AMENDMENTS FOR THE REMEDIATION AND REGENERATION OF A TRACE ELEMENT CONTAMINATED SOIL

(ENMIENDAS PARA LA RECUPERACIÓN Y REHABILITACIÓN DE UN SUELO CONTAMINADO CON ELEMENTOS TRAZA)

Trabajo de Iniciación a la Investigación

Jian Xiong

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Memoria del Trabajo de Iniciación a la Investigación
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Visado en Sevilla

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1. INTRODUCTION

1.1. Definition and origin of trace elements

The term "trace element" is widely used in the literature and may have differing meanings in various scientific disciplines. Trace elements refer generally to elements that occur in natural and perturbed environments in small amounts and that, when present in excessive bioavailable concentrations, are toxic to living organisms. Often it defines elements that are essential or toxic in small quantities to microorganisms and plant and animal organisms, including humans. However, it often includes elements with no known physiological functions. Other terms that are often used as synonymous for most "trace elements" include: "trace or heavy metals", "microelements", "trace inorganics" (Blum, 1998; Adriano, 2001; Carrillo-González *et al.*, 2006). The most used of the latter terms in environmental studies is heavy metals, referring to elements having densities greater than 5.0 g cm^3 and denote metals and metalloids that are associated with pollution and toxicity but also include essential elements. However, some authors have stressed the imprecision of the term “heavy metal” concerning which elements are included, as well as the lack of chemical consistency of using such term for any compound of a given element (Duffus, 2002; Hodson, 2004; Madrid, 2010).

In Geology trace elements are those distinct that major elements (O, Si, Al, Na, Mg, Ca and Fe) and minor elements (H, C, S, K, P, Ti, Cr, Mn). In which Soil Science this term is applied to those elements which concentration is <1000 mg kg^{-1}. This includes Mn, Cr, Ni, Pb and Zn (1 - 1500 mg kg^{-1}); Co, Cu and As (0.1 - 250 mg kg^{-1}); Cd and Hg (0.01 - 2 mg kg^{-1}). In biochemical and biomedical research, trace elements are considered to be those that are ordinarily present in plant or animal tissues in concentrations comprising <100 mg kg^{-1} of the organism dry matter. In food and nutrition science, a trace element may be defined as an element that is of common occurrence but whose concentration rarely exceeds 20 mg kg^{-1} in the foodstuffs as consumed (Blum, 1998; Adriano, 2001; Carrillo-González *et al.*, 2006).

Trace elements are ubiquitous in agroecosystems. Trace elements enter an agroecosystem through both natural and anthropogenic processes. Some soils have been found to have a high background of some trace elements, which are toxic to plants and wild life, due to extremely high concentrations of these elements in the parent materials. Anthropogenic processes include inputs of trace elements through use of fertilizers, liming materials, organic manures (municipal wastes as sewage suldges and other wastes used as soil amendments), irrigation waters and other industrial wastes. Other anthropogenic sources include mining and smelting of metallic ores, combustion of coal and other fossil fuels, use of pesticides in agriculture and timber industry, manufacturing, landfills, and so on. These processes contribute variable amounts of trace elements to the agroecosystem. Accumulation of trace elements, especially heavy metals, in the soil has potential to restrict the soil's function, cause toxicity to plants, and contaminate the food chain. (Blum, 1998; Senesi *et al.*, 1999; Adriano, 2001; He *et al.*, 2005; Carrillo-González *et al.*, 2006).

1.2. Remediation of soil contaminated with trace elements

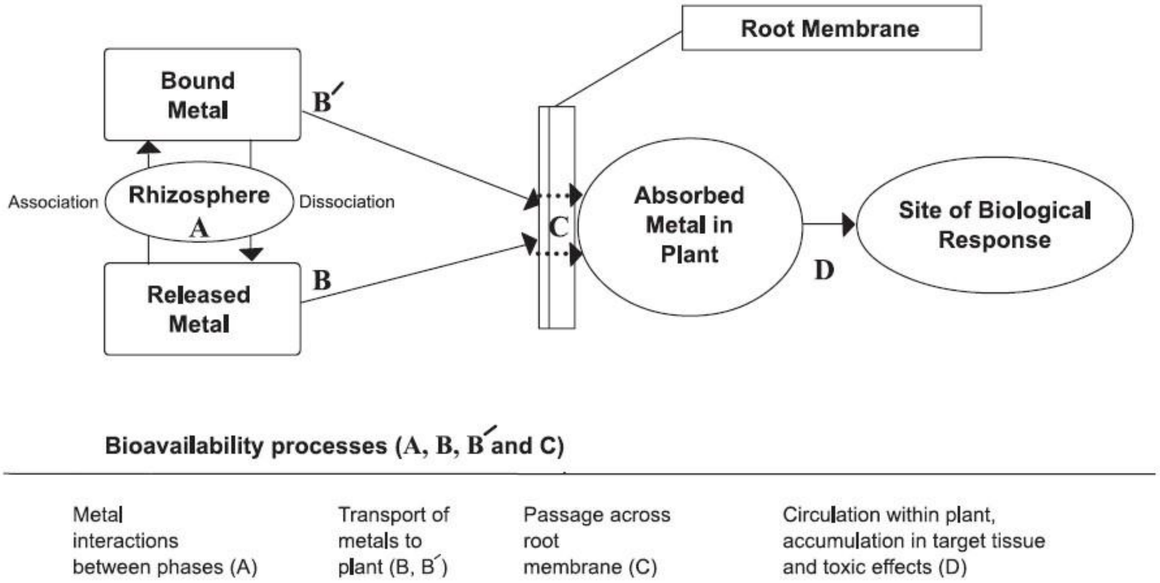


Figure 1.1 Bioavailability processes in the soil-plant system. Modified from the US-NRC (2003), (Adriano *et al.*, 2004).

Contamination of soils with trace elements is a challenging environmental issue due to: a) the non-degradable character of inorganic elements, b) the risks associated with their transfer to non-contaminated areas via run-off and leaching, and c) their potential entry in the food chain via plant uptake or direct ingestion. However, the detrimental effects in exposed organisms and ecosystems are not caused by the entire amount of trace elements released to the environment but only by the bioavailable fraction (Figure 1.1). The mobility and availability of trace elements are controlled by many chemical and biochemical processes (precipitation-dissolution, adsorption-desorption, complexation-dissociation, and oxidation-reduction, etc.) (Figure 1.2) (He *et al.*, 2005). Therefore, the studies on trace element contamination have been evolving from an old mentality, where the total concentration of the contaminants has been the main basis for risk assessment, to a modern mindset where mainly the bioavailable fraction of the total mass is considered (US-EPA, 1993; Adriano *et al.*, 2004).

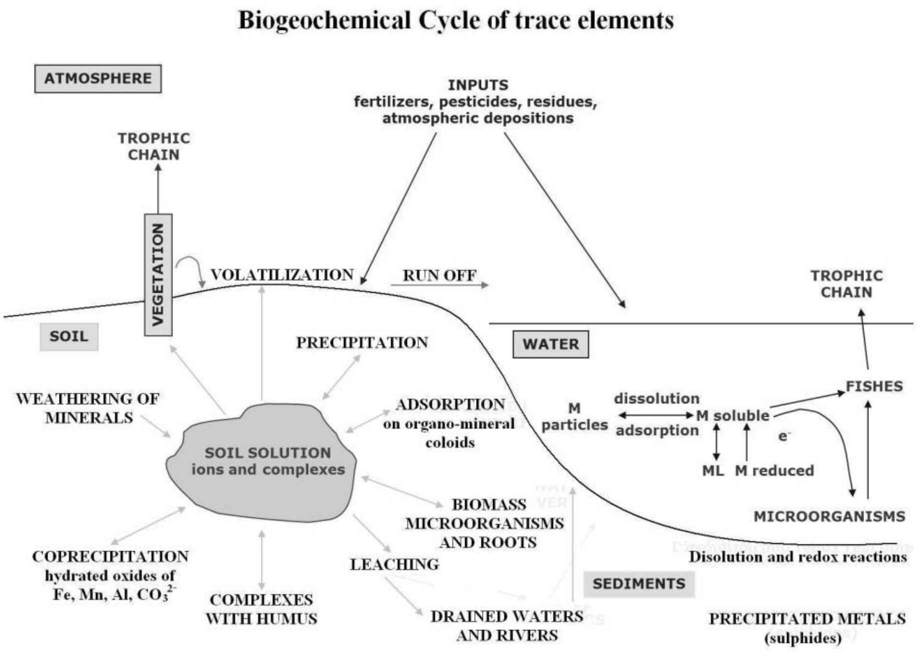


Figure 1.2 Trace element biogeochemical cycle (Alloway, 1990).

However, the definition of bioavailability and the concepts on which it is based are still unclear. The methods adopted for its measurement vary and as such there is no single standard technique for the assessment of either plant availability of contaminants or their ecotoxicological impacts on soil biota. The bioavailable contaminant fraction in soil represents the relevant exposure concentration for soil organisms (Figure 1.3) (Tobias *et al.*, 2003). According to Adriano *et al.* (2004), the bioavailable fraction of trace element refers to the readily available fraction (sum of soluble and weakly adsorbed fractions) that oftentimes refers to the most labile fraction.

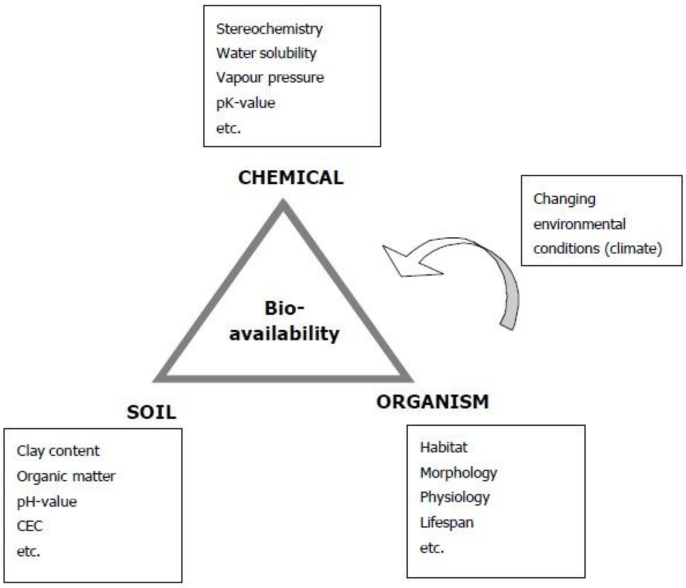


Figure 1.3 Bioavailability – complexity of parameters and processes (Tobias *et al.*, 2003).

Remediation of contaminated soil is accomplished by either destroying contaminants or by removing them from the soil or by reducing their toxicity or their bioavailability. Environmental remediation technologies use physical, chemical, or biological processes to eliminate, reduce, isolate, or stabilized contaminants. There are many classifications of the technologies of soil remediation: 1) depending on the place where the treatment is accomplished the treatment is in situ (the soil is treated in place) or ex situ (the soil is excavated and treated on the place or transported to a treatment plant); 2) depending on the type of treatment: physical, chemical or biological; 3) on the technology: isolation, immobilization, toxicity reduction, physical separation and extraction; 4) depending on the objective: remove the contaminants from the soil (“site decontamination”) or reduce the risk posed by the contaminants by reducing exposure (“site stabilization”).

Every remediation technology has certain limitations and disadvantages. Therefore, site-specific evaluations must be made to assure that the appropriate technologies are applied. If multiple contaminants are involved, it may be necessary to use a combination of techniques to reduce the concentration of pollutants to acceptable levels. The economic cost of remediation is one of the most important factors to be taken into account. Another factor is the nature and concentration of the contaminant. In this last respect is very important the cleanup goal. Within the “in situ” techniques (isolation, removal/extraction and stabilization) few of them have been fully developed to date and can be considered as cost-effective and reliable for land treating of extensive contaminated areas.

Among the different remediation techniques, biological ones have gained great importance in the last decade due to its low cost, low technical requirement and their potential to take place *in situ* (Baker & Brooks, 1989; Ensley, 1995). Bioremediation attempts to use plants and microbe to enhance the natural processes for removing or decomposing the unwanted substances. Once a favorable environmental condition is established for the selected plants or microbes to thrive, the biological processes for remediation could be sustained by the selected plants or microbes with much reduced energy inputs. After the contaminants are removed by biological means, the soil would remain biologically active and its capability to function as a medium for plant growth would not be impaired.

Therefore, while proper attention should be devoted to establishing processes and devising techniques to reduce or eliminate contaminants from soil as much rapidly as possible, equal importance should be given to understanding the impact of the treatment processes on decontaminated soil. The goal of soil remediation is not only to eliminate contamination from the soil but also to restore the quality of the soil.

Compared with physical and chemical remediation, bioremediation has several advantages: (1) it preserves the natural properties of soil; (2) it acquires energy mainly from sunlight; (3) high levels of microbial biomass in the rhizosphere can be achieved; (4) it is low in cost; and (5) it has the potential to be rapid (Huang *et al.*, 2004).

In situ bioremediation methods comprise:

Natural attenuation that occurs when physical, chemical and biological processes act to reduce the toxicity and mobility of contaminants. The predominant processes that comprise natural attenuation include biodegradation, chemical transformation, stabilization, volatilization.

Enhanced bioremediation that is directed towards stimulating the microorganisms to grow and use the contaminants as a food and energy source by creating a favorable environment for microorganisms.

Phytoremediation that refers to the technologies that use plants for treating polluted soils. Phytoremediation is a technology that should be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages, and long-term applicability. Phytoremediation is well-suited for use at very large field sites where other methods of remediation are not cost-effective or practicable; at sites with low concentrations of contaminants where only “polishing treatment” is required over long periods of time; and in conjunction with other technologies where vegetation is used as a final cap and closure of the site (Schnoor, 1997).

Plants have shown the capacity to withstand relatively high concentrations of organic chemicals without toxic effects, and they can uptake and convert chemicals quickly to less toxic metabolites in some cases. In addition, they stimulate the degradation of organic chemicals in the rhizosphere by the release of root exudates, enzymes, and the build-up of organic carbon in the soil. For metal contaminants, plants show the potential for phytoextraction (uptake and recovery of contaminants into above-ground biomass), filtering metals from water onto root systems (rhizofiltration), or stabilizing waste sites by erosion control (phytostabilization) and evapotranspiration of large quantities of water (phytovolatilization) (Schnoor, 1997).

Enhancements of phytoremediation processes (Wenzel *et al.*, 1999). Basically, three approaches may be used to enhance phytoremadioation processes:

1. Improve plant's genetic potential. Some specific targets are to improve: root morphology, root exudates involved in mobilization/immobilization of pollutants, plant enzymes that are essential for detoxification, oxidation/reduction, translocation, degradation or volatilization of pollutants, and biomass.

2. Cultural/management practices, e.g., crop management, weed/plant disease control and harvest techniques.

3. Soil conditioning aimed at providing optimal physical and nutritional conditions for plant growth, development of the root/microrhyzal system, and enhancing phytoremediation processes by adjusting soil physical, chemical, and biological properties that control pollutants mobility and bioavailability. In this sense, the use of some wastes and byproducts as amendments could enhance remediation processes.

1.3. Application of amendments to the contaminated soil

Application of soil amendments might be an environmentally friendly and cost-effective approach to restore extensive areas with a moderate-level of contamination. These "in situ" treatments aim at enhancing natural attenuation mechanisms (sorption, precipitation and complexation reactions) that occur in the soil, thus reducing the mobility and bioavailability of the contaminants rather than total concentrations of trace elements (Adriano *et al.*, 2004). In addition, amendments may facilitate plant establishment on such degraded land and enhance microbial activity (Figure 1.4) (Mora *et al.*, 2005; Madejón *et al.*, 2006a; Bolan *et al.*, 2011).

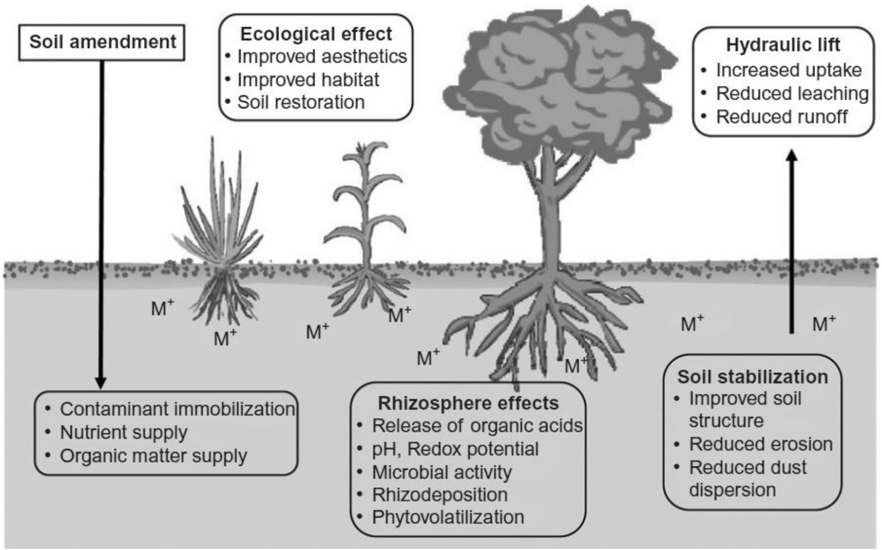


Figure 1.4 Schematic diagram illustrating the potential action of phytostabilization on contaminants in soil (Bolan *et al.*, 2011).

The use of by-products as soil amendments, such as lime or composts, also reduces waste disposal and revalues these wastes (Lombi *et al.*, 2002a; Lombi *et al.*, 2002b). However, the true potential of "in situ" treatments is still unclear, because it requires validation of long term stability data (Pérez-de-Mora *et al.*, 2007). Numerous amendments have been used to immobilize trace elements in contaminated soils (Knox *et al.*, 2001). These include lime, zeolites, apatite, Fe and Mn oxides, alkaline composted biosolids, clay minerals and industrial by-products such as beringite. Applications to soils of certain amendments that enhance key biogeochemical processes in soils that effectively immobilize metals have already been demonstrated in Europe and North America on a field scale (Adriano *et al.*, 2004). Case studies using lime, phosphate and biosolid amendments have demonstrated, under field conditions, enhanced natural remediation resulting in substantially improved vegetation growth, invigorated microbial population and diversity, and reduced offsite metal transport. Depending on soil/hydrogeochemical properties, source term and metal form/species, and land use, the immobilization efficacy induced by such assisted natural remediation may be enduring. Apart from the stabilization of trace elements, the use of these materials in soil reclamation fulfills two objectives: i) recycling of byproducts because in modern societies, adequate management of residues and wastes has become essential and ii) soil regeneration. In this second objective, the effectiveness of these remediation practices in restoring soil functionality of trace element polluted soils has been less studied (Burgos *et al.*, 2010).

Use of amendments and/or plant growth alkalinized soil pH and increased total organic C and water-soluble C concentrations. Particularly, application of lime and stabilized composts seem to be very effective to change soil pH. Addition of organic amendments is useful for increasing soil organic C, however the presence of a root system might be as important or even more than organic amendments for readily soluble C sources (Pérez-de-Mora *et al.*, 2006). In addition, application of amendments can act as a long term source of available nutrients as its release is gradual so that improve soil fertility (Alburquerque *et al.*, 2011).

1.4. Development of spontaneous vegetation in the polluted area

Vegetation plays a crucial role in the restoration of such degraded areas, because it prevents wind-blow of contaminated particles and reduces water pollution (Tordoff *et al.*, 2000). Nevertheless, trace element uptake by plants implies several hazards such as introduction into the food chain (McLaughin, 2001). Thus, a study of soil properties and vegetation development is necessary to evaluate the effectiveness of remediation measures on ecosystem ecology and risk posed by the trace element content (Madejón *et al.*, 2006a).

In semiarid areas, pioneer plant communities in trace element affected soils are well-adapted to stress due to the presence of contaminants, and poor nutrient and water availability (Freitas *et al.*, 2004; Madejón *et al.*, 2009). Natural vegetation also provides physical protection against soil erosion by wind and water (Norland & Veith, 1995). In addition, evapotranspiration limits losses to groundwater by evapotranspiration of soil water by vegetation (Tordoff *et al.*, 2000). Pioneer herbaceous vegetation may play a chief role in the cycling of trace elements during the early stages of secondary succession in contaminated areas, particularly in semiarid climates.

Minimizing accumulation of trace elements in aboveground biomass is a major challenge for any large-scale phytomanagement programme (Domínguez *et al.*, 2008). Research shows that addition of amendment may improve soil properties for colonization and growth of trace element-tolerant plants; for instance, nutrients can exert a protective effect against metal and metalloid toxicity in plants (Mengel & Kirkby, 1987).

Therefore, the study of these spontaneously developed plants is necessary to determine the associated risk of elevated trace element accumulation in plant shoots as well as the investigation of pollution indicator related to the contaminated soil.

1.5. Objective

To assess the true potential of **Assisted Natural Remediation** as a technique for the remediation of a soil moderately contaminated with trace elements it is necessary to conduct long-term studies. Such studies should consider the effect of the application of this technique on: 1) the physic-chemical properties of soil, with particular emphasis on trace element concentrations (total and bioavailable), and 2) the development of a vegetation cover and the uptake and accumulation of trace elements by plants.

Moreover, it is necessary to verify the adequacy and the effect of the amendments applied and the doses of application to determine the persistence of amendment effects over time. The use of different industrial byproduct and wastes as amendments (e.g.: sugar beet lime, biosolid compost, urban compost, etc.) has additional interest because of recycling and extending the use of these byproducts, not only in agriculture use but also in the field and landscaping in the remediation of degraded and contaminated soil under the framework of sustainable development.

The present work has two main objectives. The first is the evaluation of the effect of the application of various amendments to a trace element polluted soil on: 1) trace elements mobility and plant availability, 2) soil chemical properties, and 3) on plant colonization, growth and composition. The second objective is to assess the efficacy of repeated amendment applications on the reduction of the concentration of labile trace elements in soil.

The present study was carried out 8 years after the first application of three amendments to a moderately-polluted soil.

2. MATERIALS AND METHODS

2.1. Aznalcóllar mine spill

The Aznalcóllar mine spill occurred on the 25 April 1998; approximately 6 million cubic meters of slurry inundated both river banks of the Agrio and Guadiamar rivers (Figure 2.1). Slurry was composed of acidic water loaded with heavy metals and other toxic elements, and sludge consisting of finely divided metal sulphides: pyrite with arsenopyrite (75-80%) and sphalerite and galena (5%). Concentrations of trace elements in the sludge is shown in (Cabrera *et al.*, 1999). A strip approximately 40 km long and 300 m wide along both rivers was covered by a layer (2-30 cm thick) of the sludge (IGME, 1998). Approximately 4500 ha of agricultural land devoted to dry-land agriculture and fruit and olive tree orchards were affected by the pollution (CMA, 1998). After the flood cultivation of food crops in the spill-affected area was prohibited by law after the accident (Madejón *et al.*, 2006a).

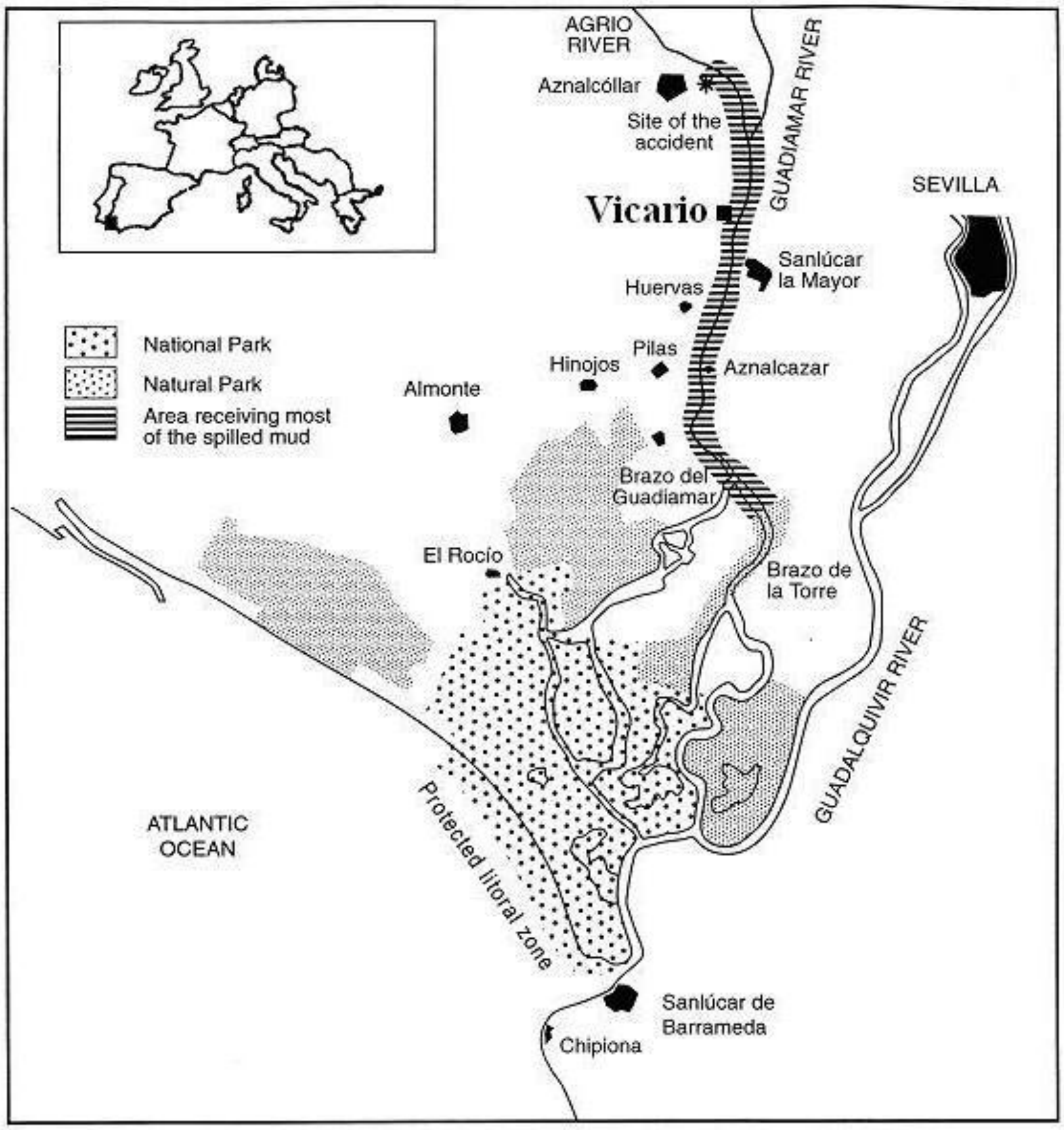


Figure 2.1 Map of the Aznalcóllar mine spill and location of the experimental plot of “El Vicario”, modified from (Grimalt *et al.*, 1999).

Soils affected by the flood showed concentration of total As, Bi, Cd, Cu, Hg, Pb, Sb, Tl y Zn in the 0-50 cm layer higher than the background levels of the Guadiamar valley soils (Cabrera *et al.*, 1999; López-Pamo *et al.*, 1999; Simón *et al.*, 1999; Cabrera *et al.*, 2008a; Cabrera *et al.*, 2008b). In most of the affected soils severe trace elements pollution was observed in the superficial layers (0-20 cm), which decreased downward in the soil profile. Generally, in soils with more than 25% of clay concentration of trace elements below the 20 cm depth decreased to values close to those of the background, while in coarser soils, heavy metal pollution penetrated below this depth, being noticeable down to a depth of at least 50-80 cm (Cabrera *et al.*, 1999).

Remediation works in the affected area consisted of: (1) removal of the sludge from the surface together with a layer of soil of around 10 cm; (2) application of amendments; (3) disk harrowing (20 cm) to mix soil within the ploughed depth. The emergency soil clean-up procedure was quickly started at the beginning of May 1998. This clean-up operation, which took about 6 months, removed the sludge and a major part of the contaminated soil surface. The toxic sludge covering the soil was mechanically removed and disposed-off in an open-cast mine. In October of the same year most of planned work was completed. The mud withdrawal left behind some amounts of sludge that was buried in the superficial layer of the soil by the machinery used in these works (Grimalt *et al.*, 1999). The amendments applied were: sugar beet lime, Fe-rich red soil and compost and manure. The application rate of sugar beet lime depended on the degree of pollution of the soils and ranged from 20 to 60 Mg ha^{-1}. Red soil was applied according to the As concentration of the soils and ranged from 500 to 900 Mg ha^{-1}. Compost or manure was applied throughout the affected soils at a rate of 15-20 Mg ha^{-1} to restore the organic matter losses in the removal of the sludge and contaminated soil layers (OTCVG, 2000; Antón Pacheco *et al.*, 2001).

The Regional Government purchased affected lands, which were farms with some fragmented forests and savannah-like woodlands and any agricultural practice was banned. The “Guadiamar Green Corridor” programme was implemented, with the aim of providing a continuous vegetation belt for wildlife to migrate along the Guadiamar River basin between the Doñana (south) and the Sierra Morena mountains (north). Revegetation on the alluvial terraces started in 1999 and consisted of the planting of more than 3 × 106 plants of bushes and trees (planting density 480-980 plants pr hectare). Depending on local habitat conditions, the target tree and shrub species selected to afforest were those typical of riparian forests (such as white poplar and willow) or those typical of drier upland forests, such as holm oak, wild olive, rosemary, broom brush and lavender oleander (Arenas *et al.*, 2008).

After the sludge removal from the soil surface and restoration activities total content of trace elements in the sludge-affected soils were still higher than background values of the area, and occasionally higher than values before restoration. This was attributed to remains of sludge left on the soil surface and buried during restoration operations (Cabrera, 2000; Cabrera *et al.*, 2008a; Cabrera *et al.*, 2008b). Several studies carried out in representative zones along the Guadiamar valley by researchers of IRNAS (Madejón *et al.*, 2002; Madejón *et al.*, 2004; Madejón *et al.*, 2006a; Madejón *et al.*, 2006b; Cabrera *et al.*, 2007) to assess the mobility and availability of trace elements in remediated soils, showed that mean values of trace elements extracted with EDTA from affected clean soils after the amendment addition were higher than those from non-affected soils (Madejón *et al.*, 2002; Madejón *et al.*, 2004), but lower than from clean soils before the amendment addition (Madejón *et al.*, 2006a).

Despite these results, Madejón *et al.* (2002) reported that eighteen months after the accident wild grasses growing in sludge-covered soils have high concentration of As, Cd, Cu, Fe, and Pb, above toxic levels, while on remediated soils only Cd reached a toxic level in grass tissue. Murillo *et al.* (2005) studying trace elements in wild olive plants five years after the accident (2003), reported that although the trace element concentrations in the soil were generally greater than those considered fitotoxic, the residual pollution within the "green corridor" seems to be stabilized by the rather low concentrations of trace elements in the different species analysed. Only the willow (*Salix*) sapling reached comparatively high concentration of Cd and Zn. According to Murillo *et al.* (2005) it is possible that the amendments applied to restore the soils after the accident together with the natural dynamics of trace elements in soils (retention is perhaps the main process) contributed to the apparent rather low mobilization of these elements, as reflected by plant uptake.

2.2. Study area and previous results

The magnitude of the Aznalcóllar mining accident, the large area of land affected, the characteristics of the landfill, as well as the need for action in the shortest possible time, did not allow to take specific measures for the remediation of the affected soils, based on the nature and content of pollutants, and on the physical-chemical characteristics of the soils. On the other hand, the monitoring of the remediated soils has not been easy due to the vast size of the contaminated area, the diversity of soils, the different degree of contamination, and even to the uncertainty of the type and dosage of amendment used in each zone. In view of these circumstances, and taking advantage of the existence of areas of contaminated soils in which the only remediation work carried out was the removal of the sludge from the soil surface, researchers of IRNAS designed experiments to evaluate 1) the effectiveness of the application of several amendments and the influence on the reestablishment of a natural plant cover, 2) the potential of several amendments and/or the development of vegetation cover and its corresponding rhizosphere to remediate a soil contaminated by various trace elements and 3) the durability of the stabilizing effect of the amendments.

The study was conducted in an experimental field area ("El Vicario") that was affected by the toxic Aznalcóllar mine spill, located on the right bank of the Guadiamar river (latitude N 37° 26′ 21″, longitude W 06° 12′ 59″), 10 km downstream from the Aznalcóllar mine in southern Spain (Figure 2.1). The only remediation work carried out in this area was the initial removal of the sludge from the surface of the soil together with a layer of underlying soil of around 10-15 cm. After the initial sludge removing work, several relevant soil characteristics were determined. Initial measurements of soil parameters showed that the field plot was very heterogeneous (Table 2.1) (Madejón *et al.*, 2006a). This could be attributed to remains of sludge left buried in the soil after the clean-up operations (Cabrera, 2000). In addition, acidic water accumulated in surface puddles could have contributed to the heterogeneity of pH, total S and trace element distribution (Clemente *et al.*, 2003).

Table 2.1 Mean, standard deviation (SD), variation coefficient (CV), minimum and maximum values of some soil chemical properties before remediation (n=48) (Madejón *et al.*, 2006a).

Variable	Mean	SD	CV (%)	Min.	Max.
pH	3.86	1.32	34.2	2.45	7.28
TOC (%)	0.92	0.16	17.6	0.62	1.33
S (mg kg^{-1})	8693	5740	66.0	529	25663
Total (mg kg^{-1})					
As	211	103	48.8	58.9	421
Cd	4.44	1.16	26.1	1.79	8.26
Cu	119	26.6	22.3	84.2	193
Pb	471	216	45.9	159	1100
Zn	381	136	35.6	134	812
Available (EDTA) (mg kg^{-1})					
As	3.58	4.88	136	0.26	24.2
Cd	0.57	0.29	51.0	0.07	1.50
Cu	34.1	9.55	28.0	17.0	60.3
Pb	5.21	4.24	81.3	0.55	15.9
Zn	96.3	35.0	36.4	26.9	187
Soluble ($CaCl_2$) (mg kg^{-1})					
Cd	0.37	0.26	70.3	0.00	1.05
Cu	15.0	12.2	81.3	0.92	47.0
Zn	98.6	64.6	65.5	0.23	233

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 (Martínez-Casasnovas et al., 2002). Average annual temperature is 19 °C (min. 9 °C in January, max. 27 °C in July) and annual average rainfall is 484 mm (Madejón et al., 2010).

2.2.1. Soil

Soils in this region of Spain are generally characterized by low water content that can alter the availability of trace elements. The soil was a clay loam (21.1% clay, 29.1% silt and 49.8% sand) classified as Typic Xerofluvent (USDA, 1996), main characteristics at 0-15cm depth were shown in Table 2.1 before the remediation.

2.2.2. Amendments

Three amendments -- two organic and one inorganic -- from different sources were used. The two organic amendments were a biosolid compost (BC) from the wastewater treatment plant of SUFISA (Jerez de la Frontera, southern Spain) and a leonardite (LE), a low rank 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) of $CaCO_3$ (EBRO-AGRÍCOLAS, San José de la Rinconada, southern Spain). The most relevant characteristics of the amendment are described followed (Table 2.2) (Madejón *et al.*, 2006a).

Table 2.2 Mean values (±SD) of some characteristics of the amendments.

	Amendment		
	BC	LE	SL
pH	6.93±0.03	6.08±0.07	9.04±0.08
Moisture (%)	45.0	27.5	25.0
TOC (%)	19.5±1.22	28.9±0.39	6.70±1.55
N (%)	1.31±0.06	1.17±0.02	0.98±0.04
P (%)	1.24±0.02	0.04±0.001	0.51±0.06
K (%)	0.93±0.02	3.97±0.08	0.53±0.05
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 (n=3) (Madejón *et al.*, 2006a).

2.2.3. Experimental design

The research was established in a field experimental plot (20×50m) which was divided into 12 subplots of 7×8m each, with a margin of 1 m (long) and 2 m (wide) between plots. The experiment was set up in October 2002. Three treatments with amendment were established SL (sugar beet lime), BC (biosolid compost), and LESL (mixture of leonardite and sugar beet lime). A treatment without amendment addition (NA) was also established. The treatments were applied as the rates (fresh basis) followed, BC: biosolid compost applied at a rate of 30 Mg ha^{-1} yr^{-1},SL: sugar beet lime applied at rate of 30 Mg ha^{-1} yr^{-1}, and LESL: leonardite at a rate of 25 Mg ha^{-1} yr^{-1} plus 10 Mg ha^{-1} yr^{-1} of sugar beet lime. The application rates were comparable to those applied to the whole of the spill-affected region of the Guadiamar Green Corridor (Antón Pacheco *et al.*, 2001) and were below the maximum permitted limits for annual trace element loading established by the European Union (Directive 86/278/EEC) (CEC, 1986). In a first stage of the experiment the amendments were applied for two consecutive years (October 2002 and October 2003) and incorporated into the top 15cm of soil using a rotary tiller (RL328 Honda). The non-amended subplots were tilled in an identical manner. The experiment was carried out in a completely randomized block design with three replicates per treatment (Figure 2.2).

Soil samples (0-15 cm and 15-30 cm) were collected from 48 sites (four sites per subplots) on a 14×15 m grid over the experimental plot. Soils were sampled three times: September 2002 (before the application of amendments), September 2003 (one year after the application of the first amendment and before the second amendment application) and September 2004 (one year after the second amendment application). At different dates (December 2003, March 2004 and June 2004) surveys of wild plants were conducted, and in (December 2003, March 2004, June 2004 and March-April 2005) samples of the most frequent grasses species were collected for chemical analysis of shoots. Plant biomass and vegetation cover were estimated and plant species were identified and listed.

In the second stage of the experiments, started in October 2005, each subplot was subdivided into two equal halves. One half remained unamending in the following years (Doses 2, DO2) (SL2, BC2 and LESL2), whereas the other half received the same amendment at the same rate than in 2002 and 2003 for two further years (October 2005 and October 2006; Doses 4, DO4) (SL4, BC4 and LESL4). Soil samples (0-15 cm and 15-30 cm) were collected in each subplot in November 2006 and December 2007. Plant surveys and samplings were carried out in the spring of 2006, 2007 and 2008 to perform the same determinations than in the first stage of the experiment.

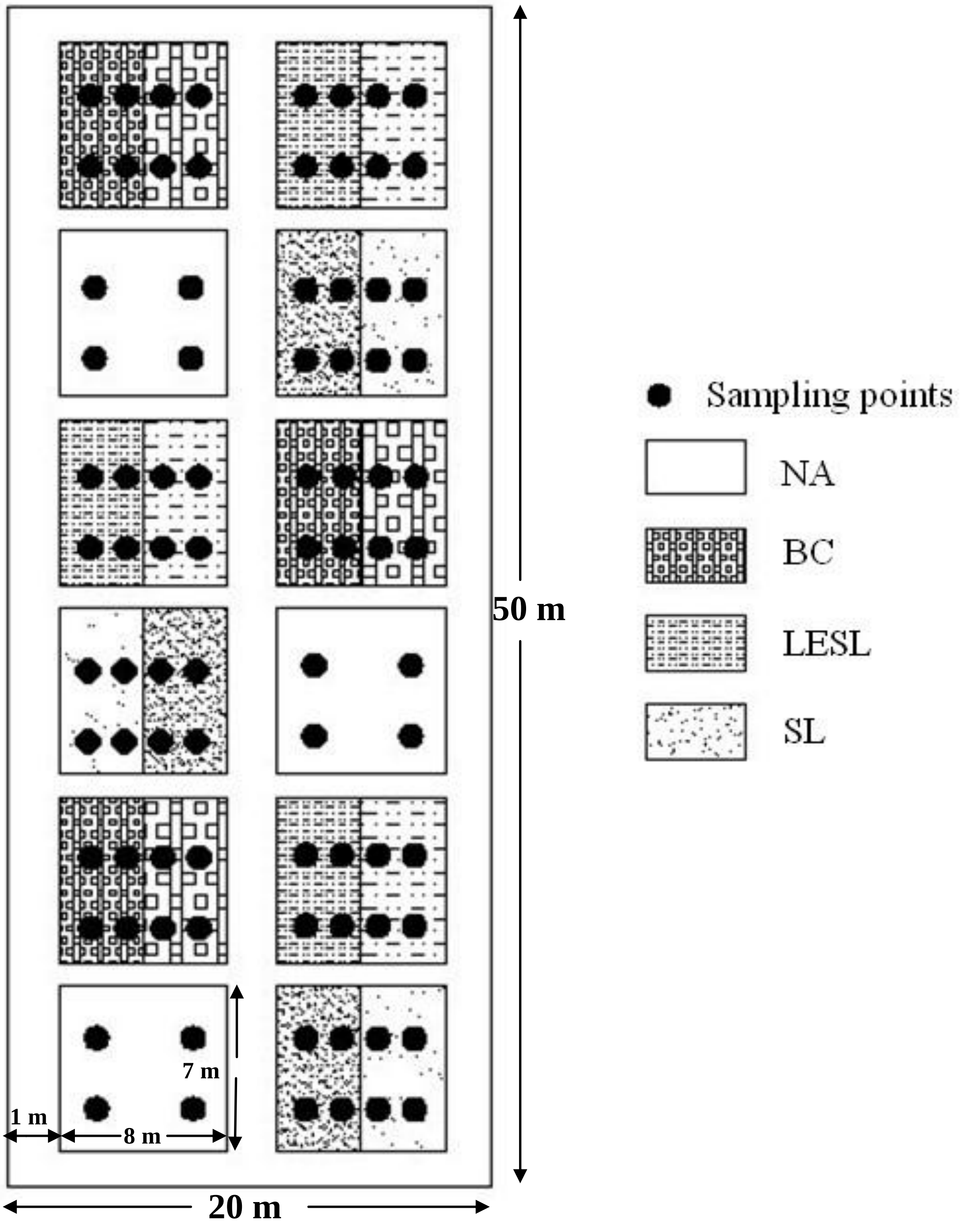


Figure 2.2 Soil sampling locations and distribution of amendments in the experimental plot.

2.2.4. Previous results

Results of the first stage of the experiment showed that the application of amendments increased soil pH values and total organic carbon (TOC) concentrations and reduced trace element $CaCl_2$ soluble concentration in comparison with non-amended soil. Amendments clearly improved the establishment and colonization of wild plants and improved in some instances the nutritional status of plants, diminishing the trace element soil-plant transfer to above ground parts (Madejón *et al.,* 2006a; Madejón *et al.*, 2009).

The second step of the experiment revealed that the need for repeated amendment additions to stabilized trace elements was dependent on the type of amendment and frequency of application. Two consecutive applications of inorganic amendment, sugarbeet lime was sufficient to maintain soil close to neutrality over a 5-year period and to reduce the $CaCl_2$-extractable pool of Cd, Ca and Zn. Repeated applications of two organic amendments were required to produce the same responses in the test soil (Madejón *et al.,* 2010). Successive applications of amendments may be necessary to improve plant growth and to reduce trace element concentrations in aboveground biomass, but this depend on the type of amendment used, the species and the element considered (Pérez-De-Mora *et al.*, 2011).

These results demonstrate that there are clear differences in the potential long-term sustainability of in situ amendments in field conditions and that treated soils require an effective monitoring program as part of any post-treatment management (Madejón *et al.*, 2010). Consequently, it is reasonable to continue the research work to obtain more information about the sustainability of the amendments. In addition, new soil sampling will allow knowing on the fertility of these soils subjected to different programs of amendments during 8 year.

2.3. New sampling

During the spring 2011 a new campaign of sampling was carried out in the experimental plot of “El Vicario”.

2.3.1. Soil sampling

Soil samples were collected in March 2011 at two depths (0–15 and 15–30 cm) from 84 sites (four sites per each subplot). The four different location samples were mixed to make a composite sample representative for each subplot. Soil samples were air-dried, crushed, sieved (< 2 mm). Prior to determination of pseudo-total trace element concentrations, < 2 mm soil samples were grounded in a agata morter and sieved to <60µm.

2.3.2. Plant sampling

Plant sampling was carried out in June 2011 in the same sites that soil sampled. In each sample site plant cover was estimated before plant sampling. The shoots of *Cynodon dactylon* (L.) Pers, one of the more representative species of wild grass existing in all the plots, were collected. Plant material was washed for 15 s with 0.1 M HCl solution, followed by 10 s with distilled water, and then oven dried at 70 °C. Dried plant materials were grounded and passed through a 500-mm stainless-steel sieve.

2.3.3. Determination of soil pH

It weighed 10 g dry soil in centrifuge tubes and added 25 ml of a 1M KCL solution. Tubes were shaken (140 agitation min^{-1}) on a reciprocating shaker (Selecta Rotabit) for 1 hour at constant temperature (22 ± 1ºC). Subsequently, the samples were left to decant and the pH was measured in the supernatant with a pH meter (CRISON micro pH 2002 with automatic temperature compensation).

2.3.4. Determination of soil total organic carbon

Total organic carbon (TOC) was analyzed by the method of Walkley and Black (1934), consisting in the oxidation of 1 g dry soil sample (< 2 mm) with 1 M K_2CrO_7 in acid media (H_2SO_4 concentrated), and subsequent tritation of excess dichromate with Mohr’ salt (0.5 M ferrous sulfate-ammonium solution) with a TOC meter (G20 compact titrator). The content of TOC was expressed in percent.

2.3.5. Determination of soil water soluble organic carbon

The extraction of soil water soluble organic carbon (WSOC) was performed with distilled water using a 1:10 (soil (<2 mm)/solution) ratio and shaking (140 agitation min^{-1}) 1 hour at room temperature (22 ± 1ºC) in a reciprocating shaker (Selecta Rotabit). The extracts were centrifuged 6 min at 20000 rpm and the supernatant was filtered through filter paper Whatman 2. Organic carbon in solution was determined in a total organic carbon analyzer (TOC-VCSH Shimadzu) (Sims & Haby, 1971). The content of WSOC was expressed in percent.

2.3.6. Determination of soil Kjeldahl N

One g dry soil sample (> 2 mm) was treated with concentrated H_2SO_4 in the presence of Se catalyst at 420 ºC in a TECATOR block digester. After the digestion and the addition of distilled water (75 ml), the samples were treated with 40% NaOH and distilled in a distillation unit Kjeltec System 1002. The distillate was collected in a 4% boric acid solution in a erlenmeyer flask and titrated with a M HCl titrated solution in the presence of methyl red and bromocresol green (Hesse P R., 1971). The content of Kjeldahl N was expressed in percent.

2.3.7. Determination of Olsen-P

The available P concentration weas determined by the method of Olsen *et al.* (1954), extracting the P with a 0.1 M $NaHCO_3$ solution at pH 8.5 (relationship soil:water/1:20), in the presence of active carbon after 30 min agitation. After filtering 2 times, the P content was determined by the method of Murphy and Riley (1962) in an auto-analyzer (Bran + Luebbe AIII). The content of Olsen-P was expressed in mg kg^{-1}.

2.3.8. Determination of soil available K

The concentration available K was determined by the method of Pratt P.F. (1965), extracting the K with a 1M ammonium acetate solution at pH 7 (soil (<2mm):water/1:10 ratio) after shaking for 30 min. The extracts were decanted, filtered, and measured by an auto-analyzer (Bran + Luebbe AIII). The content of available K was expressed in mg kg^{-1}.

2.3.9. Determination of 0.01M $CaCl_2$-soluble trace element in soil

The 0.01M $CaCl_2$-soluble trace element concentrations were determined by the method of Houba *et al.* (1996): 2.5g dry soil (<2mm) in centrifuge tubes and are extracted with 25 ml of a 0.1M $CaCl_2$ solution. Tubes were shaken (140 agitation min^{-1}) on a reciprocating shaker (Selecta Rotabit) for 3 hours at constant temperature (22 ± 1ºC). Subsequently, the samples were centrifuged during 6 min at 10000 rpm and the supernatants were filtered through filter paper Whatman 2. The determination of trace element in solution was carried out by a Varian ICP 720-ES (simultaneous ICP-OES with axially viewed plasma). The content was expressed in mg kg^{-1}.

2.3.10. Determination of soil pseudo-total trace element

The determination of total concentrations of micronutrients and elements trace in a soil sample requires the total dissolution of the sample. Mixtures of acids including the HF are used that is highly effective in breaking down most of common silicates of the soil but is a dangerous chemical requiring careful handling. However, other acidic mixtures not including HF are often used. One of the most widely aqua regia: HNO_3 conc.: HCl conc. (1: 3 v), which although not completely dissolve soil samples, dissolves most of the components of soil in which trace elements are liked in more labile forms, releasing most of these elements that could play a role in plant nutrition, pollution of surface and groundwater, etc.

The digestion of soil samples was carried out in teflon tubes, hermetically sealed, in a microwave oven (Microwave Laboratory Satation Mileston ETHOS 900, Milestone s.r.l., Sorisole, Italy). Dry soil samples (<60µm) of 0.5 g were treated with 3 ml of conc. HCl and 1 ml of conc. HNO_3 (aqua regia). After digestion and cooling of the samples, the extracts were diluted by MiliQ water to 50 ml. Finally, the diluted extracts were filtered by 0.45 NYL syringe filters (pore size 0.45 µm) and stored in PVC bottles until analysis.

Quantification of elements in the extracts was achieved using a Varian ICP 720-ES (simultaneous ICP-OES with axially viewed plasma). The accuracy and precision of the method were assessed through BCR-143R reference sample (Commission of the European Communities, Community Bureau of Reference, BCR, Reference Material No. 143R, trace elements in a sewage sludge amended soil) (Table 2.3). The concentrations of trace elements were expressed in mg kg^{-1}.

Table 2.3 Means values (mg kg^{-1}±SD) of pseudo-total trace elements concentrations for the reference sample analysis (n=2).

	BCR-143R (sewage sludge amended soil)	
	Certified (Aqua regia)	Experimental
As	ND	12±0.2
Cd	72	72±0.4
Cu	128	131±1.8
Pb	174	167±1.0
Zn	1063	1001±18

2.3.11. Plant analysis -- determination of trace element in plant shoot

The analysis of plant material was carried out by wet acid oxidation, under pressure in a microwave oven. Samples of 0.5 g dry and grounded plant material were treat with 4 ml of concentrated HNO_3 Suprapur in teflon tube, and shaking gently for completely wetting, and left overnight. After digestion and cooling, the samples were diluted with MiliQ water to 50 ml. Finally, the diluted extracts were filtered by 0.45 NYL syringe filters (pore size 0.45 µm) and stored in PVC bottles until analysis. The concentrations of different elements were determined by a Varian ICP 720-ES (simultaneous ICP-OES with axially viewed plasma). The accuracy and precision of the analytical method was assessed through analysis of a reference sample INCT-TL-1 (Polish Certified Reference Material, For Multielement Trace

Analysis, tea leaves) (Table 2.4). The concentrations of trace elements were expressed in mg kg^{-1}.

Table 2.4 Means values ($mg\ kg^{-1}$±SD) of pseudo-total trace elements concentrations for the reference plant sample analysis (n=2).

	INCT-TL-1 (tea leaves)	
	Certified	Experimental
As	0.106	0.131±0.011
Cd	0.0302	0.021±0.004
Cu	20.4	20.0±0.45
Pb	1.78	0.87±0.015
Zn	34.7	34.7±0.03

2.4. Statistical analysis

The statistic work was carried out using the software package SPSS 15.0 for Windows. Mean and standard deviation (SD) or standard error (SE) were determined for all data. Normality of the data was tested prior to analysis. The data was analyzed by one way ANOVA, considering the treatment as the independent variable. Significant statistical differences of all variables between the different treatments were established by Tukey's test ($p<0.05$). Homogeneity of variances test was performed, if it did not pass the test, the Games-Howell significance was used. Pearson's coefficient correlation analysis was performed to determine the relationship between different parameters at two significant levels ($p<0.01$ and $p<0.05$).

Principle component analysis (PCA) was carried out for the values of the different studied parameters (Plt Cov, pH, TOC, WSOC, Kj-N, Olsen-P, Avail-K, $CaCl_2$-Cd, -Cu and -Zn) of the different treatment plots, using STATISTICA 6.0 for Windows.

Sun ray plots (Dilly & Blume, 1998; Moreno *et al.*, 2009) were constructed to compare graphically the mean values of different studied parameters in each treatment plot at every soil layer. The star shape and integrated area for each treatment allow a comparison of visual and statistical presentations of multivariate data. Soil fertility and contamination profiles were designed; the integrated area of the plot for each treatment was measured using the measuring tools of Adobe Acrobat 9® (Adobe Systems Incorporated, CA, USA) (Moreno *et al.*, 2011).

Graphical works were carried out using Sigmaplot 8.0 and Microsoft Office Excel 10.0.

3. RESULTS

3.1 Soil pH

Mean values of soil pH for the two depths are shown in Figure 3.1. In general, in all amended plots pH mean values at soil upper layer (0-15cm) were higher than in NA although differences were not always significant ($p<0.05$). Mean values of pH follow the trend: SL>BC>LESL>>NA. Treatment SL2 significantly increased pH: 3.6 units of pH higher than in NA. Treatments BC2 and LESL2 also increased the soil pH, 1.9 and 1.3 units respectively, although the differences were not significant.

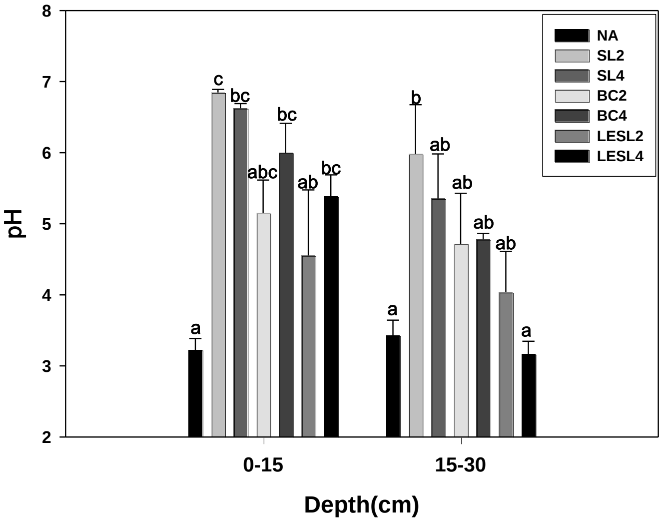


Figure 3.1 Mean values (±SE) of Soil pH at two soil layers, 0-15 and 15-30 cm (n=3). Values with the same letter do not differ significantly for each layer by one way ANOVA ($p<0.05$).

Successive amendment applications (DO4) had not significant effect in any of the treatments compared to DO2, although it was observed a small decrease in SL4, and increases of 0.83 and 0.85 units for BC4 and LESL4 respectively.

Mean pH values also increased at soil lower layer (15-30cm) but less than in the upper layer. In this layer only SL2 treatment significantly increased pH values by ca. 2.5 units. The increases in treatment BC2 and LESL2, 1.3 and 0.6 units respectively were not significant. In DO4 not significant decreases of mean pH was detected in the three treatments.

Figure 3.2 shows the evolution of the mean pH from 2003 to 2011. From 2003 up to 2007 pH values in all the treatments, both in DO2 and DO4, tend to increase, while from 2007 up to 2011 a decrease of these values was observed in all the amended plots.

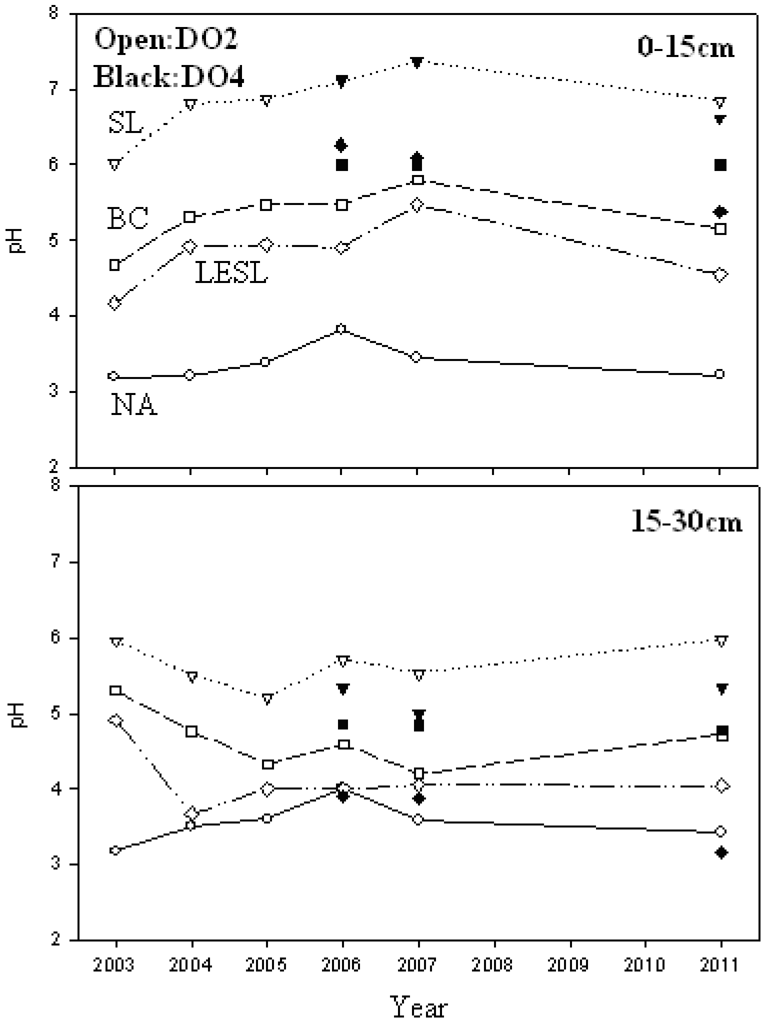


Figure 3.2 Evaluation of soil pH for every treatment at two soil layers 0-15 and 15-30 cm from 2003 to 2011. Open: amendment doses2; Black: amendment doses4.

3.2 Soil total organic carbon and water soluble organic carbon

Mean values of soil total organic carbon (TOC) increased in all the amendment plots, especially at the superficial layer (Figure 3.3). The organic treatments BC and LESL raised TOC more than the inorganic treatment SL both in DO2 and DO4. The trend of the increase of TOC was LESL>BC>SL. In DO2 the increases (BC2 and LESL2) were significant compared to NA (0.47% in SL2, 0.62% in BC2 and 0.67% in LESL2).

In DO4 different behavior was observed for each treatment: i) small no significant decrease of TOC in SL4 (0.17%), ii) small no significant increase in BC4 (0.20%) and iii) significant increase in LESL4 (0.56%). It is interesting to note that TOC values in these treatments (BC4 and LESL4) represent significant increases compared to that in NA.

No significant effect of treatment was observed in the 15-30 cm layer (Figure 3.3).

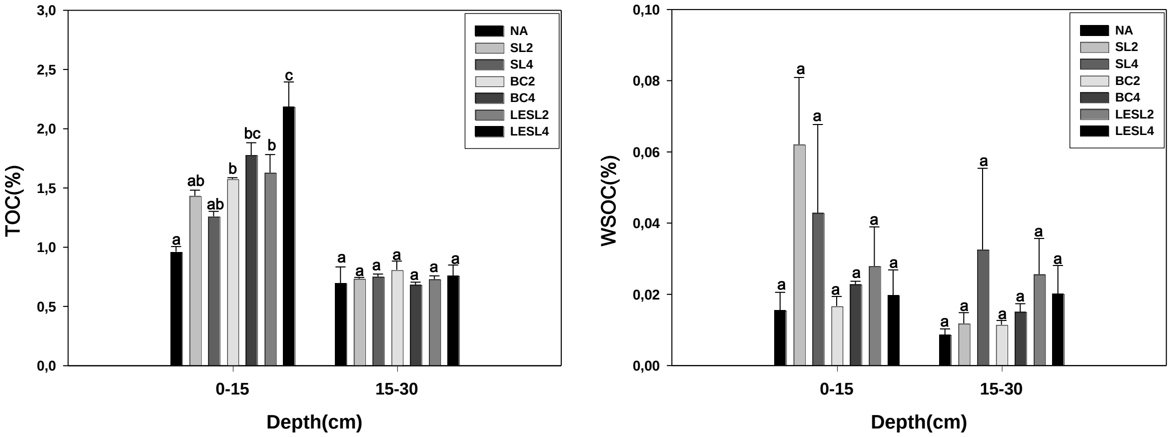


Figure 3.3 Mean values (±SE) of Total Organic Carbon (TOC) and Water Soluble Organic Carbon (WSOC) at two soil layers, 0-15 and 15-30 cm (n=3). Values with the same letter do not differ significantly for each layer by one way ANOVA ($p<0.05$).

Figure 3.4 shows the evolution of the mean values of TOC from 2003 to 2011. From 2003 up to 2007 a slight but steady increase of the mean values of TOC was observed at 0-15 cm in NA, SL2, BC2 and LESL2 plots: 1.8 g kg^{-1} for NA, 0.8 g kg^{-1} for SL2, 4.6 g kg^{-1} for BC2 and 4.5 g kg^{-1} for LESL2 compared to the values of 2003. At the end of the experiment values for BC2 and LESL2 decreased with respect to those of 2007, but in all the plots (NA, SL2, BC2 and LESL2) mean values of TOC were greater than the corresponding at the beginning of the experiment in untreated soils: 1.5 g kg^{-1} for NA, 6.2 g kg^{-1} for SL2, 7.6 g kg^{-1} for BC2 ad 8.2 g kg^{-1} for LESL2. The increase of TOC observed in the treatments without application of organic matter (NA and SL2), might be due to the indirect effect of increase of pH that enhanced vegetation cover development; plant residues from the enhanced plant growth probably gave rise to the increase soil TOC in these subplots.

Mean vales of TOC in SL4, BC4 and LESL4 subplots also decrease from 2007 up to 2011. Values of TOC in BC4 and LESL4 at 2011 were still greater than those in BC2 and LESL2.

Evolution plot of TOC at 15-30 cm (Figure 3.4) shows very low values of TOC at any treatment and sampling date. In pervious papers Madejón *et al.* (2009); Madejón *et al.* (2010) did not find significant differences due to treatment from 2003 to 2007; in the present work we neither found significant differences attributable to treatments. Therefore is difficult to discern the actual evolution of TOC in each treatment. Nevertheless, it can be observed that i) generally values in all the treated subplots were greater than in the untreated subplot; ii) at the end of the experiment TOC values are very similar for all the subplots (mean value 7.3 g kg^{-1}), and lower than the values at the starting of the experiment (mean value 9.1 g kg^{-1}).

Mean water soluble organic carbon (WSOC) concentrations at both layers increased in all the amended plots by the amendments, but no significant difference was found either the treatment or the doses (Figure 3.3).

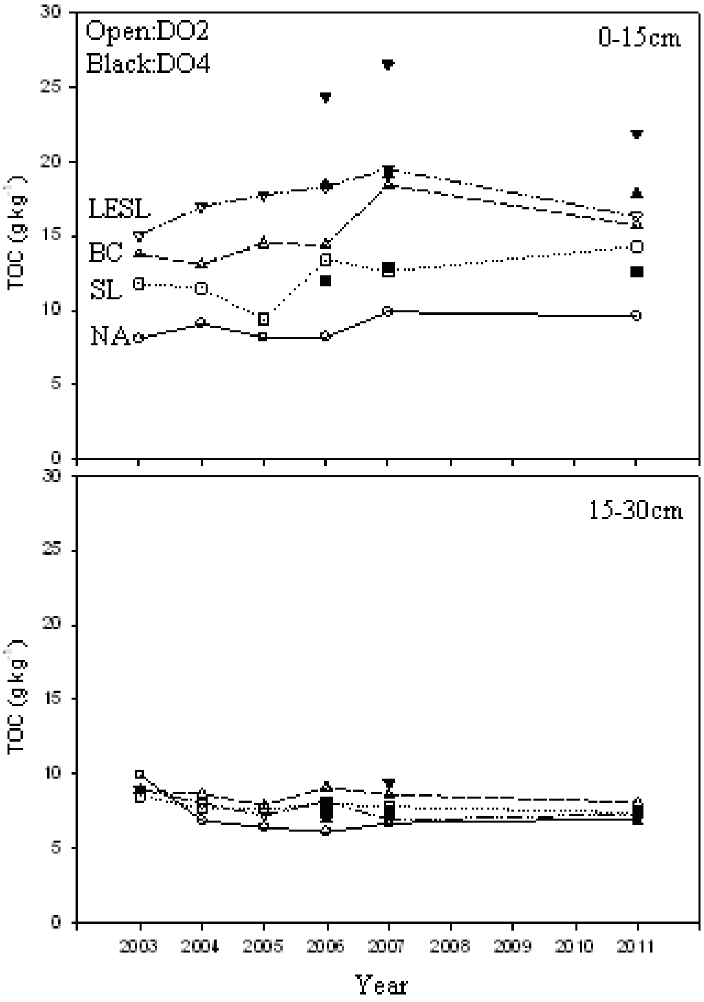


Figure 3.4 Evaluation of soil TOC (g kg^{-1}) for every treatment at two soil layers 0-15 and 15-30 cm from 2003 to 2011. Open: amendment doses2; Black: amendment doses4.

3.3 Soil Kjeldahl N, Olsen-P and available K

In general, mean values of soil Kjeldahl N (Kj-N), Olsen-P and available K (avail-K) increased in the amendment plots, especially at the soil upper layer (Figure 3.5).

In soil upper layer, in DO2 mean values of Kj-N increased in all the treated plots increased compared to NA, although differences were only significant for BC2 (Figure 3.5). In DO4 a small no significant decrease of Kj-N was observed in SL4. However increases were detected both in BC4 and LESL4 but only significant in BC4 where the Kj-N was 1.7 times greater than in NA. At 15-30 cm no effect of amended or doses was detected.

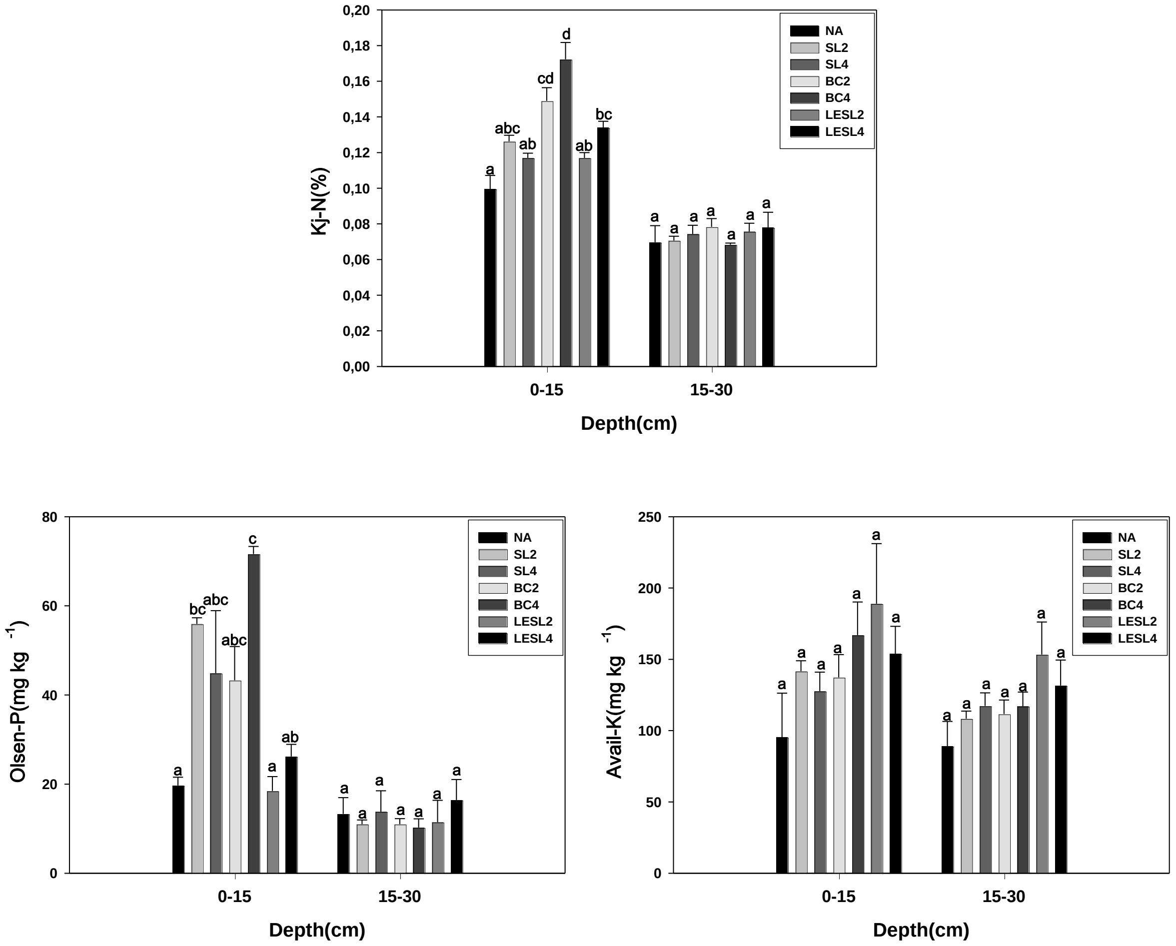


Figure 3.5 Mean values (±SE) of Kjeldahl N (Kj-N), Olsen-P and available K (avail-K) at two soil layers, 0-15 and 15-30 cm (n=3). Values with the same letter do not differ significantly for each layer by one way ANOVA ($p<0.05$).

In the upper layer, mean values of Olsen-P increased in SL2 and BC2 plots, although the differences were only significant for SL2 (Figure 3.5). In DO4 plots the more interesting result was the increase of Olsen-P in BC4 that reach values of 71.6 mg kg^{-1}, 3.6 times higher than in NA. At 15-30 cm, no effects of amendments or doses were found.

Figure 3.5 also shows that mean values of Avail-K increased in amended plots but in any case differences were significant, despite that a mean value 2 times greater in LESL2 plots than in NA was observed.

3.4 Soil trace elements concentrations (pseudo-total and $CaCl_2$ extractable trace elements concentrations)

3.4.1. Pseudo-total trace elements

Mean values of pseudo-total As, Cd, Cu, Pb and Zn concentrations are shown in (Table 3.1). In both layers no significant differences attributed to treatment or doses were found. Values at each depth were similar in all treatments irrespective of the amendment applied and of the amendment doses. In fact, difference between pseudo-total trace elements were not expected since trace elements cannot be degraded. Mean values of pseudo-total were much higher than the background values of the Guadiamar valley soils (Table 3.1) reported by Cabrera *et al.* (1999).

Table 3.1 Means values (mg kg^{-1}±SE) of pseudo-total trace element concentrations for the different treatments (n=3). In each column, values with the same latter do not differ significantly by one way ANOVA ($p<0.05$).

Treatment	Upper Layer (0-15cm)					
	As	Cd	Cu	Pb	Zn	PLI
NA	250±50a	1.2±0.20a	180±10a	600±140a	360±48a	
SL2	180±49a	2.1±0.36a	165±7.7a	350±91a	550±73a	
SL4	170±61a	2.3±0.72a	180±22a	400±110a	600±124a	
BC2	200±42a	1.7±0.33a	180±22a	430±52a	460±72a	
BC4	200±17a	2.3±0.59a	190±21a	410±46a	600±150a	
LESL2	180±54a	1.5±0.29a	180±22a	500±160a	380±68a	
LESL4	230±28a	1.4±0.23a	169±8.6a	600±93a	400±58a	
Total Mean	201±72	1.78±0.75	177±27	451±178	478±167	7.06
Background*	18.3	0.33	30.9	38.2	109	1

Treatment	Lower Layer (15-30cm)					
	As	Cd	Cu	Pb	Zn	
NA	110±39a	1.1±0.23a	120±28a	260±85a	330±36a	
SL2	50±14a	0.6±0.16a	100±24a	130±29a	260±19a	
SL4	50±14a	0.5±0.17a	100±17a	120±23a	220±24a	
BC2	73±3.4a	1.2±0.28a	130±29a	165±7.5a	380±75a	
BC4	42±6.8a	1.1±0.09a	100±13a	120±11a	330±34a	
LESL2	49±9.3a	1.0±0.07a	140±28a	130±14a	310±26a	
LESL4	120±52a	0.6±0.13a	120±28a	290±99a	250±27a	
Total Mean	71±49	0.87±0.36	116±39	173±101	297±78	3.43

*The background of Guadiamar valley soils was nearly constant throughout the profile (Cabrera *et al.*, 1999).

The degree of trace element pollution evaluated using the Pollution Load Index of (PLI) of Tomlinson *et al.* (1980). This index is based on the values of the Concentration Factors (CF) of each trace element in the soil. The CF is the quotient obtained by dividing the concentration of each trace element in the soil ($C_{\text{trace element}}$) by the base line or background value (concentration in unpolluted soil, $C_{\text{background}}$). Background values used were that reported by Cabrera *et al.* (1999) estimated from the mean concentrations of the trace elements in unaffected soils of the area. For each soil sample, PLI at one determined soil depth may be calculated as the nth root of the product of the n CF: $PLI = \sqrt[n]{(CF_1 \times CF_2 \times \cdots \times CF_n)}$

This index provides a simple, comparative means for assessing the level of heavy metal pollution. Values of PLI = 1 indicate heavy metal loads close to the background level, and values above 1 indicate pollution.

Table 3.1 shows very high values of PLI, 7.1 and 3.4 in the 0-15 and 15-30 cm layers respectively, indicating severe trace elements pollution.

3.4.2. $CaCl_2$ extractable trace elements

Figure 3.6 shows mean values concentrations of $CaCl_2$-soluble Cd, Cu Zn. Concentrations of extractable As and Pb were always below detection limits of the method (0.01mg L^{-1}; 0.1 mg L^{-1}). In general, concentrations of As and Pb were very small in comparison with total concentration in soil, suggesting little mobility of these elements.

Mean values of $CaCl_2$-soluble Cd, Cu, Zn concentrations decreased in amended plots compare to NA, especially at the upper layer. The most efficient amendment to reduce Cd and Zn values was SL; data analysis indicated that for these elements there were significant differences between NA and SL plots in both soil layers. The efficiency of the amendments in the reduction Cd and Zn concentration in the $CaCl_2$ extracts was: SL>BC>LESL. For Cu no significant differences were found in both layers.

For treatments BC and LESL repetition of the applications of amendments DO4 tended to reduce the concentration of Cd and Zn in the $CaCl_2$ extracts compared to DO2 in the soil top layer: BC4 and LESL4 significantly decreased Cd and Zn concentrations compare to BC2 and LESL2. However, no significant differences were found between SL2 and SL4 in both soil layers and for all the elements, indicating that a second application (DO4) does not continues reducing the size of the extractable pool of trace elements achieved by the lowest amendment application (DO2).

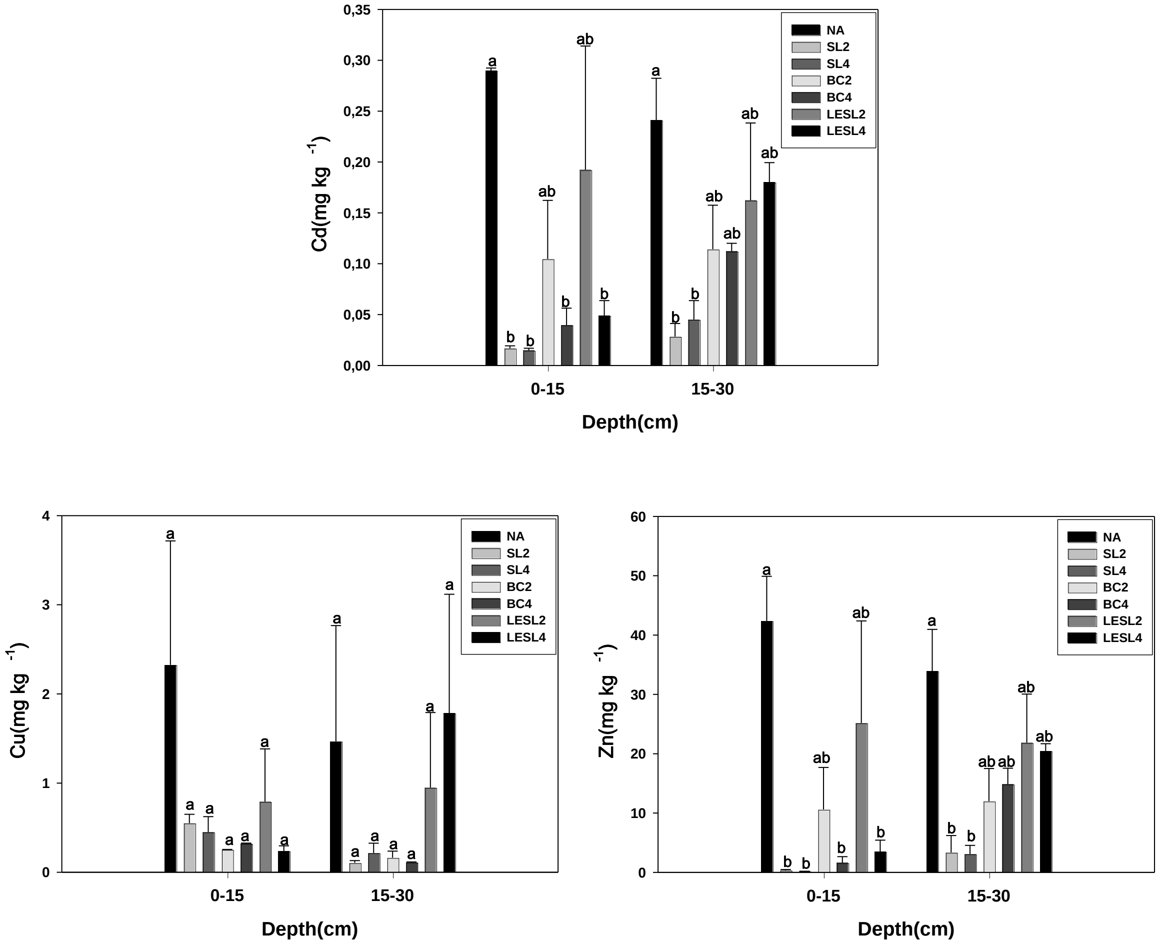


Figure 3.6 Mean values (±SE) of 0.01M $CaCl_2$-extractable trace elements concentrations at two soil layers, 0-15 and 15-30 cm (n=3). Values with the same letter do not differ significantly for each layer by one way ANOVA ($p<0.05$).

Figure 3.7 shows the evolution of the mean concentration of Cd, Cu and Zn extracted with 0.01 M $CaCl_2$. The behaviour was similar for these three elements, at 0-15 cm i) the first amendment application (2002) resulted in a significant decrease of the concentrations compared to the corresponding concentration in NA (values of 2003 in Figure 3.7); ii) the second application (2003) again produced a significant decrease in the concentrations of these trace elements extracted with $CaCl_2$ (values of 2004 in Figure 3.7). Afterwards up to 2011 in all treatments the concentrations of trace elements continued decreasing more slowly. It is interesting to note the decrease of the concentrations of trace elements extracted with $CaCl_2$ observed in the NA plots that is a sign of natural remediation; iii) New amendment applications in 2005 and 2006 (DO4) resulted in new significant decreases in the concentrations of these elements extracted with $CaCl_2$ in BC and LESL subplots, but not in SL subplots. As in DO2 treatments trace elements concentrations went on decreasing up to 2011.

In lower layer, SL treatment is the only amendment reducing significantly the Cd and Zn concentration in the $CaCl_2$ extracts (Figure 3.6). Regarding the evolution from 2003 to 2011 of the concentrations of soluble trace elements (Figure 3.7), it was observed that the decreases of the concentrations of Cd, Cu and Zn after the first and second amendment applications (DO4) were less noticeable than at the upper layer, being the greater differences those corresponding to SL in the three elements, BC in Cd and Zn. Afterwards in all the subplots of treatment DO2 decreased slowly up to 2011. Treatment DO4 supposed only small differences compared to DO2 in the concentrations of soluble Cd, Cu and Zn.

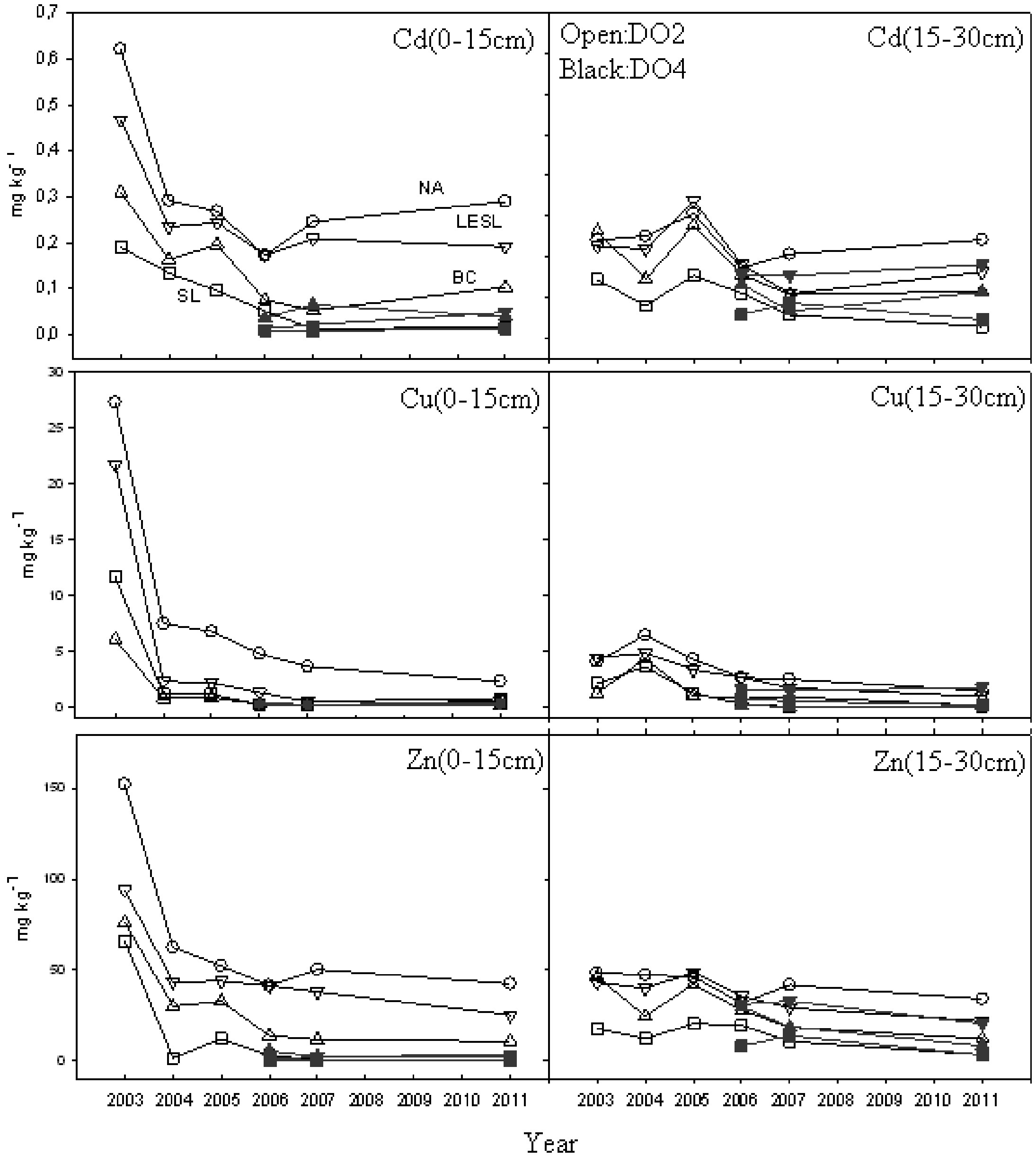


Figure 3.7 Evaluation of soil $CaCl_2$ extractable-Cd, Cu, Zn (mg kg^{-1}) for every treatment at two soil layers 0-15 and 15-30 cm from 2003 to 2011. Open: amendment doses2; Black: amendment doses4.

3.5 Plant Cover estimation and wield grass shoot trace elements concentrations

Table 3.2 shows that colonization of soil by wild plants was enhanced by amendments. However, due to high variance values no significant differences were observed for vegetation cover that followed the trend: NA 58% < LESL2 78% < LESL4 85% < SL4 88% <BC2 92% < SL2 97% < BC4 98% (Table 3.2).

Table 3.2 Mean values of Plant Cover (%; *n=3 in this column) and trace element concentrations (±SE mg kg^{-1}) in shoots of *Cynodon dactylon*. In each column values with the same latter do not differ significantly by one way ANOVA ($p<0.05$).

Treatment	Plant Cover*	As	Cd	Cu	Pb	Zn
NA(n=7)	58±20a	0.8±0.22a	0.10±0.018ab	5.4±0.22a	0.8±0.31a	80±14ab
SL2(n=6)	97±3.0a	0.6±0.14a	0.05±0.029a	4.9±0.14a	0.5±0.17a	49±4.0a
SL4(n=6)	88±6.0a	0.7±0.07a	0.07±0.019ab	5.8±0.07a	0.8±0.17a	58±6.7ab
BC2(n=4)	92±6.0a	0.7±0.06a	0.14±0.030ab	5.4±0.06a	0.3±0.10a	102±6.1b
BC4(n=4)	98±1.7a	0.5±0.17a	0.10±0.030ab	6.3±0.17a	0.3±0.11a	90±17ab
LESL2(n=7)	78±17a	1.1±0.28a	0.16±0.016b	5.8±0.28a	1.7±0.61a	78±5.0ab
LESL4(n=3)	85±7.6a	0.7±0.40a	0.10±0.024ab	4.6±0.40a	1.0±0.61a	64±6.4ab
Normal ranges**			0.1-1	3-20	2-5	15-150

**Normal ranges in plants (Madejón *et al.*, 2002).

Concentrations of As, Cd, Cu, Pb and Zn in *Cynodon dactylon* are within the normal range for plants, and generally very low in all the plots (Table 3.2). No significant differences were detected for the mean values of As, Cu or Pb in the different plots. The lowest concentrations of trace elements were found in SL treatments. Re-treatment of soil showed no consistent trends regarding trace element concentrations.

4. DISCUSSIONS

Increasing pH values is a frequent practice for remediation of trace element polluted soils, due to most trace elements are less soluble in alkaline conditions (Adriano, 2001). The data obtained shows that the most effective treatment to increase soil pH values is SL, due to the high (70-80%) $CaCO_3$ contents (Figure 3.1). The effect of SL-induced changes in pH lasted several years. In fact, in this acid soil, the first two additions of SL raised soil pH close to neutrality and these values have been maintained for at least eight years without any further amendment. However, organic amendments BC and LESL, were less effective to improve pH values and the further application DO4 was necessary to maintain full performance. Organic matter also increased soil pH, although less efficiently (Figure 3.1). It can ameliorate the effects of soil acidity in several ways: a) by binding Al^{3+} ions tightly, b) by forming soluble complexes with Al^{3+}, and c) by adding Ca^{2-} to the soil, thereby replacing H^+ and Al^{3+} ions in the soil solution and in the exchange complex (Brady & Weil, 2002; Naramabuye & Haynes, 2006). However, protons released by soil microorganisms during mineralization of organic matter can slightly acidify the soil restricting the alkalinising potential of organic amendments (Madejón *et al.*, 2010). In this experiment, repeated additions of organic amendments were necessary to maintain soil pH close to neutrality, even in the case of the LESL treatment, which contained 25% of sugar beet lime (Figure 3.1).

Due to the low TOC contents of this Mediterranean soil and the clean-up operations in the area (removing a significant amount of topsoil), it was reasonable to incorporate organic amendments to increase soil organic matter content and simultaneously increase trace element immobilization (Madejón *et al.*, 2006a; Madejón *et al.*, 2010). The organic matter of the amendment presented high stability against microbial degradation which constituted a clear advantage of soil organic amendment and guaranteed the improvement of soil fertility in the long term (Bernal *et al.*, 1998). Both organic amendments (BC and LESL) significantly increased TOC values in surface, but further applications (DO4) were necessary to maintain TOC values close or above 2% (Figure 3.3). The increase of TOC values was found in inorganic SL treatment was attributed to the indirect effect of pH improvement that enhanced plant cover development.

Nitrogen, P and K are essential nutrients for plant growth. However, Mediterranean ecosystems often suffer from nutrient deficiencies, such as nitrogen and phosphorus, which is a frequent limiting factor for soil fertility (Mayor & Rodà, 1994; Hanley & Fenner, 2001; Sardans *et al.*, 2004). Amendments acted as an available nutrient source and showed a low mineralization rate, suggesting a slow release of nutrients and thus favoring long term soil fertility (Alburquerque *et al.*, 2011). This could be attributed to the reduced stress/toxicity for the microorganisms and the positive effect of humic acids from compost on nitrifying bacteria (Vallini *et al.*, 1997).

Figure 3.5 shows that the increase of Kj-N in each treatment at 0-15 cm are related with the total N concentrations of the used amendments; Table 2.2 shows that the trend of the N concentrations in the amendments is BC > LE > SL, similar to those of the Kj-N in the treatment plots: BC > SL ≥ LESL for DO2 and BC > LESL ≥ SL for DO4 (Figure 3.5). Research shows that the continued amendment application as in our experiment (DO4) could lead to a significant increase of the total N in the soil (López-Piñeiro *et al.*, 2008). Acid pH values and trace element contamination can inhibit soil nitrification but not N mineralization (Richards, 1987; Baath, 1989).

The application of amendments in the different treatments involves increases of total P of the soil that are related to concentrations of P of each amendment (Table 2.2). Thus, in DO2 the increases of total P were 84 mg P kg^{-1} in treatment SL, 270 mg P kg^{-1} in BC and 46 mg P kg^{-1} in LESL, and in DO4 were 168 mg P kg^{-1} in SL, 540 mg P kg^{-1} in BC and 91 mg P kg^{-1} in LESL. The applied P undergoes a series of changes in soil depending on the nature of the added P and of the properties of the soil (e.g. pH, organic matter content, mineralogy, etc.). At the same time part of the soil P was imported by plant (we have estimated a maximum value of ca. 25 kg P ha^{-1} ≈ 12 mg P kg^{-1}). The result is that the increase of the concentration of P available for plants (Olsen-P) observed in Figure 3.5 is normally less than the concentration of the applied P. Alburquerque *et al.* (2011) reported that compost increased significantly the available P compared to the mineral fertilizer treatment. From Figure 3.5 it is difficult to deduce whether SL or BC was the more effective amendments to increase Olsen-P. Some researchers showed that cumulative application, rather than the source of the P, determined the available P (Eghball & Power, 1999; Cooperband *et al.*, 2002; Madejón *et al.*, 2003; Paredes *et al.*, 2005; López-Piñeiro *et al.*, 2008).

As for P, the application of amendments implies the increase of total K of the soil due to the K contents of the amendments (Table 2.2). It can be calculated that the increase are: in DO2 88 mg K kg^{-1} in treatment SL, 202 mg K kg^{-1} in BC and 783 mg K kg^{-1} in LESL, and in DO4 175 mg K kg^{-1} in treatment SL, 404 mg K kg^{-1} in BC and 1566 mg K kg^{-1} in LESL. Potassium also undergoes a series of changes tending to decrease its availability for plants. At the same time K imported by plants account for a maximum of 238 kg K ha^{-1} ≈ 120 12 mg K kg^{-1}. In fact, Figure 3.5 shows the increase of K availability in the three treatments, that as expected is less than calculated total increase, and that follow the trend LESL > BC> SL, according with the K contents of these amendments. There are many studies reporting that soil amendments can increase soil available K content (Madejón *et al.*, 2003; Montemurro *et al.*, 2004; López-Piñeiro *et al.*, 2008).

The results of the present study shows that the application of amendments to a soil moderately contaminated by trace elements in which the top soil was removed, increased soil pH and the concentrations TOC, WSOC, Kj-N, Olsen-P, and Avail-K,. This is a useful practice to improve soil fertility and help plant growth.

Nevertheless, the main aim of the present paper is the remediation of the soil reducing the trace element mobility applying amendments. When amendments that contain elevated trace element concentrations are added to soils (even if those are contaminated), their impact on total soil trace element concentrations requires investigation. In a previous study (Madejón *et al.*, 2010), both theoretical model and experimental result demonstrated that the predicted increase of total trace element concentrations from amendment application was insignificant compare to the standard error of the initial soil. Our data (Table 3.1) also support this: no effect was found between each treatment of pseudo-total trace elements concentrations neither DO2 nor DO4. Meanwhile, like the majority of polluted soils, the spatial heterogeneity in total trace element concentrations should be taken into account (Burgos *et al.*, 2006).

In the present study 0.01 M $CaCl_2$ extractable soil trace element concentrations were measured. This extract displaces cations from soil components by a process of ion-exchange, tends to reflect the readily soluble pool of trace elements and may be representative of the current bioavailability of trace elements in soil; that is considered more relevant for environmental protection and ecological risk assessment (Adriano, 2001; Kabata-Pendias, 2004; Meers *et al.*, 2006).

Our results show that, 0.01M $CaCl_2$ extractable trace element concentrations are significantly and negatively correlated with pH (Table 3.3). The alkalinizing effect of both organic and inorganic amendments can ameliorate soil acidity and reduce trace element bioavailable concentrations. The formation of insoluble and stable complexes between OM and trace element, together with absorption of trace element by organic colloids, also contributes to reduction of trace element availability in soil. Castaldi *et al.* (2005) related trace element immobilization to soil pH increases that followed amendment with compost and calcium hydroxide. Walker *et al.* (2003) attributed trace element immobilization to the formation of insoluble salts such as phosphates, as mineralization progressed after manure addition to a calcareous, contaminated soil. These results emphasize that control of soil acidification was the determining factor that permitted amendment addition to improve and recover soil quality parameters in the acidic soil, since it led to a soil pH increase and to heavy metal immobilization (Alburquerque *et al.*, 2011).

The results of the present study shows that when applying SL (Figures 3.6 and 3.7), the concentrations of soluble Cd, Cu and Zn decreased after the first treatment (DO2) and were still very low at the end of the experiment, after 8 years of the application of 2003. Further application of SL (DO4) did not change the former results. Therefore, application of SL is a reliable solution - at long term - to remediate this trace element polluted soil. Organic amendments BC and LESL, were less effective than SL. For Cd and Zn it was a second treatment (DO4) with BC and LESL was necessary to reach the same results than with SL. In the previous study (Madejón *et al.*, 2010), in a soil sampling carried out in 2007 already found that the additional amendment (DO4) contributed to control the solubilisation of trace elements, reducing risk of transfer to other environmental compartments.

4.1. Correlation between parameters

In Table 4.1, pH values were significant positively correlated with plant cover, Olsen-P and significant negatively correlated with $CaCl_2$-extractable trace element contents. This demonstrates that soil pH has a significant influence on metal solubility (Madejón *et al.*, 2010; Pérez-De-Mora *et al.*, 2011).

Table 4.1 Person´s Correlation at two soil layer (0-15 and 15-30 cm), $^{**}p<0.01$, $^{*}p<0.05$ (n=21).

Upper layer (0-15cm)

Variable	Plt Cov	pH	TOC	WSOC	Kj-N	Olsen-P	Avail-K	$CaCl_2$-Cd	$CaCl_2$-Cu	$CaCl_2$-Zn
Plt Cov	1	0.711**					0.669**	-0.755**	-0.863**	-0.853**
pH		1				0.555**		-0.918**	-0.556**	-0.897**
TOC			1		0.554**		0.483*		-0.440*	
WSOC				1						
Kj-N					1	0.640**				
Olsen-P						1				-0.443*
Avail-K							1	-0.492*	-0.633**	-0.545*
$CaCl_2$-Cd								1	0.592**	0.970**
$CaCl_2$-Cu									1	0.747**
$CaCl_2$-Zn										1

Lower layer (15-30cm)

Variable	Plt Cov	pH	TOC	WSOC	Kj-N	Olsen-P	Avail-K	$CaCl_2$-Cd	$CaCl_2$-Cu	$CaCl_2$-Zn
Plt Cov	1	0.577**				-0.576**	0.576**	-0.624**	-0.788**	-0.665**
pH		1						-0.834**	-0.567**	-0.805**
TOC			1		0.909**	0.435*			0.584**	
WSOC				1		0.617**				
Kj-N					1	0.647**			0.670**	
Olsen-P						1			0.731**	
Avail-K							1		-0.460*	
$CaCl_2$-Cd								1		0.969**
$CaCl_2$-Cu									1	0.467*
$CaCl_2$-Zn										1

Plt Cov: plant cover; TOC: total organic carbon; WSOC: water soluble organic carbon; Kj-N: Kjeldahl N; Avail-K: Available K.

4.2. Multivariate Analysis methods (**Principal Component Analysis** and **Sun Ray Plots**)

Principal Component Analysis (PCA) is a technique which uses sophisticated underlying mathematical principles to transforms a number of possibly correlated variables into a smaller number of variables called principal components. Principal component analysis of the results of soils (0-15 cm) shows that two components accounted for 67.8% (PC1 53.0%; PC2 14.8%) of the total variance (Table 4.2; Figure 4.1a). The first component (PC1) was determined mainly by the concentration of trace elements extracted with $CaCl_2$, soil pH and plant cover. These three variables are related to treatments: amendments increase soil pH and as consequently decrease solubility of trace elements, and increases plant cover. Therefore PC1 can be considered as an index of soil remediation: lower values correspond to untreated soils (NA). As increases the value of PC1, increases the degree of soil remediation. Figure 4.2 shows that the mean values of the scores of PC1 of the different treatments followed the trend: NA<LESL2<BC2<SL4<LESL4<SL2<BC4. The second component (PC2) was mainly associated positively with Olsen-P, and at less extends negatively to Avail-K and TOC, and positively to WSOC. Plot of the factor scores of the two PCA's (Figure 4.1b) shows the grouping of the treatment replicates. Despite the high variability of the results, from this plot it may be inferred that the application of the different amendments gives rise to differences in the soil variables related with contamination. This allows grouping the replicates of each treatment and assessing the efficiency of the different amendments used in this experiment. Data of the soil layer (15-30 cm) was not shown.

Table 4.2 Results of principal component analysis of some soil (0-15 cm) properties (pH, TOC, WSOC, Kj-N, Olsen-P, Avail-K, $CaCl_2$-Cd, -Cu and –Zn) and plant cover (Plt Cov).

Parameters	PC1	PC2	PC3	PC4
$CaCl_2$-Zn	**-0.957**	-0.088	-0.116	0.121
$CaCl_2$-Cd	**-0.905**	-0.163	-0.113	0.103
Plt Cov	**0.903**	-0.147	0.126	-0.189
pH	**0.876**	0.356	0.182	0.008
$CaCl_2$-Cu	**-0.811**	0.305	-0.076	0.201
Avail-K	**0.648**	-0.556	0.282	0.092
Olsen-P	0.499	**0.639**	-0.484	-0.101
Kj-N	0.568	0.021	**-0.751**	0.178
TOC	0.542	-0.451	-0.320	**0.569**
WSOC	0.206	0.535	0.536	**0.568**
Explained variance (%)	53.0	14.8	13.5	8.0

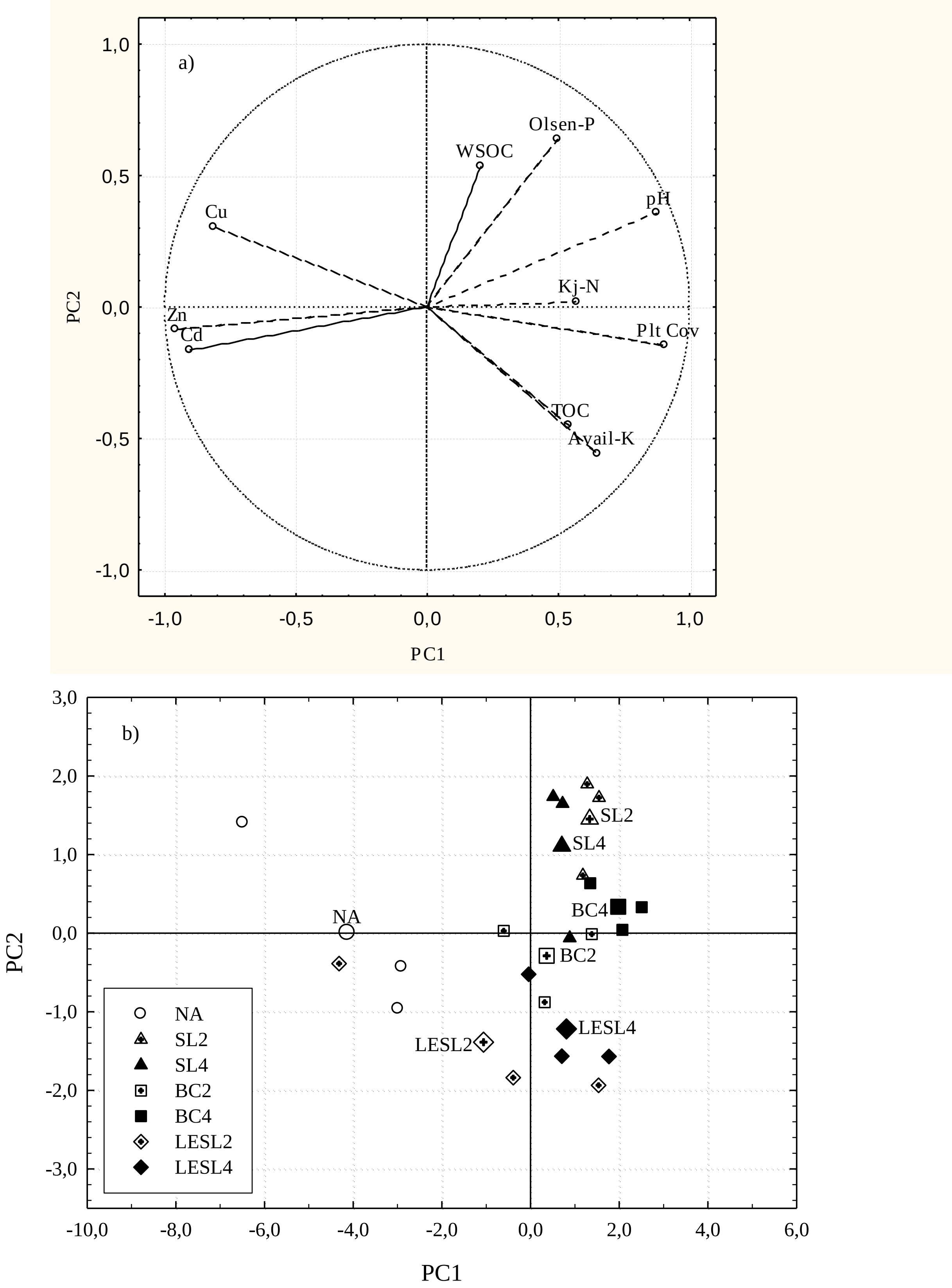


Figure 4.1 Principal components analysis (PCA) of the different studied parameters (Plt Cov, pH, TOC, WSOC, Kj-N, Olsen-P, Avail-K, $CaCl_2$-Cd, -Cu and -Zn): a) variable loading on the two first principal components, b) ordination of the treatments by the two first principal components. Big symbols with the name of treatments are the centroids of each treatment (coordinates of mean values of the PC1 and PC2 scores).

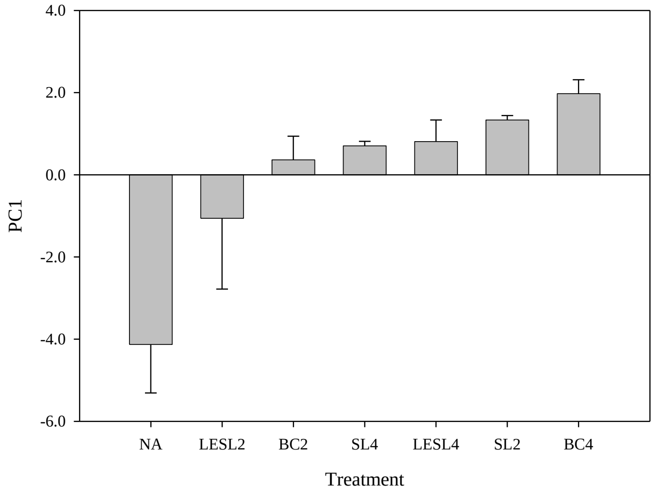
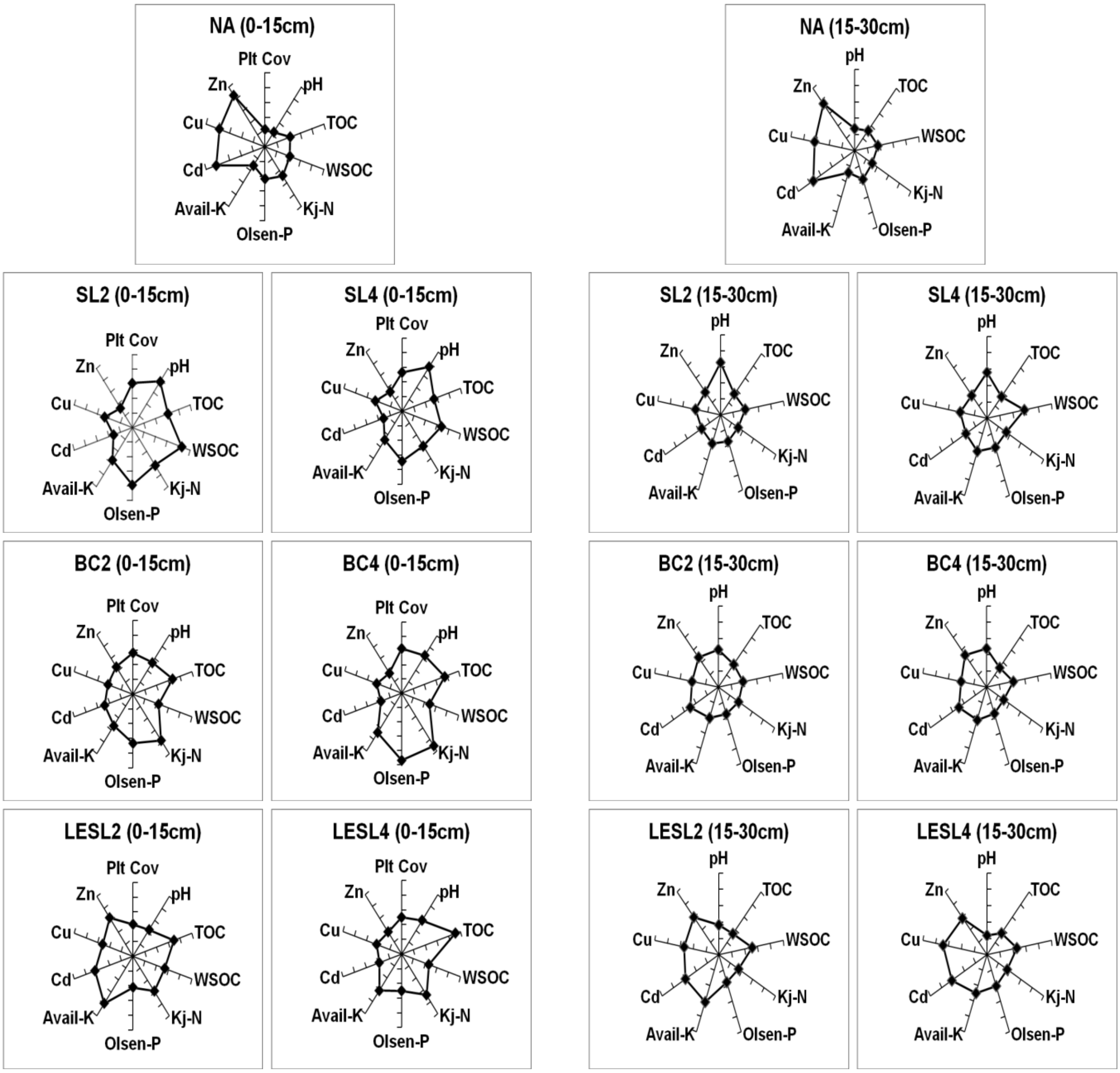


Figure 4.2 Mean values (±SE) of the factor scores of PC1 for the different treatments.

Another manner of assessing the efficiency of the different amendments is by mean of the **Sun Ray Plots**. Figure 4.3 shows the starts constructed with all the studied variables for each treatment at 0-15 and 15-30 cm depths. The shape and size of the starts is a characteristic of each treatment. In general, it can be observed that the application of amendments increased the values of those variables related with the soil fertility (soil pH and the concentrations of TOC, WSOC, Kjeldahl-N, Olsen-P and available-K) and the plant covering. At the same time amendments decreased the concentrations of Cd, Cu and Zn extracted with $CaCl_2$. Figures 4.4 and 4.5 shows the starts obtained separating both types of variables. These figures allow evaluating separately the effects of the amendments on soil fertility and soil contamination/remediation. The integrated area (IA) and relative area (RA) of each star respect to the area of the NA treatment can be considered as an index of soil fertility (Figure 4.4; Table 4.3) or as an index of soil contamination or remediation (Figure 4.5; Table 4.3). The fertility index increases as increase soil fertility. This index of soil fertility reached a maximum value of 3.5 at 0-15 cm for treatment BC4. The trend of the values of the fertility index for the different treatments was: LESL2<BC2≤SL4<LESL4<SL2<BC4. The contamination index decreases as decreases contamination. The lower value at 0-15 cm was observed for SL2 and SL4; the trend of the values of this index at this depth was: SL2=SL4≤BC4≤LESL4≤BC2<LESL2. From this trend it can be inferred that sugar beet lime (SL) is the most effective amendment to reduce mobile trace elements in the soil and that a second dose was not necessary. With the other two amendments, biosolid compost (BC) and mixture of leonerdite and sugar beet lime (LESL), the reduction of the concentration of mobile trace elements was less, and a second application improved the results.



NA (0-15cm)

NA (15-30cm)

SL2 (0-15cm)

SL4 (0-15cm)

SL2 (15-30cm)

SL4 (15-30cm)

BC2 (0-15cm)

BC4 (0-15cm)

BC2 (15-30cm)

BC4 (15-30cm)

LESL2 (0-15cm)

LESL4 (0-15cm)

LESL2 (15-30cm)

LESL4 (15-30cm)

Figure 4.3 Sun ray plots for different treatments of the two soil layers. According to the clockwise, starting at the 12 o'clock, the parameters are Plant Cover (Plt Cov), pH, Total Organic Carbon (TOC), Water Soluble Organic Carbon (WSOC), Kjeldahl N (Kj-N), Olsen-P, Available K (Avail-K), $CaCl_2$-Cd (Cd), $CaCl_2$-Cu (Cu), $CaCl_2$-Zn (Zn).

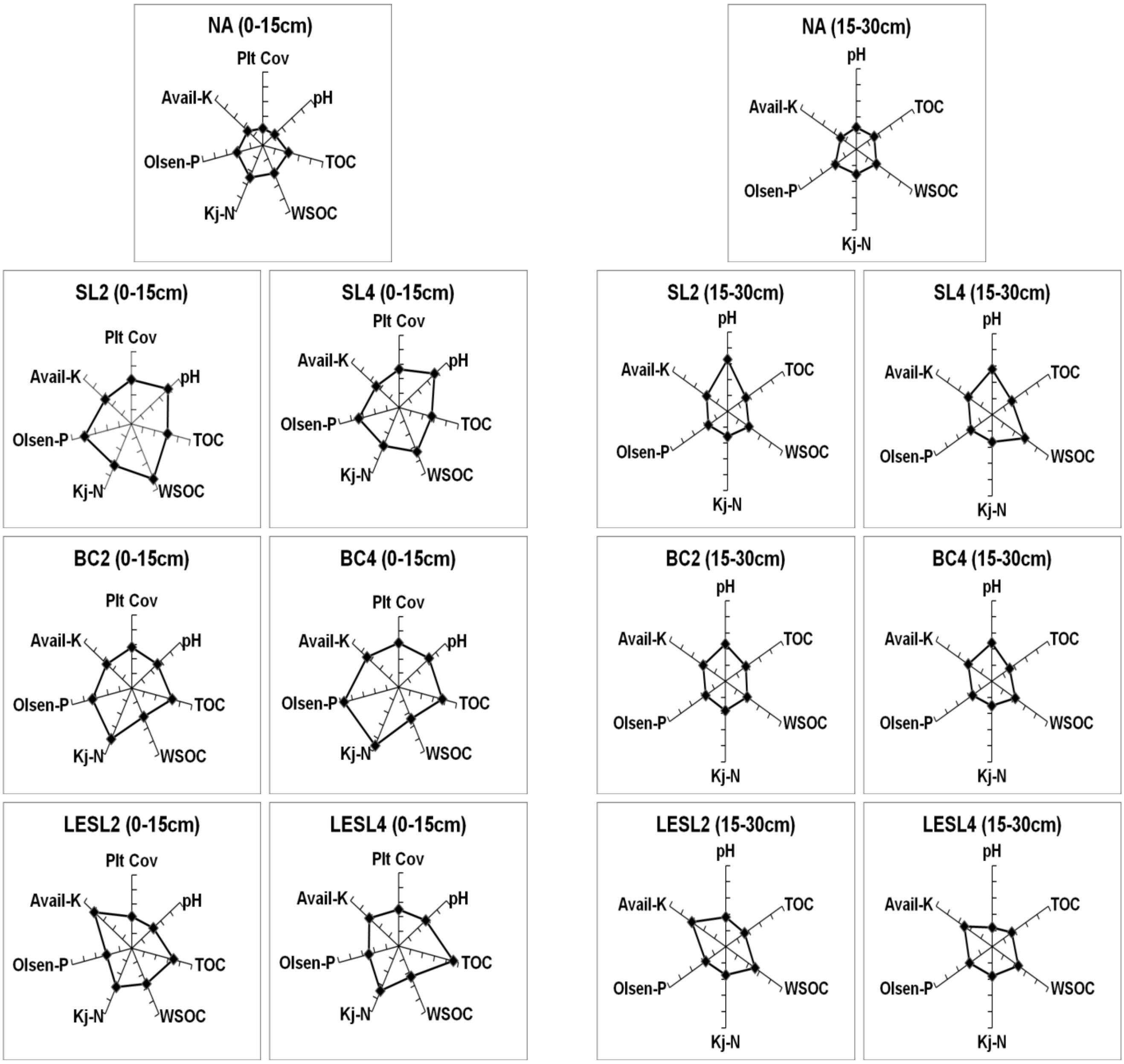


Figure 4.4 Sun ray plots of soil fertility for different treatments of the two soil layers. According to the clockwise, starting at the 12 o'clock, the parameters are Plant Cover (Plt Cov), pH, Total Organic Carbon (TOC), Water Soluble Organic Carbon (WSOC), Kjeldahl N (Kj-N), Olsen-P, Available K (Avail-K).

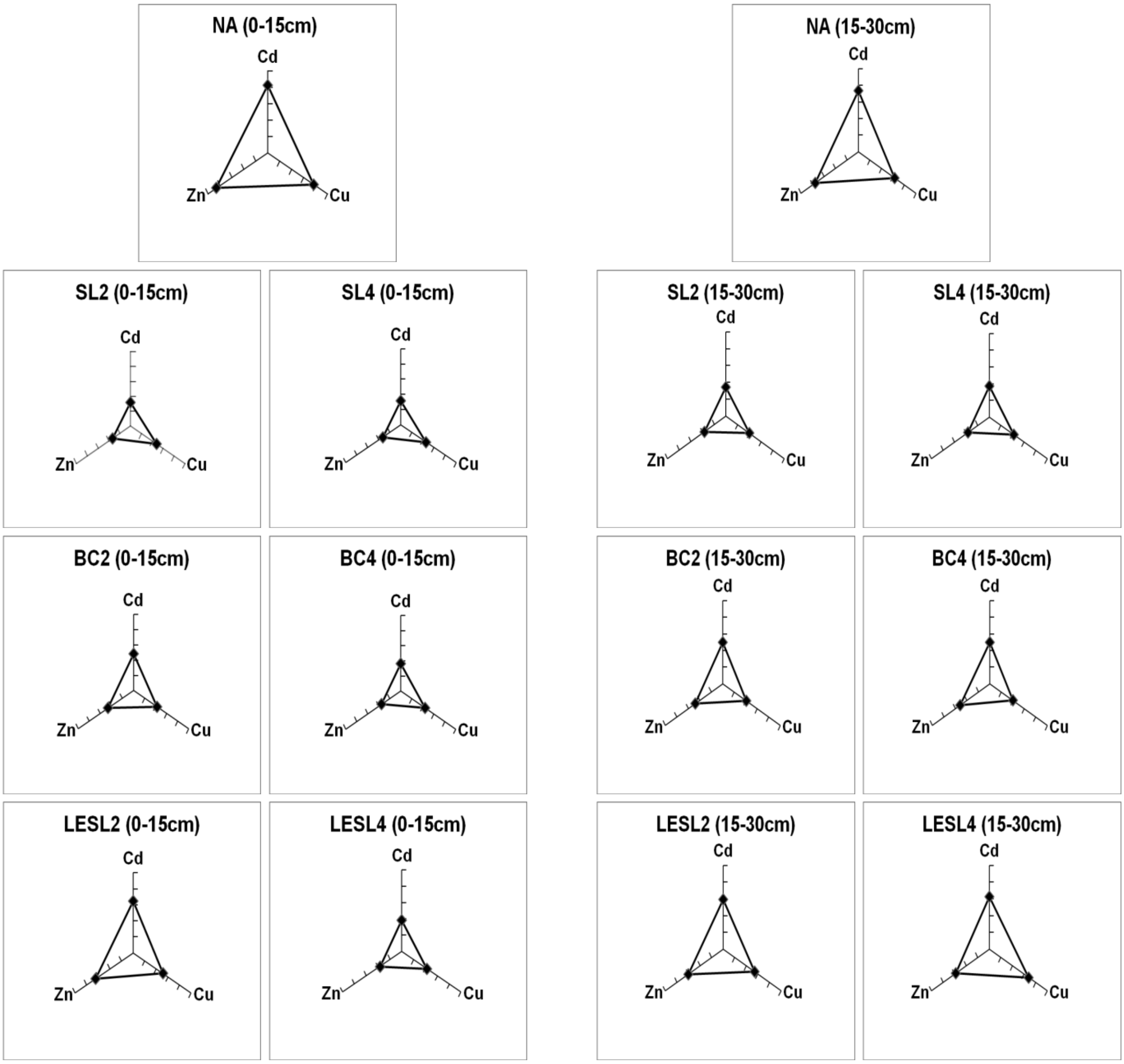


Figure 4.5 Sun ray plots of soil contamination for different treatments of the two soil layers. According to the clockwise, starting at the 12 o'clock, the parameters are $CaCl_2$-Cd (Cd), $CaCl_2$-Cu (Cu), $CaCl_2$-Zn (Zn).

Table 4.3 Index of integrated area (IA, mm^2) and relative area ($RA=IA_{Treatmen}/IA_{NA}$) values of Sun ray plots by every treatment of soil fertility and contamination at both soil layers (0-15 and 15-30cm).

Treatment	Fertility			
	0-15 cm		15-30 cm	
	IA (mm^2)	RA	IA (mm^2)	RA
NA	32.1	1.0	25.7	1.0
SL2	104.1	3.2	38.9	1.5
SL4	81.6	2.5	47.5	1.8
BC2	80.6	2.5	38.2	1.5
BC4	111.7	3.5	36.3	1.4
LESL2	69.5	2.2	44.0	1.7
LESL4	82.2	2.6	37.4	1.5

	Contamination			
	0-15 cm		15-30 cm	
	IA (mm^2)	RA	IA (mm^2)	RA
NA	90.8	1.0	66.3	1.0
SL2	16.4	0.2	19.2	0.3
SL4	15.3	0.2	20.6	0.3
BC2	25.0	0.3	28.5	0.4
BC4	16.9	0.2	29.8	0.4
LESL2	46.7	0.5	46.5	0.7
LESL4	20.9	0.2	53.8	0.8

5. CONCLUSIONS

The application of amendments to a trace element polluted soil improves its fertility, increased the pH and the concentrations of soil organic matter, of Kjeldhal-N and of Olsen-P, and reduces the concentration of readily soluble trace elements in the soil.

The repetition of the amendments two years after the first application had positive effects on most of the chemical properties of the soil; in some cases it contributes to enhance the reduction of the concentration of readily soluble trace element previously achieved with the first applications.

Amendments favoured the colonization and the establishment of spontaneous plant species, enhanced the establishment of a vegetation cover and decreased in some instances the trace element concentrations in the aerial part of plants.

Non amended soil showed clear signs of Natural Remediation.

Assisted Natural Remediation has a potential for success on field scale reducing trace element mobility and availability in soil and their entry into the food chain.

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