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Evaluation of composts and liming materials in the phytostabilization of a mine soil using perennial ryegrass

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ABSTRACT

A microcosm experiment was carried out to evaluate the effects of municipal solid waste compost (MSWC) or garden waste compost (GWC), and liming materials in the rehabilitation of a soil affected by mining activities, and to study the use of perennial ryegrass (Lolium perenne L.) for phytostabilization. The performance of the amendments was assessed by soil chemical parameters, total and bioavailable metals (Cu, Pb and Zn), soil enzymatic activities, and plant relative growth and mineral composition. In general, both composts corrected soil acidity and increased the total organic matter content of the soil, although with a better performance in the case of MSWC, especially when considering total N and available P and K levels in the amended soil. The application of both composts and liming materials led to a decrease in the mobile fractions of Cu, Pb and Zn, but mobilisable fractions of Cu and Zn increased with MSWC application. Plant biomass increased more than three times in the presence of 50 Mg MSWC ha^{-1} and with the combined use of 25 or 50 Mg MSWC ha^{-1} and CaO, but no significant differences were observed when GWC was applied. Plant tissue analysis showed that the treatments did not significantly reduce Cu, Pb and Zn uptake by the plant. Dehydrogenase, and the enzymes related to the N-cycle, urease and protease, had increased activities with increasing MSWC application rate. Conversely, the enzymatic activities of both enzymes related to the C-cycle, cellulase and β-glucosidase, were only positively affected by GWC application, a compost obtained from raw materials rich in C. Principal component analyses evidenced this clear separation between the effect of MSWC on soil enzymes related to the N-cycle and of GWC on soil enzymes related to the C-cycle. This study indicates that MSWC (50 Mg ha^{-1}, limed or unlimed) can be used successfully in the remediation of a highly acidic metal-contaminated soil, allowing the establishment of perennial ryegrass.

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1.   Introduction

Mineral resources have been exploited in Portugal for thousands of years, with records available at least since the Roman occupation. Abandoned metalliferous mine wastes, containing high concentrations of metals, were deposited on the surface, which resulted in the pollution of the surrounding terrestrial and aquatic ecosystems (Oliveira et al., 2002). Use of a vegetation cover provides a cost-effective and environmentally sustainable method of stabilizing and reclaiming toxic metal mine sites (Vangronsveld et al., 1995; Tordoff et al., 2000; Mench et al., 2003; Wong, 2003; Pérez-de-Mora et al., 2006). The purpose of revegetation is to stabilize the site, by providing a cover crop that will prevent dispersion of metal-contaminated particles by water and wind erosion and reduce metal mobility by rhizosphere-induced adsorption and precipitation processes (Vangronsveld et al., 1995; Arienzo et al., 2004). Phytostabilization also improves the chemical and biological characteristics of the contaminated soil by increasing the organic matter content, nutrient levels, cation exchange capacity and biological activity (Arienzo et al., 2004). Although revegetation is desirable, mine contaminated soils or metalliferous wastes are very unfavourable environments for plants due to the presence of many growth limiting factors, in particular residual high levels of heavy metals, soil acidity, lack of organic matter and its associated nutrients, and poor substrate structure with only skeletal materials (Tordoff et al., 2000; Johnson, 2003; Wong, 2003). That is why the use of organic biodegradable residues (e.g. sewage sludge, manure, compost) is increasingly being considered in land rehabilitation (Walker et al., 2004; Brown et al., 2005; Pérez-de-Mora et al., 2006). These play three major roles when applied to mine soils: (i) improvement of the physical nature of the rooting medium, especially improving water and nutrient-holding capacity; (ii) supply of plant nutrients in a slow-release form, facilitating plant establishment; and (3) in situ chemical immobilization of metals, reducing metal leachability and phytotoxicity (Tordoff et al., 2000).

According to Sutton and Dick (1987), soil acidity is the main constraint for the establishment of vegetation in these environments. Organic residues can correct soil acidity, but they may be insufficient to obtain a desired soil pH. In consequence, it is common practice to apply liming materials to overcome some of the problems associated with soil acidity (Sutton and Dick, 1987; Ye et al., 1999; Wong, 2003) or, alternatively, to apply lime-stabilized biosolids (Sloan and Basta, 1995; Basta and Sloan, 1999; Abbott et al., 2001; Basta et al., 2001; Bolan et al., 2003; Adriano et al., 2004). Several authors have demonstrated that the application of biosolids in combination with alkaline residues to severely metal-contaminated soils is sufficient to restore a vegetative cover (Pichtel et al., 1994; Mench et al., 2003; Brown et al., 2003, 2005). Alkaline amendments reduce the concentration of metals in soil solution by raising soil pH, thereby allowing the formation of insoluble metal precipitates, complexes and secondary minerals (Basta et al., 2001).

Finally, the choice of the plant is a very important aspect to consider in a phytostabilization-based technique (Tordoff et al., 2000; Wong, 2003; Arienzo et al., 2004; Rizzi et al.,

2004). Plants should develop an extensive root system and a large amount of biomass in the presence of high concentrations of heavy metals, while keeping the translocation of metals from roots to shoots as low as possible (Wong, 2003; Rizzi et al., 2004; Mendez and Maier, 2008). Several authors have reported perennial ryegrass (*Lolium perenne* L.) as a suitable species for revegetation of metalliferous wastes (Pichtel and Salt, 1998, Arienzo et al., 2004), producing high dry matter yields and accumulating moderate to high levels of metals in its biomass. Mendez and Maier (2008) clarified the noticeable contradiction: although metallophytes (i.e. metal-tolerant plants) have developed mechanisms to impede translocation of metals to the above-ground plant mass, there may still be an excessively high concentration of metals in the shoot material. Santibáñez et al. (2008), studying the distribution patterns of metals in perennial ryegrass for the phytostabilization of Cu mine tailings, found that metals were mainly accumulated in the roots and only a small amount of them were transported to the shoots.

Ecological restoration of mine degraded soils, achieved by the application of organic and inorganic amendments and phytostabilization, should be evaluated based not only on soil chemical characteristics and metal availability, but also on additional assays that measure soil microbial activity or community structure and enzyme activities related to the biocycles of nutrients (C, N, P and S) (Hinojosa et al., 2004a,b; ISO/DIS 17402, 2006; Zhang et al., 2006). For example, the dehydrogenase activity and the activity of hydrolytic enzymes, such as phosphatase, urease and β-glucosidase (Gil-Sotres et al., 2005), reflect microbial activity and represent a range of processes involved in decomposition and nutrient cycling.

The main objective of the present study was to evaluate the effects of a municipal solid waste compost (MSWC), a garden waste compost (GWC) and liming materials in the remediation of a soil affected by mining activities, and to determine the possibility of using perennial ryegrass in its revegetation. The improvement in soil quality was assessed by the combined use of chemical and biochemical parameters. The potential use of perennial ryegrass for soil stabilization was assessed by biomass accumulation and shoot mineral composition in response to the amendments applied.

2.   Materials and methods

2.1.   Characterization of soil and composts

The contaminated-soil was sampled in Aljustrel, a pyrite mine located in SW Portugal, in the western sector of the Iberian Pyrite Belt. The area is characterized by a Mediterranean mesothermic humid climate, with hot and dry summers and with an annual average rainfall of 500–650 mm (Reis and Gonçalves, 1987). The mine was extensively exploited from 1850 to 1980, when the production was discontinued. During all that time, pyrite and sulphides of several trace elements became exposed to the air and were responsible for the pollution observed in soils, superficial water and sediments, mainly through water erosion and aeolian dispersion. Mine soil was collected from the 20 cm topsoil, in October 2006, at a

site in the vicinity of the tailings (±50 m). A soil sub-sample was air-dried, passed through a 2 mm sieve and subjected to chemical characterization. Particle-size distribution was determined by the pipet method (Gee and Bauder, 1986). Soil pH (H$_2$O) was determined in a soil to deionised water suspension of 1:2.5 (w/v), and electrical conductivity (EC) in a soil to deionised water suspension of 1:5 (w/v). Total oxidizable organic carbon (C$_{org}$) was determined according to Walkley and Black (1934). Total nitrogen was analysed by the Kjeldahl method. Soil cation exchange capacity (CEC) was determined using the ammonium acetate (pH 7) method (Sumner and Miller, 1996). Available P and K were determined using the Egner–Riehm method (Riehm, 1958). All measurements, except particle-size distribution, were carried out in triplicate. Pseudo-total metal (Cd, Cr, Cu, Ni, Pb and Zn) concentrations were determined by either flame or electro-thermal 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 had sandy loam texture, highly acidic pH and it was low in organic matter content (OM), in essential nutrients (N, P and K) and in CEC (Table 1). Copper, Pb and Zn total concentrations present in the soil were high (Table 1), and they exceeded their limit values in agriculture soils for sewage sludge application allowed by Portuguese Legislation: 50 mg kg^{-1}, for Cu and for Pb, and 150 mg kg^{-1} for Zn, dry weight basis (Decreto-Lei n°118/ 2006). However, those metals were mostly structurally bound and, therefore, scarcely bioavailable, as discussed elsewhere (Alvarenga et al., 2008).

A compost from the organic fraction of unsorted municipal solid waste (MSWC), obtained in a composting plant near Setúbal (Portugal), and a garden waste compost (GWC) from a composting plant in Tavira (Portugal), which receives source-separated garden residues (namely grass clippings, leaves and brush), were used. Two different liming materials were tested, alone or in addition to the organic amendments: calcium oxide (CaO), and sugar beet sludge (SBS), a residue from a sugar manufacturing plant located in Coruche (Portugal). Sugar beet sludge consisted mostly of CaCO$_3$ (70–80% dry weight) but it also contained nutrients (Pérez-de-Mora et al., 2005), and is widely used by the farmers in the vicinity of the processing plant as a liming agent. Three replicates of each compost and of SBS were analysed using methodologies previously described by Alvarenga et al. (2007) and their characteristics are shown in Table 1. Considering the existing official guidelines, both composts had total metal contents below the established limits for sewage sludge (European Commission Council Directive 1986/278/EEC; Decreto-Lei n°118/ 2006), allowing their use in agricultural soils. According to the Working Document on "Biological Treatment of Biowaste — 2nd draft" (DG Env.A.2., 2001), the organic material from mechanical/biological treatment of mixed MSW should be termed "stabilised biowaste" instead of "compost", in order to avoid any confusion with compost produced from separately collected waste. The MSWC used falls in this category, with metal concentrations below the maximum limits allowed. As for GWC, which is produced from separately collected garden waste, a high-quality classification was expected. However, the Cd content in the GWC only allowed its classification as a Class 2 compost (DG Env.A.2., 2001).

2.2. Experimental set-up

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. The organic residues were applied at 25, 50 and 100 Mg ha^{-1} (12.8, 25.5 and 51.0 g of each compost per pot, respectively, on a dry weight basis) and the liming materials were used according to the soil lime requirement to pH 6.5 and the amendment liming capacity. A soil pH of 6.5 was set as the target because this is the optimum value for most arable crops (Alloway, 1995) and it is commonly recognized that, at this pH, nutrient availability to plants is at a maximum and toxicity at a minimum (Wong, 2003). CaO was applied using 4.6 Mg ha^{-1} and SBS using 8.8 Mg ha^{-1} (2.4 and 4.5 g per pot, respectively, dry weight basis). The amendments were tested alone or in combination, using a complete randomised block design with three replicates per treatment. A control, which did not receive any amendment, was included for each organic residue tested (control soil). 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 (*Lolium perenne* L., Var. Concerto) and kept in a glasshouse under day/night

Table 1 – Characteristics of the soil and of the organic and inorganic amendments used in the study (mean± standard deviation, $n=3$)				
Parameter	Soil	MSWC	GWC	SBS
pH	3.9±0.2	8.2±0.2	7.9±0.1	8.6±0.3
EC (dS m^{-1})	0.207±0.07	5.69±0.09	2.47±0.08	1.33±0.08
Organic matter (% DW)	0.37±0.02	37.2±0.4	39±2	11.6±2.7
CEC (cmol kg^{-1})	5.6±0.5	–	–	–
Texture (g kg^{-1})	Sand 704	–	–	–
	Silt 177	–	–	–
	Clay 119	–	–	–
N$_{Kjeldahl}$ (% DW)	0.07±0.1	1.8±0.4	1.0±0.4	0.37±0.08
Extractable-P (mg kg^{-1} DW)	n.d.	–	–	–
Extractable-K (mg kg^{-1} DW)	52±2	–	–	–
Total P (% DW)	–	0.61±0.09	0.094±0.004	0.6±0.2
Na (g kg^{-1} DW)	–	7.0±0.7	15.1±0.7	0.75±0.07
K (g kg^{-1} DW)	–	8.1±0.9	7.6±0.3	0.21±0.05
Ca (g kg^{-1} DW)	–	78±2	55±3	345±22
Mg (g kg^{-1} DW)	–	18.1±0.9	8.4±0.8	9.3±0.7
Cd (mg kg^{-1} DW)	2.6±0.2	4.3±1.1	1.4±0.1	<0.5
Cr (mg kg^{-1} DW)	21.8±0.6	56±13	13±1	6.4±0.2
Cu (mg kg^{-1} DW)	362±23	357±12	14±2	27±8
Ni (mg kg^{-1} DW)	15.4±0.4	56±6	16±3	<1
Pb (mg kg^{-1} DW)	4350±169	269±24	34±3	12±2
Zn (mg kg^{-1} DW)	245±64	583±26	35±10	51±4

DW: dry weight; EC: electrical conductivity; CEC: cation exchange capacity; n.d.: not detected.

temperatures of 22–30/10–16 °C, at natural light conditions. The pots were watered daily 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. Shoot dry mass was registered and used to calculate the plant relative growth, considering the value obtained in the control pots, which did not receive any amendment, as the reference. Total nitrogen was determined using the Kjeldahl method.

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. The digested samples were analysed for total P, K, Ca, Mg, Cu, Pb and Zn using the procedures described previously (Alvarenga et al., 2007).

2.4. Soil characterization after remediation

2.4.1. Chemical analysis

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 bioavailable fractions were determined using two different single step extractions: 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 biovailable metal fraction" (Gupta et al., 1996). 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 3000 g for 10 min.

2.4.2. Enzymatic activities

Soil sub-samples were kept refrigerated (4 °C) at their "field moisture content". Before analysis, samples were sieved through a 2 mm sieve, and their dry matter content was determined to express the enzymatic activity on a dry matter basis. Dehydrogenase activity was determined according to Tabatabai (1994). Acid phosphomonoesterase (EC 3.1.3.2) and β-glucosidase (EC 3.2.1.21) activities were measured by incubating the soil with a substrate containing a p-nitrophenyl group in its structure, according to Eivazi and Tabatabai (1977, 1988). Cellulase activity was determined according to Hope and Burns (1987). Cellulases are enzyme systems that degrade cellulose and release reducing sugars as the end product. In the context of this work, the term refers to the combined action of endo-1,4-β-D-glucanase (EC 3.2.1.4), exo-1,4-β-D-glucanase (EC 3.2.1.91) and β-D-glucosidase (EC 3.2.1.21) on Avicel, a purified depolymerised alpha cellulose. Urease (EC 3.5.1.5), which catalyses the hydrolysis of urea to CO_2 and NH_3, was determined according to Kandeler and Gerber (1988). Protease activity was determined spectrophotometricaly after the incubation of the soil with sodium caseinate (Ladd and Butler, 1972; Alef and Nannipieri, 1995). All analytical measurements were carried out in duplicate.

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 (HSD) test was used to further elucidate differences among means ($P < 0.05$). For statistical analysis purposes, results below the detection limit of the method used, although reported as "not detected", were assumed to be equal to the quantification limit value. Pearson correlation coefficients were calculated between soil chemical properties, mineral concentrations in the shoots and relative growth, and soil enzymatic activities. 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 and a cluster analysis. All statistical analyses were carried out with the software Statistica 6.0 (StatSoft, Inc., 2001).

3. Results and discussion

3.1. Effects of amendments on soil chemical characteristics

Considering the results at the end of the experiment, both composts corrected soil acidity, with a better performance in the case of MSWC, in particular when in combination with SBS, which, in some cases, led to greater pH values than the GWC application ($P < 0.05$) (Fig. 1a). For the treatment with GWC, a soil pH above 6 was only obtained with an application rate of 100 Mg ha^{-1} and the combined use of SBS or CaO.

All amendments led to a significant increase in EC values, more pronounced in the case of MSWC application, and especially when liming materials were also used (Fig. 1b). Total organic matter content of the soil rose to levels above 1% when 100 Mg ha^{-1} of either of the organic residues was applied (Fig. 1c). In contrast, the organic residues were significantly different in their capacity to improve soil organic nitrogen content ($N_{Kjeldahl}$) (Fig. 1d). For the highest application rates, the nitrogen content achieved with MSWC was almost twice that of GWC. That difference was even more pronounced when considering available P (Fig. 1e) and available K (Fig. 1f), which presented substantially higher values when MSWC was applied.

Soil Cu, Pb and Zn pseudo-total concentrations did not change significantly due to the amendments ($P < 0.05$), relative to the control soil (mean values: 510±49 mg kg^{-1} DW for Cu, 5431±1300 mg kg^{-1} DW for Pb, and 272±24 mg kg^{-1} DW for Zn). As a consequence, soil Cu, Pb and Zn pseudo-total concentrations were not correlated, or were only poorly
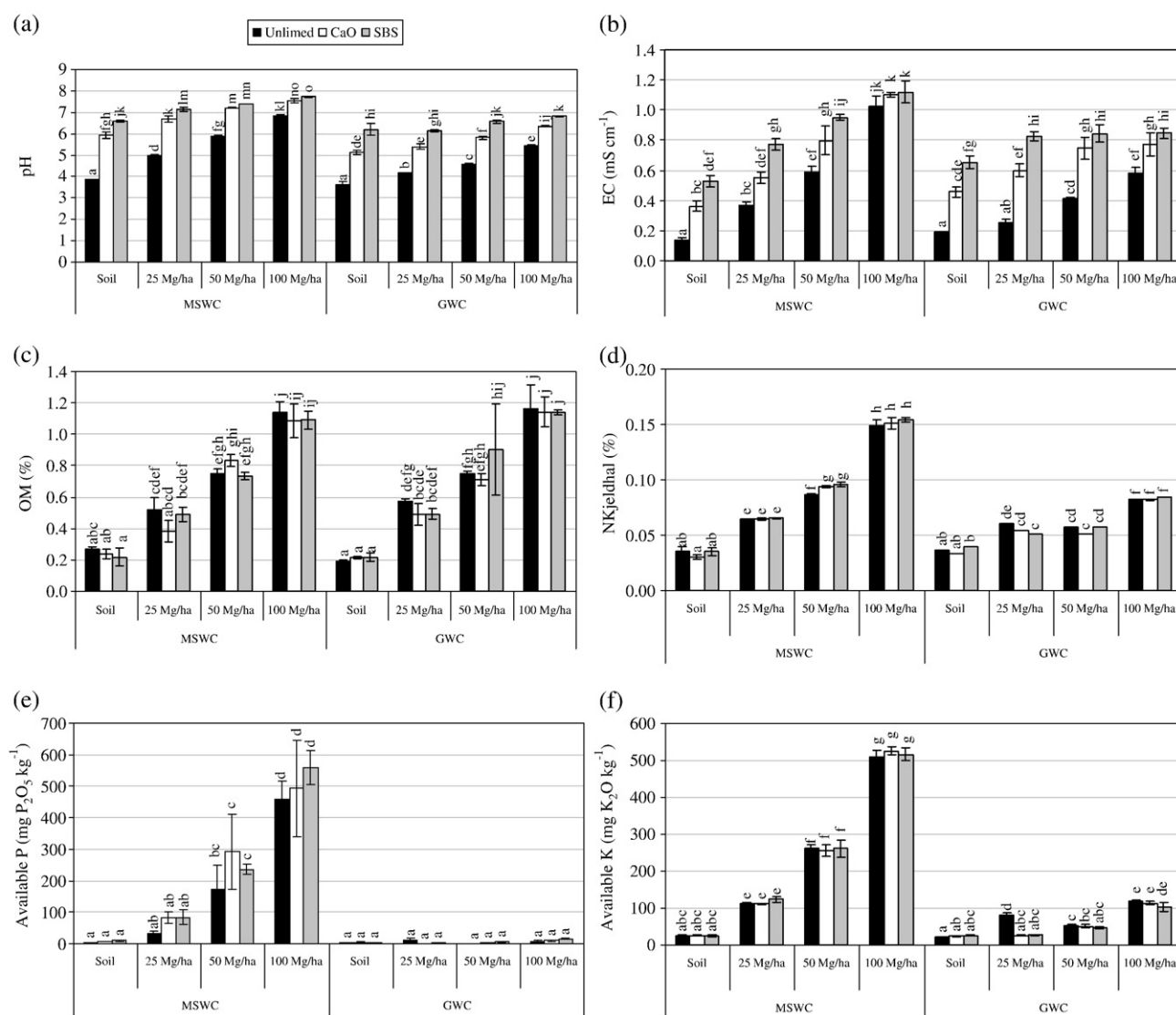
Fig. 1 – Soil chemical characteristics obtained with the different amendments: (a) pH; (b) electrical conductivity (EC) (mS cm^{-1}); (c) total organic matter content (OM) (%); (d) Kjeldahl nitrogen (%); (e) available P (mg P$_2$O$_5$ kg^{-1} DW); and (f) available K (mg K$_2$O kg^{-1} DW) (mean ± standard deviation, $n=3$). Columns marked with the same letter are not significantly different (Tukey test, $P > 0.05$). MSWC: municipal solid waste compost; GWC: garden waste compost; CaO: calcium oxide; SBS: sugar beet sludges.

correlated, with the other soil or plant characteristic (data not shown), and were not considered in the multivariate statistical analysis (Subsection 3.5.). It is important to observe that the Zn pseudo-total content in MSWC was higher than in the mine soil (Table 1), and that the Cu pseudo-total content in MSWC was similar to its value in the soil. Several authors have stressed this fact: sometimes the contents of trace metals in organic residues are much greater than in some agricultural (Pichtel and Anderson, 1997) or contaminated soils (Brown et al., 2003). In fact, many historical restoration projects used organic residues that would not be acceptable for beneficial use under current regulations. However, the sites that were restored using these materials have been able to maintain healthy plant covers with metal concentrations within an acceptable range (Brown et al., 2003).

All the amendments were able to decrease CaCl$_2$-extractable Cu, Pb and Zn, considered as effectively bioavailable or mobile metal fractions (Table 2). This was probably achieved by the combined effects of immobilization of metals by humified OM, in the compost-treated soils, and due to an increase in pH and the formation of insoluble carbonates and/or phosphates, a consequence of the application of composts and liming materials (Ross, 1994; Walker et al., 2004). In the current experiment, soil pH seems to be the major characteristic controlling CaCl$_2$-extractable metal content, as can be observed by the unlimed GWC experiments: GWC was not so effective in correcting acidity and, as a consequence, it was also not as effective in the decrease of CaCl$_2$-extractable metals.

Copper and Zn mobilisable fractions (potentially bioavailable) increased with MSWC application and the opposite was

Table 2 – Mobile metal fractions (extracted by 0.01 M calcium chloride solution) and mobilisable metal fractions (extracted by 0.5 M ammonium acetate, 0.5 M acetic acid and 0.02 m EDTA, pH 4.7) in the different soil treatments (mg kg^{-1} DW) (mean ± standard deviation, $n=3$)							
	Application rate (Mg ha^{-1})	Liming material	Mobile metal content (mg kg^{-1} DW)			Mobilisable metal content (mg kg^{-1} DW)		
			Cu	Pb	Zn	Cu	Pb	Zn
MSWC	0	Unlimed	2.7±0.4 c	124±34 c	7.6±1.0 e	8.5±0.3 abcd	1225±134 bc	9.6±0.1 efgh
		CaO	0.7±0.4 a	8±5 a	1.0±1.0 ab	6.7±0.4 ab	986±94 abc	3.4±0.1 abcd
		SBS	0.6±0.2 a	7±5 a	<QL	7.2±1.1 ab	957±62 abc	2.4±0.2 a
	25	Unlimed	0.53±0.03 a	17±2 a	2.7±0.1 c	13.0±0.5 f	958±184 abc	11.4±4.0 h
		CaO	<QL	5±1 a	<QL	13.0±0.6 f	1228±308 bc	8.3±1.4 defgh
		SBS	0.55±0.06 a	<QL	<QL	12.5±0.8 ef	684±178 bc	3.9±0.5 abcd
	50	Unlimed	0.52±0.06 a	5.1±0.4 a	0.5±0.1 c	19.9±0.7 g	759±78 abc	7.9±0.8 bcdefgh
		CaO	0.56±0.03 a	<QL	<QL	20.1±2.6 g	955±258 abc	9.8±1.5 gh
		SBS	0.60±0.05 a	<QL	<QL	21.0±0.1 g	921±73 abc	9.5±0.1 efgh
	100	Unlimed	0.6±0.1 a	<QL	<QL	20.1±3.0 g	800±50 abc	18.5±5.1 i
		CaO	0.52±0.01 a	<QL	<QL	22.7±2.3 g	842±57 abc	24.3±1.3 j
		SBS	<QL	<QL	<QL	26.3±0.3 h	964±96 abc	25.6±3.9 j
GWC	0	Unlimed	3.07±0.07 c	65±7 b	10.1±0.4 f	7.0±0.3 ab	629±386 ab	9.7±0.6 efgh
		CaO	<QL	7.7±0.2 a	2.2±0.7 bc	5.6±0.1 a	549±40 a	4.5±0.5 abcdef
		SBS	<QL	5.0±0.8 a	0.7±0.4 a	5.1±0.6 a	890±512 abc	3.9±0.8 abcd
	25	Unlimed	1.40±0.07 b	43±7 b	4.8±0.7 d	13.5±0.1 f	1387±187 c	8.1±0.7 cdefgh
		CaO	<QL	<QL	<QL	7.7±0.5 abc	579±41 ab	2.6±0.5 ab
		SBS	<QL	<QL	<QL	6.9±0.5 ab	614±104 ab	2.1±0.1 a
	50	Unlimed	<QL	10.7±0.6 a	1.9±0.1 bc	10.9±0.4 cdef	633±185 ab	5.6±0.7 abcdefg
		CaO	<QL	<QL	<QL	9.3±0.7 bcde	616±98 ab	3.7±0.8 abcd
		SBS	<QL	<QL	<QL	9.3±0.7 bcde	837±212 abc	2.7±0.5 abc
	100	Unlimed	<QL	<QL	0.6±0.1 a	11.6±0.7 def	1136±451 abc	6.1±0.5 abcdefgh
		CaO	<QL	<QL	<QL	11.3±0.2 def	895±195 abc	4.3±0.2 abcde
		SBS	<QL	<QL	<QL	11.0±1.2 cdef	804±123 abc	5.0±1.3 abcdefg

Values in the same column marked with the same letter are not significantly different (Tukey test, P>0.05). MSWC: municipal solid waste compost; GWC: garden waste compost; CaO: calcium oxide; SBS: sugar beet sludge; QL: quantification limit; QL(Cu)=0.5 mg kg^{-1}; QL(Pb)=5.0 mg kg^{-1}; QL(Zn)=0.5 mg kg^{-1}.

true following amendment with GWC. These results were in agreement 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). Mobilisable Pb content was not affected by the treatments, relative to the unamended mine soil, due to its high concentration in the original soil. As a consequence, mobilisable Pb was not significantly correlated with any other parameter analysed (data not shown), and was not considered in the multivariate statistical analysis (Subsection 3.5.). It is important to highlight that, although heavy metals become less mobile/bioavailable with this immobilization techniques, their total concentrations in soil remains unchanged, and this immobilised pool may become available again with time, through natural weathering process or through breakdown of high molecular mass organic-metal complexes (Bolan et al., 2003). That is why in situ immobilization techniques need long-term monitoring of the remediated soil.

3.2.   Effects of amendments on plant growth and mineral composition

The greatest increase in plant biomass (more than 3 times the control) was obtained in the presence of 50 Mg MSWC ha^{-1} and with the combined use of 25 or 50 Mg MSWC ha^{-1} and CaO (Fig. 2). Some phytotoxic effects were observed when using 100 Mg MSWC ha^{-1}, possibly due to the high level of soluble salts in this application rate (Rodgers and Anderson, 1995; Ye et al., 2002). The small relative growth observed with GWC application was not significantly different (P>0.05) from that obtained in the mine soil. The different capacity of MSWC and GWC to supply essential macronutrients (N, P, K) (Fig. 1), could be the main explanation for the different relative growth of the plant. Nitrogen deficiency was probably the major nutrient limitation to plant growth when GWC was applied, as N concentration in the shoots was smaller in this treatment compared with that of MSWC (Table 3). This might derive from the C:N ratio of 19.5 for GWC (a compost obtained from twigs, wood chips, dead leaves, etc, rich in C) compared with 10.3 for MSWC. As a consequence, nitrogen fertilizers should be added, in addition to GWC, as it is recommended in most soil rehabilitation projects (Tordoff et al., 2000). Potassium was the only macronutrient which increased in plant tissues, with increasing applications rates of either of the composts. Madejón et al. (2003) explained this fact considering that it may be an effect of "luxury" consumption of this element: plants tend to uptake and accumulate an excess of K when it is abundant in the soil. The concentration of Ca in the shoots rose significantly when liming materials were applied, relative
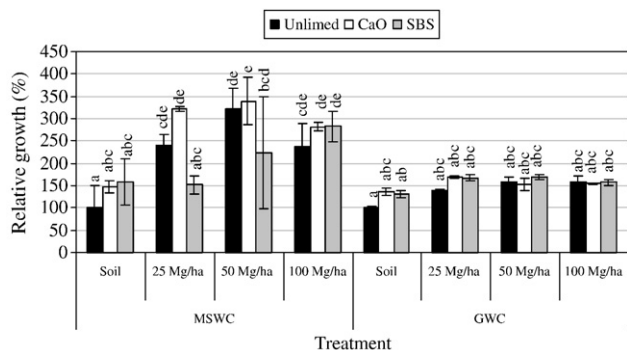
Fig. 2 – Relative growth of perennial ryegrass in the different soil treatments (mean ± SD, n = 3). Columns within each type of organic residue marked with the same letter are not significantly different (Tukey test, P > 0.05).

to the mine soil, but a positive effect of both composts was also observed. The composts or the liming materials had no effect on the concentrations of P and Mg in plant tissues.

Although essential for plant growth, Cu and Zn can have toxic effects if they reach high concentrations in plant tissues, as can non-essential trace metals such as Pb. This is of particular concern in metal contaminated sites, as is the present case. Considering the classes of soil pollution with heavy metals proposed in Poland (0- to 25-cm layer) by Kabata-Pendias and Adriano (1995), Pb and Cu pseudo-total concen-

trations in the mine soil led to a classification in class V (extremely polluted) or in class I or II (slightly to moderately polluted) when considering Zn concentration. As a consequence, Pb concentrations in the shoots were considerably higher than the values found by Arienzo et al. (2004), for the same plant grown on less Pb contaminated soil, and considered as typical concentrations of contaminated plants by Ross (1994). Moreover, comparing the results with a compilation of limit values presented by Mendez and Maier (2008), Pb concentrations in the shoots of perennial ryegrass were above the leaf tissue toxicity limits (30–100 mg kg^{-1}) and above the maximum tolerable levels for cattle (100 mg Pb kg^{-1}). Also Pichtel and Salt (1998) found elevated concentrations of Pb on perennial ryegrass and other grasses grown on mine wastes. Copper concentrations in the shoots were similar to the values found by Santibáñez et al. (2008), slightly higher than the values found by Arienzo et al. (2004), also with some concentrations typical of contaminated plants (Ross, 1994; Mendez and Maier, 2008). As for Zn concentrations, they were below the range of values found on contaminated plants (Ross, 1994; Mendez and Maier, 2008), below the values reported by Santibáñez et al. (2008) and similar to the values reported by Arienzo et al. (2004).

In this study, Cu and Pb were not affected by the imposed treatments, while Zn concentration in the shoots decreased only when GWC was applied (considering the control soil used in each experiment). Other authors have reported more promising results, showing that compost or manure application to metal-contaminated soils were effective in the

Table 3 – Mineral composition of the shoots of perennial ryegrass grown in the different soil treatments (mean, n = 3)									
	Application rate (Mg ha^{-1})	Liming material	N	P	K	Ca	Mg	Cu	Pb	Zn
			(g kg^{-1} DW)					(mg kg^{-1} DW)		
MSWC	0	Unlimed	48 cdef	3.8 a	25 a	6 ab	6.1 abcd	45 b	219 bcde	100 k
		CaO	44 cde	4.5 ab	28 ab	21 i	6.8 bcd	79 c	555 f	86 ijk
		SBS	47 cdef	5.0 abcd	32 ab	18 fghi	7.6 d	35 ab	179 abcd	76 fghij
	25	Unlimed	46 cdef	5.6 bcdef	56 c	13 cdef	4.2 ab	39 ab	324 e	79 ghij
		CaO	50 ef	4.9 abcd	57 cd	18 fghi	4.3 ab	31 ab	240 cde	72 efghij
		SBS	55 f	5.1 abcd	65 cdef	19 ghi	5.7 abcd	27 ab	115 abc	62 cdefg
	50	Unlimed	46 cdef	5.3 bcde	57 cd	13 cdef	6.2 abcd	41 ab	295 de	91 jk
		CaO	49 def	4.8 abcd	65 cdef	16 defghi	4.3 ab	35 ab	213 abcde	74 fghij
		SBS	49 cdef	5.5 bcdef	65 cdef	17 efghi	7.3 cd	36 ab	154 abc	70 efghi
	100	Unlimed	50 ef	4.9 abcd	61 cde	9 abc	4.1 ab	44 b	192 abcd	65 cdefgh
		CaO	55 f	5.6 bcdef	66 cdef	14 cdefgh	5.0 abcd	32 ab	191 abcd	84 hijk
		SBS	49 cdef	4.6 abc	57 cd	12 cde	4.4 ab	41 ab	239 cde	69 defghi
GWC	0	Unlimed	46 cdef	6.4 ef	25 a	4 a	5.9 abcd	45 b	133 abc	70 defghi
		CaO	47 cdef	5.9 cdef	35 b	20 hi	5.5 abcd	37 ab	92 ab	53 bcde
		SBS	48 cdef	6.7 f	34 ab	21 i	7.6 d	35 ab	89 ab	57 bcdef
	25	Unlimed	39 abc	5.8 bcdef	58 cd	9 abc	4.7 abc	29 ab	128 abc	50 abcd
		CaO	39 abcd	5.4 bcde	67 def	13 cdefg	4.7 abc	34 ab	120 abc	46 abc
		SBS	41 bcde	5.8 cdef	63 cde	12 cdef	5.2 abcd	35 ab	95 ab	48 abc
	50	Unlimed	34 ab	5.9 cdef	69 ef	10 abc	4.7 abc	41 ab	93 ab	39 ab
		CaO	33 ab	6.0 def	66 cdef	12 bcde	4.2 ab	25 ab	102 ab	40 ab
		SBS	32 ab	5.9 cdef	74 f	12 cde	4.1 ab	18 ab	115 abc	38 ab
	100	Unlimed	30 a	5.7 bcdef	64 cdef	10 abc	4.5 ab	19 ab	88 a	33 a
		CaO	30 a	5.4 bcdef	64 cdef	11 bcd	3.8 a	17 ab	91 ab	37 ab
		SBS	31 a	5.4 bcde	63 cde	11 bcd	5.3 abcd	15 a	87 a	31 a

Values in the same column marked with the same letter are not significantly different (Tukey test, P > 0.05). MSWC: municipal solid waste compost; GWC: garden waste compost; CaO: calcium oxide; SBS: sugar beet sludge.

reduction of metals in the shoots of some plants (Rizzi et al., 2004; Walker et al., 2004).

3.3. Effects of amendments on soil enzymatic activities

Biological oxidation of organic compounds is generally a dehydrogenation process, involving many enzymes, which are highly specific. These enzymes are an integral part of microorganisms and, therefore, the dehydrogenase activity indicates the average activity of soil microbial population (Tabatabai, 1994; Gil-Sotres et al., 2005). Dehydrogenase activity (Fig. 3a) increased significantly with increasing MSWC applications, with smaller activities in the unlimed experiments. However, in the case of GWC application, the dehydrogenase was not enhanced, except with the simultaneous application of 100 Mg ha^{-1} GWC and SBS. Protease

(Fig. 1e) and urease (Fig. 1f) activities, both related to the N-cycle (Ladd and Butler, 1972; Kandeler and Gerber, 1988; Alef and Nannipieri, 1995), also responded differently to both composts: the activities increased with increasing MSWC application, but the same was not true following GWC application. When GWC was applied, the urease activity in the amended soil decreased, with significant differences relative to the mine soil in the 100 Mg ha^{-1} application rate. Protease activity rose only slightly with GWC application, with significant differences from the mine soil only in limed treatments. As a consequence, the enzymatic activities of dehydrogenase, urease and protease were highly correlated (Table 4), and also correlated with the chemical properties that were differently affected by the two composts, i.e. soil N content, available-P and available-K, which had substantially greater values when MSWC was applied.
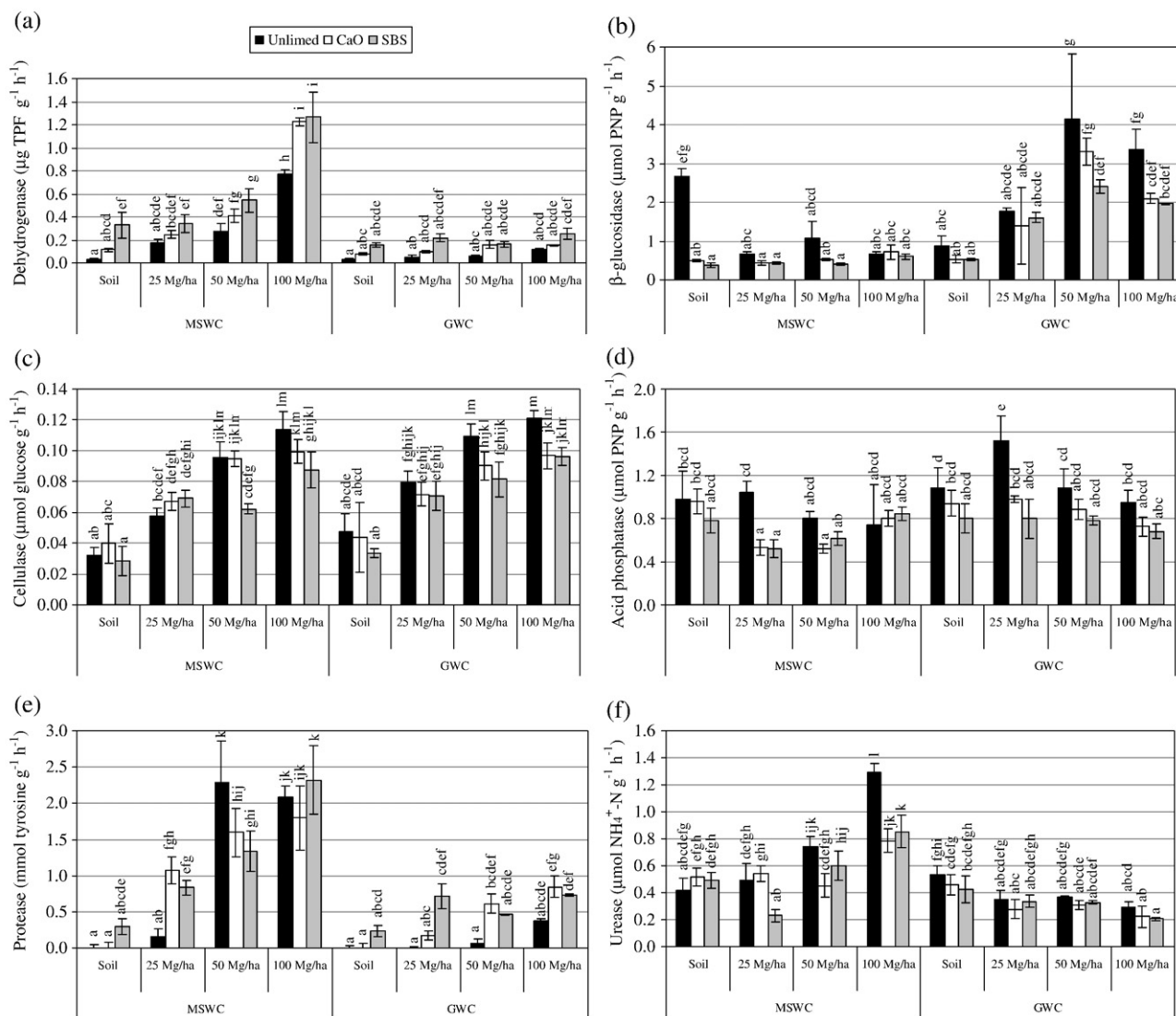

Fig. 3 – Soil enzymatic activities obtained in the different soil treatments (mean ± standard deviation, n = 6): (a) dehydrogenase (μg TPF g^{-1} h^{-1}), (b) β-glucosidase (μmol PNP g^{-1} h^{-1}); (c) cellulase (μmol glucose g^{-1} h^{-1}); (d) acid phosphatase (μmol PNP g^{-1} h^{-1}), (e) protease (mmol tyrosine g^{-1} h^{-1}), and (f) urease (μmol NH$_4^+$–N g^{-1} h^{-1}). Columns marked with the same letter are not significantly different (Tukey HSD test, P > 0.05). PNP: p-nitrophenol; TPF: 2,3,5-triphenylformazan; MSWC: municipal solid waste compost; GWC: garden waste compost; CaO: calcium oxide; SBS: sugar beet sludge.

Table 4 – Pearson's correlation coefficients between soil chemical properties, soil enzymatic activities and *L. perenne* relative growth (n=24, average values were used)

	pH	EC	OM	$N_{Kjeldahl}$	Avail_P	Avail_K	Cu_{mobile}	Pb_{mobile}	Zn_{mobile}	$Cu_{mobilisable}$	$Zn_{mobilisable}$	Dehyd	β-gluc	Ac-phos	Cellu	Prot	Ure
EC	0.89***	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
OM	0.48*	0.68***	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
$N_{Kjeldahl}$	0.62**	0.77***	0.79***	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Avail_P	0.62**	0.69***	0.52**	0.91***	–	–	–	–	–	–	–	–	–	–	–	–	–
Avail_K	0.60**	0.70***	0.64***	0.97***	0.98***	–	–	–	–	–	–	–	–	–	–	–	–
Cu_{mobile}	−0.66***	−0.61**	−0.40	−0.31	−0.19	−0.24	–	–	–	–	–	–	–	–	–	–	–
Pb_{mobile}	−0.67***	−0.63***	−0.38	−0.32	−0.22	−0.25	0.91***	–	–	–	–	–	–	–	–	–	–
Zn_{mobile}	−0.78***	−0.71***	−0.44*	−0.37	−0.27	−0.30	0.96***	0.87***	–	–	–	–	–	–	–	–	–
$Cu_{mobilisable}$	0.56**	0.61**	0.64***	0.90***	0.90***	0.92***	−0.22	−0.22	−0.28	–	–	–	–	–	–	–	–
$Zn_{mobilisable}$	0.31	0.42*	0.44*	0.83***	0.90***	0.89***	0.08	0.07	0.06	0.81***	–	–	–	–	–	–	–
Dehyd	0.71***	0.77***	0.51*	0.88***	0.95***	0.92***	−0.28	−0.30	−0.36	0.81***	0.86***	–	–	–	–	–	–
β-gluc	−0.43*	−0.19	0.32	−0.18	−0.40	−0.32	0.08	0.20	0.10	−0.25	−0.28	−0.39	–	–	–	–	–
Ac-phos	−0.78***	−0.62**	−0.22	−0.30	−0.32	−0.29	0.42*	0.43*	0.57**	−0.28	−0.02	−0.34	0.36	–	–	–	–
Cellu	0.27	0.50*	0.89***	0.66***	0.39	0.51*	−0.39	−0.40	−0.39	0.54**	0.30	0.32	0.45*	−0.10	–	–	–
Prot	0.71***	0.75***	0.60**	0.86***	0.86***	0.88***	−0.34	−0.37	−0.46*	0.87***	0.66***	0.79***	−0.33	−0.54**	0.51*	–	–
Ure	0.30	0.35	0.21	0.65***	0.79***	0.78***	−0.01	−0.07	−0.05	0.63**	0.75***	0.68***	−0.45*	−0.12	0.15	0.66***	–
Rel growth	0.57**	0.47*	0.37	0.65***	0.69***	0.69***	−0.41*	−0.40	−0.44	0.78***	0.55**	0.60**	−0.41	−0.47	0.37	0.80***	0.54**

EC: electrical conductivity; OM: total organic matter; $N_{Kjeldahl}$: soil total N (Kjeldahl); Avail_P: available P; Avail_K: available K; Cu_{mobile}, Pb_{mobile} and Zn_{mobile}: mobile metal content (extracted by 0.01 M $CaCl_2$); $Cu_{mobilisable}$, $Pb_{mobilisable}$ 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); Dehyd: dehydrogenase activity; β-gluc: β-glucosidase activity; Ac-phos: acid phosphatase activity; Cellu: cellulase activity; Prot: protease activity; Ure: urease activity; marked correlations are significant at: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Both β-glucosidase and cellulase are extracellular enzymes related to the C-cycle, which represent a very important role in organic matter degradation (Tabatabai, 1994; Eivazi and Tabatabai, 1988). Cellulase activity increased with increasing applications rates of both composts, without significant differences between them (Fig. 3c). Because of that, cellulase activity presented the highest positive correlation coefficient with soil organic matter content ($r = 0.83$, $P < 0.001$) (Table 4), which also increased with increasing applications rates of both composts, and smaller correlation coefficients to soil nitrogen, available-P and available-K than the previously discussed enzyme activities. β-glucosidase activity (Fig. 1b) was positively affected by GWC application, and was not affected following MSWC application. As a consequence, it was only weakly negatively correlated with soil pH ($r = -0.41$, $P < 0.001$) and was not appropriate to be used as a quality indicator in this particular situation. It is also interesting to observe that the activity of enzymes related to the C-cycle were positively affected by GWC application, a compost produced from carbonaceous raw materials, while the activity of enzymes related to the N-cycle were positively affected by compost produced from N-rich materials, such as MSWC.

Acid phosphatase is largely responsible for the mineralization of organic phosphate compounds in acid soils (Eivazi and Tabatabai, 1977; Huang and Shindo, 2000). As it is considered a good index of the quality and quantity of organic matter in the soil (Gil-Sotres et al., 2005), it was predicted that if the organic matter content of degraded soils were to increase, the level of this enzyme activity would also rise. However, in this study, acid phosphatase was not affected by the treatments (Fig. 3d). The raise in soil pH probably impaired its activity, which was corroborated by the negative correlation with the pH of amended soils ($r = -0.70$, $P < 0.001$), and by the fact that some of the limed treatments had smaller acid phosphatase activities than its unlimed counterparts.

3.4. Pearson's correlations

Pearson's correlations between average values of all soil and plant-related parameters were calculated, but only those with larger correlation coefficients are presented (Table 4).

The role played by the amendments in the immobilization of metals was corroborated by the negative correlation coefficients obtained between mobile Cu, Pb and Zn and soil chemical characteristics, which values increased as a consequence of the amendments application, especially with pH and EC ($P < 0.001$). Copper and Zn loadings, assessed by the mobilisable fractions, were especially relevant in the case of the MSWC application, which is evidenced by the high positive correlation coefficients between mobilisable Cu and Zn, and the soil chemical characteristics which values rose as a consequence of the MSWC application, especially soil N content, available P and available K ($P < 0.001$).

Plant relative growth was highly positively correlated with soil N content, available P, available K, mobilisable Cu and protease activity ($P < 0.001$), and moderately correlated, also with positive coefficients, with mobilisable Zn, dehydrogenase and urease activities ($P < 0.01$), i.e. with the soil characteristics with a larger increase following MSWC application.

Under the experimental conditions tested, Pb and Zn extracted with 0.01 M $CaCl_2$, which represent the soluble and easily exchangeable fractions in the soil (Houba et al., 1996; Pueyo et al., 2004; Walker et al., 2004; Pérez-de-Mora et al., 2006) were not significantly correlated with their concentration in the shoots of perennial ryegrass (data not shown). So, in this particular situation, perennial ryegrass could not serve as an indicator of Cu, Pb and Zn availability in the soil (Baker, 1981). This could also be concluded by the fact that Cu and Pb concentrations in the shoots did not decrease significantly with the imposed treatments. Pichtel and Salt (1998) previously reported that it is difficult to find an extractant that could mimic metal uptake by perennial ryegrass and other grasses in similar environments.

3.5. Multivariate exploratory techniques

Principal component analysis (PCA) is an unsupervised multivariate technique in which new variables are calculated as linear combinations of the old ones. The new variables, called principal components (PC), have two main features: (i) they are uncorrelated between themselves; (ii) the first PCs keep the main part of the variance of the original data set. In this way, it is possible to show a great part of the information by plotting the first two or three PCs. The combined plot of scores (coordinates of the objects on the new variables) and loadings (weights on the linear combination of the original variables from which a PC is built from) can be used to recognize groups of samples with similar behaviour and the correlations among the original variables, which would be more difficult to detect by a simple observation of numbers in tables (Abollino et al., 2002).

Three independent PCAs were carried out considering average results ($n = 24$ cases) and the variables: (a) plant relative growth and 11 soil chemical properties (pH, EC, OM, total N content, available P, available K, mobile Cu, Pb and Zn, and mobilisable Cu and Zn); (b) N, P, K, Ca, Mg, Cu, Pb and Zn concentrations in the shoots; and (c) soil enzymatic activities (dehydrogenase, β-glucosidade, cellulase, acid phosphatase, protease and urease). Fig. 4 shows the combined plot of scores and loadings on PC1 vs. PC2 obtained with the different analyses. The agglomerative hierarchical clustering procedure was used to evaluate similarities among samples. Fig. 4 also shows sample clustering considering a linkage distance <0.6. Pseudo total Cu, Pb and Zn and mobilisable Pb were not considered in the PCA used in this discussion, because their contents were high and they did not change with the imposed treatments. Their loadings with the different PCs generated by the PCA were always too low to be considered, regardless of the pool of parameters used in the analyses.

For the PCA obtained with relative growth and soil chemical properties (Fig. 4a), the first principal component (PC1) explained 61% of the total variance and the second principal component (PC2) almost 24%. Soil pH, EC, OM content, N content, available P, available K, mobilisable Cu and plant relative growth (all with negative values) had large loading coefficients on PC1 (loadings >0.7). Mobile metal fractions (Cu, Pb and Zn) (with negative values) had large loadings on PC2. Mobilisable Zn was not significantly correlated with any of the principal components. The unamended soil samples (M0 and
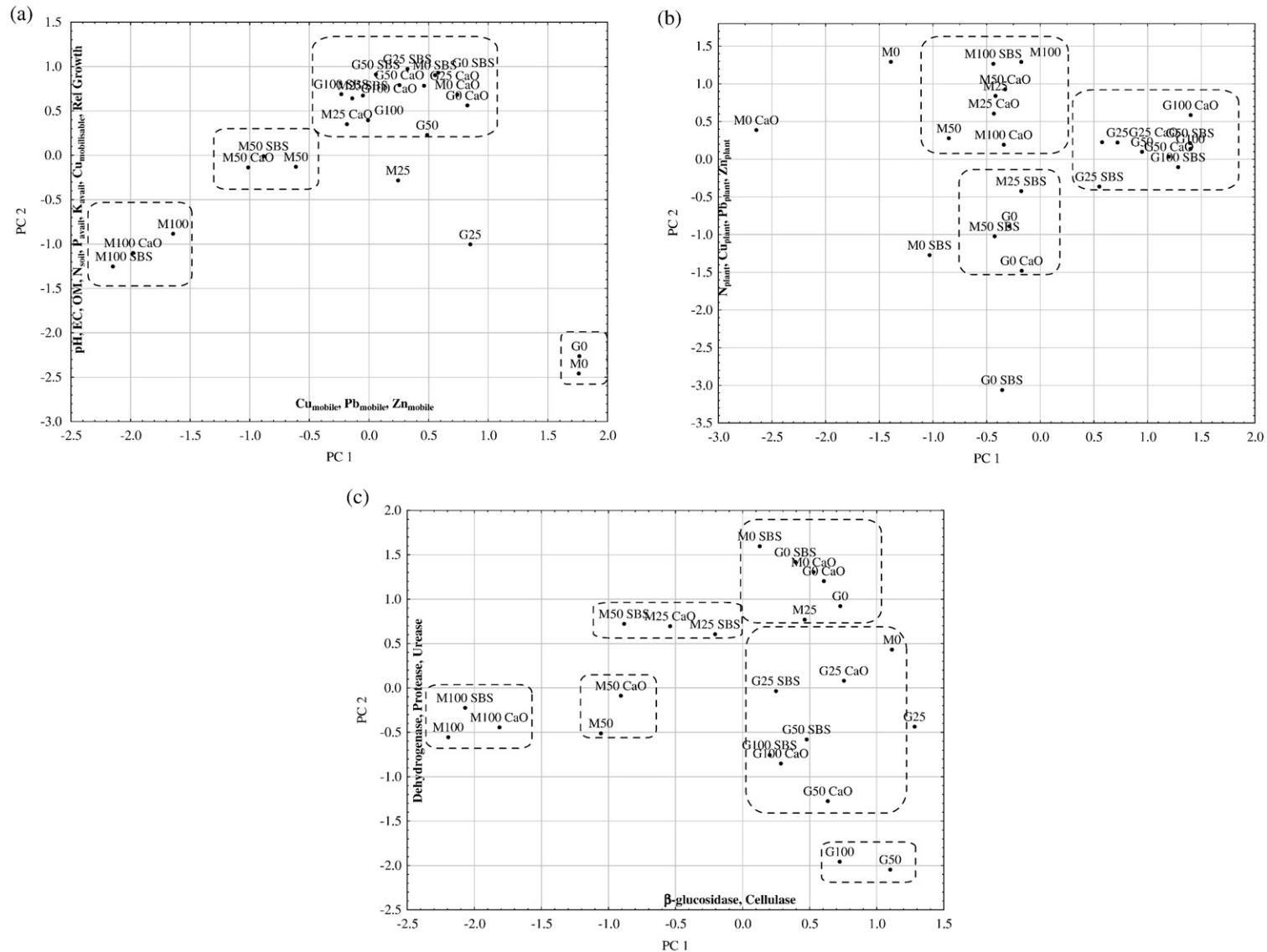
Fig. 4 – Scores of samples on two principal components PC1 versus PC2 (mean values, $n=24$ cases): (a) *L. perenne* relative growth and soil chemical properties; (b) N, P, K, Ca, Mg, Cu, Pb and Zn concentrations in the shoots; and (c) Soil enzymatic activities. The most important parameters for the definition of the two components are shown on the edge of each axis, indicating the direction in which the value of the parameter increases. Samples were clustered according to the results obtained from the hierarchical cluster analysis (linkage distance <0.6). PC1: first principal component; PC2: second principal component; G: garden waste compost, M: municipal solid waste compost; 0, 25, 50 and 100 refers to the compost application rate in Mg ha^{-1}; CaO: calcium oxide; and SBS: sugar beet.

G0) contrasted with the amended soil along PC1 and PC2: they were located on the positive section of PC1, and on the negative section of PC2. Therefore, these samples had, simultaneously, the largest mobile Cu, Pb and Zn content and the smallest values for all other variables (soil pH, EC, OM, N content, available P, available K, mobilisable Cu and plant relative growth). Composts and liming materials were able to amend those soil properties, which was obvious from the reallocation of samples in the PC1 versus PC2 map. Soil samples amended with 100 Mg ha^{-1} MSWC, with or without liming materials, were all clustered and positioned in the most negative part of PC1: they had the greatest values for the variables in which PC1 was high. However, they were still positioned in the negative part of PC2. Another important independent cluster was obtained with the soil samples amended with 50 Mg ha^{-1} MSWC, limed or unlimed, which presented less negative scores on PC1, and higher scores on PC2. All other amended treatments, except the unlimed soil amended with 25 Mg ha^{-1} MSWC or GWC, were clustered, showing that they were equivalent in their capacity to amend the mine soil.

For the PCA obtained from the mineral composition of plants (Fig. 4b), the PC1 explained 49% of the total variance and the PC2 almost 18%. However, only N, Cu, Pb and Zn concentrations were significantly correlated with PC1 (all with negative values). Total P, K, Ca, and Mg were not significantly correlated to any of the principal components. The PC1 axis allowed a clear separation between plants from soil amended with GWC and those from soil amended with MSWC. The latter were located in the negative part of PC1 axis, showing that MSWC was able to raise plant N concentration, but also its inability to decrease Cu, Zn and Pb concentrations in the shoots.

For the PCA obtained with soil enzymatic activities (Fig. 4c), the PC1 explained 50% and the PC2 explained 25% of the total variance. Dehydrogenase, protease and urease activities had large loading coefficients on PC1, all with negative values, while β-glucosidase and cellulase were significantly correlated with PC2, also with negative values. Phosphatase activity was only significantly correlated with the third principal component (PC3), which explained 15% of the total variance (data not shown). It is interesting to observe that PCA results corroborate the previous discussion: a clear separation between the effect of MSWC and GWC on soil enzymes related to the C-cycle (β-glucosidase and cellulase) and enzymes related to the N-cycle (protease and urease). Mine soil samples amended with MSWC were located in the most negative part of PC1, with higher values for dehydrogenase, protease and urease activities, clearly separated from the soil samples amended with GWC in respect to the PC1 axis. On the other hand, mine soil samples amended with 50 and 100 Mg ha^{-1} GWC were located in the most negative part of PC2, with higher values for β-glucosidase and cellulase activities. However, in this map, soil samples amended with 50 and 100 Mg ha^{-1} MSWC were centred regarding the PC2 axis, evidencing that β-glucosidase and cellulase activities were not significantly affected by MSWC application.

4.　　Conclusions

This study indicated that MSWC can be used successfully in the remediation of a highly acidic metal-contaminated mine soil, correcting soil acidity, and increasing soil OM, total N, available P and K to levels that facilitates the establishment of perennial ryegrass. The best plant growth was obtained with 25 or 50 Mg MSWC ha^{-1}, with CaO as the liming material. GWC was not as effective as MSWC, due to its inability to correct soil acidity, and to its lower contents of N, P and K. In fact, N fertilizers should be added, in addition to GWC, if a vegetation cover is to be established in the soil.

The application of both composts and liming materials led to a decrease in the level of mobile/effectively bioavailable fractions of Cu, Pb and Zn, probably as a result of increased soil pH. However, at least in our experimental time span, the treatments did not significantly reduce Cu and Pb concentrations in the plant.

Dehydrogenase and the enzymes related to the N-cycle, urease and protease, had increased activities with increasing MSWC application, but the same was not true following GWC application. Conversely, the enzymatic activities of enzymes related to the C-cycle, cellulase and β-glucosidase, were only positively affected by GWC application, a compost obtained from materials rich in C.

Although this study showed that composts and other residues, such as SBS, can be recycled in soil, avoiding their landfill disposal, it is important to emphasize that these in situ immobilization techniques need long-term monitoring of the soil, especially when organic residues have high levels of trace metals, as MSWC.

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