



Impact of fresh and composted solid olive husk and their water-soluble fractions on soil heavy metal fractionation; microbial biomass and plant uptake

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ARTICLE INFO

Article history:

Received 16 July 2010

Received in revised form

18 November 2010

Accepted 1 December 2010

Available online 8 December 2010

Keywords:

Metal-contaminated soils

Soluble organic matter

Polyphenols

Mn solubility

Phytotoxicity

ABSTRACT

The use of waste materials as organic amendments in soil remediation can affect metal solubility; this interaction will vary with the characteristics of the organic matter that is added to the soil. A pot experiment was carried out in a calcareous, metal-polluted soil, using *Beta maritima* L. as an indicator species for the treatment effects on metal solubility. The treatments were: fresh solid olive husk, a mature compost, their respective water extracts (as the most reactive and biodegradable fraction) and an unamended, control soil. The compost reduced metal availability and plant uptake, while fresh olive husk favoured Mn bioavailability and produced phytotoxicity. The water-soluble extract from fresh solid olive husk also provoked elevated Mn solubility in soil, but did not increase Mn uptake by plants. The application of water-soluble organic matter obtained from compost did not affect heavy metal solubility significantly. Therefore, composted olive husk seems to be the most-appropriate material for the development of bioremediation strategies.

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1. Introduction

Reclamation of heavy metal-polluted soils often requires the addition of organic matter (OM), as it can promote plant growth and the activity of soil microorganisms by improving the poor physical and chemical properties (e.g. poor structure and a lack of nutrients) of these soils [1,2]. In this respect, the application of some by-products traditionally used in agriculture, like animal manures and compost, has been studied in different bioremediation experiments in soils contaminated with heavy metals, due to their low cost and widespread availability [3–6].

Organic matter usually plays a decisive role in heavy metal availability through changes in soil chemical properties and by its metal-chelating ability, the latter being one of the most-important factors controlling the solubility and bioavailability of metals in the plant-soil system [7]. Although this effect depends on different parameters, such as soil pH, the type of organic matter added to soil is also a significant factor [8]. The addition of fresh organic matter with a high proportion of water-soluble organic matter (WSOM) can increase metal solubility and therefore metal bioavailability and leaching [3]. This is related to the presence of organic compounds rich in functional groups (e.g. phenols) in the WSOM,

which give this fraction its chelating ability. In contrast, humified organic matter may diminish heavy metal bioavailability in soils by redistributing these elements from soluble fractions to forms less-readily available to plants, this being related to the formation of metal-humic complexes [9].

Fresh solid olive husk (also known as “alperujo”), a wet lignocellulosic by-product of the olive oil industry, which disposal constitutes a major concern to producers (four million tonnes are generated per year in Spain), can be a good source of organic matter for soils because of its high mineral nutrient (especially K) and carbon contents [10]. This material, however, also contains organic acids and fats that may have a negative effect on both microbial activity in soil and plant growth [11,12]. Co-composting fresh solid olive husk with different bulking agents has been shown to be a suitable way of recycling this waste, since it leads to a strong reduction of the WSOM content with a concomitant increase in the partially humified organic matter fraction [13]. Moreover, composts from fresh solid olive husk have low levels of heavy metals and are non-phytotoxic and rich in K [13], indicating the potential use of this type of compost as an organic amendment in the remediation of metal-contaminated soils. Albuquerque et al. [14] reported that the improvement of different biological properties of heavy metal polluted soils was a direct consequence of enhanced chemical properties of soil (lower heavy metal solubility and higher nutrient content) after solid olive husk compost addition.

The addition to soil of liquid organic materials obtained from composts, especially of the “humic-like fraction” extractable with alkaline solutions, has been proposed as a suitable source of

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Table 1

The main characteristics of the soil and the organic amendments (SOH: solid olive husk, SOH-WE: water extract from solid olive husk, COM: compost and COM-WE: water extract obtained from compost) used in the pot experiment. EC: electrical conductivity, TOC: total organic carbon and TN: total nitrogen concentration.

	Soil	SOH	SOH-WE	COM	COM-WE
pH	7.8	6.1	6.5	8.3	8.0
CE (dS m ⁻¹)	0.3	6.0	3.0	1.7	6.8
TOC (mg g ⁻¹)	6.6	500	5.3	396	6.6
TN (mg g ⁻¹)	1.2	15	0.1	20	0.7
C/N	5.5	34	53	20	9.4
Water-soluble phenols (mg g ⁻¹)	<0.5	17	1.7	2.0	1.0
Fe (μg g ⁻¹)	112,000	1434	19	3939	23
Zn (μg g ⁻¹)	2248	30	0.2	183	1.8
Mn (μg g ⁻¹)	5000	2.4	<0.5	92	1.9
Pb (μg g ⁻¹)	2790	9.0	<0.5	8.0	<0.5

nutrients and organic matter for crop production [15]. Recently, Kohler et al. [16] showed the positive effect of the addition of an alkali-extracted fraction from compost to soil, improving plant growth whereas microbial activity and soil structure were not affected. In this context, the water-soluble organic matter from composts could be an alternative source of organic matter and nutrients for soils, as it may have a positive effect on plant growth—directly and/or through the improvement of soil properties [17]. Likewise, this fraction provides a set of microorganisms that perform a variety of beneficial functions in the soil–plant ecosystem [18], which could be relevant for the reclamation of heavy metal-polluted soils. Nevertheless, there are no specific studies regarding the impact of the WSOM from compost on the reclamation of such soils. The aim of this work was, therefore, to compare the effects of compost and fresh solid olive husk and their water-soluble fractions on soil heavy metal availability, in order to optimise their potential use as organic amendments in reclamation strategies for heavy metal-contaminated soils. The water-soluble fractions obtained from compost and fresh solid olive husk were evaluated in order to separate the effects provoked by the most-labile and reactive organic fractions of these materials, which can also affect soil heavy metal fractionation through chelation shortly after soil addition. For this purpose, a pot experiment was carried out for the evaluation of heavy metal bioavailability and microbial parameters in a heavy metal-polluted soil.

2. Material and methods

2.1. Soil and organic amendments characterisation

A soil classified as Xeric Torriorthent was collected from an agricultural area (N37°38'45", W0°50'50") neighbouring the "Sierra Minera" of Cartagena-La Unión (Murcia, SE Spain). The soil pseudo-total concentrations of Pb and Zn (Table 1) exceed greatly the European Union maximum permitted levels for agricultural soils (300 μg g⁻¹ for both Pb and Zn at soil pH 7; [19]). This soil is a calcareous, clay loam with 15% CaCO₃, 0.63% OM, 33% sand, 27% silt and 39% clay. The main mineral components were: calcite (8%), iron oxides and hydroxides (hematite, 7%, and goethite, 4%), phyllosilicates (principally illite, 3%, and kaolinite, 3%) and feldspars (albite as the major mineral, 4%). Samples were collected from the top 20 cm of the soil, air-dried and sieved to <2 mm for analysis (Table 1).

Four organic amendments were tested: fresh and composted solid olive husk and their corresponding water extracts (Table 1). The fresh solid olive husk used was slightly acidic (pH 6.1) and rich in organic matter (89%) and soluble organic matter. The compost was prepared from a mixture of fresh solid olive husk (52%, on a fresh-weight basis) and poultry manure (48%). This compost is alkaline (pH 8.3), with high levels of total (75%) and partially humified organic matter (humification index of 15%, calculated as 100 × humic acid-C/total organic-C), indicating a high degree of matu-

riety. The compost was air-dried whereas the fresh solid olive husk was frozen and freeze-dried. The dried samples were ground before analysis and addition to the soil. The extracts of the solid materials were obtained through mechanical shaking with deionised water at room temperature; the suspensions obtained were centrifuged at 7800 × g for 10 min and the supernatants were then freed of particulate matter using a nylon filter (0.45 μm), to avoid any preferential retention of soluble organic compounds.

2.2. Pot experiment

Plastic pots (250 mL) were filled with 200 g of dry soil sieved at 4 mm. Five treatments with four replicates per treatment were applied to the soils in the pots: fresh solid olive husk (SOH); compost (COM); the water-soluble fraction of fresh solid olive husk (SOH-WE) and of the compost (COM-WE); with a non-amended soil as a control (CT). Solid materials were added at rates of 1.4 g and 8.8 g dry weight of SOH and COM per pot, respectively (providing both 3.5 g TOC kg⁻¹ of soil), and the volume of liquid fractions provided the same quantity of water-soluble carbon as the SOH treatment (1.5 g TOC kg⁻¹ of soil). Each treatment was run in two separate sets of pots (20 pots each set), with and without N:P:K inorganic fertiliser (15:15:15) at a rate of 120 mg kg⁻¹ of soil.

Beta maritima L. was selected as the indicator plant species for testing changes in metal availability after the addition of the amendments [20]. Four 30-day-old seedlings of *Beta maritima* were transplanted per pot, 1 h after the addition and homogenisation of the solid materials with the soil (SOH and COM), and kept in a growth chamber (light period: 14 h, 23 °C and 58% humidity and night period: 10 h, 18 °C and 70% humidity) throughout the experiment, them being regularly watered with distilled water. The inorganic fertiliser was surface-added just after transplanting.

The addition of the liquid materials was timed to evaluate the maximum metal uptake by the plants, as the effect of their highly degradable organic matter is expected to be short-lived in the soil; they were added to the soils in the corresponding previously untreated pots once the plants had developed a good biomass (after 39 days) and the nutrient uptake was at the highest rate. Plants were harvested 46 days after transplanting (7 days after the addition of the liquid amendments). The same amount of distilled water than that applied in the liquid treatments was added to the CT, COM and SOH pots in order to keep the same soil moisture content in all the treatments.

Plants of *B. maritima* were cut just above the soil surface and washed with deionised water. The fresh and dry (72 h at 70 °C) shoot masses were recorded. Dry plant samples were ground before analysis of nutrients and heavy metals. After harvesting, soil samples were sieved to 2 mm and divided into two sub-samples: one was stored at 4 °C for microbiological analysis and the other was allowed to dry at room temperature before heavy metal fractionation.

2.3. Analytical methods

Soil particle size distribution was assessed by sieving and sedimentation, according to the hydrometer method. For the determination of CaCO_3 , CO_2 released by addition of HCl was measured with a calcimeter. The mineralogical composition of the soil was determined by X-ray diffraction in the finely powdered samples. The total-N and total organic-C (TOC) concentrations were measured in an automatic microanalyser (EuroVector, Milan, Italy). The organic matter (OM) content of the soil was calculated by multiplying TOC by 1.72; for the organic amendments, the OM was determined by loss on ignition at 430°C for 24 h. Water-soluble C (WSC) and 0.1 M NaOH-extractable C in the soil and in the solid organic amendments (COM and SOH) were determined in a 1:10 (w/v) extract, in a TOC analyser for liquid samples (TOC-V Analyzer, Shimadzu, Tokyo, Japan). Water-soluble phenolic compounds were analysed by the Folin-Ciocalteu colorimetric method [21]. The values of pH were determined in saturated soil pastes, while water extracts (1:10, w/v) of COM and SOH were obtained for pH and electrical conductivity (EC) analyses. The water extracts from the compost and fresh solid olive husk were characterised for pH and EC in the raw material. Pseudo-total heavy metals (Zn, Fe, Mn and Pb) were determined by ICP-OES (Iris Intrepid II XDL, Thermo Scientific) after nitric-perchloric acid (2:1) digestion (plants and organic amendments) or *aqua regia* digestion (soil). The sequential extraction of soil metals [22] had the following steps: 0.1 M CaCl_2 , metals in soil solution and in exchangeable forms; 0.5 M NaOH, metals associated with OM; 0.05 M $\text{Na}_2\text{H}_2\text{EDTA}$, metals that can be extracted by a strong chelator (mainly in the carbonate fraction in calcareous soils); and digestion with *aqua regia* for the residual metals. This methodology was used because it has been used successfully for sludge- and metal-polluted calcareous soils [5,23]. Metal concentrations in the different fractions were determined by ICP-OES.

The soil microbial biomass C (B_C) was measured after a fumigation-extraction procedure [24], in a TOC analyzer for liquid samples, and calculated according to Wu et al. [25]. The ninhydrin-N (B_{NIN}) was determined in the same extracts as B_C , following the procedure proposed by Joergensen and Brookes [26]. Soil respiration was calculated as the amount of $\text{CO}_2\text{-C}$ evolved during a 10-day incubation period: 20 g of soil (roots were carefully separated from the soils after harvesting) were placed in a 500-mL incubation vessel, where small vials with 10 mL of 0.1 M NaOH were placed to trap the CO_2 produced during the incubation. The soils were incubated in triplicate using empty vessels as blanks. The vials were titrated with 0.1 M HCl in an excess of BaCl_2 . Chemical analyses were performed at least in duplicate and those for microbial biomass determination at least in triplicate. Coefficients of variation were always below 2%.

2.4. Statistical analysis

The data were subjected to ANOVA and differences between means were determined using Tukey's test ($P < 0.05$). Normality and the homogeneity of the variances were checked using the Shapiro-Wilk and Levene tests, respectively, before ANOVA. Statistical analyses were performed using SPSS 17.0 software.

3. Results and discussion

3.1. Effects on soil heavy metal availability

The addition of the different amendments did not affect soil pH, all the treatments showing an alkaline pH (ranging from 7.4 to 7.6, data not shown) as a consequence of the high buffer capacity of

this soil [23]. Regardless of the treatment, the concentrations of Fe, Cu, Zn and Pb in the most-soluble forms (extractable with CaCl_2) were very low (Fig. 1), due to the calcareous character of this soil [23,27]. The most-relevant results regarding heavy metal availability in the soil were for the CaCl_2 -extractable Mn concentrations, which were increased ($P < 0.001$) in SOH and SOH-WE-amended soils compared to the rest of the treatments (Fig. 1). This could be related to the higher concentrations of water-soluble phenols of the SOH and the SOH-WE (Table 1). Several reports have demonstrated that oxidative polymerisation of phenols can be accelerated non-enzymatically by iron and manganese oxides [28]. Therefore, the soluble phenols added in the SOH and SOH-WE treatments may have oxidised in the soil, yielding water-insoluble polymers, while soil Mn and Fe oxides were reduced to their soluble, extractable forms, Mn (II) and Fe (II) [28]. This is in good agreement with de la Fuente et al. [23], who found a fast decrease in the soil redox potential, and in the soluble organic carbon and phenols concentrations from SOH, in an incubation experiment with a soil from the same area. In that experiment, the concentration of water-soluble phenols in the soil treated with SOH decreased sharply after 14 days (by 69% with respect to the initial concentration), and simultaneously soil redox potential decreased, indicating less-oxidising conditions in the SOH-treated soil. In the present experiment, the concentrations of EDTA-extractable Mn were higher ($P < 0.05$) in both the SOH and SOH-WE treatments than in the control (Fig. 1). The high content of CaCO_3 in this soil could favour the formation of insoluble carbonates with the Mn (II) released into the soil solution during phenol oxidation, increasing its EDTA-extractable concentration [23].

No differences were found between CaCl_2 -extractable Fe concentrations in control and amended soils (SOH and SOH-WE, Fig. 1). Re-oxidation of Fe (II) to Fe (III) is relatively rapid and is likely to be even faster in the presence of hematites (present in this soil), which can chemisorb molecular oxygen [29]. In a previous incubation experiment, de la Fuente et al. [23] also found a significant increase in CaCl_2 -extractable Mn concentrations shortly after SOH addition to soil (2 days), while CaCl_2 -extractable Fe concentrations were not affected throughout the experiment (56 days). Piotrowska et al. [30] showed similar results after application to an agricultural soil of different rates of olive mill-waste water: an increase in extractable Mn and Fe immediately after application, with much higher increases in extractable Mn (more than 25-times that in the control) than in Fe (3-times greater).

The addition of an inorganic fertiliser to SOH-treated soils caused a decrease ($P < 0.001$) in the concentrations of CaCl_2 -extractable Mn compared with the SOH-only treatment, reaching values close to the control soil (Fig. 1). Soluble Mn (II) re-oxidation by dissolved O_2 is quite slow in the soil, but it can be accelerated by abiotic (e.g. fast adsorption reactions on negatively charged surfaces) and, especially, biotic processes. Microbial activity is recognised widely as the main factor controlling the oxidation of Mn (II) to Mn (IV) at neutral pH (6–8) [31]. This process could have been favoured by the addition of the fertiliser in the SOH-treated soil, since it stimulated microbial activity, as discussed later in the article. In addition, in phosphorus-fertilised soils, Mn-phosphate salts are more stable than MnCO_3 [32]; this could have favoured Mn immobilisation in these soils. Contrastingly, inorganic fertiliser application did not affect the soluble and exchangeable Mn concentrations in the SOH-WE treatment, giving values close to those with SOH or SOH-WE alone (Fig. 1). This could be related to the fact that SOH-WE was applied to the soils 39 days later than SOH and no significant differences were found between microbial activities in the fertilised and non-fertilised SOH-WE soils.

The concentrations of the metals in the NaOH-extractable fraction were rather small (Fig. 1). Compost addition to soil lowered the concentrations of the metals in the NaOH-extractable

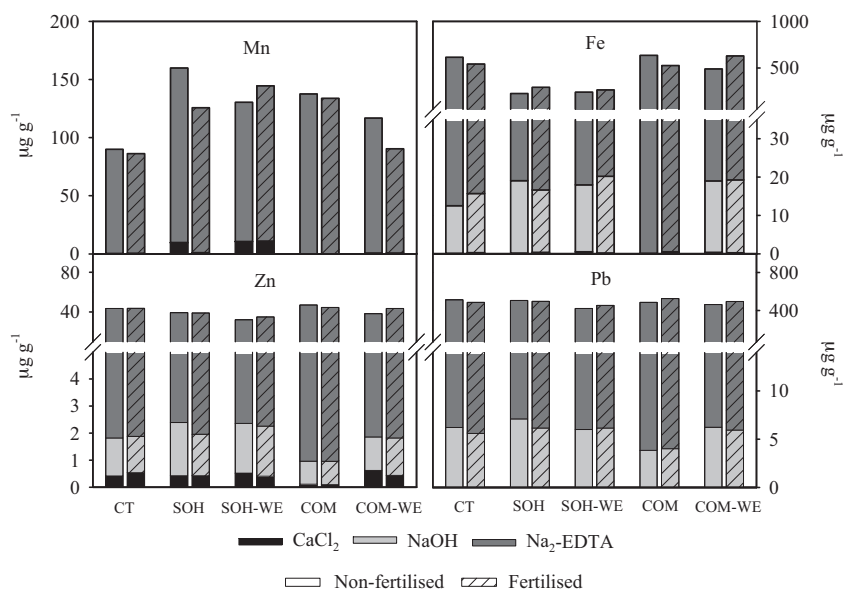


Fig. 1. Heavy metal fractionation by sequential extraction in the control (CT) and amended soils (SOH: solid olive husk, SOH-WE: water extract from fresh solid olive husk, COM: compost and COM-WE: water extract obtained from compost).

fraction ($P < 0.001$), especially Fe, Zn and Pb compared to the rest of the organic treatments (Fig. 1). Compost increased their EDTA-extractable concentrations at the expense of the NaOH fraction. The decrease in heavy metal solubility after compost addition may be related to the formation of non-NaOH-extractable compounds after metal association with humified OM [9] and/or precipitation with inorganic compounds added with the compost.

Several reports have demonstrated that soluble (low-molecular-weight) organic substances have the ability to form complexes with di- and trivalent metal cations and, accordingly, a substantial capacity to increase heavy metal mobility [33,34]. However, the addition of COM-WE in this experiment did not result in a general increase in heavy metal solubility, and only the NaOH-extractable Fe concentrations were increased slightly in the amended soil with respect to the control soil (Fig. 1). This could mean that the addition of soluble OM with the COM-WE was not sufficient to increase heavy metal solubility, perhaps due to the high carbonate content and pH of the soil, which make metal solubilisation difficult.

3.2. Effects on plants

Regardless of the addition of inorganic fertiliser, *Beta maritima* did not grow adequately in SOH-amended pots, giving the lowest yields of plant biomass ($P < 0.001$). No significant differences were observed between fertilised and non-fertilised pots, average yields per treatment following the order ($g \pm sd$, dry weight per pot): SOH (0.05 ± 0.00) < CT (0.20 ± 0.03) \approx SOH-WE (0.21 ± 0.04) \leq COM (0.26 ± 0.03) \approx COM-WE (0.29 ± 0.05). This is in agreement with the results obtained by Clemente et al. [26], in a field experiment carried out in a soil from the same area as that of the current work, where *B. maritima* did not grow adequately in SOH-amended plots. Various authors have also found negative effects on plant growth after SOH addition to soil, which often have been attributed to the high concentration of phenolic compounds in this material [35]. In this context, a similar effect to that provoked by SOH could be expected in the SOH-WE treatment, due to an equivalent addition of water-soluble phenols (about $120 \mu g g^{-1}$). Nevertheless, no negative effects on plant growth were observed with the addition of the water-soluble fraction extracted from SOH, perhaps due to the higher resistance of adult plants, compared with seedlings, to phe-

nolic compounds (SOH-WE was added to soil when plants were 39 days-old, while seedlings were transplanted directly into an SOH-soil mixture). This finding could also indicate that the phytotoxicity of SOH might be induced partly by the addition of hydrophobic compounds, such as fats and high-molecular-weight recalcitrant phenols contained in this material [30] but not present in the water extract.

In addition, elevated Mn concentrations were found in plants grown in the SOH soils (up to 668 and $881 \mu g g^{-1}$ with and without inorganic fertiliser, respectively), higher than the maximum concentration of this metal usually found in plants ($60 \mu g g^{-1}$, [36]), and they can be considered toxic [37], although this could be at least in part a consequence of the reduced biomass. The combined addition of SOH and inorganic fertiliser gave lower Mn concentrations in the leaves of *B. maritima* than for the same treatment without fertiliser (Fig. 2) even though both treatments had similar yields, in agreement with the effect on soil $CaCl_2$ -extractable concentrations. In contrast, the high solubility of Mn in the SOH-WE-treated pots (Fig. 1) did not result in an increase in the plant Mn concentration compared to control pots (Fig. 2). This finding could be due to the short time elapsed between the addition of the liquid amendment and the plant harvest (7 days). The existence of certain mechanisms, like the release of root exudates capable of complexing this metal [38], may also protect the plants against excessive Mn uptake. Walker et al. [20] showed that the increase in DTPA-extractable Mn concentrations in a metal-contaminated soil that received an EDTA solution did not affect Mn concentrations in plant tissues of *B. maritima* harvested 17 days after EDTA addition. In that experiment, plants were 76 days-old at the moment of EDTA addition, which agrees with the results found in the present experiment in that adult plants seem to resist the presence of elevated soluble Mn concentrations in the soil.

Due to metal immobilisation in the COM-amended soil, the concentrations of Fe in shoot tissues were significantly lower ($P < 0.001$) compared to control pots, although no significant effects were found for Zn and Pb (Fig. 2). Regarding Fe bioavailability, similar results were found by Albuquerque et al. [39] in an experiment involving a *Lolium perenne* culture and the addition of composted SOH; these were attributed to the formation of poorly soluble and scarcely bioavailable chelates. The lowest concentrations of Fe in the plants from the COM soils resulted in the highest Mn/Fe ratio:

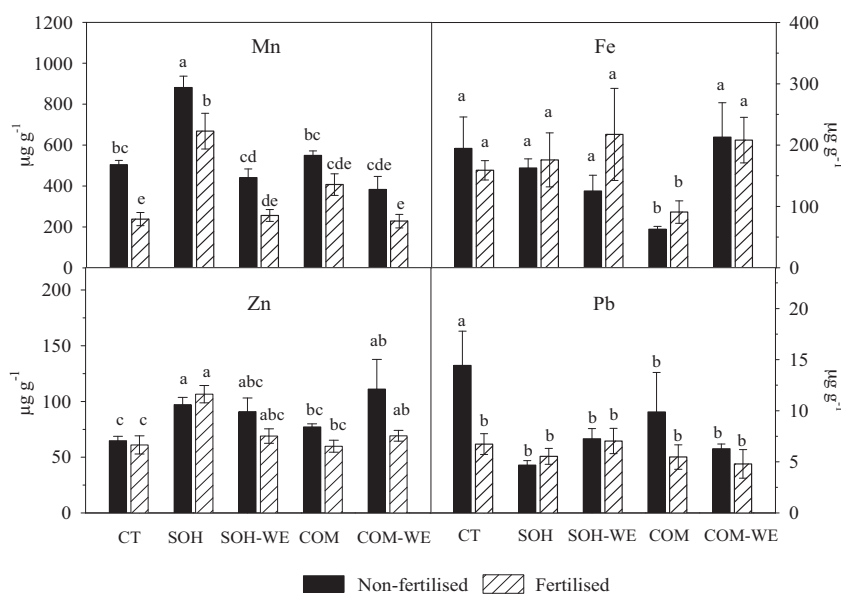


Fig. 2. Heavy metal concentrations in plants of *B. maritima* from the control soil (CT) and the different soil organic treatments (SOH: fresh solid olive husk, SOH-WE: water extract from fresh solid olive husk, COM: compost and COM-WE: water extract obtained from compost) (dry matter, $n=4$). Bars with the same letter are not significantly different according to the Tukey test ($P<0.05$).

COM (6.9) > SOH (4.4) > SOH-WE (2.9) \approx CT (2.1) > COM-WE (1.4). Poschenrieder and Barceló [40] reported phytotoxic symptoms in *Phaseolus vulgaris* for Mn/Fe values above 6. Thus, the COM treatment may have inhibited *B. maritima* growth via Mn toxicity, but no deficiency of Fe was found in these plants according to the usual concentrations of this metal for plants (18–1000 $\mu\text{g g}^{-1}$) reported by Kabata-Pendias [37].

The concentration of Zn in plants was increased by SOH and COM-WE with respect to control plants, but the values can be considered normal in plants (27–150 $\mu\text{g g}^{-1}$, [36]). Increased Zn concentrations in plant tissues were also observed by Clemente et al. [27] in *B. vulgaris* and *B. maritima* grown in a soil amended with SOH.

All the treatments, including inorganic fertilisation, lowered the shoot tissue Pb concentration ($P<0.05$) relative to plants grown in the non-fertilised, control soil (Fig. 2); anyway, all the concentrations were below the limit for animal feed (50 $\mu\text{g g}^{-1}$, [41]). Clemente et al. [27] also reported a significant decrease in the Pb concentrations of *B. maritima* plants grown in a heavy metal-polluted soil amended with SOH, compared to those obtained in a non-amended, control soil. Both the mineralisation of the amendments throughout the experiment and the addition of phosphate with the inorganic fertiliser could have favoured metal precipitation as insoluble salts. Walker et al. [20] attributed Pb immobilisation to the formation of insoluble salts after manure addition to a calcareous, contaminated soil.

3.3. Effects on soil organic matter and microbial parameters

As expected, the organic amendments led to significant increases in the TOC and WSC concentrations with respect to the control soil, especially in the COM and SOH treatments (Table 2). Although the same amount of WSC was initially added in the SOH-WE and COM-WE treatments, the latter showed lower TOC and soluble C concentrations at the end of the experiment in the non-fertilised treatment (Table 2). A higher proportion of the easily available OM added with COM-WE was therefore mineralised in the soil, compared with SOH-WE. The toxic and potentially antimicrobial compounds originally present in the SOH, which may have been reduced drastically after composting [13], could have been respon-

sible for the WSC accumulation in SOH-WE-treated soil. This is supported by the fact that the addition of inorganic fertiliser (which may have favoured microbial activity in the soil) decreased WSC concentrations in the soil compared to non-fertilised soils (Table 2).

Both COM and SOH increased biomass C concentrations and respiration ($\text{CO}_2\text{-C}$) compared to the control soil (Table 2). The capacity of compost addition to enhance microbial activity in soil (even in heavy metal-polluted soils) is well known, as composts constitute a source of available nutrients and OM, which can improve soil chemical and physical properties [6]. Contrastingly, controversial results have been found concerning the short-term influence of SOH on soil microbial biomass parameters [30,42]. Frequently, the microbial toxicity of SOH has been related to its high levels of phenolic compounds [11]. At the end of this experiment, very-low concentrations ($<0.5 \mu\text{g g}^{-1}$) of water-soluble phenols were found, even in the SOH and SOH-WE treatments, possibly due to the oxidation of these compounds; this may be why toxic effects were not apparent after the addition of these materials in the present experiment. This is in good agreement with a previous study conducted by de la Fuente et al. [23], who recorded important decreases of water-soluble phenol concentrations in an SOH-treated soil after 56 days of incubation (down to 11% of the initial concentration).

The microbial biomass C concentrations (B_C) were not significantly different in the COM-WE and SOH-WE soils with respect to the control soil (Table 2). Kohler et al. [16] also showed that neither microbial biomass C nor microbial activity (protease, β -glucosidase and phosphatase activities) were modified significantly by the addition of soluble OM extracted from composted SOH, as a consequence of its intense and rapid mineralisation. Toxicity due to the addition of soluble phenols could have also limited microbial growth in the SOH-WE-amended-soil.

The ratio biomass C/biomass N has been proposed as a useful index for the identification of the structure of soil microbial populations, high values being related to increased ratios of fungal to bacterial biomass [43]. The SOH treatment produced significantly higher B_C/B_{NIN} values than the other treatments ($P<0.001$; Table 2). Several species of fungi have shown resistance to potentially toxic phenolic compounds; consequently, their proliferation may have been favoured in the SOH treatment, even under the heavy-metal stress conditions of this soil [44]. This observation confirms

Table 2
The organic-C and microbial parameters in soil after plant harvest (dry matter basis) in the control (CT) and amended soils (SOH: solid olive husk, SOH-WE: water extract from solid olive husk, COM: compost and COM-WE: water extract obtained from compost) ($n=4$). TOC: total organic carbon. WSC: water-soluble carbon.

Treatment	Fertilisation	TOC mg g ⁻¹	WSC μg g ⁻¹	B _C μg g ⁻¹	B _{NIN} μg g ⁻¹	B _C /B _{NIN}	CO ₂ -C μg g ⁻¹ d ⁻¹
CT	No	3.9 ^c	<0.1 ^f	30 ^c	2.1 ^{bc}	15 ^{bc}	5.0 ^c
	Yes	3.8 ^c	<0.1 ^f	54 ^c	2.3 ^{bc}	24 ^{bc}	5.3 ^c
SOH	No	5.7 ^a	50 ^{cd}	105 ^a	1.2 ^c	92 ^a	12 ^b
	Yes	5.5 ^a	19 ^{de}	123 ^a	1.1 ^c	110 ^a	17 ^a
SOH-WE	No	4.9 ^b	138 ^a	53 ^{bc}	0.6 ^c	66 ^b	11 ^b
	Yes	4.7 ^b	28 ^{cde}	54 ^{bc}	1.7 ^c	34 ^b	9.3 ^{bc}
COM	No	5.6 ^a	12 ^e	80 ^{ab}	5.0 ^a	16 ^{bc}	11 ^b
	Yes	5.7 ^a	9.0 ^e	79 ^{ab}	3.6 ^a	22 ^{bc}	12 ^b
COM-WE	No	4.4 ^{bc}	88 ^b	74 ^{bc}	3.7 ^b	19 ^{bc}	8.0 ^{bc}
	Yes	4.4 ^{bc}	52 ^c	76 ^{bc}	3.4 ^{ab}	22 ^{bc}	7.6 ^{bc}
ANOVA							
Treatment	***	***	***	***	***	**	
Fertilisation	NS	***	NS	NS	NS	NS	
Treatment × fertilisation	NS	***	NS	NS	*	NS	

NS: not significant. *, **, and *** indicate significance at $P < 0.05$, 0.01 , and 0.001 , respectively.

Mean values denoted by the same letter do not differ significantly according to the Tukey test ($P < 0.05$).

previous findings by Mekki et al. [45] and Mechri et al. [46], who reported that the addition of olive-mill wastewater strongly enhanced populations of soil fungi.

The evolution of CO₂-C from soils receiving organic amendment (except COM-WE) was greater than in the control soils. Soils from the pots receiving SOH had the highest microbial respiration, especially after fertiliser addition (Table 2). This effect is in agreement with both the higher B_C and the lower WSC concentration found in the fertilised soil, compared to the non-fertilised soils (Table 2), indicating a positive effect of the fertiliser on soil microbial activity and growth. In a field experiment, Clemente et al. [42] also found a significant increase in the CO₂-C released from a heavy metal-polluted soil amended with SOH, with respect to a non-amended, control soil, in both the short- (43 days) and, especially, the long-term (more than 1 year after its addition). This suggests a slow degradation of the OM in the SOH by soil microorganisms, probably due to the presence of residual phenolic compounds and/or to the large amounts of very-resistant lignocellulosic compounds present in this material [10].

4. Conclusions

The suitability of fresh olive husk for bioremediation strategies seems to be limited, because this waste provoked phytotoxicity due to the presence of certain compounds, such as phenols. Although fresh olive husk increased the soluble Mn concentration in the soil, after the oxidation of phenolic compounds, this metal does not often represent a serious environmental risk and it is a major component of the soil, so contemplation of its phytoextraction is not commonplace. The use of an inorganic fertiliser in combination with SOH decreased Mn solubility in the long-term, related to a higher microbial activity, but phytotoxic effects were still apparent. The addition of water-soluble OM obtained from SOH also resulted in increased Mn solubility in soil, but did not increase Mn uptake or cause phytotoxic effects, suggesting that the non-soluble fraction of SOH also can be responsible for these detrimental effects. Contrastingly, compost from SOH reduced metal bioavailability, as a consequence of the strong chelating ability of the partially humified OM provided by this amendment. The addition of the water extract obtained from compost was not sufficient to affect heavy metal availability in the soil.

Therefore, composted olive husk seems to be the most appropriate material for the development of bioremediation

strategies, especially phytoimmobilisation and phytostabilisation, keeping metal bioavailability and plant absorption at control levels while improving microbial biomass activity.

Acknowledgments

This work was funded by the “Ministerio de Ciencia e Innovación” through project CTM2007-66401-C02-01/TECNO. The authors thank Dr. David J. Walker for the language revision of this manuscript and Dr. Juan Cegarra for providing the compost used in this work.

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