Universidad Politécnica de Cartagena

DEPARTAMENTO DE CIENCIA Y TECNOLOGÍA AGRARIA

The importance of edaphic niches and spontaneous vegetation for the phytomanagement of mine tailings under semiarid climate

TESIS DOCTORAL

Isabel María Párraga Aguado

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DIRECTORES:

Héctor Miguel Conesa Alcaraz

María Nazaret González Alcaraz

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CONFORMIDAD DE SOLICITUD DEAUTORIZACIÓN DE DEPÓSITO DE TESIS DOCTORAL POR EL/LA DIRECTOR/A DE LA TESIS

D. Héctor Miguel Conesa Alcaraz y Dª. María Nazaret González Alcaraz, Directores de la Tesis Doctoral "The importance of edaphic niches and spontaneous vegetation for the phytomanagement of mine tailings under semiarid climate"

INFORMAN:

Que la referida Tesis Doctoral, ha sido realizada por Dª. Isabel María Párraga Aguado, dentro del programa de doctorado Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario, dando nuestra conformidad para que sea presentada ante la Comisión de Doctorado para ser autorizado su depósito.

La rama de conocimiento en la que esta tesis ha sido desarrollada es:

- **X** Ciencias
- ☐ Ciencias Sociales y Jurídicas
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En Cartagena, a 2 de Marzo de 2015

LOS DIRECTORES DE LA TESIS

[redacted]

Fdo.: Héctor M. Conesa Alcaraz

[redacted]

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DT-17

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INFORMA:

Que la Tesis Doctoral titulada, "The importance of edaphic niches and spontaneous vegetation for the phytomanagement of mine tailings under semiarid climate" ha sido realizada, dentro del mencionado programa de doctorado, por Dª. Isabel María Párraga Aguado, bajo la dirección y supervisión del Dr. Héctor Miguel Conesa Alcaraz y la Dra. María Nazaret González Alcaraz.

En reunión de la Comisión Académica de fecha 11/03/2015, visto que en la misma se acreditan los indicios de calidad correspondientes y la autorización de los Directores de la misma, se acordó dar la conformidad, con la finalidad de que sea autorizado su depósito por la Comisión de Doctorado.

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

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☐ Ciencias Sociales y Jurídicas
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EL PRESIDENTE DE LA COMISIÓN ACADÉMICA DEL PROGRAMA

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COMISIÓN DE DOCTORADO

Esta Tesis Doctoral se ha realizado bajo la financiación de los siguientes proyectos:

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A mi padre

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No te des por vencido, ni aún vencido,
no te sientas esclavo, ni aún esclavo;
trémulo de pavor, piénsate bravo,
y acomete feroz, ya mal herido.
Ten el tesón del clavo enmohecido
que ya viejo y ruin, vuelve a ser clavo;
no la cobarde estupidez del pavo
que amaina su plumaje al primer ruido.
Procede como dios, que nunca llora;
o como lucifer, que nunca reza;
o como el robledal, cuya grandeza
necesita del agua, y no la implora...
¡Que muerda y vocifere vengadora,
ya rodando en el polvo, tu cabeza!

Almafuerte

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Resumen

Las balsas de lodos de flotación o balsas mineras son consideradas las causantes de los principales problemas ambientales asociados a la actividad minera, debido a la dispersión de partículas con alto contenido en metales y metaloides que se produce desde sus superficies desnudas a causa de la erosión hídrica y eólica. Este hecho es especialmente importante cuando las balsas mineras están situadas en las proximidades de zonas naturales protegidas o áreas urbanas, agrícolas o recreativas. El fitomanejo en términos de fitoestabilización (uso de plantas para inmovilizar metal(oid)es en la rizosfera) puede ser una opción apropiada y de bajo coste para la restauración de las balsas mineras. Sin embargo, el éxito de su aplicación aún necesita una mejor comprensión de los factores edáficos, ecológicos y fisiológicos relacionados con el establecimiento de la vegetación sobre estas balsas. La presente Tesis Doctoral se centró en el estudio de los factores edáficos y ecofisiológicos relacionados con la colonización espontánea de las balsas mineras, con el fin de proponer recomendaciones para el fitomanejo, en el contexto de clima semiárido del Distrito Minero de Cartagena-La Unión (sureste de España). Los objetivos específicos fueron:

1. Describir los gradientes edáficos y la vegetación espontánea asociados a ellos, e identificar los nichos edáficos potencialmente favorables, en balsas mineras abandonadas del Distrito Minero de Cartagena-La Unión.
2. Evaluar la idoneidad de las especies vegetales espontáneas para la restauración/estabilización de balsas mineras, de acuerdo al papel de la rizosfera en la mejora de las condiciones edáficas, al estudio de diferentes formas de vida y a las respuestas ecofisiológicas de las plantas a las condiciones de las balsas mineras.
3. Evaluar el comportamiento de especies arbóreas en las balsas mineras, para estimar sus posibilidades como candidatas para la fitoestabilización y su papel potencial en la restauración de tales ambientes.

4. Evaluar la respuesta de una especie herbácea comúnmente encontrada en las balsas (*Piptatherum miliaceum*) a la adición de una enmienda orgánica a los residuos mineros, empleando medidas de isótopos estables en planta y considerando las interacciones intraespecíficas.

Para alcanzar estos objetivos, se diseñaron dos fases: una fase de trabajo de campo y una fase experimental. El primer estudio de campo estuvo centrado en la descripción de los gradientes edáficos y ecológicos a lo largo de un transecto desde una zona no contaminada o "control" hasta las balsas mineras. La distribución en parches edáficos pareció ser determinante en el establecimiento selectivo de la vegetación espontánea que crecía en las balsas. La salinidad del suelo fue el principal factor relacionado con los cambios en el inventario de especies y con la disminución de la diversidad y riqueza ecológica desde la zona no contaminada hasta la meseta de las balsas, mientras que los metal(oid)es jugaron un papel secundario. La colonización de las balsas mineras por especies autóctonas típicas de la zona control estuvo relacionada con un gradiente de fertilidad, generado por la mejora de las condiciones edáficas debido al desarrollo previo de la vegetación pionera. Este proceso natural ha dado lugar a la creación de parches de vegetación densa, aquí llamados "islas de fertilidad", que contienen especies clímax de la zona.

La utilidad de las especies espontáneas para el fitomanejo de balsas mineras fue evaluado en primer lugar mediante un trabajo de campo enfocado en la caracterización de las rizosferas y la ecofisiología de distintas especies vegetales (herbáceas, arbustos y árboles). La rizosfera de herbáceas y arbustos espontáneos (*P. miliaceum*, *Helichrysum decumbens*) mostró una mejora significativa de la microbiología del suelo, comparada con el suelo desnudo. Además, la similitud entre la rizosfera de árboles individuales (*Tetraclinis articulata*, y sobre todo, *Pinus halepensis*) y la de las "islas de fertilidad" llevaron a sugerir que los árboles son la clave para el desarrollo de estas áreas de vegetación densa.

En relación a herbáceas y arbustos, se realizó un trabajo de campo para evaluar el diferente comportamiento de halófitas y no halófitas espontáneas, y evaluar si las halófitas podrían ser más adecuadas para sitios salinos específicos en las balsas mineras. En el caso de especies arbóreas, se llevaron a cabo tres estudios específicos sobre *P. halepensis* con el fin de conocer mejor la habilidad de los pinos para soportar las condiciones edáficas de las balsas mineras. El primero de ellos estuvo centrado en la acumulación de metal(oid)es en pinos a lo largo de un gradiente de contaminación. El segundo y el tercer trabajo trataron sobre el estado ecofisiológico, la composición foliar elemental e isotópica, y la retranslocación interna de nutrientes y metal(oid)es en poblaciones espontáneas de *P. halepensis* en balsas mineras. Los pinos mostraron una buena tolerancia a las pobres condiciones edáficas de las balsas. A pesar de una deficiencia foliar de fósforo severa, los pinos mostraron una sustancial retranslocación de macronutrientes desde las hojas senescentes, lo que puede ayudarles a tolerar algunas deficiencias nutricionales, especialmente en relación a potasio, magnesio y fósforo. El análisis de la composición isotópica foliar reveló que los pinos que crecían sobre las balsas mineras estaban menos estresados hídricamente que aquellos de la zona control, y que la asociación micorrícica podría ser crítica para el desarrollo de estos pinos sobre las balsas mineras. Además, los pinos mostraron una relativamente baja absorción de metal(oid)es y algunos mecanismos para tolerarlos, como la inmovilización en tejidos menos activos biológicamente (como Cd, Cu, Pb y Sb en las ramas) o la acumulación en órganos desechables (como As, Cd, Sb, Pb y Zn en la hojarasca).

Finalmente, se realizó un experimento en macetas usando una gramínea comúnmente encontrada en balsas mineras (*P. miliaceum*) y un residuo minero enmendado con un residuo sólido municipal obtenido del reciclado de envases metálicos. *P. miliaceum* fue capaz de crecer en el residuo minero tanto con la enmienda como sin ella. La adición del residuo sólido municipal mejoró algunos indicadores de fertilidad del suelo, aunque el principal efecto se observó sobre el crecimiento y desarrollo de la planta. Las concentraciones de metal(oid)es en las hojas estuvieron por debajo de los límites tóxicos para forraje en todos los tratamientos. El crecimiento de la planta también se vio influenciado por la competencia intraespecífica, la cual provocó un descenso de la biomasa de la planta en los tratamientos con y sin enmienda.

Por lo tanto, las conclusiones de la Tesis fueron:

1) La identificación de los nichos más favorables o apropiados para el crecimiento de la vegetación deben ser tenidos en cuenta cuando se lleven a cabo trabajos de fitoestabilización en balsas mineras. El laboreo superficial o la adición de determinadas enmiendas que podrían elevar la salinidad del suelo deben ser detenidamente evaluados para no destruir aquellos nichos favorables.

2) El uso de combinaciones de especies vegetales con diferentes formas de vida y estrategias de uso de agua y nutrientes complementarias puede resultar en un empleo más eficiente de los recursos edáficos. Esto puede favorecer la creación de una comunidad vegetal estable a largo plazo y puede proporcionar mayor resistencia a cambios ambientales como los largos períodos de sequía propios del clima semiárido.

3) El empleo de especies halófitas (*Limonium cossonianum*, *Atriplex halimus*, *Zygophyllum fabago*) puede ser una opción adecuada para revegetar y mejorar los nichos edáficos más salinos de las balsas mineras, debido a su mejor adaptación a la salinidad y a la mejora de las propiedades del suelo.

4) La especie arbórea *P. halepensis* puede ser una candidata adecuada para la fitoestabilización de balsas mineras debido a su adaptación a las condiciones de clima semiárido y a su papel potencial en la formación de las "islas de fertilidad". Sin embargo, sería recomendable la adición de fertilizantes fosfóricos para aliviar la fuerte deficiencia en este elemento.

5) La gramínea *P. miliaceum* puede ser considerada para la fitoestabilización de las balsas mineras, aunque para obtener un rápido y crecimiento y desarrollo, se deben aportar enmiendas orgánicas.

Summary

Mine tailings are considered the main responsible of the environmental problems associated to metal mining activities due to the spread of contaminants by water and wind erosion from their bare surfaces. These issues are especially critical when the tailings ponds are located in the proximities of sensible areas such as protected natural sites, urban, agricultural or recreational areas. Phytomanagement in terms of phytostabilisation (the use of plants to inmobilise metal(loid)s within the rhizosphere) might be a suitable cost-effective option for the restoration of mine tailings. However, the success of its application still needs a better understanding of the edaphic, ecological and physiological factors involved in the establishment of vegetation. The present PhD Thesis was focused on the study of the edaphic and ecophysiological factors involved in the spontaneous plant colonisation of mine tailings with the aim to obtain recommendations for the successful phytomanagement of these environments, in a semiarid climate context in the Cartagena-La Union Mining District (Southeast Spain). The specific objectives were:

1. To describe the edaphic gradients and their associated spontaneous vegetation and to identify potentially plant-favourable edaphic niches in abandoned neutral-pH mine tailings located in the Cartagena-La Union Mining District.

2. To assess the suitability of using spontaneous plant species in restoration/stabilisation of mine tailings, focusing on the role of rhizosphere in the improvement of soil conditions, the study of different life forms and the ecophysiological responses to mine tailings environment.

3. To assess the behaviour of tree species on mine tailings to estimate its possibilities as candidates for phytostabilisation and their potential role in the restoration of such environments.

4. To evaluate the response of a spontaneous grass plant species commonly found on mine wastes (*Piptatherum miliaceum*) growing under controlled conditions in an organic amended mine tailings by employing stable isotopes and considering intra-specific interactions.

To achieve these objectives, a field work stage and a pot experiment were performed. The first field survey was focused on the description of the edaphic and ecological gradients along a transect from a non-polluted control site to a mine tailings area. The edaphic patch distribution was found to be determinant for the selective establishment of spontaneous vegetation growing at the tailings. Soil salinity was the main factor involved in the shifts of the plant species inventory and the decrease of plant diversity and richness from the non-polluted area to the tailings plateau, while metal(loid)s soil concentrations played a minor role. The colonisation of the mine tailings by native late successional plant species was related with the fertility gradient generated by the enhancement of soil conditions as a result of the early development of pioneer vegetation. This natural process has provided the occurrence of high dense vegetated patches in the tailings, here called "fertility islands", which contain climax vegetation of the local area.

The usefulness of the spontaneous vegetation for the phytomanagement of mine tailings was firstly assessed through a field study focused on the characterisation of the rhizospheres and the ecophysiology of different plant species (grasses, shrubs and trees). The rhizosphere of spontaneous grasses and shrubs (*P. miliaceum*, *Helichrysum decumbens*) showed a significant enhancement of the soil microbiology compared with the bare soil. Moreover, the similarity between the rhizosphere of individual trees (*Tetraclinis articulata*, and especially *Pinus halepensis*) and that of the "fertility islands" may lead to suggest that trees are the key for the development of these dense vegetation areas.

In relation to grasses and shrubs, a field survey was conducted in order to evaluate the different performance of spontaneous halophyte and non-halophyte species and to assess if halophytes could be more suitable for specific salty sites on the tailings. In the case of tree species, three specific studies focused on *P. halepensis* were done in order to gain insight into the ability of pine trees to cope with the soil conditions at the tailings. The first study was focused on the accumulation of metal(loid)s in *P. halepensis* along a polluted gradient. The second and the third studies dealt with the ecophysiological status, elemental and isotopic foliar composition and internal nutrients and metal(loid)s retranslocation in spontaneous populations of *P. halepensis* growing on mine tailings. Pine trees showed a good tolerance to the poor soil conditions of the tailings. In spite of a severe foliar P deficiency, pine trees showed substantial macronutrient retranslocation from senescent leaves, which may help them to cope with some nutritional deficiencies, especially in relation to K, Mg and P. The analysis of the foliar isotope composition showed that pines growing at mine tailings are less water stressed that those ones from a natural forest in the area (control), and that mycorrhizal association might be critical for the development of these trees on the mine tailings. Moreover, pine trees showed a relatively low metal(loid) uptake and some mechanisms to cope with them such as the immobilisation in less biologically active tissues (Cd, Cu, Pb and Sb in woody stems) or the accumulation in deciduous parts (As, Cd, Sb, Pb and Zn in litter).

Finally, a pot experiment was performed using a grass species commonly found on tailings (*P. miliaceum*) and a mine tailings soil amended with a municipal solid waste obtained from the recycling of metallic containers. *P. miliaceum* was able to growth on tailings with and without amendment. The addition of the municipal solid waste improved some soil fertility indicators, although the principal effect was the enhancement of the growth and development of the plants. Metal(loid) concentrations in leaves were below the toxic thresholds for fodder in all treatments. Plant growth was also influenced by intra-specific competition, which provoked a decrease of plant biomass in both with and without amendment treatments.

Therefore, the conclusions of the Thesis were:

1) The identification of the most favourable/suitable niches for plant growth should be taken into account when phytostabilisation works are carried out on mine tailings. Ploughing or the addition of certain amendments that could raise soil salinity should be carefully evaluated in order to not destroy those favourable niches.

2) The use of combinations of plant species with different life forms and complementary water and nutrient acquisition strategies may result in a more efficient employment of edaphic resources. This may favour the achievement of a long-term stable plant community and may provide a higher resilience to environmental disturbances such as long drought periods typical from semiarid environments.

3) The employment of halophyte species (e.g. *Limonium cossonianum*, *Atriplex halimus*, *Zygophyllum fabago*) may result a suitable option to revegetate and ameliorate the most saline edaphic niches at the tailings due to their suitable adaptation to salinity and higher enhancement of soil properties.

4) The tree species *P. halepensis* may result a suitable candidate for the phytostabilisation of mine tailings due to its adaptation to semiarid climate conditions and the potential role for facilitating the occurrence of fertility islands. However, it is recommendable the addition of P fertilizers in order to alleviate the strong deficiency on this element at the tailings.

5) The grass species *P. miliaceum* may be considered in phytostabilisation of mine tailings, but to obtain a faster growth and development, organic amendments should be added.

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CHAPTER 1. Introduction

1.1. Mining impacts worldwide: mine tailings

Mining and processing of metal ores might be important causes of environmental degradation (Dudka and Adriano, 1997): increase area of human land use, landscape damage, multi-elemental contamination (especially metal(loid)s) and generation of acid mine drainage and acid deposition, among others. Specifically, metal mining extraction and further beneficiation processes are characterised by the generation of large quantities of wastes or unusable materials, since the valuable portion of the ore represents a small fraction of the total volume of excavated materials. In these processes, four types of wastes can be distinguished (Salomons and Forstner, 1988): mine waste (overburden, barren rocks), tailings (residual fraction after the concentration process), dump or heap leach (heap of crushed or non-crushed ore material for chemical extraction of metals) and mine water (Figure 1.1). According to Dudka and Adriano (1997), the negative impact of mining on surrounding areas has largely been related to the production of huge amounts of tailings, thus they are considered the major source of pollution from mining activity (Mendez and Maier, 2008a).

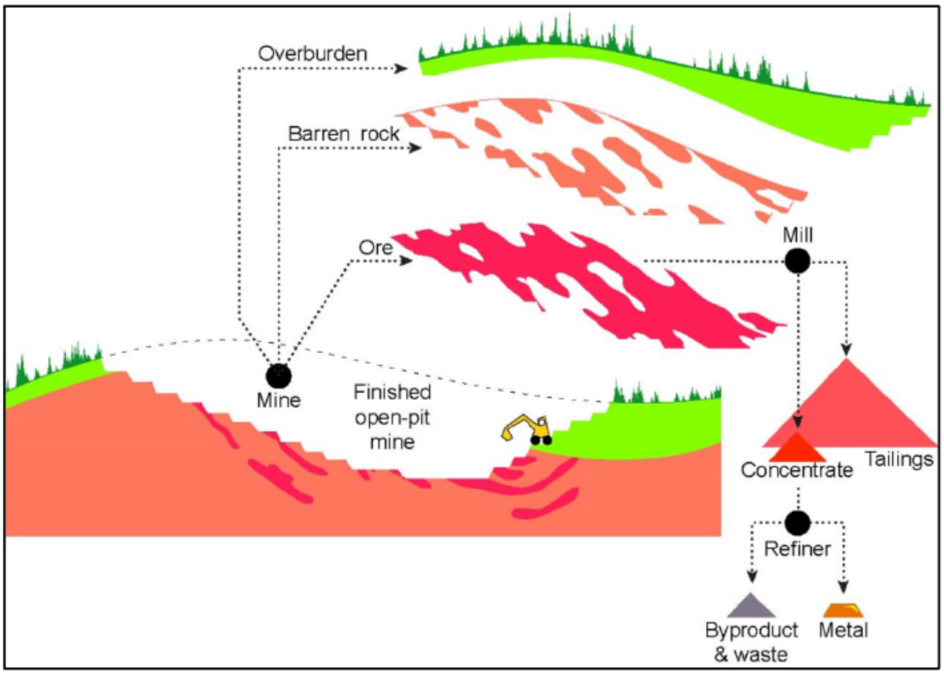

Figure 1.1. Types of wastes generated on an open-pit mine (adapted from www.groundtruthtrekking.org/Graphics/Mining-terms.html)

Mine tailings are the wastes generated in the concentration process of ore particles from the matrix of less valuable rock. This process consists of separating the mineral grains using physical (magnetic or gravimetric separation) or chemical (flotation, cyanidation, amalgamation or heap leaching) methods. The chemical methods are the most environmentally hazardous, because the use of large amounts of organic compounds, cyanide, mercury and acid reagents (Hoskin et al., 2000). The resulting wastes in form of sludge are usually disposed in ponds with the aim of precipitate the solid fraction, recovering the liquid fraction and use it again in the process. When the pond is operating, a "sedimentation lagoon" can be distinguished in the upper part, but when the activity is over, the water evaporates or is drained and a big pile of tailings stays.

Mine tailings involve two types of environmental risks: structural collapse and spread of contamination. Attending to the first one, Europe ranks the second place after the USA in reported tailings pond accidents. In Europe, these accidents are commonly caused by unusual rainfall and in the 90% of the cases have occurred in active mines (Rico et al., 2008). The failures of mine tailings ponds in the last decades have caused several episodes of contamination, with great environmental, social and economic repercussions. The most important spill in our study area, the Cartagena-La Union Mining District (SE Spain), occurred in La Union in 1972 when, after a heavy rainfall, the local tailings pond *Brunita* collapsed, causing an avalanche which killed one person and spread large amounts of mine wastes over the adjacent area (García-García, 2004). In Spain, the mine spill in Aznalcóllar (Andalusia) occurred in 1998 is considered, both from qualitative and quantitative standpoints, the largest environmental pollution accident recorded in Spanish history (Grimalt et al., 1999). In this case, the tailings were spilled into the Agrio and Guadiamar Rivers basins and spread some 40 km downstream. In addition, the polluted water reached the Guadalquivir River, affecting the UNESCO Word Heritage Site and National Park of Doñana (Simón et al., 1999). But the risk of structural collapse is not a matter of the past. Recently, in

August 2014, around 10.6 million m^3 of water and 7.3 million m^3 of tailings from the pond of the Mount Polley copper and gold mine (British Columbia, Canada) spilled over into lakes and creeks of the area, according with the information given in the website of the mine company (Imperial Metals). The huge magnitude of these disasters has frequently promoted the application of urgent measures of wastes withdrawal, restoration programmes and contamination monitoring. That was the case of the Aznalcóllar accident, whose restoration led to the development of a pioneer reclamation programme including ecological and socioeconomic parameters, with the implication of multidisciplinary research and working groups (Garrido, 2008).

Nevertheless, in a non-accident scenario, the main problem would be the spread of metal(loid)s contained in tailings particles via wind or water erosion and, in cases when sulphide oxidation occurs, also by the occurrence of metal(loid) enriched leaching. Mine tailings are characterised by very poor soil quality parameters: high metal(loid) concentrations, high salinity, low organic matter, low nutrients availability, homometric texture and lack of structure, etc. (Conesa et al., 2006). These conditions hamper the establishment of vegetation and the formation of a permanent plant cover and, therefore, the bare surfaces of tailings remain exposed to erosion, even decades after the activity stopped (Rieuwerts et al., 2009; Conesa and Sculin, 2010). Several authors have shown that, in the long-term, wind erosion might become a severe problem in adjacent areas to abandoned mine tailings (Moreno-Brotons et al., 2010), involving an environmental health issue if agricultural lands or cities are close. By other side, water erosion might be even more important since water not only transports suspended particles, causing the siltation of riverbeds, but also dissolved elements, especially in acidic conditions, that might spread contaminants to areas very distant from the tailings pond (Conesa and Schulin, 2010). In the mining impacted soils, microbiology is negatively affected. In addition, vegetation and soil organisms (e.g. earthworms, nematodes, arthropods, etc.) are exposed to toxicity and contaminants might enter into the food chain when superior organisms (birds, cattle, etc.) eat them (Dudka and Adriano, 1997). Moreover, the waterbodies (rivers, lakes, groundwater, etc.) might be also affected by metal(loid) enriched leachates or run-offs, and then, aquatic organisms could be also exposed to toxicity, with the same implications for the food chain as in the case of terrestrial organisms (Pardo et al., 2014b; Figure 1.2).

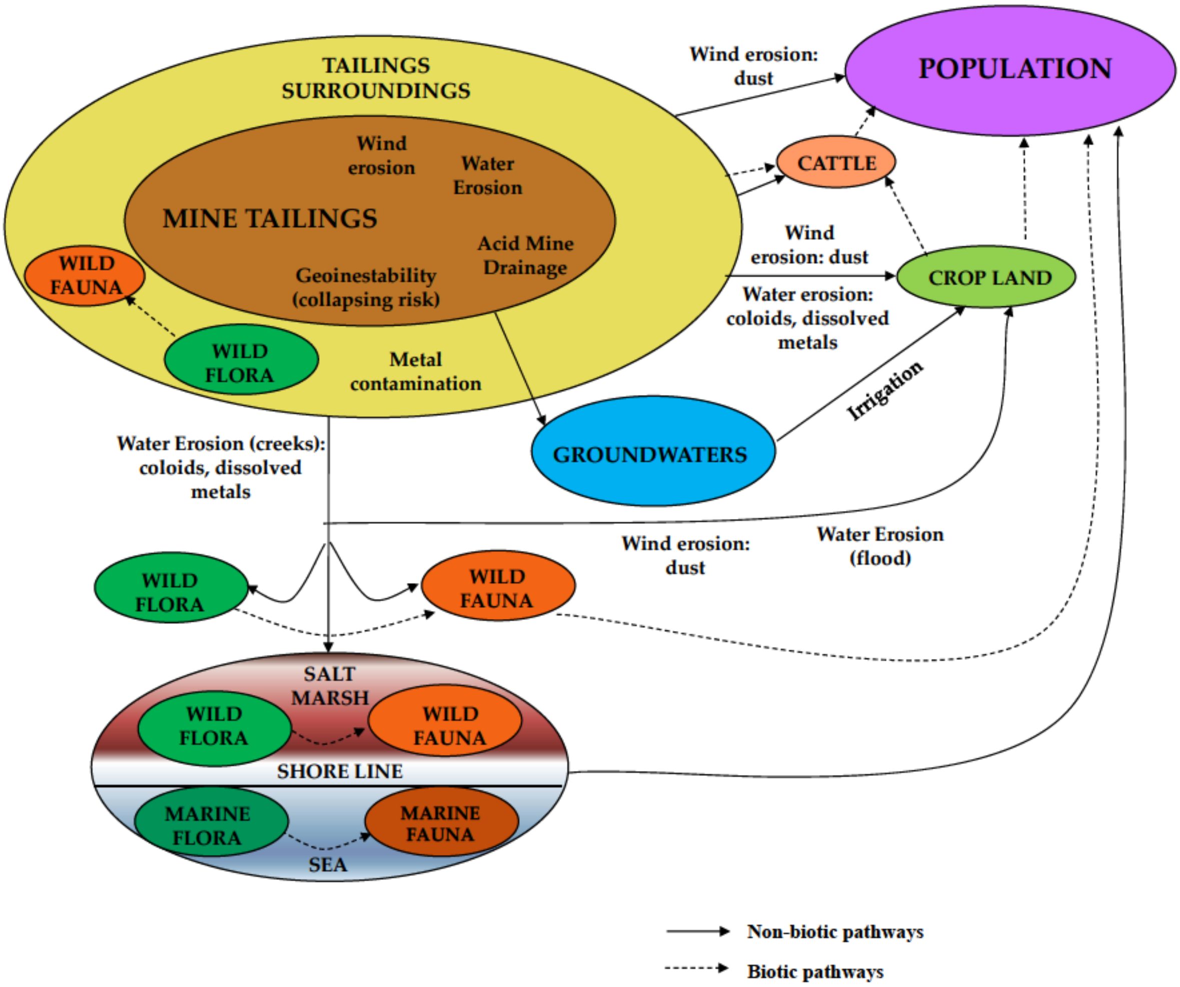

Figure 1.2. Metal exposure pathways from mining wastes disposal sites to biota. Continuous lines are non-biotic pathways. Dashed lines are biotic pathways. Adapted from Conesa and Schulin (2010).

1.2. General overview of restoration techniques for mine tailings

The most effective alternative to eliminate the risks related to mine tailings is their excavation and transport to a dumping site. This option is not usually applied due to the high costs of transporting and handling large volumes of contaminated materials, along with the need to set up a suitable and safe place to dispose them. When the structural stability of the tailings does not pose a risk of collapse, the aim of the restoration would be to minimise the spread of contamination to the nearby areas. In this case, *in situ* solutions are more accepted from a social and environmental point of view (EC, 2002). The different alternatives to achieve that aim involve the stabilisation of the tailings by physical, chemical or biological methods (Tordoff et al., 2000).

Physical stabilisation of mine tailings has been usually addressed from an engineering perspective: waterproofing of mine wastes (plastic barrier, clay capping, etc.), covering with non-polluted materials (gravel, top soil) and finally, establishment of a plant cover. These methods might be effective but are expensive and often temporary in nature because of the impermanence of the capping materials, especially in arid and semiarid environments (Mendez and Maier, 2008b). For this reason, they do not look suitable to be the best option for large areas or steep topography sites with difficult access. Moreover, importing topsoil involves an additional negative environmental impact in the area that the topsoil is mined (Brown et al., 2003). Chemical stabilisation is another method that aims to prevent wind and water erosion using a chemical agent, such as lignin sulfonate or a resinous adhesive, to create a crust in the surface of the tailings. This method could be useful as a temporary stabilisation prior to revegetation, but not for the long-term, because these "artificial crusts" can eventually fail (Tordoff et al., 2000).

In the last two decades, new cost-effective and environmental friendly techniques, involving the application of science and engineering to provide solutions using plants, have received special interest from the scientific community (Conesa et al., 2012). Moreover, in historic mining areas where industrial heritage and/or other cultural or scientific interests are present, these non-invasive techniques could be the best option to preserve the integrity of the sites and, at the same time, to satisfy the environmental regulations (Rieuwerts et al., 2009). These techniques could be grouped in the wide concept of "phytotechnologies" (UNEP, 2003; ITRC, 2009).

Phytotechnologies include several approaches related to remediation of contaminated sites, such as removal of pollutants from soil (phytoextraction) or from aqueous solutions (phytofiltration), transformation (phytovolatilisation) or immobilisation (phytostabilisation), among others (Conesa et al., 2012). In the scientific literature, the term "phytoremediation", initially focused in phytoextraction, is usually found referring to some of these techniques. For instance, phytoremediation of mine tailings is commonly understood as the application of phytoextraction or phytostabilisation techniques to these environments (Wong, 2003; Mendez and Maier, 2008a; Lambrechts et al., 2011).

Phytoremediation was initially applied in experiences in which the capacity of plants to accumulate metal(loid)s or their ability to degrade organic contaminants was the key aspect to achieve the remediation of the soil (Baker et al., 1994). These techniques have demonstrated its effectiveness for some pollutants (e.g. aromatic hydrocarbons; Gan et al., 2009), but also its inefficiency against others. For instance, in soils with very high metal(loid) concentrations, the phytoextraction strategy using hyperaccumulators (plants with high capacity to uptake metal(loid)s from the soil) has a negligible effect. Some studies have pointed that metal phytoextraction from mine tailings with high multi-metal concentrations would take from tens to several hundred of cropping cycles to achieve a non-hazardous level (Dickinson et al., 2009; Robinson et al., 2009). The latter is related to the low biomass production of plants growing in such stressful environments and the preference of hyperaccumulators for one or two metal(loid)s (Mendez and Maier, 2008a). The implementation costs would also be high due to the inputs required for the optimization of the process (irrigation, mobilising reagents) and others works like harvesting, disposal, re-seeding, etc., which would be only justified by the potential value of the land once it is restored. Finally, the use of hyperaccumulators may increase wildlife exposure to metals. For these reasons, the phytostabilisation approach (i.e. the use of plants to immobilise metal(loid)s within the rhizosphere) is receiving increasing attention, as the best option to achieve a successful restoration of mine tailings (Mendez and Maier, 2008b).

Nevertheless, the boundaries between these technologies are not distinct, e.g. the plants employed in the phytostabilisation of mine tailings may accumulate some amounts of metal(loid)s (phytoextraction) (Robinson et al., 2009). This has led scientists to propose the term "phytomanagement" which describes the engineering or manipulation of soil-plant systems to control pollutant fluxes in the environment, including the ecological interactions among plants (Robinson et al., 2009). Then, we employ phytomanagement in terms of phytostabilisation when the main goal is to immobilise pollutants within the rhizosphere but also considering the ecological sustainability of the restored ecosystem. In the following section, we will go in detail about the advantages, limitations and prospects of mine tailings phytomanagement in terms of phytostabilisation.

1.3. Phytomanagement by phytostabilisation of mine tailings in semiarid areas

In the last years, the use of phytomanagement in terms of phytostabilisation has been promoted as the more suitable alternative to stabilise soils affected by mining wastes (Mendez and Maier, 2008b; Dickinson et al., 2009; Robinson et al., 2009). This technique involves the creation of a self-sustainable plant cover for the long-term stabilisation of metals within the rhizosphere. The desirable consequences of creating a plant cover might be: (i) plant canopy reduces wind dispersion; (ii) plant roots protect the soil from water erosion; (iii) the metal(loid)s are fixed in the rhizosphere; (iv) some physical and chemical soil properties are improved (structure, organic matter, nutrient content, microbiology, etc.); (v) the risk of toxicity for wildlife and humans decreases; (vi) landscape aesthetic is ameliorated; (vii) succession from the initial plant community leads to an ecologically adapted and self-sustainable plant cover (Wong, 2003; Mendez and Maier, 2008b; Robinson et al., 2009).

The immobilisation of metal(loid)s within the rhizosphere might be achieved by the occurrence of several processes including precipitation in form of inorganic (e.g. sulphides, carbonates, phosphates) or organic (e.g. oxalates) compounds, complexation with organic matter, adsorption onto root surface and/or accumulation into root tissues (Wong, 2003; Wenzel, 2009). In addition, the plants growing on mine tailings may enhance the soil microbial community that could participate in metal(loid) stabilisation and in turn, favour plant growth. The improvement of microbial diversity is a requirement to achieve the restoration of soil ecosystem functions (Mendez and Maier, 2008b; Carrasco et al., 2006, 2010).

The implementation of phytostabilisation techniques should consider several aspects such as plant selection, irrigation, addition of amendments, fertilization and evaluation of the implementation success (Mendez and Maier, 2008b). The first requirement for the plant species to be used in phytostabilisation is that they must not translocate and/or accumulate risky concentrations of metal(loid)s of concern into above-ground tissues. Some indexes have been used to assess this issue, such as the bioconcentration or accumulation factor (BF or AF = total element concentration in shoot tissue / total element concentration in soil) and the translocation factor or shoot to root ratio (TF or S:R = total element concentration in shoot tissue / total element concentration in root tissue). These indexes should ideally have values $<<1$ (Brooks, 1998; Mendez and Maier, 2008b), meaning that metal(loid)s are not being accumulated into above-ground organs. Additionally, some references and regulations could be also used to evaluate metal(loid) concentrations in shoots or leaves (Table 1.1). For instance, maximum tolerable levels for cattle feed (NRC, 2005) could be useful to determine if there is a risk of metal exposure to fauna.

Table 1.1. Reference values of metal(loid) concentrations in plants (mg kg^{-1} dry weight).

	As	Cd	Cu	Mn	Ni	Pb	Sb
Sufficient or normal concentration in leaves[a]	1-1.7	0.05-0.2	5-30	30-300	0.1-5	5-10	7-50
Normal levels[b]	0.01-1	0.1-1	3-20	-	-	2-5	-
Normal concentration in shoots[c]		0.05-0.7	5-20	-	-	5-10	-
Tolerable in agronomic crops[a]	0.2*	0.05-0.5	5-20	300	1-10	0.5-10	-
Excessive or toxic concentration in leaves[a]	5-20	5-30	20-100	400-1000	10-100	30-300	150
Phytotoxic levels[b]	3-10	5-700	25-40	-	-	-	-
Phytotoxic levels in shoots[c]		5-30	20-100	-	-	30-300	-
Hyperaccumulator plant[d]	1000	100	1000	10,000	1000	1000	1000
Maximum levels tolerated by live stock[b]	50	0.5	25-300	-	-	30	-
Maximum tolerable levels in cattle feed[e]	30	10	40	2000	100	100	-

[a] Kabata-Pendias (2011)
[b] Chaney (1989)
[c] Barceló and Poschenreider (1992)
[d] Verbruggen et al. (2009)
[e] NRC (2005)
* fresh weight basis

Moreover, in arid and semiarid areas, drought- and salt-tolerant native species, that are naturally adapted to the environment and integrated in the landscape, could be the best candidates. In this sense, phytostabilisation should take advantage from the plant species that spontaneously colonise abandoned mine tailings, commonly named as "pioneer vegetation" (Wong, 2003). Pioneer plant species show a great capacity to tolerate the combination of limiting factors which may condition the establishment of vegetation in semiarid mine tailings (low fertility, salinity, arid conditions, toxic metal(loid)s concentrations, etc.). It is also recommended to select a variety of species, families and life forms, in order to increase the functional diversity and the plant productivity and yield (Mendez and Maier, 2008b). For instance, some halophytes, such as the Chenopodiaceae *Atriplex* sp., have shown a great potential to be used in phytostabilisation of mine tailings for its tolerance to saline soils (Clemente et al., 2012). The Leguminosae family could be interesting to increase nitrogen supply due to its symbiotic fixation (Moreno-Jiménez et al., 2012). Attending to the life forms, trees provide a better soil retention capacity, while grasses and shrubs are fast-growing and produce greater soil coverage (Mendez and Maier, 2008b). As it has been pointed before, native plant species from arid and semiarid areas are naturally adapted to drought. Therefore, once established, they should not need additional water supply for its survival. In spite of that, initial watering might be necessary during the first steps of phytostabilisation projects, in order to ensure a correct plant establishment (Tordoff et al., 2002). In addition, scheduling plantations according to the rainy seasons could be other appropriate measure. The cost of irrigation, the availability of water and the accessibility to the tailings could be key factors (Mendez and Maier, 2008b).

The addition of amendments to mine tailings becomes an indispensable requirement in most cases, since the initial conditions are so poor that the establisment of vegetation would take much time. Organic amendments have several beneficial effects on soil and plant growth. In general, metal(loid) availability decreases immediately after the addition. The soil organic matter increases soil cation-exchange capacity and thus provides a continuing release of nutrients. In addition, organic matter improves soil structure, which in turn, reduces erosion and increases infiltration and water holding capacity. There are several kinds of organic amendments that could be used or have been tested in research studies: greenwaste, olive mill, biosolids, composted urban waste, manure, pig slurry, etc. (Ippolito et al., 2005; Svendson et al., 2007; Pardo et al., 2011, 2014a; Kabas et al., 2014; Kohler et al., 2014). However, some of these organic amendments such as biosolids or composts may have some negative effects, such as the long-term risk of metal(loid) leaching (Pond et al., 2005; Clemente et al., 2010). Moreover, the fate of the metal(loid)s accumulated in plant tissues when the biomass return to soil and the long-term evolution of bioavailability of amended tailings are not yet well studied (Mendez and Maier, 2008b).

By other side, although native plant species should be able to tolerate low nutrient contents, the addition of inorganic fertilizers might be necessary in the first stages of the plant establishment (Conesa et al., 2007b). However, the type and quantity of the fertilizer to apply has to be carefully planned, as it could also have some drawbacks. For instance, nitrogen fertilizers could favour the development at the long-term of a less-diverse plant community in which "nitrophilous" species take advantage to the detriment of late successional plant species (Brown et al., 2007; Pedrol et al., 2010). In the case of phosphorus, additional inputs to overcome the deficiency of this element due to the formation of insoluble metal-phosphates in mine tailings could be justified. However, phosphorus fertilizers could have drawbacks related to the mobilisation of other elements such as arsenic and thus, the increasing plant uptake of this element (Bolan et al., 2014). This issue has to be considered when dealing with multi-elemental contamination. Finally, the addition of lime in extremely acidic tailings has been proven to be appropriate for increasing the pH, decreasing metal(loid)s bioavailability and then, facilitating plant establishment (Conesa et al., 2007a; Pardo et al., 2011; Moreno-Jiménez et al., 2012). However, in some cases, the effect of liming is temporary and continuous inputs to maintain a suitable pH could be needed (Mendez and Maier, 2008b).

Although the phytostabilisation is a promising tool for the restoration of mine tailings, long-term field studies are scarce and an evaluation of the success of these trials is difficult to assess. Most of the studies have focused on plant growth (biomass and/or plant cover) and plant metal(loid) accumulation, but other criteria must be considered (Mendez and Maier, 2008b): development of a self-sustainable late successional plant community, recovery of soil functions and microbiological status, decrease of metal(loid)s availability, mitigation of erosion, etc. In most cases, the implementation of phytostabilisation techniques in abandoned mine tailings lacks of an adequate understanding of the ecophysiology of the plant species involved. The common errors are due to (i) the integration of the vegetation in the landscape and the adaptive advantages of native pioneer plant species are not taken into account (Mendez and Maier, 2008a); (ii) the combination of factors which limit plant growth (e.g. soil salinity, nutrient content, water availability or soil microbiology) that, apart from metal(loid) toxicity, may affect the establishment of vegetation have not been well understood (Conesa et al., 2006); and (iii) the ecological issues in relation to the tolerance of the species to limiting soil factors and the interactions among pioneer species (facilitation and/or competition) are not included (Rufo and De la Fuente, 2010).

For these reasons, recent studies recommend a more ecophysiological approach in revegetation/restoration projects of mining areas, considering the dynamics of plant communities and not only the specific capacity of metal(loid) accumulation of each species (Domínguez et al., 2008; Robinson et al., 2009; Rufo and De la Fuente, 2010). The COST Action 859 (network of research groups dedicated to phytotechnologies to promote a sustainable land use and improve food safety – European Cooperation in the field of Scientific and Technical Research) has pointed the need of strengthen fundamental research related to ecological aspects, plant-soil interactions and physiological and metabolical mechanisms of plants in order to make a qualitative leap in the implementation of phytotechnologies in contaminated soils (Mench et al., 2009).

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CHAPTER 2. Study area

2.1. The Cartagena-La Union Mining District

The Cartagena-La Union Mining District has been one of the most important mining areas in Spain during the last centuries. It constitutes an interesting large-scale field site where to study the environmental and social consequences of mining activities and its long-term impacts. In the next sections, this area will be described attending to its location, physical and natural environment and its historical and socio-economical context.

2.1.1. Location, topography, climate, hydrology and geology

The ancient Cartagena – La Union Mining District is located on the coast of the "Región de Murcia" (Southeast of Spain; Figure 2.1). It comprises an approximate area of 50 km^2. The mountain range of Cartagena, called "Sierra Minera", crosses the Mining District from West to East, parallel to the Mediterranean coast (Figure 2.2), and belongs to the eastern part of the Betic Ranges that were generated by the alpine folding. The maximum height in the area is about 400 m.a.s.l. The mining district presents high slopes in the southern part next to the coast, and wide plateaus in the northern part, which extend to the "Mar Menor" lagoon (Conesa et al., 2008a). This plain is called "Campo de Cartagena" and is one of the most important agricultural areas in the Southeast of Spain.

Figure 2.1. Location of the Cartagena-La Union Mining District.

Figure 2.2. The Cartagena-La Union Mining District and its surroundings.

The climate of the area is semiarid Mediterranean, with an annual precipitation of 250-300 mm, mostly concentrated in spring and autumn, and a long dry period during the summer. The annual average temperature is 17.5 °C, with a high annual temperature range of 15.5 °C. The summers are long and warm and the winters are soft and exempt of frosts, due to the proximity of the sea. The high temperatures and the low precipitations lead to a high evapotranspiration rate of around 800 mm year^{-1}. The high relative air humidity of the air is very important for the survival of the vegetation, especially in the summer season, due to the formation of fog and crypto-precipitation. The wind is also a frequent phenomenon in this coastal area (Conesa, 2005; Jiménez-Cárceles, 2006).

The combination of the topography and the climate generates the existence of the so called *ramblas,* intermittent watercourses typical of semiarid areas (Figure 2.3). The water regime of the *ramblas* is related to the seasonal rainfall pattern. Thus, they remain dry for most of the year and only carry water after intensive rainfall events. There are several *ramblas* which drain the rain water of the "Sierra Minera" into the "Mar Menor" lagoon in the North face, or into the Mediterranean Sea in the South face, the latter being shorter and steeper (~3 km length, ~10%

slope) (Conesa and Schulin, 2010). The *ramblas* which flow into the "Mar Menor" lagoon are longer (7-12 km), have steep headwaters (~10%) but relatively long and flat lower reaches (<1%). The final stretch of these *ramblas* consists of coastal wetlands, where the sediments are deposited before water discharges into the lagoon. The "Mar Menor" lagoon is one of the biggest coastal lagoons in the Mediterranean coast, separated from the sea by a sandy barrier called "La Manga", which is an important touristic pole in Spain. The lagoon and its associated coastal wetlands occupy an area of around 15,000 ha. They are protected by several official laws and conventions. The area is a Ramsar site since 1994; it is considered a Special Protected Area of Mediterranean Interest (SPAMI) by the Convention of Barcelona in 2001; and includes five Sites of Community Importance to be integrated in the Nature 2000 Network, according to the European Union Habitats Directive (Conesa and Schulin, 2010).

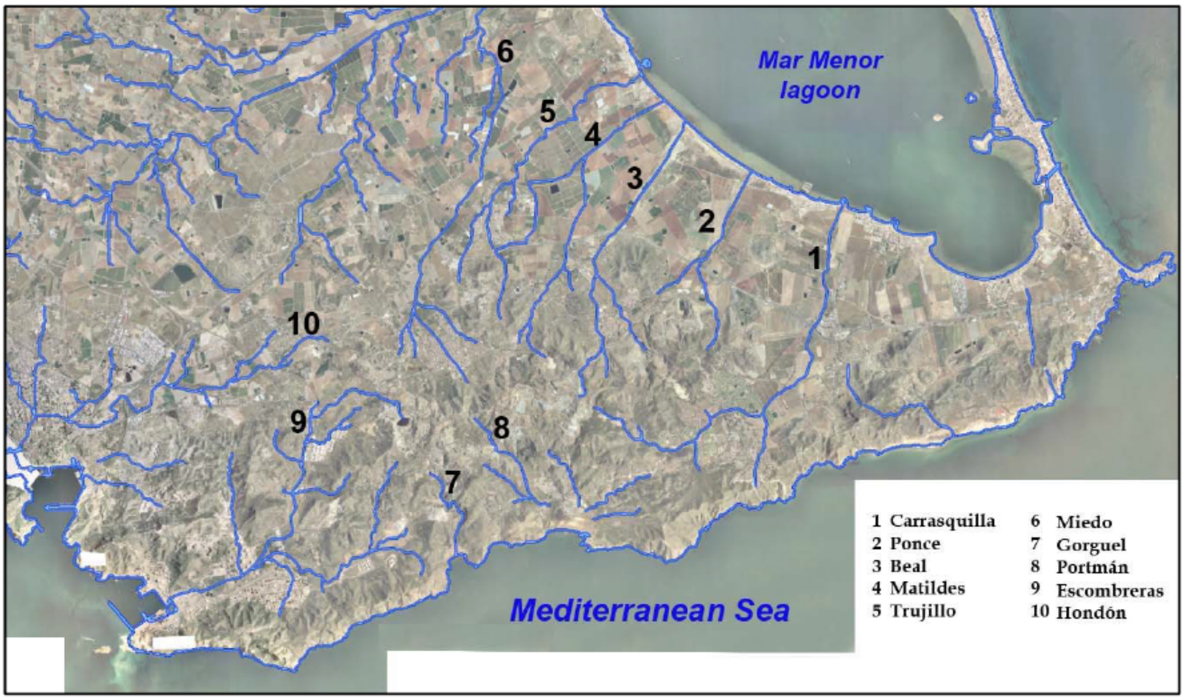

Figure 2.3. Main *ramblas* of the mining district area (CHS, 2015).

The ore deposits of the mining district have been historically exploited for iron, lead and zinc as the main metal components. The principle minerals extracted were: galena, sphalerite, cerussite, cassiterite and iron oxides. The more frequent compounds are: iron, lead, zinc, copper, calcium and magnesium carbonates; iron, zinc, silver, lead, copper, nickel, antimony and arsenic sulphides; iron, lead, tin, aluminium and manganese oxides; lead, zinc and calcium sulphates; iron, aluminium, zinc and calcium silicates; calcium phosphates (Oen et al., 1975; Conesa, 2005).

2.1.2. Vegetation and landscape

The natural vegetation of the area is mainly based on small forests dominated by *Pinus halepensis*, more or less compact according to its orientation, and thicket plant species with xerophitic characteristics (e.g. *Pistacia lentiscus*, *Helichrysum decumbens*, *Lygeum spartum*, *Rosmarinus officinalis*, *Stipa tenacissima*, *Anthyllis cytisoides*). Some of these species are endemic and, therefore, have high botanical interest (Martos-Miralles et al., 2001). For instance, *Tetraclinis articulata* (Figure 2.4) is a tree included in the red list of threatened species (IUCN, 2012) and is considered as a very rare species according to the European Habitat Directive. The Cartagena-La Union Mining District is the only area in the continental Europe where this species could be naturally found. Other locally protected species are *Limonium carthaginense*, *Teucrium carthaginense*, *Rhamnus alaternus*, *Chamaerops humilis* and *Phyllirea angustifolia* (BORM, 2003).

The high ecological diversity and exclusiveness in terms of species and habitats of this environment justify the delimitation of protected areas by several national or regional laws. In the area of the mining district, the most important site is the Natural Park of "Calblanque, Monte de las Cenizas y Peña del Águila", that is also declared Site of Community Importance in the Nature 2000 Network.

Figure 2.4. *Tetraclinis articulata* (left) and *Limonium carthaginense* (right), two of the most interesting plant species of the "Sierra Minera".

The dynamics of the landscape are strongly related to the agricultural, mining and urban development. The lower slopes of the "Sierra Minera" and the "Campo de Cartagena" plain are occupied by an intensive agricultural activity. The coastlines of the "Mar Menor" lagoon and "La Manga", as touristic poles, have suffered a strong urban development. In the "Sierra Minera", an alternation of natural and mining landscapes can be found, with drastic shifts between the two of them. The surroundings of the open pit mines are the most disturbed sites due to the high impact in the natural topography of the area (Figure 2.5.A). Mine tailings ponds generate punctual effects in the landscape, since their visual attributes (colour, shape, size) are very contrasting with the natural landscape and they are usually located on places with high visibility (Figure 2.5.A). The mining architectural heritage also introduces a number of elements in the landscape (buildings, chimneys, lifts, bridges, etc.) which, suitably restored, would be an interesting landscape enticement. Finally, the "Sierra Minera" offers several panoramic viewpoints to the "Campo de Cartagena" plain, the "Mar Menor" lagoon and the Mediterranean steep coast (Figure 2.5.B and C).

Figure 2.5. A) Transformed landscape in the surroundings of the "San Francisco Javier" mine; B) Panoramic view of the Mediterranean coast of the "Sierra Minera"; C) The "Mar Menor" lagoon and "La Manga" from the "Sierra Minera".

2.1.3. History and socio-economic context

Mining activities in the Cartagena-La Union Mining District have been developed during more than 2000 years. The mining history began during the Iberian period and continued with Phoenicians and Carthaginians, reaching a crucial point during the Roman domain (209 b.C.; Figure 2.6). This Roman mining activity (especially focused on iron, lead and silver extraction) was so intensive that, after its decline, the generated wastes were used as a source of minerals during centuries (Conesa et al., 2008a).

Figure 2.6. Lead ingots and mining tools from the Roman period conserved in the Municipal Archaeologic Museum of Cartagena.

After several centuries with a low level of activity in the area, some facts marked a turning point in the Mining District in the middle of the 19th century. By one hand, some official regulations favouring mining activities were approved (SMMPE, 1983). In addition, the development of modern metallurgy and smelting techniques and the synergistic effect of the discovery of the "Jaroso" veil in the adjacent province of Almeria provoked a "boom" of mining undertakings in the Cartagena-La Union are

a. This mining was based on a lot of small underground exploitations (Figures 2.7 and 2.8). Thousands of people came to this zone from different regions of Spain, generating a diverse cultural mix (Egea-Bruno, 2003).

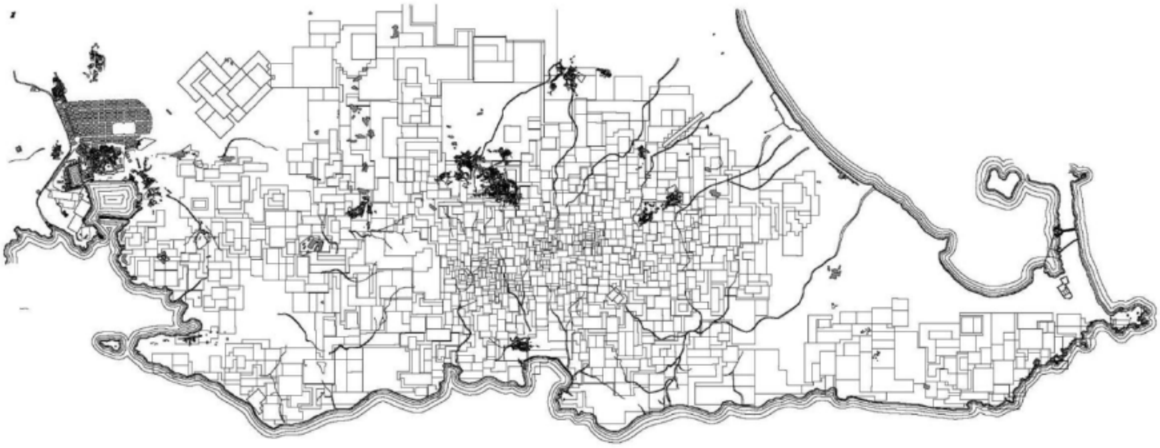

Figure 2.7. Mining concessions in 1907 (Plan of mines and transport ways of the municipalities of Cartagena and La Union by Carlos Lanzarote).

Figure 2.8. Underground exploitations: galleries and lifts.

The mining activity acquired a new dimension in the middle of the 20th century, when the multinational company "Peñarroya España S.A." began a process of concentration of mine properties. New big open cast mines (more efficient for the mining of the low metal concentration ores) were excavated, and new flotation techniques in refineries (that allowed the processing of more quantities of material) were applied (Figure 2.9). It has been estimated that the amount of rocks excavated in this period was similar to that one mined during the more than two thousands preceding years (Manteca and Ovejero, 1992; Manteca and Berrocal, 1997). The total metal production increased until the early 1980s when the exhaustion of the ore reserves, the low metal prices in the international market, the progressive elimination of the public protectionism to the mining sector, and the growing awareness of the environmental problems resulted in a deep crisis of the local mining sector (Egea-Bruno, 2003). The definitive closure of the mines came in November 1991 when the company "Portman Golf S.A." (that bought the mining exploitations from Peñarroya España S.A. in 1988) decided to stop the last open cast mine (Conesa et al., 2008b).

Figure 2.9. The open-pit mine "Emilia" (cronicasmineras.blogspot.com)

Nowadays, the Cartagena-La Union Mining District includes five small towns with a total population of 20,000 inhabitants, being La Union the principal one. The surroundings of the mining area have a population of more than 200,000 inhabitants, including the city of Cartagena and the rest of its municipality. During summer, the population dramatically increases because of tourists who come to the "Mar Menor" lagoon and the Mediterranean Sea touristic areas (Conesa and Jiménez-Cárceles, 2007; Conesa et al., 2008b).

Because mining has been the predominant economic activity during hundreds of years, the socio-economic situation in this area, and especially in the municipality of La Union, has been tied to the ups and downs of the mining activity throughout the years. The ending of the mining activity brought a socio-economic crisis to this municipality, manifested in one of the highest unemployment ratio in the region (sustained in more than 20%) and resulting in the loss of population by emigration. In the first decade of the 21st century, the local economy began to be stimulated by the nearby touristic area of "La Manga". However, the recent economic crisis occurred in Europe in the last years has provoked that the unemployment rate of La Union reaches the 49% (www.mapadelparo.com).

According to Conesa et al. (2008a), the future of La Union and its surroundings implies the restoration of the mining legacies and the generation of new economic opportunities based on tourism. The potential of the Cartagena-La Union Mining District relies in the historic and cultural values of its mining heritage combined with the nearby touristic area of "La Manga". However, the achievement of a low environmental risk level for the population and tourists should be previously solved. Although some cultural-touristic initiatives have been developed recently, such as the complex "Parque Minero" and the interpretation centre "Mina de las Matildes", an integral plan for the restoration of the whole mining district would be desirable.

2.2. Impacts derived from mining in the study area

Nowadays, mining activities in most of developed countries are carried out under environmental laws which regulate the afterward restoration of affected lands. However, it is common that ancient mining areas, where the activity ceased before the establishment of such regulations, remain abandoned, becoming a source of environmental and health risks (Rieuwerts et al., 2009; Conesa and Schulin, 2010). The latter is the case of the Cartagena-La Union Mining District, where the consequences of mining in the environment are still present. Several studies have pointed that the impacts of mining in this area not only affect directly the surface occupied by the mining facilities (100 km²), but it is extended to a wider area (about 1000 km²), including agricultural lands, shores and ecosystems of great ecological value such as the "Mar Menor" lagoon and some of its coastal wetlands (Figure 2.10; García-García, 2004; Robles-Arenas et al., 2006; Conesa and Jiménez-Cárceles, 2007; Conesa and Schulin, 2010).

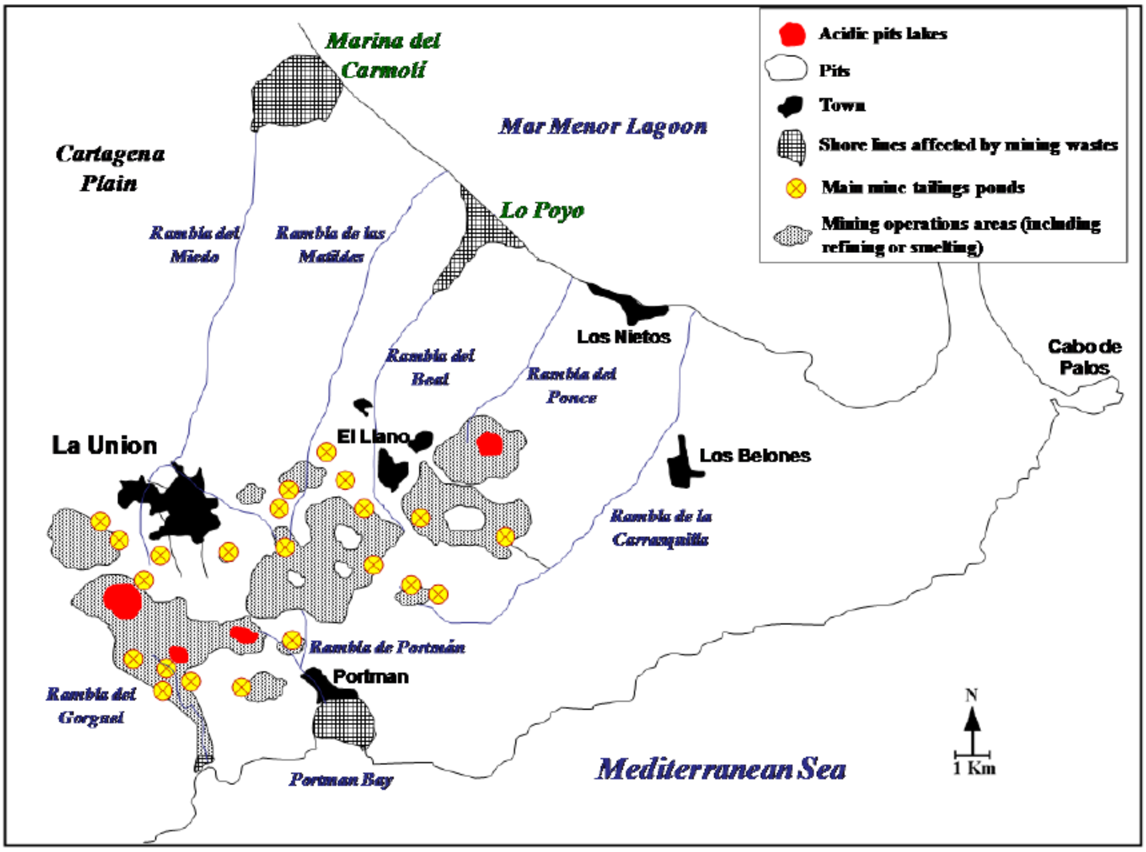

Figure 2.10. Mining impacts in the study area (adapted from Conesa and Schulin, 2010).

The first impacts on the environment began with the first mining activities in Roman times, when the need of wood for supporting structures of the galleries provoked a great deforestation (Conesa, 2005). However, the more important impacts are related with the advances of the mining and refining techniques that took place in the 19th century, but intensively in the 20th century, with the excavation of huge amounts of materials and the use of new flotation techniques.

The excavation of open cast mines led to the formation of big pits with denuded rock walls which usually provide a very hostile environment for the growth of plants, making difficult their restoration. Nowadays, there are nine open pits in the mining district which, besides the disposal of mine spoils resulting from the excavation and the wastes from the ore refining processes, substantially modify the topography and the hydrology of the mining area (Conesa, 2005; Conesa and Schulin, 2010). In this way, underground galleries, waste deposits or pits have disturbed the previous water piezometric levels. Robles-Arenas et al. (2006) stated that the Sierra de Cartagena-La Union aquifer recharge, which normally occurs through rainwater infiltration, is conditioned by the existing net of wells and mining galleries. In some cases, the excavation of pits reached the groundwater level (Figure 2.11). Then, the oxidation of rock surfaces decreases the water pH to values around 2-3, generating high dissolved metal concentrations (e.g. 159-581 mg L^{-1} Zn; Robles-Arenas et al., 2006). Conesa and Schulin (2010) pointed out that, according to the standards for drinking water recommended by the European Union (1998) and the World Health Organization (1993), the groundwater of the aquifer is not adequate for human consumption. The tap water in the villages and towns in the proximities is provided from distant sources located inland and, therefore, not affected by the local mining. Nevertheless, the uncontrolled and unmonitored use of local groundwater (e.g. for the irrigation of private gardens) may generate an undesirable transfer of metals to the food chain.

Figure 2.11. The open-pit mine "Brunita" and the acid lake formed by the excavation below groundwater level.

García-García (2004) classified the surface affected by different types of mining wastes in the Cartagena-La Union Mining District, according to their origin and characteristics (Table 2.1). Mine spoils (untreated materials from open pits) and mine tailings represented more than 70% of the total affected area and almost 80% of the total volume of wastes.

Table 2.1. Number of structures, affected area and volume of different types of wastes deposits, according to García-García (2004).

Type of wastes	Number of structures	Area (km^2)	Volume (Mm^3)
Mine spoils	32	4.21	136
Mine tailings	89	2.18	23
Mine wastes in sea	3	0.83	25
Gravimetric spoils	119	0.65	3.73
Underground spoils	176	0.48	3.01
Gossans	11	0.26	6.93
High size wastes	1	0.06	0.59
Smelting wastes	19	0.13	0.66
Excavations materials from wı	1902	0.02	0.51
Total:	2352	8.82	199

As it has been pointed out in Chapter 1, tailings could be considered the wastes of major environmental concern. The mine tailings in the Cartagena-La Union Mining District could be roughly classified in two types according to their geochemical characteristics (Conesa et al., 2008b). There are acid tailings, with pH around 3, high solubility of metals and characterised by the presence of oxidised minerals (e.g. magnetite) and reduced minerals (e.g. sphalerite). And there are neutral pH tailings, with low metal solubility and composed by non-oxidize minerals such as quartz and gypsum. The oxidation of minerals in the tailings is the main source of acidity in the mine drainage. The latter is a risk associated to acid tailings, but not to all the neutral tailings. In addition, due to the diverse origins of the materials that were disposed on the tailings ponds over the time, a high heterogeneity of geochemical characteristics could be found either in surface or in depth (Aracil et al., 2005). The metal(loid) concentrations in mine tailings in the area have been widely studied by several authors (García-García, 2004; Conesa, 2005; Robles-Arenas et al., 2006; Conesa et al., 2006, 2007, 2008b) (Table 2.2). For instance, Conesa et al. (2008b) found concentrations around 350-1900 mg kg^{-1} As, 10-34 mg kg^{-1} Cd, 80-380 mg kg^{-1} Cu, 5000-7000 mg kg^{-1} Pb and 5500-9100 mg kg^{-1} Zn, that are several orders of magnitude higher than the geochemical backgrounds of the nearby Cartagena plain (Table 2.2).

Traditionally, the tailings were dumped directly into the *ramblas* of the mining district. The accumulation of wastes in the riverbeds, that reached more than 3 m thick in some cases (Vilar and Egea-Bruno, 1990), led to the establishment of legal restrictions in 1955 prohibiting the discharge of wastes in the watercourses. The mining companies were then forced to build up tailings ponds to dispose the wastes (Figure 2.12).

Table 2.2. Metal(loid) concentrations reported in different sites in the area of the Cartagena-La Union Mining District and comparison with reference levels. All data are in mg kg^{-1}. "n.a.": not available.

Site		Reference	As	Cd	Cu	Pb	Zn
Mine tailings	"Belleza" tailings pond	Conesa (2005)	1900	8.8	380	7000	5400
	"El Gorguel" tailings pond	Conesa (2005)	350	34	84	5200	9100
	"El Lirio" tailings pond	Conesa et al. (2007)	n.a.	n.a.	150	4000	12,000
Sediments of riverbeds	"El Gorguel"	García et al. (2003)	n.a.	n.a.	200-1000	2000-10,000	10,000
	"El Gorguel"	Pavetti et al. (2006)	n.a.	n.a.	220-1700	6900-18,100	9000-14,400
	"La Carrasquilla"	García-García (2004)	n.a.	24	39	3700	3500
	"Ponce"	García-García (2004)	n.a.	25	30	1960	1990
	"Miedo"	García-García (2004)	n.a.	16	45	2250	2850
	"El Beal"	García-García (2004)	n.a.	15	35	950	650
	"El Beal"	Robles-Arenas et al. (2006)	n.a.	n.a.	n.a.	100,000	10,000
Salt marshes of the Mar Menor	"Lo Poyo"	Álvarez-Rogel et al. (2004)	n.a.	n.a.	110	8000	6900
	"Lo Poyo"	María-Cervantes et al. (2009)	500	9.1	75	7000	7100
	"Lo Poyo"	Conesa et al. (2011)					
	"Marina del Carmolí"	Jiménez-Cárceles et al. (2008)	700	80	400	16,800	62,000
Sediments in the Mar Menor		Sanchiz et al. (2000)	n.a.	0.3-0.6	n.a.	30-90	130
		Marín-Guirao et al. (2005b)	n.a.	7	n.a.	10000	7000
		Robles-Arenas et al. (2006)	n.a.	10,000	200	10,000	10,000
Mediterranean coast	Portmán Bay	García et al. (2003)	n.a.	n.a.	150	8000	20,000
	"El Gorguel" beach	Pavetti et al. (2006)	n.a.	n.a.	1050	2260	20,400
Agricultural areas	Crop land near mine tailings	Pérez-Chacón (2002)	n.a.	n.a.	8-25	156-1060	179-1391
	Crop land near mine tailings	Conesa et al. (2010)	n.a.	n.a.	21	200-500	200-900
Reference levels	Agricultural soil near mining area not affected by mining wastes	Conesa et al. (2003)	n.a.	n.a.	77-160	28-150	93-400
	Maximum metals levels allowed in soils after Aznalcollar accident	BOJA (1999)	100	10	500	500	1200
	Geochemical backgrounds in the nearby Cartagena plain	Martínez-Sánchez and Pérez-Sirvent (2007)	7.0	0.32	12.6	9.3	41.4
	Environmental thresholds in the nearby Cartagena plain	Martínez-Sánchez and Pérez-Sirvent (2007)	16	0.5	30	57	90

Figure 2.12. Left: abandoned tailings pond "Belleza". Right: remaining wood poles used to increase the height at "El Lirio" tailings pond.

To date, only few mine tailings have undergone restoration works. Most of them remain abandoned and, due to their poor conditions for plant establishment, their bare surfaces are exposed to erosion. This fact, together with their location in the headwater or the proximities of the *ramblas*, and the torrential character of the rainfalls in the area, generates a high incidence of water erosion that may transport the wastes through these *ramblas* to areas very distant from the source. Several authors (e.g. Robles-Arenas et al., 2006; Pavetti et al., 2006) have detected high concentrations of metal(loid)s in the sediments deposited in the local riverbeds and in their flood plains (Table 2.2). Shores and beaches of the "Mar Menor" lagoon located in the mouth of the *ramblas* have been receiving large amounts of wastes from the "Sierra Minera" during decades. Some coastal wetlands are strongly affected by mining wastes, reaching concentrations of more than 62,000 mg kg^{-1} Zn, 700 mg kg^{-1} As and 16,800 mg kg^{-1} Pb, as is the case of the salt marshes of "Marina del Carmolí" and "Lo Poyo" (Álvarez-Rogel et al., 2004; Jiménez-Cárceles et al., 2008; María-Cervantes et al., 2009; Conesa et al., 2011). In the latter, the occasional overflows of the *Rambla del Beal* have spread the wastes to croplands and shores on extended areas of the flood plain (Figure 2.13).

Figure 2.13. Aerial view of "Lo Poyo" salt marsh affected by mine wastes (Google Earth).

The consequences of mine wastes discharge into the "Mar Menor" lagoon have been also assessed (Sanchiz et al., 2000; Marin-Guirao et al., 2005a,b; Robles-Arenas et al., 2006). The dissolved metal(loid)s that come with the runoff precipitate rapidly in contact with the sea water of the lagoon due to the higher pH (Conesa and Jiménez-Cárceles, 2007). However, high concentrations of metals (up to 7 mg kg^{-1} Cd, 10,000 mg kg^{-1} Pb and 7000 mg kg^{-1} Zn) have been found in the sediments of the lagoon (Marin-Guirao et al., 2005b). The impact on the living organisms of the "Mar Menor" lagoon, such as macrophytes, bivalves and others, has also been studied. Sanchiz et al. (2000) and Marín-Guirao et al. (2005a,b) found high concentrations of Pb and Zn (up to 850 and 500 mg kg^{-1} respectively) in tissues of *Cymodocea nodosa*, a phanerogame that colonises the sediments of the lagoon. Auernheimer et al. (1984, 1996) found high metal concentrations in bivalve shells from the lagoon (up to 300 mg kg^{-1} Zn and 218 mg kg^{-1} Pb). María-Cervantes et al. (2009) found a positive correlation between the metal(loid) concentrations in sediments and in soft tissues of the mollusk *Hexaplex trunculus*, suggesting that this species could be used as bio-indicator of pollution by As, Mn, Pb, Cd, and Zn in the submerged sediments of the lagoon. Moreover, the concentrations of As (up to 420 mg kg^{-1} dry weight), Pb (up to 220 mg kg^{-1} dry weight) and Zn (up to 3130 mg kg^{-1} dry weight) in *H. trunculus* were much higher than the international thresholds for molluscs/shellfish compiled by FAO, leading to a risk for human health, since this species is usually collected and consumed by the population (María-Cervantes et al., 2009).

In the Mediterranean coast, the accumulation of mine wastes is also present in some beaches such as "El Gorguel". However, the most striking case is the silting of the "Portmán" bay (Figure 2.14), considered the most contaminated bay in the entire Mediterranean coast (Martínez-Frías, 1997). From 1957 to 1990, more than 57 million tons of wastes were dumped into the sea from the "Lavadero Roberto", that was the largest metal refinery in Europe when it was established in 1957 in the small fishing village of "Portmán". The filling of the bay, which originally was more than 10 m depth at its centre, the displacement of the shore line around 700 m towards the sea, and the affection of around 10 km^2 of the continental shelf are some of the environmental consequences (Martos-Miralles et al., 2001).

Figure 2.14. The "Portmán" bay filled with mine wastes.

The agricultural lands in the proximity of the mining district receive mine wastes in several ways: directly by wind or water erosion of the surrounding tailings and by overflow of the *ramblas* after occasional floodings. High metal concentrations in agricultural soils adjacent to mine tailings, in comparison to nearby non-polluted soils have been reported (Pérez-Chacón, 2002). Conesa et al. (2010) studied the metal transfer from a tailings pond into a nearby agricultural area used for lettuce crop. Despite the high metal concentrations found in the soil (e.g. 510 mg kg^{-1} Pb and 910 mg kg^{-1} Zn), metal uptake by the crop was low due to the high soil pH. However, according to these authors, a continued monitoring of the crops metal uptake is still needed in order to prevent risks in food safety.

The structural stability of mine tailings may be seriously affected by water erosion due to the presence of layers with different permeability, increasing the risk of collapse. As it has been related previously, in 1972 and after an intense rainfall, the local tailings pond called "Brunita" collapsed, causing an avalanche which killed one person and spread large amounts of mine wastes over the adjacent municipal area (García-García, 2004; Conesa, 2005; Figure 2.15).

Figure 2.15. Images of the accident of the tailings pond "Brunita" in 1972 (www.facebook.com/launionantigua).

Finally, the spread of dust by wind erosion could be considered an important risk at the long-term. García-García (2004) found dust layers of more than 50 cm on soils situated in the proximity of some tailings. Moreno-Brotons et al. (2010) showed that wind can transport high amount of materials from the bare surfaces of the tailings. Moreover, the same authors found that the finer particles, those which are easily transported by wind, had up to nine-fold higher metal concentrations than the bulk soil. This could be an important concern when cities or recreational areas are close to the tailings, as it happens in the Cartagena-La Union Mining District. Chronic exposure to toxic dust blown from tailings may result in a health risk for the populations living in the vicinity (Conesa and Schulin, 2010).

2.3. The specific mine tailings area of study

An extensive mine tailings disposal area located within the limits of the Natural Park of “Calblanque, Monte de las Cenizas y Peña del Águila” was selected for conducting the study, due to the long-term existence of mining wastes together with the occurrence of spontaneous plant colonisation. The surrounding area has a high botanical, ecological and landscape value, and the presence of mine tailings causes a great impact on it.

Four abandoned tailings piles (Agustin, Ripolles, Wikon and Mercader; Figure 2.16) included in the National Inventory of Sludge Deposits from Treatment Processes of Extractive Industries (IGME, 2002) were selected. According to this inventory, these tailings mainly come from the benefit process of galena ores. The tailings piles show a high degree of surface erosion, presence of gullies and collapsed walls (Figure 2.17). The estimated volume of wastes is 940,000 m^3, and there is a high input of fine materials into the nearby *ramblas* that flow into the “Mar Menor” lagoon. High impacts on landscape and surface water and streams (Figure 2.17) are pointed out in the cited inventory, so an integral study on the environmental restoration of the whole area is recommended.

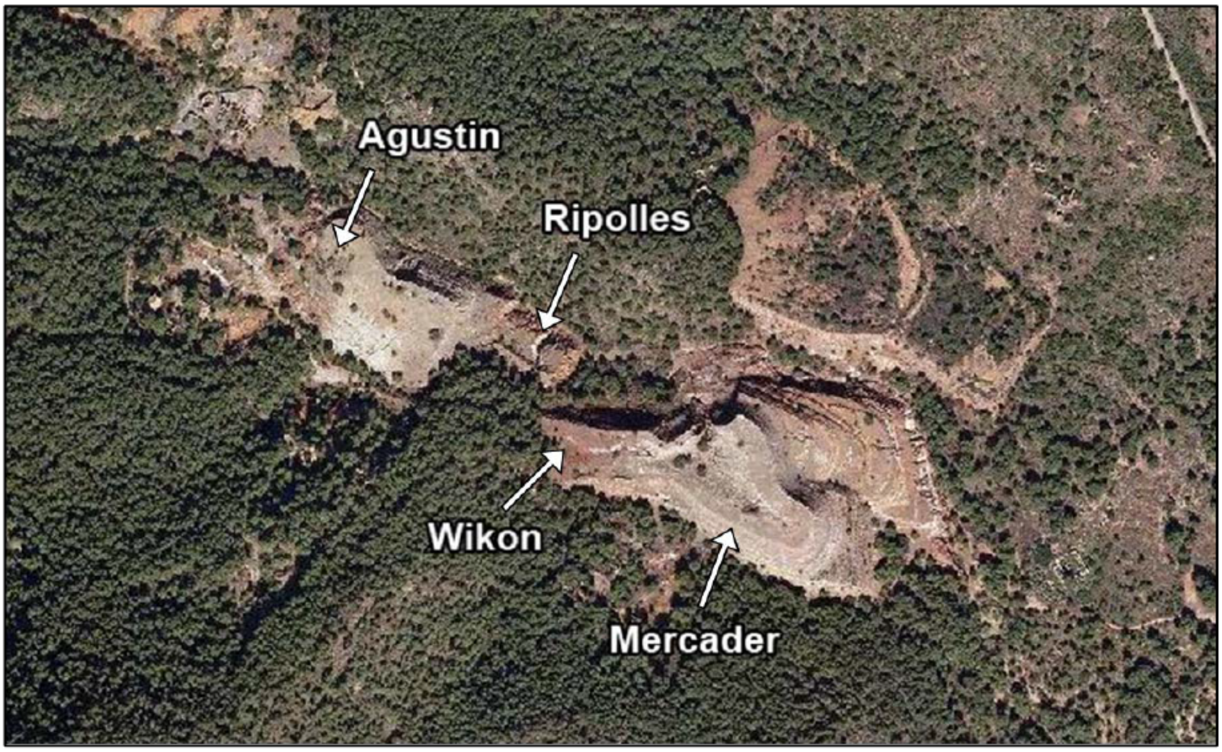

Figure 2.16. Mine tailings disposal area of study (orthophoto from IDERM, 2015).

To the best of our knowledge, there are no previous scientific studies in this specific area. Since the mining activity finished around 30-40 years ago, a natural succession process is taking place. The latter offers a great opportunity to get a better understanding of the spontaneous colonisation by pioneer plant species on this kind of mine wastes and to obtain valuable information about limiting soil factors, ecological relationships, ecophysiological adaptations of plant species, etc. Finally, the conclusions derived from the research could be applied to further restoration projects in this and others semiarid areas.

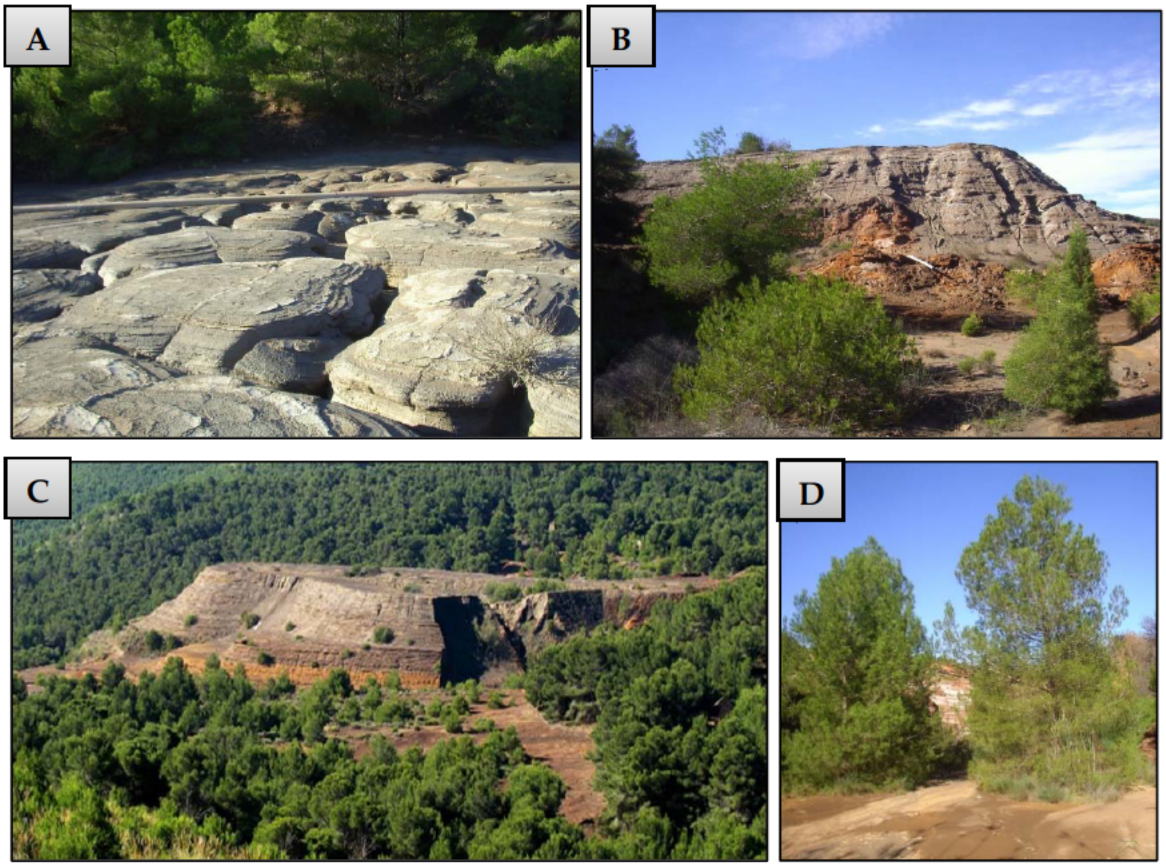

Figure 2.17. A) Surface erosion and cracks on "Agustin" tailings pile. B) Slopes erosion at "Agustin" tailings pile. C) Landscape impact of "Mercader" tailings pile. D) Mine wastes from tailings covering a close riverbed.

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CHAPTER 3. Background and objectives

3.1. Background and objectives

Previous studies carried out during the last decade in the Cartagena-La Union Mining District have been focused on different issues related to the implementation of phytostabilisation techniques (e.g. plant species selection, use of amendments; Table 3.1). However, some aspects of critical importance for the specific case of mine tailings under semiarid conditions (previously noted in Chapter 1) have not been thoroughly studied. In this section, a justification of the interest of the present PhD Thesis and the proposed objectives are explained.

Table 3.1. Some research studies on phytostabilisation of mine tailings in the study area.

Object of study	References
Field surveys on soil and plant metal(loid) content	
	Conesa et al., 2006; 2007a
	Pérez-Sirvent et al., 2012
	Martínez-López et al., 2014
Field surveys on plant communities in mine tailings	
	Conesa et al., 2007b
Effects of amendments on mine tailings properties	
Field experiments	
	Zanuzzi et al., 2009
	Zornoza et al., 2012
	Zornoza et al., 2013
Pot experiments	
	Clemente and Bernal, 2006
	Pardo et al., 2011
Effects of amendments on tailings and plant metal uptake	
Field experiments	
	Clemente et al., 2012
	Kabas et al., 2012
	Pardo et al., 2014a
Pot experiments	
	Conesa et al., 2007c; 2007d; 2009
	De la Fuente et al., 2011
	González-Alcaraz et al., 2011
	Pardo et al., 2014b
	Kohler et al., 2014
Role of soil microorganisms	
	Carrasco et al., 2009
	Carrasco et al., 2010
	Alguacil et al., 2011

It is widely accepted that high metal(loid)s concentrations and/or exteme pH are important factors that hamper the establishment of vegetation in mine tailings (e.g. Conesa et al., 2006; 2007a). In particular, under semiarid climate conditions, other edaphic parameters, such as salinity, drought, low fertility or soil microbiology may also play a critical role (Conesa et al., 2007b; Carrasco et al., 2010). Even if the individual importance of these parameters in relation to phytostabilisation in semiarid areas has been recently recognised (Mendez and Maier, 2008), most of the studies obviate how the combination of these factors are related with the establishment of vegetation (Conesa et al., 2011; 2014). This is of special importance in mine tailings, because their inherent heterogeneity (e.g. mineralogy, texture) may condition the occurrence of edaphic gradients which, in turn, may lead to the existence of more or less favourable “edaphic niches” for the establishment and growth of vegetation. The identification of the edaphic gradients and plant favourable/unfavourable niches at mine tailings could be useful in terms of phytostabilisation because: a) it would optimise the employment of amendments; b) it would question the indiscriminate ploughing of the tailings surfaces; and c) it would provide more suitable sites for the successful growth of vegetation. Moreover, the identification of the spontaneous plant species associated to those edaphic niches may provide useful information of the ecological successional processes which occurred at the tailings. To date, these issues have not been included in any study in the area.

By other side, previous studies have proposed several plant species that spontaneously colonise mine tailings in the study area as suitable candidates for phytostabilisation (Conesa et al., 2006; 2007a; Pérez-Sirvent et al., 2012; Martínez-López et al., 2014). As metal(loid) uptake in plants is an important selection criteria in order to avoid risk of transfer to the food chain, those studies have been performed in order to identify those plant species that show low metal(loid) accumulation into above-ground tissues. With a few exceptions, relatively low metal(loid) concentrations in above-ground tissues have been reported for plant species growing in local tailings dumps (e.g. <10 mg kg^{-1} Cu, <200 mg kg^{-1} Pb, <500 mg kg^{-1} Zn; Conesa and Schulin, 2010). However, the plant species selection should not be only based on the previous issue, but also on the understanding about how plants deal with the combined plant growth stress factors (including drought) of these environments, which are of critical importance for the long-term achievement of a self-sustaining plant cover. These issues cannot be assessed only employing plant uptake criteria and some ecophysiological analytics (e.g. stable isotopes) must be considered. These studies should also include an evaluation of the vegetation in terms of contribution to ameliorate tailings soil conditions (e.g. rhizosphere-induced changes, litter decomposition). For this purpose, it is useful to compare among different plant life or functional forms such as grasses, shrubs and/or trees.

Although there is an extensive scientific literature on phytostabilisation of tailings employing grasses or shrubs species, there is a lack of studies about trees. Tree species are of special interest for phytostabilisation since they may provide better soil retention (e.g. more developed root system, canopy effect) and more efficient mechanisms for dealing with high metal(loid) concentrations through their accumulation in woody stems, which allows the long-term immobilisation of these elements in less biologically-active tissues (Pulford and Watson, 2003). To date, most of the plant species proposed for phytostabilisation of tailings at the Cartagena-La Union area have been grasses and shrubs (e.g. *Lygeum spartum*, *Piptatherum miliaceum*, *Zygophyllum fabago*, *Limonium* sp, *Helichrysum decumbens*, *Dittrichia viscosa*, *Atriplex halimus*), (Conesa et al., 2006; 2007a; Pérez-Sirvent et al., 2012; Martínez-López et al., 2014) and there has not been any attempt to include some tree species in that list. For this reason, it is interesting to provide information about the spontaneous tree species which grow at local tailings and to study their behaviour in dealing with the combined growth stress factors.

Finally, the amelioration of the poor edaphic conditions of mine tailings by the addition of different types of amendments is a widely studied topic in the studied area (Table 3.1). The effects of different inorganic fertilizers, organic amendments and/or lime materials have been tested in several experimental experiences either in pots, mesocosms or field plots. In all these studies, the effects on soil fertility and metal(loid) availability have been thoroughly assessed. Moreover, the effects of the amendments on plants have been also evaluated through plant growth related parameters (e.g. biomass) and/or plant metal(loid) uptake. However, there is a lack of understanding of the effects of the amendments in the ecophysiology of plants and their ecological interactions (e.g. intra-specific and inter-specific competition or facilitation). For these reasons, it is of special importance for the critical assessment of amendments in mine tailings the evaluation of ecophysiological analytics (such as stable isotopes) and the ecological interactions among plants species (for this PhD Thesis, intra-specific interaction was considered).

The general purpose of this PhD Thesis was to increase the knowledge on the edaphic and ecophysiological factors involved in the spontaneous plant colonisation of mine tailings, with the aim of improving the feasibility of phytomanagement in terms of phytostabilisation applied to these environments in a semiarid climate context. The specific objectives were:

1. To describe the edaphic gradients and their associated spontaneous vegetation and to identify potentially plant-favourable edaphic niches in abandoned mine tailings located in the Cartagena-La Union Mining District.
2. To assess the suitability of using spontaneous plant species in restoration/stabilisation of mine tailings, focusing on the role of rhizosphere in the improvement of soil conditions, the study of different life forms and the ecophysiological responses to mine tailings environment.
3. To assess the behaviour of a tree species (*Pinus halepensis*) on mine tailings to estimate its possibilities as candidates for phytostabilisation and their potential role in the restoration of such environments.

4. To evaluate the response of a spontaneous grass plant species commonly found on mine wastes (*Piptatherum miliaceum*) growing under controlled conditions in an organic amended mine tailings by employing stable isotopes and considering intra-specific interactions.

In order to achieve these objectives, a field work stage and an experimental stage were planned. Chapters 4 to 9 correspond to six published research papers including the results of the field work stage, while Chapter 10 gathers an article "under review" about an experimental assay in pots. Figure 3.1 shows a schematic explanation about the relation between the objectives and the next chapters.

The first field survey was focused on the description of the edaphic and ecological gradients along a transect from a non-polluted control site to the mine tailings (Chapter 4). A complete study on tailings piles was performed, including the recording of the plant species inventory along the transect.

Afterwards, the usefulness of spontaneous vegetation for the phytomanagement of mine tailings was assessed through a field study focused on the characterisation of the rhizospheres and the ecophysiology of some spontaneous plant species (including grasses, shrubs and trees; Chapter 5). The use of ecophysiological tools to assess the response of plants, such as the determination of stable isotope composition, was introduced in this study.

Based on the results obtained in the study described in Chapter 5, specific research on grasses and shrubs by one side, and on trees by other side, were carried out, focusing on their aptitude as candidates for phytostabilisation (Chapters 6 to 9). As some edaphic gradients at the tailings shown in Chapter 1 were related to salinity, the objective of Chapter 6 was to evaluate the different performance of spontaneous halophyte and non-halophyte species and to assess if halophytes could be more suitable for specific salty sites on the tailings. In the case of trees, and considering the relevance of the species *Pinus halepensis* for the restoration of degraded lands in semiarid areas, three specific studies were performed to gain insight into the ability of pine trees to cope with the soil conditions at the tailings (Chapters 7 to 9). The first study was focused on the accumulation of metal(loid)s in spontaneous *P. halepensis* along a polluted gradient (Chapter 7). The second one was performed in relation to the ecophysiological status and elemental composition of spontaneous populations of *P. halepensis* growing on mine tailings (Chapter 8). The third study was focused on the internal nutrients and metal(loid)s retranslocation (Chapter 9).

Finally, to achieve the fourth specific objective, a pot experiment was performed using *Piptatherum miliaceum* and a mine tailings soil amended with a municipal solid waste (Chapter 10).

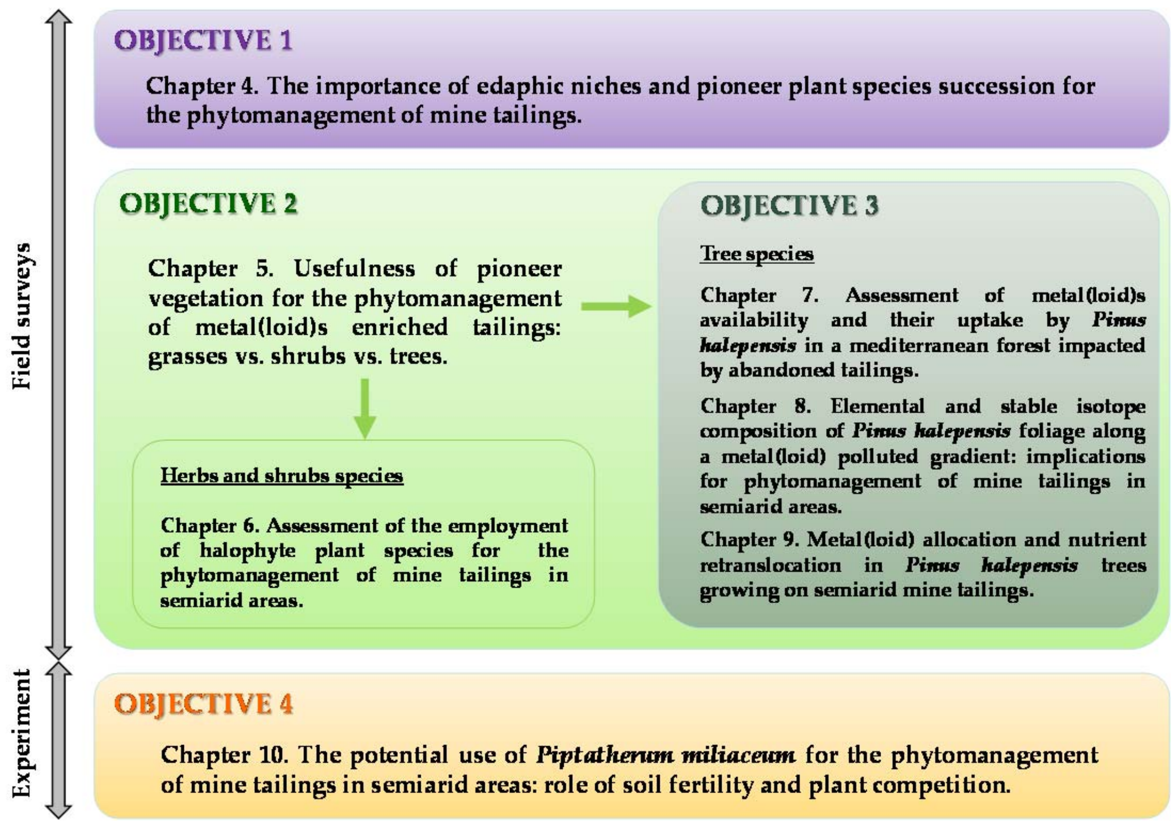

Figure 3.1. Schematic explanation about the relation between the objectives of the Thesis and the following chapters 4 to 10.

3.2. References

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CHAPTER 4. The importance of edaphic niches and pioneer plant species succession for the phytomanagement of mine tailings

Chapter 4

The importance of edaphic niches and pioneer plant species succession for the phytomanagement of mine tailings

Isabel Párraga-Aguado, María Nazaret González-Alcaraz, José Álvarez-Rogel, Francisco J. Jiménez-Cárceles, Héctor M. Conesa

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Abstract

Phytomanagement in terms of phytostabilisation is considered a suitable method to decrease environmental risks of metal(loid) enriched mine tailings. The goal of this study was to identify plant-favourable edaphic niches in mine tailings from a semiarid area, in order to obtain relevant information for further phytostabilisation procedures. For this purpose, a transect-designed sampling from non-disturbed soils to two mine tailings was performed, including the description of soil and plant ecology gradients. Plant ecological indicators showed several stages in plant succession: from weeds to stable patches of late successional plant species. PCA results revealed that plant distribution at the tailings was driven mainly by salinity while metal(loid) concentrations played a minor role. The presence of soil desiccation cracks generated low salinity patches which facilitated favourable niches for plant establishment. Edaphic patch distribution may condition phytostabilisation since ploughing or the employment of certain amendments should take into account favourable niches for plant growth.

4.1. Introduction

Mine tailings are the cause of important environmental impacts in former mining areas due to the spread of dust containing toxic metal(loid)s from their bare surfaces or to the generation of enriched metal(loid) leachates (Conesa and Schulin, 2010). Tailings relocation employing tracks (dig and dump) is an expensive and unrealistic option if huge amounts of toxic materials must be relocated. For this reason, several authors have pointed out the suitability of phytomanagement in terms of phytostabilisation as a low-cost tool to preserve the environmental risks of these sites at a safety level (Clemente et al., 2012). This technique consists of using plants to achieve the surface stabilisation of the tailings by acting as a barrier which decreases wind borne dust, fixes the soil against water erosion and decreases metal(loid) enriched leaching (Robinson et al., 2009). Most of the research carried out in this field has focused on the selection of the best adapted plant species for each mining site (following plant metal uptake criteria) (e.g. Anawar et al., 2011; Escarré et al., 2011) or cause-effect studies employing soil conditioners to favour plant establishment (Clemente et al., 2012). However, most of works fail on assuring the long-term self-sustainability of the system. For that purpose, recent studies have noted that the selection of the best adapted plant species for a phytostabilisation project should also take into account the ecological relationships among the species employed (Martínez-Ruiz and Fernández-Santos, 2005; Rufo and De la Fuente, 2010). The use of soil conditioners to improve plant growth on mine tailings is also a broad topic which has recently received increasing attention (e.g. González-Alcaraz et al., 2011; Clemente et al., 2012). Apart from the expected benefits of soil conditioning in terms of enhancement of plant biomass or decrease of metal(loid) mobility, some of these studies have also revealed drawbacks that must be also considered when evaluating the long-term suitability of phytostabilisation. For instance, the addition of organic amendments have shown the improvement of plant establishment but also the increase of labile metal(loid) concentrations (Córdova et al., 2011) or the decrease with time of plant species diversity (McGeehan, 2009). Moreover, the long-term sustain of amendment benefits have been also questioned, especially under semiarid climate (Zornoza et al., 2012; González-Alcaraz et al., 2013). This may lead to repeated applications and the establishment of a monitoring program to assure the success of the phytostabilisation project (Madejón et al., 2010), which may compromise the low-cost premise of this kind of technologies.

The establishment of a self-sustaining "ecosystem" is considered a key factor for the long-term success of a phytostabilisation program. Recent studies performed in former mining areas pointed out the option of natural colonisation as a useful tool to reach acceptable levels of plant cover in middle term scales (decades) (Kirmer et al., 2008). Pioneer vegetation, which tolerates high metal concentrations, may improve edaphic conditions by increasing soil nutrient content (Rodríguez et al., 2007) or ameliorating soil acidity (Rufo and De la Fuente, 2010), and thus may favor further establishment of other plant species. This process of plant facilitation has been considered critical to restore degraded ecosystems worldwide (Gómez-Aparicio, 2009) and is especially important under semiarid climates (Bonanomi et al., 2011). Some works have already shown the enhancement of plant establishment by ecological mechanisms of facilitation in mining wastes (Markham et al., 2011).

The goal of this study was to identify plant-favourable edaphic niches in abandoned mine tailings from a semiarid area as the basis for further phytostabilisation works. For this purpose, a transect designed sampling was performed from non-disturbed soils to the plateau at two mine tailings, including a complete description of soil and plant ecological gradients. It was hypothesized that pioneer vegetation might be mainly composed by weeds or opportunist plant species that spread in these areas due to the absence of competitors. In addition, we expected that other soil parameters different from metal(loid)s concentrations might condition plant establishment on the tailings.

4.2. Materials and methods

4.2.1. Experimental site description

The Cartagena-La Union Mining District (0-392 m.a.s.l.; 50 km^2; 37°37′20″ N, 0°50′55″W - 37°40′03″N, 0°48′12″W) is situated in the Southeast of the Iberian Peninsula (Figure 4.1). This zone has been one of the most important mining areas in Spain in the last centuries. Metal(loid) contamination in this area has been reviewed by Conesa and Schulin (2010). The climate of the zone is Mediterranean semiarid (250-300 mm annual rainfall, ~18 °C annual average temperature).

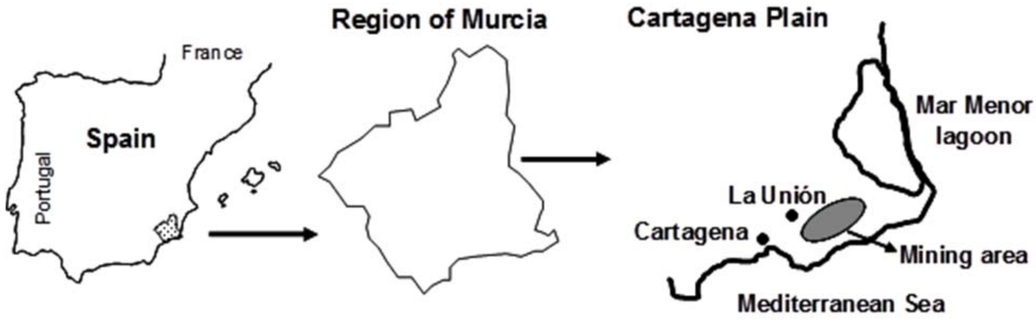

Figure 4.1. Location of the studied area.

The study was conducted at two mine tailings located within the mining area: Mercader (150 m x 100 m width and 30 m height) and Agustin (100 m x 90 m width and 20 m height). These tailings are located at a natural park which includes forests of *Pinus halepensis* and endemic xerophytic thickets.

At each mine tailings pond, a transect from non-affected forest to tailings' plateau was performed (Figure 4.2). The transects were divided into the following steps according to their topography and plant cover: *Non-Affected Forest*, which was the same for both tailings and did not show external symptoms of contamination (100% plant cover); *External Border*, which corresponded to the transitional area forest-tailings (50-100% plant cover); Slope (5-50% plant cover), which was divided into *Lower Slope* and *Upper Slope* (a *Middle Slope* was included at Mercader's slope due to its longer length). At the plateau, zoning was classified as: *Crust*, the closest area to the slope where layers of oxidized crusts were present (0% plant cover); *Flat Plateau*, a flat zone where disperse vegetation grew (~5% plant cover); *Cracks*, a zone where polygonal structures of desiccation cracks have been formed (~3% plant cover); and finally, *Fertility islands*, patches of 100% plant cover which occupied around 10% of total plateau. At each step of the transects, three plots of 5 x 5 m^2 were delimited. The samplings were performed at spring 2012.

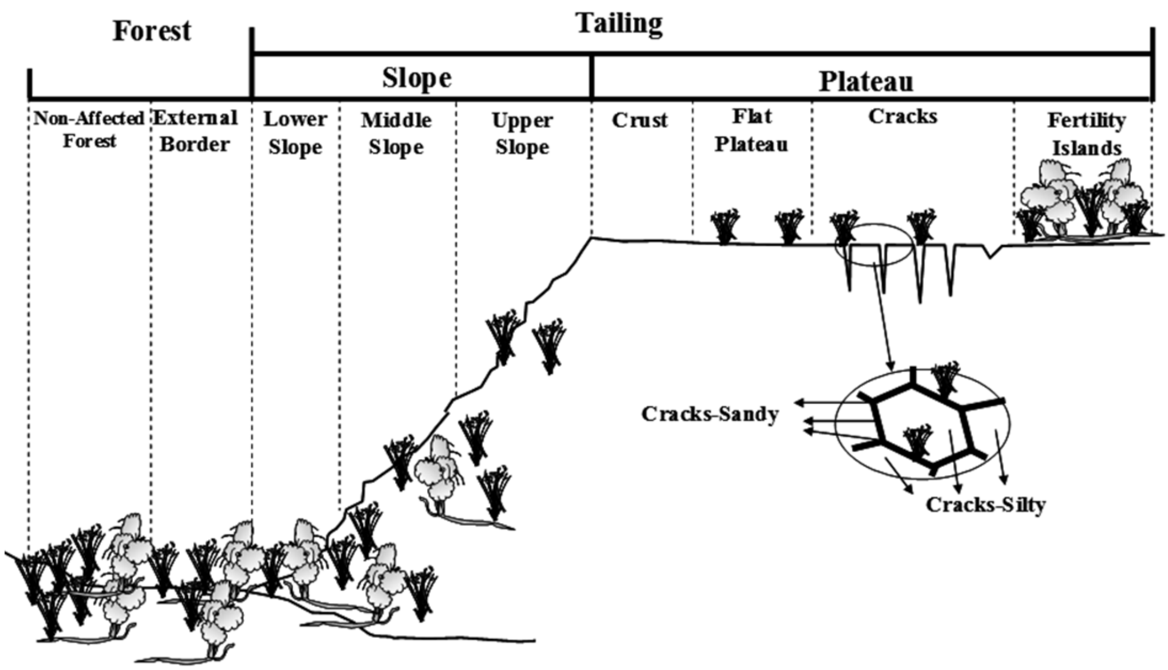

Figure 4.2. Basic scheme of transect steps at both tailings.

4.2.2. Ecological indexes

The number of different species and their corresponding individuals at each plot were recorded. With the obtained data, the Margalef index (R) (Margalef, 1958), that refers to the richness of the population, was calculated as follows:

$$R = \frac{(S - 1)}{\ln N}$$

where S is the number of species and N is the total number of individuals in the plot.

The Shannon-Weaver index (H') was calculated in order to estimate the heterogeneity of the plant communities (Shannon and Weaver, 1963) as:

$$H' = -\sum_{i=1}^{S} p_i \log_2 p_i$$

where p_i is the relative frequency of the species "i" at each plot and $\Sigma p_i = 1$.

Finally, the Pielou index (J') (Pielou, 1969) was calculated to estimate the equitability of the plant species population as:

$$J' = \frac{H'}{\log_2 S}$$

where H' is the Shannon-Weaver index and S is the number of species.

4.2.3. Soil sampling and analyses

An edaphic characterisation for each tailings transect was carried out. Four soil composite samples were taken per transect step (additionally, at *Cracks* zones soil samples were split into two groups (Figure 4.2): *Cracks-Silty*, samples of concentrated tailings from the area inside the polygons; and *Cracks-Sandy*, samples from the material which filled the cracks). Each composite sample was formed by mixing 4 subsamples.

Soil samples were air dried, sieved through a 2 mm sieve, homogenized and stored in plastic bags prior to laboratory analysis. Soil pH and Electrical Conductivity (EC) were determined in a 1:5 water/soil ratio using a Crison Basic 20 pH meter and a Crison Basic 30 conductivity meter, respectively. Particle size distribution was determined following the method of Bouyoucos densimeter (Gee and Bauder, 1986). Equivalent calcium carbonate (%$CaCO_3$) was determined using the Bernard calcimeter method. Total Nitrogen (TN) was determined using the Kjeldahl method (USDA, 1996). Organic Carbon (OC) was determined by the oxidation of organic matter using potassium dichromate. To determine the mineral and total element composition, sub-samples were ground and analysed by X-ray diffraction (Bruker D8 Advance spectrometer) and X-ray fluorescence (Bruker S4 Pioneer), respectively.

Water extractable ions (including metal(loid)s) and Dissolved Organic Carbon (DOC) were determined using a 1:5 soil/water mixture after shaking 2 h (Ernst, 1996). The resulting extracts were filtered through nylon membrane 0.45 mm syringe filters (Albet-JNY). DOC was measured in an automatic analyser (TOC-VCSH Shimadzu). Major ions (cations: Na^{+}, Ca^{2+}, Mg^{2+}, K^{+}; anions: Cl^{-}, SO_4^{2-}) were analysed using an ion chromatographer equipment (Metrohm). Water extractable metal(loid)s (As, Cd, Cu, Mn, Ni, Pb, Sb, Zn) were analysed using an ICP-MS (Agilent 7500A, detection limit 0.002 mg L^{-1}).

4.2.4. Statistics

SPSS 19.0.0 (SPSS, 2010) was used for all the statistical analysis (analysis of variance - one way ANOVA - with Tukey's Test, *t*-test, Pearson's correlations). The data were log-transformed when they did not pass the Levene test for the homogeneity of variances. Differences at p<0.05 level were considered significant. Environmental gradients were examined with a Principal Component Analysis (PCA) using the CANOCO software for Windows v4.02 (ter Braak and Smilauer, 1999).

4.3. Results

4.3.1. Ecological indexes results

Table 4.1 shows all the plant species recorded through the transects at each tailings pond. Only in the case of *Crust* zones no vegetation was found (so in this case, the ecological indexes were not calculated). The Margalef's index (*R*) (Figure 4.3.A) showed a progressive decrease towards the plateau (excluding the *Fertility Islands*, which showed R~2). The highest *R* values occurred in the *Non-Affected Forest* (*R*~3) and were similar (p>0.05) to the ones obtained at the *External Borders* (*R*~2-2.5). The *Flat Plateaus* were the only zones where significant differences between tailings occurred (p<0.05, *R*~0.8 at Mercader and *R*~1.5 at Agustin).

The Shannon-Weaver's diversity index (*H'*) (Figure 4.3.B) had a similar distribution to the Margalef index (*R*), with the exception of the *External Borders* which showed lower *H'* than the *Lower Slopes*. The *Fertility Islands* (*H'*~2.7) of both tailings showed intermediate *H'* (p>0.05) between the *Non-Affected Forest* (*H'*~3.2) and the *External Borders* (*H'*~2.2). The *H'* values did not differ between tailings, except in the *Flat Plateau* where Agustin tailings pond showed higher *H'* (*H'*~2.0) than Mercader (*H'*~0.8, p<0.05).

The Pielou's equitability index (*J'*) is shown in the Figure 4.3.C. In contrast to the Margalef index (*R*), *J'* did not show any clear trend through the transect for both tailings. At Agustin tailings pond *J'* showed similar values to *Non-Affected Forest* (p>0.05). The *Flat Plateau* and *Cracks* from Mercader tailings pond (both, *J'*~0.4) showed lower *J'* (p<0.05) than the corresponding zones at Agustin tailings pond (*J'*~0.8).

Table 4.1. List of plant species recorded at each transect step. “NA.F.” means *Non-Affected Forest*; “EXT.B.” is *External Border*; “L.S.” is *Lower Slope*; “M.S.” is *Middle Slope* (only at Mercader tailings pond); “U.S.” is *Upper Slope*; “CRST” is *Crust*; “F.P.” is *Flat Plateau*; “CRCK” is *Cracks*; “F.ISL.” is *Fertility Island*. “x” are plant species recorded at Non-Affected Forest; “▲” are plant species recorded at Mercader tailings pond; “Θ” are plant species recorded at Agustin tailings pond.

Family	**Plant Species**	**Most common environment** according to Sánchez-Gómez *et al.* (1996)	**NA.F.**	**EXT. B.**	**L.S.**	**M.S.**	**U.S**	**CRST**	**F.P.**	**CRCK**	**F. ISL.**
Anacardiaceae	*Pistacia lentiscus* L.	Shrubs and Mediterranean forests		▲ Θ	▲						▲ Θ
Araceae	*Arisarum vulgare* Targ.-Tozz	Stony soils, pasture lands, open shrubs	x	▲							Θ
Capparaceae	*Capparis spinosa* L. subsp. *Spinosa*	Slopes in marlstones, rocks, abandoned lands,widespread					Θ				
Chenopodiaceae	*Atriplex halimus* L.	Nitrified environments with some salinity				▲			▲		
Chenopodiaceae	*Salsola genistoides* Juss. Ex Poir.	Slopes in marlstone, widespread		Θ							
Chenopodiaceae	*Salsola kali* L.	Sandy soils, dry lands, abandoned lands, widespread			▲	▲	▲ Θ		▲	▲ Θ	▲ Θ
Chenopodiaceae	*Salsola oppositifolia* Desf.	Nitrified environments with some salinity									▲ Θ
Cistaceae	*Cistus monpeliensis* L.	Shrubs, widespread		▲							
Cistaceae	*Helianthemum almeriense* Pau in Mem. Mus	Shrubs	x								
Cistaceae	*Helianthemum syriacum* (Jacq.) Dum. Cours.	Shrubs, widespread		▲	▲						
Compositae	*Atractylis humilis* L.	Shrubs and altered/degraded lands	x	▲							
Compositae	*Cheirolophus intybaceus (Lam.) Dostál*	Shrubs in nitrified environments, slopes		▲							
Compositae	*Dittrichia viscosa* (L.) Greuter	Shrubs in nitrified environments, road			▲ Θ	▲			Θ		▲ Θ
Compositae	*Helichrysum decumbens* (Lag.) Camb	Shrubs in nitrified/altered env., sandy areas, widespread		▲ Θ	▲ Θ				Θ		▲ Θ
Compositae	*Inula crithmoides* L.	Shrubs in saline soils			▲						
Compositae	*Phagnalon saxatile* (L). Cass.	Shrubs in stony soils, abandoned lands	x	▲ Θ	▲						Θ
Compositae	*Reichardia tingitana* (L.) Roth	Nitrified environments, road sides									Θ
Compositae	*Sonchus asper* (L.) Hill	Nitrified environments			▲						
Compositae	*Sonchus tenerrimus* L. var. *tenerrimus*	Nitrified environments		▲	▲	▲	▲		Θ		▲ Θ
Convolvulaceae	*Convolvulus arvensis* L.	Weed, widespread		▲	▲						
Crassulaceae	*Sedum sediforme* (Jacq) Pau	Not consolidated soils, shrubs, stony soils	x								
Cupressaceae	*Tetraclinis articulata* (Vahl) Mast.	Open Forests	x		▲ Θ						
Gramineae	*Brachypodium retusum* (Pers.)Beauv.	Herbs in forests. Widespread.	x	▲ Θ							
Gramineae	*Phragmites australis* (Cav.) Trin. subsp. *australis*	dryriver sides, waterlogged soils, saline waters			Θ						

Table 4.1. (continuation)

Family	Plant Species	Most common environment according to Sánchez-Gómez *et al.* (1996)	NA.F.	EXT. B.	L.S.	M.S.	U.S	CRST	F.P.	CRCK	F. ISL.
Gramineae	*Piptatherum miliaceum* (L.) Cosson	Nitrified lands, road sides, slopes, widespread			▲ Θ	▲			Θ	Θ	▲ Θ
Gramineae	*Stipa tenacissima* L.	Not deep soils, sunny slopes, widespread	x	Θ	Θ						
Labiatae	*Lavandula dentata* L.	Shrubs in coastal areas		▲							
Labiatae	*Rosmarinus officinalis* L.	Shrubs, widespread	x	Θ							
Labiatae	*Teucrium carthaginense* Lange	Shrubs in coastal areas	x								
Labiatae	*Thymus hyemalis* Lange	Shrubs	x	▲ Θ							
Leguminosae	*Calicotome intermedia* (C. Presl) Guss.	Shrubs in coastal areas	x	Θ							
Leguminosae	*Dorycnium pentaphyllum* (Scop.)	Shrubs in nitrified/altered environments		▲	Θ				Θ		Θ
Leguminosae	*Psoralea bituminosa* (L.)C H. Stirt.	Nitrified shrubs, widespread			▲	▲					
Liliaceae	*Asparagus horridus* L. in J. A. Murray	Altered shrubs, abandoned lands, widespread	x	▲							▲
Liliaceae	*Urginea maritima* (L.) Baker	Shrubs in coastal areas		▲							
Linaceae	*Linum strictum* L.	Terophitic lands, widespread	x								
Oleaceae	*Phillyrea angustifolia* L.	Shrubs and Mediterranean forests		▲							▲
Oleaceae	*Olea europaea* L.	Shrubs and Mediterranean forests		▲							
Palmae	*Chamaerops humilis* L.	Shrubs, stony soils, above all close to the coast	x	▲ Θ	▲ Θ						Θ
Pinaceae	*Pinus halepensis* Miller	Mediterranean forests	x	▲ Θ	▲ Θ	▲	▲ Θ		▲ Θ	▲ Θ	▲ Θ
Plumbaginaceae	*Limonium carthaginense* (Rouy) Hubbard a Sandwith	Saline soils, sandy areas close to the sea					Θ		Θ	Θ	Θ
Plumbaginaceae	*Limonium cossonianum* Kunthze, Revis.	Saline soils, sandy areas close to the sea				▲	▲		▲		▲ Θ
Rhamnaceae	*Rhamnus alaternus* L.	Shrubs and Mediterranean forests	x	▲							
Rhamnaceae	*Rhamnus lycioides* L. subsp. *lycioides*	Shrubs and Mediterranean forests	x	▲ Θ							
Rubiaceae	*Rubia peregrina* L.	Shrubs and Mediterranean forests	x	▲							
Rutaceae	*Ruta angustifolia* Pers.	Shrubs, pasture lands		▲							
Solanaceae	*Lycium intricatum* Boiss.	Saline coastal soils		Θ							
Tamaricaceae	*Tamarix boveana* Bunge	Saline areas, dry rivers			Θ		▲ Θ				▲ Θ
Tamaricaceae	*Tamarix canariensis* Willd.	Saline areas, dry rivers							▲		
Umbeliferae	*Bupleurum gibraltaricum* Lam.	Shrubs and small forests	x	▲ Θ							
Umbeliferae	*Erygnium campestre* L.	Nitrified environments, abandoned lands	x								
Zygophyllaceae	*Zygophyllum fabago* L.	Slopes, road sides, altered soils				▲	▲ Θ		▲ Θ	▲ Θ	▲

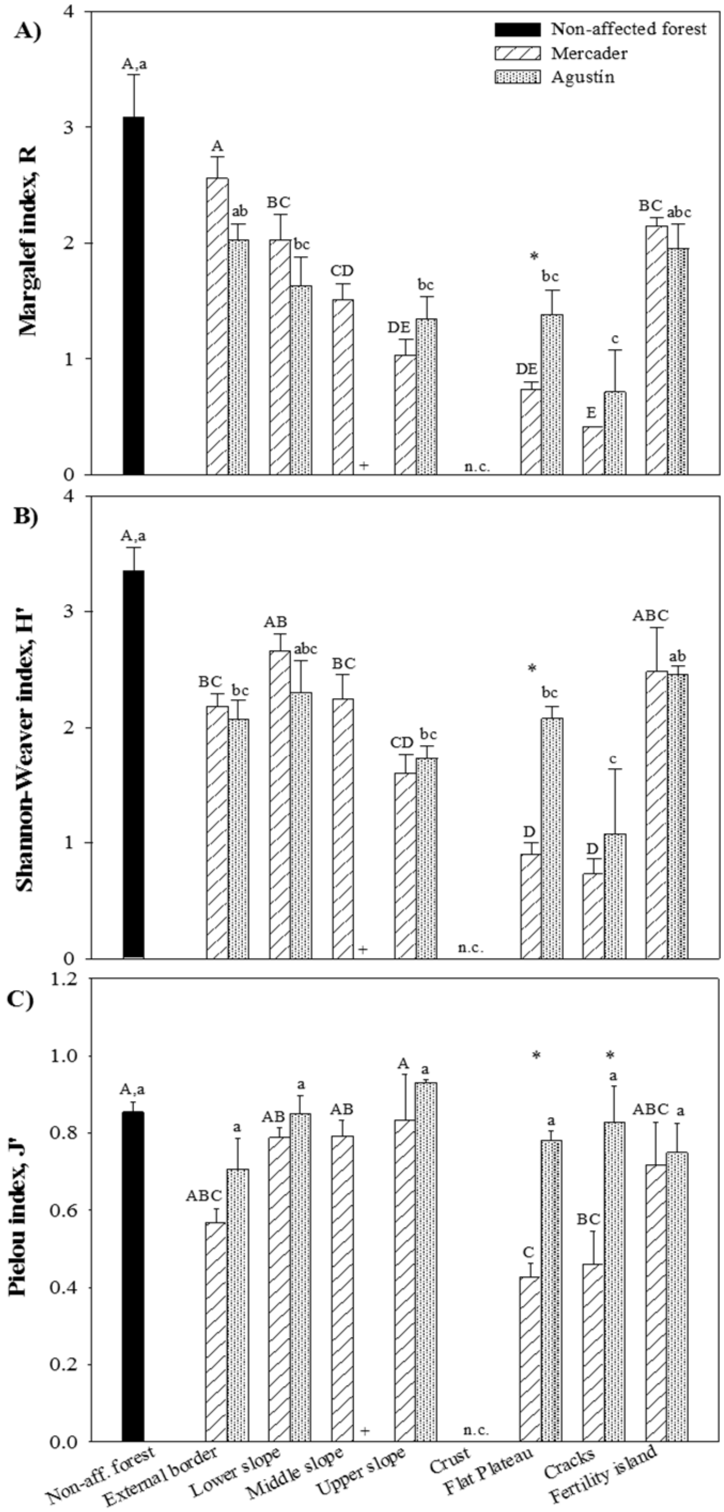

Figure 4.3. Margalef index (*R*), Shannon-Weaver index (*H'*) and Pielou index (*J'*) for each transect step. Bars on columns are standard error of three plots (*N*=3). Different letters on columns with the same case (uppercase for Mercader and lowercase for Agustin) indicate significant differences among steps at each tailings transect ($p<0.05$, Anova with Tukey test). "*" between columns indicates significant differences between tailings for the same step ($p<0.05$, *t*-test). "n.c." means not calculated due to absence of vegetation. "+" Indicates that this step was not performed at Agustin tailings pond.

4.3.2. Soil properties

At both tailings the samples from *Crust* zones showed lower pH (pH~3.5) ($p<0.05$) than the rest of zones (pH~7-8) (Figure 4.4.A). The Electrical Conductivity values (EC) (Figure 4.4.B) increased from the peripheral areas to the plateau (~0.3-0.6 dS m^{-1} to 3-5 dS m^{-1}). The *Cracks* areas showed the highest EC values, associated to the *Cracks-Silty* patches ($p<0.05$, 8-10 dS m^{-1}). The *Fertility Islands* showed slightly lower EC values (1.4-2 dS m^{-1}) than the rest of the plateau zones. Some transect steps of Agustin tailings showed higher %$CaCO_3$ values (Figure 4.4.C) ($p<0.05$ at *External Border*, *Flat Plateau*, and *Cracks-Sandy*) than the corresponding ones of Mercader tailings pond.

The Organic Carbon (OC) concentrations (Figure 4.4.D) showed values below 5 g kg^{-1} at all the tailings zones except in two ones: *Fertility Islands* (10 g kg^{-1}) and *Cracks-Silty* patches (5-7 g kg^{-1}). For the *Slopes* (*Lower* and *Upper*), OC concentrations were higher ($p<0.05$) at Agustin (3-4 g kg^{-1}) than at Mercader (~2 g kg^{-1}; the opposite occurred at *Crust*, *Flat Plateau* and *External Border* zones. The samples from *Non-Affected Forest* showed similar values to the *External Border* of Mercader tailings pond (~24 g kg^{-1}). However, they were much higher ($p<0.05$) than the ones obtained at the *External Border* of Agustin tailings pond (~12 g kg^{-1}).

The Dissolved Organic Carbon (DOC) results (Figure 4.4.E) showed similar behaviour to the OC but some exceptions occurred: the samples from Agustin tailings pond showed lower DOC concentrations ($p<0.05$) in the *Slope* (*Upper* and *Lower*) sites (10 mg kg^{-1}) than the corresponding samples from Mercader tailings pond (20 mg kg^{-1}). The distribution of Total Nitrogen (TN) concentrations (Figure 4.4.F) showed similar trend to that of DOC. The samples from *External Border* (1-1.5 g kg^{-1}) at both tailings showed lower TN values ($p<0.05$) than the ones from *Non-Affected Forest* (2.5 mg kg^{-1}).

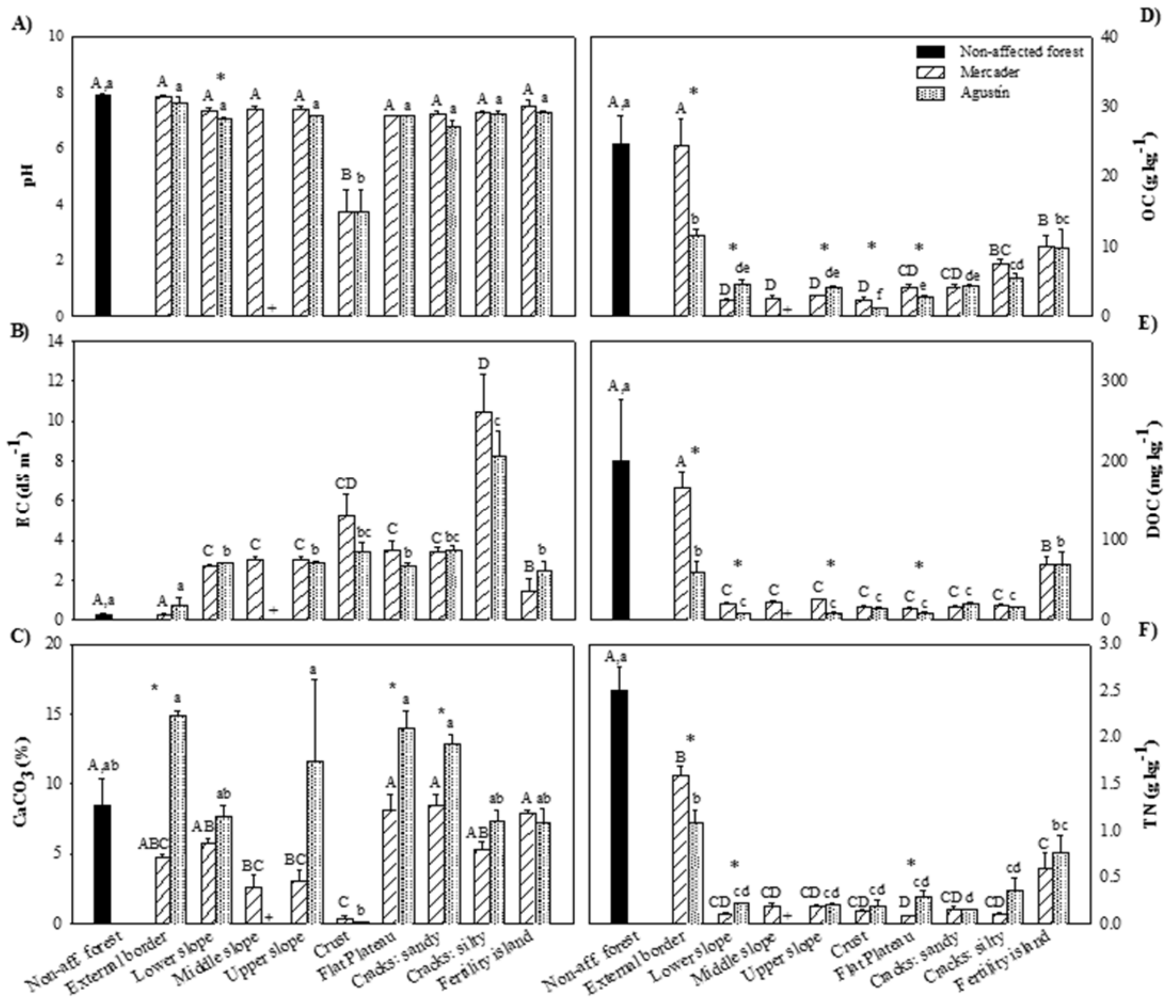

Figure 4.4. Results of the edaphic parameters measured at each step: pH, Electrical Conductivity (EC), percentage of equivalent calcium carbonate ($CaCO_3$), Organic Carbon (OC), Dissolved Organic Carbon (DOC) and Total Nitrogen (TN). Bars on columns are standard error (*N*=4). Different letters on columns with the same case (uppercase for Mercader and lowercase for Agustin) indicate significant differences among steps at each tailings transect ($p<0.05$, Anova with Tukey test). "*" between columns indicates significant differences between tailings for the same step ($p<0.05$, *t*-test). "+" Indicates that this step was not performed at Agustin tailings pond.

In relation to the particle size distribution (Figure 4.5) all the tailings zones (except the *Cracks-Silty* ones) showed around 10-20% silt and 60-80% sand. *Cracks-Silty* zones contained around 40% silt and 0-40% clay.

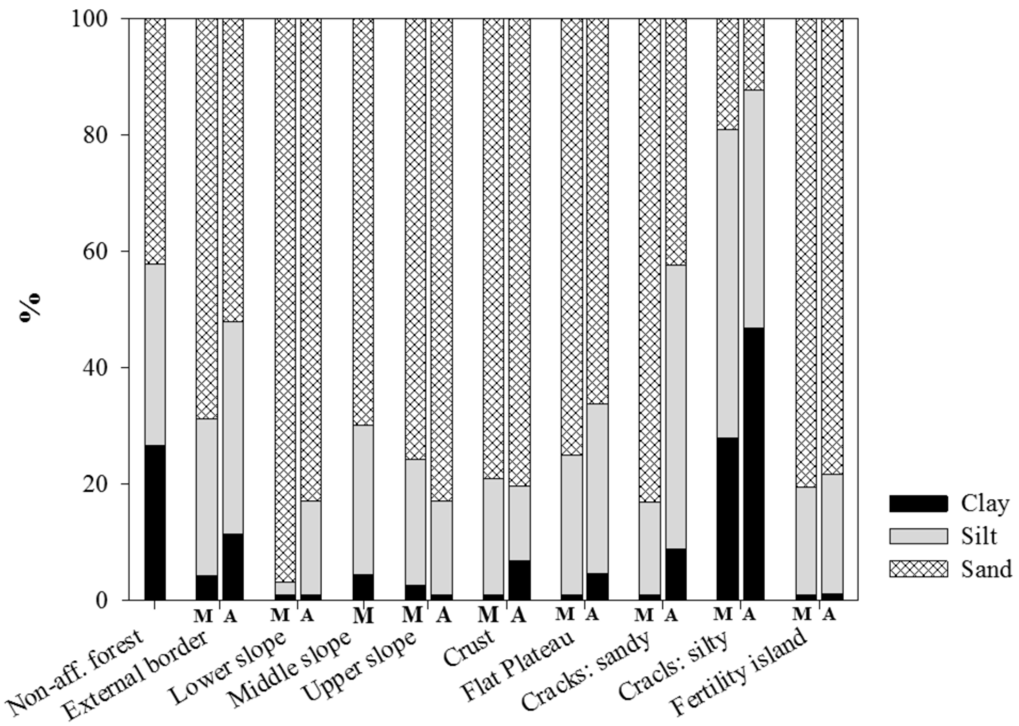

Figure 4.5. Particle size distribution for soil samples of each transect step. "M" and "A" are steps of Mercader and Agustin tailings, respectively.

4.3.3. Mineralogy and soil metal(loid) concentrations

In order to facilitate the presentation of the results of mineralogy and metal(loid) concentrations, Table 4.2 and Table 4.3 were organised by transect steps without distinguishing between tailings. The main minerals detected in the samples are shown in Table 4.2. Quartz (35-70%) was the only mineral detected in all the samples from the transects. Gypsum was not found in the *Non-Affected Forest* samples but appeared in the rest of the transect steps (in percentages between 10 and 20%), and especially in the *Crust* (up to 40%). In the latter, plumbojarosite and beudantite were additionally identified.

Trace element concentrations were high for all the tailings steps (Table 4.3), including the *External Border*. *Non-Affected Forest* also showed high values for As (~60 mg kg^{-1}), Pb (~1300 mg kg^{-1}), and Zn (~675 mg kg^{-1}) compared to the backgrounds of the nearby Cartagena Plain 7 mg kg^{-1} As, 9 mg kg^{-1} Pb and 41 mg kg^{-1} Zn (Martínez-Sánchez and Pérez-Sirvent, 2007). Nevertheless, these concentrations were within the range of the ones obtained by several authors for the mining area soils, which is known to have different geochemical background (Conesa and Schulin, 2010). The highest As and Pb concentrations (p<0.05) (Table 4.3) were found at the *Crust* zones (1770 mg kg^{-1} As and 23,100 mg kg^{-1} Pb), which was probably associated to the presence of beudantite and plumbojarosite, respectively. For the rest of the studied metals the maximums were distributed along the transect: e.g. 7250-7810 mg kg^{-1} Zn at *External Borders* and *Slopes*, respectively; ~10,000 mg kg^{-1} Mn at *Flat Plateau* and *Cracks-Sandy*.

Water extractable metal(loid) concentrations (Table 4.3) for several elements (like As, Cd, Cu, Ni, Pb, Sb) were below or in the range of the detection limit (<0.01 mg L^{-1} in 1:5 water extract) at all the transect steps except at *Crust* zones (e.g. 0.162 mg L^{-1} Cd, 0.174 mg L^{-1} Ni, data not shown). The highest concentrations of water extractable Mn and Zn were also obtained in samples from the *Crust* zones (91 mg L^{-1} and 39 mg L^{-1}, respectively, p<0.05). For the rest of samples, the concentrations of water extractable Mn ranged from 0.010 to 0.035 mg L^{-1}, while Zn ones showed a wider variation (from 0.020 to 0.075 mg L^{-1}). In relation to water extractable anions, *Cracks-Silty* samples showed the highest values for Cl^- (1420 mg L^{-1}, p<0.05) and SO_4^{2-} (2730 mg L^{-1}). The latter was also in the range of the values obtained at *Crust* (2630 mg L^{-1}). Water extractable concentrations for Mg^{2+} and Na^+ were higher (p<0.05) in the *Cracks-Silty* samples (479 mg L^{-1} and 516 mg L^{-1}, respectively) than in the rest of samples (<65 mg L^{-1}).

Table 4.2. Mineralogy at each step of the two tailings. "-" means below 5%.

Mineral	Formula	Non-affected Forest	External Border	Slope	Crust	Flat Plateau	Cracks		Fertility Islands
							Sandy	Silty	
Muscovite	$KAl_2Si_3AlO_{10}(OH)_2$	~15%	5-10%	up to 15%	up to 5%	-	-	~5%	~5%
Quartz	SiO_2	~50%	~50%	40-60%	20-70%	50-70%	~55%	40-45%	50-60%
Gypsum	$Ca(SO_4)(H_2O)_2$	-	~15%	10-20%	15-40%	~13%	~13%	10-20%	up to 10%
Clinochlore	$Mg_{4.882}Fe_{0.22}Al_{1.881}Si_{2.96}O_{10}(OH)_8$	-	~10%	15-35%	-	up to 15%	~15%	20-30%	10-20%
Magnesium calcite	$(Mg_{0.03}Ca_{0.97})(CO_3)$	~25%	~10-25%	up to 15%	-	up to 15%	10-20%	~10%	~15%
Plumbojarosite	$PbFe_6(SO4)_4(OH)_{12}$	-	-	-	up to 22%	-	-	-	-
Dolomite	$CaMg(CO_3)_2$	~10%	~10%	-	-	~5%	-	-	up to 8%
Beudantite	$Pb(Fe_{2.54}Al_{0.46})(As_{1.07}O_4)(S_{0.93}O_4)(OH)_6$	-	-	-	up to 8%	-	-	-	-

Table 4.3. Total and water-extractable concentrations for several elements and ions. Data are average ± standard error (N=4). Different letters at the same row means significant differences (p<0.05, ANOVA with Tukey test) among transect steps.

Parameter		Units	Non-Affected Forest N=4		External Border N=8		Slope N=20		Crust N=6		Flat Plateau N=8		Cracks-Sandy N=8		Cracks-Silty N=8		Fertility Islands N=8	
Total element concentrations	**Fe**	**g kg^{-1}**	37.6 ± 0.1	a	178.4 ± 1.1	b	217.2 ± 4.7	b,c	248.7 ± 8.4	c	196.3 ± 0.8	b	180.7 ± 7.6	b	187.0 ± 8.8	b	19.0 ± 16.6	b
	S		1.0 ± 0.1	a	8.0 ± 0.6	b	31.7 ± 3.6	d	97.2 ± 2.8	e	26.3 ± 1.9	d	26.4 ± 2.1	d	16.0 ± 1.6	c,d	11.4 ± 2.4	b,c
	As	**mg kg^{-1}**	59 ± 3	a	430 ± 50	b	610 ± 60	b	1770 ± 380	c	420 ± 90	b	320 ± 80	b	260 ± 43	b	450 ± 110	b
	Cd		5 ± 3	a	15 ± 5	a	16 ± 4	a	15 ± 6	a	11 ± 4	a	12 ± 4	a	13 ± 5	a	16 ± 7	a
	Cu		55 ± 2	a	100 ± 4	b,c	121 ± 5	c,d	133 ± 4	d	87 ± 6	b	80 ± 5	a,b	90 ± 9	b	84 ± 6	a,b
	Ni		35 ± 2	b	32 ± 1	b	28 ± 1	b	18 ± 1	a	27 ± 2	a,b	28 ± 2	b	32 ± 3	b	33 ± 3	b
	Mn		2370 ± 90	a	8910 ± 470	a	8900 ± 440	a	3470 ± 1070	b	9990 ± 590	a	10,250 ± 930	a	7530 ± 470	a	8650 ± 680	a
	Pb		1310 ± 160	a	5410 ± 490	b	5130 ± 600	b	23,100 ± 3230	c	3590 ± 360	b	3530 ± 370	b	3690 ± 360	b	3300 ± 280	b
	Sb		6 ± 5	a	59 ± 6	b	66 ± 3	b,c	136 ± 21	c	63 ± 7	b,c	48 ± 4	b	62 ± 11	b	56 ± 8	b
	Zn		670 ± 100	a	7810 ± 460	e	7250 ± 610	d,e	2810 ± 380	b	4330 ± 330	b,c,d	4740 ± 430	c,d,e	3930 ± 360	b,c	6320 ± 1040	c,d,e
Water extractable metals	**Cu**	**mg L^{-1}**	0.017 ± 0.014	a	<0.01		<0.01		0.158 ± 0.059	b	<0.01		<0.01		<0.01		<0.01	
	Mn		0.019 ± 0.015	a	<0.01		0.026 ± 0.008	a,b	91 ± 34	c	0.022 ± 0.002	a,b	0.013 ± 0.003	a,b	0.019 ± 0.002	a,b	0.035 ± 0.007	b
	Pb		<0.01		<0.01		<0.01		0.517 ± 0.230		<0.01		<0.01 ±		<0.01		<0.01	
	Zn		0.025 ± 0.016	a,b	0.036 ± 0.006	a,b	0.057 ± 0.008	b	39 ± 10	c	0.019 ± 0.002	a,b	0.027 ± 0.007	a,b	0.016 ± 0.008	a	0.075 ± 0.028	b
Water extractable anions and major cations	**Cl^-**	**mg L^{-1}**	23 ± 5	a	10 ± 1	a	13 ± 2	a	11 ± 1	a	65 ± 55	a	129 ± 35	b	4120 ± 215	c	11 ± 2	a
	SO_4^{2-}		27 ± 5	a	182 ± 122	b	1670 ± 31	c,d	2630 ± 324	d	1690 ± 61	c,d	1800 ± 38	c,d	2730 ± 130	d	1160 ± 274	c
	Ca^{2+}		27 ± 3	a	94 ± 44	b	599 ± 7	c,d	558 ± 6	c,d	621 ± 4	c,d	600 ± 6	c,d	672 ± 31	d	434 ± 103	c
	K^+		11 ± 3	b,c	11 ± 1	b,c	5 ± 0	a,b	56 ± 38	c	5 ± 1	a,b	3 ± 1	a,b,c	7 ± 1	a,b,c	15 ± 1	c
	Mg^{2+}		8 ± 2	a	7 ± 3	a	58 ± 9	b	117 ± 35	b	54 ± 19	b	82 ± 14	b	479 ± 83	c	40 ± 6	b
	Na^+		23 ± 5	b,c	11 ± 1	b	13 ± 1	b	5 ± 1	a	44 ± 31	b	65 ± 12	c	516 ± 77	d	25 ± 7	b,c

4.3.4. PCA results

The PCA results (Figure 4.6) reflected the physicochemical differences along the transect and showed an overall view of the parameters which drove the main edaphic gradients. The main gradient identified by the PCA was defined by OC, TN and DOC on the positive side of X-axes and water extractable SO_4^{2-} and Ca^{2+} on the negative side. The secondary gradient (Y-Axes) was related to water extractable Cl^- and Na^+ (on the positive side) and sand content (on the negative side). Samples from the *Non-Affected Forest*, *External Borders* and *Fertility Islands* were located on the positive side of X-Axes, while the rest were depicted on the negative side. Samples from the *Cracks* appeared mainly on the positive side of Y-Axes, oppositely to the most of samples.

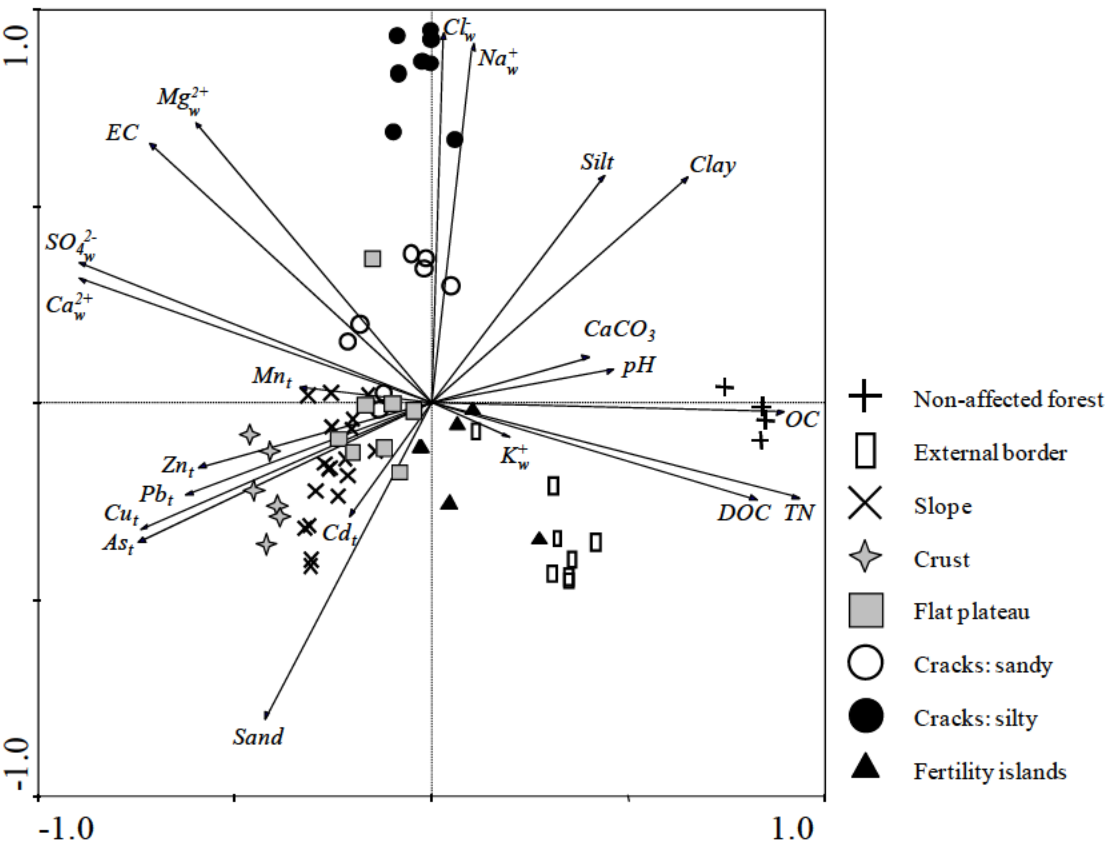

Figure 4.6. PCA results for the two studied transects and soil parameters. Variance explained by the two first components 61.0% (X-axes 38.4%; Y-axes: 22.6%)."M_t" are total element concentrations; "M_w" are water extractable ion concentrations.

4.4. Discussion

4.4.1. Changes in plant species composition through the transects

Plant species were classified into four groups (Figure 4.7) based on the environmental description performed by Sánchez-Gómez et al. (1996): A) *Shrub/Forest* plant species, which belong to the undisturbed plant communities in the area; B) *Widespread* plant species, which have regular distribution within the Mediterranean region; C) plant species of *Disturbed Ecosystems*, which have been described to appear under environmental disturbances (e.g. contamination, nitrified environments); and D) *Halophyte*, which includes the plant species whose common habitats are saline soils (salt marshes, coastal dunes or salty waterlogged soils). The ecological indexes of Margalef (*R*) and Shannon-Weaver (*H'*) at the tailings' *Borders* were lower than at the *Slopes*. This was mainly due to the decrease of the number of *Shrub/Forest* plant species (from ~60% at *External Border* to ~30% at *Slopes*) (Figure 4.7.A) since the number of *Widespread* plant species was more or less constant in all the steps (around 20-30%). Then, it might be hypothesized that the *Shrub/Forest* plant species were shifted along the transect by *Disturbed Ecosystems* plant species and *Halophyte* ones. Although the percentages of *Shrub/Forest* plant species in the *Fertility Islands* were similar to the ones obtained in the rest of the transect steps (~20%), it should be pointed out that adult trees (2-4 m height) were only recorded as part of the *Fertility Islands* while in the rest of the tailings pond most of the trees were seedlings or individuals of small size (<0.5 m height).

The Pielou equitability index (*J'*) showed different behaviour than *R* and *H'*. At some tailings zones (e.g. *Slopes*, *Cracks* at Agustin tailings pond) *J'* values were even higher than at the *Non-Affected Forest*. This might be due to the more equitable distribution in the number of individuals of each plant species at the tailings. That is to say, there was lower species richness but each species had similar population size compared to the rest. However, *Non-Affected Forest* areas, which showed higher *R*, had more heterogeneity in population sizes.

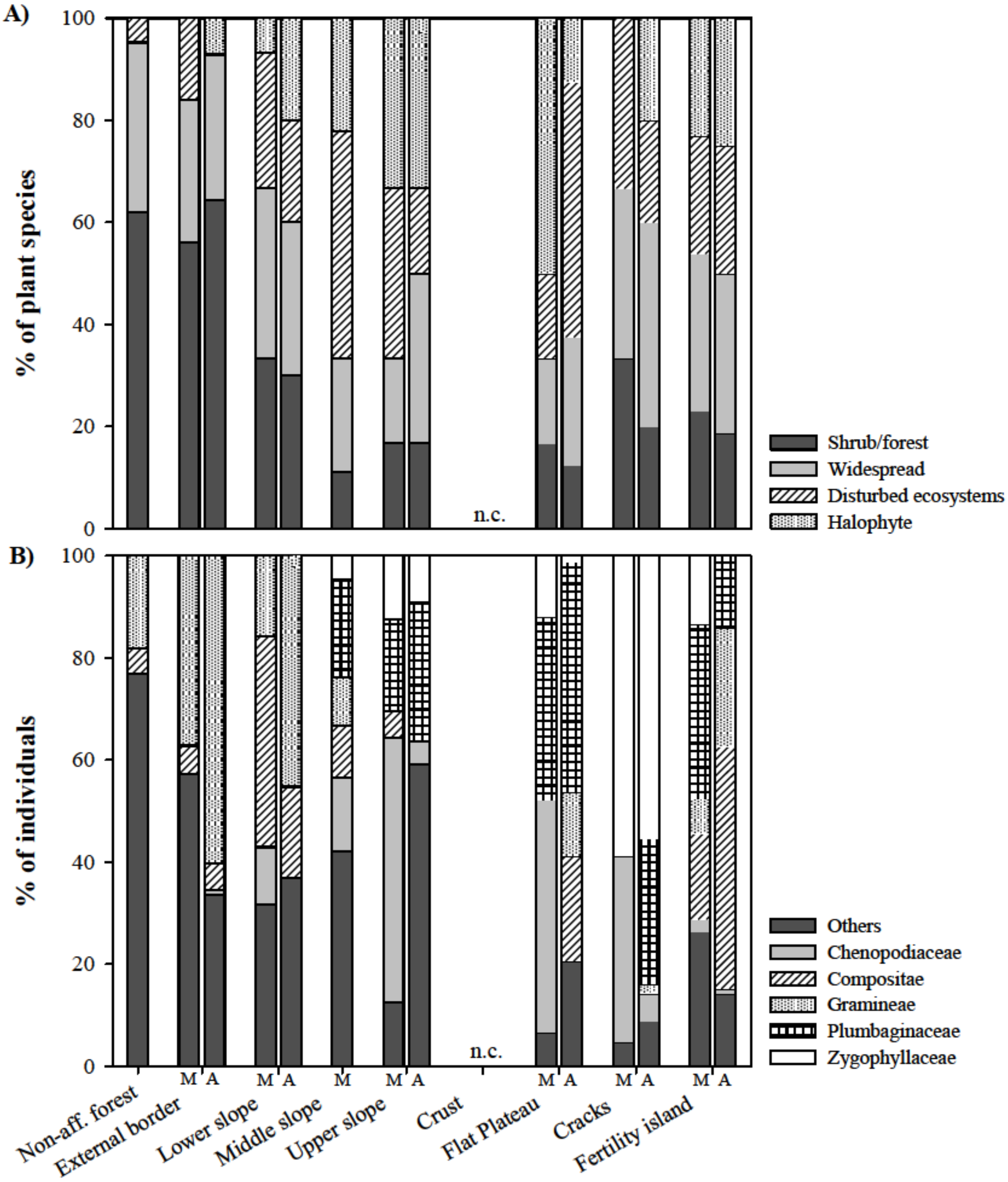

Figure 4.7. Percentage of plant species and individuals at each step of the transects according to functional groups and family, respectively. "n.c." means not calculated.

Several plant families present at Mercader and Agustin tailings have been described by previous authors such as the main contributors to pioneer vegetation in polluted ecosystems. For instance, Gramineae plant species have been long considered as ideal candidates to conduct phytostabilisation works in metalliferous soils due to their good adaptive characteristics and relatively low metal(loid) transfer from roots to shoots (Prasad, 2006; Abraham et al., 2009). At Agustin and Mercader tailings, two species belonging to this family (Figure 4.7.B), *Stipa tenacissima* and *Piptatherum miliaceum*, were recorded at the *Slopes* (counting 20-45% of total individuals at the *Lower Slopes*). Moreover, *Piptatherum miliaceum* was the only Gramineae recorded at the *Fertility Islands* (representing up to 20% of all the individuals recorded at the *Fertility Islands* of the Agustin tailings pond). Dazy et al. (2008) found around 24% of individuals belonging to Gramineae, 30% to Compositae and 30% to Chenopodicaceae, in the pioneer vegetation colonising a metal polluted soil. Shu et al. (2005) showed that around 24% of the plant species colonising Pb/Zn tailings in Southern China were Gramineae and 18% Compositae.

Compositae plant species were mainly represented at Mercader and Agustin tailings by *Helichrysum decumbens*, *Dittrichia viscosa*, *Sonchus tenerrimus* and *Sonchus asper*. In addition, Chenopodiaceae, especially *Salsola kali*, was found at Mercader tailings pond, where *Psoralea bituminosa* was also found (this one belonging to Leguminosae). Both plant species were the only annual herbs sampled at the studied tailings. It has been recognised the important role of the annual herbs in the early stages of an ecosystem development due to the enhancement of the soil properties (e.g. nutrients content), which makes possible the progressive establishment of other plant species (biannuals or perennials) (Dazy et al., 2008). Among the perennial plants sampled at Agustin and Mercader tailings, it should be pointed out *Zygophyllum fabago* (Zygophyllaceae), which has been included as an invasive weed in the Database of Spanish National List of Weed Species (Sanz Elorza et al., 2004). Weed plant species may show some properties such as lower nutrient requirements, stronger competitive behaviour or more flexible lifecycles, which make them more suitable to grow under metal stress environments (Wei et al., 2004).

Chenopodiaceae plant species, such as *Atriplex* sp. pl. are of particular interest when dealing with the revegetation of tailings under semiarid environments because of being drought- and salt-tolerant (Jefferson, 2004; Mendez and Maier, 2008). The high salinity in tailings materials may facilitate the establishment of halophytic plant species which, apart from Chenopodiaceae, may include individuals of other families such as Tamaricaceae, Plumbaginaceae, Compositae, etc. Several authors have shown that the functionality of the physiological mechanisms of salt tolerance may also be employed as tools to tolerate metal stress (Manousaki and Kalogerakis, 2011). Among the plant species recorded in the tailings some of them may fit in the scope of halophyte: *Atriplex halimus*, *Inula crithmoides*, *Limonium cossonianum*, *Tamarix boveana* and *Tamarix canariensis*. These plant species are known to be typical plant species from Mediterranean coastal salty areas and have been also found in mining polluted salt marshes from the Cartagena-La Union area (Conesa et al., 2011). Other *Tamarix* species, such as *Tamarix africana* have been shown to have good long-term response in the restoration of mining polluted habitats such as the areas close to the Doñana National Park (South Spain) which were affected by the mining toxic spill in 1998 (Domínguez et al., 2010). The presence of halophytic plant species at Agustin and Mercader tailings confirmed that seed dispersion from the nearby coastal areas (located at 5-10 km distance) had taken place. Recent works performed in former mining areas from Germany showed that the presence of migrating plant species up to a distance of at least 17 km plays an important role in colonization processes after the destruction of ecosystems (Kirmer et al., 2008). As Ash et al. (1994) affirmed, the possibility of immigration of metal tolerant plant species is critical for the natural colonisation of mine tailings and limits species richness.

Some plant species found at these tailings such as *Tetraclinis articulata* (IUCN, 2012) or *Limonium carthaginense* (BORM, 2003) have been included in red lists of threatened species. As some authors have pointed out, the natural plant colonisation of former mining areas may generate sites where local rare vegetation is able to grow (Tropek et al., 2010; Faucon et al., 2011). According to Jefferson (2004), the main goal of a restoration should be the establishment of sustainable plant communities that reflect the diversity and composition of the surrounding natural plant communities. At Mercader and Agustin tailings this goal seems to be achieved in the *Fertility Islands*. These patches contained late successional plant species such as *Chamaerops humilis*, *Olea europaea*, *Pinus halepensis* or *Pistacia lentiscus* together with *Widespread*, *Halophyte* and *Disturbed Environments* plant species, also recorded in the rest of the tailings. These *Fertility Islands* showed similar plant species composition and ecological indexes to the *External Border* and *Lower Slope* zones. These findings are in agreement with new approaches on the restoration of degraded ecosystems, which consider that different combinations or assemblages between invasive and competitor species may be of special relevance for the establishment of native perennial vegetation (Brown et al., 2008).

4.4.2. Edaphic niches for pioneer vegetation establishment

As discussed above, shifts in vegetation occurred towards the tailings' plateaus. These shifts inside the tailings (from *Lower Slope* to *Cracks*) might not be explained by changes in soil metal(loid)s contents since their concentrations were already high in the peripheral *Borders*, where plant composition was similar to the *Non-Affected Forest*. So, it is reasonable to think that there were other parameters which, together with metal(loid) concentrations, conditioned plant species composition and their distribution at the tailings. In this way, the key factor for vegetation establishment at the tailings would not be just based on showing certain metal(loid) tolerance behaviour but a wider tolerance to a combination of edaphic stresses (metal(loid)s, salinity, low nutrient content, poor soil structure, etc.). As it is shown at Table 4.4, the decrease in ecological indexes (especially, *R* and *H′*) correlated better with EC values (r = -0.752, p<0.01), water extractable SO_4^{2-} (r ~ -0.8, p<0.01) and nutrient content (OC and TN, r ~ 0.6-0.7, p<0.01) than with metal(loid)s content (total and water extractable). It should be noted that, in spite of showing high EC and high SO_4^{2-} concentrations, the low pH (~3) might have played a major role in avoiding the vegetation at the *Crust* zones (apart from the physical constraints for plant roots associated to crusting). As it has been shown in the PCA results (Figure 4.6), the main gradient, which was associated to OC and TN, did not follow the consecutive order of the steps along the transect, but was related to the presence of more/less vegetation: *Fertility Islands* symbols in the Figure 4.6 were placed close to the *External Borders* ones in spite of being located at the tailings' plateau. Assuming that OC and TN might be produced by the vegetation established (root exudates and litter) and were not inherent properties of mining wastes, then, EC (related to SO_4^{2-} and Cl^- concentrations) seemed to be the factor which controls the best pioneer plants colonisation/distribution inside the tailings pond. However, it is reasonable to think that the nutritional gradient may have played a major role in the establishment of the plant species which colonise the tailings after the "facilitation" or edaphic enhancement generated by previous plant successions. The presence of non-metal tolerant plant species, such as *Olea europaea*, growing at the tailings as part of the *Fertility Islands* might be explained by these mechanisms of facilitation.

In relation to salinity, two gradients might be distinguished at the studied tailings: a major gradient, towards the plateau, whose main shift occurred between the peripheral areas and the *Lower Slope*, and which may contribute to the shift of vegetation (increasing number of halophytic plant species and decreasing the number of non-salt tolerant plant species); and a micro-gradient (discontinuity in soil properties in centimetre scale), which occurred in the plateau generated by the occurrence of low-high salinity patches. These patches were associated to the desiccation cracks (*Cracks* zones) at the tailings' plateau and have been already described by other authors at several tailings materials (Craw et al., 1999; Deschamps et al., 2008). The cracks at Agustin and Mercader tailings had several metres depth, around 5 cm width and were filled by friable and coarse grained sandy materials. This description fits with the one that Craw et al. (2002) gave for polygonal desiccation cracks found on sulphide concentrate tailings at New Zealand. These desiccation cracks may create discontinuities in tailings properties in terms of pH, Eh, ion content, mineralogy, etc. (Craw et al., 1999) and therefore generate patches where plants may have better/worse conditions to grow. In fact, several authors have pointed out the importance of microsites to explain the pioneer vegetation establishment on mine tailings. For instance, Shu et al. (2005) affirmed that microsites with low metal concentrations or high nutrient content generated by external depositions (animals, erosion from surroundings, domestic refuses, etc.) might have contributed to the growth of several plant species on lead/zinc tailings located on Southern China. According to Skousen et al. (1994), favourable microsites on tailings could include micro-ridge or -depressions or just tiny patches with different moisture content, acidity, etc.

Samples from *Cracks-Silty* zones (40% silt, 40% clay) contained higher Cl^-, SO_4^{2-}, Mg^{2+} and Na^+ water extractable concentrations than *Cracks-Sandy* (<10% clay, 40% sand) ones. As mineralogy description showed, *Cracks-Sandy* and *-Silty* samples contained comparable amounts of gypsum. It is known that gypsum may show higher solubility under increasing Cl^- concentrations (Kaveh et al., 2011) which might explain that water extractable SO_4^{2-} concentrations were higher in *Crack-Silty* samples than in *Cracks-Sandy* ones. Better drainage in the latter may have also promoted lower salt concentrations. The lower salinity in *Cracks-Sandy* areas may have facilitated pioneer vegetation to grow in, while *Cracks-Silty* sites, due to their worse edaphic properties, remained bare. This indicates that the identification of microsites with favourable edaphic properties is critical to achieve the successful establishment of pioneer vegetation.

Table 4.4. Correlation coefficients between ecological indexes and soil properties. "*" indicates level of significance at 0.05. "**" indicates level of significance at 0.01.

Soil Parameter			***R***	***H'***	***J'***
pH			0.557**	0.562**	0.532**
$CaCO_3$		%	0.21	0.229	0.375**
Electrical Conductivity		$dS\ m^{-1}$	-0.752**	-0.735**	-0.673**
Organic Carbon		$g\ kg^{-1}$	0.695**	0.526**	0.207
Total Nitrogen			0.763**	0.624**	0.287*
Dissolved Organic Carbon		$mg\ kg^{-1}$	0.639**	0.514**	0.227
Clay		%	0.055	-0.043	-0.301*
Silt			-0.152	-0.253*	-0.333**
Sand			0.057	0.165	0.347**
Total Element Concentrations	As	$mg\ kg^{-1}$	-0.357**	-0.365**	-0.390**
	Cd		0.128	0.169	0.286*
	Cu		-0.309**	-0.273*	-0.173
	Fe		-0.578**	-0.522**	-0.320**
	Mn		-0.184	-0.106	0.111
	Ni		0.397**	0.355**	0.249*
	Pb		-0.394**	-0.424**	-0.486**
	S		-0.557**	-0.504**	-0.436**
	Sb		-0.533**	-0.537**	-0.484**
	Zn		0.06	0.118	0.268*
Water extractable metals	Cu	$mg\ L^{-1}$	-0.234*	-0.285*	-0.392**
	Mn		-0.315**	-0.354**	-0.423**
	Pb		-0.280*	-0.317**	-0.386**
	Zn		-0.356**	-0.399**	-0.477**
Water extractable ions (anions and major cations)	Cl^-	$mg\ L^{-1}$	-0.479**	-0.532**	-0.605**
	SO_4^{2-}		-0.885**	-0.797**	-0.596**
	Na^+		-0.474**	-0.526**	-0.591**
	K^+		-0.136	-0.178	-0.264*
	Ca^{2+}		-0.763**	-0.602**	-0.283*
	Mg^{2+}		-0.587**	-0.622**	-0.655**

4.5. Conclusions

Pioneer plants distribution at the tailings was driven mainly by salinity while metal(loid)s concentrations might play a minor role.

The presence of edaphic niches with contrasting soil properties for plant growth may have promoted the selective establishment of pioneer vegetation in the studied mine tailings. These niches are produced by sedimentation or geomorphology features of tailings deposits, generating areas with extreme soil properties including, pH, salinity or texture, which condition plant growth. Techniques of tailings remediation, based on phytostabilisation procedures, should identify and characterise these niches prior to ploughing or the addition of soil amendments in order to reach a suitable plant establishment.

Late successional plant species were found as part of fertility islands at tailings' plateau showing that plant natural succession within the tailings might be successful in achieving potential vegetation. Further research will focus on soil microbiology and ecophysiology issues which allow pioneer vegetation to evolve from disperse single plants to form high density vegetation patches.

4.6.Acknowledgements

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CHAPTER 5. Usefulness of pioneer vegetation for the phytomanagement of metal(loid) enriched tailings: grasses vs. shrubs vs. trees.

Chapter 5

Usefulness of pioneer vegetation for the phytomanagement of metal(loid)s enriched tailings: grasses vs. shrubs vs. trees

Isabel Párraga-Aguado, José Ignacio Querejeta, María Nazaret González-Alcaraz, Francisco J. Jiménez-Cárceles, Héctor M. Conesa

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Abstract

The goal of this work was to assess the selection of the most suitable combination of plant species for the phytomanagement of mine tailings, by comparing among different plant life-forms (grasses, shrubs and trees). A comparison on induced rhizosphere changes generated by four plant species (the grass *Piptatherum miliaceum*, the shrub *Helichrysum decumbens*, and the trees, *Pinus halepensis* and *Tetraclinis articulata*) and high density vegetation patches (fertility islands) at a mine tailings pond located at Southeast Spain and the description of their physiological status employing stable isotopes analyses were carried out. The edaphic niches for plant growth were determined by salinity, organic matter and total soil nitrogen while metal(loid)s concentrations played a minor role. Induced changes in plant rhizospheres had a significant impact in soil microbiology. While grasses and shrubs may play an important role in primary ecological succession, trees seem to be the key to the development of fertility islands. The low $\delta^{15}N$ values (-8.00‰) in *P. halepensis* needles may reflect higher ectomycorrhizal dependence. Large differences in leaf $\delta^{18}O$ among the plant species indicated contrasting and complementary water acquisition strategies. Leaf $\delta^{13}C$ values (-27.6‰) suggested that *T. articulata* had higher water use efficiency than the rest of species (-29.9‰). The implement of a diverse set of plant species with contrasting life forms for revegetating tailings may result in a more efficient employment of water resources and a higher biodiversity not only in relation to flora but soil microbiology too.

5.1. Introduction

Phytomanagement by phytostabilisation has been proposed as a suitable tool to control erosion and metal(loid) enriched leachates in metalliferous mine tailings (Robinson et al., 2009). This technique is based on the ability of plants to fix the soil and immobilize metal(loid)s within the rhizosphere. For this purpose, suitable plants to carry out phytostabilisation projects at mine tailings must be able to grow under harsh edaphic conditions, such as high metal(loid) concentrations, low pH, low water holding capacity, low nutrient content and, additionally, in semiarid regions, drought and salinity (Conesa and Schulin, 2010). Local ecotypes which spontaneously colonise metal(loid)s wastes are considered a useful tool to be employed at phytostabilisation of mine tailings (Mendez and Maier, 2008). However, the biodiversity of this pioneer vegetation is generally constricted by the aforementioned edaphic limitations, resulting in a few tolerant plant species which spread in these environments due to the lack of competitors (Macnair, 1987). These plant species may include, among others, weeds, grasses and opportunist species which create a "functional limited" ecosystem with low capacity of reaching a self-sustaining cycle (Párraga-Aguado et al., 2013a), and consequently, external factors such as drought or short rainfall events may compromise their long-term sustainability.

The establishment of sustainable plant communities which mimic the diversity of surrounding flora must be the goal in the restoration of a degraded site (Jefferson, 2004). Recent approaches on this topic point out the importance of combinations or assemblages among species (Párraga-Aguado et al., 2013a). In this way, the employment of species with different ecological functionality may assure long-term sustainability better than monospecific or few plant species combinations. For instance, grasses may provide fast growing while trees support a better soil protection against erosion (Párraga-Aguado et al., 2013b). The understanding of soil parameters which condition plant growth at mine tailings is a critical issue for the assessment of their phytostabilisation (e.g. to provide the most suitable amendment, to select the most feasible plant species combinations). Research on this topic has intensively focused in metal(loid)s availability for plants and their uptake (e.g. Conesa et al., 2009; Escarré et al., 2011). However, other parameters such as soil enzyme activity and plant isotope composition, which have been shown to have an important role as indicators of soil ecological (Fernández et al., 2012) and plant physiological (Domínguez et al., 2012) stresses, respectively, could help in the assessment of phytostabilisation schemes. For instance, dehydrogenase activity has been proposed for evaluating microbial activity in soils (García et al., 1993) while β-glucosidase is an indicator of soil organic matter decomposition (Kuperman and Carreiro, 1997). On the other hand, foliar $\delta^{13}C$ have been widely employed to characterise the Water Use Efficiency (WUE) (Ferrio et al., 2003), $\delta^{15}N$ is known to reflect the dependence of mycorrhiza interactions for N acquisition at poor N soils (Hobbie et al., 2000) and $\delta^{18}O$ may reflect the depth of soil water acquisition and/or stomatal conductance (Querejeta et al., 2006).

The goal of this work was to assess the selection of the most suitable combination of plant species for the phytomanagement of mine tailings, comparing among different plant life-forms (grasses vs. shrubs vs. trees) in relation to the nutritional status of the plants and to the role of rhizosphere for the enhancement of edaphic properties. For this purpose, and based on plant species abundance, the grass *Piptatherum miliaceum*, the shrub *Helichrysum decumbens* and the trees *Pinus halepensis* and *Tetraclinis articulata* were selected, their nutrient and stable isotope composition ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$) were studied and their rhizosphere properties were characterised, including soil enzymatic activities.

5.2. Materials and methods

5.2.1. Site description

The Cartagena-La Union Mining District (0-392 m.a.s.l.; 50 km^2; 37°37′20″ N, 0°50′55″W - 37°40′03″N, 0°48′12″W) is located on the Southeast of the Iberian Peninsula (Figure 5.1). This zone was one of the most important mining areas in Spain during the last centuries until its closure in 1991 due to economic and environmental reasons. Base metals were smelted from sulphide minerals that included galena and sphalerite. As a consequence, large volumes of wastes were generated during the mineral concentration and smelting processes and stockpiled into tailings. Nowadays, these bare tailings pose a risk to local ecosystems and human health, due to the dispersal of dust and sediments containing high metal(loid) concentrations (Conesa and Schulin, 2010).

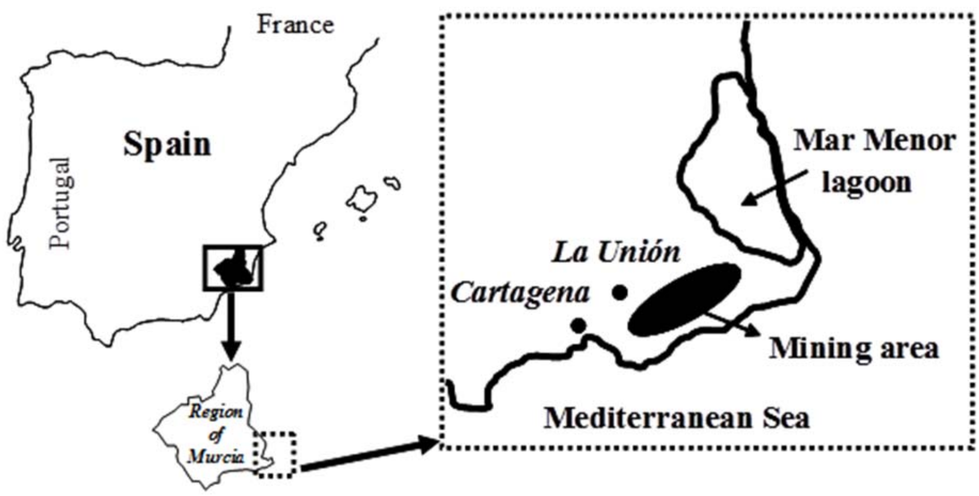


Figure 5.1. Location of studied area.

The climate of the zone is Mediterranean with annual averages of around 250-300 mm rainfall, 18 °C temperature and 850 mm evapotranspiration. The natural vegetation is composed by formations of *P. halepensis* and thicket plant species with xerophitic characteristics.

The study was conducted in spring 2012 at a mine tailings pond located at the Cartagena-La Union Mining District. This tailings pond was abandoned around 40 years ago and no man-driven revegetation works have been carried out at its surface. Nevertheless, spontaneous vegetation has colonised some parts of the tailings pond.

5.2.2. Description of selected plant species

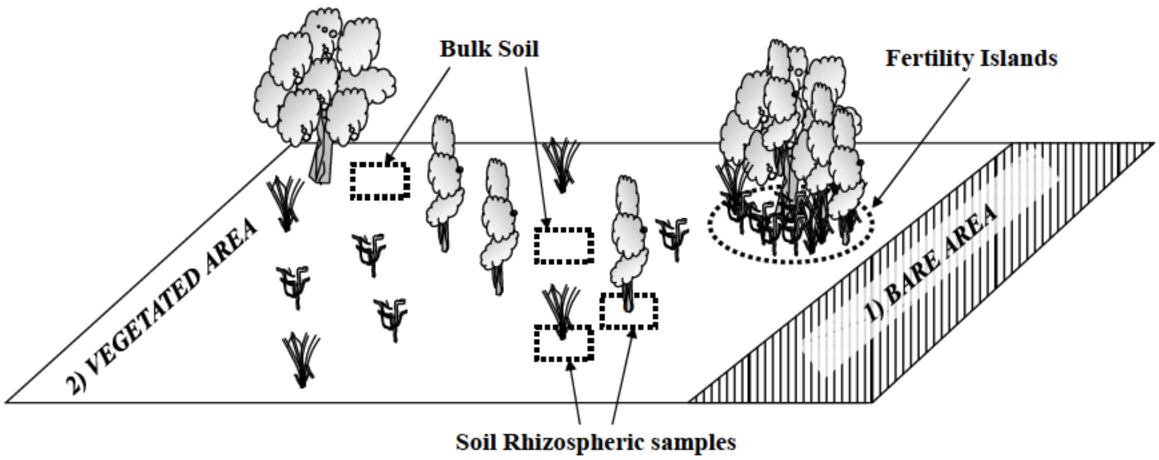
Four plant species which spontaneously grew at the tailings pond were selected. *T. articulata* (Vahl) Mast is a tree included in the red lists of threatened species (IUCN, 2012) and is considered as a very rare species according to the European Habitat Directive. The forests of *T. articulata* are protected by the Nature-2000 network and its long-term persistence requires a critical assessment (Esteve-Selma et al., 2010). *P. halepensis* Miller -Aleppo pine- is a woody plant species which has been widely employed for the restoration of degraded semiarid ecosystems in the Mediterranean area (Querejeta et al., 2008). *P. miliaceum* (L.) Cosson -smilo grass- is a widespread grass and *H. decumbens* (Lag.) Camb is a shrub, which has been found to grow in metal(loid)s enriched wastes in this area (Conesa et al., 2006).

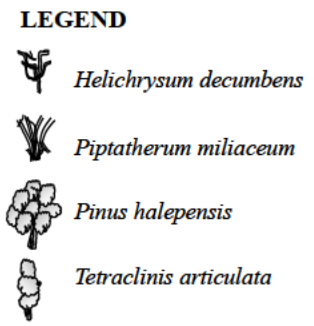
5.2.3. Soil and plant sampling

The tailings pond surface was divided in two different areas (Figure 5.2): 1) Bare Area, where no vegetation grew, and 2) Vegetated Area, where vegetation grew following two patterns of distribution: a) disperse vegetation at low soil-cover rate (plants grew isolated from each other), and b) high density vegetated patches or 'fertility islands' (>90% soil cover). The fertility islands were composed by 30-80 individuals of different plant species. The plant species composition of the three fertility islands is showed in Table 5.1.

Composite soil samples were taken from the bare area (BA, n=3). At the vegetated area, rhizospheric soil samples of *P. miliaceum* (R-Pm, n=6), *H. decumbens* (R-Hd, n=3), *T. articulata* (R-Ta, n=4) and *P. halepensis* (R-Ph, n=7) were taken, together with samples of bulk soil (BS, n=14). Finally, rhizospheric soil samples from fertility islands (FI, n=3) were also collected. All composite soil samples were taken by mixing 4 subsamples from 0 to 20 cm depth. The number of samples of rhizospheric soils and fertility islands were taken according to their occurrence at the tailings pond. For bare area and bulk soil ones, a composite sample was taken each 16 m^2.

Regarding the plant samples, the aerial parts (leaves or needles) of individuals of the four selected plant species were collected ($3 \leq n \leq 6$). No plant samples were taken from the fertility islands.





Sampling areas	Soil sample code
1) Bare area	BA
2)Vegetated area	
Bulk soil	BS
Rhizospheric soil from *Helichrysum decumbens*	R-Hd
Rhizospheric soil from *Pipthatherum miliaceum*	R-Pm
Rhizospheric soil from *Pinus halepensis*	R-Ph
Rhizospheric soil from *Tetraclinis articulata*	R-Ta
Soil from Fertility Islands	FI

Figure 5.2. Basic scheme of sampling design.

Table 5.1. Plant species composition for each one of the three fertility islands.

Scientific name	Family	Fertility Island		
		1	2	3
Asparagus horridus L. in J.A. Murray	Liliaceae		x	
Dittrichia viscosa (L.) Greuter	Compositae (Asteraceae)	x	x	x
Dorycnium pentaphyllum (Scop.)	Leguminosae			x
Helichrysum decumbens (Lag.) Camb	Compositae (Asteraceae)	x	x	x
Limonium cossonianum Kunthze, Revis.	Plumbaginaceae	x	x	
Pinus halepensis Miller	Pinaceae	x	x	x
Piptatherum miliaceum (L.) Cosson	Gramineae (Poaceae)	x	x	x
Pistacia lentiscus L.	Anacardiaceae		x	x
Rubia peregrina L.	Rubiaceae		x	
Salsola kali L.	Chenopodiaceae	*x*		
Sonchus tenerrimus L. var. *tenerrimus*	Compositae (Asteraceae)		x	
Tamarix boveana Bunge	Tamaricaceae	x	x	x
Tetraclinis articulata (Vahl) Mast.	Cupressaceae	x		
Zygophyllum fabago L.	Zygophyllaceae	x	x	x

5.2.4. Soil and plant analyses

Soil samples were air dried, sieved through 2 mm, homogenized and stored in plastic bags prior to laboratory analysis. Soil pH and Electrical Conductivity (EC) were determined in a 1:5 water/soil mixture, which was shaken for 2 h, using a Crison Basic 20 pH-meter and a Crison Basic 30 conductivity-meter, respectively. Dissolved Organic Carbon (DOC), water extractable metal(loid)s (Ernst, 1996) and ions were determined in the aforementioned 1:5 soil/water mixture after filtering through nylon membrane 0.45 mm syringe filters (WICOM). Dissolved organic carbon (DOC) was measured in an automatic analyser (TOC-VCSH Shimadzu). Water extractable metal(loid)s (As, Cd, Cu, Mn, Pb, Zn) were analysed using a ICP-MS (Agilent 7500A, detection limit 0.001 mg L^{-1}). Ions (cations: Ca^{2+}, K^{+}, Mg^{2+}, Na^{-}; and anions: Cl^{-}, SO_4^{2-}) were analysed using an Ion Chromatographer (Metrohm).

Particle size distribution was determined following the method of Bouyoucos densimeter (Gee and Bauder, 1968). Equivalent calcium carbonate was determined using the Bernard calcimeter method. Total nitrogen (TN) was determined using the Kjeldahl method (USDA, 1996). Organic carbon (OC) was determined by the oxidation of organic matter using potassium dichromate. Total element composition was measured by X-ray fluorescence (Bruker S4 Pioneer).

Soil microbiology was estimated by the determination of two enzymatic activities: dehydrogenase and β-glucosidase. Unaltered soil samples were stored at -20 °C for this purpose. Dehydrogenase activity was determined according to García et al. (1993) by measurement of INTF (iodo-nitrotetrazolium formazan) by spectrophotometry (Thermo Fisher Scientifc Multiskan GO) at 490 nm and reported as mg INTF g^{-1} dry wt^{-1} h^{-1}. β-glucosidase was determined according to the modification of Ravit et al. (2003) proposed by Reboreda and Caçador (2008) by measurement of pNP (p-Nitrophenol) by spectrophotometry at 410 nm and reported as mmol pNP g^{-1} dry wt^{-1} h^{-1}.

Plant samples were carefully washed with distilled water and dried at 65 °C for 72 h prior to grinding. For each sample, 0.1-0.5 g was incinerated followed by a redilution using concentrated nitric acid. The resulting extracts were filled to 25 ml and filtered through CHM F2041-110 ashless filter papers (20-25 μm pore diameter). Then, metal(loid)s (As, Cd, Cu, Mn, Pb, Zn) were analysed using a ICP-MS (Agilent 7500A, detection limit 0.001mg L^{-1}) and Ca, K, Mg, Cl, P and S were analysed using an Ion Chromatographer (Metrohm).

Plant analyses were referenced using a CTA-VTL-2 certified material (Virginia tobacco leaves). The percentage of recoveries were 110% for As, 89% for Cd, 119% for Cu, 104% for Mn, 96% for Pb and 100% for Zn.

Finely ground plant material was used for stable isotope measurements at the University of California-Davis Stable Isotope Facility. Leaf N, $\delta^{13}C$ and $\delta^{15}N$ analyses were conducted using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). $\delta^{13}C$ and $\delta^{15}N$ data are expressed relative to international standards V-PDB (Vienna PeeDee Belemnite). Leaf $\delta^{18}O$ analyses were performed using an elemental PyroCube (Elemental Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The final delta values are expressed relative to international standard V-SMOW (Vienna Standard Mean Ocean Water).

5.2.5. Statistics

Statistical analysis consisting of analysis of variance one way ANOVA with Tukey's Test (p<0.05) and Pearson's correlations (p<0.01) were carried out using SPSS 19.0.0 software (SPSS, 2010). Differences at p<0.05 level were considered significant. Environmental gradients were examined by a Principal Component Analysis (PCA) using the 'CANOCO for Windows' program v4.02 (ter Braak and Smilauer, 1999).

5.3. Results

5.3.1. Soil analyses

Soil parameters are shown in Table 5.2. The soil pH values ranged from slightly acid, in the case of BA samples (6.5), to slightly alkaline, for the R-Hd ones (7.5). Bare Area samples showed higher EC values (p<0.05, 5 dS m^{-1}) than the rest of samples. The rhizospheric soil samples (including FI) and the BS ones showed at least 10 fold higher $CaCO_3$ concentrations than BA samples.

Table 5.2. Results of soil samples analyses. Data are average ± standard error. $3 \leq n \leq 14$. "n.d." means not detected. Different letters within the same row indicate significant differences (ANOVA with Tukey Test, $p < 0.05$). "EC" is Electrical Conductivity; "OC" is Organic Carbon; "DOC" is Dissolved Organic Carbon; "TN" is Total Nitrogen; "BA" is Bare Area; "BS" is Bulk Soil; "FI" is Fertility Islands; "R-Xy" are soil rhizospheric samples of the corresponding plant species: Hd is *Helichrysum decumbens*; Pm is *Piptatherum miliaceum*; Ph is *Pinus halepensis* and Ta is *Tetraclinis articulata*.

Soil parameter		Units	Samples						
			BA	**BS**	**R-Hd**	**R-Pm**	**R-Ph**	**R-Ta**	**FI**
pH (1:5)			6.4 ± 0.1 a	7.2 ± 0.1 b	7.5 ± 0.1 b	7.1 ± 0.2 a,b	7.3 ± 0.1 b	7.3 ± 0.1 b	7.1 ± 0.1 b
EC (1:5)		dS m^{-1}	5 ± 0.8 c	2.9 ± 0.1 a,b	3.3 ± 0.3 b	3.4 ± 0.1 b	1.7 ± 0.4 a	2.6 ± 0.1 a,b	2.8 ± 0.1 a,b
$CaCO_3$		g kg^{-1}	4.5 ± 2 a	37.8 ± 6.2 a,b	78 ± 15 b	41.9 ± 7.6 a,b	71 ± 10 b	59.8 ± 15 a,b	46.2 ± 3.7 a,b
OC		g kg^{-1}	1.14 ± 0.3 a	3.04 ± 0.2 a,b	3.68 ± 0.3 b	3.14 ± 0.6 a,b	5.3 ± 0.6 b,c	4.46 ± 0.6 b,c	6.65 ± 0.5 c
DOC		mg kg^{-1}	7.19 ± 0.3 a	17.1 ± 1.8 b	15 ± 0.6 a,b	18.4 ± 2.7 b	50 ± 9.1 c,d	28.4 ± 2 b,c	66.5 ± 14 d
TN		g kg^{-1}	0.18 ± 0 a	0.17 ± 0 a	0.39 ± 0.1 a,b	0.33 ± 0.1 a,b	0.3 ± 0 a,b	0.28 ± 0.1 a,b	0.62 ± 0.2 b
Sand		%	62.6 ± 8.1 a	73 ± 2.9 a,b	73.6 ± 8 a,b	71.5 ± 3.9 a,b	86 ± 1.6 b	78.8 ± 4.6 a,b	75.4 ± 2.6 a,b
Silt			28.6 ± 6.1 b	25.2 ± 2.8 a,b	23.4 ± 7.5 a,b	25 ± 2.9 a,b	12 ± 1.3 a	19.2 ± 5 a,b	21 ± 1.2 a,b
Clay			8.8 ± 2 b	1.8 ± 0.3 a	3 ± 0.5 a,b	3.5 ± 1.1 a,b	2.3 ± 0.7 a	2 ± 1 a	3.6 ± 1.7 a,b
Water extractable ions (1:5)	**Cl^-**	mg L^{-1}	383 ± 152 b	10.8 ± 2.2 a	14.2 ± 6.3 a	11.3 ± 1.1 a	13 ± 1.7 a	9.7 ± 0.4 a	11.4 ± 3.4 a
	SO_4^{2-}		2280 ± 195 b	1620 ± 28 b	1630 ± 30 b	1590 ± 15 b	861 ± 270 a	1520 ± 37 b	1640 ± 21 b
	Ca^{2+}		610 ± 7 a	631 ± 3 a	632 ± 7 a	634 ± 2 a	346 ± 110 a	627 ± 16 a	614 ± 21 a
	K^+		19.8 ± 3.1 c	5.1 ± 0.9 a	12.1 ± 4 a,b,c	14 ± 2 b,c	7.1 ± 0.4 a,b	7.4 ± 0.4 a,b	12.9 ± 3 a,b,c
	Mg^{2+}		198 ± 54 b	28.5 ± 8.6 a	30.1 ± 10 a	17.9 ± 3.4 a	18 ± 3.8 a	10.5 ± 2.3 a	27.9 ± 10 a
	Na^+		289 ± 92 b	12.4 ± 2.7 a	15.7 ± 5.7 a	11.9 ± 1 a	16 ± 3.5 a	10.6 ± 0.5 a	13.9 ± 5.1 a
β-Glucosidase		µmol p-NP g^{-1} h^{-}	n.d.	0.02 ± 0 a	0.21 ± 0.1 a,b	0.28 ± 0 b	0.2 ± 0 a,b	0.31 ± 0.1 b,c	0.51 ± 0.2 c
Dehydrogenase		µg INTF g^{-1} h^{-1}	n.d.	0.04 ± 0 a	0.5 ± 0 b	0.63 ± 0.1 b,c	0.5 ± 0 b	0.63 ± 0 b,c	0.85 ± 0.1 c

Table 5.3. Total and water-extractable concentrations for several elements. Data are average ± standard error (3 ≤ n ≤ 14.). Different letters at the same column means significant differences ($p < 0.05$, ANOVA with Tukey test, except * which were compared by T-test). All units are mg kg^{-1}. Detection limit was 0.005 mg kg^{-1} for water extractable measurements. "BA" is Bare Area; "BS" is Bulk Soil; "FI" is Fertility Islands; "R-Xy" are soil rhizospheric samples of the corresponding plant species: Hd is *Helichrysum decumbens*; Pm is *Piptatherum miliaceum*; Ph is *Pinus halepensis* and Ta is *Tetraclinis articulata*.

Samples	**Element**					
	As	**Cd**	**Cu**	**Mn**	**Pb**	**Zn**
Total metal concentrations						
BA	360 ± 44 a	50 ± 4 a	127 ± 2 a	9080 ± 564 a	10,800 ± 943 b	14,100 ± 213 a
BS	561 ± 122 a	58 ± 7 a	129 ± 5 a	9360 ± 288 a	8190 ± 750 a,b	13,200 ± 988 a
R-Hd	564 ± 182 a	30 ± 4 a	109 ± 8 a	10,200 ± 418 a	5370 ± 1897 a	8600 ± 1336 a
R-Pm	507 ± 126 a	38 ± 8 a	109 ± 8 a	10,400 ± 545 a	5660 ± 787 a,b	9840 ± 1344 a
R-Ph	650 ± 63 a	36 ± 4 a	109 ± 8 a	9760 ± 312 a	5900 ± 969 a,b	9250 ± 216 a
R-Ta	417 ± 63 a	22 ± 9 a	94 ± 8 a	10,800 ± 803 a	4720 ± 650 a	8560 ± 1356 a
FI	941 ± 30 a	57 ± 3 a	117 ± 3 a	10,500 ± 249 a	7370 ± 439 a,b	11,000 ± 125 a
Water extractable concentrations						
BA	0.005 ± 0.001 a	0.026 ± 0.007 b	<0.005	0.050 ± 0.021 a	0.020 ± 0.006 a	0.874 ± 0.152 a,b
BS	0.008 ± 0.002 a,b	0.019 ± 0.003 a,b	<0.005	0.888 ± 0.655 a	0.018 ± 0.004 a	1.286 ± 0.241 b
R-Hd	0.015 ± 0.010 a,b	0.005 ± 0.000 a	<0.005	0.115 ± 0.029 a	<0.005	0.375 ± 0.044 a
R-Pm	0.010 ± 0.003 a,b	0.014 ± 0.006 a,b	<0.005	0.492 ± 0.252 a	<0.005	0.839 ± 0.307 a,b
R-Ph	0.011 ± 0.003 a,b	<0.005	0.009 ± 0.004 a*	0.142 ± 0.036 a	0.014 ± 0.004 a	0.348 ± 0.066 a,b
R-Ta	<0.005	0.006 ± 0.001 a	<0.005	0.066 ± 0.015 a	<0.005	0.352 ± 0.067 a,b
FI	0.017 ± 0.001 b	0.006 ± 0.000 a	0.028 ± 0.009 b*	0.192 ± 0.076 a	<0.005	0.374 ± 0.027 a,b

The FI samples showed better soil fertility parameters ($p<0.05$, higher OC, TN and DOC) than BA and BS ones (e.g. DOC and TN were up to 3-fold higher). However, when considering the samples of the individual plant rhizospheres, these parameters did not show a clear pattern. For instance, TN concentrations for BA and BS samples showed comparable values to those ones obtained for the individual plant rhizospheres ($p>0.05$). In relation to OC and DOC, the individual rhizospheric samples from the two studied trees (R-Ph and R-Ta) showed higher values ($p<0.05$, at least 4-fold higher) than the ones obtained for BA samples. However, these differences did not occur ($p>0.05$) when comparing the latter with those OC and DOC concentrations measured, respectively, for the rhizosphere of the grass (R-Pm) and the shrub (R-Hd). All the rhizospheric soil samples of individual plants showed similar OC and DOC concentrations to BS samples (except DOC at R-Ph).

According to the particle size distribution data, BA samples were classified as sandy loam while the rest of samples were considered as loamy sand.

The concentrations of water extractable Cl^-, Mg^{2+} and Na^+ obtained for BA samples were between 5 and 10 fold higher than the ones obtained for the rest of the samples. All the samples showed similar water extractable Ca^{2+} concentrations ($p>0.05$), while for SO_4^{2-}, BA samples showed higher concentrations (at least 10% more) than the rest. Water extractable K^+ occurred in the range of 5-20 mg L^{-1}.

Dehydrogenase and β-glucosidase could not be detected at BA samples. For both enzymatic activities, the obtained values for FI samples were of around 20-fold higher than the ones obtained for BS samples. The rhizospheric soil samples from the individual plants showed intermediate values between those ones measured for BS and FI samples.

Total As and metal concentrations are shown in Table 5.3. Significant differences only occurred for Pb when comparing BA samples (10,800 mg kg^{-1} Pb) with R-Ta ones (4720 mg kg^{-1} Pb). The highest concentrations of total As and Zn were found at FI (940 mg kg^{-1} As) and BA samples (14,100 mg kg^{-1} Zn), respectively. Cadmium ranged from 22 to 58 mg kg^{-1} while Cu and Mn showed concentrations of around 100 mg kg^{-1} and 10,000 mg kg^{-1}, respectively.

Water extractable As and metals are shown in Table 5.3. Only for Mn and Zn all the samples showed values over the detection limit (0.005 mg kg^{-1}). These metals also showed the highest concentrations for all the studied elements. For instance, water extractable Mn and Zn concentrations at BS samples were 0.88 mg kg^{-1} and 1.30 mg kg^{-1}, respectively. Water extractable Cu was only detectable in R-Ph (0.009 mg kg^{-1}) and FI (0.028 mg kg^{-1}) samples.

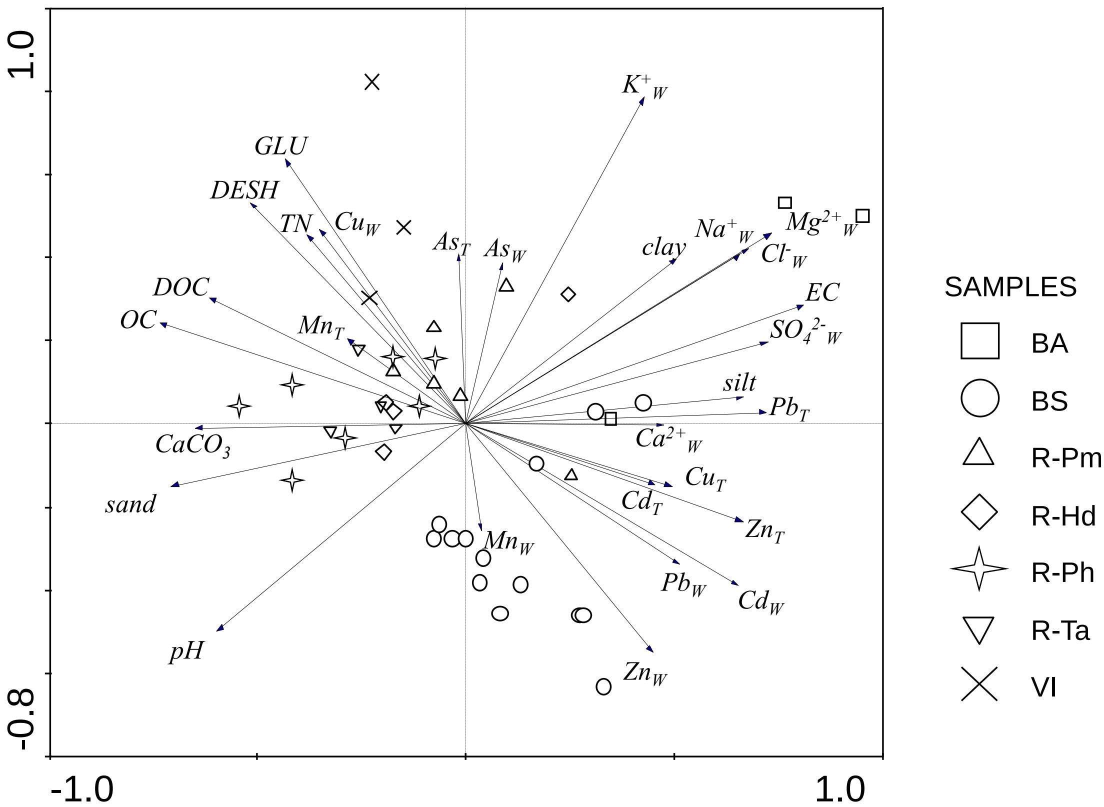


Figure 5.3. PCA results for soil parameters. "M_T" are total element concentrations; "M_W" are water extractable ion concentrations; "OC" is Organic Carbon; "TN" is Total Nitrogen; "DOC" is Dissolved Organic Carbon; "DESH" is dehydrogenase activity; "GLU" is β-glucosidase activity. "BA" is Bare Area; "BS" is Bulk Soil; "FI" is Fertility Islands; "R-Xy" are soil rhizospheric samples from the corresponding plant species: Hd is *Helichrysum decumbens*; Pm is *Piptatherum miliaceum*; Ph is *Pinus halepensis* and Ta is *Tetraclinis articulata*.

The PCA results for soil parameters are shown at Figure 5.3. The Factor 1, which explained 31.3% of the variance, was conditioned by fertility (OC and DOC), $CaCO_3$ concentrations, EC (including water extractable anions) and total metal content (specially Pb and Zn). The factor 2, was defined by soil enzymatic activities, TN and water extractable metals.

5.3.2. Plant analyses

Leaf elemental and stable isotopes composition are shown in Table 5.4. The leaves of the grass *P. miliaceum* showed around 2-fold higher N concentrations (p<0.05) than the rest of studied species. The leaves of *T. articulata* contained 2-fold the P concentrations measured at *P. halepensis* ones, being *P. miliaceum* and *H. decumbens* in between. The latter showed the highest foliar K concentrations, which were at least 3-fold higher than the ones measured in the rest of studied species.

The Ca concentrations obtained for the two trees (*P. halepensis* and *T. articulata*) were the double that the ones measured for *P. miliaceum* and *H. decumbens*. Magnesium measured at the *T. articulata* leaves (~5000 mg kg^{-1}, p<0.05) showed higher concentrations than for the rest of studied species (1800-2200 mg kg^{-1}). Sulphur was specially accumulated by the leaves of *P. miliaceum* (~5500 mg kg^{-1}).

The highest foliar Cu (~7 mg kg^{-1}), and Mn (259 mg kg^{-1}) concentrations occurred in *H. decumbens* and *P. halepensis*, respectively. For Zn, all the species showed comparable concentrations (p>0.05).

For the non-essential elements, leaves of *H. decumbens* showed the highest concentrations of As (3.43 mg kg^{-1}), Cl (~4500 mg kg^{-1}) and Pb (3.8 mg kg^{-1}) while for Cd, this occurred at the leaves of *T. articulata* (2.8 mg kg^{-1}).

Table 5.4. Leaf elemental and isotopic composition in leaves and needles of the studied plant species. Data are average ± standard error (3 ≤ n ≤ 6) Different letters at the same row means significant differences ($p < 0.05$, ANOVA with Tukey test).

Parameter		Units	Samples							
			Helichrysum decumbens		*Piptatherum miliaceum*		*Pinus halepensis*		*Tetraclinis articulata*	
Essential Elements	**N**	g kg^{-1}	13 ± 1	a	23 ± 1	b	9 ± 1	a	11 ± 0	a
	P	mg kg^{-1}	497 ± 25	a,b	488 ± 34	a,b	317 ± 35	a	617 ± 99	b
	K		14,972 ± 1963	c	5727 ± 584	b	1233 ± 237	a	5998 ± 628	b
	Ca		1119 ± 131	a	1324 ± 186	a,b	2311 ± 368	b	2316 ± 318	b
	Mg		1837 ± 69	a	2189 ± 229	a	1784 ± 123	a	4970 ± 1410	b
	S		2989 ± 163	a	5495 ± 541	b	3103 ± 354	a,b	4544 ± 1508	a,b
	Cu		6.97 ± 0.33	c	4.76 ± 0.65	b,c	2.28 ± 0.17	a	3.46 ± 0.09	a,b
	Mn		103 ± 25	a	86 ± 29	a	259 ± 45	b	152 ± 24	a,b
	Zn		132 ± 27	a	77 ± 7	a	127 ± 5	a	151 ± 55	a
Non-essential elements	**As**	mg kg^{-1}	3.43 ± 0.36	b	1.13 ± 0.28	a	1.44 ± 0.29	a	1.00 ± 0.43	a
	Cd		0.39 ± 0.23	b	0.09 ± 0.03	a	0.38 ± 0.05	b	2.82 ± 0.49	c
	Cl		4475 ± 1039	c	1031 ± 275	a,b	445 ± 106	a	2644 ± 1065	b,c
	Pb		38.4 ± 3.1	b	5.4 ± 1.2	a	12.0 ± 2.3	a	21.4 ± 11.7	a,b
Leaf isotopic composition	**$\delta^{13}C$**	‰	-29.95 ± 0.12	a	-29.94 ± 0.26	a	-29.97 ± 0.22	a	-27.57 ± 0.25	b
	$\delta^{15}N$		-3.94 ± 0.60	b	-2.84 ± 0.72	b	-8.00 ± 0.38	a	-4.00 ± 1.16	b
	$\delta^{18}O$		34.53 ± 0.45	b	39.09 ± 0.92	c	28.42 ± 0.43	a	32.42 ± 0.93	b

In relation to $\delta^{13}C$, *P. miliaceum*, *H. decumbens* and *P. halepensis* showed values around -30‰ which were significantly lower ($p<0.05$) than the ones obtained for *T. articulata* (-27.57‰). For $\delta^{15}N$, the lowest values occurred for the needles of *P. halepensis* (-8.00‰) which significantly differed from the levels measured at the rest of studied species. In relation to $\delta^{18}O$, the needles of *P. halepensis* showed the lowest values (28.42‰) while the highest occurred for the leaves of *P. miliaceum* (39.09‰).

5.4. Discussion

5.4.1. Edaphic niches for plant growth at the tailings and influence of plant rhizospheres

According to Matias et al. (2009) the generation of edaphic niches where plants may grow and facilitate the enhancement of soil microbiology is a critical factor for the feasibility of soil revegetation works. In the case of tailings, pioneer vegetation may primary colonize from niches where soil properties are more favourable for plant growth. Thus, identifying these niches is critical to get satisfactory rates of plant survival in the phytomanagement of mine tailings. At the studied tailings pond, the non-rhizospheric samples (BA and BS) showed similar metal(loid) concentrations (total and water extractable) but differed in other soil properties such as pH or EC values. These differences in EC were reflected on the higher concentrations of water extractable ions (specially Cl^- and SO_4^{2-}) of BA samples (Figure 5.3) in relation to BS ones. Then, we may explain the absence of spontaneous vegetation in BA areas in relation to BS ones as a combination of different soil stress factors including, pH, salinity, ion specific tolerance or low fertility and where metal(loid) concentrations may play a minor role. This agreed with the findings of several authors (Conesa et al., 2006; Párraga-Aguado et al., 2013a) which considered salinity and water soluble salts as critical parameters which conditioned the establishment and distribution of pioneer vegetation at tailings. The shift for better soil fertility from BA to BS soils is mainly caused by a lower EC (60% lower) and higher pH (from 6.4 to 7.2, probably due to the increase in $CaCO_3$ concentrations) but also, and although not significant, by slightly increases in OC and DOC (which may favour the occurrence of background levels of enzymatic activities). These changes in tailings' characteristics may have favoured the establishment of pioneer vegetation at the BS area. Once pioneer vegetation is established, the enhancement of soil properties takes place within plant rhizospheres through the release of organic substances by roots (which increases soil organic matter) and the enhancement of soil microbiology (Wenzel, 2009). Thus, the enzymatic activities were positively correlated with DOC, OC and TN (e.g. for β-glucosidase r~0.5 p<0.01 for OC and DOC and r~0.6 p<0.01 for TN; Figure 5.3) which showed the strong dependence of soil microbiology to organic matter decomposition. This agreed with previous authors who affirmed that in polluted soils the competition for soil nitrogen (Unterbrunner et al., 2007) or DOC (Song et al., 2012) represent limiting factors for microorganisms. It is noticeable that although the increases of OC, DOC and TN in most of the individual plant rhizospheres compared to BS were less than 2-fold, soil microbiology improvement, measured by enzymatic activities, was at least of 10-fold. This showed that a slight improvement in soil fertility parameters at a metal(loid) enriched tailings may induce a significant improvement of soil microbiology. Therefore, and as several authors have shown, the understanding of microbial activity dynamics in metal polluted soils is not only explained by direct toxicity on microorganisms but also by the lower input of organic matter due to the absence of vegetation (Kuperman and Carreiro, 1997; Konopka et al., 1999). In relation to the effect of metal(loid)s on enzymatic activities, and as it is shown at Figure 5.3, all the water extractable metals (except Cu) showed a negative correlation. It is known that high metal(loid)s (e.g. As, Cd, Pb) concentrations in soils may alter the functional stability of microbial communities, favouring detoxification instead of growth or reproduction processes (Tobor-Kaplon et al., 2005). This may be reflected in the decrease of respiration rates and enzymatic activities (Marzadori et al., 1996) and in changes in the structure of the microbial community, containing a lower number of microorganisms but with higher resistance to disturbances (Konopka et al., 1999). The positive correlation of the enzymatic activities with water extractable Cu (e.g. $r>0.5$, $p<0.01$ for β-glucosidase) may be explained by the lower toxicity of this metal for soil microbiology (Bernard et al., 2009).

The differences, in terms of soil fertility and microbiology, among the rhizospheres of the four plant species considered were not significant in most of cases (only DOC at R-Ph was higher than for the rest of plant species). The fertility islands showed closer soil fertility indicators to the two studied trees (*P. halepensis* and *T. articulata*) than the other two plant species studied (*H. decumbens* and *P. miliaceum*), especially when comparing OC, DOC and β-glucosidase. This may suggest that trees might act as precursors of the fertility islands (e.g. favouring the establishment of vegetation under the canopy) while grasses and shrubs may have a more important role as pioneer colonizers of the tailings.

Comparatively, the rhizospheres of the fertility islands (which are the result of the assemblage of grasses, shrubs and trees) did not contain the sum of the individual plant species contribution. This point up that the shift for the establishment of fertility islands from individual plants would not require high inputs of nutrients. In fact, individual plant rhizospheres counted around 40-80% of the values of OC, DOC or TN measured at the fertility islands. We may hypothesize that the edaphic improvement in plant rhizospheres (especially in trees) may enhance soil parameters till surpassing a "facilitating threshold" which allows the germination and further establishment of a more diverse number of plant species within the fertility islands.

On the other hand, when comparing FI samples with BS ones, all the soil fertility parameters considered (including pH, EC, OC, DOC, TN and the two enzymatic activities) showed significant differences. This demonstrates that natural recolonisation of the tailings by spontaneous vegetation may improve significantly soil fertility indicators and this is strongly linked with ecological successional processes which may lead to the formation of fertility islands. These fertility islands may be considered a key factor in the revegetation of tailings since they support high biodiversity and therefore may provide higher resilience against environmental stresses.

5.4.2. Plant nutrient and metal(loid) concentrations

P. halepensis is a tree species which has been widely used in the restoration of degraded semiarid areas in the Mediterranean region due to its drought resistance and low nutrient requirements (Querejeta et al., 2008). However, its employment for revegetating mining polluted soils is not extended yet since its tolerance to metal(loid)s has not been completely shown. Several studies worldwide (Sun et al., 2009; Kord et al., 2010) have used pine trees for biomonitoring the metal pollution in soils by means of metal uptake into needles showing concentrations (3-10 mg kg^{-1} Cu, 10-50 mg kg^{-1} Pb, 100-200 mg kg^{-1} Zn) in the range of the ones obtained in this work. It is noticeable to point out, that in the present work pine trees were directly growing in the tailings and not in areas with diffuse pollution, which might imply some level of tolerance.

The metal(loid)s tolerance of *T. articulata* has not been comprehensively studied. For instance, Disante et al. (2010) working under lab conditions and hydroponics, showed high toxicity in *T. articulata* seedlings when growing under solutions with increasing Zn concentrations (up to 90 mM ~ 5.88 mg L^{-1}) concluding that this plant species could not be adequate to restore metal polluted soils. However, the high mycorrhization potential of *T. articulata* under field conditions may modify this behaviour and therefore improve their metal(loid) tolerant properties (Fernández et al., 2012). In addition, this plant species showed a specific affinity for uptaking Cd into leaves (5-fold higher in relation to *P. halepensis*), which should be taken into account for further research in Cd-polluted ecosystems.

Zinc and Pb concentrations obtained in this study for *H. decumbens* were lower (1.5 fold for Zn and 9 fold for Pb) than those found by Conesa et al. (2006) in acidic tailings of the same mining district, while Cu concentration was in the same order. Other similar plant species had shown different behaviour in metal accumulation. For instance, Leita et al. (1989) found higher Cd concentration in shoots of *Helichrysum italica* growing in a mining site at SW Sardinia while Pratas et al. (2013) found lower concentration of Zn (60 mg kg^{-1}) and Pb (10 mg kg^{-1}) and similar of Cu (6 mg kg^{-1}) for *Helichrysum stoechas* growing in an abandoned Pb mine in Central Portugal.

P. miliaceum showed the lowest metal(loid) uptake of the four species studied (except for Cu). However, this plant species has shown high ability for accumulating metals (Pb, above all) under lab conditions (Conesa et al., 2009).

The ratios N:P:K in plant leaves have been employed to evaluate the existence of nutrient co-limitation. Attending to the thresholds proposed by Olde-Venterink et al. (2003), N:P > 14.5 and K:P > 3.4 (which is obtained in all the plant studied in the present work) may indicate N and P co-limitation. The large differences in leaf stoichiometry among the plant species colonizing the mine tailings (e.g. in the C:N:P:K ratios of foliar tissue) will tend to increase diversity in the chemical composition of litter inputs to soil. Chemically diverse litter inputs to soil are expected to foster the diversity, abundance and activity of invertebrate and microbial decomposers in the soil, which in turn should enhance organic matter turnover and biogeochemical cycling rates. For example, soil respiration and net N mineralization rates often correlate positively with the chemical diversity of litter mixtures (Meier and Bowman, 2008). The diversity of plant litter species and decomposer organisms may have important feedback effects to plant growth, community composition and ecosystem processes (Hättenschwiler et al., 2005).

5.4.3. Plant stable isotope composition

The employment of stable isotope data analysis to describe the response of plant species to environmental changes has been widely included in plant physiology studies (Querejeta et al., 2006, 2008; Domínguez et al., 2012). At the present study, it was assumed that differences due to changes in climate or water availability conditions were negligible and then, the differences could be attributed to the physiology of the different species. According to ecological theory, colonization of the mine tailings by a taxonomically and functionally diverse set of plant species representing contrasting life forms (perennial grass, small shrub, small tree, large tree) should enhance key ecosystem processes including primary productivity, nutrient retention, and resilience against disturbance (Hooper et al., 2005). For example, large differences in leaf $\delta^{18}O$ among the plant species colonizing the mine tailings indicated that they may have widely contrasting water use strategies, likely including differences in depth of water uptake and in degree of stomatal control of transpiration (Moreno-Gutiérrez et al., 2012). *P. miliaceum* showed higher leaf $\delta^{18}O$ values indicating utilization of water stored in shallow soil layers, which is subjected to intense evaporative isotopic fractionation and enrichment in this semiarid environment (Barnes and Allison, 1983). The high leaf $\delta^{18}O$ of *P. miliaceum* also suggested tight stomatal control of transpiration and low stomatal conductance during the growing season (Barbour, 2007). In contrast, *P. halepensis* showed much lower leaf $\delta^{18}O$ suggesting utilization of non-enriched, deeper sources of water and higher stomatal conductance and transpiration rates during the growing season. *H. decumbens* and *T. articulata* showed intermediate $\delta^{18}O$ values suggesting that their depths of water extraction and/or degree of stomatal control of transpiration were also intermediate between the two former species. Leaf $\delta^{13}C$ values suggested that *T. articulata* had higher water use efficiency (WUE, the ratio of photosynthesis to transpiration) than the other target species, with little differences in WUE among *P. miliaceum*, *H. decumbens* and *P. halepensis* (Farquhar et al., 1989). The potentially complementary water use strategies of the plant species colonizing the mine tailings could minimize competition for soil water in this semiarid environment, thus favouring plant species coexistence (Nippert and Knapp, 2007). Moreover, vertical partitioning of soil water resources among coexisting plant species should lead to a more thorough and efficient use of the limited available water, which may enhance the overall primary productivity of the vegetation colonizing the mine tailings.

Oliveras et al. (2003) obtained comparable values of $\delta^{13}C$ in leaves of *T. articulata* and *P. halepensis* growing in a dune Mediterranean area in South East Spain. According to these authors, *T. articulata* may tolerate better extremely dry conditions than *P. halepensis* because of having a more resistant xylem to drought embolism.

On the other hand, under nitrogen limiting soils low values of $\delta^{15}N$ may reflect the existence of mycorrhizal associations (Dave-Evans, 2001). The large difference in leaf $\delta^{15}N$ between *P. halepensis* and the other target species reflected the fact that pines were ectomycorrhizal (EM), whereas *P. miliaceum*, *H. decumbens* and *T. articulata* were all arbuscular mycorrhizal species (AM). EM and AM plants may use different sources of N, and the N that symbiotic ectomycorrhizal fungi transfer to their host plants is depleted in the heavy isotope ($\delta^{15}N$), both of which may contribute to different $\delta^{15}N$ signatures in EM and AM plants (Craine et al., 2009). The coexistence of AM and EM plant species in the mine tailings should foster the functional diversity of microbial populations in soil, because AM and EM fungi differ widely in key traits such as hyphal biomass production and turnover rates, enzymatic capabilities, access to organic nutrient sources, tolerance of abiotic stress, etc. (Smith and Read, 2008).

5.5. Conclusions

From the results obtained in the present work, we may conclude that the phytomanagement of mine tailings would benefit from the assembling of different functional plant species. While grasses and shrubs may play an important role in primary succession and soil fertility improvement, trees seem to be the key to the development of fertility islands. The latter may support a self-sustaining vegetation and therefore, set the base for the successful revegetation following natural plant succession processes. The development of fertility islands would not require high inputs of nutrients since the differences in soil fertility parameters were not significant in relation to individual plant rhizospheres, especially, in relation to trees.

The implementation of a diverse set of plant species with contrasting life forms for revegetating tailings may result in a more efficient employment of water resources and higher biodiversity not only in relation to flora but soil microbiology too. This may support the long-term stability of the system and provide a higher resilience to environmental disturbances such as long drought periods or short intense rainfalls, typical from semiarid environments.

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CHAPTER 6. Assessment of the employment of halophyte plant species for the phytomanagement of mine tailings in semiarid areas

Chapter 6

Assessment of the employment of halophyte plant species for the phytomanagement of mine tailings in semiarid areas

Isabel Párraga-Aguado, María Nazaret González-Alcaraz,
José Álvarez-Rogel, Héctor M. Conesa

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Abstract

Plant selection is a critical issue for the long-term success of phytomanagement projects. The presence of "microenvironments" related to salinity in mine tailings under semiarid climates make halophytes a suitable alternative for phytostabilisation. The goal of this work was to assess the criteria for plant species selection for the phytostabilisation of mining wastes in semiarid areas, focusing on the suitability of the employment of spontaneous halophyte plant species. For this purpose, a comprehensive soil below-plant and plant survey including spontaneous halophyte and non-halophyte plant species were performed in an extensive area covered by mining wastes in SE Spain. The soil samples collected below halophyte plants showed higher electrical conductivity, organic carbon and water extractable salts concentrations than the non-halophyte ones. *Zygophyllum fabago* and *Limonium cossonianum* were the most suitable species to colonise the salty micro niches at the tailings while *Tamarix canariensis* and *Atriplex halimus* showed the best soil fertility indicators under moderate electrical conductivity values. In general, the halophyte species showed lower metal(loid) concentrations in leaves or shoots than the non-halophyte ones (e.g. *Cistus monspeliensis*, *Dittrichia viscosa* and *Helichrysum decumbens*). Oppositely, the leaves of halophyte plant species, specially *A. halimus and Z. fabago*, showed higher accumulation of Na and Cl which may be of interest to effect the long-term desalination of the tailings. The interest of employing halophytes is not only focused on metal(loid)s tolerance but also in the high potential for soil improvement (organic matter accumulation, desalination). The positive effects of combining halophyte with non-halophyte plant species may enhance the long-term sustainability of the plant community.

6.1. Introduction

Plant selection is a critical issue for the long-term success of phytomanagement projects (employment of plants to restore degraded ecosystems). In the particular case of the phytomanagement by phytostabilisation of mining wastes in semiarid areas, apart from showing tolerance to extreme pH, high metal(loid) concentrations or low fertility, plants must deal with additional soil stresses such as salinity or drought (Mendez and Maier, 2008).

Plants which spontaneously colonise mining wastes are suited for phytostabilisation purposes due to their adaptation to contamination but also to local climate conditions (Martínez-Sánchez et al., 2012). However, these polluted areas are normally characterised by low biodiversity, and therefore, abiotic factors (e.g. drought) may risk their long-term sustainability (Párraga-Aguado et al., 2013a). For this reason, current approaches have highlighted the need of favouring the employment of several plant species with different ecological functions instead of monospecific plantations or few plant species combinations (Párraga-Aguado et al., 2014).

The Cartagena-La Union mining district, a former mining area located in semiarid south-east Spain (Figure 6.1) and with more than 200 ha of tailings, has been proposed as a suitable scenario for applying phytostabilisation techniques (Conesa and Schulin, 2010; Martínez-Sánchez et al., 2012). A recent study performed by Párraga-Aguado et al. (2013a) in this mining area showed that soil pH and salinity were the main factors driving plant distribution at mine tailings. These authors suggested that high evaporation rates, typical of this semiarid area, might have favoured the existence of high salinity patches inside the tailings in which plants must cope with a combination of stresses (e.g. salinity, metals, low fertility). In order to overcome this problems, salt tolerant plant species (halophytes) may result a good alternative. Several authors have shown the connection between salt and metal(loid) tolerance mechanisms in halophytes (e.g.

Otte, 2001). For instance, halophytes have a better system for the compartmentalisation of salts, well developed antioxidant system and osmoprotectants that may also act under metal(loid) stress situations (Manousaki and Kalogerakis, 2011).

Although there is an extensive research in grasses, weeds and trees in relation to metal tolerance, studies on halophytes are restricted to metal(loid) pollution in salt marshes (Conesa et al., 2011; González-Alcaraz et al., 2011). Therefore, the goal of this study was to assess the criteria for plant species selection in the phytostabilisation of mining wastes in semiarid areas, focusing on the suitability of spontaneous halophyte plant species. To achieve this goal, a comprehensive survey including aerial parts of selected plant species and the soil below-plants was performed in an extensive area covered by mining wastes at the Cartagena-La Union Mining District.

6.2. Materials and methods

6.2.1. Site description

The Cartagena-La Union Mining District (0–392 m.a.s.l.; 50 km^2) was one of the most important mining areas in Spain during the last centuries. Metal(loid) contamination in this area has been recently reviewed by Conesa and Schulin (2010). The semiarid Mediterranean climate of the zone is characterised by an annual precipitation of 250–300 mm, average temperature of 18 °C and evapotranspiration rate of 850 mm. The natural vegetation is mainly based on small formations of *Pinus halepensis* and thicket plant species with xerophitic characteristics.

A former tailings disposal area, covering approximately 18,000 m^2 and located at the *Huerta de la Calesa* site (37°60′40′′N, 0°83′42′′W) was selected for the sampling (Figure 6.1). This site has been selected due to the long-term existence of mining wastes together with the occurrence of pioneer vegetation adapted to the local climatic conditions (Párraga-Aguado et al., 2013a).

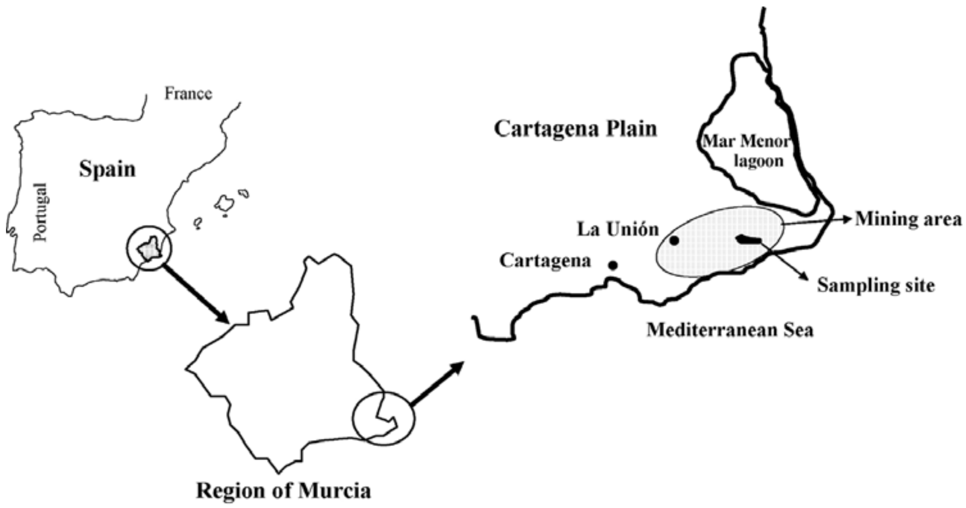

Figure 6.1. Location of the study area

6.2.2. Plant and soil sampling and analyses

Twelve plant species were chosen (Table 6.1) based on the species abundance found by Párraga-Aguado et al. (2013a) when describing the spontaneous vegetation growing at the selected tailings. The plants species were classified into halophytes and non-halophytes following the classification proposed by Menzel and Lieth (2003). Depending on plant morphology, leaves or shoots were collected. In all the cases, four replicates were taken at least.

Plant samples were carefully washed with distilled water and dried at 65 °C for 72 h. For each sample, an aliquot of 0.1–0.5 g was incinerated (550 °C, 3 h) prior to ashes redissolution with concentrated nitric acid. The resulting extracts were diluted to 25 ml with distilled water and filtered through a CHM 2041 ashless filter paper (20–25 μm pore diameter). Then, metal(loid)s (As, Cd, Cu, Mn, Pb, Sb and Zn) were analysed using an ICP-MS (Agilent 7500A), Cl and S (calculated from SO_4^{2-} concentrations) were analysed using an Ion Chromatographer (Metrohm), and Ca, Mg, Na and K were measured using a flame atomic absorption spectrometer (UNICAM 969 AA). Plant analyses were referenced using a CTA-VTL-2 certified material (Virginia tobacco leaves). The recovery percentages were between 90% and 110%.

Table 6.1. List of plant species collected.

Type of species according to Menzel and Lieth (2003)	Plant Species	Family	Most common environment according to Sánchez-Gómez et al. (1996)
Halophyte	*Atriplex halimus* L.	Chenopodiaceae	Nitrified environments with some salinity
	Inula crithmoides L.	Compositae	Shrubs in saline soils
	Limonium cossonianum Kunthze, Revis.	Plumbaginaceae	Saline soils, sandy areas close to the sea
	Salsola kali L.	Chenopodiaceae	Sandy soils, dry lands, abandoned lands, widespread
	Tamarix canariensis Willd.	Tamaricaceae	Saline areas, dry rivers
	Zygophyllum fabago L.	Zygophyllaceae	Slopes, roads, altered soils
Non-halophyte	*Cistus monspeliensis* L.	Cistaceae	Shrubs, widespread
	Dittrichia viscosa (L.) Greuter *	Compositae	Shrubs in nitrified environments, road sides
	Dorycnium pentaphyllum (Scop.)	Leguminoseae	Shrubs in nitrified/altered environments
	Helichrysum decumbens (Lag.) Camb	Compositae	Shrubs in nitrified/altered env., sandy areas, widespread
	Piptatherum miliaceum (L.) Cosson	Gramineae	Nitrified lands, road sides, slopes, widespread
	Pistacia lentiscus L.	Anacardiaceae	Shrubs and Mediterranean forests

* According to Curadi et al., (2005)

Additionally, the corresponding soil-below plants was collected, completing 32 soil samples for the halophyte species and 28 for the non-halophyte ones. Soil samples were air dried, sieved through a 2 mm mesh, homogenised and stored in plastic bags prior to laboratory analysis. Soil pH and Electrical Conductivity (EC) were determined in a 1:5 soil to water mixture after 2 h shaking using a Crison Basic 20 pH-meter and a Crison Basic 30 conductivity meter, respectively. The resulting extracts were filtered through nylon membrane 0.45 μm syringe filters (Wicom), and then, analysed for major ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}) and dissolved organic carbon (DOC) using an Ion Chromatographer (Metrohm) and a TOC-automatic analyser (TOC-VCSH Shimadzu), respectively. Equivalent calcium carbonate ($CaCO_3$) was estimated using the Bernard calcimeter method. Particle size distribution was determined following the method of Bouyoucos densimeter (Gee and Bauder, 1986). Total nitrogen (TN) was determined using the Kjeldahl method (USDA, 1996). Organic carbon (OC) was determined by oxidation of the organic matter using potassium dichromate (Duchaufour, 1970). Total element composition was measured by X-Ray Fluorescence (Bruker S4 Pioneer).

Extractable (0.01 M $CaCl_2$) metal(loid) concentrations were employed to assess their bioavailability for plants (González et al., 2011). For this purpose, a 1:10 soil to 0.01 M $CaCl_2$ solution was shaken for 2 h. The resulting extracts were filtered through nylon membrane 0.45 μm syringe filters (Wicom) and analysed for metal(loid) concentrations (As, Cd, Cu, Mn, Pb, Sb,Zn) using an ICP-MS (Agilent 7500A).

6.2.3. Statistical analyses

Statistics were performed with SPSS 20 software (SPSS, Chicago, IL, USA). The averages of soil parameters for the halophytes and non-halophytes were compared using the Student *t*-test (for normally distributed data) or the Mann–Whitney *U* test (for non-normally distributed data). Correlations among parameters were evaluated by Pearson's (for normally distributed data) or Spearman's (for non-normally distributed data) coefficients. Differences at $p<0.05$ level were considered significant. Additionally, soil gradients and elemental contents in plants were examined for square root-transformed data by a principal component analysis (PCA) using Canoco for Windows v.4.5 software (ter Braak and Smilauer, 1999).

6.3. Results and discussion

6.3.1. Soil parameters

The results of soil analyses are shown in the Table 6.2. The pH values of the tailings were in the range of neutral soils and the fertility properties were deficient (around 10-fold higher EC values and up to 10-fold lower OC, DOC and TN concentrations) compared to other non-mining impacted soils reported at the local area by Párraga-Aguado et al. (2013a). In addition, the tailings contained high total metal(loid) concentrations which surpassed the geochemical backgrounds of the region (Martínez-Sánchez and Pérez-Sirvent, 2007). The $CaCl_2$-extractable concentrations were under the detection limit (20 μg kg^{-1}) for As, Cd, Pb and Sb, and showed low values for Cu, Mn and Zn (<1500 μg kg^{-1}), probably because of the tailings' neutral pH. The higher EC values of the tailings in relation to the local non-mining impacted soils (Table 6.2) were also reflected in the higher water extractable ions concentrations, especially SO_4^{2-} and Ca^{2+}. The high SO_4^{2-} water extractable concentrations (~1500–1800 mg L^{-1}) may be related to the presence of gypsum (and not to acid generation due to the neutral pH), which is known to be secondary formed in the tailings (García-Lorenzo et al., 2012). The EC values of the tailings positively correlated (p<0.01) with clay and silt (for both, r > 0.38), and negatively with sand (r = −0.48). The latter also correlated with the total Cu concentrations (r = 0.56, p<0.01). This suggests that the arrangement of the textural size fractions at the tailings may condition some edaphic properties such as salinity and/or metal(loid) concentrations (Párraga-Aguado et al., 2013a) and therefore, it may determine the occurrence of niches or microenvironments with specific soil conditions for plant growth.

The comparison between the soil samples collected below halophyte (H-samples) and non-halophyte plants (nH-samples) showed significant differences (p<0.05) for some of the studied parameters. For instance, the higher EC values (p<0.05) of H-samples (3.12 dS m^{-1}) in relation to nH-samples (2.92 dS m^{-1}) were probably due to the higher concentrations of water extractable salts (e.g. ~5-fold for Cl^- and 4-fold for Na^+). The higher affinity/ability of halophytes for growing at these high EC spots in the tailings should be considered for the phytomanagement of salty niches, where most of plants are not able to grow. Then, approaches in the phytostabilisation of tailings which include ploughing or seed distribution should be preceded by the identification of those areas with specific edaphic constraints (Párraga-Aguado et al., 2013a).

Table 6.2. Physicochemical characteristics of soil below plant samples for halophyte and non-halophyte plant species (average ± standard errors). Data between brackets are first quartile (Q1) and third quartile (Q3); "n.a.": not available; "*" indicates significant differences ($p < 0.05$) between halophyte and non-halophyte samples.

Soil parameter			Soil below plant samples					Non mining impacted local soils (Párraga-Aguado et al. 2013a)
			Halophyte plant species (H-samples)			Non-halophye plant species (nH-samples)		
pH (1:5)			7.21 ± 0.03	(7.13 - 7.29)		7.23 ± 0.05	(7.16 - 7.39)	7.89
EC (1:5)		dS m^{-1}	3.12 ± 0.12	(2.89 - 3.12)	*	2.92 ± 0.07	(2.76 - 2.95)	0.28
$CaCO_3$		g kg^{-1}	78 ± 4	(65 - 91)	*	66 ± 8	(47 - 68)	84
OC			5.9 ± 0.5	(4.0 - 6.8)	*	2.9 ± 0.2	(2.1 - 3.4)	25
TN			0.31 ± 0.05	(0.10 - 0.41)		0.24 ± 0.03	(0.10 - 0.3)	2.50
DOC		mg kg^{-1}	33 ± 4.1	(17 - 39)		21 ± 1.5	(18 - 24)	200
Sand		%	73 ± 3	(62 - 83)	*	82 ± 2	(76 - 92)	26
Silt			23 ± 2	(16 - 31)	*	16 ± 2	(7 - 22)	31
Clay			3 ± 1	(1 - 7)	*	2 ± 0	(1 - 1)	43
Total metal(loid)s concentations	As	mg kg^{-1}	580 ± 46	(450 - 760)		500 ± 44	(400 - 560)	59
	Cd		30 ± 4	(6 - 45)		31 ± 3	(27 - 39)	5
	Cu		96 ± 4	(84 - 113)	*	112 ± 4	(104 - 125)	55
	Mn		10,320 ± 350	(9660 - 10,950)		10,110 ± 260	(9530 - 10,530)	2370
	Pb		3970 ± 280	(2960 - 4390)	*	5440 ± 290	(4330 - 6500)	1310
	Sb		71 ± 7	(44 - 90)		77 ± 7	(59 - 86)	6
	Zn		5730 ± 430	(3810 - 6370)	*	8460 ± 460	(6950 - 9690)	670
0.01M $CaCl_2$-extractable metal(loid)s concentrations (1:10)	As	µg kg^{-1}	<20			<20		n.a.
	Cd		<20			<20		n.a.
	Cu		34 ± 9	(<20 - 52)		111 ± 31	(<20 - 350)	n.a.
	Mn		240 ± 30	(135 - 320)		230 ± 38	(88 - 345)	n.a.
	Pb		<20			<20		n.a.
	Sb		<20			<20		n.a.
	Zn		350 ± 39	(220 - 460)	*	1090 ± 130	(550 - 1540)	n.a.
Water extractable ions (1:5)	Cl^-	mg L^{-1}	51 ± 20	(10 - 22)	*	8.2 ± 0.3	(7.0 - 8.9)	23
	SO_4^{2-}		1720 ± 35	(1630 - 1800)	*	1580 ± 13	(1560 - 1600)	27
	Ca^{2+}		610 ± 7	(600 - 630)		620 ± 5	(620 - 635)	27
	K^+		12 ± 1.7	(4.0 - 16)		8.6 ± 1.0	(4.5 - 11)	11
	Mg^{2+}		64 ± 8	(37 - 78)	*	22 ± 2	(16 - 25)	8
	Na^+		48 ± 14	(16 - 46)	*	11 ± 0.9	(8 - 11)	23

The potential interest of halophytes for the phytomanagement of the tailings was also supported by the higher concentration of OC ($p<0.05$) in the H-samples. Even though salinity may be considered an intrinsic property of the tailings, fertility parameters such as OC or TN are related to plant growth and therefore, they are plant species-dependent. Then, it may be expected that, under the salty conditions of the tailings, halophytes could reach optimal growth rates which, in turn, may promote a higher enhancement of soil properties. This improvement of soil organic matter could also stimulate the microbiological activity leading to a higher CO_2 production and therefore, favouring carbonate precipitation (Hammes and Verstraete, 2002), that could explain the higher $CaCO_3$ concentrations (p<0.05) which were found in the H-samples in relation to nH-ones. Besides, higher calcium carbonate contents would contribute to decrease metal availability (McLean and Bledsoe, 1992), which is in agreement with the lower extractable Zn and Cu concentrations found in H-samples. The improvement of soil fertility provided by the studied halophytes may result a critical issue at the tailings, since it has been shown that under the deficient edaphic conditions of mine tailings, slight organic matter inputs may be enough to trigger plant successional dynamics (Párraga-Aguado et al., 2014).

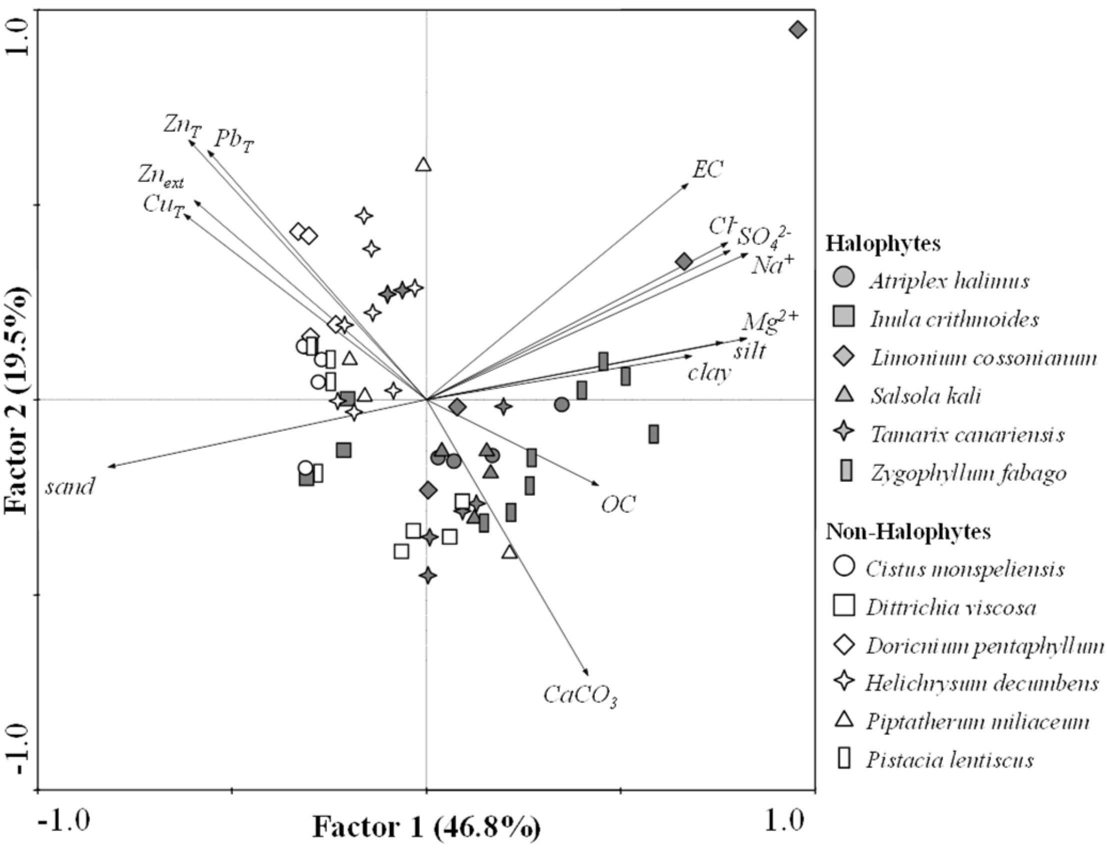

Figure 6.2. Principal components analysis (PCA) of soil parameters. "XT" refers to total metal concentration; "Xext" refers to metal extractable concentrations; "OC" to organic carbon and "EC" to electrical conductivity.

The PCA (Figure 6.2) was performed for those edaphic parameters which showed significant differences between H- and nH-samples (Table 6.2). The Factor 1 (X axis) was defined by a salinity gradient that was in turn determined by higher water extractable salts and EC and finer texture on the positive side, and higher sand content on the negative side. The Factor 2 (Y axis) was mainly related to higher $CaCO_3$ concentration on the negative side, and higher metal concentration (especially Pb and Zn) on the positive side. The samples from halophytes were mostly depicted on the positive side of the X axis (more saline sites with higher $CaCO_3$ and OC contents) while the non-halophytes appeared on the negative side (the sandier, less saline sites with higher metal contents). Some halophytes, such as *Limonium cossonianum* and *Zygophyllum fabago*, were able to grow in the saltier micro-niches at the tailings, while *Tamarix canariensis* and *Atriplex halimus* showed better soil fertility indicators under moderate EC values. The halophyte *Inula crithmoides* appeared on sandier sites (>90% sand) than the rest of studied halophytes (~70% sand), probably because of its affinity for growing in sandy soils of saline ecosystems (Álvarez-Rogel et al., 2007; Al-Oudat and Qadir, 2011). The soil samples of *Dittrichia viscosa* showed similar characteristics of halophytes', which agree with previous findings that attributed a facultative halophyte behavior to this plant species (Menzel and Lieth, 2003; Al-Oudat and Qadir, 2011). However, several studies have reported a substantial decrease in its biomass under increasing salt concentrations (Curadi et al., 2005; Maciá-Vicente et al., 2012) which, in turn, may affect its ability to cope with combined soil stress factors.

6.3.2. Elemental contents in plants

The metal(loid) concentrations accumulated by the studied plants showed high variability between species (Table 6.3 and Figure 6.3). The highest metal(loid) concentrations (except for Cu which occurred for the shoots of the halophyte *I.*

crithmoides) were found in some non-halophyte species: *Cistus monspeliensis* (As, Cd), *D. viscosa* (Mn, Pb, Zn) and *Helichrysum decumbens* (Sb).

The total and $CaCl_2$-extractable metal(loid) concentrations did not correlate (p>0.05) with their corresponding contents in plant tissues for both, halophyte and non-halophyte species. However, for the non-halophytes, the metal(loid) concentrations in leaves/shoots correlated positively among them ($r > 0.4$, $p<0.05$), which probably might suggest a low selectivity in metal(loid) plant uptake. For the halophytes, As, Pb and Sb concentrations positively correlated among them ($r > 0.57$, $p<0.01$), Mn correlated with As and Pb ($r > 0.45$, $p<0.01$), while Cd, Cu and Zn did not show any correlation with other metal(loid) concentrations.

Sulphur concentrations in non-halophytes species positively correlated with most of the metal(loid)s analysed (As, Cd, Mn, Pb and Zn; $r > 0.45$, $p<0.01$). This could be related to the role of S as a component of phytochelatins, which are known to be involved in the physiological mechanisms for metal(loid)s sequestration and detoxification (Rauser, 1995). However, in halophytes species, those positive correlations were not found, possibly because their tolerance to metal(loid)s may rely on other physiological mechanisms related to salt tolerance such as a better compartmentalisation, synthesis of osmoprotectants (such as proline) or inducement of other antioxidant compounds (Manousaki and Kalogerakis, 2011).

Sodium accumulation may indicate halophytic properties (Zurayk et al., 2001), since Na^+ is the foremost inorganic ion and a common tool for halophytes to maintain the osmotic balance (Tester and Davenport, 2003). In general, most of the studied halophytes presented higher Cl and Na concentrations than the non-halophytes: *Z. fabago* showed the highest values of Na (26,200 mg kg^{-1}) and *A. halimus* the highest concentrations of Cl (38,600 mg kg^{-1}) and K (23,800 mg kg^{-1}). For both halophytes and non-halophytes, a significant positive correlation ($r > 0.55$, $p<0.01$) between Cl and K occurred. However, only in halophytes a significant correlation between Cl and Na was found ($r = 0.55$, $p<0.01$).

Table 6.3. Metal(loid)s and other elements uptake in leaves/shoots of halophyte and non-halophyte species growing at the selected tailings (average ± standard error). Values are expressed as mg kg^{-1} (dry weight).

Type of species	**Elements**		**Concentrations in leaves/shoots**					
Halophytes								
			A.halimus	*I. crithmoides*	*L. cossonianum*	*S. kali*	*T.canariensis*	*Z. fabago*
	Metal(loid)s	**As**	10.08 ± 0.76	5.22 ± 0.78	8.01 ± 0.81	11.84 ± 1.00	6.09 ± 0.87	1.70 ± 0.25
		Cd	0.65 ± 0.19	2.28 ± 0.55	0.86 ± 0.04	1.44 ± 0.17	1.87 ± 0.31	2.48 ± 1.25
		Cu	10.72 ± 1.15	16.26 ± 0.66	8.05 ± 0.70	4.89 ± 0.41	8.12 ± 1.12	9.07 ± 1.12
		Mn	339 ± 39	98 ± 15	73 ± 3	268 ± 34	179 ± 26	151 ± 12
		Pb	84.1 ± 5.9	25.4 ± 7.0	23.3 ± 4.1	60.1 ± 2.6	64.1 ± 6.1	14.9 ± 3.5
		Sb	0.72 ± 0.07	0.53 ± 0.08	0.58 ± 0.02	0.60 ± 0.02	0.57 ± 0.11	0.35 ± 0.03
		Zn	273 ± 19	98 ± 15	108 ± 11	237 ± 12	503 ± 91	433 ± 66
	Other elements	**Ca**	3840 ± 596	3240 ± 766	3710 ± 160	13,500 ± 4330	4550 ± 285	30,400 ± 2410
		Cl	28,600 ± 5280	22,600 ± 1870	1900 ± 185	6570 ± 1440	2140 ± 316	7270 ± 2230
		K	23,800 ± 5080	10,800 ± 2380	7300 ± 900	28,500 ± 3120	4660 ± 386	12,200 ± 1930
		Mg	11,600 ± 1220	18,200 ± 312	10,100 ± 467	15,500 ± 188	15,300 ± 857	15,500 ± 494
		Na	20,500 ± 3280	19,000 ± 2010	2830 ± 488	4680 ± 870	5980 ± 666	26,200 ± 3260
		S	6140 ± 1000	9800 ± 1640	13,800 ± 334	11,600 ± 1030	14,780 ± 892	35,900 ± 5560
Non Halophytes								
			C. monpeliensis	*D.viscosa*	*). penthaphyllun*	*H. decumbens*	*P.miliaceum*	*P. lentiscus*
	Metal(loid)s	**As**	87.0 ± 7.28	31.60 ± 2.54	1.28 ± 0.23	13.11 ± 4.07	3.07 ± 0.85	1.79 ± 0.19
		Cd	4.77 ± 0.98	2.82 ± 0.65	0.10 ± 0.04	0.94 ± 0.30	0.11 ± 0.03	0.20 ± 0.06
		Cu	5.61 ± 0.68	12.01 ± 1.51	3.93 ± 0.42	11.06 ± 1.13	4.39 ± 0.61	7.08 ± 1.68
		Mn	420 ± 49	677 ± 87	72 ± 27	157 ± 7	71 ± 9	79 ± 11
		Pb	349 ± 54.2	371 ± 29.3	20.0 ± 3.0	107 ± 38.4	5.8 ± 1.2	30.4 ± 1.8
		Sb	1.62 ± 0.11	1.66 ± 0.23	0.21 ± 0.04	2.07 ± 1.06	0.32 ± 0.09	0.25 ± 0.02
		Zn	303 ± 62	685 ± 23	147 ± 59	249 ± 24	113 ± 13	77 ± 3
	Other elements	**Ca**	2840 ± 259	5630 ± 804	1810 ± 173	1790 ± 71	1460 ± 145	3450 ± 438
		Cl	4010 ± 445	3840 ± 462	1440 ± 344	4310 ± 88	3990 ± 1230	1140 ± 89
		K	5970 ± 349	5910 ± 761	7640 ± 1370	17,200 ± 329	14,100 ± 2690	6180 ± 1040
		Mg	3530 ± 517	7110 ± 204	2610 ± 334	1840 ± 119	2120 ± 145	3180 ± 403
		Na	1640 ± 219	6020 ± 1110	558 ± 218	1580 ± 297	627 ± 195	813 ± 127
		S	5890 ± 515	7730 ± 438	2530 ± 331	3280 ± 278	5530 ± 331	1610 ± 106

The PCA in Figure 6.3 showed the distribution in elemental composition in the studied plant species. Plants belonging to both halophytes and non-halophytes groups showed different patterns of accumulation, probably related with specific strategies to salt and metal(loid)s tolerance. According to Figure 6.3, three groups of plant species can be distinguished: a first group of non-halophytes, including *Dorycnium penthaphyllum*, *Piptatherum miliaceum* and *Pistacia lentiscus*, which were depicted in the X-negative/Y-positive quadrant, characterized by the lowest element concentrations. A second group of non-halophytes, which appeared in the X-positive/Y-positive quadrant, and were characterized by showing the highest metal(oid) concentrations. This group included *C. monspeliensis* and *D. viscosa* which have been reported to be colonizers of mining wastes in the Mediterranean area by Freitas et al. (2004) and Pérez-Sirvent et al. (2012), respectively. Finally, most of the halophytes (except *L. cossonianum*) were depicted in the X-positive/Y-negative quadrant, which was mainly defined by the highest macroelement (Ca, Cl, Na, Mg, S) concentrations. In spite of growing in the saltiest soil spots (Figure 6.2), *L. cossonianum* showed the lowest salt concentrations of all the studied halophytes. This may be related to the mechanisms for salt tolerance employed by this plant species which include the elimination of salts through excretion by leaves (Mohr and Schopfer, 1995). A similar strategy has been also reported for the Tamaricaceae *T. canariensis* (Kadukova et al., 2008). Oppositely, *Z. fabago* and *I. crithmoides* were found to accumulate the highest salt concentrations.

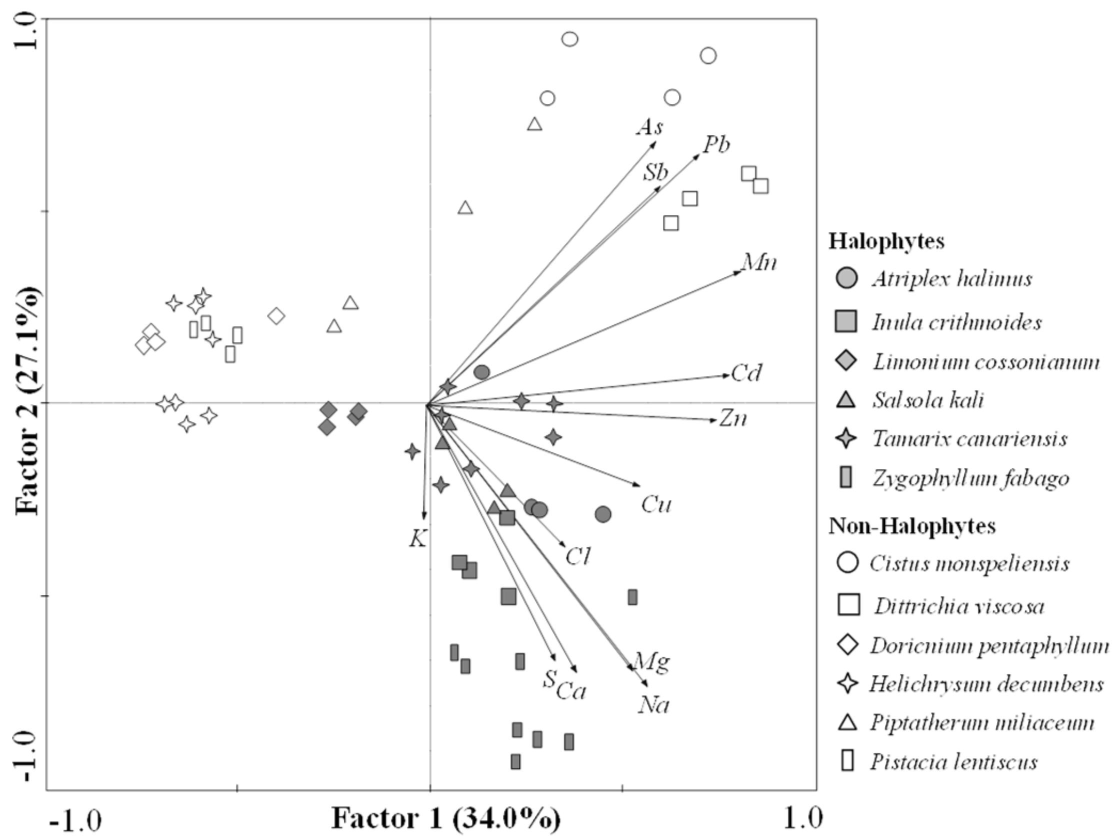

Figure 6.3. Principal components analysis (PCA) of plant elemental composition. "X" refers to total concentrations in leaves/shoots.

6.3.3. Suitability of halophytes for the phytomanagement of semiarid mine tailings

The potential use of halophytes for phytoremedation purposes has been widely accepted because of their metal(loid) tolerance capacity (Manousaki and Kalogerakis, 2011). However, when dealing with salty mine tailings at semiarid areas, the interest of halophytes species is not only focused on metal(loid) tolerance but also in their high potential for soil improvement. As discussed previously, halophytes were related with higher OC concentrations in the soil below-plants, which may be critical for the improvement of soil fertility and microbiology. The enhancement of soil properties may also include the amelioration of soil salinity by desalination. It is extended the cultivation of halophytes for the restoration of degraded saline areas (Ravindran et al., 2007; Ali et al., 2013). Therefore, the use of halophytes with high rates of salt uptake in those niches with high salinity at the tailings could also bring the positive effect of decreasing soil salinity at middle/long-term. This is of high interest for the phytomanagement of tailings in semiarid areas, since salinity has been considered as a key factor for the establishment of vegetation (Párraga-Aguado et al., 2013a). Among the halophytes which were found at the tailings, *A. halimus* has been described as a suitable plant species for the phytoremediation of metal polluted areas (Lutts et al., 2004; Clemente et al., 2012; Pardo et al., 2014) but also to be used in the restoration of saline environments (Manousaki and Kalogerakis, 2009). Assuming an annual yield of 5 t dry weight ha^{-1} y^{-1} for *A. halimus* in natural Mediterranean ecosystems (Lutts et al., 2004), and based on the data obtained at the current study, it may be expected to extract around 100 kg ha^{-1} y^{-1} Na and 140 kg ha^{-1} y^{-1} Cl.

The Compositae *I. crithmoides* (Zurayk and Baalbaki, 1996), the Plumbaginaceae *L. cossonianum* and the Zygophyllaceae *Z. fagabo* (Al-Oudat and Qadir, 2011) have been also proposed as suitable saline crops, although their biomass production might be lower than *A. halimus*. Nevertheless, the interest of these halophytes in the phytomanagement of mine tailings may lie in their ability to grow in the highest EC spots, such as *L. cossonianum* (also reported by Conesa et al., 2011), or their fast spread and lower nutrient requirements such as *Z. fabago* (Boojar and Tavakkoli, 2011; Martínez-Sánchez et al., 2012).

Although it has not been included in the previous group of saline crops, the interest of the tree *T. canariensis*, may lie in the ecological relevance of this plant species. The introduction of trees in remediation projects provides the possibility of improving metal(loid) immobilisation by their large and deep root systems and the enhancement of soil microbiological and fertility conditions. This could facilitate the establishment of other plant species by a "nurse effect", as it has been previously shown by Párraga-Aguado et al. (2013b, 2014).

The variety of plant species studied in this work included small trees (*T. canariensis*, *P. lentiscus*), woody perennial shrubs (*A. halimus*, *C. monspeliensis*, *D. viscosa, H. decumbens*), perennial herbs (*I. crithmoides*, *L. cossonianum*, *Z. fabago*, *D. pentaphyllum*), annual plant species (*Salsola kali*) and perennial grasses (*P. miliaceum*). The diversity of life forms in plant communities may result in a more efficient use of water and nutrients and a higher biodiversity (Párraga-Aguado et al., 2014), providing higher long-term sustainability. Annual herbs and grasses may supply fast soil coverage during the first stages of phytostabilisation. Then, perennial shrubs and herbs may provide a permanent canopy, thus reducing erosion and metal(loid)s dispersion by airborne dust.

The potential risk of transferring metal(loid)s into the food chain is one of the main constraints when dealing with phytomanagement in terms of phytostabilisation (Wolfe and Bjornstad, 2002; Robinson et al., 2009). Those selected plants species should present low concentrations of metal(loid)s in their aerial or edible parts. Some halophytes have been reported for showing hiperaccumulator properties (>10,000 mg kg^{-1} uptake for metal(loid)s) (Redondo-Gómez et al., 2010). Then, it is necessary to evaluate the proposed halophyte species in order to meet the requirement of low metal(loid) uptake into aerial/edible parts. For this purpose, it is employed the bioconcentration factor (BF), calculated as the ratio between total element concentration in shoots and the corresponding total element concentration in the soil (Brooks, 1998; Mendez and Maier, 2008). When BF is less than 1, which was the case for all the halophytes an non-halophytes reported at this study, plants may be considered as no hyperaccumulators.

Finally, some of the reported plant species (halophytes such as *A. halimus*, *I. crithmoides* and *Z. fabago*, and non-halophytes such as *P. miliaceum* or *P. lentiscus*) could have a potential interest for being grazed by livestock or wild animals. According to the toxicity thresholds proposed by the National Research Council of

U.S. (NRC, 2005) for safe cattle feeding, the plant metal(loid) concentrations obtained in our study do not imply toxicological risk for animals.

6.4. Conclusions

This work has assessed the advantages of introducing halophyte plant species in the phytomanagement of mine tailings under semi-arid climate. The studied halophyte plant species may result a good alternative to overcome the combined stress factors which constraint the plant growth at mine tailings (e.g. salinity, metal(loid) toxicity).The positive effects of combining halophytes (which may decrease the salt content in the soil and show higher OC inputs) and non-halophyte plant species may enhance the long-term sustainability of the plant communities within the phytomanagement scheme. Further research should focus on the ecological succession and facilitation/competition processes which control the plant ecological relationships at mine tailings.

6.5. Acknowledgements

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CHAPTER 7. Assessment of metal(loid)s availability and their uptake by *Pinus halepensis* in a Mediterranean forest impacted by abandoned tailings

Chapter 7

Assessment of metal(loid)s availability and their uptake by *Pinus halepensis* in a Mediterranean forest impacted by abandoned tailings

Isabel Párraga-Aguado, José Álvarez-Rogel, María Nazaret González-Alcaraz, Francisco J. Jiménez-Cárceles, Héctor M. Conesa

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Abstract

Tailings are frequently source of pollution in mining areas due to the spread of metal(loid)s from their bare surfaces via wind, water run-off and/or leaching. For this reason, areas surrounding tailings may be affected by high concentrations of those toxic chemical elements. The aim of this study was to determine the influence of soil parameters on metal availability in a Mediterranean forest affected by mining contamination and the potential employment of *Pinus halepensis* as a suitable plant species for phytostabilising mining polluted sites under semiarid climates. Five tailings ponds, including their surroundings were selected. At the same sampling area, additional soil samples were taken in less impacted zones (up to 1 km and 3 km far from tailings). The highest total concentrations occurred indistinctly at some forest samples closed to tailings (e.g. ~12,000 mg kg^{-1} Pb) or at tailings ones (~790 mg kg^{-1} As). The alkaline soil pH and some carbonate minerals conditioned low $CaCl_2$-extractable metal(loid) concentrations and therefore low risk of pollutants leaching. $CaCl_2$-extractable As and metal concentrations did not correlate with the corresponding concentrations in pine needles indicating that this procedure might not be suitable to predict metal(loid) availability in pine trees. Needles of pine trees from the less impacted areas showed lower Mn and Zn concentrations (40–100 mg kg^{-1} Mn, 25–55 mg kg^{-1} Zn) in relation with the ones taken from the tailings. *P. halepensis* Miller looks a suitable plant species to be employed in the phytostabilisation of tailings due to a larger root system, which may provide a better soil retention, and its relative low metal(loid) accumulation.

7.1. Introduction

Mining contamination is considered a critical issue in many sites worldwide due to its negative effects on environment (Conesa and Schulin, 2010). Mine tailings are known to produce most of the environmental impacts in former mining sites due to the spread of metal(loid)s enriched particles from their bare surfaces via wind, water run-off and/or leaching. In some cases, mining contamination may reach regional scales affecting urban areas, agricultural lands or ecological protected sites (García-Lorenzo et al., 2012). This may increase the risk of transferring pollutants into food chain. For this reason, it is necessary to keep the environmental risks of these areas under a safety level and to perform a periodic monitoring of the geochemistry of the pollutants, not only at the tailings sites but also at their surroundings. Soil parameters such as pH, Eh, organic compounds, mineralogy or electrical conductivity have been shown to play an important role in the metal(loid) spetiation and, therefore, in their uptake by biota (María-Cervantes et al., 2010).

Phytostabilisation (using plants to immobilise metal(loid)s) have been proposed as a feasible tool to effect the surface stabilisation of mine tailings under semiarid climate (Mendez and Maier, 2008). For this purpose, suitable plant species must show good adaptation to drought, metal(loid)s soil concentrations, salinity or low metal(loid) accumulation into shoots. In order to meet these requirements, the employment of indigenous plant species seems a good alternative (Rufo and De la Fuente, 2010). The former Cartagena-La Union Mining District (Figure 7.1) located at Southern Spain has been widely researched for dynamics of metal(loid) contamination (Martínez-Sánchez et al., 2008) and emerging soil remediation low cost technologies, including phytostabilisation (Conesa and Schulin, 2010). Research on plant candidates for the in situ phytostabilisation has proposed the use of grasses (e.g. *Lygeum spartum* L., *Piptatherum miliaceum* (L.) Cosson), halophytes (e.g. *Arthrocnemum macrostachyum*

(Moric.) Moris), weeds (e.g. *Zygophyllum fabago* L.) or shrubs (e.g. *Dittrichia viscosa* (L.) Greuter) (Conesa et al., 2011; Martínez-Sánchez et al., 2012). According to Jefferson (2004), the main goal of a restoration project should be to recreate sustainable plant communities which reflect the diversity and composition of the surrounding natural plant communities. For this reason, the additional employment of tree species to the already listed plants would provide a better ecological-landscape assembling. For instance, *Pinus halepensis* Miller is a woody plant species which has been widely employed on the restoration of degraded semiarid ecosystems in the Mediterranean area (Fuentes et al., 2007). In addition, pine tree species have been proposed to be used as bioindicators of metal(loid) availability in polluted sites (Sun et al., 2009).

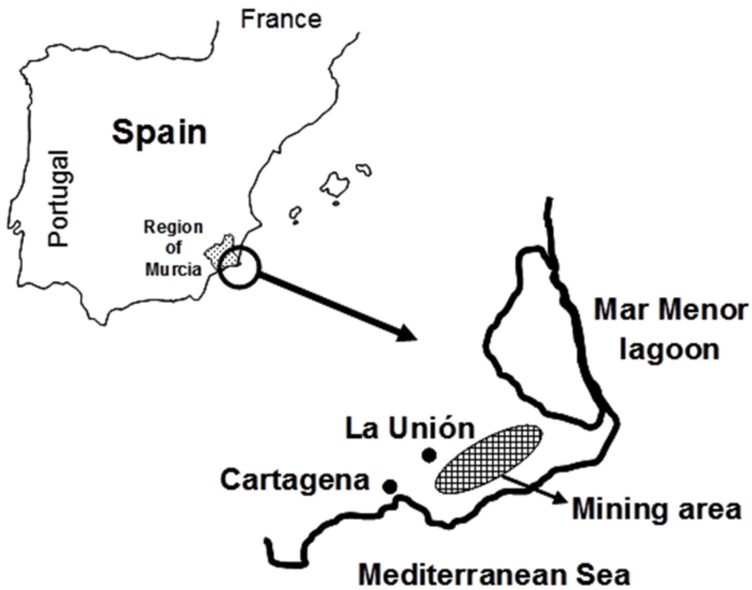

Figure 7.1. Location of studied area (0 – 400 m above sea level; 37°37′20′′ N, 0°50′55′′ W–37°40′03′′ N, 0°48′12′′ W).

The aim of this study was to determine the influence of soil parameters on metal availability in a Mediterranean forest affected by mining contamination and the potential employment of *P. halepensis* for monitoring metal pollution and phytostabilising mining polluted sites under semiarid climates.

7.2. Materials and methods

Sampling was conducted at a tailings disposal area located at the Cartagena-La Union Mining District. Five tailings sites, where *P. halepensis* grew spontaneously, were selected (T1–T5; Figure 7.2). In addition, the spread of contamination into the surrounding areas was evaluated by performing a sampling at the tailings' surroundings (F0), 100 m far from tailings (F1), up to 1 km (F2), and up to 3 km (F3). Tailings samples (T1–T5) were waste materials, F0 and F1 were soils developed on waste materials and F2 and F3 were natural soils. All forest (F) areas had at least 90% plant cover, mainly composed of *P. halepensis* Miller and other plant species typical from Mediterranean areas (e.g. *Rosmarinus officinalis* L., *Thymus hyemalis* Lange, *Chamaerops humilis* L.). At each sampling site three or four composite samples were taken by mixing sub-samples from four points randomly distributed. Each sampled point corresponded with the rhizosphere of the pine trees.

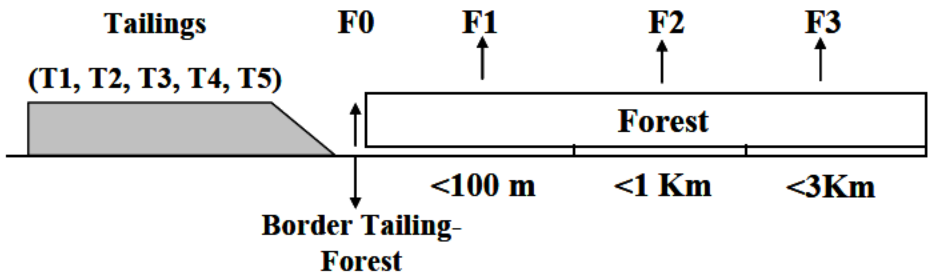

Figure 7.2. Basic scheme of sampling design.

Soil samples were air dried, sieved through 2 mm grain size, homogenised and stored in plastic bags prior to laboratory analysis. The particle size distribution was determined using the Bouyoucos densimeter method. Equivalent calcium carbonate ($CaCO_3$) was determined using a Bernard calcimeter. Electrical conductivity and pH in the 1:5 (soil:water) extract were determined using a Crison Basic 30 conductivity meter and a Crison Basic 20 pH-meter, respectively. In the same 1:5 extract, dissolved organic carbon was measured using an automatic TOC analyzer (TOC-VCSH Shimadzu). Organic carbon was determined by the oxidation of organic matter using potassium dichromate and total nitrogen was determined using the Kjeldahl method. To determine the total element concentrations, sub-samples were ground and analysed by X-ray fluorescence (Bruker S4 Pioneer). An aliquot of these sub-samples was used for the determination of mineralogy by X-ray diffraction (Bruker D8 Advance spectrometer).

Plant available arsenic and metal (Cd, Cu, Mn, Pb and Zn) concentrations were measured after shaking 5 g soil sample in 50 ml 0.01 M $CaCl_2$ solution for 2 h (González et al., 2011). The resulting extracts were filtered through nylon membrane 0.45 μm syringe filters (Albet-JNY) and measured by ICP-MS (Agilent 7500A, detection limit 0.002 mg L^{-1})

At each sampling site, pine trees of similar size (3–4 m height) were chosen. From each selected pine, three or four samples of adult needles were taken. The needles were washed with distilled water, dried at 65 °C for 72 h, and then, ground. An aliquot of 0.5 g was incinerated during 5 h at 450 °C prior to a redilution using concentrated nitric acid. Arsenic and metal concentrations (Cd, Cu, Mn, Pb and Zn) were measured by ICP-MS (Agilent 7500A, detection limit 0.002 mg L^{-1}). Plant analyses were referenced using a CTA-VTL-2 certified material (Virginia tobacco leaves). The percentages of recovery were 110% for As, 89% for Cd, 119% for Cu, 104% for Mn, 96% for Pb and 100% for Zn.

All the statistical analysis (ANOVA with Tukey test, Pearson's correlations) was carried out with SPSS 19.0.0 (SPSS, Chicago, IL, USA). The data were log-transformed when they did not meet the Levene test for homogeneity of variances. Differences at $p<0.05$ level were considered significant. Principal component analysis (PCA) was performed using the 'CANOCO for Windows' programme v4.02 (ter Braak and Smilauer, 1999).

7.3. Results

7.3.1. Soil parameters and metal(loid) concentrations

Soil properties are shown in Figure 7.3. The pH of all the samples was in the range of neutral-slightly alkaline soils (pH 7–8). The soil samples from forest zones showed lower electrical conductivity values (~0.3 dS m-1) than the tailings ones (0.9–2.8 dS m^{-1}). Organic carbon (OC), total nitrogen (TN), and dissolved organic carbon (DOC) values for tailings samples were about the half of those obtained at forest samples. Tailings samples had sand percentages close to 80%, while F-samples were below 70% for F0 and F1 and of around 40% for F2 and F3. The total and $CaCl_2$-extractable concentrations of the studied elements are shown in Table 7.1. The highest total concentrations occurred indistinctly at some forest samples (especially for F1, ~12,000 mg kg^{-1} Pb and ~10,700 mg kg^{-1} Zn) or at tailings ones (~790 mg kg^{-1} As for T2 or 115 mg kg^{-1} Cu for T1). F2 and F3 showed the lowest metal(oid) concentrations, especially for As, Cu, Mn and Zn ($p<0.05$). The highest concentrations for $CaCl_2$-extractable metals occurred for Mn and Zn indistinctly at tailings (0.552 mg kg^{-1} Mn for T2) or forest samples (2.506 mg kg^{-1} Zn for F1).

As it is shown in Figure 7.4, and according to the soil parameters measured, samples were organised into three groups: a first one, composed of tailings samples, was mainly defined by Factor 1, showing increasing sand percentages, higher electrical conductivity and metal and As concentrations (except $CaCl_2$-extractable Cu); a second one, defined by Factor 2, was formed by F0 and F1 samples (the ones from the tailings' surroundings), showing increasing $CaCl_2$-extractable metal (Cd, Cu, Pb and Zn) concentrations and organic matter content; and a third one, which included F2 and F3 samples (1–3 km from tailings), was mainly defined by Factor 1 but opposite to tailings samples, showing higher percentage of $CaCO_3$ and clay content.

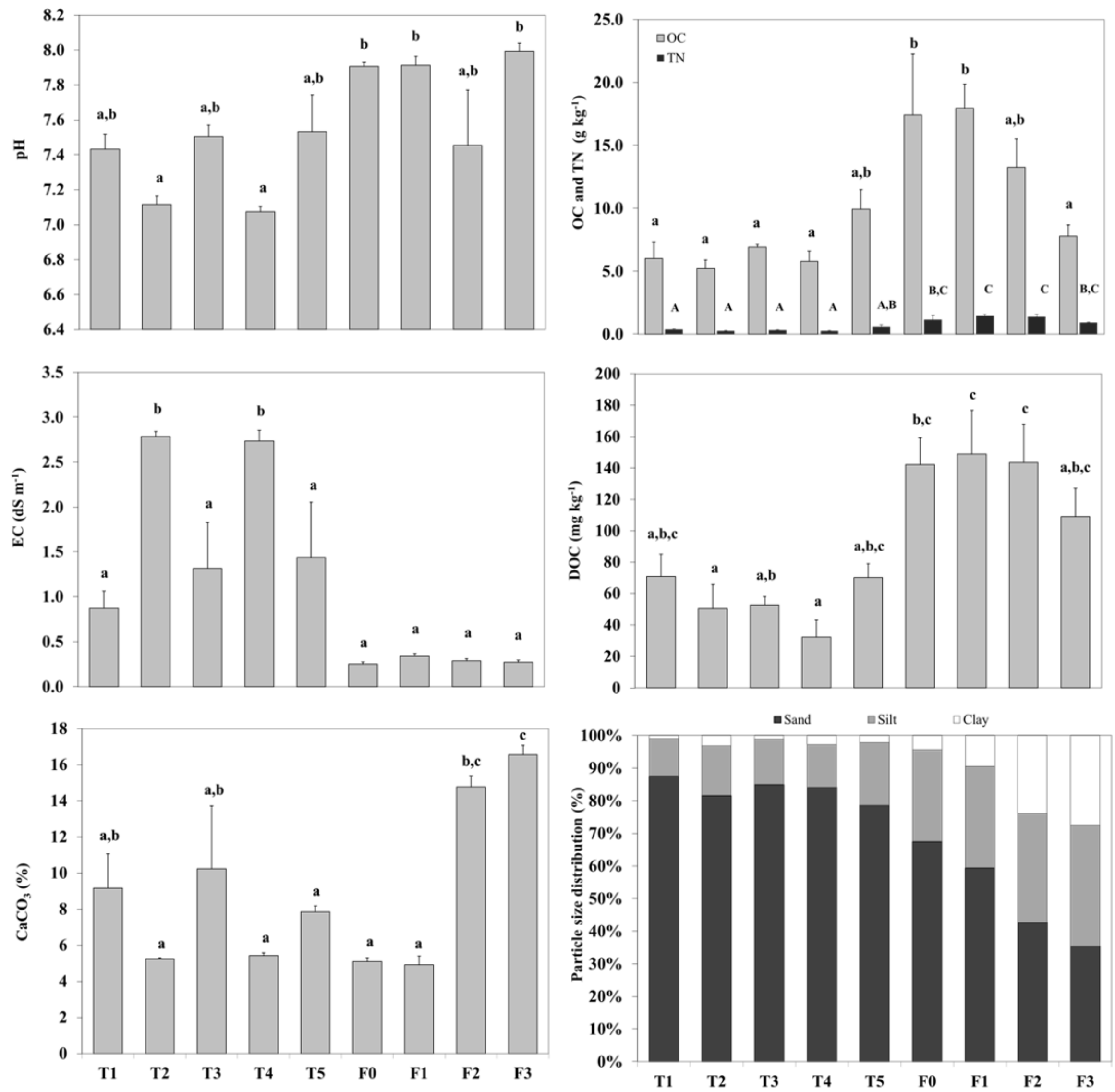

Figure 7.3. Soil properties from tailings (T1–T5) and soil forest samples (F0, F1 – up to 100 m, F2 – up to 1 km, F3 – up to 3 km). EC is electrical conductivity; $CaCO_3$ is percentage of equivalent calcium carbonate; OC is organic carbon; TN is total nitrogen, DOC is dissolved organic carbon. Bars on columns are standard error. *N* = 3 or 4. Different letters indicate significant differences (p<0.05).

Table 7.1. Total and $CaCl_2$ -extractable As and metal concentrations for tailings (T1–T5) and soil forest samples (F0–F3). Data (average ± standard error) are mg kg^{-1}. N = 3 or 4. Different letters indicate significant differences for each element and measurement ($p<0.05$). Detection limit was 0.010 mg kg^{-1} for $CaCl_2$-extractable concentrations.

Sampling Site	As		Cd		Cu		Mn		Pb		Zn	
Total Concentrations												
T1	541 ± 107	c,d	29 ± 3	a,b	115 ± 21	b	9732 ± 794	c	6833 ± 2396	c,d	9516 ± 502	c
T2	793 ± 64	d	47 ± 10	b	107 ± 4	b	10,329 ± 342	c	5850 ± 424	c,d	9655 ± 586	c
T3	227 ± 27	b	36 ± 12	a,b	86 ± 3	b	7639 ± 511	b	4665 ± 356	c,d	8685 ± 1265	c
T4	675 ± 34	d	28 ± 7	a,b	115 ± 2	b	10,813 ± 116	c	5924 ± 462	c,d	8616 ± 548	c
T5	698 ± 36	c,d	23 ± 8	a,b	95 ± 1	b	10,058 ± 111	c	2873 ± 241	c	4356 ± 284	b
F0	657 ± 45	d	27 ± 12	a,b	112 ± 6	b	10,343 ± 335	c	6288 ± 313	c,d	8653 ± 473	c
F1	320 ± 86	b,c	47 ± 2	b	94 ± 4	b	9915 ± 230	c	11,854 ± 2340	d	10,715 ± 959	c
F2	47 ± 1	a	6 ± 0	a	42 ± 4	a	2143 ± 366	a	1051 ± 164	b	979 ± 169	a
F3	18 ± 11	a	17 ± 11	a,b	45 ± 3	a	1635 ± 62	a	398 ± 23	a	264 ± 16	a
$CaCl_2$-extractable Concentrations												
T1	<0.010		0.017 ± 0.002	a	<0.010		0.298 ± 0.162	a,b	<0.010		0.726 ± 0.093	a,b
T2	0.034 ± 0.006	b	0.029 ± 0.003	a,b	<0.010		0.552 ± 0.103	b	<0.010		1.140 ± 0.123	a,b,c
T3	0.011 ± 0.000	a	0.044 ± 0.008	b,c	<0.010		0.435 ± 0.172	b	0.018 ± 0.005	a	1.624 ± 0.760	b,c
T4	0.017 ± 0.002	a,b	0.021 ± 0.004	a,b	<0.010		0.164 ± 0.033	a,b	<0.010		0.819 ± 0.146	a,b
T5	0.027 ± 0.001	b	0.028 ± 0.006	a,b	0.063 ± 0.006	a	0.255 ± 0.096	a,b	<0.010		1.609 ± 0.521	b,c
F0	0.017 ± 0.002	a,b	0.017 ± 0.002	a	0.045 ± 0.006	a	0.361 ± 0.132	b	<0.010		0.757 ± 0.072	a,b
F1	0.017 ± 0.003	a,b	0.060 ± 0.002	c	0.046 ± 0.015	a	0.396 ± 0.099	b	0.049 ± 0.004	b	2.506 ± 0.082	c
F2	0.019 ± 0.003	a,b	<0.010		0.038 ± 0.007	a	0.238 ± 0.080	a,b	<0.010		0.144 ± 0.023	a
F3	0.015 ± 0.001	a,b	<0.010		0.051 ± 0.008	a	0.062 ± 0.028	a	<0.010		0.094 ± 0.002	a

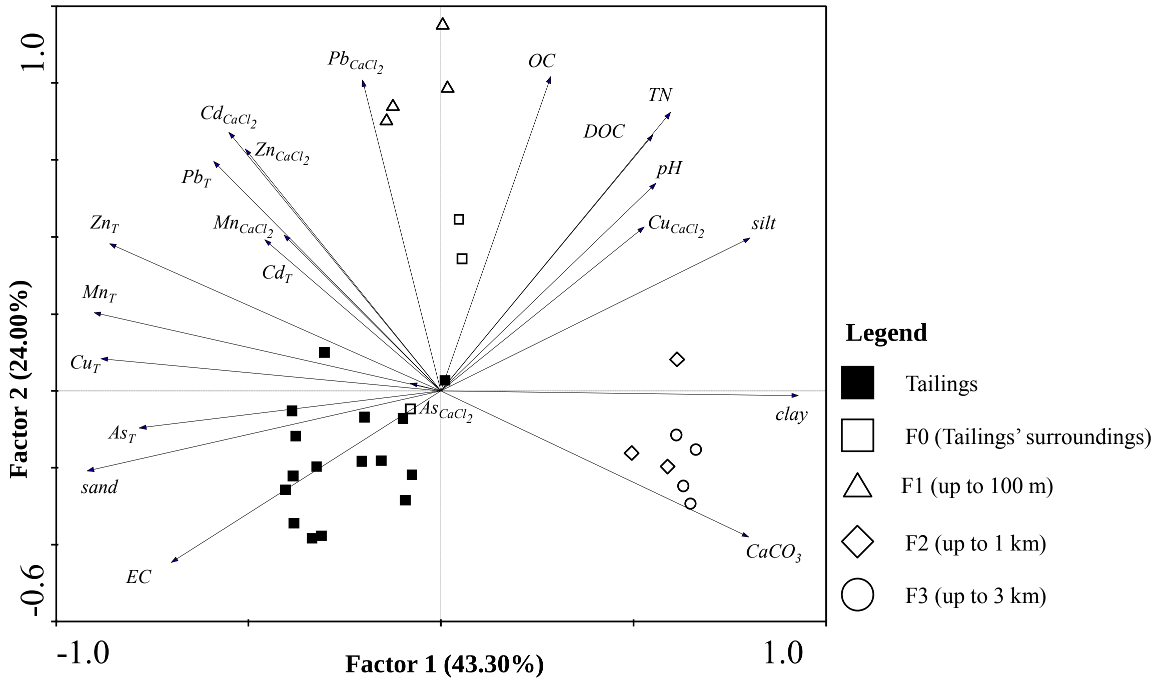

Figure 7.4. PCA analysis for the studied samples and soil parameters. "M_T" are total element concentrations; "M_{CaCl2} " are $CaCl_2$-extractable concentrations; "OC" is organic carbon; "TN" is total nitrogen.

7.3.2. Plant metal uptake

Arsenic and metal concentrations in pine needles are shown in Figure 7.5. Needles of the sampled forest trees (F0–F1) showed lower Mn and Zn concentrations (40–100 mg kg^{-1} Mn, 25–55 mg kg^{-1} Zn) than the ones taken from the tailings (up to 320 mg kg^{-1} Mn, 80–130 mg kg^{-1} Zn). For Cu, all the samples were in the same range (~2 mg kg^{-1}) except the ones from T5 which showed 3.5 mg kg^{-1} Cu. In relation to Pb, pine needles from the forest areas corresponding to F1, F2 and F3 had concentrations of around 4 mg kg^{-1}, which significantly differed from the ones obtained at the tailings (from 7 to 28 mg kg^{-1}) and F0 (~20 mg kg^{-1}).

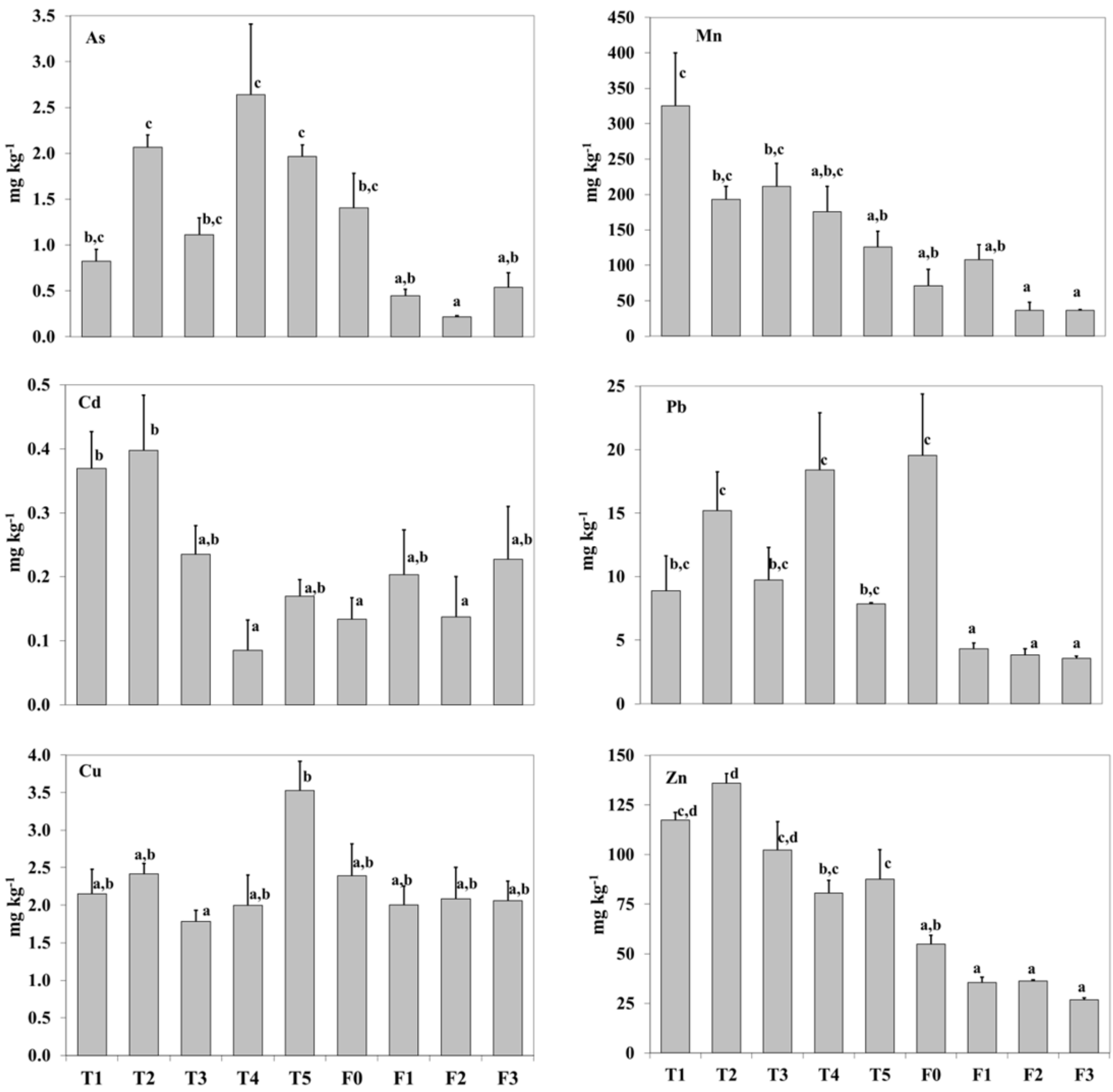

Figure 7.5. Arsenic and metal (Cd, Cu, Mn, Pb, Zn) concentrations in pine needles from each studied zone. Bars on columns are standard error. Different letters indicate significant differences (p<0.05).

7.4. Discussion

7.4.1. Metal(loid) content in the studied soils and influence of soil parameters on metal(loid) availability

According to the Spanish guidelines for soil contamination, the thresholds to determine whether a soil is polluted or not must be established regionally, based on the site-specific geochemical backgrounds. A comprehensive report of the geochemical backgrounds of Murcia Region performed by Martínez-Sánchez and Pérez-Sirvent (2007) showed that the soils from the nearby Cartagena plain had a geochemical basis of 12.6 mg kg^{-1} Cu, 9 mg kg^{-1} Pb and 41 mg kg^{-1} Zn. The same authors affirmed that the thresholds in the soils from the Cartagena-La Union area should be established separately due to their different geochemical basis. For comparing, it is meaningful to employ technical reports or studies on local soils. Following this criteria, some agricultural soils located around La Union town have shown concentrations of 21 mg kg^{-1} Cu, 500 mg kg^{-1} Pb and 900 mg kg^{-1} Zn (Conesa et al., 2010) which were very similar to the concentrations obtained for F3 samples but lower than the ones obtained at F0, F1 or F2.

Areas surrounding tailings have been shown to be impacted by high concentrations of metal(loid)s (Navarro et al., 2012). At the present study, the soil samples closest to tailings sites (F0 and F1) significantly differed from F2 and F3 in relation to As and metal concentrations (except Cd). García-Lorenzo et al. (2012) classified the extent of the pollution in this kind of situations as it follows: primary contamination, those areas surrounding tailings where soils develops directly on waste materials (represented by F0); secondary contamination, areas where parent materials have been mixed with previously transported tailings wastes (F1); and tertiary contamination, areas where the transported tailings materials appear as negligible fractions in comparison with the parent materials (F2 and F3). The employment of this classification in the present study was supported by the mineralogical results obtained (Table 7.2). Gypsum was found at F0 samples (not at F1). Although this mineral does not form part of the main mineral paragenesis in this area, composed by greenalite-clinochlore-magnetite-sulfides-carbonates-silica (Pérez-Sirvent et al., 2012), it has been found to appear as a secondary mineral in local tailings and soils deeply impacted by tailings materials (García-Lorenzo et al., 2012). The mineralogy of F0 and F1 also showed, among other minerals, clinochlore, which has been related to As-enriched mining wastes in this area (Pérez-Sirvent et al., 2012) However, the presence of dolomite (carbonate rock which occur in local unaltered soils) at F1 samples confirmed that the soils developed in these sampling points contained a mixture with the surrounding parent carbonated materials, which were not present at F0.

Table 7.2. Mineralogy of the tailings and forest soil samples studied.

Mineral	T1-T5	F0	F1	F2	F3
Quartz	50-70%	53	68	60	70
Muscovite	up to 5%	10	15	7	10
Clinochlore	5-20%	9	5		
Magnesium calcite	up to 20%	10	5	20	9
Dolomite			5	9	5
Gypsum	10-20%	16			
Beudantite	up to 8%				
Plumbojarosite	up to 20%				
Others		2	2	4	6

In spite of showing the closest metal(loid) concentrations to the ones obtained for tailings samples, F0 and F1 areas were able to support a similar vegetation to F2 and F3. It is known that some plants may grow in soils containing high metal(loid) concentrations without showing visible symptoms, which could be a risk in case the impacted soils support agricultural, grazing or forest use (Conesa et al., 2009).

As it is known, metal availability in soils is conditioned by several factors such as pH, redox potential, organic matter or electrical conductivity (María-Cervantes et al., 2010). In the studied samples, the neutral to alkaline pH may have contributed to keep low $CaCl_2$-extractable concentrations. In addition, the percentages of $CaCO_3$ between 5 and 10 in most of samples might provide enough buffer capacity to prevent the drop of pH and thus, metal leaching.

Arsenic is a metalloid which is known to show a different geochemistry than metals (e.g. its mobility is generally favoured with higher pH). The studied samples (tailings and forest ones) showed lower $CaCl_2$-extractable As concentrations than the rest of metals (except Cd, which were in the same range) in spite of having slightly alkaline pH. However it is also known that minerals such as gypsum or calcite may act precipitating As and decreasing its availability (Fernández-Martínez et al., 2006), which may have occurred at the present study.

Organic matter may also play an important role in enhancing metal availability especially in relation to Cu and, to a lesser extent, Pb (Strobel et al., 2001). For instance, $CaCl_2$-extractable Cu concentrations in the studied samples showed significant correlations ($r > 0.5$, $p<0.01$) with OC, DOC and TN (Figure 7.4). In this way, forest samples, which contained higher DOC concentrations, shower higher $CaCl_2$-extractable Cu concentrations than tailings samples (except T5). Nevertheless, these concentrations were much lower than the ones obtained for Zn or Mn, and therefore it should not be considered Cu leaching an environmental issue at these sites.

7.4.2. Employment of *P. halepensis* to monitor metal pollution and as a feasible plant species for phytostabilisation

Several authors have employed *P. halepensis* to monitor metal contamination in industrial areas (Sun et al., 2009; Cicek and Koparal, 2004). Then, the analysis of pine needles may be employed as a tool to monitor the availability of pollutants at a specific site. In the case of the current work, the differences of metal(loid)s accumulation among the selected trees might be attributed to the differences in soil parameters, since the variations in relation to microclimate or physiological status were considered negligible. In this way, only for Pb and Zn, the needles taken at F2 and F3 sites (the less impacted by metal(loid)s) showed significant lower concentrations ($p<0.05$) than the ones analysed from tailings. Although the highest concentrations normally occurred in needles from tailings trees (except Pb, which occurred at F0), these samples showed high variability (above all for As, Cd and Mn), giving comparable concentrations to the ones obtained at the forest samples. This may question the employment of pine needles to monitor metal(loid) pollution under these soil conditions. Moreover, $CaCl_2$-extractable metal(loid)s concentrations did not show any significant correlation (p>0.05) with their corresponding concentrations in needles indicating that this extraction procedure might not be suitable to predict metal accumulation by pine trees. For instance, the highest $CaCl_2$-extractable Zn obtained for F1 samples did not correspond with the highest Zn uptake.

The concentrations of As and metals accumulated in the pine needles of the present work were in the ranges of those obtained by several authors in other polluted sites. For instance, Sun et al. (2009) obtained similar metal concentrations when comparing pine needles of industrial and natural areas in China: e.g. Pb ranged from 1.5 to 20 mg kg^{-1} and Cu, from 3 to 10 mg kg^{-1}. Studies conducted in Turkey by Cicek and Koparal (2004) showed concentrations up to 5 mg kg^{-1} Cd, 50 mg kg^{-1} Pb and 100–200 mg kg^{-1} Zn in needles of *Pinus nigra* which grew at polluted soils around a thermal power plant.

It is known that high biomass plant species may enhance the phytodilution of metals (Robinson et al., 1998). In comparison to other grasses or shrub species proposed for phytostabilisation in Mediterranean semiarid climates (Martínez-Sánchez et al., 2012) trees such as *P. halepensis* may have the advantage, when dealing with phytostabilisation, of providing better soil retention (Pulford and Watson, 2003). In case of pine trees, the addition of organic matter from needles falling and the wide canopy, may contribute to decrease the erosion by creating a mulching which favours soil microbiology and protects the soil from rain drops. However, it is reasonable to think that the slower growth of trees may suppose a constraint in relation to other plant species such as grasses. Then, the assembling of herbs/shrubs at the first stages of a phytostabilisation scheme combined to trees at long-term, could result a good option. In this case, *P. halepensis* could suppose a suitable candidate, since in comparison to the available tree species employed for restoration of degraded semiarid areas, it has shown a relatively faster growth.

7.5. Conclusions

The risk assessment of potential land uses (agricultural or forest) of areas surrounding the studied tailings should take into account the high metal(loid)s concentrations which contain. The alkaline pH and some carbonate minerals of the surrounding soils may keep low metal(loid) available concentrations and therefore, decrease the risk of pollutants leaching. $CaCl_2$-extractable As and metal concentrations did not correlate with the corresponding concentrations in pine needles indicating that this procedure might not be suitable to predict metal(loid) availability in pine trees.

Compared to low biomass plant species, P. halepensis looks a suitable plant species to be employed in the phytostabilisation of tailings due to a larger root system, which may provide a better soil retention, and its relative low metal uptake. Further research must be focused in the growth of *P. halepensis* in tailings by understanding the physiological mechanisms and soil parameters involved in plant establishment (e.g. soil microbiology, antioxidative response).

7.6. Acknowledgements

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CHAPTER 8. Elemental and stable isotope composition of *Pinus halepensis* foliage along a metal(loid) polluted gradient: implications for phytomanagement of mine tailings in semiarid areas

Chapter 8

Elemental and stable isotope composition of *Pinus halepensis* foliage along a metal(loid) polluted gradient: implications for phytomanagement of mine tailings in semiarid areas

Isabel Párraga-Aguado, José-Ignacio Querejeta, María Nazaret González-Alcaraz, Francisco J. Jiménez-Cárceles, Héctor M. Conesa

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Abstract

Aleppo pine (*Pinus halepensis* Mill.) is a widely used species for restoring degraded semiarid areas, but its use for the revegetation of metal(loid) polluted soils has not been thoroughly investigated. The main goal of this research was to study the ecophysiological status and elemental composition of spontaneous populations of *Pinus halepensis* growing on mine tailings to assess their use in phytomanagement of mine spoils in semiarid climates. Edaphic characteristics and the physiological (by stable isotopes) and nutritional status of pine trees were determined on mine tailings, in the metal(loid)-polluted surroundings and a non-polluted control area. Low soil phosphorus availability at the tailings was found to be a more important limiting factor for pine physiological performance than high soil metal(loid)s concentrations. Foliar phosphorus concentrations showed a strong negative correlation with foliar sulphur concentrations along the studied transect. The carbon and oxygen isotopic composition ($\delta^{13}C$ and $\delta^{18}O$) of pine needles indicated that trees at the tailings were less water stressed than those in surroundings or control areas. The low foliar $\delta^{15}N$ of pines growing at the tailings was due to low soil fertility and/or a heavy reliance on symbiotic ectomycorrhizal fungi for nitrogen uptake. The results of this study indicate that *Pinus halepensis* is a suitable tree species for the phytostablisation of neutral or slightly-alkaline mining wastes in semiarid environments, thanks to its drought hardiness and good adaptation to low soil fertility and salinity.

8.1. Introduction

Aleppo pine (*Pinus halepensis* Mill.) is a widely used tree species for restoring degraded soils in semiarid regions due to its remarkable drought resistance and tolerance to low fertility soils, particularly in the Mediterranean region where it is naturally distributed (Querejeta et al., 2008). The extensive use of Aleppo pine in monoculture afforestation and reforestation schemes has been questioned due to the resulting low biodiversity of the restored sites (Maestre and Cortina, 2004). However, many studies have shown an enhancement of key soil properties such as carbon soil contents (Maestre et al., 2003) or aggregate stability (Caravaca et al., 2002) following pine afforestation.

Mining pollution has become an important environmental issue in many areas worldwide due to the large amount of tailings containing high concentrations of toxic metal(loid)s (e.g. Bhattacharya et al., 2006; García-Lorenzo et al., 2012; Courtney, 2013). Traditionally, the restoration of mine tailings has used engineering approaches based on technical reclamation techniques (dig and dump, capping layers) which entail high economic costs (Conesa and Schulin, 2010). For this reason, low intervention techniques based on the use of vegetation to achieve the surface stabilisation of mine tailings (phytostabilisation) have been proposed as an effective alternative (Mendez and Maier, 2008). Due to the unfavourable conditions of mine tailings for vegetation establishment and growth (e.g. high salinity, high phytotoxic metal(loid)s concentrations, low water holding capacity, low fertility), the range of suitable species for phytostabilisation is restricted to stress-tolerant plant species. This may hamper the goal of creating a permanent vegetation cover which assures the long-term self-sustainability of the system. To overcome this problem, current research approaches to this topic have pointed out the importance of favouring natural processes of ecological succession to increase the long-term stability and biodiversity of plant communities colonizing post-mined landscapes (Tropek et al., 2012). The rhizospheres of pioneer plant species usually show enhanced soil microbiological activity and increased fertility in relation to bulk soil of tailings (Wenzel, 2009). The magnitude of this soil fertility improvement is plant species-dependent and is of great interest when dealing with the revegetation of mining areas, since the long-term vegetation succession may benefit from facilitation processes provided by the rhizospheres of the pioneer vegetation. Plant facilitation is known to occur through several mechanisms in which pioneer plants favour the establishment of other plant species whether by increasing soil fertility (Norland and Veith, 1995) or by ameliorating limiting factors such as soil acidity (Rufo and De la Fuente, 2010) or salinity (Bonanomi et al., 2011).

For the long-term success of phytostabilisation, it is important to use assemblages of plant species that show complementary ecological functions: grasses are fast growing species that can provide rapid soil cover, while trees can provide a more enduring protection against erosion (Párraga-Aguado et al., 2013a). In spite of the potential of *Pinus halepensis* for restoring degraded lands in semiarid areas, its use as part of plant assemblages in metal(loid) polluted soils has not been thoroughly investigated. There have been some attempts at employing pine trees for biomonitoring environmental pollution (Sun et al., 2009; Kord et al., 2010). Preliminary studies have reported the spontaneous colonization of metal(loid) enriched tailings by *Pinus halepensis* in the semiarid area of Cartagena-La Union (Southeast Spain) (Párraga-Aguado et al., 2013a). However, a more comprehensive study of the edaphic factors and plant physiological parameters which determine pine establishment and growth in mine tailings is urgently needed before this species can be routinely used for the phytostabilisation of mine tailings.

Foliar elemental analysis is considered an important tool to assess the nutrient status of conifer trees (López-Serrano et al., 2005). Analysing the carbon, oxygen and nitrogen stable isotope composition of plant leaves can provide valuable insights into the physiological responses of plants to a wide array of biotic and abiotic environmental factors (Dawson et al., 2002), particularly in semiarid ecosystems (e.g. Querejeta et al., 2008; 2007; Ramírez et al., 2009). The carbon isotope composition of plants ($\delta^{13}C$) provides a useful index for assessing intrinsic water use efficiency, i.e. the ratio of photosynthetic carbon fixation to stomatal conductance (Farquhar et al., 1989). The oxygen isotope composition of plants ($\delta^{18}O$) reflects the isotopic composition of the source water used by the plant, evaporative effects at leaf level, and isotopic exchange between oxygen atoms in organic molecules and plant water (Barbour, 2007). Leaf $\delta^{18}O$ can provide a time-integrated measure of plant stomatal conductance when other sources of variation (mainly source water $\delta^{18}O$) are negligible (Barbour and Farquhar, 2000; Barbour, 2007; Farquhar et al., 2007). Simultaneous measurement of both $\delta^{13}C$ and $\delta^{18}O$ in leaves may help separate the independent effects of carbon fixation and stomatal conductance on $\delta^{13}C$, since $\delta^{18}O$ shares dependence on stomatal conductance with $\delta^{13}C$, but is unaffected by photosynthetic rate (Scheidegger et al., 2000). The nitrogen isotope composition of leaves ($\delta^{15}N$) reflects the net effect of a wide range of processes, including the isotopic signature of the soil N sources used by the plant, mycorrhizal associations, soil fertility levels, temporal and spatial variation in N availability or changes in plant demand (Dawson et al., 2002; Craine et al., 2009).

The main goal of this work was to assess the nutritional and ecophysiological status of *Pinus halepensis* trees growing on metal(loid) polluted tailings in a semiarid environment. For this purpose, we used a transect sampling design encompassing a mine tailings deposit, the polluted surrounding area and a non-polluted control area to assess variations in soil properties and the physiological and nutritional status of pine trees. We hypothesized that pine trees growing on tailings would show a poorer nutrient and altered physiological status compared to those in unpolluted control areas, which might compromise the suitability of *Pinus halepensis* for mine tailings phytostabilisation.

8.2. Materials and methods

8.2.1. Study area

The study was conducted at a tailings deposit located in the Cartagena-La Union Mining District (Southeast Spain; Figure 8.1). This area has been intensively mined since ancient times for metals such as Pb or Zn. Mining activity ceased in the last decade of the 20th century, but hundred of hectares remain affected by mine tailings (Conesa and Schulin, 2010). The climate is semiarid Mediterranean (250–300 mm annual rainfall, ~18 °C annual average temperature, 856.8 mm year^{-1} of evapotranspiration). Some tailings in the area have been spontaneously colonized by pioneer vegetation including grasses (*Piptatherum miliaceum* (L.) Cosson), weeds (*Zygophyllum fabago* L.), shrubs (*Atriplex halimus* L.) and trees (*Pinus halepensis*) (Párraga-Aguado et al., 2013b).

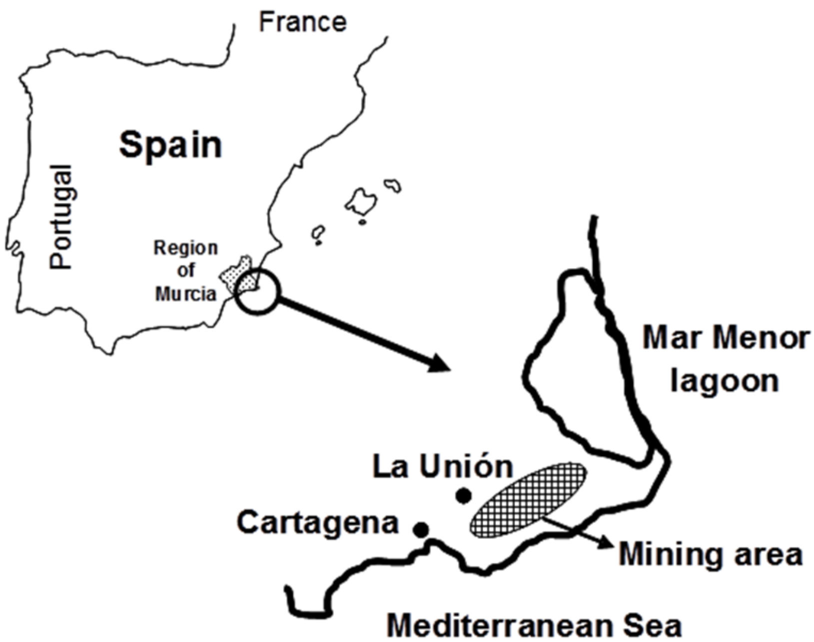

Figure 8.1. Location of studied area (0 – 400 m above sea level; 37°37′20″N, 0°50′55″W–37°40′03″N, 0°48′12″W).

8.2.2. Sampling design

Sampling sites were distributed between the selected tailings deposit, the surrounding polluted area and a non-polluted control area (Figure 8.2). At the tailings, soil samples were taken from bare areas (Bulk-T, *N*=12), the rhizosphere of isolated *Pinus halepensis* trees (IP-T, *N*=4) and Fertility Islands (vegetation patches composed by associations of several plant species including pine trees, FI-T, *N*=3). Soil samples were also taken from the polluted areas surrounding the tailings (*Surroundings*, *N*=7), within 100 m distance from the tailings' deposit border. Finally, a non-mining-impacted forest located 1 km away from the tailings (*Control*, *N*=4) was used as a control. A listing of the plant species found at each sampling site is provided as Table 8.1. At each site, at least four adult pine trees of similar height (~3 m) were sampled for their needles. Foliage samples were taken at 1.5-2.0 m height from South-facing (i.e. sun exposed) pine canopies on September 2012.

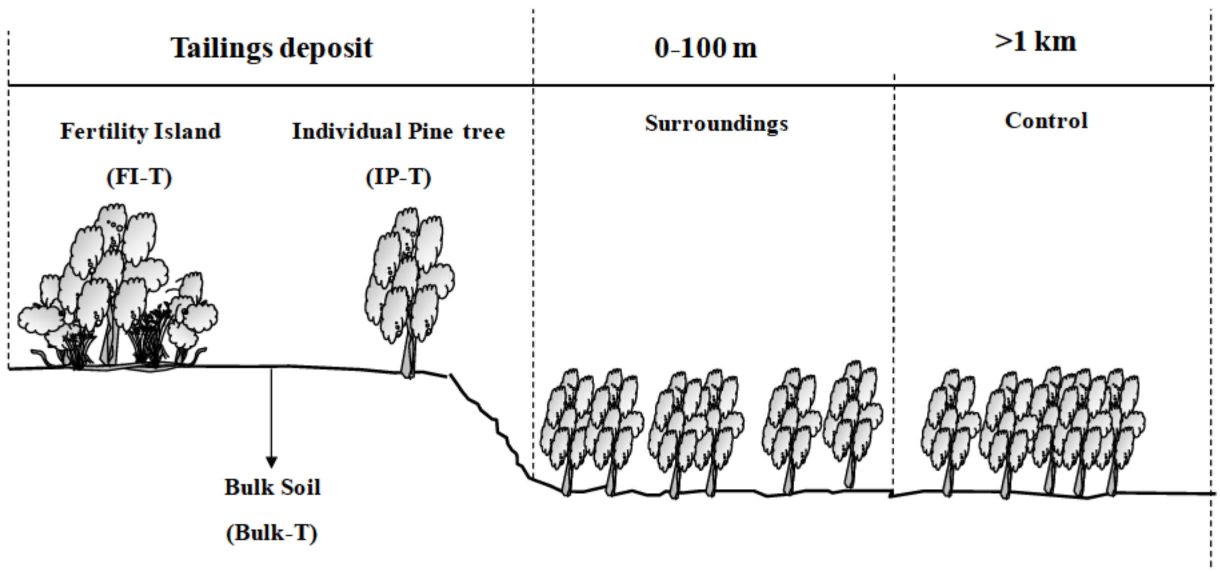

Figure 8.2. Scheme of sampling transect.

Table 8.1. Relation of plant species recorded at each sampling site.

Family	Plant species	Sampling site			
		Fertility Islands	Rest of tailing	Surroundings	Control
Anacardiaceae	*Pistacea lentiscus* L.	*x*		*x*	*x*
Araceae	*Arisarum vulgare* Targ.-Tozz			*x*	*x*
Chenopodiaceae	*Salsola kali* L		*x*		
	Salsola oppositifolia Desf.	*x*			
Cistaceae	*Cistus monspeliensis* L.			*x*	*x*
	Helianthemum almeriense Pau in Mem. Mus				*x*
Compositae	*Atractylis humilis* L.				*x*
	Helichrysum decumbens (Lag.) Camb	*x*		*x*	
	Phagnalon saxatile (L.) Cass.			*x*	*x*
	Sonchus tenerrimus L. var. *tenerrimus*	*x*	*x*	*x*	
Crassulaceae	*Sedum sediforme* (Jacq) Pau				*x*
Cupressaceae	*Tetraclinis articulata* (Vahl) Mast.				*x*
Gramineae	*Brachypodium retusum* (Pers.) Beauv.			x	*x*
	Piptatherum miliaceum (L.) Cosson	*x*			
	Stipa tenacissima L.				*x*
Labiatae	*Lavandula dentata* L.			*x*	*x*
	Rosmarinus officinalis L.				*x*
	Satureja obovata Lag,				*x*
	Teucrium carthaginense Lange			x	*x*
	Thymus hyemalis Lange			*x*	*x*
Leguminosae	*Calicotome intermedia* (C. Presl) Guss.			*x*	*x*
	Dorycnium pentaphyllum Scop.			*x*	
	Hippocrepis ciliata Willd.				*x*
	Retama sphaerocarpa (L.) Boiss.			*x*	
Liliaceae	*Asparagus stipularis* L. in J. A. Murray			*x*	*x*
	Urginea maritima (L.) Baker			*x*	
	Linum strictum L.				*x*
Olaceae	*Olea europaea* L.	*x*		*x*	*x*
Palmae	*Chamaerops humilis* L.			*x*	*x*
Pinaceae	*Pinus halepensis* Miller	*x*	*x*	*x*	*x*
Plumbaginaceae	*Limonium cossonianum* Kunthze, Revis.	*x*	*x*		
Rhamnaceae	*Rhamnus lycioides* L. subsp. *lycioides*			*x*	*x*
Rubiaceae	*Rubia peregrina* L.			*x*	*x*
Tamaricaeae	*Tamarix canariensis* Willd.	*x*			
Thymelaeaceae	*Thymelaea hirsuta* (L.) Endl				*x*
Umbeliferae	*Bupleurum gibraltaricum* Lam.			*x*	*x*
	Eryngium campestre L.				*x*
Zygophyllaceae	*Zygophyllum fabago* L.	*x*	*x*		

8.2.3. Soil and plant analyses

Soil samples were air dried, sieved through 2 mm sieves, homogenized manually in plastic trays and stored in plastic bags prior to laboratory analysis. Soil analyses included: 1) the determination of pH and Electrical Conductivity (EC) in a 1:5 soil:water ratio using a Crison Basic 20 pH meter and a Crison Basic 30 conductivity meter, respectively; 2) particle size distribution, following the method of Bouyoucos densimeter (Gee and Bauder, 1986); 3) equivalent calcium carbonate (%$CaCO_3$), using the Bernard calcimeter method; 4) Total Nitrogen (TN), using the Kjeldahl method (USDA, 1996); and 5) Organic Carbon (OC), by the oxidation of organic carbon using potassium dichromate. To determine total element composition, soil sub-samples were ground and analysed by X-ray fluorescence (Bruker S4 Pioneer).

Water extractable ions (including metal(loid)s) and Dissolved Organic Carbon (DOC) were determined using the previous 1:5 soil:water mixture (Ernst 1996). The resulting extracts were filtered through nylon membrane 0.45 μm syringe filters (WICOM). DOC was measured by a TOC-automatic analyzer (TOC-VCSH Shimadzu). Major ions (cations: Na^+, Ca^{2+}, Mg^{2+}, K^+; anions: Cl^-, SO_4^{2-}) were analyzed using an ion chromatograph (Metrohm). Water extractable metal(loid)s (As, Cd, Cu, Mn, Pb, Zn) were analysed using an ICP-MS (Agilent 7500A). Available phosphorus (Available-P) was measured following the Olsen method (Olsen et al., 1954) after using 0.5 M $NaHCO_3$ solution at pH 8.5 as extractant and measuring the extracted PO_4^- by a Lambda 25 UV/VIS spectrometer (Perkinelmer) at λ= 820 nm.

Soil microbiology was assessed by quantifying soil microbial biomass and two enzymatic activities: dehydrogenase and β-glucosidase. Unaltered portions of soil samples were stored at −20 °C for this purpose. Dehydrogenase activity was determined according to García et al. (1993) by measurement of INTF (iodo-nitrotetrazolium formazan) by spectrophotometry at 490 nm (Thermo Fisher Scientifc Multiskan GO) and reported as μg INTF g dry wt^{-1} h^{-1}. β-glucosidase activity was determined according to the modification of Ravit et al. (2003) proposed by Reboreda and Caçador (2008) by measurement of pNP (p-Nitrophenol) by spectrophotometry at 410 nm and reported as μmol pNP g dry wt^{-1} h^{-1}. Soil Microbial Biomass Carbon (MBC) was estimated by the measurement of the extractable organic C by 0.5 M K_2SO_4 after a 24 h $CHCl_3$-fumigation (Vance et al., 1987; Wu et al., 1990). Carbon in the resulting extracts was measured employing a TOC-automatic analyzer (TOC-VCSH Shimadzu).

Plant samples were carefully washed with distilled water and dried at 65 °C for 72 h prior to grinding. For each sample, 0.1–0.5 g was incinerated prior to a suspension of the ash using concentrated nitric acid. The resulting extracts were filled to 25 ml and filtered through CHM F2041-110 ashless filter papers (20–25 μm pore diameter). Then, metal(loid)s (As, Cd, Cu, Mn, Ni, Pb, Zn) were analysed using a ICP-MS (Agilent 7500A) and Cl, P and S were analysed using an ion chromatograph (Metrohm) while Ca, K, Mg and Na were analysed using a flame atomic absorption spectrometer (UNICAM 969 AA). Plant analyses were referenced using a CTA-VTL-2 certified material (Virginia tobacco leaves). The percentages of recovery were 110 % for As, 89 % for Cd, 119 % for Cu, 104 % for Mn, 96 % for Pb and 100 % for Zn.

Finely ground plant material was used for stable isotope measurements at the University of California-Davis Stable Isotope Facility. Leaf C, N, $\delta^{13}C$ and $\delta^{15}N$ analyses were conducted using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). $\delta^{13}C$ and $\delta^{15}N$ data are expressed relative to international standards V-PDB (Vienna PeeDee Belemnite). Leaf $\delta^{18}O$ analyses were performed using an elemental PyroCube (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The final delta values are expressed relative to the international standard V-SMOW (Vienna Standard Mean Ocean Water). The long-term standard deviation is 0.2 permil for ^{13}C and 0.3 permil for ^{15}N.

8.2.4. Statistics

Analysis of variance (one way ANOVA) with Tukey's Test (p<0.05) and Pearson's correlations (p<0.01) were done with SPSS 19.0.0 software (SPSS, 2010).

The data were log-transformed when they did not meet the Levene test for the homogeneity of variances. Environmental gradients were examined by a Principal Component Analysis (PCA) using the 'CANOCO for Windows' program v4.02 (ter Braak and Smilauer, 1999). This analysis can be used to determine the main parameters which cause the variability in a set of data.

8.3. Results

8.3.1. Soil parameters

We found significant differences (p<0.05) between tailings soils (Bulk-T, IP-T and FI-T) and *Control* soils for most of the measured parameters, whereas *Surroundings* soils generally showed intermediate values between those obtained for *Control* and tailings samples. Soil fertility was much lower at the tailings than in *Control* areas (lower OC, TN, DOC and available-P, and higher EC; Table 8.2). For instance, DOC and TN concentrations in *Control* soil samples were up to 10-fold and 20-fold higher, respectively, than in tailings samples. The tailings soil samples also showed much lower microbial biomass and extracellular enzymatic activity values than *Control* soils: β-glucosidase and dehydrogenase activity at the tailings were half or less than half respectively to those in *Control* soils, whereas MBC was around 10-fold lower. Available-P concentration in tailings soil was about half of that in the *Control* area. On the other hand, EC of tailings samples was at least five times higher than the values obtained for *Control* samples. The tailings contained around 80% of sand, more than 2-fold the percentages measured in the *Control* area. In fact, sand percentages correlated negatively with all the soil fertility parameters, including microbial biomass and enzymatic activity (p<0.01, r<−0.8), and positively with EC (p<0.01, r=0.75). Soil fertility parameters such as OC, TN, DOC and available-P were positively correlated among themselves (p<0.01, r>0.8). However, only TN and available-P showed significant correlations (p<0.01, r>0.7) with soil microbial biomass and activity

(MBC and β-glucosidase and dehydrogenase activities). Soil pH varied within the range of 7–8 for all the soil samples along the transect (Table 8.2). The highest pH values were found in *Control* samples (pH~8), coinciding with the highest $CaCO_3$ contents (~200 g kg^{-1}).

Table 8.2. Results of soil samples. Data are average ± standard error. 3≤*n*≤12. "nd" means not detected. Different letters within the same row indicate significant differences (ANOVA with Tukey Test, *p*<0.05). "Bulk-T" are bulk soil samples from the tailings; "IP-T" are rhizospheres of isolated pine trees at the tailings; "FI-T" are fertility islands of the tailings; "EC" is Electrical Conductivity; "OC" is Organic Carbon; "TN" is Total Nitrogen; "DOC" is Dissolved Organic Carbon; "MBC" is Soil Microbial Biomass Carbon", "Available-P" is Available Phosphorus.

Soil Parameter		Units	Samples									
			Tailings						Surroundings		Control	
			Bulk-T		IP-T		FI-T					
pH (1:5)			7.23 ± 0.02	a	7.07 ± 0.03	a	7.53 ± 0.21	b	7.88 ± 0.04	c	7.99 ± 0.05	c
EC (1:5)		dS m^{-1}	3.06 ± 0.05	c	2.74 ± 0.12	c	1.44 ± 0.62	b	0.36 ± 0.02	a	0.27 ± 0.02	a
$CaCO_3$		g kg^{-1}	58.96 ± 10.36	a	65.09 ± 2.01	a	92.31 ± 3.90	a,b	56.71 ± 3.98	a	202.99 ± 6.41	b
OC			3.29 ± 0.31	a	5.80 ± 0.79	a,b	9.94 ± 1.54	b	22.35 ± 2.94	c	24.66 ± 4.18	c
TN			0.13 ± 0.02	a	0.25 ± 0.04	a	0.59 ± 0.17	b	1.57 ± 0.10	c	2.50 ± 0.25	c
DOC		mg kg^{-1}	22.8 ± 1.8	a	32.3 ± 10.9	a	70.1 ± 8.7	a,b	155.8 ± 18.52	b,c	200.6 ± 77.0	c
Available-P			5.9 ± 0.3	a	7.0 ± 0.1	a,b	7.4 ± 0.5	a,b	9.0 ± 0.45	b,c	10.9 ± 1.0	c
Sand		%	78.0 ± 1.8	c	84.1 ± 1.6	c	78.6 ± 3.0	c	62.0 ± 1.46	b	35.4 ± 3.2	a
Silt			20.2 ± 1.6	a	13.1 ± 1.7	a	19.3 ± 2.9	a	31.6 ± 0.58	b	37.1 ± 4.3	b
Clay			1.8 ± 0.3	a	2.8 ± 0.6	a,b	2.1 ± 0.5	a,b	6.4 ± 1.51	b	27.5 ± 1.2	c
MBC		μg kg^{-1}	17.9 ± 4.8	a	36.2 ± 3.5	a	30.6 ± 6.8	a	183.0 ± 20.47	b	305.1 ± 106.4	b
β-Glucosidase		μmol p-NP g^{-1} h^{-1}	nd		0.12 ± 0.04	a	1.24 ± 0.22	b	1.26 ± 0.05	b	2.35 ± 0.22	c
Dehydrogenase		μg INTF g^{-1} h^{-1}	nd		0.63 ± 0.13	a	0.88 ± 0.19	a	1.83 ± 0.15	b	5.06 ± 0.39	c
Total Metal(loid)s Concentrations	**As**	mg kg^{-1}	621 ± 62	b	675 ± 34	b	698 ± 36	b	396 ± 60	b	18 ± 11	a
	Cd		32 ± 7	a	28 ± 7	a	23 ± 8	a	29 ± 6	a	17 ± 11	a
	Cu		111 ± 7	b	115 ± 2	b	95 ± 1	b	107 ± 4	b	45 ± 3	a
	Mn		9564 ± 507	b	10,813 ± 116	b	10,058 ± 111	b	9560 ± 160	b	1635 ± 62	a
	Pb		5879 ± 882	b,c	5924 ± 462	b,c	2873 ± 241	b	6979 ± 1592	c	398 ± 23	a
	Zn		7488 ± 1126	b,c	8616 ± 548	b	4356 ± 284	b	9183 ± 599	c	264 ± 16	a
Water Extractable Metal(loid)s (1:5)	**As**	μg kg^{-1}	9.6 ± 1.1	a,b	5.9 ± 1.1	a	31.1 ± 13.9	c	21.7 ± 2.9	b,c	26.1 ± 0.9	c
	Cd		4.6 ± 1.3	a	5.2 ± 0.7	a	1.2 ± 0.3	a	2.8 ± 0.5	a	<1	
	Cu		<1		5.4 ± 2.1	a	69.3 ± 32.4	b	56.2 ± 9.6	b	33.0 ± 1.9	b
	Mn		59.8 ± 7.5	b	61.7 ± 9.4	b	206.9 ± 65.8	c	93.3 ± 20.5	b,c	14.5 ± 5.0	a
	Pb		2.3 ± 0.4	a	<1		48.9 ± 44.5	a,b	50.7 ± 12.0	b	2.3 ± 1.9	a
	Zn		262.6 ± 61.8	a,b	390.6 ± 58.0	b	97.0 ± 53.7	a	459.2 ± 82.2	b	<1	
Water Extractable Major Ions (1:5)	**Cl^-**	mg L^{-1}	15.4 ± 2.7	a	16.2 ± 0.5	a	8.7 ± 0.8	a	15.7 ± 2.07	a	19.8 ± 2.9	a
	NO_3^-		7.8 ± 0.2	a	8.9 ± 1.1	a	7.7 0.4	a	15.6 ± 2.18	b	10.5 ± 0.5	a,b
	SO_4^{2-}		1773 ± 37	c	1722 ± 59	c	676 ± 368	b	277 ± 220	a,b	21 ± 1	a
	Ca^{2+}		600 ± 4	c	671 ± 35	c	241 ± 121	b	130 ± 79.5	a,b	35 ± 0	a
	K^+		4.1 ± 0.4	a	10.2 ± 1.0	b	13.1 ± 0.4	b	10.6 ± 0.62	b	12.7 ± 1.2	b
	Mg^{2+}		83.4 ± 12.0	c	35.2 ± 5.3	c	35.1 ± 11.3	c	8.6 ± 2.84	b	2.7 ± 0.4	a
	Na^+		19.3 ± 2.8	a	16.3 ± 1.9	a	17.4 ± 1.0	a	15.8 ± 1.10	a	17.2 ± 2.8	a

Within the tailings, samples collected from the rhizospheres of individual pines and vegetation patches (IP-T and, especially FI-T) showed a significant improvement in soil fertility relative to Bulk-T samples. For instance, OC, TN and DOC were around 3-fold higher in FI-T than in Bulk-T samples. This improvement of soil fertility in rhizosphere samples was also evident from soil microbial measurements, especially for β-glucosidase and dehydrogenase, which were not detected at all in Bulk-T samples.

The *Control* samples showed lower total metal(loid)s concentrations ($p<0.05$) than the rest of sampled sites (including the tailings' surroundings), except for Cd. The highest total metal(loid)s concentrations occurred indistinctly in the tailings samples (e.g. 700 mg kg^{-1} As at FI-T samples) or in *Surroundings* samples (e.g. ~9,200 mg kg^{-1} Pb). As for water extractable metal(loid)s concentrations, *Control* samples showed comparable values ($p<0.05$) to tailings' samples for As, Cu and Pb. The highest water-extractable metal(loid)s concentrations also occurred indistinctly in tailings samples (e.g. 390 mg kg^{-1} Zn at IP-T) or in *Surroundings* samples (e.g. 206.9 mg kg^{-1} Mn).

The tailings samples showed up to 20–30 times higher concentrations of water extractable divalent ions (SO_4^{2-}, Ca^{2+}, Mg^{2+}) than the samples collected from the *Control* area, while for the monovalent ions (Cl^-, NO_3^-, K^+, Na^+) there were no differences between these areas. Divalent ions concentrations negatively correlated ($p<0.01$, $r<-0.6$) with fertility parameters (OC, TN, DOC and available-P). Sulphate concentrations positively correlated with Ca^{2+} ($p<0.01$, $r=0.98$) and Mg^{2+} ($p<0.01$, $r=0.76$) while Cl^- correlated with Na^+ ($p<0.01$, $r=0.81$).

The PCA results for soil parameter variation along the transect are shown in Figure 8.3. Factor 1, which explained 48.3% of the variance, clearly separated the *Control* samples from the tailings samples. The group of *Control* samples was defined by higher soil fertility and higher microbial biomass and activity, whereas tailings samples (Bulk-T and IP-T ones) were characterised by higher salinity (mainly due to SO_4^{2-}), higher total concentrations of some metal(loid) (specially As) and higher sand percentages. Factor 2 was mainly defined by total and extractable metal(loid) concentrations (except As).

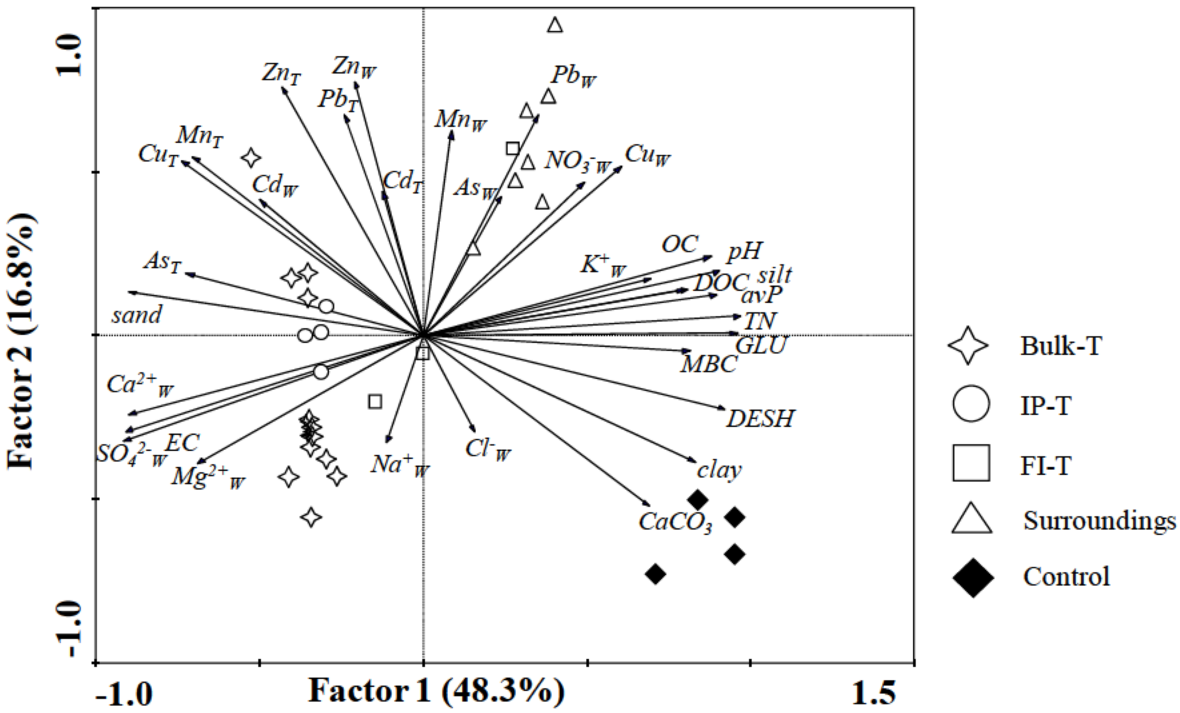

Figure 8.3. PCA results for soil parameters. "M_T"are total element concentrations; "M_W" are water extractable ion concentrations; "OC" is Organic Carbon; "TN" is Total Nitrogen; "DOC" is Dissolved Organic Carbon; "DESH" is dehydrogenase activity; "GLU" is β-glucosidase activity, "MBC" is Soil Microbial Biomass Carbon", "AvP" is Available Phosphorus. "FI-T" are soil samples from the fertility islands at the tailings; "IP-T" are rhizospheric soils from isolated pines growing at the tailings; "Bulk-T" are bulk soil samples from the tailings.

Table 8.3. Leaf nutritional content and isotopic composition in needles of the studied pines. "IP-T" are needles from isolated pines growing at the tailings; "FI-T" are needles from pines growing at fertility islands of the tailings. Data are average ± standard error (N = 4 or 7). Different letters at the same row means significant differences (p<0.05, ANOVA with Tukey test).

Parameter		Units	Plausible range in pine foliage samples[a]		Concentrations in Aleppo pines in Murcia Region[b]	Samples							
						Tailings				Surroundings		Control	
			min.	*max.*		*IP-T*		*FI-T*					
Esssential nutrients	**C**	%	47	57	39.7	48.1 ± 0.2	a	48.0 ± 0.3	a	49.3 ± 0.3	b	48.9 ± 0.3	a,b
	N		0.922	1.428	1.070	0.759 ± 0.03	a,b	0.961 ± 0.09	b	0.756 ± 0.0	a,b	0.643 ± 0.02	a
	P	mg kg^{-1}	800	1790	710	364 ± 9	a	307 ± 13	a	503 ± 36	b	565 ± 21	b
	K		3200	8670	3600	3274 ± 351	a	3716 ± 405	a,b	4194 ± 352	a,b	4974 ± 433	b
	Ca		2120	8040	7500	940 ± 64	a	1226 ± 97	a	957 ± 115	a	865 ± 101	a
	Mg		1840	2890	2020	2096 ± 121	b	1793 ± 128	b	1254 ± 91	a	1186 ± 72	a
	S		920	1680	-	1833 ± 71	b	2190 ± 137	c	1221 ± 51	a	965 ± 54	a
	Cu		1.96*	6.88*	-	1.98 ± 0.31	a	2.62 ± 0.24	a	3.19 ± 0.4	a	2.06 ± 0.26	a
	Mn		32	1331*	-	170 ± 28.46	b	91 ± 4.22	a,b	85 ± 21.2	a,b	36 ± 0.88	a
	Zn		23	96*	-	80 ± 5.01	c	64 ± 5.92	b,c	47 ± 3.7	b	27 ± 1.04	a
Other elements	**As**		-	-	-	2.48 ± 0.62	b,c	2.63 ± 0.46	c	0.81 ± 0.3	a,b	0.54 ± 0.16	a
	Cd		0.06*	0.507*	-	0.08 ± 0.04	a	<0.01		0.34 ± 0.1	a	0.23 ± 0.08	a
	Cl		-	-	-	337 ± 51	a	637 ± 61	b	431 ± 54	a,b	580 ± 48	b
	Na		-	-	-	601 ± 158	a	679 ± 80	a	368 ± 40	a	434 ± 61	a
	Pb		0.14*	5.59*	-	16.1 ± 4.17	b	8.4 ± 2.35	a,b	12.6 ± 3.1	b	3.6 ± 0.17	a
Leaf isotopic composition	**δ^{13}C**	‰	-	-	-	-27.65 ± 0.30	a	-26.95 ± 0.34	a,b	-25.16 ± 0.3	c	-25.87 ± 0.36	b,c
	δ^{15}N		-	-	-	-8.83 ± 0.45	a	-5.44 ± 0.22	b	-3.19 ± 0.2	c	-3.76 ± 0.53	c
	δ^{18}O		-	-	-	26.65 ± 0.48	a,b	25.71 ± 0.23	a	27.58 ± 0.3	b	26.43 ± 0.07	a,b

[a](Clarke et al., 2008; Reference levels for *Pinus halepensis* except * which are referred to *Pinus sylvestris*).
[b](López-Serrano et al., 2005).

8.3.1. Elemental and isotopic composition of pine foliage

Along the sampled transect, foliar N concentrations were highest in pines growing in vegetation patches at the tailings (*Fertility Islands*), and lowest in *Control* pines. The foliar concentrations of the essential macronutrients P and K were 30–50% lower in pines growing at the tailings than in those growing in *Surroundings* or *Control* areas (Table 8.3). For other essential plant nutrients such as Ca and Cu, foliar concentrations did not vary significantly along the transect. However, for Mg, S and the essential micronutrients Mn and Zn, the foliar concentrations of pines growing at the tailings were between 2- and 4-fold higher than those measured in *Control* pines.

Foliar concentrations of non-essential elements such as As and Pb were 5-fold higher in pines growing at the tailings than in those growing in *Control* areas. The foliar concentrations of Na did not change significantly along the sampled transect. The results obtained for Cd showed higher foliar concentrations in the pines of *Surroundings* areas than in some of the tailings' pines, whereas Cl was found at higher concentrations in the foliage of *Control* pines than in some of the isolated pines growing at the tailings (IP-T).

The pines growing at the tailings generally showed lower leaf $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ values than those growing in *Surroundings* and *Control* Areas. Across sites, pine leaf $\delta^{13}C$ and $\delta^{15}N$ were both strongly positively correlated with all measures of soil fertility (OC, TN, available-P, MBC and enzymatic activities), and negatively correlated with soil salinity and water extractable SO_4^{2-}, Ca^{2+} and Mg^{2+} concentrations (Table 8.4). Along the studied gradient, leaf $\delta^{13}C$ and $\delta^{15}N$ were positively correlated with each other and with foliar P, K, C and Cu concentrations, and were both negatively correlated with foliar S, Mg, Mn, Zn, Na and As. Finally, leaf $\delta^{13}C$ and $\delta^{18}O$ were also positively correlated with each other along the studied gradient.

Table 8.4. Pearson's correlation coefficients between selected plant parameters with selected soil properties. "EC" is Electrical Conductivity; "OC" is Organic Carbon; "TN" is Total Nitrogen; "DOC" is Dissolved Organic Carbon; "MBC" is Soil Microbial Biomass Carbon", "Available-P" is Available Phosphorus.

Soil parameter		**Plant parameter**										
		N	**P**	**K**	**S**	**Ca**	**Mg**	**Mn**	**Zn**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{18}O$**
pH		-0.29	0.66**	0.64**	-0.65**	0.12	-0.76**	-0.61**	-0.83**	0.80**	0.87**	0.36
EC		0.32	-0.67**	-0.64**	0.65**	0.00	0.79**	0.62**	0.81**	-0.86**	-0.92**	-0.47
OC		-0.46	0-5'*	0.38	-0.73**	-0.25	-0.65**	-0.64**	-0.64**	0.59*	0.66**	0.62**
TN		-0.54*	0-72**	0.56*	-0.84**	-0.28	-0.77**	-0.69**	-0.85**	0.62**	0.72**	0.38
DOC		-0.38	0.56*	0.31	-0.63**	-0.01	-0.65**	-0.55*	-0.61**	0.50*	0.53*	0.26
Available-P		-0.46	0.60**	0.40	-0,71**	-0.32	-0.69**	-0.63**	-0.67**	0.50*	0.53*	0.36
MBC		-0.48*	0.60**	0.34	-0,71**	-0.02	-0.61**	-0.38	-0.60**	0.47	0.44	0.25
β-glucosidase		-0.31	0.52*	0.63**	-0.56*	-0.15	-0.67**	-0.65**	-0.84**	0.52*	0.67**	0.05
Dehydrogenase		-0.51*	0.71**	0.60**	-0.71**	-0.22	-0.59**	-0.56*	-0.80**	0.44	0.50*	0.05
Water extractable ions	**SO_4^{2-}**	0.25	-0.63**	-0.73**	0.54*	0.21	0.71**	0.77**	0.75**	-0.69**	-0.79**	-0.44
	K^+	0.19	0.05	0.21	0.06	0.12	-0.09	-0.49*	-0.35	0.00	0.21	-0.12
	Ca^{2+}	0.20	-0.61**	-0.68**	0.52*	0.17	0.70**	0.82**	0.75**	-0.67**	-0.80**	-0.40
	Mg^{2+}	0.46	-0.82**	-0.63**	0.71**	0.22	0.67**	0.65**	0.71**	-0.72**	-0.74**	-0.46

The PCA results for pine foliage are shown at Figure 8.4. Factor 1, which explained 48.3% of the total variance, clearly separated the pines from *Control* and *Surroundings* areas from those growing at the tailings. The pines from *Control* and *Surroundings* areas were defined by their higher foliar P and K status and also higher isotope ratios ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$), whereas the pines growing at the tailings (IP-T and FI-T) were characterized by higher foliar S, Mg and metal(loid)s concentrations. Factor 2 was mainly defined by foliar N, Ca and Cu concentrations, and separated the pines growing at the tailings' FI-T and *Surroundings* areas from the rest.

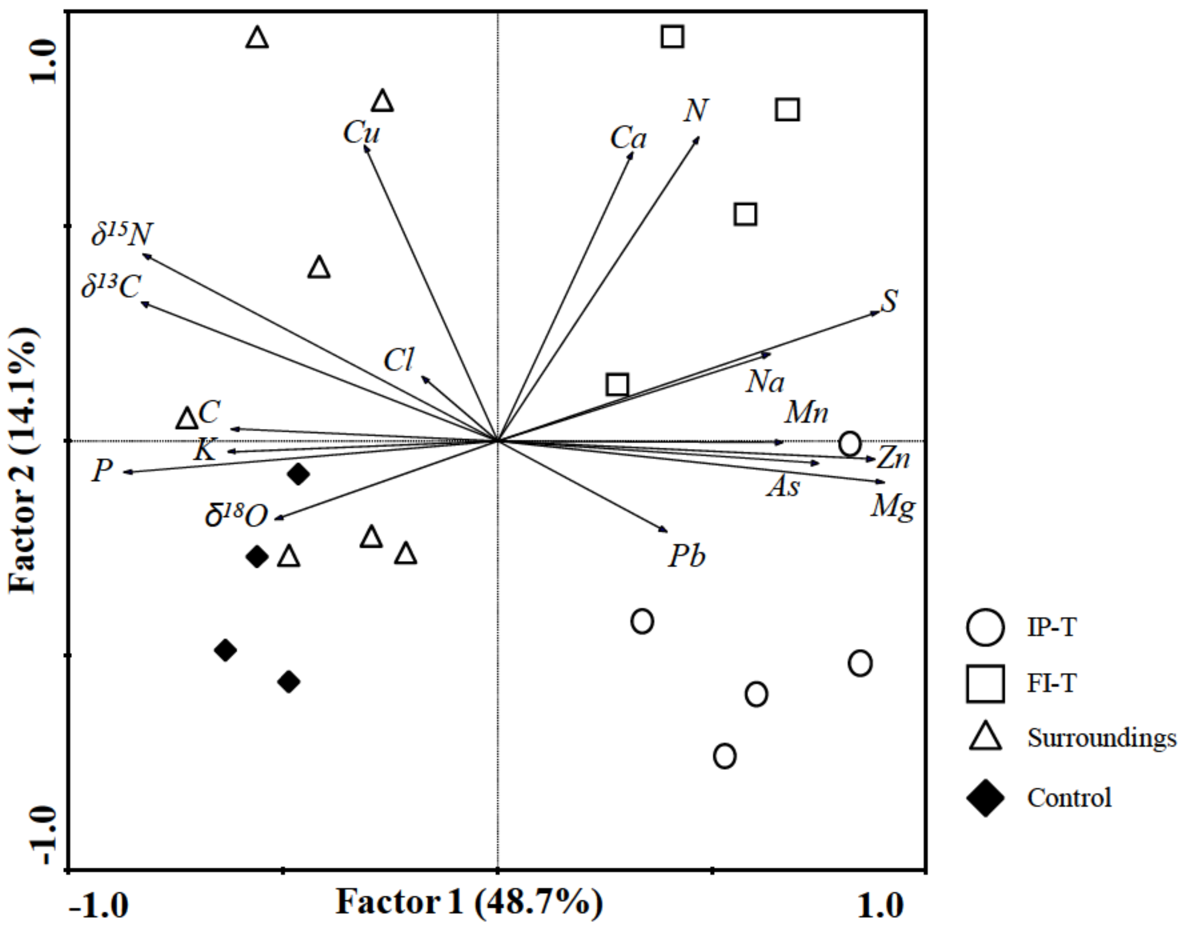

Figure 8.4. PCA results for plant parameters. All elements are total element concentrations; δ^{13}C, δ^{15}N and δ^{18}O are stable isotopic contents; "FI-T" are needles from pine trees growing at fertility islands of the tailings; "IP-T" are needles from isolated pine trees growing at the tailings. Cd is omitted because some of the samples showed concentrations below the detection limit.

8.4. Discussion

8.4.1. Limiting factors for plant performance at the mine tailings

Spontaneous colonisation of mine tailings by natural vegetation in this semiarid environment has been found to be conditioned by multiple soil stresses and nutrient co-limitation in which parameters such as soil pH, salinity, fertility and metal(loid)s concentrations played important roles (Párraga-Aguado et al., 2013b). However, some of these limiting factors (e.g. soil metal(loid)s concentrations) played a greater role in selecting or "filtering" plant species capable of colonizing the tailings, than in determining the spatial distribution of vegetation at the tailings. In this study, the main differences when comparing the tailings with *Control* soils were mainly determined by salinity (and the corresponding water extractable ion concentrations), soil fertility, microbial biomass and activity and metal(loid) concentrations (total and water extractable). Variations in soil pH areas along the studied gradient were modest, and the neutral to slightly alkaline soil found at all sites would not be expected to represent a constraint for Aleppo pine establishment and growth.

With regards to soil salinity and its effect on plant growth and survival, it is necessary to distinguish between salinity tolerance and ion-specific toxicity. In general, pine species are known to be moderately tolerant (*Pinus halepensis*, Aleppo pine: 6–8 dS m^{-1}) or tolerant (Italian stone pine, *Pinus pinea*: >8 dS m^{-1}) to salinity (Francois and Maas, 1999). Water extractable Cl^- and Na^+ concentrations in soil were similar along the transect (including the *Control* area), but the large changes in EC along the transect were strongly correlated with changes in SO_4^{2-} concentrations (p<0.01, r=0.95; Figure 8.3). The tolerance of pine species to high soil SO_4^{2-} concentrations was demonstrated by Renault et al. (1998), who found that *Pinus contorta* seedlings could be grown in water solutions containing up to 3000 mg L^{-1} of SO_4^{2-}. Water extractable SO_4^{2-} concentrations at the tailings were lower than this threshold, even though it should be noted that data reported here refer to 1:5 extract and not to soil solution. The tight correlation between water extractable SO_4^{2-} and water extractable Ca^{2+} (p<0.01, r=0.99) along the studied transect was remarkable, indicating that the high SO_4^{2-} concentrations at the tailings were not associated to soil acidity (pH was over 7), but instead, to secondary formation of gypsum (García-Lorenzo, et al., 2012).

For evaluating metal(loid) phytotoxicity at the tailings, we focused on water extractable concentrations rather than on total concentrations, which are known to be less relevant for phytotoxicity (Ernst, 1996). The neutral to slightly alkaline soil pH may have contributed to maintain low concentrations of water extractable metal(loid)s at the tailings. In areas affected by the Aznalcóllar toxic mining spill at the Guadiamar valley (South Spain), Domínguez et al. (2010) found that soil pH strongly determined the pool of plant-available metal(loid)s (and therefore phytotoxicity), and did so to a greater extent than other key soil parameters such as organic matter content or texture. Moreover, As, which is known to increase its mobility with alkaline pH (Smith et al., 1998) was also maintained at similar or lower water extractable concentrations than other metal(loid)s in the tailings. The water extractable metal(loid)s concentrations measured at the tailings were far below those reported as phytotoxic for pines trees. As an example, the highest Zn water extractable concentrations found at the tailings (390 μg kg^{-1} ~0.078 mg Zn L^{-1}) were far lower than the ones that Fuentes et al. (2007) found to be phytotoxic (100 μM Zn, ~6.5 mg Zn L^{-1} in soil solution) for *Pinus halepensis* seedlings, which are expected to be less tolerant than the adult trees sampled in this study.

8.4.2. Influence of pine rhizopsheres on soil properties within the mine tailings

According to the PCA for soil data (Figure 8.3), all measures of soil fertility (OC, DOC, TN, available-P, MBC, β-glucosidase and dehydrogenase activity) along the studied transect were lowest in bare soil areas of the tailings, following the order: Bulk-T < IP-T < FI-T < Surroundings < Control. The occurrence of gradients in soil fertility within mine tailings in semiarid areas has been shown to be strongly determined by vegetation distribution and development (Párraga-Aguado et al., 2013b). Within the tailings, significantly improved soil conditions (higher fertility and enhanced microbiology) were encountered in those rhizosphere areas with a more biodiverse and structurally complex vegetation: the FI-T patches showed higher soil fertility than IP-T patches, which in turn showed higher soil fertility than Bulk-T. The soil fertility improvement found in IP-T patches relative to Bulk-T areas may have been due to the biogeochemical processes which take place within the rhizospheres of isolated pines. Despite only modest (largely non-significant) increases in soil OC, DOC, TN and available-P contents in IP-T relative to Bulk-T, soil microbial activity (evaluated by β-glucosidase and dehydrogenase activities) was detectable in the rhizospheres of isolated pines, but not in Bulk-T areas. *Pinus halepensis* is obligately ectomycorrhizal (e.g. Querejeta et al., 1998), and we found evidence of extensive ectomycorrhizal fungal (EMF) colonization in the pine trees growing on mine tailings (including abundant production of *Pisolithus* sp. fruiting bodies, and presence of EMF roots and extramatrical hyphae in pine rhizospheres; see Figures 8.5 and 8.6). Microbial biomass and activity in the rhizospheres of the pine trees growing on the tailings are likely enhanced by EMF colonization of roots. Previous research on the effects of Aleppo pine afforestations on non metal(loid) polluted soils in Southeast Spain (Maestre et al., 2003; Ruiz-Navarro et al., 2009) has reported negligible increases in soil organic carbon and total nitrogen contents following afforestation. However, given the harsh initial soil conditions prevailing at the tailings, even small increases in soil fertility and microbial activity might be sufficient to trigger successional ecological processes. The enhancement of soil properties (especially microbial activity) by plant rhizospheres is considered a critical factor in the revegetation of mining wastes which may reduce the need for additional soil conditioners to foster vegetation establishment and growth (Grandlic et al., 2008).

Within the tailings, soil properties in fertility islands (FI-T) appeared to “mimic” those found in Surroundings area (with similar values of OC, TN and β-glucosidase) better than those found in the rhizospheres of isolated pine trees (IP-T). The assemblage of different plant species in the fertility islands may have resulted in a greater amelioration of soil stress factors, thus triggering positive feedbacks on the establishment of new incoming plant species. It is often assumed that the balance between plant-plant competition and facilitation shifts towards facilitation under stressful abiotic conditions (Maestre and Cortina, 2004). The favourable microsites provided by the nurse vegetation in fertility islands may thus provide a key facilitating role for incoming plant species in semiarid tailings (Bonanomi et al., 2011). In addition to Aleppo pine and a few other species that were relatively frequent throughout the tailings (Table 8.1; e.g. the grass *Piptatherum miliaceum* (L.) Cosson or the halophyte *Limonium cossonianum* Kunthze, Revis.), the fertility islands also included other species from *Surroundings*, and *Control* areas, (such as the shrubs *Helichrysum decumbens* (Lag.) Camb and *Pistacia lentiscus* L. and the tree *Olea europaea* L.). The establishment and further spread of fertility islands at the tailings following a natural successional process may be a good option to achieve acceptable levels of tailings stabilisation, instead of the application of technical remediation schemes. This agrees with current approaches to the revegetation of former mining areas based on non-intervention methods (Tropek et al., 2012).

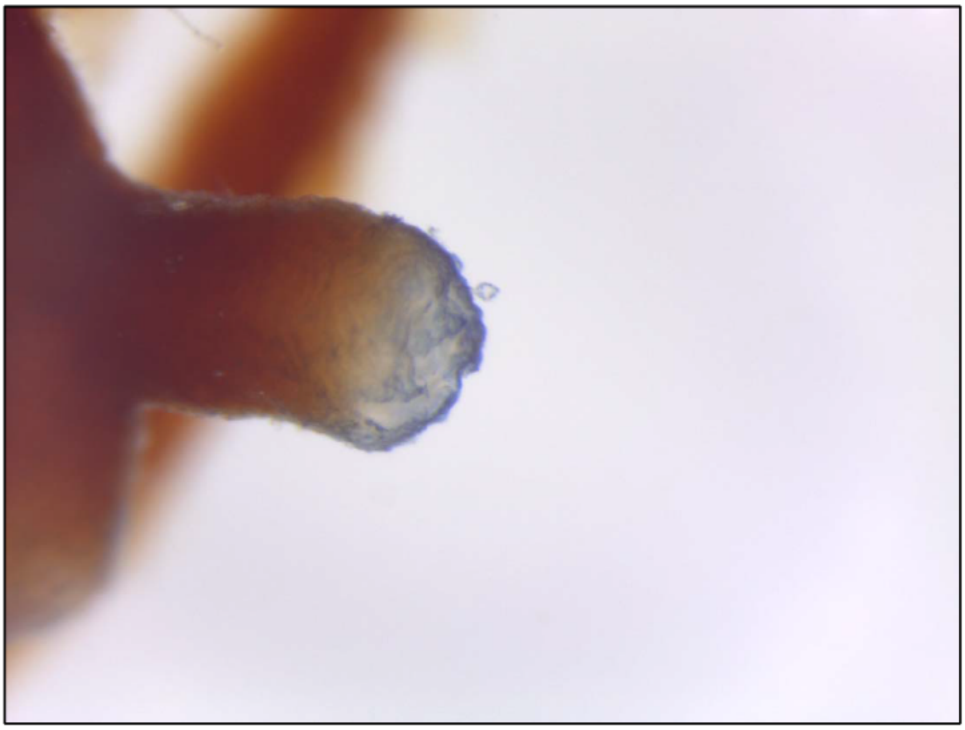

Figure 8.5. Presence of mycorrhiza hifae (blue color) on tailings' single pines roots after staining by trypan blue.

Figure 8.6. Presence of Pisolithus sp. close to pine trees at tailings surface.

8.4.3. Changes in the elemental composition of pine foliage along the studied transect

Several studies worldwide (e.g. Sun et al., 2009; Kord et al., 2010) have used pine species for biomonitoring environmental metal(loid)s pollution by assessing their concentrations in pine needles. However, our study did not show good correlations between metal(loid) concentrations in soil (total and water extractable) and their corresponding concentrations in pine needles (r<0.25) along the studied transect. Actually, the soil water extractable metal(loid) concentrations measured at the tailings were negligible compared to those obtained for major ions such as SO_4^{2-}, Ca^{2+} or Mg^{2+}. Along the studied transect, the concentrations of these major ions in the soil solution were highest at the tailings and *Surroundings* areas (Table 8.2 and Figure 8.3, e.g. SO_4^{2-} concentrations were highest at the tailings ≥ *Surroundings* >> *Control*), which also led to high concentrations in pine needles. Accordingly, we found good correlations between soil water extractable concentrations of SO_4^{2-} and Mg^{2+} and their corresponding S and Mg concentrations in pine needles (e.g. for Mg $p<0.01$, $r=0.67$, Table 8.4), which reflected more accurately than metal(loid)s concentrations the changes in the soil solution along the studied transect.

The foliar N concentrations found in this study were below the normal range for pine species (0.922–1.428%; Clarke et al., 2008), except for trees in the fertility islands (FI-T). The leaf sampling period, which was coincidental with the driest season (end of summer), may explain the low foliar concentrations of key macronutrients such as N or P (López-Serrano et al., 2005). Phosphorus, which is known to be an important soil limiting factor for the growth of pine trees in the Mediterranean region (Sardans et al., 2005), showed significantly lower foliar concentrations in pines growing on tailings than in those from *Control* and *Surroundings* areas. Further, large variations in foliar N:P ratios along the studied gradient revealed unusually high ratios in the pines growing at the tailings (N:P ratios ≈ 20–31) which indicate strong phosphorus limitation (Güsewell, 2004). The low foliar P concentrations of pines growing on tailings materials can be explained by the low available-P measured in tailings soil (significant correlation between foliar P and available-P in soil along the transect; $p<0.01$; $r{\sim}0.6$, Table 8.4). Foliar P concentration showed a strong negative correlation with both foliar S ($p<0.01$, $r=-0.82$, Figure 8.4 and Table 8.6) and soil water extractable SO_4^{2-} ($p<0.01$, $r=0.63$, Table 8.4) along the studied transect, suggesting that antagonism or interference with S may have contributed to low P availability and uptake at the tailings (Sardans et al., 2006). Foliar S concentrations in the trees growing on tailings exceeded the normal thresholds for pines (Table 8.3), so that foliar S/P ratios (Table 8.5) were up to four-fold higher in pines at the tailings ($p<0.05$) than in pines from *Control* and *Surroundings* areas. Domínguez et al. (2010) found a strong correlation between metal(loid) soil concentrations and P foliar deficiency in trees (*Olea europaea*) planted on areas affected by the Aznalcóllar mining spill (South Spain), but did not appreciate any negative effects on the foliar concentrations of other plant macronutrients. Interestingly, pines in *Surroundings* area showed higher foliar P concentrations that were similar to those of *Control* pines, despite the much higher soil metal(loid) concentrations (total and water extractable) in *Surroundings* area. This suggests that high soil metal(loid) concentrations can not explain by themselves why the pines growing at the tailings had such low foliar P concentrations.

Table 8.5. Nutrient ratios for the analysed needles. “IP-T” ” are needles from isolated pines growing at the tailings; “FI-T” are needles from pines growing at fertility islands of the tailings.

Ratio	Average levels reported by Lopez-Serrano et al. (2005)	Ratios calculated from FFCC report averages (Stefan et al., 1997)	Samples			
			Tailing		Surroundings	Control
			IP-T	FI-T		
C/N	-	-	63.72 ± 2.63 a,b	51.31 ± 4.75 a	66.28 ± 3.71 a,b	76.18 ± 2.14 b
Ca/Mg	4.30	2.56	0.46 ± 0.05 a	0.68 ± 0.02 a,b	0.78 ± 0.09 b	0.74 ± 0.09 b
K/Ca	0.46	0.95	3.59 ± 0.54 a	3.05 ± 0.26 a	4.93 ± 0.87 a	6.03 ± 0.96 a
K/Mg	1.82	2.44	1.56 ± 0.13 a	2.09 ± 0.22 a,b	3.50 ± 0.47 b,c	4.18 ± 0.16 c
K/P	5.04	5.64	9.09 ± 1.17 a,b	12.06 ± 0.91 b	8.34 ± 0.49 a	8.77 ± 0.49 a,b
N/Ca	1.49	2.37	8.25 ± 0.69 a	7.94 ± 0.82 a	9.08 ± 1.30 a	7.70 ± 0.78 a
N/K	3.30	2.47	2.43 ± 0.29 a,b	2.67 ± 0.37 b	1.95 ± 0.23 a,b	1.32 ± 0.10 a
N/Mg	5.97	6.03	3.65 ± 0.16 a	5.39 ± 0.43 a,b	6.49 ± 0.91 b	5.46 ± 0.28 a,b
N/P	16.64	13.94	20.89 ± 0.90 c	31.40 ± 2.97 d	15.66 ± 1.22 b	11.41 ± 0.37 a
S/P	-	1.59	5.05 ± 0.27 b	7.12 ± 0.17 c	2.58 ± 0.26 a	1.71 ± 0.04 a

Table 8.6. Pearson’s correlation coefficients among selected plant parameters. * indicates level of significance at 0.05 level; ** indicates level of significance at 0.01 level.

	N	P	K	C	S	Ca	Mg	Mn	Cu	Zn	Cl	Na	As	Pb	$\delta^{13}C$	$\delta^{15}N$	$\delta^{18}O$
N	1																
P	-0.39	1															
K	-0.26	0.68**	1														
C	-0.47*	0.36	0.12	1													
S	0.62**	-0.82**	-0.39	-0.60**	1												
Ca	0.45*	-0.38	-0.31	0.05	0.44	1											
Mg	0.30	-0.73**	-0.38	-0.53*	0.82**	0.24	1										
Mn	0.13	-0.50*	-0.42	-0.19	0.52*	0.16	0.67**	1									
Cu	0.30	0.29	0.16	0.17	-0.13	0.16	-0.33	0.05	1								
Zn	0.33	-0.72**	-0.51*	-0.462*	0.80**	0.13	0.90**	0.78**	-0.14	1							
Cl	-0.01	0.11	0.48*	-0.04	0.10	0.08	-0.18	-0.44	-0.12	-0.25	1						
Na	0.49*	-0.52*	-0.26	-0.55*	0.56*	0.14	0.52	0.50*	0.07	0.44	-0.15	1					
As	0.23	-0.66**	-0.35	-0.41	0.79**	0.14	0.73**	0.47*	-0.32	0.75**	0.07	0.35	1				
Pb	-0.09	-0.29	-0.11	-0.11	0.31	-0.20	0.54*	0.58**	0.00	0.68**	-0.14	0.29	0.41	1			
$\delta^{13}C$	-0.18	0.71**	0.59**	0.55**	-0.59**	-0.10	-0.70**	-0.40	0.50*	-0.60**	0.21	-0.58*	-0.58*	-0.23	1		
$\delta^{15}N$	-0.06	0.64**	0.53*	0.58**	-0.56*	0.00	-0.76**	-0.58**	0.46*	-0.74**	0.28	-0.41	-0.52*	-0.33	0.85**	1	
$\delta^{18}O$	-0.31	0.37	0.24	0.42	-0.50*	-0.36	-0.34	-0.22	0.23	-0.21	-0.30	-0.63**	-0.43	0.02	0.54*	0.25	1

Along the studied transect, foliar Mg and Ca concentrations were highest in the pines growing at the tailings, where the concentrations of these cations in the soil solution were one order of magnitude higher than in *Control* soils.

According to the thresholds that Clarke et al. (2008) reported for pine foliage in Europe, all foliar metal concentrations along the sampled transect were within the normal ranges except for Pb, which surpassed the upper critical threshold of 5.59 mg kg^{-1} at all the sites along the pollution gradient except in the *Control* area. However, the results of the PCA (Figure 8.4) showed that pines growing at the tailings were clearly characterized by their higher foliar metal and As concentrations (except Cu). The high foliar Cu concentrations in the foliage of pines from FI-T and *Surroundings* areas may be related to the high water extractable Cu concentrations in the soils of these areas. It is known that Cu shows high affinity for dissolved organic carbon, so a higher soil organic carbon contents in FI-T and *Surroundings* sites than in IP-T sites may have led to higher Cu availability for plants (Strobel et al., 2001).

8.4.4. Changes in the isotopic composition of pine foliage along the studied transect

Changes in climatic conditions were negligible along the studied transect (<1 km long), so it is reasonable to assume that any changes in the isotopic composition of pine foliage along the transect might be plausibly related to differences in water and/or nutrient availability and/or soil edaphic conditions.

The low leaf $\delta^{13}C$ values found in the pines growing at the tailings indicate lower water use efficiency due to greater soil water availability and higher leaf stomatal conductance and transpiration, relative to *Control* or *Surroundings* areas. Lower inter-tree competition for soil water at the tailings due to a sparse vegetation cover may have lead to greater water availability for tree transpiration, compared to *Control* and *Surroundings* areas where vegetation density (and therefore, competition for soil water) is much higher (Ramírez et al., 2009; Moreno-Gutiérrez et al., 2011). Despite the low water holding capacity of the upper soil layers at the tailings (due to their sandy texture), the tailings contain impermeable clay layers at depth which may create moist soil pockets and layers (Conesa and Schulin, 2010). The low resistance of sandy materials to penetration by roots may have facilitated water acquisition from deep layers at the tailings. The positive correlation between leaf $\delta^{13}C$ and $\delta^{18}O$ along the studied gradient (Pearson $p<0.05$, $r= 0.54$, Table 8.6) further suggests that water availability for tree transpiration was higher at the tailings than in *Surroundings* or *Control* areas. The low leaf $\delta^{18}O$ values of pines growing at the tailings (particularly those in FI-T) would be consistent with both increased stomatal conductance due to low inter-tree competition for water (Barbour, 2007; Moreno-Gutiérrez et al., 2011), and/or use of deeper soil water (which is less exposed to evaporation and thus is more depleted in ^{18}O; Barnes and Allison, 1983).

Along the studied transect, leaf $\delta^{13}C$ in *Pinus halepensis* was positively correlated with all measures of soil fertility (Table 8.4), as well as with foliar P concentration ($p<0.01$, $r=0.71$, Table 8.6), which suggests that low soil fertility and P deficiency may have contributed to low Water Use Efficieny (WUE) in the pines growing at the tailings. Querejeta et al. (2008) showed that low soil fertility and low foliar P status were both tightly associated with low WUE and $\delta^{13}C$ in *Pinus halepensis*. It is widely reported that P deficiency decreases plant photosynthetic rates (Conroy et al., 1988; Warren, 2011) and is associated with low WUE caused by poor stomatal control of transpirational water loss (Singh et al., 2000).

Foliar $\delta^{15}N$ was strongly positively correlated with all measures of soil fertility along the studied transect (Table 8.4), which is in good agreement with observed trends at local, regional and global scales (Evans, 2001; Craine et al., 2009). Further, leaf $\delta^{15}N$ was positively correlated with foliar P and K concentrations along the transect ($p<0.01$–0.05, $r=0.64$–0.53, respectively). The low leaf $\delta^{15}N$ of pines growing at the tailings may therefore reflect low soil nutrient availability and significant foliar P and K deficiencies. A heavy dependence on symbiotic ectomycorrhizal fungi (EMF) for nitrogen uptake due to low soil fertility may have further contributed to low foliar $\delta^{15}N$ in the pines growing at the tailings, as EMF preferentially transfer ^{15}N-depleted nitrogen to their host trees (Hobbie et al., 2000; Craine et al., 2009).

Whereas both leaf $\delta^{13}C$ and $\delta^{15}N$ were strongly correlated with nearly all soil variables along the studied transect (including strong positive correlations with soil fertility, and negative correlations with electrical conductivity and cation and metal(loid) concentrations), leaf $\delta^{18}O$ showed very few significant correlations with soil properties (Table 8.4). This points that the large changes in the ecophysiological status of *Pinus halepensis* trees along the studied transect were more strongly determined by variations in soil physico-chemical properties than by differences in soil water availability along the transect. This suggests that soil water availability at the tailings is adequate for the establishment and growth of *Pinus halepensis* trees in this semiarid environment, and that reclamation efforts should thus focus on enhancing soil fertility and amelioriating the harsh physical-chemical conditions of the tailings.

8.5. Conclusions

The results of this study indicate that *Pinus halepensis* is a suitable tree species for the phytostablization of neutral or slightly alkaline mine tailings in semiarid environments, thanks to its drought hardiness and good adaptation to low soil fertility and high salinity conditions. Given the neutral to slightly alkaline soil pH values along the studied transect, soil fertility parameters (especially P availability) exerted a stronger control on pine nutritional and ecophysiological status than soil metal(loid) concentrations (water extractable or total). Low soil P availability and severe foliar P deficiency may be currently limiting the spontaneous colonization, expansion and growth of *Pinus halepensis* on the tailings, but single or repeated addition of phosphorus fertilizers would help alleviate this constraint.

Within the tailings, soil organic matter content, fertility levels and microbial activity were higher, and salinity and sulphate concentration were lower, in the rhizospheres of *Pinus halepensis* trees than in adjacent bare soil areas, thus illustrating the potential of this species for the reclamation of mine spoils. Furthermore, larger and more taxonomically diverse vegetation patches/fertility islands showed better soil properties than the rhizospheres of isolated pine trees, which highlights the crucial importance of high-diversity plant assemblages for improving soil fertility conditions in mine tailings.

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CHAPTER 9. Metal(loid) allocation and nutrient retranslocation in *Pinus halepensis* trees growing on semiarid mine tailings

Chapter 9

Metal(loid) allocation and nutrient retranslocation in *Pinus halepensis* trees growing in semiarid mine tailings

Isabel Párraga-Aguado, José-Ignacio Querejeta,
María Nazaret González-Alcaraz, Héctor M. Conesa

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Abstract

The goal of this study was to evaluate internal metal(loid) cycling and the risk of metal(loid) accumulation in litter from *Pinus halepensis* trees growing at a mine tailings disposal site in semiarid Southeast Spain. Internal nutrient retranslocation was also evaluated in order to gain insight into the ability of pine trees to cope with the low fertility soil conditions at the tailings. We measured metal(loid) concentrations in the foliage (young and old needles), woody stems and fresh leaf litter of pine trees growing on tailings. The nutrient status and stable isotope composition of pine foliage ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ as indicators of photosynthesis and water use efficiency) were also analyzed. Tailings soil properties in vegetation patches and in adjacent bare soil patches were characterized as well. Significant amounts of metal(loid)s such us Cd, Cu, Pb and Sb were immobilized in the woody stems of *Pinus halepensis* trees growing on tailings. Leaf litterfall showed high concentrations of As, Cd, Sb, Pb and Zn, which thereby return to the soil. However, water extractable metal(loid) concentrations in tailings soils were similar between vegetation patches (mineral soil under the litter layer) and bare soil patches. The pines growing on mine tailings showed very low foliar P concentrations in all leaf age classes, which suggests severe P deficiency. Young (current year) needles showed lower accumulation of metal(loid)s, higher nutrient concentrations (P and K), and higher water use efficiency (as indicated by and $\delta^{13}C$ and $\delta^{18}O$ data) than older needles. Substantial nutrient resorption occurred before leaf litterfall, with 46% retranslocation efficiency for P and 89% for K. In conclusion, phytostabilization of semiarid mine tailings with *Pinus halepensis* is feasible but would require careful monitoring of the trace elements released from litterfall, in order to assess the long-term risk of metal(loid) transfer to the food chain.

9.1. Introduction

Mine tailings resulting from metal ore mining activities pose a major threat to the surrounding environment due to their high metal(loid) concentrations. The phytomanagment of mine tailings by phytostabilization (metal(loid) containment by the establishment of a permanent vegetation cover on the tailings' surface) has been proposed as an effective and low-cost option to decrease the environmental risks that tailings pose to surrounding areas (Conesa and Schulin, 2010). Suitable plant species for phytostabilization should tolerate high metal(loid) soil concentrations, low pH, high salinity, low fertility and, in the case of semiarid areas, drought (Mendez and Maier, 2008). In addition, it is desirable that candidate plant species show low metal(loid) accumulation in edible organs (to prevent metal(loid) incorporation into the trophic chain) and deciduous parts (to decrease metal(loid) return to the soil) (Robinson et al., 2009). In general, phytostabilization is considered a suitable technique for long-term projects in which the goal is to reach some acceptable level of environmental risks and not their complete elimination (Conesa et al., 2012). For this reason, it is important to use assemblages of native, locally adapted plant species with complementary ecological functions, in order to create self-sustaining plant communities. For instance, grasses are fast growing species that can provide rapid soil cover, while woody species (trees and shrubs) can provide a more enduring soil protection against eolian and water erosion (Párraga-Aguado et al., 2013a). While there is extensive research on the use of grasses, herbs and forbs for phytostabilization of metal(loid) polluted soils in semiarid areas (Clemente et al., 2012; Martínez-Sánchez et al., 2012), less attention has been paid to trees despite their great potential for stabilization of mining spoils (Domínguez et al., 2008).

The high metal(loid) resistance of Aleppo pine (*Pinus halepensis* Mill.) and its ability to spontaneously colonize metal(loid) enriched tailings have been recently noted (Párraga-Aguado et al., 2013a, 2014). This tree species is widely used in the restoration of degraded drylands throughout the Mediterranean basin (Querejeta et al., 2008), and has shown high efficiency in the use of water and nutrients in low fertility soils (Sardans et al., 2005). However, high metal(loid) concentrations in soil may negatively affect plant physiological processes (Kabata-Pendias and Pendias, 2001), and may therefore compromise the acclimatory responses of pines to the nutrient-deficient soil conditions of mine tailings. Plant mechanisms to cope with the uptake of high metal(loid) concentrations include compartmentalisation (e.g. by long-term storage in bark or stems; Pulford and Watson, 2003) or elimination (e.g. through shedding of metal(loid)-loaded senescent leaves; Robinson et al., 2009). The latter mechanism should be carefully evaluated in phytostabilization projects, as the leaf litter returning to the soil could cause the accumulation of metal(loid)s in the upper layers of the soil profile (Evangelou et al., 2012).

The C, O and N isotopic composition of plant tissues has been used to characterize the resource acquisition strategy of plant species (Dawson et al., 2002) as well as to infer plant physiological responses to environmental gradients in water and nutrient availability (Domínguez et al., 2012; Ferrio et al., 2003; Ramírez et al, 2009). The combined measurement of foliar $\delta^{13}C$ and $\delta^{18}O$ can provide time-integrated information on plant stomatal conductance, photosynthetic activity and intrinsic water use efficiency (WUEi, which is given by the ratio between photosynthetic rate and stomatal conductance) throughout the lifespan of the leaf (Scheidegger et al., 2000; Dawson et al., 2002; Querejeta et al., 2006, 2008; Ramírez et al., 2009; Domínguez et al., 2012). Leaf $\delta^{13}C$ provides a useful time-integrated index for assessing plant water use efficiency (Farquhar et al., 1989; Dawson et al., 2002), whereas leaf $\delta^{18}O$ can provide a time-integrated measure of stomatal conductance (Barbour, 2007). Foliar $\delta^{18}O$ is inversely related to stomatal conductance, but is not affected by changes in photosynthetic rate (Keitel et al., 2003; Pendall et al., 2005), so the joint interpretation of $\delta^{13}C$ and $\delta^{18}O$ signals in leaf material allows separation of the independent effects of carbon fixation and stomatal conductance on $\delta^{13}C$ (Scheidegger et al., 2000). On the other hand, plant $\delta^{15}N$ reflects the net effect of a wide range of processes, including the isotopic signature of the soil N sources used by the plant, mycorrhizal associations, soil fertility levels, temporal and spatial variation in N availability or changes in plant demand (Dawson et al., 2002).

The goal of this study was to evaluate internal metal(loid) cycling in *Pinus halepensis* trees growing on mine tailings in Southeast Spain, as well as to assess the risk of metal(loid) accumulation in litter. Internal nutrient retranslocation was also investigated to gain insight into the ability of pine trees to cope with the low soil fertility conditions at the tailings. For that purpose, we sampled the foliage (young and old needles), woody stems and fresh litter needles of pine trees growing on tailings in a semiarid area of Southeast Spain. We carried out a comprehensive characterisation of the metal(loid) concentrations, nutrient status, and stable isotope (C, N, O) composition of pine foliage. Tailings soil properties were also characterized in vegetation patches and in bare soil patches.

9.2. Materials and methods

9.2.1. Site description

The Cartagena-La Union Mining District (0–392 m a.s.l.; 50 km^2; 37°37′20″ N, 0°50′55″ W–37°40′03″ N, 0°48′12″ W) is located on the Southeast of the Iberian Peninsula (Figure 9.1). This zone was one of the most important mining areas in Spain in the last centuries due to its metal ore mining. Metal(loid) contamination in this area has been recently reviewed by Conesa and Schulin (2010). The climate of the area is semiarid Mediterranean with an annual rainfall around 250– 300 mm and an annual average temperature of 18 °C. The natural vegetation is *Pinus halepensis* woodlands and shrubland species with xerophytic characteristics. The study was conducted in a tailings disposal area covering ~18,000 m^2, which is located at the Huerta de la Calesa site at the Cartagena–La Union Mining District.

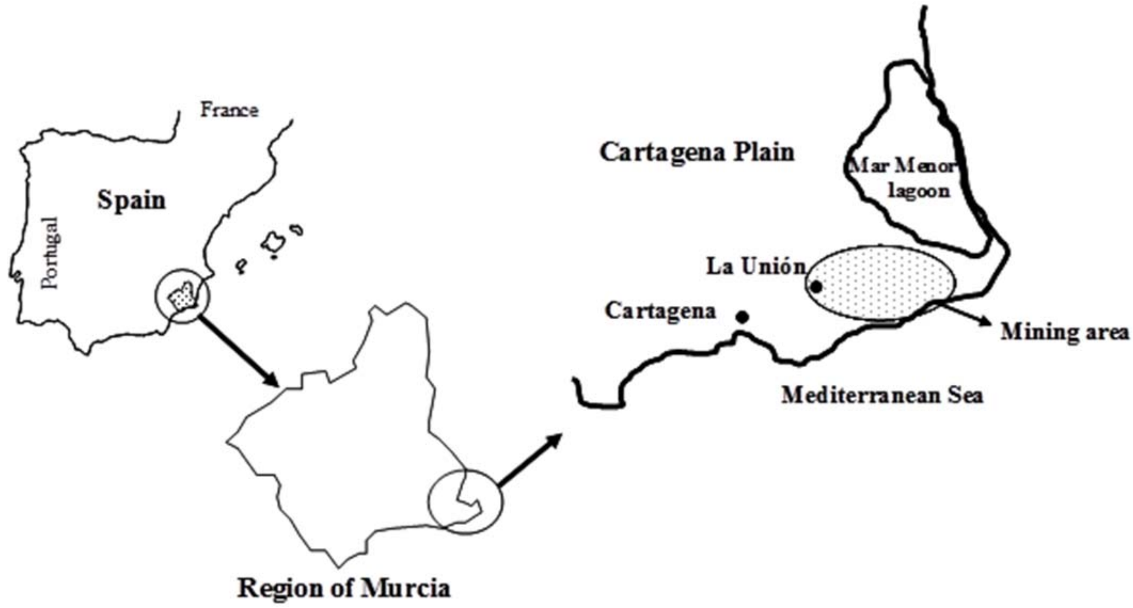

Figure 9.1. Location of the studied mining area.

9.2.2. Soil and plant sampling and analyses

Fifteen pine trees that spontaneously colonized the tailings disposal area were chosen for this study. Sampling was conducted during September 2012. From each individual pine tree, we sampled young (current year) needles, one year old needles, woody stems and fresh leaf litter (from the uppermost litter layer). Young needles were also taken from nearby pine trees (n = 4) growing on non-polluted soils (Párraga-Aguado et al., 2013b), in order to serve as reference background values of metal(loid) accumulation, nutrient status and leaf isotopic composition for *Pinus halepensis* (together with background values reported in the literature; López-Serrano et al., 2005; Sardanset al., 2005, 2006; Clarke et al., 2008). In order to evaluate the effects of pine leaf litterfall on the water extractability of metal(loid)s in tailings soils, we sampled the mineral soil below the litter layer under pine trees (0–40 cm depth) as well as the soil in adjacent bare areas.

Soil samples were air dried, sieved (2 mm), homogenized and stored in plastic bags prior to laboratory analysis. Soil pH and Electrical Conductivity (EC) were determined in a 1:5 soil to water mixture that was shaken during 2 h, using a Crison Basic 20 pH meter and a Crison Basic 30 conductivity meter, respectively. In the same 1:5 extract, Dissolved Organic Carbon (DOC), major ions and water extractable metal(loid)s were determined after filtering through nylon membrane 0.45 μm syringe filters (Albet-JNY). DOC was measured in an automatic analyzer (TOC-VCSH Shimadzu). Major ions (cations: Na^+, Ca^{2+}, Mg^{2+}; anions: Cl^-, SO_4^{-2}) were analyzed using an Ion Chromatographer (Metrohm). Water extractable metal(loid)s (As, Cd, Cu, Fe, Mn, Ni, Pb, Sb, Zn) were analyzed using a ICP-MS (Agilent 7500A, detection limit 0.002 mg L^{-1}). Particle size distribution was determined following the method of Bouyoucos densimeter. Total nitrogen (TN) was determined using the Kjeldahl method. Organic carbon (OC) was determined by the oxidation of organic matter using potassium dichromate. Total element composition was measured in stored soil samples using XRF (Bruker S4 Pioneer). Available phosphorus (Available-P) was measured following the Olsen method (Olsen et al., 1954).

The needles and woody stems were carefully washed with distilled water, dried at 65 °C for 72 h, and finely ground. For each sample, 0.1–0.5 g was combusted in a muffle furnace (550 °C, 3 h) prior to a redilution using concentrated nitric acid. The resulting extracts were filled to 25 ml and filtered through CHM F2041-110 ashless filter papers (20–25 μm pore diameter). Then, metal(loid)s (As, Cd, Cu, Fe, Mn, Ni, Pb, Sb, Zn) were analyzed using a ICP-MS (Agilent 7500A, detection limit 0.002 mg L^{-1}) and Ca, K, Mg, Na, Cl, P and S were analyzed using an Ion Chromatographer (Metrohm). Plant analyses were referenced using a CTA-VTL-2 certified material (Virginia tobacco leaves). The percentages of recovery were between 90 and 110%.

Young and old needles and fresh leaf litter material were finely ground and were analyzed for C and N concentrations using a C/N Flash EA 1112 Series-Leco Truspec analyzer. Additionally, the C, N and O stable isotope composition of young and old needle samples was measured at the University of California-Davis Stable Isotope Facility. Leaf $\delta^{13}C$ and $\delta^{15}N$ analyses were conducted using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Leaf $\delta^{18}O$ analyses were performed using an elemental PyroCube (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

9.2.3. Statistics

All statistical analyses were done with SPSS 19.0.0 software (SPSS, 2010). The differences in soil properties between soil below litter and bare soil were evaluated by a *T*-test. A one way ANOVA with Tukey's test was used to evaluate differences in elemental composition and nutrient ratios between pine samples (woody stems, young and old needles and leaf litter). The differences in C, O and N isotopic composition between young and old needles were examined using a *T*-test. Differences in leaf metalloid and nutrient concentrations and isotopic composition between pine foliage from the tailings and from the non-polluted local area were also examined using a *T*-test. The data were log-transformed when they failed to pass the Levene test for homogeneity of variances. Differences at $p<0.05$ level were considered significant. Principal component analysis (PCA) was used to reduce the number of variables and summarize the data for the different leaf age classes, using the 'CANOCO for Windows' program v4.02 (ter Braak and Smilauer, 1999).

9.3. Results

9.3.1. Soil analyses

The mine tailings soil showed lower nutrient contents (total nitrogen, available-P, water extractable K and Mg), lower organic carbon content, higher electrical conductivity and higher total metal(loid) concentrations than the control soil (Table 9.1). Soil fertility values at the tailings were at the low end of the range reported for semiarid soils in the Murcia Region (López-Serrano et al., 2005; Querejeta et al., 2006; Párraga-Aguado et al., 2013b). Tailings soils showed high total metal(loid) concentrations that by far surpassed the geochemical backgrounds of the area (Table 9.1; Martínez-Sánchez and Pérez-Sirvent, 2007). For instance, total As was 100-fold higher and total Cd, Mn, Pb, Sb and Zn were between 4 and 12 fold higher than the values found in non-mining impacted local soils (Párraga-Aguado et al., 2013b). However, the slightly alkaline pH of tailings materials may have contributed to maintain the concentrations of water extractable metals (which play a key role in phytotoxicity; Ernst, 1996) at relatively low levels. According to the thresholds proposed by Alarcón-Vera (2007) for an adequate ion balance in soil solution for plant nutrition, the tailings were deficient in K^+ and Mg^{2+}, and showed very high concentrations of SO_4^{2-}. In addition, the tailings had adequate concentrations of Ca^{2+} and low concentrations of Na^+ and Cl^-. The tailings soil below the pine litter layer showed higher fertility (p<0.05, higher OC, TN and DOC concentrations, lower EC) than the soil in adjacent bare patches. However, no significant differences (p<0.05) in total or water extractable metal(loid) concentrations were found between the soil below the pine litter layer and the soil in adjacent bare patches.

Table 9.1. Results of soil samples analyses. Data are average ± standard error (*n* = 15). "n.a." means not available. "*" means significant differences between soil below litter and bare soil ($p<0.05$, *T*-test).

Soil parameter		Units	Tailings samples: Soil below litter		Tailings samples: Bare soil	Non mining impacted local soils (Párraga-Aguado et al. 2013b)
pH (1:5)			7.37 ± 0.06	*	7.15 ± 0.05	7.89
EC (1:5)		dS m^{-1}	1.70 ± 0.26	*	2.85 ± 0.07	0.28
$CaCO_3$		g kg^{-1}	79 ± 8		78 ± 13	84
Organic Carbon		g kg^{-1}	6.60 ± 0.61	*	3.25 ± 0.29	25
Total Nitrogen		g kg^{-1}	0.35 ± 0.01	*	0.16 ± 0.03	2.50
Available-P		mg kg^{-1}	6.37 ± 0.23		6.07 ± 0.50	-
DOC (1:5)		mg kg^{-1}	56.7 ± 1.0	*	16.4 ± 1.7	200.0
Sand		%	83.8 ± 0.3	*	74.1 ± 2.5	26
Silt			14.1 ± 1.5	*	24.4 ± 2.4	31
Clay			2.1 ± 1.6		1.5 ± 0.3	43
Total Metal(loid) Concentations	**As**	mg kg^{-1}	590 ± 52		567 ± 101	59
	Cd		34 ± 4		42 ± 8	5
	Cu		100 ± 5		113 ± 8	55
	Fe		205,000 ± 7400		207,920 ± 7555	376,000
	Mn		9500 ± 330		10141 ± 308	2370
	Ni		29 ± 1		27 ± 2	35
	Pb		5000 ± 490		5909 ± 762	1310
	Sb		71 ± 7		113 ± 22	6
	Zn		8100 ± 650		8485 ± 1122	670
Water Extractable Metal(loid)s (1:5)	**As**	μg kg^{-1}	14 ± 4		<10	<10
	Cd		<10		<10	<10
	Cu		26 ± 9		<10	17
	Fe		100 ± 89		584 ± 114	<10
	Mn		163 ± 27		138 ± 33	19
	Ni		<10		<10	<10
	Pb		<10		<10	<10
	Sb		<10		<10	<10
	Zn		385 ± 70		423 ± 89	25
Water extractable ions (1:5)	**Cl^-**	meq L^{-1}	0.33 ± 0.02		0.33 ± 0.06	0.65
	SO_4^{2-}		26.37 ± 2.60	*	34.61 ± 0.63	0.56
	Ca^{2+}		22.47 ± 2.30	*	31.26 ± 0.14	1.37
	K^+		0.26 ± 0.03	*	0.14 ± 0.02	0.27
	Mg^{2+}		2.03 ± 0.30		3.49 ± 0.78	0.64
	Na^+		1.37 ± 0.40		0.64 ± 0.11	1.00

9.3.2. Plant analyses

As expected, the foliage of pines growing on tailings showed higher metal(loid) concentrations than the foliage of pines growing in the nearby non-polluted control area (Table 9.2). Arsenic and Mn concentrations in young needles were more than 5.4-fold higher in pines growing on tailings than in the control pines. Fe, Ni and Zn concentrations in current-year needles were also more than 2-fold higher in the pines growing on tailings than in the pines from the non-polluted control area. In contrast, N, K, Ca and Mg concentrations in young needles were similar between pines growing on tailings and control pines. However, the pines at the tailings showed 1.8-fold lower foliar P and 1.3-fold higher S concentrations than those in the non-polluted area.

Attending to differences among leaf age classes within the pines growing on tailings, we found that the concentrations of the essential macronutrients K and P were higher in current year needles than in older needles (p<0.05, e.g. 2.5-fold higher for K). These differences were much greater when comparing young needles with fresh leaf litter (e.g. up to 25-fold higher for K). However, old needles showed higher concentrations of Mg and S (p<0.05, at least 2-fold) than young or fresh leaf litter. Calcium concentrations in the latter were 2-fold higher (p<0.05) than those in old needles, and 5-fold higher than those in young needles.

Table 9.2. Leaf isotopic and elemental composition for pine samples. Data are average ± standard error. Different letters in the same row means significant differences between samples from pine trees at tailings (p<0.05, ANOVA with Tukey test). "*" means significant differences between young and old needles (p<0.05, T-test); "x" means significant differences between young needles from the tailings and young needles from a non-polluted local area (p<0.05, T-test). "n.a." means not available.

Parameter		Units	Samples from pine trees at tailings								Young needles from non-polluted local area	
			Young Needles		Old Needles		Fresh Leaf Litter		Woody Stems			
Essential Macroelements	**C**	%	50.47 ± 0.23	a	50.62 ± 0.13	a	48.76 ± 0.22	b	n.a.		50.01 ± 0.53	
	N		0.71 ± 0.03	a	0.69 ± 0.03	a	0.69 ± 0.05	a	n.a.		0.71 ± 0.02	
	P	mg kg^{-1}	378 ± 17	d	303 ± 12	c	163 ± 8	a	254 ± 10	b	668 ± 46	x
	K		4900 ± 300	d	1870 ± 205	b	203 ± 12	a	2745 ± 165	c	5270 ± 149	
	Ca		1190 ± 85	a	3070 ± 190	b	6315 ± 405	c	3395 ± 215	b	1430 ± 125	
	Mg		1880 ± 80	b	2685 ± 140	c	1795 ± 99	b	1250 ± 85	a	1660 ± 116	
	S		1970 ± 55	a	3460 ± 275	b	1770 ± 72	a	1425 ± 52	a	1500 ± 201	x
Essential Microelements	**Cl**	mg kg^{-1}	1150 ± 83	c	432 ± 76	b	231 ± 8	a	374 ± 34	b	965 ± 126	
	Cu		3.08 ± 0.15	a	2.83 ± 0.27	a	2.99 ± 0.18	a	4.93 ± 0.23	b	2.64 ± 0.24	
	Fe		245 ± 22	a	348 ± 31	a,b	489 ± 46	b,c	547 ± 65	c	99 ± 33	x
	Mn		221 ± 28	b	364 ± 40	c	303 ± 19	c	115 ± 16	a	41 ± 2.9	x
	Ni		0.31 ± 0.05	a	0.24 ± 0.06	a	0.74 ± 0.07	b	0.86 ± 0.06	b	0.14 ± 0.02	x
	Zn		79 ± 5	a	153 ± 15	b,c	196 ± 13	c	127 ± 10	b	35 ± 5	x
Other Elements	**As**	mg kg^{-1}	1.49 ± 0.13	a	2.97 ± 0.38	b	7.91 ± 0.85	c	4.36 ± 0.61	b	0.25 ± 0.04	x
	Cd		0.15 ± 0.02	a	0.16 ± 0.03	a	0.78 ± 0.06	b	1.3 ± 0.14	b	0.12 ± 0.05	
	Na		926 ± 125	b	823 ± 104	b	146 ± 11	a	790 ± 126	b	440 ± 115	x
	Pb		8.00 ± 1.15	a	13.7 ± 2.0	b	87 ± 9.48	c	67 ± 7.11	c	3.4 ± 1.33	
	Sb		0.29 ± 0.08	a	0.21 ± 0.03	a	0.37 ± 0.03	b	0.34 ± 0.04	a,b	0.26 ± 0.03	
Stable Isotopes	**$\delta^{13}C$**	‰	-27.28 ± 0.33	*	-29.03 ± 0.22		n.a.		n.a.		-23.58 ± 0.21	x
	$\delta^{15}N$		-6.92 ± 0.29		-7.16 ± 0.29		n.a.		n.a.		-2.51 ± 0.57	x
	$\delta^{18}O$		26.77 ± 0.25		26.43 ± 0.18		n.a.		n.a.		26.50 ± 0.08	

Fresh leaf litter and woody stems showed significantly higher concentrations of non-essential trace elements (As, Cd, Pb and Sb) than young or old needles (e.g. 2-fold higher for As, ~5-fold higher for Cd and Pb). Woody stems showed the highest concentrations of Cu, Fe and Ni of all the plant parts evaluated: for instance, Cu concentration was 1.5-fold higher (p<0.05) in woody stems than in any of the leaf samples (young, old or fresh litter), whereas Fe concentration was more than 1.6-fold higher (p<0.05) in woody stems than in young or old needles. Fresh leaf litter showed more than 2-fold higher (p<0.05) Fe, Ni and Zn concentrations than young needles. Sodium concentration in fresh leaf litter was at least 5-fold lower (p<0.05) than in the rest of the plant materials. Old needles showed the highest Mn concentration of all needle age classes (365 mg kg^{-1}). Young needles showed more than 2-fold higher Cl concentrations (p<0.05) than old needles or fresh litter.

The PCA results for the elemental composition of pine needles across leaf age classes are shown in Figure 9.2. Factor 1, which explained 52.2% of the total variance, clearly separated the young needles from the fresh leaf litter. The young leaves were characterized by their higher foliar K and P status, whereas fresh leaf litter was characterized by its high Ca and metal(loid) concentrations (except Mn). Factor 2 was determined by foliar S, Mg and Mn concentrations, which were higher in the old needle samples.

Foliar $\delta^{13}C$ and $\delta^{15}N$ values were significantly lower (p<0.05) in pines growing on tailings than in the control pines from the non-polluted area, with no difference in leaf $\delta^{18}O$ values between them. Within the pines growing on tailings, foliar $\delta^{13}C$ was significantly higher (p<0.05) in young needles than in older needles (Table 9.2), but there were no differences in $\delta^{18}O$ or $\delta^{15}N$ between needle age classes. Foliar $\delta^{13}C$ and $\delta^{18}O$ were strongly correlated across (r = 0.67; p<0.001) and within needle age classes (r = 0.73, p = 0.002 for young needles; r = 0.64, p = 0.032 for old needles). Foliar $\delta^{13}C$ was also strongly correlated with foliar P (r = 0.70; p<0.001) and K (r = 0.61, p = 0.001) concentrations across needle age classes. In contrast, foliar $\delta^{13}C$ was tightly negatively correlated with Ca concentration across needle cohorts (r = −0.70, p<0.001). Foliar $\delta^{15}N$ correlated with $\delta^{13}C$ and foliar P concentrations across needle age classes (r = 0.47, p = 0.016 and r = 0.37, p = 0.061), but was negatively correlated with foliar C:N ratio (r = −0.37, p = 0.069).

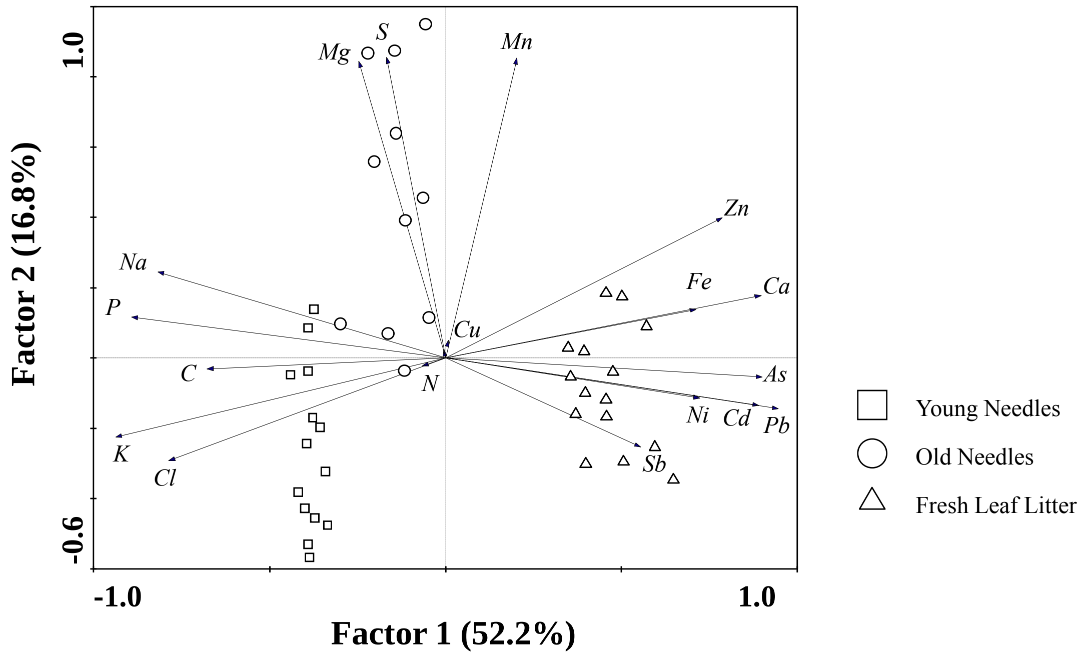

Figure 9.2. PCA results for elemental needle sample composition.

9.4. Discussion

9.4.1. Comparison of foliar elemental composition between pines growing on tailings and control pines

The foliage of pine trees growing on tailings showed higher metal(loid) and S concentrations, and lower P concentration, than the foliage of control pines from a nearby non-polluted area. However, the foliar concentrations of other important macronutrients such as N, K or Mg were very similar between the two groups of trees. The high metalloid (e.g. Pb, As, Fe, Mn, Zn) and S concentrations in the foliage of pines growing on tailings reflected the high water extractable SO_4^{2-} and metal(loid) concentrations in the tailings soils (Table 9.1). However, metal(loid) concentrations in the young foliage of pines growing on tailings were within the normal ranges reported in the literature for young needles of *Pinus halepensis* growing on non-polluted soils (Clarke et al., 2008), and therefore may not reach phytotoxic levels for most metalloids (except for Pb, for which the upper limit of the normal range was clearly surpassed; 5.6 mg kg^{-1} Pb). Foliar S concentrations in the pines growing at the tailings were also higher than the maximum normal values (~1600 mg kg^{-1}) reported by Clarke et al. (2008). Foliar N and P concentrations in the pines at both the tailings and control sites were lower than those reported in most published studies on *Pinus halenpensis* in the Mediterranean area (López-Serrano et al., 2005; Sardans et al., 2005, 2006; Querejeta et al., 2008). In particular, the foliar P concentrations found in the pine trees growing at the tailings were exceedingly low, and were well below the minimum threshold (500 mg kg^{-1}) reported for *Pinus halepensis* in non-metal-polluted Mediterranean ecosystems (e.g. López-Serrano et al., 2005; Sardans et al., 2005, 2006; Querejeta et al., 2008). According to the reference nutrient ratios (N:P, N:K, K:P) proposed by Olde-Venterink et al. (2003), the pines at the non-polluted area are primarily N-limited, whereas the pines at the tailings are primarily P limited or N and P co-limited. However, the concentrations of K and Mg in the foliage of pines growing on tailings were within or near the normal ranges reported for *Pinus halepensis* trees from non-metal polluted Mediterranean ecosystems (e.g. López-Serrano et al., 2005; Sardans et al., 2005, 2006).

9.4.2. Internal nutrient cycling in pines growing on tailings

Fresh leaf litter contained around 96% of the C concentration present in young and old needles, which indicates that they were still in the initial stages of decomposition. Furthermore, foliar C:N ratios did not differ (p>0.05) between litterfall material and green needles (Table 9.3). Moore et al. (2006) found a sharp decrease in leaf litter C concentration during the early stages of litter decomposition in *Pinus banksiana* Lamb, with no change in litter N concentration until ~25% of C was lost from the litter material. So it is safe to assume that nutrient losses by decomposition were still negligible in our fresh litter material at time of collection, and that nutrient concentrations in fresh litterfall can thus be used to evaluate nutrient and metal(loid) cycling in the pine trees growing on tailings.

Table 9.3. Nutrient ratios for the analyzed needles. Data are average ± standard error. Different letters in the same row means significant differences between samples from pine trees at tailings (p<0.05, ANOVA with Tukey test). "*" means significant differences between young needles from the tailings and young needles from a non-polluted local area (p<0.05, T-test). "n.a" means not available.

Ratio	Samples from pine trees at tailings				Young needles from non-polluted local area
	Young Needles	Old Needles	Fresh Leaf Litter	Woody Stems	
C:N	73 ± 3.1 a	75 ± 3 a	76 ± 6 a	n.a	70 ± 2
N:P	19 ± 1.2 a	23 ± 2 a	43 ± 3 b	n.a	11 ± 1 *
N:K	1.5 ± 0.1 a	3.6 ± 0.7 a	35 ± 3.2 b	n.a	1.4 ± 0.1
N:S	3.7 ± 0.2 b	2.2 ± 0.2 a	4.0 ± 0.3 b	n.a	5.0 ± 0.7 *
K:P	13.4 ± 1.0 c	6.2 ± 0.7 b	1.2 ± 0.0 a	10.9 ± 0.7 c	8.1 ± 0.8 *
Ca:Mg	0.6 ± 0.0 a	1.2 ± 0.1 a	3.6 ± 0.3 b	2.9 ± 0.3 b	0.9 ± 0.0 *

Although mine tailings with neutral pH values (such as our study site) generally show low water metal(loid) extractability and moderate phytotoxicity, high soil electrical conductivity (including ion specific toxicity, especially SO_4^{2-}) or severe nutrient deficiency (e.g. P) may still represent major constraints for plant establishment and growth on tailings materials (Párraga-Aguado et al., 2014). It is thus important to evaluate the ability of the pine trees at the tailings to cope with salts excess in the soil solution (especially SO_4^{2-}) and severe soil nutrient de ficiency (especially P).

The pines at the tailings must cope with an excess of S, as indicated by high S concentrations in young and, especially, old needles. Within the plant, S is known to form compounds which play important roles in the mechanisms of plant response to biotic and abiotic stresses, including metal tolerance (Wu et al., 2010). In S deficient soils, the uptake of SO_4^{2-} is enhanced by the transcription of genes coding for specific transporters. However, when SO_4^{2-} appears at high concentrations in the soil solution (such as in mine tailings) the repression of the activity of S assimilation enzymes is not completely effective (Hawkesford, 2007), and therefore large amounts of SO_4^{2-} can be taken up by the plant. The SO_4^{2-} excess is normally stored in vacuoles (Hoefgen andHesse, 2007). Old needles have been found to store S in the form of glutathione, which can be later translocated to other plant organs (Rennenberg and Herschbach, 1995; Herschbach and Rennenberg, 2001). According to Tausz (2007), young needles have low activities of S reduction enzymes and thus import the reduced S (in the form of glutathione) from older leaves. This may explain the higher S concentrations found in old needles relative to young needles in our study. In spite of the high S concentrations found in old needles, the low S concentrations in fresh leaf litter indicates resorption of S from senescent leaves and redistribution to other plant organs before needle fall (Figure 9.3). This may indicate that high S concentrations in soil and foliage do not pose major constraints to *Pinus halepensis* performance and growth in these semiarid mine tailings. In fact, previous studies have demonstrated the high tolerance of other pine species (*Pinus contorta*) to high SO_4^{2-} concentrations in the soil solution (Renault et al., 1998).

Nutrient resorption and retranslocation are key mechanism to alleviate nutrient deficiencies in plants growing on nutrient-poor soils (Pugnaire and Chapin, 1993; Rapp et al., 1999; Sardans et al., 2005) as they involve redistribution of essential nutrients from senescent organs to younger ones (López-Serrano et al., 2005), and/or nutrient storage in perennial plant parts for their later use (Rapp et al., 1999). In evergreen species, most of the foliar macronutrients, except Ca, and micronutrients, except Mn or Fe, may show some internal redistribution within the plant before leaf fall (Marschner, 1995). Essential nutrients such as N, P or K (which are known to be important limiting factors for *Pinus halepensis* photosynthesis and growth in Mediterranean forests) can be efficiently redistributed before leaf fall, as shown by Sardans et al. (2005). These authors reported retranslocation percentages of 20–30% for N, 60% for P and 80% for K in *Pinus halepensis*. These percentages are similar to the ones that we found in *Pinus halepensis* trees growing on tailings, which were 46% for P and 89% for K (Figure 9.3). The absence of significant N retranslocation before litterfall in the pines growing on tailings may be the consequence of severe N deficiency and a small pool of mobile N.

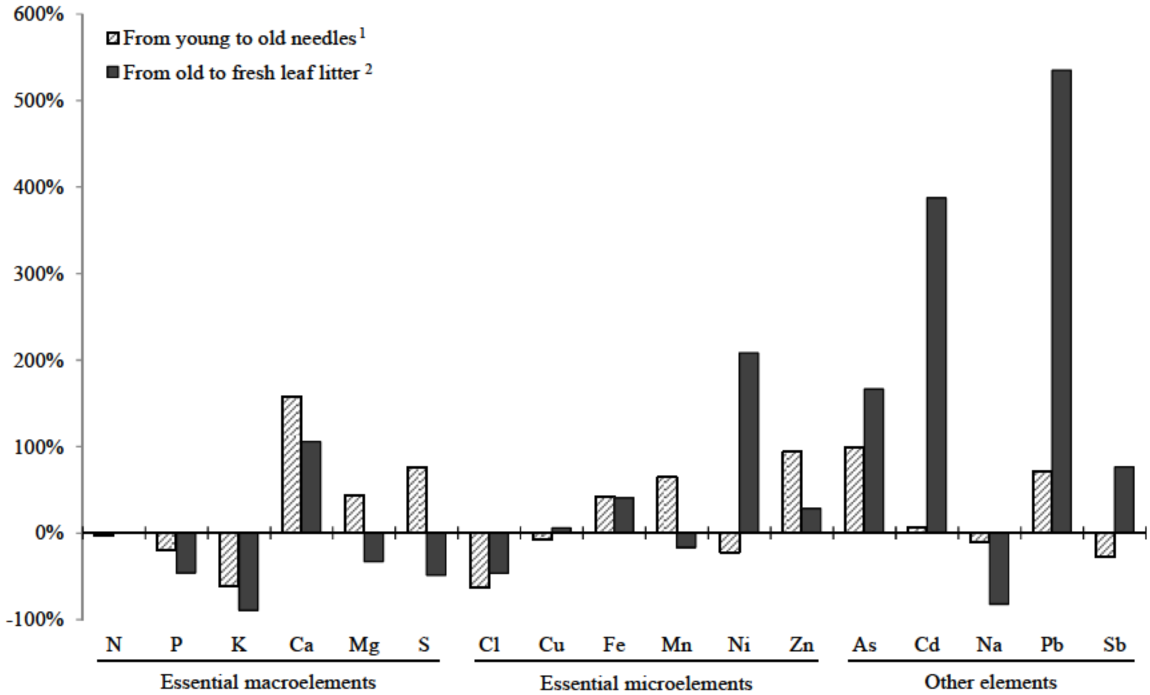

Figure 9.3. Percentages of translocation from young to old needles and from old to fresh leaf litter. ([1]) % = (O − Y)/Y, where O and Y are the concentration in old and young needles respectively. ([2]) %= (L − O)/O, where L and O are the concentration in litter and old needles respectively.

Magnesium is known to play an important role in photosynthesis (between 6 and 25% of total leaf Mg is bound up in chlorophyll), and is stored in vacuoles (Marschner, 1995). Despite the low Mg concentration in the soil solution, its concentration in pine needles was within the normal range reported for *Pinus halepensis* (López-Serrano et al., 2005; Sardans et al., 2005, 2006). Old needles showed higher Mg concentrations than fresh litter material, which suggests retranslocation (~50%) prior to leaf abscission (Marschner, 1995). Proe et al. (2000) reported that up to 52% of the Mg in newly formed plant organs came from internal nutrient retranslocation in *Pinus sylvestris* growing on Mg-deficient soil. Although Mg is highly mobile in the phloem, this essential nutrient has often been overlooked in studies on nutrient retranslocation (Miloud and Ali, 2012). In contrast to some studies which have reported Mg accumulation in senescent leaves (Killingbeck, 2004), we found that the Ca:Mg ratios (Table 9.3) of pine foliage increased with leaf age and reached a maximum in fresh leaf litter, which indicates that Mg, unlike Ca, is being retranslocated before needle abscission in pines growing on tailings.

Calcium uptake and transport to leaves are closely related to and enhanced by transpiration fluxes (Gilliham et al., 2011). Foliar Ca concentration tends to increase with cumulative transpiration flux throughout the leaf lifespan, and thus, older leaves usually contain higher Ca concentrations than younger ones (López-Serrano et al., 2005). Indeed, at the present study, foliar Ca concentrations increased sharply with needle age (young needles < old needles < fresh leaf litter) in *Pinus halepensis* trees growing on semiarid tailings. The tight link between transpiration flux and foliar Ca accumulation was further supported by the strong negative correlations found between foliar Ca concentration and leaf $\delta^{13}C$ and $\delta^{18}O$ (see paragraph 9.4.4).

The foliar concentrations of Cl, Na and K in the pines growing on tailings were positively correlated among each other (r~0.5, p<0.01; Figure 9.2), which is in agreement with previous studies on pine seedling nutrition (Fostad and Pedersen, 2000), and may indicate uptake of these ions in the form of neutral salts (Khaldi et al., 2011).

9.4.3. Metal(loid) allocation in pines growing on tailings

Metalloid translocation patterns from young to old needles, and from the latter to fresh litterfall are shown in Figure 9.3. A pattern of increasing foliar accumulation with increasing leaf age was evident for some of the metal(loid)s such as As, Cd, Fe, Pb and Zn. Although metalloid concentrations in young needles were within the normal ranges reported by Clarke et al. (2008; except for Pb), metalloid concentrations in old needles were clearly above normal thresholds (e.g. >170 mg kg^{-1} Fe; >5.6 mg kg^{-1} Pb; >96 mg kg^{-1} Zn). Furthermore, high accumulation percentages (75–535%) were found in fresh litterfall for Ni, As, Cd, Pb and Sb, relative to old needles (Figure 9.3). This suggests detoxification of metal(loid)s by their active/passive accumulation in senescent leaves and subsequent abscission, which allows pines to get rid of these potentially toxic elements (Baker and Walker, 1990). Another plant mechanism for dealing with high metal(loid) concentrations is their accumulation in woody stems, which allows the long-term immobilization of these elements in less biologically-active tissues (Pulford and Watson, 2003). For example, Ots and Mandre (2012) found that both woody shoots and old senescent leaves were sinks for Cu accumulation in *Pinus sylvestris* trees growing on metal polluted soils in Estonia, and we observed a similar pattern in the present study.

The pines growing on tailings showed a combination of metal(loid) immobilization in woody stems and elimination via leaf abscission. Cu primarily accumulated in woody tissues, Cd, Fe, Ni, Pb and Sb accumulated in both woody stems and litterfall, whereas Zn and As accumulated in litterfall. After leaf abscission, the metal(loid)s contained in litterfall may return to the soil, where they can be incorporated into biogeochemical cycles and may thus enter into the food chain (Robinson et al., 2009). However, the decomposition of litterfall in semiarid tailings is expected to be strongly hampered by high pine litterfall recalcitrance (Navarro-Cano et al., 2010) and by unfavorable environmental conditions for decomposition, such as high metal content in soils (which may hamper microbiological activity) (Berg et al., 1991) and low soil moisture (Pausas, 1997).

Within the tailings area, the soil below the litter layer under pine trees showed higher fertility (higher TN, OC and DOC contents and lower EC) than the soil in surrounding bare areas. Litterfall inputs to the soil (along with belowground rhizosphere processes) may have contributed to the higher fertility of tailings soils under the pine trees, relative to adjacent bare areas.

Most of the metal(loid)s remained at low water extractable concentrations in both soil below litter and bare soil areas. Only Cu and As, whose availability may be enhanced by soil organic matter (Strobel et al., 2001; Wang and Mulligan, 2006), were slightly higher in the soil below litter. Water extractable metalloid concentrations in soil are good indicators of metal(loid) phytotoxicity, but may obviate some important metal(loid) fractions (e.g. metal(loids) bound to organic matter). For this reason, more comprehensive studies are warranted to further investigate the long-term release of metal(loid)s from the litterfall layer in semiarid tailings.

9.4.4. Stable isotope composition of pine foliage

Pine trees growing on tailings showed significantly lower $\delta^{13}C$ values than control pines growing in a nearby non-polluted area. However, the similar leaf $\delta^{18}O$ values in both tree groups suggest that lower $\delta^{13}C$ and WUE in the pines growing on tailings was the result of a lower photosynthetic activity caused by severe nutrient deficiency (and possibly metalloid toxicity as well), relative to the pines growing on non-polluted soils (Scheidegger et al., 2000).

Foliar $\delta^{13}C$ and $\delta^{18}O$ were tightly positively correlated with each other across needle age classes in the pines growing on tailings, indicating strict stomatal regulation of both photosynthesis and transpiration in these pine trees. This tight correlation may be interpreted as evidence that the photosynthetic activity of *Pinus halepensis* is strongly limited by water availability in this semiarid tailings (Barbour et al., 2002; Moreno-Gutiérrez et al., 2011). Interestingly, pine trees growing on tailings showed lower $\delta^{13}C$ values in old needles than in young needles, which is in agreement with the findings of Choi et al. (2005) and Barszczowska and Jedrysek (2005). Lower $\delta^{13}C$ values in older needles suggest a decrease in water use efficiency (WUEi) with increasing leaf age, due to either higher stomatal conductance or lower photosynthesis in older leaves. A higher transpiration rate in older needles would also be supported by their higher Ca concentrations, as Ca accumulation in leaves is related to and enhanced by transpiration flux (Gilliham et al., 2011). However, since foliar $\delta^{18}O$ did not differ between young and old needles, it is more likely that the lower $\delta^{13}C$ and water use efficiency of older foliage was more the result of decreased photosynthetic rate than of increased stomatal conductance (Scheidegger et al., 2000). A higher photosynthetic activity in young needles (relative to old needles) is further supported by the tight positive correlations found across needles age classes between leaf $\delta^{13}C$ and the foliar concentrations of two key nutrients for photosynthesis, namely P (Warren, 2011) and K (Jin et al., 2011). Higher metalloid concentrations may have hampered photosynthesis and water use efficiency in older needles relative to younger needles (Stoeva et al., 2005).

Foliar $\delta^{15}N$ values were much lower in pines growing on tailings than in control pines growing on non-polluted soils. Low leaf $\delta^{15}N$ may be the result of severe nutrient deficiency at the mine tailings, as plant $\delta^{15}N$ generally increases with increasing soil fertility and plant nutrient status at local, regional and global scales (Craine et al., 2009). The correlations of leaf $\delta^{15}N$ with $\delta^{13}C$ and foliar P concentrations (positive) and C:N ratio (negative) across needle age classes in pines growing on tailings further indicate that low foliar $\delta^{15}N$ values are linked to severe nutrient deficiency. Depleted foliar $\delta^{15}N$ values in the pines growing on tailings also point to a heavier dependence on symbiotic ectomycorrhizal fungi (EMF) for N uptake (relative to control pines), since EMF strongly fractionate against ^{15}N during N transfer to the host plant (Hobbie and Högberg, 2012).

9.5. Conclusions

Significant amounts of metal(loid)s such us Cd, Cu, Pb and Sb were immobilized in the woody stems of *Pinus halepensis* trees growing on tailings, but leaf litterfall also showed high concentrations of As, Cd, Sb, Pb and Zn that thereby may return to the soil. Water extractable concentrations did not reveal a greater release of metal(loid)s in the soil below the litter layer under pine trees compared to bare tailings soil. Nevertheless, more comprehensive studies that include additional metal(loid) speciation procedures will be necessary in order to evaluate the long-term release of metal(loid)s from pine litter and their fate. Therefore, the phytostabilization of tailings using *Pinus halepensis* will require the careful monitoring of the trace elements released from litterfall in order to assess the long-term risk of metal(loid) transfer to the food chain.

Pinus halepensis trees growing on semiarid mine tailings show substantial macronutrient retranslocation from senescent leaves and resorption before leaf fall, which may help them to cope with severe soil nutritional deficiencies, especially in relation to K, Mg and P. However, P concentrations in pine needles were well below optimum levels across leaf cohorts, despite substantial P retranslocation before needle fall. Phosphorus fertilization should thus be considered as a promising option to favor the establishment and growth of pine trees on these semiarid tailings.

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CHAPTER 10. The potential use of *Piptatherum miliaceum* for the phytomanagement of mine tailings in semiarid areas: role of soil fertility and plant competition

Chapter 10

The potential use of *Piptatherum miliaceum* for the phytomanagement of mine tailings in semiarid areas: role of soil fertility and plant competition

Isabel Párraga-Aguado, María Nazaret González-Alcaraz, Rainer Schulin, Héctor M. Conesa

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Abstract

Phytomanagement in terms of phytostabilisation has been proposed as a suitable technique to decrease the environmental risks of metal(loid) enriched mine tailings. Nevertheless, at these sites some issues must be solved to assure the long-term establishment of vegetation (e.g. salinity, low fertility, metal(loid) phytotoxicity, etc.) The objective of this study was to assess the effects of the addition of a municipal solid waste on a mine tailings soil and on the growth and metal(loid) accumulation of a grass plant species (*Piptatherum miliaceum*). In addition, the effects of intra-specific interactions were evaluated. A pot experiment was performed during eight months, including two soil treatments: the mine soil and its combination with a municipal solid waste. For each treatment, pots without plants, pots with one plant, and pots with two plants were arranged. The addition of municipal solid waste improved the soil fertility and plant growth in the mine soil, but also increased the mobile fractions of Zn, Pb, Cd, Mn and Ni. Plants in the amended treatments showed better nutritional status (higher P and K). Stable isotope $\delta^{15}N$ was associated to the better nutritional status, while $\delta^{13}C$ and $\delta^{18}O$ indicated higher photosynthetic efficiency and stomatal conductance in amended treatments. Although the accumulation in leaves of most metal(loid)s decreased with the municipal waste application, the concentrations in both treatments did not exceed toxic limits for fodder. There was an effect of intra-specific competition in plant growth, probably due to lack of nutrients in the mining soil or limited pots volume in the treatments with municipal waste.

10.1. Introduction

Phytomanagement in terms of phytostabilisation has been proposed as a suitable technique to decrease the environmental risks of metal(loid) enriched mine tailings. Phytostabilisation provides a long-term vegetal cover which may mitigate erosion and immobilise metal(loid)s within the rhizosphere. Nevertheless mine tailings usually show several edaphic constraints which may condition plant growth, including high salinity, low fertility, limited water holding capacity, high metal(loid) concentrations, etc. (Conesa et al., 2006). In addition, in semiarid areas, drought and high temperatures should be taken into account (Mendez and Maier, 2008).

Previous research at abandoned mine tailings have pointed out the importance of pioneer plant species for enhancing soil properties and promoting plant successional processes (Antonsiewicz et al., 2008; Jiménez et al., 2011; Testiati et al., 2013; Páraga-Aguado et al., 2013). Pioneer vegetation growing in tailings has shown to be tolerant to site specific edaphic and climate conditions (Conesa et al., 2006). However, plant successional processes in these high stressed environments are usually slow and their related long-term schedules may be considered unacceptable for most restoration projects (Conesa et al., 2007a). Then, it is critical to accelerate these processes by favouring plant growth. Among the different alternatives, the use of organic amendments has been widely employed because of stimulating soil fertility and decreasing metal(loid) mobility (Norland and Veith, 1995; Clemente et al., 2010; Kohler et al., 2014). The performance of organic amendments depends on their nature, the dose and the properties of the polluted soil. Although there is a general assumption that organic amendments may enhance soil fertility, structure and plant cover (Norland and Veith, 1995; Zanuzzi et al., 2009), recent studies have pointed out the need of specific assessments in order to avoid undesirable long-term effects such as metal(loid) leaching or disturbances in plant ecological relationships (Madejón et al., 2010; Yang et al., 2013; Pardo et al., 2014).

Linking phytomanagement projects to the employment of municipal organic wastes as amendments might have positive economic and environmental feedbacks. The increasing amounts of organic wastes generated in cities makes necessary to find alternatives different from depositing in landfills (He et al., 1992). However, prior to the use of these wastes in phytomanagement projects, some environmental issues must be solved, including the assessment of the effects on soil fertility properties or the fate of metal(loid)s in the environment.

Ecological interactions (facilitation/competition) among pioneer plant species at mine tailings have not been thoroughly studied. Pioneer vegetation is essential for the achievement of a long-term self-sustaining vegetal cover. The growth of pioneer species improves soil conditions by providing nutrients and organic matter, and thus, favouring the introduction and survival of new plant species. This process could be referred as 'facilitation' (Markham et al., 2011). Recent studies in Mediterranean areas have revealed that the combined use of local grasses ecotypes and Fabaceae species had positive effects in biomass production and plant successional issues (Frérot et al., 2006; Arco et al., 2014). However, some studies have pointed out the role of inter and/or intraspecific competition in the establishment of vegetation in mining impacted soils (Jefferson, 2004) and in the specific response to phytotoxicity by metal(loid)s (Koelbener et al., 2008). Therefore, it is still necessary to get a better understanding of the role of ecological interactions in the phytostabilisation of mine tailings.

The objective of this study was to assess the effects of the addition of a municipal solid waste on a mine tailings soil, attending to soil fertility and metal(loid)s behaviour (leaching, changes in speciation, etc.). At the same time, the response of a grass plant species (*Piptatherum miliaceum*), commonly found among pioneer vegetation of mine tailings in semiarid areas, was evaluated. In this case, the effects of intra-specific interactions (facilitation/competition) were also considered. This will provide useful information to determine whether or not the establishment of this plant species is influenced by intra-specific competition/facilitation. Finally, some strategies for its employment in the revegetation of mine tailings in semiarid areas will be proposed. For this purpose, a pot experiment was performed, using the plant species *P. miliaceum* and including two soil treatments (the mine soil and its combination with municipal solid wastes). For each of these treatments, pots with no plant, pots with one plant and pots with two plants were arranged.

10.2. Materials and methods

10.2.1. Experimental set-up

A pot experiment using the Graminae *P. miliaceum* growing on a mine tailings soil, with and without the addition of a municipal solid waste, was carried out for eight months in a greenhouse located in Southeast Spain. The tailings soil (T) was taken from an abandoned mine tailings disposal area at the Cartagena-La Union mining (SE Spain). Information on the mining history and their related environmental impacts was reviewed by Conesa and Schulin (2010). The selected organic amendment was a municipal solid waste (MW) provided by a recycling plant of urban metallic containers (Pedro Segura SL).

The soil treatments tested during the experiment were the single mine tailings soil (T) and the combination of the mine tailings soil plus the municipal solid waste (T+MW) following the rate of 10 g of MW per 100 g of T (Tercero et al., 2012). Each resulting treatment was homogenised and then, stabilised for two weeks. A complete physical-chemical characterisation of T, MW and T+MW was performed. Plastic pots of 20 cm diameter were filled with ~3.5-4 kg of soil.

The selected plant species, *Piptatherum miliaceum* (L.) Coss. is a widespread perennial grass usually found among the pioneer vegetation which grow at mine tailings in SE Spain (Conesa et al., 2007a; Carrasco et al., 2010). It has a well-extended root system and developed rhizomes, which provides a suitable protection against soil erosion (De Baets et al., 2007). The seeds of *P. miliaceum* were collected in September 2012 from plants growing in situ at the aforementioned mine tailings area and stored in plastic bags. In February 2013, around one hundred seeds were germinated in plastic trays containing the mine tailings soil in a growth chamber (26 °C, 16/8 h light/dark). In March 2013, four week old seedlings were transplanted into the pots. For each soil treatment, eight pots with one plant (named T/1 and T+MW/1) and eight pots with two plants (named T/2 and T+MW/2) were prepared. In addition, four pots without plants were also prepared for each soil treatment (T/0 and T+MW/0), giving a total of 40 pots.

All the pots were randomly distributed on a gridded table inside a greenhouse and watered by an automatic programmed drip irrigation system to maintain the soils at field capacity during the experiment. The irrigation water had a pH~7.5-8, low electrical conductivity (EC~0.45 dS m^{-1}), ~2 mg L^{-1} of dissolved organic carbon (DOC), ~100 mg L^{-1} Cl^-, ~2.5 mg L^{-1} SO_4^{2-}, ~20 mg L^{-1} Ca^{2+}, ~2.5 mg L^{-1} K^+, ~1 mg L^{-1} Mg^{2+}, and ~65 mg L^{-1} Na^+. Metal(loid) concentrations were below 2 μg L^{-1} except for Zn, that were ~15 μg L^{-1}. Inorganic fertilizers were not applied to not mask the effect of the MW.

Two Rhizon® samplers (pore diameter = 0.1 μm) were placed in each pot to collect soil solution samples. The leachates from each pot were collected in opaque bottles. Soil solution samples were collected at the beginning of the experiment (S0) and once a month (S1-S8) while leachates were collected at the beginning of the experiment (S0) and alternating months (S2, S4, S6 and S8). At the end of the experiment, plants and soils of each pot were employed for analysis.

10.2.2. Soil analyses

10.2.2.1. Soil and municipal solid waste characterisation

A comprehensive soil characterisation was performed at the beginning and at the end of the experiment. At the beginning, aliquot samples for the mine tailings soil (T, N=5), the municipal solid waste (MW, N=5) and the tested combination T+MW (N=5) were taken. At the end, soil samples were taken from each pot (N=4 or 8, for each treatment).

The soil pH and EC were measured in a 1:5 soil:water w/v suspension after shaking for 2 h and filtered through CHM F2041 filter papers (20-25 μm). The measurements were done with a Crison Basic 20 pH-meter and a Crison Basic 30 conductivity meter, respectively. Afterwards, these water extracts were re-filtered through nylon membrane 0.45 μm syringe filters (Wicom) and were analysed for major ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, NH_4^+, HPO_4^-, NO_3^-, Cl^- and SO_4^{2-}), metal(loid)s (As, Cd, Cu, Fe, Mn, Ni, Pb, Sb and Zn) and dissolved organic carbon and nitrogen (DOC, DON) employing an Ion Chromatographer (Metrohm), an ICP-MS (Agilent 7500 CE) and a TOC-automatic analyser (TOC-VCSH Shimadzu), respectively.

Particle size distribution was determined by Bouyoucos' densimeter method (Gee and Bauder, 1986). Equivalent calcium carbonate ($CaCO_3$) was estimated using the Bernard calcimeter method. Total nitrogen content (TN) was determined using the Kjeldahl method (USDA, 1996) and Organic carbon content (OC) by the oxidation of the organic matter using potassium dichromate (Duchaufour, 1970). Total metal(loid) concentrations (As, Cd, Cu, Fe, Mn, Ni, Pb, Sb and Zn) were measured by X-Ray Fluorescence (Bruker S4 Pioneer).

Dehydrogenase activity was used for describing the soil microbiological status and was determined according to García et al. (1993), measuring the amount of iodo-nitrotetrazolium formazan (INTF) by spectrophotometry at λ=490 nm.

In the soil samples taken at the end of the experiment, the following selected soil analyses were performed: pH, EC, major ions, water extractable metal(loid)s, DOC, and dehydrogenase activity.

10.2.2.2. Metal(oid) spetiation

The sequential extraction procedure proposed by Zeien and Brümmer (1989) was carried out in soil samples taken at the beginning and the end of the experiment for each tested treatment (*N*=3). Table 10.1 shows the definition of the fractions obtained as well as the components of the extraction solutions. All the suspensions of steps 1 to 6 were centrifuged at 2500 rpm for 10 min and passed through Whatman 42 Ashless filter papers (2.5 μm). Several metal(loid)s (As, Cd, Cu, Mn, Ni, Pb, Sb and Zn) were measured by means of ICP-OES (Vista-MPX Varian; detection limit 0.010–0.005 mg L^{-1}). The residual fraction was analysed by means of X-ray fluorescence spectrometry (SPECTRO XEPOS EDXRF). The average percentages of recovery (sum of all steps/total concentration) were 124% for As, 91% for Cd, 107% for Cu, 96% for Mn, 100% for Ni, 102% for Pb, 125% for Sb and 109% for Zn.

Table 10.1. Chemical interpretation and operational definition of the fractions obtained from the sequential extraction scheme of Zeien and Brümmer (1989).

Fraction	Chemical interpretation	Extractant	Duration, temperature
F1	Mobile (water soluble and easily exchangeable metals)	1 M NH_4NO_3 (pH 5.5)	24 hours, 20 °C
F2	Easily mobilisable (specifically adsorbed, bound to $CaCO_3$)	1 M NH_4-acetate (pH 6)	24 hours, 20 °C
F3	Bound to Mn oxides	0.1 M NH_2OH-HCl + 1 M NH_4 acetate (pH 6)	30 minutes, 20 °C
F4	Bound to organic matter	0.025 M NH_4EDTA (pH 4.6)	90 minutes, 20 °C
F5	Bound to amorphous Fe oxides	0.2 M NH_4-oxalate (pH 3.25)	4 hours in the dark, 20 °C
F6	Bound to crystalline Fe oxides	0.1 M ascorbic acidic + 0.2 M NH_4 oxalate (pH 3.25)	30 minutes at 96 °C
F7	Residual fraction	X-ray fluorescence	-

10.2.3. Soil solution and leachates analyses

The pH and EC were measured using a Crison Basic 20 pH-meter and a Crison Basic 30 conductivity meter. Then, the samples were filtered through a nylon membrane 0.45 μm syringe filters (Wicom). In the resulting extracts, DOC was determined in an automatic TOC analyser (TOC-V CSH, Shimadzu), major ions (K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^- and SO_4^{2-}) were analysed in an Ion Chromatographer (Metrohm) and dissolved metal(loid)s (As, Cd, Cu, Mn, Ni, Pb, Sb and Zn) in an ICP-MS (Agilent 7500 CE).

10.2.4. Plant Analyses

Plants collected at the end of the experiment from each pot were separated into roots, stalk and leaves. Then, each sample was carefully washed with deionised water and dried in an oven (65°C, 72h). Dry weight was recorded and samples were finely ground with an IKA-A11 mill. Around 0.5 g of ground material was incinerated (550 °C, 3 h). The resulting ashes were dissolved with 1 mL of concentrated nitric acid and filled to 25 ml with distilled water. Then, the elemental content was measured: Cl, P (calculated from PO_4^{3-} concentrations) and S (calculated from SO_4^{2-} concentrations) were analysed using an Ion Chromatographer (Metrohm); Ca, K, Mg and Na were analysed using a flame AAS (UNICAM 969 AA); and metal(loid)s As, Cd, Cu, Mn, Ni, Pb, Sb and Zn were analysed using a ICP-MS (Agilent 7500A). Plant analyses were referenced using a CTA-VTL-2 certified material (Virginia tobacco leaves). The percentages of recovery were between 75% and 125%.

The ground leaf samples were also used for stable isotope measurements at the University of California-Davis Stable Isotope Facility. Carbon, Nitrogen, $\delta^{13}C$ and $\delta^{15}N$ analyses were conducted using a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). $\delta^{13}C$ and $\delta^{15}N$ data are expressed relative to international standards V-PDB (Vienna PeeDee Belemnite). Leaf $\delta^{18}O$ analyses were performed using an elemental PyroCube (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The final delta values are expressed relative to international standard V-SMOW (Vienna Standard Mean Ocean Water).

10.2.5. Statistical analyses

Statistical analyses were performed with the software IBM SPSS Statistics 22. Homogeneity of variances was tested using Levene test. Data that were not normally distributed were log transformed. Means were compared by *t*-test (for 2 independent groups) or one-way ANOVA with Tukey's test (for >2 independent groups). When homogeneity of variances could not be corrected by log transformation, non-parametric tests (Mann-Whitney for 2 groups or Kruskal-Wallis and a non-parametric post-hoc test for >2 groups) were used. In addition, a repeated measures ANOVA was carried out in order to evaluate differences along time and among treatments for soil solution and leachates parameters.

The presence of plants did not cause any significant differences in the soil parameters neither in leachates or soil solution. Therefore, the results were statistically evaluated obviating the plant treatments, as follows: T/0, T/1 and T/2 were evaluated as T, while T+MW/0, T+MW/1 and T+MW/2 were grouped as T+MW. Then, the treatments considered were T (N=20) and T+MW (N=20).

10.3. Results

10.3.1. Soil characterisation

The main characteristics of the mine tailings soil (T), the municipal solid waste (MW) and their combination (T+MW) are shown in the Table 10.2. The addition of the MW significantly (p<0.05) increased the EC of T (up to 3 dS m^{-1}), due to the raise (p<0.05) of the concentrations of water extractable ions, especially

Cl^- (from 1.5 to 102.9 mg L^{-1}). Likewise, some fertility-related parameters significantly ($p<0.05$) increased: around 10-fold for OC and DON; more than 20-fold for DOC; soil microbiology (dehydrogenase activity) also showed a positive enhancement (up to 0.23 μg INTF g^{-1} h^{-1}). Finally, other soil parameters such as pH, $CaCO_3$, TN or particle size distribution were not affected.

Attending to the total metal(loid) concentrations, some significant effects were found. For instance, total Cu and Ni concentrations significantly increased ($p<0.05$) with the addition of the MW (e.g. total Ni was 2-fold higher in T+MW), while Pb and Zn significantly decreased ($p<0.05$), probably due to a dilution effect. The water extractable metal(loid) concentrations significantly increased ($p<0.05$) with the addition of the MW. Compared to T, the T+MW treatment showed detectable values of As, Cd, Cu, Ni, Pb and Sb (that were < 10 μg kg^{-1} in T) and 100-fold and 4-fold higher concentrations for Mn (up to 11,321 μg kg^{-1}) and Zn (up to 1870 μg kg^{-1}), respectively.

At the end of the experiment (Table 10.3), all the studied parameters (except water extractable HPO_4^{2-} and Cd, which were under their detection limit) showed significant differences ($p<0.05$) when comparing T and T+MW treatments. Thus, the effects of the addition of the MW were detectable even after eight months.

Compared to the initial characterisation, and considering that the plants factor had no effect in soil parameters ($p>0.05$), the changes found at the end of the experiment could be attributed to the effects of irrigation and time. For instance, pH was higher in both treatments (although only significant in T+MW, $p<0.05$) which might be explained because of the pH 8 of the irrigation water. The EC was significantly lower in both treatments at the end of the experiment (mainly due to the significant decrease of SO_4^{2-} concentrations; $p<0.05$) probably due to the decrease of SO_4^{2-} and Cl^- in T and T+MW treatments, respectively. The water extractable Cu, Sb and Zn concentrations in T samples were higher ($p<0.05$) at the end of the experiment. On the other hand, T+MW samples showed a significant decrease ($p<0.05$) of As, Cd, Cu, Ni and Zn concentrations.

Table 10.2. Soil characterisation at the beginning of the experiment: T is the mine tailings soil; MW is the municipal soil waste employed as amendment; and T+MW is the combined treatment of mine tailings soil and municipal solid waste. The results are average ± standard error (*N*=5). "*" indicates significant differences (p<0.05) between T and T+MW treatments. EC (electrical conductivity); TN (total nitrogen); DON (dissolved organic nitrogen); OC (organic carbon); DOC (dissolved organic carbon).

Soil parameter		Units	Treatments			MW
			T		T+MW	
pH (1:5)		-	7.1 ± 0.1		7.0 ± 0.0	5.3 ± 0.1
EC (1:5)		dS m^{-1}	2.4 ± 0.0	*	3.0 ± 0.0	4.1 ± 0.1
$CaCO_3$		g kg^{-1}	43 ± 2.1		44 ± 2.7	70 ± 1.9
TN		g kg^{-1}	0.7 ± 0.1		0.9 ± 0.07	12 ± 0.5
DON		mg kg^{-1}	5.1 ± 0.1	*	47.4 ± 2.7	1571 ± 41.8
OC		g kg^{-1}	1.4 ± 0.1	*	12.6 ± 0.4	162 ± 5.1
DOC		mg kg^{-1}	29.0 ± 0.7	*	686 ± 40	25,218 ± 1032
Particle size distribution	**Sand**	%	82 ± 1		81 ± 2	n.d.
	Silt		20 ± 1		19 ± 1	n.d.
	Clay		<1		<1	n.d.
Dehydrogenase activity		μg INTF g^{-1} h^{-1}	0.06 ± 0.02	*	0.23 ± 0.02	0.67 ± 0.05
Water extractable ions (1:5)	**Cl^-**	mg L^{-1}	1.5 ± 0.0	*	102.9 ± 3.0	1155 ± 20.4
	HPO_4^{2-}		<0,7		<0,7	17.2 ± 0.4
	NO_3^-		1.1 ± 0.1		1.0 ± 0.1	10.4 ± 0.3
	SO_4^{2-}		1472 ± 8.5	*	1540 ± 10.7	691 ± 19.2
	Ca^{2+}		577 ± 3.4		575 ± 3.2	314 ± 12.3
	K^+		1.7 ± 0.1	*	45.8 ± 1.6	638 ± 13.1
	Mg^{2+}		18.5 ± 0.4	*	31.6 ± 0.7	151 ± 6.1
	Na^+		1.4 ± 0.0	*	86.9 ± 2.9	972 ± 18.5
	NH_4^+		0.4 ± 0.0		0.6 ± 0.1	12.0 ± 2.4
Total metal(loid)s	**Fe**	g kg-1	218 ± 1	*	202 ± 1	117 ± 4
	As	mg kg^{-1}	426 ± 28.2		406 ± 12.2	<20
	Cd		35.2 ± 4.1		43.4 ± 3.7	141 ± 10.1
	Cu		119 ± 2.1	*	180 ± 3.1	2625 ± 239
	Mn		10,100 ± 54		9700 ± 119	5380 ± 140
	Ni		31.0 ± 2.5	*	64.2 ± 2.3	746 ± 22.8
	Pb		6200 ± 72.6	*	5800 ± 94.6	743 ± 31.8
	Sb		74.8 ± 5.1		64.2 ± 4.2	108 ± 66.4
	Zn		10,000 ± 60.9	*	9800 ± 74.0	7918 ± 354
Water extractable metal(loid)s (1:5)	**As**	μg kg^{-1}	<10	-	149 ± 9.4	199 ± 10.8
	Cd		<10	-	33.7 ± 1.2	3041 ± 61.7
	Cu		<10	-	816 ± 57	44,149 ± 4703
	Fe		248 ± 53.7	*	1567 ± 195	757,669 ± 26,237
	Mn		106 ± 9.0	*	11,321 ± 721	161,731 ± 9255
	Ni		<10	-	704.3 ± 65.8	35,539 ± 1416
	Pb		<10	-	102.9 ± 5.7	6604 ± 321
	Sb		<10	-	15.4 ± 0.6	627 ± 27.0
	Zn		428 ± 26.7	*	1870 ± 110	196,010 ± 5686

Table 10.3. Soil characterisation at the end of the experiment: T is the mine tailings soil, and T+MW is the combined treatment of mine tailings soil and municipal solid waste. The results are average ± standard error (*N*=20). "*" indicates significant differences (p<0.05) between T and T+MW treatments. The symbols "+" or "-" indicate that the parameter is significantly higher or lower (p<0.05) than in the initial characterisation. EC (electrical conductivity); DOC (dissolved organic carbon); DON (dissolved organic nitrogen).

Soil parameter		Units	Treatments			Differences with initial characterisation	
			T		T+MW	T	T+MW
pH (1:5)		-	7.4 ± 0.01	*	7.5 ± 0.01		+
EC (1:5)		dS m^{-1}	1.8 ± 0.00	*	1.9 ± 0.01	-	-
DOC		mg kg^{-1}	24.1 ± 0.42	*	77.8 ± 1.83	-	-
DON		mg kg^{-1}	5.1 ± 0.06	*	8.5 ± 0.37		-
Dehydrogenase activity		μg INTF g^{-1} h^{-1}	0.06 ± 0.01	*	0.53 ± 0.02		+
Water extractable ions (1:5)	**Cl^-**	mg L^{-1}	7.6 ± 0.39	*	16.5 ± 0.8	+	-
	HPO_4^{2-}		<0.7		<0.7		
	NO_3^-		1.0 ± 0.02	*	1.2 ± 0.03		
	SO_4^{2-}		810 ± 18.3	*	1286 ± 22	-	-
	Ca^{2+}		546 ± 7.46	*	600 ± 3	-	+
	K^+		2.4 ± 0.08	*	3.4 ± 0.3	+	-
	Mg^{2+}		2.4 ± 0.12	*	1.8 ± 0.1	-	-
	Na^+		15.8 ± 0.51	*	19.9 ± 0.8	+	-
	NH_4^+		0.5 ± 0.02	*	0.8 ± 0.02		
Water extractable metal(loid)s (1:5)	**As**	μg kg^{-1}	<10	*	10.3 ± 0.6		-
	Cd		<10		<10		-
	Cu		40.4 ± 0.53	*	49.1 ± 1.4	+	-
	Mn		52 ± 3.17	*	26340 ± 821	-	+
	Ni		<10	*	26.7 ± 1.5		-
	Pb		<10	*	105.9 ± 3.5		
	Sb		10.6 ± 0.29	*	27.9 ± 0.7	+	+
	Zn		518 ± 9.06	*	1101 ± 25	+	-

10.3.2. Changes in soil solution throughout the experiment

The repeated measures ANOVA showed a significant effect (p<0.05) for the samplings (time effect), the treatments and the interaction sampling x treatment for all the studied parameters (Figure 10.1). The pH was significantly lower (p<0.05) in T+MW throughout the experiment (Figure 10.1.a). In both treatments, the pH tended to decrease during the first five months, showing a slight increase from the sixth month onwards. The EC was significantly higher (p<0.05) in T+MW at the beginning of the experiment, but it tended to decrease in both treatments throughout the samplings (Figure 10.1.b). The slight increases of EC values in S4 and S5 were attributed to the higher evapotranspiration rates in summer months, which could have favoured the concentration of salts in the soil solution.

The DOC concentrations were significantly higher (p<0.05) in T+MW during all the experiment (T: ~2-4 mg L^{-1}; T+MW: ~700 mg L^{-1} at S0 to ~10 mg L^{-1} at S8) (Figure 10.1.c) in spite of showing a sharp decrease during the first months.

Both treatments showed As, Cd, Cu, Ni, Pb and Sb soil solution concentrations lower than 15 μg L^{-1} in all the samplings, except at S0 in T+MW for Cu (475 μg L^{-1}), Ni (425 μg L^{-1}) and Pb (55 μg L^{-1}) (data not shown). Throughout the experiment, the Mn concentrations of T+MW treatment were higher (p<0.05) (up to 46,000 μg L^{-1}; Figure 10.1.d) than those obtained for T treatment. Likewise, the concentrations of Zn were significantly higher (p<0.05) in T+MW at the beginning of the experiment (up to 1400 μg L^{-1}), but, after four months (S4), the concentrations dropped to similar concentrations (p>0.05) than those measured in T treatment (~200 μg L^{-1}; Figure 10.1.e).

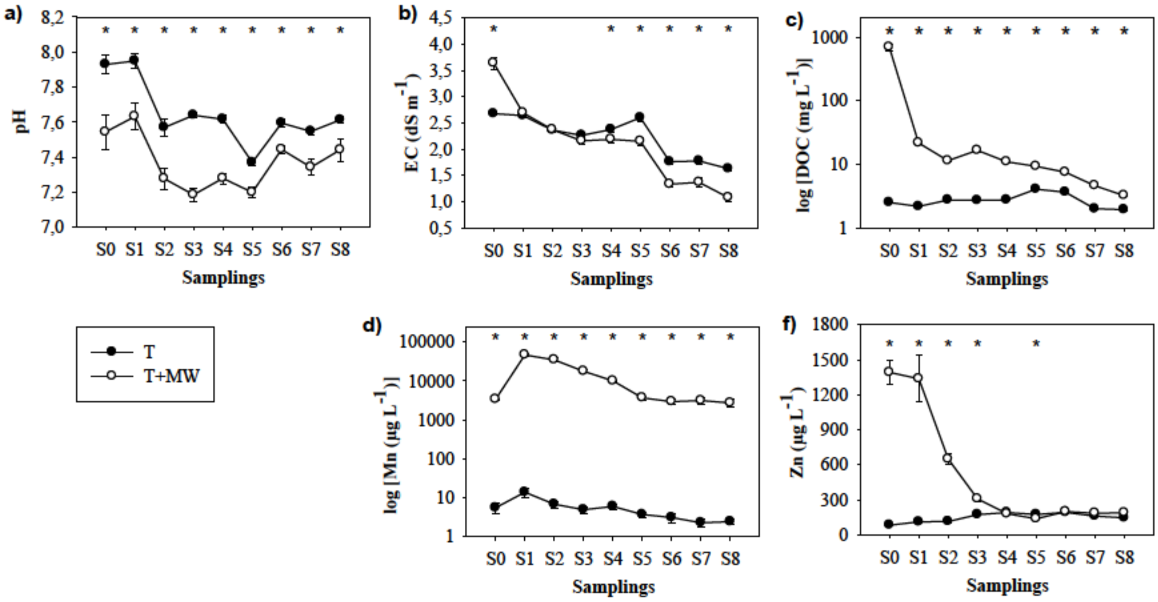

Figure 10.1. pH, EC, DOC, and Mn and Zn concentration in soil solution throughout the experiment (average ± standard error; *N*=20). "*" on the upper part indicates significant differences ($p<0.05$) between T and T+MW treatments in the corresponding sampling. EC (electrical conductivity), DOC (dissolved organic carbon).

10.3.3. Changes in leachates throughout the experiment

Similar to the soil solution parameters, the repeated measures ANOVA showed a significant effect ($p<0.05$) for the samplings (time effect), the treatments and the interaction sampling x treatment for the parameters measured in the leachates (Figure 10.2). The pH of T+MW increased throughout the experiment (from 6.9 to 7.8), reaching similar values than T treatment (pH ~7.8; Figure 10.2.a). The EC of T+MW dropped in the first month of the experiment, from 12 to 2.6 dS m^{-1} (Figure 10.2.b).

The DOC concentrations in T treatment slightly decrease at the beginning (from 7.5 to 3.5 mg L^{-1}) and showed significantly ($p<0.05$) lower values than T+MW throughout all the experiment (Figure 10.2.c). In the T+MW treatment, the concentrations of DOC dropped from 5000 mg L^{-1} (beginning of the experiment) to around 10 mg L^{-1} after S4.

With the exception of the initial sampling (S0) in T+MW, in which 90 μg L^{-1} As, 125 μg L^{-1} Cd, 2370 μg L^{-1} Cu, 2100 μg L^{-1} Ni, 500 μg L^{-1} Pb and 40 μg L^{-1} Sb were found, these elements were below 10 μg L^{-1} in all the treatments from S2 onwards (data not shown). However, the concentrations of Mn were significantly higher (p<0.05) in T+MW throughout the experiment (Figure 10.2.d). The Zn concentrations were in the range of 30–150 μg L^{-1} in both treatments after S2 (Figure 10.2.e).

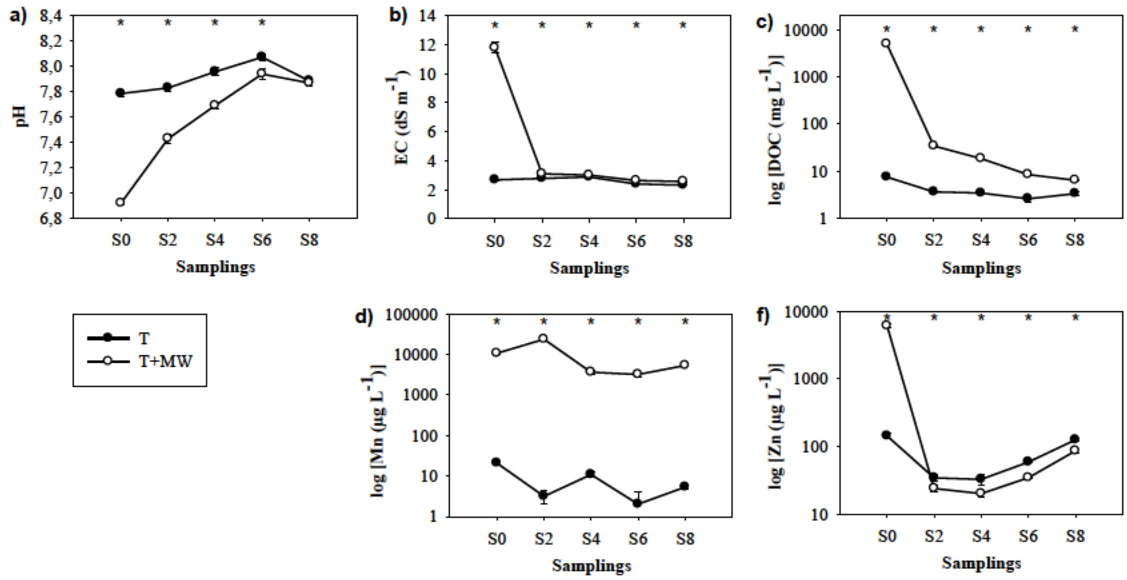

Figure 10.2. pH, EC, DOC, and Mn and Zn concentration in leachates throughout the experiment (average ± standard error; N=20). "*" on the upper part indicates significant differences (p<0.05) between T and T+MW treatments in the corresponding sampling. EC (electrical conductivity), DOC (dissolved organic carbon).

10.3.4. Metal(loid) fractionation in soils

There were no differences in metal(loid) speciation when comparing T samples at the beginning and at the end of the experiment (Figure 10.3). The sum of the three most recalcitrant fractions (F5+F6+F7) was 90-100% for As, Cu and Sb, 77% for Zn, 50% for Cd, Mn and Pb and 30% for Ni. Therefore, it can be stated that Cu, As, Sb and Zn were mainly bound to the soil matrix, indicating a lower risk of mobilisation, while Cd, Pb, Mn and Ni were bound to more mobile fractions (F1+F2+F3+F4).

The initial effect of the addition of MW to the T soil only caused significant changes ($p<0.05$) in Cu and Ni speciation (T initial vs. T+MW initial; Figure 10.3). For Cu, there was an increase in highly mobile fractions such as F1 and F2 (Figure 10.3.c). In addition, the residual fraction increased from ~40 to ~60%. In the case of Ni, the fraction bound to organic matter (F4) occurred in the T+MW treatment (Figure 10.3.e). Other metals such as Cd and Mn showed a slight increase of the more mobile fractions (F1 and/or F2; Figure 10.3.b and 10.3.d). Lead and Zn distribution did not show significant changes in metal fractionation.

Contrary to the T samples, some differences occurred when comparing T+MW treatment at the beginning and at the end of the experiment (T+MW initial vs T+MW final). For instance, T+MW final samples showed lower percentages of recalcitrant fractions (F5+F6+F7) for Cu and an increase in the fraction bound to organic matter (F4) (Figure 10.3.b). In relation to Cu, at the end of the experiment F1 and F2 fractions were not detected (Figure 10.3.c). Finally, similar percentages were obtained when evaluating F4 to F7 for Mn, Ni and Zn in the T+MW initial and T+MW final. However, a re-distribution among F1, F2 and F3 fractions occurred for the aforementioned metals, with gains in the more mobile fractions (F1+F2).

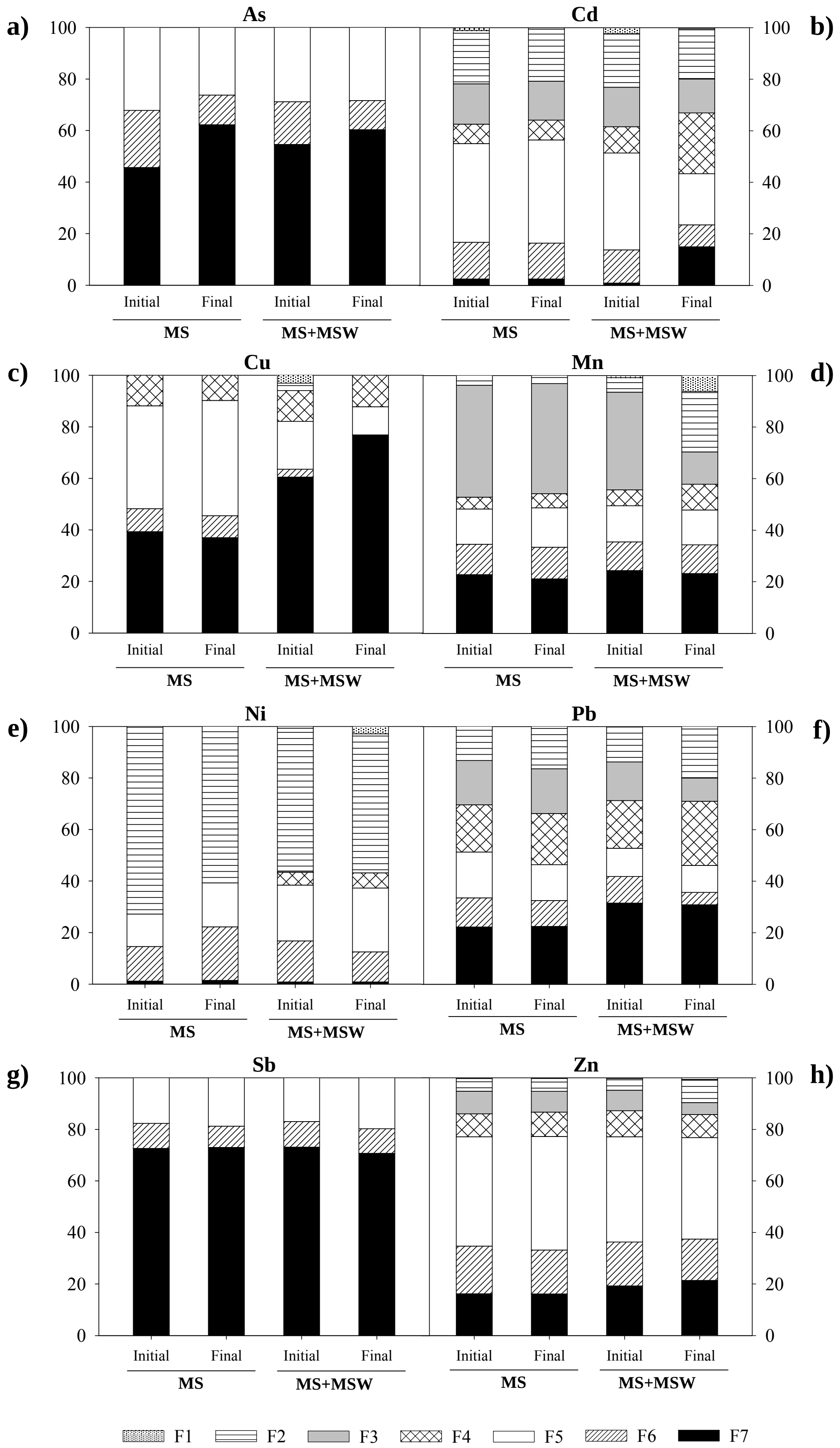

Figure 10.3. Percent distribution of As, Cd, Cu, Mn, Ni, Pb, Sb and Zn fractions in the sequentially extracted samples of T and T+MW from the initial and final characterisation (N=3).

10.3.5. Plant biomass

During the experiment, one pot of the T/2 and one of the T+MW/2 treatments were discarded because some plants died.

The total dry biomass of T+MW plants was ~40 fold higher (~8 g, $p<0.05$ Table 10.4) than T plants (≤0.2 g). The improvement of the fertility conditions in T+MW pots also allowed plants to complete their life cycle (flowering), while plants in T pots stayed in vegetative growth (authors visual observation).

The total dry biomass in those pots with two plants (T/2 and T+MW/2) was similar to the corresponding treatments in the single plant pots (T/1 and T+MW/1). Consequently, the dry biomass of each individual plant from the two plants pots was lower (significant for T+MW; $p<0.05$) than the biomass of each plant from the single plant pots.

In most of the two plants pots either with MW or not, one of the individual plants showed higher biomass (1.1-2.2 fold) than the other (data not shown). The differences were due either to higher root biomass, higher aboveground biomass or both.

Table 10.4. Dry biomass per pot and plant for the different treatments tested (average ± standard error; N=8 for T/1 and T+MW/1; N=7 pots for T/2 and T+MW/2; and N=14 plants for T/2 and T+MW/2). Different letters in the same column indicate significant differences ($p<0.05$) among treatments.

Dry biomass (g)	N	Root	Stalk	Leaves	Total	Root: Stalk+Leaves
Total biomass of pots						
T/1	8	0.08 ± 0.01 b	0.02 ± 0.01 b	0.09 ± 0.01 b	0.19 ± 0.02 b	0.78 ± 0.09 a
T/2	7	0.11 ± 0.01 b	0.01 ± 0.01 b	0.12 ± 0.01 b	0.24 ± 0.02 b	0.89 ± 0.07 a
T+MW/1	8	3.70 ± 0.32 a	1.53 ± 0.46 a	2.92 ± 0.27 a	8.14 ± 0.89 a	0.91 ± 0.12 a
T+MW/2	7	4.06 ± 0.55 a	1.61 ± 0.19 a	3.56 ± 0.30 a	9.23 ± 0.82 a	0.88 ± 0.06 a
Biomass of individual plants						
T/1	8	0.08 ± 0.01 c	0.02 ± 0.02 c	0.09 ± 0.01 c	0.19 ± 0.02 c	0.78 ± 0.10 a
T/2	14	0.05 ± 0.00 c	0.01 ± 0.01 c	0.06 ± 0.01 c	0.12 ± 0.01 c	0.87 ± 0.06 a
T+MW/1	8	3.70 ± 0.32 a	0.46 ± 0.46 a	2.92 ± 0.27 a	8.14 ± 0.89 a	0.91 ± 0.11 a
T+MW/2	14	2.03 ± 0.23 b	0.10 ± 0.10 b	1.78 ± 0.13 b	4.62 ± 0.35 b	0.87 ± 0.09 a

10.3.6. Elemental and stable isotope leaf composition

The main differences in elemental and stable isotope composition were found between T and T+MW treatments (Table 10.5). Carbon concentration was similar (~400 g kg^{-1}) for all treatments, while N concentration was significantly lower (p<0.05) in the leaves of T+MW treatments (~7.5 g kg^{-1}), probably because these plants completed their life cycle and they were senescent at the end of the experiment (authors visual observation).

Compared to the T treatments, the plants from T+MW ones, both single and two plants pots, showed better nutritional foliar status when considering macronutrients such as P and K (Table 10.5). Chloride concentrations were significantly higher (p<0.05) as well, while those ones obtained for S, Mg and Na were significantly lower (p<0.05).

Table 10.5. Elemental and stable isotope leaf composition for the different treatments tested (average ± standard error; N=8 plants for T/1 and T+MW/1; N=14 plants for T/2 and T+MW/2). Different letters in the same row indicates significant differences (p<0.05) among treatments

Element	Units	Treatments							
		T/1		T/2		T+MW/1		T+MW/2	
C	g kg^{-1}	410 ± 2.4	a	410 ± 0.0	a	400 ± 13	a	390 ± 30	a
N		10.6 ± 0.8	a	10.9 ± 0.8	a	7.5 ± 0.3	b	7.2 ± 0.5	b
P	mg kg^{-1}	<400		<400		690 ± 70	a	650 ± 90	a
K		6280 ± 840	ab	5060 ± 660	b	8430 ± 450	a	7950 ± 510	a
S		9900 ± 310	b	11,020 ± 460	a	4690 ± 390	c	4720 ± 340	c
Ca		4050 ± 420	a	3490 ± 260	a	3410 ± 180	a	3630 ± 190	a
Mg		1100 ± 90	a	1040 ± 40	a	820 ± 30	b	760 ± 20	b
Na		860 ± 160	b	1470 ± 150	a	350 ± 40	c	390 ± 40	c
Cl		<250		<250		1100 ± 110	a	980 ± 130	a
$\delta^{13}C$	‰	-31.40 ± 0.28	a	-30.80 ± 0.33	a	-31.25 ± 0.09	a	-31.15 ± 0.08	a
$\delta^{18}O$		34.16 ± 0.76	ab	34.79 ± 0.35	a	33.23 ± 0.17	b	33.22 ± 0.21	b
$\delta^{15}N$		-11.02 ± 0.50	b	-10.36 ± 0.36	b	0.33 ± 0.37	a	-0.11 ± 0.34	a

Carbon isotopic composition ($\delta^{13}C$) did not show any significant difference among treatments (Table 10.5). However, $\delta^{18}O$ was higher in T treatments (especially for MS/2; p<0.05). The higher $\delta^{15}N$ values (p<0.05) for leaves of T+MW treatments was related to their better nutritional status in relation to the plants of T treatments.

In relation to metal(loid) uptake, the plants of the T+WM treatments showed significant lower concentrations (p<0.05) of Mn (leaves), Cu (leaves and stalks), and Pb and Zn (leaves, stalks and roots) (Figure 10.4). Arsenic, Cd and Sb were only detected in roots (~40 mg kg^{-1} As, ~5 mg kg^{-1} Cd and ~1.4 mg kg^{-1} Sb for T treatments; <9 mg kg^{-1} As, ~2 mg kg^{-1} Cd and ~1 mg kg^{-1} Sb in T+MW treatments). Nickel was only detected in roots and leaves of T+MW (4-6 mg kg^{-1}).

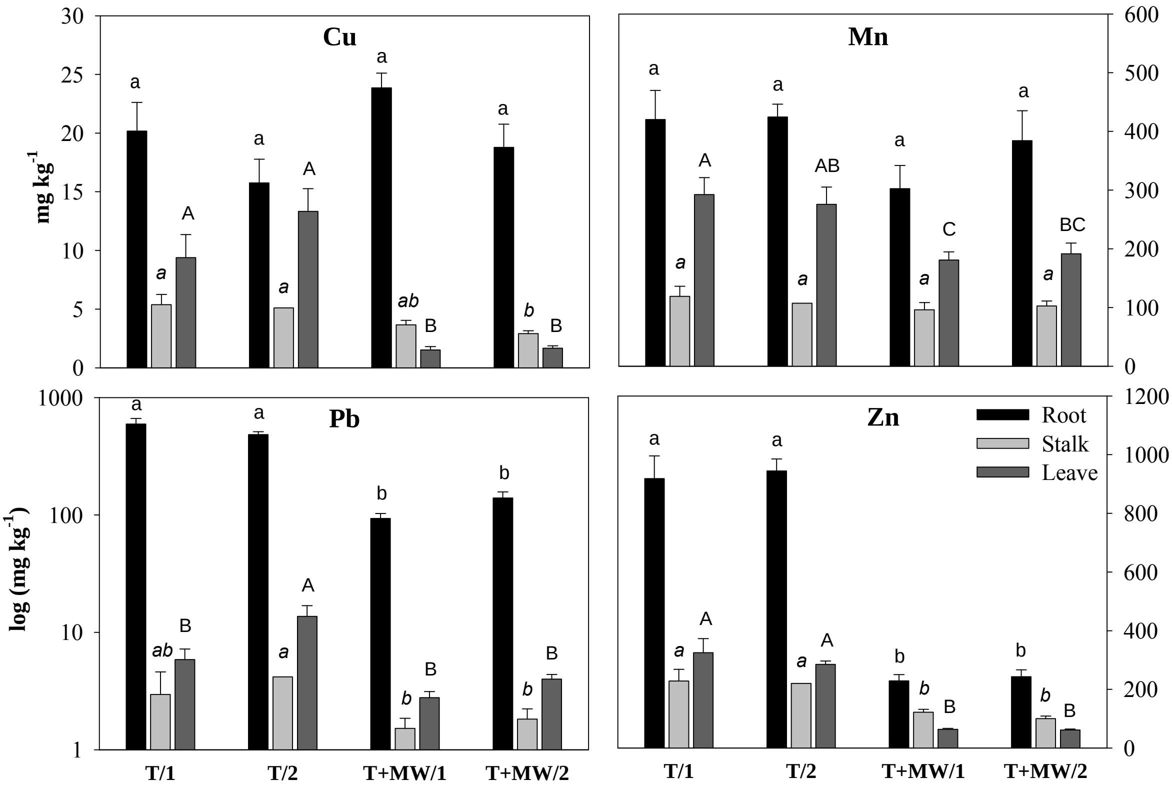

Figure 10.4. Concentration of Cu, Mn, Pb and Zn in *Piptatherum miliaceum* leaves, stalk and roots. Columns represent the average values and bars over columns indicate the standard errors (N=8 for T/1 and T+MW/1, N=14 for T/2 and T+MW/2). Different letters indicate significant differences (p<0.05) among treatments for the same plant organ (lower case for roots, italics for stalks and upper case for leaves).

10.4. Discussion

10.4.1. Suitability of the addition of municipal solid wastes on mine soils properties

Several authors have evaluated the use of organic amendments (biosolids, composts, manures, agro-industrial residues, etc.) to alleviate the deficient fertility conditions for plant growth in mine tailings (e.g. Farrel et al., 2010; Soler-Rovira et al., 2010; Clemente et al., 2012; Kohler et al., 2014). Due to their heterogeneous nature, municipal solid wastes may show high variability in composition and properties. Thus, prior to field-scale applications, specific assessment tests in controlled conditions must be carried out (Kohler et al., 2014). In our study, the addition of a municipal solid waste to a mine soil caused an increase in salinity and some of the metal(loid)s concentrations. While the rise in soil salinity was diluted in time after irrigation, the increases in some metal(loid) total concentrations and in their mobility lasted during all the experiment. The latter, then, needs to be assessed due to its environmental health concern.

Although the total metal(loid) concentrations of the selected municipal solid waste were above the thresholds stated for its use in agricultural soils in Spain (BOE, 2005), it may be acceptable its employment for the reclamation of metal(loid) enriched tailings, due to the low impact in the total metal(loid) concentrations and the benefits on soil fertility and plant growth. Some studies have pointed out that some metal enriched amendments, such as biosolids or urban sludges, could be efficiently used to restore mining affected sites, by supporting a long-term stable plant cover and low metal(loid) transfer into the food chain (Sopper, 1993; Brown et al., 2003). In our experiment, the addition of the municipal solid waste to a mine tailings soil promoted better soil fertility conditions and higher plant biomass with no increases in plant metal(loid) uptake (except Ni). However, it is necessary to state that the high the metal(loid) concentrations in the soil solution and leachates could condition the environmental suitability of this amendment. Although there was a dramatic decrease of metal(oid)s concentrations in the leachates of amended treatments after three months of the experiment (except Mn), the sequential extraction procedure revealed an increase of the easily mobile fractions of Mn, Ni, Pb and Zn at the end of the experiment. Therefore, the environmental risks posed by this municipal solid waste could be stated in a short-term lixiviation of the mobile metal(loid)s pool concentrations and, in the medium-term, a redistribution of some metals spetiation into more easily mobile fractions. The latter was especially critical for Mn since the water-extractable fractions increased almost 2-fold while maintaining high concentrations in soil solution and leachates throughout the experiment. It might be hypothesized that the degradation of fresh organic matter and the high water content in the pots could have promoted a limited oxygen supply into the soil and thus a decrease in redox potential (Bernal et al., 2007). This could have led to the reduction of Mn and its transformation into mobile forms, which is supported by the decrease in the sequential extraction procedure of the fraction bound to Mn oxides in most of metals (Cd, Ni, Pb and Zn). González-Alcaraz et al. (2013) found a similar effect in an acidic mine soil amended with marble wastes.

The increase in dehydrogenase activity in the T+MW treatment at the end of the experiment could be attributed to the stimulation of microbiological activity due to the combined effect of the labile organic carbon and microbial biomass contained in the municipal soil waste and the water availability (Kohler et al., 2014). Several studies have reported increased microbial biodiversity, biomass and activity when different organic materials were added to metal contaminated soils (Clemente et al., 2012; Pardo et al., 2011; Farrell et al., 2010).

Organic amendments may have contrasting effects on metal(loid) mobility depending on its degree of humification. For instance, highly humified organic matter might decrease metals availability, by forming stable insoluble complexes while low-molecular-weight organic compounds might favour the occurrence of mobile organometallic complexes (Bernal et al., 2007). In addition, Madejón et al. (2010) found that time-related decomposition of organic matter led to an increase of metal mobility in contaminated field plots treated with organic amendments, and that repeated applications were needed to stabilise metal(loid)s in soil. In our experiment, the municipal solid waste contained high concentrations of DOC, that could have favoured the high extractability of metal(loid)s at the beginning. To avoid this issue, the composting of fresh organic materials are useful to increase the complexation and immobilisation of metal(oid)s, so metals are strongly bound to the compost matrix and organic matter, limiting their solubility and potential bioavailability in soil (Smith, 2009). Nevertheless, it may be hypothesized that the composting of the municipal soil waste employed in our study is not likely to decrease its metal(loid) availability under acceptable ranges due to the high concentrations which contained. There are additional techniques (but rather expensive) to remove metal(loid)s, such as calcinations followed by ashes washing (Fedje et al., 2010), but this treatment might compromise the cost-effective requirement of phytostabilisation. Therefore, the use of low metal(loid) composted organic amendments, such as agro-industrial residues, green wastes composts or biochar, besides a thorough monitoring programme for metal(loid) mobilisation, would suit better the goals of phytostabilisation. It may be concluded that the municipal solid waste used in this experiment would not be a priori suitable for ecological restoration in spite of the benefits on soil fertility and plant growth.

10.4.2. Effects of municipal solid waste addition and plant competition on plant growth, nutritional and physiological status and metal(loid) accumulation in plant tissues.

Piptatherum miliaceum has been shown to be a slow growing plant species under not aided growth conditions at mine tailings soils (Conesa et al., 2007b; Conesa et al., 2009; Kohler et al., 2014). However, at neutral pH mine tailings, Conesa et al. (2009) found a significant increase in the biomass production of this plant species after adding an enriched N-P-K solution while Kohler et al. (2014) obtained similar results employing urban organic waste compost.

The dry biomass of *P. miliaceum* was negatively affected by the presence of two individuals per pot (whether with or without municipal soil waste), which may indicate the occurrence of intra-specific competition. When competition takes place, plants may compete for above- or belowground resources (Jefferson, 2004). Competition for limited aboveground resources (e.g. light), involves fast and significant increases in aboveground biomass, while competition for limited belowground resources, such as nutrients and water, involves fast root growth rates and greater root biomass. Thus, in plant species capable to adjust their biomass allocation patterns, the root to shoot (or above-ground organs) biomass ratio may provide a clear indication of the type of competition (Dietz et al., 1998). In our study, there was not a clear pattern of allocation of biomass in below- or aboveground organs. Although the root to shoot (stalk plus leaves) ratio did not show any significant differences among treatments (Table 10.4), a slight increase in this ratio in the non-amended treatment was found (T/1 vs T/2), which could indicate belowground competition for soil resources. In the amended treatments, other factors such as the available soil volume per pot, could constraint plant growth, affecting equally to above- and below- ground biomass and thus, reflecting no differences in root to shoot (stalk plus leaves) ratio.

Isotope leaf composition is a useful tool to assess some physiological traits such as drought tolerance, water use efficiency or use of N sources. The $\delta^{15}N$ isotopic signature has been tested to assess the contribution from different sources of soil N, but its interpretation may be conditioned by several factors such as the large variability of soil $\delta^{15}N$ sources (NH_4^+, NO_3^- or dissolved organic nitrogen, with different preference according to plant species, age, soil contents, etc.), N fractionation during uptake or metabolic processes or interactions with mycorrhizal fungi, among others (Högberg, 1997). The differences found in our study might be mainly due to the different sources of N in each treatment and the higher uptake of N (and consequently, higher uptake of $\delta^{15}N$) in the case of the plants from the T+MW treatment. Carbon isotopic composition, $\delta^{13}C$, constitutes an integrated record of the climatic and physiological long-term conditions that affect carbon assimilation and/or stomatal conductance (Ferrio at al., 2003) and therefore, it can provide an estimation of water use efficiency, WUE (Farquhar et al., 1989). In addition, simultaneous measurements of $\delta^{13}C$ and $\delta^{18}O$ in plant material may help to discriminate the effects of carbon fixation and stomatal conductance in $\delta^{13}C$, since $\delta^{18}O$ depends on stomatal conductance but not on rubisco activity (Scheidegger et al., 2000; Keitel, 2003). The $\delta^{18}O$ signature of plant organic matter decreases in response to increased stomatal conductance (Barbour et al., 2002). In our experiment, no differences were found in $\delta^{13}C$, i.e. the relation between carbon assimilation and stomatal conductance was similar in all the treatments, but the lower $\delta^{18}O$ in the amended treatments indicated that stomatal conductance was higher in these plants, so consequently carbon assimilation should be also higher in the latter. The continuous water supply during all the experiment prevented them from suffering water stress, so the WUE was not affected by stomatal regulation. However, in the amended treatments, the availability of nutrients may have promoted plant growth, keeping the transpirative flux and improving carbon assimilation and photosynthesis.

Phytomanagement by phytostabilisation aims to achieve the establishment of a long-term plant cover to decrease erosion and stabilise the tailings, maintaining low metal(loid) accumulation in above-ground plant tissues. For this reason, besides plant growth and nutritional and physiological status, the accumulation of metal(loid)s in plants should be also critically evaluated. In this study, the accumulation of metal(loid)s in plants growing in T treatments were in the range of those found by Conesa et al. (2007) in a pot experiment with a mine soil, and also with those found by Párraga-Aguado et al. (2014) in a field survey in a mining area of Southeast Spain. The lower concentrations of Cu, Mn, Pb and Zn in leaves from the T+MW treatments were in accordance with the results obtained by Kohler et al. (2014). These results seem to be related to the "dilution effect" which comes with higher biomass production if metal uptake is not proportional to the biomass increase (Robinson et al., 1998), and not to a decrease in soil metal(loid) bioavailability.

Toxic limits for fodder were only surpassed for Pb (40 mg kg^{-1}; BOE, 2001) in roots of both treatments, and for Zn (500 mg kg^{-1}; NRC, 2005) in roots of T treatments. Thus, the low metal(loid) concentrations accumulated in the aerial parts of *P. miliaceum* makes this species suitable for phytostabilisation of neutral mine tailings, showing a better response if organic amendments are incorporated. The intra-specific competition for soil resources did not affect metal(loid) uptake which remained low also in two plants pots.

10.5. Conclusions

The municipal solid waste added to a mine tailings soil showed positive effects in improving soil fertility indicators (OC, DOC, dehydrogenase activity) and plant growth. However, its extended use as an amendment for the reclamation of mine tailings should be discarded in the current form due to some undesirable environmental effects like the increase of the metal(loid) availability, either at short and middle term. The role of plants showed to be irrelevant for affecting the availability/fractionation of metal(loid)s in soil.

Piptatherum miliaceum could be a suitable species for phytostabilisation purposes since metal(loid) accumulation in leaves was below toxic limit for fodder in all soil treatments. However, in order to obtain an acceptable plant growth and development of this plant species on mine tailings, the addition of organic amendments should be considered. Apart from soil fertility, plant growth was also influenced by intra-specific competition which should be taken into account in the management of phytostabilisation projects.

10.6. Acknowledgements

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CHAPTER 11. Final Conclusions

11.1. Final conclusions

Based on the results of the previous chapters and in relation with the proposed objectives, the general conclusions of the PhD Thesis are the following:

1) The identification of the most favourable/suitable niches for plant growth should be taken into account when phytostabilisation works are carried out on mine tailings. Ploughing or the addition of certain amendments that could raise soil salinity should be carefully evaluated in order to not destroy those favourable niches.
2) The use of combinations of plant species with different life forms and complementary water and nutrient acquisition strategies may result in a more efficient employment of edaphic resources. This may favour the achievement of a long-term stable plant community and may provide a higher resilience to environmental disturbances such as long drought periods typical from semiarid environments.
3) The employment of halophyte species (*Limonium cossonianum*, *Atriplex halimus*, *Zygophyllum fabago*) may result a suitable option to revegetate and ameliorate the most saline edaphic niches at the tailings due to their suitable adaptation to salinity and higher enhancement of soil properties.
4) The tree species *Pinus halepensis* may result a suitable candidate for the phytostabilisation of mine tailings due to its adaptation to semiarid climate conditions and the potential role for facilitating the occurrence of fertility islands. However, it is recommendable the addition of phosphorus fertilizers in order to alleviate the strong deficiency on this element at the tailings.
5) The grass species *Piptatherum miliaceum* may be considered in phytostabilisation of mine tailings, but to obtain a faster growth and development, organic amendments should be added.

Finally, future research should be oriented to the next issues:

1) Study of the long-term dynamic of metal(loid) availability on rhizosphere soil and litter from “fertility islands”.
2) Study of the influence of inter-seasonal variations in ecological indicators of spontaneous plant communities at mine tailings.
3) Assessment of the suitability of other tree species, such as *Tetraclinis articulata*, for the phytomanagement of mine tailings.
4) How to promote the occurrence of the “fertility islands” on mine tailings.
5) Study of the inter-specific interactions among spontaneous plant species.