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In situ phytoextraction of copper and cadmium and its biological impacts in acidic soil

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HIGHLIGHTS

- *Pennisetum sinese* produced the highest biomass among five plant species.
- *Pennisetum sinese* was the best species for Cu and Cd removal when biomass was considered.
- *Elsholtzia splendens* soil had the highest enzyme activities and microbial populations.

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ABSTRACT

Phytoremediation is a potential cost-effective technology for remediating heavy metal-contaminated soils. In this study, we evaluated the biomass and accumulation of copper (Cu) and cadmium (Cd) of plant species grown in a contaminated acidic soil treated with limestone. Five species produced biomass in the order: *Pennisetum sinese* > *Elsholtzia splendens* > *Vetiveria zizanioides* > *Setaria pumila* > *Sedum plumbizincicola*. Over one growing season, the best accumulators for Cu and Cd were *Pennisetum sinese* and *Sedum plumbizincicola*, respectively. Overall, *Pennisetum sinese* was the best species for Cu and Cd removal when biomass was considered. However, *Elsholtzia splendens* soil had the highest enzyme activities and microbial populations, while the biological properties in *Pennisetum sinese* soil were moderately enhanced. Results would provide valuable insights for phytoremediation of metal-contaminated soils.

1. Introduction

Environmental pollution by heavy metals has become a global concern due to human activities such as burning of fossil fuels, mining and smelting of metalliferous ores, metallurgical industries, disposal of municipal wastes, applications of fertilizers, pesticides, and sewage (Hazrat et al., 2013; Li et al., 2014a). In China, there is a total area of 2.88×10^6 ha lands that have been impaired, and additional 4.67×10^4 ha of impaired land are produced each year, as a result of mining and smelting of metalliferous ores (Hazrat et al., 2013). Such impaired lands are almost completely without vegetation due to soil contamination and may also be subjected to severe soil erosion and off-site pollution (Xia, 2003). Many physical, chemical and biological technologies have been studied in an effort to remediate such contaminated soils (Matsunaga and Yasuhara, 2003; Yang et al., 2007). Among those technologies, phytoremediation is considered as one of the most environmentally friendly and cost-effective approach for the remediation of contaminated soils and sediments (Marques et al., 2009).

Phytoremediation relies on plant uptake to remove contaminants and reduce metal content in soil (McGrath et al., 2002). However, metal hyperaccumulators are usually characterized by slow growth with limited biomass and contaminant specific accumulation (Pedron et al., 2009). Moreover, phytoremediation usually is a long-term process and limited by weather conditions. One approach for effective removal is to use chemicals such as ethylenediaminetetraacetic acid (EDTA), ethylenediaminedisuccinic acid (EDDS), or cytokinins in combination with high biomass plant

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species for phytoextraction (Doumett et al., 2008; Tassi et al., 2008; Vamerali et al., 2015). On the other hand, high metal availability, low soil pH, organic matter (OM) and nutrients, and poor soil structure in contaminated soils could also prevent plant establishment and growth (Fellet et al., 2011; Pardo et al., 2011). Therefore, appropriate soil amendments such as limestone, apatite, and zeolite have been applied in order to enhance plant biomass production (Castaldi et al., 2005; Madrid et al., 2008; Karami et al., 2011). For example, Gray et al. (2006) found that red fescue (*Festucar ubrare*) can be only established in heavy metal-contaminated soil with the applications of lime and red mud.

The ultimate objective of phytoremediation should be not only to remove contaminants from soil but more importantly to improve soil quality (Jusselme et al., 2013). The assessment of soil quality, therefore, needs to include the evaluation for the efficacy of phytoremediation. Brookes and McGrath (1984) proposed using soil microbiological properties as indicators for assessing soil pollution. Several studies have reported that soil enzyme activity is a good indicator of soil quality because of its significance in nutrient cycles, organic matter turnover, soil characteristics, microbial activity and biomass (Gil-Sotres et al., 2005; Wasilkowski et al., 2014).

The goal of this study was to investigate the metal removal effects of five plant species in combination with limestone application in an acidic, heavy-metals contaminated soil. Objectives included: (1) monitoring the changes of soil chemical properties and heavy metal availability; (2) determining the plant biomass and heavy metal accumulation; and (3) measuring the soil enzyme activities and microbial populations.

2. Materials and methods

2.1. Study site

The study site is located in Jiuniugang Village, Binjiang Country, Guixi City, Jiangxi Province, China (117°12′ E, 28°19′N), which is near a large copper smelter and a fertilizer plant and has been contaminated for more than 30 years by wastewater irrigation. The study area has a typical warm and humid subtropical monsoon climate with an annual mean temperature of 17.8 °C and an annual rainfall of 1706 mm (50% of rainfall occurring from March to early July). Soils in this region are primarily derived from Quaternary red clay, which are classified as Ultisols by the USDA Soil Taxonomy (Soil Survey Staff, 2010). Prior to land abandonment, the land in this area was mainly used as paddy fields. Currently, the soil is no longer polluted by the contaminated irrigation water, but it still subjected to dust and exhaust gas from the plants. The primary pollutants in the soil are Cu and Cd, with concentrations of 719 and 1.0 mg kg^{-1}, respectively (Cui et al., 2013). Moreover, the site soil is very acidic (pH = 4.20), having soil organic carbon (SOC) content, cation exchange capacity (CEC), exchangeable acid and exchangeable Al of 17 mg kg^{-1}, 94, 33 and 25 mmol kg^{-1}, respectively. Total N and total P in the soil were 1.28 and 0.57 g kg^{-1}, respectively.

2.2. Experimental design

The field experiment consisted of 5 m (long) x 4 m (wide) plots arranged in a completely random plot design with three replicates per treatment. The treatments included five selected plant species: *Sedum plumbizincicola* (*Sedum plumbizincicola X.H. Guo et S.B. Zhou ex L.H. Wu*), *Elsholtzia splendens* (*Elsholtzia splendens Nakai ex F. Maekawa*), *Vetiveria zizanioides* (*Vetiveria zizanioides (L.) Nash*), *Pennisetum sinese* (*Pennisetum sinese Roxb*) *and Setaria pumila* (*Setaria pumila (Poir.) Roem. & Schult. subsp. pumila*). A control treatment without plants or added limestone was conducted in paralleled. These plant species were selected because *S. plumbizincicola* is a Cd-hyperaccumulator (Ma et al., 2015); *E. splendens* is a Cu-tolerant species (Sun et al., 2010), *V. zizanioides* and *P. sinese* are high biomass species; and *S. pumila* is a native weed. Moreover, *S. plumbizincicola*, *V. zizanioides*, and *P. sinese* are perennial species while *E. splendens* and *S. pumila* are annual species.

Prior to planting, limestone (pH = 12.2, 46.1% calcium carbonate, 1.4 mg Cu kg^{-1}, 0.9 mg Cd kg^{-1}) was applied and mixed with topsoil (0–17 cm) at a rate of 2.2 tons ha^{-1} (0.1%, w/w) on October 30, 2013. The plots were then irrigated with tap water (5×10^5 l ha^{-1}). After a week of equilibration, a compound fertilizer ($N:P_2O_5:K_2O$ = 15:15:15, 834 kg ha^{-1}) was applied to each plot. *S. plumbizincicola* was planted with a density of 15 × 15 cm on November 8, 2013, and *E. splendens*, *V. zizanioides* and *P. sinese* were planted with a density of 15 × 15, 30 × 20 and 50 × 40 cm on March 10, 2014, respectively.

2.3. Sample collection

The shoots of *S. plumbizincicola* were collected on June 23, 2014, and the above ground tissues of the other plants were harvested on November 3, 2014. The roots of *S. pumila* were not collected because they were decayed when the shoots were harvested, although it was an annual species. Only *E. splendens* roots were collected while other plant roots remained in the plots for next year growth (Li et al., 2014b). All plant samples were first washed with tap water and then with deionized water. The washed samples were oven-dried at 80 °C for 24 h, and ground for chemical analysis.

Soil samples were collected from the top 17 cm at three representative locations per plot and then mixed together to form a composited sample on November 3, 2014. The samples were dried and the roots and other visible plant residues removed. All samples were passed through a <2 mm sieve and were divided into two subsamples: one subsample was stored at 2 °C for biological analysis, and another subsample was air dried at room temperature for chemical analysis. Meanwhile, moisture content of soil was determined.

2.4. Chemical analysis

Soil and dry limestone pH was measured by a pH electrode in suspension of distilled water at a liquid to solid ratio of 2.5 (E-201-C, Shanghai Truelab Instrument Company, China). Total soil N was determined by semimicro-Kjeldahl method after soil was digested by $HClO_4$ and HF. Total soil P was determined colorimetrically by acidic molybdate–ascorbic acid blue color method (Van Veldhoven and Mannaerts, 1987) after the soil digestion with nitric acid/perchloric acid mixture (4:1). SOC was determined by digesting soil with $K_2Cr_2O_7$ and concentrated H_2SO_4 at 170–180 °C, and then titrating with $FeSO_4$ (Walkley and Black, 1934). Soil alkali-hydrolyzable N was analyzed using the method described by Lu (2000), in which alkali-hydrolyzable N was released and transformed to NH_4-N by 1.0 M NaOH and $FeSO_4$ powder at 40 °C for 24 h, and then absorbed with 2% (w/v) H_3BO_3 and titrated with 0.01 M H_2SO_4. The alkali-hydrolyzable N was calculated as the difference in the amount of NH_4-N before and after NaOH treatment. Soil Olsen P was extracted with 0.5 M $NaHCO_3$ at pH = 8.5 and determined by colorimetry using ammonium molybdate and ascorbic acid (Olsen et al., 1954). Soil-test K was extracted with 1.0 M NH_4OAc at pH = 7.0 and determined by atomic absorption spectroscopy (Pratt, 1965). The cation exchange capacity was determined using the ammonium acetate method (Pansu and Gautheyrou, 2006). Soil exchangeable acidity and aluminum (Al) were extracted with 1.0 M KCl, followed by titration with standard NaOH solution in an N_2 atmosphere (Pansu and Gautheyrou, 2006).