toxicity (Weber, 2008), e.g. *Arthrocnemum*, *Sarcocornia*, *Suaeda*.
- Salt demand (Sabovljevic and Sabovljevic, 2007)
    - Obligates: halophytes that need some salt for their growth, e.g. *Arthrocnemum*, *Halimione*, *Sarcocornia*.
    - Facultatives: halophytes that can grow under saline stress, but can also grow without salt or at least in an environment where the concentration of salt in the soil is low, e.g. *Frankenia*, *Mesembryanthemum*, *Salsola*.
- Habitat (Youseff, 2009)
    - Hydro-halophytes: halophytes which can grow in aquatic soil or in wet conditions, e.g. *Juncus*, *Phragmites*, *Scirpus*.
    - Xero-halophytes: halophytes which can grow in soils where the water content is low due to evaporation, e.g. *Atriplex*, *Frankenia*, *Suaeda*.

The distribution of wetland plants depends on the flooding regime, wetland soil properties (such as pH, organic matter, texture, and salinity), relationship between intensity and capacity of reduction, and root aeration capacity (Reddy and Delaune, 2008). The U.S. Fish and Wildlife Service classifies wetland plants, based on the probably of occurrence, into the following groups (Reed, 1988):

- Obligate wetland plants (only occur in wetland environments, e.g. *Lemna* spp., *Rhizophora mangle* L., *Salicornia perennis* (Mill.) A.J. Scott, *Spartina alterniflora* Loisel., *Typha latifolia* L.).
- Facultative wetland plants (occur in wetlands, but occasionally in non-wetlands, e.g. *Juncus acutus* L., *Phragmites australis* (Cav.) Trin. ex Steud., *Tamarix africana* Poir.).
- Facultative plants (occur in wetlands and non-wetlands, e.g. *Atriplex semibaccata* R. Br., *Ranunculus hispidus* Michx., *Ulmus rubra* Muhl.).
- Facultative upland plants (occur in uplands, but occasionally in wetlands, e.g. *Asparagus officinalis* L., *Bromus* spp., *Senecio vulgaris* L.).

1.4. Wetland eutrophication

Eutrophication is defined as an increase of chemical nutrients in aquatic systems leading to an excessive growth of vegetation/phytoplankton, which disrupts the normal functioning of the system, and to a lack of oxygen, which reduces water quality. High concentrations of nitrogen and phosphorus are the main causes of eutrophication, and they frequently occur through the release of sewage effluent and/or run-off of fertilizers.

Wetlands are among the ecosystems most affected by eutrophication worldwide. However, these environments have also high capacity to combat eutrophication, transforming and/or reducing harmful compounds, acting as green filters and thus contributing to water quality improvement (Mitsch and Gosselink, 2000). The capacity of natural (White et al., 2000; Newman and Pietro, 2001; Rummer, 2004) and constructed (Knight et al., 2000; Nairn and Mitsch, 2000; Braskerud, 2002a, 2002b; Poe et al., 2003) wetlands to filter wastewater and nutrients from arable lands have been demonstrated. To understand the role of wetlands in the reduction of nutrient contents in water, it is necessary to know the cycles of N and P in these systems.

1.4.1. Nitrogen dynamics in wetlands

Nitrogen, like P, is found in two forms: a soluble form, that is used directly by the organisms and can contribute significantly to eutrophication processes, and a particulate form, that does not have any significant effect on eutrophication (Jones et al., 2004; Christou et al., 2005). The particulate forms include N in organic debris. The soluble forms include:

- Dissolved inorganic nitrogen, which corresponds to NO_3^-, NO_2^-, and NH_4^+.
- Dissolved organic nitrogen, which corresponds to simple compounds (free amino acids, amino-sugars, and urea) that can be easily mineralized and contribute actively to eutrophication, as well as high molecular weight compounds (proteins and polyphenols) that are resistant to the attack by microorganisms and their contribution to eutrophications processes is low.

It has been estimated that between 80 and 90% of N in wetlands occurs in soil (Vepraskas and Faulkner, 2001), and that there are seven possible major changes that N may undergo (Figure 1.2): 1) fixation of N_2 from atmosphere, 2) ammonification of organic N, 3) nitrification of NH_4^+, 4) denitrification to N_2, 5) dessasimilatory reduction of NO_3^-, 6) volatilization of NH_4^+, and 7) absorption by organisms.

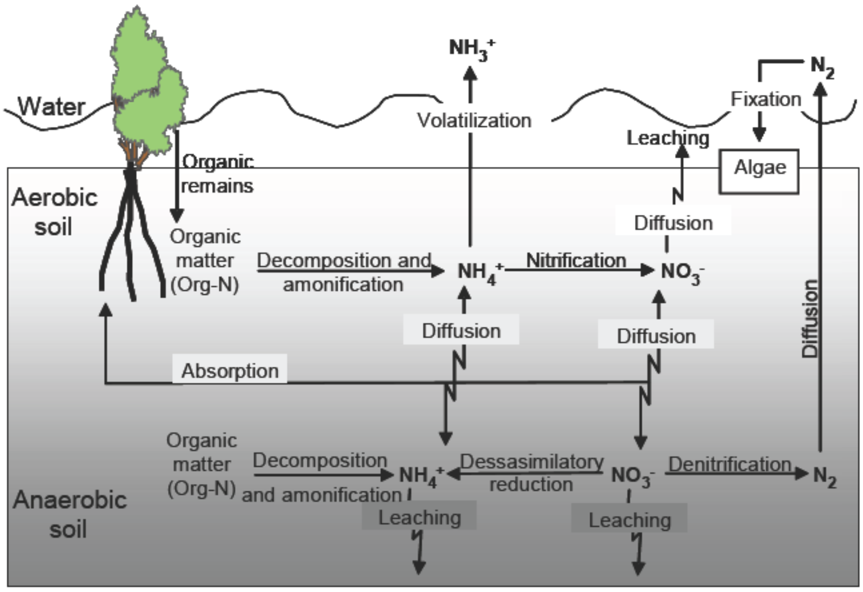

Figure 1.2. Nitrogen cycling in wetlands (modified from Vepraskas and Faulkner, 2001). Org-N = organic nitrogen.

1.4.2. Phosphorus dynamics in wetlands

The main soluble forms of P are inorganic phosphates and organic forms such as phospholipids, nucleic acids, inositol phosphates, and phosphor-sugars. Particulate P may appear as mineral particles or organic debris.

It has been estimated that between 80 and 90% of the P in wetlands is retained in the soils (Vepraskas and Faulkner, 2001), and that there are four possible major changes that P may undergo (Figure 1.3): 1) mineralisation of organic P, 2) absorption by organisms, 3) fixation to soil compounds, and 4) solubilisation and leaching.

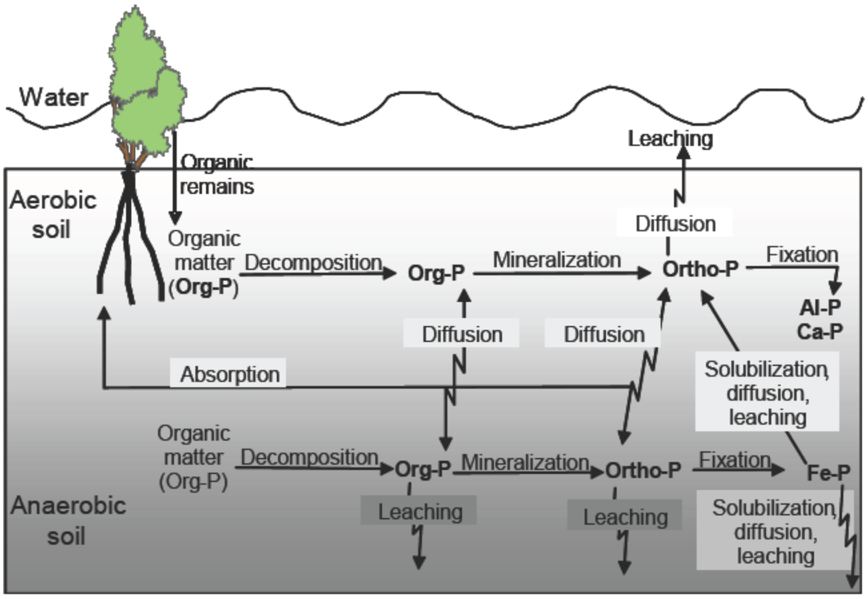

Figure 1.3. Phosphorus cycling in wetlands (modified from Vepraskas and Faulkner, 2001). Org-P = organic phosphorus.

The main mechanisms of P retention in soils are as follows:

- Ionic adsorption to soil particles (clay, humic substances, and mineral-organic complexes) by cation bridges (Ca^{2+}, Mg^{2+}, Al^{3+}, or Fe^{3+}).
- Adsorption to the surface of clay particles and oxihydroxides by covalent bonds with OH^- groups (Kirk, 2004).
- Precipitation as insoluble Ca-phosphates, such as apatite, and/or co-precipitation with carbonates in basic soils.
- Precipitation as Fe and Al phosphates in acid soils.
- Immobilization due to assimilation by organisms.

1.5. Wetland pollution by metals: the case of mine wastes

Soils contain metals of various origins (Kabata-Pendias, 2001): lithogenic (metals that are directly inherited from the lithosphere), pedogenic (metals of lithogenic origin, but their concentration and distribution in soil are changed due to soil formation processes), and anthropogenic (metals that are deposited into soil as direct or indirect result of human activity). The latter is the major cause of the environmental problems associated with metal emissions, being urban, industrial, and mining activities the main pollution sources.

Mining has deleterious effects on the environment through the deposition of huge amounts of wastes (Dudka and Adriano, 1997). Mine wastes usually present unfavourable conditions for plant establishment such as low pH (Wong et al., 1998), toxic metal concentrations (Norland and Veith, 1995; Ye et al., 2000), high salt content (Norland and Veith, 1995; Wong et al., 1998), low water retention capacity (Henriques and Fernandes, 1991; Norland and Veith, 1995), nutrient deficiencies (Wong, 2003), and steep slopes that are unstable and prone to erosion (Henriques and Fernandes, 1991).

Metals with and elemental density greater than 5 g cm^{-3} or with and atomic number above 20 are defined as heavy metals. Usually, most heavy metals are found in very low concentrations (trace elements), although some can occur naturally at high levels such as Fe, Mn, or Cr (Conesa, 2005). Some heavy metals are essential for living organisms, to be part of biological molecules (Co, Cr, Cu, Fe, Mn, Ni, Pb, or Zn) (Kosolapov et al., 2004), although most do not have biological known functions, being highly toxic at very low concentrations and being able to accumulate in live tissues (Conesa, 2005).

The impacts of metal mine wastes not only occur in the mine zone, but also in the surrounding areas when sediments are carried by rivers and runoff. Thus, wetlands are among those environment most affected, due to their location in topographically depressed positions. Several authors have pointed out the role of wetland soils as sink for heavy metals (Williams et al., 1994; Doyle and Otte, 1997; O'Sullivan et al., 2000; Mays and Edwards, 2001; Cheng et al., 2002) and hence these ecosystems can be used for rehabilitating metal mine wastes (O'Sullivan et al., 2000). However, under some environmental conditions, these ecosystems can also be source of metal pollution

(Carbonell et al., 1998; Weis et al., 2002; Peltier et al., 2003; Kirk, 2004; Du Laing et al., 2007) because changes in pH and Eh can liberate metals retained in soils (Kirk, 2004).

1.5.1. Metal dynamics in wetlands

Metals can be present in solid or aqueous phases, the latter being the most toxic (Reddy and DeLaune, 2008). Metals in wetland soils are present in various forms: dissolved, adsorbed, bound to carbonates, Fe and Mn oxides, sulphides and insoluble organic matter forms, and within the crystalline structure of minerals (Shannon and White, 1991). The ranking of metal chemical forms, from most available to least available, is as follows (Gambrell and Patrick, 1991; Shannon and White, 1991):

1) Readily available (dissolved and exchangeable forms).
2) Potentially available (metal carbonates, metal oxides and hydroxides, metals adsorbed on or occluded with Fe and Mn oxides, metals strongly adsorbed or chelated with insoluble humic materials, and metals precipitated as sulphides).
3) Unavailable (metals within the crystalline structure of minerals).

Metal solubility in upland soils is known to be mainly controlled by pH. However, the factors that control metal dynamics in wetlands include several interrelated parameters such as pH, redox potential (Eh), temperature, salinity, clay, organic matter, oxides, hydroxides, carbonates, and microbial activity (Filgueiras et al., 2002; Du Laing et al., 2009b). Among these, pH and Eh have the greatest effect on metal transformation.

Low pH and Eh conditions tend to favour the formation of soluble species of many metals, whereas in oxidised and non-acid systems, slightly soluble or insoluble forms tend to predominate (Reddy and DeLaune, 2008). However, pH and particularly Eh may regulate other processes, such as sulphide formation, which indirectly affect the solubility of metals. Under reduced conditions, sulphides can act as sink for metals (Eq. 9) (van der Berg, 1999; Otero et al., 2000). Oxidation of metal sulphides release associated metals (Eq. 10), increasing its concentration in pore water and, therefore, its bioavailability (Otero et al., 2000; Otero and Macías, 2002, 2003).

$H_2S + M^{2+} \rightarrow MS\downarrow + 4H^+$ (Eq. 9) (Kosolapov et al., 2004)

$MS + 2O_2 \rightarrow M^{2+} + SO_4^{2-} + 2H^+$ (Eq. 10) (Reddy and DeLaune, 2008)

It is assumed that maximum metal solubility occurs when Eh values are low enough (Eh < +300 mV) for Fe and Mn oxide-hydroxides reduction, thus releasing metals into the soil solution, but high enough (Eh > +100 mV) to prevent metal sulphides formation (Jacob and Otte, 2003) (Figure 1.4).

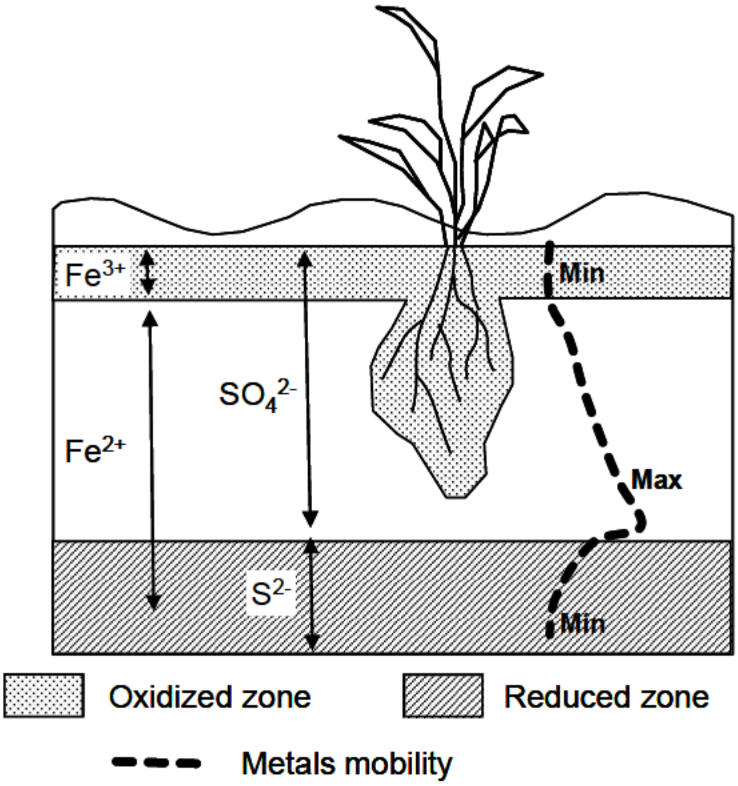

Figure 1.4. Relationship among vegetation, oxidation status of soil, and metals mobility in wetland soils (modified from Jacob and Otte, 2003).

1.5.1.1. Iron dynamics in wetlands

Iron in wetland soils is present in various forms: water soluble (pore water), exchangeable (sorbed on cation exchangeable complex), easily reducible (insoluble ferric and ferrous compounds), and residual (crystalline stable pool) (Reddy and DeLaune, 2008). Mobile pools of Fe are present in water soluble or dissolved forms, whereas immobile forms include insoluble precipitates and mineral phases (amorphous and crystalline). It has been estimated that between 30 and 60% of the Fe in wetlands is in amorphous forms (Vepraskas and Faulkner, 2001).

Metals (such as Cd, Co, Cr, Cu, Mn, Ni, and Zn) are often immobilised by Fe oxides in soil aerobic zones, precipitating as coatings on the surface of minerals (Reddy and DeLaune, 2008). Amorphous and crystalline forms of Fe oxides can both sorb or coprecipitate metals. Amorphous Fe oxides (e.g. ferrihydrite –$Fe(OH)_3$-) are more active to retain metals than crystalline forms by the higher surface/area relation and the greater exchange positions (Golterman, 1988). The solubility and reactivity of Fe oxides are regulated by pH and Eh. They are soluble under acidic or reducing conditions, thus resulting in the release of the associated metals.

The biogeochemical cycling of Fe in wetland soils involves various oxidation and reduction reactions (Figure 1.5).

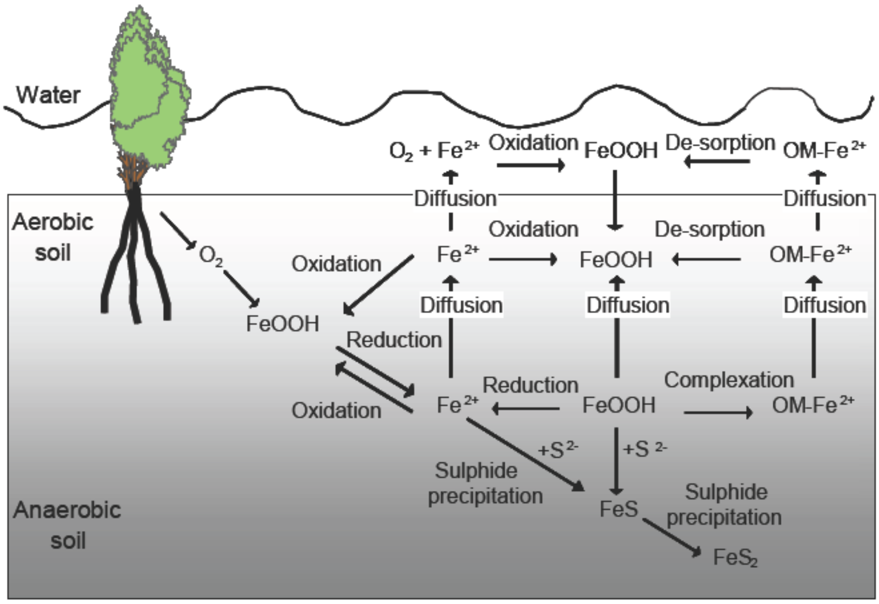

Figure 1.5. Iron cycling in wetlands (modified from Reddy and DeLaune, 2008). $OM-Fe^{2+}$ = Fe^{2+} complexed with organic materials.

The reduction of Fe^{3+} to Fe^{2+} can occur enzymatically (linked to microbial activity) and non-enzymatically, although the significance of the latter process is minimal (Lovley, 1991, 2004). The reduction of the oxidised forms of Fe depends on the quality of organic substrates (electron donors), bioavailability and crystallinity of Fe minerals, presence of electron acceptors with higher reduction potentials, pH, and temperature (Reddy and DeLaune, 2008). Iron reduction not only contributes to the solubilisation of the metal, it also has an indirect impact on wetland soil chemistry (Reddy and DeLaune, 2008):

- Soil pH tends to increase as a result of the consumption of protons during reduction.
- The increase of Fe^{2+} may displace other soil cations (such as Ca^{2+}, Mg^{2+}, and NH_4^+) from the exchangeable complex to soil solution.
- Solubility of P increases as a result of ferric-phosphate compounds reduction.
- Sulphide levels decrease as a result of precipitation with Fe^{2+}.

The oxidation of Fe^{2+} to Fe^{3+} can take place through enzymatic reactions (mediated by microorganisms) and non-enzymatic processes (spontaneous reactions with oxidants such as oxygen and nitrate). In flooded soils, the oxidation of the reduced forms of Fe is restricted to the aerobic thin layer at the soil-water interface or in the root zone, thus the extent of the oxidation process depends on the flux of the dissolved species from the anaerobic to the aerobic soil zones. At circumneutral pH, the concentrations of dissolved Fe^{2+} are low since most of Fe^{2+} compounds are present as immobile solid phases such as $Fe(OH)_2$, $FeCO_3$, and FeS_2 (Figure 1.6) (Reddy and DeLaune, 2008). The oxidation of these compounds only occurs when the water table is lowered and the top soil is exposed to aerobic conditions. The oxidation of Fe^{2+} is regulated by the following factors (Reddy and DeLaune, 2008):

- Soil pH and Eh.
- Oxygen flux and the thickness of the aerobic layer.
- Presence of oxidants with higher reduction potentials.
- Flux of soluble Fe^{2+}.
- Soil cation exchange capacity.
- Metal-dissolved organic matter complexation.

Some authors (e.g. Trolard et al., 1997; Hansen, 2001; Génin et al., 2006) have indicated the role of green rust minerals in the redox cycling of Fe in hydric soils. Green rust minerals are layered Fe^{2+}-Fe^{3+} hydroxyl salts responsible for the bluish-green colours of these soils. The crystalline structure (Figure 1.7) consists in the stacking of brucite-like layers carrying positive charges and interlayers constituted by anions (Cl^-, SO_4^{2-}, CO_3^{2-}, or OH^-) and water molecules (Génin et al., 1998; Bourrié et al., 1999; Brocher et al., 2004).

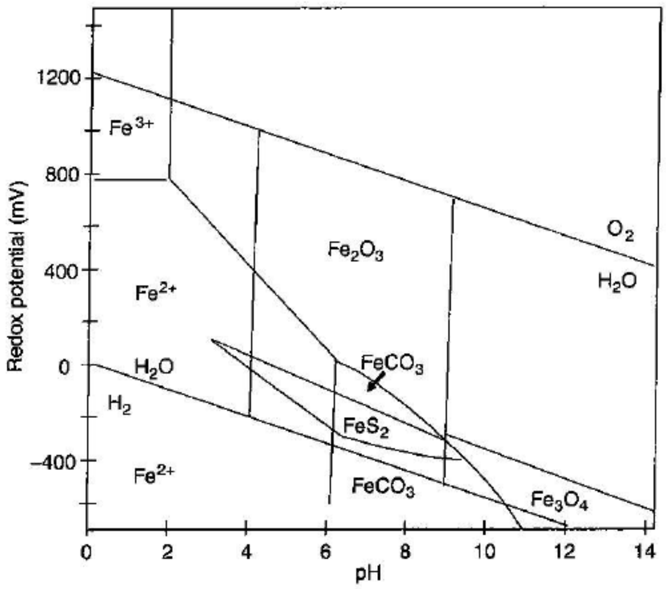

Figure 1.6. Eh-pH diagram for Fe^{3+}-Fe^{2+} redox couple (modified from Reddy and DeLaune, 2008).

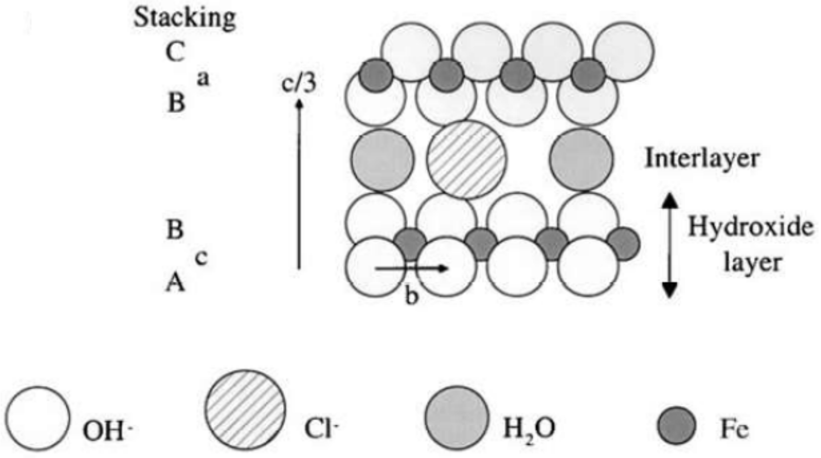

Figure 1.7. Crystal structure of green rust mineral incorporating Cl^- (modified from Refait et al., 1998).

In anoxic environments (Eh~0 mV at pH = 7) (Figure 1.8), the interaction of soluble Fe^{2+} species with ferric minerals favours the formation of green rust minerals (Usman et al., 2011). These Fe^{2+}-bearing minerals can alter the oxidation state, and hence the mobility and toxicity, of inorganic contaminants (Mitsunobu et al., 2008; Wang et al., 2010; Bearcock et al., 2011).

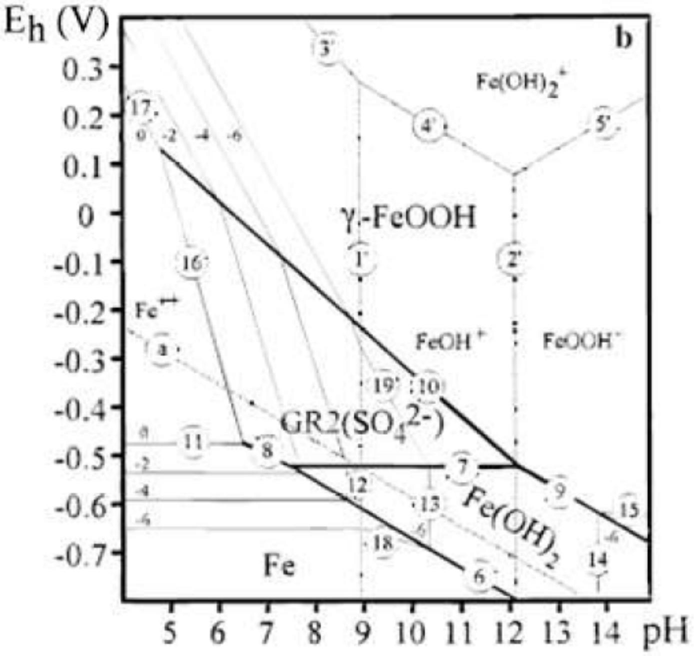

Figure 1.8. Eh-pH diagram of green rust (SO_4^{2-}) (modified from Génin et al., 1998).

1.6. Management strategies of soils polluted by mine wastes

Numerous mechanisms (physical, chemical, and biological) have been studied for the recovery of soils polluted by mine wastes (Scullion, 2006). Among all the techniques, revegetation is considered the most suitable alternative for long term reclamation (Tordoff et al., 2000). Phytomanagement describes the engineering or manipulation of soil-plant systems to control the fluxes of contaminants in the environment (Robinson et al., 2009). When refers to soils polluted by metals is based on the use of green plants and their associated micribiota, soil amendments, and agronomic techniques to remove, retain, or reduce environmental contaminants (Cunningham and Ow, 1996; Chaney et al., 1997).

The aim of phytomanagement is not only to mitigate the negative effects associated with pollutants, but also to improve the quality of ecosystems enhancing the possibilities of plant development and recovering the functions of soil. Hence, a successful phytomanagement program must:

- Decrease leachate of contaminants to groundwater and/or watercourses.
- Limit accumulation of contaminants in above-ground biomass of plants, because this would facilitate their entry into the food chain (Salomons et al., 1995) and

increase their concentration on the soil surface after litter fall (Johnson et al., 2003; Watmough et al., 2005).

- Stabilise soil surface so that wind and water erosion are minimised, reducing the risk of spread of pollutants (Robinson et al., 2003).
- Increase nutrient levels in the soil (Cobb et al., 2000) and promote the establishment of a self-sustaining plant cover (Norland and Veith, 1995).
- Improve the aesthetic value of degraded environments (Tordoff et al., 2000).

Among the phytomanagement alternatives, phytoextraction and phytostabilisation are the most widely employed techniques to reduce the environmental risk posed by metals. Phytoextraction or phytoaccumulation involves the uptake and translocation of metals by roots into the above-ground biomass of plants (Wong et al., 2003). This technique requires the repeated harvesting and processing of metal-rich biomass until the concentration of soil contaminants reach acceptable levels (Robinson et al., 2003, 2009). Phytostabilisation involves the use of plants to immobilise metals through absorption and accumulation by roots, adsorption onto roots, or precipitation within the rhizosphere (Wong, 2003). Plants roots help maintain aerobic conditions in the rhizosphere zone, which prevent the formation of reduced metal species, and also provide organic substances that bind metals (Robinson et al., 2009). However, the flux of carbon compounds from plant roots may also enhance the activity of microorganisms within the rhizosphere (Hinsinger, 2001), leading to a decrease of redox potential (Eh). Hence, attention must be taken when phytostabilisation techniques are applied to environments subject to saturation soil conditions since we can promote metals solubilisation instead of immobilisation. (Du Laing et al., 2009a; Wenzel et al., 2009; María-Cervantes et al., 2010, 2011). In addition, plant roots may create macropores in the soil that increase the leachate of contaminants through preferential flow processes (Robinson et al., 2009).

The bioconcentration factor (BCF) is the ratio between metal concentration in plant and metal concentration in soil (Mattina et al., 2003). The BCF can be used as an index to express the ability of plants to accumulate metals from the soil and, according to Baker (1981), there are three different types of plants: accumulators (BCF > 1), indicators (BCF ~ 1) and excluders (BCF < 1). Accumulator or hyperaccumulator plants concentrate metals, even those that are non-essential, in their shoots at both low and high metal concentrations in soils (Baker, 1981). Accumulator plants are ideal candidates for the phytoextraction of metal-contaminated soils (Wong et al., 2003), but may pose a risk of entry of metals into the food chain if the above-ground biomass are not removed. Indicator plants uptake metals in proportion to their concentrations in soil (Baker, 1981). Excluder plants limit the translocation of metals within them over a wide range of soil metals concentrations (Baker, 1981), thus being ideal candidates for the phytostabilisation of soils polluted by metals because they can stabilise the soil surface and reduce erosion and leachate, while minimising the entry of metals into the food chain (Robinson et al., 2009). Nevertheless, the effectiveness of selected vegetation depends on environmental conditions and their introduction should enhance the ecological value of the site, by increasing biodiversity and providing a habitat for other species (Robinson et al., 2009).

The solubility and speciation of metals in soil determines their toxicity, availability for plants, and downward mobility. Understand the factors' controlling these processes is essential to select the most appropriate soil amendments. In the phytomanagement of soils polluted by metals, the application of soil amendments such as liming materials, phosphates, zeolite, bentonite, clay, Fe and Mn oxides, and organic matter may reduce metal solubility (Cheng and Hseu, 2002). The immobilisation of metals can be achieved by promoting the formation of insoluble precipitates or enhancing the capacity of soils to bind metals, directly through the addition of adsorbent materials or indirectly by adjusting the pH-Eh soil conditions to promote metal sorption onto the soil matrix (Robinson et al., 2009).

The effectiveness of the selected amendment must be balanced with its potential negative effects on plant growth and soil characteristics such as essential nutrients immobilisation, salinity increases, metal mobilisation, and any physical changes that disrupt root penetration, oxygenation, and water uptake (Robinson et al., 2009). In this sense, the use of lime amendments enhances the physical, chemical, and biological properties of soils through its direct effect on soil acidity amelioration and its indirect effect on the mobilisation of plant nutrients, immobilisation of heavy metals, and improvement in soil structure and hydraulic conductivity (Haynes and Naidu, 1998). Thus, liming is the most common management practice used to neutralise soil acidity (Bolan et al., 2003) and to reduce the solubility of metals by increasing the negative charge on oxides, clays, and organic matter (Kirkham, 2006). This results in an increase in metal sorption and a decrease in desorption, thus diminishing the soluble metal concentrations in soils (Oste et al., 2002; Basta and McGowen, 2004).

When the soil to be managed is contaminated by more than one metal, choosing the most appropriated amendment is often complicated. Sometimes, an amendment may be effective immobilising one metal, but may increase the mobility of others, for example: lime amendments can favour As solubility (González et al., 2012) and organic materials can increase Cu (Hsu and Lo, 2000) and Al mobility (del Moral et al., 2010). In these cases, the combination of different amendments may be a possibility. Nevertheless, in order to improve the remediation program, a preliminary evaluation of the combination is necessary because the effectiveness of adding two or more amendments may be reduced in relation when using each individual amendment (Gräfe et al., 2002; del Moral et al., 2010).

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Chapter 2

Study Area

2.1. The Mar Menor lagoon

The Mar Menor (maximum depth 6 meters) is one of the largest (135 km^2) coastal lagoons of the Mediterranean basin (Figure 2.1). It is located in southeastern Spain, under a semiarid Mediterranean climate with long warm summers and short moderate winters. The annual mean temperature is 17 ºC, the annual mean precipitation is 275 mm, most of which falls in autumn and spring, and the annual mean evapotranspiration rate is 856 mm year^{-1}.

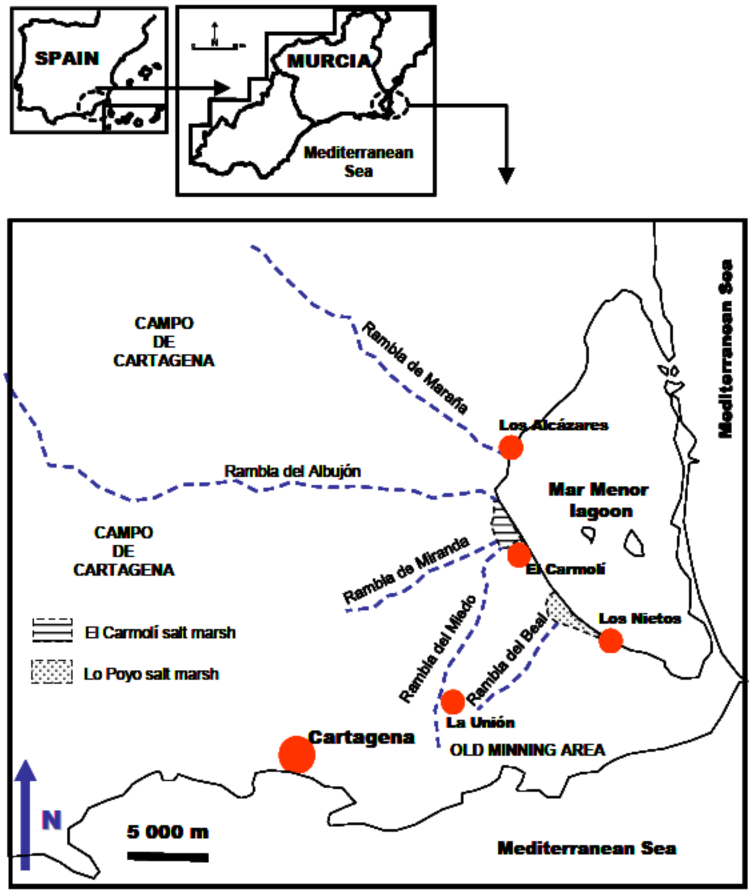

Figure 2.1. Location map of the Mar Menor lagoon in SE Spain.

The lagoon is isolated from the Mediterranean Sea by a sand bar (La Manga) which is 2 km long and 100 to 1500 m wide, and is intersected by five shallow channels. Hence, it can be classified as a choked lagoon with restricted circulation and long water residence times (Gilabert, 2001). These characteristics make the lagoon especially sensitive to the effects of human activity (Pérez-Ruzafa et al., 2002), such as tourism and intensive agriculture. Only few relatively non-altered zones remain on its coast, most of them salt marshes.

The Mar Menor and its associated wetlands (salt marshes, saltworks, and reedbeds) are the main wet area of the Murcia Region (150 km^2) (Vidal-Abarca et al., 2003) and are included in the Ramsar Convention on Wetlands. The zone is also a Special Protected Area of Mediterranean Interest (SPAMI), Specially Protected Area under the EU Wild Birds Directive (SPA), and Site of Community Importance (SCI) to be integrated in the Nature 2000 Network (EU Habitats Directive).

2.1.1. Main environmental threats on the Mar Menor lagoon

2.1.1.1. Impacts of agricultural activities

The Mar Menor lagoon receives water runoff from the streams flowing from the agricultural area of the Campo de Cartagena. The Campo de Cartagena has been used by humans since ancient times. At the end of the Middle Ages the area was deforested, thereby increasing the rate of deposition of sediments by the watercourses that flow into the lagoon (García-Doroy and Maldonado-Inocencio, 1980). Until the early 1970s, food production was based on a non-irrigated agriculture, but the increase in the use of groundwater and the construction of the Tajo-Segura aqueduct (to bring water from central to southeastern Spain) changed the land use, and new, modern irrigation systems were established. Today, the Campo de Cartagena is one of the main agricultural areas in Europe with 30,000 ha of intensive production (vegetables, greenhouse plants, citrus, and other fruits).

The increase in irrigation has generated an important rise of the groundwater level of the Campo de Cartagena and a continuous supply of water in the former dry streams of the zone (Martínez and Esteve, 2000). Moreover, the scarcity of water in the region has also led to the construction of desalination plants to support agriculture, which has generated a new problem related to the disposal of brine. These effluents, rich in salts and nutrients (mainly nitrates), are usually dumped into streams (Martínez and Esteve, 2002; Velasco et al., 2006).

Several studies have found high concentration of nitrates, ammonium, and phosphates in some of the watercourses reaching the Mar Menor (Table 2.1). In addition to the nutrient enriched effluents, these streams also carry waters with high concentrations of pesticides (Pérez-Ruzafa et al., 2000, 2002; Pérez-Ruzafa and Aragón, 2002). This situation led the Mar Menor to be declared an area sensitive to eutrophication in June 2001 under the European Directive 91/271/EEC, concerning urban waste treatment, and the Campo de Cartagena a vulnerable area in December 2002 under the European Directive 91/676/EEC, concerning protection of waters against pollution caused by nitrates from agricultural sources.

Table 2.1. Nitrate, ammonium, and phosphate concentrations (mg L^{-1}) in some of the watercourses flowing into the Mar Menor (modified from Conesa and Jiménez-Cárceles, 2007).

Watercourse	NO_3^-	NH_4^+	PO_4^{3-}	Reference
Rambla de Miranda	33.1	n.a.	n.a.	SACYR S.A. (1997)[1]
	>281	n.a.	>2	Álvarez-Rogel et al. (2006) Jiménez-Cárceles et al. (2006)
Rambla del Albujón	62.6	n.a.	6.7	SACYR S.A. 1997[1]
	72.7	4.4	6.2	Velasco et al. (2006)
	54.0	6.3	1.2	Ruíz-Martínez (2006)
	>186	>11.9	>16.1	Álvarez-Rogel et al. (2006) Jiménez-Cárceles et al. (2006)
Rambla del Miedo	>53.5	>31.1	>37.8	Álvarez-Rogel et al. (2006) Jiménez-Cárceles et al. (2006)

n.a. = not available data, [1] data cited in Martínez and Esteve (2000, 2002).

2.1.1.2. Impacts of urban growing and tourist development

The Mediterranean Sea shelters a substantial human population and is a suitable zone for tourism. From 1960, the tourist development in the Mar Menor began to modify the orography of the shoreline, including the construction of recreational ports and the enlargement of the communication channels with the Mediterranean Sea. The latter has caused a progressive "Mediterranisation" process in the water properties of the lagoon. During the 1970s, the water temperature of the Mar Menor varied according to the different seasons of the year, between 7.5 ºC in winter and 29 ºC in summer, the salinity between 48.5 and 53.4 g L^{-1}, and the water had a residence time of 1.28 years (Pérez-Ruzafa et al., 1991; Díaz del Río, 1993). Nowadays, the temperature ranges between 10 and 32 ºC, the salinity between 42 and 47 g L^{-1}, and the water residence time is 0.79 years (Pérez-Ruzafa et al., 1991, 2002). The modification of the shoreline by urban development has also damaged the characteristics dune systems and broad beaches from La Manga, and over 60% of the surface area of sand stretches has disappeared during the last 30 years (ESA, 2003).

The increase in the population over the last few decades, consequence of the high tourist development, has led to the installation of wastewater treatment plants in the nearby urban areas of the lagoon. However, these systems have important deficiencies, and as a consequence, there are some wastewater discharges (rich in nutrients such as ammonium and phosphates, Table 2.1) to the lagoon during the high tourist season in summer, when there is a significant increase in the inhabitants (Martínez et al., 2004; Álvarez-Rogel et al., 2006a; Jiménez-Cárceles et al., 2006; Velasco et al., 2006). After rainfalls is usually when the overflow water from the treatment plants enters the lagoon (Álvarez-Rogel et al., 2006a; Velasco et al., 2006; González-Alcaraz et al., 2012). In addition, motor oil and petrol spillages from boats are source of hydrocarbures to the lagoon, with average values between 0.4-0.9 $\mu g\ L^{-1}$ in water and 1-5 $\mu g\ L^{-1}$ in sediments (Martínez et al., 2004).

2.1.1.3. Impacts of mining activities

The Mar Menor lagoon receives water runoff from the streams flowing from the nearby mining area of La Unión-Sierra de Cartagena. This mining district was an important mine area for more than 2500 years until 1991, when the mine activity ceased (Conesa et al., 2008). The main minerals extracted were pyrite (FeS_2), blend (ZnS), and galene (PbS). Most of the mine wastes spills were caused by direct discharge into the local streams. In 1955 some public restrictions prohibited mining companies to dump mine wastes into watercourses, storing the wastes in form of mine tailings (Vilar et al., 1991). However, many tailings are located at the headwaters or along the riverbeds of the streams, most without any environmental restoration and with erosion problems due to their bare surfaces. Then, the short, intensive rainfall, typical of this area, can relocate large volumes of mine wastes with high concentrations of metals from these sites to the Mar Menor. Conesa et al. (2006) found concentrations of 7670-11,600 $mg\ kg^{-1}$ Zn, 94-530 $mg\ kg^{-1}$ Cu, and 4900-7900 $mg\ kg^{-1}$ Pb in tailings located at the headwater of some streams. Robles-Arenas et al. (2006) obtained average concentrations of 77 $mg\ L^{-1}$ Zn, 1.3 $mg\ L^{-1}$ Cd, and 0.54 $mg\ L^{-1}$ Pb in the runoff waters from the mining area.

Some studies have found metal-rich sediments in the Mar Menor (Table 2.2). The highest concentrations of metals are in the southern part of the lagoon, near the mouths of the watercourses draining the mining area. Once the acid runoff waters (pH 2-4) of these streams comes into contact with the alkaline water (pH > 8) of the lagoon, a portion of the soluble metals precipitate. In addition, suspended particles and colloids, rich in metals, are carried by the sea currents to the middle part of the lagoon (García-García, 2004).

Table 2.2. Heavy metal concentrations in soils and sediments (mg kg^{-1}) and water (mg L^{-1}) of the Mar Menor lagoon and surrounding watercourses and salt marshes (modified from Conesa and Jiménez-Cárceles, 2007).

Site		Metal				Reference
		Cd	Cu	Pb	Zn	
Sediment	Mar Menor	0.31-0.60	n.a.	30-90	130	1
	Mar Menor	>7	n.a.	>10,000	>7000	2
	Rambla de la Carrasquilla	24	39	3690	3500	3
	Rambla de Ponce	25	30	1960	1990	
	Rambla del Beal	15	35	950	650	
	Rambla del Miedo	16	42	2250	2850	
Soil	Lo Poyo salt marsh	n.a.	110	8000	6940	4
	El Carmolí salt marsh	>100	>450	>15,000	>60,000	5
Water	Rambla de la Carrasquilla	0.03	n.a.	0.04	0.05	3
	Rambla del Beal	0.02	n.a.	0.07	0.41	

n.a. = not available data, [1] Sanchiz et al. (2000), [2] Marín-Guirao et al. (2005c), [3] García-García (2004), [4] Álvarez-Rogel et al. (2004), [5] Jiménez-Cárceles et al. (2006).

The presence of sediments with high concentrations of metals in the Mar Menor has affected living organisms. Sanchiz et al. (2000) found higher levels of Cd, Pb, and Zn in the sediments from the Mar Menor (Table 2.2) than ones obtained in different points of the Spanish coast, and that *Cymodocea nodosa* (Ucria) Asch. (and aquatic macrophyte) had the highest contents of Pb and Zn. Marín-Guirao et al. (2005a,c) reported higher values of metals in sediments and *C. nodosa* than Sanchiz et al. (2000). María-Cervantes et al. (2009) found high levels of metals and As in sediments and molluscs collected from coastal areas of the Mar Menor, pointed the existence of a human health risk.

Álvarez-Rogel et al. (2004) and Jiménez-Cárceles et al. (2006) reported high concentrations of heavy metals (mainly Pb and Zn) in Lo Poyo and Marina del Carmolí salt marshes of the Mar Menor (Table 2.2). Conesa et al. (2011) also found high levels of heavy metals and As in soils and plants from Lo Poyo salt marsh. These metal contents greatly exceed the thresholds proposed by some European legislations to perform cleaning activities (VBBo, 1998; MHSPE, 2000), as well as the intervention levels proposed by the Andalucía region after the toxic spill in Aznalcóllar in 1998 (BOJA, 1999).

2.2. Salt marshes under study

2.2.1. The Marina del Carmolí salt marsh

The Marina del Carmolí salt marsh (N37º41'42'', W0º51'52'') is the largest salt marsh (318.6 ha) on the coast of the Mar Menor lagoon (Figures 2.1 and 2.2).

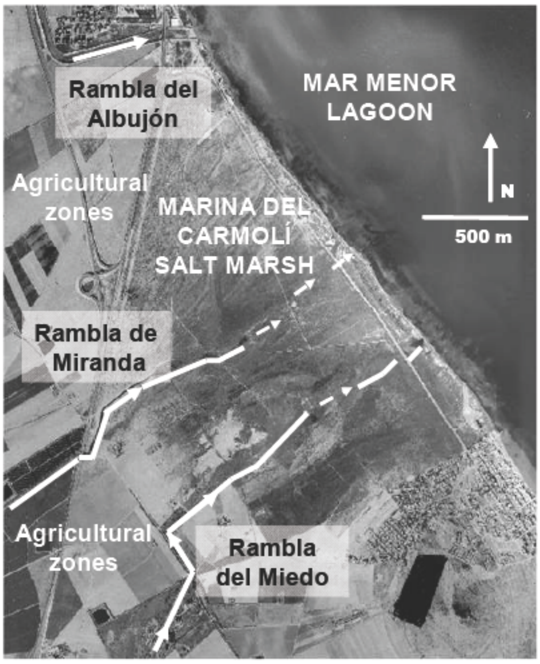

Figure 2.2. The Marina del Carmolí salt marsh.

This salt marsh receives eutrophic waters from two surface watercourses: the Rambla de Miranda and the Rambla del Miedo (Figure 2.2). The first one flows across areas of intensive agriculture of the Campo de Cartagena and so receives drainage waters from agricultural exploitations (Álvarez-Rogel et al., 2006a; González-Alcaraz et al., 2012). The second one received, until few years ago, wastewater from an urban water treatment plant (Photo 2.1.) (Álvarez-Rogel et al., 2006a). In addition, the Rambla del Miedo comes from the nearby mining area of La Unión-Sierra de Cartagena and carries large quantities of metal mine wastes to the salt marsh (Jiménez-Cárceles et al., 2008). A third watercourse, the Rambla del Albujón, directly reaches the Mar Menor at the northern limit of the salt marsh, without flowing across the marsh (Figure 2.2). This watercourse receives effluents from both agricultural areas and depuration stations

(Ruíz-Martínez, 2006; Velasco et al., 2006; García-Pintado et al., 2007) and has been cited as one of the main sources of polluted water flowing into the Mar Menor (Pérez-Ruzafa et al., 2002).

Photo 2.1. Wastewater discharge in the Rambla del Miedo in April 2004.

Soil typology and soil-plant relationships in the Marina del Carmolí salt marsh have been previously studied by Ortiz et al. (1995) and Álvarez-Rogel et al. (2000, 2001). The soils are mainly Calcic and Gleyic Solonchaks (WRB, 2006). In some soils it has been observed the existence of a very fine-textured horizon over a calcareous crust (<1 m deep in some areas). In many areas of the marsh the infiltration is reduced, causing their flooding for prolonged periods of the year.

Plant distribution in the salt marsh is related to soil salinity and flooding gradients, factors that are affected by the micro-topography of the land (Álvarez-Rogel, 1997). The watercourses and nearby zones are almost always colonised by monocultures of *Phragmites australis* (Cav.) Trin. ex Steudel, while *Juncus maritimus* Lam., *Juncus acutus* L., and *Scirpus holoschoenus* L. grow in flooded areas farthest from the main watercourses. In the most saline soils, subject to flooding during some periods of the year, the dominant species are *Arthrocnemum macrostachyum* (Moric.) Moris and *Sarcocornia fruticosa* (L.) A.J. Scott, while in non flooded saline areas, the dominant species is *Limonium delicatulum* (Girard) O. Kuntze. Drier. Less saline soils are occupied by *Lygeum spartum* L. and several shrub species such as *Halimione portulacoides* (L.) Aellen, *Suaeda vera* Forsskal ex. J.F. Gmelin, *Frankenia corymbosa*

Desf., *Asparagus horridus* L. in J.A. Murray, *Sedum sediforme* (Jacq.) Pau, *Bromus* sp., among others (Álvarez-Rogel, 1997; Álvarez-Rogel et al., 2000).

Photo 2.2. Channeling of the Rambla de Miranda in September 2003.

Álvarez-Rogel et al. (2007) pointed to a rise of the water table in the salt marsh, from 1991 to 2004, which was attributable to the increase of flowing water in the surface watercourses crossing the marsh. This is in accordance with Lloret et al. (2005) and Velasco et al. (2006), who indicated that the typical intermittent regime of the watercourses of the zone changed into a continuous flow due to the surplus of anthropogenic water from the Campo de Cartagena. This situation modified the spatial and temporal regime of flooding and soil salinity of the salt marsh: in 2004 flooding periods had increased and soil salinity had dropped in the most saline sites and had increased in the less saline ones (Álvarez-Rogel et al., 2007). Following these changes, *Sarcocornia fruticosa*, *Phragmites australis*, and *Juncus maritimus* strongly expanded at the wettest sites, while *Limonium delicatulum* decreased with increasing moisture and increased with increasing salinity, modifying the original zonation pattern. In addition, the vegetation of the marsh has also been affected due to different actions taken by the Regional Government such as: reedbeds harvesting, riverbeds cleaning, streams channeling (Photo 2.2), and ponds construction to improve the diversity of the habitat and enhance the presence of birds in the area (Photo 2.3). Generally, these actions are often accompanied of earth movements and changes in flooding-drying conditions, which not only affect vegetation, favouring the expansion of *Phragmites australis* (Photo 2.4 and 2.5), but also chemical and biogeochemical processes of the system.

Photo 2.3. Ponds construction in the Rambla de Miranda in November 2006.

Photo 2.4 and 2.5. *Phragmites australis* growing in the waterbed of the Rambla de Miranda in December 2009 and May 2010, respectively.

2.2.2. Lo Poyo salt marsh

The Lo Poyo salt marsh (N37º39’52’’, W0º49’02’’), with a surface area of 210.6 ha, is located on the coast of the Mar Menor lagoon (Figures 2.1 and 2.3). The salt marsh was used for salt extraction in former times but it was abandoned at the beginning of the 20th century.

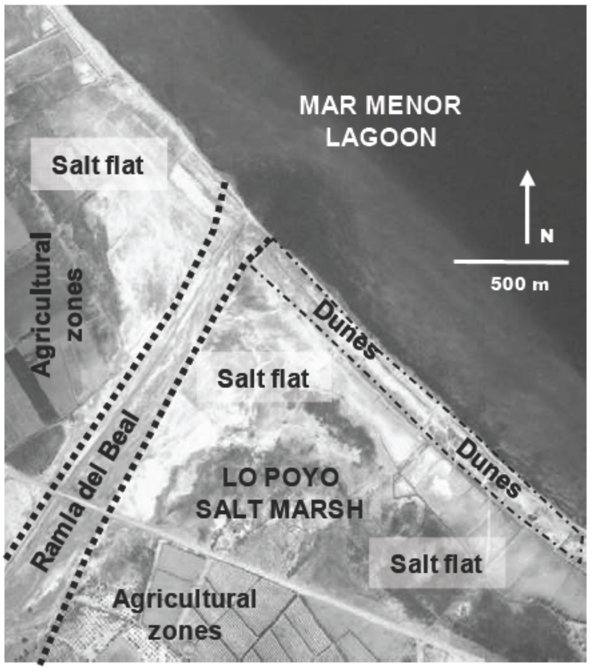

Figure 2.3. Lo Poyo salt marsh with different environments (modified from Conesa et al., 2011).

This salt marsh receives waters from two ephemeral surface watercourses coming from the nearby mining area of La Unión-Sierra de Cartagena: the Rambla del Beal and the Rambla de Ponce. Both are strongly affected by metal mine wastes in all its length, but the Rambla del Beal has been determined as the main source of mine wastes going into the marsh (Photo 2.6) (Marín-Guirao et al., 2005b). As a consequence, the old salt ponds and part of the soils were buried under several millions of tons of wastes resulting in one of the most polluted salt marshes around the Mar Menor (Photo 2.7). Despite this, the salt marsh is surrounded by agricultural lands, and the beach between the marsh and the Mar Menor is used for recreational activities.

Conesa et al. (2011), attending to soil type, micro-topography, and vegetation, distinguished four different zones within the Lo Poyo salt marsh (Figure 2.3): the area occupied by the riverbed, the plain centre occupied by the former salt ponds, and a belt of sandy soils along the shore line, partially affected by the mine wastes, with degraded dunes and shrubs systems. According to WRB (2006), the former soils were Fluvisols in the riverbed zone, Solonchaks in the salt ponds, and Arenosols in the degraded dunes and shrubs zones. However, it is estimated that more than 2 m of mine wastes are above the old soils and nowadays most of them can be classified as Spolic Technosols (WRB, 2006), which suffer seasonal flooding-drying conditions due to the natural dynamics of the marsh. The contents of heavy metals in soils are extremely high (188-530 mg kg^{-1}

As, 11-51 mg kg^{-1} Cd, 56-137 mg kg^{-1} Cu, 708-5640 mg kg^{-1} Mn, 4990-11,600 mg kg^{-1} Pb, and 3550-20,600 mg kg^{-1} Zn) (Conesa et al., 2011) and they are transferred to the vegetation (Álvarez-Rogel et al., 2004; Carrasco et al., 2006; Conesa et al., 2011).

Photo 2.6. Rambla del Beal crossing Lo Poyo salt marsh.

Photo 2.7. Mine wastes in Lo Poyo salt marsh.

The plant cover is scarce in the riverbed area, probably due to the continuous transport and sedimentation of metal-rich sediments which makes unsuitable the establishment of many species. At the salt flat, plants appear in mono-specific dense patches of *Phragmites australis*, *Juncus maritimus*, *Arthrocnemum macrostachyum*, and *Sarcocornia fruticosa* in the wettest zones, leaving bare the rest of soils (Conesa et al., 2011). In the areas with lower salinity and metal concentrations the vegetation is scattered, formed by species typical of dune systems such as *Crucianella maritima* L., *Pancratium maritimum* L., *Asteriscus maritimus* (L.) Less., *Elymus farctus* (Viv.) Runemark ex Melderis, and *Sporobolus pungens* (Schreber) Kunth (Conesa et al., 2011). In addition, the disturbance caused by the mine wastes has led to the introduction of exogenous/invasive plants species in the former environments and in the riverbed area such as *Zygophyllum fabago* L., *Dittrichia viscosa* (L.) Greuter, *Helicrysum stoechas* (L.) Moench, or *Foeniculum vulgare* Mill., among others (Conesa et al., 2011).

At the salt flat area, closed to the patches of *Arthrocnemum macrostachym* and *Sarcocornia fruticosa*, surface reddish crusts with high content of salts appear, especially during the warmer and drier months (Photo 2.8 and 2.9). These type-crusts may be related to the capillary upward movement of water and salts (Álvarez-Rogel et al., 2006b), together with solubilised metals like Fe, which precipitate in the surface. The pumping of water by plants actively contributes to the latter process (Barrett-Lennard and Malcolm, 1999).

Photo 2.8. Reddish crusts in Lo Poyo salt marsh.

Photo 2.9. Reddish crusts in Lo Poyo salt marsh.

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Chapter 3

Background and Objectives

This doctoral thesis was developed within the context of the studies of nutrients and metals dynamics in eutrophic wetlands polluted by metal mine wastes, on the coast of the Mar Menor lagoon, that the scientific group of Agroquímica, Tecnología y Manejo de Suelos y Sustratos (Escuela Técnica Superior de Ingeniería Agronómica of the Universidad Politécnica de Cartagena) has been conducting since 2000. Previous field studies, conducted in the Marina del Carmolí and the Lo Poyo salt marshes, have pointed the presence of soils strongly affected by mine wastes with high levels of metals (Álvarez-Rogel et al., 2004; Jiménez-Cárceles et al., 2008; Conesa et al., 2011). The latter has negatively affected the natural colonisation of vegetation in some of the polluted areas, leading to the existence of bare soils, as well as the transferred of metals to plants (Conesa et al., 2011). The unfavourable soil conditions have also allowed the entry of some exogenous species, typical from disturbance environments, which naturally do not occur (Conesa et al., 2011).

In addition to the metal mine wastes pollution and its deleterious effect on vegetation, the biogeochemical behaviour of the soils can determine if the salt marsh act as sink or source of metals to the environment. In this sense, a previous greenhouse pot experiment, conducted with polluted soil from both salt marshes and with two plant species (*Sarcocornia fruticosa* (L.) A.J. Scott and *Phragmites australis* (Cav.) Trin ex Steudel), showed that an alternating flooding regime (wet-dry cycles) affected the concentrations of water soluble metals (María-Cervantes et al., 2010, 2011). These authors found that 3 months of flooding favoured the decrease of water soluble metal contents, due to the lowering of Eh, and that *S. fruticosa* favoured the mobilisation of metals in wastes from the Marina del Carmolí salt marsh (María-Cervantes et al., 2010). In contrast, in wastes from the Lo Poyo salt marsh, the concentrations of metals were lower in vegetated pots in relation to bare soil, probably due to the precipitation/adsorption of metals within plant rhizosphere (María-Cervantes et al., 2011). They also found that alternating flooding-drying conditions induced changes in Eh and pH that mobilised metals into the soil solution (María-Cervantes et al., 2010).

María-Cervantes et al. (2010) pointed that changes in pH/Eh, organic matter content, and microorganisms' activity play an important role in the biogeochemical processes within the salt marsh system. The latter may enhanced if eutrophic water reaches the marsh, due to the availability of substrates for microorganisms, leading to more dynamic systems in relation to metals and nutrients cycling. In this sense, previous field studies, conducted in the Marina del Carmolí salt marsh, have shown that the marsh receives eutrophic waters with high loads of N and P from agricultural and urban origin (Álvarez-Rogel et al., 2006; González-Alcaraz et al., 2012) and these nutrients are retained before surface waters overflow into the Mar Menor lagoon (Álvarez-Rogel et al., 2007). Jiménez-Cárceles and Álvarez-Rogel (2008) found that P retention by soil was the main mechanism involved in the removal of P from wastewater, being Ca and Mg compounds the most active in P retention, except in those areas polluted by metal mine wastes where Fe/Mn/Al oxihydroxides bound P were more abundant.

Based on these premises, two general objectives were established for this doctoral thesis:

1. To study the biogeochemical processes related to the dynamics of nitrogen and phosphorus in the soil-water-plant system of eutrophic wetlands polluted by metal mine wastes, in order to identify their possible role as sinks or sources of both nutrients.
2. To assay the effect of liming, presence of plants, and hydric/flooding conditions on metals mobility in the soil-water-plant system of eutrophic wetlands polluted by metal mine wastes, in order to identify the advantages and drawbacks of these conventional remediation treatments.

To achieve these objectives, three experimental studies were performed under greenhouse conditions. In the experiments, soils polluted by metal mine wastes from the Marina del Carmolí and the Lo Poyo salt marshes, and plant species typical of these environments (*Sarcocornia fruticosa* and *Phragmites australis*) were used. In addition, eutrophic water from local surface watercourses and eutrophic water synthetically prepared were employed. A brief description of the experiments is present as follows:

Experiment 1. Loam soils from the Marina del Carmolí salt marsh (pH~7.8) and sandy-loam soils from the Lo Poyo salt marsh (pH~6.2) were used. Three pot treatments were assayed: *S. fruticosa*, *P. australis*, and bare soil. The plants were grown for 6 months for its establishment and were flooded for 15 weeks with eutrophic water (NO_3^-~180 mg L^{-1}, PO_4^{3-}~7 mg L^{-1}). During the flooding period, pH and Eh were weekly measured and pore water samples were taken for the analysis of NO_3^-, PO_4^{3-}, and water soluble organic carbon concentrations. Before and after the flood stage, soil samples were taken for analysing total organic carbon, total nitrogen, and total phosphorus content, together with a phosphorus fractionation procedure. The results of this experiment are present in *Chapter 4* (Nitrogen dynamics under continuous flooding) and *Chapter 5* (Phosphorus dynamics under continuous flooding) of the thesis.

Experiment 2. Soils with fine texture from the Marina del Carmolí salt marsh (in this case of pH~6.4) and sandy soils from the Lo Poyo salt marsh (in this case of pH~3.1) were used. Each type of polluted soil was mixed with a lime amendment (dose of 20 g kg^{-1}), assaying two treatments -non-limed and limed soil-, and cuttings of *S. fruticosa* were planted in pots prepared with the soil treatments. Plants were irrigated for 10 months with eutrophic water (NO_3^-~370 mg L^{-1}, PO_4^{3-}~1 mg L^{-1}). Pore water samples were taken for the analysis of soluble metals concentrations (Al, Cd, Mn, Pb, and Zn) and plant survival, plant biomass, and plant metals content were determined. The results of this experiment are present in *Chapter 6* (Liming application in pot experiment) of this thesis.

Experiment 3. The same treatments of the second experiment were assayed in simulated soil profiles constructed with methacrylate cylinders (60 cm depth), together with non-vegetated profiles. Soil profiles were placed inside large, opaque containers and were irrigated for 5 months with the same eutrophic water than in the second experiment. Then, soil profiles were subjected during 18 weeks to a regime of rise and fall of the water level, using synthetic eutrophic water with high content of organic carbon (150 mg L^{-1} $C_6H_{12}O_6$, 100 mg L^{-1} meat extract). The water level in the containers was maintained at 20 cm below soil surface for five weeks and at 45 cm during the following five weeks, repeating this cycle once more along the experiment. Hence, the upper 20 cm (A horizons) were never under water, between 20 and 45 cm (C1 horizons) were flooded ten weeks with two alternating drying periods of five and three weeks, respectively, and the deeper 45 cm (C2 horizons) were always under water. At the three depths, pH and Eh were weekly measured and each two weeks pore water samples were taken for the analysis, among others, of soluble metal concentrations (Cd, Cu, Fe, Mn, Pb, and Zn). After the flooding period, soil profiles were opened, redoximorphic features were described, and soil samples were taken for a metal speciation procedure. The results of this experiment are present in *Chapter 7* (Iron dynamics in simulated soil profiles), *Chapter 8* (Metal dynamics in simulated slightly acidic soil profiles), and *Chapter 9* (Metal dynamics in simulated strongly acidic soil profiles) of this thesis.

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Part II

Chapter 4

Nitrogen dynamics under continuous flooding

Effects of eutrophic water flooding on nitrate concentrations in mine wastes

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Abstract

Salt marshes near urban, industrial, and mining areas are often affected both by heavy metals and by eutrophic water. The aim of this study was to assess and evaluate the main processes involved in the decrease of nitrate concentration in pore water of mine wastes flooded with eutrophic water, considering the presence or absence of plant rhizhosphere. Basic (pH~7.8) carbonated loam mine wastes and free-carbonated acidic (pH~6.2) sandy-loam mine wastes were collected from polluted coastal salt marshes of SE Spain which regularly receive nutrient-enriched water. The wastes were put in pots and flooded for 15 weeks with eutrophic water (dissolved organic carbon ~26 mg L^{-1}, PO_4^{3-} ~7 mg L^{-1}, NO_3^- ~180 mg L^{-1}). Three treatments were assayed for each type of waste: pots with *Sarcocornia fruticosa*, pots with *Phragmites australis* and unvegetated pots. Soluble organic carbon, nitrate, soluble Cd, Pb and Zn, pH, and Eh were monitored. But the 2nd day of flooding, nitrate concentrations had decreased between 70 and 90% (equivalent to 1.01-1.12 g of $N\text{-}NO_3^-$ m^{-2} day^{-1}) with respect to the content in the water used for flooding, except in unvegetated pots with acidic wastes. Denitrification was the main mechanism associated to the removal of nitrate. The role of vegetation in improving the rhizospheric environment was relevant in the acidic wastes because higher sand content, lower pH, and higher soluble metal concentrations might strongly hinder microbial activity. Hence, revegetation of salt marshes polluted by acidic sandy mining wastes might improve the capacity of this type of environments to act as a green filter against excessive nitrate contents flowing through them.

Keywords: Denitrification, Mar Menor, Microcosms, Redox potential, *Phragmites australis*, *Sarcocornia fruticosa*

4.1. Introduction

Wetlands are often transitional zones between aquatic and terrestrial ecosystems, and are thus prone to receive polluted sediments and a wide array of dissolved substances through erosion and water runoff. Contamination by trace metals is one of the main impacts on aquatic environments and many salt marshes near urban, industrial, and mining areas are affected both by heavy metals and by eutrophic water. Although such systems are among those most affected by pollution, they are at the same time capable of transforming and/or reducing harmful compounds, acting as green filters and thus contributing to water quality improvement (Mitsch and Gosselink, 2000).

Among dissolved nutrients, nitrogen plays a major role in coastal eutrophication (Elser et al., 1990). Field data and studies under experimental conditions have shown that vegetated and de-vegetated wetlands show a different response when it comes to sequestering large amounts of nitrate and other nutrients from their environment (Romero et al., 1999; Teal and Howes, 2002). Nitrogen in wetlands is removed through two main biologically mediated pathways: uptake by plants and denitrificacion (White and Reddy, 2003; DeLaune et al., 2005). Therefore, denitrification is an important component of the N cycle in wetlands, as it lessens the impact of an excessive N load on downstream aquatic systems (Saunders and Kalff, 2001). Denitrification is negatively affected by the existence of mine wastes, since heavy metals are harmful for micro-organisms and hence disrupt biogeochemical cycles (Konopka et al., 1999). The negative effect of trace metals on nitrogen transformation pathways in aquatic habitats has been studied extensively (Reber, 1992; Sandaa et al., 1999), but, to our knowledge, there are not any studies on the response of wetlands to high loads of nitrate when the upper soil horizons are formed by mine tailings. Both the presence of mine wastes in soils and the flow of eutrophic water strongly affect the coastal marshes of the Mar Menor lagoon, SE Spain, (Conesa and Jiménez-Cárceles, 2007).

The Mar Menor lagoon is one of the largest coastal lagoons (135 km^2 surface) of the Mediterranean basin. It receives water runoff from streams originating at the intensive agricultural area of Campo de Cartagena, which have led to the lagoon being declared a sensitive area subject to eutrophication in June 2001 under European Directive 91/271/EEC and also to Campo de Cartagena being declared a zone vulnerable to nitrate from agricultural origin in December 2002 under Directive

91/676/EEC. Moreover, the existence of abandoned dumps in the nearby mining area of La Unión-Sierra de Cartagena, has caused metal polluted wastes have been transported by several watercourses and deposited on some Mar Menor coastal areas for more than fifty years. As a consequence, these soils are buried by wastes on extensive zones, and although most of them have been colonised by vegetation, others remain unvegetated. Previous field studies in these salt marshes have found high content of Cd, Pb, and Zn in the soil (Jiménez-Cárceles et al., 2008). We also found that the marshes receive eutrophic water with a high load of nitrogen and phosphorous from agricultural and urban origin (Álvarez-Rogel et al., 2006) and these nutrients are retained before surface water overflows into the Mar Menor lagoon (Álvarez-Rogel et al., 2007a). Hence, knowing that these salt marshes have an important role in reducing the eutrophication of the lagoon, it is important to increase our knowledge about the mechanisms influencing nutrient removal in order to implement the best management practices. Our study can also have a broader interest, because eutrophication and metal pollution are two worldwide wetland problems.

Based on the field studies cited before, an experimental set up was designed in order to best ascertain the possible mechanisms for reducing nitrate concentrations in marshes polluted by mining wastes under vegetated and de-vegetated conditions. Microcosms and mesocosms are extremely useful scenarios for controlled investigations (Fraser and Keddy, 1997) and have previously been used to test soil-plant systems' ability to treat wastewater (Gersberg et al., 1986; Coleman et al., 2001). Over the last years, the use of these methodologies has been common, particularly in wetland science, in studies on the effects of pollutants on biogeochemical cycles and on the effects of nutrients on ecosystem dynamics. Although extrapolation of greenhouse data to field conditions is difficult, Adey and Loveland (1991) argued that microcosms contain the same relative forcing functions (sunlight, water, and nutrient inflow) and main components (plants, sediments, and water) as those in natural wetlands which allow for self-organisation of the system over a relatively short period of time. In that sense, data obtained from experiments such as ours give a reasonable model of a natural system in the time scale, in spite of reducing its complexity.

The aim of this study was to evaluate under experimental conditions the main processes involved in decreasing nitrate concentration in pore water of salt marshes affected by basic and acidic mining wastes, when the soil is flooded for extended periods, with and without presence of plant rhizosphere. Based on our results we propose some guidelines for management of salt marshes affected by mine wastes and eutrophic water.

4.2. Material and methods

4.2.1. Preparatory phase

This phase consisted of the production of enough living plants to develop the experiment of prolonged flooding.

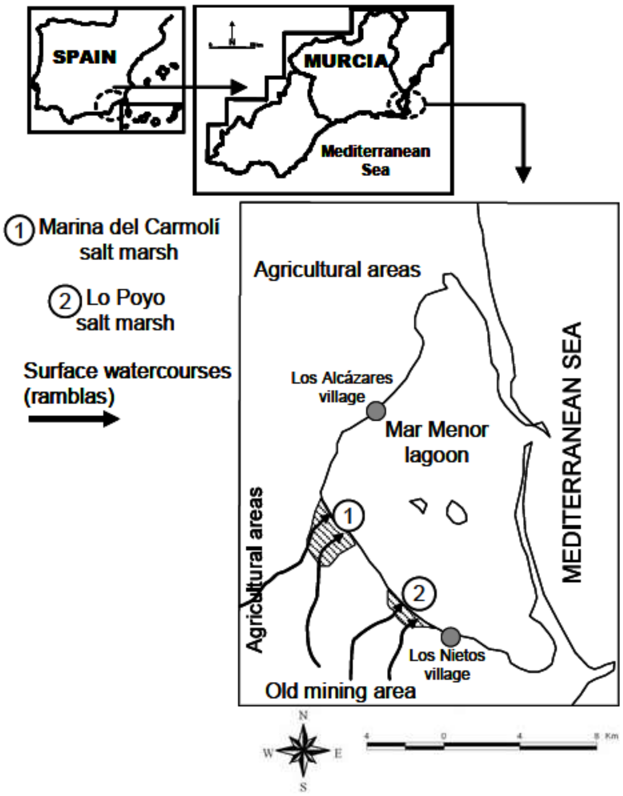

Figure 4.1. Location map of the sampling sites in SE Spain.

Wastes were collected from bare areas of the Marina del Carmolí (carbonated wastes with basic pH) and Lo Poyo (de-carbonated wastes with acidic pH) salt marshes (Figure 4.1) (Table 4.1). Waste samples were then taken to a greenhouse and homogenised separately, but not sieved. After that, 13.5 cm x 14 cm pots were filled up to ¾ of their capacity (~1.5 kg of soil per pot).

Table 4.1. Main characteristics of the mining wastes used in the experimental set up. The values are mean ± SD (n = 9). EC: electrical conductivity; n.d.: not detected.

	Salt marsh	
	Marina del Carmolí	Lo Poyo
pH	7.8 ± 0.2	6.3 ± 0.6
EC (dS m^{-1})	42 ± 5	37 ± 8
Total organic carbon (g kg^{-1})	12.4 ± 0.7	3.5 ± 0.7
Total nitrogen (g kg^{-1})	0.64 ± 0.13	0.37 ± 0.04
Total phosphorus (g kg^{-1})	0.64 ± 0.04	0.36 ± 0.01
Sand (%)	47.7 ± 3.4	83.8 ± 0.2
Silt (%)	39.1 ± 4.5	11.6 ± 0.2
Clay (%)	13.2 ± 4.2	4.6 ± 0.1
Calcium carbonate (g kg^{-1})	42.5 ± 19.5	n.d.
Total Cd (mg kg^{-1})	36.8 ± 2.9	5.9 ± 1.2
Total Pb (mg kg^{-1})	6746 ± 646	5640 ± 221
Total Zn (mg kg^{-1})	15,320 ± 1282	5299 ± 745

Rhizomes of *Phragmites australis* (Cav.) Trin ex Steudel and cuttings of *Sarcocornia fruticosa* (L.) A. J. Scott were collected from non-polluted areas of the Marina del Carmolí salt marsh. This material was washed with deionised water and roots and rhizomes were submerged in a solution of indolebutyric acid at 50% in order to facilitate root development. They were then planted in pots prepared with mining wastes. At this stage, a large number of pots were planted with just one individual in order to guarantee the survival of enough living plants for the experiment. Pots were spatially distributed at random in the greenhouse and irrigated twice a week during six months until plants were fully established (based on visual observation). Pots without plants were handled in the same manner.

The water used was collected from two eutrophic surface watercourses flowing into the area and stored in an opaque tank to prevent its degradation. Its characteristics appear in Table 4.2.

Table 4.2. Main characteristics of the flooding water (n = 3). EC: electrical conductivity; WSOC: water soluble organic carbon.

	Irrigating water (average ± SD)
pH	7.9 ± 0.1
EC (dS m^{-1})	13 ± 2
WSOC (mg L^{-1})	26 ± 2
NO_3^- (mg L^{-1})	183 ± 21
PO_4^{3-}(mg L^{-1})	6.9 ± 1.1
Cd (µg L^{-1})	0.1 ± 0.1
Pb (µg L^{-1})	1.9 ± 0.7
Zn (µg L^{-1})	139 ± 71

Just before starting the flooding period aliquots were collected from the pots and were analysed to characterise wastes at the zero-point of the experiment (Table 4.1). Redox potential in the pots was 369 ± 52 mV at that moment.

4.2.2. Experimental set up

The experimental design was a two (types of mining wastes) by three (plant types) factorial combination (n = 4 for each treatment).

Mining wastes (Table 4.1) had two “levels”: basic carbonated loam wastes from Marina del Carmolí; and acidic free-carbonated sandy-loam wastes from Lo Poyo. Basic wastes were richer in nutrients (with 3.55 fold more total organic carbon, 1.73 fold more total nitrogen, and 2.75 fold more total phosphorous) than acidic wastes. Cd, Pb, and Zn contents were extremely high in both types of wastes, with higher concentrations of total Cd and Zn in basic wastes.

Plant type had three “levels”: bare pots, pots with *P. australis*, and pots with *S. fruticosa*. We chose these species because they are among the most abundant in saline Mediterranean wetlands, especially in the coastal salt marshes of the Mar Menor lagoon where their expansion is being favoured by the impacts of anthropogenic inputs of eutrophic water (Álvarez-Rogel et al., 2007b).

Each pot was placed inside a 16 cm x 17 cm container which was filled with the water collected from the study area (Table 4.2) until the pots were submerged 5 cm, maintaining water level during 15 weeks, from October 2006 to February 2007. To compensate for water loss by evaporation and plant absorption, the level in the containers was maintained by adding water when necessary. After 15 weeks of flooding, pots were left to dry for two weeks and soil samples were collected and analysed for total nitrogen and total organic carbon.

Two 10 cm vertical porous Rhizon ® type samplers were installed in each pot to extract pore water every two weeks during the experiment. In addition, pH and Eh were measured weekly from each pot by using a previously calibrated Crison 507 portable Eh/pH-meter. Measurements of Eh were corrected according to Vepraskas and Faulkner (2001) by adding 200 mV to pot voltage (the value of the Ag/AgCl reference electrode). Temperature, light, and humidity in the greenhouse were not controlled. However, the temperature inside the pots where recorded inserting thermometers and performing periodical random monitoring among pots. The data were (in ºC, average ± SD): 21.3 ± 0.2 October 2006, 11.3 ± 0.1 November 2006, 14.9 ± 0.4 January 2007, and 21.9 ± 1.5 February 2007.

Soil solution samples were stored in plastic vials and electrical conductivity was measured immediately. After that, samples were acidified at pH~2 with a drop of concentrated sulphuric acid, hermetically closed, and frozen at -20ºC until analysis.

4.2.3. Sample preparation and analysis

4.2.3.1. Wastes

Samples collected from the pots were air-dried and passed through a 2 mm mesh and an aliquot was crushed in an Agata mortar.

Particle size distribution was determined in the 2 mm fraction using the Bouyoucos Hydrometer Method (Gee and Bauder, 1986; Filgueira et al., 2006).

Total organic carbon (TOC), total nitrogen (TN), total calcium carbonate ($CaCO_3$), and total metals were determined in the crushed aliquots. TOC was measured in an automatic analyzer (TOC-V_{CSH}, Shimadzu Corporation, Tokio, Japan). TN was measured by the Kjeldahl method (USDA, 1996). $CaCO_3$ content was determined by the Bernard calcimeter method, with 4M HCl.

Total metal contents were determined after wet digestion in a microwave according to method 3052, proposed by the United States Environmental Protection Agency (USEPA, 1996) using HNO3 and HF (3:1 v/v). Pb and Zn were measured in a Unicam 969 Atomic Absorption Spectrometer and Cd in an Inductively Couple Plasma Mass Spectrometer (ICP-MS Agilent 7500a). The accuracy of measurements was compared with European Reference Material CRM027-050. Results were in agreement with certified values: certified Cd = 12 ± 1.7 mg kg^{-1}, measured Cd = 10 ± 1.27 mg kg^{-1}; certified Pb = 51.9 ± 9.28 mg kg^{-1}; measured Pb = 40.3 ± 7.95 mg kg^{-1}; certified Zn = 51.3 ± 7.76 mg kg^{-1}; measured Zn = 51.1 ± 4.64 mg kg^{-1}.

4.2.3.2. Water samples

Electrical conductivity (EC) was measured at 25 ºC with a Crison Basic 30 conductimeter. Soluble Cd, Pb, and Zn, water soluble organic carbon (WSOC), and nitrate were determined in the acidified samples.

Soluble Cd, Pb, and Zn were measured in the samples taken at the 8th week in order to achieve a better understanding of the different responses of the soil-plant system in the two types of mine wastes. Those metals were measured with an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent 7500a).

WSOC was measured using a Total Organic Carbon Analyzer (TOC-V_{CSH} Shimadzu). NO_3^- were measured with a V/UV Spectrometer at λ = 220 nm and the interference due to organic matter was corrected by measuring absorbance at λ = 275 nm (AOAC, 1975).

4.2.4. Statistical methods

Repeated ANOVA measurements were carried out in order to compare the evolution of pH, Eh, EC, WSOC, and nitrate over time and between different treatments for each type of waste. When data did not fulfil the sphericity requirement for Maulchly's sphericity test, univariate F statistics using a corrector index of epsilon were applied, based on either Greenhuse-Geisser, Huynh-Feldt, or Lower-bound corrections (SPSS Inc, 2006).

Factors included in the analysis were the following: 1) an inter-subjects factor (plant species, with three levels, *S. fruticosa*, *P. australis*, and bare soil) and 2) an intra-subject factor (time -the repeated factor, with seventeen levels corresponding to 17 weeks of the experiment) affecting all subjects. The dependent variables were pH, Eh, EC, and concentration of WSOC and nitrate. When species was a significant factor, Bonferroni post-hoc tests were performed to identify differences. Also, significant "species vs. time" ANOVA interactions were further separated by simple effects comparisons between pairs of means using a Bonferroni adjustment for multiple comparisons (SPSS Inc, 2006). Total nitrogen and total organic carbon contents, before and after the flooding period, were compared with a non-parametric test (U Mann-Whitney).

4.3. Results

4.3.1. Pore water characteristics

4.3.1.1. pH, Eh, EC, water soluble organic carbon (WSOC) and soluble Cd, Pb and Zn

In acidic wastes, pH significantly increased during the experiment (effect of time, $F_{1.10} = 9.394$; $p = 0.012$) regardless the species (time vs. species not significant), although the ranges of values for each species were statistically different (effect of species, $F_{2.10} = 16.819$; $p = 0.001$). However, in basic wastes, temporal evolution of pH was not significant (effect of time) regardless the species (time vs. species), although again the species had different ranges of pH values (effect of species, $F_{2.9} = 22.922$; $p = 0.0000$). The Bonferroni post-hoc test revealed that vegetated pots had significantly lower pH than bare pots in both types of wastes ($p \leq 0.030$ for El Carmolí, and $p \leq 0.003$ for Lo Poyo).

The increase observed in acidic wastes led them to become neutral-basic after 17 weeks, with increases from 6.62 ± 0.43 to 7.32 ± 0.35 in bare pots, from 6.01 ± 0.14 to 7.47 ± 0.69 in pots with *S. fruticosa* and from 6.11 ± 0.25 to 7.12 ± 0.35 in pots with *P. australis* (Figure 4.2).

In basic wastes without plants, pH had increased from 7.86 ± 0.07 to 8.25 ± 0.14 in the 5th week and remained higher than in vegetated pots until the end of the experiment (Figure 4.2). The opposite tendency took place in pots with *S. fruticosa*, with lower pH values than in the other treatments, reaching a minimum average value of 7.37 ± 0.17 in the 11th week. The treatment with *P. australis* showed an intermediate behaviour.

For both types of wastes, drying in the last two weeks caused an increase of pH in pots with *S. fruticosa* and a decrease in the other two treatments.

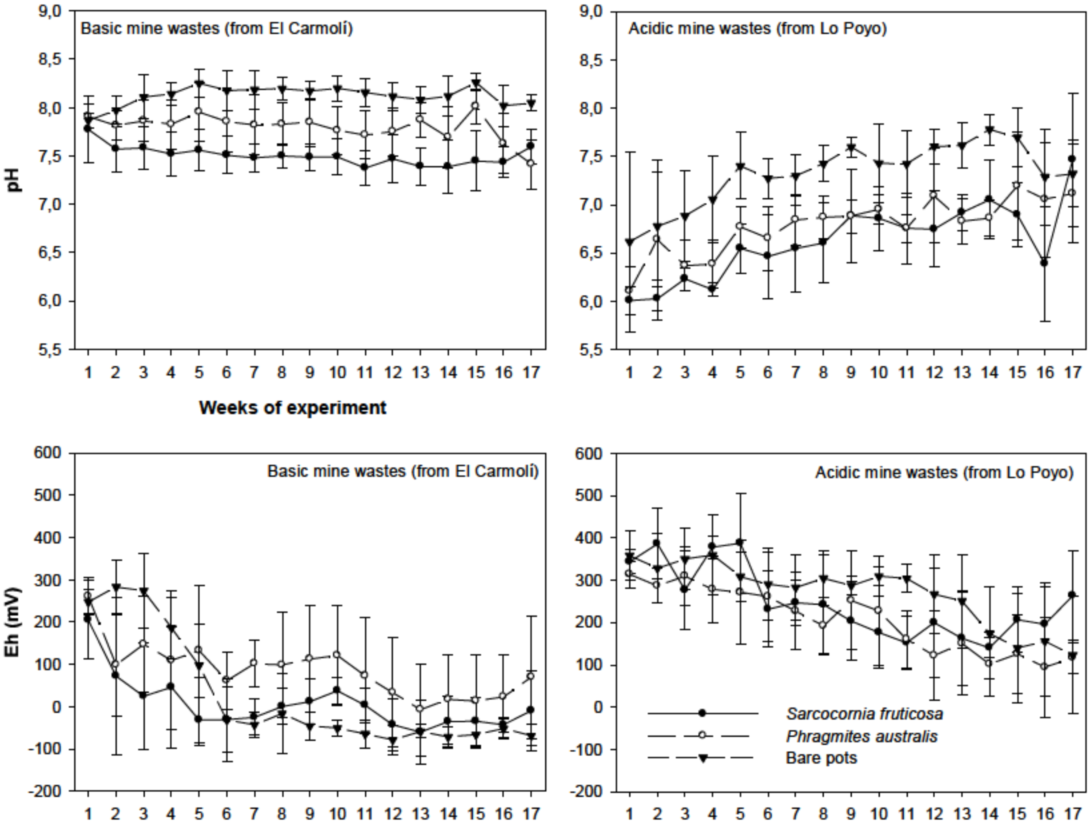

Figure 4.2. Evolution of pH and Eh in pore water of acidic (from Lo Poyo salt marsh) and basic (from El Carmolí salt marsh) mine wastes flooded with eutrophic water. Bars represent ± 1SD (n = 4).

Redox potential (Figure 4.2) significantly tended to fall throughout the flooding period for both types of wastes (effect of time, $F_{1.10} = 8.845$; $p = 0.014$ for acidic; $F_{1.8} = 11.021$; $p = 0.001$ for basic), without effects neither from species nor from the time vs. species interaction. Bare pots with wastes from El Carmolí tended to be anoxic (Eh<+100 mV, Otero and Macías, 2003) from the 5th week onwards and they had the lowest values of redox potential of all three treatments (-68.5 ± 25.2 mV) at the end of the experiment. Pots with *P. australis* had higher Eh values than the other two treatments most of the time. Wastes tended to be anoxic for all treatments from the 6th week onwards.

Acidic wastes had an initial Eh value close to +350 mV (oxic conditions according to Otero and Macías, 2003) in the three treatments, showing a much more gradual decline than in the case of basic wastes. In vegetated pots, values fell below +300mV in the 6th-7th week while in bare pots it remained about +300 mV until the 11th week.

During the drying period, Eh tended to increase in vegetated pots and decrease in bare pots for both types of wastes.

The evolution of electrical conductivity over time was not significant for neither type of waste. Ranges were similar in all cases, with initial values of about 30-45 dS m^{-1} and final values of about 25-30 dS m^{-1} (data not shown).

WSOC significantly decreased in basic (effect of time, $F_{1.7}$ = 51.049; p = 0.0000) and acidic wastes (effect of time, $F_{1.9}$ = 40.660; p = 0.0000), regardless of species and of the time vs. species interaction. Initial WSOC contents were higher in basic wastes (between ~50 to ~80 mg L^{-1}) than in acidic ones (between ~30 to ~40 mg L^{-1}) and the decrease was about 4-fold in the former and about 2-fold in the latter (Figure 4.3).

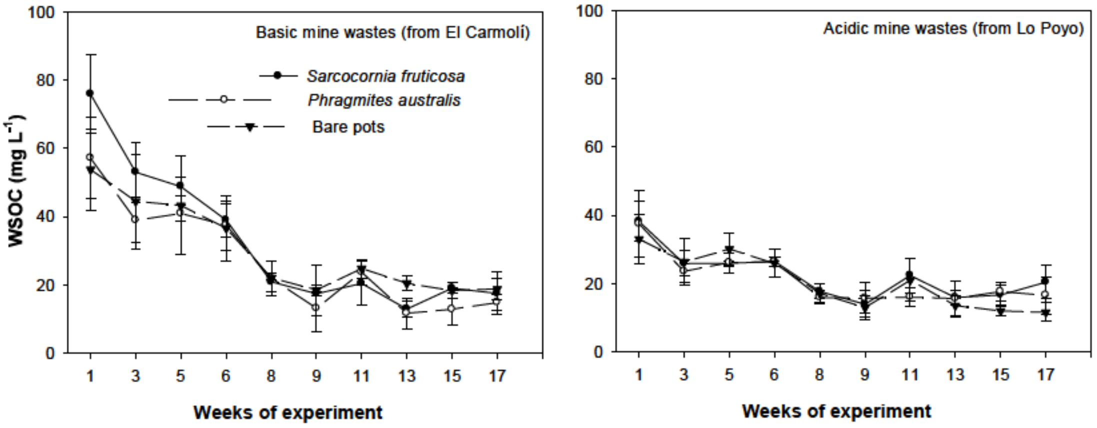

Figure 4.3. Evolution of the concentration of water soluble organic carbon (WSOC) in pore water of acidic (from Lo Poyo salt marsh) and basic (from El Carmolí salt marsh) mine wastes flooded with eutrophic water. Bars represent ± 1SD (n = 4).

Contents of Cd, Zn, and Pb in pore water (Table 4.3) were largely higher in acidic wastes, with Zn as the most abundant metal. In those treatments from El Carmoli, unvegetated pots showed lower metal concentrations than vegetated ones, while in case of acidic wastes from Lo Poyo, the opposite happened.

Table 4.3. Metals in pore water at the 8th week of experiment. Values are mean ± SD (n = 4).

	Pots with *S. fruticosa*	Pots with *P. australis*	Bare pots
Concentrations (µg L^{-1})			
Cd			
Acidic wastes	368 ± 23	237 ± 133	433 ± 135
Basic wastes	51 ± 31	18 ± 3	16 ± 9
Zn			
Acidic wastes	32,698 ± 10,574	26,995 ± 13,680	69,905 ± 43,871
Basic wastes	5145 ± 3001	2169 ± 1393	530 ± 167
Pb			
Acidic wastes	72 ± 26	47 ± 27	70 ± 17
Basic wastes	27 ± 7	6.6 ± 5.5	2.4 ± 1.3

4.3.1.2. Nitrate

Two days after flooding, nitrate concentration in pore water of pots from El Carmolí was between 15 and 35 mg L^{-1}, which represented a reduction from 80 to 90% with respect to the concentration in water used for flooding (Figure 4.4). Throughout the successive weeks of flooding, concentrations experienced some variation without ever rising above 60 mg L^{-1}, reaching values lower than 1 mg L^{-1} in certain weeks in pots with *S. fruticosa*. Changes in concentration from the second day onwards were not significant; neither for time nor for the time vs. species interaction (that is, temporal changes were not significant regardless the species). However, the effect of species was significant ($F_{2.9}$ = 8.722; p = 0.008) and Bonferroni post-hoc tests revealed that pots with *S. fruticosa* had lower nitrate concentrations than bare pots (p = 0.007).

In wastes from Lo Poyo, nitrate concentrations in treatments with plants had decreased to about 30-50 mg L^{-1} after two days of flooding, which represented 70-80% lower than concentrations in irrigation water. Repeated ANOVA measurements indicated that the decrease of nitrate concentration was significantly affected by time ($F_{1.7}$ = 13.680, p = 0.008), time vs. species ($F_{2.7}$ = 5.799; p = 0.033) and species ($F_{2.7}$ = 43.893, p = 0.000). Bonferroni post-hoc tests showed that pots with *S. fruticosa* (p = 0.001) and pots with *P. australis* (p = 0.0000) were different from bare pots.

Concentration in the latter was above 200 mg L^{-1} on the second day, falling below 100 mg L^{-1} after the period of flooding.

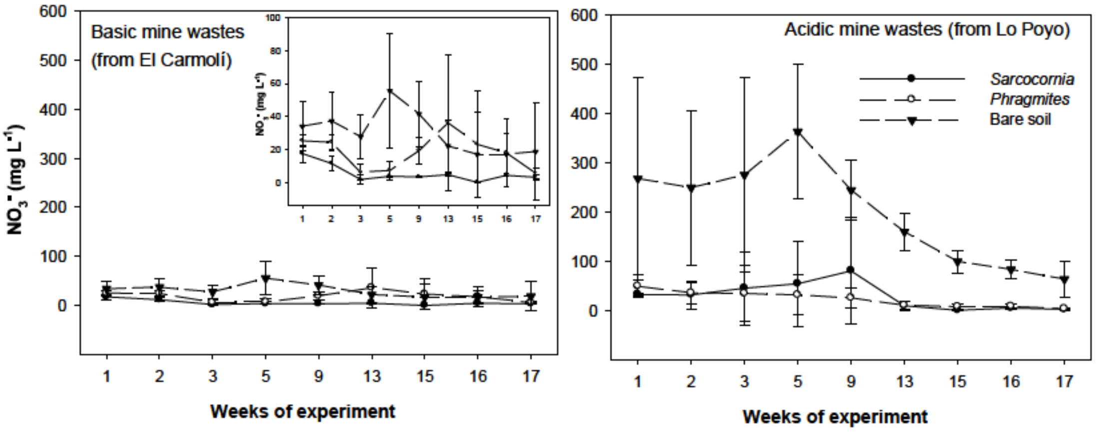

Figure 4.4. Evolution of the concentration of nitrate (NO_3^-) in pore water of acidic (from Lo Poyo salt marsh) and basic (from El Carmolí salt marsh) mine wastes flooded with eutrophic water. Bars represent ± 1SD (n = 4).

4.3.2. Changes in total organic carbon (TOC) and total nitrogen (TN) in wastes

Although changes in the concentration of TOC and TN in pots during the experiment were not significant (U-Mann Whitney tests, $p>0.05$), probably due to the high standard deviation, results were different for each type of waste (Figure 4.5). Initial TOC contents in basic wastes were of about 11-13 g kg^{-1}, which had fallen to values between 3 and 6 g kg^{-1} after the 17 weeks. However, in pots with acidic wastes, the initial contents (about 3–4 g kg^{-1}) had hardly increased during the experiment in pots with plants but not in bare pots (Figure 4.5).

Pots with wastes from El Carmolí had initial concentrations of TN of about 0.5-0.7 g kg^{-1} with a reduction of about 10-20% in treatments with plant, and of about 40% in bare pots at the end of the experiment (Figure 4.5). In turn, the initial content of nitrogen in wastes from Lo Poyo was of about 0.37 g kg^{-1} which had hardly changed after 17 weeks.

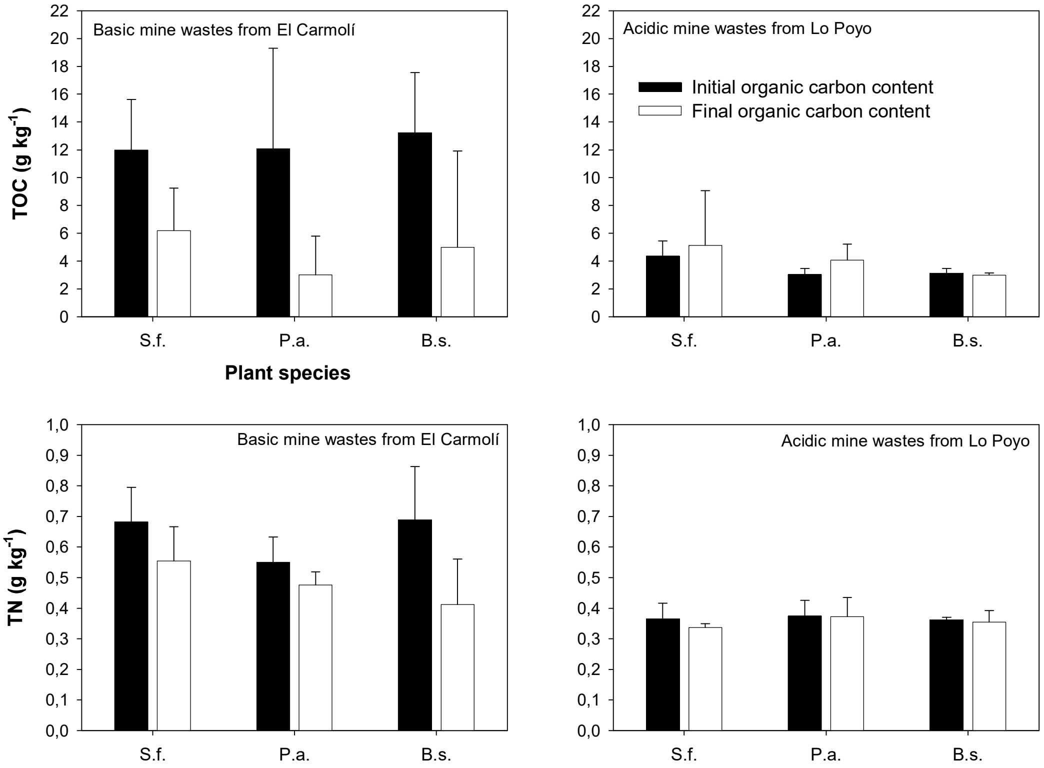

Figure 4.5. Initial and final concentrations of total organic carbon (TOC) and nitrogen (TN) in acidic (from Lo Poyo salt marsh) and basic (from El Carmolí salt marsh) mine wastes flooded with eutrophic water. Bars represent ± 1SD (n = 4). S.f.: *Sarcocornia fruticosa*; P.a.: *Phragmites australis*; B.s.: Bare soil.

4.4. Discussion

The significant negative Spearman rank correlations between pH and Eh in wastes from Lo Poyo (r= -0.318 $p<0.05$ in *S. fruticosa*, r= -0.260 $p<0.05$ in *P. australis*; and r= -0.310 $p<0.01$ in bare pots) suggest that the increase of pH was coupled to the decrease of Eh due to H^+ consumption during reduction processes (Ponnamperuma, 1972), but it is clear that the use of water with pH~7.7 must also have contributed to this increase. In pots filled with wastes from El Carmolí, Eh vs. pH correlation was not significant, probably due to the buffer capacity of this type of waste, in which the concentration of calcium carbonate was 42.5 ± 19.5 g kg^{-1} and initial pH~7.8.

In both types of wastes, there were significant differences in pH between pots with and without plants, with lower values in the former for most of the experiment, which can be explained by several factors. Some authors have found a more oxidant environment in rhizospheric soils due to the radial loss of oxygen (ROL) from the roots of wetland plants (Wright and Otte, 1999; Armstrong et al., 2000; Jacob and Otte, 2004), which may contribute to a decrease of pH as a consequence of the oxidation of metal sulphides (Otero and Macías, 2002; Otero et al. 2009). The higher Eh values in wastes from El Carmolí planted with *P. australis* (Figure 4.2) seem to be in accordance with that finding, possibly due to the existence of aerenchyma -highly developed in this species (Armstrong and Armstrong, 1987)- which would increase ROL and hence decrease pH. The lower development of aerenchyma in dicotyledonous plants such as *S. fruticosa* would reduce the effect of ROL (Reboreda and Caçador, 2007) while other factors -such as excretion of organic acids (Dakora and Phillips, 2002), favoured by the large quantity of small roots that this species develops (author's visual observation)- could be more important in pH reduction. In basic wastes from El Carmolí a slightly more acidic pH of vegetated treatments (Figure 4.2) could have led to a greater solubility of heavy metals. In wastes from Lo Poyo, this effect was not observed probably due to the own acidic pH of this type of mining wastes.

When a soil is inundated or saturated, respiring microorganisms quickly consume O_2 that is dissolved in the water or trapped in the soil. This causes to organic matter consumption to occur via anaerobic pathways using other terminal electron acceptors such as nitrate, oxides of iron and manganese, and sulphates (Craft, 2001). This process leads to a decrease in the redox potential of flooded soil.

During our experiment there were two possible sources of organic carbon for microorganisms: soil organic matter and dissolved organic compounds added with the water used for flooding. We did not identify carbon from both sources, but due to unfavourable conditions of mining wastes for microbial activity, it is expected that microbes consume more easily soluble organic compounds from the eutrophic water. The latter agrees with the significant decrease of WSOC in pore water (70% in pots with basic wastes and 50% in pots with acidic wastes), which correlates with the decrease in Eh for both types of wastes ($r= 0.406$ $p<0.01$ for El Carmolí and $r = 0.444$ $p<0.01$ for Lo Poyo). This relation between Eh and soluble organic carbon is well known and has been observed in many other studies (e.g. Allen et al., 2002). Soil organic matter mineralisation was relatively notable in basic wastes and could have contributed to the accumulation of WSOC observed at Eh below 0 mV (Figure 4.6). This effect was not observed in acidic wastes where organic matter mineralisation was negligible and Eh never decreased below 0 mV.

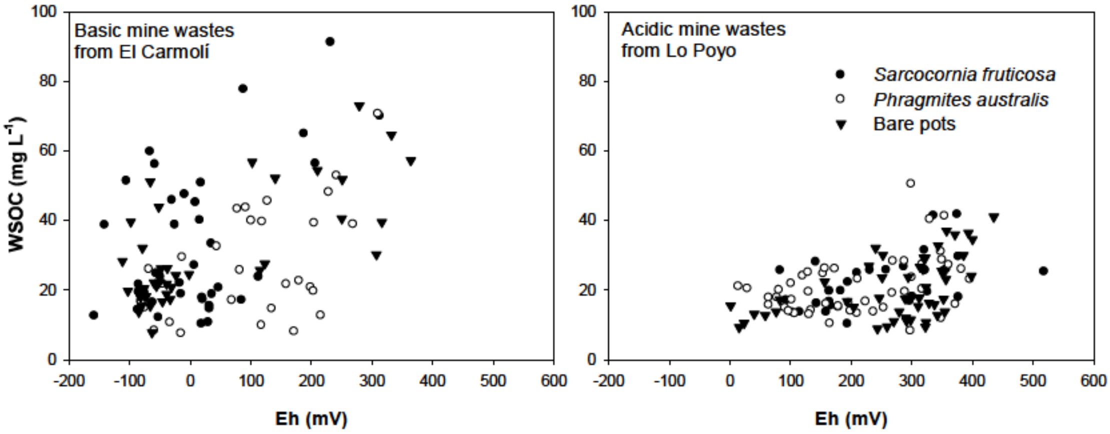

Figure 4.6. Changes in concentration of water soluble organic carbon (WSOC) vs. changes in Eh in pore water of acidic (from Lo Poyo salt marsh) and basic (from El Carmolí salt marsh) mine wastes flooded with eutrophic water.

Lower pH and organic carbon content and higher concentrations of water soluble metals in wastes from Lo Poyo would hinder microbial activity, hence leading to a lower consumption of organic matter and slowing the decline in Eh. In fact, while there was a strong decrease of TOC in pots with wastes from El Carmolí after 17 weeks, the concentration increased or remained constant in wastes from Lo Poyo (Figure 4.5). In a field study at the Lo Poyo salt marsh, Carrasco et al. (2005) found that the phosphatase, dehydrogenase, and protease activities were lower in acidic zones more contaminated by heavy metals than in basic areas with less pollution. The latter may be ascribed to either the direct effect of toxic metals or –indirectly- to the scarce synthesis of these enzymes associated with the inhibition of microbial growth. Sakadevan et al. (1999) found that the addition of Cd, Cu, and Zn significantly inhibited denitrification in surface wetland sediments receiving wastewater. Magalhaes et al. (2007) tested the effect of Cu, Cr, Zn, Cd, and Pb in the denitrification process in subtidal and intertidal sediments from the Duero Estuary. These authors found that the zones more likely to be affected by metals were the ones with lower organic matter content and sandier texture, with total inhibition of denitrification at high concentrations of Cr, Cu, and Zn. Hattori (1992) demonstrated that metals inhibit microbial respiration to a higher extent in wastes with less organic matter, as compared to what happens in rich organic wastes. Similarly, Maliszewska et al. (1985) found that metals were, in general, less toxic to microorganisms in fine-texture rather than in sandy wastes, attributing this finding to a lower absorption capacity of the latter. According to this, the lower organic matter content and higher sand content in wastes from Lo Poyo is bound to have enhanced the toxicity of metals.

The evolution of nitrate concentrations was in agreement with previous findings. After two days of flooding in pots with wastes from El Carmolí, all treatments had reduced the concentration of added nitrate in more than 80%. Since this decline was very fast the main mechanism involved should be denitrification. The similar results obtained for bare and vegetated pots (Figure 4.4) indicate that the rhizosphere was probably not a decisive factor in the latter process. However, the significantly lower nitrate concentration in *S. fruticosa* seems to indicate a more active contribution of this species to nitrate removal either by absorption or by favouring the microbial activity in the rhizosphere, according to a more developed root system than in *P. australis* (author's visual observation).

However, in pots with wastes from Lo Poyo nitrate concentration had decreased only in treatments with plants (decline exceeding 70%). This suggests a leading role of plants in nitrate removal in this type of wastes, and at the same time that the intensity of the process is geared rather towards denitrification -favoured in a root environment- than to uptake by plants.

Although we did not have data for direct measurements of denitrification, this process is considered to be a major removal mechanism for nitrogen in constructed wetlands (Vymazal, 2007). van Oostrom (1994) indicated that nitrate removal by plant uptake is fairly low, and unless plants are harvested and the biomass is removed from the system, denitrification is the main significant mechanism for nitrogen removal. Xue et al. (1999) measured denitrification *in situ* in a constructed wetland and found that approximately 90% of nitrate nitrogen lost was denitrified while 10% was stored in wetland plants. The initial Eh values (lower than +350 mV) and temperatures (about 25 ºC) in our pots support our interpretation since those were optimal conditions to provoke denitrification (Vymazal, 2007; Spiels and Mitsch, 2000). Our results agree with Sakadevan et al. (1999) who found that most denitrification occurs within the first 24 hours after the addition of nitrate to a sediment-water system.

Relationships between external supply and uptake of nitrogen by wetland plants are difficult to detect in sediments that are periodically flooded, and where nitrification-denitrification reactions are likely to remove a significant amount of inorganic nitrogen

(Patrick et al., 1989). However, macrophytic plants have the capacity to not only bind certain amounts of nutrients within their system, but also to create an environment conductive to decrease nutrients (Brix, 1997). For example, their roots provide a structure for microorganisms to adhere to and perform the processes necessary for nutrients' transformation. Besides, the increase of downward oxygen flux facilitates a rich microbial community that will foster sedimenting of organic carbon and coupling of nitrification/denitrification (Caffery and Kemp, 1990).

In bare pots with acidic wastes, nitrate concentration did not get lower than 200 mg L^{-1}, until after 13 weeks of flooding, reaching 100 mg L^{-1} in the 15th week. This reduction in nitrate content coincided with the decrease of Eh below +200 mV, a potential that may be effective for nitrate removal by denitrification. In wetlands, denitrification rates are generally limited by the availability of NO_3^- and organic carbon (Cornwell et al., 1999; Greenan et al., 2006). Various studies have shown that when sufficient NO_3^- is available to induce the denitrifying activity, an increase in soil organic matter increases the soil's denitrification rate (Bijay-Singh et al., 1988; Gale et al., 1993). Hence, it would be interesting to test if higher organic matter content might increase denitrification rates in acidic wastes (such as those from Lo Poyo).

On the basis of the size of the pots and the decline of nitrate concentration in the first two days, we calculated rates of nitrate removal between 1.01 (bare pots, except for acidic wastes) and 1.12 (*S. fruticosa* pots) g $N\text{-}NO_3^-$ m^{-2} day^{-1}. These rates are in the range (0.003 to 1.02 g N m^{-2} day^{-1}) suggested by Vymazal (2007) and were lower than the rates found by van Oostrom (1995) in planted wetlands (up to 5.5 g N m^{-2} day^{-1}).

4.5. Conclusions and recommendations for management

According to the results of our experiment, denitrification seems to be the main mechanism associated to nitrate release in mining wastes flooded with eutrophic water. In fine textured basic wastes the process is intense regardless of the existence of plant rhizosphere, while the role of vegetation is more relevant in sandier acidic wastes. A better environment for microorganisms in basic wastes would allow for microbial activity in any case. However, due to more unfavourable conditions in acidic wastes, the rhizosphere would become more decisive for improving these conditions and promoting microbial activity. Hence, revegetation of the acidic sandier zones of salt marshes might improve the capacity of this type of environment to act as a green filter against excessive nitrate contents flowing through them.

Nevertheless, the introduction of vegetation in these marshes must be planned as part of regeneration projects in which the role as green filters should be considered jointly with the reduction of the risk associated with the high heavy metals content. For this purpose, management practices such as use of amendments have to be considered. For example, liming increases pH and decreases metals solubility (Aguilar et al., 2004) facilitating plant growth (Conesa et al., 2007). This option would be relatively cheap and easy to implement due to the large amounts of residues from marble industry that are produced in nearby areas of the Mar Menor. In fact, researches in progress (González-Alcaraz et al., 2009) have shown a greater growth of *S. fruticosa* and a lower soluble metal content in acidic wastes from Lo Poyo salt marsh amended with marble industry residue than wastes without liming. Dosage of lime amendment should be calculated according to the acidity and neutralisation capacity of each waste. Flooding regime and water flow should also be taken into account since a slow water circulation is necessary in order to achieve a low oxygen concentration for denitrification. In addition, a stagnant water regime would reduce the risk of metal leaching and favour the precipitation of metal sulphides as showed by María-Cervantes (2009). Details about how and when to act are decisions that should be taken after analysing each particular case.

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Chapter 5

Phosphorus dynamics under continuous flooding

Evolution and phosphorus fractionation in saline Spolic Technosols flooded with eutrophic water

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Abstract

Purpose: The aim of this study was to evaluate the behaviour of P in saline Spolic Technosols flooded with eutrophic water, with and without plant rhizosphere, in order to assess the role of these soils as sinks or sources of this nutrient.

Materials and methods: Samples were taken from basic (pH~7.8), carbonated and acidic (pH~6.2), de-carbonated soils of salt marshes polluted by mine wastes. Three treatments were assayed: pots with *Sarcocornia fruticosa*, pots with *Phragmites australis*, and pots without plants (bare soil). The pots were flooded for 15 weeks with eutrophic water (PO_4^{3-}~6.92 mg L^{-1}) and pH, Eh, and water-soluble organic carbon and PO_4^{3-} concentrations were monitored in the soil solution. A soil P-fractionation was applied before and after the flooding period.

Results and discussion: The PO_4^{3-} concentration in the soil solution decreased rapidly in both soils, with and without plant, being diminished by 80-90% after 3 hours of flooding. The Fe/Mn/Al oxides and the Ca/Mg compounds played an important role in soil P retention. In pots with *S.* fruticosa, the reductive conditions due to flooding induced P release from metal oxides and P retention to Ca/Mg compounds. In turn, *P. australis* may have favoured the release of P from carbonates, which was transferred to Fe/Mn/Al compounds.

Conclusions: The retention of P by the soil was the main mechanism involved in the removal of PO_4^{3-} from the eutrophic flooding water but, to evaluate the capacity of these systems as long-term P sinks, the combined effect of metals, Ca/Mg compounds, and specific plant species should be considered.

Keywords: Mar Menor, Phosphorus fractionation, *Phragmites australis*, Redox potential, Salt marsh soils, *Sarcocornia fruticosa*

5.1. Introduction

Wetlands close to agricultural, urban, industrial, or mining areas receive pollutants in the incoming waters and/or sediments, including high concentrations of trace metals and/or nutrient-rich leachates. The latter is a worldwide problem and wetlands are among the systems most affected by pollution. Fortunately, these environments show some buffering capacity against environmental stresses, being able to transform and/or deplete harmful compounds, thus acting as green filters and improving water quality (Mitsch and Gosselink, 2000). However, biogeochemical processes in flooded soils may favour the release of contaminants and these systems might act as sources of dangerous substances (Song et al., 2007).

Wetlands may act as phosphorus sinks (Fisher and Acreman, 2004; Wang and Li, 2010). Vepraskas and Faulkner (2001) pointed out that between 80 and 90% of the P in wetlands is in the soil, due to the long turnover time of nutrients in these environments (Kadlec, 1999). The retention of this nutrient is mainly controlled by soil physico-chemical conditions, but also by other factors such as vegetation, plant litter and detritus accumulation, water-table depth and water-flow velocity, hydraulic retention time, and hydrological fluctuations (Reddy et al., 1999). Wetlands may also be sources of P (Song et al., 2007) through biogeochemical processes, sediment erosion, or dredging (Reddy et al., 1999), hence increasing the eutrophication risk for coastal waters. When pollution problems include the presence of nutrient-rich water in combination with high concentrations of heavy metal in the soils, the management of the affected areas is complex and it is necessary to know in detail the behaviour of the system to take the better decisions. The Mar Menor lagoon (SE Spain) and surrounding areas are a representative example of a territory with several important environmental problems, including heavy metal pollution and eutrophication.

The Mar Menor is one of the largest coastal lagoons (135 km^2 surface area) of the Mediterranean basin. The area is characterised by a semi-arid Mediterranean climate with an annual mean rainfall of 275 mm, most of which falls in the autumn and spring, an annual mean temperature of 17ºC, and an annual mean rate of evapotranspiration of 856.8 mm/year. The lagoon and its associated salt marshes are included in the Ramsar Convention of Wetlands. The zone is a Special Protected Area of Mediterranean Interest (SPAMI), a Special Protected Area (SPA) under the EU Wild Birds Directive, and a

Site of Community Importance (SCI) to be integrated into the Nature 2000 Network (EU Habitats Directive). The dominant vegetation in the most saline areas of the salt marshes consists of shrubs of *Sarcocornia fruticosa* (L.) A. J. Scott. and *Arthrocnemum macrostachyum* (Moric.) Moris, while reedbeds of *Phragmites australis* (Cav.) Trin. ex Steudel predominate in the wettest sites (Álvarez-Rogel et al., 2007). Previous studies have classified the soils of some of these marshes as Spolic Technosols (WRB, 2006) because they are polluted by mine wastes from the nearby mining area of La Unión-Sierra de Cartagena (Álvarez-Rogel et al., 2004; Jiménez-Cárceles et al., 2008). Also, it has been shown that eutrophic water with high loads of N and P, of agricultural and urban origin (Álvarez-Rogel et al., 2006), flows across these marshes, and that these nutrients are retained by the salt marshes before reaching the lagoon (Jiménez-Cárceles and Álvarez-Rogel, 2008).

The aim of this study was to evaluate the behaviour of P in saline Spolic Technosols flooded with eutrophic water, with and without plant rhizosphere, in order to assess the role of these soils as sinks or sources of this nutrient. The specific objectives were: 1) to ascertain the mechanisms of and the capacity for P retention, for saline Spolic Technosols, and 2) to identify possible P transferences among different soil components during flooding and if the presence of plant rhizosphere may influence these transferences. For this purpose, a greenhouse experiment was conducted with polluted soils of different characteristics, with and without vegetation, subjected to prolonged flooding conditions. The results will contribute to improvements in the management of polluted salt marshes.

5.2. Material and methods

5.2.1. Experimental set up

Soil samples from saline Spolic Technosols (Table 5.1) were collected from bare areas of two coastal salt marshes of the Mar Menor lagoon (Figure 5.1): the Marina del Carmolí (318.6 ha, N37º41’42’’, W0º51’31’’) -hereafter El Carmolí- (carbonated soils with basic pH) and Lo Poyo (210.6 ha, N37º39’52’’, W0º49’02’’) (de-carbonated soils with acidic pH). The contents of metals were very high for both soils (Table 5.1). Soil samples were taken to a greenhouse and homogenised separately, but not sieved, in order to simulate, as far as possible, the field conditions. After this, 13.5 cm x 14 cm pots were filled to ¾ of their capacity (~1.5 kg of soil per pot).

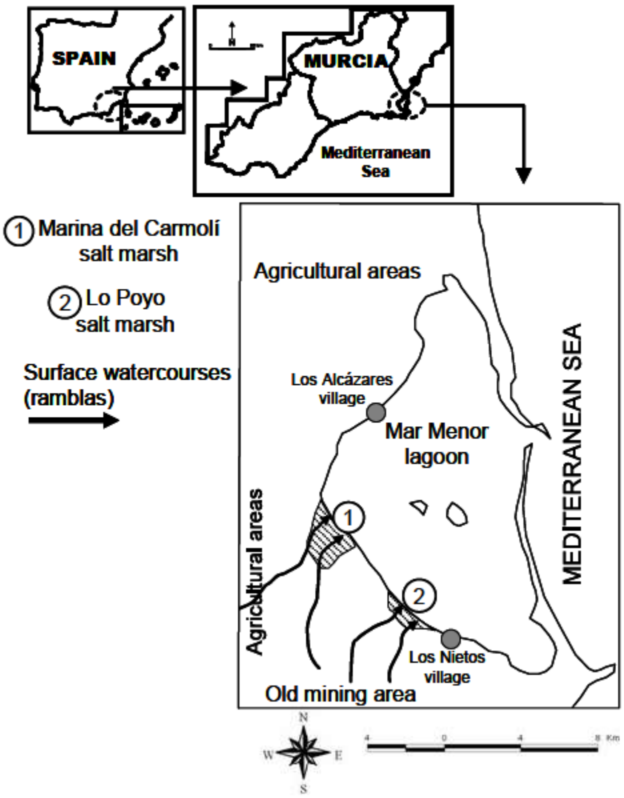

Figure 5.1. Location map of the sampling sites in SE Spain.

Cuttings of *Phragmites australis* and *Sarcocornia fruticosa*, collected from non-polluted areas of El Carmolí salt marsh, were planted in the pots and grown for six months inside the greenhouse until the plants were well established. Pots without plants (bare soil) were handled in the same manner. For more details of this phase, see González-Alcaraz et al. (2011).

At the 7th month, four pots of each treatment were placed inside larger containers (16 cm x 17 cm), fitted with waterproof plastic. The pots were flooded for 15 weeks, from October 2006 to February 2007, with eutrophic water (2 litres per pot) collected from the study area (Figure 5.1, Table 5.2). During the flooding period, the water level was maintained ~5 cm above the soil surface by adding, when it was necessary and to all pots equally, small amounts of eutrophic water to compensate for loss by evaporation and, to a lesser extent, plant transpiration.

In each pot, two 10-cm Rhizon soil moisture samplers (Rhizon MOM 19.21.21) were placed vertically in order to extract soil solution at different time intervals, for analysis of the phosphate (PO_4^{3-}) and water-soluble organic carbon (WSOC) concentrations. Water samples were taken every 30 minutes during the first three hours of pot inundation, and then at 5, 7, 9, and 24 hours. After the first day of flooding, soil solution samplings were performed every 2 weeks until the complete 15 weeks had elapsed (after 15, 30, 45, 60, 75, 90, and 105 days). Additionally, to better understand the physico-chemical characteristics of the system, pH and Eh were measured for each pot, using a previously-calibrated Crison 507 portable pH/Eh-meter. The measurements of Eh were corrected according to Vepraskas and Faulkner (2001), by adding 200 mV to the pot voltage (the value of the Ag/AgCl reference electrode).

Table 5.1. Soil characteristics (González-Alcaraz et al. 2011). Values are mean (SD) (n = 9).

	La Marina del Carmolí	Lo Poyo
pH	7.8 (0.2)	6.3 (0.6)
EC (dS m^{-1})	42 (5)	37 (8)
Total organic carbon (g kg^{-1})	12.4 (0.7)	3.5 (0.7)
Total nitrogen (g kg^{-1})	0.64 (0.13)	0.37 (0.04)
Total phosphorus (g kg^{-1})	0.64 (0.04)	0.36 (0.01)
Total $CaCO_3$ (g kg^{-1})	42.5 (19.5)	n.d
Total Ca (g kg^{-1})	55.50 (3.97)	23.33 (3.43)
Total Mg (g kg^{-1})	10.05 (0.40)	6.28 (0.71)
Total Al (g kg^{-1})	49.20 (0.88)	34.85 (0.91)
Total Cd (g kg^{-1})	0.037 (0.003)	0.006 (0.001)
Total Cu (g kg^{-1})	0.25 (0.01)	0.17 (0.01)
Total Fe (g kg^{-1})	148.18 (7.90)	158.74 (33.23)
Total Mn (g kg^{-1})	3.06 (0.36)	1.58 (0.26)
Total Pb (g kg^{-1})	6.75 (0.65)	5.64 (0.22)
Total Zn (g kg^{-1})	15.3 (1.28)	5.30 (0.74)
Sand (%)	47.7 (3.4)	83.8 (0.2)
Silt (%)	39.1 (4.5)	11.6 (0.2)
Clay (%)	13.2 (4.2)	4.6 (0.1)

EC is electrical conductivity. n.d.: not detected.

Soil samples from the pots were taken just before the beginning of the 15 weeks of flooding and immediately after the flood stage, in order to determine the $CaCO_3$ content and carry out a sequential extraction of P.

Table 5.2. Flooding water characteristics. Values are mean (SD) (n = 3).

	Flooding water
pH	7.87 (0.13)
EC (dS m^{-1})	13.5 (1.2)
WSOC (mg L^{-1})	26.5 (1.54)
NO_3^- (mg L^{-1})	183 (21)
PO_4^{3-}(mg L^{-1})	6.92 (1.11)
Na^+ (mg L^{-1})	2286 (232)
K^+ (mg L^{-1})	67 (7.7)
Ca^{2+} (mg L^{-1})	706 (73)
Mg^{2+} (mg L^{-1})	832 (64)
Cl^- (mg L^{-1})	2496 (904)
SO_4^{2-} (mg L^{-1})	2256 (85)
HCO_3^- (mg L^{-1})	1070 (59)

EC is electrical conductivity. WSOC is water soluble organic carbon.

5.2.2. Sample analysis

5.2.2.1. Soil solution samples

The PO_4^{3-} was measured with a V/UV spectrometer at λ=820 nm, using the molybdenum-blue complex method (Murphy and Riley, 1962). The WSOC was measured using a Total Organic Carbon Analyzer (TOC-V_{CSH} Shimadzu).

5.2.2.2. Soil samples

Aliquots of the soil samples were air-dried, sieved through a 2-mm mesh, and ground in an Agatha mortar for the determination of total $CaCO_3$ by the Bernard calcimeter method, with 4M HCl (Hulseman, 1966; Muller and Gatsner, 1971).

Phosphorus fractionation was performed with un-sieved, fresh soil samples in order to avoid P redistribution due to drying (Poach and Faulkner, 1998) and visible roots were removed from the soil before the fractionation (Condron and Newman, 2011). The sequential extraction procedure consisted of a four-step scheme, modified from Jensen and Thramdrup (1993), Paludan and Jensen (1995), and Paludan and Morris (1999), in which the Fe/Mn oxides-bound P fraction was extracted simultaneously with the Al oxides/clay-bound P, using 0.1N NaOH, instead of separately (Ann et al., 2000a, b; Schlichting et al., 2002; Hogan et al., 2004). The fractions considered were the following (Figure 5.2): 1) After 2 hours of shaking with 2M KCl (pH = 7), a dissolved reactive P fraction (DRP), corresponding to the KCl-exchangeable inorganic P (Exc-Pi), and a dissolved non-reactive P fraction ($DNRP_1$), corresponding to organic P, were obtained; 2) After 17 hours of shaking with 0.1M NaOH, a new DRP fraction, corresponding to the inorganic P adsorbed onto Fe, Mn, and Al minerals (Fe/Mn/Al-Pi), such as oxyhydroxides, and a second organic fraction ($DNRP_2$), were extracted; 3) After 24 hours of shaking with 0.5M HCl, a DRP fraction, corresponding to the inorganic P associated with Ca and Mg compounds (Ca/Mg-Pi), was extracted. Because the DNRP fraction present in this extract is considered negligible (Paludan and Morris, 1999; Schlichting et al., 2002), only the DRP fraction was considered; 4) Residual soil was combusted at 520ºC for 2 hours, according to Holmboe et al., (2001). The ashes were dissolved in boiled 1M HCl (Paludan and Jensen, 1995) and the P was measured and referred to as residual P (Res-Po). Although this P fraction is considered to be mainly refractory organic matter-bound P (Schlichting et al., 2002; Largeau, 2004), it can also include some mineral-bound P not extracted with alkali or acid (Reddy et al., 1999).

All of the DRP extractions (Exc-Pi, Fe/Mn/Al-Pi, and Ca/Mg-Pi) are considered inorganic P forms (Pi) and $DNRP_1$, $DNRP_2$, and Res-P are considered organic P forms (Po). The DNRP fractions were determined as the difference between total dissolved P and DRP, although it is assumed that NaOH extracts far more DNRP than any other step, including some fulvic acids (Paludan and Morris, 1999). Total dissolved P was measured after alkaline oxidation of the supernatants with $K_2S_2O_8$ in an autoclave for 30 minutes, according to Koroleff (1983). The amount of DRP in all the extracts was determined spectrophotometrically at λ=820 nm, according to Murphy and Riley (1962). The fractions $DNRP_1$ and $DNRP_2$ were combined, to constitute a single pool of dissolved organic phosphorus (DNRPo).

Aliquots of the un-sieved, fresh soil samples were oven-dried at 105ºC to constant weight and soil moisture was determined. The latter data was used to calculate the concentration of P on a dry weight basis.

The same P fractionation scheme was applied by Jiménez-Cárceles and Álvarez-Rogel (2008) in a field study conducted in El Carmolí salt marsh. They determined that the total P content, calculated as the sum of the different fractions, had a variation of ±14% with respect to the total P obtained by acid digestion. This result was of the same magnitude as the 10% variation obtained by Paludan and Morris (1999) in a P fractionation study in salt marshes of the USA. Hence, we calculated total P (Total-P) as the sum of the different fractions extracted.

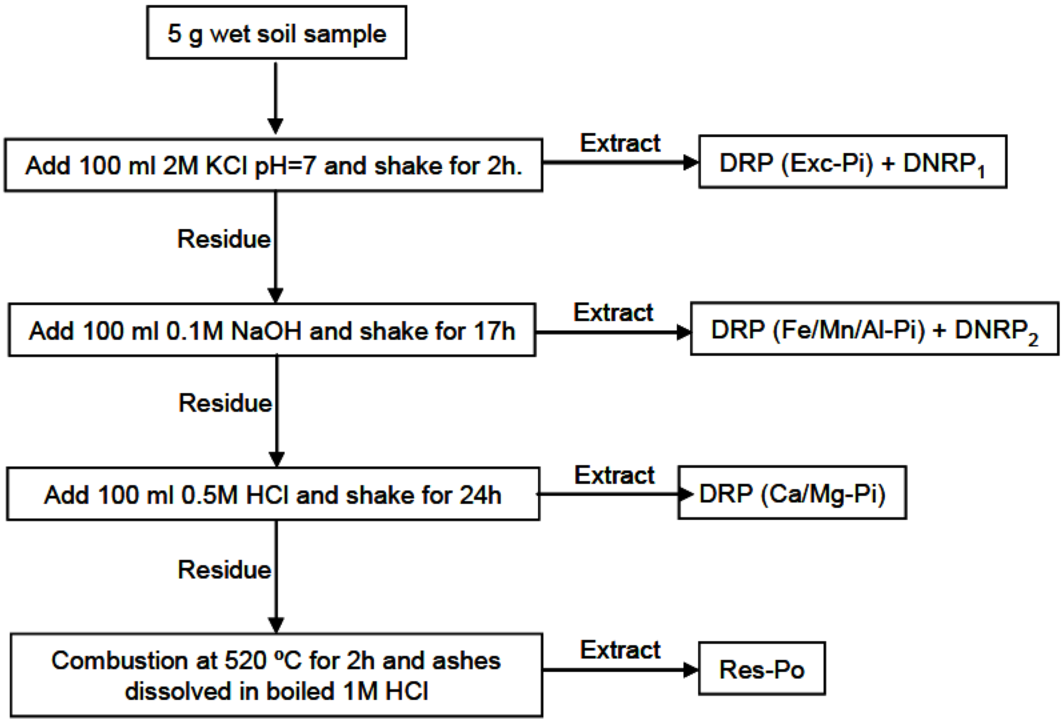

Figure 5.2. Soil phosphorus fractionation modified from Jensen and Thramdrup (1993), Paludan and Jensen (1995), and Paludan and Morris (1999). DRP: dissolved reactive P. DNRP: dissolved non reactive P. Exc-Pi: loosely adsorbed P. Fe/Mn/Al-Pi: P bound to Fe, Mn, and Al oxides. Ca/Mg-Pi: P bound to Ca and Mg compounds. Res-Po: residual organic P

5.2.3. Statistical analysis

The statistical analyses were performed with SPSS 17.0 (SPSS Inc., 2008). To compare the evolution of pH, Eh, and the WSOC and PO_4^{3-} concentrations in the soil solution over time and among treatments, repeated measures ANOVA was performed for each type of soil. The factors included in the analyses were an inter-subjects factor (plant species, with three levels: pots with *S. fruticosa*, pots with *P. australis*, and bare soil) and an intra-subjects factor (time-the repeated factor-, with many levels corresponding to the soil solution samplings). The dependent variables were pH, Eh, and the WSOC and PO_4^{3-} concentrations. When data did not fulfil the sphericity requirement for Mauchly's sphericity test, univariate F statistics were applied, using a corrector index of epsilon based on Greenhouse-Geisser, Huynh-Feldt, or Lower-bound estimations. The $CaCO_3$ content and P fractionation data, before and after the flooding period, were compared by a non-parametric test (U Mann-Whitney, significance level = 0.05).

5.3. Results

5.3.1. pH, Eh, and WSOC and PO_4^{3-} concentrations in the soil solution

At the beginning of the flooding period, the pH was between 7.6-7.8 in the pots from El Carmolí and between 5.8-6.5 in those from Lo Poyo (Figure 5.3A and 5.3B). In both soils, the evolution of the pH was significantly affected by time ($p \leq 0.019$) and species ($p \leq 0.04$), but the interaction time vs. species was only significant for El Carmolí ($p = 0.045$). The pots with *S. fruticosa* of El Carmolí and those with plant of Lo Poyo showed significant lower pH values than bare soil ($p \leq 0.015$).

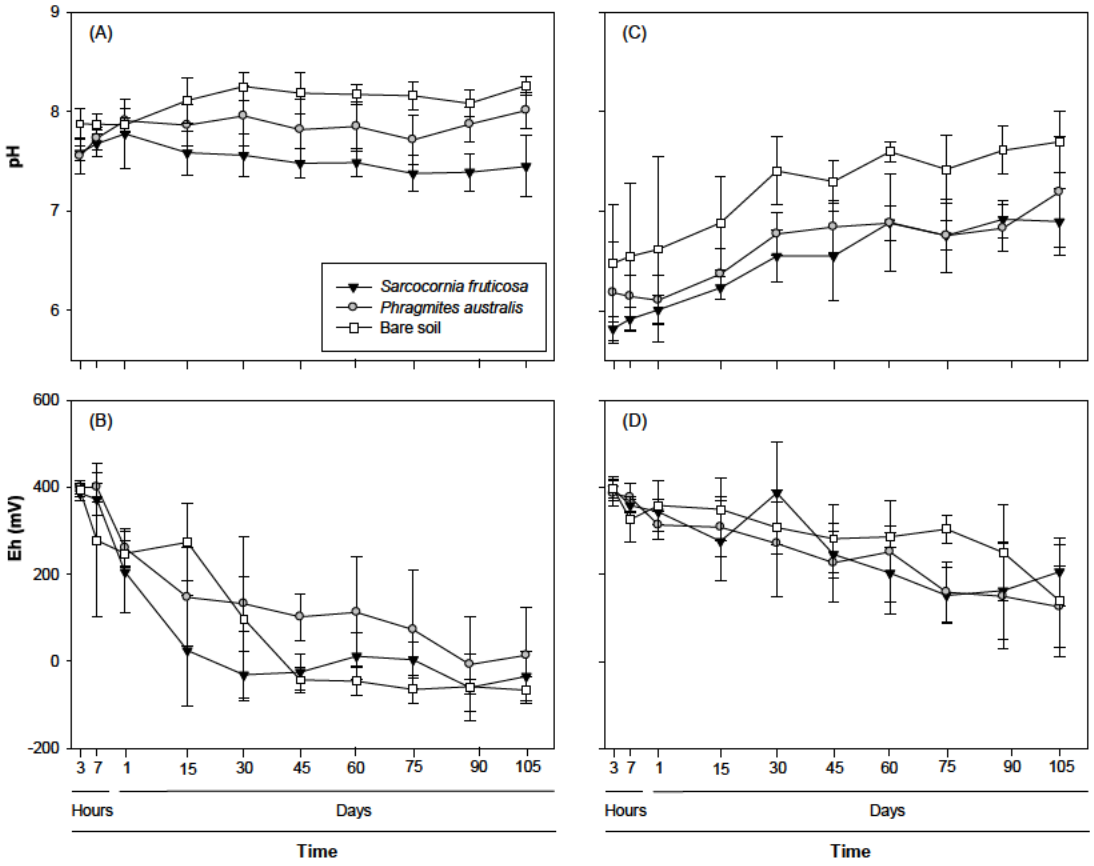

Figure 5.3. Evolution of pH and Eh in soil solution of La Marina del Carmolí salt marsh (A and B) and Lo Poyo salt marsh (C and D). Bars represent standard deviation (n = 4).

The Eh was around +400 mV at the beginning of the experiment in both soils, with and without vegetation (Figure 5.3C and 5.3D). During the flooding period, the Eh decreased significantly over time ($p \leq 0.003$), with no effect of species or the time vs. species interaction. The pots containing soil from El Carmolí became suboxic (+300 mV < Eh < +100 mV, Otero and Macías, 2003) after 24 hours of flooding and anoxic (Eh < +100 mV, Otero and Macías, 2003) after 30 days, showing higher Eh values the pots with *P. australis*. The pots with soil from Lo Poyo were suboxic after 45 days, although the Eh did not decrease below +100 mV in most cases.

In both soils, the concentration of WSOC (Figure 5.4A and 5.4B) significantly decreased during the flooding period ($p<0.001$), regardless of species and the time vs. species interaction.

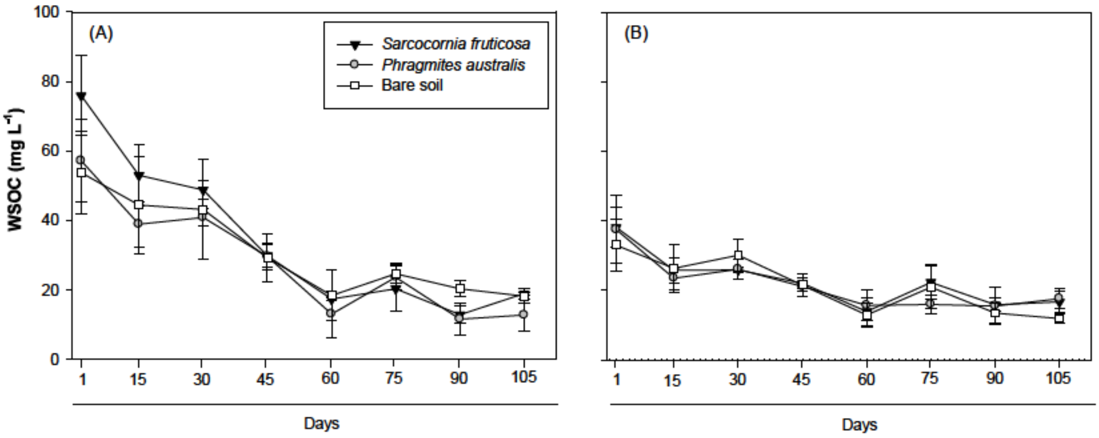

Figure 5.4. Evolution of the concentration of water soluble organic carbon (WSOC) in soil solution of La Marina del Carmolí salt marsh (A) and Lo Poyo salt marsh (B). Bars represent standard deviation (n = 4).

The evolution of the PO_4^{3-} concentration in the soil solution (Figure 5.5A and 5.5B) was similar in both soils, decreasing significantly along the experiment ($p \leq 0.001$), with no effect of species or the time vs. species interaction. In the pots containing soil from El Carmolí, the PO_4^{3-} concentration was around 2.58±1.09 mg L^{-1} after 30 minutes of flooding, a decrease of more than 60% respect to the concentration in the flooding water (Table 5.2). After one hour, the PO_4^{3-} concentration had decreased below 2 mg L^{-1} (>71% decrease), reaching values below 1 mg L^{-1} in 3 hours (>86% decrease). Thereafter, the PO_4^{3-} concentration remained low (<0.50 mg L^{-1}). In the pots with soil from Lo Poyo, after 30 minutes of flooding, the PO_4^{3-} concentration was around 2.75±1.34 mg L^{-1} (60% decrease). After one hour, this concentration had decreased to

2.04±0.98 mg L^{-1} (70% decrease), reaching values below 2 and 1 mg L^{-1} after 2 and 5 hours, respectively. Over successive samplings, the PO_4^{3-} concentration did not exceed 0.5 mg L^{-1}.

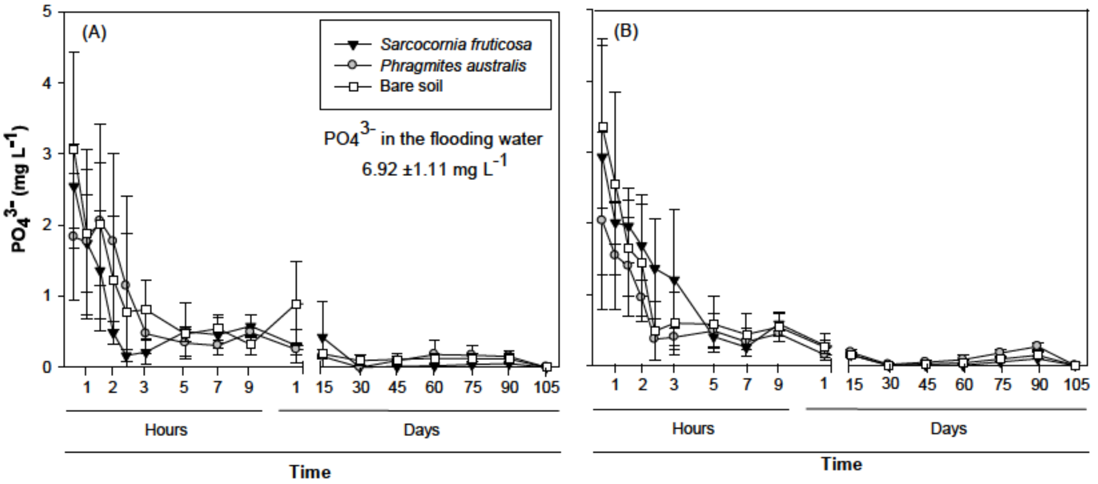

Figure 5.5. Evolution of the concentration of PO_4^{3-} in soil solution of La Marina del Carmolí salt marsh (A) and Lo Poyo salt marsh (B). Bars represent standard deviation (n = 4).

5.3.2. $CaCO_3$ content and P fractionation in soil

5.3.2.1. $CaCO_3$ content

In pots with soil from El Carmolí, the $CaCO_3$ content increased, but not significantly, in *S. fruticosa* (from 43±7 to 52±4 g kg^{-1}) and bare soil (from 53±5 to 59±7 g kg^{-1}) and decreased, significantly, from 64±4 to 52±4 g kg^{-1} in pots with *P. australis*. In pots with soil from Lo Poyo, $CaCO_3$ was not detected.

5.3.2.2. Phosphorus fractionation

5.3.2.2.1. Soil from El Carmolí salt marsh

Initially, most of the P was found in the Res-Po fraction (51-58%), followed by the Ca/Mg-Pi fraction (22-32%), the Fe/Mn/Al-Pi fraction (14-19%), the DNRPo fraction (2-3%), and the Exc-Pi fraction (<1%) (Figure 5.6A, 5.6B, and 5.6C). After 15 weeks of flooding, the P concentrations in the soil varied in all the treatments. The changes were not significant for the Total-P (from 639±2.5 to 665±73 mg kg^{-1} in *S. fruticosa*, from 637±34 to 649±9 mg kg^{-1} in *P. australis*, and from 654±15 to 664±11 mg kg^{-1} in bare soil), but they were for some of the fractions (Figure 5.6A, 5.6B, and 5.6C). The Res-Po tended to increase in both treatment with plant (significant for pots with *P. asutralis*) and hardly changed in bare soil, while DNRPo significant decreased in all the treatments. The behaviour of major inorganic P fractions was opposite for *S. fruticosa* and *P. australis* pots: the Ca/Mg-Pi fraction increased in the first and decreased in the second, and the Fe/Mn/Al-Pi fraction increased in the second and decreased in the first (Figure 5.6A and 5.6B). The Ca/Mg-Pi fraction significant increased in bare soil.

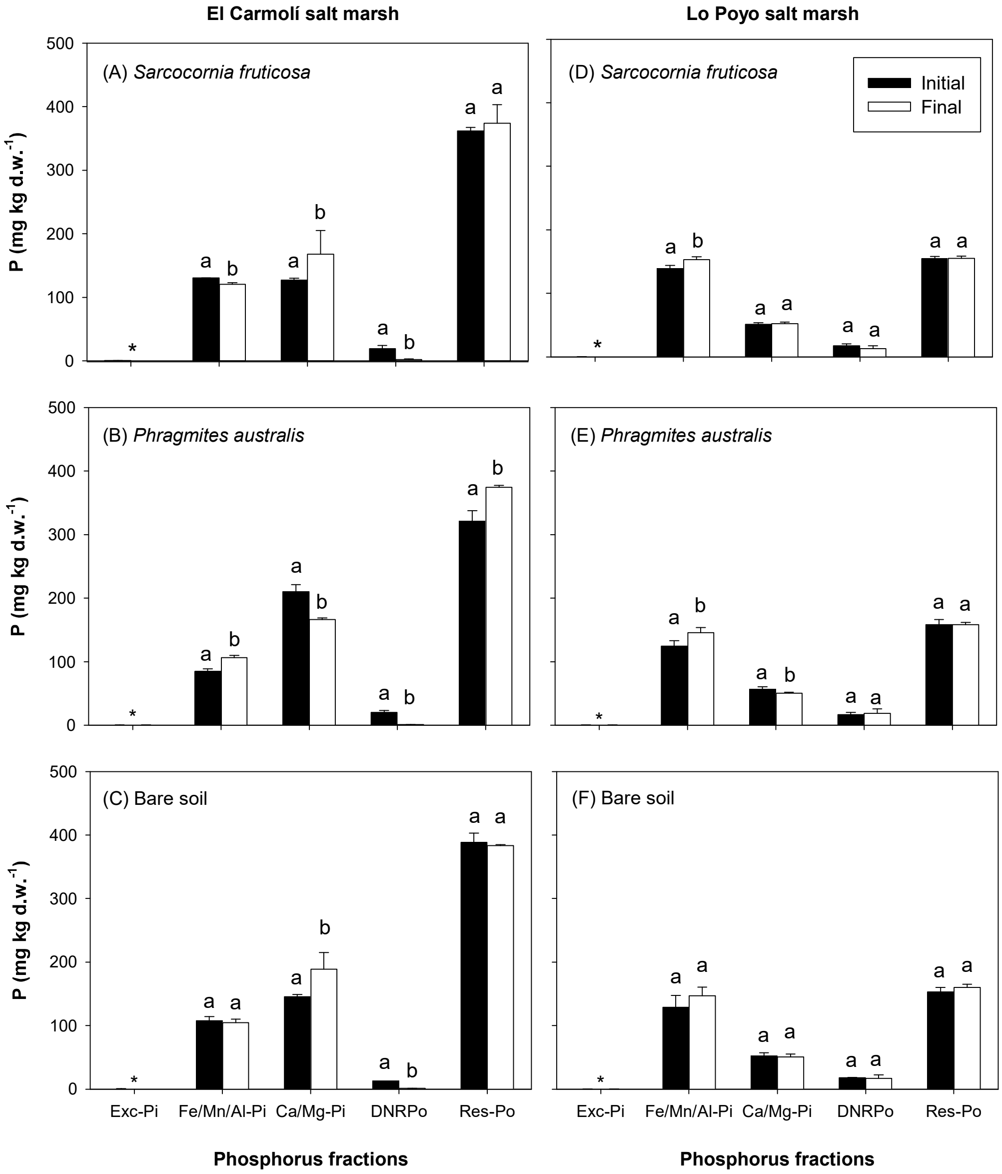

Figure 5.6. Phosphorus concentration (expressed on a dry weight basis) of different fractions (Exc-Pi = loosely adsorbed P, Fe/Mn/Al-Pi = P bound to Fe, Mn and Al oxides, Ca/Mg-Pi = P bound to Ca and Mg compounds, DNRPo = dissolve non reactive P, Res-Po = Residual organic P) in the soil from La Marina del Carmolí salt marsh (A, B, and C) and from Lo Poyo salt marsh (D, E, and F). Bars on columns represent standard deviation (n = 4). For each phosphorus fraction, different letters indicate significant differences before and after the flooding period (U Mann-Whitney, $p < 0.05$). * < 1 mg kg^{-1}.

5.3.2.2.2. Soil from Lo Poyo salt marsh

Initially, Fe/Mn/Al-Pi and Res-Po fractions accounted for more than 70% of the phosphorus in the soils (Figure 5.6D, 5.6E, and 5.6F). After 15 weeks of flooding, the Total-P was about 4-5% higher, but not significant, in the soil (from 362±6 to 376±6 mg kg^{-1} in *S. fruticosa*, from 357±9 to 373±15 mg kg^{-1} in *P. australis*, and from 353±16 to 372±21 mg kg^{-1} in bare soil). The increase in the Fe/Mn/Al-Pi was significant in pots with *S. fruticosa* and *P. australis*, but not in bare soil. The Ca/Mg-Pi and DNR-Po fractions had a minor role in P retention in this soil and the changes after flooding were generally not significant (Figure 5.6D, 5.6E, and 5.6F).

5.4. Discussion

5.4.1. Retention of PO_4^{3-} from flooding water

The retention of P by soil in wetlands is related to P loading and various physico-chemical properties of the soil including the pH, Eh, mineralogy, clay content, Fe, Al, and Ca contents, organic matter content, and P content (Reddy et al., 1996; Reddy and DeLaune, 2008). In our experiment, after flooding the pots with eutrophic water (PO_4^{3-}~6.92 mg L^{-1}), the concentration of PO_4^{3-} in the soil solution decreased rapidly in all soils, regardless of the presence or absence of vegetation (no significant effect of species or the time vs. species interaction) (Figure 5.5). This dynamic suggests that the P in the flooding water was retained by the soil and that the role of the plant was scarce. This agrees with other studies (e.g. Nairn and Mitsch, 2000; Yang et al., 2001) which showed no differences in P removal between vegetated and non-vegetated wetlands.

Several studies (e.g. Yoo et al., 2006; Wang et al., 2009) have shown that the PO_4^{3-} adsorption kinetic on sediments has two steps: an initial, rapid adsorption, when most of the PO_4^{3-} is retained, and a slow adsorption, prolonged in time, leading to a steady state. In our experiment, both soils showed similar behaviour, regardless of the presence or absence of plants (Figure 5.5). In the first 30 minutes of flooding, more than half of the PO_4^{3-} in the flooding water was retained by the soil and by 80-90% during the first 3 hours. From this point on, the concentration of PO_4^{3-} in the soil solution tended to stabilise until the end of the experiment (Figure 5.5). Although we had, in theory, favourable conditions for P release into the soil solution (low Eh during the flooding period, Figure 5.3), this release was not detected. This agrees with Gomez et al. (1999) who found that the decrease of Eh led to the adsorption of P onto sediments when a high P load was added (10 mg L^{-1}).

Although some variations of the Total-P in the soils were observed after the flooding period, the addition of P with the eutrophic water was not enough to modify, significant, the content of phosphorus in soil. The latter can be explained taken into account the concentration of phosphorus in the water (~6.92 mg L^{-1} of PO_4^{3-} = ~2.26 mg L^{-1} of P) and the quantity of water used during the experiment. To flood the pots we used 2 litres of water, which would be about 4.52 mg of P added to each pot. During the flooding period we replenished only small quantities of water when it was necessary and this was not reflected not even in an increase of the PO_4^{3-} concentrations in soil solution. If we consider the initial P contents in both soils (570-690 mg kg^{-1} in El Carmolí and 340-370 mg kg^{-1} in Lo Poyo), the P added with the flooding water would be a small amount in relation to the initial content of the soil and the changes could be not significant. However, the significant changes in the different P-fractions indicate that there were transferences of phosphorus among different soil components during the flooding period and that the plant rhizosphere contributed to the latter. The rhizosphere exhibits a unique biophysics and biogeochemistry (Hinsinger et al., 2009) that contrasts from that of the bulk soil, influencing some geochemical parameters, such as pH and Eh (Wenzel, 2009), and thus affect the mobility of P in the soil (Hinsinger, 2001). Moreover, Hinsinger (2001) indicated that the influence of the plant rhizosphere might depend on each plant species and even of the species genotype.

5.4.2. Phosphorus fractionation and transferences during flooding

Sequential extraction procedures allow one to identify different inorganic P forms, based on differences of solubility in various chemical extractants. Inorganic P accounted for 41-46% of Total-P in the soil from El Carmolí and for 51-53% in the soil from Lo Poyo (Figure 5.6). These values are in the range of those obtained for inorganic P forms in mineral wetland soils (20-50%) (Reddy and DeLaune, 2008). In the soil from El Carmolí, the main pool of inorganic P was bound to Ca/Mg compounds, while in Lo Poyo the main pool was bound to Fe/Mn/Al compounds (Figure 5.6). This agrees with field data obtained by Jiménez-Cárceles and Álvarez-Rogel (2008).

Organic P in soil is associated with living organisms (microorganisms, algae, macrophytes, and detritus) and consists of easily-decomposable compounds such as nucleic acids, phospholipids, or sugar phosphates, and other recalcitrant forms such as inositol phosphates or phytin (Reddy et al., 1999). Organic P accounted for 50-60% and 43-45% of Total-P in the pots with soil from El Carmolí and Lo Poyo, respectively (Figure 5.6). According to Reddy and DeLaune (2008), in mineral wetland soils, the organic P content may range from 10 to 50%. In both soils studied here, 95% of organic P was found in highly-resistant/refractory forms (Res-Po) and the rest as easily-decomposable forms (DNRPo) (Figure 5.6). Caution must be taken when interpreting the high content of Res-Po since this fraction can be overestimated when using the fractionation procedure applied in this study (as explained in section 2.2.2).

The comparison of P fractions in the soils before and after 15 weeks of flooding allowed the determination of whether inundation caused noticeable changes in soil P distribution. The evolution of Eh through the experiment indicates that prolonged flooding affected more the soil from El Carmolí than that from Lo Poyo, as shown by the Eh values (Figure 5.3). González-Alcaraz et al. (2011) and María-Cervantes et al. (2010) attributed these differences to the lower pH, lower organic carbon content, higher sand content, and higher concentration of water-soluble metals in the soil from Lo Poyo. The differing behaviour of both soils, regarding the evolution of the physico-chemical conditions, was reflected in the changes of the soil P fractions, which were more pronounced in the soil from El Carmolí.

In the soil from El Carmolí, the P bound to Fe/Mn/Al compounds had decreased after 15 weeks of flooding in pots with *S. fruticosa*, while the P bound to Ca/Mg compounds had increased (Figure 5.6). The opposite behaviour was shown by the *P. australis* pots (Figure 5.6). The Fe/Mn-Pi pool is redox-sensitive and, under anaerobic conditions, the reactive, oxidised species of both metals are reduced and chemically-sorbed PO_4^{3-} is released into the soil solution (Jensen and Thamdrup, 1993; Reddy et al., 1995). The decrease in this P fraction agrees with the increase in water-soluble Fe and Mn observed by María-Cervantes et al. (2010) in the same treatments, due to the dissolution of oxy-hydroxides at the beginning of a flooding period. Probably, inorganic P released by metal oxides was transferred to the Ca and Mg fraction, which had increased at the end of the experiment. Gomez et al. (1999) demonstrated that P mobilisation between different fractions is possible without P release into water being detected.

The existence of a highly-developed aerenchyma in *P. australis* (Armstrong and Armstrong, 1987) could be responsible for the different evolution of inorganic P fractions, through promotion of greater gas exchange within the rhizosphere. Plant roots provide a significant proportion of the CO_2 input into the soil through respiration and exudation of organic substances that are degraded by microorganisms, and higher CO_2 concentrations should be expected in soils with lower gas permeability, such as compacted or water-saturated soils (Hinsinger et al., 2003). A greater CO_2 flux into the soil from *P. australis* may have contributed to a decrease of pH in the rhizosphere due to the formation of carbonic acid ($CO_2 + H_2O = H_2CO_3$), promoting the dissolution of Ca carbonates and releasing the P bound to these compounds. This agrees with the decrease in the total $CaCO_3$ content in these pots after flooding.

In general, P bound to Ca/Mg compounds is considered as a storage fraction, unavailable to biological assimilation (Reddy et al., 1995; Aminot and Andrieux, 1996). Nevertheless, some studies have shown Ca/Mg-Pi mobilisation as a result of pH decrease (Gomez et al., 1999; Peng et al., 2007). Inorganic P released from Ca and Mg compounds in the *P. australis* pots could have been transferred to the Fe/Mn/Al fraction. However, in both treatments with plants, it cannot completely exclude that some of the P released from soil fractions could be taken by the vegetation.

In pots with soil from Lo Poyo, the main inorganic P fraction was Fe/Mn/Al-Pi (Figure 5.6). This fraction of P bound to metal oxides did not decrease with flooding, unlike in the El Carmolí pots, which agrees with the slow and gradual decline of Eh in the Lo Poyo pots (Figure 5.3). The formation of Ca/Mg-Pi compounds, in spite of the absence of $CaCO_3$ in this soil, can be explained by the abundance of Ca^{2+} and Mg^{2+} in the flooding water.

Regarding the evolution of organic P, the residual fraction in the soil from El Carmolí tended to increased in pots with *S. fruticosa* and *P. australis* (Figure 5.6). This could be related to the detrital material produced by plants and deposited on the soil surface, becoming an integral part of the soil solid phase and providing long-term P storage. As the plants produce detrital matter P, it is rapidly released through leaching and decomposition, although the rates of mineralisation are reduced under conditions of low O_2 content, which leads to the accumulation of moderately-decomposable compounds together with lignins and other recalcitrant compounds (Reddy and DeLaune, 2008).

In the pots with soil from El Carmolí, with and without vegetation, the DNRPo fraction decreased markedly (>80%) after the flooding period (Figure 5.6). This fraction is considered as easily-degradable parts of organic P together with P stored in bacteria (such as polyphosphate) and more-stable compounds like phytate (Emil, 2000). The contribution of the DNRPo fraction to the total content of P is less under low oxygen conditions (Paludan and Morris, 1999; Emil, 2000) which agrees with the decrease of the P content of this fraction in our experiment. Some microorganisms have the ability to accumulate polyphosphate intracellularly under aerobic conditions and to release it as orthophosphate under anaerobic conditions, allowing them to be dominant under fluctuating redox conditions at the soil-water interface (Khoshmanesh et al., 2002). The latter process could be intense in the soil from El Carmolí, which reached Eh values below +100 mV (Figure 5.3), indicative of anaerobic conditions (Otero and Macías ,2003). Goedkoop and Pettersson (2000) found a significant relationship of the DNRPo fraction with sediment bacterial biomass in lake sediments. The stronger decrease in WSOC in pots with soil from El Carmolí (Figure 5.4) agrees with a higher microbial activity. The lowering of the DNRPo fraction could have contributed to the increase of the Fe/Mn/Al-Pi and Ca/Mg-Pi fractions after flooding in some pots, together with the aforementioned P transfer between these inorganic fractions.

5.5. Conclusions

Based on the dynamics of PO_4^{3-} in the soil solution (rapid decrease in all soils and no significant effect of species or the time vs. species interaction), our results suggest that the retention of P by the soil was the main mechanism involved in the removal of PO_4^{3-} from eutrophic flooding water in saline Spolic Technosols. Organic and inorganic soil components were both highly efficient as sinks of P, and if release of P from the soil solid phase occurred, the transferences among different soil components prevented the increase of the P concentration in the soil solution. Despite the high content of metals in the saline Spolic Technosols studied, and even with a low content of $CaCO_3$, Ca/Mg compounds played an important role in soil P retention. Our results pointed that the role of plants in P retention was scarce. However, *P. australis* could have influenced P transferences between carbonates and Fe/Mn/Al compounds. Hence, to evaluate the capacity of these systems as long-term P sinks, the combined effect of metals, Ca/Mg compounds, and specific plant species should be considered.

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Chapter 6

Liming application in pot experiment

The combined use of liming and *Sarcocornia fruticosa* development for phytomanagement of salt marsh soils polluted by mine wastes

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Abstract

The aim of this study was to evaluate the combined effects of liming and behaviour of *Sarcocornia fruticosa* as a strategy of phytomanagement of metal polluted salt marsh soils. Soils were taken from two polluted salt marshes (one with fine texture and pH~6.4 and the other one with sandy texture and pH~3.1). A lime amendment derived from the marble industry was added to each soil at a rate of 20 g kg^{-1}, giving four treatments: neutral soil with/without liming and acidic soil with/without liming. Cuttings of *S. fruticosa* were planted in pots filled with these substrates and grown for 10 months. The pots were irrigated with eutrophic water. As expected, lime amendment decreased the soluble metal concentrations. In both soils, liming favoured the growth of *S. fruticosa* and enhanced the capacity of the plants to phytostabilise metals in roots.

Keywords: *Sarcocornia fruticosa*, Mine wastes, Liming, Phytomanagement, Mar Menor

6.1. Introduction

Wetlands exposed to pollution may act as sinks for hazardous substances such as heavy metals (Ramsar, 2001; Doyle and Otte, 1997; Williams et al., 1994) and may be seriously threatened by ecotoxicological effects (Vega et al., 2009). Metal mobility is dependent on multiple parameters: temperature, pH, redox potential, salinity, texture, distribution and organic matter content (Otte et al., 1993; Williams et al., 1994; Almeida et al., 2004). Marsh vegetation may immobilise and store metals in the rhizosphere or take them up (Jacob and Otte, 2003; Weis and Weis, 2004; Vardanyan and Ignole, 2006).

Soils polluted by mine wastes often have deficient properties (Wong, 2003; Liu et al., 2006). The spontaneous colonisation of the contaminated areas by plants is slow and only metal-tolerant ones can spread (Macnair, 1987). Hence, revegetation of metal-polluted, bare soils requires specific management techniques. One of the most-common practices is the application of soil amendments. Liming materials, like wastes from the marble industry, can raise pH and overcome some of the problems associated with acidity (Ye et al., 2000; Wong, 1987).

When polluted areas occur in salt marshes, the high salinity further reduces the number of candidate species for revegetation. Nevertheless, halophytes are adapted to high salinity and have an inherent capacity to tolerate high concentrations of heavy metals in the soil (Otte et al., 1991; Otte et al., 1993).

Territories affected by the impact of mining activities are common in Europe. Among the most-impacted areas is the Cartagena-La Unión Mining District, which is located in the SE of the Iberian Peninsula and covers an area of 50 km^2 (Conesa and Schulin, 2010). Abandoned dumps have been eroded strongly and contaminated sediments transported to the nearby Mar Menor lagoon (one of the largest coastal lagoons in the Mediterranean basin) (Figure 6.1). This lagoon and its associated salt marshes are included in the Ramsar Convention of Wetlands. Also, it is Special Protected Area (SPA) under the EU Wild Birds Directive and a Site of Community Importance (SCI). Previous studies have shown that several coastal salt marshes are polluted by mine wastes (Álvarez-Rogel et al., 2004; Jiménez-Cárceles et al., 2008) and eutrophic water (Álvarez-Rogel et al., 2007; Jiménez-Cárceles and Álvarez-Rogel, 2008). Extensive areas of these salt marshes lack a vegetation cover, which favours the transport of hazardous compounds into the lagoon and thence to nearby beaches (Conesa and Jiménez-Cárceles, 2007). Hence, molluscs and other organisms collected by hand by the local human population, and consumed without sanitary control, accumulate toxic concentrations of heavy metals (María-Cervantes et al., 2009). Reduction of soluble metal concentrations in the soils of the salt marshes in combination with revegetation of bare areas, are key aspects for reducing the risks of pollutants spread and entry into the food web.

The aim of this study was to evaluate the combined effects of liming and behaviour of *Sarcocornia fruticosa* (L.) A. J. Scott (a typical halophyte species in Mediterranean salt marshes) as a strategy of phytomanagement of metal contaminated salt marsh soils. For this purpose we assayed the effect of liming on soluble metal concentrations and on the development and metal uptake of this species in salt marsh soils polluted by mine wastes. There are numerous studies that evaluate the effectiveness of liming in the remediation of metal-contaminated soils (e.g. Simón et al., 2010) and many works dealing with metal accumulation in wild plants (e.g. Moreno-Jiménez et al., 2009), particularly in wetland species (e.g. Almeida et al., 2006; Reboreda and Caçador, 2007; Caetano et al., 2008; Vymazal et al., 2007). However, as far as we know, there are no reported data about the use of lime amendments in metal-polluted salt marsh soils and the effect of this on the development and behaviour of halophytic plants.

6.2. Material and methods

6.2.1. Field soil sampling and initial characterisation

Soil samples (from the top 20 cm) were collected from two polluted salt marshes: neutral soils from La Marina del Carmolí (318.6 ha, N37º41’42’’, W0º51’31’’), and acidic soils from Lo Poyo salt marsh (210.6 ha, N37º39’52’’, W0º49’02’’) (Figure 6.1). These marshes receive sediments of two surface watercourses, called ramblas, which flow from the nearby mining area. Both watercourses have bare tailings at their headwaters or along their riverbeds (Robles-Arenas et al., 2006).

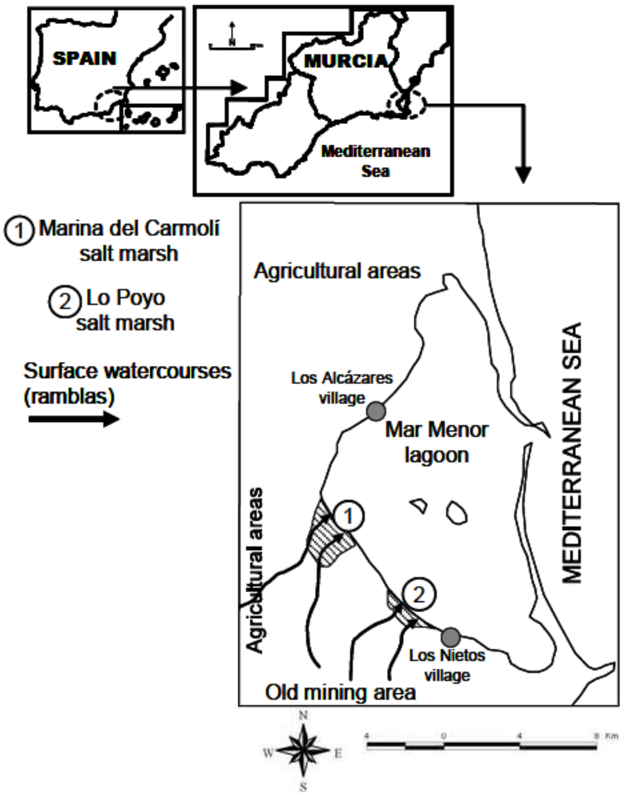

Figure 6.1. Location map of the sampling sites in SE Spain.

Samples from each salt marsh were air-dried, mixed mechanically, and stored in plastic bags. Three sub-samples of each site were analysed in order to perform an initial soil characterisation (Table 6.1). The samples were sieved through a 2-mm mesh for the determination of pH, electrical conductivity (EC), and particle size distribution. Soil pH and EC were measured at 25 ºC in a 1:2.5 soil:water suspension (Cobertera, 1993). Particle size distribution was determined by passing 50 g of soil through a 50-µm mesh, in order to separate the fine fraction (silt + clay) from the sand fraction, prior to sample dispersion with a solution of $(NaPO_3)_n$ and Na_2CO_3. Each fraction was weighed and expressed as a percentage of the total weight. Aliquots of soil were ground in an Agatha mortar and used for measuring total organic carbon (TOC), total nitrogen (TN), total calcium carbonate ($CaCO_3$), and total metals. The TOC was measured in an automatic analyzer (TOC-V CSH, Shimadzu Corporation, Tokyo, Japan), the TN by Kjeldahl's method as described by USDA (1996) and the $CaCO_3$ by the Bernard calcimeter method, using 4M HCl (Hulseman, 1966; Muller and Gatsner, 1971). The total metals were extracted by nitric – perchloric (1:1) acid digestion at 210ºC for 90 minutes (Álvarez-Rogel et al., 2004). The Al, As, Cd, Cu, Mn and Pb were measured with an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7500A) and the Fe and Zn with a Unicam 969 atomic absorption spectrometer. The mineralogical composition of both soils was conducted by a semi-quantitative estimation of the crystalline fraction by power X-ray diffraction (XRD) on a Bruker-AXS D8-Advance diffractometer, using monochromatic CuKα radiation at 40 kV and 30 mA.

Table 6.1. Soil characterisation. Average (SD) (n = 3).

	Marina del Carmolí	Lo Poyo
pH	6.43 (0.06)	3.09 (0.01)
EC (dS m^{-1})[a]	20.3 (2.84)	24.8 (1.31)
$CaCO_3$ (g kg^{-1})	5.88 (1.15)	n.d.
TOC (g kg^{-1})[b]	4.83 (0.46)	3.11 (0.49)
TN (g kg^{-1})[c]	0.28 (0.02)	0.41 (0.06)
Sand (%)	40 (3.7)	67 (2.5)
Silt + Clay (%)	60 (3.7)	33 (2.5)
Al (g kg^{-1})	13.2 (1.72)	16.4 (2.42)
As (g kg^{-1})	0.22 (0.03)	0.31 (0.03)
Cd (g kg^{-1})	0.016 (0.006)	0.001 (0.0006)
Cu (g kg^{-1})	0.18 (0.06)	0.16 (0.07)
Fe (g kg^{-1})	61 (12.3)	118 (26.7)
Mn (g kg^{-1})	3.51 (0.67)	0.40 (0.09)
Pb (g kg^{-1})	6.75 (0.86)	5.03 (0.24)
Zn (g kg^{-1})	52.3 (10.2)	3.78 (1.10)

n.d.: not detected. [a] Electrical conductivity. [b] Total organic carbon. [c] Total nitrogen.

6.2.2. Experimental set up

A lime amendment (pH 8.9 in a 1:2.5 water suspension) derived from the marble industry was added to each soil at 20g kg^{-1} soil, giving four treatments: neutral soil from La Marina del Carmolí with and without liming and acidic soil from the Lo Poyo salt marsh with and without liming. The mineralogical composition of the amendment was carried out in a manner similar to that of both soils, with 54% magnesium calcite-($Mg_{0.03}Ca_{0.97}$)(CO_3)- and 37% dolomite-$CaMg(CO_3)_2$-.

For each treatment, 13.5 cm x 14 cm plastic pots (40 replicates) were filled to ¾ of their capacity (~1.5 kg of soil per pot). Cuttings of *S. fruticosa* were collected from non-polluted areas of the La Marina del Carmolí salt marsh. Special care was taken so that all the cuttings had similar biomass (<5 g fresh weight). The plants were washed with de-ionised water, their roots submerged in a solution of indolebutyric acid (50 %) in order to facilitate root growth, and then transplanted to the aforementioned pots (1 plant per plot). The pots were distributed randomly inside a greenhouse and watered 2-3 times per week for 10 months. The water from a local stream was used as irrigation water in order to simulate as far as possible the conditions in the salt marshes. This water was poured into an opaque tank to prevent its degradation (Table 6.2).

Table 6.2. Characteristics of irrigation water (n = 5).

	Average (SD)
pH	8.13 (0.23)
EC (dS m^{-1})[a]	21.6 (5.3)
NO_3^- (mg L^{-1})	366 (50)
PO_4^{3-}(mg L^{-1})	0.82 (0.32)
WSOC (mg L^{-1})[b]	10.5 (1.12)
Na^+ (mg L^{-1})	3286 (232)
K^+ (mg L^{-1})	67 (7.7)
Ca^{2+} (mg L^{-1})	706 (73)
Mg^{2+} (mg L^{-1})	832 (64)
Al (µg L^{-1})	n.d.
Cd (µg L^{-1})	n.d.
Mn (µg L^{-1})	12.6 (10.7)
Pb (µg L^{-1})	4.15 (0.91)
Zn (µg L^{-1})	69.3 (7.66)

n.d.: not detected. [a] Electrical conductivity. [b] Water soluble organic carbon.

Pore water samples were collected in the 18th week of the experiment from four randomly selected plots of each treatment. Two 10-cm Rhizon soil moisture samplers (Rhizon MOM 19.21.21) were placed vertically in each pot to extract soil solution. The two sub-samples obtained were poured into the same plastic vial. In addition, during this sampling, we also measured the pH in each pot, using a previously calibrated Crison 507 portable pH-meter. At the end of the experimental period, the plant survival rate for each treatment (expressed as the percentage of living plants of the 40 replicates) was calculated. Then, the shoots and roots of *S. fruticosa* were separated from the soil by hand in the four pots previously selected for sampling pore water. This plant material and the corresponding soil were analysed.

6.2.3. Sample preparation and analysis

6.2.3.1. Pore water samples

Pore water samples were stored in plastic vials and the EC at 25 ºC was measured immediately using a Crison Basic 30 conductimeter. After that, the samples were acidified using sulphuric acid, hermetically stored and frozen at -20 ºC until analysis. After thawing, the concentrations of macronutrients (K^+, Na^+, Ca^{2+}, and Mg^{2+}) and soluble metals (Al, Cd, Mn, Pb, and Zn) were determined. Macronutrients were measured using a Dionex ionic chromatographer, the Al, Cd, and Pb with an ICP-MS (Agilent 7500A) and the Mn and Zn with a Unicam 969 atomic absorption spectrometer.

6.2.3.2. Plant samples

Shoots and roots were separated and then carefully washed using de-ionised water. In addition, shoots were separated into swollen photosynthetic stems and dry, perennial, non-photosynthetic shoots. Afterwards, the plants were oven-dried at 65 °C to constant weight and then weighed and ground.

The resulting samples were analysed for their total element contents using X-ray fluorescence (Spectro X-Lab 2000). Quality control was carried out using the Polish Certified Reference Material Virginia Tobacco Leaves (CTA-VTL-2). We obtained recoveries of 100 % for Fe, 99 % for Ca, K and P, 98 % for Zn, 96 % for Al, 86 % for Cd, and 82 % for Pb.

6.2.3.3. Soil Samples

Soil samples were air-dried and sieved to <2mm; then, aliquots were ground for measuring total $CaCO_3$, TOC, and TN as explained in section 6.2.1.

6.2.4. Statistical methods

Statistical analyses were performed with SPSS 17.0 (SPSS, 2008). The data were log-transformed when necessary to achieve homogeneity of variance (Levene's test). The pore water, plant, and soil data were analysed by t-tests to compare the means of different treatments of the soil, at the 0.05 significance level.

6.3. Results

6.3.1. Initial soil characterisation

The soil from the La Marina del Carmolí salt marsh had pH~6.4 and a fine texture while that from the Lo Poyo salt marsh was very acidic (pH~3.1) and sandy (Table 6.1). Both soils showed high EC, up to 20 dS m^{-1}, calcium carbonate contents below 6 g kg^{-1}, and low concentrations of TOC and TN. In both soils, the total heavy metal concentrations were high, especially for Al, Fe, Pb, and Zn (Table 6.1). The major minerals identified by XRD were: soil from La Marina del Carmolí (quartz 36%, phyllosilicates 22 %, gypsum 14%, goethite 3%, and natrojarosite 2%) and soil from Lo Poyo salt marsh (quartz 46%, phyllosilicates 18%, natrojarosite 13%, gypsum 7%, and goethite 4%).

6.3.2. Effect of liming on soil pH and $CaCO_3$ content

Lime amendment significantly increased the pH and $CaCO_3$ content of both soils ($p \leq 0.004$). The neutral soil from La Marina del Carmolí reached a pH of 7.34 ± 0.01, one unit above its former value, while its initial $CaCO_3$ content was doubled (to 11.4 ± 0.4 g kg^{-1}). In the case of the acidic soil from Lo Poyo, pH increased by more than two units, with a final value of 5.79 ± 0.65, while the $CaCO_3$ content became 4.3 ± 1.1 g kg^{-1} after liming.

6.3.3. pH in the pots and characteristics of the soil solution

At the 18th week, lime amendment had significantly increased pH in the acidic soil (6.42 ± 0.46 without liming and 7.88 ± 0.14 with liming, $p = 0.002$), but no effect occurred in the neutral soil, which showed similar pH values with and without liming (7.84 ± 0.16 without liming and 7.87 ± 0.40 with liming, $p > 0.05$).

The neutral soil had EC values above 40 dS m^{-1}, with and without liming (Figure 6.2). In the case of the acidic soil, the amendment produced a significant increase of EC (60 dS m^{-1}, $p = 0.004$) compared to the unamended soil (40 dS m^{-1}).

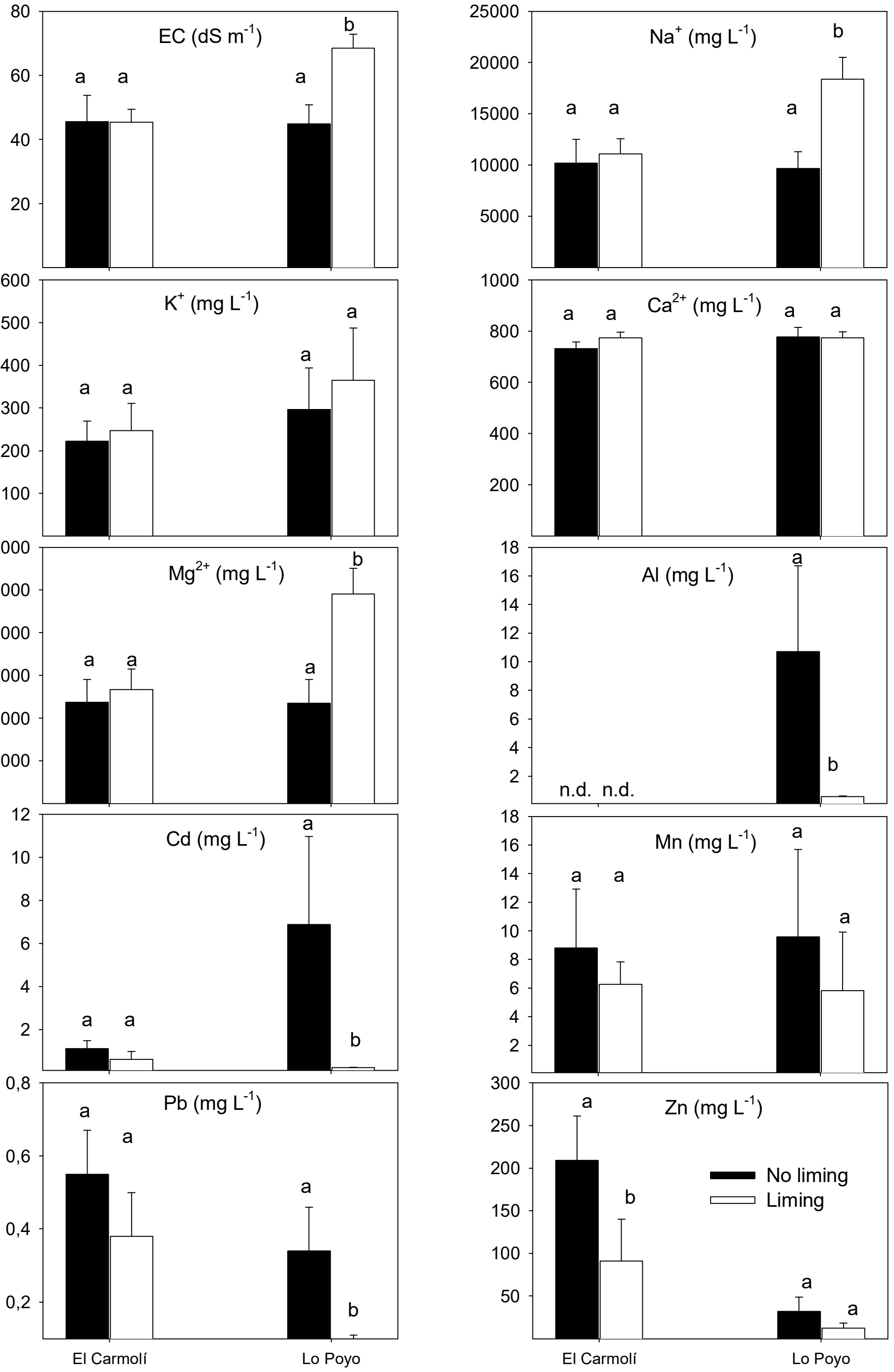

Figure 6.2. Characteristics of pore water at 18th week. Bars on columns are standard deviation (n = 4). Different letters indicate significant differences between treatments for a type of soil ($p<0.05$). EC is electrical conductivity. n.d.: not detected.

The concentrations of K^+, Na^+, Ca^{2+}, and Mg^{2+} were high in all the treatments (Figure 6.2). The Na^+ and Mg^{2+} behaved similarly to EC, with significantly ($p \leq 0.016$) higher values in the amended, acidic soil ($Na^+ \approx 18{,}000$ mg L^{-1} and $Mg^{2+} \approx 5000$ mg L^{-1}). In the neutral soil, there were no significant differences between treatments. Concentrations of K^+ and Ca^{2+}were similar in the two treatments for both types of soil.

In both types of soil, soluble metal concentrations were lower with the amendments (Figure 6.2). Soluble Al was only detected in samples from the acidic soil, with a significant reduction ($p = 0.004$) of more than 90% of its concentration due to the application of lime. The Cd concentration decreased, but not significantly, by over 40% in the amended, neutral soil and significantly ($p = 0.009$) in the amended, acidic soil (by 98%). The soluble Mn concentration was reduced by 30 and 40% in the amended, neutral and acidic soil, respectively, but without significant differences from their respective treatments without liming. The concentration of Pb decreased by 30% in the neutral, amended soil (no significant reduction) and by more than 90% in the acidic soil (significant reduction, $p = 0.014$). The reduction of the Zn concentration with liming was similar in both amended soils, with declines of more than 50%, but was only significant in the case of the neutral soil ($p = 0.047$).

6.3.4. Plant growth and metal uptake

6.3.4.1. Plant survival and biomass production

Liming favoured the survival of *S. fruticosa*. At the end of the experiment, the unamended neutral and acidic soils had percentages of living plants of 13 and 18%, respectively, while the amendment gave a survival rate of 83% in the neutral soil and 53% in the acidic one.

The total biomass production was higher in the amended soils (Figure 6.3). In the neutral soil, total biomass was 3.98 ± 2.43 g d.w. plant^{-1} without liming and 15.81 ± 8.44 g d.w. plant^{-1} with liming (an increase of 75%, not significant, $p = 0.060$). In the acidic soil, total biomass was 4.11 ± 1.79 g d.w. plant^{-1} without liming and 37.41 ± 5.17 g d.w. plant^{-1} with liming (an increase of 90%, significant, $p = 0.000$). Lime amendment increased root weight (Figure 6.3), but only significantly so in the acidic soil ($p = 0.003$). Amendment produced higher dry, perennial shoot biomass (Figure 6.3), with a significantly higher values ($p = 0.011$) in the case of the acidic soil (increase of 82%).

The biomass of swollen photosynthetic stems was significantly higher for both amended soils (p = 0.044 in the neutral soil and p = 0.001 in the acidic soil) (Figure 6.3).

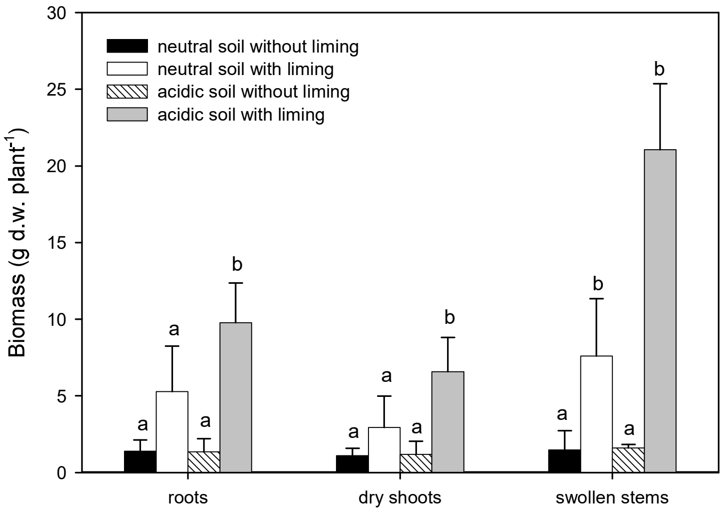

Figure 6.3. Dry weight of the plants. Bars on columns are standard deviation (n = 4). Different letters indicate significant differences between treatments for a type of soil ($p<0.05$). Neutral soil is from El Carmolí salt marsh and acidic soil from Lo Poyo salt marsh.

6.3.4.2. Plant metal concentration

The roots had higher concentrations of Al, Mn, Pb, and Zn than the aerial shoots (Figure 6.4). Plants grown in both amended soils accumulated higher concentrations of these four metals in roots than the corresponding plants grown without liming. In the neutral soil, the concentrations were 35% higher for Al, 57% higher for Mn, and 45% higher for Pb, while in the acidic soil, the concentrations were 57% higher for Al, 22% higher for Mn, and 84% higher (significantly different, p = 0.005) for Pb. In the case of Zn, amendment produced root concentration between 20 and 24% higher than for the unamended soils.

By contrast, in the above-ground tissues, the Al, Mn, Pb, and Zn concentrations were lower with the application of liming, both in dry, perennial, non-photosynthetic shoots and in swollen, photosynthetic stems (Figure 6.4). The dry, perennial shoots showed concentration reductions in the amended, neutral soil between 51% for Mn and 70% for Zn, with respect to the treatment without liming. In the case of the acidic soil, the amendment gave concentration reductions in dry, perennial shoots between 58% for Mn and 90% for Zn. The lime amendment also decreased the metal concentrations in swollen stems, between 37% for Pb and 62% for Zn in the neutral soil and between 12% for Mn and 75% for Zn in the acidic one. However, in the case of Al, the concentration in swollen stems was 18% lower in the neutral, amended soil than in the unamended soil, while the contrary happened in the acidic soil: the accumulation of Al was 55% higher with liming.

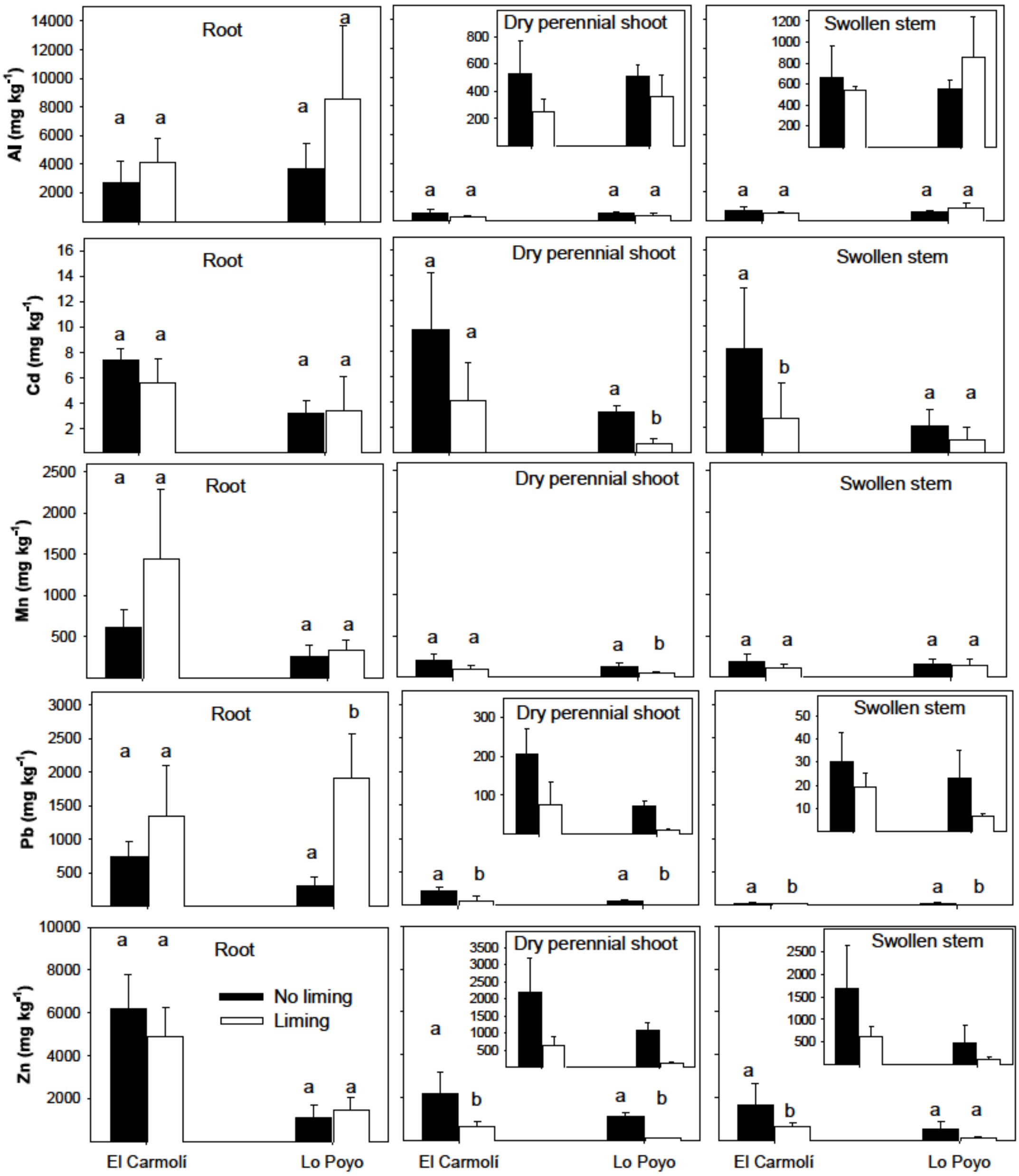

Figure 6.4. Metal concentrations in plants. Bars on columns are standard deviation (n = 4). For each type of soil, vegetal tissue and metal, different letters indicate significant differences between treatments ($p<0.05$). n.d.: not detected.

The Cd accumulation in plant tissues showed a behaviour different to that of the other metals, with similar concentrations in the roots and aerial shoots in all the treatments (Figure 6.4). Plants grown in both amended soils had lower Cd concentrations than those in the unamended ones. Liming decreased the accumulation of Cd below 50% in above-ground tissues. This reduction was significant in swollen stems of the neutral, amended soil ($p = 0.016$) and in dry, perennial shoots of the acidic, amended soil ($p = 0.001$).

6.3.5. Total $CaCO_3$, total organic carbon (TOC) and total nitrogen (TN) in soils

Samples from the neutral soil showed, at the end of the experimental period, $CaCO_3$ contents of 4.3 ± 1.9 g kg^{-1} when unamended and 11.3 ± 3.9 g kg^{-1} when amended (significantly different, $p = 0.005$). In the acidic soil, $CaCO_3$ was not detected, with or without liming.

After 10 months of *S. fruticosa* growth in the pots, the TOC concentrations were significantly higher in both amended soils (neutral soil, $p = 0.008$ and acidic soil, $p = 0.039$): in the neutral soil 4.51 ± 0.21 g kg^{-1} without liming and 6.70 ± 1.11 g kg^{-1} with liming and in the acidic soil 3.95 ± 0.49 g kg^{-1}without liming and 5.09 ± 0.72 g kg^{-1} with liming.

Unlike TOC, the concentrations of TN in soils were not significant different between treatments of the same soil, with values around 0.3 and 0.5 g kg^{-1} in all cases.

6.4. Discussion

6.4.1. Effect of liming on soluble metal concentrations

Conventional soil remediation methods involve excavation and land-filling and the application of engineering-type techniques like chemical washing or thermal desorption (Kumpiene et al., 2008; Fernández-Caliani and Barba-Brioso, 2010). The high cost of these methods, which are environmentally unfriendly, has led to the development of alternative techniques to remediate contaminated sites, such as chemical stabilisation of heavy metals induced by immobilising soil additives (Kumpiene et al., 2008; Gray et al., 2006). Liming improves the characteristics of soil through its direct effect on the amelioration of soil acidity and through its indirect effects on the mobilisation of plant nutrients (Bolan et al., 2003), the immobilisation of toxic heavy metals and the improvements in soil structure and hydraulic conductivity (Haynes and Naidu, 1998). Hence, the aim of metals immobilisation is to reduce their availability, since the soluble forms are generally considered to be the most-readily available for plant uptake (McLaughlin et al., 2000).

For our experiment, we used as the soil liming amendment a waste generated during the cutting, sawing and polishing of marble. It is made up of finely divided particles and thus has a high surface area, advantageous for the reduction of soil acidity. After the application of this material, the calcium carbonate content and pH increased, which in turn led to a decrease in the concentration of soluble metals. In the acidic soil the concentrations following amendment being 90% lower for most of the metals analysed, compared with the unlimed soil. An increase in soil pH leads to an increase in the net negative charge of variably charged colloids in soils, such as clays, organic matter and Fe/Al oxides. This can result in an increase in heavy metal sorption and a decrease in desorption, thus diminishing the soluble metal concentrations in soils (Oste et al., 2002; Basta and McGowen, 2004). Also, it has been suggested that the metals in limed soils are precipitated as carbonates or hydroxides (Gray et al., 2006). In the neutral soil, with a higher initial pH (~6.4), the addition of the lime amendment resulted in less-marked reductions in the concentrations of metals in the soil solution, with less than 50% difference between the limed and unlimed soils.

6.4.2. Plant survival and growth

The establishment of vegetation in areas affected by mine activity has several advantages over the conventional remediation methods: it is aesthetically pleasing, relatively low-cost and less disruptive to the environment, it provides a beneficial habitat for wildlife, it stimulates microbial immobilisation of heavy metals in the rhizosphere and it reduces the risk of ingestion and inhalation of wind-blown toxic particles (Fernández-Caliani and Barba-Brioso, 2010). *S. fruticosa*, a common halophyte in Mediterranean salt marshes, can occur in areas with high metal loads (Álvarez-Rogel et al., 2004; Carrasco et al., 2006; Caetano et al., 2007), so it could be a good option for the regeneration of saline areas polluted by mining wastes - as in the case of the coastal salt marshes of the Mar Menor lagoon.

Survival rates of *S. fruticosa* were 6-fold greater in the neutral, amended soil and 3-fold greater in the acidic, amended soil, with respect to the corresponding unamended treatments, the higher biomass values occurring in the swollen, photosynthetic stems. This was accompanied by an increase in the TOC content in the soils The shoot:root biomass ratio can be used as an index to evaluate the health and vigour of plants and to determine the success or failure of their establishment in a habitat (Conesa et al., 2009). In our experiment, lime addition increased this index in both soils, but to differing extents: from 1.9 to 2.0 in the neutral soil and from 2.0 to 2.8 in the acidic one. The greater growth increase followed the lime application to the acidic soil, although the highest survival rate occurred in the neutral, amended soil. The soil physical properties may help to explain this result.

The mine wastes used are unstructured. When wet, they tend to loosen and settle. When dry, they become very hard, which, together with the high metal load, makes them very difficult substrates for rooting of plants. According to Bradshaw et al. (1978), fine tailings are prone to compaction and cementation processes which can combine to form an impenetrable surface barrier. At a constant soil water content, as in our experiment, penetration resistance increases with an increase in the silt + clay fraction or degree of compaction (Taylor et al., 1966; Bennie, 1991). Penetration resistance is defined as the resistance offered by the soil matrix against deformation by roots growing in a homogeneous, non-structured soil (Bennie, 1991). Probably, after neutralisation of the high acidity of the Lo Poyo soil and plant establishment, the sandier texture of this soil could have favoured a greater development and penetration of the root system of *S. fruticosa*, which was thus able to support a greater aerial biomass, than the neutral soil from El Carmolí with a finer texture. This is in accordance with Wang et al. (2008), who proposed the mixing of tailings with sand, as an alternative to achieve a favourable physical structure.

The greater biomass production led to a higher concentration of salts (Na^+ and Mg^{2+}) in the soil solution of the acid, amended soil, since, through their growth, the plants extracted more water from the soil, causing greater accumulation of salts in their rhizospheric environment. This is in accordance with Barrett-Lennard and Malcolm (1999), who found accumulation of salt in the rhizosphere of salt tolerant, perennial shrubs as a consequence of the uptake of the saline groundwater by the roots.

6.4.3. Metal uptake and translocation

Those plant species that accumulate more metals in below-ground tissues and in their surrounding sediments contribute more effectively to reduction of their bioavailability (Weis and Weis, 2004), while metals accumulated in aerial tissues may be excreted through leaves (Weis et al., 2002) or accumulated in leaf and stem litter (Windham et al., 2003), returning to the soil.

Metals in halophytes are mainly accumulated in the roots, with small quantities translocated to the aerial parts (Weis et al., 2002; Windham et al., 2003). We found higher metal concentrations in roots of *S. fruticosa* than in above-ground tissues (except for Cd), which is in accordance with (Caetano et al., 2007, 2008; Caçador et al. 2009; Duarte et al., 2010).

The translocation factor (TF) (Mattina et al., 2003) is the ratio between the concentrations of metals in shoots and roots and defines the ability of the plant to translocate metals to the above-ground tissues. Hyperaccumulator plants have ratios higher than 1 and excluders less than 1. In both soils, we found TF<1 for Al, Mn, Pb and Zn, both for the ratio swollen, photosynthetic stems:roots and the ratio dry, perennial shoots:roots. Most plants restrict the movement of metals into photosynthetic tissues (Windham et al., 2003). This is achieved by restricting metal transport across the root endodermis and upwards in the xylem, by storing in cell walls and vacuoles or through binding by metallothionins or phytochelatins (Vesk et al., 1999; MacFarlane and Burchett, 2000). Although the application of the lime amendment to our soils lowered the content of metals in the soil solution, some of the metals remained at high levels with respect to unpolluted soils. So, the plants that grew in the amended soils and produced roots of greater surface area (greater biomass production) had to have taken up significant quantities of water and nutrients along with other solutes like metals, compared to the plants of the unamended soils. These metals were transported towards the roots, with only small amounts being translocated upwards. Conesa et al. (2007) found high contents of some metals in plants grown in acid mine tailings amended with $CaCO_3$, in spite of the fact that most metals were not detectable in the soil solution.

6.4.4. Implications for management

Our results show numerous advantages of the combined effects of liming on soluble metal concentrations and *S. fruticosa* development in polluted, salt marsh soils. The first and most-positive effect is the decrease in soil soluble metal concentrations. This is a key point because flooding (and/or frequent soil water saturation) has a main role in biogeochemical cycles in wetlands and may convert these systems into sources of toxic metals dissolved in the water (Vega et al., 2009).

The second effect is the higher plant biomass production with liming, which can have some drawbacks but also many positive implications for reducing environmental risks in metals–polluted, salt marsh soils. *S. fruticosa*, unlike other halophytes of the Chenopodiaceae such as *Arthrocnemum macrostachyum*, grows relatively quickly, forming dense stands that can have 100% cover and more than 1.5m height (Álvarez-Rogel et al., 2006). Hence, stands of this plant can be a pool of metals in aerial tissues. However, at the same time, the closed canopy greatly reduces water and wind erosion and makes it difficult (and eventually impossible) for the Limnicolae and other species to feed in these vegetated soils, by eliminating the pathway of exposure for many water birds that the ingestion of soil particles along with food represents (Beyer et al., 1994).

High metal accumulation in the above-ground parts of plants may pose a risk for the food web if aerial organs are consumed by animals. Hence, it is useful to reduce metal accumulation in aerial shoots. In our experiment, translocation factors decreased with the application of lime in both types of soil, indicating that higher-biomass plants tended to accumulate more metals in roots and to restrict their movement to aerial tissues. This is a very-interesting result, since liming not only favoured plant growth, but also improved the ability of *S. fruticosa* to phytostabilise metals in its below-ground parts. In fact, the limed, acidic soil was the only case in which the Zn concentration in the aerial swollen stems was below 178 mg kg^{-1}, the maximum Zn level recommended in order to prevent marginal, sub-lethal effects in birds (Eisler, 1993).

Our findings meet the objectives of phytomanagement techniques (Robinson et al., 2009), since liming decreased metal solubility in polluted, salt marsh soils and increased the growth and the capacity for phytostabilisation of heavy metals of *S. fruticosa*. We currently have field researches in progress in order to corroborate our greenhouse results.

6.5. Acknowledgments

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Chapter 7

Iron dynamics in simulated soil profiles

Liming and vegetation favour Fe-mobilisation in eutrophic wetland soils affected by mine wastes

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Abstract

Purpose: The aim of this study was to evaluate the effects of liming and vegetation on the dynamic of Fe in eutrophic wetland soils affected by mine wastes, under different flooding regimes.

Materials and methods: Simulated soil profiles were constructed with two types of wastes (pH~3.1 and ~6.4) collected from polluted salt marshes and four treatments were assayed: without liming + without plant, without liming + with plant, with liming + without plant, and with liming + with plant. The plant species was the halophyte *Sarcocornia fruticosa*. Three depths were differentiated in the profiles: A horizons (never under water), C1 (alternating flooding-drying conditions), and C2 (always under water). The pH, Eh, and soluble Fe concentrations were measured regularly at each depth for 18 weeks.

Results and discussion: Liming decreased Fe solubility, mainly in the acidic waste, but also facilitated a drop in Eh, favouring the dissolution of amorphous Fe oxides and hence increasing the concentration of soluble Fe. The plant rhizosphere contributed to the decrease of Eh and actively re-distributed the dissolved Fe along the profiles.

Conclusions: We conclude that the combined use of liming and vegetation enhance the mobilisation of Fe in eutrophic wetland soils polluted by mine wastes. This should be considered for the environmental management of these sites due to the role of Fe-minerals as sink for potentially-harmful compounds.

Keywords: Mar Menor, Phytomanagement, Redox potential, *Sarcocornia fruticosa*, Spolic Technosols

7.1. Introduction

The accumulation of mine wastes in tailings and old ore deposits is one of the main consequences of the closure of mines, worldwide (Dudka and Adriano, 1997). Mining also affects land far away from the mine site, when the tailings are eroded. Wetlands are among those environments most affected due to their location in the most-depressed positions, topographically. Biogeochemical processes in wetlands soils may favour the removal of contaminants (Mitsch and Gosselink, 2000; Ramsar, 2001), however these systems might also act as sources of hazardous substances (Kirk, 2004; Du Laing et al., 2007). The mobility of metals is dependent on multiple factors: temperature, pH, redox potential, salinity, texture, distribution, and organic matter content (Otte et al., 1993; Williams et al., 1994; Almeida et al., 2004). Wetlands vegetation may modify these parameters and influence metal dynamics (Jacob and Otte, 2003; Weis and Weis, 2004; Vardanyan and Ignole, 2006).

Mine wastes have high contents of metals and pose a risk for environmental and human health; for this reason, their restoration and geochemical stabilisation have been studied extensively (Simón et al., 2010). The use of strategies such as phytomanagement has gained interest in recent years. Phytomanagement consists of the engineering or manipulation of soil–plant systems to control pollutant fluxes in the environment (Robinson et al., 2009). In particular, it has been demonstrated that the application of lime amendments is a viable method for reducing the risks of metals leaching and for facilitating plant growth (Davis et al., 1999; Simón et al., 2010). For example, a study conducted in microcosms demonstrated that liming reduced the concentrations of soluble metals and increased the growth of *Sarcocornia fruticosa* (L.) A. J. Scott (a typical halophyte species from Mediterranean environments) in saline mine wastes (González-Alcaraz et al., 2011a). However, little is known of the consequences of combining liming and vegetation in flooded soils polluted by mine wastes.

The aim of this study was to evaluate the combined effect of liming and the presence of *Sarcocornia fruticosa* on the dynamic of Fe under distinct flooding regimes using eutrophic water, in simulated soil profiles constructed with mine wastes collected from polluted salt marshes. The biogeochemistry of Fe is related to the behaviour of other metals and to a great number of potentially-harmful substances that can be retained on oxides, oxyhydroxides, and sulphides of this metal (Otero and Macías, 2002, 2003; Du Laing et al., 2007). In fact, the behaviour of Fe is a key aspect in the processes occurring at the sediment-water interface and an understanding of its dynamic is basic for the improvement of remediation strategies (Koschorreck et al., 2007). If an inadequate management strategy is implemented, it might transform these systems into sources of potentially-harmful substances. The identification of drawbacks in the conventional treatments (e.g. liming and revegetation) employed in the remediation of wetland soils affected by mine wastes should help to improve the management strategies and to reduce the environmental risks.

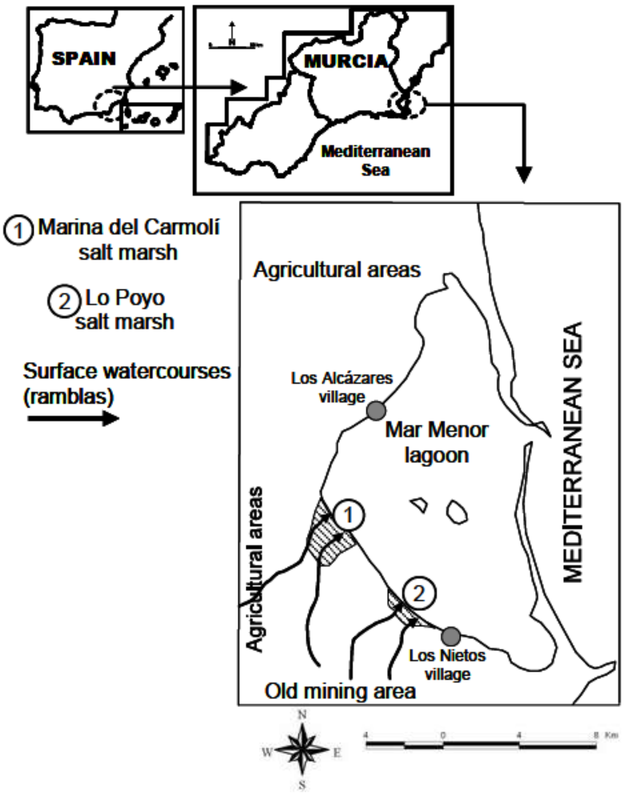


Figure 7.1. Location map of the sampling sites in SE Spain.

7.2. Material and methods

7.2.1. Sampling sites and characteristics of the mine wastes

Mine wastes were collected from two coastal salt marshes (top 20 cm of soil) of the Mar Menor lagoon in SE Spain (Figure 7.1): El Carmolí salt marsh (318.6 ha, N37º41´42´´, W0º51´31´´) and Lo Poyo salt marsh (210.6 ha, N37º39´52´´, W0º49´02´´). These marshes are affected by eutrophic water flowing from agricultural and urban areas and by mine wastes derived from erosive processes in the nearby mining area of La Unión-Sierra de Cartagena (Conesa and Jiménez-Cárceles, 2007). The latter territory is among those most affected by the impact of mining activities in Europe (Conesa and Schulin, 2010).

Table 7.1. General characteristics of the mine wastes (González-Alcaraz et al., 2011a). The values are average ± SD (n = 3). n.d.: not detected.

	Salt marsh	
	El Carmolí	Lo Poyo
pH	6.43 ± 0.06	3.09 ± 0.01
EC[a)] (dS m^{-1})	20.3 ± 2.84	24.8 ± 1.31
$CaCO_3$ (g kg^{-1})	5.88 ± 1.15	n.d.
TOC[b)] (g kg^{-1})	4.83 ± 0.46	3.11 ± 0.49
TN[c)] (g kg^{-1})	0.28 ± 0.02	0.41 ± 0.06
Sand (%)	40 ± 3.7	67 ± 2.5
Silt + Clay (%)	60 ± 3.7	33 ± 2.5
Al (g kg^{-1})	13.2 ± 1.72	16.4 ± 2.42
As (g kg^{-1})	0.22 ± 0.03	0.31 ± 0.03
Cd (g kg^{-1})	0.016 ± 0.006	0.001 ± 0.0006
Cu (g kg^{-1})	0.18 ± 0.06	0.16 ± 0.07
Fe (g kg^{-1})	61 ± 12.3	118 ± 26.7
Mn (g kg^{-1})	3.51 ± 0.67	0.40 ± 0.09
Pb (g kg^{-1})	6.75 ± 0.86	5.03 ± 0.24
Zn (g kg^{-1})	52.3 ± 10.2	3.78 ± 1.10

[a)] Electrical conductivity, [b)] Total organic carbon, [c)] Total nitrogen

The mine wastes from El Carmolí were fine textured (60% silt+clay), slightly acidic (pH~6.4), and with a low calcium carbonate content (~6 g kg^{-1}) (Table 7.1). The wastes from Lo Poyo were sandy (67% sand), strongly acidic (pH~3.1), and without carbonates (Table 7.1). The contents of Fe and other metals were very high for the wastes from both sites. The major minerals identified (semi-quantitative estimation of the crystalline fraction by power X-Ray Diffraction) in the wastes from El Carmolí were quartz (36%), phyllosilicates (22 %), gypsum (14%), goethite (3%), and natrojarosite (2%), and in the wastes from Lo Poyo, quartz (46%), phyllosilicates (18%), natrojarosite (13%), gypsum (7%), and goethite (4%) (González-Alcaraz et al., 2011a).

7.2.2. Experimental set up

A lime amendment (54% magnesium calcite-($Mg_{0.03}Ca_{0.97}$)(CO_3)- and 37% dolomite-$CaMg(CO_3)_2$-) of pH 8.9 (1:2.5 water suspension) and particle size <50 µm, derived from the marble industry, was added to each waste at a dose of 20 g kg^{-1}, giving four waste treatments: waste from El Carmolí with and without liming and waste from Lo Poyo with and without liming. Liming increased the pH of both wastes: to ~7.3 in El Carmolí and to ~5.8 in Lo Poyo. Plastic pots (13.5 cm diameter and 14 cm height) and methacrylate cylinders (20 cm diameter and 60 cm height) were filled with each waste treatment. The cylinders were covered with opaque plastic to simulate the darkness in the soil profiles.

Table 7.2. Characteristics of irrigation water. Values are average ± SD (n = 5). n.d. = not detected.

	Average (SD)
pH	8.13 ± 0.23
EC[a)] (dS m^{-1})[a]	21.6 ± 5.3
NO_3^- (mg L^{-1})	366 ± 50
PO_4^{3-}(mg L^{-1})	0.82 ± 0.32
DOC[b)] (mg L^{-1})[b]	10.5 ± 1.12
Na^+ (mg L^{-1})	3286 ± 232
K^+ (mg L^{-1})	67 ± 7.7
Ca^{2+} (mg L^{-1})	706 ± 73
Mg^{2+} (mg L^{-1})	832 ± 64
Al (µg L^{-1})	n.d.
As (µg L^{-1})	n.d.
Cd (µg L^{-1})	n.d.
Cu (µg L^{-1})	11.1 ± 4.77
Fe (µg L^{-1})	121 ± 47.9
Mn (µg L^{-1})	12.6 ± 10.7
Pb (µg L^{-1})	4.15 ± 0.91
Zn (µg L^{-1})	69.3 ± 7.66

[a)] Electrical conductivity [b)] Dissolved organic carbon.

The experiment was performed in two phases (always under natural light and temperature ranging between 5 and 35 ºC). In the first phase, cuttings of *Sarcocornia fruticosa* were collected from non-polluted areas of El Carmolí salt marsh and planted in the pots (one plant per pot). The pots were distributed randomly inside a greenhouse and irrigated (but not flooded) 2-3 times per week for 10 months, with eutrophic water from a local stream (Table 7.2). In both types of mine waste, liming favoured the growth of *S. fruticosa* and the largest plants were found in the limed waste from Lo Poyo (González-Alcaraz et al., 2011a). Pots without plants were handled in the same way for each waste treatment.

After ten months, the second phase began. The contents of six pots (three with *S. fruticosa* and three without plants) for each waste treatment were transferred to the corresponding cylinders, by mixing and homogenising the soil in the root ball with the soil in the cylinder. The cylinders were irrigated (but not flooded) for 5 months with the preceding eutrophic water in order to facilitate plant establishment.

In each cylinder (hereafter soil profile), two 10-cm Rhizon ® type samplers (MOM 19.21.21, diameter = 2.5 mm, pore diameter = 0.1 μm) and Eh and pH electrodes (Crison 50-55 and Crison 50-50, respectively) were installed horizontally at depths of 5, 30, and 55 cm (hereafter A, C1, and C2 horizons). The Rhizon ® samplers were connected to 50-mL plastic syringes (BD Plastipak) by means of extension tubes, to extract soil solution. Figure 7.2 shows a scheme of the experimental set up.

Each soil profile was placed inside a larger container filled with synthetic eutrophic water with a pH~7.5 and a electrical conductivity~11 dS m^{-1}, enriched in organic carbon (0.31 g L^{-1} $Ca(NO_3)_2$, 3.8 g L^{-1} NaCl, 3 g L^{-1} $MgSO_4$, 0.8 g L^{-1} $CaCl_2$, 0.2 g L^{-1} KCl, 0.043 g L^{-1} H_3PO_4 85%, 0.15 g L^{-1} glucose, and 0.1 g L^{-1} meat extract with 46% organic carbon). The water level (WL) in the containers was maintained at 20 cm below the soil surface for five weeks -weeks 1 to 5- (1st High WL) and at 45 cm below during the following five weeks - weeks 6 to 10- (1st Low WL), repeating this cycle two times along the study (2nd High WL -weeks 11 to 15- and 2nd Low WL - weeks 16 to 18-). Hence, the upper 20 cm (A horizons) were never under water, the soil between 20 and 45 cm (C1 horizons) was under water for 10 weeks with two alternating drying periods of 5 and 3 weeks, respectively, and the deepest 45 cm (C2 horizons) were always under water.

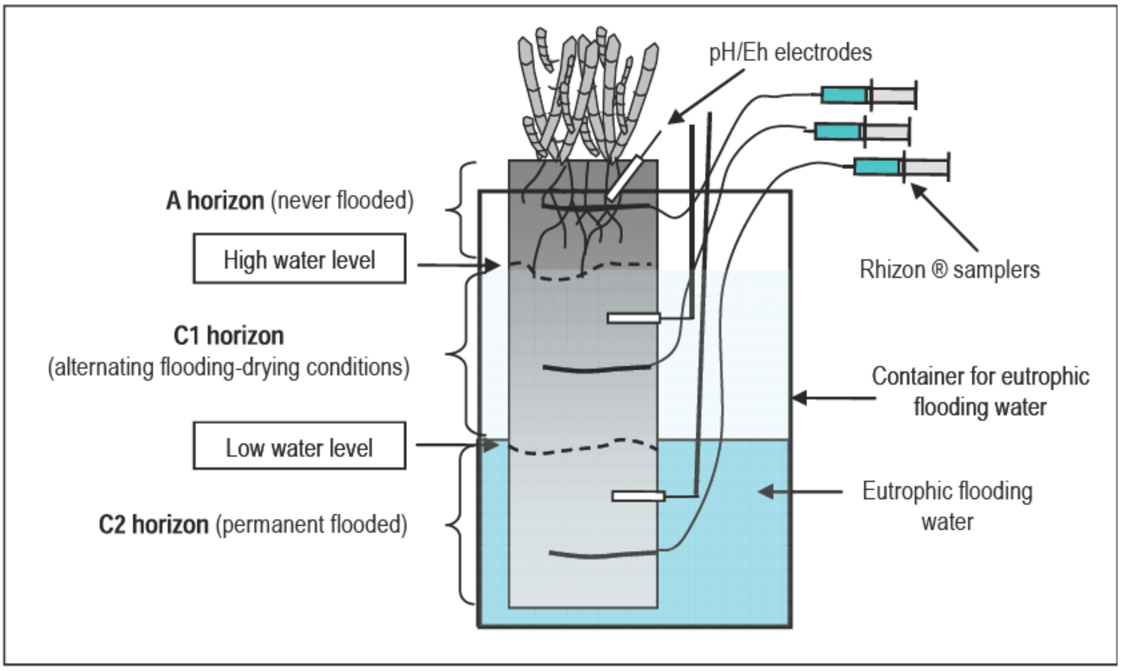


Figure 7.2. Scheme of the experimental set up.

So, the work in the soil profiles lasted for a total of 38 weeks. At the end, the total fresh biomass of the plants in the different treatments was 25±14 g for El Carmoli without liming, 502±74 g for El Carmolí with liming, 20±4 g for Lo Poyo without liming, and 326±150 g for Lo Poyo with liming.

7.2.3. Monitoring of parameters and water sampling and analysis

The pH and Eh were weekly measured at each depth in each soil profile. The data for these two parameters were recorded after 10 minutes of stabilisation of the electrodes. The Eh measurements were adjusted according to (Vepraskas and Faulkner, 2001), by adding +200 mV to the soil voltage (the value of the Ag/AgCl reference electrode at 20 ºC).

Pore water samples were collected every two weeks. The two sub-samples obtained for each depth in each soil profile were poured into the same plastic vial for subsequent analysis. The electrical conductivity (EC) at 25 ºC was measured immediately using a Crison Basic 30 conductimeter. Then, the samples were acidified with sulphuric acid, stored hermetically, and frozen at -20 ºC until analysis. After thawing, the concentration of soluble Fe was determined by ICP-MS (Agilent 7500A).

Additionally, the Eh and pH were weekly measured in the eutrophic flooding water at ~15 cm under water, in three containers for each waste treatment chosen at random, and samples were taken and analysed for EC, Cl^-, SO_4^{2-}, dissolved organic carbon (DOC), and soluble Fe, in order to monitor the changes in the physical-chemical characteristics outside of the soil profiles. The Cl^- and SO_4^{2-} were determined with a Dionex Ionic Chromatographer and the DOC using a Total Organic Carbon Analyzer (TOC-V_{CSH} Shimadzu). The EC and soluble Fe were measured as in the pore water samples.

7.2.4. Soil sampling and analysis

At the end of the experiment, the methacrylate cylinders were cut longitudinally and opened into two halves. The colours of the soil matrix and the mottles were described according to (Munsell, 1994; FAO, 2006) in order to identify redoximorphic features (Vepraskas, 2001) that indicated Fe-mobilisation.

The sequential extraction procedure proposed by Zeien and Brümmer (1989, 1991) was applied to fresh waste samples taken at each depth from two soil profiles per treatment. Table 7.3 presents the definition of the fractions and the characteristics of the extracting solutions. All the suspensions were centrifuged at 2500 rpm for 15 min, filtered, and the Fe concentration measured by ICP-MS (Agilent 7500A). Aliquots of the fresh samples were oven-dried at 65ºC to constant weight and soil moisture was calculated. The concentration of Fe was expressed on a dry weight basis. In order to evaluate the efficiency of the sequential extraction procedure, a total digestion of fresh samples was made in parallel with HNO_3 (69%) + $HClO_4$ (60%). The recoveries for the sum of the seven fractions compared to the total analysis were between 75 and 125%. These values were similar to those reported by other authors that used the same extraction procedure in similar soils (e.g. Conesa et al. 2008). The accuracy of the HNO_3 + $HClO_4$ digestion was checked by analysing the certified reference soil CRM027-050 and the recovery obtained for total Fe was 101%. Unfortunately, the samples from the treatment without liming + without plant for El Carmolí were lost before analysis.

Table 7.3. Zeien and Brümer (1989, 1991).

Fraction	Chemical interpretation	Extractant	Duration
F1	Mobile (water soluble and easily exchangeable)	1M NH_4NO_3	24 h, 25 ºC
F2	Easily mobilise (specifically adsorbed, bound to $CaCO_3$)	1M NH_4OAc (pH 6.0)	24 h, 25 ºC
F3	Bound to Mn oxides	0.1M NH_2OH-HCl + 1M NH_4OAc (pH 6.0)	30 min, 25 ºC
F4	Bound to organic complexes	0.025M NH_4-EDTA (pH 4.6)	90 min, 25 ºC
F5	Bound to amorphous Fe oxides	0.2M NH_4-oxalate (pH 3.25)	60 min, 25 ºC, dark
F6	Bound to crystalline Fe oxides	0.1 M ascorbic acid + 0.2M NH_4-oxalate (pH 3.25)	30 min, 96 ºC
F7	Residual	HNO_3 (69%) + $HClO_4$ (60%)	90 min, 210 ºC

7.2.5. Statistical analysis

The statistical analysis was performed with SPSS 17.0 (SPSS Inc., 2008). For each type of waste and for each horizon (A, C1, and C2), repeated measures ANOVA were performed in order to compare the pH, Eh, EC, and soluble Fe concentrations in the soil profiles among the different treatments (without liming + without plant, without liming + with plant, with liming + without plant, and with liming + with plant). When the ANOVA was significant, the post-hoc Bonferroni test was used to identify different groups ($\alpha = 0.05$).

7.3. Results

7.3.1. Eh, soluble Fe, DOC, and pH in the eutrophic flooding water

The changes in the Eh, soluble Fe, DOC, and pH in the eutrophic flooding water were in accordance with the variations in the water level, for all the treatments (Figure 7.3). The lowest Eh values were found during the phases of High WL (minimum ~-100 mV at the 13th week) and the highest during the phases of Low WL (maximum ~+400 mV at the 10th week). The opposite occurred with soluble Fe: the concentrations were low during the Low WL phases (minimum ~100 µg L^{-1} at the 10th week) and strongly increased during the High WL phases (maximum ~440 µg L^{-1} at the 12th week).

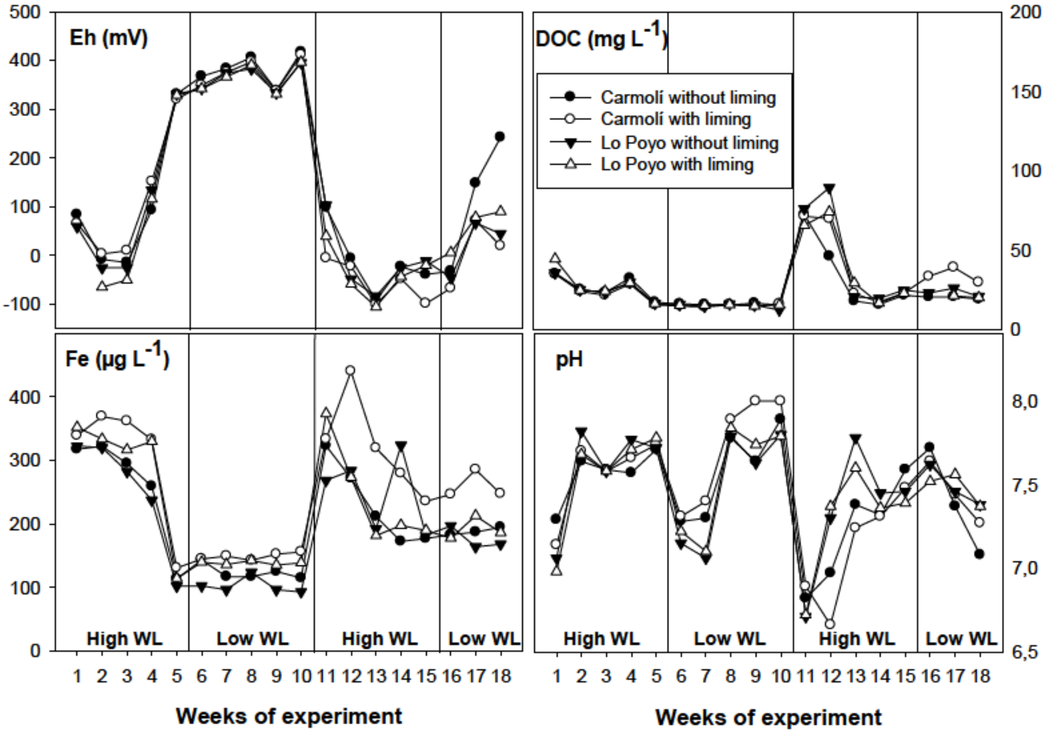


Figure 7.3. Changes of Eh, DOC, soluble Fe, and pH in the flooding water. Each value is the average for each sampling date. n = 3. Bars of standard deviation were omitted for clarity.

The concentration of DOC was high (~40 mg L^{-1}) in the first week and decreased progressively (to a minimum of ~10 mg L^{-1} at the 10th week) until the new addition of eutrophic water at the beginning of the second period of High WL (Figure 7.3), before decreasing again during the last weeks.

Unlike the other parameters, the pH not only varied when the water level changed, but also within each period of High and Low WL (Figure 7.3). In the first week, the pH was ~7 and then increased until ~7.7 at the 5th week. When the water level dropped, the pH decreased again to ~7 (7th week) before increasing to ~8 at the 10th week. When the water level rose again, the pH dropped to ~6.7 between weeks 11 and 12 but increased to ~7.6 in the 15th week. Finally, the pH tended to drop again in the last period of Low WL. These changes may be related to the consumption of DOC and to the physical-chemical processes occurring in the flooding water, as will be discussed later.

The EC was nearly constant during the 18 weeks of the experiment, with a mean value of 11±0.79 dS m^{-1} (data not shown).

7.3.2. Electrical conductivity, pH, and Eh in the soil profiles

The profiles with waste from El Carmolí had lower EC values than those with waste from Lo Poyo; in both cases, the accumulation of salts was higher in the A horizons of all the treatments (Figure 7.4). For each type of waste, the A horizon of the treatment with liming + with plant showed significantly higher EC than the other treatments ($p \leq 0.007$ for El Carmolí and $p \leq 0.006$ for Lo Poyo), reaching values greater than 60 dS m^{-1}.

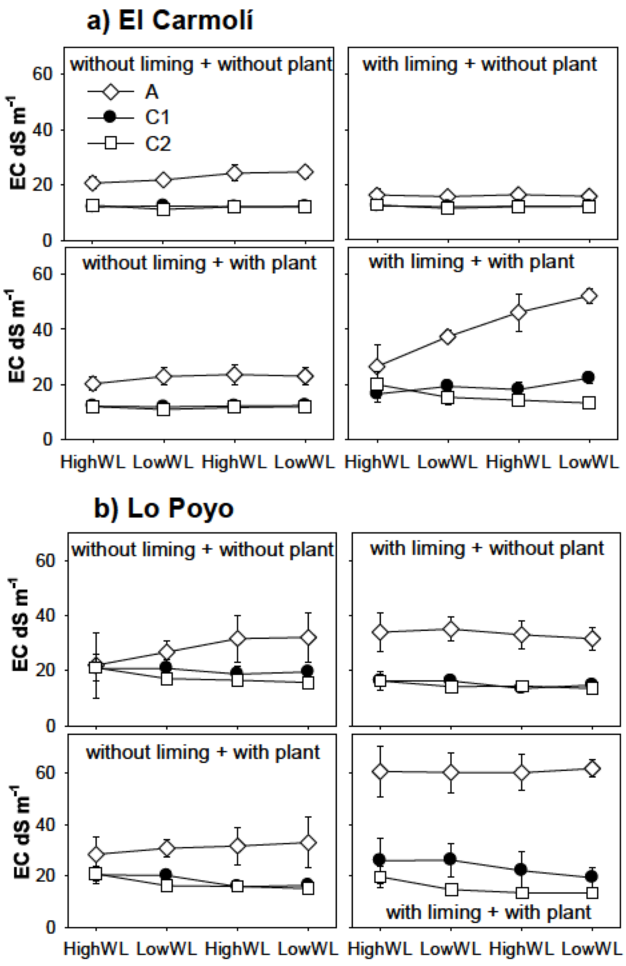


Figure 7.4. Electrical conductivity (EC) within the soil profiles. Each value is the average for each period of High and Low Water Level –WL- (±SD). n = 9. Where SD is not shown, the bars are the size of symbols or smaller.

For both types of wastes, liming favored a significant increase of the pH ($p \leq 0.027$ for El Carmolí and $p \leq 0.001$ for Lo Poyo), except in the C1 horizon of the treatment with liming + with plant from El Carmolí (Figure 7.5). In the profiles with waste from El Carmolí, the pH values were between 6 and 7 without liming and between 7 and 8 with liming. In the profiles with waste from Lo Poyo, the effect of the lime amendment was more evident: the pH ranged from 3.5 to 5 without liming and from 6 to 7 with liming.

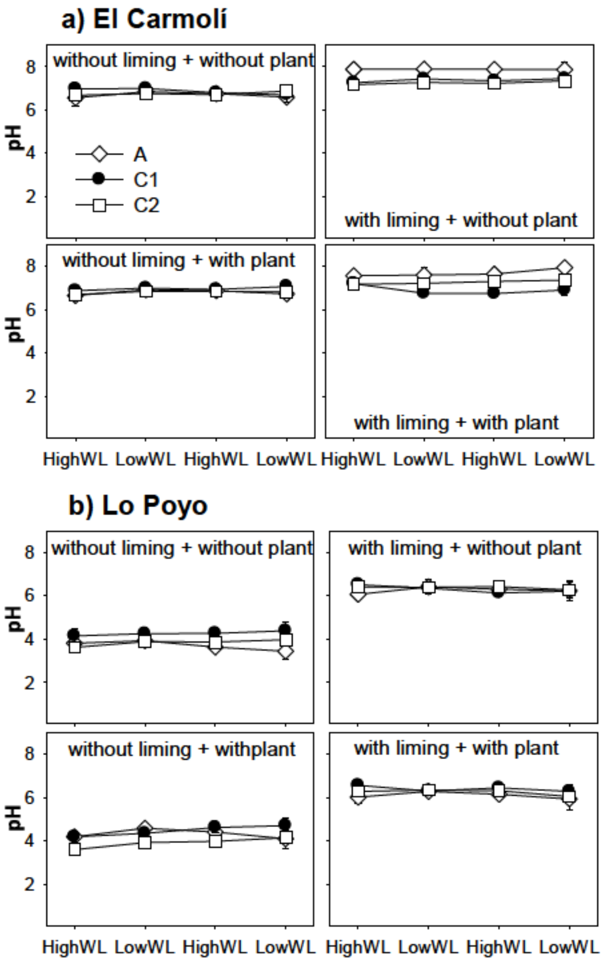


Figure 7.5. pH within the soil profiles. Each value is the average for each period of High and Low Water Level –WL- (±SD). n = 9. Where SD is not shown, the bars are the size of symbols or smaller.

In the profiles with waste from Lo Poyo, liming favoured significantly lower Eh values at all depths ($p \leq 0.048$), while in the profiles with waste from El Carmolí, the latter was true for the A and C1 horizons ($p \leq 0.013$) (Figure 7.6). In addition, the Eh was related to the changes in the water level. In the A horizons, the Eh was always higher than +300 mV. In the C1 horizons, it decreased with flooding and increased with drying, but these changes varied among the different treatments. The strongest fall of the Eh values (reaching ~-200 mV) occurred for the liming treatments of El Carmolí, with or without plant, while the lowest variations happened in the waste from Lo Poyo without liming + without plant. In the C2 horizons, the Eh did not decrease in the unlimed waste from Lo Poyo, with or without plant, while in the other treatments the fall was progressive, with the lowest Eh values occurring from the ~11th week onwards.

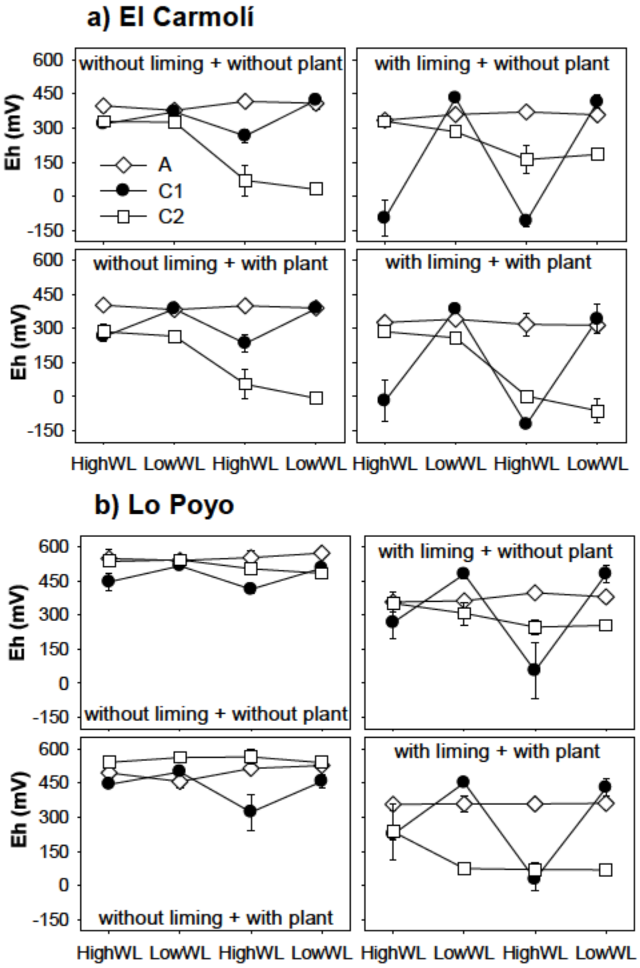
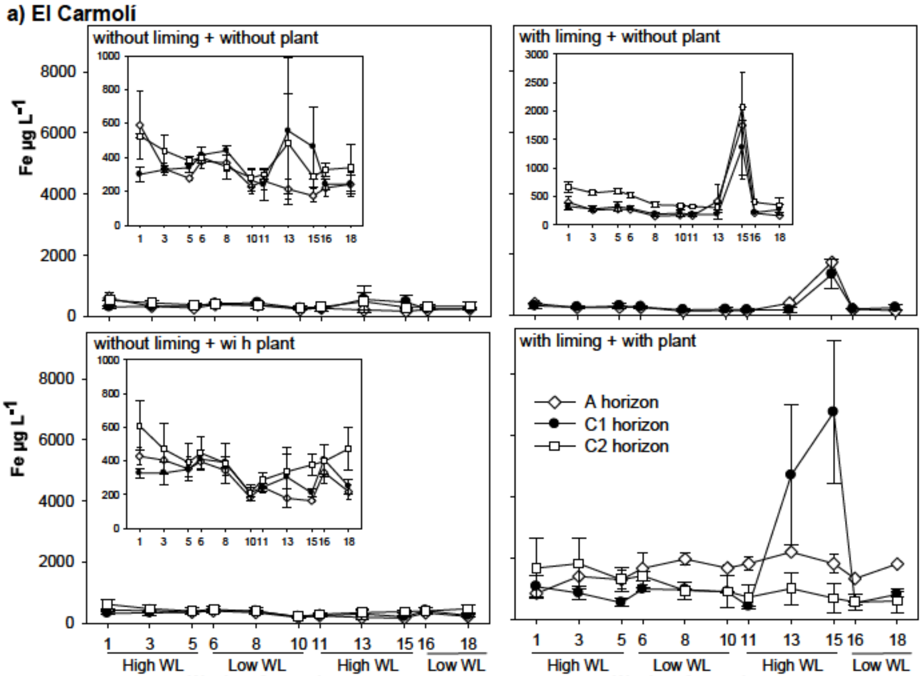


Figure 7.6. Eh within the soil profiles. Each value is the average for each period of High and Low Water Level –WL- (±SD). n = 9. Where SD is not shown, the bars are the size of symbols or smaller.

7.3.3. Changes in the pore water soluble Fe concentrations in the soil profiles

The concentrations of soluble Fe changed throughout the experiment at different depths and treatments and the effect of liming –decreasing them- was evident in the wastes of Lo Poyo but not in those of El Carmolí (Figure 7.7). The concentrations were largely higher in the treatments without liming with waste from Lo Poyo and dropped in the treatment with liming + without plant (significant differences in C1 and C2 horizons, p≤0.011). The latter effect was not observed when liming was combined with the presence of plants. In the profiles with waste from El Carmolí, the treatment with liming + with plant (A and C1 horizons) showed significant higher soluble Fe concentrations than the other treatments (p≤0.012).

a) El Carmolí



b) Lo Poyo

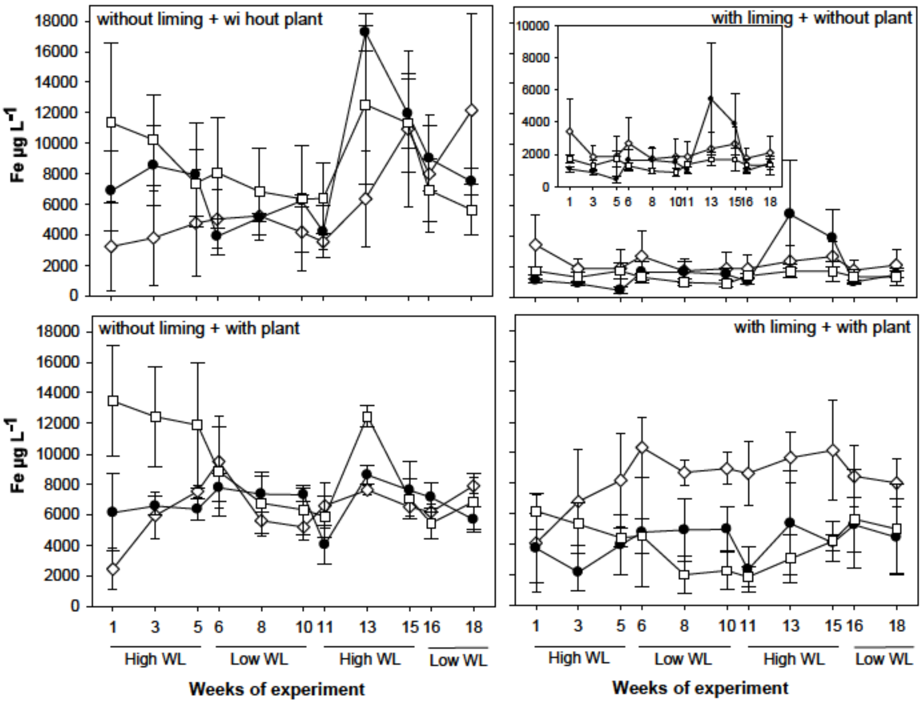


Figure 7.7. Soluble Fe concentrations in pore water within the soil profiles. Each value is the average for each sampling date (±SD). n = 3. WL is water level.

In many of the treatments, the soluble Fe concentrations tended to decrease or hardly varied until the second period of High WL, when sharp increases were observed, especially in the C1 and C2 horizons (Figure 7.7). An increase of the soluble Fe concentrations in the A horizons of all the profiles with waste from Lo Poyo, and in the profile with liming + with plant with waste from El Carmolí was also observed.

7.3.4. Development of redoximorphic features in the soil profiles

The Fe dynamic in the soil profiles led to the development of redoximorphic features (Table 7.4). Regardless of the treatment and depth, the soil matrix was brown to yellowish-brown in the profiles with waste from El Carmolí (HUE 10YR, VALUE 4 to 4.5, and CHROME 3.5 to 6). In the profiles with waste from Lo Poyo, the soil matrix was dark yellowish-brown (10YR 3.5/6) in the A horizon and olive-brown to light yellowish-brown (HUE 2.5Y, VALUE 4 to 6, and CHROME 4 to 5) in the C1 and C2 horizons. In both types of wastes, features with gley colours appeared; these were more abundant in the presence of plants. The abundance and size of these features tended to increase with depth, particularly in the waste from Lo Poyo, where grey spots (5Y 7/2.5) - indicative of Fe-depletion - were prominent (Figure 7.8). Bluish-black (10B-5B 2.5/1) and bluish-gray mottles (10B-5PB 5/1) were also observed in the C2 horizons of both wastes.

Figure 7.8. Left: grey spots due to Fe-depletion in the matrix of C1 horizons in a profile with waste from Lo Poyo. Right: detailed view of depletion.

Table 7.4. Mottling description in each horizon: colour, abundance (N = none, V = very few, F = few, C = common, M = many, A = abundant), size (V = very fine, F = fine, M = medium, A = coarse), contrast (F = faint, D = distinct, P = prominent), and boundary (S = sharp, C = clear, D = diffuse) (FAO 2006).

Mine waste	Horizon	Treatment			
		No liming + No plant	Liming + No plant	No liming + Plant	Liming + Plant
El Carmolí	A	Without mottles	Without mottles	*10YR 6/8, F, M, D, C	*10YR 7/8, F, F, D, D *2.5Y 8/8, F, F, D, D
	C1	*10B-5PB 2.5/1, V, V, P, S	Without mottles	*10YR 6/8, C, M, D, C	*10YR 7/8, M, F, D, D
	C2	*10B-5PB 2.5/1, V, F, P, S *2.5Y 6.5/1, F, F-M, D, D	*10B-5PB 2.5/1, V, F, P, S	*10YR 6/8, A, M, D, C *2.5Y 6.5/1, C, F, D, D *10B-5PB 2.5/1, F-V, V, P, D	*10YR 7/8, M-A, F, D, C-D *2.5Y 6.5/1, C, F, D, D *10B-5PB 2.5/1, F-V, V, P, S
Lo Poyo	A	Without mottles	Without mottles	*5Y 7/2.5, F, V, D, D	*5Y 8/4, V, V, D, D
	C1	*5Y 7/2.5, F, M, D, C-D	*5Y 7/2.5, F-C, M-F, D, D *10B 5/1, F-C, V-F, P, S	*5Y 7/2.5, C-F, F, D, C *5BG 7/1, F-C, V-F, D, S *10B 5/1, F-C, V-F, D, S	*5Y 8/4, C, V, D, D *5Y 7/2.5, C, F, D, D
	C2	*5Y 8/4, M, F, D, C *10B 5/1, V, V, D, S	*5Y 7/2.5, C, M, D, D *10B 5/1, C, F-M, P, C-S	*10YR 6/8, C, V, F, S *5Y 8/4, M, F, D-P, D *5Y 7/2.5, M, F-M, D-P, D *10B 5/1, C, V, P, S	*10YR 6/8, C, M, D, D *5Y 7/2.5, C, M, D, D *5Y 8/4, C, F, D, D *10B 5/1, V-F, V, P, S

7.3.5. Sequential extraction

In the soil profiles with waste from El Carmolí, the highest content of Fe was found in the crystalline Fe oxides fraction (58-76%), followed by the amorphous Fe oxides fraction (12-30%), the residual fraction (9-13%), and the organic matter fraction (1-3%) (Figure 7.9). The other fractions accounted for less than 1%. In the case of Lo Poyo the highest contents of Fe were in the crystalline Fe oxides fraction (36-62%) and the residual fraction (35-62%). The amorphous Fe oxides fraction accounted for 1-3% and the others for less than 1% (Figure 7.9).

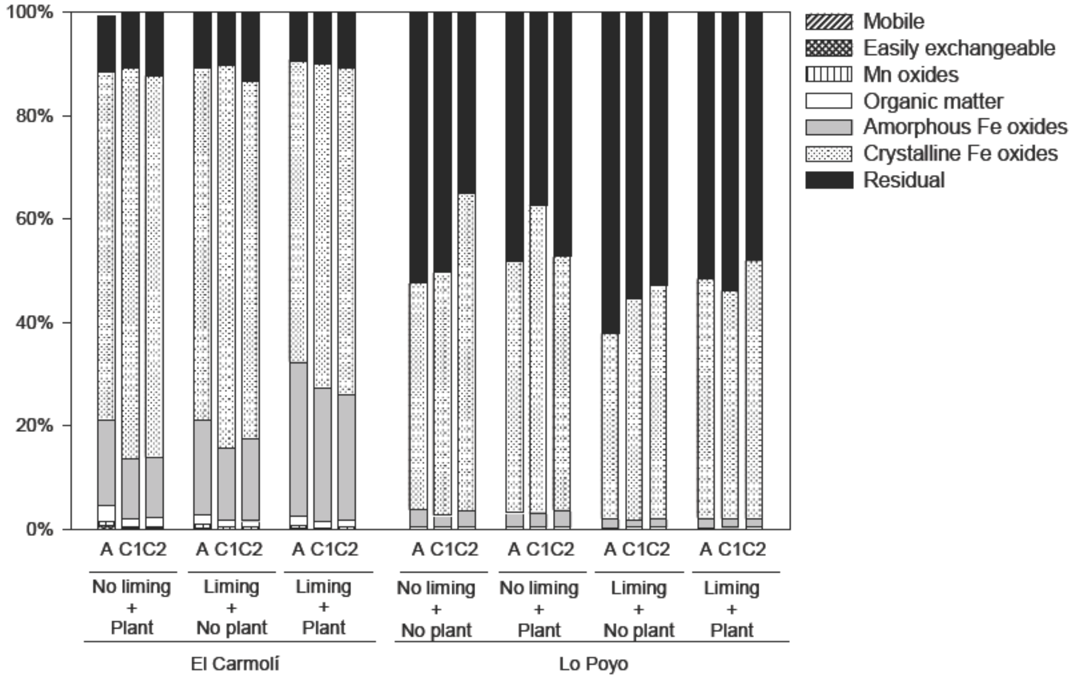


Figure 7.9. Results of the sequential extraction.

7.4. Discussion

7.4.1. Processes in the flooding water

The conditions in the water of the containers, outside the soil profiles, are important because they shed light on the processes governing the dynamic of Fe in the free water. In fact, the results indicate that the type of waste and the waste treatment (with or without liming) had a negligible influence on this dynamic and on the associated biogeochemical processes. Hence, these data could be considered as controls that may help in the interpretation of the processes occurring in the pore water within the soil profiles.

The results clearly show a close relationship among the consumption of DOC, the Eh, and the concentration of soluble Fe. When oxygen disappears or its concentration falls below 4% (Eh~+300 mV at pH=7), microorganisms use other terminal electron acceptors for their metabolism, such as Fe^{3+} that is reduced to Fe^{2+} (soluble form) (Otero et al., 2009). In our experiment, the High WL phases favoured the consumption of DOC in the eutrophic flooding water, leading to anoxic conditions (manifested as the low Eh values) which favoured the dissolution of Fe-oxides (as shown by the high concentrations of soluble Fe). When the water level dropped, the opposite occurred: the Eh increased and the concentrations of soluble Fe decreased. The highest soluble Fe concentrations were obtained at Eh values between 0 and +100 mV (Figure 7.10): this indicates Fe-solubilisation. At Eh values higher than +300 mV, the concentrations of soluble Fe were the lowest (Figure 7.10), which is attributable to the formation of Fe-oxides, while at Eh values lower than 0 mV a tendency towards Fe-immobilisation was again observed, now attributable to the reduction of sulphates and the formation of Fe-sulphides (Otero et al., 2009). The changes in the SO_4^{2-}/Cl^- ratios were in accordance with the latter process: 0.19±0.01 (1st High WL), 0.22±0.01 (1st Low WL), 0.17±0.01 (2nd High WL), and 0.21±0.01 (2nd Low WL). The chloride ion is a conservative element (biologically and chemically non-reactive) used as a tracer to determine the variations in the SO_4^{2-} concentration due to either hydrological or biological and chemical processes (Sherman et al., 1998). Increased ratios reflect the oxidation of metal-sulphides, mainly pyrite (FeS_2), the major end-product of the sulphate reduction in salt marshes, and decreased ratios, in turn, are consistent with sulphate reduction (Otero and Macías, 2003).

The evolution of the pH was in accordance with the behaviour of the Eh and the processes involved in the consumption of DOC by microorganisms. The pH was low at the beginning of both phases of High WL, which is attributable to the production of protons and organic acids when microorganisms consume labile organic carbon (Gounou et al., 2010). During the following weeks, the Eh dropped, leading to an increase in pH due to the consumption of protons (Reddy and DeLaune, 2008) and to the production of HCO_3^- (Otero et al., 2009) during reduction processes. In the phases of Low WL, the diffusion of oxygen into the water led to the oxidation of the sulphides formed during the High WL phases, decreasing the pH (Otero et al., 2006). Later, the values of pH tended to increase again due to the buffer capacity of the eutrophic flooding water (evident between weeks 6 and 10).

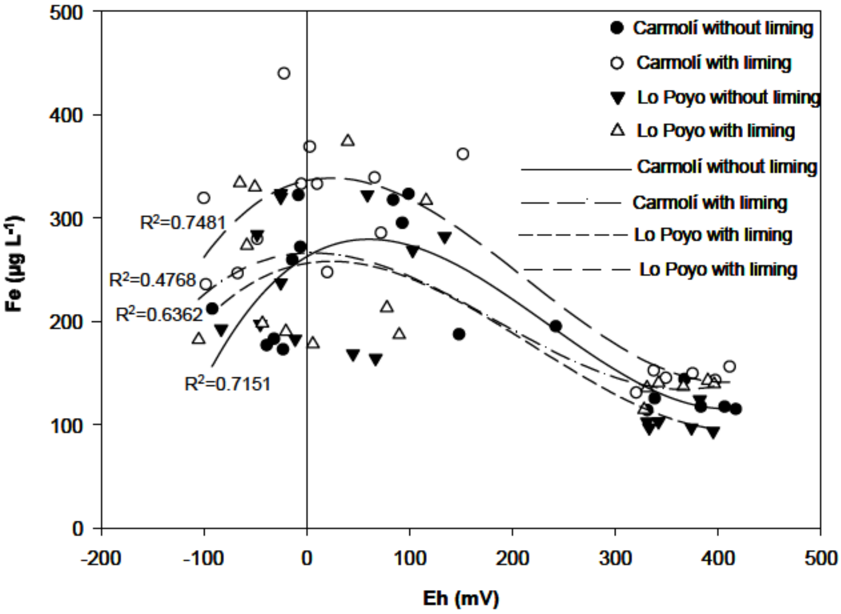


Figure 7.10. Soluble Fe vs. Eh in the flooding water. Lines represent the trend of each waste treatment.

7.4.2. Effects of the treatments on the physical-chemical conditions and the pore water soluble Fe concentrations in the soil profiles

The pH, Eh, and concentrations of soluble Fe within the soil profiles were clearly influenced by the liming and plants. Many studies have recommended liming as a strategy to immobilise metals in mine soils (e.g. Fernández-Caliani and Barba-Brioso, 2010; Simón et al., 2010). However, these studies were conducted under oxic conditions and, therefore, the results may not be applicable to flooded soils.

The representation of the individual values of pH vs. soluble Fe concentrations (Figure 7.11) shows that liming favoured the increase of the pH and the decrease of the soluble Fe concentrations in the waste from Lo Poyo, while the latter was not evident for the waste from El Carmolí. However, the relationship between the Eh and soluble Fe concentrations (Figure 7.12) indicates that liming led to a more-anoxic environment in the C1 and C2 horizons of both wastes, probably because the increase in pH could have promoted the activity of microorganisms, then stimulating the consumption of oxygen (Otero et al., 2009) and lowering the Eh. In fact, Eh reflects soil microbial activity (Fiedler et al., 2007). In addition, pH and Eh are parameters inversely related (Patrick et al., 1996), so that the increase in pH by liming could have favoured a fall in Eh. The lower Eh facilitated the solubilisation of Fe and hence reduced the effectiveness of the lime amendment with regard to Fe-immobilisation. This phenomenon was more evident in the treatments with liming + with plant, in which large amounts of Fe were dissolved (Figure 7.12d and 7.12h), originating numerous Fe-depletions.

Our results agree with other studies conducted in mine soils flooded for long periods, in which the Eh reached lower values in the presence of vegetation (González-Alcaraz et al., 2011b; María-Cervantes et al., 2010, 2011). It is well known that plant roots may condition some geochemical parameters, such as pH and Eh, relative to the bulk soil (Wenzel, 2009). In fact, the C1 horizon of the treatment with liming + with plant from El Carmolí, with greater growth of *S. fruticosa*, showed pH values similar to those without liming, probably due to the acidic roots exudates (Dakora and Phillips, 2002). The rhizosphere not only favours microbial activity through the excretion of organic substances (Hinsinger, 2001), but also provides a structure for microorganisms to adhere to and to perform the processes necessary for element transformation (Caffery, 1990).

The low Eh and the existence of bluish-black mottles indicate strongly-reducing conditions under which the sulphate-reduction process could have occurred in some phases of the experiment, although the high flux of soluble Fe shows that the Fe solubilisation rate exceeded the rate of sulphate reduction (Koschorreck, 2007). We can also not discard the formation of green rust minerals (layered Fe^{2+}-Fe^{3+} hydroxyl salts) (Trolard et al., 1997; Hansen, 2001; Génin et al., 2006) due to the high salinity of the pore water within the soil profiles.

A consequence of the greater solubilisation of Fe in the C1 and C2 horizons brought about by liming was the higher accumulation of this element in the A horizons due to the capillary upward movement of water and salts (Álvarez-Rogel et al., 2006), shown clearly by the higher EC in the upper part of the soil profiles. The pumping of water by plants actively contributes to the latter process (Barrett-Lennard and Malcolm, 1999). In our experiment, the key role of *S. fruticosa* in the re-distribution of soluble Fe along the soil profiles was evident (Figure 7.12d and 7.12h).

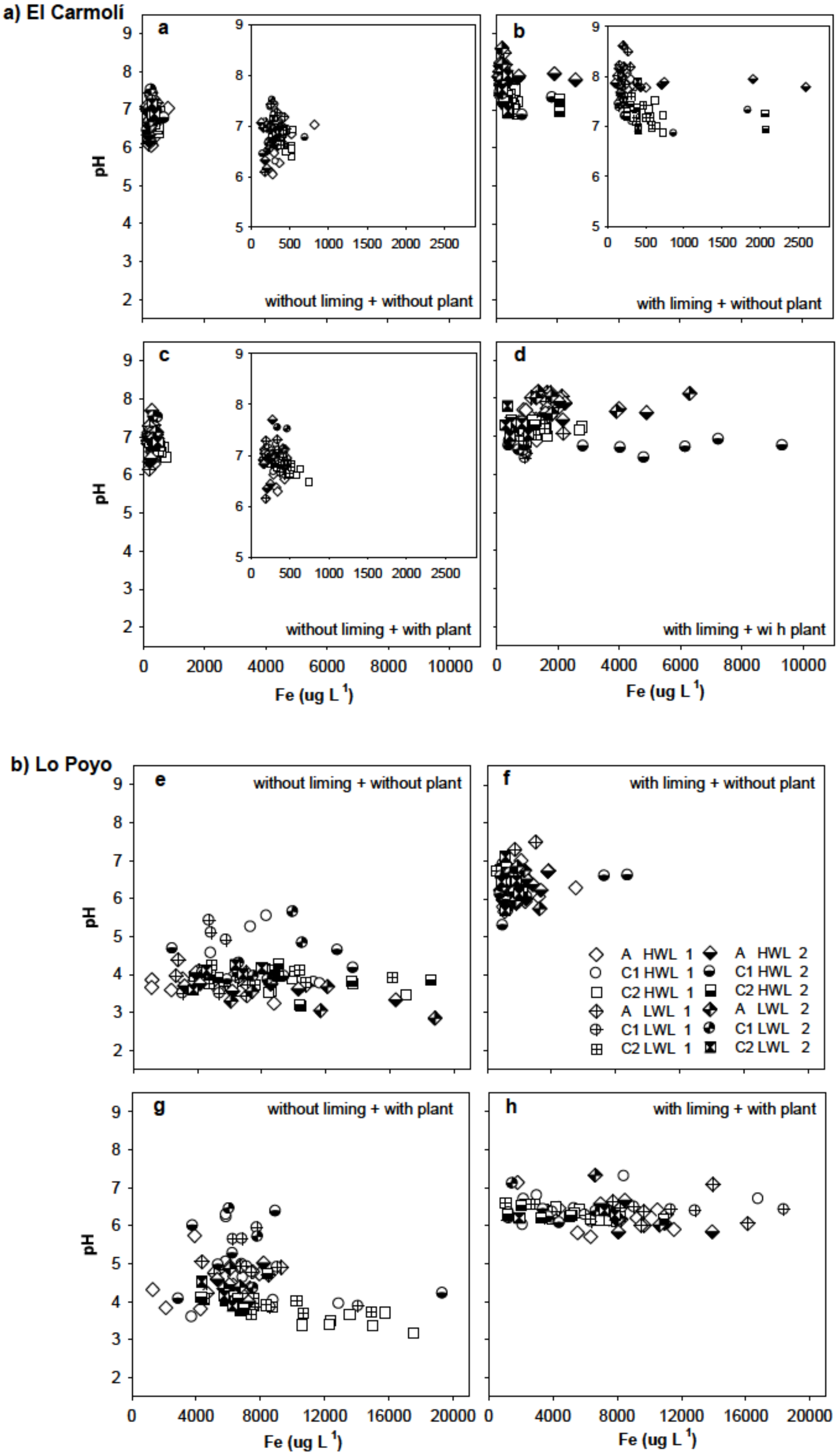


Figure 7.11. pH vs. soluble Fe in pore water within the soil profiles. Each symbol corresponds to an individual value.

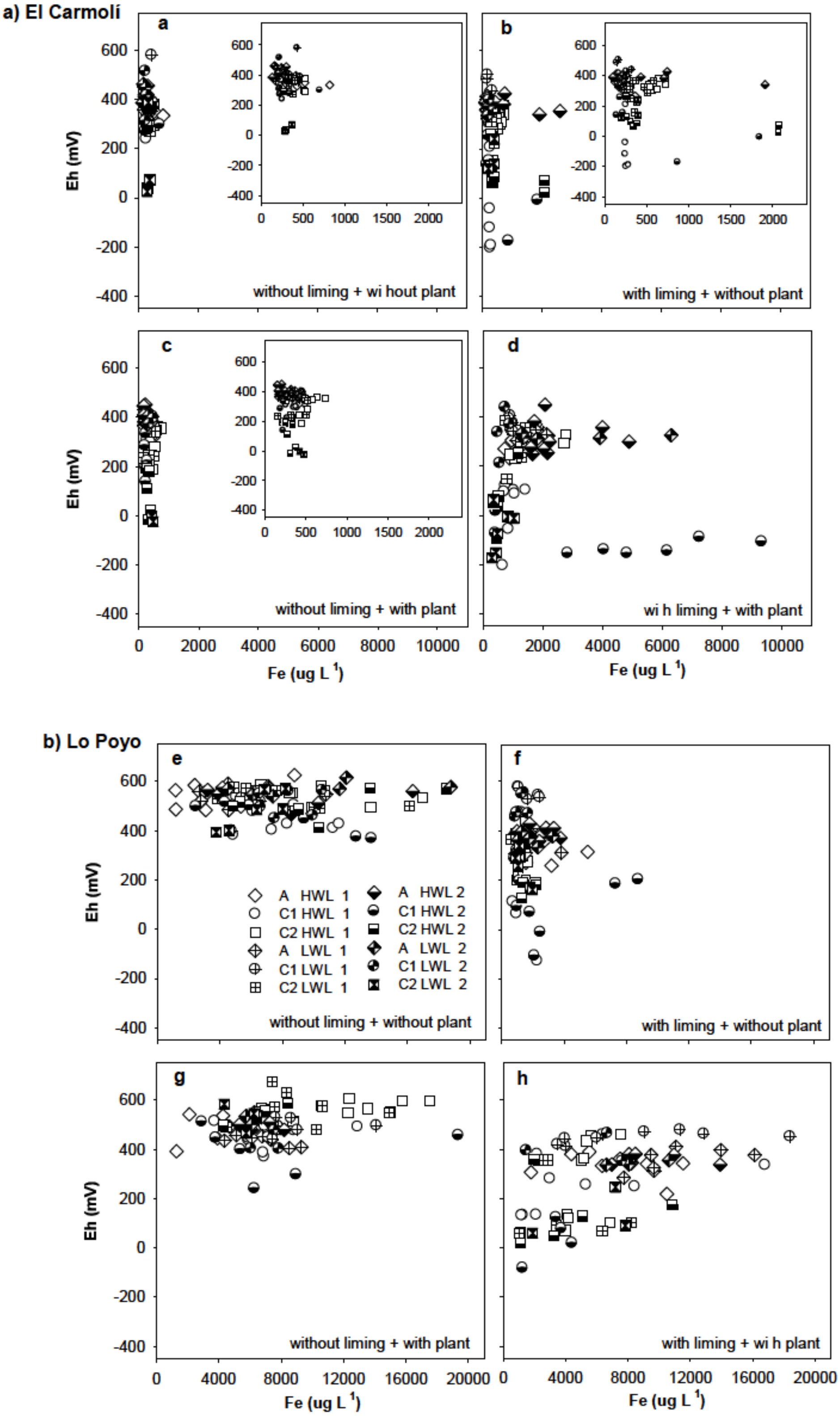


Figure 7.12. Eh vs. soluble Fe in pore water within the soil profiles. Each symbol corresponds to an individual value.

The sequential extraction procedure showed that a great amount of the Fe in the soil profiles of both wastes was in the amorphous and crystalline Fe oxides fractions. Some studies have pointed that amorphous Fe oxides are reduced preferentially over crystalline Fe oxides, due to the energy economy by the microorganisms involved (Munch and Ottow, 1983), and that the crystalline Fe oxides are transformed into amorphous oxides before the reduction of Fe^{3+} (Wahid and Kamalam, 1993). In systems with alternating flooding-drying periods, the Fe dissolved during flooding precipitates as amorphous forms during drying (Otero et al., 2009). Then, the amorphous/crystalline Fe oxides ratio could be used as an indicator of the dynamic of Fe in the soil profiles. A higher ratio implies an increase of the amorphous phases at the expense of the crystalline ones. The amorphous/crystalline Fe oxides ratios obtained in the profiles for El Carmolí were: without liming + with plant (A=0.24, C1=0.15, C2=0.16), with liming + without plant (A=0.27, C1=0.19, C2=0.23), and with liming + with plant (A=0.51, C1=0.41, C2=0.38), and for Lo Poyo: without liming + without plant (A=0.072, C1=0.048, C2=0.050), without liming + with plant (A=0.058, C1=0.045, C2=0.065), with liming + without plant (A=0.050, C1=0.034, C2=0.038), and with liming + with plant (A=0.040, C1=0.036, C2=0.033). Although it was not possible to analyse the samples of the treatment without liming + without plant for El Carmolí, the results of the other seven treatments showed that, regardless of the abundance of amorphous or crystalline Fe forms in both types of wastes, the amorphous/crystalline Fe oxides ratios were higher in the A horizons as a consequence of the capillary upward movement of the soluble Fe to the surface and its precipitation as amorphous oxides, a process that is favoured in saline environments (Hansel et al., 2005; Otero et al., 2009).

The difference in magnitude of the amorphous/crystalline Fe oxides ratio between the two types of waste indicates some possible differences. In the waste from El Carmolí, the greater decline in the Eh during the phases of High WL, mainly in the treatment with liming + with plant, would have promoted the transformation of crystalline Fe oxides into amorphous forms before the reduction of Fe^{3+} to Fe^{2+}, that would have precipitated as amorphous oxides during the periods of Low WL. These oxides appear to have dissolved easily when the water level rose again, producing sharp increases of soluble Fe, mainly in the C1 horizons. Compared with El Carmolí, in the soil profiles with waste from Lo Poyo the concentrations of soluble Fe were higher, due to the lower pH of the waste, and the amorphous Fe oxides fraction was minor compared to the Fe in the crystalline forms. This agrees with Conesa et al. (2008), who found higher amounts of soluble metals and lower amounts of metals bound to amorphous Fe oxides than to crystalline oxides when comparing acidic (similar to Lo Poyo) with neutral (similar to El Carmolí) mine wastes. These authors indicated a stronger short-term environmental risk in the acidic wastes due to the higher solubility of metals, but a greater long-term risk in the neutral ones due to the superior binding of metals in potentially-available forms.

7.5. Conclusions and management recommendations

The results of our experiment show that liming may not always be a suitable alternative for the reclamation of eutrophic wetlands soils polluted by mine wastes: it decreases the solubility of Fe, mainly in acidic wastes, but at the same time it may promote microbial activity (Bolan et al., 2003), hence increasing the potential capacity of the system to reach reductive conditions, which favour the dissolution of Fe-minerals. The use of plants is not necessarily favourable because: 1) the plant rhizosphere may enhance redox processes, and 2) plants redistribute compounds dissolved in the soil solution along the soil profile. The alternation of oxidising and reducing conditions poses another drawback since it facilitates the precipitation of soluble Fe - during drying periods - as amorphous oxides that will re-dissolve more easily in wet periods, releasing high concentrations of soluble Fe, and, possibly, associated harmful compounds, into the environment (Das et al., 2011).

Therefore, the combined use of liming and vegetation might not be an appropriate management strategy for eutrophic wetland soils affected by mine wastes, differently from non-flooded tailings where the latter has been recommended (Solis-Dominguez et al., 2012).

7.6. Acknowledgments

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Chapter 8

Metal dynamics in simulated slightly acidic soil profiles

When liming and revegetation contribute to the mobilisation of metals: learning lessons for the phytomanagement of metal-polluted wetlands

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Abstract

The aim of this study was to identify the effectiveness of liming and revegetation for the recovery of slightly acidic, saline soils of eutrophic wetlands affected by mine wastes, under fluctuating flooding conditions. Simulated soil profiles were constructed and four treatments were assayed: without liming + without plant, without liming + with plant, with liming + without plant, and with liming + with plant. The plant species was the halophyte *Sarcocornia fruticosa*. Three depths were differentiated: A horizons (never under water), C1 (alternating flooding-drying conditions), and C2 (always under water). Several parameters were measured regularly for 18 weeks. Liming favoured the growth of *S. fruticosa*, an increase in pH and a fall in Eh. The amendment was effective for immobilising Mn, Zn, and Cd, but not Cu and Pb. The growth of *S. fruticosa* counteracted the effect of liming, strongly increasing the concentrations of soluble metals and distributing them along the soil profile. Hence, the combined use of liming and plants may increase the risk of metals mobilisation.

Keywords: *Sarcocornia fruticosa*, Mine wastes, Spolic Technosols, Redox potential, Liming

8.1. Introduction

The negative effects of mining on soils, waters, and organisms are well known. The persistence of these pollutants and their release into ecosystems in ecotoxicological forms are currently major concerns worldwide (Batty et al., 2008). The consequences of mining also affect areas far away from the mine site, when tailings are eroded and wastes transported and deposited in lowland areas. Wetlands are among those environments most affected due to their location in topographically depressed positions. These environments have the capacity to remove and/or immobilise contaminants and are considered as green filters against pollution and eutrophication (Williams et al., 1994; Mitsch and Gosselink, 2000; Vega et al., 2009). However, since metals mobility depends on multiple factors, wetlands might also act as sources of hazardous substances (Du Laing et al., 2007; Frohne et al., 2011).

Liming is a common management practice to recover soils polluted by mine wastes, since it promotes a rise in the pH and thus reduces metals mobility (Fernández-Caliani and Barba-Brioso, 2010; Simón et al., 2010). By-products from the marble industry are some of the materials used. In fact, their use is a suitable strategy to reduce waste disposal and revalue the wastes generated during marble processing (Fernández-Caliani and Barba-Brioso, 2010). There is extensive literature demonstrating the effectiveness of liming with regard to improvement of soil conditions and enhancement of plant growth (e.g. Haynes and Naidu, 1998; Davis et al., 1999; Bolan et al., 2003). Since phytomanagement consists of the engineering or manipulation of soil-plant systems in order to control pollutant fluxes in the environment (Robinson et al., 2009), the combined use of soil amendments and revegetation is a strategy recommended for the remediation of mine soils.

A study conducted in microcosms showed that liming reduced the concentrations of soluble metals and increased the growth of *Sarcocornia fruticosa* (L.) A.J. Scott (a Mediterranean halophyte) in salt marsh soils polluted by mine wastes (González-Alcaraz et al., 2011). Nevertheless, several reports have indicated that the plant rhizosphere may contribute to metals mobilisation (Du Laing et al., 2009b; Wenzel, 2009), particularly in waterlogged soils (María-Cervantes et al., 2010). Moreover, as far as we know, the consequences of combining liming and plants in soils polluted by mine wastes and regularly flooded are poorly understood. We hypothesise that the benefits of liming may depend on the moisture content and/or soil flooding regime and may be modulated, in turn, by the presence or absence of vegetation. In addition, different depths in the soil profile may behave differently. Hence, if an inadequate management strategy is implemented, the release of metals might be favoured, transforming these systems into sources of contaminants.

The aim of this study was to identify the effectiveness of combining liming and vegetation for the remediation of slightly acidic, saline soils of eutrophic wetlands affected by mine wastes, under fluctuating flooding conditions. The latter shall help to improve the phytomanagement strategies of these polluted systems and to reduce the potential environmental risks. For this purpose, we tested the effects of marble sludge application and the growth of *Sarcocornia fruticosa* on the dynamics of metals in simulated soil profiles constructed with soil polluted by mine wastes from a saline, eutrophic wetland.

8.2. Material and methods

8.2.1. Field soil sampling and initial characterisation

Soil samples were collected from the top 20 cm of a Spolic Technosol (WRB, 2006) in a coastal salt marsh -Marina del Carmolí- (318.6 ha, N37º41’42’’, W0º51’31’’) of the Mar Menor lagoon, south-eastern Spain (Figure 8.1). This marsh is affected by eutrophic water of agricultural and urban origin (Álvarez-Rogel et al., 2006a) and by metal-mine wastes carried there by the surface watercourses coming from the nearby mining area of Sierra de Cartagena-La Unión (Conesa and Jiménez-Cárceles, 2007; Jiménez-Cárceles et al., 2008). This area is among those most affected by the impact of mining activities in Europe (Conesa and Schulin, 2010).

Soil material was homogenised mechanically and three sub-samples were analysed to perform an initial characterisation (for details see González-Alcaraz et al., 2011). The soil was fine textured (60% silt+clay) and slightly acidic (pH~6.4), with a low $CaCO_3$ content (~6 g kg^{-1}) and high concentrations of total metals (g kg^{-1}: Cd~0.016, Cu~0.18, Mn~3.51, Pb~6.75, Zn~52.3). The major minerals identified (by semi-quantitative estimation of the crystalline fraction, by power X-Ray Diffraction) were quartz (36%), phyllosilicates (22 %), gypsum (14%), goethite (3%), and natrojarosite (2%).

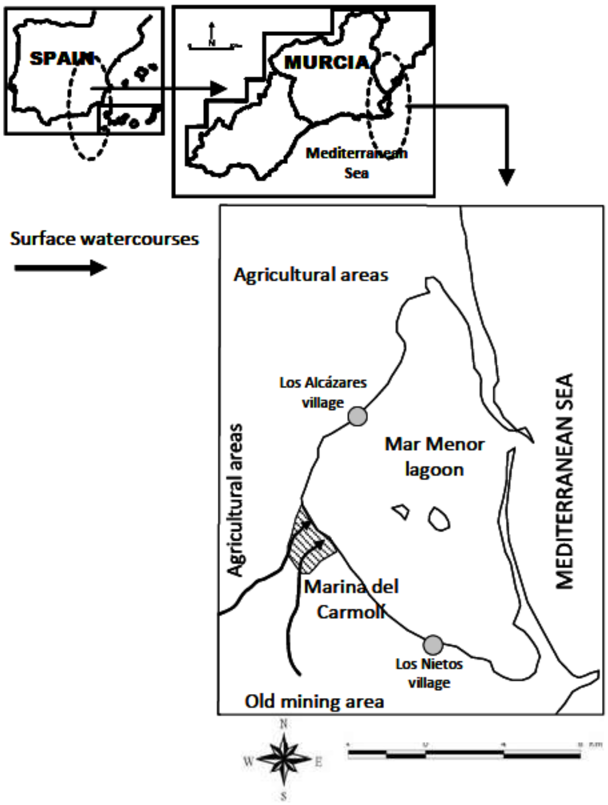

Figure 8.1. Location map of the sampling site (SE Spain).

8.2.2. Experimental set up

The soil was amended with a marble sludge (54% magnesium calcite-$(Mg_{0.03}Ca_{0.97})(CO_3)$- and 37% dolomite-$CaMg(CO_3)_2$-) of pH 8.9 (1:2.5 water suspension), at a dose of 20 g kg^{-1} soil, giving two soil treatments: 1) soil without liming and 2) soil with liming. Liming increased the soil pH, from ~6.4 to ~7.3. Plastic pots (13.5 cm in diameter and 14 cm high) and methacrylate cylinders (20 cm in diameter and 60 cm high) were filled with each soil treatment. The cylinders were covered with opaque plastic, to prevent light exposure of the soil profile.

The experiment was performed in a greenhouse in two phases, both under natural light conditions and with temperatures ranging from 5 to 35 ºC. In the first phase, cuttings of *Sarcocornia fruticosa* (collected from non-polluted areas of the Marina del Carmolí) were planted in the pots (one plant per pot) (for details see González-Alcaraz et al., 2011). The pots were watered (but not flooded) 2-3 times per week for 10 months, with eutrophic water from a local stream reaching the salt marsh (pH~8.1, electrical conductivity~22 dS m^{-1}, NO_3^-~370 mg L^{-1}, PO_4^{3-}~1 mg L^{-1}, dissolved organic carbon~10 mg L^{-1}). Pots without plants were handled in the same way. Liming favoured the decrease of soluble metal concentrations and enhanced the growth of *S. fruticosa* (González-Alcaraz et al., 2011).

After 10 months of plant growth, the contents of six pots per soil treatment (three with plants and three without) were transferred to their corresponding cylinders, by mixing and homogenising the soil in the root ball with the soil in the cylinder. The cylinders were irrigated (but not flooded) for 5 months with the same eutrophic water, to facilitate plant establishment. After this period of plant adaptation, the second phase of the experiment began.

In each cylinder (hereafter soil profile), two 10-cm Rhizon ® type samplers (pore diameter = 0.1 μm) and pH and Eh electrodes (Crison 50-50 and 50-55, respectively) were installed horizontally at depths of 5, 30, and 55 cm (hereafter horizons A, C1 and C2, respectively) (Figure 8.2). Hence, each horizon had two Rhizon ® samplers and two electrodes. The Rhizon ® samplers were connected to 50-mL plastic syringes by means of extension tubes, to extract pore water (Figure 8.2).

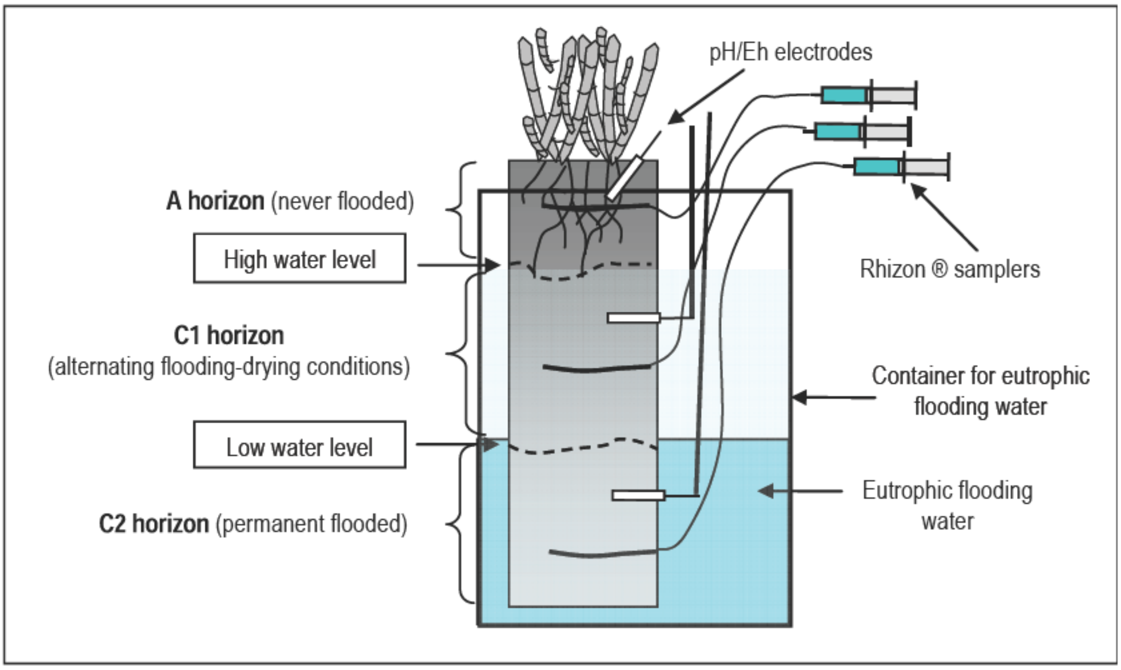

Figure 8.2. Scheme of the experimental set up.

Each soil profile was placed inside a larger container filled with synthetic eutrophic water with a pH~7.5 and a electrical conductivity~11 dS m^{-1}, enriched in organic carbon (g L^{-1}: 0.31 $Ca(NO_3)_2$, 3.8 NaCl, 3 $MgSO_4$, 0.8 $CaCl_2$, 0.2 KCl, 0.043 H_3PO_4 (85%), 0.15 $C_6H_{12}O_6$, and 0.1 meat extract (46% organic carbon)). The water level (WL) in the containers was maintained at 20 cm below the soil surface for five weeks -weeks 1 to 5- (1st High WL) and then at 45 cm during the following five weeks - weeks 6 to 10- (1st Low WL), repeating this cycle once more along the study (2nd High WL -weeks 11 to 15- and 2nd Low WL -weeks 16 to 18-). Hence, the upper 20 cm (A horizons) were never under water, the soil between 20 and 45 cm (C1 horizons) was under water for 10 weeks with two alternating drying periods of 5 and 3 weeks, respectively, and the deepest 45 cm (C2 horizons) were always under water.

As in the first phase, at the end of the cylinders phase (that lasted 38 weeks) the total fresh biomass of *S. fruticosa* was greater with liming (aerial part = 477±91 g; roots = 25±17 g; root length ~46±6 cm) than without liming (aerial part = 14±1 g; roots = 10±0.5 g; root length ~26±1 cm).

8.2.3. Monitoring of parameters and water sampling and analysis

The pH and Eh were monitored weekly at each depth in each soil profile: the data were recorded after 10 minutes of electrode stabilisation. The Eh measurements were adjusted according to Vepraskas and Faulkner (2001), by adding +200 mV to the soil voltage (the value of the Ag/AgCl reference electrode at 20 ºC).

Pore water samples were collected every two weeks. The two sub-samples, obtained for each depth in each soil profile, were poured together into a plastic vial for subsequent analysis. An aliquot of each sample was acidified with sulphuric acid and the rest remained non-acidified. In the acidified portion, the concentrations of Cd, Cu, Mn, and Pb were measured using an Inductively Coupled Plasma Mass Spectrometer (Agilent 7500A, detection limit 0.002 mg L^{-1}) and the concentration of Zn with an Atomic Absorption Spectrometer (Unicam 969). In the non-acidified portion, the electrical conductivity (EC) at 25 ºC was measured with a Conductimeter (Crison Basic 30), the Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-} concentrations with an Ionic Chromatographer (Methrom 681) and the dissolved organic carbon (DOC) with a Total Organic Carbon Analyser (V_{CSH} Shimadzu). The accuracy of the measurements was checked with certified standards.

8.2.4. Statistical analysis

The statistical analyses were performed with SPSS 17.0 (SPSS Inc., 2008). For each horizon (A, C1, and C2), repeated measures ANOVA was performed in order to compare the pH, Eh, EC, DOC, salts (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-}), and metals (Cd, Cu, Mn, Pb, and Zn) for the different treatments (without liming + without plant, without liming + with plant, with liming + without plant, with liming + with plant). When the ANOVA was significant, the post-hoc Bonferroni test was used to identify different groups ($\alpha = 0.05$). The relations among pore water parameters were analysed by Spearman's rank correlations.

8.3. Results

8.3.1. Evolution of Eh and electrical conductivity (EC)

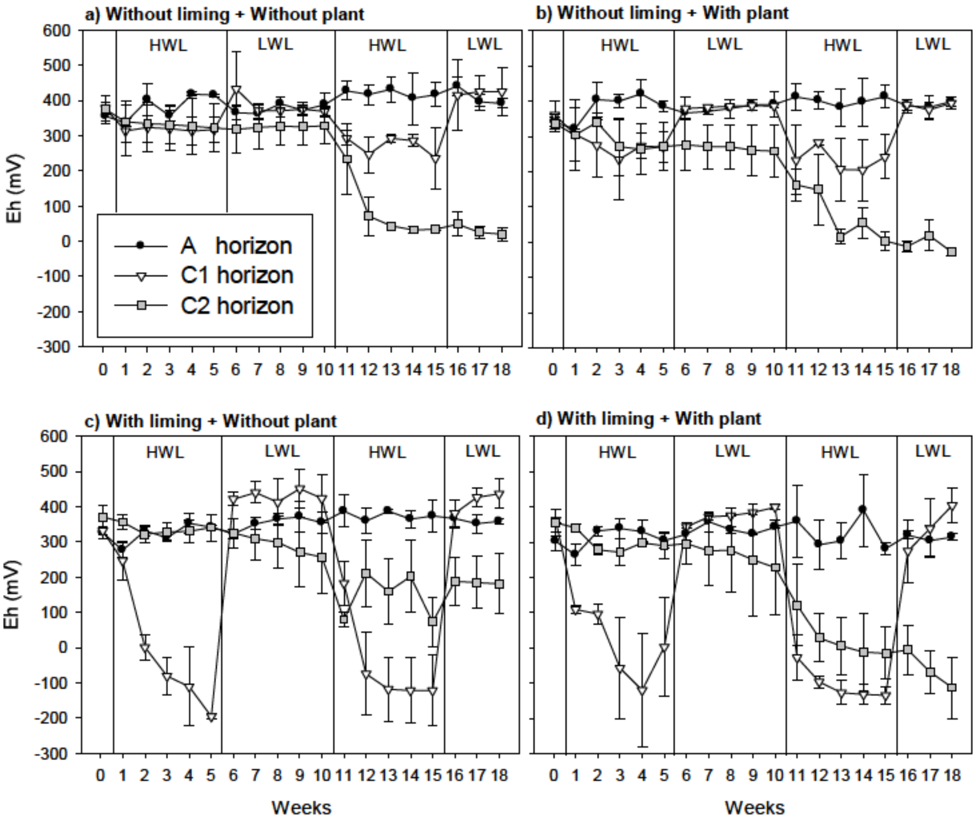

Figure 8.3. Changes in redox potential (Eh) during the phases of High Water Level (HWL) and Low Water Level (LWL). Bars represent the standard deviations (n = 3).

Before the beginning of the experiment, the Eh values were similar among horizons and treatments (>300 mV) (Figure 8.3, week 0). The evolution of Eh generally differed among both depths and treatments, showing significantly lower values the A and C1 horizons of the treatments with liming ($p \leq 0.013$) (Figure 8.3). In the A horizons, the Eh was always ~+350 mV, regardless of the water level or the treatment. In the C1 horizons, the Eh fluctuated in relation to the changes in the water level, decreasing when flooded (High WL) and increasing when dried (Low WL). Liming treatments showed the strongest variations, reaching Eh values below -100 mV in High WL and above ~+400 mV in Low WL (Figure 8.3c and 8.3d). In the C2 horizons, the Eh tended to decrease along the experiment. This was more evident from the beginning of the 2nd High WL (11th week): the Eh had declined to below -100 mV in the treatment with liming + with plant at the end of the experiment (18th week) (Figure 8.3d).

The EC was always higher in the A horizons, regardless of the water level and the treatment (Figure 8.4). All the treatments assayed gave values between 10 and 25 dS m^{-1}, except with liming + with plant where the EC increased throughout the experiment in the A horizon, showing significantly higher values ($p \leq 0.007$), above 50 dS m^{-1} at the end (Figure 8.4d).

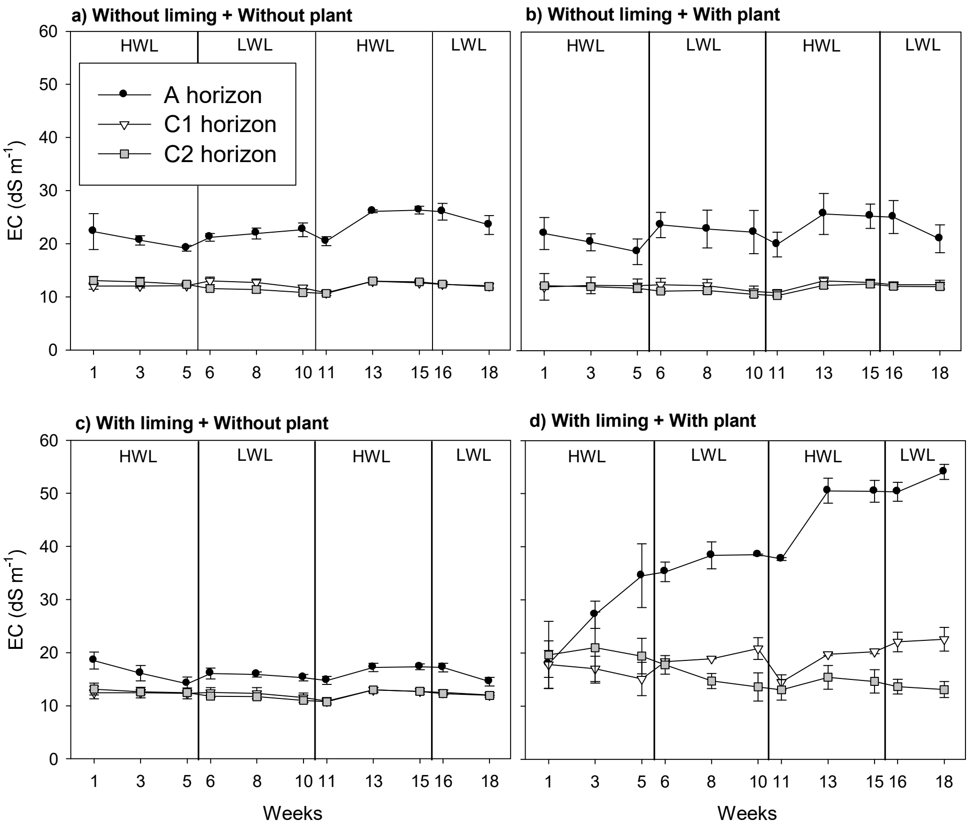

Figure 8.4. Changes in electrical conductivity (EC) during the phases of High Water Level (HWL) and Low Water Level (LWL). Bars represent the standard deviations (n = 3).

8.3.2. Changes in pH and in the concentrations of DOC and soluble salts

The pH was significantly higher in both treatments with liming, except in the C1 horizon of the treatment with liming + with plant (Figure 8.5). For liming, the A horizons showed higher pH values than the deep horizons (C1 and C2), regardless of the presence or absence of plants, with maximum values close to 8. In all cases, little variation was observed between the High and Low WL.

The concentrations of DOC were significantly higher in the treatment with liming + with plant, where the A horizon showed the greatest values (mg L^{-1}: 84±28 in High WL and 102±30 in Low WL) (Figure 8.5). For the other treatments, the DOC concentrations increased slightly with depth.

The concentrations of Cl^- were higher than those of SO_4^{2-} and, in relation to cations, the order of abundance was $Na^+ > Mg^{2+} > Ca^{2+} > K^+$ (Figure 8.5). The treatment with liming + with plant gave significantly higher values of soluble salts, except for Ca^{2+}; the A horizons showed the highest values. The concentrations of most of the soluble salts tended to increase during the Low WL.

8.3.3. Changes in soluble metal concentrations

The concentrations of soluble metals were affected by the liming and plants, showing variations related to the depth in the soil profile (Figure 8.6), and they evolved in relation to changes in the water level (Figure 8.7). The concentrations of Cd, Mn, and Zn were significantly lower with liming, except in the C1 horizon of the treatment with liming + with plant, where Mn reached its highest value (32±24 mg L^{-1}). For Cd and Zn, the A horizons of both treatments without liming showed the greatest values (Cd>800 µg L^{-1}, Zn>350 mg L^{-1}) (Figure 8.6), while Cu and Pb showed significantly higher concentrations in the treatment with liming + with plant, with greater values in the C2 (~400 µg L^{-1}) and A (~870 µg L^{-1}) horizons, respectively (Figure 8.6).

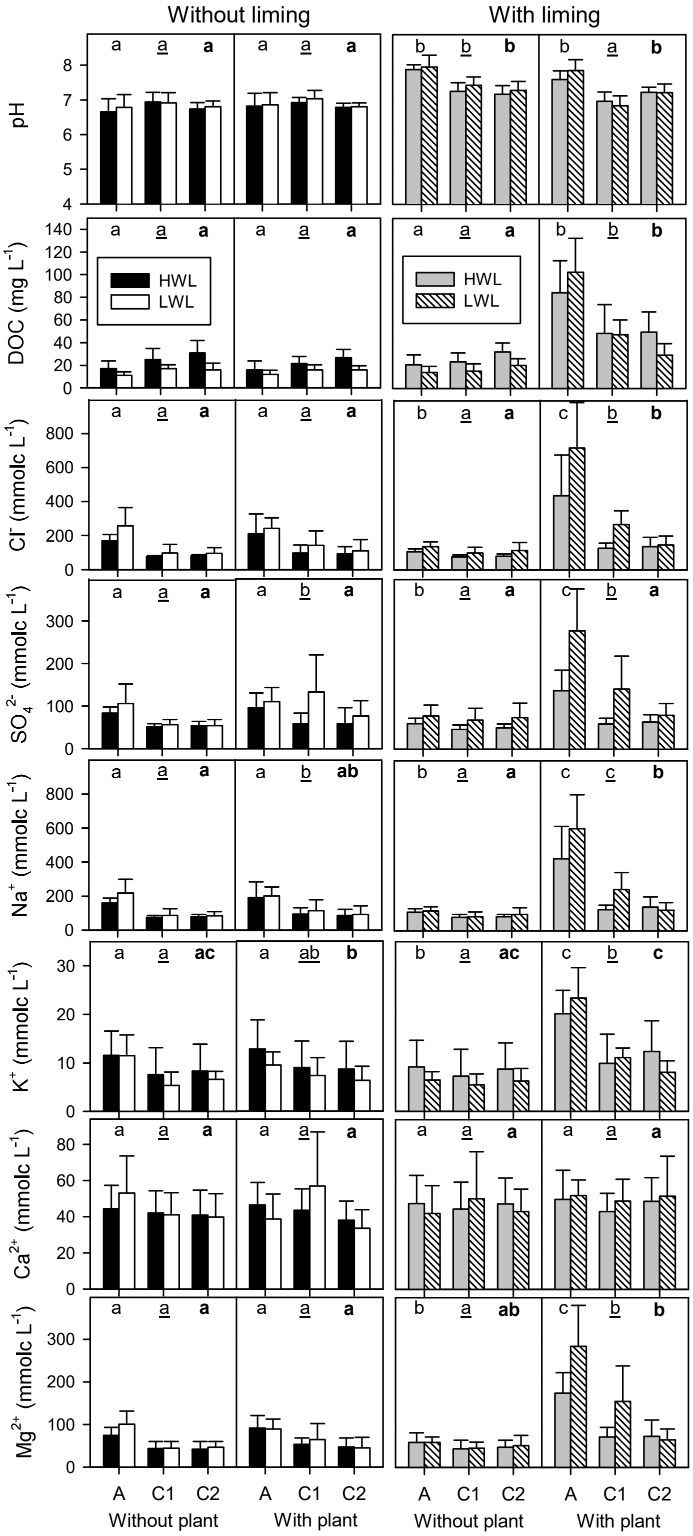

Figure 8.5. Average values of pH (HWL, n = 30; LWL, n = 24) and concentrations of dissolved organic carbon (DOC) and soluble salts (HWL, n = 18; LWL, n = 15) in pore water during the phases of High Water Level (HWL) and Low Water Level (LWL). Bars above columns are standard deviations. For each horizon, different letters above the bars indicate significant differences among treatments, regardless of the flooding period ($p<0.05$). Regular characters: A horizons (normal), C1 (underlined), C2 (bold).

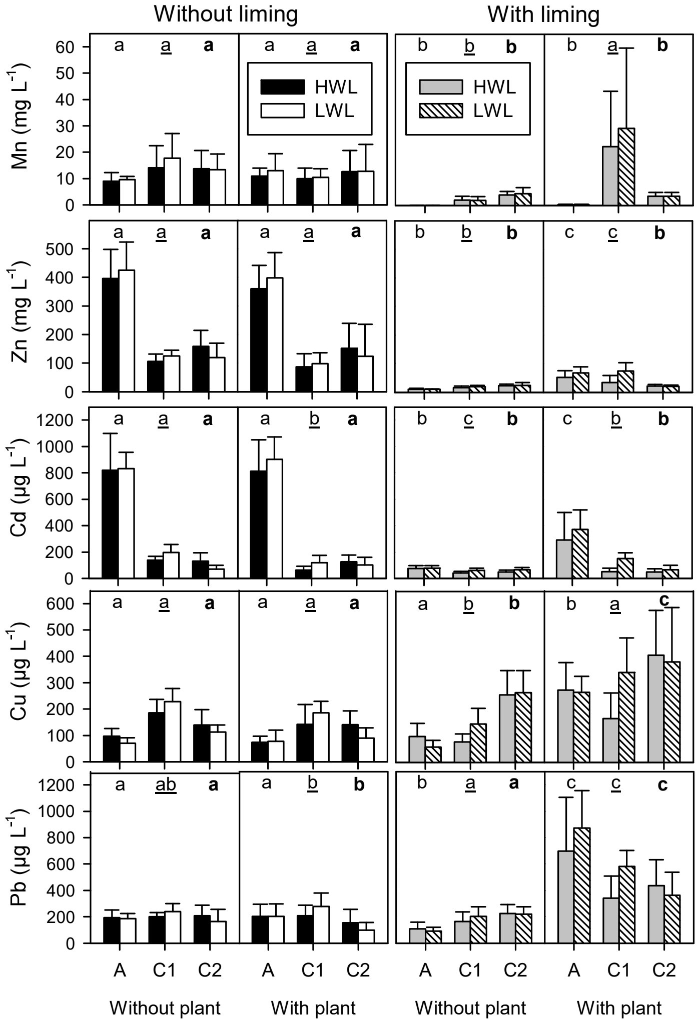

Figure 8.6. Average values of metal concentrations (HWL, n = 18; LWL, n = 15) in pore water during the phases of High Water Level (HWL) and Low Water Level (LWL). Bars above columns are standard deviations. For each horizon, different letters above the bars indicate significant differences among treatments, regardless of the flooding period ($p<0.05$). Regular characters: A horizons (normal), C1 (underlined), C2 (bold).

For all the treatments, the concentrations of most of the soluble metals analysed tended to increase in the A horizons throughout the experiment (Figure 8.7). In the C1 horizons, the concentrations of soluble metals decreased with flooding (High WL) and increased with drying (Low WL), except for Mn which tended to increase throughout the experiment (Figure 8.7). In the C2 horizons, the concentrations of Cd, Cu, Pb, and Zn tended to decrease, while Mn increased (Figure 8.7).

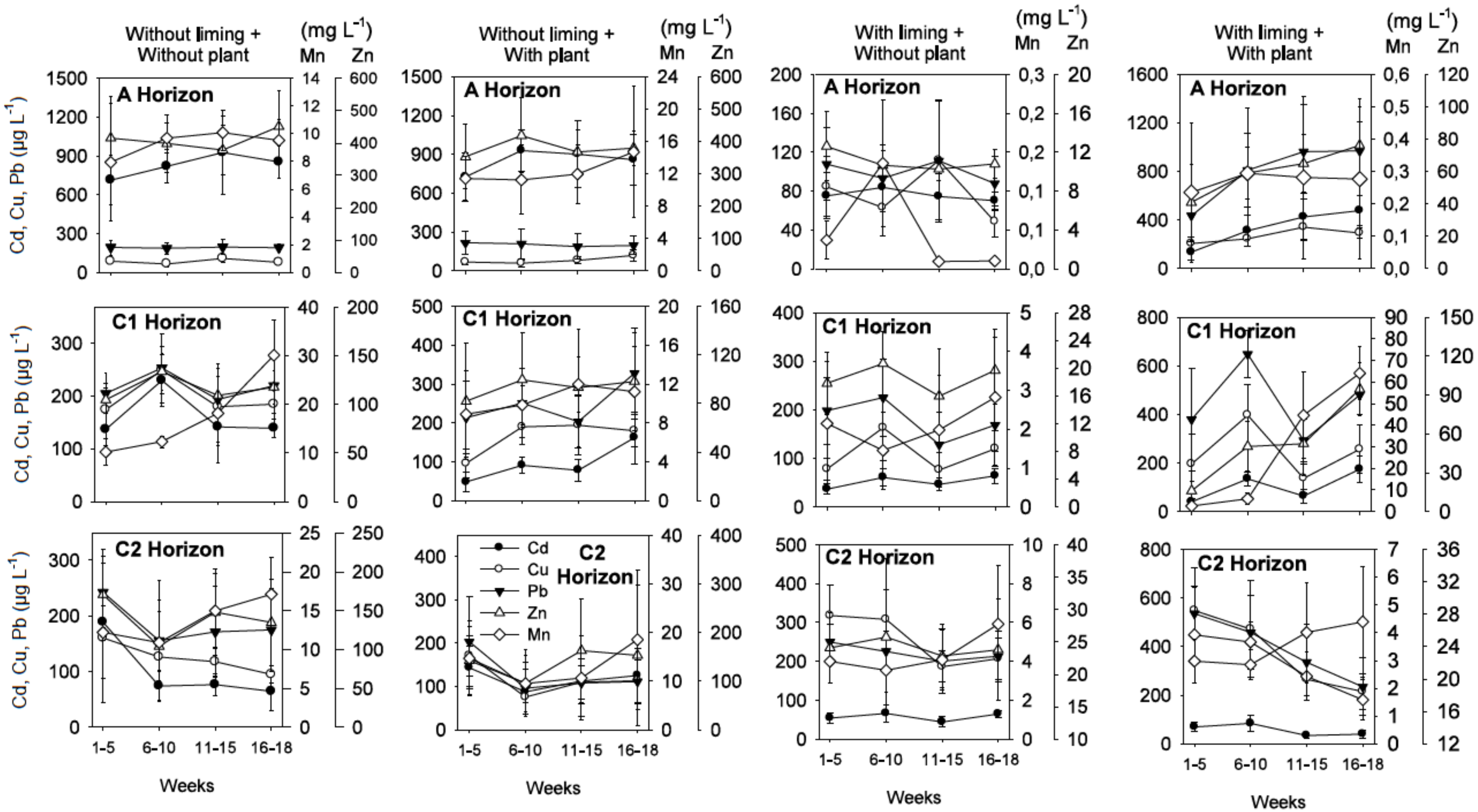

Figure 8.7. Changes in the concentrations of metals in the pore water during the phases of High Water Level (weeks 1-5 and 11-15, n = 9) and Low Water Level (weeks 6-10 and 16-18, n = 9 and 6, respectively). Bars represent the standard deviations.

8.4. Discussion

8.4.1. Variation of the physical-chemical conditions, DOC and salinity in the soil profiles

When the soil is aerated, microorganisms use free oxygen for their metabolism and the redox potential (Eh) remains high. Hence, the A and C1 horizons during the Low WL were oxic (Eh>+300 mV, according to Vepraskas and Faulkner, 2001 and Otero and Macías, 2003). However, when the oxygen concentration falls below 4% (Eh~+300 mV at pH = 7) due to flooding, microorganisms may use other electron acceptors for the mineralisation of organic matter (Otero et al., 2009). This leads to a gradual decrease of Eh (Otero et al., 2009), as occurred in the C1 horizons during the High WL and in the C2 horizons from the 11th week onwards. The Eh reached lower values in both treatments with liming, especially in the C1 horizons, probably because the increase in pH promoted the activity of microorganisms, stimulating the consumption of oxygen (Otero et al., 2009). In addition, pH and Eh are inversely related (Patrick et al., 1996), so that the rise in pH by liming would have favoured a fall in Eh. It should be noted that in the C1 horizon of the treatment with liming + with plant, where the Eh reached values below -100 mV during the High WL, the pH values were lower and similar to those without liming. This could be attributable to the greater growth of *S. fruticosa* with this treatment, since, relative to the bulk soil, plant roots may condition some geochemical parameters such as pH (Wenzel, 2009) and acidic root exudates may lowering the pH in the rhizosphere (Dakora and Phillips, 2002). The greater biomass of *S. fruticosa* in the treatment with liming + with plant could also explain the higher concentrations of DOC, due to the excretion of organic compounds by plant roots (Dakora and Phillips, 2002).

When the environmental conditions are not favourable for microorganisms, oxygen is not consumed and the Eh remains high, even under saturated soil conditions (Vepraskas and Faulkner, 2001). As previously explained, the Eh values of the C2 horizons did not drop until the 2nd High WL. This dynamics could be related to the new addition of eutrophic water (rich in easily degradable carbon sources) with the second elevation of the water level, which would have improved the microbiology of the deepest horizon, after 10 weeks under water. According to Fiedler et al. (2007), the Eh reflects soil microbial activity and is therefore affected by a range of environmental factors such as pH, temperature and organic matter availability. Several studies (e.g. The et al., 2005; Pett-Ridge et al., 2006; DeAngelis et al., 2010)) have observed that microbial populations can adapt rapidly to fluctuating redox conditions, resulting in communities that are ready to use new resources and that impact significantly on the biogeochemical cycling of the systems.

For most of the soluble salts, the highest concentrations occurred in the A horizons, mainly during the Low WL. When the soil water content decreases the solution concentrates. At the same time, the lowering of water potential with desiccation stimulates the capillary upward movement of water and salts (Álvarez-Rogel et al., 2006b), leading to a greater EC in the surface horizons. The latter was more evident in the treatment with liming + with plant, where the greater biomass of *S. fruticosa* could have favoured the pumping of saline water to the surface (Barrett-Lennard and Malcolm, 1999). In fact, in this treatment, DOC could be also moved with soluble salts towards the A horizons (significant correlation of DOC vs. soluble salts, $r \geq 0.647$, $p = 0.000$).

The dynamics of salts are governed mainly by dissolution and precipitation processes, but changes in the concentrations of ions are also influenced by the particular behaviour of each one; this is especially the case for cations, whose dynamics are influenced by their adsorption onto the soil exchangeable complex. For example, the precipitation of Ca^{2+} as gypsum ($CaSO_4 \cdot 2H_2O$) is a common process in saline mine wastes in the presence of SO_4^{2-} (Simón et al., 2010)but, in addition, Ca^{2+} is strongly adsorbed onto soil particles, lowering its mobility. This agrees with its homogeneous concentrations along the experiment, regardless of the treatment.

Under saturated soil conditions, the SO_4^{2-} concentration may be influenced by sulphate-reduction processes (Otero et al., 2009). An index of the sulphate reduction is the ratio SO_4^{2-}/Cl^-. The chloride ion is a conservative element used as a tracer to determine the variations in the concentrations of SO_4^{2-} (Sherman et al., 1998): low ratios reflect the reduction of sulphates and high ratios the oxidation of sulphides (Otero and Macías, 2003). Figure 8.8 shows that, in the absence of the plant, little difference was observed between the High and Low WL in relation to SO_4^{2-} vs. Cl^- (most values grouped at the bottom of the graphs). However, the plants, both with and without liming, modified this behaviour (giving rise to regression lines with different slopes on the graphs). For a given concentration of Cl^-, that of SO_4^{2-} was lower during the High WL and higher during the Low WL. Since the solubility of sulphate salts is generally lower than that of chloride salts (Porta et al., 2003), it could be expected that, during the Low WL, sulphate salts would precipitate before chloride salts and thus the concentration of SO_4^{2-} in the pore water would decrease faster than that of Cl^-. However, in our experiment, this did not occur, probably due to the continuous supply of SO_4^{2-} from sulphide-oxidation processes during the Low WL.

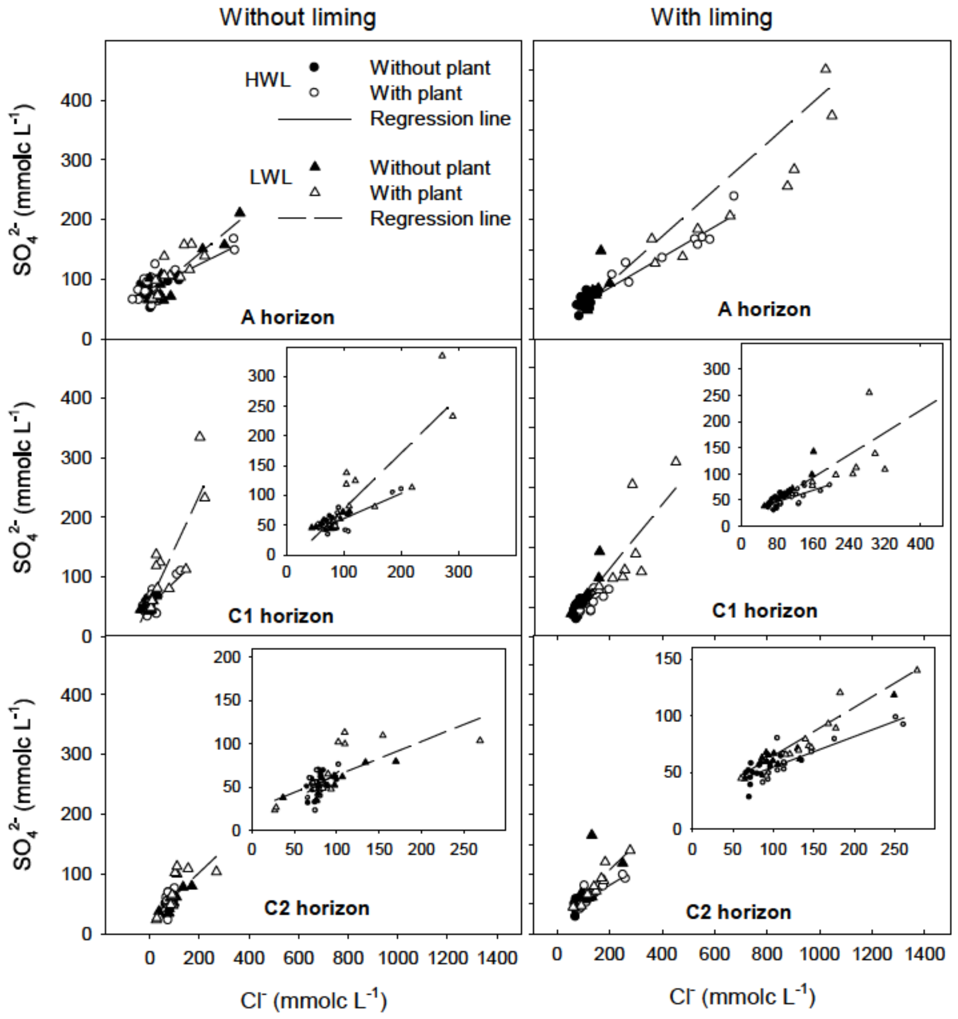

Figure 8.8. Relationships between the concentrations of SO_4^{2-} and Cl^- in pore water.

8.4.2. Metal dynamics and the effectiveness of the treatments with respect to soluble metal concentrations

The dynamics of metals in wetland soils are influenced by several factors such as pH, Eh, temperature, salinity, adsorption-desorption processes, metal oxides and hydroxides, carbonates, complexation with organic matter, and microbial activity (Filgueiras et al., 2002; Du Laing et al., 2009b).

In all the treatments, the rise and fall of the water level induced changes in the concentrations of soluble metals (Figure 8.7). These variations could be related to the changes in Eh; directly, through changes in the valence state of the metals, and/or indirectly, through changes in Fe/Mn oxyhydroxides and the formation/oxidation of insoluble metal sulphides (Du Laing et al., 2008a, 2009a). Moderate reducing conditions lead to the dissolution of metal oxyhydroxides, hence increasing the concentrations of soluble metals which, under strongly reducing conditions, can be insolubilised as metal sulphides (Otero et al., 2000; Du Laing et al., 2007). These meta-stable sulphides may be re-oxidised when oxygen availability increases, leading to the release of metals into the pore water (Otero et al., 2000; Otero and Macías, 2002, 2003). Based on the Eh values and changes in the SO_4^{2-}/Cl^- ratios, the latter could have occurred and this would explain the general tendency of the metal concentrations of the C1 horizons to decrease during the High WL and to increase during the Low WL, particularly in the treatment with liming + with plant (Figure 8.7). In fact, in this treatment, the Zn, Cd, Cu, and Pb concentrations were correlated strongly with Eh ($r \geq 0.391$, $p = 0.000$). María-Cervantes et al. (2010, 2011) found increases in soluble metals under alternating flooding-drying conditions in pot experiments with salt marsh soils polluted by mine wastes. Otero and Macías (2002) and Du Laing et al. (2009b) also observed increases in soluble metals when drying submerged soils, due to the oxidation of metal sulphides. In the C2 horizons, in turn, permanent water saturation could have contributed to the decreases in the concentrations of soluble metals (except for Mn), probably through the progressive formation of metal sulphides.

In both treatments without liming, the higher concentrations of Zn and Cd in the A horizons, which were never under water, were related to the capillary upward movement of water, carrying dissolved salts and metals. In fact, these increases in metal concentrations were correlated with the increases in Cl^- and SO_4^{2-} of the surface horizons ($r \geq 0.350$, $p \leq 0.005$). This agrees with Du Laing et al. (2008b), who found that salinity enhanced the mobility of Cd, and with Comans and Van Dijk (1988), who pointed out that the stability of $CdCl_2$ complexes was higher than the sorption affinity of the metal to soil particles, hence favouring a high concentration of soluble Cd. Gerringa et al. (2001) showed that high salinity may lead to a faster dissolution of Zn and Cd sulphides. In addition, according to Balls et al. (1994) and Tam and Wong (1999), the higher concentrations of major cations in the A horizons could hinder the adsorption of metals onto the soil exchange sites. Nevertheless, our results are contrary to those of Riba et al. (2010), who observed that Zn mobilisation in overlying water increased when salinity decreased.

Unlike Zn and Cd, in the treatments without liming, the concentrations of Mn, Cu, and Pb were lower in the A horizons, probably due to the higher Eh in the surface soil. This agrees with Gambrell et al. (1991), who observed low mobilisation of Pb upon oxidation of brackish, polluted sediments. Du Laing et al. (2008b) found that salinity did not affect Cu mobility in oxidised sediments. In fact, in our experiment, the Mn and Pb concentrations were not correlated with soluble salts and Cu was correlated negatively with them ($r \geq -0.246$, $p \leq 0.042$). Similar to Gambrell et al. (1991) and Du Laing et al. (2008b), both Cu and Pb were correlated with the DOC ($r \geq 0.229$, $p \leq 0.047$), which probably can be attributed to the complexation of the metals with soluble organic compounds.

In many cases, liming has been proposed as a useful tool for the remediation of metal polluted soils (e.g. Simón et al., 2010). It was shown that liming decreased the concentrations of soluble metals in polluted salt marsh soils and increased the growth of *S. fruticosa* and its capacity to phytostabilise metals (González-Alcaraz et al., 2011). However, our data demonstrate that the effectiveness of liming with regard to metal immobilisation, under fluctuating flooding-drying conditions, was different for each of the metals studied and that it decreased when the amendment was combined with *S. fruticosa*.

In our experiment, when *S. fruticosa* was not present, liming increased the soil pH above 7, leading to strong decreases in soluble Mn, Zn, and Cd. The solubility of these metals is related strongly to pH, dropping sharply when pH is above 6 (Brown et al., 2005). Since our soil had an initial pH~6.4, the increase in pH produced by liming was not the only factor involved in the decrease of metal concentrations in the pore water. According to the Eh-pH stability diagrams for metals (Reddy and DeLaune, 2008), the combination of Eh and pH values in the treatment with liming + without plant could have promoted the precipitation of Mn, Zn, and Cd as carbonates. This agrees with Otero et al. (2009), who found that Mn preferentially precipitates as carbonates or is co-precipitated with Ca compounds, and with Jiménez-Cárceles et al. (2008), who found a relationship between Mn content and the presence of $CaCO_3$ in soils from El Carmolí. Charlatchka and Cambier (2000), Otero et al. (2009), and González et al. (2012) also showed that Cd and Zn precipitation with carbonates was the main mechanism of their immobilisation when marble sludge was used to improve metal polluted soils. However, in our experiment, liming was not so effective at decreasing the concentrations of soluble Pb and Cu, especially the latter, whose mobilisation increased. This could be related to the drop of Eh, which facilitates the dissolution of Fe-compounds, hence contributing to the release of the metals bound to them (Otero and Macías 2002, 2003; Du Laing et al., 2007). In addition, the effectiveness of $CaCO_3$ can be limited by the precipitation of Fe-compounds on their surface, reducing their capacity to react with the solution (Simón et al., 2010).

As in the treatment without liming, Cu and Pb were both correlated with DOC ($r \geq 0.503$, $p = 0.000$), indicating that metal complexation with organic compounds contributed to the higher concentrations of these metals in pore water. This agrees with Strobel et al. (2001), who pointed out that, at $pH>6$, almost all the soluble Cu is complexed by dissolved organic ligands, and with Khokhotva and Waara (2010), who found that metal affinity to dissolved organic matter decreases in the sequence Cu>Pb>Ni>Zn.

When liming was combined with the presence of the plant, the growth of *S. fruticosa* (20-fold greater) attenuated the effectiveness of the amendment. The highest Mn concentrations were found in the C1 horizon of this treatment, where the combination of pH and Eh values was outside the zone of $MnCO_3$ formation (according to Reddy and DeLaune, 2008). In fact, there was a significant, negative correlation for Mn vs. pH ($r = -0.766$, $p = 0.000$) and Mn vs. Eh ($r = -0.450$, $p = 0.000$). In addition, the greater pumping of saline water to the surface by the plants promoted the distribution of metals along the soil profile. Although Zn and Cd were pumped towards the A horizons (significant correlation for Zn and Cd vs. Cl^- and SO_4^{2-}, $r \geq 0.698$, $p = 0.000$), the greatest mobilisation occurred for Cu and Pb, which were redistributed throughout the profile. Again, as in the other treatments, Pb was correlated with DOC ($r = 0.694$, $p = 0.000$); the A horizons showed high Pb concentrations together with the highest DOC concentration. This redistribution of metals within the soil profile was also observed by Du Laing et al. (2008b), although some authors, such as Jacob and Otte (2003), suggested that it is not clear if the presence of plants leads to large scale metal mobilisation.

8.5. Conclusions

Changes in the water level of the slightly acidic, saline soils of eutrophic wetlands affected by mine wastes led to variations in the physical-chemical conditions, especially the Eh, and increased metal solubilisation. In addition, the capillary upward movement of soluble metals, together with soluble salts, favoured their accumulation in the surface soil.

Liming contributed to the growth of *Sarcocornia fruticosa* and modified the physical-chemical conditions of the soil, increasing pH and favouring a fall in the Eh. The amendment was effective at immobilising Mn, Zn, and Cd, but not Cu or Pb. The growth of *S. fruticosa* counteracted the effect of liming, increasing the concentrations of soluble metals and distributing them along the soil profile.

Although some authors (e.g. Solis-Dominguez et al., 2012) have indicated that stimulation of the biota of the soil-plant system is an appropriate management strategy for the recovery of non-flooded tailings, our findings show that the effectiveness of this phytomanagement option changes depending on the metal, the soil hydric conditions, and the presence or absence of plants. Hence, one must be careful when choosing the remediation technique for eutrophic, saline mine soils which are regularly flooded, since the use of liming together with the introduction of plants may increase the risk of metals mobilisation.

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Chapter 9

Metal dynamics in simulated strongly acidic soil profiles

Phytomanagement of strongly acidic, saline eutrophic wetlands polluted by mine wastes: the influence of liming and *Sarcocornia fruticosa* on metals mobility

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Abstract

The aim of this study was to assess the effectiveness of combining liming and vegetation for the phytomanagement of strongly acidic, saline eutrophic wetlands polluted by mine wastes. Simulated soil profiles were constructed and four treatments were assayed: without liming + without plant, without liming + with plant, with liming + without plant, and with liming + with plant. The plant species was the halophyte *Sarcocornia fruticosa*. Three horizons were differentiated: A (never under water), C1 (alternating flooding-drying conditions), and C2 (always under water). The soluble Cd, Cu, Mn, Pb, and Zn concentrations were measured regularly for 18 weeks and a sequential extraction procedure was applied at the end of the experiment. Liming was effective (between ~70 and ~100%) in reducing the soluble Zn, Cu, and Pb. In contrast, soluble Mn and Cd increased with liming, especially in the treatment with liming + with plant, where the concentrations were 2-fold higher than in the non-limed treatments. The amendment increased the contents of Zn, Mn, and Cd bound to potentially-mobilisable soil fractions at the expense of the most-environmentally-inert fractions. Hence, the combined use of liming and vegetation may increase the long-term environmental risk of metals solubilisation.

Keywords: Mar Menor, Redox potential, Spolic Technosols, Metal speciation

9.1. Introduction

Soils polluted by mine wastes often have properties such as acidity and high metal content (Wong et al., 2003) and thus require specific management strategies for restoration. Phytomanagement is one of the most promising techniques, consisting of the engineering or manipulation of the soil-plant system to control pollutant fluxes in the environment (Robinson et al., 2009). The effectiveness of liming for phytomanagement of upland soils has been demonstrated, since it promotes a rise in pH, reduces metals mobility, and enhances plant growth (Bolan et al., 2003; Fernández-Caliani and Barba-Brioso, 2010). However, there is little literature regarding hydric soils. Wetlands generally appear in lowland areas and hence are systems prone to receiving mine wastes from topographically higher zones due to erosion and sedimentation processes. These environments have the capacity to remove and/or immobilise metals, acting as green filters (Vega et al., 2009). However, since the mobility of metals depends on multiple factors, such as the flooding regime or vegetation (Almeida et al., 2004; Weis and Weis, 2004), wetlands might be transformed into sources of potentially toxic metals (Frohne et al., 2011) if an unsuitable management strategy is implemented.

The aim of this study was to assess the effectiveness of combining liming and vegetation for the phytomanagement of strongly acidic, saline eutrophic wetlands polluted by mine wastes. For this purpose, we assayed the effect of lime application and growth of the halophyte *Sarcocornia fruticosa* (L.) A. J. Scott on metal dynamics in simulated soil profiles.

9.2. Material and methods

9.2.1. Field soil sampling and initial soil characterisation

Soil samples (top 20 cm) were collected from a Spolic Technosol (WRB, 2006) in a coastal salt marsh -Lo Poyo- (210.6 ha, N37º39´52´´, W0º49´02´´, SE Spain, Figure 9.1) of the Mar Menor lagoon (the largest coastal lagoon of the Mediterranean basin). Territories around the Mar Menor are affected by metal pollution due to previous mining activities in the nearby Sierra de Cartagena-La Unión (Conesa and Jiménez-Cárceles, 2007; Conesa and Schulin, 2010; Conesa et al., 2011). In addition, coastal marshes are also affected by eutrophic water of agricultural and urban origin (Álvarez-Rogel et al., 2006).

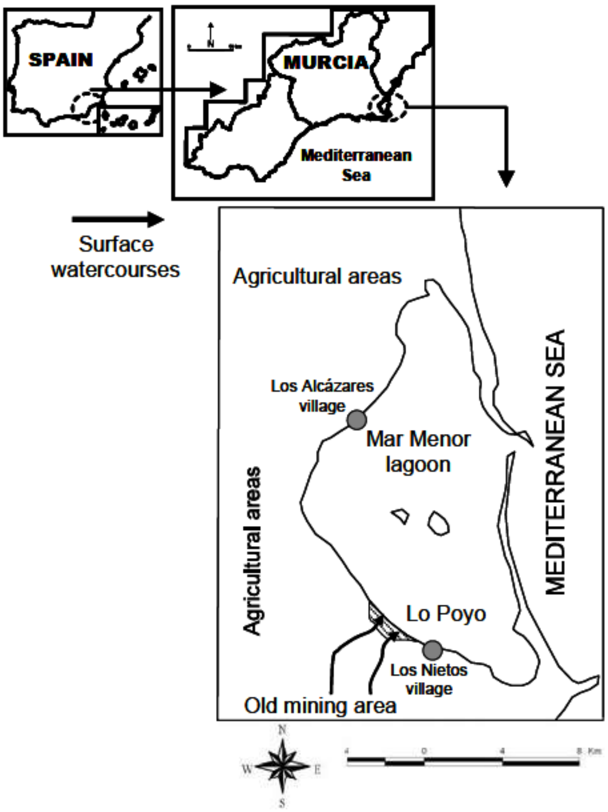

Figure 9.1. Location map of the sampling site (SE Spain).

The soil was sandy (67% sand), strongly acidic (pH~3.1), free of carbonates, and with high levels of total metals (g kg^{-1}: Cd~0.001, Cu~0.16, Mn~0.40, Pb~5.03, Zn~3.78) (González-Alcaraz et al., 2011).

9.2.2. Experimental set up

A lime amendment (pH~8.9) was added to the soil at a dose of 20 g kg^{-1}, giving two soil treatments: 1) soil without liming and 2) soil with liming. Liming increased the soil pH up to ~5.8. Plastic pots (13.5 cm in diameter and 14 cm high) and methacrylate cylinders (20 cm in diameter and 60 cm high) were filled with each soil treatment. The cylinders were covered with opaque plastics to simulate darkness.

The experiment was performed in a greenhouse, in two phases. In the first phase, cuttings of *Sarcocornia fruticosa* (collected from a nearby, non-polluted salt marsh) were planted in pots that were maintained at field capacity with saline water collected from a local stream. Pots without plants were handled in the same way. Liming gave rise to lower concentrations of metals in the soil solution and enhanced the growth of *S. fruticosa* (details of this phase appear in González-Alcaraz et al., 2011). After ten months, the contents of six pots per soil treatment (three with plants and three without) were transferred to their corresponding cylinders, which were watered for five months, as described for the pots, in order to facilitate plant establishment.

In each cylinder (hereafter soil profile), two 10-cm Rhizon ® type samplers (pore diameter = 0.1 μm) and pH and Eh electrodes (Crison 50-50 and 50-55, respectively) were installed horizontally at depths of 5, 30, and 55 cm (hereafter A, C1 and C2 horizons, respectively). The Rhizon ® samplers were connected to 50-mL plastic syringes by means of polyethylene extension tubes, to extract soil solution.

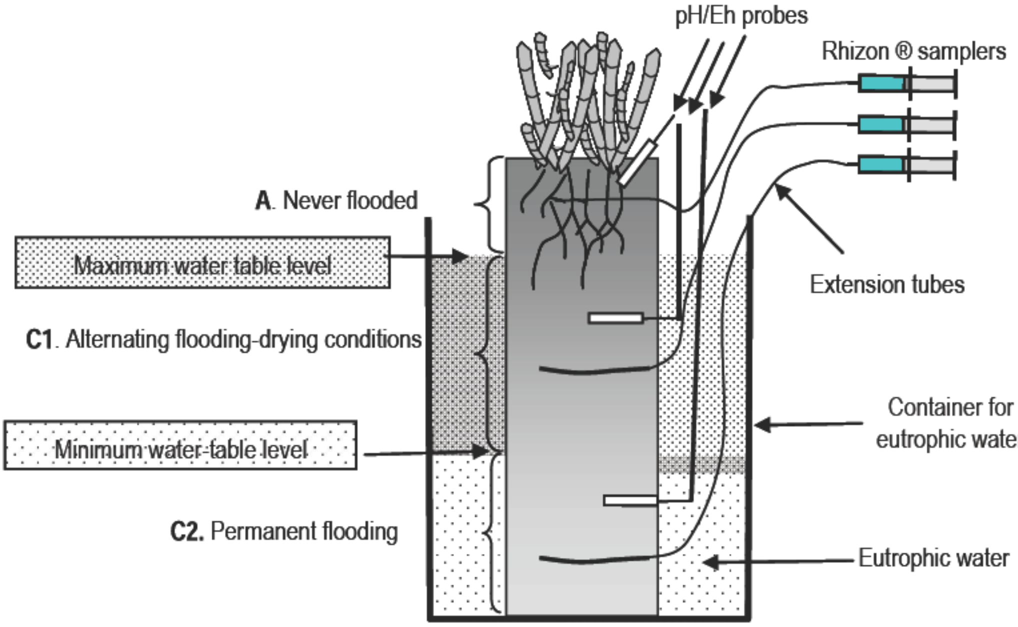

Figure 9.2. Experimental set up.

Each soil profile was placed inside a larger container filled with synthetic eutrophic water with a pH~7.5 and a electrical conductivity~11 dS m^{-1}, enriched in organic carbon (g L^{-1}: 0.31 $Ca(NO_3)_2$, 3.8 NaCl, 3 $MgSO_4$, 0.8 $CaCl_2$, 0.2 KCl, 0.043 H_3PO_4 -85%-, 0.15 $C_6H_{12}O_6$, 0.1 meat extract -46% organic carbon-) (Figure 9.2). The water level (WL) in the containers was maintained at 20 cm below the soil surface for five weeks -weeks 1 to 5- (1st High WL) and at 45 cm below during the following five weeks - weeks 6 to 10- (1st Low WL), repeating this cycle once more along the experiment (2nd High WL -weeks 11 to 15- and 2nd Low WL -weeks 16 to 18-). Hence, the upper 20 cm (A horizons) were never under water, the soil between 20 and 45 cm

(C1 horizons) was under water for 10 weeks with two alternating drying periods of 5 and 3 weeks, respectively, and the deepest 45 cm (C2 horizons) were always under water.

At the end of the profiles phase (that lasted a total of 38 weeks), the fresh biomass of *S. fruticosa* was greater with liming (above ground: 290±124 g; below ground: 44±17 g) than without liming (above ground: 10±0.8 g; below ground: 9.8±3.8 g).

9.2.3. Monitoring of parameters and sampling and analysis of water and soil

The pH and redox potential (Eh) were measured weekly at each depth in each soil profile. The Eh measurements were adjusted according to Vepraskas and Faulkner (2001), by adding +200 mV to the soil voltage (the value of the Ag/AgCl reference electrode at 20 ºC).

Pore water samples were collected every two weeks, at each depth in each profile, for the analysis of electrical conductivity (EC), dissolved organic carbon (DOC) (TOC-V_{CSH} Shimadzu Analyser), Cl^- and SO_4^{2-} (Dionex Ionic Chromatographer), soluble Cd, Cu, Mn, and Pb (ICP-MS Agilent 7500A), and soluble Zn (AAS-Unicam 969).

At the end of the experiment, a sequential extraction procedure (Zeien and Brümmer, 1989, 1991) was applied to fresh samples taken at each depth from two profiles per treatment. The fractions obtained were the following: F1) water soluble and easily exchangeable metals (1M NH_4NO_3-extractable); F2) easily mobilisable metals bound to $CaCO_3$ (1M NH_4OAc-extractable); F3) metals bound to Mn oxides (0.1M NH_2OH-HCl + 1M NH_4OAc-extractable); F4) metals bound to organic complexes (0.025M NH_4-EDTA-extractable); F5) metals bound to amorphous Fe oxides (0.2M NH_4-oxalate-extractable); F6) metals bound to crystalline Fe oxides (0.1M ascorbic acid + 0.2M NH_4-oxalate-extractable); and F7) a residual fraction –metals in refractory minerals-(HNO_3+$HClO_4$-extractable).

All the suspensions were centrifuged at 2500 rpm for 15 min, filtered, and the metal concentrations measured (ICP-MS Agilent 7500A). Aliquots of fresh samples were oven-dried at 65ºC and soil moisture was calculated. The concentrations of metals were expressed on a dry weight basis. In order to evaluate the efficiency of the sequential extraction, a total digestion of fresh samples was made in parallel with the residual fraction. All efficiencies were between 100 and 120%. The accuracy of the measurements was checked by analysing the certified reference soil CRM027-050. The recoveries obtained were between 80 and 120%.

9.2.4. Statistical analysis

The statistical analysis was performed with SPSS 17.0 (SPSS Inc., 2008). For each horizon (A, C1, and C2), repeated measures ANOVA was performed in order to compare the pH, Eh, EC, Cl^-, SO_4^{2-}, DOC, and soluble metals for the different treatments (without liming + without plant, without liming + with plant, with liming + without plant, with liming + with plant). When the ANOVA was significant, the post-hoc Bonferroni test was used to identify different groups ($\alpha=0.05$). Relations among pore water parameters were analysed by Spearman's rank correlations.

9.3. Results

9.3.1. Evolution of Eh

At the beginning, the Eh values were between +400 and +550 mV (Figure 9.3, week 0). Along the experiment, the treatments involving liming gave significantly lower values at all depths ($p \leq 0.048$).

In the treatments without liming, the Eh values of the A and C2 horizons hardly changed along the study. However, in the C1 horizons, the Eh slightly decreased when flooded (High WL), especially in the treatment without liming + with plant (Eh~+300 mV during the 2nd High WL) (Figure 9.3).

In the treatments with liming, the Eh values of the A horizons showed little change, while in the C2 horizons they decreased from the 7th week onwards for the treatment with liming + without plant and from the 1st week for the treatment with liming + with plant (Figure 9.3). The C1 horizons showed the strongest oscillations of Eh related to the changes in the water level: Eh decreased during High WL and increased during Low WL. The lowest values (Eh~-100 mV) were reached during the 2nd phase of High WL (Figure 9.3).

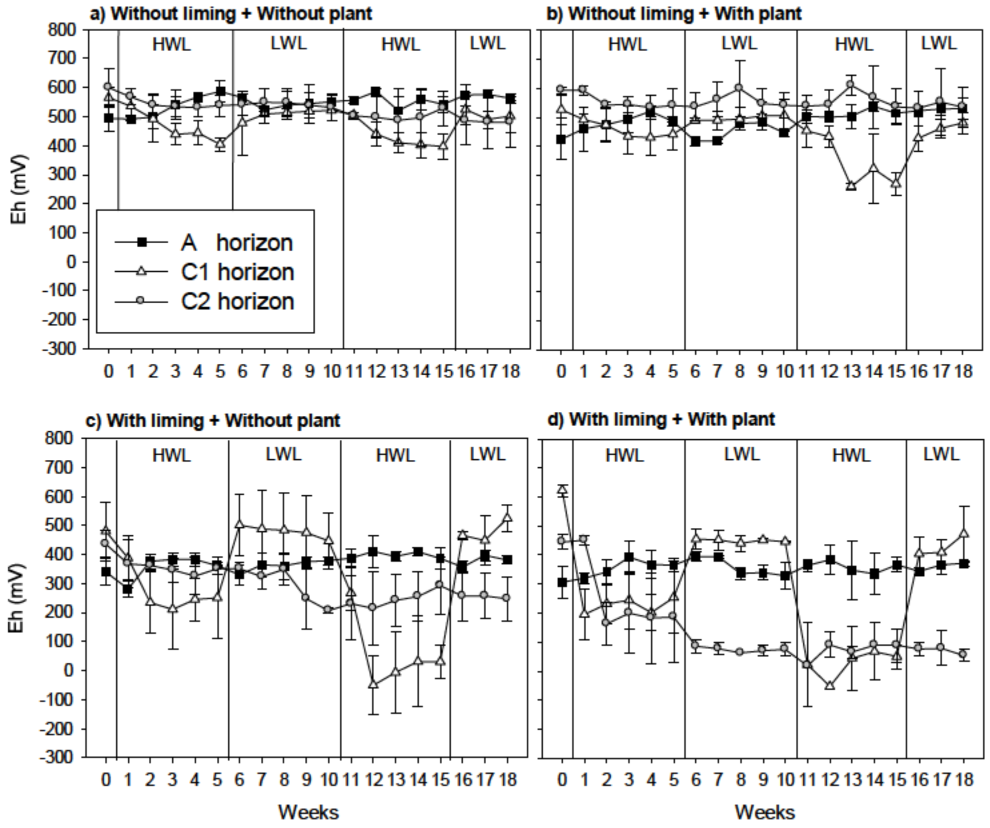

Figure 9.3. Variation of redox potential (Eh) during the phases of High Water Level (HWL) and Low Water Level (LWL). Bars represent the standard deviation (n=3).

9.3.2. Changes in pH, DOC, EC, Cl^- and SO_4^{2-}

Liming increased the pH significantly at all depths ($p \leq 0.001$), from values between 3.5 and 5 without liming to values between 6 and 7 with liming (Figure 9.4). The concentrations of DOC increased during High WL and were significantly higher in the A horizon of the treatment with liming + with plant ($p=0.000$), showing concentrations of ~100 mg L^{-1} (Figure 9.4).

The EC was always higher in the A horizons for all the treatments, regardless of the water level (Figure 9.4). The values varied between 15 and 30 dS m^{-1}, except in the A horizon of the treatment with liming + with plant, which showed significantly higher values (~60 dS m^{-1}) ($p \leq 0.049$) (Figure 9.4). Related to the EC, the concentrations of Cl^- and SO_4^{2-} were significantly higher in the A horizons of the treatment with liming + with plant ($p \leq 0.046$) (Figure 9.4). The concentrations of SO_4^{2-} tended to increase during Low WL, especially in the A and C1 horizons, except in the treatment with liming + without plant (Figure 9.4).

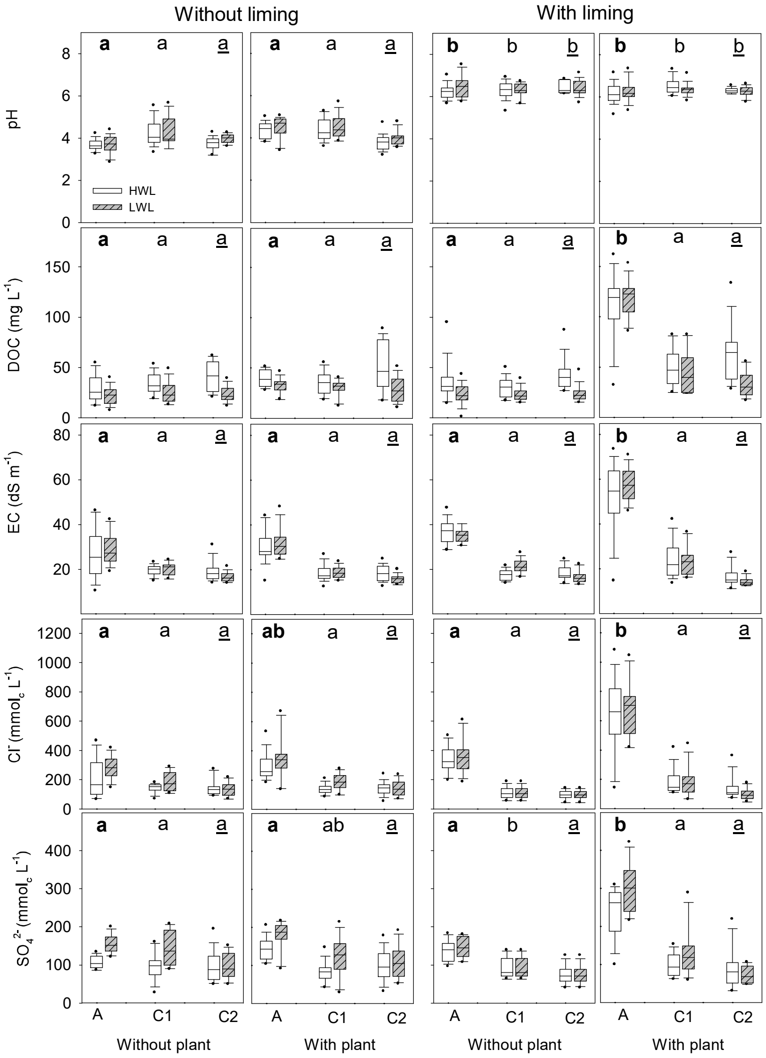

Figure 9.4. Whisker plots for the pH, DOC, EC, Cl^-, and SO_4^{2-} in the soil solution during the phases of High Water Level (HWL) and Low Water Level (LWL). Solid lines within boxes indicate median value. Whisker lines refer to 5th and 95th percentile points. For each horizon, different letters above boxes indicate significant differences among treatments, regardless of the flooding period ($p<0.05$). Regular characters: A horizons (bold), C1 (normal), C2 (underlined).

9.3.3. Changes in soluble metal concentrations

The concentrations of Zn, Cu, and Pb were significantly lower with liming at all depths ($p \leq 0.045$), as were those of Cd in the C1 and C2 horizons ($p \leq 0.017$) (Figure 9.5). However, the A horizon of the treatment with liming + with plant showed significantly higher concentrations of soluble Cd ($p \leq 0.006$) (Figure 9.5). Soluble Mn was hardly affected by the amendment, except in the A horizon of the treatment with liming + with plant, which clearly showed significantly higher concentrations ($p \leq 0.045$) (Figure 9.5).

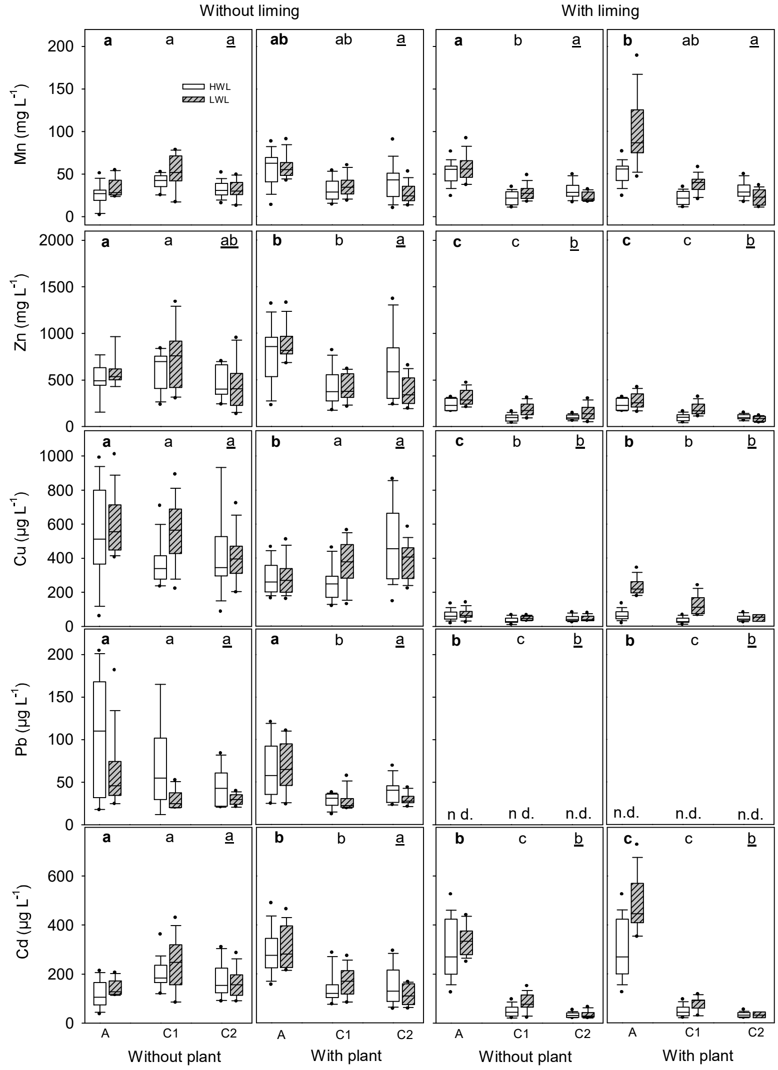

Figure 9.5. Whisker plots for metals in the soil solution during the phases of High Water Level (HWL) and Low Water Level (LWL). Solid lines within boxes indicate median value. Whisker lines refer to 5th and 95th percentile points. For each horizon, different letters above boxes indicate significant differences among treatments, regardless of the flooding period ($p<0.05$). Regular characters: A horizons (bold), C1 (normal), C2 (underlined). n.d.: not detected.

Changes in the water level influenced the soluble metal concentrations, mainly for Mn, Zn, Cu, and Cd (Figure 9.6). Most of them showed a general tendency to increase in the A horizons and to decrease in the C2 horizons, throughout the experiment (Figure 9.6). In the C1 horizons, the concentrations of soluble metals tended to decrease during the phases of High WL and to increase during Low WL phases (Figure 9.6).

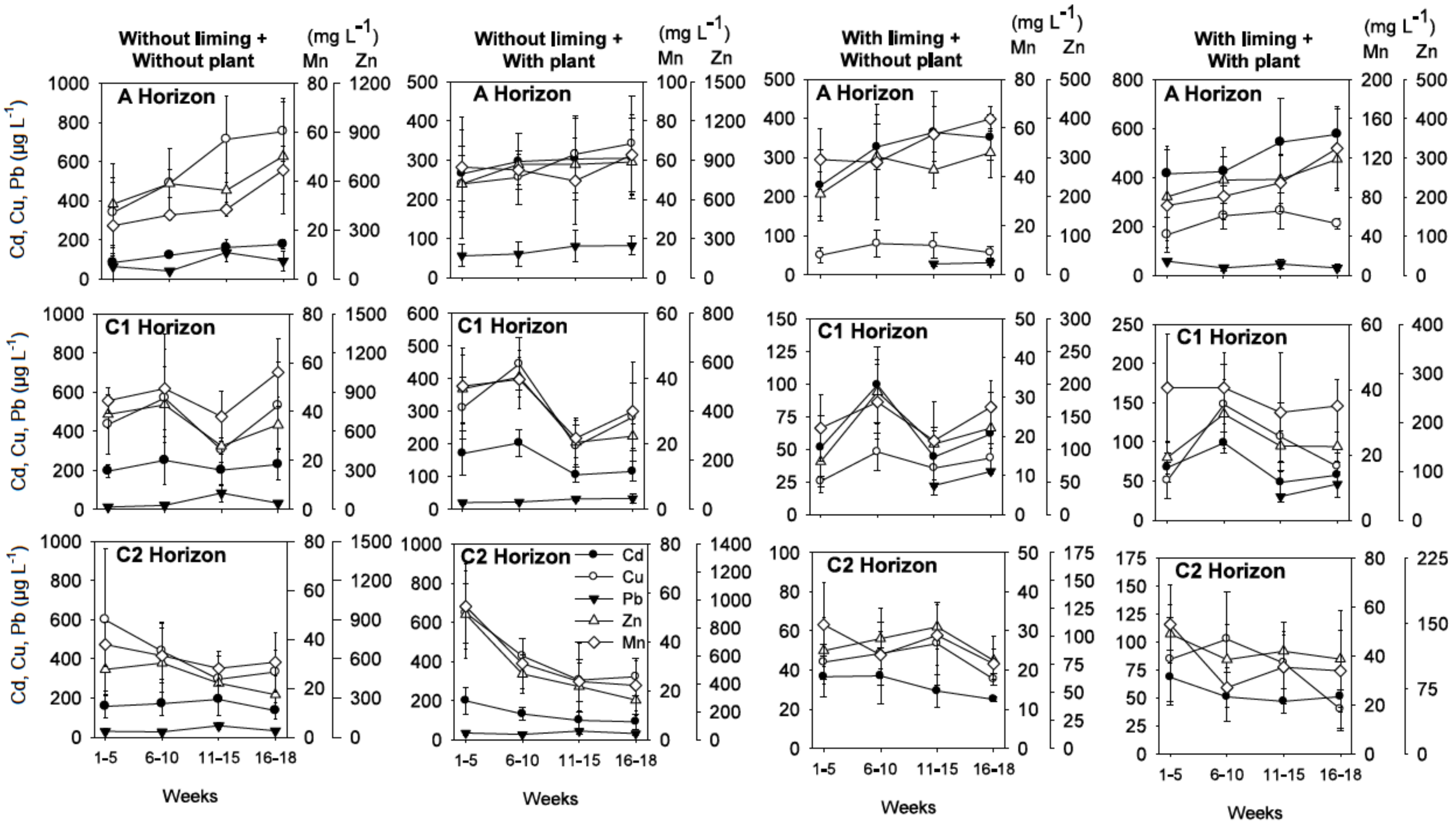

Figure 9.6. Changes in the concentrations of metals in the soils solution during the phases of High Water Level (weeks: 1-5 and 11-15, n=9) and Low Water Level (weeks: 6-10 and 16-18, n=9 and 6, respectively). Bars represent the standard deviations.

9.3.4. Metals speciation in the soil

In all the treatments assayed, the metals in the soil were bound mainly to the residual and crystalline Fe oxides fractions (Figure 9.7). The concentrations of Mn, Zn, and Cd in the mobile fraction were higher than those of Cu and Pb, and tended to increase in the presence of the plant, especially in the A horizons (Figure 9.7).

Liming modified the speciation of Mn, Zn, and Cd, changing their concentrations in the mobile, easily mobilisable, Mn oxides, organic matter, and amorphous Fe oxides fractions (Figure 9.7). In contrast, Cu and Pb speciation was not affected by liming (Figure 9.7).

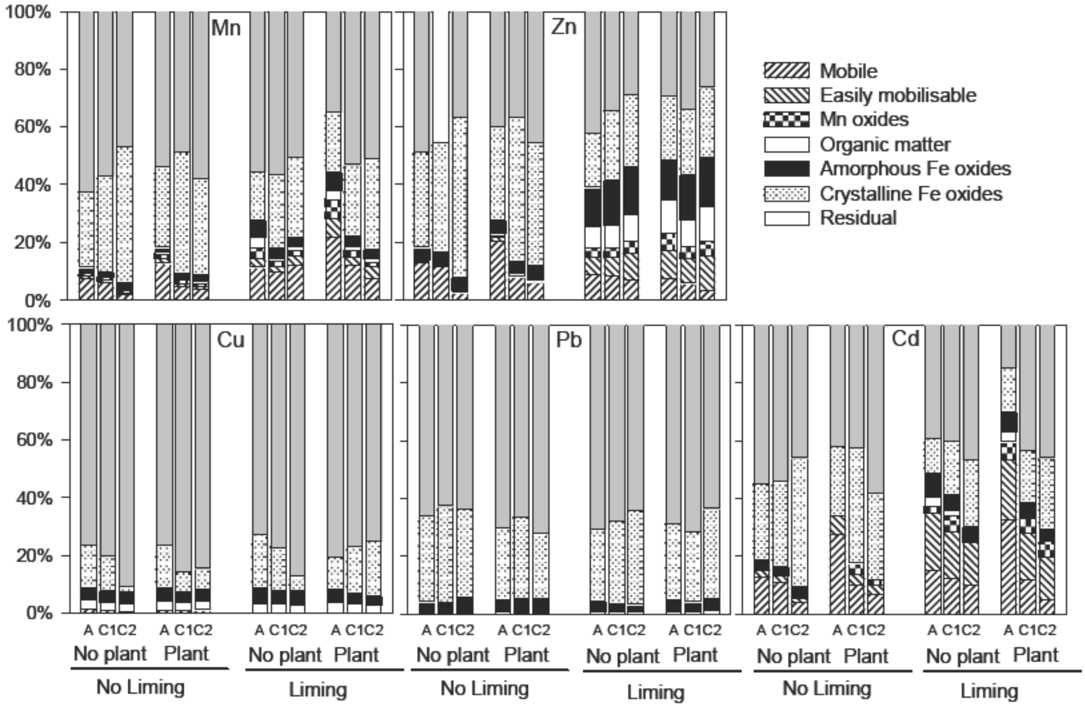

Figure 9.7. Speciation of metals in soil.

9.4. Discussion

9.4.1. Variation of physical-chemical conditions, DOC and salinity in the soil profiles

The redox potential reflects the activity of soil microorganisms (Fiedler et al., 2007). Its values are high in well-aerated soils, where microorganisms use free oxygen for their metabolism. This explains the Eh values above +300 mV (the threshold of oxic soil according to Vepraskas and Faulkner, 2001 and Otero and Macías, 2003) in the A horizons, which were never under water. However, when a soil is flooded and the oxygen concentration falls below 4% (Eh~+300 mV), microorganisms use other electron acceptors for the mineralisation of organic matter (Craft, 2001). This leads to a gradual decrease of Eh, as happened in the C1 horizons during High WL and in the C2 horizons of the treatments with liming. Probably, the increase in pH produced by liming promoted the activity of microorganisms, stimulating the consumption of oxygen (Otero et al., 2009). Moreover, since pH and Eh are related inversely (Patrick et al., 1996), the rise in pH due to liming would have favoured the decline of Eh.

When the environmental conditions are not favourable for microbial activity, oxygen is not consumed and the Eh remains high, even under saturated soil conditions (Vepraskas and Faulkner, 2001). The latter explains the high Eh values in the C2 horizons of the non-limed treatments, which did not drop below +500 mV. In the treatment without liming + with plant, the presence of *S. fruticosa* could have stimulated soil microbiological activity, leading to Eh values of ~+300 mV in the C1 horizon during the 2nd High WL. The facilitative effect of the plant rhizosphere on soil microbial activity through the excretion of organic compounds is widely known (Hinsinger, 2001), but it also provides a structure for microorganisms to adhere to and perform the processes necessary for transformation of elements (Caffery and Kemp, 1990). In addition, the fluctuating redox conditions of the C1 horizon could have promoted the acclimation of soil microorganisms (DeAngelis et al., 2010), leading to the development of microbial populations able to use the organic substrates added with the second elevation of the water level. This effect was also shown in the C1 horizons of the treatments with liming, where the Eh reached values close to -100 mV during the 2nd phase of High WL.

For all the treatments assayed, the values of EC were higher in the A horizons. This is related to the capillary upward movement of saline water to the surface, which is favoured by the pumping of water by plants (Barrett-Lennard and Malcolm, 1999). In fact, the highest EC was produced by the treatment with liming + with plant, where *S. fruticosa* reached its greatest biomass. The greater growth of roots in the latter treatment also explains the higher concentration of DOC in the A horizon, due to increased excretion of organic compounds (Dakora and Phillips, 2002).

9.4.2. Metal dynamics in the soil profiles

The solubility of metals in wetland soils is controlled by numerous factors such as pH, Eh, salinity, adsorption-desorption processes, complexation with organic matter, and microbial activity (Du Laing et al., 2009b).

Except for Pb, the variations in the flooding regime led to changes in the concentrations of soluble metals. This could be related to the changes in the Eh, which may regulate the solubility of metals through changes in the valence state of the metals, changes in metal oxyhydroxides, and the formation/oxidation of metal sulphides (Du Laing et al., 2009a). In all the treatments, the concentrations of SO_4^{2-} were correlated positively with the Eh ($r \geq 0.232$; $p \leq 0.044$) and with the concentrations of soluble Mn, Zn, Cu, and Cd ($r \geq 0.396$; $p \leq 0.001$). Under reduced conditions, sulphates are reduced to sulphides, which can act as sink for metals (Otero et al., 2000). When the oxygen availability increases, the oxidation of metal sulphides releases the associated metals, increasing their concentrations in the soil solution (Otero and Macías, 2003). The molar ratio SO_4^{2-}/Cl^- can be used as an index of the sulphate reduction processes because the chloride ion is a conservative element, used as a tracer to determine variations in the SO_4^{2-} concentration (Sherman et al., 1998). Otero and Macías (2003) pointed out that decreases in this ratio reflect the reduction of sulphates while increases indicate the oxidation of sulphides. The SO_4^{2-}/Cl^- ratios obtained in the C1 and C2 horizons are in accordance with the latter processes, decreasing (ratios between 0.56 and 0.65) during High WL and increasing (ratios between 0.74 and 0.88) during Low WL. In the C1 horizons, direct exposure to air during Low WL periods induced sulphide oxidation, while in the C2 horizons, always under water, oxygen diffusion from the upper horizons caused this process.

In all the treatments assayed, the increases in the concentrations of soluble metals in the A horizons were related to the capillary upward movement of water, carrying soluble salts and metals. In fact, the concentrations of soluble metals were correlated positively with the EC ($r \geq 0.364$; $p \leq 0.001$) and with the concentrations of Cl^- and SO_4^{2-} ($r \geq 0.273$; $p \leq 0.021$). This agrees with Du Laing et al. (2008), who found that salinity favoured metals mobility. The latter was more evident in the treatments with plants, where the A horizons showed higher concentrations of soluble metals in relation to their corresponding non-planted treatments, due to the pumping of water by the plant.

In addition, in the treatments with *S. fruticosa*, the concentrations of soluble Mn, Zn, Cu, and Cd were correlated positively with DOC ($r \geq 0.389$; $p \leq 0.001$), due to complexation of the metals by root exudates (Sager et al., 2007).

Liming was effective with regard to reducing the contents of soluble Zn, Cu, and Pb, whose concentrations were 71, 78, and ~100% lower, respectively, in the soil solution of the limed treatments. In the case of Zn, the decrease in the soluble concentration was accompanied by a decrease in the mobile fraction (F1). In contrast, soluble Mn and Cd increased with liming, especially in the A horizon of the treatment with liming + with plant, where the concentrations were 2-fold higher than in the non-limed treatments. These increases were accompanied by increases in the Mn and Cd mobile fractions (F1) with liming.

For Zn, Mn, and Cd, the liming induced an increase in the fraction bound to organic complexes (F4). This could be related to the rise of pH, leading to an increase in the binding of metals to organic compounds. Similarly, Claff et al. (2011) found higher contents of metals bound to organic materials with increasing pH in acid sulphate sediments of tidally re-inundated wetlands.

The treatments involving liming also resulted in higher concentrations of Zn, Mn, and Cd in the easily mobilisable fraction (F2) and in the fractions bound to Mn oxides (F3) and bound to amorphous Fe oxides (F5). This agrees with Claff et al. (2011), who, upon tidal inundation, found higher storage of metals in the fraction bound to carbonates and poorly crystalline Fe minerals. According to Gambrell and Patrick (1991), F2, F3, F4, and F5 can be considered as potentially-mobilisable fractions, since changes in the pH and Eh conditions may solubilise their associated metals. Hence, the ratio (F2+F3+F4+F5)/(F6+F7) can be used as an index to predict the long-term risk of metals leaching in the environment. Higher ratios imply an increase of the potentially-mobilisable fractions at the expense of the most-environmentally-inert fractions. The ratios obtained in the soil profiles of the current study were about one order of magnitude higher with liming (between 0.15 and 0.79) than without liming (between 0.04 and 0.08), the maximum values being reached in the treatment with liming + with plant, which implies a greater long-term environmental risk of Zn, Mn, and Cd leaching. This is in agreement with Conesa et al. (2008), who found higher short-term metals mobility in acidic mine tailings due to their higher solubility, but a greater risk of long-term mobility in neutral tailings due to the greater binding of metals in potentially-available forms.

9.5. Conclusions

The lime amendment was effective at decreasing the concentrations of soluble Zn, Cu, and Pb, but increased those of soluble Mn and Cd. In addition, the capillary upward movement of soluble metals, together with soluble salts, favoured their accumulation in the surface soil, especially in the presence of plants. Liming induced changes in the speciation of Zn, Mn, and Cd in the soil, increasing the content of metals bound to potentially-mobilisable fractions at the expense of the most-environmentally-inert fractions.

Hence, the combined used of liming and vegetation for the phytomanagement of strongly acidic, saline eutrophic wetlands polluted by mine wastes might not always be an appropriate strategy: it can reduce soluble metal levels in the short-term, but may also favour the storage of metals in potentially-mobilisable fractions, hence increasing the long-term environmental risk of metals solubilisation.

9.6. Acknowledgments

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Chapter 10

Conclusions

The general conclusion of this work is that the choice of the most appropriate phytomanagement techniques for recovering eutrophic wetlands affected by metal mine wastes depends on the particular characteristics of the soil-water-plant system, the hydric/flooding regime, and the type of pollutant (nitrogen, phosphorus, and metals). This general statement is supported by the following specific findings:

1. The presence of plants in flooded eutrophic wetlands polluted by metal mine wastes improves the capacity of these systems to remove nitrates through denitrification processes, especially in sandier acidic soils where the vegetation may promote the microbial activity in the rhizospheric environment.

2. The retention of phosphorus by soil is the main mechanism associated to the removal of phosphates in flooded eutrophic wetlands polluted by metal mine wastes, although the capacity of these systems as long-term phosphorus sink depends on the presence of metal oxides and Ca/Mg compounds in soil and the plant species.

3. Regarding metals dynamics, liming and presence of plants (at least *Sarcocornia fruticosa*) is a suitable management alternative in eutrophic wetlands polluted by metal mine wastes under non-flooded conditions, since it decreases the concentrations of soluble metals and favours their immobilisation within plant roots. However, under alternating flooding-drying conditions, the combination of liming and vegetation do not seems to be a suitable alternative since it enhances the solubilisation and redistribution of metals through the soil profile, especially in slightly acidic soils, and increases the potentially-mobilisable metal fractions in strongly acidic soils thereby increasing the long-term environmental risks.