

## Postprint: Yeast Promotes *Jatropha curcas* Growth Under Lead and Cadmium Stress

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### Abstract

Screening yeast strains with resistance and adsorption capacity to lead and cadmium, constructing a *Jatropha curcas* root-yeast combined remediation system, and promoting the growth of *Jatropha curcas* under high-concentration lead and cadmium stress is of great significance for improving the efficiency of *Jatropha curcas* in remediating lead- and cadmium-contaminated soils. In this study, three yeast strains with lead and cadmium resistance were isolated from *Jatropha curcas* root segments, *Davidia involucrata* stem segments, and *Davidia involucrata* root segments, designated as Jc, Di1, and Di2, respectively, and their resistance and adsorption capacities to lead and cadmium were determined. Two screened yeast strains capable of adsorbing lead and cadmium were inoculated into *Jatropha curcas* seedlings to investigate the response of *Jatropha curcas* plants inoculated with these two yeast strains to lead and cadmium stress. The results showed that based on morphological, physiological, and biochemical characteristics, Jc was preliminarily identified as *Rhodotorula* sp., Di1 as *Candida* sp., and Di2 as *Debaryomyces* sp. All three yeast strains exhibited certain resistance to lead and cadmium, with the resistance capacity following the order of Jc > Di2 > Di1. Di1 and Jc both possessed certain adsorption capacities for lead and cadmium and were used for inoculating *Jatropha curcas* seedlings. Compared with *Jatropha curcas* plants without yeast inoculation (ck), those inoculated with Di1 and Jc showed significant increases in root, stem, leaf, and whole-plant dry weight, significant increases in chlorophyll, whole-plant nitrogen, and whole-plant phosphorus concentrations, enhanced activities of SOD, POD, and CAT, and a significant decrease in MDA concentration. From the perspective of comprehensive inoculation effects: Jc and Di1, as passivating agents for lead and cadmium, are candidate strains for promoting *Jatropha curcas* growth under lead and cadmium stress.

## Full Text

### Preamble

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### Plant Growth-Promoting Traits of Yeasts on *Jatropha curcas* Under Lead and Cadmium Stress

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### Abstract

Screening yeasts with resistance and adsorption capacity to lead and cadmium, constructing a *Jatropha curcas* root-yeast combined remediation system, and promoting the growth of *J. curcas* under high concentrations of lead and cadmium stress are important for improving the efficiency of lead and cadmium phytoremediation in contaminated soils. In this study, three endophytic yeast strains with lead and cadmium resistance were isolated from healthy *J. curcas* roots, *Davidia involucrata* stems, and *D. involucrata* roots, designated as Jc, Di1, and Di2. Their resistance and adsorption capacities for lead and cadmium were determined. Two strains capable of adsorbing lead and cadmium were selected for inoculating *J. curcas* seedlings to investigate the response of inoculated plants to lead and cadmium stress. Morphological and physiological-biochemical characterization preliminarily identified Jc as *Rhodotorula* sp., Di1 as *Candida* sp., and Di2 as *Debaryomyces* sp. All three yeasts exhibited certain resistance to lead and cadmium, with resistance levels following the order Jc > Di2 > Di1. Di1 and Jc showed adsorption capacity for both lead and cadmium and were used to inoculate *J. curcas* seedlings. Compared with non-inoculated control plants, *J. curcas* inoculated with Di1 and Jc showed significant increases in dry weight of roots, stems, leaves, and whole plants, along with significant increases in chlorophyll, total nitrogen, and total phosphorus concentrations. The activities of SOD, POD, and CAT were enhanced, while MDA concentration decreased significantly. Based on the comprehensive inoculation effects, Jc and Di1 can serve as passivating agents for lead and cadmium and represent promising candidate strains for promoting *J. curcas* growth under lead and cadmium stress.

**Keywords:** endophytic yeast, lead and cadmium resistant yeasts, growth-promoting effect, phytoremediation, *Jatropha curcas*

## Introduction

Lead and cadmium are two of the most common heavy metal pollutants released into the environment through agricultural and industrial activities. They can cause toxic effects in plants, affecting growth and development, and can enter the human body through the food chain, posing health risks. Consequently, lead and cadmium contamination has attracted worldwide attention (Wong & Selvam, 2006; Tchounwou et al., 2012). Compared with traditional chemical and physical remediation technologies, phytoremediation of contaminated soils offers advantages including non-destructiveness, safety, in-situ application, lack of secondary pollution, and cost-effectiveness, making it a promising approach for soil remediation. Successful phytoremediation requires plants with multiple characteristics, such as the ability to grow on heavy metal-contaminated land, rapid growth, high biomass production, and accumulation of heavy metals in harvestable plant parts (Bhargava, 2012).

*Jatropha curcas*, a member of the Euphorbiaceae family, is a multipurpose, drought-resistant deciduous perennial shrub distributed across tropical and subtropical regions of America, Africa, and Asia. The seeds contain 30–35% oil that can be converted into high-quality, environmentally friendly biodiesel, making it a promising energy crop (Foidl et al., 1996). Furthermore, numerous studies have demonstrated that *J. curcas* is an excellent candidate for environmental remediation and can be used to restore lead- and cadmium-contaminated soils (Pandey et al., 2016; Jamil et al., 2009; Mangkoedihardjo, 2008). However, high concentrations of lead and cadmium reduce the growth rate of *J. curcas* and decrease aboveground biomass (Liang et al., 2012), which diminishes its remediation efficiency and limits its environmental applications.

Microbial remediation technology for heavy metals utilizes microbial metabolic functions to immobilize heavy metal ions or convert toxic heavy metal ions into non-toxic or less toxic forms. However, due to the difficulty of using microorganisms alone to remove heavy metals from soil, the application scope of microbial remediation technology is limited (Li et al., 2015). In soil micro-ecosystems, the functional interactions between plants and rhizosphere microorganisms can be combined to establish a plant-microbe combined remediation system that leverages the advantages of both approaches to improve the efficiency of heavy metal-contaminated soil remediation (Li et al., 2015). Yeasts are unicellular fungi that reproduce asexually primarily through budding. Some yeasts from the rhizosphere and surrounding soil, such as *Candida*, *Rhodotorula*, *Sporobolomyces*, *Trichosporon*, *Williopsis*, and *Yarrowia*, can promote plant growth (Cloete et al., 2009). Additionally, some endophytic yeasts such as *Williopsis saturnus* and *Rhodotorula graminis* can produce auxins that promote plant growth (Nassar et al., 2005; Xin et al., 2009). Deng et al. (2012) reported that an endophytic yeast strain *Cryptococcus* sp. CBSB78, resistant to Cu, Zn, Pb, and Cd, could promote the growth of *Brassica* species. Screening yeast strains that can promote *J. curcas* growth is important for constructing a *J. curcas*-microbe remediation system to improve the efficiency of lead and cadmium phytoreme-

diation. This study isolated lead- and cadmium-resistant yeasts from plants growing under stress conditions, determined their adsorption capacity for lead and cadmium, inoculated lead- and cadmium-adsorbing yeasts into *J. curcas* seedlings, observed their effects on plant growth, screened for strains that promote *J. curcas* growth, and explored their growth-promoting mechanisms to provide a theoretical and practical foundation for constructing a *J. curcas*-yeast combined remediation system.

## Materials and Methods

### 1.1 Yeast Isolation and Characterization

#### 1.1.1 Culture Media

- 1. Isolation and purification medium:** Potato dextrose agar (PDA) containing 200 g peeled potato, 20 g glucose, 20 g agar powder, and 1000 mL purified water.
- 2. Screening medium:** PDA supplemented with  $50 \text{ mg} \cdot \text{L}^{-1} \text{ CdCl}_2 \cdot 2.5\text{H}_2\text{O}$  and  $500 \text{ mg} \cdot \text{L}^{-1} \text{ Pb}(\text{NO}_3)_2$ .
- 3. Sugar-peptone-yeast medium:** 20 g glucose, 10 g peptone, 5 g yeast powder, 20 g agar, and 1000 mL distilled water.
- 4. Resistance medium:** PDA supplemented with analytical grade  $\text{Pb}(\text{NO}_3)_2$  or  $\text{CdCl}_2$ . Lead concentration gradients were 250, 500, 750, and  $1000 \text{ mg} \cdot \text{L}^{-1}$  (as  $\text{Pb}(\text{NO}_3)_2$ ); cadmium concentrations were 25, 50, 75, and  $100 \text{ mg} \cdot \text{L}^{-1}$  (as  $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ ).
- 5. Adsorption medium:** PDA supplemented with analytical grade  $\text{Pb}(\text{NO}_3)_2$  or  $\text{CdCl}_2$ . Liquid medium contained  $250 \text{ mg} \cdot \text{L}^{-1}$  lead (as  $\text{Pb}(\text{NO}_3)_2$ ) and  $50 \text{ mg} \cdot \text{L}^{-1}$  cadmium (as  $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ ); the lead-cadmium mixed medium contained  $250 \text{ mg} \cdot \text{L}^{-1} \text{ Pb}$  and  $50 \text{ mg} \cdot \text{L}^{-1} \text{ Cd}$ .

**1.1.2 Yeast Isolation and Purification** Two-year-old healthy seedlings of *J. curcas* and *Davidia involucrata* were used as source material for roots and stems. Plant materials were washed with tap water, surface-sterilized by soaking in 0.5% mercuric chloride for 3 min, rinsed four times with sterile water, soaked in 75% ethanol for 1 min, and rinsed several times with sterile water (Shao et al., 2001). The sterilized materials were placed on screening medium and incubated in the dark at 30 °C. Surface-sterilized stem and root segments were pressed onto PDA plates to ensure contact with the medium. After 2 days, the segments were removed, and the PDA plates were used for isolation and purification of strains by streak dilution method.

**1.1.3 Yeast Identification** Selected strains were inoculated on sugar-peptone-yeast medium and incubated in the dark at 30 °C for 5 days. Colony morphology was observed, and yeasts were stained with lactophenol cotton blue staining solution for microscopic examination and photography using a digital imaging system. Physiological and biochemical characteristics were identified using yeast micro-biochemical reaction tubes (Hangzhou Tianhe Microbial Reagent Co., Ltd.).

**1.1.4 Determination of Lead and Cadmium Resistance and Adsorption** Prior to experiments, strains were activated on PDA medium and inoculated onto slanted resistance medium at each concentration (three replicates per concentration). Cultures were incubated in the dark at 30 °C for one week, after which colony growth was observed. The three yeast strains were inoculated into liquid adsorption medium, with uninoculated medium serving as a blank control. Inoculated flasks were placed in a constant temperature shaker at 30 °C and 137 r · min<sup>-1</sup> for 7 days. Samples were acidified with nitric acid to pH 1-2, filtered through medium-speed filter paper, and the filtrate was digested with nitric acid and perchloric acid. Lead and cadmium concentrations in the digested solution were determined by flame atomic absorption spectrophotometry.

The adsorption rate was calculated using the formula:

$$\text{Adsorption rate (RE)} = \frac{(\text{Initial concentration} - \text{Final concentration})}{\text{Initial concentration}} \times 100\%$$

## 1.2 Plant Experiments

**1.2.1 Experimental Soil** Soil was collected from the surface layer at the edge of a forest on the North Campus of Xichang University. Soil physicochemical properties were: pH 6.10 (water), available phosphorus 6.29 mg · kg<sup>-1</sup>, hydrolyzable nitrogen 170.84 mg · kg<sup>-1</sup>, available potassium 787.16 mg · kg<sup>-1</sup>, organic matter 12.34 mg · kg<sup>-1</sup>, Pb concentration 49.7 mg · kg<sup>-1</sup>, and Cd concentration 0.94 mg · kg<sup>-1</sup>. Before the experiment, the soil was treated with 500 mg · kg<sup>-1</sup> Pb (as Pb(NO<sub>3</sub>)) and 50 mg · kg<sup>-1</sup> Cd (as CdCl<sub>2</sub> · 5H<sub>2</sub>O) (Pb500-Cd50) and aged for at least two weeks before transplanting *J. curcas* seedlings.

**1.2.2 Yeast Activation and Inoculation** Strains were activated on solid medium and then inoculated into liquid PDA medium. Cultures were shaken in a constant temperature incubator at 30 °C and 100 r · min<sup>-1</sup> for one week, with uninoculated medium serving as a control. After 7 days, the bacterial suspension was diluted five-fold with sterilized pure water and applied to germinating *J. curcas* seeds. Each yeast strain had six replicates, and the control was treated with five-fold diluted sterile PDA medium. A second application of yeast suspension was performed one week later. Seedlings were transplanted into lead- and cadmium-treated soil 30 days after inoculation.

*Jatropha curcas* seeds were sown in plastic pots containing sterilized nursery substrate (autoclaved at 121 °C for 2 h). Seeds were soaked in 1% potassium

permanganate for 1 h and rinsed with tap water before planting (five seeds per pot).

**1.2.3 Plant Cultivation** A pot experiment was conducted by transplanting inoculated *J. curcas* seedlings into Pb500-Cd50 treated soil (three plants per pot, five pots per treatment). Water was supplemented as needed during the experimental period. Both inoculation and seedling cultivation were performed in an artificial climate chamber at 30 °C, with 16 h light and 50% humidity.

**1.2.4 Determination of Biochemical Indicators in *Jatropha* Seedlings** Samples were collected 30 days after treatment. Six plants from each inoculation treatment were selected and divided into three groups. Roots, stems, and leaves were excised and weighed for fresh weight, then placed in a drying oven at 105 °C for 30 min, followed by drying at 80 °C to constant weight for dry weight determination.

Dried roots, stems, and leaves from each treatment were combined, ground, and sieved for nitrogen and phosphorus analysis. Phosphorus concentration was determined by molybdenum-antimony colorimetry (Li, 2012), and nitrogen concentration by Nessler' s colorimetry (McDonald, 1978).

The second or third leaves from the top of remaining plants were used for other physiological analyses. Chlorophyll concentration was determined by acetone-ethanol extraction (Li, 2003). Malondialdehyde (MDA) concentration was measured by thiobarbituric acid (TBA) method (Li, 2003; Lagrimini, 1991). Superoxide dismutase (SOD) activity was determined by nitroblue tetrazolium (NBT) method (Beauchamp & Fridovich, 1971). Total lead and cadmium concentrations were measured by atomic absorption spectrophotometry (Zhang & Tang, 2011).

## Results

### 2.1 Screening, Identification, and Resistance Analysis of Lead/Cadmium-Adsorbing Yeasts

Three yeast strains were isolated from the preliminary screening medium: Jc from *J. curcas* root segments, Di1 from *D. involucrata* stem segments, and Di2 from *D. involucrata* root segments.

#### 2.1.1 Identification of Lead/Cadmium-Adsorbing Yeasts

**2.1.1.1 Morphological characteristics:** Preliminary observation identified Jc as belonging to the genus *Rhodotorula*, with round or oval shapes and red colonies. Di1 colonies were pure white with a raised center and fluffy surface [Figure 1c: see original paper], and microscopic examination revealed blue-stained long elliptical cells [Figure 1d: see original paper].

**2.1.1.2 Biochemical characteristics:** Physiological and biochemical features of the three yeasts were identified using yeast micro-biochemical reaction tubes, following the *Yeasts: Characteristics and Identification* handbook (Barnett et al., 1983; Hu, 1990). Based on morphological and physiological-biochemical analysis, Jc was identified as *Rhodotorula sp.* (specifically *Rhodotorula dif-fusens*), Di1 as *Candida sp.* (specifically *Candida hydrocarbofumarica*), and Di2 as *Debaryomyces sp.* (specifically *Debaryomyces castellii*).

**2.1.2 Analysis of Lead and Cadmium Resistance in Three Yeast Strains** All three yeast strains could grow on medium containing  $500 \text{ mg} \cdot \text{L}^{-1}$  Pb and  $50 \text{ mg} \cdot \text{L}^{-1}$  Cd. Jc showed the strongest resistance, capable of growing at  $1000 \text{ mg} \cdot \text{L}^{-1}$  Pb and  $200 \text{ mg} \cdot \text{L}^{-1}$  Cd. Di2 could grow at  $1000 \text{ mg} \cdot \text{L}^{-1}$  Pb and  $100 \text{ mg} \cdot \text{L}^{-1}$  Cd. The results indicated that all three yeasts possessed certain resistance to lead and cadmium, with resistance capacity following the order  $\text{Jc} > \text{Di2} > \text{Di1}$ .

All three yeasts exhibited some adsorption capacity for lead and cadmium. Di2 and Jc showed high adsorption rates for lead, while Di1 differed significantly from Di2 and Jc in lead adsorption. Although Di2 could grow in  $50 \text{ mg} \cdot \text{L}^{-1}$  Cd<sup>2</sup> or  $250 \text{ mg} \cdot \text{L}^{-1}$  Pb +  $50 \text{ mg} \cdot \text{L}^{-1}$  Cd, both Di1 and Jc showed adsorption capacity for Cd and Pb. Jc's cadmium adsorption was significantly higher than Di1's, while Di2's cadmium adsorption was nearly zero. Therefore, Di1 and Jc were selected as inoculants for *J. curcas* seedlings in pot experiments to observe plant responses to lead and cadmium stress.

## 2.2 Effects of Different Inoculation Treatments on Jatropha Plants

**2.2.1 Effects on Plant Biomass** Inoculation with Di1 and Jc significantly increased fresh and dry weights of *J. curcas* plants. Compared with non-inoculated controls, root fresh weight increased by 29% and 35%, stem fresh weight by 38% and 47%, leaf fresh weight by 74% and 78%, and whole-plant fresh weight by 49% and 56% after Di1 and Jc inoculation, respectively.

Similarly, root dry weight increased by 8% and 27%, stem dry weight by 30% and 39%, leaf dry weight by 32% and 55%, and whole-plant dry weight by 29% and 44% after Di1 and Jc inoculation, respectively. These results demonstrate that inoculation with Jc and Di1 promoted *J. curcas* growth under lead and cadmium stress.

**2.2.2 Effects on Chlorophyll, Nitrogen, and Phosphorus Concentrations** Inoculation with Di1 and Jc significantly increased chlorophyll a, chlorophyll b, total chlorophyll (a+b), and carotenoid concentrations. Chlorophyll a increased by 46.3% and 46.8%, chlorophyll b by 66% and 55%, total chlorophyll by 50% and 48%, and carotenoids by 28% and 44% after Di1 and Jc inoculation, respectively. The greater increase in chlorophyll b led to increases in chlorophyll/carotenoid ratio by 8.5% and 4.4%, while chlorophyll a/b ratio decreased by 9.7% and 13.6%.

Nitrogen and phosphorus concentrations in roots, stems, leaves, and whole plants also increased significantly after inoculation. Nitrogen concentration increased by 36.7% and 86.9% in roots, 18.8% and 62.5% in stems, 20.7% and 64.6% in leaves, and 57.84% and 145.22% in whole plants after Di1 and Jc inoculation, respectively. Phosphorus concentration increased by 35.9% and 60.7% in roots, 46.1% and 63.3% in stems, 5.5% and 6.3% in leaves, and 56.3% and 86.7% in whole plants, respectively.

**2.2.3 Effects on Lead and Cadmium Concentrations** Inoculation with Di1 and Jc significantly reduced lead and cadmium concentrations in roots, stems, and leaves. Lead concentration decreased by 20.1% and 27.9% in roots, 22.5% and 25.9% in stems, and 33.5% and 34.3% in leaves after Di1 and Jc inoculation, respectively. Cadmium concentration decreased by 23.9% and 29.5% in roots, 18.8% and 25.2% in stems, and 31.5% and 33.5% in leaves, respectively.

**2.2.4 Effects on Enzymatic and Non-enzymatic Antioxidants** Inoculation with Di1 and Jc enhanced the activities of antioxidant enzymes SOD, POD, and CAT, with Di1-inoculated plants showing significantly higher activities of all three enzymes and significantly lower concentrations of the lipid peroxidation product MDA compared with controls. MDA concentration decreased by 55.34% and 43.59%, CAT activity increased by 23.9% and 11.8%, POD activity increased by 10.8% and 18.3%, and SOD activity increased by 129.8% and 49.0% after Di1 and Jc inoculation, respectively. The Di1 treatment particularly improved antioxidant capacity, with SOD activity increasing 1.29-fold compared with the control.

## Discussion and Conclusion

The combined Cd50-Pb500 pollution exhibited toxic effects on *J. curcas*, inhibiting growth and reducing fresh and dry weights of roots, stems, and leaves (Liang et al., 2012). In this study, inoculation with Di1 and Jc significantly increased root, stem, leaf, and whole-plant fresh and dry weights compared with non-inoculated plants, indicating that Di1 and Jc inoculation alleviated the toxicity of combined Cd50-Pb500 pollution and promoted *J. curcas* growth.

Lead and cadmium concentration measurements revealed that inoculation with Di1 and Jc significantly reduced lead and cadmium concentrations in roots, stems, and leaves compared with controls. However, due to increased dry weights of roots, stems, and leaves, total lead and cadmium concentrations per plant did not differ significantly from controls. Further investigation showed that Di1 and Jc did not colonize the interior of *J. curcas* roots but were transferred to the contaminated soil as rhizosphere microorganisms along with the nursery substrate during seedling transplantation. Therefore, Di1 and Jc may function as effective passivating agents for lead and cadmium, causing precipitation or adsorption fixation of these metals in soil, altering their speciation, and

“passivating” their bioavailability, thereby reducing *J. curcas* uptake and promoting plant growth. Plant growth-promoting bacteria can serve as excellent heavy metal passivating agents by changing heavy metal bioavailability in the rhizosphere microenvironment, promoting plant growth, and regulating heavy metal uptake and accumulation efficiency (Babu et al., 2015; Han et al., 2019). Thus, Di1 and Jc can also act as lead and cadmium passivating agents to prevent heavy metal uptake by crops and promote crop growth.

Nitrogen and phosphorus are essential macronutrients for plants. Lead and cadmium toxicity also includes impaired nitrogen and phosphorus absorption and transport, reducing their uptake by plants (Ramón et al., 2003; Kibria et al., 2009; Farouk et al., 2011). Inoculation with Di1 and Jc passivated lead and cadmium in the soil surrounding *J. curcas*, reducing their toxic effects and increasing nitrogen and phosphorus absorption and transport. Consequently, nitrogen and phosphorus concentrations in roots, stems, leaves, and whole plants increased significantly after Di1 and Jc inoculation compared with controls.

Lead and cadmium toxicity also manifests through induction of oxygen free radical production or reduced antioxidant enzyme activity, generating oxidative stress (Benavides et al., 2005) and damaging cell walls, plasma membranes, mitochondria, and chloroplasts (Liang et al., 2012). Reactive oxygen species (ROS), including superoxide radicals ( $O \cdot^-$ ) and hydroxyl radicals ( $OH \cdot$ ), are normal metabolic byproducts that plants can scavenge through an antioxidant system. However, under stress, these radicals accumulate due to an imbalance between ROS production and scavenging (Mittler, 2002). Malondialdehyde (MDA), a final product of unsaturated fatty acid oxidation, is commonly used as a marker for lipid peroxidation damage (Zhang et al., 2009). As a lead- and cadmium-resistant plant, *J. curcas* can enhance antioxidant enzyme activities (SOD, POD, CAT) to cope with stress, though these activities may decrease under severe Cd50-Pb500 stress (Liang et al., 2012).

Inoculation with Di1 and Jc significantly reduced lead and cadmium concentrations in leaves, alleviating oxidative damage. Simultaneously, the activities of antioxidant enzymes SOD, POD, and CAT increased significantly, scavenging more free radicals. This reduction in ROS and oxidative damage was reflected by significantly decreased MDA concentrations.

The toxic effects of combined Cd50-Pb500 pollution on *J. curcas* also included significantly reduced photosynthetic pigment concentrations, with chlorophyll b showing a more pronounced decrease, leading to increased chlorophyll a/b ratio and decreased chlorophyll/carotenoid ratio (Liang et al., 2012). Inoculation with Di1 and Jc significantly increased chlorophyll concentrations, decreased chlorophyll a/b ratio, and increased chlorophyll/carotenoid ratio. The increased chlorophyll concentration in inoculated plants may be attributed to enhanced nitrogen uptake (Farouk et al., 2011).

This study isolated two lead- and cadmium-adsorbing yeast strains, *Rhodotorula* sp. (Jc) and *Candida* sp. (Di1), from *D. involucrata* stems and *J. curcas*

roots. These yeasts can form a special rhizosphere microenvironment with *J. curcas* roots and soil, reducing the bioavailability of lead and cadmium in soil, alleviating the toxic effects of high-concentration combined lead and cadmium pollution, promoting *J. curcas* growth, and enhancing the prospects for heavy metal phytoremediation applications.

## References

- AEBI H, 1984. Catalase in vitro[J]. *Method Enzymol.* 105:121-126.
- BABU AG, SHEA PJ, SUDHAKAR D, et al., 2015. Potential use of *Pseudomonas koreensis* AGB-1 in association with *Miscanthus sinensis* to remediate heavy metal(loid)-contaminated mining site soil[J]. *J Environ Manag*, 151: 160-166.
- BEAUCHAMP C, FRIDOVICH I, 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels[J]. *Anal Biochem*, 44(1):276-287.
- BENAVIDES MP, GALLEGO SM, TOMARO ML, 2005. Cadmium Toxicity in Plants[J]. *Braz J Plant Physiol*, 17(1): 21-34.
- BHARGAVA A, CARMONA FF, BHARGAVA M, et al., 2012. Approaches for enhanced phytoextraction of heavy metals[J]. *J Environ Manag*, 105:103-20.
- CLOETE KJ, VALENTINE AJ, STANDER MA, et al., 2009. Evidence of symbiosis between the soil yeast *Cryptococcus laurentii* and a sclerophyllous medicinal shrub, *Agathosma betulina* (Berg.) Pillans[J]. *Microb Ecol*, 57:624-632.
- DENG Z, WANG W, TAN H, et al., 2012. Characterization of heavy metal-resistant endophytic yeast *Cryptococcus* sp. CBSB78 from rapes (*Brassica chinensis*) and its potential in promoting the growth of *Brassica* spp. in metal-contaminated soils[J]. *Water Air Soil Poll*, 223:5321-5329.
- FAROUK S, MOSA A, TAHA A, et al., 2011. Protective effect of humic acid and chitosan on radish (*Raphanus sativus*, L. var. *sativus*) plants subjected to cadmium stress[J]. *J Stress Physiol Biochem*, 7(2):99-116.
- FOIDL N, FOIDL G, SANCHEZ M, et al., 1996. *Jatropha curcas* L. as a source for the production of biofuel in Nicaragua[J]. *Bioresour Technol*, 58(1):77-82.
- HAN H, WANG XY, CAI H, et al., 2019. Isolation of heavy metal immobilized and plant growth-promoting bacteria and its effects on reducing heavy metal accumulation in wheat[J]. *Environ Sci*, 7:1-15.
- HU RQ, 1990. *Yeasts: Characteristics and Identification*[M]. Qingdao: Qingdao Ocean University Press: 34;36;45;76-79;112-115;164-167.
- JAMIL S, ABHILASH PC, SINGH N, et al., 2009. *Jatropha curcas*: A potential crop for phytoremediation of coal fly ash[J]. *J Hazard Mater*, 172(1):269-75.
- KIBRIA MG, ISLAM M, OSMAN KT, 2009. Effects of lead on growth and mineral nutrition of *Amaranthus gangeticus* L. and *Amaranthus oleracea* L.[J].

Soil Environ, 28(1): 1-6.

LAGRIMINI LM, 1991. Wound-induced deposition of polyphenols in transgenic plants overexpressing peroxidase[J]. *Plant Physiol*, 96(2):577-83.

LI HJ, 2012. Comparative study on determination of phosphorus content in two kinds of plants[J]. *Mod Agric Sci Technol*, 11:16-17.

LI HS, 2000. *The Principle and Measuring Technique of Plant Physiology and Chemistry*[M]. Beijing: Beijing Higher Education Press: 134-137;195-197;260-261.

LIANG J, YANG ZH, TAN L, et al., 2012. Growth performance and tolerance responses of jatropha (*Jatropha curcas*) seedling subjected to isolated or combined cadmium and lead stresses[J]. *Int J Agric Biol*, 14: 861-869.

LI YS, FENG CL, WU XF, et al., 2015. A review on the functions of microorganisms in the phytoremediation of heavy metal-contaminated soils[J]. *Acta Ecol*, 35(20): 6881-6890.

MANGKOEDIHARDJO S, 2008. *Jatropha curcas* L. for phytoremediation of lead and cadmium polluted soil[J]. *World Appl Sci J*, 4(4):519-522.

MCDONALD M, 1978. A simple and improved method for the determination of microgram quantities of nitrogen in plant material[J]. *Ann Bot*, 42(2):363-366.

MITTLER R, 2002. Oxidative stress, antioxidants and stress tolerance[J]. *Trends Plant Sci*, 7(9):405-10.

NASSAR AH, EL-TARABILY KA, SIVASITHAMPARAM K, 2005. Promotion of plant growth by an auxin-producing isolate of the yeast *Williopsis saturnus* endophytic in maize (*Zea mays* L.) roots[J]. *Biol Fert Soils*, 42, 97-108.

PANDEY VC, BAJPAI O, SINGH N, 2016. Energy crops in sustainable phytoremediation[J]. *Renew Sust Energ Rev*, 54:58-73.

RAMON OC, SAUL V, ESTEBAN E, et al., 2003. Cadmium-stress in white lupine: Effect on nodule structure and function[J]. *Plant Physiol*, 161: 911-919.

SHAO AJ, LIN SF, ZHANG SJ, et al., 2001. Isolation of a kind of endophytic fungus which can produce taxol compounds[J]. *Acta Acad Med Sin*, 23(6):642-644.

TCHOUNWOU PB, YEDJOU CG, PALLOLLA AK, et al., 2012. Heavy metal toxicity and environment[J]. *Exp Suppl*, 101: 133-164.

WANG LJ, HE XS, 2006. Research methods of the isolation and sublimation of plant endophytical fungi[J]. *J Microbiol*, 26(4): 55-60.

WONG JWC, SELVAM A, 2006. Speciation of heavy metals during composting of sewage sludge with lime[J]. *Chemosphere*, 63:980-986.

XIN G, GLAWE D, DOTY SL, 2009. Characterization of three endophytic, indole-3-acetic acid-producing yeasts occurring in *Populus* trees[J]. Mycolog Res, 11(3): 973-980.

ZHANG H, TANG J, 2011. Determination of Fe, Mn, Cu, Pb and Cd in vegetables by atomic absorption spectrometry[J]. Chin J Spectrosc Lab, 28(1):72-74.

ZHANG SS, ZHANG HM, QIN R, et al., 2009. Cadmium induction of lipid peroxidation and effects on root tip cells and antioxidant enzyme activities in *Vicia faba* L.[J]. Ecotoxicology, 18(7):814-823

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