Chapter 6
Reclamation of Contaminated Mine Ponds Using Marble Wastes, Organic Amendments, and Phytoremediation

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6.1 Introduction

Mining activities in the Region of Murcia – SE Spain, which started 2,500 years ago and ended in the 1990s, generated several tailing ponds, which store residues from the extraction of lead and zinc (Pb/Zn). These tailing ponds contain materials of high Fe-oxyhydroxides, sulphates, and elevated contents of potentially leachable heavy metals (mainly Cd, Pb, Cu, and Zn) due to extreme acidic conditions. Since a long time those mine residues have been transported downstream during periods of high flow, erosion is evident in these areas, causing migration of pollutants into surface and ground water. These metal-contaminated soils also contribute to human

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and animal metal exposure, through food chain transfer or inhalation of wind-blown dust (Pierzinski 1997). In terms of stabilizing metal contaminated mine sites, a lower metal concentration in vegetation shoot is preferred, in order to prevent metal from entering the ecosystem through food chain (Pichtel et al. 2000). Thus, efforts are needed for long-term reclamation of these contaminated areas to stabilize soil metals, create a structured soil, promote cover vegetation, and avoid health risks in the ecosystems.

In response to a growing concern for human health and environmental quality, many technologies have been developed to treat and remediate metal-contaminated soils. One of the remediation options gaining considerable interest over the last decade is the in situ immobilization of metals using metals immobilizing agents (Vangronsveld and Cunnungham 1998). Thus, the transformation of metals into harmless species or their removal in a suitable recycled mineral form such as carbonates using marble wastes or lime (Geebelen et al. 2003) is a possible solution for the remediation of a mining area. In addition, incorporation of organic amendments into contaminated mine soils has been proposed as feasible, inexpensive, and environmentally sound disposal practice, as generally such wastes can improve soil physical and chemical properties, and contain nutrients beneficial to initialize plant colonization (Barker 1997), favoring the reactivation of biogeochemical cycles and the natural establishment of vegetation. The increment in vegetation cover reduces or even prevents the dispersion of the contamination through wind and water erosion, and improves the aesthetic value of formerly bare areas (Vangronsveld and Cunnungham 1998). Besides, vegetation itself may contribute to metal immobilization processes through biological activities in the production of organic matter (Bouwman and Vangronsveld 2004), an emerging technology called phytostabilization.

Although there is a general consensus that efficiency of soil remediation also depends on the presence and activity of microorganisms, the long-term ecological consequences of inorganic and organic amendments for these features have received little attention (Mench et al. 2006). Biochemical properties may indicate the potential of a soil to sustain microbiological activity, which can be used to assess the effectiveness of a soil remediation process (Pérez de Mora et al. 2005). In this sense, soil enzymes have been reported to be highly sensitive to heavy metals, and, therefore, have been recommended as standard biochemical indicators to assess quality of heavy metal-polluted soils (Hinojosa et al. 2004).

The main objective of this study is to evaluate of the long-term effectiveness of different inorganic and organic amendments for remediation of contaminated mine soils by means of (1) monitoring the evolution of some physicochemical properties and availability of heavy metals, (2) determination of soil quality using biochemical properties as indicators, and (3) assessing the establishment of spontaneous vegetation and bioaccumulation of heavy metals in plants in order to avoid the risk of mobility in the food chain.

6.2 Material and Methods

6.2.1 Study Site

The study was conducted in the Region of Murcia (SE Spain), in the Cartagena-La Unión Mining District, which covers an area of ~50 km^2 with an elevation range from 0 to 110 m asl (Fig. 6.1). Great mining activity has been carried out for more than 2,500 years, the activity being stopped in the nineties. The climate of the area is semiarid Mediterranean, with annual average temperature of 18°C and mean annual rainfall of 200–300 mm. Two tailing ponds generated by mining activities were selected: El Lirio (L) and Brunita (B), representative of the 85 rest of existent tailing ponds in this Mining District. This mining area was an important center for the extraction of mineral ores such as sphalerite [(Zn,Fe)S], galena (PbS), and pyrite ($Fe^{2+}S_2$).

6.2.2 Field Experimental Set-Up

Twenty field plots (4 m^2) were established in 2004 in a completely randomized design to evaluate influence of the combined additions of industrial and organic wastes in soil development in tailing ponds. Two different organic amendments were used to reclaim the soils, pig manure (P), and sewage sludge (S). In addition, three different doses per amendment were applied. Thus, the treatments were: Untreated contaminated soil (C), soil treated with pig manure at dose 1 (P1), dose

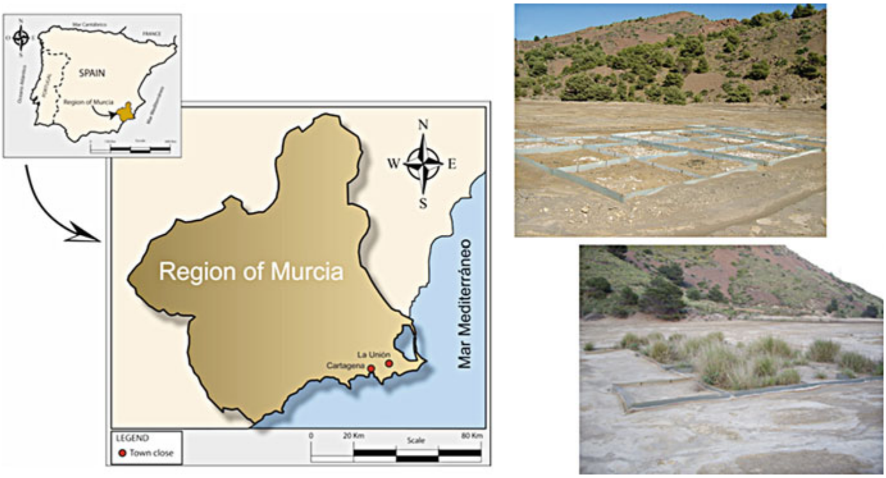

Fig. 6.1 Study site and images of El Lirio tailing pond during the application of amendments (*above*) and 5 years after application (*below*)

2 (P2), and dose 3 (P3); and soil treated with sewage sludge at dose 1 (S1), dose 2 (S2), and dose 3 (S3). For pig manure, doses were 2.5, 5, and 10 kg per plot, respectively. For sewage sludge, doses were 1.99, 3.98, and 7.97 kg per plot, respectively. Marble waste was added at 22 kg per plot in all plots except for the control. Calcium carbonate from marble waste was applied to correct the acidity in the mine soils. The particle size distribution in marble waste was 26% <2 mm and the rest from 2 to 5 mm in size. Marble waste came from marble industry located in Cehegín (NE of Murcia Region). The anaerobically digested sewage sludge was collected from the drying process of Cartagena wastewater treatment plant, and pig manure came from a pig farm in the Cuevas de Reillo (SE of Murcia).

Dose of organic amendments (dry-weight) were calculated on the bases of European and Spanish legislation regarding the addition of N to soil (Directive 91/676/EEC 1991; Real Decreto 261/1996). Organic amendments were applied with the purpose of increasing soil pH to immobilize metals and create better conditions for microbial and plant development. Dose of marble waste was determined based on Eq $CaCO_3$ required to neutralize the acidity and reach soil pH ~7, according to the Sobek method (Sobek et al. 1978).

Amendments were manually applied. First, we added marble wastes and let it dry for 24 h. Then, we mixed the materials with the soil to a depth of 0–15 cm. Following this, we applied the organic amendments using the same procedure. After the addition of soil amendments, plots were exposed to the semiarid climatic conditions in the study area for long-term observations.

6.2.3 Soil and Vegetation Sampling and Analytical Methods

The soil samplings were carried out previously to the application of amendments (time 0), and at 6 months, 1, 2, and 5 years after application of amendments. Composite soil samples from five sub-samples were taken from the 0–15 cm layer for each treatment plot. Samples were air-dried for 7 days, passed through a 2-mm sieve and stored at room temperature prior to laboratory analyses.

Although the vegetation in the tailing pond of study was absent, the application of amendments in the plots conducted to spontaneous colonization of vegetation by the surrounding environment. Thus, at the same time of the last soil sampling (5 years after application), the identification of all plants species present in the plots was carried out (richness), as well as the percentage of vegetation cover. In addition, shoots of the most dominant species in P3 (the treatment with highest vegetation cover and richness) were collected in each of the three replicated plots, making a composite sample, for analyses of metals concentrations. These species were *Piptatherum miliaceum*, *Zygophylum fabago*, *Dactilis glomerata*, and *Brassica fruticulosa*.

Soil pH and electrical conductivity (EC) were measured in distilled water (1:1 and 1:5 w/v, respectively) (Peech 1965). Soil organic carbon (SOC) was determined by chemical oxidation using dichromate solution (Walkley and Black 1934), while total nitrogen (N_t) was determined according to Duchaufour (1970). Equivalent calcium carbonate (inorganic carbon) was estimated using a Bernard calcimeter. Total metals content were determined by acid digestion with conc. $HNO_3/HClO_4$ at 210°C for 1.5 h and addition in HCl 0.1 N (Risser and Baker 1990). Soluble metals were extracted by water (soil:distilled water ratio = 1:2) (Buurman et al. 1996), and available metals extracted using DTPA (for soils with $pH > 6$) or EDTA (for soils with $pH < 6$) (soil:DTPA = 1:2; soil:EDTA = 1:5) (Lindsay and Norvell 1978). For plant metals concentration, 1 g of dried plant sample was ashed in a muffle furnace at 450°C for 24 h. After that, the digested material was dissolved in HNO_3 and filtered. Measurements of metals (Cd, Cu, Pb, Zn) were carried out using flame atomic absorption spectrophotometer (AAnalyst 800, Perkin Elmer).

Microbial biomass carbon (MBC) was determined using the fumigation–extraction procedure (Vance et al. 1987); basal soil respiration (BSR) was determined according to Anderson (1982); β-glucosidase activity was measured following the method of Tabatabai (1982); arylesterase activity was established according to Zornoza et al. (2009); acid phosphatase activity was determined according to Tabatabai and Bremner (1969); phosphodiesterase was measured following the method of Browman and Tabatabai (1978); arylsulphatase activity was measured by the method of Tabatabai and Bremner (1970). In addition, the metabolic quotient qCO_2 (BSR/MBC) was calculated.

In order to assess the efficiency of plants for phytostabilization, the bioaccumulation factor (BF) was also calculated as $[\text{metal}]_{\text{shoot}}/[\text{bioavailable metal}]_{\text{soil}}$ (Kumar et al. 1995). Ideally this value would be $<<1$, but it should not exceed a ratio of 1, which would indicate that the plant is useful for phytoextraction (accumulation of metals in shoot tissue) but should not be used in phytostabilization (Brooks 1998).

6.2.4 *Statistical Analyses*

The fitting of the data to a normal distribution for all properties measured was checked with the Kolmogorov–Smirnov test. The data were submitted to one-way ANOVA to assess the differences among treatment and doses. The separation of means was made according to Tukey's verified significant difference at $P < 0.05$. Relationships among properties were studied using Pearson correlations. Soil chemical and biochemical properties were subjected to principal components analysis (PCA) to elucidate major variation patterns in terms of amendments and doses. Statistical analyses were performed with the software SPSS for Windows, Version 17.0.

6.3 Results

6.3.1 General Physicochemical Properties of Tailing Ponds and Amendments

Mine soils samples from El Lirio and Brunita can be classified as Anthropic Spolic Regosol according to WRB (2007), and Haplic Torriarent according to USDA (2010). Both tailing ponds El Lirio and Brunita present a similar particle size distribution corresponding to sandy loam textural class (sand: 83%, silt: 4%, clay: 13%).

Selected chemical properties of the studied tailing ponds at time 0 showed that lowest pH (2.6) and moderate salinity was measured in Brunita, while El Lirio had the highest pH (6.7) and high salinity (Table 6.1). Both SOC and N_t contents were absent, what indicate inhospitable conditions for vegetation growth. Tailing ponds had higher total contents of Pb and Zn compared to Cu and Cd, being higher in El Lirio. Both El Lirio and Brunita can be considered very contaminated, exceeding limit values of the European legislation for soil contamination (Zn: 300, Pb: 300, Cu: 140, Cd: 30, in mg/kg). These levels indicated high toxicity in mine soils which could adversely affect the biological activity (Nwachukwu and Pulford 2010).

With regards to amendments, pig manure and sewage sludge had high pH that favors increases in mine soil pH, and high levels of total N and C, needed to promote the activation of biochemical cycles. In terms of heavy metals, sewage sludge showed higher contents of Cd, Cu, and Zn than the studied tailing ponds. In the case of pig manure, only total Cu was high.

Table 6.1 Selected properties of amendments and mine soils

Parameter	S	P	MW	Soil L	Soil B
pH	7.57	8.58	7.88	6.72	2.65
EC (dS/m)	2.49	9.00	2.18	11.46	4.17
$CaCO_3$ (%)	4.8	19.6	97.9	0.49	0.33
N_t (%)	5.05	2.17	–	0.01	0.00
SOC (%)	34.0	32.0	–	0.0	0.0
C/N	7	14	–	–	–
Moisture (%)	75.0	40.0	2.1	–	–
Total metals					
Cd (mg/kg)	192	1.51	0.94	23.6	1.44
Cu (mg/kg)	357	832	4.98	93.4	39.7
Pb (mg/kg)	39.6	26.6	11.6	13973	1539
Zn (mg/kg)	8659	261	1.36	2351	1157
Bioavailable metals					
Cd (mg/kg)	–	–	–	10.46	0.10
Cu (mg/kg)	–	–	–	1.52	0.14
Pb (mg/kg)	–	–	–	436.9	5.57
Zn (mg/kg)	–	–	–	236.4	30.4

Marble waste (MW), Sewage sludge (S), Pig manure (P), Lirio (L), Brunita (B) on dry-weight basis. Soil samples (0–15 cm)*EC* electrical conductivity, N_t total nitrogen, *SOC* soil organic carbon

6.3.2 Evolution of Soil Properties in Field-Plots Trial

The evolution of soil properties and available metals for 5 years are represented in Figs. 6.2 (El Lirio) and 6.3 (Brunita). After the application of amendments, we observed an increase in pH in both ponds, being higher in Brunita, since the initial

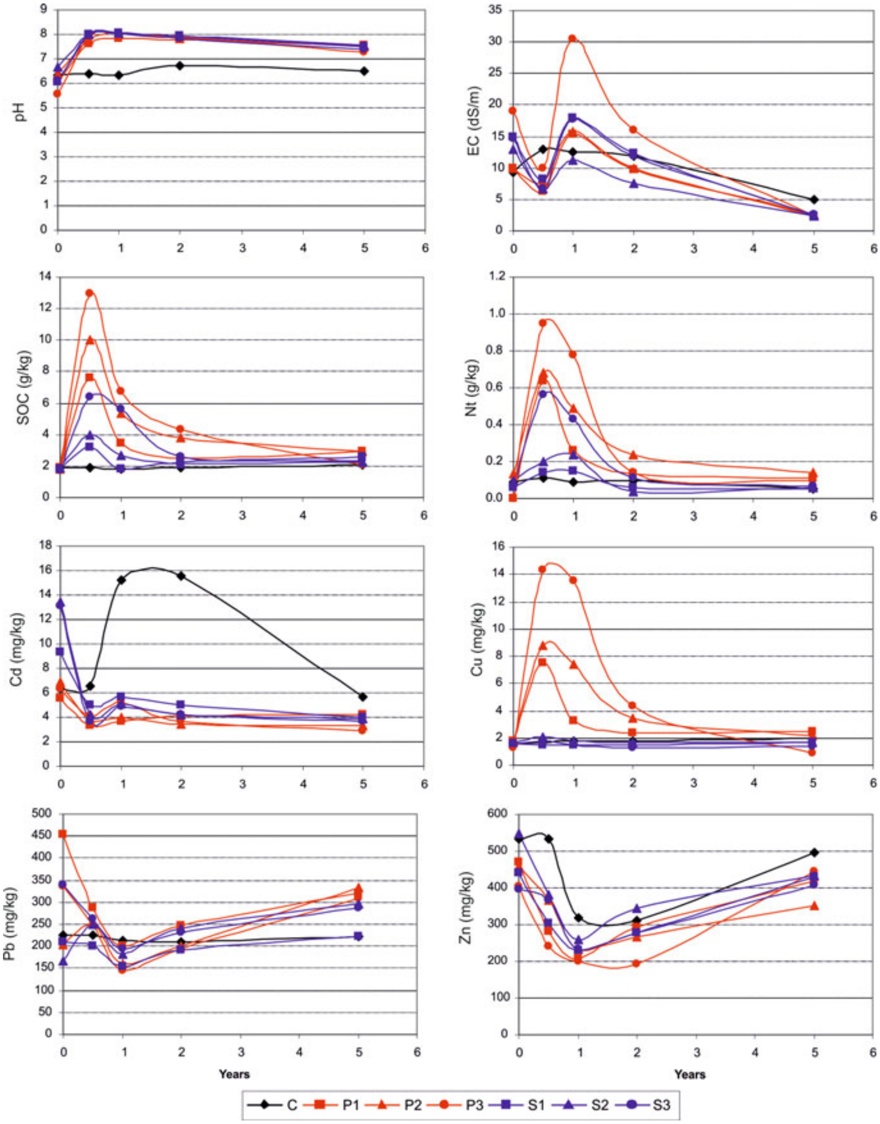

Fig. 6.2 Evolution of pH, electrical conductivity (EC), soil organic carbon (SOC), total nitrogen (N_t) and bioavailable metals in El Lirio plots (see the text for the meaning of plots abbreviations)

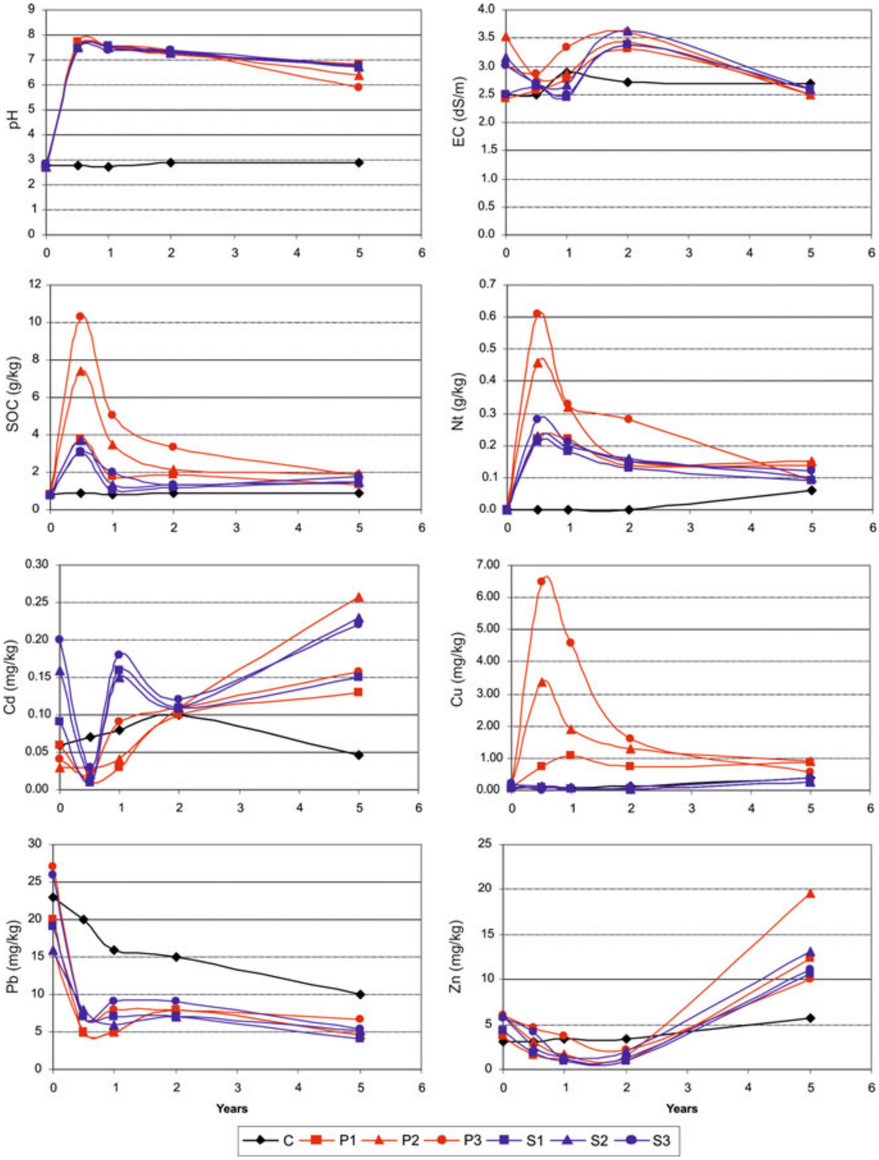

Fig. 6.3 Evolution of pH, electrical conductivity (EC), soil organic carbon (SOC), total nitrogen (N_t) and bioavailable metals in Brunita plots (see the text for the meaning of plots abbreviations)

pH value in this pond was extremely low (<3.0). The pH remained practically stable with time, without differences among treatments, although it tended to decrease after 5 years, mainly due to decreases in carbonates content (data not shown). With regards to EC, we observed an increment after 1 year of amendments application, owing to the high quantity of salts provided by the organic amendments and the solution of carbonates. After 2 years, there is a decreasing trend in EC, due to leaching of sulphates (easily soluble) from mine soils and soluble ions from organic amendments. SOC and N_t initially increased with the application of amendments, mainly in P plots. This increase was in general terms related with the dose of application. However, the values of these two properties decreased owing to leaching and mineralization, shifting down after 5 years of applications to values slightly higher to control.

Regarding total metals, Cd, Cu, Zn, and Pb were above European legislation thresholds, and did not change with time. Bioavailable metals decreased as general pattern in the amended plots in both tailing ponds. However, we detected slight increments in Pb and Zn in El Lirio, and in Cd and Zn in Brunita in amended plots after 5 years of monitoring. The possible explanation for this behavior is difficult owing to the different factors implied in the mobility of heavy metals and their interactions with soil properties. The detected slight decreases in pH and solubilization of carbonates may have likely contributed to increments in bioavailability of some metals. Moreover, the fact that we have also detected increments in the control plot in some metals could be indicating changes in bioavailability of these metals owing to water and wind erosion of surface particles that migrate to other zones, thereby exposing subsurface soils. In addition, the parent material is rich in Cd, Pb, Cu, and Zn sulphides. Thus, oxidation processes of these sulphides and dissolution of secondary sulphates may have also released some metals to the soil. On the other hand, decreases in soil organic matter could have had an important effect, since the application of organic amendments initially immobilized metals by complexation (Zanuzzi 2007). Biovailable Pb and Zn in El Lirio were positively correlated with plant richness and vegetation cover, whilst Cd and Zn were also positively correlated with plant richness in Brunita. This could indicate that the spontaneous establishment of vegetation could be influencing the availability of these metals. In fact, plant roots are known to exude organic compounds capable of complexing metals, which can increment the metals availability in the rhizosphere, and this process differs among different plant species (Jones 1998; Almeida et al. 2006). Plants release some labile compounds to soil to promote the availability and uptake of nutrients, provoking also the availability and uptake of heavy metals (Séguin et al. 2004).

6.3.3 Biochemical Properties

Results of the different biochemical properties determined in the plots emplaced in El Lirio 5 years after the application of the amendments are shown in Figs. 6.4 and 6.5. According to the general trends, all biochemical properties were higher in treated soils than in control, despite the fact that SOC and N_t were similar amongst the treatments after 5 years of the application in El Lirio plots (Fig. 6.2).

The highest increases with respect to control were for MBC in P plots (100%), β-glucosidase in P plots (250%), phosphodiesterase in P plots (210%), and

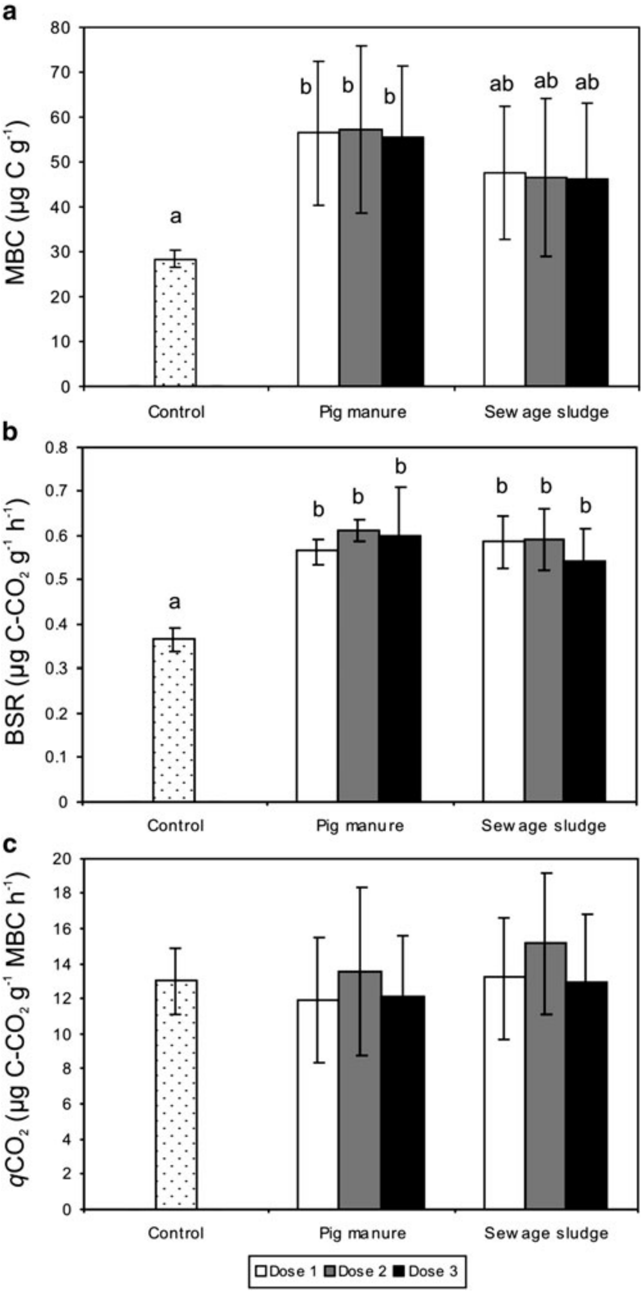

Fig. 6.4 Microbial biomass carbon, soil respiration and metabolic quotient ($q\text{CO}_2$) of the control soil and remediated plots with different organic amendments at three different doses. Different letters indicate mean values significantly different after Tukey's honestly significant difference at $P < 0.05$

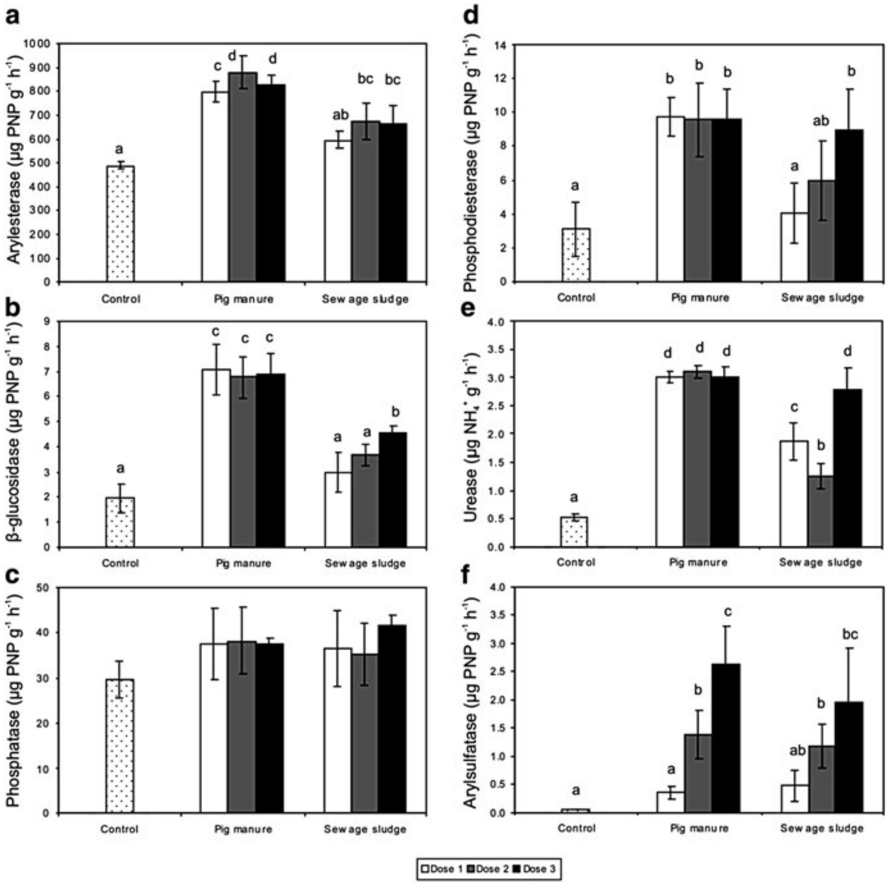

Fig. 6.5 Enzyme activities of the control soil and remediated plots with different organic amendments at three different doses. Different letters indicate mean values significantly different after Tukey's honestly significant difference at $P < 0.05$

arylsulfatase in P3 (4,000%). This confirms the high sensitivity of biochemical properties to evaluate soil quality (Nannipieri et al. 1990), as undetected shifts occurred with other chemical properties. In addition, also as general pattern, biochemical properties showed higher values after application of pig manure than after application of sewage sludge. These results are promising in an area like Murcia province where more than 10% of pig production in Spain is located. These industries generate a large volume of pig slurry that continuously increases with high demands for pork, and consequently creates disposal problem for many pig producers. However, doses did not have a great effect, being only significant for β-glucosidase, phosphodiesterase, and arylsulfatase.

Metabolic quotient has often been used as an indicator of efficiency in carbon mineralization (Insam and Haselwandter 1989). These authors postulated that the efficiency in the use of carbon of the microbial communities increases as the ecosystems succession progresses, resulting in decreases in $q\mathrm{CO}_2$. Nonetheless, in this research, the metabolic quotient did not show this expected trend (lower values in amended plots, which have higher vegetation cover and richness). In fact, values of $q\mathrm{CO}_2$ in this study are high, what can likely indicate a stressful situation for microorganisms, maybe due to high contents in heavy metals and salinity.

The fact that SOC remains similar in all plots after 5 years of application (Fig. 6.2), indicates a mineralization or leaching of the organic amendments, since treated plots had initially significantly higher values of SOC (Zanuzzi 2007). Nonetheless, this initial incorporation of organic matter has triggered the activation of microbial populations which has increased their activity, favoring the recovery of soils and the establishment of vegetation. However, the values of microbial biomass, respiration, and enzyme activities are still low comparing with noncontaminated soils from other zones from SE Spain with the same climatic conditions (Zornoza et al. 2006, 2007; Bastida et al. 2008). This can be explained by the still extreme edaphic conditions, like the already moderate levels of heavy metals, low organic matter, and high salinity.

6.3.4 Factor Analyses

With the PCA performed on the soil chemical and biochemical properties, 70.4% of the total variance could be explained by the first two principal components (Fig. 6.6). Soil samples were clearly clustered by the first principal component (PC1), which explained 48.7% of the variation. PC1 separated all P samples and S3 from the rest of

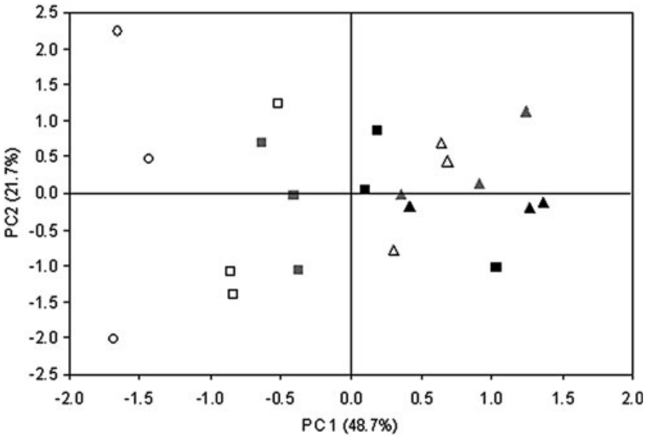

Fig. 6.6 PCA factor scores from chemical and biochemical properties for all treatments and doses. Treatments: control (*circles*), pig manure (*triangles*), sewage sludge (*squares*). Doses: dose 1 (*open symbols*), dose 2 (*gray symbols*), dose 3 (*black symbols*)

samples. Additionally, PC1 also separated control soils from S1 and S2, which clustered together. Samples were not separated by doses by any principal component; solely S3 was separated from S1 and S2 by PC1. This component was associated with all enzyme activities (except for acid phosphatase) and BSR. Second principal component was associated with bioavailable Zn, Pb, and Cd and acid phosphatase.

In general, the factor analyses performed with all samples showed a slight improvement in soil quality in amended soils, mainly in plots amended with pig manure (independently of the dose) and plots amended with the highest dose of sewage sludge. Thus, the use of organic amendments has been proved as a suitable and beneficial procedure to restore mine soils with high contents of heavy metals. The soil properties associated with the separation of treatments were the enzyme and metabolic activities, reinforcing the evidence that biochemical properties are the most sensitive to assess changes in soil quality after soil mine reclamation. Hence, the overall improvement in soil quality of mine sites should be evaluated based not only on soil chemical properties, but also with additional biochemical or biological assays that measure restoration of habitat functions (Hinojosa et al. 2008; Alvarenga et al. 2008).

6.3.5 Vegetation

The untreated plots for both sites remained without vegetation, while natural plant species spontaneously colonized the amended plots (Fig. 6.1). The vegetation cover increased with the dose as a general trend, although plots amended with pig manure showed highest vegetation cover (Table 6.2). Richness also increased with the application dose of amendments, with highest values in plots amended with pig manure. In fact, vegetation cover and richness were significantly positively correlated in both zones, El Lirio ($r = 0.78$; $P < 0.001$) and Brunita ($r = 0.67$; $P < 0.01$).

Thus, the initial and unique incorporation of organic matter has triggered the establishment of vegetation, which remains after 5 years of amendments application. The maintenance of this vegetation cover is essential for true landscape reclamation, activating nutrient cycles and microbial activity (Bouwman and Vangronsveld 2004).

Accumulation and distribution of heavy metals in plant tissues are important aspects to evaluate the role of plant in remediation of metalliferous soils (Friedland 1989). The present results showed that the contents of the different metals in shoots were similar in the most dominant plant species in the P3 plots, except for *Zygophylum fabago*, which had significantly higher values of Cd, Cu, and Zn (Table 6.3).

The BF showed that Cd, Cu, and Zn bioaccumulation occurred in most species (with values of BF > 1), while for Pb no accumulation was observed (Table 6.4). Moreover, *P. miliaceum* only showed bioaccumulation for Cu, being the plant species with lower BF factors for the rest of metals. It is important to highlight

Table 6.2 Natural colonization of plant species on the plots

Treatment	Vegetation cover (%)	Richness	Plant species
El Lirio			
Control	0	0	–
LP1	43	4	*Zigophyllum fabago, Piptatherum miliaceum, Dittrichia viscosa, Phragmites australis*
LP2	45	4	*Zigophyllum fabago, Piptatherum miliaceum, Helichrysum decumbens, Sonchus tenerrimus*
LP3	60	5	*Zigophyllum fabago, Piptatherum miliaceum, Helichrysum decumbens, Dittrichia viscosa, Phragmites australis*
LS1	13	2	*Zigophyllum fabago, Piptatherum miliaceum,*
LS2	27	3	*Zigophyllum fabago, Piptatherum miliaceum, Helichrysum decumbens,*
LS3	32	4	*Zigophyllum fabago, Piptatherum miliaceum, Helichrysum decumbens, Sonchus tenerrimus*
Brunita			
Control	0	0	–
BP1	23	5	*Dactylis glomerata, Brassica fruticulosa, Piptatherum miliaceum, Bromus rubens, Helichrysum decumbens*
BP2	30	8	*Bromus rubens, Brassica fruticulosa, Helichrysum decumbens, Sonchus tenerrimus, Phagnalon saxalite, Dactylis glomerata, Zigophyllum fabago, Spergularia bocconei*
BP3	47	7	*Dactylis glomerata, Bromus rubens, Helichrysum decumbens, Dittrichia viscose, Phagnalon saxalite, Phallaris canariensis, Sonchus tenerrimus*
BS1	19	5	*Dactylis glomerata, Brassica fruticulosa, Bromus rubens, Phagnalon saxalite, Phallaris canariensis*
BS2	25	6	*Brassica fruticulosa, Phallaris canariensis, Bromus rubens, Sedum sediforme, Dactylis glomerata, Piptatherum miliaceum*
BS3	26	7	*Dactylis glomerata, Brassica fruticulosa, Sonchus tenerrimus, Bromus rubens, Helichrysum decumbens, Phallaris canariensis, Spergularia bocconei*

Table 6.3 Metal concentrations in shoots for the most dominant plant species in P3 plots

Metal in shoots (mg/kg)	Plant species				
	Dactilis glomerata	*Piptatherum miliaceum*	*Brassica fruticulosa*	*Zygophylum fabago*	*F* value[a]
Cd	4.2 ± 0.0a	4.4 ± 0.1a	4.2 ± 0.1a	13.2 ± 2.0 b	61.9***
Cu	3.4 ± 0.4a	3.8 ± 0.4a	4.4 ± 0.7a	12.0 ± 0.9 b	116.2***
Pb	23.7 ± 6.2 ab	13.1 ± 6.6a	49.9 ± 18.5 b	21.6 ± 4.8a	6.7*
Zn	21.8 ± 5.7a	90.9 ± 27.0 b	49.0 ± 8.4 ab	288.2 ± 30.2 c	100.0***
Fe	171 ± 53	325 ± 116	225 ± 119	180 ± 38	1.8 ns
Mn	46.1 ± 10.0a	28.0 ± 2.0 b	31.0 ± 6.9 ab	30.4 ± 4.5 ab	4.8*

Values are mean ± standard deviation ($n = 3$)

[a] Significant at: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ after one-way ANOVA; *ns* not significant ($P > 0.05$). Different letters indicate significant differences ($P < 0.05$) among means in each location after Tukey's honestly significant difference

Table 6.4 Bioaccumulation factors (BF) of each metal in the most dominant plant species in P3 plots

	Plant species				
BF	*Dactilis glomerata*	*Piptatherum miliaceum*	*Brassica fruticulosa*	*Zygophylum fabago*	*F* value
Cd	18.7 ± 7.8^{a}	1.0 ± 0.1 b	18.8 ± 6.7^{a}	3.1 ±0.9 b	4.9*
Cu	6.2 ± 0.8^{a}	5.9 ± 3.7^{a}	8.0 ± 0.9 ab	12.7 ± 1.5 b	6.8*
Pb	0.4 ± 0.1^{a}	0.1 ± 0.0 b	0.7 ± 0.2 c	0.1 ± 0.0 b	23.4***
Zn	3.2 ± 1.1 ab	0.3 ± 0.1^{a}	5.0 ± 1.5 b	1.0 ± 0.3^{a}	10.1**

Values are mean ± standard deviation ($n = 3$)

[a] Significant at: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ after one-way ANOVA; ns: not significant ($P > 0.05$). Different letters indicate significant differences ($P < 0.05$) among means in each location after Tukey's honestly significant difference

the high values of BF for Cd in *B. fruticulosa* and *D. glomerata*, indicating the high bioaccumulation of this metal in these concrete species. Thus, these species may be more suitable for phytoextraction technique, rather than phytostabilization, since the most suitable species are those that show mechanisms for protecting themselves against uptake of metals and restricting their transport within the plant (Lefèvre et al. 2005). However, most species presents in these plots are not eaten by herbivores (Zanuzzi 2007), acting like a sink for metals and preventing it from becoming available to other organisms.

6.4 Conclusion

1. The application of pig manure and sewage sludge together with marble wastes has proved to be effective for long-term decrease in the bioavailability of most toxic heavy metals present in two tailing ponds from SE Spain, besides maintaining pH close to neutrality. Despite the initial decrease in SOC and N_t, mineralization and leaching have led to levels of organic matter only slightly higher in comparison to control plots. Since increments in some bioavailable metals have been monitored, future studies are needed to determine the causes, or mitigate this trend by new applications of organic amendments, until succession of vegetation progresses to provide enough litter to increase and maintain soil organic matter.
2. After 5 years of applications of amendments, plots with pig manure presented the best effects on microbial biomass and activity. However, the values of the biochemical properties are still low even for a semiarid environment. Besides, pig manure plots have proved to be more effective to initialize natural spontaneous vegetation colonization, richness, and vegetation cover. *Zygophylum fabago* accumulated moderated quantities of metals, not observed in the other plant species. However, BF was high for all plants except for *P. miliaceum*. Thus, even though most species grew in this study are refused by herbivores, a better

through selection of the most suitable species to continue with phytostabilization progress and mine soils remediation in SE Spain should be developed in the immediate future, focusing on reduction of erosion, tolerance to metals and salinity, nitrogen fixation and low accumulations of metals so that risks in the food chain are minimized.

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