Environ Sci Pollut Res (2014) 21:1029–1038
DOI 10.1007/s11356-013-1988-z

RESEARCH ARTICLE

The use of olive-mill waste compost to promote the plant vegetation cover in a trace-element-contaminated soil

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Received: 13 April 2013 / Accepted: 4 July 2013 / Published online: 19 July 2013

Abstract The applicability of a mature compost as a soil amendment to promote the growth of native species for the phytorestoration of a mine-affected soil from a semi-arid area (SE Spain), contaminated with trace elements (As, Cd, Cu, Mn, Pb and Zn), was evaluated in a 2-year field experiment. The effects of an inorganic fertiliser were also determined for comparison. *Bituminaria bituminosa* was the selected native plant since it is a leguminous species adapted to the particular local pedoclimatic conditions. Compost addition increased total organic-C concentrations in soil with respect to the control and fertiliser treatments, maintained elevated available P concentrations throughout the duration of the experiment and stimulated soil microbial biomass, while trace elements extractability in the soil was rather low due to the calcareous nature of the soil and almost unaltered in the different treatments. Tissue concentrations of P and K in *B. bituminosa* increased after the addition of compost, associated with growth stimulation. Leaf Cu concentration was also increased by the amendments, although overall the trace elements concentrations can be considered non-toxic. In addition, the spontaneous colonisation of the plots by a total of 29 species of 15 different families at the end of the experiment produced a greater vegetation cover, especially in plots amended with compost. Therefore, the use of compost as a soil amendment appears to be useful for the promotion of a vegetation cover and the phytostabilisation of moderately contaminated soils under semi-arid conditions.

Keywords Soil remediation · Organic amendments · Metals · Arsenic · Phytorestoration · *Bituminaria bituminosa*

Responsible editor: Zhihong Xu

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Introduction

The intense mining activity carried out for over 2,500 years in the Sierra Minera of La Unión-Cartagena (Murcia, southern Spain) has led to an area of over 50 km^2 of land with high concentration of trace elements (TEs) (Conesa et al. 2009). The adverse conditions of these soils (high levels of TEs, low organic matter and nutrients content, poor physical structure etc.) and the typically Mediterranean semi-arid climate (hot and dry summers, annual rainfall <300 mm with occasional heavy storms) hinders plant growth and exposes soils to erosion via wind and water (Marqués et al. 2005; Mendez et al. 2007).

The establishment of a vegetation cover adapted to site-particular characteristics of environmental stress (TE toxicity, poor nutrient and water availability) would physically stabilise the soils ("phytostabilisation") and minimise the erosion and dissemination of the contaminants (Mench et al. 2007; Mendez and Maier 2008), this being a potentially cost-effective and environmentally friendly strategy to remediate TEs-contaminated sites. The use of native plants in phytostabilisation ("phytorestoration") is of special interest because they demonstrate pedoclimatic tolerance and provide the basis for natural ecological succession (Mendez et al. 2007). *Bituminaria bituminosa* (L.) C.H. Stirton (Fabaceae) is a biennial–perennial herbaceous which grows in many Mediterranean countries (Walker et al. 2006) and is found at TEs-contaminated sites in the Sierra Minera in Murcia (Walker et al. 2007) and in other parts of the Iberian Peninsula (Poschenrieder et al. 2001; del Río et al. 2002).

Soil organic amendments are often necessary for the improvement of soil properties in the reclamation of TEs-polluted soils, to allow the establishment of the vegetation (Tordoff et al. 2000). Several agroindustrial and farm residues produced in large amounts and with important management problems (animal manures and slurries, urban residues etc.) have been used for the bioremediation of TEs-contaminated soils (Bernal et al. 2007; Clemente et al. 2007), which represents a sustainable way of recycling them (de la Fuente et al. 2011; Pardo et al. 2011). Using these materials as soil amendments may not only provide essential nutrients and organic matter (OM) and improve soil structure, but also modify the solubility, both directly (direct interaction TEs-OM) and indirectly (changes in soil pH, redox conditions, salinity, etc.) and thus the phytotoxicity of TEs and stimulate the soil microbial biomass (Bernal et al. 2007). In Spain, the olive oil industry generates vast amounts of residues from the extraction process in a short period of time, usually from November to January, causing a great environmental concern (López-Piñeiro et al. 2008). The main by-product is alperujo (two-phase olive-mill waste), which can be composted before its application to the soil in order to obtain a high-quality organic amendment (Alburquerque et al. 2006). The beneficial short-term effects of the addition of alperujo compost to TEs-contaminated soils have been reported in laboratory and greenhouse experiments (Fornes et al. 2009; Alburquerque et al. 2011; Pardo et al. 2011; Martínez-Fernández and Walker 2012) as well as in field applications (Clemente et al. 2012). However, the long-term viability and effectiveness of this amendment under field conditions in semi-arid areas and its effect on the establishment and growth of native plant species must be studied further. Long-term (years) monitoring of the contaminants will be crucial for the success of any phytostabilisation scheme (Vangronsveld et al. 2009). Natural attenuation, and its promotion and monitoring, can be a realistic remediation action under certain circumstances (Dickinson et al. 2009), like the ones in derelict and abandoned TEs-contaminated sites.

The purpose of this work was to study the effects in time of olive-mill waste (alperujo) compost on the field scale phytostabilisation of a TEs-polluted soil within the mining area of La Unión-Cartagena using the native species *B. bituminosa*, comparing with those of a mineral fertiliser. Its influence on the soil conditions, with respect to pH, nutrient and contaminant availability, on plant growth and on the development of the soil microbial biomass was evaluated. Additionally, the colonisation by a spontaneous natural vegetation cover was documented, in relation to the different treatments.

Materials and methods

Experimental site and soil amendments

The experiment was carried out in the field in a Xeric Petrocalcic soil (Soil Survey Staff 2010) close to the locality of San Ginés de la Jara (Murcia, Spain) (37°38′22.3″ N, 0°50′1.9″ W). This soil was indirectly affected by mine activities and has moderate to high As, Pb and Zn concentrations compared to the background concentrations of these elements in soils of this area (Table 1). The average rainfall and potential evapotranspiration in this site for the period 2000–2010 were 382 and 1,217 mm, respectively, demonstrating the semi-arid nature of the climate (Clemente et al. 2012). A mature compost and an N/P/K fertiliser (15:15:15, acquired commercially) were used as soil amendments. The compost was made of a mixture of alperujo and cow manure (10:1, fresh weight), and provided highly stabilised organic matter and elevated concentrations of essential nutrients (Table 1).

Experimental procedures and treatments

Three soil treatments were set up: control (no treatment), compost (30 t ha^{-1}) and N/P/K (15:15:15) fertiliser (1.3 t ha^{-1}).

Table 1 Characteristics of the soil and of the compost used in the experiment

	Soil	Compost
pH	7.60±0.1	8.8±0.01
EC (dS m^{-1})	0.15±0.01	6.1±0.2
$CaCO_3$ (%)	19.5±0.5	nd
OM (%)	3.9±0.03	73.1±0.3
TOC (g kg^{-1})	22.5±0.2	438.6±7.9
Total-N (g kg^{-1})	0.9±0.03	31.7±0.8
Total-P (g kg^{-1})	nd	4.9±0.1
As (mg kg^{-1})	65.4±5	<0.01
Cd (mg kg^{-1})	<0.01	<0.01
Cu (mg kg^{-1})	28±0.5	48±0.5
Fe (g kg^{-1})	36±0.1	1.62±0.1
Mn (mg kg^{-1})	6774±223	92±1
Pb (mg kg^{-1})	651±18	36±2
Zn (mg kg^{-1})	632±7	141±4

Background concentrations of local soils (mg kg^{-1}): 12–16 As; 0.5–0.6 Cd; 23–30 Cu; 43–57 Pb; 90–96 Zn (Martínez and Pérez-Sirvent 2007)

EC electrical conductivity, *CEC* cation exchange capacity, *OM* organic matter, *TOC* total organic carbon, *nd* not determined.

These doses of compost and fertiliser were chosen in order to add to the soil the same amount of available N, taking into account that the N provided by the fertiliser is completely available, whereas that for compost would be only up to 20 % of its organic-N. Four replicates per treatment were distributed in 12 plots of 3 m^2 (3×1 m) in a fully randomised design. An empty strip, 0.5 m wide, was left between plots. Inside each plot, one sub-plot (1 m^2) was left without plants. The compost was added to the corresponding plots in autumn 2008 (9 kg per plot). The surface soil (0–20 cm) was mixed with the amendment and left to stabilise for 1 month before transplanting. The mineral fertiliser N/P/K was added to the soil 2 weeks after the plants were transplanted to the soil, at a rate of 390 g per plot.

Seeds of *B. bituminosa* were collected from plants within a contaminated site in the Sierra Minera (Walker et al. 2007; Martínez-Fernández et al. 2011), sown in trays containing perlite for 6 weeks before transplanting them in the corresponding sub-plots (16 plants m^{-2}) in the field. All plots were irrigated (4 l m^{-2}) three times with tap water (pH 8.8, 2.75 mM Ca, 0.06 mM K, 3.37 mM Mg 2. 99 mM Na, EC=0.88 dS m^{-1}) at the time of transplantation and after 1 and 2 months. The assay was carried out for 2.5 years, sampling composite soil samples from each subplot (0–20 cm) four times: 4, 12, 16 and 24 months after transplanting the plants. Four different plant samplings (4, 16, 18 and 24 months after transplanting) were carried out throughout the experiment. Above-ground biomass production was estimated after 16 months by harvesting one to three “typical” plants per sub-plot and measuring the oven-dry weight (65 °C, 3 days), which was scaled-up according to the number of surviving plants to give the biomass production per sub-plot.

At the end of the experiment (28 months after transplanting *B. bituminosa*), a floristic inventory of the plant species that had spontaneously colonised the plots throughout the second year (number of species in each treatment plot, percentage soil coverage) was carried out.

The timing of the experiment was:

- October 2008: addition of compost to the soil
- November 2008: transplanting of *B. bituminosa* seedlings
- December 2008: mineral fertiliser addition
- April 2009 (4 months after transplanting): sampling 1 (soil, leaves of *B. bituminosa* and plant biomass estimation)
- November 2009 (12 months): sampling 2 (soil, estimate plant biomass and percentage of ground cover)
- March 2010 (16 months): sampling 3 (soil, leaves of *B. bituminosa*)
- May 2010 (18 months): seeds of *B. bituminosa*
- November 2010 (24 months): sampling 4 (soil, leaves of *B. bituminosa*)
- April 2011: floristic inventory and percentage of ground cover

Chemical analysis

The physico-chemical characteristics of the soils and the compost (pH, electrical conductivity (EC), $CaCO_3$ and OM content, total organic-C (TOC), total-N (TN) and total-P concentrations) were determined routinely according to the methods described in Pardo et al. (2011). Microbial biomass-C (B_C) and biomass-N (B_N) were measured after a fumigation–extraction procedure (Vance et al. 1987) in an automatic microanalyser (TOC-V CSN+TNM-1 Analyzer, Shimadzu, Tokyo, Japan) and calculated according to Wu et al. (1990) and Jenkinson (1988). Soil and organic amendment pseudo-total metals (Cd, Cu, Fe, Mn, Pb and Zn) and As concentrations were determined by flame atomic absorption spectrometry (AAS) in a UNICAM 969 atomic absorption spectrometer or by inductively coupled plasma optical emission spectroscopy (ICP-OES; Iris Intrepid II XDL, Thermo Scientific) after microwave assisted (Ethos1, Milestone) aqua regia digestion. The analytical accuracy was checked with a certified reference material (SRM 2711 Montana Soil); recoveries obtained were between 83 and 95 % for the elements analysed.

Soil available metal concentrations (DTPA-extractable; Lindsay and Norvell 1978) were determined by AAS. Soil available-As was determined in a 0.5 M $NaHCO_3$ extract (1:10 *w*/*v*) by hydride generation atomic fluorescence spectrometry (PSAnalytical Millennium Excalibur, UK). All the analyses were done at least in duplicate and adjusted to values for oven-dried soil (105 °C for 24 h). Analytical errors (standard error) were below 2 %.

Samples of fully grown leaves and samples of mature seeds were taken for the determination of TEs accumulation. Leaves and seeds were rinsed with deionised water, washed with 0.1 % Triton to remove surface depositions and rinsed four times with deionised water. They were oven-dried (65 °C, 3 days) and milled to a fine powder; 100 mg samples were digested at 210 °C with a 1:1 mixture of nitric and perchloric acids (3 ml). For each set of samples, a reference plant material (Bowen’s Kale; IUPAC 1979) was digested also. The trace elements (As, Cd, Cu, Fe, Mn, Pb and Zn), and nutrients (K and P) concentrations were determined in the extracts by ICP-OES. For the reference material, the recoveries were 80–105 %. The N concentration was determined by an automatic microanalyser (Euro EA; EuroVector, Milan, Italy). The percentage ground cover for each sub-plot was estimated after 12 (for *B. bituminosa* plants, providing an estimation of the % of plant survival) and 28 (for the spontaneous vegetation) months processing photographs taken 2 m above the soil surface using ImageJ software (downloaded from http://rsb.info.nih.gov/ij/, 7 May 2009).

Statistical analysis

Soil and plant characterisation and composition data from each sampling time were subjected to ANOVA to determine the significance of treatment effects, and differences between means were determined using Tukey's test (performed with IBM SPSS Statistics 19 software). Treatment and time effects were also evaluated, considering data from all the samplings together. Simple correlations between the different variables were performed. When necessary, values were transformed to satisfy normality and variance homogeneity tests.

Results and discussion

No significant differences in the analysed soil-related parameters were found between planted and non-planted sub-plots in any of the samplings as a consequence of the overall scarce growth of *B. bituminosa* plants in the plots, so the mean values of them were considered as plots data and only treatment effects are discussed.

Effects on soil physico-chemical properties and TEs availability

The addition of compost caused a slight initial increase of soil pH ($p<0.001$; Table 2), related to the alkaline nature of this material. However, in all treatments soil pH remained within a narrow range (7.8–8.1) throughout the experiment. Soil EC was higher in compost-treated plots in all samplings, reaching double the values observed in control plots at the first sampling ($p<0.001$; Table 2) due to the elevated soluble salts content of this amendment (Fornes et al. 2009), although values were still rather low (<0.21 dS m^{-1}) and soils can be considered non-saline (Bernal et al. 1992). As expected, compost addition significantly improved TOC concentrations in soil throughout the experiment with respect to the control and fertiliser treatments ($p<0.001$; Table 2), although overall concentrations of TOC decreased with time, indicating certain mineralisation of compost's organic matter in the soil ($p<0.01$; Table 2). Additionally, this amendment increased soil TN concentrations ($p<0.001$, Table 2), which remained above those in control and fertiliser-treated soils until the last sampling. In contrast, the mineral fertiliser did not increase soil TN, showing the fast plant uptake of its readily available inorganic-N. The fertiliser and, especially, the compost (rich in K; Alburquerque et al. 2006) treatment increased available-K and available-P concentrations in the soil, reaching concentrations 30-fold that in control plots in the first sampling for K, and 5-fold that in control plots for P in the last sampling (Table 2). The slow mineralisation of compost in the soil

Table 2 The pH, electrical conductivity (EC; dS m^{-1}), total organic-C (TOC) and total N (TN; g kg^{-1}) and available K ($NaNO_3$-extractable) and P ($NaHCO_3$-extractable) concentrations (mg kg^{-1}) in the soil from the differently treated plots at the four sampling times

Sampling time	Treatment	pH	EC	TOC	TN	$NaNO_3$–K	$NaHCO_3$–P
4 months	Control	7.8±0.05b	0.090±0.010b	7.9±1.6b	1.6±0.4ab	8±0.9c	0.3±0.1b
	Compost	8.1±0.03a	0.205±0.013a	35.7±5.1a	2.4±0.5a	253±21a	8.9±1.4a
	Fertiliser	7.8±0.03b	0.123±0.009b	8.8±1.4b	0.7±0.2b	34±7b	6.1±0.6a
	ANOVA	***	***	***	**	***	***
12 months	Control	8.0±0.02	0.124±0.005b	9.5±0.6b	1.3±0.2b	26±2.8c	0.3±0.2b
	Compost	8.0±0.01	0.178±0.011a	27.4±5.4a	2.1±0.2a	435±57a	8.5±1.6a
	Fertiliser	8.0±0.01	0.115±0.007b	7.6±1.0b	1.0±0.1b	73±9.9b	7.3±1.6a
	ANOVA	ns	***	***	**	***	**
16 months	Control	8.0±0.02	0.101±0.002b	6.3±1.0b	1.3±0.0b	16±1.5c	0.5±0.2c
	Compost	8.0±0.03	0.160±0.007a	16.4±3.4a	2.2±0.3a	312±31a	7.9±1.3a
	Fertiliser	8.0±0.03	0.105±0.001b	7.3±0.6b	2.4±0.1a	61±6.5b	4.3±0.6b
	ANOVA	ns	**	**	**	***	***
24 months	Control	7.9±0.03	0.132±0.008b	12.7±0.8b	1.3±0.1ab	10±1.3c	1.2±0.1c
	Compost	7.9±0.02	0.154±0.004a	19.1±2.6a	1.7±0.4a	110±9a	4.9±0.4a
	Fertiliser	7.9±0.01	0.109±0.001c	8.3±0.5b	0.9±0.1b	20±1.5b	3.0±0.2b
	ANOVA	ns	***	**	*	***	***
ANOVA	Treatment	***	***	***	***	***	***
	Time	***	**	**	**	***	ns
	T×t	***	***	***	***	***	***

Mean values denoted by the same letter in a column for each sampling time do not differ significantly according to Tukey's test ($p>0.05$)

ns not significant

*$p<0.05$; **$p<0.01$; ***$p<0.001$

meant that the P (mainly present in organic forms; Jorgensen et al. 2010) was released gradually (Clemente et al. 2012), whilst available P in the fertiliser treatment decreased faster with time. In agreement with this, Alburquerque et al. (2011) also reported an increase of $NaHCO_3$-extractable P in two contaminated soils treated with alperujo compost compared to N/P/K fertiliser (15:15:15) application after 56 days of incubation.

The concentrations of DTPA-extractable Cd, Cu, Mn, Pb and Zn and $NaHCO_3$-extractable As (Fig. 1) were relatively low in all plots and all samplings, and were presumably not in the phytotoxic range, since previous reports indicate phytotoxicity thresholds of TEs available concentrations >1, ≥5, ≥40, >35, >20 and ≥1.0 mg kg^{-1} for Cd, Cu, Mn, Pb, Zn and As, respectively, depending on the species and soil (Poschenrieder et al. 2001; Menzies et al. 2007; Martínez-Fernández and Walker 2012). The calcareous character of the soil favoured the occurrence of TEs in forms of low solubility (Kabata-Pendias 2001). No effects of the treatments were found on TEs availability throughout the experiment, with the only exception of Mn in compost-treated plots in the first three samplings (Fig. 1). Arsenic extractable concentrations were also generally higher in compost plots throughout the experiment, although differences were not statistically significant in any of the samplings. Numerous studies have shown that the addition of stabilised organic matter to contaminated soils promotes the immobilisation of the metals (Alvarenga et al. 2008; Gadepalle et al. 2009). However, the unaltered solubility of TEs in this soil is in agreement with the results reported previously by Pardo et al. (2011) and Martínez-Fernández and Walker (2012) from incubation and pot experiments using the same soil and amendments. Also, it has been previously reported from field experiments carried out in calcareous soils of nearby areas that TEs available concentrations in the soil were rather low, and that the addition of organic amendments did not change significantly the available fraction of TEs (Clemente et al. 2007; de la Fuente et al. 2011). As mentioned above, compost application led to a slight increase of the DTPA-extractable Mn concentration (Fig. 1), this effect disappearing in the last sampling (November 2010). The oxidative polymerisation of certain phenolic compounds present in the olive mill

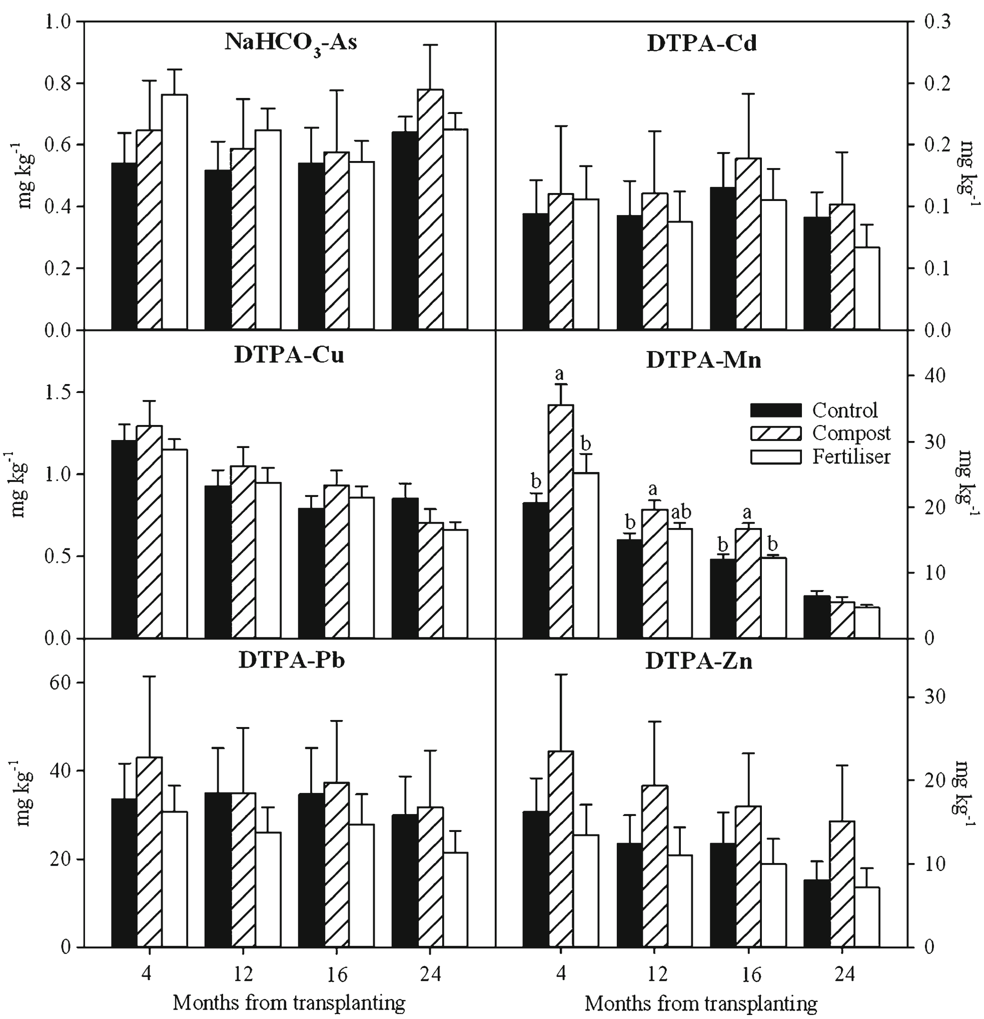

Fig. 1 Soil available concentrations of metals (DTPA-extractable) and As ($NaHCO_3$-extractable) in the differently treated plots, at the different sampling times. Bars marked with the same letter at each sampling time do not differ significantly according to Tukey's test (p>0.05)

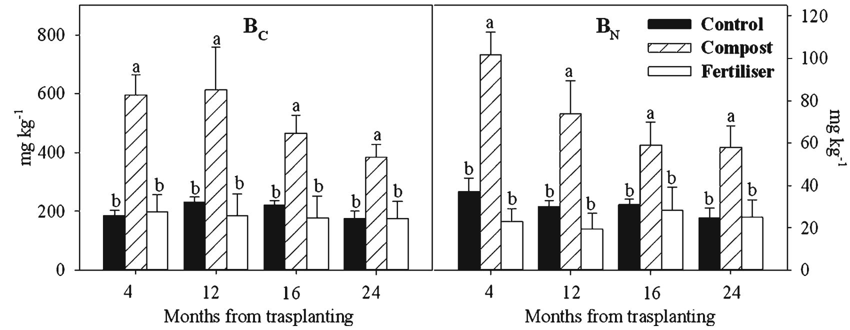

Fig. 2 Soil microbial biomass-C (B_C) and biomass-N (B_N) concentrations in the differently treated plots, at the different sampling times. Bars marked with the same letter at each sampling time do not differ significantly according to Tukey's test ($p>0.05$)

waste—that may resist the composting process—could provoke Mn mobilisation, as Mn oxides reduction in the soil releases highly soluble Mn^{2+} ions (Clemente et al. 2007; de la Fuente et al. 2011; Piotrowska et al. 2006) that are slowly re-oxidised in the soil. Extractable concentrations of As were not increased (nor decreased) after the addition of the compost (Fig. 1), which contrasts with results from the incubation experiment where a slight mobilisation of this element was reported (Pardo et al. 2011), showing how mobilisation and/or immobilisation effects can vary from laboratory experiments to field scale ones.

Soil biomass-C and biomass-N concentrations

The addition of compost significantly increased the soil microbial biomass-C (B_C) and biomass-N (B_N) concentrations ($p<0.001$), resulting in values much higher than those in the control and fertiliser treatments (Fig. 2). These results corroborate those observed in the previously mentioned incubation experiment with this soil (Pardo et al. 2011), where compost application doubled B_C concentrations in untreated soil after 56 days of incubation. Indeed, values found here were similar to those observed in mine-contaminated soils of a nearby area after the addition of different organic amendments (Clemente et al. 2007; de la Fuente et al. 2011).

In the present experiment, no significant effect of compost on TEs availability was observed, so the increased microbial biomass must be related to the important load of OM and nutrients provided with this material. This is supported by the significant, positive correlations found between these parameters and the soil TOC and available K and P concentrations (Table 3). The positive significant correlations found between available Pb and Zn and B_C and B_N (Table 3) must be a consequence of the concomitant increase caused by the compost in the DTPA-extractable concentrations of those metals (not statistically significant due to data variability) and in microbial biomass concentrations. It could also be considered a sign of non-toxicity of the metal available concentrations in this soil. Baker et al. (2011) also found no significant correlations between the decrease of TEs availability and B_C concentrations after the addition of cattle manure compost to a mine-contaminated soil, and suggested that available-C was the key factor for the establishment of a healthy microbial community.

Biomass production and mineral composition in *B. bituminosa*

Compost improved plant biomass production, estimated as the dry weight (DW) of the aerial part of the plants per plot ($p<0.05$), although values were actually quite low (Table 4), showing impaired growth of the plants in the soil. This was also observed for the percentage of ground cover reached by *B. bituminosa* that was rather low (<20 %) but higher in compost plots, although due to variability of the data between plots of the same treatment differences were not statistically significant. In fact, a positive significant correlation was found between plant biomass production and percentage ground cover ($r=0.87$; $p<0.01$).

The addition of compost significantly increased tissue P concentrations, compared to the control, in the first and the second samplings and concentrations did not change significantly with time (Table 5). In *B. bituminosa*, P deficiency appears at tissue concentrations below 80 μmol g^{-1} (Walker et al. 2007; Pang et al. 2010), so a certain deficiency of this nutrient was found in the plants, which may account for the limited plant size and number of leaves (Marschner 1995), and consequently ground cover.

No significant changes in tissue concentrations were detected for N in plants from the different plots, and a significant increase in K concentrations was only observed in plants from compost-treated plots in the last sampling (Table 5). For *B. bituminosa*, shoot K and N concentrations ≥300 μmol g^{-1} (Martínez-Fernández and Walker 2012) and 25–37 g kg^{-1}

Table 3 Simple correlations between soil TOC, available K, P and trace elements (only significant correlations are shown) and microbial biomass-C (B_C) and biomass-N (B_N) concentrations (mg kg^{-1}; $n=12$)

	TOC	K	P	Pb	Zn
B_C	0.930***	0.931***	0.709*	0.705*	0.857***
B_N	0.955***	0.790**	0.605*	0.764**	0.898***

*$p<0.05$; **$p<0.01$; ***$p<0.001$

Table 4 Percentage of plant (*B. bituminosa*) ground cover per plot 12 months after transplanting, and aerial part dry biomass of *B. bituminosa* plants 16 months after transplanting, for the different treatments (n=4)

Treatments	Cover (%)	Aerial part biomass (g m^{-2})
Control	6.2±2.1	39.0±13.9b
Compost	16.6±5.8	135.7±37.4a
Fertiliser	8.7±1.2	52.5±5.3ab
ANOVA	ns	*

Mean values denoted by the same letter in a column do not differ significantly according to Tukey's test (p>0.05)

ns not significant

*p<0.05

(Walker et al. 2007), respectively, are required for maximum growth. So N deficiency did not occur in the present experiment (not even in the control plots) reflecting the ability to trap atmospheric N_2 of this leguminous species, whilst the apparent K deficiency declined with time and after 24 months the compost treatment resulted in K sufficiency (Table 5).

Regarding TEs, the concentration of Cu was the only one significantly affected by the amendments, showing higher values in plants from compost-treated plots in the second sampling and in plants from fertiliser-treated plots in the third one, than in those from control plots (Table 5); nonetheless, those values cannot be considered toxic (<20 mg kg^{-1}) (Kabata-Pendias 2001). Although this soil contained high levels of Zn, the population of *B. bituminosa* used, collected from a site greatly contaminated by Zn and other TEs, seems able to restrict its uptake (and that of the other TEs in the soil) and transport (Walker et al. 2007). In addition, available Zn concentrations in the soil were low (<25 mg kg^{-1}), indicating the high metal retention capacity of this calcareous soil. The concentrations of Fe, Mn and Pb in the plants cannot be considered toxic either (<80–350 Fe, 400–1,000 Mn and 30–100 Pb mg kg^{-1}; Kabata-Pendias 2001; Mendez and Maier 2008).

An important fact was that despite the scarce growth of plants of *B. bituminosa* these were able to complete their life cycle, producing flowers with fertile seeds. The concentration of TEs in the seeds did not differ among treatments and were even lower than those in plant leaves (5.1–6.4 Cu, 40–112 Fe, 19–22 Mn, 0.5–0.9 Pb and 19–21 Zn mg kg^{-1}DW). Restricting TEs transport to the fruits limits their movement through the food chain, since the seeds of *B. bituminosa* are a food source for some insects (Oliveras et al. 2008).

The bioconcentration factor (BCF; leaf concentration of TE/soil total concentration) values (e.g. 0.006–0.008, 0.22–0.47, 0.011–0.013, 0.003–0.005 and 0.056–0.068 for As, Cu, Mn, Pb and Zn, respectively, in the control soil in the last sampling) confirm *B. bituminosa* as a species capable of restricting TEs accumulation in its aerial parts and hence potentially suitable for phytostabilisation of contaminated sites. Walker et al. (2007), in a more-highly contaminated soil,

Table 5 Nitrogen (%), P, K and Na (μmol g^{-1}) and trace elements (μg g^{-1}) concentrations in *B. bituminosa* leaves from plots receiving the different treatments, at the different sampling times

Sampling time	Treatment	N	P	K	As	Cd	Cu	Fe	Mn	Pb	Zn
4 months	Control	3.83±0.47	58±4 b	159±6	0.38±0.13	bdl	6.8±0.4	320±1	75.0±4.4	3.5±0.7	38±2
	Compost	4.14±0.42	76±4 a	159±2	0.31±0.15	bdl	8.6±0.5	232±28	82.6±9.2	3.4±1.3	43±4
	Fertiliser	4.70±0.25	62±4 ab	162±9	0.41±0.05	bdl	7.6±0.3	320±94	76.6±6.2	3.2±1.0	38±2
	ANOVA	ns	*	ns	ns		ns	ns	ns	ns	ns
16 months	Control	5.10±0.09	67±3 b	241±17	bdl	bdl	13.2±0.6 ab	195±18	89.7±5.2	2.1±0.1	40±3
	Compost	4.73±0.25	83±4 a	246±29	bdl	bdl	14.4±1.3 a	158±6	87.3±6.9	2.1±0.4	36±1
	Fertiliser	5.40±0.22	67±4 b	239±11	bdl	bdl	11.1±0.5 b	232±48	95.9±10.1	2.0±0.9	38±4
	ANOVA	ns	*	ns			*	ns	ns	ns	ns
24 months	Control	4.15±0.22	64±13	245±16 b	0.55±0.34	0.12±0.01	6.0±0.5 b	179±11	90.7±11.0	2.4±0.3	43±2
	Compost	4.36±0.10	77±7	325±11 a	1.51±0.61	0.13±0.02	7.2±0.1 ab	191±18	87.9±5.32	3.2±1.4	42±5
	Fertiliser	4.33±0.09	74±4	287±9 ab	1.31±0.68	0.13±0.03	7.6±0.4 a	167±17	94.8±1.1	1.9±0.5	47±3
	ANOVA	ns	ns	***	ns	ns	*	ns	ns	ns	ns
ANOVA	Treatment	ns	*	ns	ns		*	ns	ns	ns	ns
	Time	***	ns	***	*		***	***	ns	ns	ns
	T×t	ns	ns	ns	ns		*	ns	ns	ns	ns

Mean values denoted by the same letter in a column for each sampling time do not differ significantly according to Tukey's test (p>0.05)

ns not significant, *bdl* below detection limit

*p<0.05; **p<0.01; ***p<0.001

found BCF values for *B. bituminosa* of ≤0.16, ≤0.11, ≤0.019, ≤0.008 and ≤0.02 for Cd, Cu, Mn, Pb and Zn, respectively, similar to those found in the present experiment. Poschenrieder et al. (2001) found that of 32 species growing on Cu-contaminated soils, *B. bituminosa* was one of those which accumulated least Cu in its shoot (BCF 0.04–0.30).

Spontaneous vegetation

Different plant species spontaneously colonised plots during the second year of the experiment. One of the ultimate objectives of TEs-contaminated sites reclamation is to facilitate the development of the plant community and to restore the ability of soil to function as a self-sustaining ecosystem (Holl 2002). In this sense, the study of species richness and plant cover in the plots can be a first approach to estimating the successional dynamics of vegetation (Alday et al. 2011).

Two and a half years after the addition of the amendments, a total of 29 species of 15 different families (Table 6) were recorded in the plots; 20.1 % were woody species and 79.9 % were herbaceous. The largest family was Asteraceae with eight taxa (27.6 % of total), followed by the Fabaceae (10.3 %) and Boraginaceae (10.3 %). The plant species recorded belong to the first stages of the vegetative succession of the zone (Rivas Martínez 1987). The most frequent species were (% of total plant number) *Convolvulus althaeoides* (13.5 %), *Valantia hispida* (13.1 %), *Anagallis arvensis* (11.8 %) and *Galium verrucosum* (9.4 %).

Several native species were unique to the compost-treated plots: *Echium sabulicolum* (Boraginaceae), *Reichardia tingitana* (Asteraceae), *Sedum sediforme* (Crassulaceae), *Scorpiurus sulcatus* (Fabaceae) and *Phalaris brachystachys* (Poaceae). Despite the large variability observed, plots amended with compost showed higher percentages of plant ground cover than control and fertiliser treatments (control, 5–14 %; compost, 8–54 %; fertiliser, 4–18 %), likely related to the long-term supply of essential nutrients through the mineralisation of compost OM, and the stimulation of the microbial community. Previous field studies have reported that the application of organic materials to mine soils favours the establishment of vegetation (regarding ground cover, biomass production and species richness) as a consequence of the improvement in soil biochemical properties and nutrients availability, reflecting the complex relationships that exist between plants, soil microorganisms and the reactivation of soil biogeochemical cycles (Pérez de Mora et al. 2011; Clemente et al. 2012; Zornoza et al. 2012).

Colonising vegetation can play an important role in the early stages of succession in contaminated soils, regarding TEs translocation to aerial parts, and soil re-entry after plant decomposition (Pérez de Mora et al. 2011; Conesa et al. 2011). In this sense, excluder species whose tissues may take time to degrade, could immobilise TEs over a long period and limit their entry into the food chain (Vangronsveld et al. 2009). Therefore, among the taxa found in this soil, shrubs as *Lavandula multifida*, *Thymus hyemalis* or *Fumana laevis* and perennial herbs such as *Eryngium campestre* may have a potential use in phytostabilisation. In addition, combinations involving leguminous plants as *Coronilla scorpioides* or *Scorpiurus sulcatus* may be of interest in oligotrophic soils because their ability to fix atmospheric N_2 can decrease N deficiencies in soil (Guo et al. 2008).

Table 6 Plant species inventory of the plots 28 months after transplanting *B. bituminosa*

Family	Species
Amaranthaceae	*Atriplex halimus*
Apiaceae	*Eryngium campestre*
Asteraceae	*Atractyllis cancellata*
	Carduus bourgeanus
	Carthannus lanatus
	Crespis vesicaria subsp haenseleri
	Dittrichia viscosa
	Leontodon taraxacoides
	Phagnalon saxatile
	Reichardia tingitana
Boraginaceae	*Cynoglossum creticum*
	Echium creticum subsp. coincyanum
	Echium sabulicolum
Brassicaceae	*Lobularia maritima*
Cistaceae	*Fumana laevis*
Convolvulaceae	*Convolvulus althaeoides*
Crassulaceae	*Sedum sediforme*
Fabaceae	*Bituminaria bituminosa*
	Coronilla scorpioides
	Scorpiurus sulcatus
Frankeniaceae	*Frankenia corymbosa*
Lamiaceae	*Lavandula multifida*
	Thymus hyemalis
Myrsinaceae	*Anagallis arvensis*
Poaceae	*Phalaris brachystachys*
	Stipa capensis
Rubiaceae	*Galium verrucosum*
	Valantia hispida
Xanthorrhoeaceae	*Asphodelus fistulosus*

Conclusions

The calcareous character of the studied soil meant that TEs were present mostly in hardly available forms in the soil and the addition of compost or fertiliser did not significantly alter

TEs (As, Cd, Cu, Pb and Zn) availability. The application of compost allowed the increase of soil microbial biomass C and N throughout the experiment, and favoured plant growth due to a combination of C, N, K and P supply, and did not affect plant tissue TEs concentrations. In contrast, mineral fertiliser addition did not stimulate the microbial biomass and/or plant development, showing the importance of the presence of organic matter in soil amendments and its persistence with time. In spite of the low solubility of the TEs and the apparent absence of phytotoxicity, the total soil concentrations of Pb and Zn were above background concentrations; this suggests that toxicity thresholds should, instead, be based on "plant-available" TE concentrations.

The combination of compost amendment and native plants appears to be useful for the phytostabilisation of moderately contaminated calcareous soils similar to the one studied here, under semi-arid conditions. In contrast, inorganic fertilisation was not so efficient. The different species that spontaneously colonised the area during the last part of the experiment helped to achieve a greater vegetation cover in plots amended with compost, and were a clear sign of improved soil health.

Acknowledgements This work was co-funded by the Spanish 'Ministerio de Ciencia e Innovación' (MICINN) and the EU through FEDER funds (projects CTM2007-66401-C02-01 and CTM2010-21922-C02-01). Part of R. Clemente and D. Walker's salaries is paid by the European Social Fund of the European Union. D. Martínez-Fernández received a pre-doctoral grant from the IMIDA. T. Pardo thanks the Spanish MICINN for the FPI grant awarded (BES-2008-009480). The authors thank J. A. Alburquerque and I. M. Martínez-Alcalá for their help throughout the experiment.

References

Alburquerque JA, González D, Cegarra J (2006) Composting of a solid olive-mill by-product and the potential of the resulting compost for cultivating pepper under commercial conditions. Waste Manage 6:620–626

Alburquerque JA, de la Fuente C, Bernal MP (2011) Improvement of soil quality after "alperujo" compost application to two contaminated soils characterised by differing heavy metal solubility. J Environ Manage 92:733–741

Alday JG, Pallavicini Y, Marrs RH, Martínez-Ruiz C (2011) Functional groups and dispersal strategies as guides for predicting vegetation dynamics on reclaimed mines. Plant Ecol 212:1759–1775

Alvarenga P, Gonçalves AP, Fernandes RM, de Varennes A, Vallini G, Duarte E, Cunha-Queda AC (2008) Evaluation of compost and liming materials in the phytostabilization of a mine soil using perennial ryegrass. Sci Total Environ 406:43–56

Baker LR, White PM, Pierzynski GM (2011) Changes in microbial properties after manure, lime, and bentonite application to a heavy metal-contaminated mine waste. Appl Soil Ecol 48:1–10

Bernal MP, Clemente R, Walker DJ (2007) The role of organic amendments in the bioremediation of heavy metal-polluted soils. In: Gore RB (ed) Environmental research at the leading edge. Nova, New York, pp 1–57

Bernal MP, Roig A, Madrid R, Navarro AF (1992) Salinity risks on calcareous soils following pig slurry applications. Soil Use Manage 8:125–130

Clemente R, de la Fuente C, Moral R, Bernal MP (2007) Changes in microbial biomass parameters of a heavy metal-contaminated calcareous soil during a field remediation experiment. J Environ Qual 36:1137–1144

Clemente R, Walker DJ, Pardo T, Martínez-Fernández D, Bernal MP (2012) The use of a halophytic plant species and organic amendments for the remediation of a trace elements-contaminated soil under semi-arid conditions. J Hazard Mater 223–224:63–71

Conesa H, Moradi AB, Robinson BH, Kühne G, Lehmann E, Schulin R (2009) Responses of native grasses and *Cicer arietinum* to soil polluted with mining wastes: implications for the management of land adjacent to mine sites. Environ Exp Bot 65:198–204

Conesa HM, María-Cervantes A, Álvarez-Rogel J, González-Alcaraz MN (2011) Influence of soil properties on trace element availability and plant accumulation in a Mediterranean salt marsh polluted by mining wastes: implications for phytomanagement. Sci Total Environ 409:4470–4479

de la Fuente C, Clemente R, Martínez-Alcalá I, Tortosa G, Bernal MP (2011) Impact of fresh and composted solid olive husk and their water-soluble fractions on soil heavy metal fractionation, microbial biomass and plant uptake. J Hazard Mater 186:1283–1289

del Río M, Font R, Almela C, Vélez D, Montoro R, de Haro BA (2002) Heavy metals and arsenic uptake by wild vegetation in the Guadiamar river area after the toxic spill of the Aznalcóllar mine. J Biotechnol 98:125–137

Dickinson NM, Baker AJM, Doronila A, Laidlaw A, Reeves RD (2009) Phytoremediation of inorganics, realism and synergies. Int J Phytorem 11:97–114

Fornes F, García de la Fuente R, Belda RM, Abad M (2009) "Alperujo" compost amendment of contaminated calcareous and acidic soils: effects on growth and element uptake by five *Brassica* species. Bioresour Technol 100:3982–3990

Gadepalle VP, Ouki SK, Hutchings T (2009) Remediation of copper and cadmium in contaminated soils using compost with inorganic amendments. Water Air Soil Pollut 196:355–368

Guo ZL, Zhong C, Cai CF, Ding SW, Wang ZM (2008) Nitrogen competition in contour hedgerow systems in subtropical China. Nutr Cycl Agroecosyst 81:71–83

Holl KD (2002) Long-term vegetation recovery on reclaimed coal surface mines in the eastern USA. J Appl Ecol 39:960–970

IUPAC (1979) Reference material for trace analysis by radioanalytical methods: Bowen's kale. Pure Appl Chem 51:1183–1193

Jenkinson DS (1988) The determination of microbial biomass carbon and nitrogen in soil. In: Wilson JR (ed) Advances in nitrogen cycling in agricultural ecosystems. CAB International, Wallingford, pp 368–386

Jorgensen K, Magid J, Luxhoi J, Jensen LS (2010) Phosphorus distribution in untreated and composted solid fractions from slurry separation. J Environ Qual 39:393–401

Kabata-Pendias A (2001) Trace elements in soils and plants, 3rd edn. CRC Press LLC, Boca Raton

Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci Soc Am J 42:421–428

López-Piñeiro A, Albarrán A, Rato Nunes JM, Barreto C (2008) Short and medium-term effects of two-phase olive mill waste application on olive grove production and soil properties under semiarid Mediterranean conditions. Bioresour Technol 99:7982–7987

Marqués MJ, Jiménez L, Pérez-Rodríguez R, García-Ormaechea S, Bienes R (2005) Reducing water erosion in a gypsic soil by combined use of organic amendment and shrub revegetation. Land Degrad Develop 16:339–350

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London

Martínez-Fernández D, Walker DJ, Romero-Espinar P, Flores P, del Río JA (2011) Physiological responses of *Bituminaria bituminosa* to heavy metals. J Plant Physiol 168:2206–2211

Martínez-Fernández D, Walker DJ (2012) The effects of soil amendments on the growth of *Atriplex halimus* and *Bituminaria bituminosa* in heavy metal-contaminated soils. Water Air Soil Pollut 223:63–72

Martínez MJ, Pérez-Sirvent C (2007) Niveles de fondo y niveles genéricos de referencia de metales pesados en suelos de la Región de Murcia. University of Murcia, Murcia

Mench M, Vangronsveld J, Lepp N, Ruttens A, Bleeker P, Geebelen W (2007) Use of soil amendments to attenuate trace element exposure: sustainability, side effects, and failures. In: Hamon R, McLaughlin M, Lombi E (eds) Natural attenuation of trace element availability in soils. SETAC, Pensacola, pp 197–228

Mendez MO, Maier R (2008) Phytostabilization of mine tailings in arid and semiarid environments—an emerging remediation technology. Environ Health Persp 116:278–283

Mendez MO, Glenn EP, Maier R (2007) Phytostabilization potential of quailbush for mine tailings: growth, metal accumulation, and microbial community changes. J Environ Qual 36:245–253

Menzies NW, Donn MJ, Kopittke PM (2007) Evaluation of extractants for the estimation of the phytoavailable trace metals in soils. Environ Pollut 145:121–130

Oliveras J, Gómez C, Bas JM, Espadaler X (2008) Mechanical defence in seeds to avoid predation by a granivorous ant. Naturwissenschaften 98:501–506

Pang J, Tibbett M, Denton MD, Lambers H, Siddique KHM, Bolland MDA, Revell CK, Ryan MH (2010) Variation in seedling growth of 11 perennial legumes in response to phosphorus supply. Plant Soil 328:133–143

Pardo T, Clemente R, Bernal MP (2011) Effects of compost, pig slurry and lime on trace element solubility and toxicity in two soils differently affected by mining activities. Chemosphere 84:642–650

Pérez de Mora A, Madejón P, Burgos P, Cabrera F, Lepp N, Madejón E (2011) Phytostabilization of semiarid soils residually contaminated with trace elements using by-products: sustainability and risks. Environ Pollut 159:3018–3027

Piotrowska A, Iamarino G, Rao MA, Gianfreda L (2006) Short-term effects of olive mill waste water (OMW) on chemical and biochemical properties of a semiarid Mediterranean soil. Soil Biol Biochem 38:600–610

Poschenrieder C, Bech J, Llugany M, Pace A, Fenés E, Barceló J (2001) Copper in plant species in a copper gradient in Catalonia (North East Spain) and their potential for phytoremediation. Plant Soil 230:247–256

Rivas Martínez S (1987) Memoria del Mapa de Series de Vegetación de España. Ministerio de Agricultura, Pesca y Alimentación, Madrid

Soil Survey Staff (2010) Keys to soil taxonomy, 11th edn. USDA-Natural Resources Conservation Service, Washington

Tordoff GM, Baker AJM, Willis AJ (2000) Current approaches to the vegetation and reclamation of metalliferous mine wastes. Chemosphere 41:219–228

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:689–696

Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnevajova E, van der Lie D, Mench M (2009) Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ Sci Pollut Res 16:765–794

Walker DJ, Bernal MP, Correal E (2007) The influence of heavy metals and mineral nutrient supply on *Bituminaria bituminosa*. Water Air Soil Pollut 184:335–345

Walker DJ, Moñino I, Correal E (2006) Genome size in *Bituminaria bituminosa* (L.) C.H. Stirton (Fabaceae) populations: separation of “true” differences from environmental effects on DNA determination. Environ Exp Bot 55:258–265

Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990) Measurement of soil microbial biomass C by fumigation-extraction. An automated procedure. Soil Biol Biochem 22:1167–1169

Zornoza R, Faz A, Carmona DM, Kabas S, Martínez-Martínez S, Acosta JA (2012) Plant cover and soil biochemical properties in a mine tailing pond five years after application of marble wastes and organic amendments. Pedosphere 22:22–32