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Different behaviours in phytoremediation capacity of two heavy metal tolerant poplar clones in relation to iron and other trace elements

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ABSTRACT

Plant biodiversity and intra-population genetic variability have not yet been properly exploited in the framework of phytoremediation and soil reclamation. For this reason, iron and other metal accumulation capacity of two Cu and Zn tolerant poplar clones, namely AL22 (*Populus alba* L.) and N12 (*Populus nigra* L.), was investigated in a pot experiment. Cuttings of the two clones were planted in iron rich soil collected from an urban-industrial area. Concentrations of Cd, Cu, Fe, Pb and Zn were analysed in leaves (at different times), as well as in stems and in roots (at the end of the experiment), both in control plants and in plants grown on a soil whose Fe availability was artificially enhanced. Results showed that Cd and Zn were preferentially accumulated in leaves, whereas Cu, Fe and Pb were mainly accumulated in roots. The main differences in metal accumulation between clones were related to Cd (about tenfold higher concentrations in N12) and Cu (higher concentrations in AL22). Once soil Fe availability was enhanced, the uptake and accumulation of all metals declined, with the exception of Fe at the first sampling time in AL22 leaves. The different behaviour of the two poplar clones suggests that a thoughtful choice should be made for their use in relation to soil heavy metal remediation.

1. Introduction

Iron is one of the most abundant elements on Earth and it is essential to all organisms, being involved in crucial processes like electron transport in photosynthesis and respiration, and blood oxygen exchange in vertebrates. On a global scale, it plays a key role in producing the Earth magnetic field, which protects the biosphere from geomagnetic storms and streams of charged particles ejected from the sun surface (Birkeland, 1916), that are extremely dangerous to all living beings. Even the evolution of the human society has been tightly related to iron use since the early stages of human civilization, and it still heavily relies on iron industry. The huge scale of iron processing, amounting to several millions of tons in many countries worldwide, determines both local and broad scale contamination issues to comply with. Iron, indeed, can enter the food chains and affect human beings through the biomagnification process (Wong, 2003). High ingested doses of Fe can cause serious health problems including DNA, cellular and tissue damages, oncogene activations, hemorrhagic necrosis, sloughing of the stomach mucosa, diabetes, atherosclerosis, and hormonal and immune system abnormalities (Gurzau et al., 2003). The problems related to environmental contamination, however, are not limited to iron. The environmental pollution caused by heavy metals (HMs) in general has been accelerated dramatically over the last decades, since emerging countries, which recently appeared on the global market, have increased their own mining and metallurgical industries. This resulted in a significant increase of polluted sites, both due to the metallurgical deposits and to solid, liquid and gaseous industrial wastes (Wong, 2003). In this context, the reduction of environmental contaminations and human health risks are primary challenges, and appropriate reclamation techniques are needed. Among these, a green technology, known as phytoremediation, underwent a remarkable development and gained broad consensus as an environmentally friendly alternative to the commonly used chemical and physical methods. Phytoremediation involves the use of plants for the reclamation of air, soil and water contaminated by organic and inorganic pollutants (Pilon-Smits, 2005). Phytoremediation is a general term including phyto-extraction, -stabilization, -degradation, -stimulation, -volatilization and rhizo-filtration. In particular, phytoextraction and phytostabilization refer to the preferential accumulation of pollutants in epigeous organs or in roots, respectively (Pilon-Smits, 2005). Phytoremediation has increased its importance during the last decade (Pilon-Smits, 2005; Golubev, 2011), becoming the

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leading technology for soil and water reclamation in developed countries. It has several advantages, such as cost effectiveness, beneficial environmental impact, good acceptance from public opinion and politicians. However, this technique has also some drawbacks, mainly related to the long time needed to obtain an effective cleaning of the polluted areas, as compared to traditional engineering technologies, that are much faster but also extremely expensive for the municipalities and the environment (Mulligan et al., 2001). Phytoremediation, and phytoextraction in particular, can be achieved through hyperaccumulator plants, which have the capability to take up large quantities of HMs and to translocate them to the epigeous parts (Baker et al., 1994a; Wong, 2003), as in the case of *Thlaspi caerulescens* (Baker et al., 1994b) and *Sedum alfredii* (Yang et al., 2004). Hyperaccumulators, however, have strong limitations, due to the slow plant growth and the small biomass they produce, which limit their capacity to effectively reclaim contaminated soils. In the last ten years, to overcome these limitations, scientists have focused their attention on high biomass producing plants, such as maize, sunflower, rice, etc. (Baldantoni et al., 2011b; Komarek et al., 2007; Murakami and Ae, 2009), and on highly productive trees such as willow and poplar (Cicatelli et al., 2010; Tognetti et al., 2013). Poplar, in particular, has several interesting features that make it a good candidate for phytoremediation purposes. It is a fast growing tree with a deep and wide root system, it has a marked adaptability to different soils and climates and a unique capability to vegetative reproduction, which makes its propagation easy. Moreover, it has a very good tolerance to different contaminants, both organics and inorganics; can be pre-inoculated with *Arbuscular mycorrhizal* fungi (Baldantoni et al., 2011a; Lingua et al., 2008), and has high genetic biodiversity at both the species and the population level (Castiglione et al., 2009). Its capability to be used in short rotation coppices, in addition, allows its use in biomass production.

Plant genetic biodiversity has a great potential for phytoremediation. Nehnevajova et al. (2005) demonstrated that different cultivars of sunflower have different HM tolerances, and some of them show pronounced phytoextractive and HM accumulating capacities. However, this potential was not properly exploited yet in the case of poplar and *Salicaceae* in general. Many papers in the scientific literature, indeed, focus their attention on a limited numbers of clones, or compare one or few clones of the same species with one or few belonging to different species (Pietrini et al., 2010; Migeon et al., 2012). Thus there has not been a clear evaluation of the phytoremediation capacities associated to the high genetic biodiversity present in the different poplar populations and species (Castiglione et al., 2009; Pietrini et al., 2010; Migeon et al., 2012).

In this context, the purposes of the present research were to:

i) compare the capacity of two poplar clones, namely AL22 (*Populus alba* L.) and N12 (*Populus nigra* L.), previously selected for their high tolerance to HMs, to phytostabilize or phytoextract, Cd, Cu, Fe, Pb and Zn from an artificially Fe polluted soil;
ii) evaluate the influence of high concentrations of soil available Fe, supplied as iron sulphate, on uptake and bioaccumulation of the analysed metals in roots, stems and leaves of the two poplar clones.

2. Materials and methods

2.1. Experimental setting and analyses

Six cuttings for each metal tolerant (Castiglione et al., 2009) *P. alba* (AL22) and *P. nigra* (N12) clones were planted (March 2007) in pots filled with soil collected at a depth of 0–20 cm from an urban-industrial area (40°42′40″ N; 14°46′49″ E) close to the town of Salerno (Italy). Pot filling soil was characterised at the beginning of the experiment (t_0) for pH in distilled water (electrometric method on soil solution at a ratio of 1.0:2.5 w:w = soil:water; HI 4212, Hanna, Italy), organic matter content (loss on ignition at 550 °C for 4 h; Controller B 170, Nabertherm, Germany) and total and available concentrations of Cd, Cu, Fe, Pb and Zn (see for details Baldantoni et al., 2011a, b). Iron sulphate, $Fe_2(SO_4)_3$, was added to the soil of three pots for each clone (T = Treated plants) three times at weekly intervals, starting one month before the first leaf sampling (June 2007), reaching a final concentration of 450 $\mu g\ g^{-1}$ d.w. The plants grown in the untreated pots were kept as controls (C = Control plants).

Poplar leaves were collected after four (July 2007), five (August 2007), seven (October 2007) and sixteen (July 2008) months from cutting plantation. At the end of the experimental period (July 2008), stems and roots, as well as soils, were collected from all the pots. All the matrices were analysed for Cd, Cu, Fe, Pb and Zn concentrations. Metal total concentrations in leaves were determined on pulverised (liquid nitrogen) and dried (75 °C, up to constant weight) samples, in stems and roots on ashes obtained from loss on ignition (550 °C for 4 h; Controller B 170, Nabertherm, Germany), and in soil granulometric fractions (<2 mm) on samples dried (75 °C up to constant weight) and pulverized in a planetary ball mill (PM4, Retsch, Germany) with agate mortars. The samples were digested with an acid mixture (65% HNO_3: 50% HF = 2:1 v:v) in a microwave oven (Ethos, Milestone, Shelton, CT, USA) as reported in detail by Baldantoni et al. (2009). The soil available (DTPA-extractable) fraction of metals was extracted from the granulometric fractions with a diethylenetriamine-pentaacetic acid (DTPA) solution (0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA, pH 7.3) at room temperature in continuous agitation for 2 h (Lindsay and Norvell, 1978). Metal concentrations were determined by atomic absorption spectrometry (AAnalyst 100, PerkinElmer, Wellesley, MA, USA), via air-acetylene flame (Fe and Zn), or a graphite atomizer (Cu, Pb and Cd). Three replicates of each sample were carried out. To test the accuracy of the obtained data, standard reference materials (*Olea europaea* leaves BCR 62 – Commission of the European Communities, 1982; pine needles 1575a – NIST, 2004; calcareous loam soil BCR CRM 141R – European Commission, 1996) were also analysed, obtaining good recoveries.

2.2. Data processing

Heavy metal translocation in plants was evaluated using the Translocation Factors (TFs), calculated as C_l/C_r, C_s/C_r and C_l/C_s, where C_l is the metal concentrations in the leaves, C_s in the stems and C_r in the roots. TFs allow to define the phytostabilization or phytoextraction behaviour in respect to each HM (An, 2004; Lingua et al., 2014). Metal Bioavailability Factors (MBFs) were calculated as the percentage of each element available fraction with respect to the total concentration of the same element in the analysed soil.

The HM Bio-Accumulation Factors (BAFs) were calculated as the ratio of metal concentrations in the three different organs of the plants to soil total concentrations, C_p/C_{soil}, where C_p is the metal concentration in the different plant organs and C_{soil} is the metal concentration in the soil at the end of the experiment.

All the statistical analyses were performed with the R 3.0.2 programming environment (R Core Team, 2013), using the functions of the “lmtest” (Zeileis and Torsten, 2002), “nortest” (Gross and Ligges, 2012), “stats” (R Core Team, 2013) and “vegan” (Oksanen et al., 2013) packages. The differences between treatments, among organs, among the samplings and between the two poplar clones were evaluated by two different three way ANOVA