**Phytostabilization of amended soils polluted with trace elements using the
Mediterranean shrub: *Rosmarinus officinalis***

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Abstract

We evaluate the mid-term effects of two amendments and the establishment of *R. officinalis* on chemical and biochemical properties in a trace element contaminated soil by a mine spill and the possible use of this plant for stabilization purposes. The experiment was carried out using containers filled with trace element polluted soil, where four treatments were established: organic treatment (biosolid compost, OAR), inorganic treatment (sugar beet lime, IAR), control with plant (NAR) and control without plant (NA). Amendment addition and plant establishment contributed to restore soil chemical (pH, total organic carbon and water soluble carbon) and biochemical properties (microbial biomass carbon and the enzymatic activities: aryl-sulphatase and protease). The presence of rosemary did not affect soluble (0.01 M CaCl₂) Cd and Zn and decreased trace element EDTA extractability in amended soils. There were no negative effects found on plant growth and nutrient content on polluted soils (NAR, OAR and IAR). Trace element contents were within normal levels in plants. Therefore

rosemary might be a reliable option for successful phytostabilization of moderate trace element contaminated soils.

Keywords: phytoremediation, aryl-sulphatase activity, protease activity; microbial biomass; trace element bioavailability, transfer coefficient

1. Introduction

The importance of plant cover in reducing water and wind erosion and protecting the soil from degradation is well documented (White, Loftin and Aguilar, 1997; Rostagno and Sosebee, 2001; Martínez et al., 2003). Reforestation is a useful method of erosion control.

The revegetation of trace element polluted areas is a difficult task because of the presence of many-limiting factors. Ecotoxic effects of soil pollution can be an important factor; in addition, other factors such as high irradiance, nutrient deficiencies and poor substrate structure can be also detrimental for plants (Tordoff, Baker and Willis, 2000; Walker, 2002). Moreover, the accumulation of high concentrations of trace elements in aerial plant biomass should be avoided because that would facilitate their entry into the food chain (Salomons, Förstner and Mader, 1995) and increase their concentration on the soil surface upon litter fall (Watmough, Dillon and Epova, 2005, Pérez de Mora et al., 2006a).

Rosmarinus officinalis L., a shrub of the Lamiaceae family, native of the Mediterranean Basin and commonly known as rosemary, is a useful species in erosion control (Aydin, Celik and Berkman, 1999). This drought-tolerant plant grows from rocky to sandy soils. This plant has also multiple uses including production of oil, food

1 products, perfumes and cosmetics. As a medical plant, rosemary has been used as an
2 external stimulant, a soothing herbal brew and it is also known for its powerful
3 antibacterial, antimutagenic properties, and as a chemo-preventive agent (Oluwatuyi,
4 Kaatz, and Gibbons, 2004). This plant has been widely accepted as one of the species
5 with the highest antioxidant activity concentrated in leaves (Peng et al., 2005).

6 Due to its multiple utilities and its ability to grow in rough environments, such as
7 semi-arid zones of South Spain, rosemary could be an alternative crop for growing on
8 organic waste-amended or metal-contaminated soils as long as growth and oil
9 composition are not affected. In fact, this plant is being used successfully for
10 phytostabilisation in the Green Corridor of de Guadiamar, an area contaminated with
11 several trace elements (Murillo et al., 2005).

12 In this work, we evaluated the mid-term effects of two amendments and the
13 establishment of *Rosmarinus officinalis* and its associated rhizosphere on chemical and
14 biochemical properties on a trace element contaminated soil. We also discuss the use of
15 this plant for phytostabilization purposes based on its potential trace element
16 accumulation.

17 18 **2. Materials and methods**

19 Ten tons of soil were collected by an excavator from “El Vicario” (N 37°26’21”;
20 W 06°12’59”) an area affected by the Aznalcóllar mine accident (Southern Spain, 1998).
21 In this area the sludge layer from the mine spill had been removed together with the
22 upper 15 cm of top soil. The soil was transported to the experimental farm “La Hampa”
23 (IRNAS-CSIC) in Coria del Río (Southern Spain). Soil was homogeneized and the

fracction > 30 mm was removed using a sieve. General soil characteristics are given in Table 1.

2.1 Experimental design

The experiment was carried out in 16 containers (made of corrugated iron) (70 cm long x 60 cm wide x 40 cm deep) that were placed outdoors in the experimental farm “La Hampa”. The containers were filled with the upper 20 cm of the soil (1.32 g cm^{-3} bulk density). Containers were arranged according to a complete randomised block design with four treatments (one organic, one inorganic and two controls) and four replicates per treatment. The organic treatment was a biosolid compost (OAR) constituted from wastewater sludge from a water treatment plant and green waste from parks and gardens (EGMASA, Sevilla, Southern Spain). The inorganic treatment was sugar beet lime (IAR), a residual material from the sugar manufacturing process with 70-80% of CaCO_3 (dry basis) (AZUCARERA EBRO, San José de la Rinconada, Southern Spain). Amendments were applied and mixed with the top soil (10 cm) in the containers to simulate potential field application. Each amendment was applied twice: initially (February 2002) ($70 \text{ Mg ha}^{-1} \text{ dw}$ for biosolid compost and $50 \text{ Mg ha}^{-1} \text{ dw}$ for sugar beet lime) and after 12 months (February 2003) ($35 \text{ Mg ha}^{-1} \text{ dw}$ for biosolid composts and $25 \text{ Mg ha}^{-1} \text{ dw}$ for sugar beet lime). Two control treatments without amendments were also established: control with plant (NAR) and control without plant (NA). The characteristics of the amendments are shown in Table 2.

Two grasses were established in the containers: *Agrostis stolonifera* L. was sown for 3 consecutive seasons (2002, 2003 and 2004) while *Cynodon Dactilon* was sown in 2005. More details of the previous crops in the containers are reported in Pérez de Mora

et al., (2006 a, b). Previous plant residues were removed two years before the current experiment for *Agrostis stolonifera* and *Cynodon dactylon* was removed 6 months before the first soil sampling.

2.2. Sampling and chemical analysis

Rosemary saplings (one year old) were grown in a local nursery until transplant. In October 2006, two rosemary saplings were transplanted into every container, except in the NA containers. Rosemary leaves were sampled in October 2006 and in October 2007. On the other hand, four rosemary saplings were planted in a non polluted soil to be used as control plants. Each rosemary plant was monthly measured for shoot height (stretched distance from ground level to the highest living bud).

Soil samples were taken in September 2006 and 2007, four and five years respectively, after the first amendment addition. In each container, 2 soil cores were taken with a auger of 2 cm (15 cm depth) at a distance of 15-20 cm from the stem to make a composite sample. Samples were air-dried, crushed and sieved (2 mm) prior to preparation for chemical analysis. Prior to determination of total trace element concentrations, soil and amendment samples were also ground to < 60 μm particle size.

Soil pH was measured in a 1:2.5 sample/1 M KCl extract after shaking for 1 h. Total organic C (TOC) was analysed by dichromate oxidation and titration with ferrous ammonium sulphate (Walkley and Black, 1934). Water-soluble Carbon (WSC) was obtained from an aqueous solution (1:10, soil/water) after shaking for 1 h and centrifugation at 5000 rev min⁻¹ for 15 min. The extract was analyzed using a TOC-VE Shimazu analyser.

Extractable trace element concentrations with 0.01M CaCl_2 (Ure et al., 1993) and with 0.05 M EDTA (pH 7) (Quevauviller et al., 1998) were determined in 1/10 soil sample (<2 mm)/ extractant. Both extracts were analysed by ICP-OES (inductively coupled plasma spectrophotometry). Total trace element concentrations in soil (<60 μm) were determined by ICP-OES following *aqua regia* digestion in a microwave oven. The accuracy of the analytical method was assessed by carrying out analyses of the BCR (Community Bureau of Reference) reference samples: BCR 320R (Channel sediment; Table 3).

Plant samples were washed (for 10 s approximately) with a solution 0.1 N HCl solution and then washed in deionised water. Plant material was then dried at 70° C, ground and passed through a 500- μm stainless steel sieve.

Plant material was analysed for N by Kjeldahl digestion. Nutrients and trace elements were extracted by wet oxidation with ultrapure concentrated HNO_3 (65 % w/w) under pressure in a microwave digester. Analysis of mineral nutrients and trace elements was performed by ICP-OES. The accuracy of the analytical method was assessed by carrying out analyses of the BCR (Community Bureau of Reference) reference sample DC 73350 (Populus leaves; Table 3).

2.3. Microbiological analysis

A homogeneous soil subsample of each container was sieved (2 mm) and stored at 4 °C at its field moisture content.

Microbial biomass carbon (MBC) content was determined by the chloroform fumigation–extraction method modified by Gregorich et al. (1990). Concentration of C in the extract was measured by a TOC-VE Shimazu analyser. An extraction efficiency

coefficient of 0.38 was used to convert the difference in soluble C between the fumigated and the unfumigated soil to MBC (Vance, Brookes and Jenkinson, 1987).

Two hydrolases closely related to nutrient cycling were assessed. Arylsulphatase activity was determined after soil incubation with p-nitrophenyl sulphate and measurement of p-nitrophenol (PNF) absorbance at 400 nm (Tabatabai and Bremme, 1970). The enzyme activity is expressed as mg p-nitrophenol PNF kg⁻¹ h⁻¹. Protease activity was measured after incubation of soil with casein and measurement of the absorbance of the extracted tyrosine at 700 nm (Ladd and Butler, 1972). Protease activity is expressed as mg of Tyrosin kg⁻¹ 2h⁻¹.

2.4 Metal transfer coefficient

In order to find out what proportion of the total soil metal concentration was available and transferred to plant aboveground parts, the transfer coefficient (TC) was calculated. This is defined as the ratio of metal concentration in the plant [M]_{plant} to the total metal concentration in the soil [M]_{soil} (Adriano, 2001).

2.4 Statistical analysis

Statistical analyses were carried out with the program SPSS 11.5 for Windows. All values are expressed as mean values. One way ANOVAs were used for the comparison of the treatments on soil chemical and biochemical properties and trace element in soils and plants. When significant differences were found, a multiple comparison of mean values was determined by the Tukey test at P < 0.05. Data normality was tested prior to analysis, and when necessary, variables were transformed

logarithmically to achieve a normal distribution. If after transformation, the data did not fit a normal distribution, we used the non-parametric Kruskal-Wallis analysis of ranks.

3. Results

3.1 Soil pH, Total Organic Carbon and Water Soluble Carbon

In both years pH values were significantly higher in amended soils (OAR and especially IAR) than in non amended soils (NA and NAR) (Table 4). In 2007, NA soils showed a slight acidification (5.20 to 5.05) compared to the previous year whereas in NAR soils a slight increase of pH values (5.09 to 5.48) was observed. At OAR and IAR soil pH showed also a slight decrease in time.

The highest values of TOC and WSC were found in OAR soil where the organic amendment was applied (Table 4). Values of organic matter in IAR were in general higher than those of the non amended soils (NA and NAR) although the differences were not always significant. In the second year TOC values of all treatments were similar to those found in the first year except for NA in which a significant decrease in TOC values was observed between both years. WCS values were higher in the second sampling (2007) than in the first sampling (2006) (Table 4).

3.2 Trace elements in soils

Table 5 shows total contents of trace elements in soils in 2007 (values of 2006 were similar to those of the later year). For all treatments these contents were higher than background values in the area (Madejón et al., 2006) and no significant differences among treatments were found (Table 5). Arsenic concentration was 20-fold higher than normal levels in soils and Cd and Pb concentrations were 6-fold higher than normal

1 levels. Copper and Zn contents were 2.5- fold and 2-fold higher respectively than those
2 values in normal soils. The intervention values in agricultural soils for the Andalucía
3 Regional Government are shown in Table 5. As, Cu, Cd and Pb were above this
4 legislative limit therefore we had to remediate this soil according to authorities. Only As
5 contents in soils were above this range (Table 5; Kabata Pendias and Pendias, 1992).

6 7 *3.2.1 Available trace elements in soils*

8 *CaCl₂ extraction*

9 CaCl₂-extractable concentrations of As and Pb (Table 6) were always below
10 detection limits (0.1 mg L⁻¹). Amended soils (OAR and IAR), showed CaCl₂-extractable
11 Cd and Zn contents lower than non amended soils (NA and NAR) especially in the
12 second sampling in which the differences where statistically significant (Table 6). A
13 significant increase in both elements (from 0.01 to 0.035 mg kg⁻¹ for Cd and from 1.79
14 to 3.69 mg kg⁻¹ for Zn) was found in NAR soils with time, whereas in the amended soils
15 these contents tended to decrease with time. No changes in NA soils were detected in
16 Cd and Zn concentrations between both years.

17 On the contrary, values of CaCl₂-extractable Cu were significantly higher in
18 amended than in non amended soil (Table 6) in both years. However, in non amended
19 soils a significant increase between years was noticed whereas in amended soils
20 concentrations were similar in time. Correlation coefficients were significant between
21 pH values and extractable Cd, Cu and Zn concentrations ($r=-0.52$ for Cd, $r= 0.47$ for Cu
22 and Zn $r= -0.81$ for Zn $p<0.01$ for all cases).

23 24 *EDTA extraction*

1 In general, the highest concentrations of trace elements extracted by EDTA were
2 found in amended treatments and in most of cases the differences compared to non
3 amended treatments were significant (Figure 1). As a rule, there were no significant
4 differences in trace metal concentrations extracted by EDTA over time, although a
5 slightly decrease was observed in 2007 compared to 2006 (Figure 1).

6 For Cd, Cu and Zn, the percentage of metal extracted with EDTA compared to
7 the total content (Table 5) was between 21-25 %. For other elements with low mobility
8 as As and Pb, this percentage was between 1.4-2.5 %.

9 These values were significantly correlated with TOC ($r = 0.82$ for As, $r = 0.66$ for
10 Cd, $r = 0.61$ for Cu, $r = 0.62$ for Mn, $r = 0.82$ for Pb and $r = 0.80$ for Zn, $p < 0.001$) and to a
11 lesser extent with pH (data non shown).

12 13 3.3 Biochemical analyses

14 In both years, the greatest values of Microbial Biomass Carbon (MBC) were
15 found in amended soils (OAR and IAR) with significant differences compared to NA
16 and NAR (Figure 2). All soils with rosemary plants (NAR, OAR and IAR) increased
17 their MBC values in 2007 whereas in NA soil a very slight decrease in MCB content
18 was measured (Figure 2).

19 Enzymatic activities among treatments depended on the parameter (Figure 2),
20 but highest activity values were found in amended soils. In case of aryl-sulphatase
21 activity maximum mean values were found in soils with rosemary (NAR, OAR, IAR)
22 after one year of growth, and showed a significant increase for OAR (2.06 to 5.62 mg
23 PNF $\text{kg}^{-1}\text{h}^{-1}$) and IAR (3.00 to 6.30 mg PNF $\text{kg}^{-1}\text{h}^{-1}$; Figure 2). Protease activity also

reached the highest value in IAR treatment (55.8 mg Tyrosin kg⁻¹2h⁻¹). In general, for all treatments, protease values tended to increase with time (Figure 2).

Both hydrolyses were positively correlated with pH ($r=0.45$ for arylsulphatase and $r=0.53$ for protease, $p<0.001$) and WSC ($r=0.59$ for arylsulphatase and $r=0.43$ for protease, $p<0.001$).

3.4 Plant growth

Rosemary survival in all treatments after one year of growth was 100 %. No visual symptoms of toxicity or injury were observed. Plant height at the beginning of the experiment was 23.0 cm (mean value, $n=24$) and after one year rosemary height for each treatment was 61.1 cm (NAR), 64.5 cm (OAR) and 61.0 cm (IAR) and differences among treatments were not significant. Monthly increase of rosemary is shown in Table 7 and these increases were similar between treatments.

3.5. Plant chemical composition

3.5.1 Macronutrients

Figure 3 shows the main macronutrients in rosemary leaves at the three treatments (NAR, OAR and IAR) and in control plants (CR) growing on soils non-contaminated with trace elements. Concentrations of nutrients, except for Mg and P, were significantly higher in CR plants than in plants growing in trace element polluted soils. Concentrations of nutrients in rosemary leaves were similar in non amended and amended polluted soils.

3.5.2 Trace elements

Concentrations of trace elements in rosemary leaves are shown in Figure 4. Values of Pb were below the detection limit (1 mg kg^{-1}). Trace elements concentrations were always within the normal range for plants of all study treatments (Figure 4; Chaney, 1989). In general, concentrations of As, Cd, Cu and Zn in plants growing in polluted soils were very similar in all treatments and significantly greater than in control plants (CR).

No correlations were found between extractable or total trace element concentrations in soil and trace element concentrations in plants.

3.5.3 Trace element Transfer Coefficient (TC)

Table 8 shows Transfer Coefficients (TC) of As, Cd, Cu, and Zn. Values of TC of Pb could not be calculated due to low content in leaves, indicating the low TC of this element in plants. Arsenic TC values were similar in all treatments and showed low values, smaller than 0.02 (Table 8). For Cd, TC values were slightly higher in OAR than in NAR and IAR plants, although these values were always very low (<0.02). TC values for Cu and Zn were lower at OAR and IAR rosemary leaves than in NAR leaves, showing significant differences for Zn. All values were below 1.

4. Discussion

4.1 Soil chemical properties

Although many studies have shown positive effects of amendment application in increasing pH of acid and trace element contaminated soils (Madejón et al., 2006), there is still concern regarding the longevity of this effect. Re-acidification of soil may reverse the action of amendments that make soils alkaline. On the other hand, plant

1 roots and their associated microorganisms can be responsible of substantial changes of
2 rhizosphere pH and could have a distinct impact on bioavailability of many pH-
3 dependent nutrients and potentially toxic metals (Hinsinger, Plassard and Jaillard,
4 2005). Roots can alter local pH soil conditions through various processes such as
5 assimilation/production of anions/cations, release of organic acids and CO₂, O₂
6 consumption and redox reactions (Jones, Hodge and Kuzyakov, 2004).

7 Both amendments (IAR and OAR) and the previous grasses grown in the
8 containers were effective for increasing soil pH (Pérez de Mora et al., 2006 a, b). This
9 alkaline effect of the amendments lasted for five years after the first application,
10 pointing out their effectiveness on pH increase. As occurred in previous years and with
11 other species, pH values tended to increase in time in NAR soils (Pérez de Mora et al.,
12 2006 a, b). This could be attributed to the presence of a rhizosphere in these acid soils
13 that could contribute to the alkalization processes. The effect of the rhizosphere on
14 soil pH could be different depending on the initial value of pH of the soil. Youssef and
15 Chino (1989) showed that barley and soybean alkalised the soil when the initial pH was
16 4.8, however, a slight acidification was observed when initial pH was 7.1.

17 As expected, addition of organic amendment caused the highest increase in TOC
18 and WSC values in OAR soils. Turnover of organic matter in soil caused a slight
19 decrease in TOC and the increase of WSC was probably due to the production of labile
20 fractions of organic matter as result of the mineralization processes. Values of TOC and
21 WSC in soils with inorganic amendment (IAR) and non amended (NAR) tended to be
22 similar in time (Table 4). Previous grasses and the presence of rosemary influence the
23 quality of exudates and plant debris a soil receives which could maintain the
24 equilibrium despite the mineralization losses.

4.2 Trace elements in soils

Determination of total metal content of soils is not strong enough to estimate their mobility and availability for ecological processes. According to Kabata-Pendias and Pendias (2004) extraction with 0.01 M CaCl_2 is an adequate method to extract bioavailable trace elements. The lower availability of Cd and Zn in the amended soils could have been due mainly to the alkalinisation of these soils due to amendment addition. The negative and significant correlation coefficients between pH values and Cd and Zn concentration confirmed this hypothesis. Despite the slight increase in pH values in NAR soils between 2006 and 2007, extractable Cd and Zn increased in time in these containers. This fact would imply the convenience of adding amendments in polluted soils before its reforestation to increase pH and also TOC, which could help to retain metals in soils.

Concentration of Cu, in OAR and IAR soils was slightly higher than in non amended soils likely due to the input of this metal added to soil through the amendments (Table 2). Moreover, Cu could form soluble complexes with organic matter that increase its solubility. In fact, a positive significant correlation was found between Cu and WSC ($r= 0.70$ $p<0.001$).

In any case concentration of CaCl_2 extractable trace elements was very low (Table 6); in the same levels or even lower than those found by Pérez de Mora et al. (2006 a) which indicates no sign of re-solubilization of trace elements in these soils.

Extraction with EDTA has been used to determine availability of trace elements in soils affected by the Aznalcóllar mine spill (Aguilar et al., 2004). Our study produced similar results to those reported by Madejón et al. (2004) in soils of the Guadiamar area.

EDTA-extractability has been reported to either increase or decrease in the presence of organic amendment (Bolan and Duraisamy, 2003). In contrast to extraction with 0.01 M CaCl_2 , values of trace elements extracted by EDTA were higher in amended soil. These values were significantly correlated with TOC and in a lesser extend with pH. Trace elements may form stable complexes with organic ligands that are soluble in EDTA. Nevertheless, these values were in the same range or slightly lower then those found by Pérez de Mora et al., (2006a) three years before in the same containers. The presence of rosemary did not increase the availability of trace elements and even tended to decrease availability with time (Figure 1).

4.3 Biochemical properties

Microbial biomass carbon (MBC) has been widely used as an option to evaluate soil quality (Gil-Sotres et al., 2005). This C fraction is one of the most labile pools of organic matter and it serves as an important reservoir of plant nutrients such as N and P (Marumoto, Anderson, and Domsch, 1982). Apart from the effect of the amendments, in the increase of MBC values in 2006 (Figure 2), rhizosphere has a positive effect on improving this parameter in soils, as can be observed by the increase of MBC values in NAR in 2007 whilst in non amended containers (NA), with no plant was growing, MBC values decreased in time slightly.

Enzyme activities have been used in a variety of ways to assess issues of agronomic and environmental quality. They have been tested as indices of soil fertility, soil quality, soil productivity, pollution effect and nutrient cycling potential (Dick, 1994). Arylsulphatase is an extracellular enzyme that catalyses the hydrolysis of organic sulphate esters, releasing plant available SO_4^{-2} . Mean values of this activity increased, in

treatments with plants, after one year of rosemary growth. Protease activity is involved in the cycle of N. For this activity the presence of rosemary in the containers (NAR, OAR and IAR) is positive, at least keeping the activity levels with time whereas in the case of NA containers, protease activity tended to decrease with time (Figure 2).

The presence of a root system could have contributed to the increase in MBC and hydrolases activities observed in all treatments in the second sampling. Plants excrete 10-20 % of their photosynthetates as root exudates (Lambers and Porter, 1992), which can serve as substrates for the microbial community, thus increasing the number of microorganisms in the soil (Salt, Smith and Raskin, 1998).

4.4. Growth, nutritional status and trace elements in plants

Rosemary growing under the trace element polluted soils did not show visual signs of injury. We did not find any difference between treatments in length along the first year of study (Table 7), although other authors (Cala, Cases and Walter, 2005), have found increases in biomass production of this plant growing in biowaste treated soils, after eight months growing in these polluted soils. Normally, longer periods are necessary to detect differences in growing parameters in Mediterranean shrubs. Dominguez et al. (in press) found no relationship between woody plants performance and soil contamination.

In general, nutrient contents of rosemary growing in polluted soils were lower than those in control plants except for P and Mg concentrations. The higher P content at OAR and IAR plants may be related to the P contents of the organic (OAR) and inorganic amendments (IAR) (Table 2). Metals could limit uptake of some macronutrients by plants, reducing their concentrations in plant tissues (Hagemeyer,

1999; Stolt and Oscarson, 2002). However, N, Ca and K contents are in the same range of values obtained by Cala, Cases and Walter (2005) and even higher than the values in control rosemary of the cited study. Marañón, Murillo and Madejón (2003) found similar nutrient values in rosemary leaves growing in trace element polluted and non polluted soils in the Green Corridor of the Guadiamar.

Rosemary growing on trace element polluted soils accumulated higher contents of these elements than control plants (Figure 4). However, contents were never above normal levels in plants, and therefore maximum levels tolerated by livestock (Chaney, 1989). Although our semi-field conditions are different to field conditions, trace element concentration in rosemary leaves in this study were similar to those founded by Murillo et al. (2005) in field conditions in the Green Corridor of the Guadiamar. This area has similar trace element levels and the same amendment addition than our semi-field experiment.

Moreover, the low Transfer Coefficient (TC) (Table 8) obtained for all trace elements indicates that this plant could be an option for phytostabilization of trace element polluted soils.

Other advantage of the use of this plant is for oil production. In experiments using other plants to extract aromatic oils it has been shown that the presence of trace elements in highly contaminated soils did not affected oil quality and its chemical composition. (Scora and Chang ,1997).

5. Conclusions

The use of amendments improved quality in trace element contaminated soils by the increase of pH and organic matter content and enhanced biochemical properties.

Establishment of a plant cover as rosemary helped the effectiveness of the amendment addition in the improvement of soil quality. Although EDTA extractability was greater in amended containers compare to non amended, rosemary growth, in general, reduced these values in time. Despite of the presence of trace elements in soils negative effects were not found in plant performance. Nutrient content in rosemary growing on contaminated soils (NAR, OAR and IAR) were adequate and concentrations of trace elements in plant was not above normal contents in plants.

Under Mediterranean conditions, rosemary is able to grow on moderate contaminated soils, and does not accumulate significant levels of trace elements in its aerial part. Therefore, the use of this plant for stabilization purposes might be a reliable option in the long-term for successful trace element contaminated soil reclamation and for industrial purposes.

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Figure captions

Figure 1. Mean values and standard deviation (SD) of EDTA-extractable trace element concentrations. For each treatment, bars with the same colour and different letter differ significantly. Lower case letters corresponds to 2006 (black bars) and capital letter for 2007 (white bars). For the same treatment the asterisk indicates significant differences between years.

Figure 2. Mean values and standard deviation (SD) of microbial biomass carbon and enzymatic activities. For each treatment bars with the same colour and different letter differ significantly. Lower case letters corresponds to 2006 (black bars) and capital letter for 2007 (white bars). For the same treatment the asterisk indicates significant differences between years.

Figure 3. Nutrient concentration (mean values and standard deviation, SD) in rosemary leaves growing in experimental substrates (NAR, OAR, IAR) and in control soil (CR). Bars with different letters are significantly different ($P < 0.05$).

Figure 4. Trace element concentration (mean values and standard deviation, SD) in rosemary leaves growing in experimental substrates (NAR, OAR, IAR) and in control soil (CR). Bars with different letters are significantly different ($P < 0.05$). Normal ranges in plants are shown for each element according to Chaney (1989).

1 Table 1. General characteristics of ‘El Vicario’ soil.

	Average	SD	Total concentration	Average	SD
pH	3.32	0.76	As (mg kg ⁻¹)	120	2.65
TOC (g kg ⁻¹)	5.40	0.07	Cd (mg kg ⁻¹)	2.43	0.04
N (g kg ⁻¹)	0.90	0.10	Cu (mg kg ⁻¹)	78.3	1.41
P (g kg ⁻¹)	0.42	0.08	Mn (mg kg ⁻¹)	645	24.6
K (g kg ⁻¹)	2.30	0.40	Pb (mg kg ⁻¹)	201	5.51
Ca (g kg ⁻¹)	4.70	0.40	Zn (mg kg ⁻¹)	226	1.53

2 SD: standard deviation; TOC: total organic Carbon (n=3)

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Table 2. General characteristics of amendments. Mean and standard deviation values are shown

	pH	EC (dS m ⁻¹)	TOC	N (g kg ⁻¹)	P (g kg ⁻¹)	K	As	Cd	Cu (mg kg ⁻¹)	Mn	Pb	Zn
OA	6.93 <i>0.03</i>	2.91 <i>0.18</i>	195 <i>12.2</i>	13.1 <i>0.60</i>	12.4 <i>1.80</i>	9.30 <i>2.30</i>	5.63 <i>1.47</i>	0.73 <i>0.40</i>	121 <i>5.60</i>	257 <i>24.7</i>	137 <i>26.2</i>	258 <i>18.4</i>
IA	9.04 <i>0.08</i>	0.93 <i>0.02</i>	67.0 <i>15.4</i>	9.80 <i>0.40</i>	5.10 <i>0.60</i>	5.30 <i>0.50</i>	1.63 <i>0.34</i>	0.43 <i>0.14</i>	51.0 <i>8.20</i>	297 <i>10.3</i>	39.2 <i>6.67</i>	138 <i>31.0</i>

OA: biosolid compost; IA: sugar beet lime.
EC: electrical conductivity; TOC total organic carbon
Standard deviation in cursive.

Table 3. Analysis of soil and plant reference samples (mean values $\pm$ 95 %confidence interval; g 100g⁻¹ dry matter for macronutrient and mg kg⁻¹ dry matter for trace elements)

Element	BCR 320 R (Channel sediment)		DC 73350 (Populus leaves)	
	Certified	Experimental	Certified	Experimental
Ca			1.81 $\pm$ 0.07	1.56 $\pm$ 0.02
K			1.38 $\pm$ 0.04	1.25 $\pm$ 0.05
Mg			0.65 $\pm$ 0.03	0.56 $\pm$ 0.01
N				
P			0.17 $\pm$ 0.004	0.15 $\pm$ 0.02
S			0.35 $\pm$ 0.03	0.33 $\pm$ 0.03
As	21.7 $\pm$ 2.00	17.2 $\pm$ 1.46	0.37 $\pm$ 0.06	0.50 $\pm$ 0.45
Cd	2.64 $\pm$ 0.18	2.42 $\pm$ 0.27	0.32 $\pm$ 0.05	0.31 $\pm$ 0.05
Cu	46.3 $\pm$ 2.90	47.7 $\pm$ 0.45	9.3 $\pm$ 0.50	9.66 $\pm$ 0.50
Fe	25700 $\pm$ 1300	2500 $\pm$ 305	274 $\pm$ 10	222 $\pm$ 1.57
Mn	910 $\pm$ 50	867 $\pm$ 3.80	45.2 $\pm$ 2.0	43.5 $\pm$ 0.36
Pb		75.9 $\pm$ 2.48	1.50 $\pm$ 0.20	0.95 $\pm$ 0.60
Zn	319 $\pm$ 20	273 $\pm$ 4.00	37.1 $\pm$ 1.00	36.4 $\pm$ 0.90

Table 4. Values of pH, Total Organic Carbon (TOC) and Water Soluble C (WSC) at NA (non amended) soils, NAR (non amended with plants) soils, OAR (organic amended soil with plants) and IAR soils (inorganic amended soil with plants) soils in the two studied years (2006 and 2007). For each parameter and year values followed by the same letter do not differ significantly ($P < 0.05$).

Treatment	Year	pH	TOC (%)	WSC (mg kg^{-1})
NA (n= 4)	2006	5.20 ± 0.40 a	0.84 ± 0.04 a/*	78.5 ± 13.2 a
NAR (n=4)	2006	5.09 ± 0.45 a	0.92 ± 0.09 a	81.8 ± 14.2 a
OAR (n=4)	2006	6.84 ± 0.23 b	1.35 ± 0.18 b	155 ± 15.8 b
IAR (n=4)	2006	7.75 ± 0.02 c	1.04 ± 0.04 a	147 ± 11.3 b
NA (n= 4)	2007	5.05 ± 0.36 a	0.76 ± 0.04 a	78.3 ± 14.4 a
NAP (n=4)	2007	5.48 ± 0.31 a	0.97 ± 0.06 b	113 ± 44.5 ab
OAR (n=4)	2007	6.63 ± 0.38 b	1.29 ± 0.05 c	181 ± 28.5 c
IAR (n=4)	2007	7.39 ± 0.032 c	1.04 ± 0.20 b	150 ± 7.91 bc

Significant differences between years are marked with asterisk

Table 5. Total concentration (mg kg⁻¹) of trace elements at NA (non amended soils), NAR (non amended with plants), OAR (organic amended with plants) and IAR (inorganic amended with plants) containers in 2007.). For each parameter values followed by the same letter do not differ significantly (P<0.05).

Treatment	As	Cd	Cu	Mn	Pb	Zn
NA (n= 4)	124 ± 24.8a	2.30 ± 0.16a	84.1 ± 2.41a	360 ± 22.9a	219 ± 9.61a	167 ± 6.75a
NAR (n=4)	115 ± 9.15a	2.18 ± 0.14a	79.9 ± 2.62a	339 ± 26.3a	222 ± 16.7a	150 ± 15.5a
OAR (n=4)	109 ± 3.32a	2.12 ± 0.44a	88.3 ± 4.81a	487 ± 87.5a	213 ± 5.68a	208 ± 30.7a
IAR (n=4)	107 ± 6.13a	2.68 ± 0.24a	87.7 ± 5.60a	607 ± 89.0a	210 ± 12.8a	247 ± 34.8a
Median values in soils *	9.00	1.00	11.50	497	10.0	47.6
Toxic range for plants **	15-50	3-8	60-125	1500-3000	100-400	70-400
Intervention values***	20	2-3	50	-	100-200	200-500

* Madejón et al., 2006

** Kabata-Pendias and Pendias 1992

***Aguilar et al., 1999

Table 6. CaCl₂-extractable trace element concentrations at NA (non amended soils), NAR (non amended with plants), OAR (organic amended with plants) and IAR (inorganic amended with plants) soils in two study years (2006 and 2007). For each parameter and year values followed by the same letter do not differ significantly (P<0.05).

Treatment	Year	Cd	Cu (mg kg ⁻¹)	Zn
NA (n= 4)	2006	0.020 ± 0.020 a	0.042 ± 0.021 a	2.89 ± 2.45 a
NAR (n=4)	2006	0.009 ± 0.003 a	0.047 ± 0.019 a	1.79 ± 1.18 a
OAR (n=4)	2006	0.010 ± 0.000 a	0.165 ± 0.038 b	0.793 ± 0.699 a
IAR (n=4)	2006	0.010 ± 0.000 a	0.114 ± 0.034 b	0.078 ± 0.024 a
NA (n= 4)	2007	0.026 ± 0.006 c	0.089 ± 0.033 a	1.67 ± 1.61 b
NAR (n=4)	2007	0.033 ± 0.014 bc	0.101 ± 0.022 a	3.69 ± 0.119 b
OAR (n=4)	2007	0.009 ± 0.001 b	0.187 ± 0.064 b	0.229 ± 0.119 b
IAR (n=4)	2007	0.003 ± 0.002 a	0.106 ± 0.029 b	0.100 ± 0.010 a

1 Table 7. Monthly increase of rosemary growth (cm) (Mean values $\pm$ Standard deviation, SD, n= 8)

Treatment	November	December	January	February	March	April
NAR	4.45 $\pm$ 0.65	3.05 $\pm$ 0.45	1.00 $\pm$ 0.41	4.25 $\pm$ 0.93	2.38 $\pm$ 1.22	3.19 $\pm$ 1.13
OAR	4.30 $\pm$ 0.93	3.70 $\pm$ 0.74	0.38 $\pm$ 0.44*	4.94 $\pm$ 0.94	2.94 $\pm$ 0.68	2.79 $\pm$ 0.81
IAR	4.54 $\pm$ 0.97	3.84 $\pm$ 0.58	0.19 $\pm$ 0.26*	3.42 $\pm$ 1.83	2.21 $\pm$ 1.11	2.71 $\pm$ 1.25
	May	June	July	August	September	October
NAR	1.63 $\pm$ 1.09	0.81 $\pm$ 0.96	4.14 $\pm$ 3.20	7.71 $\pm$ 2.22	7.50 $\pm$ 1.50	2.25 $\pm$ 1.16
OAR	1.94 $\pm$ 0.98	1.31 $\pm$ 0.96	4.00 $\pm$ 2.46	7.88 $\pm$ 4.64	6.83 $\pm$ 1.94	1.88 $\pm$ 1.96
IAR	1.63 $\pm$ 1.62	1.81 $\pm$ 1.28	3.50 $\pm$ 1.44	7.57 $\pm$ 2.37	7.50 $\pm$ 2.51	3.29 $\pm$ 2.14

2 Significant differences between treatments are marked with asterisk

Table 8. Transfer Coefficient (TC) for trace elements in rosemary leaves

Treatment	As	Cd	Cu	Zn
NAR	0.016	0.010	0.20	0.54*
OAR	0.014	0.018	0.17	0.39
IAR	0.014	0.008	0.16	0.34

Significant differences between treatments are marked with asterisk

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Figure 3

Figure 4