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Original article

Effect of biodegradable chelating agents on heavy metals phytoextraction with *Mirabilis jalapa* and on its associated bacteria

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Abstract

The application of chelating agents can be associated with phytoremediation in order to reduce the time required for remediation of heavy metal contaminated soils. The present work has the purpose to test the use of easily biodegradable chelating agents in the assisted phytoextraction process and to evaluate their effect on soil and *Mirabilis jalapa* plant associated bacteria. Two easily biodegradable chelating agents were used (EDDS and MGDA) in two different dosages (4 and 8 mmol/kg of soil). Metal concentration in soil solution, in leaves and in leachate were determined during the phytoextraction process, while at the end of the experiment metal concentration was evaluated separately in roots, stalk and leaves. In untreated reactors Zn and Pb were accumulated in the roots, but only Zn was translocated to the shoots. Both chelating agents demonstrated to be very effective in Pb and Zn mobilization in soil solution. After chelate treatment, accumulation and translocation of the two metals was different: unaffected with regard to Zn and enhanced with regard to Pb. The chelating agents seem to have a positive influence on bacterial communities of bulk soil and rhizosphere by mitigating the selective pressure caused by Pb and Zn, whereas the endophytes are less affected.

Keywords: Chelates; EDDS; Heavy metals; MGDA; *Mirabilis jalapa*; Phytoremediation; Plant-associated bacteria

1. Introduction

Abandoned mining areas represent a relevant environmental problem all over Europe. The Montevecchio mine district (Sardinia, Italy) during the last 150 years of activity until its closure was the richest Pb and Zn source in Italy. Poor management after the mine closure produced an intense heavy metal contamination. Mine wastes have been transported up to 20 km downstream from the main source, the Piccalinna flotation tailing dam [5]. The wide extension of the contaminated area and the low contamination depth makes phytoextraction a valuable cost-effective alternative to traditional disruptive technologies. Plant uptake capacity towards heavy metals and, as a consequence, metal removal efficiency from soil, is limited by the bioavailable metal fraction presence and abundance [14]. Pb has a limited solubility in soil solution and a low availability for plant

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uptake [12]. Synthetic chelates have been shown to desorb heavy metals from the soil matrix into the soil solution, to facilitate Pb transport into xylem, and to increase Pb translocation from roots to shoots of several high biomass producing plants [9]. Recent research verified the possibility of using biodegradable chelates for soil washing processes [16] and to enhance phytoextraction of heavy metal contaminated soils [11,17]. The aim of the present study is to verify the possibility of using easily biodegradable chelating agents, EDDS and MGDA, to enhance phytoextraction and to investigate their influence on soil and plant-associated bacterial communities.

2. Materials and methods

2.1. Soil characterization

The soil used in the experiment was collected from a site called Pauli Giuncus, 15 km downstream Piccalinna flotation tailing dam in the Montevecchio valley. The mining activity has deeply changed land-use in the Montevecchio valley. Traditionally along the valley the main land-use was sheep- and goat-grazing and limited agricultural activity. The original soils of the valley, vertisoils, and of the catchment basin of the river were considered fertile. The contamination has produced a loss of fertility and desertification across a wide area, estimated around 20 km^2. The width of contaminated soil ranges from 0.2 to 2.0 m [5].

The soil characterization is shown in Table 1. The soil is characterized by a low fertility. This can cause poor plant growth and a low tolerance of the high concentration of contaminants.

2.2. Plant selection

The selection of plant species was made considering the plant adaptability to the Mediterranean climate, its capacity to accumulate metals and biomass production. The fast growing species *Mirabilis jalapa* was selected for its capacity to accumulate 1500 mg of Pb/kg of plant from soils with a total Pb content of 3000 mg/kg [10] and its adaptability to the Mediterranean climate.

2.3. Experiment set-up and pot monitoring

Polyethylene pots used during the experiment had a volume of 3.2 l and were filled with 0.5 l of expanded clay pebbles and 2.5 l of contaminated soil.

In order to evaluate the potential bioavailable metal fraction and the potential risks of chelate application, metal content in soil solution and in leachate was measured during the experiment. In each pot a Rhizon soil moisture sampler was installed (Eijkellkamp, Agrisearch Equipment, Wageningen, Netherlands). Every pot was equipped with a leachate sampling device. *M. jalapa* seeds were grown in Petri dishes in a controlled environment with a 12 h photoperiod and with 25–20 °C day–night temperature. After 15 days every pot was planted with one seedling of *M. jalapa*. The plants were grown in a greenhouse and watered daily using tap water to maintain optimal growth conditions.

Soil solution and leachate in the reactors treated with chelates were sampled one day before and after chelate treatment, twice a week in the first two weeks and weekly until the end of the experiment. Soil solution and leachate were extracted weekly from the untreated reactors.

Samples of plants were taken one day before and after chelate treatment and monthly after the treatment. At the end of the experiment the plants were extracted from the growing media. Soil and root samples were collected in order to evaluate the susceptibility of bacterial populations to heavy metals. The plants were washed accurately, the aerial part was divided from the roots and the two parts were analysed separately to determine the metal content.

Soil samples were taken from each pot to determine both total and bioavailable soil Zn and Pb content.

2.4. Chelate treatment

Chelate influence on phytoextraction was evaluated using two biodegradable chelating agents, [*S*,*S*]-ethylenediaminedisuccinic acid (EDDS; Octel) and methylglycinediacetic acid (MGDA; BASF), and two doses, 4 and 8 mmol/kg of soil. Untreated reactors were used as control. The experiment was performed in triple and lasted six months.

Table 1
Montevecchio soil characterization

CEC (cmol/kg)	pH		N (%)	C (%)	Total (ppm)		H_2O (ppm)		KNO_3 (ppm)		EDTA (ppm)	
	H_2O	KCl			Pb	Zn	Pb	Zn	Pb	Zn	Pb	Zn
17.84	6.40	5.35	0.20	1.95	5526	1717	2	3	16	105	3278	443