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Investigation on metal tolerance and phytoremoval activity in the poplar hybrid clone “Monviso” under Cu-spiked water: Potential use for wastewater treatment

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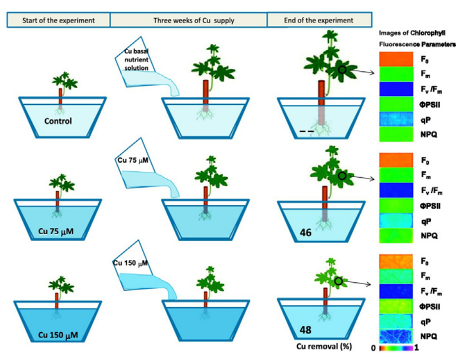
HIGHLIGHTS

- Poplars were exposed to higher Cu levels (75 and 150 μM) than those allowed by Italian law for water.
- Plants showed good Cu tolerance especially at the lowest concentration supplied.
- Photosynthetic data were consistent with the growth responses at both Cu levels.
- High Cu accumulation in the roots allowed the 50% Cu removal from spiked solution.
- Perspectives for using such plants for Cu removal from wastewater were highlighted.

GRAPHICAL ABSTRACT



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ABSTRACT

A serious concern for the environmental and human health is represented by the increasing copper (Cu) occurrence in agricultural soils and waters, because of the possible food contamination and bioaugmentation along the trophic chain. The request for the decontamination of different matrices with an environmentally sustainable technology as the phytoremediation should be addressed by selecting plant materials with improved pollutant tolerance and removal capability. With this purpose, plants of the hybrid poplar clone “Monviso” (*Populus* × *generosa* A. Henry × *P. nigra* L.) were grown in growth chamber under hydroponics and exposed to excess Cu concentrations (T_1, 75 μM Cu; T_2, 150 μM Cu), selected as about 5 and 10 times higher than those allowed by the Italian regulation on water use. Results evidenced a notable Cu tolerance by poplar plants, particularly at the lowest Cu concentration. At organ level, the root system was the most affected by Cu treatment, especially in T_2-exposed plants. Copper determinations revealed that the metal was mostly bioaccumulated in the roots, with a limited amount reaching the shoots. Chlorophyll content and fluorescence analyses confirmed the visible symptoms in leaves, highlighting a good physiological status in T_1-exposed plants. Contrarily, an impairment of the main processes associated to photosynthesis was observed in T_2-exposed plants also by gas exchange measurements. Remarkably, the Cu content analysis of the spiked water solutions revealed that poplar plants

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succeeded in removing almost the 50% of the total Cu amount added. These results strengthen the evidence that poplar plants represent a useful eco-friendly bio-tool for the decontamination of metal polluted waters.

1. Introduction

The increasing levels of heavy metals in soil and waters represent a primary environmental concern worldwide. In fact, in these last decades, the anthropogenic activities have resulted in a massive heavy metal enrichment of the ecosystems, far over the release due to the natural biogeocycles. The alteration of the natural metal occurrence in soils and waters has led to threaten the stability of the ecosystem and the human health. Particularly, in the agricultural soils the excess levels of some heavy metals, such as copper, zinc, iron, lead, manganese, cadmium, commonly present in the fertilization and pesticide formulation, pose serious concerns over the contamination of the food crops and the possible bioconcentration effect along the food chain. In addition, in many countries water shortage have led farmers to irrigate crops with wastewaters to fulfill the water requirements for agricultural production. Long-term irrigation with wastewater may result in the contamination of hazardous substances, including heavy metals, over soils and increase the risk of metal transfer to food plants (Alam et al., 2003; Pimentel et al., 2004; Zhuang et al., 2009). In this regard, wastewater reclamation and reuse have been receiving increasing attention to address the demand for water for crop irrigation while experiencing water scarcity (Shah et al., 2010). Recently, several approaches have been studied for the development of low cost, low energetic input and environmentally friendly technologies. Among them, the use of plants to remove contaminants from different matrices, *i.e.* the phytoremediation, has attracted a great deal of interest in recent years (Giachetti and Sebastiani, 2006; Guidi Nissim et al., 2014; Macek et al., 2004). To be really effective, phytoremediation should rely on selected plant materials for improved heavy metal tolerance and accumulation (Marmiroli et al., 2011; Pietrini et al., 2010; Stoláriková-Vaculíková et al., 2015). Different plant species with a natural capability to hypertolerate and hyperaccumulate heavy metals have been extensively studied (Prasad and Freitas, 2003; Van der Ent et al., 2013). Unfortunately, most of these species, called hyperaccumulators, are characterized by a low biomass and a limited soil exploration by roots and therefore they are recognized as very useful for studying the biological mechanisms underlying the metal tolerance and accumulation (Galardi et al., 2007). On the contrary, a great deal of interest is actually paid to non metal hyperaccumulating plants (Brunner et al., 2008; Iori et al., 2013). Such plants, both herbaceous and woody, can show a good metal tolerance, allowing them to produce large biomass through a fast growing process, a large rooting system, an adaptability to the severe soil conditions characterizing the contaminated sites. All these biological properties are well expressed in the fast growing forest plants like poplars and willows (Di Baccio et al., 2011a; Fischerová et al., 2006; Guerra et al., 2011; Marmiroli et al., 2011), making them good candidates for successful phytoremediation applications. Moreover, being these riparian plant species notably tolerant to the submersion, their utilisation in the decontamination of polluted waters has been proposed (Guidi Nissim et al., 2014; Iori et al., 2015; Skłodowski et al., 2014; Zacchini et al., 2009). Finally, these plants are not recognized as taking part in the human food chain. Therefore, their use as phytoremediation agents can allow to obtain the decontamination of polluted substrates while producing biomass for energy or timber products, realizing multiple ecological services and an income for farmers suffering from a restriction of the soil cultivation.

In this work, the attention was focused on the poplar clone "Monviso" (*Populus* × *generosa* A. Henry × *P. nigra* L.), which is largely utilised in the Mediterranean area under short rotation coppice plantation for high biomass production in adverse environments (Di Baccio et al., 2011b; Di Matteo et al., 2015; Sixto et al., 2014). The goal was both to study some traits related to the metal tolerance and accumulation ability, useful as predictors of the phytoremediation potential of plant material, and to evaluate the effective metal removal by plants under controlled conditions. In order to investigate the possible use of this plant species for the decontamination of metal polluted waters, a hydroponic growth system was chosen, being this system largely utilised in similar laboratory trials (Fernàndez-Martínez et al., 2014; Migeon et al., 2012). Copper (Cu) was chosen as a model heavy metal for this study, as the contamination of soil and waters by this metal is largely diffused, especially in Mediterranean countries, due to the massive utilisation of Cu sulphate in the pest management in agriculture, particularly for vine cultivation. Copper is easily taken up by plants, being an essential micronutrient for growth and development, even if at high concentrations it can become extremely toxic causing symptoms such as chlorosis and necrosis, stunting, leaf discoloration and inhibition of root growth (Borghi et al., 2008; Cuypers et al., 2000). To assay the ability of the plants to cope with and remove Cu from spiked water solutions, the concentrations utilised in this work ranged from the plant physiological level to about 5 and 10 times the threshold allowed by the regulatory limit for civil and ground water widespread in many European countries including Italy ($1\ mg \cdot L^{-1}$, D.Lgs n.152/2006, 2006).

2. Materials and methods

2.1. Plant material, experimental conditions and biometric analyses

Rooted cuttings of poplar (*Populus* × *generosa* A. Henry × *P. nigra* L. clone "Monviso") were grown under hydroponics using a modified third strength Hoagland nutrient solution (pH 6.5), prepared as described in Zacchini et al. (2009), in a growth chamber (t: 25 ± 3 °C; Photosynthetic Photon Flux Density, PPFD: 370 $\mu mol \cdot m^{-2}\ s^{-1}$; photoperiod: 14-h light/10-h dark). After three weeks of acclimation, three randomized groups of homogeneously grown plants were distributed to control (C, basal nutrient solution, 0.107 μM Cu), T_1 (75 μM Cu) and T_2 (150 μM Cu) pots. Two plastic pots containing four plants and 5 L Hoagland solution each per treatment were prepared. Copper was supplied as $CuSO_4 \cdot 5H_2O$. The solutions were renewed every three days to avoid nutrient and oxygen deprival throughout the experimental trial. At each step, aliquots of the solution taken from the pots were filtered, acidified and stored until Cu determination. At the end of three weeks of treatment, after physiological analyses, leaf number was assayed and stem length was measured by a ruler while root volume was determined according to Shevyakova et al. (2011). Then, plants were harvested, carefully washed with distilled water, separated into leaves, stem, cutting and roots and dried in an oven at 105 °C and finally weighed. These determinations were used to calculate also the shoot to root ratio.

2.2. Copper determination

Copper concentration in the plant organs and in the solution was measured by an atomic absorption spectrophotometer (Perkin Elmer, Norwalk, CT, USA). Plant tissues were previously mineralized by treating powdered samples with concentrated HNO_3, distilled water, and H_2O_2 (30% vol/vol in water) and heating (EXCEL Microwave Chemistry Workstation, Preekem Scientific Instruments Co., Ltd., Shangai), as reported by Iori et al. (2013). The solutions were filtered and acidified to pH 2–3 with concentrated HCl before Cu determination.