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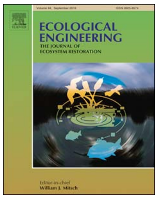

Removal of cadmium in subsurface vertical flow constructed wetlands planted with *Iris sibirica* in the low-temperature season

Na Ma[a], Wenlong Wang[b], Jingqing Gao[c,*], Jie Chen[d]

[a] *Research Institute of Environmental Sciences, College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou 450001, China*
[b] *Zhengzhou University Multi-functional Design and Research Academy Co., Ltd., Zhengzhou 450001, China*
[c] *School of Water Conservancy and Environment, Zhengzhou University, Zhengzhou 450001, China*
[d] *Zhengzhou University Library, Zhengzhou University, Zhengzhou 450001, China*

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ABSTRACT

In this study, the evergreen *Iris sibirica* plant was planted in a constructed wetland during the low-temperature season. Microcosmic subsurface vertical flow constructed wetlands (MVFCWs) were fed with simulated Cd polluted river water for three months under different Cd supply and the seasonal growth changes and Cd uptake ability of *Iris sibirica* were investigated. The treatment performance of MVFCWs and removal mechanisms and distribution of Cd were evaluated. The amount of rhizospheric microorganisms (bacteria, fungi, and actinomyces) and the enzyme activities (urease, sucrase, alkaline phosphatase and catalase) were also monitored. *Iris sibirica* proves to be a good accumulator of Cd with the highest accumulation of Cd in below-ground parts of 443.3 mg/kg when exposed to 8 mg/L Cd for three months. Under 1, 2, 4 and 8 mg/L Cd supply in low-temperature seasons, the MVFCWs exhibit average Cd removals of 94.9%, 97.4%, 95.6% and 96.2%, respectively. The predominant removal process of Cd in MVFCWs is substrate adsorption, which accounts for about 50% of Cd removal. Under the same Cd supply, the removal rates of Cd, amount of rhizospheric microbes and activities of enzymes in planted wetland are higher than those in unplanted wetland.

1. Introduction

Pollution of water environments such as rivers and lakes by heavy metals (HMs) is becoming increasingly serious in China (Schaller et al., 2013). Of all the HMs, cadmium, a naturally occurring non-nutrient HM, which is considered to be highly toxic to most organisms, enters the environment mainly from industrial processes and phosphate fertilizers. It presents a serious threat to the health of people and animals and can enter the body through the food chain, causing chronic poisoning (Liu et al., 2007). The constructed wetlands (CWs) offer a cost-effective, eco-friendly and technically feasible technology (Lv et al., 2017; Wang et al., 2012) and have proven effective and successful in remediation of heavy metal pollution (Ali et al., 2013; Guittonny-Philippe et al., 2014).

The mechanism of metal removal from wastewater in CWs can involve binding to sediments and media, precipitation as insoluble salts, plant uptake and microbial metabolism (Yadav et al., 2010). A study by Song et al. (2011) demonstrated that 64.9% of Cd at an initial concentration of 2.4 mg/L was removed in laboratory-scale CWs planted with *Canna generalis* in springtime. Another study by Arroyo et al. (2010) showed average removal efficiencies of Cd of 6.4% for CW planted with *Typha latifolia* and 16.6% for CW planted with *Iris pseudacorus* and *Salix atrocinera*.

The vegetation, especially macrophytes, covering CWs is considered to play an important role in accumulation and sequestration of metals by storing them mainly in their oxygenated rhizosphere and their roots or shoots. However, phytoremediation in constructed wetlands is a slow process, and climate or seasonal conditions may interfere with or inhibit plant growth (Lee, 2013). Moreover, aquatic plants tend to wither easily and become dormant in winter when temperatures are extremely low (Faulwetter et al., 2009). Plant species selection may be more important in locations with low winter temperatures and extended periods of plant dormancy than in milder climates (Taylor et al., 2011).

The *Iris sibirica* (*I. sibirica*), a flowering plant species in the family *Iridaceae*, is more resistant to low temperatures than other species of *Iris* (e.g. *Iris pseudacorus*) (Wang et al., 2012). It is an evergreen emergent plant with a one-month recovery period (after harvesting) and an eleven-month water purification period. Its well-developed root system can provide a large surface area for attached microorganisms, thus increasing the potential for uptake of HMs. Although information about the Cd accumulation of *I. sibirica* and its influence on plant growth in spring was reported in our previous study (Gao et al., 2015), there is limited information on removal mechanisms and distribution of Cd in vertical flow constructed wetlands (VFCWs) planted *I. sibirica* in winter, let alone about variations in

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[*] Corresponding author.
E-mail address: jingqinggao@zzu.edu.cn (J. Gao).

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rhizosphere conditions of *I. sibirica*. Consequently, more research is needed to explore further the Cd removal performance of VFCWs planted with *I. sibirica* and new insights into variations in rhizosphere conditions in low-temperature season are necessary.

In this paper, microcosmic subsurface vertical flow constructed wetland systems (MVFCWs) planted with *I. sibirica* were developed to treat Cd-contained simulated polluted river water with different Cd levels in the low-temperature season. The main objectives of this study are (1) evaluation of the treatment performance of VFCWs planted with *I. sibirica*, (2) elucidation of the removal mechanisms and distribution of Cd in VFCWs, (3) investigation of the relationship between Cd removal and microbial amount and enzyme activities in the rhizosphere, and (4) providing information bearing on plant selection in CWs at the low-temperature season in various areas. The main findings of this research could provide reference for the utilization of *I. sibirica* in the phytoremediation of heavy metals in wetland ecosystems at the low-temperature season.

2. Materials and methods

2.1. Experimental wetland system

2.1.1. Plant culture and operation

Whole plants of *I. sibirica* (Fig. 1a) with essentially similar biomass were collected from two-stage baffled surface-flow CWs in Jialu River, Zhengzhou City, Northern China, on September 2016. They were cultivated in a container with 10% modified Hoagland's solution (Hoagland and Arnon, 1950) after their roots had been washed to remove soil and dead plant tissue. After a week of acclimation, the plants were transplanted into MVFCW units (six plants per unit) on September 2016.

2.1.2. Composition of polluted river water

In order to minimize variability, the experiment was undertaken with synthetic Cd-containing inflow water intended to simulate the characteristics of polluted river water based on Chinese environmental quality standards for different grades of surface water (MEPC, 2002). The "test solution" of Cd-containing simulated polluted river water was prepared with tap water composed of macro-nutrient solution, microelement solution and working solution (Cd) according to the methods described by Yadav et al. (2010). The "test solution" was used immediately after it was ready.

2.1.3. Design and operation of wetland

The experimental site is located in a transparent rain shelter at the Zhengzhou University, Northern China (112°42′–114°14′ E, 34°16′–34°58′ N). The microcosmic wetland systems were designed in a subsurface vertical flow style, and the MVFCW unit was constructed with a plastic reactor. The schematic cross section of the MVFCW unit is presented in Fig. 1b. The treatment unit is 50 cm long, 36 cm wide and 25 cm deep and has a 45 L capacity, and a surface area of 0.18 m^2. To improve the significance of the study, three replicates per microcosm were operated from September to December 2016 for a period of about 3 months. The set-up parameters for these units are similar to those described in our previous research (Gao et al., 2012). The theoretical hydraulic retention time is approximately 1.8 days, and approximately 10 L of simulated river water is added per day with a hydraulic loading frequency of 0.056 m^3/(m^{2}d). Design parameters of different microcosm units are shown in Table 1. The MVFCWs were divided into six groups (U0: Blank treatment; U1: 1 mg/L Cd; U2: 2 mg/L Cd; Uc: 2 mg/L Cd; U4: 4 mg/L Cd: U8: 8 mg/L Cd). U0, U1, U2, U4 and U8 were planted with *I. sibirica* and Uc remained unplanted as a control.

Table 1
Design parameters of the microcosm wetland units.

Microcosm units	U0	U1	U2	Uc	U4	U8
Cd inflow concentration (mg/L)	0	1	2	2	4	8
Plant number (rhizome per unit)	6	6	6	0 (unplanted)	6	6
Influent flow (L/d)	10	10	10	10	10	10
Hydraulic retention time (d)	1.8	1.8	1.8	1.8	1.8	1.8

The MVFCW units were first managed with tap water for two weeks to clean the systems. Then they were watered immediately with Cd-containing simulated polluted river water to maintain a water level consistent with a 2–3 cm layer up to the substrate surface. The experiment began on September 14, 2016, and sequencing fill-and-draw batch mode was applied to the influent. The Cd-contained simulated polluted river water was intermittently pumped from a storage tank into the MVFCW units by a peristaltic pump at the same speed. Each unit was manually drained twice a day through a valve at the bottom of the unit.

2.2. Sampling and analytical methods

2.2.1. Water quality

Water samples were collected weekly from September to December 2016 in all sampling sites to determine the main parameters of water quality. All the samples were manually taken at 10:00 a.m., analyzed immediately for pH, water temperature and dissolved oxygen (DO). The pH value was monitored using a pH meter (pHS-3C, Shanghai INESA Scientific Instrument, China). Water temperature and DO were measured with a DO meter (HQ30d53LEDTM, HACH, USA) in situ. Water samples were filtered through a standard filter paper (ALBET, particle retention is 7–12 μm), acidified with HNO$_3$ (APHA, 2005), and stored at 4 °C until laboratory analysis for metal determination. Metal concentrations were measured using inductively coupled plasma mass spectrometry (ICPMS) (ICAP 6500 DUO, Thermo Electron Corporation, USA).

2.2.2. Plants and sediments

Plant samples and rhizospheric sediments were collected in the middle of the experiment on November 7, during the autumn growth period, and at the end of the experiment on December 28, during the winter growth period. Plants removed from the experimental site for measurement of biomass and Cd content were replaced by cultured plants marked with a rope. Harvested plant samples were separated into aboveground and belowground portions. The corresponding fractions of plants were dried in an oven at 105 °C for 1 h to deactivate enzymes and then dried at 80 °C for

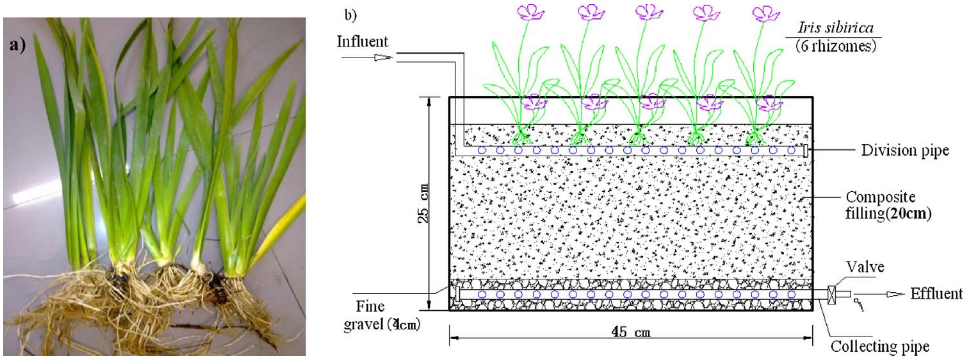

Fig. 1. a) *Iris sibirica plants*; b) Schematic section of the MVFCWs.