Organic residues as immobilizing agents in aided phytostabilization: (I) Effects on soil chemical characteristics

P. Alvarenga [a,*], A.P. Gonçalves [a], R.M. Fernandes [a], A. de Varennes [b], G. Vallini [c], E. Duarte [b], A.C. Cunha-Queda [b]

[a] Department of Environmental Sciences, Escola Superior Agrária de Beja, Rua Pedro Soares, Apartado 6158, 7801-908 Beja, Portugal
[b] Department of Agricultural and Environmental Chemistry, Instituto Superior de Agronomia, Technical University of Lisbon (TULisbon), Tapada da Ajuda, 1349-017 Lisboa, Portugal
[c] Department of Science and Technology, Laboratories of Microbial Biotechnology and Environmental Microbiology, University of Verona – Strada Le Grazie 15, Ca' Vignal, 37134 Verona, Italy

ARTICLE INFO

ABSTRACT

A greenhouse experiment was conducted to evaluate the effect of three different organic residues, sewage sludge (SS), municipal solid waste compost (MSWC), and garden waste compost (GWC), as immobilizing agents in aided phytostabilization of a highly acidic metal-contaminated soil, affected by mining activities, using perennial ryegrass (*Lolium perenne* L.). The organic residues were applied at 25, 50 and 100 Mg ha^{-1} (dry weight basis), and their effects on soil chemical characteristics and on relative plant growth and metal concentrations were assessed. All the organic residues tested immobilized Cu, Pb and Zn, decreasing their mobile fractions. This was corroborated by negative correlations obtained between mobile Cu, Pb and Zn and other soil chemical characteristics, which rose as a consequence of the amendments applied (i.e., pH, electrical conductivity, organic matter, nitrogen content, available P and available K), and by the multivariate exploratory techniques performed that showed an inverse correlation between these groups of variables. The greatest increase in ryegrass relative growth (more than three times) was obtained in the presence of 50 Mg MSWC ha^{-1}, followed by SS at the same application dosage. GWC did not contribute to an increase in shoot growth, due to its small capacity to correct soil acidity and to supply essential macronutrients (N, P, K). No extractant was able of demonstrating by a linear correlation the uptake of Cu, Pb and Zn by ryegrass. This plant was therefore not a good "indicator" of Cu, Pb and Zn availability in the soil. The results obtained in this study suggest that ryegrass can be used in aided phytostabilization for this type of mine contaminated soils and that MSWC, and to a minor extent SS, applied at 50 Mg ha^{-1}, were effective in the *in situ* immobilization of metals, improving soil chemical properties and leading to a large increase in plant biomass.

1. Introduction

Rehabilitation projects for lands impacted by mining activities are being developed, leaving the contaminated soils in place and using amendments, like organic residues and materials rich in carbonates, to reduce the bioavailability of metals and to restore the ecological function of the sites (Basta et al., 2001; Geebelen et al., 2002; Brown et al., 2003). Organic residues are able to improve soil physical, chemical and biological properties by: (i) raising the pH, (ii) increasing the organic matter content, (iii) adding essential nutrients for plant growth, (iv) increasing the water holding capacity, and (v) modifying heavy metals bioavailability. Furthermore, *in situ* immobilization of metals can be combined with phytostabi-

lization, using plants to establish a "green cover" that will prevent dispersion of metal-contaminated particles by water or wind erosion, and reduce metal mobility by rhizosphere-induced adsorption and precipitation processes (Vangronsveld et al., 1995; Arienzo et al., 2004). If this revegetation is performed in combination with immobilizing agents it can be considered as "aided phytostabilization".

The choice of the plant is a very important aspect to consider in phytostabilization (Tordoff et al., 2000; Wong, 2003; Rizzi et al., 2004; Arienzo et al., 2004). Plants should develop an extensive root system and produce a large amount of biomass in the presence of high concentrations of trace metals, while keeping metal translocation from roots to shoots as small as possible (Wong, 2003; Rizzi et al., 2004). Several authors reported perennial ryegrass (*Lolium perenne* L.) as a suitable species for revegetation of metalliferous wastes (Pichtel and Salt, 1998), metal-contaminated soils from

* Corresponding author. Tel.: +351 284314312; fax: +351 284314388.
E-mail address: paula.alvarenga@esab.ipbeja.pt (P. Alvarenga).

metallurgical sites (Arienzo et al., 2004), and mine tailings (Santi-báñez et al., 2008).

The addition of immobilizing agents to soil, as well as its reveg-etation, will modify the dynamic equilibrium between total content of metals with their mobility (soluble, very active and bioavailable) and mobilisable fractions (potentially bioavailable, leachable and partly active) (Gupta et al., 1996). In fact, some organic residues can decrease metal mobility/bioavailability in these soils, but they can also be responsible for large metal loadings, and the potential risk derived from its application can be estimated from the mobilis-able metal content (Alvarenga et al., in press). van Herwijnen et al. (2007) also discussed that composts can increase or decrease the bioavailability of metals in soils, depending on the compost, the soil, and the level of contaminants. They recommended that plant and leaching tests should be performed before composts are applied for the reclamation of metal-contaminated soils at a field scale.

In most European countries, soil quality standards are based on the pseudo-total metal content, measured with the help of strong acids (e.g. boiling HNO_3 or aqua regia), which are excellent criteria to define the extent of metal build up in a soil, but of little value for the prediction of ecological impact (Gupta et al., 1996). In this sense, the use of selective chemical extractions is the usual ap-proach to understand the interaction of trace metals with soils, or to assess their mobility and availability to plants (Ure, 1996; Sahuquillo et al., 2003). Single step extractions are mainly used to evaluate the exchangeable fraction of trace metals in soils, while sequential extraction procedures, which are more time-consum-ing, are usually applied to estimate metal association with the dif-ferent solid-phase components (Sahuquillo et al., 2003).

The aim of this experiment was to assess the effect of three dif-ferent organic residues as immobilizing agents in aided phytostabi-lization of a highly acidic metal-contaminated soil, evaluating: (i) soil physico-chemical characteristics, (ii) metal pseudo-total and bioavailable content, using single step extractions (iii) metal speci-ation, using a sequential extraction procedure, and (iv) *L. perenne* relative growth and its mineral composition. From the results it will be possible to further elucidate the benefits and/or potential risks derived from the application of different types of organic residues as amendments in the remediation of a contaminated mine soil.

The effects of the amendments were also assessed considering the soil biochemical and ecotoxicological characteristics. These re-sults will be discussed in Part II of this study and we hypothesized that it will be possible to evidence the importance of an integrated evaluation of soil quality, considering the soil chemical, biochemi-cal and ecotoxicological characteristics, in a remediation process.

2. Materials and methods

2.1. Soil characterization

Topsoil (<20 cm depth) from a metal-contaminated site in the Aljustrel mining area (a pyrite mine located in SW Portugal in the Iberian Pyrite Belt) was collected in October 2006. A soil sub-sample was air-dried, passed through a 2-mm sieve and character-ized for: particle-size distribution by the pipet method (Gee and Bauder, 1986); soil pH (H_2O) in deionised water suspension of 1:2.5 (w/v); electrical conductivity (EC) in deionised water suspen-sion of 1:5 (w/v); total nitrogen by the Kjeldahl method ($N_{Kjeldahl}$); total oxidizable organic carbon according to Walkley and Black (1934); soil cation exchange capacity (CEC) using the ammonium acetate (pH 7) method (Sumner and Miller, 1996); and available P and K using the Egner–Riehm method (Riehm, 1958). All mea-surements, except particle-size distribution, were carried out in triplicate. Pseudo-total metal (Cd, Cr, Cu, Ni, Pb and Zn) concentra-tions were determined by either flame or electrothermal atomic

absorption spectrometry after digestion of the samples with aqua regia according to ISO 11466 (1995), using a Varian apparatus (SpectrAA 220FS, 220Z, and 110Z). Three independent replicates were performed for each sample and blanks were measured in parallel.

The soil used was a sandy loam, highly acidic, low in organic matter (OM) content, in essential nutrients (N, P and K) and in CEC (Table 1). Copper, Pb and Zn total concentrations were high and exceeded many times their limit value in agriculture soils for sewage sludge application, allowed by Portuguese Legislation (Decreto-Lei n° 118/2006). However, previous studies showed that these metals were mostly structurally bound and, therefore, scar-cely bioavailable (Alvarenga et al., 2008b).

2.2. Experimental set-up

The residues used were: an anaerobically digested sewage sludge (SS), a compost from the organic fraction of unsorted muni-cipal solid waste (MSWC) and a garden waste compost (GWC). SS was collected from the drying bed of an urban wastewater treat-ment plant located in Portalegre (Portugal) and dried outdoors un-til a moisture content of 5% was reached. It was then ground and mixed before analysis and use. MSWC was obtained in a compost-ing plant near Setúbal (Portugal), and GWC came from a compost-ing plant in Tavira (Portugal), which receives source-separated garden residues (namely grass clippings, leaves and brush). Three replicates of each organic residue were analysed using methodolo-gies previously described by Alvarenga et al. (2007) and their char-acteristics are shown in Table 1.

All the organic residues had total metal contents below the lim-its established by official guidelines for SS (Decreto-Lei n° 118/ 2006), allowing their use in agricultural soils.

The soil was homogenised, without sieving, and stored at field moisture content. Pots were prepared with 3000 ± 100 g of soil (dry weight basis), plus the amendments, which were mixed with the soil. The organic residues were applied at 25, 50 and 100 Mg ha^{-1} (dry weight basis), using three replicates per treat-ment. A control, which did not receive any amendment, was also

Table 1
Characterization of the soil and of the organic amendments used in the study (mean ± SD, $n = 3$).

Parameter	Soil	MSWC	GWC	SS
pH	3.7–4.1	8.0–8.4	7.8–8.0	6.5–6.9
EC (dS m^{-1})	0.21 ± 0.07	5.69 ± 0.09	2.47 ± 0.08	2.90 ± 0.09
Organic matter (%)	0.37 ± 0.02	37.2 ± 0.4	39 ± 2	72 ± 2
CEC (cmol kg^{-1})	5.6 ± 0.5	–	–	–
Texture (g kg^{-1})	Sand 704	–	–	–
	Silt 177	–	–	–
	Clay 119	–	–	–
N$_{Kjeldahl}$ (%)	0.07 ± 0.01	1.8 ± 0.4	1.0 ± 0.4	7.2 ± 0.3
C/N	–	10.3	19.5	5.0
Available P (mg kg^{-1})	n.d.	–	–	–
Available K (mg kg^{-1})	52 ± 2	–	–	–
Total P (%)	–	0.6 ± 0.09	0.094 ± 0.004	1.36 ± 0.06
Na (g kg^{-1})	–	7.0 ± 0.7	15.1 ± 0.7	4.6 ± 0.9
K (g kg^{-1})	–	8.1 ± 0.9	7.6 ± 0.3	1.3 ± 0.3
Ca (g kg^{-1})	–	78 ± 2	55 ± 3	21.7 ± 0.8
Mg (g kg^{-1})	–	18.1 ± 0.9	8.4 ± 0.8	3.4 ± 0.2
Cd (mg kg^{-1})	2.6 ± 0.2	4.3 ± 1.1	1.4 ± 0.1	1.46 ± 0.04
Cr (mg kg^{-1})	21.8 ± 0.6	56 ± 13	13 ± 1	15.3 ± 0.2
Cu (mg kg^{-1})	362 ± 23	357 ± 12	14 ± 2	98 ± 5
Ni (mg kg^{-1})	15.4 ± 0.4	56 ± 6	16 ± 3	10.0 ± 0.1
Pb (mg kg^{-1})	4350 ± 169	269 ± 24	34 ± 3	37 ± 1
Zn (mg kg^{-1})	245 ± 64	583 ± 26	35 ± 10	491 ± 12

MSWC, municipal solid waste compost; GWC, garden waste compost; SS, sewage sludge; EC, electrical conductivity; CEC, cation exchange capacity; n.d.: not detec-ted; SD: standard deviation; concentrations refer to a dry weight basis.

included (nine replicates). The soil mixtures were then adjusted to 70% of their maximum water holding capacity, with deionised water, transferred to a greenhouse, and allowed to equilibrate for 28 d prior to sowing.

After the incubation period, the pots were sown with 5 g of seeds per pot of perennial ryegrass (*L. perenne* L.) and kept in a greenhouse. The pots were daily watered to maintain their initial water content.

2.3. Plant analysis

Two months after sowing the shoots were cut, washed thoroughly with tap water to remove any attached particles, and then rinsed three times with deionised water. The samples were dried at 70 °C for 48 h, weighed, and ground in an electric mill. Approximately 1 g of dried plant sample was ashed in a muffle furnace at 500 °C for 6 h, dissolved with 10 mL of 3 M HCl and evaporated to near dryness twice, dissolved again with the same acid solution, filtered (Whatman 40), and adjusted to a volume of 100 mL with ultra-pure water. Plant digested samples were analysed for total Cu, Pb and Zn by flame atomic absorption spectrometry, using a Varian apparatus (SpectrAA 220FS).

2.4. Soil characterization after remediation

The soil mixtures were air-dried, passed through a 2-mm sieve and subjected to chemical characterization (pH, EC, OM content, $N_{Kjeldahl}$, available P and available K) and to pseudo-total heavy metal quantification (Cu, Pb and Zn) as described before.

Metal available fractions were determined using two different single step extractions, and the definitions suggested by Gupta et al. (1996): a mobile fraction (extracted by 0.01 M $CaCl_2$, pH 5.7, without buffer) (Houba et al., 1996; Pueyo et al., 2004), sometimes referred as the "effective bioavailable metal fraction", and a mobilisable fraction (extracted by a solution of 0.5 M NH_4CH_3COO, 0.5 M CH_3COOH and 0.02 M EDTA, pH 4.7) (Hammer and Keller, 2002), considered as a "potentially bioavailable metal fraction". Extractions were performed with 2 h horizontal reciprocate shaking, on a 1:10 (w/v) soil to solution ratio, at room temperature. The extract was separated from the solid residue by centrifugation at 3000g for 10 min.

Metal speciation was performed using the modified BCR (Community Bureau of Reference) sequential extraction (three step) procedure (Rauret et al., 2000): first step – extraction with 0.11 M acetic acid to obtain exchangeable, water and acid soluble species; second step – solubilization using 0.5 M hydroxylamine hydrochloride to obtain metals associated with the reducible phases, e.g. bound to Fe/Mn oxyhydroxides; third step – oxidation with 8.8 M hydrogen peroxide, followed by extraction using 1.0 M ammonium acetate to obtain the oxidisable metal fraction, e.g. bound to organic matter or sulphides.

2.5. Statistical treatment of data

All data were checked for homogeneity of variance and normality (Kolmogorov–Smirnov test) and, when possible, subjected to one-way analysis of variance (ANOVA). Data not satisfying assumptions for ANOVA were analysed non-parametrically using Kruskal–Wallis ANOVA by Ranks test. Whenever significant differences where found ($P < 0.05$) a post hoc Tukey honest significant difference test was used to further elucidate differences among means ($P < 0.05$).

For statistical analysis purposes, results below the method detection limit, although reported as "not detected", were assumed to be equal to the method quantification limit value. Pearson correlation coefficients (r) were calculated between soil physico-chemical properties, soil metal concentrations and plant related parameters (three levels of significance were considered: $P < 0.05$, $P < 0.01$ and $P < 0.001$).

Two different multivariate exploratory techniques were performed on the results: a principal component analysis (PCA), to identify the key parameters describing data variability, and a cluster analysis that, performing a sample clustering, will contribute to the discussion of the differences and similarities between the organic residues tested as soil amendments. All statistical analysis were carried out with the software Statistica 6.0 (StatSoft, Inc., 2001).

3. Results and discussion

3.1. Effects of organic residues on soil chemical properties

As expected from preliminary incubation studies (Alvarenga et al., in press), the addition of organic residues to the mine soil led to a significant increase in pH values compared with control soil ($P < 0.05$) (Fig. 1a), with a better performance in the case of MSWC and SS application: pH values above 6.0 were only possible with the application of 100 Mg ha^{-1} of SS or MSWC. It is also important to note that, although SS had the lowest initial pH (6.7) it presented a higher capacity to modify soil acidity. This likely derived from an inadequate or incomplete stabilization at the wastewater treatment plant. As a consequence, when applied to the soil, it will undergo a biological digestion leading to a rapid rise in pH. On the other hand, although the composts used had higher initial pH values, they were no longer prone to sudden biological transformations and had less effect on soil pH, especially GWC.

The original mine soil had low levels of salts (EC = 0.21 dS m^{-1}), as a consequence of its exposure to weathering and leaching. The application of organic amendments led to a significant increase in EC values, compared to control soil ($P < 0.05$) (Fig. 1b), more pronounced in the case of SS and MSWC application. In contrast, the low salinity of GWC, typical of wood and yard waste-based composts (Zmora-Nahum et al., 2007), made it an attractive organic amendment to be used in soil remediation. Secondary salinisation of soils, due to application of organic residues, can be a major constraint in a phytoremediaton strategy, as discussed by Rodgers and Anderson (1995) who stated that sludge amendments should not exceed a one-time application of 100 Mg ha^{-1}, on a dry weight basis, to prevent inhibition of plant growth during the first few years following application. Alvarenga et al. (2008a) also found a considerable phytotoxic effect when using 200 Mg ha^{-1} of the same type of SS used in this experiment.

Both composts, MSWC and GWC, were equivalent in OM content (Table 1), and in their capacity to raise soil OM (Fig. 1c), but SS application led to the greatest soil OM values, due to its large OM content.

The organic residues tested were significantly different in their capacity to supply essential macronutrients (N, P, K) (Fig. 1d–f). SS application led to the greatest soil N values, followed by MSWC. This result can be explained if we consider the C:N ratio of the residues (Table 1), with the order SS < MSWC < GWC. The latter was poor in nitrogen as it was produced from carbonaceous raw materials (e.g. twigs, wood chips, dead leaves and residues of dead plants). Therefore, GWC did not correct soil nitrogen deficiency and, as a consequence, nitrogen fertilizers should be added together with GWC, as it is recommended in most soil rehabilitation projects (Tordoff et al., 2000). GWC cannot be thus considered a N fertilizer (Keeling et al., 2003). Substantially higher values of available P and available K were obtained when MSWC was applied, compared to other treatments.
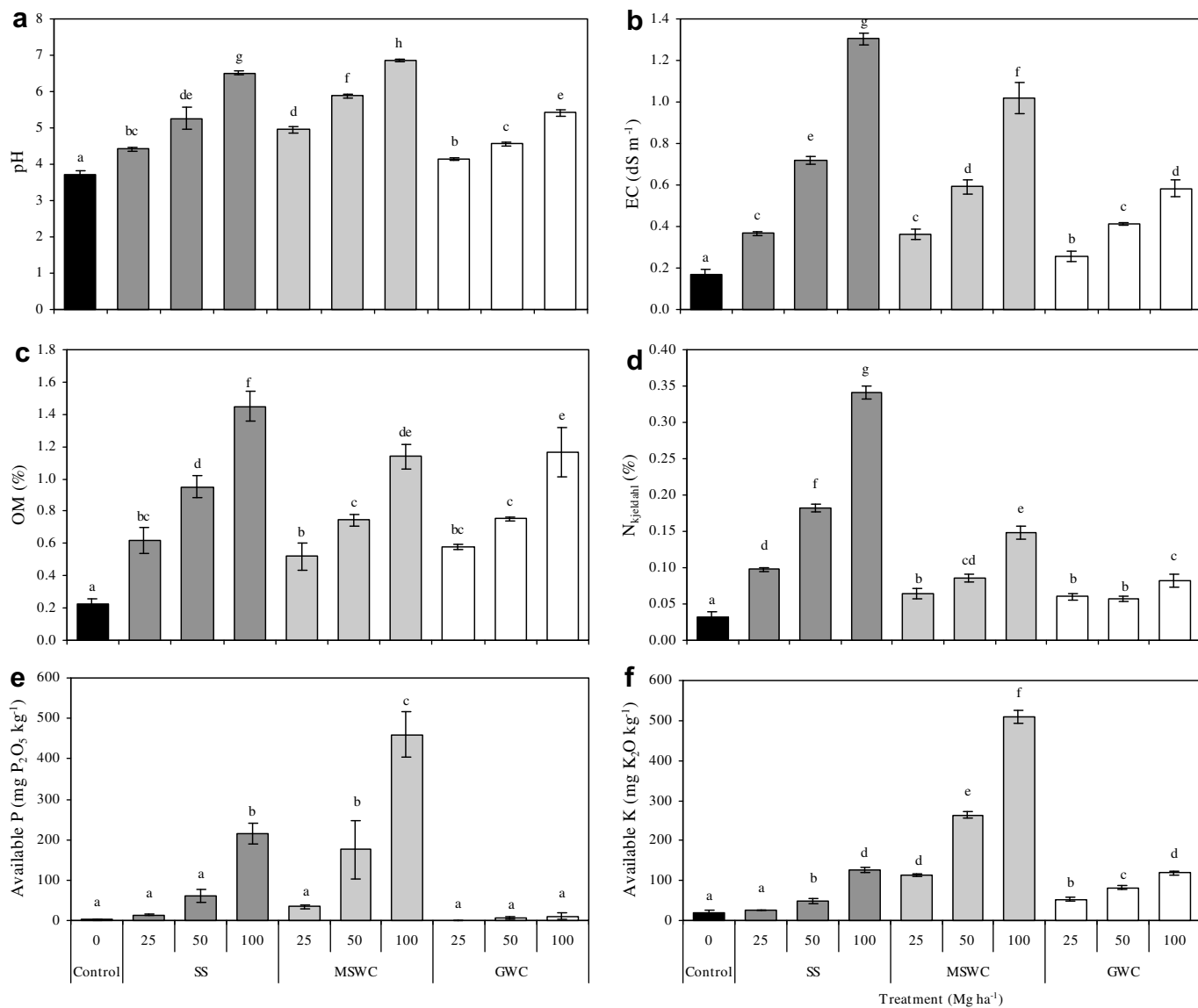
Fig. 1. Soil chemical characteristics obtained with the different amendments tested: (a) pH; (b) electrical conductivity (EC) (dS m^{-1}); (c) total organic matter content (OM) (%); (d) Kjeldahl nitrogen (%); (e) available P (mg P$_2$O$_5$ kg^{-1} DW); and (f) available K (mg K$_2$O kg^{-1} DW) (mean ± SD, $n = 3$, $n = 9$ for the control). Columns marked with the same letter are not significantly different (Tukey test, $P > 0.05$). MSWC, municipal solid waste compost; GWC, garden waste compost; SS, sewage sludge.

3.2. Effects of organic residues on soil metal concentrations

Soil Cu, Pb and Zn pseudo-total concentrations did not change significantly due to the application of organic residues, when compared to the control soil (Table 2). However, all the amendments decreased CaCl$_2$-extractable Cu, Pb and Zn (mobile fractions), sometimes to values below the quantification limit of the method used (Table 2). These results can be explained by the fact that acidity is one of the most important factors controlling solubility and adsorption–desorption of metal in soils (Ross, 1994; Kabata-Pendias and Adriano, 1995; Walker et al., 2003). Consequently, the residues decreased metal mobility/bioavailability mainly because they raised soil pH. Another important factor controlling metal bioavailability is the quantity and quality of the OM present (Ross, 1994). Organic amendments that contain a high proportion of humified OM can also decrease the mobility of some heavy metals due to the formation of stable chelates (Walker et al., 2003; Clemente et al., 2006).

The large metal loadings into the soil, resulting from addition of low quality organic residues could represent a long-term risk (Basta and Sloan, 1999). This potential risk can be estimated from the mobilisable metal content (Table 2). In the present experiment, Cu and Zn mobilisable fractions increased with MSWC (all doses) and 100 Mg ha^{-1} SS application. Generally, GWC application did not produced any significant difference, except for Cu with 25 Mg ha^{-1} dose. These results were in accordance with the characteristics of the residues: a noticeable difference in Cu, Pb and Zn contents between GWC, a compost obtained from separately collected garden waste, and MSWC, a compost from the organic fraction of unsorted municipal solid waste (Alvarenga et al., 2007). van Herwijnen et al. (2007) also observed that Zn leaching was reduced by the addition of GWC but increased by the addition of composted sewage sludge to soils. Hence, the use of organic residues in the remediation of mine derelict soils is in accordance with sustainable management practices, but they should be applied at the right doses and the extractability/mobil-

Table 2
Pseudo-total, mobile and mobilisable metal fractions in soil with the different soil treatments (mean ± SD, $n = 3$, $n = 9$ for the control). Values in each column marked with the same letter are not significantly different (Tukey test, $P > 0.05$).

Organic amendment (Mg ha^{-1})		Metal concentrations (mg kg^{-1} DW)								
		Cu			Pb			Zn		
		Pseudo-total	Mobile[a]	Mobilisable[b]	Pseudo-total	Mobile[a]	Mobilisable[b]	Pseudo-total	Mobile[a]	Mobilisable[b]
Control soil		525 ± 50a	3.5 ± 0.9b	9 ± 2a	5491 ± 1338abc	99 ± 32b	1001 ± 354ab	253 ± 17a	8.0 ± 0.8c	9 ± 1a
SS	25	493 ± 22abc	1.0 ± 0.1a	9.3 ± 0.3ab	5601 ± 64abc	26 ± 4a	856 ± 109ab	231 ± 6a	5.0 ± 0.4b	8.8 ± 0.2a
	50	489 ± 12abc	<QL	10.5 ± 0.6abc	5640 ± 470abc	6 ± 1a	1011 ± 294ab	256 ± 17a	2.4 ± 0.5ab	13 ± 1ab
	100	444 ± 22a	0.7 ± 0.1a	17.3 ± 0.6 de	5310 ± 307abc	<QL	1033 ± 65ab	239 ± 19a	<QL	33 ± 6c
MSWC	25	450 ± 7a	0.5 ± 0.1a	13.0 ± 0.5bc	6248 ± 472bc	17 ± 2a	958 ± 184ab	240 ± 15a	2.7 ± 0.1ab	11 ± 4ab
	50	446 ± 23a	0.5 ± 0.1a	19.9 ± 0.7 e	6202 ± 578bc	<QL	759 ± 78ab	264 ± 26ab	<QL	7.9 ± 0.8a
	100	495 ± 22abc	0.6 ± 0.1a	20 ± 3 e	6599 ± 520c	<QL	800 ± 50ab	304 ± 33b	<QL	18 ± 5b
GWC	25	480 ± 6ab	1.4 ± 0.3a	13.5 ± 0.1cd	5631 ± 416abc	43 ± 7a	1387 ± 187b	252 ± 7a	4.8 ± 0.7b	8.1 ± 0.7a
	50	516 ± 17abc	<QL	10.9 ± 0.4abc	3699 ± 97[a]	11 ± 1a	633 ± 185a	281 ± 15ab	1.9 ± 0.1a	5.6 ± 0.7a
	100	578 ± 28c	<QL	11.6 ± 0.7abc	4151 ± 402ab	<QL	1136 ± 451ab	262 ± 15ab	0.6 ± 0.1a	6.1 ± 0.5a

QL, quantification limit; QL (Cu) = 0.5 mg kg^{-1} DW; QL (Pb) = 5 mg kg^{-1} DW; QL (Zn) = 0.5 mg kg^{-1} DW; MSWC, municipal solid waste compost; GWC, garden waste compost; SS, sewage sludge; DW, dry weight; SD, standard deviation.
[a] Extracted by 0.01 M calcium chloride.
[b] Extracted by 0.5 M ammonium acetate, 0.5 M acetic acid and 0.02 M EDTA, pH 4.7.

ity/bioavailability of metals in soils should be monitored regularly.

3.3. Effects of organic residues on Cu, Pb and Zn speciation

Copper, Pb and Zn, the three most abundant metals in the mine-contaminated soil, were mainly found in the residual fraction (94.6 ± 0.6% for Cu, 85.7 ± 1.7% for Pb and 93.6 ± 0.1% for Zn, mean ± standard deviation, $n = 9$), with less than 2% of their total content extracted in the first step (water-soluble, exchangeable, and weakly bound to OM), which can be considered a relative empirical estimate of metal bioavailability, and 2–3% of their total content released by oxidation of organic matter and sulphur (extracted in the third step). Lead was also found associated with Fe–Mn oxides (10.6 ± 1.0% of the total content, extracted in the second step). Usually this metal is only sparingly mobile in soils, and probably mostly unavailable to plants (Kabata-Pendias and Adriano, 1995), which also explains the low Pb mobile/bioavailable content, despite its extremely high pseudo-total concentrations.

Copper and Pb extracted in the first step decreased as a consequence of the application of all the organic residues (Fig. 2a and b), in agreement with the results obtained by the CaCl$_2$ extraction. The decrease in Pb was not achieved at the expense of an increase in its content in the fraction associated with the reducible phases (e.g. bound to Fe/Mn oxyhydroxides) or in the fraction of oxidisable metals (e.g. bound to organic matter or sulphides). In fact, the sum of the Pb concentrations extracted by the three steps decreased, irrespectively of the organic residue applied, which can be attributed to its precipitation at the higher pH values achieved with soil amendments.

In this study, as in others (Narwal and Singh, 1998), Cu extractable fraction is predominantly associated with organic matter (Fig. 2a), which is consistent with the known affinity of Cu for organic ligands (Hue, 1995). Amending the soil with MSWC led to an increase in the Cu bound to organic matter or sulphides (i.e., extracted in the third step). This was probably due to the similar Cu concentrations found in MSWC and in the soil (Table 1), but also to the high-humified organic OM content of this type of compost, in opposition to the less stabilized OM content of the SS used.

Zinc speciation showed a different pattern from that of Cu and Pb (Fig. 2c). Zinc extracted in the first step was enhanced following SS application and when using MSWC at the highest application rate. In fact, the sum of Zn extracted in the three steps increased as a consequence of both SS and MSWC application.

Zinc is more "mobile metal" and may exist mainly as an inorganically complexed, exchangeable, and water-soluble form (Kabata-Pendias and Adriano, 1995). As a consequence, a single extraction procedure, with a neutral unbuffered salt solution, like CaCl$_2$, might underestimate the potential risks arising from the application of organic residues with high Zn content.

3.4. Effects of organic residues on plant growth and on Cu, Pb and Zn concentrations

Ryegrass grew well on all pots, except in those where 100 Mg ha^{-1} of SS or MSWC were applied, without any visible symptoms of metal toxicity or nutrient deficiency. The greatest increase in its relative growth (more than three times) was obtained in the presence of 50 Mg MSWC ha^{-1}, followed by SS at the same application rate (Table 3). The relative growth observed with GWC application was not significantly different from that obtained in the mine soil ($P > 0.05$). The fact that GWC was less capable of correcting soil acidity and of supplying essential macronutrients (N, P, K), should be the main explanation for the different relative growth observed. Other authors also mentioned the low N content of this type of compost, and the need for additional N fertilization (Keeling et al., 2003).

An impaired growth was registered when using 100 Mg ha^{-1} of both SS and MSWC that, in the case of SS application, it was very notorious. This probably derived from soluble salts present in the residues, rather than to the presence of trace metals that, as discussed above, were less bioavailable after soil amendments addition.

Copper and Pb concentrations in ryegrass shoots were not significantly different following organic residues addition, compared with the control soil (data not shown). The same was true for Zn when applying SS and MSWC. However, Zn concentration in the ryegrass shoots decreased significantly following the application of 100 Mg GWC ha^{-1}. So, in this particular situation, *L. perenne* could not be considered as a good "indicator" of Cu, Pb and Zn availability in the soil (Baker, 1981), because the effective bioavailable/mobile fractions of Cu, Pb and Zn decreased in the soil, as a consequence of the treatments and the same was not true for their concentrations in the aboveground tissue.

Lead concentrations in the shoots (140 ± 97 mg kg^{-1} DW, $n = 36$) were considerably higher than the values found by Arienzo et al. (2004) for the same plant, grown on less Pb contaminated soil, considered as typical concentrations of contaminated plants
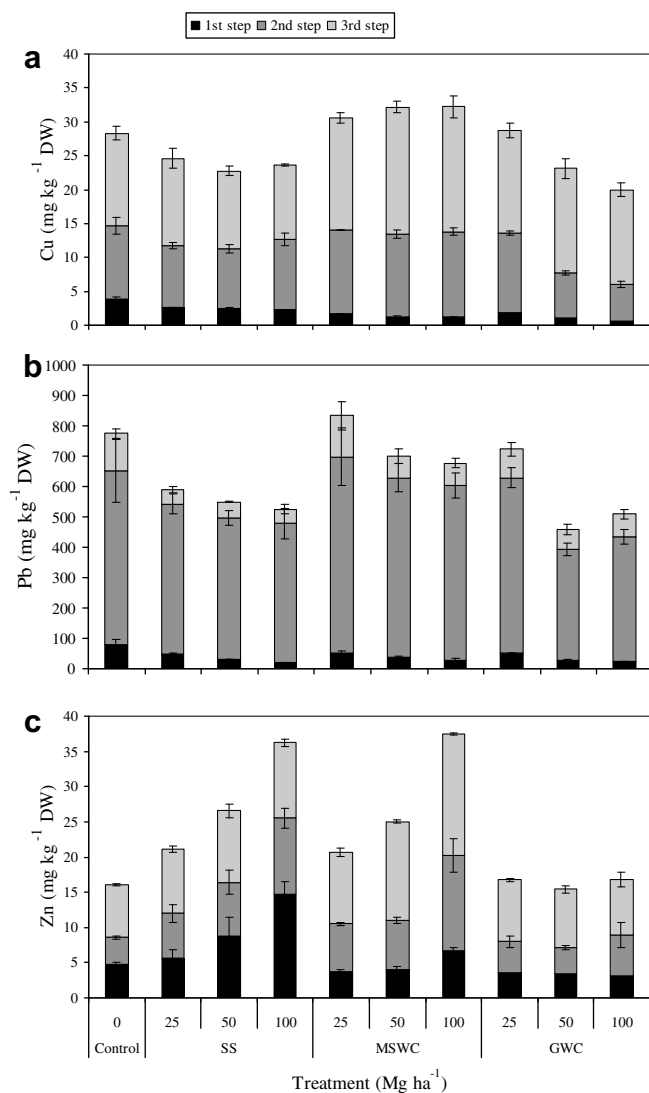
Fig. 2. Metal concentrations in the soil (mg kg⁻¹ DW) after fractionation by the BCR procedure (mean ± SD, $n = 3$, $n = 9$ for the control): 1st Step (exchangeable fraction), 2nd step (reducible fraction), 3rd step (oxidizable fraction) (residual fraction not shown): (a) Cu, (b) Pb, and (c) Zn. MSWC: municipal solid waste compost; GWC: garden waste compost; SS: sewage sludge.

Table 3
Relative growth of perennial ryegrass grown in the different soil treatments (mean ± SD, $n = 3$, $n = 9$ for the control). Values marked with the same letter are not significantly different (Tukey test, $P > 0.05$).

Organic amendment (Mg ha⁻¹)	Relative growth (%)
Control soil	100 ± 23a
SS	
25	197 ± 15bc
50	277 ± 57de
100	118 ± 7a
MSWC	
25	240 ± 22cd
50	323 ± 46e
100	238 ± 49cd
GWC	
25	138 ± 4ab
50	159 ± 10ab
100	158 ± 13ab

MSWC, municipal solid waste compost; GWC, garden waste compost; SS, sewage sludge; SD, standard deviation.

by Ross (1994). Pichtel and Salt (1998) also found elevated concentrations of Pb on *L. perenne* and other grasses grown on mine wastes. Copper concentrations (29 ± 15 mg kg⁻¹ DW, $n = 36$) were similar to, or slightly higher than the values found by Arienzo et al. (2004), but also with concentrations typical of contaminated plants (Ross, 1994). Zinc concentrations in the shoots (59 ± 22 mg kg⁻¹ DW, $n = 36$) were below the values found on contaminated plants (Ross, 1994), and below the values reported by Arienzo et al. (2004). Santibáñez et al. (2008), when using *L. perenne* for the phystabilization of Cu mine tailings amended with biosolids, found that Cu, Zn, Mo and Cd were mainly accumulated in the roots and that only a small amount was translocated to shoots.

The results obtained in this study suggest that, although perennial ryegrass seems appropriate to be used in aided phytostabilization programs, the high Pb concentrations found in the shoots increases the risk of metal transfer to the food chain, which should be further elucidated.

3.5. Pearson's correlations

Pearson's correlations between soil physico-chemical properties and plant related parameters were calculated ($n = 36$; Table 4). The strength of the associations was interpreted according to the Hopkins' correlation classification (Hopkins, 2002): insubstantial (0.0–0.1), low (0.1–0.3), moderate (0.3–0.5), high (0.5–0.7), very high (0.7–0.9) and nearly perfect (0.9–1.0). Correlation analysis showed high positive relationship between soil pH and plant relative growth, and high negative correlations between mobile Cu, Pb and Zn and plant growth. In fact, the acidity correction allowed by SS and MSWC application was a strong factor contributing to the establishment of a healthy plant cover. Moreover, available P and available K were moderately correlated to plant growth.

The role played by the organic amendments in the *in situ* immobilization of metals was corroborated by the moderate, high or very high negative correlation coefficients obtained between mobile Cu, Pb and Zn and other soil chemical characteristics that rose as a consequence of the treatments, i.e., pH, EC, soil OM, soil N, available P and available K. Conversely, mobilisable Cu and Zn were high or very high positively correlated with the same properties, due to their high content both in SS and MSWC.

Neither the 0.01 M CaCl₂-extractable Cu, Pb and Zn concentrations, nor the metal concentrations obtained in the first step of the BCR sequential extraction procedure, were correlated with their contents in the plant. This fact is indicating that Cu, Pb and Zn concentrations in the plant were not markedly affected by treatments, and corroborates the fact that *L. perenne* could not be considered as a good "indicator" of Cu, Pb and Zn availability in the soil (Baker, 1981). Similar results were obtained by Pichtel and Salt (1998), using *L. perenne* and other grasses grown on metal-contaminated wastes: no extractant was able to represent metal uptake by a particular grass in a linear fashion, and metal concentrations in the aboveground plant material were not correlated or were only poorly correlated with the soil physico-chemical properties or metal concentrations.

Copper and Pb concentrations extracted with a 0.01 M CaCl₂ solution, which represent the soluble and easily exchangeable metal fraction in the soil (Houba et al., 1996; Pueyo et al., 2004; Walker et al., 2004; Pérez-de-Mora et al., 2006) were highly positively correlated with their concentrations obtained in the first step of BCR sequential extraction procedure. The same was not true for Zn, which behaved differently regarding the sequential extraction procedure, as already evidenced: Zn extracted in the first step of BCR sequential extraction procedure was very highly correlated ($r = 0.85$) with the mobilisable Zn content, and both increased with increasing SS and MSWC application rates (Table 2 and Fig. 2c).

Table 4
Pearson's correlation coefficients between soil physico-chemical properties and plant related parameters ($n = 36$).

	pH	EC	OM	N_{soil}	P_{avail}	K_{avail}	Cu_{mobile}	Pb_{mobile}	Zn_{mobile}	$Cu_{mobilisable}$	$Zn_{mobilisable}$	Cu_{plants}	Pb_{plants}	Zn_{plants}	Cu_{1BCR}	Pb_{1BCR}	Zn_{1BCR}
EC	0.92*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
OM	0.87*	0.91*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
N_{soil}	0.73*	0.91*	0.81*	–	–	–	–	–	–	–	–	–	–	–	–	–	–
P_{avail}	0.83*	0.75*	0.57*	0.53*	–	–	–	–	–	–	–	–	–	–	–	–	–
K_{avail}	0.79*	0.59*	0.50*	0.28	0.91*	–	–	–	–	–	–	–	–	–	–	–	–
Cu_{mobile}	−0.71*	−0.58*	−0.73*	−0.48*	−0.35*	−0.43*	–	–	–	–	–	–	–	–	–	–	–
Pb_{mobile}	−0.74*	−0.66*	−0.76*	−0.53*	−0.41*	−0.46*	0.90*	–	–	–	–	–	–	–	–	–	–
Zn_{mobile}	−0.86*	−0.76*	−0.85*	−0.57*	−0.54*	−0.58*	0.79*	0.81*	–	–	–	–	–	–	–	–	–
$Cu_{mobilisable}$	0.81*	0.67*	0.57*	0.47*	0.79*	0.83*	−0.44*	−0.50*	−0.73*	–	–	–	–	–	–	–	–
$Zn_{mobilisable}$	0.62*	0.79*	0.59*	0.88*	0.57*	0.31	−0.23	−0.28	−0.35*	0.46*	–	–	–	–	–	–	–
Cu_{plants}	0.02	−0.13	−0.20	−0.27	0.19	0.33	−0.03	0.14	0.13	0.17	−0.08	–	–	–	–	–	–
Pb_{plants}	0.13	−0.15	−0.21	−0.29	0.15	0.38*	−0.18	−0.01	−0.07	0.31	−0.13	0.64*	–	–	–	–	–
Zn_{plants}	−0.11	−0.30	−0.45*	−0.32	0.08	0.17	0.12	0.34*	0.29	0.06	−0.11	0.57*	0.80*	–	–	–	–
Cu_{1BCR}	−0.64*	−0.44*	−0.65*	−0.20	−0.34*	−0.54*	0.85*	0.79*	0.80*	−0.53*	0.00	−0.18	−0.23	0.22	–	–	–
Pb_{1BCR}	−0.67*	−0.67*	−0.77*	−0.54*	−0.38*	−0.38*	0.63*	0.75*	0.77*	−0.49*	−0.29	0.24	0.26	0.57*	0.62*	–	–
Zn_{1BCR}	0.51*	0.75*	0.57*	0.90*	0.43*	0.10	−0.17	−0.25	−0.28	0.26	0.85*	−0.29	−0.36*	−0.24	0.15	−0.31	–
Rel growth	0.51*	0.31	0.30	0.14	0.36*	0.48*	−0.61*	−0.58*	−0.55*	0.46*	−0.07	0.05	0.45	0.27	−0.50*	−0.35*	−0.06

* Marked correlations are significant at $P < 0.05$; EC, electrical conductivity; OM, soil organic matter; N_{soil}, soil total nitrogen; P_{avail}, available P; K_{avail}, available K; Cu_{mobile}, Pb_{mobile} and Zn_{mobile}, mobile metal content (extracted by 0.01 M $CaCl_2$); $Cu_{mobilisable}$ and $Zn_{mobilisable}$, mobilisable metal content (extracted by a solution of 0.5 M NH_4CH_3COO, 0.5 M CH_3COOH and 0.02 M EDTA, pH 4.7); Cu_{plants}, Pb_{plants} and Zn_{plants}, metal concentrations in the aboveground plant material; Cu_{1BCR}, Pb_{1BCR}, and Zn_{1BCR}, metal fraction obtained using the 1st step of BCR sequential extraction procedure (extracted by 0.11 M acetic acid).

3.6. Evaluation of treatments using PCA

PCA allows associations between variables, reducing the dimension of the data matrix. This is accomplished by diagonalization of the correlation matrix of the data, which transforms the original variables into the same number of uncorrelated ones called PCs. The eigenvalues of the PCs are a measure of their variance, the participation of the original variables in the PCs is given by the loadings, and the individual transformed observations are called scores (Helena et al., 2000). The PCs provide information on the most meaningful parameters, which describe the whole data set, affording a reduction of data with minimum loss of the original information (Helena et al., 2000; Abollino et al., 2002).

PCA was carried out on 15 soil and plant variables to assess the influence of each treatment on soil parameters (pH, EC, OM content, soil $N_{Kjeldahl}$, available P, available K, mobile Cu, Pb and Zn, mobilisable Cu and Zn, *L. perenne* relative growth, and Cu, Pb and Zn concentrations in the shoots).

The first component (PC1) explained 52% of the variance of the original variables and the second component (PC2) explained 21%. The original 15 variables could thus be reduced to two principal components, as they could explain about 73% of the total variance. pH, EC, OM content, soil $N_{Kjeldahl}$, available P, available K, and mobilisable Cu, all with positive values, had large factor loadings on PC1 (loadings > 0.70). Mobile Cu, Pb and Zn also had large loadings on PC1, but with negative values. Copper, Pb and Zn concentrations in the aboveground plant material had large factor loadings on PC2. Neither mobilisable Zn nor *L. perenne* relative growth had significant correlations to the principal components.

Fig. 3 shows the combined plot of scores and loadings on PC1 *versus* PC2. The agglomerative hierarchical clustering procedure was used to evaluate similarities among samples. Fig. 3 also shows sample clustering considering a linkage distance <0.75. Unamended soil samples were located on the left side of the map, with nine points (replicates) (Fig. 3). This means that they had the highest mobile/soluble Cu, Pb and Zn contents and the lowest values for all other soil chemical parameters, which rose with incorporation of organic residues, shifting the amended soil samples to the right, relative to the unamended soil samples. Treatments corresponding to 100 Mg SS ha^{-1} or to 100 Mg MSWC ha^{-1} were located on the right side of the map, on the most positive part of PC1 axis, showing that they had the greatest values for pH, EC, OM content, soil
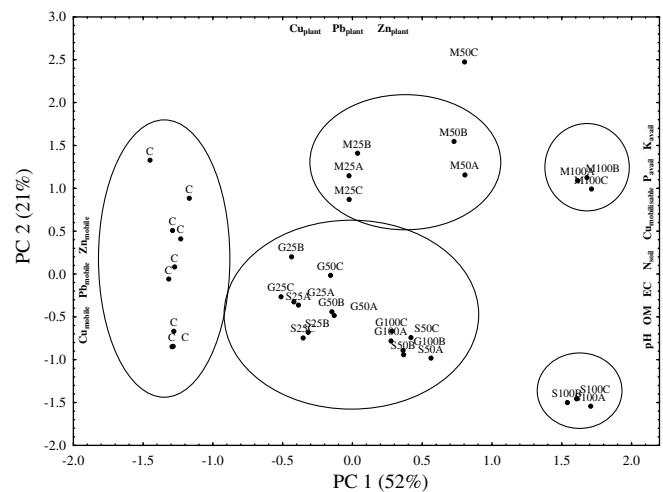

Fig. 3. Scores of each sample on the two main principal components. The most important parameters for the definition of the two components are shown on the edge of each axis, indicating the direction in which the value of the parameter increases (EC, electrical conductivity; OM, soil organic matter; N_{soil}, soil total nitrogen; P_{avail}, available P; K_{avail}, available K; Cu_{mobile}, Pb_{mobile} and Zn_{mobile}, mobile metal content; $Cu_{mobilisable}$, mobilisable Cu; Cu_{plants}, Pb_{plants} and Zn_{plants}, metal concentrations in the aboveground plant material). Samples were clustered according to the results obtained from the hierarchical cluster analysis (linkage distance < 0.75) PC1: first principal component; PC2, second principal component; S, sewage sludge; M, municipal solid waste compost; and G, garden waste compost; followed by 25, 50 and 100, indicating the application rate in Mg ha^{-1}. A, B and C refers to each replica (each treatment was replicated three times, generating three points on the PCA plot). C: control soil (nine replicates).

$N_{Kjeldahl}$, available P, available K, and mobilisable Cu, and the smallest values for mobile Cu, Pb and Zn content.

Soil samples amended with GWC and with 25 and 50 Mg SS ha^{-1} were clustered, centred relatively to the PC1 axis. This means that we were not able to differentiate among these amendments based on the variables used to describe PC1. The same was true for the soil samples amended with 25 and 50 Mg MSWC ha^{-1}, that were also clustered (with one replicate outside the cluster), similarly positioned concerning PC1. However, these samples were situated in the positive part of PC2 axis, evidencing a tendency to present higher overall Cu, Pb and Zn content in the above ground plant material.

4. Conclusions

The organic residues tested corrected soil acidity, with a better performance in the case of MSWC and SS. Both composts raised soil OM, but SS led to greater soil OM values, and provided the highest soil N content, due to its lower C:N ratio. In contrast, GWC was very ineffective in correcting soil N, and nitrogen fertilizers should be added to overcome this situation. However, its low salinity makes it an attractive organic amendment to be used in soil remediation.

The organic amendments were also effective in the *in situ* immobilization of metals, as shown by the negative Pearson's correlation coefficients obtained between mobile Cu, Pb and Zn fractions and other soil chemical characteristics, which rose as a consequence of the amendments addition, i.e., pH, EC, soil OM, soil N, available P and available K. This was confirmed by the multivariate exploratory techniques performed on the results, evidencing a clear negative correlation between the two groups of variables.

The greatest increase in *L. perenne* relative growth was obtained in the presence of 50 Mg MSWC ha^{-1}, and to a lesser extent SS at the same application rate. Amending the soil with 100 Mg ha^{-1} of either SS or MSWC did not lead to a greater biomass accumulation, probably due to the high soluble salt content present in these residues.

The results obtained in this study suggest that *L. perenne* seems appropriate for aided phytostabilization programs. However, the high Pb concentrations found in the aboveground plant material could increase the risk of metal transfer to the food chain, which should be further elucidated.

MSWC, followed by SS, applied at 50 Mg ha^{-1}, were effective organic residues to be used in the immobilization of metals, in this type of mine contaminated soil, improving the soil chemical properties and allowing the greatest increase in plant biomass. Further research is needed to evaluate long-term effects of the organic residues under field conditions.

Acknowledgements

References

Abollino, O., Aceto, M., Malandrino, M., Mentasti, E., Sarzanini, C., Petrella, F., 2002. Heavy metals in agricultural soils from Piedmont, Italy. Distribution, speciation and chemometric data treatment. Chemosphere 49, 545–557.

Alvarenga, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., in press. Reclamation of a mine contaminated soil using biologically reactive organic matrices. Waste Manage. Res. doi: 10.1177/0734242X08091556.

Alvarenga, P., Palma, P., Gonçalves, A.P., Fernandes, R.M., Baião, N., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008a. Assessment of chemical, biochemical and ecotoxicological aspects in a mine soil amended with sludge of either urban or industrial origin. Chemosphere 72, 1774–1781.

Alvarenga, P., Palma, P., Gonçalves, A.P., Fernandes, R.M., Cunha-Queda, A.C., Duarte, E., Vallini, G., 2007. Evaluation of chemical and ecotoxicological characteristics of biodegradable organic residues for application to agricultural land. Environ. Int. 33, 505–513.

Alvarenga, P., Palma, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008b. Evaluation of tests to assess the quality of mine contaminated soils. Environ. Geochem. Hlth. 30, 95–99.

Arienzo, M., Adamo, P., Cozzolino, V., 2004. The potential of *Lolium perenne* for revegetation of contaminated soils from a metallurgical site. Sci. Total Environ. 319, 13–25.

Baker, A.J.M., 1981. Accumulators and excluders – strategies in the response of plants to heavy metals. J. Plant Nutr. 3, 643–654.

Basta, N.T., Gradwohl, R., Snethen, K.L., Schroder, J.L., 2001. Chemical immobilization of lead, zinc, and cadmium in smelter-contaminated soils using biosolids and rock phosphate. J. Environ. Qual. 30, 1222–1230.

Basta, N.T., Sloan, J.J., 1999. Bioavailability of heavy metals in strongly acidic soils treated with exceptional quality biosolids. J. Environ. Qual. 28, 633–638.

Brown, S.L., Henry, C.H., Chaney, R., Compton, H., Volder, P.S.D., 2003. Using municipal biosolids in combination with other residuals to restore metal-contaminated areas. Plant Soil 249, 203–215.

Clemente, R., Escolar, Á., Bernal, M.P., 2006. Heavy metals fractionation and organic matter mineralisation in contaminated calcareous soil amended with organic materials. Bioresource Technol. 97, 1894–1901.

Decreto-Lei n° 118/2006, de 21 de Junho, 2006. Diário da República n° 118 – I Série A. Ministério do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional. Lisboa.

Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In: Klute, A. (Ed.), Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods. Soil Science Society of America, Madison, Wisconsin, USA, pp. 383–412.

Geebelen, W., Vangronsveld, J., Adriano, D.C., Carleer, R., Clijsters, H., 2002. Amendment-induced immobilization of lead in a lead-spiked soil: evidence from phytotoxicity studies. Water Air Soil Pollut. 140, 261–277.

Gupta, S.K., Vollmer, M.K., Krebs, R., 1996. The importance of mobile, mobilisable and pseudo total heavy metal fractions in soil for three-level risk assessment and risk management. Sci. Total Environ. 178, 11–20.

Hammer, D., Keller, C., 2002. Changes in the rhizosphere of metal-accumulating plants evidenced by chemical extractants. J. Environ. Qual. 31, 1561–1569.

Helena, B., Pardo, R., Vega, M., Barrado, E., Fernández, L., 2000. Temporal evolution of groundwater composition in an alluvial aquifer (Pisuerga River, Spain) by principal component analysis. Water Res. 34, 807–816.

Hopkins, W.G., 2002. A scale of magnitudes for effect statistics. In: Internet Society of Sport Science. A New View of Statistics. <http://www.sportsci.org/resource/stats/effectmag.html> (accessed 02.05.08).

Houba, V.J.G., Lexmond, T.M., Novozamsky, I., van der Lee, J.J., 1996. State of the art and future developments in soil analysis for bioavailability assessment. Sci. Total Environ. 178, 21–28.

Hue, N.V., 1995. Sewage sludge. In: Rechcigl, J.E. (Ed.), Soil Amendments and Environmental Quality. Lewis Publishers, Florida, pp. 199–247.

ISO 11466, 1995. Soil quality – Extraction of Trace Elements Soluble in Aqua Regia. International Organisation for Standardisation. Geneva, Switzerland.

Kabata-Pendias, A., Adriano, D.C., 1995. Trace metals. In: Rechcigl, J.E. (Ed.), Soil Amendments and Environmental Quality. Lewis Publishers, Florida, pp. 139–167.

Keeling, A.A., McCallum, K.R., Beckwith, C.P., 2003. Mature green waste compost enhances growth and nitrogen uptake in wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.) through the action of water-extractable factors. Bioresource Technol. 90, 127–132.

Narwal, R.P., Singh, B.R., 1998. Effect of organic materials on partitioning, extractability and plant uptake of metals in an alum shale soil. Water Air Soil Pollut. 103, 405–421.

Pérez-de-Mora, A., Burgos, P., Madejón, E., Cabrera, F., Jaeckel, P., Schloter, M., 2006. Microbial community structure and function in a soil contaminated by heavy metals: effects of plant growth and different amendments. Soil Biol. Biochem. 38, 327–341.

Pichtel, J., Salt, C.A., 1998. Vegetative growth and trace metal accumulation on metaliferous waste. J. Environ. Qual. 27, 618–624.

Pueyo, M., López-Sanchez, J.F., Rauret, G., 2004. Assessment of CaCl$_2$, NaNO$_3$ and NH$_4$NO$_3$ extraction procedures for the study of Cd, Cu, Pb and Zn extractability in contaminated soils. Anal. Chim. Acta 504, 217–226.

Rauret, G., López-Sanchez, J.-F., Sahuquillo, A., Barahona, E., Lachica, M., Ure, A.M., Davidson, C.M., Gomez, A., Lück, D., Bacon, J., Yli-Halla, M., Muntau, H., Quevauviller, P., 2000. Application of a modified BCR sequential extraction (three-step) procedure for the determination of extractable trace metal contents in a sewage sludge amended soil reference material (CRM 483), complemented by a three-year study of acetic acid and EDTA extractable metal content. J. Environ. Monitor. 2, 228–233.

Riehm, H., 1958. Die ammoniumlaktatessigsäure-methode zur bestimmung der leichtloeslichen phosphosäure in karbonathaltigen boden. Agrochimica 3, 49–65.

Rizzi, L., Petruzzelli, G., Poggio, G., Guidi, G.V., 2004. Soil physical changes and plant availability of Zn and Pb in a treatability test of phytostabilization. Chemosphere 57, 1039–1046.

Rodgers, C.S., Anderson, R.C., 1995. Plant growth inhibition by soluble salts in sewage sludge-amended mine spoils. J. Environ. Qual. 24, 627–630.

Ross, S.M., 1994. Retention, transformation and mobility of toxic metals in soils. In: Ross, S.M. (Ed.), Toxic Metals in Soil–Plant Systems. John Wiley & Sons, Chichester, pp. 63–153.

Sahuquillo, A., Rigol, A., Rauret, G., 2003. Overview of the use of leaching/extraction tests for risk assessment of trace metals in contaminated soils and sediments. Trends Anal. Chem. 22, 152–159.

Santibáñez, C., Verdugo, C., Ginocchio, R., 2008. Phytostabilization of copper mine tailings with biosolids: Implications for metal uptake and productivity of *Lolium perenne*. Sci. Total Environ. 395, 1–10.

StatSoft, Inc., 2001. STATISTICA 6.0 – Data Analysis Software System.

Sumner, M.E., Miller, W.P., 1996. Cation exchange capacity and exchange coefficients. In: Bartels, J.M. (Ed.), Methods of Soil Analysis. Part 3 – Chemical Methods. Soil Science Society of America, Madison, Wisconsin, pp. 1201–1230.

Tordoff, G.M., Baker, A.J.M., Willis, A.J., 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere 41, 219–228.

Ure, A., 1996. Single extraction schemes for soil analysis and related applications. Sci. Total Environ. 178, 3–10.

van Herwijnen, R., Hutchings, T.R., Al-Tabbaa, A., Moffat, A.J., Johns, M.L., Ouki, S.K., 2007. Remediation of metal contaminated soil with mineral-amended composts. Environ. Pollut. 150, 347–354.

Vangronsveld, J., Assche, F.V., Clijsters, H., 1995. Reclamation of a bare industrial area contaminated by non-ferrous metals: *in situ* metal immobilization and revegetation. Environ. Pollut. 87, 51–59.

Walker, D.J., Clemente, R., Bernal, M.P., 2004. Contrasting effects of manure and compost on soil pH, heavy metal availability and growth of *Chenopodium album* L. in soil contaminated by pyritic mine waste. Chemosphere 57, 215–224.

Walker, D.J., Clemente, R., Roig, A., Bernal, M.P., 2003. The effects of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. Environ. Pollut. 122, 303–312.

Walkley, A., Black, J.A., 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29–38.

Wong, M.H., 2003. Ecological restoration of degraded soils, with emphasis on metal contaminated soils. Chemosphere 50, 775–780.

Zmora-Nahum, S., Hadar, Y., Chen, Y., 2007. Physico-chemical properties of commercial composts varying in their source materials and country of origin. Soil Biol. Biochem. 39, 1263–1276.