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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.