Ecological Engineering 109 (2017) 35–40

Contents lists available at ScienceDirect

Ecological Engineering

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

Research paper

Role of co-planting and chitosan in phytoextraction of As and heavy metals by *Pteris vittata* and castor bean – A field case

Junxing Yang[a], Jun Yang[a,*], Jun Huang[b,*]

[a] Center for Environmental Remediation, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, PR China

[b] Key Laboratory of Agricultural Products Chemical and Biological Processing Technology of Zhejiang Province, School of Biological and Chemical Engineering, Zhejiang University of Science and Technology, Hangzhou, 310023, PR China

ARTICLE INFO

Keywords:
Co-planting
Metal chelator
Phytoextraction
Pteris vittata
Castor

ABSTRACT

Phytoextraction is a promising technology that uses hyperaccumulating plants to remove arsenic (As) and heavy metals (HMs) from soil. A field experiment has been conducted to evaluate impacts of water-soluble chitosan (WC) upon the growth and phytoextraction of As, Cd and Pb by the hyperaccumulator *Pteris vittata* L. and the economic plant castor bean (*Ricinus communis* L.) in a co-planting system in polymetallic contamination of agricultural soil. The co-planting system was comprised of As-hyperaccumulator and Pb high-accumulating *P. vittata* and Cd high-accumulating *R. communis*. The results showed that yields of *P. vittata* were significantly increased by co-planting. The results revealed that the uptake of As and Pb by *P. vittata* was also significantly increased by co-planting. When the WC was applied to the co-planting system, the uptake of Cd and Pb by *P. vittata* and *R. communis* was significantly increased, but the uptake of As was not increased obviously. This study suggested that the combination of co-planting system with WC is a more promising approach than single hyperaccumulator. This approach not only enhanced the removal rates of soil As and HMs, but also simultaneously offered agricultural practices with no edible products in the As and HMs contaminated farmland soils.

1. Introduction

Contamination of soils by arsenic (As) and heavy metals (HMs) is a major global issue that affects a large number of sites. The accumulation of As and HMs in soil may have adverse effects on food quality, soil health, and the environment (Gray et al., 2006; Zhuang et al., 2009; Liu et al., 2013). Thus, it is essential to remediate As and HMs contaminated soil to reduce the risk of human exposure *via* consumption of contaminated produce. (McLaughlin et al., 1999).

Phytoremediation is a cost-effective and environmentally friendly technology, which can remove As and HMs from polluted soil or to render them harmless (Shi and Cai, 2009). A successful phytoremediation technology requires a suitable plant to use on the toxicant. *In situ* phytoextraction projects using As-hyperaccumulator and Pb high-accumulating *Pteris vittata* L. have been established on farmlands and residential areas in China and America, and high As and Pb removal rates are achieved (Ma et al., 2001; Chen et al., 2002; Xie et al., 2010). Castor bean (*Ricinus communis* L.), a C3 plant of the Euphorbiaceae, has great potential for phytoremediation of Cd contaminated soil (Huang et al., 2011; Zhang et al., 2015, 2016). Castor bean can be cultivated to provide phytoremediation and also for bioenergy production, making it a highly valuable, renewable resource (Olivares et al., 2013).

Chelate enhanced phytoremediation is an effective approach for the removal of HMs from soils by plants (Romkens et al., 2002). Chitosan, biodegradable and non-toxic (Muzzarelli et al., 1988), possessing a better ability to chelate ions of HMs, due to the free amine function, and it has been widely used in removing of cations from waste solution (Piron et al., 1997; Huang et al., 2016; Lin et al., 2017). In addition, low molecular weight and water-soluble chitosan can chelate HMs to enhance their mobility and taken up and/or transported more easily by plants. Wang et al. (2007) reported that water-soluble chitosan application remarkably increased Zn, Cd and Pb concentrations in both shoots and roots of *Elsholtzia splendens* grown in HM-contaminated soil under field conditions. Thus, chitosan has been regarded as an alternative choice to synthetic chelates for enhancement of phytoremediation (Lin et al., 2017).

Recently, intercropping of hyperaccumulator with economic crops is now widely utilized in slightly or moderately As and heavy metal-polluted farmlands (Wu et al., 2002; 2007). The results showed that the co-planting system can effectively remove the As or HMs (Cd and Pb) from the polluted soil by the hyperaccumulator plant (*P. vittata* or

* Corresponding authors.
E-mail addresses: yangj@igsnrr.ac.cn (J. Yang), Huangjun@zust.edu.cn (J. Huang).

http://dx.doi.org/10.1016/j.ecoleng.2017.09.001
Received 28 December 2016; Received in revised form 16 August 2017; Accepted 5 September 2017
Available online 19 September 2017

Sedum alfredii) while the harvested products from the agricultural crop meet the Chinese food standards (Wu et al., 2007; Li 2011; Wan et al., 2016a). However, most hyperaccumulator plants only accumulating specific metalloid or metal, it suggests a limited applicability to remediate farmlands simultaneously polluted by As and HMs. Therefore, we have demonstrated a co-planting system including *P. vittata* and *R. communis*, it might be further enhance phytoextraction of As and HMs (Cd and Pb) in farmland soil simultaneously with chitosan application. The goal of this study is to evaluate the feasibility of the removal efficiency of As and HMs simultaneously from the contaminated soils by using a co-planting system associated with the addition of the WC. Our specific objectives were: (1) assess the ability of a WC to enhancing phytoextraction of As and HMs (Cd and Pb) under field conditions; (2) estimate the effects of the co-cropping system to the growth of *P. vittata* and *R. communis*, the extraction and accumulation of As and Pb by *P. vittata*, and Cd by *R. communis* and the production of inedible products by *R. communis* in a contaminated field site; (3) evaluate the feasibility of the combined co-planting system and with the WC for remediation of As and heavy metal contaminated soils.

2. Materials and methods

2.1. Site description

The field plot experiments were conducted on a farmland soil (35°13′N; 112°54′E) located in Jiyuan District in Henan Province, China. This site has a history of atmospheric sedimentation by local Lead Mining plant. Jiyuan situates in a warm temperate zone with average annual rainfall 860 mm and mean annual temperature 14.6 °C. Physico-chemical properties of the soil used for the field plot experiment were pH 7.73 (2.5:1 distilled water: soil, v/w), organic matter: 16.98 $g\,kg^{-1}$ (K_2CrO_7-H_2SO_4), total N: 0.89 $g\,kg^{-1}$ (semi-quantitative titration), Olsen-P: 0.51 $g\,kg^{-1}$ 0.5 M $NaHCO_3$, available K: 128.05 $mg\,kg^{-1}$ (1.0 M NH_4OAc) total As: 26.4 $mg\,kg^{-1}$, total Cd: 3.51 $mg\,kg^{-1}$.and total Pb: 281 $mg\,kg^{-1}$. According to the Chinese national standards of soils (GB15618–1995), this soil is contaminated by Cd and As. The total As and metal concentrations of top soils vary little among field blocks.

2.2. Materials and chemical chelators

Selection of As hyperaccumulator and Pb high-accumulating *P. vittata* and Cd high-accumulating *R. communis* were based on our early experiments. Mature spores of *P. vittata* were collected from mature plants growing in a tin mine spoil, in Gejiu County of Yunnan Province, China (Wan et al., 2017a). Spores of *P. vittata* were mixed with fine sand and then floured onto a seedling tray filled with substrate. The substrate was maintained wet, and the light/dark cycle was maintained at 16 h/8 h (day/night) in the green house. The temperature was kept at 25 ± 1 °C during the day and 20 ± 1 °C during the night. The relative humidity was maintained at 70%. The seedlings were thinned out when they were 5 cm high. *R. communi* (Zibo-8) was a high Cd accumulating cultivar with high concentrations of Cd in its leaves (Zhang et al., 2016). The water-soluble chitosan used in the experiment was kindly provided by Professor Jun Huang of Zhejiang University of Science and Technology (Lin et al., 2017). It is a low-molecular weight (< 2000) and water-soluble chelator that can chelate metals such as Pb and Cd. It is biodegradable and nonphytotoxic and may be used in phytoextraction of HMs.

2.3. Treatments and experimental design

The planting treatments were described as: Control (CK), *R. communis* (*R. communis*), *P. vittata* (*P. vittata*), Co-crop of *R. communis* (*R. communis*) and *P. vittata* (Co-crop), *R. communis* amended with the WC (*R. communis* + WC), *P. vittata* amended with MC (*P. vittata* + WC), Co-crop amended with WC (Co-crop + WC). There were seven treatments in total and each with triplicates during the experiments.

In April 2016, 21 field plots with each size 5 m × 2 m were set up with at least 1 m protecting zone all around the experimental plots and there was a ditch in 0.10 m width between two plots. There were seven plots in each block and each plot randomly distributed within a block. Forty-eight healthy and equal-size *P. vittata* were planted per plot at four columns and 12 rows and 12 plants of *R. communis* per plot were added in 2 columns × 6 rows in April 2016. Twenty days before harvest, 2 $L\,m^{-2}$ of 1% chitosan solution was supplied to the amended treatments (*R. communis* + WC, *P. vittata* + WC, Co-crop + MC). No fertilizers and pesticides were applied but interval weeding was made and seedlings were watered when required during the growth stage.

2.4. Sample collection and analysis

Soil samples from the topsoil (0–20 cm depth) were collected from each plot and harvested in September 2016. At harvest, three *R. communis* and three *P. vittata* were collected from each plot and each plant species was composite to one mixed plant sample. *R. communis* was divided into roots, stems, leaves and beans whereas *P. vittata* was collected only shoots. The plant parts were dried at 70 °C for 76 h to obtain the dry weights. The oven-dried materials were finely grinded in an agate mill.

Soil pH was measured at a soil to water ratio of 1:2.5. Available Pb and Cd in soils were extracted by diethylenetriamine pentaacetic acid (DTPA: 0.005 M DTPA, 0.1 M triethanolamine and 0.01 M $CaCl_2$ at pH 7.3) in a soil: solution ration 1:2 (v/v) (Lindsay and Norvell, 1978). Available As in soils were extracted by sodium bicarbonate ($NaHCO_3$: 0.05 M $NaHCO_3$) in a soil: solution ration 1:10 (v/v) (Bandyopadhyay et al., 2004). Oven-dried plant shoot or root samples were grinded using a Retsch grinder (Type: 2 mm, Retsch Company, made in Germany). As, Cd and Pb in plant tissues were extracted by a digestion method using a mixture of $HNO_3/HClO_4$ (85/15, v/v). The total concentrations of Cd and Pb and DTPA-extractable Pb and Cd were determined by inductively coupled plasma mass spectrometry (ICP-MS, Elan 5000, Perkin Elmer, USA). Total concentration of As was determined by liquid chromatography coupled to atomic fluorescence spectroscopy (LC-AFS, AFS-9130, Titan Instrument, Beijing, China). The temperature of digestion was controlled under 110 °C to avoid As volatilization (Cai et al., 2000). Blank and bush leaf material (BGW-07603) (China Standard Materials Research Center, Beijing, PR China) were used for quality control. The recovery rates of As, Pb and Cd were 90 ± 10%.

The results were presented as arithmetic means with standard errors. A statistical comparison of means of plant data was examined with one-way ANOVA followed by LSD test as available in the SPSS 12.0 statistical package. If the differences among different planting treatments for *P. vittata* or *R. communis* were significant at 5% level, the least significant difference (LSD) was calculated as the post hoc test to determine where differences lay.

3. Results

3.1. Plant yields

Yields of stems and leaves of *R. communis* showed no significant differences among the planting treatments (Table 1). However, C and C + W treatments have both resulted a significant increment in roots and fruits of *R. communis* and the shoots of *P. vittata* compared with the control.

3.2. As, Cd and Pb concentrations

Compared with the control, C treatment significantly increased shoot As concentrations of *P. vittata* and decreased As concentrations in roots, stems, leaves and fruits of *R. communis* (Table 2). Significant