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Tolerance and bioaccumulation of Cd and Cu in *Sesuvium portulacastrum*

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ABSTRACT

In order to investigate the tolerance and bioaccumulation of Cd and Cu in the halophyte *Sesuvium portulacastrum*, seedlings were hydroponically cultured for 30 days using the modified 1/2 Hoagland nutrient solution with different concentrations of Cd (0, 5, 10, 15, and 20 mg L^{-1}) and Cu (0, 2.5, 5, 7.5, and 10 mg L^{-1}). Afterwards, the seedling height, leaf area, biomass, and mineral element contents (Fe, Mg, Cu, and Zn) in the roots, stems and leaves were measured, and the tolerance index, bioconcentration factor (BCF), transportation index, and removal rate were calculated. The effects of salinity (0‰–30‰) on the growth and bioaccumulation ability of *S. portulacastrum* under combined Cu/Cd (5 mg L^{-1}) exposure were also determined. The results showed that, with an increasing Cd concentration, the biomass and seedling height of *S. portulacastrum* initially increased and then decreased. The highest leaf biomass and seedlings height was observed in the 10 mg L^{-1} and 5 mg L^{-1} Cd treatment group, respectively. Salinity did not affect the biomass of *S. portulacastrum* but decreased Cd concentration in roots and aboveground tissues and Cu concentration in roots of *S. portulacastrum.* Cu treatment significantly facilitated the absorption of Mg, Cu, and Zn in roots. With an increasing Cu concentration, the Mg and Fe contents increased in the leaves of *S. portulacastrum*. In comparison to the above-ground portions, the root showed a higher bioaccumulation ability of Cd and Cu, with the BCF of 341.5 and 211.9, respectively. The BCF and translocation factor (TF) values indicated that *S. portulacastrum* was not a hyperaccumulator for Cd and Cu, but could be used as a phytostablization plant in heavy metal contaminated coastal environments.

1. Introduction

Due to the rise in agriculture, industry and urban development, a large amount of wastewater is discharged into the sea, which contributes to severe heavy metal pollution in coastal areas, and endangers the coastal marine ecosystem (Bai et al., 2011; Feng et al., 2017; Wang et al., 2013). Heavy metals are highly stable, difficult to degrade, and therefore easily transported and accumulated through the food web, which can significantly threaten the safety of seafood and thus human health (Feng et al., 2016; Wang, 2002). The removal and treatment of heavy metal pollution is urgent for the remediation of coastal marine ecosystems. The utilization of natural marine organisms during remediation is considered safe and efficient (Huang et al., 2013). Moreover, this method needs a low economic investment and can avoid secondary pollution, compared with traditional physical and chemical treatments. This method is suitable for aquaculture seawater ponds and coastal areas where high water quality is required (He et al., 2008; Huang et al., 2013; Valipour et al., 2009).

Many types of organisms have shown heavy metal remediation potential. For example, both marine macro and microalgae can remove heavy metals (Kumar et al., 2013). However, their heavy metal accumulation capabilities, which vary among species, are sensitive to various environmental changes. As an economical, eco-friendly and green technology to remove heavy metals from contaminated aquatic body (Pandey, 2013; Pandey et al., 2014), the phytoremediation has obtained increasing attention (Pandey, 2012). In comparison to algae, halophytes are generally highly tolerant to salinity and have high heavy metal accumulation ability. Moreover, they display a high growth rate (Ali et al., 2002; Taamalli et al., 2014; Yoon et al., 2006). These advantages of halophytes make them a promising choice for the remediation of heavy metal in polluted coastal marine ecosystems.

Cu and Cd are two major heavy metal pollutants (Ali et al., 2002;

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Forte and Mutiti, 2017; Ghnaya et al., 2005; Yoon et al., 2006). Cu and Cd demonstrate their toxicity by competing with other essential elements to bind the active sites of enzymes (Taamalli et al., 2014; Yruela, 2009). They can cause serious damages to marine organisms and become potential hazards to marine ecosystems (Pan and Wang, 2011; Wang et al., 2013). Since Cu and Cd cannot be effectively degraded by marine organisms, their removal using halophytes might be a feasible way to remediate contaminated areas.

Sesuvium portulacastrum is a succulent herbaceous halophyte that grows in the coastal sand of tropical and sub-tropical areas. It is valuable for both economic production and ecosystem remediation (Ghnaya et al., 2005; Lokhande et al., 2010). Ecological floating beds growing *S. portulacastrum* in aquaculture ponds not only decontaminate nutrient salts in seawater, but also improve the quality of the aquacultural products (Huang et al., 2013; Zhai et al., 2017). A few studies have investigated the tolerance of *S. portulacastrum* to heavy metals and its potential application for heavy metal remediation (Ghnaya et al., 2007; Mariem et al., 2014). For example, *S. portulacastrum* is highly tolerant of Zn, and can be used for Zn removal in contaminated areas with Zn concentration < 90 mg L^{-1} (Lin et al., 2016). However, the tolerance and bioaccumulation of Cd and Cu in *S.portulacastrum* were largely unknown, which hindered the further applications of *S. portulacastrum* in phytoremediation.

Salinity is an important factor affecting the tolerance ability, the uptake and transportation of heavy metals in halophyte species (Mariem et al., 2014). Many studies have demonstrated that high salinity could alleviate the toxic effects and reduce the accumulation of Cd in *S.portulacastrum*, which can tolerate high salinity up to 30‰ without severe suppress of survival rate and growth (Ghnaya et al., 2007; Han et al., 2012a, 2012b; Mariem et al., 2014; Zeng et al., 2017). However, in most studies, the concentration of NaCl (200 mmol L^{-1}) was lower than that in real field conditions. Reports of Cu accumulation, especially regarding the influences of salinity on the absorption of Cu, in halophyte are scarce (Forte and Mutiti, 2017; Han et al., 2012a).

In this study, we aimed to assess the potential of *S. portulacastrum* in Cu and Cd phytoremediation and the interference caused by salinity. *S. portulacastrum* seedlings were water cultivated and the effects of Cu and Cd on their growth and mineral absorption were investigated. Moreover, the ability of *S. portulacastrum* to remove Cu and Cd was evaluated. The effects of salinity on the growth and bioaccumulation ability of *S. portulacastrum* under combined Cu/Cd exposure were also determined. These results provide a theoretical reference for the application of *S. portulacastrum* in the remediation of heavy metal pollution in coastal marine ecosystems.

2. Materials and methods

2.1. Experimental design

S. portulacastrum was collected from the Hailing Levee, Shatoulong Village, Yangjiang City, Guangdong Province, PR China (21°42′05.22″N 111°55′09.68″E) and transported to the greenhouse of the Sun Yat-Sen University (Guangzhou, China). Prior to experiments, *S. portulacastrum* plants were washed to remove any contaminants. Next, segments were prepared and inserted in white pearl cottons (59 cm × 39 cm × 1.5 cm) and placed in plastic containers (60 cm × 40 cm × 20 cm) with 20 L of ½ Hoagland nutrient solution. The segments were cultivated until their roots reached 10–15 cm.

The Cd and Cu treatments were carried out from April to May and July to August 2015, respectively. Cd treatments were prepared by dissolving $CdCl_2 \cdot 2.5H_2O$ in culture medium at 5, 10, 15, and 20 mg L^{-1} concentrations. Cu treatments were prepared using $Cu(NO_3)_2 \cdot 3H_2O$ and the concentrations were set at 2.5, 5, 7.5, and 10 mg L^{-1}. Culture medium without Cu or Cd was used as the control. For each treatment, 10 healthy *S. portulacastrum* individuals (length: 18–20 cm, biomass: 65 ± 5 g, and diameter: 5 ± 1 mm) with complete root systems and 2–3 pairs of leaves were selected. After washing, these individuals were inserted in white pearl cotton (18 cm diameter, 5 mm thickness) and then placed in plastic containers with 2.5 L culture medium. Three replicates were set for each treatment. The growth of *S. portulacastrum* was monitored every week and the nutrient solutions were supplemented without the addition of Cu or Cd to balance the natural evaporation. Samples were harvested after 30 days of treatment.

During July to September 2015, to test the interference of salinity, seedlings was cultured in the ½ Hoagland nutrient solution prepared with four salinities (0‰, 5‰, 15‰ and 30‰), containing 5 mg L^{-1} Cu and 5 mg L^{-1} Cd. After 30 days, samples were harvested for analyses.

2.2. Sample process and metal element analyses

Prior to harvest, one of the top third leaves was collected from each plant for the measurement of leaf area.

After measuring the seeding height, the leaves, roots, and stems were separated. The roots were soaked in EDTA-Na_2 solution to remove all absorbed heavy metals and then washed with Milli-Q water. Stems and leaves were also washed with Milli-Q water. The roots, stems, and leaves were dried at 105 °C for 30 min in an oven, and then dried at 75 °C until the samples reached a constant weight. Afterwards, the samples were weighed and then ground into powder. The samples were sieved using a 60-mesh sieve, and sealed and preserved in plastic bags.

The samples were digested using HNO_3 at 140 °C and then the concentrations of Cd, Cu, Zn, Fe, and Mg were measured using an Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). The detection limits were all 0.01 mg L^{-1}. The detection wavelengths were 206, 238, 285, 229 and 325 nm for Zn, Fe, Mg, Cd and Cu, respectively.

2.3. Data analyses

The following indices were calculated to compare the tolerance and bioaccumulation potential of *S. portulacastrum* to heavy metals.

Tolerance index (TI) = (LS + WR + WS + WL) / 4,

where LS, WR, WS, and WL are the ratio for height, root biomass, shoot biomass, and leaf biomass between the treatment and control group, respectively.

Bioconcentration factor (BCR) = the heavy metal concentration in samples/heavy metal concentration in solution (Ali et al., 2002).

Translocation factor (TF) = the heavy metal concentration in the above-ground tissues / the heavy metal concentration in the roots.

Removal rate = the total amount of heavy metal in plants/the total amount heavy metal amount in container × 100%

One-way ANOVA analysis was performed, followed by the Tukey's test, to detect significant differences among different treatments. These statistical analyses were conducted using SPSS 19.0 (SPSS for windows, SPSS, Inc.).

3. Results

3.1. Effects of Cd and Cu on the growth of S. portulacastrum

Cd treatments did not significantly affect the total biomass, the root biomass, the ratio of the root biomass to total biomass, or the stem biomass (Table 1). The highest value of leaf biomass and seedlings height was observed in treatment with 10 mg L^{-1} and 5 mg L^{-1} Cd, respectively ($p < 0.05$), indicating that low concentrations of Cd might facilitate the growth of *S. portulacastrum*. No significant differences were found in TF among all treatments. In the treatment with 20 mg L^{-1} Cd, the leaf biomass and seedlings height were 22.9% and 10.4% lower than those for the control group, respectively ($p < 0.05$), demonstrating that the growth of *S. portulacastrum* was suppressed. However, no death, defoliation, yellowing, or other toxicological symptoms were found in these treatments.