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Research article

Effects of heavy metals and chelants on phytoremediation capacity and on rhizobacterial communities of maize

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A B S T R A C T

Heavy metals (HMs) are one of the major ecological problem related to human activities. Phytoremediation is a promising "green technology" for soil and water reclamation, and it can be improved by means of the use of chelants. In the past particular attention was paid on the effects of HMs and/or chelants on plant health, but much less on their effects on rhizosphere communities. To shed light on the interaction among plant-HM-chelant-rhizobacterial community a pot experiment was set up. Maize plants were grown on uncontaminated, multi-metal (copper and zinc) contaminated and chelants artificially amended soils. A high concentration of HMs was detected in the different maize organs; chelants improved the accumulation capacity of the maize plants. The rhizosphere bacterial community isolated from control plants showed the largest biodiversity in terms of bacterial genera. However, the addition of HMs reduced the number of taxa to three: *Bacillus*, *Lysinibacillus* and *Pseudomonas*. The effects of HM treatment were counteracted by the addition of chelants in terms of the genetic biodiversity. Furthermore, several bacterial strains particularly resistant to HMs and chelants were isolated and selected. Our study suggests that the combined use of resistant bacteria and chelants could improve the phytoremediation capacity of maize.

1. Introduction

Heavy metal contamination of soils is historically one of the most severe environmental problems. HMs and especially their persistent toxicity cause severe damages to ecosystems, economic losses and negatively impact the human food chain and human health (McLaughlin et al., 2000). Among the many methodologies used for the reclamation of contaminated sites, phytoremediation is currently considered to be a promising and cost-effective solution. This approach involves the use of plants to improve soil and/or water quality by inactivating or translocating pollutants in the different plant organs without negative effects on soil biological activity, structure and fertility (Ebbs et al., 1997; Salt et al., 1995). Several efforts have been made to develop sustainable and environmentally friendly technologies useful to extract and remove toxic HMs from water and soil. The hyperaccumulator plants, with an exceptional metal accumulating capacity in their aboveground organs (Kumar et al., 1995) were initially investigated. However, because of their slow growth rate and low biomass production, they were not the right solution for cleaning up contaminated sites (Lasat, 2002). As an alternative, high biomass crops (*e.g.* maize, sunflower, etc.) and chelant-assisted phytoremediation were proposed for improving HM solubilization and availability (Baldantoni et al., 2011; Chen et al., 2004; Grcman et al., 2003). Chelating agents such as EDTA [ethylenediaminetetraacetic acid (Gheju and Stelescu, 2013; Maxted et al., 2007; Saifullah et al., 2009)], EDDS [[S,S]-ethylenediaminedisuccinate (Komarek et al., 2009)] and polyaspartic acid (Lingua et al., 2014) have been tested due to their high effectiveness in HM soil mobilization. Several studies have reported the use of EDTA and its biodegradable structural isomer EDDS as chelating agents suitable for enhancing metal extraction from contaminated substrates, or metal uptake by different plant species (Komarek et al., 2011). However, their use presents several disadvantages, including the competition of major soil cations for the ligand (Komarek et al., 2009; Nowack et al., 2006), the risk of leaching (Nowack, 2002), and, last but not least, the limitation of plant growth due to toxicity and low biodegradation rate.

The success of phytoremediation also depends upon beneficial

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associations between microorganisms and plants (Baker et al., 2000). Plants and soil microorganisms, in particular the rhizosphere bacteria, can establish specific associations in which the plant provides a carbon source (Belimov et al., 2004) for bacteria and, in turn, the rhizobacteria produce plant growth-promoting substances, which help to decrease the negative impact of HM toxicity (Glick, 1995; Kloepper et al., 1989; Kumar et al., 2008). Plant root-microorganism interaction is strongly influenced by the presence of pollutants in the soil. The high HM soil contamination can affect the dimension and structure of bacteria communities, and overall, the activity of soil rhizobacteria (Belyaeva et al., 2005). Although microbial communities in HM-polluted soils have been studied (Margesin et al., 2011; Piotrowska-Seget et al., 2005), little is known about their complexity in maize grown on metal contaminated sites (Khan, 2005). Currently, great attention is being focused on the microbial treatment of HM contaminated sites (Glick, 2010) and there is a need for greater knowledge of metal resistant bacterial strains.

A high ability to remove and accumulate HMs was reported for the cereal crop maize [*Zea mays* L. (Hernandez-Allica et al., 2008; Murakami and Ae, 2009)]. Maize is an annual cereal crop characterized by rapid growth, an extensive fibrous root system with large biomass, resistance to adverse conditions and abundant seed production, easy to cultivate and useful for repeated cropping (Garbisu and Alkorta, 2001; Zhang et al., 2008). Although not listed as a hyperaccumulator, maize can be used for phytoextraction (Ebbs et al., 1997; Meers et al., 2005). Thus, greater knowledge of the indigenous rhizosphere soil bacteria of maize might be crucial for clarifying the mechanism of the plant-bacteria interactions and accelerating the efficiency of phytoremediation.

The aims of the present study were to:

- evaluate the effect of chelating agents on the phytoextraction potential of *Z. mays* (cultivar DDK 743) grown on artificially multi-metal (copper and zinc) polluted soil;
- investigate the effects of HM contamination and chelants (EDTA and/or EDDS) on rhizobacterial communities associated to maize;
- isolate and characterize HM tolerant maize rhizosphere bacterial strains that are potentially useful for phytoremediation.

2. Materials and methods

2.1. Experiment set-up

The experiments were conducted in pots using a garden soil collected at a depth of about 10 cm in the vicinity of the Campus of the University of Salerno (Fisciano, SA-Italy). The soil was characterized for several parameters (Table 1). The methods used for the determination of organic carbon and available phosphate content in the soil have been described by Walkley and Black (1934) and by Olsen et al. (1954), respectively. The ammonium content of the soil was determined with an ammonia electrode as ammonium formed by Kjeldahl digestion (Bremner, 1965). The total carbon, total nitrogen and C:N ratio were determined through the combustion method of elemental analyzer. The barium chloride-triethanolamine method of Mehlich (Mehlich, 1938) was employed to estimate the Cation Exchange Capacity (CEC) of the used soil. Eleven kg of dry weight (dw) soil were transferred in six liter rectangular plastic pots and 10 seeds of the *Z. mays* (cultivar DDK 743) were sown in each pot. After germination, the soil of four pots was artificially contaminated with $Cu(NO_3)_2 \cdot 3H_2O$ and $Zn(NO_3)_2 \cdot 6H_2O$ to a final concentration of 150 and 450 mg kg^{-1} dw, respectively; while one pot was fertilized with ammonium nitrate (control sample), using the same amount of N employed for HM contamination of the pot soil. Seven weeks after sowing, EDTA and/or EDDS were added to the HM contaminated soils as follows: 5.0 mmol kg^{-1} dw soil of EDTA or EDDS, 3.33 mmol kg^{-1} dw soil of EDTA plus 1.67 mmol kg^{-1} dw soil of EDDS. Chelants were added three times in order to reach the final concentrations reported above and in this way to reduce their toxicity towards maize plants and microbial communities. At the end of the experiment (approx.150 days after seed sowing), plants were harvested, weighed and separated into leaves, stems and roots. Stems, leaves and part of the roots were dried at 70 °C until constant weight, while the remaining roots were used for bacteria isolation (see paragraph 2.4).

Table 1
Selected physico-chemical parameters of soil (mean values ± standard deviations of 3 replicates), collected near the University Campus of Salerno and used for pot experiment.

Soil parameter	Value
pH	7.30 ± 0.8
Organic C (g kg^{-1})	36.2 ± 0.7
Available P (mg L^{-1})	47.2 ± 1.5
Ammonium (mg L^{-1})	33.9 ± 1.1
C (%)	5.98 ± 0.8
N (%)	0.46 ± 0.05
C/N	13
CEC (cmol kg^{-1})	28.9 ± 1.29
Organic matter content (g kg^{-1})	62.4 ± 0.75

2.2. Plant and soil elemental analysis

Pot filling soil was characterized for total and available Cu and Zn content (Table 2). Cu and Zn content was determined in pot soils (controls and treatments) and in plant organs at the end of the pot experiment. The soil granulometric fraction (2.0 mm) was pulverized in an agate mortar (Eatchs, Retsch, Germany). Dried stems and roots were reduced to ashes at 550 °C for 2 h in a muffle furnace (Nabertherm, Controller B170, Germany) while leaves were pulverized in liquid nitrogen and re-dried for a half day, at 75 °C to eliminate traces of water. Soil and plant matrices were digested with an acid mixture (HNO_3 65%:HF 50% = 2:1 = v:v) in a microwave oven (Ethos, Milestone, Shelton, CT-USA), as described by Baldantoni et al. (2009). The method of Lindsay (Lindsay and Norvell, 1978) was used to estimate Cu and Zn soil bioavailable fractions. Metal concentrations were determined by means of an atomic absorption spectrophotometer (AAnalyst 100, Perkin Elmer, Wellesley, MA-USA), via a graphite atomizer (Cu), or air-acetylene flame (Zn). Two replicates of each sample were carried out in order to evaluate variability among sample preparations. Standard reference materials (calcareous loam soil BCR CRM 141R – European Commission, 1996, and pine needles 1575a – Mackey et al., 2004) were also analyzed to obtain accurate data, and Cu and Zn concentrations were calculated considering the recoveries of the certified metals.

2.3. Statistical analysis

The data were processed by statistical tests (Sigma Plot 12.0 software package; Systat Software, Inc). The significance of differences in biomass production, metal soil content and metal accumulation among plant organs and groups, was tested by two way ANOVA and the analysis was followed by the Holm-Sidak post hoc test.