Journal of Geochemical Exploration 182 (2017) 201–205

Contents lists available at ScienceDirect

Journal of Geochemical Exploration

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

Enhanced phytoremediation capacity of a mixed-species plantation of *Eucalyptus globulus* and *Chickpeas*

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A R T I C L E   I N F O

Article history:
Received 16 April 2015
Revised 6 September 2016
Accepted 7 January 2017
Available online 9 January 2017

Keywords:
Eucalyptus globulus
Chickpea
Phytoremediation
Heavy metals

A B S T R A C T

The objective of this research was to determine the phytoremediation efficiency for a mixed plantation of non-nitrogen-fixing *Eucalyptus globulus* with nitrogen-fixing *Chickpeas* in soil contaminated by heavy metals including Cd, Cu, Hg and Pb. Plants in the mixed-species system produced more biomass, circulated more nutrients and water and absorbed more toxic materials due to the nitrogen fixation of *Chickpeas* and the generation of root and canopy stratification, which reduced nutrients and light competition.
It would take, respectively, 40, 68, 4225 and 127 years to reduce the concentration of Cd, Cu, Hg and Pb in the soil to safe levels, which is approximately half the time needed to achieve the same goal for *Eucalyptus globulus* or *Chickpeas* monocultures. The mixed cultivation enhances the phytoremediation efficiency of Cd and Cu contaminated soil significantly, but it is ineffective in reducing the Hg level in soil because of its low bioavailability.

1. Introduction

The continuous industrialization of countries and growth of the world's population have led to the dramatically elevated release of a wide variety of chemicals into the environment, which leads to severe public health problems. Soil, water, and air can be contaminated with heavy metals that cannot be degraded by microbial or chemical processes.

Techniques used to remove heavy metal ions include vitrification, soil excavation, soil flushing, solidification, chemical precipitation, electrokinetics, and adsorption among others (Polechońska and Klink, 2014). Most of the conventional methods are either extremely expensive and labor intensive or have deleterious effects, such as causing irreversible changes in soil structure, the destruction of native soil microflora and secondary pollution. Therefore, after using traditional techniques, agricultural activities would no longer be possible. Alternatively, phytoremediation has long been recognized as a cost-effective and environmentally friendly method that utilizes plants that are capable of extracting heavy metals from the soil. Furthermore, phytoremediation can prevent soil erosion by both wind and water, replace the use of fossil fuels as an energy supply and decrease greenhouse gas emissions by storing carbon in non-harvesting parts. Additionally, this process can be cultivated on marginal land and does not compete for agricultural soils. Species that can be utilized for the remediation of contaminated soil are those with high biomass production, which are resistant to extreme weather, have a high tolerance to heavy metal accumulation and are easy to establish. Some non-hyperaccumulators can extract comparative quantity of pollutants as hyperaccumulators due to greater biomass production despite the fact that the target heavy metals in tissues of them does not conform to the criteria of a hyperaccumulator (Bech et al., 2012).

In recent years, there have been numerous studies on heavy metal tolerance and their uptake by plants. However, almost all of these studies focused on single species and neglected the phytoremediation potential of mixed species either as part of an in-situ or ex-situ experiment.

Eucalyptus globulus is the most adaptive species used for the phytoremediation of heavy metals owing to its high aboveground biomass, fast growth and high tolerance to heavy metals. This species stores a mass of heavy metals in its roots (Mughini et al., 2013) and, therefore, the elements in the trunks are not high enough to impact the market value of its wood. Forrester et al. (2010) found that mixed-

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http://dx.doi.org/10.1016/j.gexplo.2017.01.006
0375-6742/

species plantations containing non-nitrogen-fixing and nitrogen-fixing species can increase the productivity of each species by increasing the N availability and decreasing intra-specific competition. In the present study, the contents of heavy metals in different plant organs of two species planted in monoculture and together are discussed and the remediation efficiency of using mixed plantations of *Eucalyptus globulus* with *Chickpeas* for phytoremediation were investigated.

2. Materials and methods

2.1. Study area description and growth conditions

The experimental site, Guiyu, locates in southeast Guangdong Province, China, with a subtropical continental climate. Its mean annual air temperature is approximately 21.5 °C, and annual rainfall varies from 980 to 1550 mm. Guiyu has a local population of approximately 150,000 and has been involved in e-waste dismantling activities for >30 years. It has accepted massive amounts of imported e-waste annually and approximately 6000 family artisanal enterprises participate in this high-polluting industry, using primitive techniques including incineration, sorting, employing strong acid to recover rare metals, burning cables to recycle copper wires and melting circuit boards on a coal stove to separate precious elements. In the process of recycling, fly ash and effluent laden with toxic materials are usually discharged, resulting in contaminated air, soil, surface water and crops. Although uncontrolled open burning and strong acid leaching of e-waste are considered illegal in Guiyu and have been banned for many years, the pollution can still be detected in farmland soils and streams.

Three experimental contaminated soil disposal sites (20 × 24 m) were established on otherwise valuable arable land in Guiyu, a size above the threshold of 100–150 m^2 established for real remediation situations. The experimental field was divided into six 10 × 8 m subplots including a surrounding row of buffer trees to prevent the transfer of heavy metals and nutrients between plots by rain. The experimental sites were plowed three times using normal agricultural machinery to drastically homogenize the soil after the e-waste was manually removed.

Plant transpiration was counted based on the Penman–Monteith equation and the modified Jarvis–Stewart model (Estévez et al., 2009; Whitley et al., 2009).

2.2. Soil characterization

The soil in this experiment is a typical Ferric Acrisol, according to previous regional geochemical surveys. 60 soil samples were collected from the surface soil (20 cm) before air dried and sieved (2 mm) for analysis. The pH of the soil was measured in situ with water at 1:2 (w/v) ratio using a pH meter. The CEC of the soil was determined using the ammonium acetate saturation method and the total organic carbon (TOC) was determined using ferrous ammonium sulfate titration after the oxidation of the organic matter in soil through potassium permanganate. Table 1 presents the physical and chemical properties of the soil.

Table 1
Physical and chemical properties of the soil.

Soil type		Ferric Acrisols
pH		6.4 ± 1.1
CEC	cmol kg^{-1}	12.7 ± 3.3
TOC	g kg^{-1}	42 ± 6
Cd	mg kg^{-1}	0.61 ± 0.17
Hg	mg kg^{-1}	0.44 ± 0.18
Pb	mg kg^{-1}	69.5 ± 19.4
Cu	mg kg^{-1}	56.2 ± 21.7
Cr	mg kg^{-1}	57.1 ± 13.5
Zn	mg kg^{-1}	111.27 ± 35.08

2.3. Experimental design

Three experiments were performed, E1, E2 and E3. In early April 2010, healthy, three-year-old *Eucalyptus globulus* were transplanted by hand into E1 from another experiment site that served similar purposes. Planted at a density of 2500 per ha with a low level application of phosphate fertilizer (200 kg per ha of calcium superphosphate), the trees were spaced at 4 m^2 intervals, which is the recommended planting density when phytoremediation is the main plantation objective (Guoa et al., 2002). For the E2 experiment, *Chickpea* seeds were obtained commercially from Guangzhou and planted at the same time at a planting density of 45 × 5 cm, for a total of 445 thousand crops per ha, which results in the best comprehensive performance (Wu et al., 2008). *Eucalyptus globulus* and *Chickpeas* were planted together (50% *Eucalyptus globulus* and 50% *Chickpeas*) in the E3 experiment. All plants were harvested manually in late August.

2.4. Laboratory analyses

Five plants of each subplot were collected randomly and separated into roots and shoots to determine the biomass and concentrations of heavy metals. Plant parts were washed with tap water to remove any adhering sediments and dried to constant weight in an oven at 80 °C. Dried plant samples were pulverized for digestion. The powder samples were pressed into pellets after being packaged with boric acid and the Pb, Cu, Cr and Zn content in the pellets was estimated using an X-ray fluorescence spectrometer. Soil or plant samples were digested with a solution containing 75% concentrated HCl and 25% concentrated HNO_3 (v/v) in a 50-mL Teflon crucible. A 1-mL aliquot of solution was then placed into a 10-mL colorimetric tube and diluted with 3% nitric acid. The concentrations of Cd were analysed using inductively coupled plasma-mass spectrometry (ICP-MS). The Hg in soil or plant samples was determined by cold vapor generation atomic fluorescence spectrometry and the samples were digested with a mixed medium of potassium permanganate, sulfuric acid and potassium persulfate.

2.5. Statistical analyses

The data were processed for two-way analysis of variance (ANOVA) using SPSS 15.0. All results were expressed as the mean ± S.D. of the six replicates. Mean separation was conducted based on Duncan's multiple range tests. Significant differences were assessed at the level $P < 0.05$.

To study heavy metals uptake and bio-concentration behavior, the following indices were used: BCF = the element in the plant tissue / the element in the soil. A BCF > 1 elucidates the plant is suitable for phytoremediation purposes. TF = the element in shoot / the element in root. The TF value is used to determine the internal transport of metals from root to shoot. The total metal extraction by roots/shoots (TE) = the metal in part of the plant × the dry biomass yield of the part. The TE value represents the total heavy metals absorbed from the soil by the plant.

3. Results

3.1. Impact of heavy metals in the soil on plant growth

As shown in Table 1, the Cd, Hg, Pb, and Cu contents in the top 20 cm of soil exceeded the upper limits set by the US Environmental Protection Agency and the Grade II environmental quality standard for soils used for agricultural purposes in China, which signifies that the soil was moderately polluted by these heavy metals. The concentrations of Cr and Zn in the surface soil were below the safe limit.

There were no visual symptoms of phytotoxicity such as yellowing, chlorosis, leaf necrosis or reduced growth on *Eucalyptus globulus* during the experiment, which is in agreement with the findings of Arriagada et al. (2007), who found that *Eucalyptus* is one of the most promising