International Biodeterioration & Biodegradation xxx (2016) 1–7

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Citric acid and EDTA on the growth, photosynthetic properties and heavy metal accumulation of *Iris halophila* Pall. cultivated in Pb mine tailings

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ARTICLE INFO

Article history:
Received 14 April 2016
Received in revised form
8 May 2016
Accepted 10 May 2016
Available online xxx

Keywords:
Iris halophila Pall.
Organic acids
Pb mine tailings
Phytoremediation

ABSTRACT

The effect of citric acid (CA) and EDTA on the growth, root and leaf anatomical structure, photosynthetic properties and lead (Pb) accumulation in *Iris halophila* seedlings cultivated in Pb mine tailings for 30 days were studied. Results showed that the dry weights of above-ground parts and underground parts of *I. halophila* under the treatments of 2 mmol kg^{-1} CA increased significantly and the tolerant index (IT) value treated with 2 mmol kg^{-1} EDTA was 19% lower than the control. The contents of photosynthetic pigments increased and decreased significantly with 0.5 mmol kg^{-1} CA and 2.0 mmol kg^{-1} EDTA, respectively. The leaf anatomic structure was negatively impacted by 2.0 mmol kg^{-1} EDTA. Treatment of 2 mmol kg^{-1} CA enhanced Cd and Zn accumulation in the above-ground parts, and Pb, Cu, Cd and Zn accumulation in the underground parts. EDTA treatments promoted Pb and Cd accumulation in both parts of *I. halophila*. The results indicated that the addition of organic acids could promote Pb and Cd accumulation and *I. halophila* could serve as pioneer species to be used in remediation of Pb mine tailings to improve the harsh environment.

1. Introduction

Many industrial processes contribute to extensive environmental pollution (Chen et al., 2015; Cieśliński et al., 1998; Dash and Das, 2015; Bahar et al., 2016; Wang et al., 2016). The solid wastes generated by mining processes are sources of contaminants to the environment (Adriano et al., 2004). Some heavy metal mine tailings which usually left exposed and dumped into nearby valley, streams and rivers are the most difficult to clean up, especially the mine tailings containing high concentrations of heavy metals (Richmond, 2000). Moreover, the leachate of the tailings may contaminate the aquifer and ground water and significantly impacts water quality of downstream river and natural ecosystems.

Pb is known as an environmentally important metal because of its toxicity and its widespread pollution. Pb mine tailings contain high concentrations of Pb together with other heavy metals (Han et al., 2013a). When the tailings are neglected for a long period of time, these pollutants begin to migrate and move into the surrounding soil, and water. Many studies have shown that plant tissue and cell structure will be altered or even destroyed, metabolic processes will be disrupted and normal growth will be significantly inhibited when plants are exposed to Pb contamination (Han et al., 2008, 2013b). Excessive Pb enters the food chain through the absorption and accumulation in the plants, disrupting the balance of the ecosystem and impairing the ecological function and threating human health.

The use of plants for remediation of mine tailings is proposed as a cost-effective and long-term applicable technique. The plants can be used to remove pollutants from the contaminated environment or to render them harmless, which are defined as phytoremediation (Huang and Cunningham, 1996). Plants used for phytoremediation should ideally combine the characteristics of withstanding the detrimental effects of heavy metals, high biomass

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http://dx.doi.org/10.1016/j.ibiod.2016.05.011
0964-8305/

Please cite this article in press as: Han, Y., et al., Citric acid and EDTA on the growth, photosynthetic properties and heavy metal accumulation of *Iris halophila* Pall. cultivated in Pb mine tailings, International Biodeterioration & Biodegradation (2016), http://dx.doi.org/10.1016/j.ibiod.2016.05.011

production and high metal accumulation in shoots and/or stems, but minimum in leaves (Han et al., 2013a). Therefore, the selection of pioneer species is very important in improving the key characteristics of the physical environment by increasing organic matter contents and possibly reducing heavy metals toxicity of the tailing sites. In this way, more and more sensitive plants can gradually establish their growth and a healthy ecosystem can be eventually achieved. However, efficiency of plants to accumulate heavy metals varies with plant species and environmental conditions (Yu and Gu, 2007, 2008a,b; Yu et al., 2007, 2008; Yang et al., 2010).

Under normal circumstances, most of the heavy metals are adsorbed on the soil particles and precipitated as mineral as part of the soil aggregates, which are difficult to be assimilated by plants. Recently, researchers have been paying more attention to using some chelating agents, such as low molecular organic acids, to change the chemical activity and availability of heavy metals and enhance the phytoextraction of metals (Evangelou et al., 2006; Kim et al., 2010). Studies found that the application of citric acid (CA), oxalic acid and other natural organic acids or some synthetic ones, such as ethylene diaminetetra acetic acid (EDTA), to the soil can improve the bioavailability of heavy metals for accumulation in plants (Piechalak et al., 2003; Neugschwandtner et al., 2008). The methods using organic acids to assist phytoextraction of heavy metals have improved the efficiency in the remediation of heavy metals contaminated soil (Evangelou et al., 2006; Wang et al., 2009).

Iris halophila Pall., a hardy perennial species of Iridaceae, is widely distributed in the dry meadow steppe, gravel slope and saline-alkali land in Gansu and Xinjiang of China, Roumania, Ukraine and Russia (Mathew, 1981). *I. halophila* is cold and drought tolerant species with relatively high biomass, nice leaves and flowers, and has been widely cultivated in northern China and the regions along the middle and lower reaches of the Yangtze River (Huang et al., 2003). Han (2008) found that *I. halophila* is a salt and Pb tolerant species. The objectives of this study were to assess: the growth and tolerant index of plants grown under different treatments, the root and leaf anatomical structure of the seedling, Pb and some other heavy metals absorption and translocation in the seedlings of *I. halophila*. The goal of this study was to find an effective method in increasing organic matter inputs at mine tailing sites and decreasing Pb content in the tailings.

2. Materials and methods

2.1. Physicochemical properties of Pb tailings and seedling cultivation

Pb mine tailings were collected from tailing sites of Dexing Pb Mine, Jiangxi Province, China. Sample collection and treatment were based on the method of Jiang et al. (2012). The physicochemical properties of Pb tailings were as follow: available P 8.46 mg kg^{-1}, available K 23.27 mg kg^{-1}, available N 5.075 mg kg^{-1}, pH ($CaCl_2$) 6.23, and Pb 706.93 mg kg^{-1}.

The seeds of *I. halophila* were collected from the vegetative propagated plants in the Iris Resource Collection Garden, Jiangsu Province and Chinese Academy of Science, Nanjing, China. Seedlings were cultured according to the methods of Han et al. (2007).

2.2. Experimental set up

When the seedlings reached 10 cm in height, they were removed from the plastic plates and the roots of similar size seedlings were gently washed and transferred to 12 cm in diameter plastic pots with Pb mine tailing, six seedlings per pot. The insides of the pots were covered with plastic bags to avoid solution and tailings leaked out. After a week of adaptation in Pb tailings, the seedlings were treated with an equal quantity (100 ml) of tap water (CK) or treatments containing 0.5 mmol kg^{-1} CA (CA0.5), 2 mmol kg^{-1} CA (CA2), 0.5 mmol kg^{-1} EDTA (EDTA0.5) or 2 mmol kg^{-1} EDTA (EDTA2), respectively. Each treatment was in triplicate. The experiment was conducted at 15–25 °C and under natural light at the Experimental Teaching Center of Ecological Environment of Jiangxi Province, Jiangxi University of Finance and Economics, Nanchang. Equal quantity of tap water was supplemented into each pot when the tailings were relatively dry until the seedlings were harvested.

2.3. Determination of biomass and tolerance index

After the seedlings of *I. halophila* treated with CA and EDTA for 30 days, they were harvested. The roots of the seedlings were immersed in 20 mmol l^{-1} EDTA for 20 min to remove Pb adsorbed on the surface of roots and then all the seedlings of *I. halophila* were washed thoroughly with running tap water and divided into aboveground parts and underground parts. The root length was measured with the ruler accurate to millimetre. The dry weights (DWs) were measured after drying in an oven at 80 °C until a constant weight was reached.

The tolerant index (IT) was calculated according to Wilkins (1957):

$$IT = R_{tested}/R_{control}$$

where R_{tested} represents the average length of roots in tested, $R_{control}$ represents the average length of roots in the control.

2.4. Determination of photosynthetic pigments

The leaves on the same position were taken from different seedlings at 30 days of organic acid treatments and used for the determination of photosynthetic pigments. After extraction with 80% acetone, photosynthetic pigments in the extracts of the leaves of *I. halophila* were measured spectrophotometrically at 470, 647 and 664.5 nm, respectively, according to the method of Lichtenthaler (1987).

2.5. Observation of root and leaf structure

After 30 days of treatment with organic acids, the same part of the roots and leaves of different treatments were chosen and the thin-section slices were made by freehand section and root slices were stained with Safranin and Fast Green. The slices were observed under the Leica microscope (DME(T/CA)) and photographed.

2.6. Measure of HM contents and translocation factor

The dry materials of aboveground and underground parts of *I. halophila* treated with different organic acids were ground separately to fine powder (<1 mm) for metal analysis. Approximately 0.1–0.2 g samples of the fine powders were digested in HNO_3:ClO_4 (87:13, v/v). The digested samples were dissolved in 5% HNO_3 to the volume of 25 ml and analyzed with flame atomic absorption spectrophotometer (TAS-990) for the contents of Pb, Cu, Zn and Cd in the different parts of *I. halophila*. Translocation factor (TF) was calculated to evaluate the capability of plant to accumulate the metal in the aboveground parts from underground parts:

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