



Trace elements, pH and organic matter evolution in contaminated soils under assisted natural remediation: A 4-year field study

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ABSTRACT

A 4-year study was undertaken on the effect of three amendments (biosolid compost (BC), sugar beet lime (SL), and combination of leonardite plus sugar beet lime (LESL)) on reclamation of a moderately trace element-contaminated soil under field conditions. Results showed that organic C increased in BC and LESL treatments. BC and SL treatments increased soil pH and reduced CaCl₂-extractable metal concentrations more efficiently. At the end of the experiment, CaCl₂-extractable metal concentrations decreased and were similar in all treatments pointing out the importance of the natural remediation processes in contaminated soils. Addition of amendments showed no clear reduction in EDTA-extractable trace element concentrations, even, BC and LESL subplots showed some little increase of these elements with time. Amendments did not cause any change in total trace element concentration in soil. Addition of amendments could be a successful and reliable long term technique for stabilization of trace elements in contaminated soils at a field scale with minimum maintenance.

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1. Introduction

Natural remediation of trace element-contaminated soils can be improved by in situ inactivation of contaminants through the use of soil amendments [1,2]. Generally, formation of insoluble trace element chemical species reduces both leaching through the soil profile and the labile pool available for biological interaction [3,4].

The use of soil amendments has been proposed as a low input alternative for remediation of metal polluted soils. However, little information is available concerning the stability and the longevity of the remediation treatments when important soil parameters change [5]. It is well documented that some amendments (lime, phosphates, organic and inorganic waste products) are effective in reducing mobility and availability of trace elements in soils [2,3], although there is little information on the sustainability of such treatments when soil properties change with time.

Many studies on the effectiveness of soil amendments have been carried out in controlled conditions, utilizing finite volume of treated soils [5,6]. There is relatively less information on the long term effectiveness of soil amendments in regulating trace element speciation under real-world field conditions. Such field evaluations are essential before any in situ remediation approach could be

considered as a potentially safe option to treat metal polluted soils [7].

The study of solubility and bioavailability might be more important in remediation activities than the study of total or pseudo-total concentrations of these elements in contaminated soils, because they represent the most labile fractions subject to leaching and to being uptaken by plants and microorganisms [8]. The utilization of chemical extractants has become the most common approach to estimate the available fraction of trace elements in soil. Despite some weakness, these methods may provide rapid and valuable information of changes in trace element availability of soils. Extraction with 0.01 M CaCl₂ seems to be adequate to assess the impact of trace elements on plants and soil bioactivity [9]. The EDTA-extractable pool will be composed of those ions present in the soil solution, as well as those that are readily solubilised and potentially able to move into the plant root system [10]. Mild soil extractants such as EDTA are currently used to estimate this available fraction (although this method can overestimate the availability of Cu and Pb [11]). This particular extraction was also used for comparability with other studies of soils affected by the Aznalcóllar mine spill [12].

The aim of this work was to evaluate the efficiency and longevity of three amendments in regulating trace element bioavailability in a mine-spill contaminated soil under field conditions. Both organic and inorganic amendments were evaluated, as their addition may change key soil properties, such as pH and organic C content that are known to affect trace element bioavailability. pH, TOC, and

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bioavailable trace element content (CaCl₂-extractable and EDTA-extractable As, Cd, Cu, Pb and Zn concentrations) and total trace element concentrations were studied in amended soils at two depths (0–15 and 15–30 cm) over a 4-year period under prevailing field conditions.

2. Materials and methods

2.1. Study area

The study site is an experimental field (“El Vicario”) that was affected by the toxic Aznalcóllar mine spill [13]. The soils affected by the spill had a layer of toxic sludge and approximately 10–15 cm of underlying topsoil mechanically removed shortly after the accident. “El Vicario” is located on the right bank of the Guadiamar river (latitude N 37°26'21", longitude W 06°12'59"), a distance of 10 km downstream from the Aznalcóllar mine. The only remediation work carried out in this field was the initial removal of the sludge from the surface of the soil together with a layer of underlying soil. The soil was a clay loam (21.1% clay, 29.1% silt and 49.8% sand) classified as Typic Xerofluvent [14]. Prior to the spill, land use in this area was mainly cropland (sunflower, wheat, sorghum) and orchards.

The area has a semi-arid Mediterranean climate that shows a complex pattern of spatial and seasonal variability, with wide and unpredictable rainfall fluctuation from year to year [15]. The soils in the Mediterranean area are generally characterized by low water content that can alter the availability of trace elements. The annual rainfall for each period of the study was as follows: 371 mm for the period January 2002–September 2002, 567 mm for the period October 2002–September 2003, 848 mm for the period October 2003–September 2004 and 311 mm for the period October 2004–September 2005.

2.2. Experimental design and soil sampling

The experimental plot (20 m × 50 m) was divided into 12 subplots of 7 m × 8 m each, with a margin of 1 m (long) and 2 m (wide) between plots. Three amendments – two organic and one inorganic – were used. The two organic amendments were biosolid compost (BC) from the wastewater treatment plant of SUFISA (Jerez de la Frontera, Southern Spain) and leonardite (LE), a low grade coal rich in humic acids (DAYMSA, Zaragoza, Northern Spain). The inorganic amendment was sugar beet lime (SL), a residual material from the sugar beet manufacturing process with 70–80% (dry basis) CaCO₃ (EBRO-AGRÍCOLAS, San José de la Rinconada, Southern Spain). The most relevant characteristics of the amendments are shown in Table 1.

The following rates (fresh basis) were applied twice (October 2002 and October 2003): (i) 30 Mg ha⁻¹ of BC (treatment BC), (ii) 25 Mg ha⁻¹ of LE mixed with 10 Mg ha⁻¹ of SL (treatment LESL, mix-

Table 2

Mean values (±S.D.) of the input of TOC and trace elements added through amendments after two applications

	Amendment		
	BC	LESL	SL
TOC (kg ⁻¹ ha ⁻¹)	8190 ± 512	10920 ± 332	2412 ± 558
As (kg ⁻¹ ha ⁻¹)	0.24 ± 0.06	1.24 ± 0.12	0.06 ± 0.012
Cd (kg ⁻¹ ha ⁻¹)	0.03 ± 0.017	0.03 ± 0.006	0.015 ± 0.005
Cu (kg ⁻¹ ha ⁻¹)	5.08 ± 0.24	1.60 ± 0.18	1.83 ± 0.30
Mn (kg ⁻¹ ha ⁻¹)	10.8 ± 1.04	5.88 ± 0.17	10.7 ± 0.37
Pb (kg ⁻¹ ha ⁻¹)	5.75 ± 1.10	1.24 ± 0.16	1.41 ± 0.24
Zn (kg ⁻¹ ha ⁻¹)	10.8 ± 0.77	3.91 ± 0.40	4.97 ± 1.11

TOC, total organic carbon; BC, biosolid compost; LE, leonardite; SL, sugar beet lime.

ture of LE and SL), and (iii) 30 Mg ha⁻¹ of SL (treatment SL). The application rates were of the same magnitude as those applied to the whole of the spill-affected region of the Guadiamar Green Corridor [16]. Table 2 indicates the total quantity of organic matter and trace elements applied with each amendment. A treatment without amendment addition (NA) was also established. The amendments were mixed with the top-soil (0–15 cm) of each subplot using a rotary tiller (RL328 Honda). The non-amended subplots were tilled in an identical manner. The experiment was carried out in a completely randomised block design with three replicates per treatment.

Soil samples were taken at two depths (0–15 and 15–30 cm) from 48 sites (four sites per plot) on a 14 m × 45 m grid over the experimental area (Fig. 1). At each location three soil cores were taken to make a composite sample representative of each location and depth. Soils were sampled for four consecutive years: first sampling in September 2002 (before the application of amendments); second sampling in September 2003 (1 year after the application of amendment and before the second amendment application); third sampling October 2004 (2 years after the first amendment application and 1 year after the second application); fourth sampling in October 2005 (3 years after the first amendments application and 2 years after the second application).

2.3. Chemical analysis

Soil samples were air-dried, crushed and sieved (<2 mm) prior to preparation for chemical analysis. Prior to determination of pseudo-total trace element concentrations, air-dried soil and amendment samples were ground to <60 μm.

Soil pH and amendment pH values were determined using a pH meter (CRISON micro pH 2002). 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 [17].

Soil 0.01 M CaCl₂-extractable trace element concentrations were determined in 1/10 soil sample (<2 mm)/0.01 M CaCl₂ extracts after shaking for three and 0.05 M EDTA-extractable trace element concentrations were determined in 1/10 soil sample (<2 mm)/0.05 M EDTA (pH 7.00) extracts after 1 h shaking using ICP-OES [18]. Pseudo-total trace element concentrations in soil and amendments (<60 μm) were determined by ICP-OES following *aqua regia* digestion in a microwave oven (Microwave Laboratory Station Mileston ETHOS 900, Mileston s.r.l., Sorisole, Italy). The term pseudo-total accounts for the *aqua-regia* digestion, because it does not completely destroy silicates. Recovery of pseudo-total elements was assessed against total elements in a BCR reference sample (CRN 277). Recovery rates were: As 83%, Cd 66%, Cu 103%, Pb 72% and Zn 93%. Total trace elements were calculated on dry wt basis using the above recovery factors.

Table 1

Mean values (±S.D.) of some characteristics of the amendments (n = 3)

	Amendment		
	BC	LE	SL
pH	6.93 ± 0.03	6.08 ± 0.07	9.04 ± 0.08
TOC (%)	19.5 ± 1.22	28.9 ± 0.39	6.70 ± 1.55
As (mg kg ⁻¹)	5.63 ± 1.48	34.9 ± 3.46	1.63 ± 0.34
Cd (mg kg ⁻¹)	0.73 ± 0.40	0.83 ± 0.11	0.43 ± 0.15
Cu (mg kg ⁻¹)	121 ± 5.66	28.2 ± 2.40	51.0 ± 8.20
Mn (mg kg ⁻¹)	257 ± 24.8	66.2 ± 1.41	297 ± 10.3
Pb (mg kg ⁻¹)	137 ± 26.2	22.0 ± 2.33	39.2 ± 6.70
Zn (mg kg ⁻¹)	258 ± 18.4	64.5 ± 1.06	138 ± 31.0

TOC, total organic carbon; BC, biosolid compost; LE, leonardite; SL, sugar beet lime.

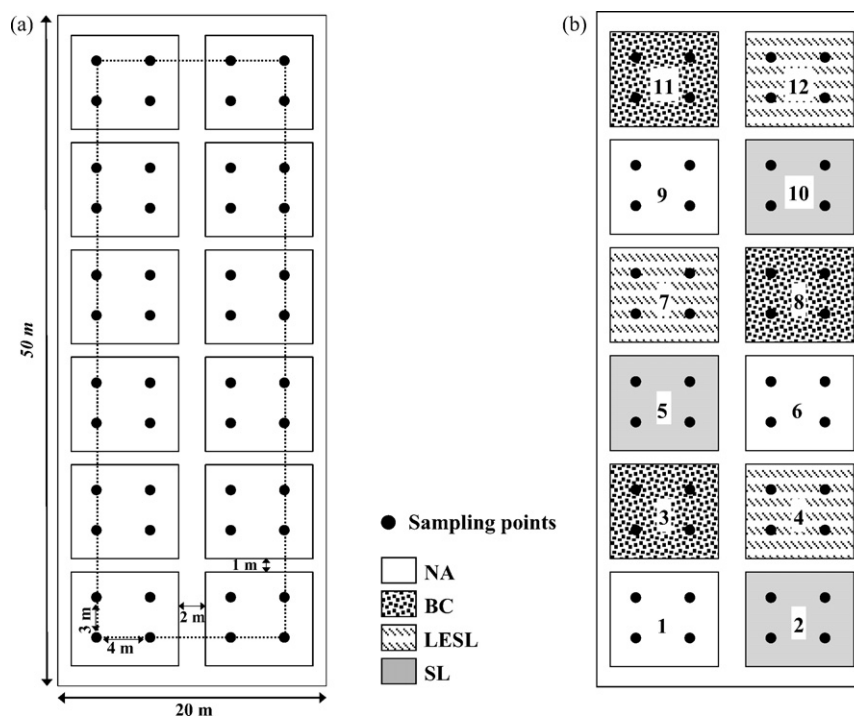


Fig. 1. (a) Soil sampling locations in the experimental plot. (b) Distribution of amendments in the experimental plot.

2.4. Statistical analysis

Mean and standard error (S.E.) were determined for all data. Normality of the data was tested prior to analysis and, when necessary, variables were transformed logarithmically. The data was analysed by ANOVA, considering the treatment and year sampling as the independent variable. Significant statistical differences of all variables between the different amendments were established by Tukey's test. If, after transformation, the data did not fit a normal distribution, we used the non-parametric Kruskal–Wallis analysis of ranks.

Correlation analysis (Pearson's coefficients) was performed to determine the relationship between the different parameters analysed.

3. Results and discussion

While every effort was made to remove the sludge, there were inevitable instances where soils were inadvertently left in a contaminated state due to difficulties in mechanical removal or where sludge was physically incorporated into the exposed subsoil. This left a very heterogeneous distribution of trace elements in the soils that inevitably resulted in a considerable degree of variability between and within the different subplots in terms of soil metal concentrations.

3.1. Soil pH and TOC

Changing the pH is a frequent remediation practice for trace element polluted soils, as the majority of the elements (apart from As) are less soluble in alkaline conditions [8]. Mean values of pH for the 4-year study are shown in Fig. 2a and b. Values were similar at both depths at the beginning of the study (2002). All the amendments had noticeable effects on soil pH, particularly in the 0–15 cm horizon. Here, the greatest change in pH was observed for the SL treatment: this raised the pH by 2 units in 2003 and 3 units at the

end of the experiment, giving a final pH value of 6.8 (Fig. 2a). Significant differences between NA and amended subplots were found in years 2003, 2004 and even in 2005 after 24 months of the last amendment application. Amendments had both a direct and residual effect on soil pH. The greatest effect on pH was found in the SL subplots due to the high CaCO_3 content of this amendment. In 2004 and 2005 there were significant differences between SL subplots and the organically amended subplots (BC and LESL). In the BC subplots pH increase could be attributed to the elevated content of basic cations in the compost [19]. In the LESL subplots, pH increase is related to the SL fraction of this amendment, as leonardite is an acidic material. The pH changes at depth (15–30 cm) were different compared to the superficial layer (Fig. 2b). In general, amendments raised pH abruptly in 2003, with values of 6 for SL, 5.5 for BC and 5.0 for LESL. In 2004, pH slightly reduced in both in BC and LESL treatments, but values were always higher than those found in the NA.

Increasing the organic matter content of the original soil was one of the main objectives of applying organic amendments and growing a plant cover [20] as the clean-up operations had removed the upper 15 cm of top soil and its associated organic matter content. As found for pH, the amendments also increased TOC (Fig. 3a). The addition of organic amendments (BC and LESL) (0–15 cm depth) raised TOC more than the inorganic (SL) treatment. There were significant differences for TOC between the BC and LESL plots when compared to the NA plots. At the end of the study, (2005) TOC was 18 g kg^{-1} , in the LESL plots, representing a twofold increase. This fact is related to the high TOC content of these amendments. For BC and SL subplots TOC in 2005 was 14.5 and 11.0 g kg^{-1} , respectively. This is especially important here, where the treated soil was highly degraded, totally or partially devoid of surface litter and upper soil horizon. A sustainable increase in soil TOC will potentially enhance fertility, soil structure and water retention.

The increase in TOC observed in the SL (a mainly inorganic amendment) treatment might be due to the indirect effect of pH that enhanced plant cover development [21]. Residues from

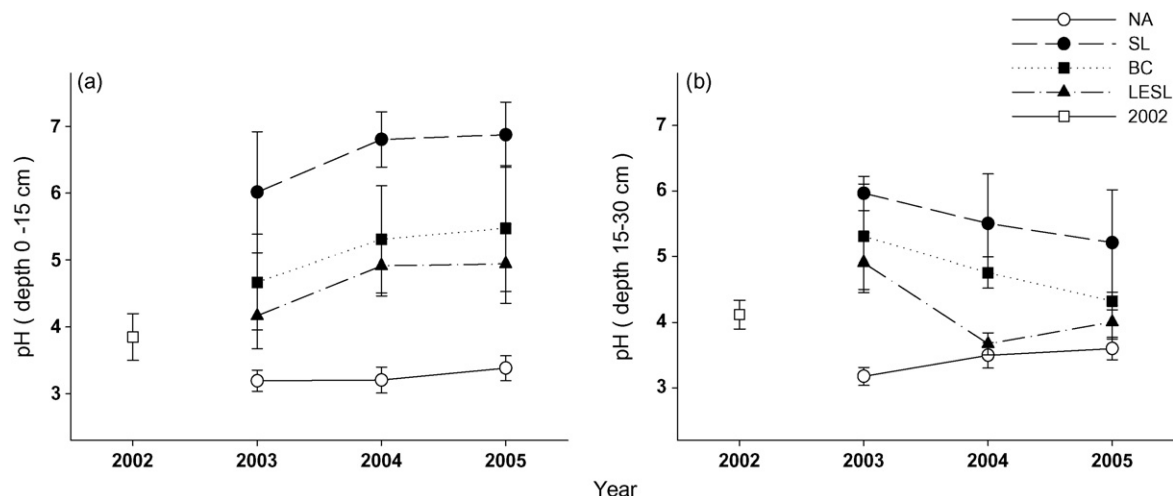


Fig. 2. Changes in soil pH during the study. (a) 0–15 cm depth and (b) 15–30 cm depth. Mean values \pm S.E. are shown ($n=3$).

enhanced plant growth probably gave rise to the higher TOC in these subplots. At lower horizon (15–30 cm) TOC followed similar evolution to pH in time, decreasing this parameter after the year 2003 (Fig. 3b).

These results indicate that positive effects of these amendments on pH and TOC are more evident in the upper soil horizon. Both parameters were significant and positively correlated throughout the study; ($r=0.51$ $p<0.001$ (2003), $r=0.34$ $p<0.05$ (2004) and $r=0.38$ $p<0.001$ (2005)). In previous experiments with this soil in semi-field conditions, there were more significant changes in pH and TOC following amendment because of the scale and conditions of the former experiment and the lower variability of the treated soils [20].

3.2. Extractable- CaCl_2 Cd, Cu and Zn evolution

After the first amendment addition in 2003, mean CaCl_2 -extractable concentrations of Cd, Cu and Zn decreased in all treatments, compared to the NA (Fig. 4a–c) in the upper soil horizon (0–15 cm). Cadmium concentration decreased from 0.37 mg kg^{-1} in 2002 to 0.10 mg kg^{-1} (SL treatment) in 2005. Cu content in soil decreased from 15.0 mg kg^{-1} in 2002 to 1.00 mg kg^{-1} in 2005 (for all amended treatments) and Zn content in soil decreased from 98.6 mg kg^{-1} in 2002 to 11.9 (for SL treatment). These results show that SL and, to a lesser extent, BC are the most effective amend-

ments for reducing CaCl_2 extractability of trace elements in soil, although the differences between treatments were not significant (on a year to year basis) due to the high spatial variability of these trace elements in the test soils. Change in soil pH in these treatments seems to be the main reason for the reduction. The greater reduction in subplots treated with SL could be related to the greater alkalinity after amendment (Fig. 2a). The pH of the soil-residual system is often the most important chemical property governing trace element sorption, precipitation, solubility, and availability [22]. The importance of pH in trace element solubility is shown by the correlation coefficient between pH and CaCl_2 -extractable trace elements. Cadmium, Cu and Zn were negatively and significantly correlated with pH all the years of the study. In amended subplots correlations had a significance level of $p<0.001$ while in NA subplots this level was always lower than $p<0.05$ (data not shown).

CaCl_2 -extractable concentrations of As and Pb were always below detection limits (0.1 mg L^{-1}).

As a rule, extractable CaCl_2 trace elements (Cd, Cu and Zn) followed the same behaviour in time: the greater concentrations in 2003 drastically dropped in 2004 and maintained low values in 2005 (Fig. 4a–c) when amendments were not applied. The residual year in which amendments were not applied did not reverse trace element stabilization in soil, at least in the mid-term.

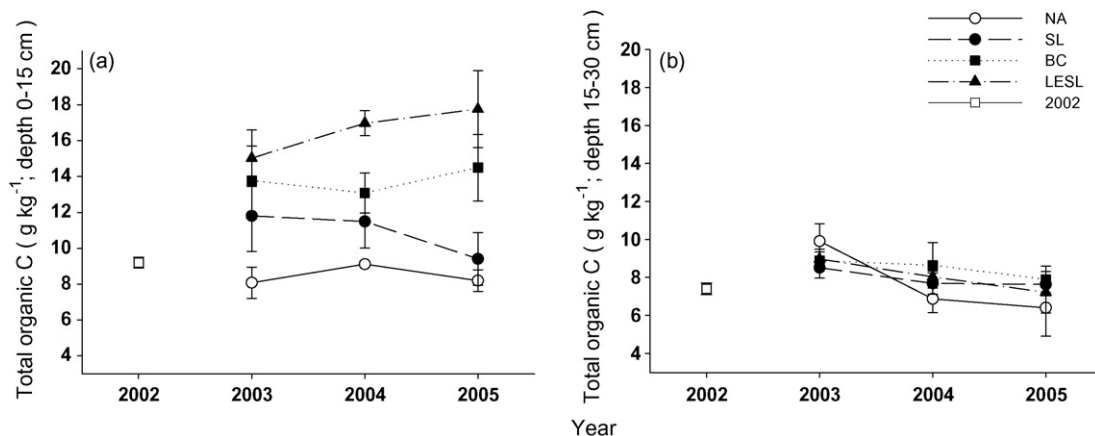


Fig. 3. Changes in total organic carbon (TOC) during the course of the study. (a) TOC at depth 0–15 cm and (b) TOC at depth 15–30 cm. Mean values \pm S.E. are shown ($n=3$).

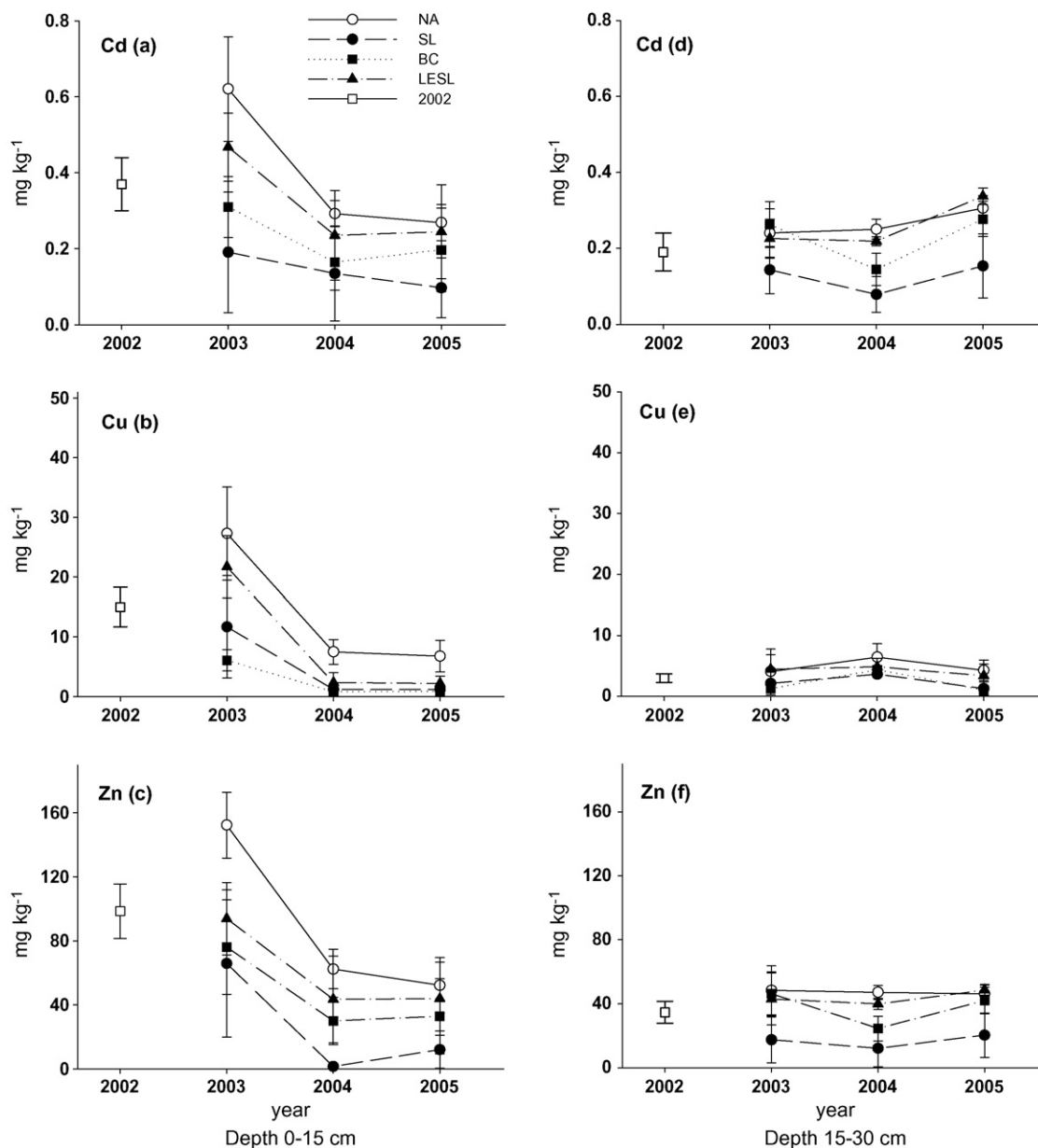


Fig. 4. 0.01 M CaCl₂-extractable trace element concentrations at two depths, 0–15 and 15–30 cm. (a) CaCl₂-extractable Cd at 0–15 cm, (b) CaCl₂-extractable Cu at 0–15 cm, (c) CaCl₂-extractable Zn at 0–15 cm, (d) CaCl₂-extractable Cd at 15–30 cm, (e) CaCl₂-extractable Cu at 15–30 cm and (f) CaCl₂-extractable Zn at 15–30 cm. Mean values ± S.E. are shown (*n* = 3).

The behaviour observed in NA indicated that natural attenuation processes could have also played an important role in diminishing trace element solubility [23].

Mean values CaCl₂-extractable at depth 15–30 cm were lower than in the surface soil (0–15 cm; Fig. 4d–f) suggesting that main pollution level was in the top soil. Differential incorporation of the sludge at depth is shown by the high standard error found in each subplot (Fig. 4d–f), higher than for the 0–15 cm horizon (Fig. 4a–c). These results also suggest that the reduction of CaCl₂-extractable Cd, Cu and Zn found for top soils in 2004 was not due to a leaching effect into the lower horizon (15–30 cm). There was no time-related pattern of changes in trace element extractability in this horizon (mean CaCl₂-extractable values were lower, so their reduction was less significant than in the superficial soils). The amendment application was performed in superficial soil therefore their effects were mainly detected in the upper layer.

3.3. Changes in EDTA-extractable trace element concentrations

In contrast to the 0.01 M CaCl₂ extraction, EDTA-extractable trace element concentrations did not decrease for all treatments with time in the upper soil horizon (0–15 cm) (Fig. 5a–e). The same result was found by Pérez de Mora et al. [20] in semi-field conditions. We could not find any clear time-dependant pattern in reduction of trace element extractability in this horizon. In general, trace elements tended to decrease in 2004 but at the end of the experiment in 2005 after 24 months from the last amendment application, values were higher than in the previous year.

There was no difference in the behaviour of As between treatments (Fig. 5a). After the second amendment application (2003), As content dropped for all subplots to 1.00 mg kg⁻¹ in 2004 (Fig. 5a). At the end of the study (2005), As extractability slightly increased in BC and SL subplots. This maybe due in part to the higher pH

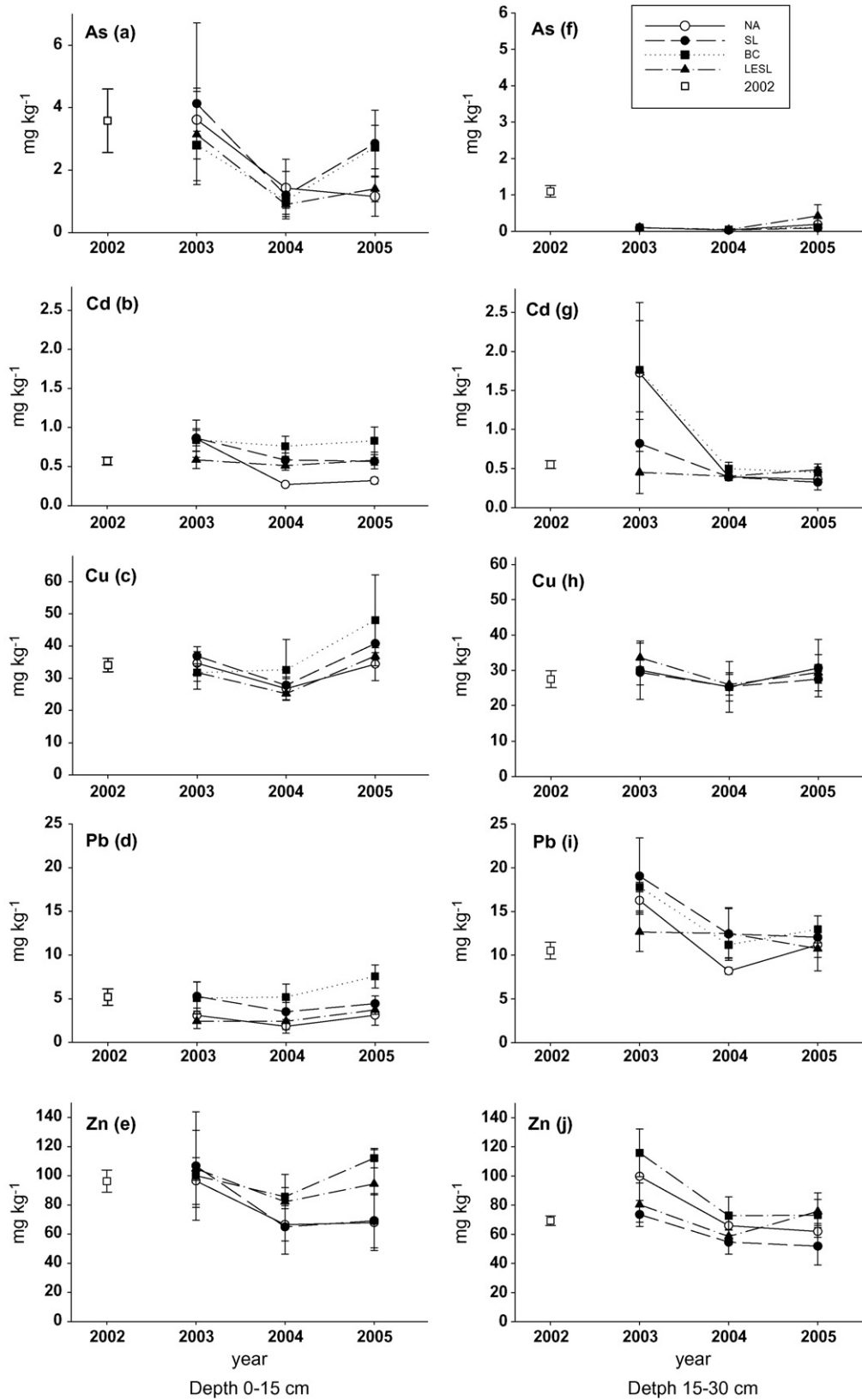


Fig. 5. 0.05 M EDTA-extractable trace element concentrations at two depths, 0–15 and 15–30 cm. (a) EDTA-extractable As at 0–15 cm, (b) EDTA-extractable Cd at 0–15 cm, (c) EDTA-extractable Cu at 0–15 cm, (d) EDTA-extractable Pb at 0–15 cm, (e) EDTA-extractable Zn at 0–15 cm, (f) EDTA-extractable As at 15–30 cm, (g) EDTA-extractable Cd at 15–30 cm, (h) EDTA-extractable Cu at 15–30 cm, (i) EDTA-extractable Pb 15–30 cm and (j) EDTA-extractable Zn at 15–30 cm. Mean values ± S.E. are shown (n=3).

of both subplots; incorporation of lime to As contaminated soil may increase As mobility due to the pH increase [24]. Higher EDTA-extractable Cd content was found in 2003 compared to the initial value (2002; Fig. 5b). The only significant decrease in EDTA-extractable Cd was in the NA subplots (0.9 mg kg^{-1} in 2002 to 0.3 mg kg^{-1} in 2004). In general, in the amended subplots, Cd content remained constant or showed a non-significant reduction. Mean EDTA-extractable Cu concentrations showed similar values in 2002 (34.1 mg kg^{-1}) and 2003 ($30.0\text{--}36.8 \text{ mg kg}^{-1}$) (Fig. 5c). After two consecutive years of amendment addition (2004) EDTA-extractable Cu slightly decreased except in BC plots, although at the end of the study these levels rose again (maximum value 48 mg kg^{-1} in CB subplots). Pérez de Mora et al. [20] found similar behaviour in a semi-field experiment using the same amendments. The EDTA-extractable Pb content was lower in NA subplots than in amended subplots throughout the study (Fig. 5d). The highest values were found in CB subplots, where concentrations increased in time (maximum of 7.55 mg kg^{-1} in 2005). This could be related to the higher Pb content of this amendment (mean value 137 mg kg^{-1}). The lowest concentrations of Zn (extracted by EDTA) were found in NA and SL subplots, both showed similar behaviour throughout the study. In both treatments, Zn content decreased non-significantly from 96.3 to 70 mg kg^{-1} , however, subplots treated with organic amendments (LESL and BC) showed also in this case a slight increase of Zn extractability in 2005 (Fig. 5e).

In general, subplots amended with BC and to a lesser extent with LESL showed some increase in EDTA-extractable trace elements. This result could exclude this kind of materials for reclamation of trace element polluted soils, however results obtained using CaCl_2 showed a clear decrease of bioavailability. Trace element bioavailability has been reported to either increase or decrease in the presence of organic amendments [25], however, this depends on the extractant used. It is known that EDTA can remove trace elements bound to organic matter that are not available for plants and/or microorganisms. For that reason biomonitoring is a vital component of bioavailability measurements. In previous studies [21,26,27] it has been shown that EDTA-extractable trace element concentrations were not as well correlated as CaCl_2 -extractable trace element concentrations with trace element content in plants grown in the soil. This agrees with Kabata-Pendias [9], who emphasizes the importance of using extractants that simulate the natural soil solution, such as 0.01 M CaCl_2 , for bioavailability measurements.

At lower soil horizon (15–30 cm), EDTA-extractable elements had different behaviour in time compare with upper horizon and it could not found any pattern of variability in time (Fig. 5f–j). Lead values were always higher at this depth compared to top soils, even before the amendment addition (5.2 mg kg^{-1} at 0–15 cm and 10.5 mg kg^{-1} at 15–30 cm; Fig. 4i). This result could be due to heterogeneity of pollution penetration, although total Pb content was always higher in the upper horizon (Table 2).

3.4. Total soil As, Cd, Cu, Pb and Zn content in relation to the incorporation of amendments

When amendments that contain elevated trace element concentrations are added to soils (even if soils themselves contain high concentrations) it is necessary to study their impact on total soil metal concentrations.

Table 3 shows pseudo-total trace element content in soil (depth 0–15 and 15–30 cm) at the beginning of the experiment in 2002 (before amendment addition) and at the end of the experiment in 2005. As a rule pseudo-total As, Cd, Cu, Pb and Zn concentrations in soils measured in 2005 (after two amendment additions) were similar or even lower in some cases to those measured in 2002 (Table 3).

Cadmium content in all subplots was between 4.0 and 4.8 mg kg^{-1} ; still around 14-fold higher than background values in this area (0.33 mg kg^{-1}) [28]. Total soil Cu concentrations at the end of the study were threefold higher than background values for this area (31 mg kg^{-1}) [28]. Total Zn content in soils showed the same behaviour for all amendment subplots, with total Zn content increasing in the order $\text{BC} > \text{SL} > \text{LESL}$, the same order as the Zn content of the amendments (258 mg kg^{-1} $\text{CB} > 138 \text{ mg kg}^{-1}$ $\text{SL} > 65 \text{ mg kg}^{-1}$ LESL). Total soil Zn content at the end of the study was two- to fourfold higher than background values reported for the area (109 mg kg^{-1}) [28]. Total arsenic and lead concentrations followed the pattern of change for each treatment (Table 2), although the proportion of both elements was different for each amendment. Higher total As and Pb contents were found in the LESL subplots, and lower contents in the BC and SL subplots. Content of both elements in BC and SL subplots were even lower than NA subplots (Table 2). Total contents of both elements at the end of the study were 8–10-fold higher than background values in the area (18.9 mg kg^{-1} for As and 38.2 mg kg^{-1} for Pb) [28]. These results suggest that the addition of different amendments did not increase total trace element in the soil.

Table 3

Total trace element content (mg kg^{-1}) in soils at the beginning of the experiment (2002, $n = 12$) and at the end of the experiment (2005, for each treatment $n = 3$)

Year	Treatment	Upper layer (0–15 cm)				
		As	Cd	Cu	Pb ^a	Zn
2002		$202 \pm 19 \text{ a}$	$4.4 \pm 0.21 \text{ a}$	$119 \pm 5.2 \text{ a}$	$471 \pm 38 \text{ b}$	$381 \pm 27 \text{ b}$
2005	NA	$177 \pm 52 \text{ a}$	$4.0 \pm 0.40 \text{ a}$	$99 \pm 17 \text{ a}$	$377 \pm 116 \text{ ab}$	$218 \pm 19 \text{ a}$
	SL	$168 \pm 63 \text{ a}$	$4.4 \pm 0.40 \text{ a}$	$105 \pm 12 \text{ a}$	$327 \pm 100 \text{ a}$	$320 \pm 32 \text{ ab}$
	BC	$145 \pm 8.8 \text{ a}$	$4.8 \pm 0.36 \text{ a}$	$113 \pm 15 \text{ a}$	$291 \pm 13 \text{ a}$	$398 \pm 61 \text{ b}$
	LESL	$186 \pm 46 \text{ a}$	$4.4 \pm 0.50 \text{ a}$	$110 \pm 11 \text{ a}$	$404 \pm 119 \text{ ab}$	$290 \pm 36 \text{ ab}$
Year	Treatment	Lower layer (15–30 cm)				
		As	Cd	Cu	Pb ^a	Zn ^a
2002		$91 \pm 58 \text{ a}$	$2.7 \pm 0.80 \text{ a}$	$90 \pm 29 \text{ a}$	$218 \pm 113 \text{ b}$	$272 \pm 72 \text{ b}$
2005	NA	$55.7 \pm 15.4 \text{ a}$	$2.9 \pm 0.25 \text{ a}$	$67.2 \pm 32 \text{ a}$	$122 \pm 29 \text{ ab}$	$234 \pm 42 \text{ a}$
	SL	$48.9 \pm 13.6 \text{ a}$	$2.8 \pm 0.12 \text{ a}$	$65.0 \pm 7.4 \text{ a}$	$133 \pm 19 \text{ a}$	$200 \pm 20 \text{ a}$
	BC	$49.0 \pm 14.4 \text{ a}$	$3.0 \pm 0.21 \text{ a}$	$74.7 \pm 30 \text{ a}$	$140 \pm 38 \text{ a}$	$253 \pm 46 \text{ ab}$
	LESL	$71.5 \pm 35.1 \text{ a}$	$3.1 \pm 0.17 \text{ a}$	$75.3 \pm 21 \text{ a}$	$171 \pm 67 \text{ ab}$	$206 \pm 22 \text{ a}$

For each layer values followed by the same letter in the same column do not differ significantly ($p < 0.05$).

^a Non-parametric test.

For depth 15–30 cm total trace element contents were lower than in upper soils (Table 2), and still higher than in concentrations of non-affected soils in the area [28].

4. Conclusion

The two successive applications of amendments alkalinized soil pH and increased total organic C concentrations in soil. Particularly, application of lime seems to be very effective to change soil pH. Addition of organic amendments is useful for increasing soil organic C. These improvements of soil properties were maintained after 1 year of residual effect.

Results on bioavailability of trace elements were highly influenced by the extractant used. Whereas 0.01 M CaCl₂-extraction showed a noticeable reduction of trace element bioavailability induced by application of amendments, 0.05 M EDTA-extraction indicated that organic matter addition slightly increased trace element extractability with time. Nevertheless results of EDTA extraction do not always have a clear correlation with bioavailability. For this reason, monitoring in soil and vegetation is required to ensure trace element stabilization and evaluate the efficiency of the treatments and the need for further applications.

Trace element total concentrations were not significantly increased respect to initial values of the soil and changes might be also influenced by field heterogeneity.

Assisted natural remediation proved to be a successful and reliable technique for remediation of a trace element-contaminated soil at a field scale with minimum maintenance. Based on their total trace element concentrations, soils still present high contamination. However, this technique could reduce trace element availability and thus the risk of entry in the food chain even after 2 years after the last amendment application.

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