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Soil plant interactions of *Populus alba* in contrasting environments

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ABSTRACT

The effects of the *Populus alba* tree on different biochemical soil properties, growing in a contaminated area, were studied for two years under field conditions. Two types of trace element contaminated soils were studied: a neutral contaminated soil (NC) and an acid contaminated soil (AC). One neutral non-contaminated area was studied as control. Soil samples were collected at depths of 0–20 cm and 20–40 cm. Leaves and litter samples were analysed. The addition of organic matter, through root exudates and litter, contributed to an increase in soil pH, especially in acid soil. Microbial Biomass Carbon (MBC) was significantly increased by the presence of the trees in all studied areas, especially in the upper soil layer. Similar results were also observed for protease activity. Both MBC and Protease activity were more sensitive to contamination than β-glucosidase activity. These changes resulted in a decrease of available trace element concentrations in soil and in an improvement of soil quality after a 2-year study. The total concentration of Cd and Zn in soil did not increase over time due to litter deposition. Analysis of *P. alba* leaves did not show a significant nutritional imbalance and trace element concentrations were normal for plants, except for Cd and Zn. These results indicate that *P. alba* is suitable for the improvement of soil quality in riparian contaminated areas. However, due to the high Cd and Zn concentrations in leaves, further monitoring of this area is required.

1. Introduction

Afforesting polluted sites is part of a low-cost, ecologically and sustainable reclamation strategy to gain value from otherwise derelict land (Dickinson, 2000). Planting trees on these sites promotes soil development and nutrient cycling. The success of phytoremediation trials cannot only be evaluated in terms of reduction bioavailable trace elements in soils but also soil biological quality improvements that can restore multifuntionality of the soil (Hartley et al., 2011). A successfully phytomanaged area should have limited leaching and limited plant uptake of contaminants. The soil surface must be stabilised so that wind and water erosion are minimised and there is a reduced risk of direct soil consumption by humans and animals (Robinson et al., 2003).

A large-scale phytomanagement programme was implemented after a toxic sludge spill in the Guadiamar River Valley (Southwestern Spain). This programme was one of the largest soil remediation operations in Europe. It included the use of soil amendments and the revegetation of the affected area (about 55 km^2) with native woody plants (Domínguez et al., 2008). This vegetation acts as a sink for contaminants by uptake or assimilation, reducing the amount of available contaminant for transport to groundwater. Poplar occurs in the riparian forest in the area of the phytomanagement programme of Guadiamar River Valley. Its response as biomonitor was analysed in surviving trees shortly after the mine-spill (Madejón et al., 2004). Poplars are deciduous trees that can accumulate inordinate concentrations of B, Cd and Zn in their leaves (Lepp and Madejón, 2007; Robinson et al., 2005) with little or no visual toxicity (Punshon and Dickinson, 1997). Their plantation in trace element contaminated areas may cause, after the autumnal fall, the presence of an extensive 'carpet' of litter loaded with heavy metals. Some studies have investigated the possible long-term effects on soil quality of establishment of these trees in soils, focussing mostly on changes in soil organic carbon and soil microbial colonization and activity (Baum et al., 2009). However, there is little information regarding the influence of this heavy metal-rich litter on the properties of the afforested soils using these trees (Scheid et al., 2009), especially the biochemical properties involved in the dynamics of nutrients and organic matter under semi-arid conditions.

We aimed to determine whether the elevated concentrations of metals in the leaves of deciduous trees used in the Guadiamar

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phytomanagement programme would detrimentally affect soil quality. Specifically, we sought to (1) determine the effects of *P. alba* on contaminated soil investigating the chemical (pH, TOC, WSC, nutrients, pseudo-total trace elements and $CaCl_2$ extractable trace elements) and biochemical properties (MBC, β-glucosidase and protease) of the soils; (2) study the nutrients and pseudo-total trace elements in leaves and litter of the studied trees and (3) evaluate the quality of these soils after 2 years study.

2. Materials and methods

2.1. Sampling sites

Soil and *P. alba* leaves were collected at two riparian contaminated areas of the Guadiamar river valley affected by the Aznalcóllar mine spill. A neutral contaminated area (NC, 37° 18′ 12″ N, 6° 15′ 38″ W) and an acid contaminated area (AC, 37° 23′ 45″ N, 6° 13′ 35″ W) where *P. alba* was growing. For comparison, soil and *P. alba* leaves from a non-contaminated area (Control, 37° 17′ 08″ N, 6° 04′ 1.5″ W) were also collected. Control and AC soils are sandy loams whereas NC could be classified as loam. Figure S1 shows the precipitations and temperatures in the areas of study during the two years of monitoring.

Soil samples were collected at each sampling site in October 2009 (sampling 1), April 2010 (sampling 2), October 2010 (sampling 3), April 2011 (sampling 4) and October 2011 (sampling 5). Three soil samples were taken around the six selected trees at two depths (0–20 cm and 20–40 cm) to make a composite sample per tree. The field-moist soil was passed through a 2 mm sieve, homogenized and then divided in two subsamples: one oven-dried at 40 °C, for at least 48 h for various chemical analyses; a second subsample was stored at 4 °C in plastic bags for analysis of microbial biomass and enzymatic activities. Dry samples were ground to <60 μm for trace element analysis.

At each sampling site, leaves of the six selected trees at about 5 m height, from the outer canopy were collected in autumn 2010 and 2011, just before abscission. Leaf samples were washed for 15 s with a 0.1 N HCl solution, and finally with distilled water. Next, they were dried at 70 °C for at least 48 h, and ground using a stainless-steel mill.

In autumn 2010 and 2011, three samples per site of litter were collected and the amount of litter (kg (dw) m^{-2}) at each site was calculated by weighing the litter cover in an area of 30 cm × 30 cm. Litter was washed for 15 s with a 0.1 N HCl solution then for 10 s with distilled water. Washed samples were oven dried at 70 °C. Dried plant material was ground and passed through a 500 μm stainless-steel sieve prior to preparation for analysis.

2.2. Soil and plant analysis

Soil and litter pH were measured using a pH meter (CRISON micro pH 2002) in a 1/2.5 sample/1M KCl extract after shaking for 1 h. Pseudo-total trace element concentrations in soil (<60 μm) were determined by ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy) following *aqua regia* digestion in a microwave oven (ISO, 1995). Available trace element concentrations from the soil were estimated using a 0.01M $CaCl_2$-extraction using a sample extraction ratio of 1:10 (Houba et al., 1996). Analysis of trace elements was performed by ICP-OES. Total organic carbon (TOC) in soil was analysed by dichromate oxidation and titration with ferrous ammonium sulphate (Walkley and Black, 1934). The water-soluble carbon (WSC) content was determined on using a TOC-VE Shimadzu analyser after extraction with water using a sample-to-extractant ratio of 1:10.

Microbial biomass carbon (MBC) content was determined by the chloroform fumigation–extraction method modified by Gregorich et al. (1990). The C concentration in the extract was measured by a TOC-VE Shimadzu analyser. An extraction efficiency coefficient of 0.38 was used to convert the difference in soluble C between the fumigated and the unfumigated soil to MBC (Vance et al., 1987).

β-glucosidase activity in soil was measured as described by the method of Tabatabai (1982) after soil incubation of soil with p-nitrophenyl-β-D-glucopyranoside and measurement of PNP absorbance at 400 nm.

Protease activity in soil was measured after incubation of soil with casein and measurement of the absorbance of the extracted tyrosine at 700 nm (Ladd and Butler, 1972). Protease activity is expressed as mg of tyrosine kg^{-1} 2 h^{-1}.

Total N in leaves was determined by Kjeldahl digestion (Hesse, 1971). Nutrients (Ca, K, Mg, P y S) and trace elements (As, Cd, Cu, Fe, Mn, Pb and Zn) in leaves and litter were determined by wet oxidation with concentrated HNO_3 under pressure in a microwave digester. The analysis of these elements in the extracts was performed by ICP-OES. The accuracy of the analytical method was determined using the plant reference materials. The recovery rates were: Cd 105%, Cu 105%, Pb 82%, Mn 101% and Zn 96% in INCT-TL-1 (Tea leaves), and As 103% in NCSDC 73348 (Bush branches and leaves).

2.3. Statistical analysis

Statistical analyses were carried out using SPSS 15.0 for Windows and the results are expressed as mean values with standard errors. The results of nutrients and trace elements in leaves were analysed by ANOVA considering the site as the independent variable. Significant statistical differences of soil variables between depths were established by the Tukey test at $p < 0.05$.

3. Results

The amount of litter found in the different soils were 0.66 kg (dw) m^{-2} in Control soil, 1.71 kg (dw) m^{-2} in the Neutral Contaminated soil (NC) and 1.10 kg (dw) m^{-2} in the Acid Contaminated soil (AC). These results show that contamination did not affect plant growth and, indirectly, plant biomass.

3.1. Changes on pH, TOC and pseudototal trace elements

Fig. 1 shows the change in soil pH during the study. In neutral soils (Control and NC) pH values decreased in time although in Control soil an increase was recorded in the last sampling (October 2011). In the AC, a continuous increase in values of soil pH was observed in the superficial layer (0–20 cm).

The TOC contents in all soils were below 2% (Table 1). In the first sampling, values were higher in neutral (Control and NC) than in acid soil (AC). However, in the final sampling a noticeable decrease in TOC was observed in Control soil whereas in contaminated soils (NC and AC) TOC was maintained or even increased slightly. The values in the superficial layer were higher than those of deepest layer, presumably due to litter fall. Significant differences in time were found only for TOC in Control soil at the deepest layer, may be due to the minor provision of litter for this soil that produced a strong decrease in time for this parameter.

Concentrations of the pseudototal trace elements in NC and AC were significantly higher than in Control soil (except for Mn) (Table 1). In general, values were lower at depth 20–40 cm compared to the upper layer. Values in 2011 were similar to those of 2009, especially for Cd and Zn.

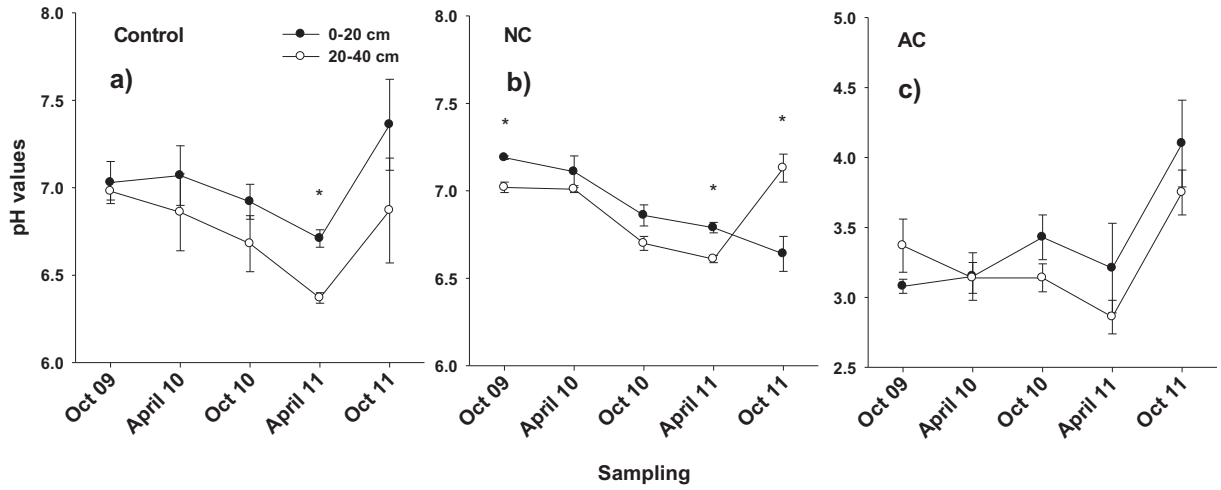

Fig. 1. Evolution in time of soil pH (graphs a, b and c) at the three studied soils and at two different depths (0–20 cm and 20–40 cm). Mean values ± standard error ($n = 3$). Significant differences between depths for each soil and time are marked with an asterisk.

3.2. Available trace elements in soil

Availability was evaluated for several trace elements (As, Cd, Cu, Mn, Pb and Zn), however in this work only Cd and Zn values are presented (Fig. 2).

In neutral soils (Control and NC), no significant differences were found between Control and NC soils of Cd and Zn for both depths and general lower values of these elements were obtained compared to acid soil (AC) (Fig. 2). However, in the AC soil, the concentration of these two elements decreased over time. Extractable concentrations in the final samplings were just 23.5% for Cd and 17.5% for Zn of the initial concentrations. Moreover, concentrations of Cd and Zn in the deepest layer (20–40 cm) were higher than in the superficial layer (0–20 cm). This result highlights the important role that pH plays in trace element bioavailability. The most significant and negative correlations were found between pH and Cd and Zn availability ($r = -0.733$ for Cd and $r = -0.677$ for Zn at 0–20 cm depth and $r = -0.751$ for Cd and $r = -0.696$ for Zn at 20–40 cm depth; $p < 0.01$).

For neutral soils (Control and NC), values of As and Pb were below the detection limit (As 0.1 mg kg^{-1} and Pb 0.3 mg kg^{-1}) at both depths (data no shown). However, values above these limits were obtained for AC soil in the first samplings (Figure S2). Values of Cu (Figure S3) at both depths ranged between 0.01 and 0.06 mg kg^{-1} for Control, between 0.06 and 0.12 mg kg^{-1} for NC and between 3.50 and 26.1 mg kg^{-1} for AC. Values of Mn (Figure S3) at both depths ranged between 0.08 and 1.03 mg kg^{-1} for Control, between 0.08 and 0.72 mg kg^{-1} for NC and between 0 and 90 mg kg^{-1} for AC. In general, for these elements, available concentration tended to decrease during the experimental period.

3.3. Water soluble carbon (WSC) and microbial biomass carbon (MBC)

As with the TOC, the concentrations of WSC were higher in the upper than in deeper layer (Fig. 3).

Maximum values were found in Control and NC soils in the sampling of April 2011, especially comparing these values to those obtained in the same sampling in the previous year (April 2010). In the AC soils, the concentrations of WSC were similar at depth and stable over time. These values were lower than those found in neutral soils (Fig. 3).

A similar increase was observed for MBC values in the three analysed soil during the study period, however, values obtained in the non-contaminated (Control) soil were higher than those found in contaminated soils (NC and AC) (Fig. 4). The largest increases occurred in the Control soil (up to 500 mg kg^{-1}, Fig. 4), while in NC and AC soils values were not above 200 mg kg^{-1}. In all the soils values tended to be higher in the 0–20 cm layer, especially in the control soil. The MBC was significantly and negatively correlated

Table 1
Pseudo-total trace elements concentrations (mg kg^{-1}) and total organic carbon (TOC %) at the beginning (October 2009) and at the end of the study (October 2011) at the three studied sites at two depths.

Year	Site	Depth	TOC	As	Ca	Cd	Cu	Fe	Mn	Pb	Zn
2009	Control	0–20	1.5 ± 0.3	9.2 ± 0.5	27636 ± 3498	0.1 ± 0.02	26 ± 1.6	30032 ± 1203	550 ± 18[a]	18 ± 0.5	69 ± 4
		20–40	0.7 ± 0.1[a]	9 ± 0.4	15589 ± 2873	0.2 ± 0.01[a]	26 ± 2.2	29461 ± 1220	532 ± 20[a]	18 ± 1.8	63 ± 4
	NC	0–20	1.3 ± 0.2	80 ± 36	43013 ± 57.0	1.6 ± 0.6	132 ± 41	31609 ± 2136	602 ± 20[a]	167 ± 72	476 ± 140
		20–40	0.8 ± 0.1	42 ± 17	37235 ± 3836	1.1 ± 0.7	112 ± 53	29658 ± 1870	625 ± 40[a]	108 ± 49	327 ± 162
	AC	0–20	1.1 ± 0.02	165 ± 19	6921 ± 364	1.2 ± 0.04	246 ± 12	44247 ± 978	557 ± 16	264 ± 37	315 ± 32
		20–40	0.8 ± 0.1	118 ± 14	6856 ± 733	1.2 ± 0.4	270 ± 17	36829 ± 1269	640 ± 88	135 ± 19	326 ± 54
2011	Control	0–20	1 ± 0.1	8.2 ± 0.5	17173 ± 3339	0.1 ± 0.1	67 ± 39	26242 ± 878	455 ± 20	49.2 ± 33	136 ± 77
		20–40	0.5 ± 0.1	7.5 ± 0.5	8090 ± 454	0.02 ± 0.01	21 ± 2	25897 ± 809	411 ± 27	16.7 ± 6	52.9 ± 3
	NC	0–20	1.5 ± 0.1	75 ± 4	44041 ± 317	1.9 ± 0.1	131 ± 4	26930 ± 721	454 ± 11	149 ± 6	501 ± 29
		20–40	1 ± 0.03	60 ± 5	38187 ± 1551	1.9 ± 0.2	152 ± 10	27347 ± 287	493 ± 12	69.5 ± 12	483 ± 42
	AC	0–20	1.1 ± 0.2	157 ± 22	6510 ± 298	0.9 ± 0.2	226 ± 12	40242 ± 1937	465 ± 106	243 ± 35	248 ± 10
		20–40	1 ± 0.1	106 ± 8	7181 ± 313	1.4 ± 0.5	248 ± 31	36071 ± 1530	668 ± 85	101 ± 18	308 ± 71

Mean values ± standard error ($n = 3$).
[a] Significant differences ($p < 0.05$) between the same soil and depth in time.

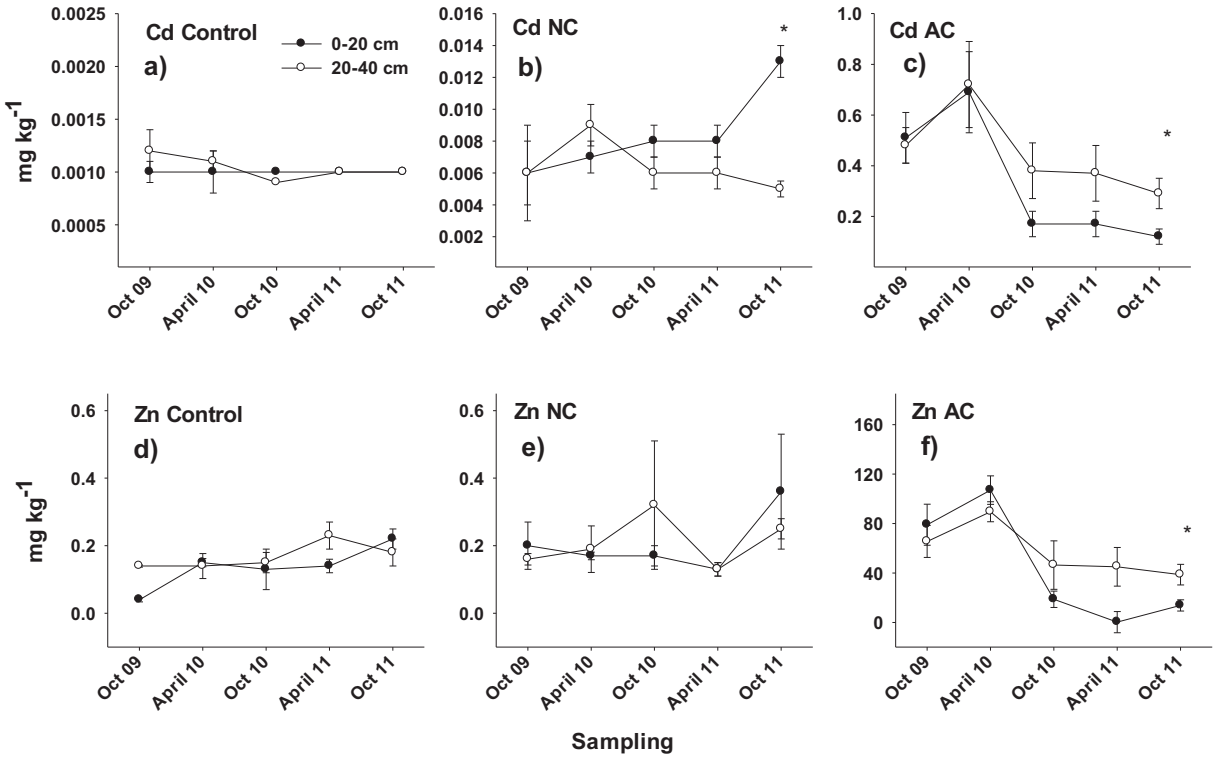

Fig. 2. Evolution in time of available Cd and Zn extracted with 0.01M $CaCl_2$ (graphs a, b and c) at the three studied soils and at two different depths (0–20 cm and 20–40 cm). Mean values ± standard error ($n = 3$). Significant differences between depths for each soil and time are marked with an asterisk.

with all available trace elements concentrations in soils, especially with Cd and Zn, in the upper layer ($r = -0.519$ for Cd and $r = -0.482$ for Zn; $p < 0.01$).

3.4. β-glucosidase and protease activities

Values of β-glucosidase activity were higher in the neutral soils (Control and NC) than in acid soil (AC) (Fig. 5). Values observed in NC were higher than those of Control soils. In each soil, the values of this activity were significantly higher in superficial layer than in deeper layers during the whole study period (Fig. 5).

Significant and positive correlations were found between this activity at depth 0–20 cm and pH, TOC, WSC and MBC ($r = 0.612$ for pH, $r = 0.698$ for TOC, $r = 0.471$ for WSC and $r = 0.366$ for MBC; $p < 0.01$); at depth 20–40 cm only were found correlations with pH and WSC ($r = 0.562$ for pH and $r = 0.235$ for WSC; $p < 0.01$ and $p < 0.05$, respectively).

Protease activity (Fig. 6) was significantly higher in the superficial layer although differences between depths were lower compared to those observed for β-glucosidase activity. This activity was clearly higher in Control than in contaminated soils (Fig. 6) and tended to increase in time with maximum values at the last sampling. Significant and positive correlations were found between protease activity at depth 0–20 cm and pH and MBC ($r = 0.729$ for pH and $r = 0.628$ for MBC; $p < 0.01$).

Correlations between available trace elements (especially Cd and Zn) in soils for both activities were significant and negative ($p < 0.01$), showing higher values for protease activity ($r = -0.367$

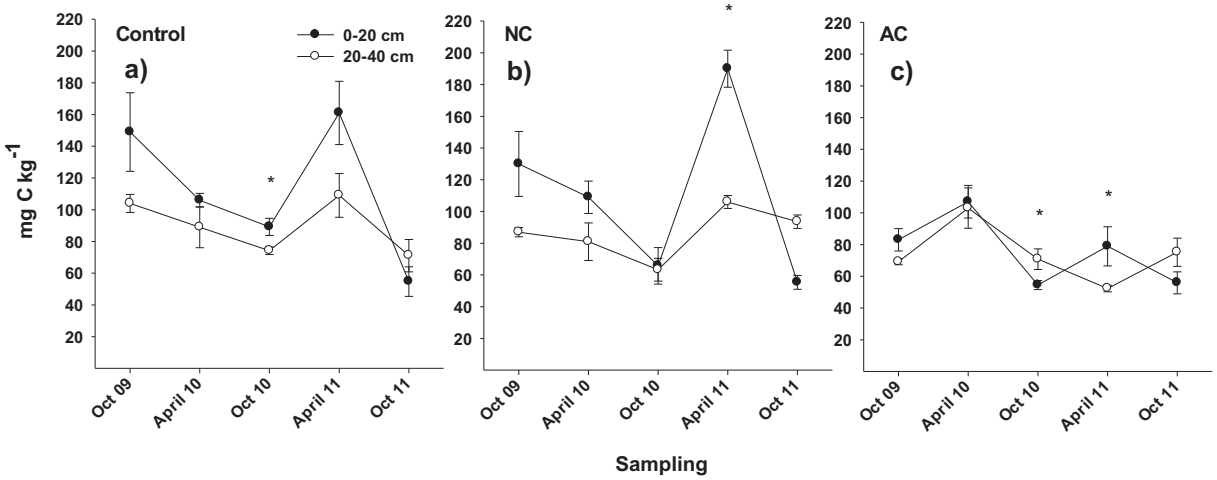

Fig. 3. Evolution in time of water soluble carbon (WSC) in the three studied soils and at two different depths (0–20 cm and 20–40 cm). Mean values ± standard error ($n = 3$). Significant differences between depths for each soil and time are marked with an asterisk.

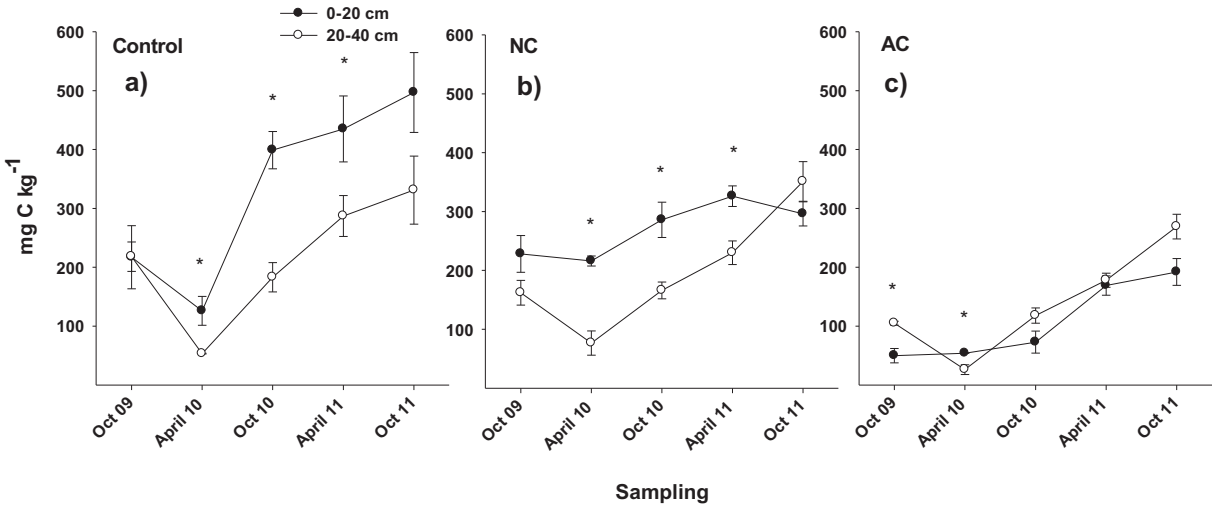

Fig. 4. Evolution in time of Microbial Biomass Carbon (MBC) in the three studied soils and at two different depths (0–20 cm and 20–40 cm). Mean values ± standard error ($n = 3$). Significant differences between depths for each soil and time are marked with an asterisk.

for Cd and $r = -0.326$ for Zn with β-glucosidase and $r = -0.572$ for Cd and $r = -0.527$ for Zn with protease, at 0–20 cm depth respectively; $r = -0.377$ for Cd and $r = -0.317$ for Zn with β-glucosidase and $r = -0.448$ for Cd and $r = -0.422$ for Zn with protease, at 20–40 cm depth respectively; $p < 0.01$). The sampling season had no influence on either β-glucosidase or protease activity.

3.5. Nutrient and trace elements in P. alba leaves

Concentrations of nutrients in leaves are shown in Table 2. In general, in the trees of the Control soil the concentrations of N, K and P in leaves were significantly higher than in leaves of contaminated soils. Values of these three elements in Control leaves were slightly lower than the proposed by Reuter and Robinson (1997) for young leaves of *P. deltoides* (2.2% N, 1.4% K and 0.3% P) and lower than leaves of contaminated soils used in this experiment. In contrast, the concentration of Ca, Mg and S tended to be significantly higher in contaminated soils compared to Control soils in both years. Djingova et al. (1995) reported concentrations of 1.82% of Ca and 0.42% of Mg, similar to our control leaves, and lower than in both contaminated soils (NC and AC).

Trace element concentrations in leaves either did not change or decreased with respect to time, except for Cd and Zn (Table 3). In general, concentrations of As, Cu, Fe Mn and Pb were similar or even greater in the Control soil than in the NC soil. For all these elements, concentrations were always within the normal range considered in plants (Table 3).

In case of Cd and Zn, concentrations were significantly higher in both contaminated soils. Concentrations of these elements in the leaves were above normal levels in plants, except for Control soil (Table 3).

4. Discussion

4.1. pH and trace elements in soils

The effect of plants on soil pH depends on the soil and the plant species (Pulford and Watson, 2003). Tree species influence soil acidity and exchangeable cations by several mechanisms. There are interspecific differences in the uptake of exchangeable cations and anions (Alban, 1982), nitrogen fixation and nitrification (Van Miegroet and Cole, 1984), the production of litter high in organic

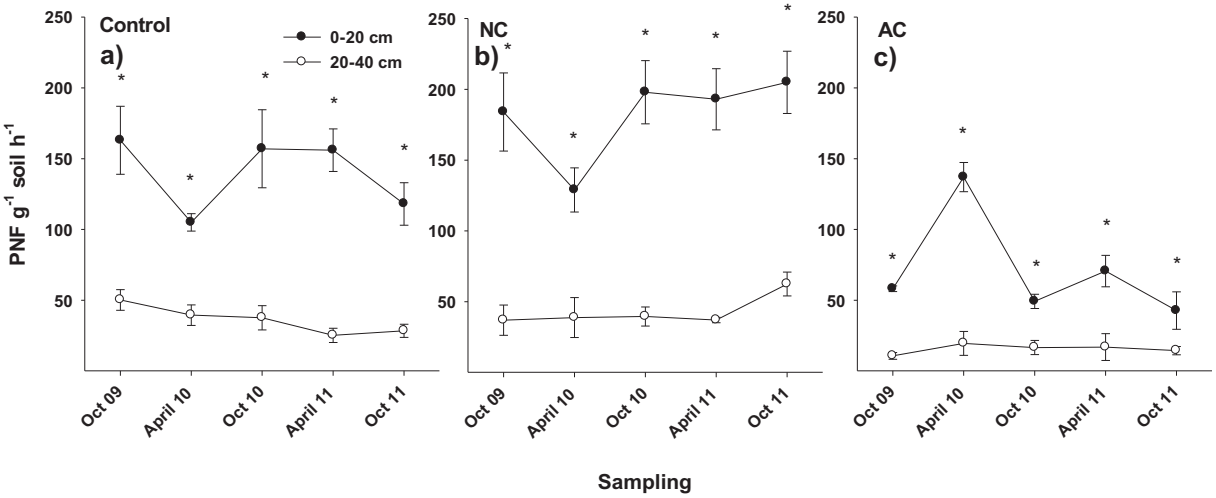

Fig. 5. Evolution in time of β-glucosidase activity in the three studied soils and at two different depths (0–20 cm and 20–40 cm). Mean values ± standard error ($n = 3$). Significant differences between depths for each soil and time are marked with an asterisk.

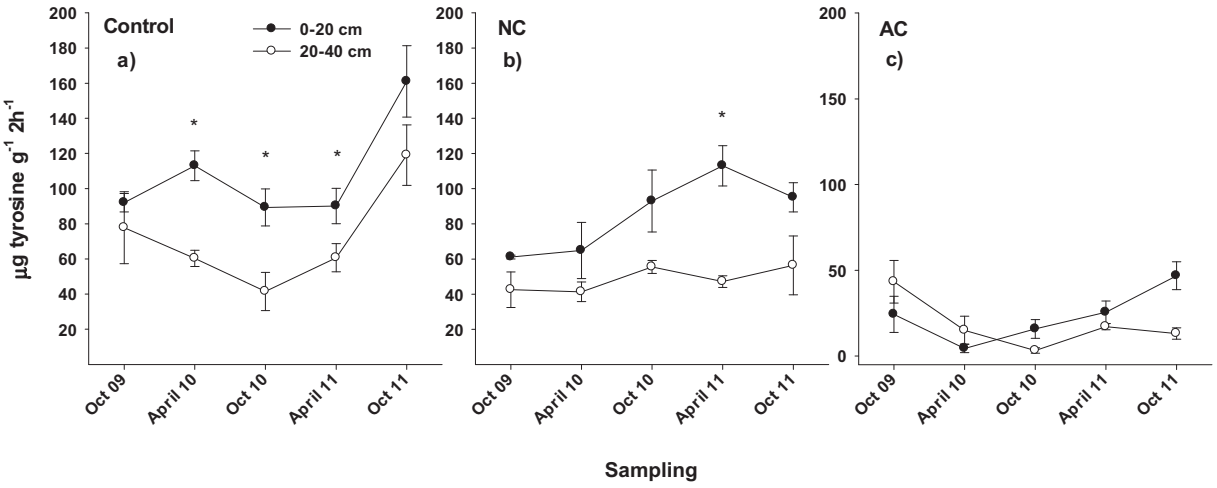

Fig. 6. Evolution in time of protease activity in the three studied soils and at two different depths (0–20 cm and 20–40 cm). Mean values ± standard error ($n = 3$). Significant differences between depths for each soil and time are marked with an asterisk.

acid content (Ovington, 1953) and the stimulation of mineral weathering (Tice et al., 1996). Zinke (1962) found significant variation in soil pH around tree bases of *Pinus contorta* and Crozier and Boerner (1986) demonstrated that stem-flow influenced soil pH and ex-changeable calcium around trees.

In this study, we only found a pH decrease in case of neutral contaminated soils in the superficial layer (Fig. 1). This result may be related to the acidification process due to the litter "carpet" with a pH more acid than the soil (Table S1). Moreover, the high concentrations of Cd and Zn in the litter (Table S1) may retard its decomposition which could result in a decreased pH (Mertens et al., 2007). In a previous study (Madejón et al., 2012), similar results were obtained in a microcosm experiment using the same soils. In this case, the moderately acidic nature of the litter minimized the alkalinizing effect in soils.

However, in the acid contaminated soil, values tended to increase. The same result was obtained in semi-field experiment by Ciadamidaro et al. (2013), in which poplar growth increased soil pH values of the same acid soil. This may be due to the action of the root exudates that can form soluble complexes, for e.g. with Al^{3+}, reducing the acidity of the medium (Shi et al., 2011) or the adjacent rhizosphere soil may become more alkaline (Nye, 1981), mainly because of H^+ consumption and/or OH^- exudation (Jones et al., 2004; Nye, 1981). In this case, the value of the litter was higher than that of the soil there may raise soil pH (Table S1).

Despite the presence of this litter rich in trace elements in the contaminated soils, the pseudototal trace element concentrations of the soil at depths <40 cm did not increase during the experimental period (Table 1). Furthermore, a stabilization or general reduction of extractable Cd and Zn was observed in contaminated soils (Fig. 3). Likewise, a significant decrease occurred in the AC soil, related with the increase on soil pH.

The concentrations of Cd and Zn in NC soil during the study were affected by the pH. Although in this soil values of Cd and Zn in the litter were higher than in Control soil, values of these two elements extracted with $CaCl_2$ were not significant higher than those obtained in Control soil. Madejón et al. (2012) demonstrated that, although the time-related decomposition of litter could lead to an increase of trace element bioavailability, after 40 weeks of incubation, no increase in trace element concentration with time were found in any of the contaminated soils. It is well known that an increase in soil pH reduces metal availability through precipitation reactions and by increasing adsorption by variable charge colloids (Selin and Amacher, 1997).

In general, differences in trace element concentrations between the two depths were not observed; therefore it seems that there were negligible lixiviation process during the experimentation period, except in the last sampling of the AC soil. Here, the values of these elements at 20–40 cm were lower than in previous samplings but still significantly higher than at the superficial layer (Fig. 2). Nevertheless, continual monitoring is advisable to ensure that the enrichment of the deepest layer does not pose a risk to groundwater, which is crucial for the sustainability of both natural and agricultural systems in the Mediterranean area (Burgos et al., 2013).

4.2. Soil quality

In forest ecosystems, litter fall is the main source of organic matter and nutrients for the humus layer (Kuzyakov and Domanski, 2000). Low decomposition, resulting from acidic conditions, limited supply of essential nutrients and the presence of organic or inorganic pollutants, can lead to an accumulation of organic matter in soil and to immobilization of essential nutrients (Swift et al., 1979).

Table 2
Nutrients concentrations (g 100 g^{-1}) in leaves at two consecutive years (October 2010–2011) at the three studied sites.

Leaves	Year	N	Ca	K	Mg	P	S
Control	2010	2 ± 0.1 b	1.7 ± 0.3 a	1 ± 0.1 b	0.3 ± 0.04 a	0.2 ± 0.01 b	0.2 ± 0.01 a
NC		1.3 ± 0.03 a	3.7 ± 0.4 b	0.8 ± 0.1 b	0.5 ± 0.1 b	0.2 ± 0.03 b	0.3 ± 0.02 a
AC		1.5 ± 0.1 a	3.2 ± 0.2 b	0.5 ± 0.04 a	0.7 ± 0.02 c	0.1 ± 0.01 a	0.6 ± 0.1 b
Control	2011	1.8 ± 0.02 a	2 ± 0.2 a	0.7 ± 0.1 a	0.3 ± 0.02 a	0.2 ± 0.02 b	0.2 ± 0.01 a
NC		1.3 ± 0.1 b	2.7 ± 0.2 a	0.7 ± 0.1 a	0.4 ± 0.02 b	0.2 ± 0.01 ab	0.3 ± 0.01 a
AC		1.4 ± 0.2 ab	2.7 ± 0.2 a	0.6 ± 0.1 a	0.5 ± 0.04 c	0.1 ± 0.01 a	0.5 ± 0.1 b

Mean values ± standard error ($n = 3$). In each column, values with the same letter do not differ significantly by one way ANOVA ($p < 0.05$).

Table 3
Trace elements concentrations (mg kg^{-1}) in leaves at two consecutive years (October 2010–2011) at the three studied sites.

Leaves	Year	As	Cd	Cu	Fe	Mn	Pb	Zn
Control	2010	0.7 ± 0.1 a	0.2 ± 0.02 a	8.3 ± 0.3 a	203 ± 50 a	48 ± 14 a	0.7 ± 0.2 a	78.6 ± 5 a
NC		0.7 ± 0.2 a	3.1 ± 0.3 b	7.5 ± 0.3 a	170 ± 25 a	56 ± 12 a	0.3 ± 0.1 a	283 ± 39 a
AC		1 ± 0.1 a	5.5 ± 0.5 c	8.8 ± 1.2 a	218 ± 15 a	204 ± 35 b	1.2 ± 0.1 b	860 ± 111 b
Control	2011	0.3 ± 0.04 a	0.5 ± 0.2 a	11 ± 0.7 b	205 ± 14 b	43 ± 3 a	0.9 ± 0.1 b	97 ± 16 a
NC		0.1 ± 0.03 a	3.7 ± 0.6 b	8.4 ± 0.5 a	119 ± 9 a	48 ± 5 a	0.5 ± 0.1 a	301 ± 48 a
AC		0.5 ± 0.1 b	3.8 ± 0.6 b	9.2 ± 0.6 ab	195 ± 16 b	138 ± 23 b	1.1 ± 0.1 b	603 ± 106 b
Normal		1.0–1.7	0.05–0.2	5–30	2–250	30–300	5–10	27–150

Mean values ± standard error ($n = 3$). Normal values (range) for trace elements in plants, according to Kabata-Pendias and Pendias (1992), and for Fe according to Bowen (1979). In each column, values with the same letter do not differ significantly by one way ANOVA ($p < 0.05$).

In the contaminated soils (NC and AC) the addition of organic matter through the litter fall resulted in the maintenance or increase of organic matter in these soils during the 2 years of the study. It is well known that maintenance of OM levels in Mediterranean soils is important for adequate water holding capacity. Madejón et al. (2012) obtained similar results in microcosm experiments. Several authors (Tyler et al., 1989; Gadd, 1993) demonstrated that organic matter sustains microbial growth and immobilizes trace elements.

Water-soluble organic carbon (WSC) is the most mobile and reactive soil carbon source which modulates a number of physical, chemical and biological processes (Marschner and Kalbitz, 2003; Halvorson and Gonzalez, 2008). The decrease of WSC in the soils during the first year may be due to the consumption of labile C by the microorganisms and the mineralization process, especially in April 2010 due to the lixiviation process after an intense period of precipitation (Figure S1). The increase in April 2011 could be related with the lower precipitation occurred in the previous autumn–winter period that probably tended to maintain the WSC coming from the decomposition of organic matter. Alternatively, the lower WSC content in AC soil might be related to the low pH and with the retard of litter decomposition. Several authors state that many environmental factors affect WSC in soils, including ecosystem acidification (Shamrikova et al., 2006; Karavanova et al., 2007), litter quality as influenced by forest composition (Shamrikova et al., 2006; Ohno et al., 2007) and soil temperature (Tao and Lin, 2000).

Microbial biomass is one of the most sensitive indicators of soil changes (Perucci, 1993). The effect of contamination was more evident for this parameter than for the others (Fig. 4). The general increase in time of this value in all soils may be linked with the organic matter through the presence of extra C from plant root exudates and from litter decomposition, especially in the superficial soil layer. The plants may have contributed to the activation of the biochemical and microbial functionality of the Control and the polluted soils (Hernández-Allica et al., 2006).

In a microcosm experiment using the same soils, Madejón et al. (2012) observed that litter addition promoted the growth of microorganism and increased WSC and MBC content in soils. Otherwise, Madejón et al. (2012)demonstrated that WSC and MBC were affected by soil conditions and positively correlated with soil pH.

β-glucosidase activity was less sensitive to trace element contamination (Fig. 5) than MBC. This activity, involved in the C cycle, is more likely to be influenced by the amount of WSC and TOC rather than the degree of contamination. This hypothesis is supported by the strong positive correlations between β-glucosidase activity and WSC and TOC and by the higher β-glucosidase activity found in the upper layer soils. This would explain why the NC soil had a higher β-glucosidase activity compared to the Control. The behaviour of β-glucosidase activity is consistent with the results obtained by Madejón et al. (2012). In this study, in fact, the nature of organic input was the major cause of increase of β-glucosidase activity lesser affected by soil contamination. This hypothesis does not account for AC soil because of its acidity, since soil pH is crucial for enzymatic survival and functioning (Acosta-Martínez and Tabatabai, 2000). In the AC soil, β-glucosidase activity was significantly lower than in the other soils, demonstrating the effects of soil pH on this parameter: there was a significant positive correlation between pH and β-glucosidase activity. Furthermore, differences in microbial activity between the two depths may due to the litter that was in contact with the upper soil layer and the differences in the composition of microbiota.

In general, for all soils and different depths the values of protease increased with time (Fig. 6). This demonstrates the positive effect of *P. alba* on soil quality. The protease activity may be stimulated in soil by the plant presence via litter fall and root exudates (Madejón et al., 2009). As with β-glucosidase, trees would be expected to introduce organic C and microorganisms into the soil by litter, resulting in a large difference in enzymatic activity between the upper and the deepest soil layers. Moreover, the organic matter added through litter addition increased pH that might be responsible for the final increase of protease activity, especially in acid contaminated soil (AC). The provision of organic matter in form of litter caused an important increase in MBC and consequently an increase in this activity. The significant and positive correlation coefficient between protease activity and MBC supports this hypothesis. Likewise, the highest values of protease activity was found for uncontaminated soil (Control), proving that soil pH was not the only interacting parameter but also trace element concentrations could influence this enzymatic activity. Therefore, in this study, protease activity was sensitive to the trace element toxicity and high negative correlations with Cd and Zn confirm the adverse effects provoked on this parameter.

4.3. Nutrients and trace elements in P. alba leaves

When plants are used to stabilize contaminants and improve soil quality, it is important to monitor the nutritional levels in these plants to determine their health status. The nutrients in leaves affected by the soil contamination were N, P and K only in 2010, showing lower values than in Control soil. These results indicate that trace elements interfere with the uptake processes, possibly because of the competitive interaction that may be taking place at the root level (Madejón et al., 2004; Domínguez et al., 2009) or the interference with the mechanisms of absorption of the essential nutrients for the plant, altering its nutritional balance (Kabata-Pendias and Pendias, 1992).

In contrast, higher concentrations of Ca, Mg and S in contaminated soils were found (Table 2). Higher foliar Ca concentrations could be related to the protective role of this element against trace elements toxicity (Carbonell et al., 1998). The higher Mg concentrations in AC soils were directly correlated with soil pH. Moreover S values were also correlated to the higher concentration of this

nutrient in the contaminated soil, due to the sludge that contaminated the soils was from the pyrite extraction.

For all the trace elements studied, the concentrations were at or under the normal values established reported for plants, except for Cd and Zn. The highest concentrations of trace elements were found in contaminated soils (NC and AC) compared to the Control soil, especially for Zn and in the AC trees. These data confirm that soil pH is one of the most important parameters explaining nutrient variability in the leaves of trees. Basta et al. (2005) showed that even small changes in soil pH could result in large changes in trace element phytoavailability. The highest differences due pH were found in case of Mn and Zn, being both elements known for their highly dependence to pH and to be easily taken up by plants when they occurs in soil (Kabata-Pendias, 2001). Moreover Domínguez et al. (2009) proved with their study that the bioavailability of the cationic trace elements in the Guadiamar area (same study area) is primary determined by soil pH.

The acidity of the AC soil could be attenuated by litter presence and this factor influenced the bioavailability of trace elements for plant uptake in the last sampling (Table 3). The rhizosphere enriched with biomolecules of plant and microbial origins that include organic acids, sugars, amino acids, proteins, carbohydrates and other substances could also influence trace element availability (Chang et al., 2002).

5. Conclusions

Despite some researches stating that *P. alba* is not advisable for phytostabilization, due to the accumulation of high concentrations of Cd and Zn in its leaves that might cause a risk of soil contamination because of autumn litter, our study showed that pseudototal concentrations remained similar over time and the bioavailavility of Cd and Zn decreased.

The chemical and biochemical properties of soil were not negatively affected by litter contamination in NC soils and were improved in AC soil. Natural processes appear to be enhanced by the addition of organic matter content coming from the litter that influence soil pH. Soil pH was the most important factor affecting the soil contamination and quality. All these factors may make white poplar suitable in biomonitoring and in maintaining the stability of riparian zones.

The use of *P. alba* for phytomanagement in the study area may increase the risk of Cd and Zn entering the food chain. For this reason, even if our results confirm that *P. alba* is recognized to improve soil quality, further long-term analysis are required in this area to establish potential risk due to the accumulation of trace element concentrations in the topsoil due to litter fall.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2013.11.010.

References

Acosta-Martínez, V., Tabatabai, M.A., 2000. Enzyme activities in a limed agricultural soil. Biol. Fert. Soils 31, 85–91.
Alban, D.H., 1982. Effects of nutrient accumulation by aspen, spruce, and pine on soil properties. Soil Sci. Soc. Am. J 46, 853–861.
Basta, N.T., Ryan, J.A., Chaney, R.L., 2005. Trace element chemistry in residual treated soil: key concepts and metal bioavailability. Environ. Qual. 34, 49–63.
Baum, C., Leinweber, P., Lamersdorf, N., Dimitriou, I., 2009. Effects of short rotation coppice with willows and poplar on soil ecology. LBF 3, 183–196.
Bowen, H.J.M., 1979. Environmental Chemistry of the Elements. Academic Press, London.
Burgos, P., Madejón, P., Madejón, E., Girón, I., Cabrera, F., Murillo, J.M., 2013. Natural remediation of an unremediated soil twelve years after a mine accident: trace element mobility and plant composition. J. Environ. Manage. 114, 36–45.
Carbonell, A.A., Aarabi, M.A., DeLaune, R.P., Grambell, R.P., Patrick, W.H., 1998. Arsenic in wetland vegetation: availability, phytotoxicity, uptake and effects on plant growth and nutrition. Sci. Total Environ. 217, 189–199.
Chang, A.C., Page, A.L., Koo, B.J., 2002. Biogeochemistry of phosphorus, iron, and trace elements in soils as influenced by soil-plant-microbial interactions. Dev. Soil Sci. 28, 43–57.
Ciadamidaro, L., Madejón, P., Puschenreiter, M., Madejón, E., 2013. Growth of *Populus alba* and its influence on soil trace element availability. Sci. Total Environ. 454–455, 337–347.
Crozier, C.R., Boerner, R.E.J., 1986. Stemflow induced soil heterogeneity in a mixed mesophytic forest. Bartonia 52, 1–8.
Dickinson, N.M., 2000. Strategies for sustainable woodland on contaminated soils. Chemosphere 41, 259–263.
Djingova, R., Wagner, G., Peshev, D., 1995. Heavy metal distribution in Bulgaria using *Populus nigra* "Italica" as a biomonitor. Sci. Total Environ. 172, 151–158.
Domínguez, M.T., Marañon, T., Murillo, J.M., Schulin, R., Robinson, B.H., 2008. Trace element accumulation in woody plants of the Guadiamar valley, SW Spain: a large scale phytomanagement case study. Environ. Pollut. 152, 50–59.
Domínguez, M.T., Madrid, F., Marañon, T., Murillo, J.M., 2009. Cadmium availability in soil and retention in oak roots: potential for phytostabilization. Chemosphere 76, 480–486.
Gadd, G.M., 1993. Interactions of fungi with toxic metals. New Phytol. 124, 25–60. Transley, Review 47. pp. 25–60.
Gregorich, E.G., Wen, G., Voroney, R.P., Kachanoski, R.G., 1990. Calibration of rapid direct chloroform extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 22, 1009–1011.
Halvorson, J.J., Gonzalez, J.M., 2008. Tannic acid reduces recovery of water-soluble carbon and nitrogen from soil and affects the composition of Bradford-reactive soil protein. Soil Biol. Biochem. 40, 186–197.
Hartley, W., Riby, P., Dickinson, N.M., Shutes, B., Sparke, S., Scholz, M., 2011. Planting woody crops on dredged contaminated sediment provides both positive and negative effects in terms of remediation. Environ. Pollut. 159, 3416–3424.
Hernández-Allica, J., Becerril, J.M., Zárate, O., Garbisu, C., 2006. Assessment of the efficiency of a metal phytoextraction process with biological indicators of soil health. Plant Soil 281, 147–158.
Hesse, P.R., 1971. A Textbook of Soil Chemical Analysis. John Murray, London.
Houba, V.J., Lexmond, Th.M., Novozamsky, I., Van der Lee, J.J., 1996. The state of the art and future developments in soil analysis for bioavailability assessment. Sci. Total Environ. 178, 21–28.
ISO, 1995. Soil Quality. Extraction of Trace Elements Soluble in Aqua Regia. International Organization for Standardization. ISO 11466:1995.
Kabata-Pendias, A., 2001. Trace Elements in Soils and Plants, third ed. CRC Press, Boca Raton, p. 315.
Kabata-Pendias, A., Pendias, H., 1992. Trace Elements in Soils and Plants. CRC Press, Boca Raton, Florida, USA, p. 365.
Karavanova, E.I., Belyanina, L.A., Stepanov, A.A., 2007. Water-soluble organic matter and soil solution acidity in the mainsoil types of the central forest state biosphere reserve. Eurasian Soil Sci. 40, 493–504.
Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. J. Plant Nutrit. Soil Sci. 163, 421–431.
Ladd, J.N., Butler, J.H.A., 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivates as substrates. Soil Biol. Biochem. 4, 19–30.
Lepp, N.W., Madejón, P., 2007. Cadmium and Zinc in vegetation and litter of a voluntary woodland that has developed on contaminated sediment-derived soil. J. Environ. Qual. 36, 1123–1131.
Madejón, P., Marañon, T., Murillo, J.M., Robinson, B., 2004. White poplar (*Populus alba*) as a biomonitor of trace elements in contaminated riparian forests. Environ. Pollut. 132, 145–155.
Madejón, P., Burgos, P., Cabrera, F., Madejón, E., 2009. Phytostabilization on amended soils polluted with trace elements using the mediterranean shrub: Rosmarinus officinalis. Intern. J. Phytor. 11, 542–557.
Madejón, P., Soler-Rovira, P., Ciadamidaro, L., Cabrera, F., Madejón, E., 2012. Trace element-rich litter in soils: influence on biochemical properties related to the carbon cycle. J. Soils Sedim 12, 663–673.
Marschner, B., Kalbitz, K., 2003. Controls of bioavailability and biodegradability of dissolved organic matters in soils. Geoderma 113, 211–235.
Mertens, J., Van Nevel, L., De Schrijver, A., Piesschaert, F., Osterbaan, A., Tack, F.M.G., Verheyen, K., 2007. Tree species effect on the redistibution of soil metals. Environ. Pollut. 149, 173–181.
Nye, P.H., 1981. Changes of pH across the rhizosphere induced by roots. Plant Soil 61, 7–26.
Ohno, T., Fernandez, I.J., Hiradate, S., Shermann, J.F., 2007. Effects of soil acidification and forest type on water soluble soil organic matter properties. Geoderma 140, 176–187.

Ovington, J.D., 1953. Studies of the development of wood- land conditions under different trees. I. Soil pH. J. Ecol. 41, 13–52.
Perucci, P., 1993. Enzyme activity and microbial biomass in a field soil amended with municipal refuse. Biol. Fertil. Soils 14, 125–137.
Pulford, I.D., Watson, C., 2003. Phytoremediation of heavy metal-contaminated land by trees – a review. Environ. Int. 29, 529–540.
Punshon, T., Dickinson, N.M., 1997. Acclimation of Salix to metal stress. New Phytol. 137, 303–314.
Reuter, D.J., Robinson, J.B., 1997. Plant Analysis. An Interpretation Manual. CSIRO Publishing, Colling-wood, Australia, p. 572.
Robinson, B., Fernández, J.-E., Madejón, P., Marañón, T., Murillo, J.M., Green, S., Clothier, B., 2003. Phytoextraction: an assessment of biogeochemical and economic viability. Plant Soil 249, 117–125.
Robinson, B., Mills, T., Green, S., Chancerel, B., Clothier, B., Fung, L., Hurst, S., McIvor, I., 2005. Trace element accumulation by poplars and willows used for stock fodder. New Zeal. J. Agr. Res. 48, 489–497.
Scheid, S., Günthardt-Goerg, M.S., Schulin, R., Nowack, B., 2009. Accumulation and solubility of metals during leaf litter decomposition in non-polluted and polluted soil. Eur. J. Soil Sci. 60, 613–621.
Selin, H.M., Amacher, M.C., 1997. Reactivity and Transport of Heavy Metals in Soils. CRC, Lewis, Boca Raton, Flo, p. 201.
Shamrikova, E.V., Ryazanov, M.A., Vanchikova, E.V., 2006. Acid–base properties of water-soluble organic matter of forest soils, studied by the pK-spectroscopy method. Chemosphere 65, 1426–1431.
Shi, S., Richardson, A.E., O'Callaghan, M., Deangelis, K.M., Jones, E.E., Stewart, A., Firestone, M.K., Condron, L.M., 2011. Effects of selected root exudate components on soil bacterial communities. FEMS Microbiol. Ecol. 77, 600–610.
Swift, M.J., Heal, O.W., Anderson, J.M., 1979. Decomposition in Terrestrial Ecosystems. Blackwell, Oxford, pp. 130–140.
Tabatabai, M.A., 1982. Soil enzymes. In: Page, A.L., Miller, E.M., Keeney, D.R. (Eds.), Methods of Soil Analyses, Part 2, Chemical and Microbiological Properties. American Society of Agronomy, Madison, pp. 903–947.
Tao, S., Lin, B., 2000. Water soluble organic carbon and its measurement in soil and sediment. Water Res. 34, 1751–1755.
Tice, K.R., Graham, R.C., Wood, H.B., 1996. Transformations of 2:1 phyllosilicates in 41-year-old soils under oak and pine. Geoderma 70, 49–62.
Tyler, G., Pahlsson, A., Bengtsson, G., Bååth, E., Tranvik, L., 1989. Heavy metal ecology of terrestrial plants, microorganism and invertebrates. Water Air Soil Pollut. 47, 189–215.
Van Miegroet, H., Cole, D.W., 1984. The impact of nitrification on soil acidification and cation leaching in a red alder ecosystem. J. Environ. Qual. 13, 586–590.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. Microbial biomass measurements in forests soils: determination of Kc values and test of hypothesis to explain the failure of the chloroform fumigation-incubation method in acid soils. Soil Biol. Biochem. 19, 381–387.
Walkley, A., Black, J.A., 1934. An examination of the Degtjareff method for determining soil organic matter and proposed modification of the chromic acid titration method. Soil Sci. 37, 29–38.
Zinke, P.J., 1962. The pattern of influence of individual forest trees on soil properties. Ecology 43, 130–133.