Constructed Wetlands Planted with Iris for Treatment of Wastewater Simulating a Typical Mine Drainage in Japan: Effects of Organic-Feeding on Removal of Zn and Cd

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Abstract:

This study investigated the removal of heavy metals from synthetic acid mine drainage (AMD) using lab-scale constructed wetlands (CWs) packed with limestone and loamy soil and planted with iris/cattail. The AMD contained 7.60 mg/L Zn, 0.07 mg/L Cd, 37.20 mg/L Fe, and other metals with a pH of 3.60. The CWs were operated with hydraulic retention times (HRT) of 4–7 days in sequencing batch mode for 14.5 months. Metal removal in the CWs was gradually saturated. The Zn and Cd removals were, respectively, 56.99–72.15% and 74.50–86.22% before being supplemented with sludge and domestic wastewater (DW). Synthetic DW containing organic matter and nutrients was added to promote the activity of sulfate-reducing bacteria (SRB), which can precipitate metal sulfides such as CdS, FeS, and ZnS. Subsequently, the Zn and Cd removals increased to 67.60–88.41% and 94.31–96.28%, respectively. Fe removal was successful at more than 98.00% in all CWs with/without synthetic domestic wastewater components. The treatment performance of CWs planted with iris as an ornamental plant was comparable to those planted with cattail as a typical wetland plant. The findings of this research suggest metal removal in the CWs can be enhanced significantly by creating a favorable condition for SRB, such as supplementing external carbon sources. In addition, using ornamental flowering plants such as iris in CWs may create beautiful landscapes.

Keywords: *heavy metals, constructed wetlands, acid mine drainage, domestic wastewater, iris*

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1. Introduction

Japan has a long history of mining, having previously held a distinguished position as a leading global producer of metals, thereby accumulating considerable economic affluence. However, since the middle of the 20th century, the depletion of mineral reserves, the escalation of labor costs, the liberalization of mineral resource imports, and environmental concerns have led to the closure or abandonment of most mines in Japan (Ueda and Masuda 2005). Nevertheless, the continuous existence of acid mine drainage (AMD) streams with high levels of heavy metals and low pH necessitates ongoing remediation efforts (Kato et al. 2020; Koide et al. 2012). In pursuance of environmental protection and the preservation of human health, the Japanese government allocates substantial financial resources, estimated at billions of yen annually, to the treatment of mine wastewater (Kato et al. 2017; Otsuka et al. 2014). Several conventional approaches have been employed for the treatment of AMD, including coagulation, filtration, and neutralization. However, these approaches suffer from the disadvantage of substantial capital, operational, and maintenance costs (Kato et al. 2020; Rodríguez-Galán et al. 2019). Furthermore, the generation of secondary waste, such as sludge, adds to the drawbacks of these methods. Therefore, in order to reduce the financial burden and achieve sustainable AMD treatment, the development of a low-cost and environmentally friendly alternative technology is deemed necessary.

Constructed wetlands (CWs) are artificial systems designed to purify wastewater by utilizing the inherent capabilities of filter media, plants, and organisms. Their operational simplicity, low cost, and remarkable biodiversity make them a potential eco-friendly solution for mine drainage treatment (Hassan et al. 2021; Nguyen et al. 2021). The involvement of plants and microorganisms in the process of heavy metal removal from wastewater within CWs is of utmost importance. Microorganisms can oxidize and reduce metals, thereby causing their precipitation and subsequent elimination from water. Plants actively contribute to metal removal through phytostabilisation, phytoextraction, and rhizofiltration (Hamad 2020). Besides these, plants significantly enhance bacterial activity in the rhizosphere through the secretion of substances from their roots (Chen et al. 2021; Wang et al. 2018).

Mine drainage in Japan can be classified into eight types based on the pH and the ratio of the metal concentrations to the effluent standard (Soda and Nguyen 2023). One of the eight types is weakly acidic with high Zn concentration. For example, the AMD of a mine in Kyoto prefecture displayed a low pH of about four, with two and three times higher concentrations of Zn, Cd, and Fe, respectively, than the effluent standard values. In our earlier study, a lab-scale batch experiment was conducted to evaluate the treatment performance of CWs filled with limestone and planted with cattails as a typical wetland plant for actual and synthetic mine drainage (Nguyen et al. 2022b). The lab-scale CWs demonstrated effective removal of heavy metals. Sulfate-reducing bacteria (SRB) in the CWs implied metal removal through sulfide precipitation.

In CWs, the stimulation of sulfate-reducing bacteria (SRB) activities is desirable for sustainable AMD treatment because SRB can simultaneously generate carbonate to neutralize the acidity and sulfide to stably precipitate dissolved metals (Ayangbenro et al. 2018; Kiran et al. 2017). However, the electron donors and nutrients in AMD are generally low. Thus, supplementation of external carbon sources is a feasible way to stimulate SRB activities to achieve effective treatment of AMD. Domestic wastewater (DW) containing organic matter and nutrients in mining areas can be used as an external carbon source to promote bacterial activities. Nutrients in DW can significantly promote the growth of wetland plants. Many studies showed that DW can be effectively treated by CWs (Ji et al. 2020; Ho et al. 2022).

In order to achieve high metal removal, the plants used in CWs should be selected carefully (Hamad 2020; Tangahu et al. 2011). Additionally, to provide social and economic benefits, such as creating landscapes and reducing environmental stress and disease, ornamental flowering plants are encouraged to be used as wetland plants. Iris species are widely cultivated ornamentals in Asian and European countries. They have high metal-removing ability, strong tolerance, and great aesthetic appeal (Naing et al. 2023). The metal phytoremediation capacity of *I. pseudacorus* was reported in previous studies (Branković et al. 2018; Małachowska-Jutsz and Gumińska 2018; Parzych et al. 2016; Schück and Greger 2020). However, data on its role in AMD remediation is still lacking.

For this case study in a lab-scale batch experiment, synthetic DW components were added to AMD to promote the bioprocesses. *I. pseudacorus* was used to create more value for CWs. The main purposes of this study are (1) to evaluate the treatment performance of CWs for heavy metal removal from AMD containing DW components, (2) to compare the use of a typical wetland plant (cattail) and an ornamental flowering plant (iris) for heavy metal removal from AMD, and (3) to clarify the pathway of heavy metal removal from AMD in CWs.

2. Materials and Methods

(1) Synthetic Wastewater and Substrates

The synthetic wastewater was prepared based on the characteristics of a mine in Kyoto prefecture, Japan (Nguyen et al. 2022). Table 1 presents the chemical composition of The AMD used in Phases I–III and Japan effluent standards. The concentrations of Cd, Fe, and Zn were found to exceed the Japanese effluent standard limits of 0.03 mg/L, 10.00 mg/L, and 2.00 mg/L, respectively.

AMD containing DW components used in Phase IV was synthesized by the chemicals shown in Table 1 and additionally bonito extract (Fujifilm Wako Pure Chemical Co. Ltd., Japan) (40.00 mg/L), polypeptone (Fujifilm Wako Pure Chemical Co. Ltd., Japan) (60.00 mg/L), $Na_2HPO_4 \cdot 12H_2O$ (8.36 mg/L), KH_2PO_4 (1.36 mg/L), KCl (0.70 mg/L), NaCl (1.50 mg/L), $MgSO_4 \cdot 7H_2O$ (1.03 mg/L), $CaCl_2 \cdot 2H_2O$ (9.27 mg/L) (Soda et al. 2021a). The final concentrations of total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP) in the influent were, respectively, 32.00 ± 9.45, 10.11 ± 1.31, and 1.75 ± 0.02 mg /L.

Limestone and loamy soil were the substrates employed in the current study. Limestone (Konan Shoji Co., Ltd., Tokyo, Japan) and loamy soil (Akadama, Hirota Shokai, Co., Japan) had the same particle size of 10 mm, porosity of 45.0% and 55.0%, and bulk density of 1.64 g/cm^3 and 0.83 g/cm^3, respectively. Loamy soil includes many metal oxides such as SiO_2, Al_2O_3, FeO, CuO, K_2O and CaO (Soda et al. 2021b). The main component of limestone is calcium carbonate.

Table 1. Chemical composition of simulated AMD in Phases I–III (Avg. ±SD, *n* = 75)

	Reagents	Concentration (mg/L)	Effluent standard (mg/L)
Zn	$ZnSO_4 \cdot 7H_2O$	7.61±0.40	< 2.00
Cu	$CuSO_4 \cdot 5H_2O$	0.22±0.02	< 3.00
Cd	$CdCl_2$	0.07±0.01	< 0.03
Mn	$MnSO_4 \cdot 5H_2O$	0.89±0.10	< 10.00
Pb	$PbCl_2$	0.08±0.05	< 0.10
Fe	$FeSO_4 \cdot 7H_2O$	37.20±2.40	< 10.00
As	$AsNaO_2$	0.07±0.05	< 0.10
Ca	$CaSO_4 \cdot 2H_2O$	30.00±3.00	
Na	NaCl	6.70±0.60	
Mg	$MgSO_4 \cdot 7H_2O$	10.00±1.00	
K	KCl	1.50±0.15	
Al	$AlNa(SO_4)_2 \cdot 12H_2O$	2.01±0.21	
pH		3.58±0.12	5.80–8.60

(2) Lab-scale CWs Setup and Operation

The laboratory-scale wetlands were installed at the greenhouse located at Biwako Kusatsu Campus (BKC), Ritsumeikan University (Shiga Prefecture, Japan) for a duration of 14.5 months (April 30, 2021–July 15, 2022). The experimental setup is depicted in Figure 1.

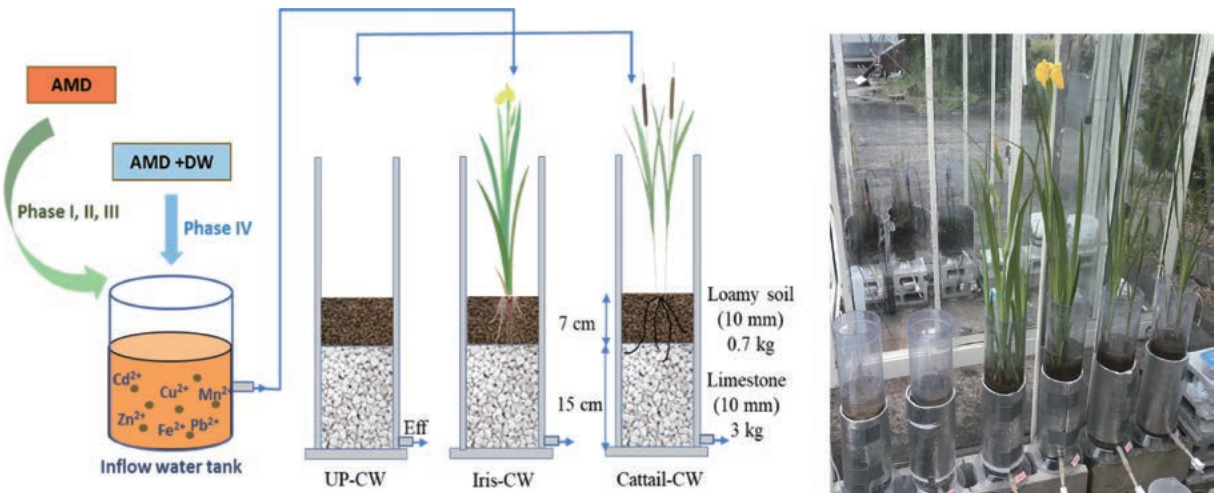

Figure 1. Diagram of CWs for removing heavy metals from AMD.

For each CW, a plastic column (12.5 cm diameter × 50 cm height) was filled from the bottom to top with limestone (15 cm depth, 3 kg) and loamy soil (7 cm depth, 0.7 kg). To simulate a real system, all CWs were wrapped in aluminum foil to prevent light penetration into the substrate. Cattails (*T. orientalis*, 76.0±4.0 cm shoot, 14.0±2.0 cm root, 91.2±2.4 g-wet) and Iris (*I. pseudacorus*, 60±2 cm shoot, 18±2 cm root, 98.3±2.8 g-wet) were planted in CWs (referred to as cattail-CW and iris-CW, respectively), while another set of wetlands (referred to as UP-CW) were left unplanted. These plants were purchased from Tojaku Engei Co. Ltd., Japan. Prior to being planted in the CWs, the plant roots underwent a gentle washing process using tap water to eliminate the original culture soil. Each CW was replicated twice. The temperature in greenhouse was recorded by using a weather recorder (TR-7Ui, T&D Corp. Japan). All CWs were filled with tap water for one week before being provided with synthetic AMD.

In the sequencing batch experiment, each CW was supplied with 1.5 L synthetic AMD. Upon completion of the designated timeframe, the treated water was fully discharged from the bottom. Subsequently, a fresh batch of AMD was introduced into each CW. Samples were collected from both the inlet and outlet to assess the water quality. The experiment was conducted over 14.5 months, divided into four Phases: Phase I (April 30–August 6, 2021, late spring and summer) with a hydraulic retention time (HRT) of 7 days, Phase II (August 10–November 26, 2021, late summer and autumn) with an HRT of 4 days, Phase III (December 3, 2021–March 18, 2022, winter) with an HRT of 7 days. Before starting Phase IV, a mixture of 400 mL of pond sediment (26.0±4.1g of volatile suspended solids) and 100 mL activated sludge (3.0±1.1g_VSS, dry weight) was added to each CW for inoculating microorganisms for two weeks. The pond sediment was collected at a pond near BKC. The activated sludge sample was collected from a sewage treatment plant. In Phase IV (March 25–July 15, 2022, spring and early summer), AMD containing the DW component was treated with an HRT of 7 days.

(3) Sampling and Analysis

Throughout the experimental period, there was a total of 75 batches. The influent and effluent samples in each batch were collected to analyze heavy metal contents and other water parameters. Heavy metals were determined by using inductively coupled plasma spectroscopy (ICP-OES 700 series; Agilent Technologies Japan, Ltd., Tokyo, Japan). The pH, dissolved oxygen (DO), oxidation-reduction potential (ORP), total dissolved solids (TDS), and total organic carbon (TOC) were measured using specific instruments: a pH meter (Laqua; Horiba Ltd., Kyoto, Japan), a DO meter (Hach HQ30D, CA, USA), an ORP meter (YK-23RP, Mother Tool Co. Ltd., Ueda, Japan), a TDS meter (ASTDS1; AS One Corp., Osaka, Japan), and a TOC analyzer (TOC-V CSH/CSN, Shimadzu Corp., Japan), respectively. Suspended solids (SS), sulfate (SO_4^{2-}), and sulfide (S^{2-}) were analyzed following standard procedures (APHA, 2012). Total nitrogen (TN) and total phosphorus (TP) levels were determined through alkaline potassium persulfate digestion followed by UV-vis spectrophotometry, and the ascorbic acid reduction molybdenum blue method after potassium peroxodisulfate decomposition, respectively.

Substrate and plant samples were collected before and after 14.5 months of operation for the purpose of analyzing the heavy metal contents. Plant samples were divided into two parts, including aboveground (shoots) and belowground (roots and rhizomes). Both substrate and plant samples were dried to a constant weight and then ground to less than 50.0 µm for further analysis. The determination of heavy metal contents in plant and substrate samples followed the method described by Nguyen et al., 2022a. For soil and limestone samples, 0.5 g of the dried sample underwent digestion in a solution containing 3.75 mL of hydrochloric acid (35–37%) and 1.25 mL of nitric acid (65%) for 24 hours. The mixture was heated to 95°C for one hour and then cooled to 25°C, before adding 5 mL of distilled water. As for the plant samples, 0.1 g of the dried sample underwent digestion in 1 mL of nitric acid (65%) for 48 hours, followed by the addition of 9 mL of distilled water. After digestion, all supernatants from both substrate and plant samples were filtered through a 0.45 µm filter paper. The resulting filtrates were then analyzed for their heavy metal content using ICP-OES. The experiments were performed in triplicate, and the mean values were recorded.

The plate-count technique, employing R2A agar medium (Merck KGaA, Germany) and Postgate's medium F (Nguyen et al. 2021), was utilized to enumerate heterotrophic bacteria and SRB individually.

In addition, the volumes of input (V_i) and output (V_o) wastewater for each batch were measured to calculate water evaporation using the equation:

$$\text{Evapotranspiration (\%)} = (V_i - V_o) \times 100 / V_i \qquad \text{(Eq.1)}$$

The efficiency of heavy metal removal (%) was computed as:

$$\text{Removal efficiency (\%)} = (V_iC_i - V_oC_o) \times 100 / V_iC_i \qquad \text{(Eq.2)}$$

where C_i and C_o represent the concentrations of heavy metals in the input and output samples, respectively.

Bioconcentration factor (BCF) and translocation factor (TF) are parameters used to evaluate the uptake and movement of contaminants by plants. The BCF and TF for heavy metals in iris and cattail were determined as follows:

$$\text{BCF} = C_p / C_s \qquad \text{(Eq.3)}$$
$$\text{TF} = C_{ps} / C_{pr} \qquad \text{(Eq.4)}$$

where C_p (mg/kg) and C_s (mg/kg) represent the metal concentration in the whole plant and loamy soil, respectively. Similarly, C_{ps} (mg/kg) and C_{pr} (mg/kg) denote the concentration of the metal in shoots and roots, respectively.

3. Results

(1) Environmental Conditions and Water Parameters

1) Environmental conditions

The greenhouse temperatures in Phases I, II, III, and IV were 28.0±10.6, 22.0±8.1, 6.6±7.1, and 23.4±11.7°C, respectively. The plants grew well in summer and spring (Phase I-middle Phase II). The drop in temperature in winter (Phase III) led to the death of the aboveground part of the plants, and only the roots remained alive until the next spring when the young shoots of cattails started to grow. Supplemented by the DW components, the plants grew quickly during Phase IV.

The difference between inflow and outflow of water in the CWs was recorded during the experiment. The water volume of the CWs decreased in each batch treatment. The evaporated amount was insignificant in the late autumn and winter. The highest evaporation (44.0–98.0%) was recorded in summer as the plants grew rapidly under the high temperature. The average evapotranspiration in the unplanted, iris-, and cattail-CWs were 13.5, 35.8, and 25.3%, respectively. The higher evapotranspiration in the iris-planted CW was possibly due to the higher biomass. The high evapotranspiration significantly affected the mass removal of heavy metals in summer, as depicted in Eq.2.

2) Water quality

The pH value, ORP, DO, TDS, sulfate, and dissolved sulfide concentrations before and after CW treatment are depicted in Figure 2.

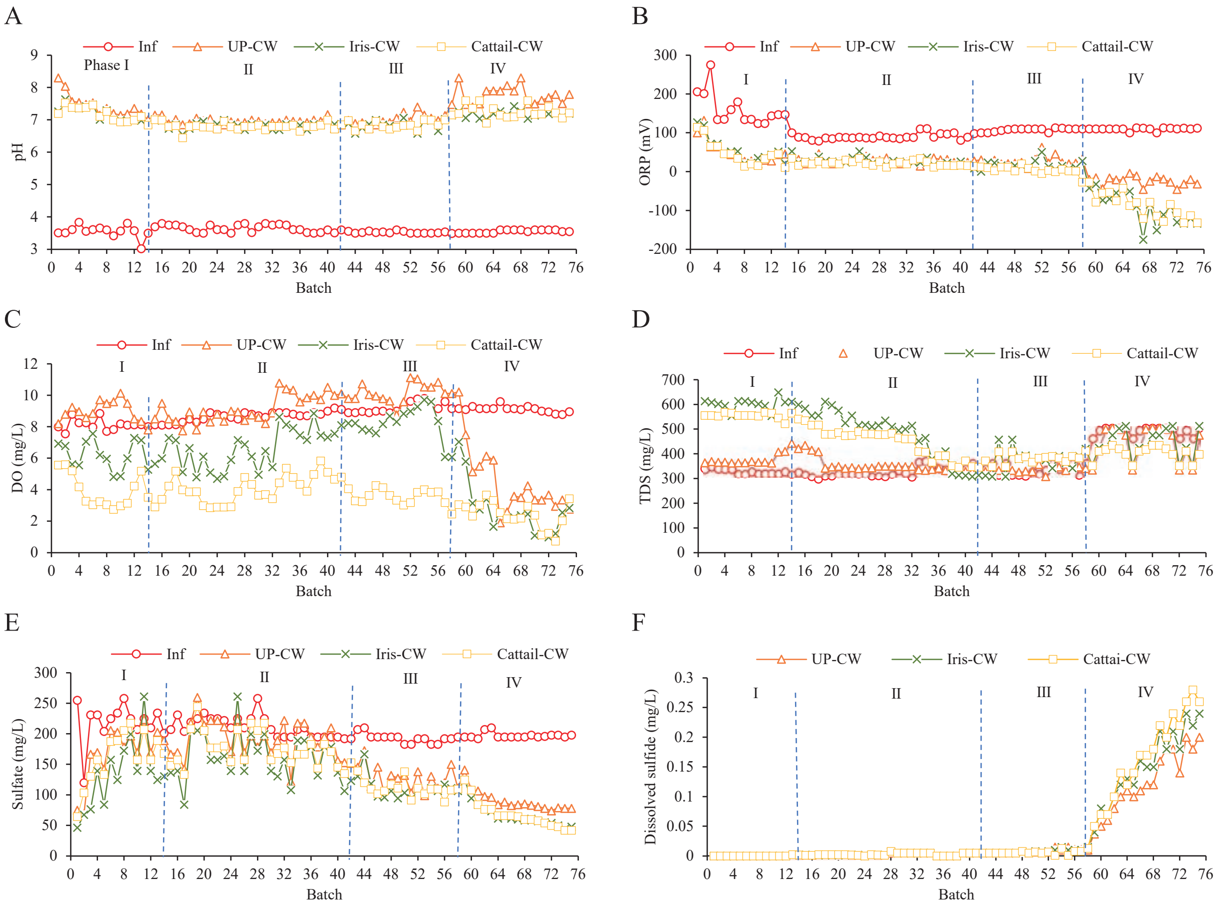

Figure 2. Water parameters in the influents and effluents of the unplanted, iris-, and cattail-CWs treating AMD in the sequencing batch mode: (A) pH, (B) ORP, (C) DO, (D) TDS, (E) sulfate, and (F) dissolved sulfide. April 30, 2021, was defined as day 0.

The pH values of the effluent in all CWs during all phases ranged from 6.50 to 8.31. They slightly increased in Phase IV (Fig. 2A). This was probably because of the acidity buffer capacity of DW, and bicarbonate produced by SRB activities. The pH value in the unplanted CWs tended to be slightly higher than that of the planted CWs. This was probably because the unplanted CWs lacked live plant material, thus, the decomposition of the organic matter mostly occurred through microbial action. This decomposition process might lead to the release of carbon dioxide, which could raise the pH of water. On the other hand, during photosynthesis in the planted CWs, plants absorbed carbon dioxide and released oxygen, which occasionally lowered the pH of the surrounding water.

The ORP values were generally positive in Phases I–III, and dropped drastically in Phase IV, extending from -175.00 mV to 27.50 mV after three months (Fig. 2B). The effluent DO levels in the planted CWs were lower than those of the unplanted CWs, suggesting high oxygen consumption by the roots and microorganisms in the rhizosphere (Fig. 2C). Its values in Phase I-III in the unplanted CWs, iris-, and cattail-CWs were 7.69–11.10, 4.70–9.71, and 2.41–5.82, respectively. The DO values dropped in Phase IV because of the high oxygen demand for decomposing organic matter. The effluent TDS concentration was generally higher than in the influent, except for Phase IV (Fig. 2D). The sulfate concentrations decreased in the CWs effluents, especially in Phase IV (Fig. 2E). The dissolved sulfide concentrations were very low (0.00–0.01 mg/L) in all CWs in Phases I–III, and gradually increased (0.04–0.21 mg/L) in Phase IV (Fig. 2F). These results suggested that SRB reduced sulfate to sulfide.

In Phase IV, the TOC, TN, TP were also removed effectively by all CWs. The average TOC removal was 84.00–88.50% in all systems. In the CWs, nitrogen was removed dominantly through microbial nitrification and denitrification processes, while phosphorus was effectively removed mainly through adsorption and precipitation processes. Besides this, the plants contributed to nutrient uptake. This was proven by the higher nutrient removal in the planted CWs. The TN and TP removals were, respectively, 57.50–92.29% and 86.71–96.70% in all CWs. The results indicated that the CWs can effectively remove multiple contaminants from AMD and DW simultaneously.

(2) Heavy Metal Removal

The time courses of Zn, Cd, Cu, Pb, Mn, and Fe concentrations in the CWs and the average removal efficiencies of each Phase are presented in Figure 3 and Table 2.

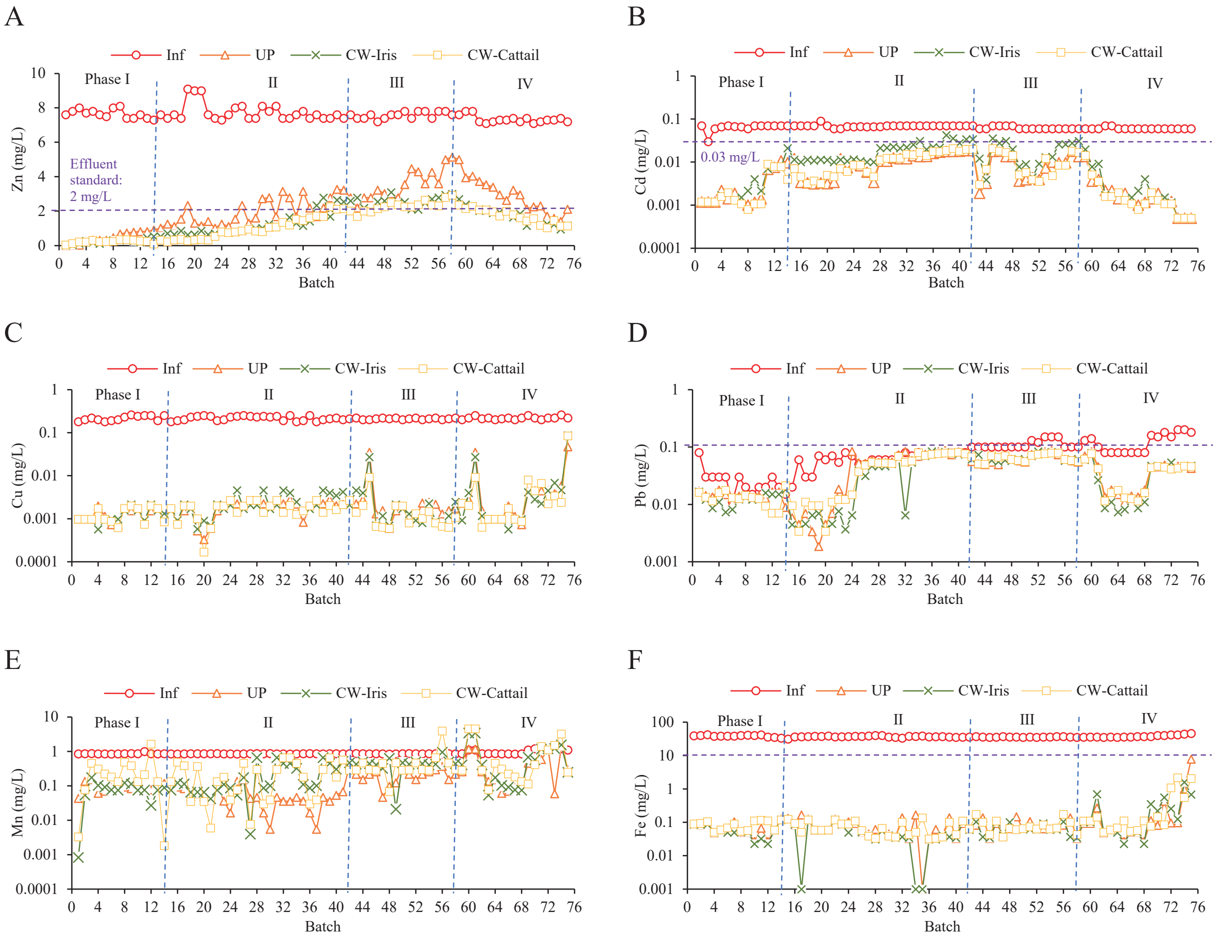

Figure 3. Heavy metal concentrations in the influents and effluents of the unplanted, iris-, and cattail-CWs treating AMD in the sequencing batch mode: (A) Zn, (B) Cd, (C) Cu, (D) Pb, (E) Mn, and (F) Fe. April 30, 2022, was defined as day 0.

Table 2. Removal (%) of heavy metals from AMD in the unplanted, iris-, and cattail-CWs (Avg ± SD)

Metals		Phase I HRT=7days without DW	Phase II HRT=4days without DW	Phase III HRT=7days without DW	Phase IV HRT=7days with DW	Avg ±SD
	UP-CWs	95.00±3.60	77.47±9.52	56.99±9.87	67.55±13.47	74.50±15.41
Zn	Iris-CWs	98.73±0.91	88.13±8.34	70.43±4.07	88.43±10.58	86.70±11.60
	Cattail-CWs	98.02±0.94	88.71±7.92	72.15±3.30	88.05±10.53	87.10±10.80
	UP-CWs	95.84±3.78	86.16±7.01	86.22±7.69	95.96±6.08	91.04±7.90
Cd	Iris-CWs	97.46±3.17	78.90±12.89	74.45±13.83	94.31±11.66	85.41±14.40
	Cattail-CWs	97.37±2.34	86.25±8.14	85.00±8.26	96.28±7.24	90.60±8.80
	UP-CWs	99.52±0.15	99.24±0.28	98.28±3.89	97.54±4.46	98.71±2.91
Cu	Iris-CWs	99.77±0.10	99.18±0.51	98.61±2.81	98.06±4.38	98.90±2.50
	Cattail-CWs	99.62±0.14	99.31±0.29	99.27±0.96	98.51±3.44	99.21±1.80
	UP-CWs	50.13±15.78	37.16±35.97	47.29±6.47	75.89±13.80	51.00±28.10
Pb	Iris-CWs	76.96±13.64	55.71±33.20	48.63±5.91	85.29±15.85	65.40±26.71
	Cattail-CWs	60.64±21.94	43.95±33.31	46.58±6.92	83.88±16.67	57.12±28.80
	UP-CWs	91.08±3.04	93.19±7.65	77.28±9.74	65.12±33.10	82.90±20.70
Mn	Iris-CWs	95.19±3.50	78.97±21.40	53.45±18.46	49.36±81.67	69.90±45.70
	Cattail-CWs	78.49±22.40	75.69±22.18	37.89±95.90	35.37±98.00	58.61±75.86
	UP-CWs	99.86±0.07	99.81±0.04	99.80±0.05	98.95±3.14	99.62±1.60
Fe	Iris-CWs	99.93±0.11	99.99±0.06	99.84±0.10	99.63±0.54	99.82±0.30
	Cattail-CWs	99.86±0.10	99.85±0.07	99.79±0.10	99.62±0.56	99.94±0.30

1) Heavy metal removal from AMD in CWs in Phases I–III

In Phases I, II, and III, the heavy metal concentrations generally decreased after treatment. The highest removal was recorded in Phase I with an HRT of 7 days. The heavy metal removal was decreased at Phase II with an HRT of 4 days. Although the HRT remained at 7 days, the removal slightly decreased at Phase III. The planted CWs showed higher removal in all metals except for Mn.

The average concentration of Cd (0.07 mg/L) and Zn (7.61 mg/L) in influent were higher than the Japan effluent standards of 0.03 mg/L and 2.00 mg/L, respectively. In Phase I, the effluent Zn and Cd concentrations in all CWs were satisfactorily well within the standards. The Zn concentration in effluents exceeded the standard in the middle of Phase II and Phase III for the unplanted CWs, and those in the planted CWs in Phase III (Fig. 3A). The Cd effluent concentration in the iris-CWs also exceeded the standard several times in Phases II and III (Fig. 3B). Among the heavy metals, the Fe had the highest level in the influent. The average Fe concentration (37.40 mg/L) was approximately four-fold higher than the Japan effluent standard (10 mg/L). After treatment, more than 99.00% Fe from AMD was removed from all CWs.

The average Cu, Mn and Pb concentrations were generally less than the effluent standards. In the CWs, Cu was removed successfully from AMD. The concentration of Mn and Pb was also reduced in the CWs. The Mn removal (37.89–95.79%) varied significantly in all CWs during the three phases. The treatment performance for Mn in the planted CWs was greatly decreased in Phase III. The Pb was removed effectively in Phase I. Its removal efficiency was less than 50.00% in Phases II and III, except for those in the iris-CWs (Table 2). The substrate saturation probably led to the reduction in the treatment performance of the CWs. Thus, biological processes should be stimulated to achieve sustainable and effective heavy metal treatment. In the present study, sludge and DW were added to boost the microbial activities and plant growth in Phase IV.

2) Heavy metal removal from AMD in CWs in Phase IV

After inoculating sludge into the CWs and supplementing DW components to AMD, the removal for Zn, Cd and Pb increased significantly in Phase IV, especially in the planted CWs (Fig. 3 and Table 2). The Zn concentration in the planted CWs in Phase IV decreased below the effluent standard and remained at about 1.70 mg/L on average. The Zn removal increased from 56.99–72.15% in Phase III to 67.6–88.4% in Phase IV. Similar to Zn, the Cd level decreased significantly in Phase IV. The effluent Cd concentration (0.002–0.003 mg/L) was below the standard. The average Cd removal in Phase IV increased by 11.31–19.80% compared with Phase III. In this phase, the CWs still exhibited high removal efficiencies for Cu (97.50–98.50%) and Fe (99.00–99.60%) in all the CWs. Among investigated metals, Mn was removed less effectively since being added with DW. Its removal efficiency was slightly reduced from 37.89–77.28% in Phase III to 35.37–65.12% in Phase IV.

(3) Metals Accumulated in CW Media and Plants

1) Accumulation of heavy metal in substrates

During the experiment, sediment was also formed due to substrate breakdown and leaching, and the formation of suspended solids. The heavy metal contents in the sediment and the substrate before and after treatment are presented in Table 3. The total sediment collected in each unplanted CW and planted CW were approximately 100g and 110g, respectively. In general, the levels of investigated heavy metals in substrates increased in all the CWs during the experimental period.

Table 3. Heavy metal contents (mg/kg) in sediment and substrate before and after treatment

	Before use		After use								
			UP-CW			Iris-CW			Cattail-CW		
	Loamy soil	Limestone	Loamy soil	Limestone	Sediment	Loamy soil	Limestone	Sediment	Loamy soil	Limestone	Sediment
Zn	53.60	20.20	498.89	98.86	744.90	377.42	67.50	671.30	365.07	69.01	735.20
Cd	0.10	0.02	5.21	0.63	11.00	4.52	0.49	7.31	4.12	0.54	8.29
Cu	36.60	2.09	67.20	2.81	57.10	54.23	2.41	44.38	53.26	2.38	43.95
Pb	16.58	7.26	20.30	8.41	17.43	19.31	8.21	18.94	19.51	8.01	16.59
Mn	297.50	25.40	389.51	32.15	341.00	347.14	31.19	240.60	346.70	29.52	191.50
Fe	566.00	346.50	3883.00	629.40	13430.00	2879.00	562.80	9790.00	2881.00	497.70	9980.00

2) Accumulation of heavy metal in iris and cattail

The iris and cattail were harvested at the end of the experiment. The amount harvested from iris was 250 g-dry (81 g-dry of the aboveground part and 169 g-dry of the belowground part) from each iris-CW. It was 208 g-dry for cattail from each cattail-CW, including about 60 g-dry of the aboveground part and 148 g-dry of the belowground part. The heavy metal contents in iris and cattail biomass before and after the experiment are shown in Table 4.

Table 4: Contents of heavy metals in iris and cattail biomass in CWs before and after AMD treatment

	Before Use				After Use			
	Iris		Cattail		Iris		Cattail	
	Above-ground	Below-ground	Above-ground	Below-ground	Above-ground	Below-ground	Above-ground	Below-ground
Zn (mg/kg)	99.00	146.00	102.20	158.00	312.50	1899.00	601.50	2039.00
Cd (mg/kg)	1.98	3.68	2.01	4.12	2.61	7.89	2.64	11.20
Cu (mg/kg)	17.00	76.00	86.23	104.00	23.50	94.00	97.70	111.00
Pb (mg/kg)	2.47	9.04	3.01	11.00	5.29	12.89	3.41	17.86
Mn (mg/kg)	140.00	168.00	216.20	211.50	151.00	181.00	221.50	215.40
Fe (mg/kg)	599.00	1082.00	734.00	5887.87	1187.00	6823.00	1663.00	10997.00

The metal amounts in iris and cattail increased significantly during the experiment, especially in the belowground parts. The Zn, Cu, Fe, and Mn concentrations, as essential elements, showed higher levels than Pb and Cd in the plant biomass.

After 14.5 months of operation, the amounts of Zn, Cd, Cu, Pb, Mn, and Fe accumulated in the iris biomass were, respectively, 329.76, 1.15, 10.88, 1.76, 22.59, and 1133.61 mg in the iris-CW. Those were, respectively, 322.55, 1.44, 11.40, 1.95, 21.44, and 1282.47 mg in the cattail-CW. This indicates that iris was comparable with cattail in removing heavy metals from AMD.

Table 5. BCF and TF values of iris and cattail in the planted CWs after the experiment

CWs	Metal	TF		BCF
		Before Use	After Use	
Iris-CW	Zn	0.68	0.16	5.86
	Cd	0.54	0.33	2.32
	Cu	0.22	0.25	2.17
	Pb	0.27	0.41	0.94
	Mn	0.83	0.83	0.96
	Fe	0.55	0.17	2.78
Cattail-CW	Zn	0.65	0.29	7.23
	Cd	0.49	0.24	3.36
	Cu	0.83	0.88	3.92
	Pb	0.27	0.19	1.09
	Mn	1.02	1.03	1.26
	Fe	0.12	0.15	4.39

The BCF and TF of iris and cattail, both before and after their utilization in the CWs, are displayed in Table 5. The BCF values for all heavy metals examined were observed to be between 0.94 and 7.23 across all the planted CWs, indicating the effective uptake of heavy metals from loamy soil by iris and cattails. Notably, the cattail-CWs exhibited higher BCF values.

In all the investigated metals in the planted CWs, the TF values were consistently lower than 1, except for Mn in the cattail-CWs. This finding indicates that the belowground parts of the iris and cattail plants were the main contributors to the phytoextraction and the accumulation of heavy metals.

(4) Bacterial Communities in the CWs

In all CWs, the population of heterotrophic bacteria detected from water samples during Phases I–III ranged from 5×10^3 to 10^4 CFU/mL. This population can be supposed to be proportional to that in the substrate in the CWs. Its population slightly decreased in Phase III due to the low temperature, the death of plants, and lacking organic matter. In Phase IV, the numbers of heterotrophs in effluent in all the CWs increased to 1.4×10^5–1.4×10^5 CFU/mL after being supplemented with sludge and DW.

Table 6. Population of SRB in effluents during four phases and soil samples in Phase IV

	UP-CW	Iris-CW	Cattail-CW
Effluent samples (CFU/mL)			
Phase I (*n*=4)	$0 – 10^1$	$5\times10^0 – 10^1$	$4\times10^0 – 10^1$
Phase II (*n*=4)	$5\times10^0 – 10^1$	$2\times10^1 – 3\times10^1$	$10^1 – 3\times10^1$
Phase III (*n*=3)	$10^0 – 2\times10^1$	$3\times10^1 – 3\times10^1$	$2\times10^1 – 4\times10^1$
Phase IV (*n*=3)	$10^2 – 3\times10^3$	$4\times10^2 – 2\times10^3$	$3\times10^2 – 2\times10^3$
Soil samples (CFU/g-dry)			
	3×10^3	2×10^3	1.5×10^3

The population of SRB in the effluents throughout the experimental period and soil samples at the end of Phase IV are presented in Table 6. A small number of SRB (0– 4×10^1 CFU/mL) were observed in the effluent during Phases I-III, and its population increased to (10^2 – 3×10^3 CFU/mL) in the effluent at Phase IV. The addition of sludge and DW stimulated SRB growth. At the end of the experiment, a considerable quantity of SRB (1.5×10^3 – 3×10^3 CFU/g-dry) was found in the substrate.

(5) Mass Balance in the CWs

In this experiment, the removal of heavy metal from AMD can be achieved through substrate, plant uptake, sedimentation, and other processes. Throughout the experiment, the total amount of Zn, Cd, Cu, Pb, Mn, and Fe provided to each wetland column was, respectively, 855.15, 7.33, 24.37, 9.25, 100.31, and 4207.55 mg.

As depicted in Figure 4, the accumulation in the substrate within the unplanted CWs served as the principal pathway for heavy metal removal, accounting for 44.00–64.50% of the total metals. In the planted CWs, the levels of Zn, Cu, and Mn accumulated in the plants were found to be comparable to those in the substrate. Generally, metal accumulation in the substrates and the plant biomass were the main pathways in removing heavy metal from AMD. The Zn, Cd, Cu, Pb, Mn and Fe accumulated in substrates were, respectively, 37.81–38.28%, 53.74–55.10%, 30.57–33.36%, 17.12–22.10%, 12.02–17.08%, and 41.71–46.10%, while those in plants were 37.72–38.56%, 15.74–19.69%, 44.65–46.95%, 19.07–21.09%, 21.38–22.52%, and 26.94–30.48%, respectively. In addition, the metal accumulation in the sediment contributed significantly to heavy metal removal from AMD, accounting for 8.64–33.99%. This was more in the case of Mn and Fe, which can be precipitated by forming metal oxide/hydroxide under oxidative conditions (Soda and Nguyen, 2023). Other processes were the smallest among all the heavy metal removal routes, accounting for 0.86–2.13% for the planted CWs and 1.62–8.98% for the unplanted CWs for all metals.

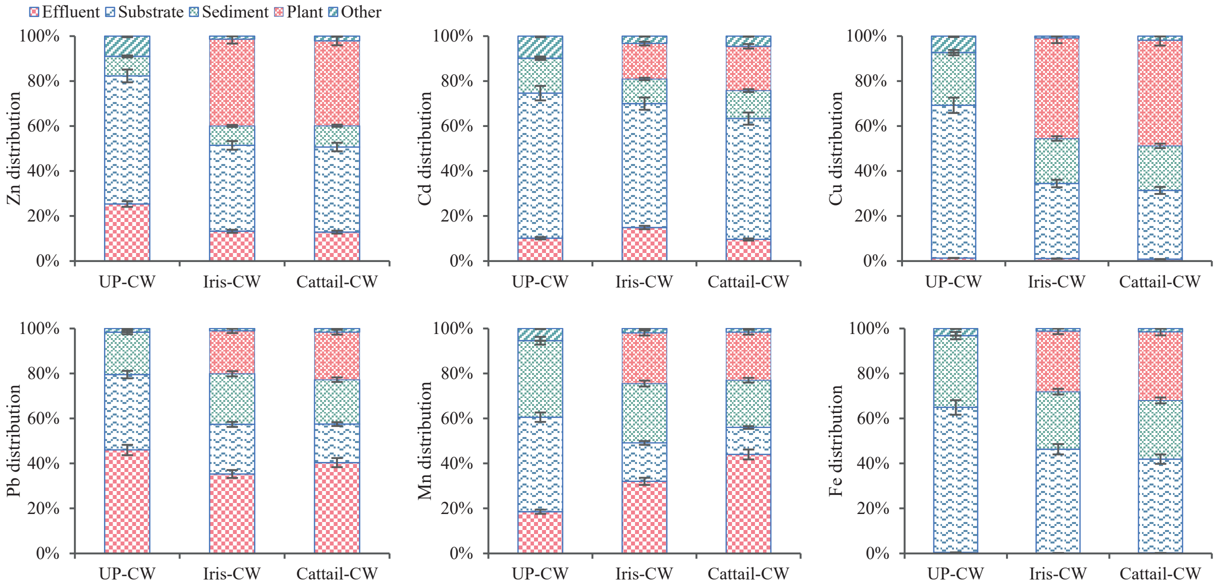

Figure 4. Heavy metal removal pathway in each CW during the experiment

4. Discussion

(1) Performance of CWs for Heavy Metal Removal from AMD

Similar to the previous study (Nguyen et al. 2022b), the lab-scale CWs showed a high capacity for removing heavy metals and neutralizing synthetic AMD. In the present study, loamy soil and limestone were used as substrates in the CWs. Loamy soil has characteristics of high permeability, porosity, water retention, and adsorbing metal ions. Limestone generates hydroxide ions, leading to increased pH. Metals can be removed through neutralization or precipitation as metal hydroxides, such as $Zn(OH)_2$ and $Cu(OH)_2$. Fe can be removed easily by the formation of insoluble oxides in CWs (Hara et al. 2021; Nguyen et al. 2022a). In Phases I and II, heavy metal removal was lower with the shorter HRT for metals interacting with the substrate. Adsorption was a dominant process in the Cd removal in CWs in previous studies (Nguyen et al. 2021; Soda et al. 2021b). Although the HRT remained at 7 days, the Cd, Zn, and Pb removal decreased in Phase III. This result implied that the substrate was gradually saturated by adsorbing the metals. Also, the wilted plants in late autumn and winter reduced the treatment performance in Phases II and III.

To achieve effective AMD treatment, the stimulation of bioprocesses was necessary. Thus, sludge was inoculated into the CWs and DW components were added to the AMD to boost the microbial activities in Phase IV. The removal for Zn, Cd and Pb increased significantly especially in the planted CWs (Fig.3A and Table 2). A higher population of heterotrophic bacteria and SRB was found in Phase IV (Table 6). Heavy metal removal can be promoted through adsorption on the biofilm in CWs (Carpio et al. 2014; Edwards and Kjellerup 2013). Some types of heterotrophic bacteria degrade complex organic substances into simple organic substances, such as formic, lactic, and butyric acids, for SRB utilization (Chen et al. 2021). SRB enhanced the conversion of sulfate into sulfide in Phase IV. The produced sulfide reacted with dissolved metals, leading to the formation of highly insoluble metal sulfides. Metal sulfide precipitation is an effective way to remove metals in CWs (Nguyen et al. 2021; Nguyen et al. 2022a). Additionally, the reduction of sulfate by SRB facilitated the increase of pH in Phase IV, which promoted precipitation in the metal hydroxides/sulfides. Supplementing nutrients in DW also promoted the growth of the plants. The roots and rhizomes could remove organic matter and nutrients in DW without secondary pollution.

Mn was the only metal removed less effectively in Phase IV. Mn is often removed through oxidation in CWs. Thus, the addition of DW led to a drop in the DO concentration and ORP value in the flooded CWs (Figure 2), leading to a decrease in Mn removal efficiency (Nguyen et al. 2022b).

Overall, the planted CWs exhibited high effectiveness in heavy metal removal from AMD mixed with DW, with the effluents generally meeting the Japan effluent standards in Phase IV. This treated wastewater can be repurposed for various uses such as irrigation and car washing. Throughout the experiment, approximately 3.60% of limestone and 14.20% of loamy soil were lost due to substrate breakdown and leaching. Therefore, it is crucial to estimate the lifespan of CWs to ensure timely maintenance and replacement. Additionally, calculating the cost of substrate is essential for determining the most cost-effective approach and developing sustainable strategies for managing the wetland throughout its lifecycle. Moreover, implementing a strategy for harvesting and treating dead plants for recovering metals can enhance treatment performance, minimize environmental impact, and maximize the recovery of valuable resources.

(2) Potential Use of Ornamental Flowering Plants in AMD Remediation

In this study iris, an ornamental flowering plant was used for AMD treatment. Iris demonstrated a high tolerance with low pH and high metal concentrations in AMD. With characteristics of fast growth, a large root system, high biomass, and ecological flexibility, the iris has great potential for phytoremediation (Crisan et al. 2021; Naing et al. 2023). After 14.5 months of operation, the concentration of heavy metals in iris increased significantly, especially in the belowground part, being comparable with cattail (Table 4). The BCF values in iris for the metals were 0.94–5.86, implying its ability in the uptake of metals from the loamy soil. The accumulated metals can be collected easily by harvesting the aboveground parts of the plant biomass. At the end of the experiment, the contribution to heavy metal removal by iris was 15.74–44.65% in all the CWs (Figure 4).

Given the attractive flowers and foliage, iris has advantages in the ornamental and commercial industries. By incorporating iris into mine wastewater treatment systems, the aesthetic appeal of these plants can enhance the visual appearance of the treatment area. This can have positive social and economic impacts by improving the site's overall aesthetics, potentially attracting visitors or tourists, and increasing property values. With this approach, an old dump in Colombia was reshaped into a beautiful park by planting different ornamental species, and it has now become a tourist destination and recreational area (Hogland et al. 2019). Similarly, in Sweden, a polluted soil site was transformed into Orrefors Park using ornamental plants (Hogland et al. 2019). In addition, establishing iris-based AMD treatment systems can create employment opportunities, particularly in areas where mining activities are prevalent. Local communities can benefit from job creation, whether it involves the cultivation and maintenance of iris plants, monitoring water quality, or managing the treatment systems.

5. Conclusion

This study demonstrated that CWs can effectively remove Zn, Cd, Fe, and other metals from AMD. Heavy metal removal in the CWs was enhanced significantly by supplementing DW components with AMD to stimulate SRB activities for achieving the effluent standards. The pH values in the effluents reached 6.50–8.30 in all the CWs. The average removal rates for Zn, Cd, and Fe in the planted CWs were, respectively, 86.03%, 86.57%, and 99.90% during the experiment. The treatment performance of the CWs planted with iris as an ornamental plant was comparable to those with cattail as a typical wetland plant. In addition, the iris has the advantage of creating beautiful landscapes. The mass balance also showed that accumulation in substrates and plants was the main pathway for removing heavy metals from AMD. The findings of this research encourage the application of ornamental flowering plants in AMD treatment.

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