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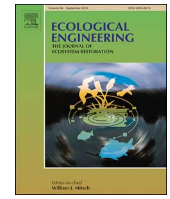

Effects of cropping patterns of four plants on the phytoremediation of vanadium-containing synthetic wastewater

Liang Wang[a,b], Hai Lin[a,b,*], Yingbo Dong[a,b,*], Yinhai He[a,b]

[a] School of Energy and Environmental Engineering, University of Science and Technology Beijing, Beijing 100083, China
[b] Beijing Key Laboratory on Resource-oriented Treatment of Industrial Pollutants, Beijing 100083, China

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ABSTRACT

The effects of cropping patterns of four terrestrial plants (*Artemisia selengensis*, *Trifolium repens*, *Houttuynia cordata* and *Medicago sativa*) on purifying vanadium-containing wastewater co-polluted by V, Cr, Cd and Pb along with their impact pathway was investigated. The results revealed that reasonable cropping pattern could significantly improve the efficiency of phytoremediation for wastewater, which was depended on three factors, namely, total biomass, metal concentration in plants and metal adsorption at root surface. Pattern VII (3:4:1:2, plants mass ratio) showed the best purification effect with 74.15% V, 78.78% Cr, 53.09% Cd and 91.25% Pb removal for the first influent. The root adsorption contributed 57.96%, 46.66%, 41.27% and 49.35% to the total removal of V, Cr, Cd, and Pb. By contrast, in pattern III (2:2:4:2), the maximum plant uptake pattern, only 33.84% V, 29.68% Cr, 18.33% Cd and 35.84% Pb removed from the wastewater were contributed by root adsorption. Our research may open up further scope of utilizing co-cropping of plants for enhancing phytoremediation of metals co-contaminated wastewater. The possible mechanism involves processes that would improve rhizosphere environment, such as the activities of rhizosphere microbes, root exudates, pH and redox potential, by reasonable co-planting.

1. Introduction

Vanadium (V) is an important alloying element and widely used in metallurgy, aerospace, chemical, and other industrial sectors in the form of ferrovanadium, V compounds and metallic V (Zhang et al., 2011). However, the relevant anthropogenic heavy metal in the environment has significantly increased in recent years due to the high-temperature vanadium industrial activities (Yang et al., 2014). Multiple heavy metals (V, Cr, Cd and Pb etc.) have been found in wastewater discharged from mining and smelting for vanadium-containing minerals (Leiviskä et al., 2017), stormwater runoffs from contaminated district (Yang et al., 2014) and the leachate from vanadium-containing stone coal slag (Lin et al., 2017). Recently, more studies have warned against the carcinogenic and toxic effects of high V upon excessive exposure (Khan et al., 2011; Xiao et al., 2015). Overall, multiple heavy metals co-pollution from vanadium industry cannot be ignored and probably induces potential risks to the ecosystem and human health. However, there are few studies addressing technologies to remove V and its associated metals from wastewater (Leiviskä et al., 2017). Moreover, Vanadium-containing wastewater usually involves multiple heavy metals co-pollution, thus making the water remediation more challenging.

Physicochemical remediation methods such as chemical precipitation, ion exchange, adsorption, membrane filtration, coagulation and flocculation, flotation and electrochemical treatment have been widely used in many studies (Fu and Wang, 2011; Zeng et al., 2016; Kaczala et al., 2009). Despite the great progress achieved, these conventional technologies have limiting factors of high cost, generation of secondary pollution and negative effect on the environment (Olguín and Sánchez-Galván, 2012). As a cost-effective and eco-friendly technology with high public acceptance, phytoremediation is considered to be a promising purification method for contaminated water (Bedabati Chanu and Gupta, 2016; Rezania et al., 2016). Aquatic macrophyte such as *Lemna minor*, *Pistia stratiotes*, *Eichhornia crassipes*, *Salvinia natans*, *Elodea Canadensis* and *Limnocharis flava* have been widely utilized for remediating contaminated water in recent decades (Sekomo et al., 2012; Marrugo-Negrete et al., 2017; Rezania et al., 2015). However, the practical application perspective of these plants is subject to their inherent defects including their small, slow-growing roots and high water content of aquatic plants making the disposal process of drying, composting, or incineration more complicated and difficult (Roy et al., 2015). Terrestrial plants are recently counted as alternatives for

* Corresponding authors at: University of Science and Technology Beijing, 100083, China.
E-mail addresses: linhai@ces.ustb.edu.cn (H. Lin), ybdong@ustb.edu.cn (Y. Dong).

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phytoremediation of wastewater due to their longer, fibrous root systems offering huge surface areas for increased metal adsorption. *Sedum alfredii Hance* was used to remove Zn, Cd, Cu and Pb in synthetic wastewater (Xiong et al., 2011) and the roots surface adsorption of *S. alfredii Hance* accounted for most of the total metal removal. However, a single plant species has limited ability to efficiently treat wastewater in which various metals co-exist. Research has shown that co-cropping of some plant species is effective in improving the phytoremediation of multi-metals polluted water (Syukor et al., 2016; Dong et al., 2014). For example, when *Typha angustifolia* and *Limnocharis flava* were co-cropping, the removal rates of multiple metals were all increased in comparison with monoculture (Syukor et al., 2016). However, some research argues that a combination of plant species may not improve removal efficiency for any pollutant taken individually (Rodriguez and Brisson, 2016). Thus, we determine that the cropping patterns of remediation plants need to be further studied especially for multi-metal co-contamination treatment.

We hypothesized that the reasonable co-planting of four selected terrestrial plants could promote growth for each other and enhance the synergistic removal of multiple heavy metals in vanadium-containing wastewater by improving rhizosphere environment. Eight cropping patterns of four plants were designed to investigate the effects of co-cropping on removing combined heavy metals (V, Cd, Cr, and Pb). Our objectives were to i investigate the effect of different cropping patterns on phytoremediation, ii obtain the best cropping pattern of the four plants for purifying vanadium-containing wastewater discharged from a stone coal smelter in Hubei province, China and iii explore the feasibility of using plants co-cropping for metal co-contamination treatment.

2. Materials and methods

2.1. Plant materials

Four plant species, namely, *Artemisia selengensis*, *Trifolium repens*, *Houttuynia cordata* and *Medicago sativa* were selected in our experiment. They were obtained from a nursery garden in Hubei Province, China. Seedlings were cultivated in Hoagland nutrient solution (Hoagland and Arnon, 1950) and plants with consistency in size and vigor were selected for the experiments after pre-culturing for 30 d. These four plants were chosen for this study, because they have strong enrichment ability to V, Cr, Cd or Pb, and they are native plants of this study area.

2.2. Preparation of heavy metal loaded solution

V, Cr, Cd and Pb solutions were prepared by dissolving appropriate quantities of $NaVO_3$, $K_2Cr_2O_7$, $Cd(NO_3)_2{\cdot}4H_2O$ and $Pb(NO_3)_2$ in Hoagland nutrient solution at concentrations of 14.18, 6.12, 0.79 and 4.08 mg/L based on the concentrations of metals in leachate derived from roasted stone coal slag near a stone coal smelter in Hubei province, China (Wang et al., 2018). The pH of the solution was adjusted to 6.0 using 0.1 M NaOH or 0.1 M HCl, and the solution was prepared just before use.

2.3. Heavy metals removal by eight cropping patterns of four herbaceous plants

According to our previous study (Lin et al., 2016a), heavy metal enrichment characteristics of these four plants could be significantly influenced by coexistence to one another under hydroponic culture, which was closely related to plant species. Therefore, to further study the effects of plant species coexistence, the experiment was designed as eight different co-cropping patterns of four herbaceous plants (Table 1). All the plant samples from pre-cultures were rinsed three times with deionized water to clean any adhered substances. The total mass of all plants in each pattern was uniformly 40 g. Then the plants for each pattern were grown in a 1-L black plastic pot with 500 mL of heavy metal loaded solution. The water level reduced due to uptake and evaporation in each pot was maintained daily by adding tap water. To ensure a continuous study of the removal efficiency, the heavy metal loaded solution was continuously aerated and replaced every week. The containers for cropping patterns were arranged in a greenhouse for 28 d in a complete randomized block design under natural light and temperature conditions. The pots added with heavy metal loaded solution without any plants were used as a control to observe metals precipitation.

Table 1
Design of eight different cropping patterns according to mass ratio of four plants.

Cropping pattern	*A. selengensis*	*T. repens*	*H. cordata*	*M. sativa*
I	4	2	2	2
II	2	4	2	2
III	2	2	4	2
IV	2	2	2	4
V	1	2	3	4
VI	4	1	2	3
VII	3	4	1	2
VIII	2	3	4	1

2.4. Sampling and analysis

Water samples were collected from every pot before renewal of heavy metal loaded solution and analyzed for V, Cr, Cd and Pb concentrations using inductively coupled plasma optical emission spectrometry (ICP-OES; Thermo Scientific ICAP 6000 series, Thermo Fisher Scientific Inc., USA).

After a growth period of 4 weeks in the metal-enriched nutrient solution, plant roots were exposed to a certain amount of 0.02 mol/L Na_2EDTA solution for 15 min to remove the adsorbed metals on the root surface (Xiong et al., 2011). The total contents of V, Cr, Cd, and Pb in the eluant were quantified using ICP-OES for obtaining the amount of metals adsorbed on the root surface. Plants were then washed three times with deionized water, separated into roots and shoots and oven dried at 80 °C for 24 h, after which dry weight was recorded. Dried plant material was ground in a metal-free mill. About 0.2 g of subsamples was digested with 5 mL HNO_3 and 1 mL $HClO_4$ in closed Teflon vessels at 180 °C for 8 h. Digested material was diluted up to 50 mL with distilled water and filtered prior to determining metal concentrations by ICP-OES. For quality control, certified reference peach leaf (GBW08501) was also analyzed in triplicate using the same method in each batch of analyses. Results were only accepted when the measured standard concentrations were within 95% to 105% of the certified value (Huang et al., 2012).

2.5. Data analysis

The individual metal uptake of all plants in each pattern was calculated by the equation given below:

$$W = \sum_{i=1}^{4} (C_{ia}M_{ia} + C_{ir}M_{ir}) \quad \text{(a)}$$

where W (μg) is the plant uptake of V (or Cr/Cd/Pb) by all plants in each pattern. C_{is} and C_{ir} (μg/g) are the concentration of V (or Cr/Cd/Pb) in aerial part and root of *A. selengensis*, *T. repens*, *H. cordata* and *M. sativa*, respectively. M_{ia} and M_{ir} (g) are the dry weight of aerial part and root of *A. selengensis*, *T. repens*, *H. cordata* and *M. sativa*, respectively.

All values in this paper are presented as the mean of at least three replicates. One-way ANOVA and LSD's multiple comparisons were applied at a significant level of $P < 0.05$ to verify the statistical significances on the effect of different cropping patterns on plant biomass, contents of V, Cr, Cd and Pb in four plants and accumulation of all metals in one culture pattern using SPSS 22.0.