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Growth and trace metal accumulation of two *Salix* clones on sediment-derived soils with increasing contamination levels

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Abstract

The growth and metal uptake of two willow clones (*Salix fragilis* 'Belgisch Rood' and *Salix viminalis* 'Aage') was evaluated in a greenhouse pot experiment with six sediment-derived soils with increasing field Cd levels (0.9–41.4 $mg\,kg^{-1}$). Metal concentrations of eight elements were measured in roots, stems and leaves and correlated to total and soil water metal concentrations. Dry weight root biomass, number of leaves and shoot length were measured to identify eventual negative responses of the trees.

No growth inhibition was observed for both clones for any of the treatments (max. 41.4 $mg\,kg^{-1}$ Cd, 1914 $mg\,kg^{-1}$ Cr, 2422 $mg\,kg^{-1}$ Zn, 655 $mg\,kg^{-1}$ Pb), allowing their use for phytoextraction on a broad range of contaminated sediments. However, dry weight root biomass and total shoot length were significantly lower for *S. viminalis* compared to *S. fragilis* for all treatments.

Willow foliar Cd concentrations were strongly correlated with soil and soil water Cd concentrations. Both clones exhibited high accumulation levels of Cd and Zn in aboveground plant parts, making them suitable subjects for phytoextraction research. Cu, Cr, Pb, Fe, Mn and Ni were found mainly in the roots. Bioconcentration factors of Cd and Zn in the leaves were highest for the treatments with the lowest soil Cd and Zn concentration.

Keywords: Phytoremediation; Willows; Contaminated sediment; Dredged; Bioavailability

1. Introduction

Several studies have shown that many species or clones of *Salix* have the capacity to accumulate high levels of Cd in aboveground biomass compartments (Landberg and Greger, 1994; Landberg and Greger, 1996; Felix, 1997; Lunácková et al., 2003; Rosselli et al., 2003; Vandecasteele et al., 2004). *Salix* trees on calcareous metal contaminated sites can thus mobilize Cd from the subsoil and recycle it to the stand surface with leaf fall (Beyer et al., 1990). This raises concerns of increased Cd mobility in natural ecosystems and the risk of food chain contamination. Conversely, this also opens prospects of cleaning Cd contaminated soils through repeated harvesting of the produced wood

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and leaf biomass in carefully managed and monitored short rotation forestry systems (Östman, 1994).

Dredged sediment is a highly suitable substrate for willow growth (Vervaeke et al., 2001). *Salix* is an early colonizer of contaminated dredged sediment-derived soils (Vandecasteele et al., 2002b). However, due to poor national water management in the past and transboundary industrial fluxes from Northern France, most fine-grained sediments of the Belgian inland waterways contain elevated levels of heavy metals. Vandecasteele et al. (2002b) showed that, on historically polluted dredged-sediment disposal sites, concentrations of Cd and Zn in willow leaves increased with levels in the soil. This suggested that foliar Cd and Zn concentrations could be a good indicator of Cd and Zn availability in contaminated sediment-derived soils. Willows can be easily reproduced with cuttings and grow fast, which makes them well suited for testing metal bioavailability and accumulation.

This paper presents results of a greenhouse study which further investigates this correlation. The aim of this experiment was to determine if metal uptake by two willow clones was correlated to the total and soil water metal concentrations over a pollution gradient. The assessment was made for the root, stem, and leaf compartments. Root biomass, number of leaves, and shoot length were determined to identify eventual effects of soil contamination.

2. Materials and methods

2.1. Soil characteristics

Six sites on sediment-derived soils with a range of Cd concentrations were selected for sampling (Table 1). On each site, the 0–30 cm soil horizon was sampled in quadruplicate. Soil material was intensively mixed and homogenized, while stones, plastics and branches if any were removed. Total Cd, Cr, Cu, Ni, Pb, Mn, Fe and Zn concentrations were measured in *aqua regia* extracts on ICP-AES (Varian Liberty Series II, Varian, Palo Alto, CA). Soil pH_{H_2O}, pH_{CaCl_2} and electrical conductivity (EC) were measured in a 1:5 soil to water suspension after stirring for 2 h. Organic carbon (OC) was determined by the method of Walkley-Black, assuming that this method measures 75% of the total organic carbon. $CaCO_3$ content was determined by back-titration with 0.5 M NaOH of an excess of H_2SO_4 added to 1 g air-dried sediment. The grain size distribution of the soil samples was determined using laser diffractometry (Coulter LS200, Miami, FL) with the clay fraction defined as the 0–6 μm fraction (Vandecasteele et al., 2002a). Soil organic and NH_4^+–N was measured by a NH_4^+–N distillation method and then titrated with boric acid. All soils were characterized as calcareous, fertile, heavy clay soils.

2.2. Greenhouse experiment

Containers (LWH: 11 × 11 × 11 cm) were filled with 1.4 kg of each of the six soils at field capacity. Twenty centimeter cuttings of *Salix fragilis* L. 'Belgisch Rood' and *S. viminalis* L. 'Aage' were planted in six replicates for each soil type. Both clones were selected for their elevated metal uptake. All containers were placed in a greenhouse with regular irrigation to keep the soil at a constant moisture content (approx. 30%). Each container was fitted with a 10 cm RhizoMOM soil moisture sampler (Eijkelkamp, Giesbeek, NL) to extract the soil solution. Soil solution was sampled in vacuum tubes after 100 days (d) of plants growth.

Leaves, stems and roots were sampled 100 d after the start of the experiment. For each replicate, the number of leaves, the total shoot length per cutting, the length of the first shoot per cutting, and the dry weight (DW) root biomass were determined. Roots were soaked in water for 24 h and gently washed with abundant water to remove soil particles. They were subsequently dried for 7 d at 40 °C and weighed. To assure a sufficient quantity of roots, stems and leaves for tissue analysis, the replicates per clone and per soil type were combined.

2.3. Chemical analyses of plant compartments and soil solution

Foliar, stem and root samples were dried for 7 d at 40 °C, mechanically ground (Pulverisette 14, Fritsch, Idar-Oberstein, Germany), and stored in dark vials before analysis. Total element concentrations in roots, stems and leaves are extracted with HNO_3 (p.a. 65%) and H_2O_2 (ultrapure) in a 3:1 ratio using microwave digestion and measured with ICP-AES (Varian Liberty Series II, Varian, Palo Alto, CA). The accuracy of the tissue element analysis was checked using BCR 60 (Aquatic plant) for Cd, Cu, Mn and Zn, and CRM 100 (Beech leaves) for Cr.

Soil solution was analyzed for Cd, Zn, and Cu using Flame Atomic Absorption Spectrometry (Varian SpectrAA 10) and ET Atomic Absorption Spectrometry with Zeeman correction (Varian SpectrAA 800, Varian, Palo Alto, CA).

2.4. Statistical analysis

The metal concentration in the different tissue compartments of the plants was studied by a general linear model with one continuous variable (concentration in the soil) and two factors (clone and tissue compartment). This allowed to test statistically if the relation with the soil concentration depended on clone or tissue