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Selection of Plant Species–Organic Amendment Combinations to Assure Plant Establishment and Soil Microbial Function Recovery in the Phytostabilization of a Metal-Contaminated Soil

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Abstract A mesocosm experiment was established to evaluate the effect of two organic wastes: fermented sugar beet residue (SBR) and urban waste compost on the stimulation of plant growth, phytoaccumulation of heavy metals (HM) and soil biological quality and their possible use in phytostabilization tasks with native (*Piptatherum miliaceum*, *Retama sphaerocarpa*, *Bituminaria bituminosa*, *Coronilla juncea* and *Anthyllis cytisoides*) and non-native (*Lolium perenne*) plants in a heavy metal-contaminated semiarid soil. Except *R. sphaerocarpa*, SBR increased the contents of shoot N, P and K and shoot biomass of all plants. The percentage of mycorrhizal colonization was not affected by the organic amendments. The highest increase in dehydrogenase and β-glucosidase activities was recorded in SBR-amended *P. miliaceum*. SBR decreased toxic levels of HM in shoot of *P. miliaceum*, mainly decreasing Fe and Pb uptake to plants. This study pointed out that the SBR was the most effective amendment for enhancing the plant performance and for improving soil quality. The combination of SBR and *P. miliaceum* can be regarded the most effective strategy for being employed in phytostabilization projects of this contaminated site.

Keywords Phytostabilization · Heavy metal · Mining tailings · Organic residues · Soil restoration · Mediterranean native plants

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1 Introduction

Mine tailings are produced in often high volumes due to mining activities and are exposed to water and air erosion. These tailings contain huge amount of diverse heavy metals and there is a high risk that adjacent soils are polluted by transport of fine particles by wind (dry deposition) (Mendez and Maier 2008; Pardo et al. 2011). Such mine soils are usually lacking in organic matter, are often highly acidic (with high variations), contain high salt concentrations, have poor structure and possess a low water holding capacity and cation exchange capacity. All these factors impede a natural revegetation which, in turn, could minimize the risk of erosion and transport to adjacent soils (Clemente et al. 2006; Mendez and Maier 2008; Madejón et al. 2009; de la Fuente et al. 2011).

A possible solution for this problem is the phytostabilization, which is an effective, minimally environmentally damaging and relatively inexpensive method to stabilize these sites and to minimize the risk of toxicity (Mendez and Maier 2008). A stable vegetation cover is able to immobilize heavy metals via binding and sorption processes in the rhizosphere. There is a growing trend toward using indigenous native plant species for phytostabilization purposes, which are often better able to survive, grow and reproduce under such hostile environments than introduced plants originating in other environments (Mendez and Maier 2008). In semiarid regions, revegetation of the tailings is additionally hampered not only by highly phytotoxic concentrations of metals and lack of nutrients but also by shortage of water supply (Carrasco et al. 2010). Therefore, soil conditions have to be improved, so that plant establishment and growth is favoured.

With the aim to achieve an improvement of the soil conditions, the addition of organic matter (OM) is a widespread method to establish a stable vegetation cover of degraded soils in semiarid regions (Tejada et al. 2007) and more specific also in mine tailings; thus, it supplies nutrients and carbon for the plants and improves soil structure. Furthermore, organic matter decreases the plant availability of heavy metals (Tordoff et al. 2000; Walker et al. 2003; Alvarenga et al. 2009; Pardo et al. 2011). Organic wastes could be an ideal source of organic matter, but its application can affect metal solubility, depending mainly on the characteristics of the organic matter. Therefore, its application to degraded soil has to be tested previously (de la Fuente et al. 2011). Importantly, the effect of adding organic matter from wastes on plant growth and soil properties depends on amount, type and treatment of the organic matter (Tejada et al. 2007; de la Fuente et al. 2011). For instance, sugar beet residue (SBR), an organic waste from agroindustry, is rich in polysaccharide compounds and has been shown that, after fermentation with *Aspergillus niger* and supplementation with rock phosphate, can effectively improve the structural stability of mine tailings in semiarid regions (Carrasco et al. 2009; Vassileva et al. 2010). The fermentation with *A. niger* eliminates the compound ferulic acid, which inhibits both the growth of plants and important soil organisms as arbuscular mycorrhizal fungi (Medina et al. 2011). Another organic source example is compost made from solid urban wastes, which also has been reported as an organic amendment in heavy metal-contaminated sites, facilitating plant establishment and increasing soil organic matter, nutrients like P and K, and buffering soil acidity (Wong 2003; Alvarenga et al. 2008).

Several studies have demonstrated the adverse effect of elevated heavy metal levels on soil microbial biomass and its activity, varying with the kind of heavy metal and with the type of soil (Hattori 1992). Remediation strategies of contaminated soils should seek to reduce the negative impact of metals on soil microbial communities, preserving the microbial processes involved in soil fertility and plant growth. Meanwhile, the microbial activity can be used to assess the effectiveness of the soil reclamation efforts, as soil enzymes allow us to get insight the biogeochemical cycling of elements in the soil (Pérez-de-Mora et al. 2005; Lee et al. 2009; Melgar-Ramírez et al. 2012).

In spite of this, little attention has been paid on the selection of plant species–organic amendment combinations which not only assures the development of the vegetation cover in terms of phytostabilization but also the recovery of soil quality and microbial rhizosphere functions. Therefore, we hypothesized that the efficacy of the type of organic amendment on plant growth and soil biological quality in a heavy metal-contaminated soil will vary depending on the plant species. For this purpose, we compared in a two-factorial experiment the efficiency of fermented SBR and composted urban residue on the establishment of native and nonnative plant species in a semiarid mine tailing, as well as the effect of residue–plant combinations on soil microbial activities in order to achieve the best results for phytoremediation purposes.

2 Materials and Methods

2.1 Study Site

The Cartagena–La Unión mining district "Sierra Minera" (SE Spain) has been exploited since Roman times up to the twentieth century for mining of silver, zinc and lead and produced huge amounts of mine tailings and a severe landscape transformation (Conesa and Schulin 2010). The tailings contain iron, lead and zinc as main heavy metal components (Carrasco et al.

2009). The area is close to the coast with slopes between 20 and 30 %. Due to its semiarid climate, there is low annual precipitation (250–300 mm year^{-1}) and a high potential evapo-transpiration (1,000 mm year^{-1}) with the mean annual temperature reaching 17.5 °C. In this area, an approximately 50-year-old mine tailing was selected (UTM X687480 Y4162800 Z135, length 200–300 m, width 95 m, height 25 m, volume 750,000 m^3; IGME 1999). For substrate characterization, we took randomly from the top 20-cm depth of soil three soil samples each consisting of a mixture of six subsamples. The analytical characteristics of the mine tailing are indicated in Table 1.

2.2 Plants

We collected in the nearby area to the mine tailings mature seeds of the following five native species, including shrubs and grasses: *Retama sphaerocarpa* L., *Coronilla juncea* L., *Anthyllis cytisoides* L., *Bituminaria bituminosa* (L.) C.H. Stirt and *Piptatherum miliaceum* (L.) Coss. Carrasco et al. (2010) found the grass *P. miliaceum* to be suitable for revegetation of these types of mine tailings. The perennial herb *B. bituminosa* is mainly limited by nutrient availability when it grows in heavy metal-contaminated sites (Walker et al. 2007). *R. sphaerocarpa* was recently shown to be able to increase soil health and quality during revegetation in a pyrite mine soil (Moreno-Jiménez et al. 2012). *C. juncea* (Carrasco et al. 2011) and *A. cytisoides* (Díaz et al. 1996) were both shown to grow in heavy metal-contaminated soils only in symbiosis with AM fungi. Additionally, we selected also a non-native grass *Lolium perenne* L., as it is highly mycorrhizal (Wang and Qiu 2006) and also known as a heavy metal-tolerant species (Santibáñez et al. 2008).

All seeds were surface sterilized by soaking in 1 % sodium hypochlorite (NaOCl) for 5 min and subsequently rinsed thoroughly with sterilized water prior to a wetting treatment with sterilized water for 2 h.

Table 1 Chemical, biochemical, microbiological and physical characteristics of the soil used in the experiment (*N*=3)

pH (H_20)	7.7±0.3
EC (1:5, dS m^{-1})	2.5±0.4
$CaCO_3$ (%)	<5
Total organic C (g kg^{-1})	4.3±0.6
Total N (g kg^{-1})	0.21±0.03
Clay (%)	5±2
Silt (%)	24±5
Sand (%)	71±6
Water-soluble C (μg g^{-1})	41±2
Water-soluble carbohydrates (μg g^{-1})	10±1
Dehydrogenase (μg INTF g^{-1})	6.9±1.3
Aggregate stability (%)	24.7±1.6
Fe_2O_3 (%)	16±1
Al_2O_3 (%)	8±1
Total Zn (mg kg^{-1})	12,100±900
Total Pb (mg kg^{-1})	8,950±300
Total Cu (mg kg^{-1})	221±20
Total Cd (mg kg^{-1})	61±11
Total Ni (mg kg^{-1})	26±5

2.3 Organic Amendments

The urban organic waste compost (OWC) used was the organic fraction of a municipal solid waste obtained from a treatment plant in Murcia, Spain. The composted residue was produced mechanically by fast fermentation (60 days) and mixing the waste heap daily under aerobic conditions. The composted residue was sieved, ground to 0.5 mm particles and air-dried for analysis. The main characteristics of the composted residue, determined by standard methods (Page et al. 1982), were: pH (1:10) 6.70, electrical conductivity 4,700 μS cm^{-1}, total organic C 27.6 %, total N 14.5 g kg^{-1}, water soluble C 1,950 μg g^{-1}, total P 3.8 g kg^{-1}, Cu 146 μg g^{-1}, Zn 261 μg g^{-1}, Ni 25 μg g^{-1}, Cr 63 μg g^{-1}, Cd 3 μg g^{-1}, Pb 98 μg g^{-1}. Total heavy metals contents of OWC used were below the limits imposed by the Spanish legislation for use of an organic residue as fertilizer in agricultural soils (B.O.E. 2013) and by the European legislation for the use of sewage sludge in agriculture (C.E.C. 1986).

SBR was fermented mixing 15 g of SBR with 40 ml of Czapek solution (agar 15.0 g l^{-1}, di-potassium hydrogen phosphate 1.0 g l^{-1}, iron(II) sulphate heptahydrate 0.01 g l^{-1}, potassium chloride 0.5 g l^{-1}, magnesium sulphate heptahydrate 0.5 g l^{-1}, sodium nitrate 3.0 g l^{-1}, sucrose 30.0 g l^{-1}, pH=7.3) and 0.75 g of rock phosphate (Morocco fluorapatite, 12.8 % P, 1 mm mesh) in 250 ml Erlenmeyer flasks. The mixture was inoculated with *Aspergillus niger* strain NB2 (initial spore suspension 1.2×10^7 flask^{-1}) and was allowed to ferment at 30 °C for 20 days without shaking. The concentration of P was determined after digestion in nitric–perchloric acid using an Inductively Coupled

Plasma Mass Spectrometry (ICP-MS) (Thermo electron corporation Mod. IRIS intrepid II XDL). Total N was determined by the Kjeldahl method. Lignin, cellulose and hemicellulose contents were measured according to the method of Goering and Van Soest (1970). The fermented SBR is composed of 224 μg ml^{-1} total P, 1.2 % total N, 11.3 % cellulose, 3.1 % hemicellulose, 4.1 % lignin and 0.25 g l^{-1} reducing sugar.

2.4 Experimental Design

The experiment was arranged in a completely randomized two-factorial design with eight replicates having a total of 144 pots. The first factor was the addition or not of two different organic amendments to soil (urban organic waste compost and fermented sugar beet). The second factor consisted in the different plant species (*B. bituminosa*, *R. sphaerocarpa*, *C. juncea*, *A. cytisoides*, *P. miliaceum* and *L. perenne*). For each plant species, the experimental treatments were as follows: soil without organic amendment addition (control), soil with urban organic waste compost addition (OWC) and soil with fermented sugar beet addition (SBR).

Five hundred grams of air-dried soil was placed in 600 ml pots, where seeds of the selected plants were sowed. The fermented SBW and OWC were mixed manually with the experimental soil at a rate of 2.5 % (*w*/*w*). The experiment was conducted in a greenhouse, located in the Campus of Espinardo (Murcia, Spain). During the experiment, the average maximum temperature reached 22 °C. Plants were watered regularly with sterile water to a 60 % water holding capacity, without any fertilizer treatment. Eight months after sowing, the plants were harvested, separating the soil carefully from the roots. Soil was stored at 4 °C for further analysis.

2.5 Plant Analyses

Fresh and dry mass of shoots and roots (105 °C, 5 h) were recorded.

Foliar tissues were ground before chemical analysis. Sub-samples were oven-dried at 480 °C, then the ashes were dissolved in 0.6 M nitric acid and were filtered through an Albert® 145 ashless filter paper. P, K, Cd, Cu, Fe, Mn, Pb and Zn were quantified using an ICP-MS (Thermo electron corporation Mod. IRIS intrepid II XDL). The precision and accuracy of this method were tested by analysing (five replicates) the CTA-VTL-2 certified material, corresponding to Virginia Tobacco leaves. Heavy metal recoveries from plant standards ranged between 89 and 110 %. Shoot N was determined by dry combustion using a LECO Tru-Spec CN analyzer (Leco Corp., St. Joseph, MI, USA).

The percentage of root length colonized by arbuscular mycorrhizal fungi was calculated by the gridline intersect method (Giovanetti and Mosse 1980) after staining with trypan blue (Phillips and Hayman 1970).

2.6 Soil Analyses

Total N was determined by the Kjeldahl method. Available P, extracted with sodium bicarbonate, was determined by colorimetry, according to Murphy and Riley (1962). Total metal contents were determined by nitric–perchloric digestion: 1 g of crushed sample was placed in a Kjeldahl flask, and 10 ml of concentrated HNO_3 plus 10 ml of concentrated $HClO_4$ were added. The mixture was heated at 210 °C for 90 min, and then left to cool down at room temperature. When cool, the content of the tubes was filtered through an Albert® 145 ashless filter paper, and the volume completed at 50 ml by washing the Kjeldahl flasks with 0.5 N HCl several times. All metals were quantified using an ICP-MS (Thermo electron corporation Mod. IRIS intrepid II XDL). This methodology was referenced using the CRM027-050 Certified Material (Resource Technology Corporation, USA). The recoveries from soil standard were between 84 and 112 %.

Dehydrogenase activity was determined using 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT) as oxidizing agent (García et al. 1997). Briefly, soil samples adjusted to 60 % of its water holding capacity were incubated with 0.4 % INT for 20 h at 22 °C in the dark. The iodo-nitrotetrazolium formazan (INTF) formed was extracted by methanol and measured on a spectrophotometer at 490 nm. Controls were made with water instead of INT.

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate according to Naseby and Lynch (1997). Half a gram of soil was buffered with 2 ml of 0.5 M sodium acetate at pH 5.5. Substrate (0.5 ml) was added and incubated shaking at 37 °C for 90 min. The reaction was stopped by cooling to 2 °C and adding 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH. The mixture was centrifuged at 4,000 rpm for 8 min. The *p*-nitrophenol

(PNP) formed was determined by spectrophotometry at 398 nm. Controls were made adding the PNPP after incubation.

β-Glucosidase was determined using *p*-nitrophenyl-β-D-glucopyranoside (PNG, 0.05 M) as substrate. This assay is again based on the release and detection of PNP spectrophotometrically at 398 nm after incubation in water bath of 0.5 g soil with 2 ml maleate buffer (0.1 M, pH 6.5) and 0.5 ml of substrate for 90 min. The reaction was stopped with tris-hydroxymethyl aminomethane (THAM) according to Tabatabai (1994).

2.7 Statistical Analysis

Percentage colonization was arcsin-transformed, and the other parameters were log-transformed to compensate for heterogeneity of variance, before analysis of variance. Plant species, organic amendment and their interactions effects on measured variables were tested by a two-way analysis of variance and comparisons among means were made using the Tukey's HSD test calculated at $p<0.05$. Statistical procedures were carried out with the software package SPSS 10.0 for Windows.

3 Results

Both organic amendments and plant species had a significant effect on plant growth parameters (Fig. 1, Table 2). Except *R. sphaerocarpa*, SBR increased significantly shoot biomass of all plants species. The highest increase in shoot biomass was recorded in SBR-amended *P. miliaceum* plants, resulting in a 43-fold increase in biomass compared to control plants (Fig. 1). Amendment with OWC had a positive significant effect only in *B. bituminosa* and *P. miliaceum*, increasing by sixfold compared with their control plants. Root growth was increased in *P. miliaceum* after amendment with both organic residues and in *L. perenne* plants after amendment with SBR (Fig. 1, Table 2).

Shoot N, P and K contents were significantly affected by both plant species and organic amendments (Table 2). For all plant species, SBR was more effective than OWC for increasing the contents of shoot N, P and K (Table 3). SBR enhanced shoot N content in all plants except in *R. spaerocarpa*, with a maximum increase in *P. miliaceum* plants (28.5-fold). The addition of OWC resulted in 3.4- and 2.5-fold increases in shoot N of *B. bituminosa* and *A. cytisoides*, respectively. Both organic amendments increased shoot P content in almost all plants assayed, the exception was OWC that had no effect in *C. juncea* (Table 3). The highest enhancement in shoot P contents after addition of SBR was observed in *P. miliaceum* plants, increasing by 41-fold compared with control plants. Shoot K contents were significantly increased with SBR in *C. juncea* (17-fold), *A. cytisoides* (3.5-fold) and *P. miliaceum* (20-fold) with respect to their control plants (Table 3). However, the control plants and OWC-amended plants had similar contents of shoot K. The percentage of root colonization by local indigenous arbuscular mycorrhizal fungi (AMF) varied with plant species, but not by the addition of organic amendments (Tables 2 and 3). *R. spaerocarpa* and *L. perenne* presented the lowest values of AM root colonization followed by *C. juncea*. The highest levels of mycorrhization were recorded in roots of *B. bituminosa*, *A. cytisoides* and *P. miliaceum*, without significant differences among such plant species (on average about 67 %).

The effect of organic amendments on the heavy metal concentrations in shoot tissues depended on the plant species (Tables 2 and 4). SBR provoked a significant decrease in heavy metal uptake of at least one or more heavy metals in all plants except *B. bituminosa* and *C. juncea*, where no differences were observed with respect to control plants (Table 4). The highest decreases in the concentrations of heavy metals produced by the addition of SBR were observed in *P. miliaceum* plants, mainly in the levels of shoot Fe and Pb (about 90 % compared with control plants). In *L. perenne*, we observed decreased heavy metal uptake in SBR-amended plants only for Mn (about 17 %). In comparison, SOW produced a decrease in heavy metal uptake of Mn and Zn in shoots of *R. sphaerocarpa* and *A. cytisoides*. In this latter plant, the concentration of Pb also decreased due to the addition of SOW (Table 4).

Soils amended with SBR presented the highest concentrations of total N in the rhizosphere soil of all plants (Table 5), whereas soils amended with OWC generally showed similar concentrations to control soil. Only the type of plant species had a significant effect on available P concentrations of rhizosphere of plants (Table 2), recording the highest concentrations in *L. perenne* and the lowest concentrations in *C. juncea*. All enzymatic activities assayed were significantly affected by both plant species and organic amendment and their interaction (Table 2). SBR amendment enhanced

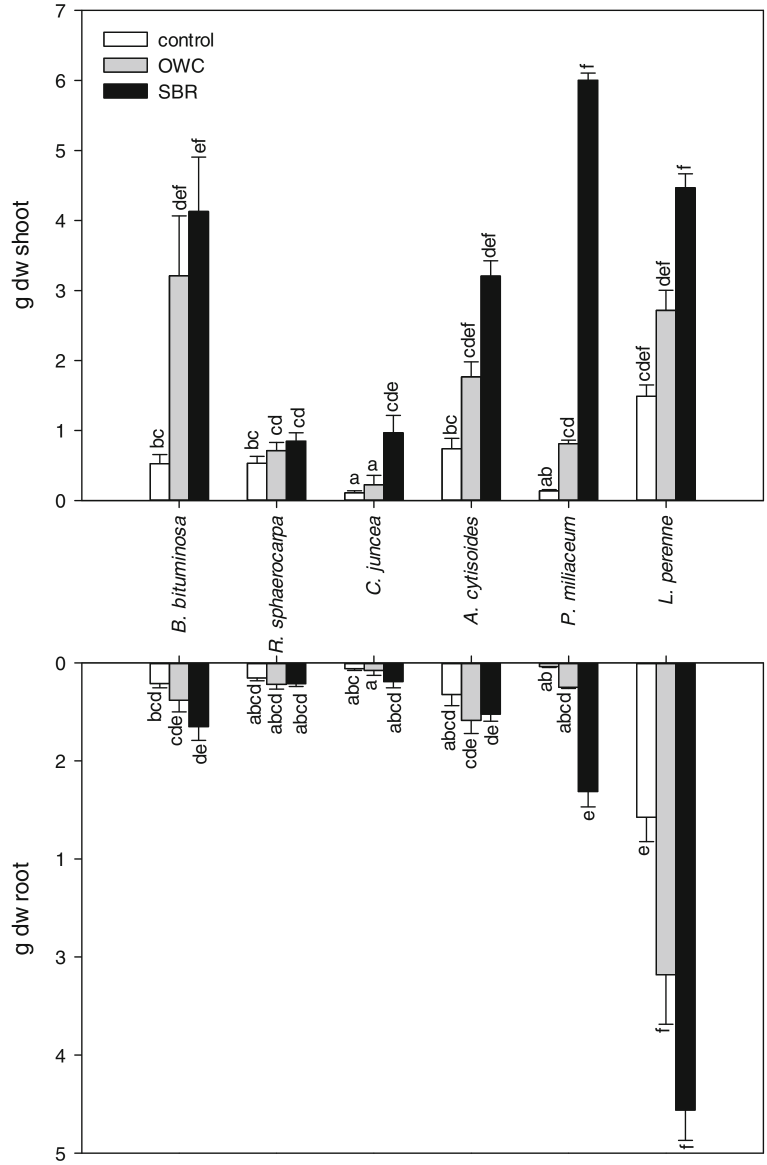

Fig. 1 Shoot and root dry weight in response to addition of organic waste compost (OWC) and fermented sugar beet residue (SBR) to soil of six plant species 8 months after planting (means ± standard errors, $N=8$). *Values in columns, followed by the same letter*, do not differ significantly within the same plant species ($p<0.05$) as determined by the Tukey test

dehydrogenase activity of rhizosphere soil in all plants with respect to control plants, but not OWC amendment (Table 5). The highest increase in dehydrogenase activity was recorded in SBR-amended *P. miliaceum* (by about 175 % compared with control plants). The addition of SBR did not affect phosphatase activity of rhizosphere soil of all plants. OWC decreased phosphatase activity in rhizosphere of *L. perenne*, and no effect in all other species. Except *B. bituminosa* and *A. cytisoides*, β-glucosidase activity was increased after SBR amendment in all plants, particularly in plants of *P. miliaceum* (about 50 % higher than control plants). However, the addition of OWC did not affect such enzymatic activity.

4 Discussion

The present study confirms the different ability of two organic amendments: microbially treated sugar beet and urban organic waste compost to promote plant growth in a semiarid mine tailing. While SBR had a positive effect on growth of the majority of plants tested, confirming its ability for soil restoration and phytostabilization (Carrasco et al. 2009), OWC had only a positive effect in two of the six species. The effectiveness of fermented sugar beet with respect to stimulation of plant growth could be due to an improvement in the available nutrient supply in soil. During the SBR fermentation process

Table 2 Two factor ANOVA (plant species and organic amendments) for plant biomass, plant nutrients, soil enzymatic activities and heavy metals

	Plant (*P*)	Amendment (*A*)	*P*×*A*
Shoot biomass	25.70***	47.38***	3.96***
Root biomass	38.63***	19.06***	2.47*
Shoot N	17.59***	47.38***	3.44***
Shoot P	19.54***	126.92***	4.15***
Shoot K	30.94***	44.26***	3.57***
AM root colonization	12.42***	1.08	1.69
Cd	34.78***	2.09	11.24***
Cu	46.06***	21.19***	26.09***
Fe	37.17***	35.16***	11.41***
Mn	219.94***	101.95***	23.61***
Pb	50.59***	27.45***	8.92***
Zn	72.88***	199.53***	24.29***
Total N	3.13	31.02***	3.08
Available P	22.02***	2.18	2.49
Dehydrogenase	44.52***	116.28***	1.65
Phosphatase	9.76***	20.87***	2.37*
β-Glucosidase	9.09***	68.47***	2.17*

*$p<0.05$; ***$p<0.001$ (significant)

A. niger is known to solubilize the rock phosphate, which improves P availability to plants (Vassilev et al. 1995). We have previously showed that the addition of this organic amendment increased the total N, available P and extractable K contents of non-contaminated soil (Alguacil et al. 2003), favouring the plant growth. In our study, the soils amended with SBR exhibited the highest concentrations of total N in all plants assayed, which resulted in enhanced uptake of N in shoots. However, the SBR did not increase soil available P, showing that plants readily assimilated the available-P. Simultaneously, shoot P content of most of the plants was significantly enhanced by the addition of SBR to soil. These findings agree with those obtained by Pardo et al. (2013) in a contaminated soil fertilized with N/P/K fertilizer. On the other hand, the absence of a negative effect of this fermented residue on plant growth could be related to the elimination of the toxic substances like ferulic acid during the fermentation process (Medina et al. 2011).

The OWC showed a low effectiveness for stimulating growth of the most of plants. Thus, for this organic amendment, the utilization of a higher dose than 2.5 % might be recommended in order to increase the input of nutrients added to soil and obtain similar results to SBR. However, this kind of amendment applied in the same dose that in our study has been successful in the remediation of a highly acidic metal-contaminated soil, allowing the establishment of perennial ryegrass (Alvarenga et al. 2008, 2009). The very high variability of the composition and properties of compost and different application rates may be responsible for the differences between our results and those previously reported for composted urban waste. This points to the need to carry out mesocosm experiments for assessing the effectiveness of organic amendments before their large-scale field application.

Organic amendments can also help to plant establishment in contaminated soils by decreasing heavy metal bioavailability by adsorption and by forming stable complexes with their organic matter (Shuman 1999; Alvarenga et al. 2008). SBR was also more effective than OWC for reducing the uptake of heavy metals to shoot tissues. Previously, Medina et al. (2006) recorded that *Aspergillus*-treated sugar beet waste was effective for decreasing Zn accumulation in plants of *T. repens*. In this study, the effect of SBR on heavy metal concentrations in shoot tissues was dependent on the plant species. For most plants, Cd, Cu and Mn concentrations in shoots were within the normal ranges for plants (Kabata-Pendias and Pendias 2001). Although this soil contained high levels of total heavy metal concentrations, its slightly alkaline pH favoured low solubility and in consequence bioavailability of soil heavy metals (Pérez-de-Mora et al. 2006). Moreover, *L. perenne* is able to survive when metal availability to the plant is low (Arienzo et al. 2004). However, excessive or toxic levels of Zn were recorded in non-amended *A. cytisoides*, *P. miliaceum* and *L. perenne* plants and of Pb in the same plants together with *R. sphaerocarpa* (Kabata-Pendias and Pendias 2001). Lower concentrations of Zn and Cu have been reported by Conesa et al. (2006) in *P. miliaceum* plants growing on a neutral tailing under natural conditions. Meanwhile, the addition of SBR was able to decrease the concentrations of heavy metals to normal levels. Thus, the low metal concentrations accumulated in shoots of plants amended with SBR could minimize the risk of metal uptake in the food chain.

The benefits of organic amendments are due also to the improvement of soil microbial function (Alguacil et al. 2008), which is negatively affected by high heavy metal concentrations in soil. Dehydrogenase and

Table 3 Shoot nutrient contents and mycorrhizal colonization in response to addition of organic waste compost (OWC) and fermented sugar beet residue (SBR) to soil of six plant species 8 months after planting (means ± standard errors, n=8). Values in columns followed by the same letter do not differ significantly within the same plant species (p<0.05) as determined by the Tukey test

Plant	Treatment	N (mg plant^{-1})	P (mg plant^{-1})	K (mg plant^{-1})	AM root colonization (%)
B. bituminosa	Control	11±2abcd	0.5±0.1bc	13±2efg	57±5abcde
	OWC	37±9efgh	2.7±0.5fg	28±9fgh	74±2de
	SBR	66±9gh	3.8±0.3fg	30±2fgh	73±2cde
R.sphaerocarpa	Control	12±1bcde	0.4±0.1bcd	6±1bcde	45±5abcd
	OWC	13±2bcdef	1.7±0.3efg	7±1cdef	47±8abcd
	SBR	19±2cdefg	1.7±0.2defg	9±1def	34±7a
C. juncea	Control	4±1ab	0.1±0.0a	1±0ab	57±5abcde
	OWC	5±2a	0.4±0.2ab	4±3abc	49±4abcde
	SBR	18±4cdefgh	1.6±0.4defg	17±3efg	51±6abcde
A. cytisoides	Control	11±2abc	0.4±0.1ab	11±2cdef	68±4bcde
	OWC	27±3defgh	3.1±0.3fg	21±3efgh	64±4abcde
	SBR	53±4fgh	4.7±0.4g	38±2gh	70±2cde
P. miliaceum	Control	2±0a	0.1±0.0a	1±0a	53±6abcde
	OWC	9±1abc	1.2±0.1cdef	3±0abcd	67±3bcde
	SBR	57±4efgh	4.1±0.3g	20±2efgh	79±1e
L. perenne	Control	16±2cdefg	0.6±0.1bcde	17±1efgh	42±2abc
	OWC	31±3defgh	3.5±0.4fg	36±2gh	37±3ab
	SBR	62±14h	5.2±0.4g	56±4h	38±4ab

hydrolases activities have been proposed as sensitive indicator of heavy metals toxicity (Pérez-de-Mora et al. 2006). Only SBR was effective for promoting soil microbial activity indicated by dehydrogenase activity, being the highest values in *B. bituminosa* and *L. perenne*. The input of bioavailable C compounds and microbial biomass as consequence of organic amendment addition could account for increased soil microbial activity. In contrast, the loss of labile fraction of organic matter during composting course of urban organic residue, which is used as carbon and energy sources by soil microflora, could explain why this type of residue did not stimulate soil microbial activity.

Phosphatases catalyse the hydrolysis of organic phosphorus compounds to inorganic phosphorus. No significant variations occurred with the addition of organic amendments studied to rhizosphere soil of all plants, which could indicate that they had a scarce content of substrates capable of activating the synthesis of this enzyme. In the case of SBR, these results can also be ascribed to the available P provided with this amendment, which can lead to an inhibition synthesis of this enzyme (Nannipieri 1994). Similar results were obtained by Pérez-de-Mora et al. (2006) after four organic amendments addition to soil. These authors suggested limiting the use of phosphatase activity as potential indicator of soil quality in bioremediation tasks based on the application of organic amendments with high content of bioavailable P.

β-Glucosidase is a hydrolase of C cycling, involved in the last limiting step of cellulose degratation, hydrolysing β-glycoside links in large carbohydrate chains (Alef and Nannipieri 1995). The high β-glucosidase activity in the soils amended with SBR can be attributed to the cellulose contained in this amendment.

The improved functionality of soils amended with SBR could be also attributed to shifts in soil microbial populations (Pérez-de-Mora et al. 2006). In fact, we have previously demonstrated the ability of SBR for altering microbial community composition in the rhizosphere of *T. articulata* and *C. maritimum* plants grown in a soil from this slightly alkaline mine tailing (Fernández et al. 2012). The symbiosis between AMF and plants has been shown to alleviate heavy metal toxicity of plants by improving their nutrient uptake

Table 4 Shoot heavy metal concentrations in response to addition of organic waste compost (OWC) and fermented sugar beet residue (SBR) to soil of six plant species 8 months after planting (means ± standard errors, $n=8$). Values in columns followed by the same letter do not differ significantly within the same plant species ($p<0.05$) as determined by the Tukey test

Plant	Treatment	Cd (μg g^{-1})	Cu (μg g^{-1})	Fe (μg g^{-1})	Mn (μg g^{-1})	Pb (μg g^{-1})	Zn (μg g^{-1})
B. bituminosa	Control	0.37±0.00bcd	5.4±0.0ab	73.0±2.3a	8.5±0.2a	17.0±0.5ab	70.1±0.9ab
	OWC	0.37±0.02bcd	5.1±0.2a	97.6±14.1a	12.5±0.7ab	14.3±1.8ab	45.6±2.5a
	SBR	0.38±0.01bcd	5.0±0.1a	91.9±7.3a	14.4±0.4abc	14.3±1.0ab	42.5±1.0a
R.sphaerocarpa	Control	0.61±0.01ef	10.1±0.1ef	49.0±1.0a	44.4±0.1e	15.4±1.1ab	235.9±4.6f
	OWC	0.42±0.02cde	9.3±0.2de	73.0±4.0a	27.6±0.9bcd	15.1±1.0ab	138.4±4.1cde
	SBR	0.37±0.00bcd	8.5±0.0cd	33.6±0.0a	27.3±0.0bcd	22.0±0.0ab	113.0±0.0bcd
C. juncea	Control	0.26±0.01abc	8.6±0.0cde	62.0±0.0a	26.1±0.0bcd	23.0±0.0ab	137.0±0.0cde
	OWC	0.29±0.01bc	9.3±0.0de	113.8±0.0a	19.8±0.0abcd	21.2±0.0ab	106.6±0.0bc
	SBR	0.26±0.01abc	8.7±0.1cde	128.2±2.3a	24.5±0.7abcd	22.7±0.7ab	118.8±1.5cd
A. cytisoides	Control	0.43±0.01c	8.6±0.0cde	503.0±15.0d	64.4±2.4f	115.1±0.1e	247.6±5.2fg
	OWC	0.43±0.02cde	7.2±0.1bc	318.0±18.6bc	29.4±1.0cde	67.6±4.6d	126.2±3.9cd
	SBR	0.52±0.02cde	8.2±0.2cd	176.6±16.6ab	26.5±1.1bcd	37.3±4.4bc	127.5±2.6cde
P. miliaceum	Control	0.29±0.01bc	11.4±0.0f	409.7±0.0cd	91.2±0.0gh	49.3±0.0cd	283.6±0.0g
	OWC	0.28±0.01bc	4.2±0.2a	184.1±6.2ab	34.2±1.4de	26.6±1.6abc	119.8±4.0cd
	SBR	0.19±0.01a	4.6±0.2a	39.9±2.1a	20.1±0.4abcd	5.3±0.3a	72.0±1.9ab
L. perenne	Control	0.67±0.03f	6.1±0.1ab	381.9±19.7cd	98.3±1.2h	34.4±2.4bc	171.5±4.6e
	OWC	0.33±0.01bcd	7.2±0.1bc	141.0±7.71a	84.1±1.1gh	12.0±0.5ab	110.4±1.3bcd
	SBR	0.43±0.01cde	9.0±0.3cde	145.1±14.89a	81.5±2.2g	10.8±1.2ab	152.1±3.4de

and/or by restricting heavy metals translocation from roots to shoot (Hildebrandt et al. 2007). Both organic amendments did not affect the degree of root colonization of all plants by indigenous AMF present in the experimental soil. Previous studies have confirmed that SBR increased the diversity of AMF colonizing roots of all plants tested, except *B. bituminosa* (Alguacil et al. 2011). Based on the data obtained in our study, it is not possible to know whether the native AMF in conjunction with SBR contributed to stimulate the growth of host plants.

Comparing all tested plants, the native grass *P. miliaceum* responded more effectively to the addition of SBR than the rest of plants confirming its ability to withstand these harsh environments (Carrasco et al. 2009). Being a perennial grass with a well-extended root system forming short woody rhizomes, it is useful to create a vegetal layer in the polluted soil to reduce mobilization and erosion (Conesa et al. 2007) and it can be considered for phytoremediation purposes (García et al. 2004).

B. bituminosa is known to be a native heavy metal-tolerant plant and seed germination is not inhibited by heavy metal stress (Walker et al. 2007; Martínez-Fernández et al. 2011). *B. bituminosa* produces large amounts of different furanocoumarins against pathogens and it is hypothesized that stress stimulates production of these substances, regardless if it is biotic or abiotic (Martínez-Fernández et al. 2011). In this study, we have confirmed the suitability of this legume for phytostabilization showing limited heavy metals transfer to its aerial parts, even in the soil non-amended, and enhanced biomass after both organic amendments addition.

R. sphaerocarpa was the only plant that could not improve its nutrient content and growth, despite the reduced accumulation of foliar Zn promoted by the addition of organic amendments. The lack of response to bioremediation techniques proposed could be related to the ability of this evergreen legume shrub for raising the fertility of its own rhizosphere due to its associated rhizobial symbioses. It is also possible that the benefits of stabilized organic residues are not immediate but are evident in long-term basis. In this regard, the effectiveness of a composted municipal residue for improving the performance of *R. sphaerocarpa* plants was

Table 5 Nutrients and enzymatic activities in response to addition of organic waste compost (OWC) and fermented sugar beet residue (SBR) to soil of six plant species 8 months after planting (means ± standard errors, $n=8$). Values in columns followed by the same letter do not differ significantly within the same plant species ($p<0.05$) as determined by the Tukey test

Plant	Treatment	Total N (g kg^{-1})	Available P (μg g)	Dehydrogenase (μg INTF g^{-1} soil)	Phosphatase (μmol PNP g^{-1} h^{-1})	β-Glucosidase (μmol PNP g^{-1} h^{-1})
B. bituminosa	Control	0.57±0.02bc	15±1bc	22.6±1.4def	1.6±0.1abcde	11.2±0.2cdefg
	OWC	0.69±0.01bc	18±0cd	27.3±1.9fg	1.6±0.1ab	9.5±0.1abcd
	SBR	0.90±0.03d	17±0cd	42.6±2.3h	1.8±0.1abcd	12.6±0.2defg
R. sphaerocarpa	Control	0.60±0.01bc	14±1bc	17.4±0.2bcd	2.4±0.1cde	10.2±0.3abcde
	OWC	0.71±0.01bc	15±0bc	17.1±0.7bcd	2.0±0.1abcd	8.8±0.2abc
	SBR	0.89±0.02d	19±1cd	27.3±1.8efg	2.4±0.1e	13.9±0.2fgh
C. juncea	Control	0.52±0.01a	10±0a	10.8±0.3a	1.7±0.0abc	9.6±0.1abcd
	OWC	0.62±0.02bc	11±1ab	11.4±0.4a	1.5±0.0ab	8.4±0.0ab
	SBR	0.83±0.01cd	11±0a	19.2±0.7cde	2.0±0.0bcde	13.0±0.1fg
A. cytisoides	Control	0.49±0.01a	15±0bc	18.3±0.7bcd	1.2±0.1ab	10.9±0.1bcdefg
	OWC	0.63±0.01bc	21±1de	17.3±0.4bc	1.1±0.0a	7.8±0.2a
	SBR	0.88±0.02d	20±0cd	29.7±0.5fg	2.0±0.0bcde	12.8±0.2defg
P. miliaceum	Control	0.57±0.01bc	15±0bc	10.9±0.7a	2.1±0.0bcde	10.2±0.1bcdef
	OWC	0.75±0.02cd	16±0cd	13.1±1.0ab	1.9±0.0abcde	9.2±0.1abcd
	SBR	0.91±0.02d	16±0bc	30.0±1.0fg	2.6±0.0de	15.3±0.3gh
L. perenne	Control	0.62±0.01bc	30±1f	18.2±1.0cde	2.0±0.0bcde	13.7±0.3efg
	OWC	0.56±0.01ab	32±1f	18.0±1.3cde	1.2±0.0a	10.2±0.2bcdef
	SBR	0.92±0.03d	28±1ef	39.2±0.6gh	1.6±0.1abc	17.8±0.2h

confirmed 18 months after planting in a degraded soil (Caravaca et al. 2003). Interestingly, the establishment of *R. sphaerocarpa* can facilitate the introduction of other species in the surrounding area by acting as a "nurse plant" (Pugnaire et al. 1996). *R. sphaerocarpa* has been widely used in revegetation of semiarid degraded ecosystem (Caravaca et al. 2003; Alguacil et al. 2004) and its use as a phytostabilizer species in restoration of Mediterranean soils affected by As has recently been encouraged due to its high As-resistance (Moreno-Jiménez et al. 2008).

The non-native grass *L. perenne* had the highest root development and comparable aerial biomass to the native grass *P. miliaceum*, confirming its suitability to be used in phytoremediation of contaminated sites (Arienzo et al. 2004; Santibáñez et al. 2008). Nevertheless, there is a risk that non-native species become established in the area after remediation of the site and displace native tolerant species (Gressel and Al-Ahmad 2005). Furthermore, *L. perenne* has already been shown to be an invasive species (Sherwood and Jasieniuk 2009).

In conclusion, the effect of organic amendments addition on performance of plants is not predicable and it should be previously tested in mesocosm experiments before including their use in bioremediation tasks. The application of fermented sugar beet residue was the most effective treatment for improving soil biochemical and chemical fertility and for reducing metal phytoaccumulation in aerial parts, leading improved nutritional status and plant growth. The plants assayed showed contrasted abilities to thrive in contaminated soil but the character native and non-native of plant species was not a key factor in the establishment of the same. The combination of SBR and *P. miliaceum* plants can be regarded the most effective strategy for being employed in phytostabilization projects of slightly alkaline mine tailings in semiarid environments. Further studies should be performed for assessing the effectiveness of these restoration biotechnologies in acidic mine tailings.

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