Influence of inorganic and organic amendments for mine soils reclamation on spontaneous vegetation colonization and metal plant bioaccumulation

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Abstract
The effectiveness of two organic amendments (pig manure and sewage sludge) and marble mud for the in situ remediation of contaminated soils by heavy metals owing to former mining activities in SE Spain was assessed. Concretely, we evaluated the spontaneous colonization of plant species and increments in richness and vegetation cover in the amended plots, as well as the bioaccumulation of heavy metals in plants, to avoid the risk of mobility in the food chain. Results showed that although the untreated plots remained without vegetation, natural plant species spontaneously colonised the amended plots. Increases in vegetation cover and richness were related with the application dose, with pig manure plots having the highest values. Metal concentrations in shoots of plant species were similar except for *Zygophylum fabago* which had highest levels of Cd, Cu and Zn. The calculation of the bioaccumulation factor (BF) showed that Cd, Cu and Zn bioaccumulation was carried out in most species, while for Pb no bioaccumulation was observed. Moreover, *Piptatherum hordeum* only showed bioaccumulation for Cu, although it showed lower BF factors for all the other studied metals.

Key Words
Metal pollution; in situ remediation; phytostabilization; sewage sludge; pig slurry; marble waste.

Introduction
The environmental impacts of the long-history of mining activities in southeast Spain include large areas of soils characterized by strong acidification processes, high salinity and accumulation of metals. These mining activities have generated high amounts of sterile materials for many years; the wastes being accumulated in pyramidal structures called tailing ponds. Mine sites contain materials of high Fe-oxyhydroxides, sulphates, and potentially leachable elevated contents of heavy metals (mainly Cd, Pb, Cu and Zn) due to extreme acidic conditions. As a consequence, these mine soils have scarce or null vegetation due to very poor properties, including extremely low soil organic matter. These metal-contaminated soils contribute to human and animal metal exposure, through food chain transfer or inhalation of wind blown dust (Pierzynski 1997). 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 promising solution for the remediation of a mining area, decreasing the concentration of available metal pools in the amended soil. 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). 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. Thus, the goal of this work is to assess the establishment of spontaneous vegetation cover after the remediation of contaminated mine soils with different inorganic and organic amendments, and to evaluate the bioaccumulation of heavy metals in plants, to avoid the risk of mobility in the food chain.

Methods

Study site and experimental design

The study was conducted in the province of Murcia (SE Spain), in the Cartagena-La Unión Mining District, where extensive mining activity has been carried out for more than 2500 years, till the nineties. The climate of the area is semiarid Mediterranean type with mean annual temperature of 18ºC and mean annual rainfall of 275 mm. Two tailing ponds generated by mining activities were selected: El Lirio (L) and Brunita (B), representative of the rest of existent ponds in Cartagena-La Unión Mining District.

The field trial was established in 2004. Plots (2 m x 2 m) were randomised and replicated 3 times. Two different organic amendments were used to reclaim the soils, pig manure (P) and sewage sludge (S). In addition, 3 different doses per amendment were applied. Thus, the treatments were: Untreated contaminated soil (Control: C), soil treated with pig manure at dose 1 (P1), dose 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, dose 1, 2 and 3 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. Doses were established by thresholds imposed by legislation regarding the addition of N to soil (Council Directive 91/676/EEC, 1991) With the purpose of increasing soil pH to immobilise metals and create better conditions for microbial and plant development, marble mud was applied in all plots except for control, adding 22 kg of this inorganic waste per plot.

Soil and plant samplings and analytical methods

The soil sampling was carried out in May 2009 (5 years after application of amendments). One sample (0-15 cm depth) was collected for each plot, taken to the lab, air-dried for 7 days, passed through a 2-mm sieve and stored at room temperature prior to laboratory analyses. The physico-chemical characterization of the plots is shown in Table 1. At the same time, the identification of all plants that spontaneously colonized the plots was carried out (richness), as well as the percentage of vegetation cover. In addition, the shoot of one of the two most dominant species in P3 (the treatment with highest vegetation cover and richness) was collected in each of the three replicated plots for analyses of metal concentration. These species were *Piptatherum hordeum* and *Zygophylum fabago* in El Lirio, *Dactilis glomerata* and *Brassica fruticulosa* in Brunita.

To determine the concentration of bioavailable metals in soils DTPA was used in the ratio of 1:2 as a soil-extractant (Lindsay and Norwell, 1978). For plant metals concentration, dry plant material was ground and combusted at 450ºC for 24 h. After that, the digested material was suspended in HNO_3 and filtered. Measurements of metals were carried out using atomic absorption spectrophotometer (AAnalyst 800, Perkin Elmer). The bioaccumulation factor (BF) was also calculated as $[\text{metal}]_{shoot}/[\text{bioavailable metal}]_{soil}$.

Table 1. Main physico-chemical properties and bioavailable metals of control and amended plots.

Treatment	pH	EC (dS/m)	SOC (g/kg)	Nt (g/kg)	Texture	Cd (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
El Lirio									
Control	7.1	2.5	2.13	0.05	Sandy loam	5.63	1.94	177	495
LP1	7.6	2.4	2.96	0.12	Sandy loam	4.21	2.45	320	418
LP2	7.5	2.5	2.92	0.15	Sandy loam	3.29	2.21	333	352
LP3	7.3	2.4	2.14	0.06	Sandy loam	2.92	0.84	309	446
LS1	7.5	2.4	2.66	0.05	Sandy loam	4.02	1.72	223	428
LS2	7.6	2.3	2.29	0.06	Sandy loam	3.86	1.68	295	433
LS3	7.4	2.5	2.33	0.06	Sandy loam	3.70	1.38	288	406
Brunita									
Control	2.8	2.7	0.91	0.06	Sandy loam	0.02	0.40	2.13	5.74
BP1	6.8	2.5	1.34	0.15	Sandy loam	0.13	0.85	4.72	12.39
BP2	6.4	2.5	1.92	0.16	Sandy loam	0.32	0.91	4.66	19.65
BP3	5.0	2.5	1.84	0.09	Sandy loam	0.16	0.55	6.67	10.02
BS1	6.7	2.5	1.52	0.10	Sandy loam	0.15	0.26	4.03	10.67
BS2	6.7	2.5	1.60	0.11	Sandy loam	0.23	0.28	5.26	13.04
BS3	6.7	2.5	1.81	0.12	Sandy loam	0.22	0.40	5.34	11.14

EC: Electrical conductivity, SOC: soil organic carbon, Nt: total nitrogen..

Results and discussion

The untreated plots for both sites remained without vegetation, while natural plant species spontaneously colonised the amended plots. The vegetation cover increased with the dose as a general trend, although plots amended with pig manure showed highest vegetation cover (Table 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). These results are promising in an area like Murcia province where more than 10% of pig production in Spain is located. Annually, Murcia province generates an estimated 8 millions m^3 of waste residues from the pork industry (CAAMA, 2003), which is continuously increase with high demands for pork, and consequently creating disposal problem for many pig producers. SOC presents similar values in all plots in each different zone (Table 1), which 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 establishment of vegetation, which remains after 5 years of amendments application. The maintenance of this vegetation cover is essential for true landscape reclamation, reactivating nutrient cycles and microbial activity (Bouwman and Vangronsveld, 2004).

Table 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 hordeum, Dittrichia viscosa, Phragmites australis*
LP2	45	4	*Zigophyllum fabago, Piptatherum hordeum, Helichrysum decumbens, Sonchus tenerrimus*
LP3	60	5	*Zigophyllum fabago, Piptatherum hordeum, Helichrysum decumbens, Dittrichia viscosa, Phragmites australis*
LS1	13	2	*Zigophyllum fabago, Piptatherum hordeum,*
LS2	27	3	*Zigophyllum fabago, Piptatherum hordeum, Helichrysum decumbens,*
LS3	32	4	*Zigophyllum fabago, Piptatherum hordeum, 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*

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 3).

Table 3. Metal concentrations in shoots for the most dominant plant species in P3 plots. Values are mean ± Standard Deviation (n=3).

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

[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 BF showed that Cd, Cu and Zn bioaccumulation occurred in most species, (with values >1), while for Pb no accumulation was observed. Moreover, *P. hordeum* only showed bioaccumulation for Cu, being the plant species with lower BF factors for the rest of metals. In this sense, plant species studied here (except for *P. hordeum*), translocated to the shoots high quantities of heavy metals, being *B. fruticulosa* and *D. glomerata* the species with highest BF for Cd, especially toxic for the food chain. 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 used in this study are not eaten by herbivores (Zaunuzzi, 2007), acting like a sink for metals and preventing it from becoming available to other organisms.

Table 4. Bioaccumulation factors (BF) of each metal in the most dominant plant species in P3 plots. Values are mean ± Standard Deviation (n=3).

BF	Plant species				
	Dactilis glomerata	*Piptatherum hordeum*	*Brassica fruticulosa*	*Zygophylum fabago*	*F* value[a]
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 **

[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.

Conclusion

The application of pig manure and sewage sludge together with marble wastes has proved to be effective to initialize natural spontaneous vegetation colonization, richness and vegetation cover being highest in pig manure plots, also increasing with the dose of application. *Zygophylum fabago* accumulated moderated quantities of metals, not observed in the other plant species. However, BF was high for all plants except for *P. hordeum*. Thus, even though most species used 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 (Zanuzzi, 2007), so that risks in the food chain are minimised.

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