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Poplar response to cadmium and lead soil contamination

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ABSTRACT

An outdoor pot experiment was designed to study the potential of poplar (*Populus nigra* 'Italica') in phytoremediation of cadmium (Cd) and lead (Pb). Poplar was treated with a combination of different concentrations of Cd (w = 10, 25, 50 mg kg^{-1} soil) and Pb (400, 800, 1200 mg kg^{-1} soil) and several physiological and biochemical parameters were monitored including the accumulation and distribution of metals in different plant parts (leaf, stem, root). Simultaneously, the changes in the antioxidant system in roots and leaves were monitored to be able to follow synergistic effects of both heavy metals. Moreover, a statistical analysis based on the Random Forests Analysis (RFA) was performed in order to determine the most important predictors affecting growth and antioxidative machinery activities of poplar under heavy metal stress. The study demonstrated that tested poplar could be a good candidate for phytoextraction processes of Cd in moderately contaminated soils, while in heavily contaminated soil it could be only considered as a phytostabilisator. For Pb remediation only phytostabilisation process could be considered. By using RFA we pointed out that it is important to conduct the experiments in an outdoor space and include environmental conditions in order to study more realistic changes of growth parameters and accumulation and distribution of heavy metals. Also, to be able to better understand the interactions among previously mentioned parameters, it is important to conduct the experiments during prolonged time exposure., This is especially important for the long life cycle woody species.

1. Introduction

Heavy metals are considered one of the most serious environmental pollutants worldwide. Among all heavy metals, the most common are cadmium and lead, which can occur as by-products of petrochemical industries and traffic. In response to a growing need to address environmental contamination, many mechanically or physiochemically based remediation technologies have been developed to treat contaminated soil (Marques et al., 2009). However, these technologies are usually expensive and soil disturbing, sometimes rendering the land useless as a medium for further activities such as plant growth. Consequently, phytoremediation, a biologically based technology is gaining attention of both soil remediation scientists and the general public. Phytoremediation is a promising, inexpensive and aesthetically acceptable, in situ clean-up technology that exploits the natural ability of green plants to remove contaminants from the environment or render toxic compounds harmless (Polle et al., 2013; Carbisu and Alkorta, 2001).

The ability of plants to absorb and accumulate significant quantities of heavy metals in aboveground parts offers the possibility to use them for removing heavy metals from their substrate. Plant accumulation of heavy metals from soil depends on a large number of factors and varies significantly among plant species (Pietrini et al., 2010; Evangelou et al., 2013). The bioavailability of metals in soil and various physiological processes in plants (e.g. plant growth stage, exposure time and heavy metal concentrations in soil, soil pH) significantly affect heavy metal toxicity, adsorption and distribution in the plant (Bhargava et al., 2012; Zárubová et al., 2015). The most common symptoms of heavy metal phytotoxicity are leaf chlorosis, growth inhibition, disturbance of respiration and nitrogen metabolism, and the reduction of photosynthesis and water and nutrient uptake (Benavides et al., 2005; Wang et al., 2008). Additionally, heavy metal generates oxidative stress through the overproduction of reactive oxygen species (ROS) which can lead to cell death due to membrane lipid peroxidation, protein oxidation, enzyme inhibition and damage of nucleic acid. To ameliorate this oxidative

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stress, plants employ the antioxidative machinery by alternations in activities of antioxidative enzymes (e.g. superoxide dismutase SOD, ascorbate peroxidase APX, catalase CAT, glutathione reductase GR, guaiacol peroxidase GPX, and glutathione S-transferase GST) and the level of low molecular weight antioxidants (ascorbic acid, reduced glutathione, carotenoids, phenolics) (Benavides et al., 2005; Hossain et al., 2012). The maintaining of the balance between ROS and the antioxidative system is crucial for plant adaptation and its survival under stressful conditions such as heavy metal contamination (He et al., 2011; Sharma et al., 2012).

One of the phytoremediation research strategies is to identify plant species which are able to tolerate and accumulate a range of heavy metals in their harvestable parts coupled with higher biomass yield (Bhargava et al., 2012). Accordingly, special attention is focused on the potential use of trees in phytoremediation of heavy metals due to a number of good characteristics, such as fast growth accompanied with high biomass yield, a deep root system, aesthetically acceptable and cheap cultivation (French et al., 2006; Brunner et al., 2008; Luo et al., 2016). From a diverse range of tree species used for phytoremediation, the most extensively investigated species are some fast-growing trees belonging to the genus *Salix* and *Populus*. Many studies indicated that various *Populus* species could tolerate and accumulate high concentrations of heavy metals such as cadmium (Cd), nickel (Ni), zinc (Zn), copper (Cu) and lead (Pb) in the aboveground tissues, making them good candidates for phytoremediation processes (Nikolić et al., 2008; Pietrini et al., 2010; Wu et al., 2010; Gaudet et al., 2011; He et al., 2011, Bhargava et al., 2012; Chen et al., 2014, Dai et al., 2013; He et al., 2013a; Jakovljević et al., 2013).

Furthermore, the majority of the studies reported plant performance under stress caused by one of heavy metal despite the fact that polluted soils are typically contaminated by more than one metal. (He et al., 2013a, 2013b, 2011; Domínguez et al., 2011; Chen et al., 2014). Based on the aforementioned, in order to get more information about the potential of poplar (*Populus nigra* 'Italica') in phytoremediation of synergistic effects of cadmium (Cd) and lead (Pb), the outdoor pot experiment was designed. Poplar was treated with the combination of different concentrations of Cd (w = 10, 25, 50 mg kg^{-1} soil) and Pb (400, 800, 1200 mg kg^{-1} soil) and several physiological and biochemical parameters were monitored including the accumulation and distribution of metals in different plant parts (leaf, stem, root). Simultaneously, the changes in the antioxidant system in roots and leaves were monitored. Moreover, a statistical analysis based on the Random Forests Analysis (RFA) a new and powerful statistical classifier, was performed in order to determine the most important predictors affecting growth and antioxidative machinery of poplar under heavy metal stress.

2. Material and methods

2.1. Plant material and experimental design

The cuttings of poplar (*Populus nigra* 'Italica') were collected and grown at the experimental station of Croatian Forest Research Institute (CFRI) in Jastrebarsko, Croatia (N 45°40'18.71", E 15°39'4.27"). For experiment purposes, rooted one-year old cuttings were used. To simulate field conditions, the pot experiment was conducted outdoors from 1st June 2012 to 30th September 2012 and the meteorological data (total rainfall, relative humidity, and average day/night temperature, average light time) for the experimental station were obtained from Croatian Meteorological and Hydrological Service (Supplementary Table 1s). The soil used in this study was Durpeta, Universal Substrate, Lithuania made from eco-friendly peat, enriched with nutritional supplements and microelements (pH (H_2O) 5.5–6.5, N 140–200 mg L^{-1}, P_2O_5 120–300 mg L^{-1}, K_2O 150–250 mg L^{-1}, total organic 92–96%). The concentration of cadmium and lead in soil was analyzed before the treatment. The results showed that metal concentration in soil is less than LOQ (LOQ_{Cd} < 0.5 mg kg^{-1} and LOQ_{Pb} < 2.4 mg kg^{-1}). The soil was placed in containers and spiked with a solution of $CdCl_2{\cdot}H_2O$ and $Pb(NO_3)_2$ in order to obtain appropriate Cd (0, 10, 25 and 50 mg Cd kg^{-1} soil) and Pb (0, 400, 800 and 1200 mg Pb kg^{-1} soil) concentrations in soil and then left to equilibrate outdoors under a waterproof tarpaulin for two months. Soil samples were placed into 10 L plastic pots, while single cuttings of poplar were planted in 24 pots per 6 treatment groups (Table 1). The additional water irrigation was not performed. The irrigating water was not additionally or not The plants grown in untreated pots were kept as control plants (C = control plants). The growing period (from GP1 to GP4) was four months and each month six plants were harvested (from each growing period and each treatment group). After each growing period plant biomass was recorded. During the experiment no growth restriction due to limited pot volume was observed.

Table 1
Abbreviations of treatment groups with corresponding Cd and Pb content.

Treatment group	Cd [mg kg^{-1} soil]	Pb [mg kg^{-1} soil]
C	0	0
LCdLPb	10	400
MCdMPb	25	800
HCdHPb	50	1200
LCdHPb	10	1200
HCdLPb	50	400

2.2. Analysis of metals in plant material and soil

Harvested plants were separated into roots, stems and leaves. They were washed twice, first with tap water to remove soil, and then with deionised water. Plant samples were oven-dried at 80 °C for 24 h (Rautio et al., 2010). Dry samples were ground to obtain a homogeneous powder in a metal-free mill (Ika-Werke, M20, Germany). Collected soil samples were air-dried, ground using a mortar and pestle and passed through a stainless steel test sieve (Retsch, Germany) into fractions < 2 mm (Cools and De Vos, 2010). Approximately 0.3 g of sub-samples were digested using a mixture of 1 mL 30% H_2O_2 and 65% HNO_3 (5 mL) for plants (Rautio et al., 2010) and a mixture of 3 mL 65% HNO_3 and 9 mL 37% HCl for soil (ISO 11 047, 1998) in a closed high-pressure microwave system (Anton-Paar, Multiwave 3000, Germany). Sub-samples were analyzed for Cd and Pb using Graphite Furnace Atomic Absorption Spectroscopy (Perkin-Elmer, Aanalyst 600, USA).

2.3. Evaluation of Cd and Pb phytoextraction ability of poplar

In order to evaluate the Cd phytoextraction ability in poplar cadmium accumulation, the bioconcentration factors (BCF), translocation factors from concentration (TF) and from accumulation (TF’) were calculated at the end of the experiment (Wu et al., 2010). The bioconcentration factor (BCF) was calculated as the ratio of the Cd concentration in plant shoots to the Cd concentration in soil as follows:

$$BCF = \frac{C_{shoots}}{C_{soil}} \quad (1)$$

where, C_{shoot} is the concentration in plant shoots and C_{soil} is the concentration in soil.

The translocation factor from concentration (TF) was calculated as the ratio of the Cd concentration in plant shoots to the Cd concentration in plant roots as follows:

$$TF = \frac{C_{shoot}}{C_{root}} \quad (2)$$

where, C_{shoot} is the concentration in plant shoots and C_{root} is the concentration in plant root.

The translocation factor from accumulation (TF´) was calculated as