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Limestone-Based Constructed Wetlands for High-Strength Mn-Rich Mine Drainage: Effects of Reed Vegetation on Mn and Zn Removal

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Abstract

Mine drainage containing manganese (Mn) and zinc (Zn) poses a major environmental challenge. In Japan, Mn-rich mine drainage frequently exceeds the discharge standards of 10 mg/L for Mn and 2 mg/L for Zn. This study evaluated laboratory-scale continuous-flow constructed wetlands (CWs) for the treatment of both synthetic and actual mine drainage without addition of external organic carbon. The actual mine drainage was collected from an anonymous former Cu–Pb–Zn mine in Japan. Two limestone-filled CWs (2 L), one planted with *Phragmites australis* and the other unplanted, were operated at hydraulic retention times of 1–2 d. The influent mine drainage contained approximately 66 mg/L Mn and 10–15 mg/L Zn together with Fe, Cu, Cd, and Pb. During 170 days of continuous operation, the planted CW consistently achieved lower effluent Mn and Zn concentrations than the unplanted CW under both synthetic and actual mine drainage conditions. Effluent Mn concentrations in the planted CW ranged from 5.5 to 20.0 mg/L, compared with 46.1–54.2 mg/L in the unplanted CW. Likewise, effluent Zn concentrations ranged from 0.21 to 3.0 mg/L in the planted CW and from 2.4 to 10.1 mg/L in the unplanted CW. The superior performance of the planted CW was associated with enhanced limestone dissolution, elevated pH, and favorable rhizosphere conditions that likely promoted biologically mediated Mn(II) oxidation and the formation of Mn oxides, while Mn carbonate precipitation may also have contributed to Mn removal. These findings demonstrate that limestone-based planted CWs can effectively treat Mn-rich mine drainage without addition of external organic carbon and highlight their potential as sustainable, low-energy passive treatment systems for the long-term management of abandoned mine drainage.

Keywords: constructed wetland; mine drainage; manganese oxidation; limestone; rhizosphere; passive treatment

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1. Introduction

The mining industry generates large volumes of wastewater containing elevated concentrations of dissolved metals. In Japan, mine drainage can be broadly classified into several categories based on its chemical composition, one of which is characterized by elevated manganese (Mn) concentrations [1,2]. Typical Mn-rich mine drainage in Japan is

acidic (pH 3.5–6.5) and contains elevated levels of not only Mn but also zinc (Zn), often exceeding the Japanese discharge standards (10 mg/L for Mn and 2 mg/L for Zn) by 3–7 and 1.5–6 times, respectively [2]. Elevated Mn and coexisting Zn concentrations pose potential risks to aquatic ecosystems and water quality; therefore, the development of effective and low-cost treatment technologies remains a critical challenge.

Physicochemical methods such as neutralization, coagulation, and filtration have been widely applied for mine drainage treatment; however, these approaches require high capital and operational costs [3]. Mn removal typically involves the oxidation of Mn(II), followed by precipitation as Mn oxides such as MnO_2 or MnOOH. However, direct oxidation by dissolved oxygen (DO) is extremely slow under circumneutral pH conditions and becomes appreciable only above pH 8.5. In contrast, Mn-oxidizing bacteria (MnOB) can rapidly oxidize Mn(II) within a pH range of 6.5–8.5 [4–6]. During biological oxidation, Mn(II) is converted to Mn(III, IV) and subsequently forms biogenic Mn oxides, which can adsorb Mn(II) and coexisting metals [7]. Compared with physicochemical methods, biological processes offer advantages such as reduced chemical input and lower operational costs. However, MnOB are typically heterotrophic, and their activity in mine drainage is often limited by low organic carbon availability.

Constructed wetlands (CWs) have attracted increasing attention as a low-cost and environmentally sustainable approach for acid mine drainage treatment [8]. CWs remove metals through the combined actions of substrates, microorganisms, and vegetation. Alkaline substrates increase the pH and promote metal removal through adsorption and precipitation of metal hydroxides and oxides, including $Zn(OH)_2$, $Fe(OH)_3$, and Mn oxides. In addition, plants enhance microbial activity by supplying oxygen and releasing organic compounds into the rhizosphere, which can serve as carbon sources for MnOB. These processes also modify environmental conditions such as the pH and oxidation–reduction potential (ORP), thereby facilitating Mn oxidation and metal removal. Conventional CWs rely mainly on biological processes, including microbial activity and plant-associated processes, together with physical filtration and adsorption. When alkaline substrates such as limestone are incorporated, however, chemical processes such as neutralization, precipitation, and surface sorption also become important. Therefore, limestone-based CWs can be regarded as biochemical treatment systems in which biological and chemical processes act concurrently.

Several studies have demonstrated the applicability of CWs for treating Mn-containing wastewater (Table 1). For example, a pilot-scale surface-flow CW in the USA using soil and compost substrates reduced Mn concentrations in coal combustion leachate containing up to 3.3 mg/L Mn to 0.0005–0.44 mg/L [9]. A pilot-scale CW and prototype bioreactors in the UK treated mine drainage containing 10–40 mg/L Mn, achieving Mn concentrations below 1 mg/L only when circumneutral pH conditions were successfully maintained [10]. A full-scale CW in the UK achieved 76.8% Mn removal from mine drainage containing 2 mg/L Mn and 45 mg/L Fe [11]. In China, a pilot-scale CW using gravel and Mn ore as substrates was applied to steel wastewater containing 0.11–2.23 mg/L Mn and 0.16–2.24 mg/L Fe, reducing both metals to <0.05 mg/L at a hydraulic retention time (HRT) of 2–5 days [12]. A bench-scale hydroponic CW treating synthetic wastewater containing 1.0 mg/L Mn reduced Mn concentrations to <0.1 mg/L, although Mn re-release was observed after 7 days [13]. A lab-scale Mn polishing CW treating synthetic mine drainage and domestic sewage reduced Mn concentrations from 4.8 mg/L to 0.12 ± 0.11 mg/L at an HRT of 4 d under neutral to alkaline conditions using limestone-based substrates and aquatic plants [14]. More recently, a full-scale CW in France treating mine drainage containing 3.6 ± 0.5 mg/L Mn achieved Mn removal efficiencies of 43–92% [15]. However, most previous studies have focused on relatively low Mn concentrations, and the treat-

ment of high-strength Mn-rich mine drainage without external carbon addition remains insufficiently investigated.

Table 1. Constructed wetlands (CWs) for removing Mn from wastewater.

Wastewater	CW Configuration	Effluent	References
Coal combustion leachate in USA (Mn < 3.3 mg/L, Fe < 4.7 mg/L, pH 6.2–7.7)	A pilot-scale surface flow CW; compost + soil; *Typha*, *Scirpus*, *Carex*; HRT 20 h; 0–30 °C	Mn 0.0005–0.44 mg/L, Fe 0.002–0.49 mg/L, pH 6.2–7.7	[9]
Pretreated mine drainage in UK (Mn 10–40 mg/L, pH 5.0–7.0)	Pilot-scale rock filters following pretreatment; granite; algae; HRT 21 h; 5–13 °C	Mn < 1 mg/L (pH 7.0), unchanged (pH < 5.0)	[10]
Alkaline mine waters in UK (Mn 2 mg/L, Fe 45 mg/L, sulfate 3000 mg/L, pH ≈ 7)	Full-scale lagoon + surface-flow CWs; natural sediments; *Typha*, *Phragmites*	Mn < 0.4 mg/L, pH 7.8, Fe < 1 mg/L	[11]
Steel wastewater in China (Mn 0.11–2.23 mg/L, Fe 0.16–2.24 mg/L, pH 6.5–7.2)	Lab-scale vertical flow CWs; Mn ore or gravel; *Phragmites*; HRT 2–5 d	Mn < 0.05 mg/L, Fe < 0.05 mg/L, pH 7.5–8.2	[12]
(Mn < 2.23 mg/L)	A pilot-scale subsurface horizontal flow CW; gravel Mn ore; *Phragmites*; HRT 1.5–3 d	Mn < 0.1 mg/L, Fe removal 94%, pH 6.5–7.2	
Synthetic wastewater in Sri Lanka (Mn 1.0 mg/L, pH 6.2–7.1)	Bench-scale hydroponic CWs; water hyacinth; 3-week cycle batch; 2–22 °C	Mn < 0.1 mg/L (re-release after 7 d); 0.2–0.4 mg/L (nutrient deficient), pH 7.3–7.8	[13]
Synthetic mine drainage and domestic sewage in China (Mn 4.8 mg/L, Fe < 1 mg/L, pH 7.4–8.0)	Lab-scale polishing CW after anaerobic pretreatment; limestone, cobblestone; *Salvinia*, *Alternanthera*; HRT 4 d	Mn 0.12 ± 0.11 mg/L, Fe < 0.1 mg/L, Zn < 0.1 mg/L, pH 7.8–8.6	[14]
Mine wastewater in France (Mn 3.6 mg/L, Fe 28.4 mg/L, pH 6.5)	Full-scale surface-flow CWs; natural sediments; *Phragmites* 11–28 °C	Mn removal 43–92%	[15]
Synthetic and actual mine drainage in Japan (Mn 66 mg/L, Zn 10–15 mg/L, pH 5.0–6.5)	Lab-scale horizontal subsurface-flow CWs; limestone; *Phragmites*; HRT 1–2 d; greenhouse temperature 15–30 °C	Mn 5.5–20.0 mg/L, Zn 0.2–3.0 mg/L, pH 7.0–7.9	This study

In this study, a continuous-flow CW system using limestone as a substrate was developed to treat mine drainage containing approximately 66 mg/L Mn and 12 mg/L Zn, representing high-strength Mn-rich mine drainage in Japan. Two parallel systems were operated: a planted system with reeds and an unplanted system, to evaluate the effect of vegetation. Both synthetic mine drainage and actual mine drainage collected from a mine in Japan were treated under HRTs of 1 and 2 d and seasonal conditions. The objectives of this study were (i) to assess the applicability and robustness of limestone-based CWs for treating Mn- and Zn-rich mine drainage using both actual and synthetic mine drainage, (ii) to evaluate the effect of plant presence on Mn and Zn removal in CWs, and (iii) to investigate the influence of temperature and HRT on treatment performance.

2. Materials and Methods

2.1. Actual and Synthetic Mine Drainage

Actual mine drainage was collected from an anonymous former Cu–Pb–Zn mine located in the Tohoku region of Japan in 2023. The actual mine drainage exhibited a near-neutral pH of approximately 6.5. Its metal concentrations were Mn, 63.5 ± 2.8 mg/L; Zn, 9.3 ± 2.2 mg/L; Fe, 2.0 ± 1.6 mg/L; Pb, 0.18 ± 0.07 mg/L; Cu, 0.02–0.03 mg/L; and Cd, 0.014 ± 0.005 mg/L.

Because the amount of actual mine drainage available for the long-term experiment was limited, synthetic mine drainage was used to provide a controlled and reproducible influent composition, enabling systematic evaluation of the effects of HRT, seasonal conditions, and plant presence on metal removal. In contrast, actual mine drainage varies depending on site conditions, making it difficult to isolate the effects of individual operational factors. The composition was designed to represent Mn-rich mine drainage typically found in Japan [2] as follows: $MnSO_4 \cdot 5H_2O$, 290.71 mg/L; $ZnCl_2$, 24.46 mg/L; $FeSO_4 \cdot 7H_2O$, 44.81 mg/L; $PbCl_2$, 0.15 mg/L; $CuSO_4 \cdot 5H_2O$, 0.08 mg/L; and $CdCl_2$, 0.03 mg/L. These corresponded to dissolved metal concentrations of Mn (66.4 mg/L), Zn (11.7 mg/L), Fe (6.5 mg/L), Pb (0.05 mg/L), Cu (0.03 mg/L), and Cd (0.02 mg/L). The concentrations of Mn and Zn exceeded the Japanese discharge standards (10 and 2 mg/L, respectively), whereas those of Fe, Pb, Cu, and Cd were below the corresponding standards (10, 0.1, 3, and 0.03 mg/L, respectively). To evaluate the robustness of the CWs under acidic conditions, the pH of the synthetic mine drainage was adjusted to 5.0 using diluted hydrochloric acid (1:30, *v*/*v*), which was lower than the Japanese discharge standard range (pH 5.8–8.6). No external organic carbon source was added throughout the experiment.

2.2. CW Setup

To evaluate the effect of vegetation on Mn removal, two CWs were established: a planted CW using common reed (*Phragmites australis*) and an unplanted CW. A schematic diagram of the experimental CW setup is provided in Figure S1. Plants were obtained from a commercial nursery (Tojaku Engei Co., Ltd., Joyo, Japan). All CWs were constructed using plastic containers (W 655 × L 230 × D 180 mm) and filled with 5 kg of limestone (particle size: 10 mm, Tokai Jari Co., Ltd., Hamamatsu, Japan). The working volume of each CW was approximately 2 L. In the planted CW, four reed seedlings (shoot height, 45 cm; root length, 13 cm) were introduced on 26 May 2023.

Mine drainage was continuously pumped into the bottom of each CW using a cassette tube pump (SMP-23, EYELA, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The influent flowed upward through the limestone bed and was discharged from the outlet, approximating plug–flow conditions. A single pump equipped with a flow splitter was used to supply both CWs at identical flow rates. To minimize the effects of rainfall and other environmental factors, all CWs were installed in a greenhouse at the Biwako Kusatsu Campus, Ritsumeikan University, Japan.

2.3. Operating Conditions of the CWs

To allow plant acclimation, all CWs were operated with tap water for one week before the mine drainage treatment. The experiment was conducted over 170 d, from 4 June to 21 November 2023. As summarized in Table 2, the operation was divided into five phases according to the wastewater type and hydraulic loading conditions. Synthetic mine drainage was treated during Phases I, III, and IV, whereas actual mine drainage was treated during Phases II and V. The influent flow rate was maintained at 1 L/d during Phases I–III and V, corresponding to a hydraulic loading rate of 6.6 mm/d and an HRT of 2 d,

assuming no evapotranspiration. In Phase IV, the influent flow rate was increased to 2 L/d, corresponding to a hydraulic loading rate of 13.2 mm/d and an HRT of 1 d.

Table 2. Operating period and operational conditions of CWs.

Phase	Date	Influent Flow Rate (L/d)	Mine Drainage	Mean Air Temperature (°C)	Mean Daily Integrated Illuminance (klux·h d^{-1})
I	4 June–26 August 2023 (Day 0–85)	1.0 (HRT = 2 d)	Synthetic	30.7 ± 8.7	306 ± 126
II	27 August–15 September 2023 (Day 86–101)		Actual	27.9 ± 7.4	241 ± 98
III	16 September–3 October 2023 (Day 102–121)		Synthetic	24.9 ± 5.0	186 ± 74
IV	4–22 October 2023 (Day 122–142)	2.0 (HRT = 1 d)	Synthetic	20.8 ± 4.2	121 ± 59
V	23 October–21 November 2023 (Day 143–170)	1.0 (HRT = 2 d)	Actual	15.1 ± 7.6	196 ± 82

An HRT of 2 d was selected as the baseline operating condition to provide sufficient contact time between the mine drainage, limestone substrate, plant roots, and associated microorganisms. The HRT was shortened to 1 d in Phase IV to evaluate the response of the CWs to increased hydraulic loading and reduced contact time. The HRT was subsequently restored to 2 d in Phase V to examine treatment performance under a longer contact time during the lower-temperature period.

2.4. Sampling and Analysis

Greenhouse air temperature and illuminance were recorded at 10 min intervals using a data logger (TR-74Ui, T&D Corporation, Matsumoto, Japan). Daily integrated illuminance was calculated from the 10 min interval illuminance measurements.

Water samples (50 mL) were collected from the influent and effluent of each CW. The pH, DO, ORP, and total dissolved solids (TDS) were measured using a pH meter (LAQUA, Horiba Ltd., Kyoto, Japan), a portable DO meter (HQ30d equipped with an LDO101 luminescent DO probe, Hach, Loveland, CO, USA), an ORP meter (ORP57, Milwaukee Electronics, Rocky Mount, NC, USA), and a TDS meter (ASTDS1, AS ONE Corporation, Osaka, Japan), respectively.

For metal analysis, a 10 mL aliquot of each sample was filtered through a 1.0 µm pore-size filter and analyzed for Mn, Fe, Zn, Cu, Pb, and Cd using inductively coupled plasma–optical emission spectrometry (ICP–OES; 700 Series, Agilent Technologies, Santa Clara, CA, USA).

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

Because water loss through evapotranspiration and evaporation could vary with seasonal temperature, the treatment performance was evaluated not only from concentration changes but also on a mass basis. The effluent flow volume was measured, and the mass-based removal efficiencies of Mn and Zn were calculated from the influent and effluent metal loads as follows:

$$\text{Mass-based removal } (\%) = \frac{Q_{in}C_{in} - Q_{out}C_{out}}{Q_{in}C_{in}} \times 100, \quad (1)$$

where Q_{in} and Q_{out} are the influent and effluent flow rates, respectively, and C_{in} and C_{out} are the corresponding metal concentrations. This calculation accounts for water loss through evapotranspiration and evaporation.

3. Results

3.1. Performance of CWs During Phased Operation

The CWs were operated under five experimental phases using either synthetic or actual mine drainage. Table 2 summarizes the operational conditions, including the mean air temperature and daily integrated illuminance. During summer daytime, the greenhouse air temperature occasionally exceeded 50 °C because of solar heat accumulation within the enclosed greenhouse. The observed flow rate during each operational phase is shown in Figure S2, whereas pH, ORP, DO, and TDS are summarized in Figure S3. Temporal changes in pH, Mn, and Zn concentrations are presented in Figure 1, and those of Fe, Cu, Pb, and Cd are shown in Figure S4. The appearance of the CWs during the experimental period is shown in Figure 2.

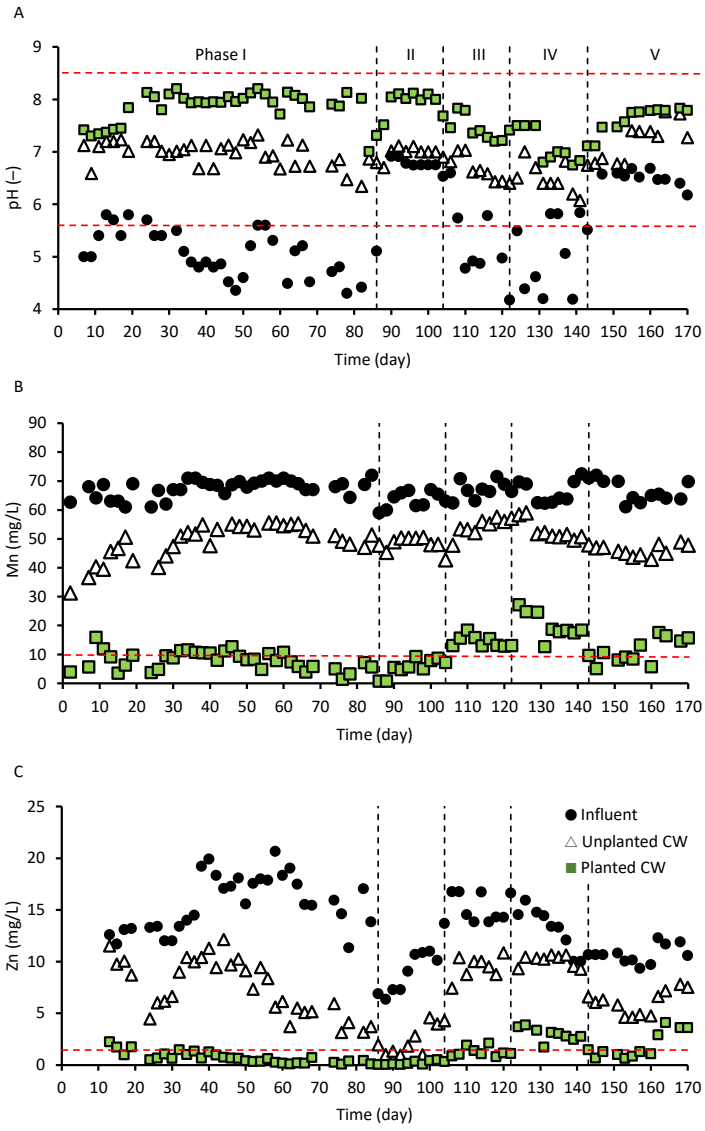


Figure 1. Changes in (**A**) pH, (**B**) Mn concentration, and (**C**) Zn concentration during the operation of the CWs. Vertical dashed lines indicate transitions between operational phases (Phases I–V). Horizontal dashed lines indicate the Japanese discharge standards for pH (5.8–8.6), Mn (10 mg/L), and Zn (2 mg/L).

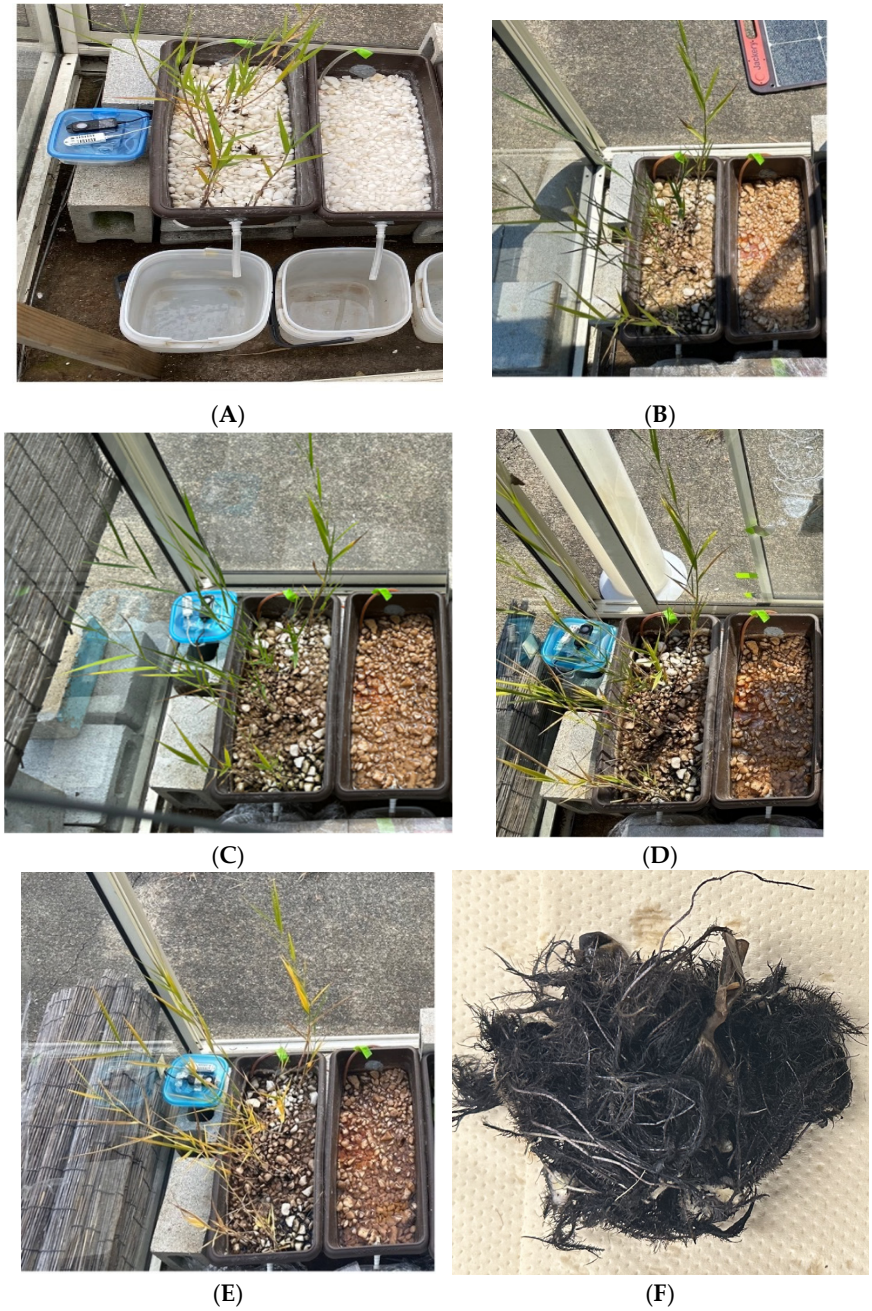


Figure 2. Photographs of the CWs used for the treatment of Mn-containing mine drainage and the harvested belowground plant biomass. The planted CW (**left**) and unplanted CW (**right**) on (**A**) day 0, (**B**) day 85, (**C**) day 101, (**D**) day 121, and (**E**) day 170, and (**F**) roots and rhizomes recovered from the planted CW at the end of the experiment.

3.1.1. Phase I

During Phase I (Day 0–85), the CWs treated synthetic mine drainage under hot conditions (30.7 ± 8.7 °C). The influent flow rate was generally maintained at the target level (Figure S2), averaging 933 ± 99 mL/d. The effluent flow rates were 499 ± 125 and 407 ± 144 mL/d for the planted and unplanted CWs, respectively, corresponding to water losses through evapotranspiration and evaporation of 56.9 ± 11.2% and 46.7 ± 12.0%. Correspondingly, the TDS increased from 214 ± 28 mg/L in the influent to 267 ± 27 mg/L in the unplanted CW and 343 ± 37 mg/L in the planted CW (Figure S3C), indicating concentration of dissolved constituents in the effluent. The influent pH averaged 5.1 ± 0.4 and increased to 7.9 ± 0.3 in the planted CW and 7.0 ± 0.2 in the unplanted CW (Figure 1A).

The ORP and DO showed no clear differences between the planted and unplanted CWs, whereas the TDS was generally higher in the planted CW (Figure S3). The ORP decreased from 236 ± 31 mV in the influent to 129 ± 40 mV in the planted CW and 160 ± 33 mV in the unplanted CW. The DO concentrations remained at approximately 8 mg/L in both CW effluents.

The influent Mn concentration averaged 67.5 ± 3.0 mg/L, which was consistent with the target concentration (Figure 1B). The effluent Mn concentration averaged 48.6 ± 6.4 mg/L in the unplanted CW and 7.9 ± 3.2 mg/L in the planted CW. The influent Zn concentration averaged 15.7 ± 2.6 mg/L, slightly exceeding the target concentration (Figure 1C). The effluent Zn concentration gradually decreased to 5.2 ± 2.0 mg/L in the unplanted CW and to 0.7 ± 0.5 mg/L in the planted CW. The limestone substrate was initially white (Figure 2A), but brown precipitates gradually accumulated on its surface during Phase I (Figure 2B). These precipitates likely included Fe (oxyhydr)oxides and possibly Mn-containing phases, although their mineralogical composition was not determined.

The effluent Fe, Pb, and Cu concentrations remained consistently low, averaging less than 0.1, 0.06, and 0.025 mg/L, respectively, in both CWs (Figure S4). The effluent Cd concentration averaged 0.0085 ± 0.0047 mg/L in the unplanted CW and 0.0015 ± 0.0012 mg/L in the planted CW.

3.1.2. Phase II

During Phase II (Day 86–101), actual mine drainage was treated under warm conditions (27.9 ± 7.4 °C). The reeds continued to grow and exhibited vigorous growth by day 101 (Figure 2C). The influent flow rate averaged 938 ± 62 mL/d, whereas the water losses through evapotranspiration and evaporation were 46.7 ± 7.5% and 42.1 ± 9.2% in the planted and unplanted CWs, respectively (Figure S2). Compared with the synthetic mine drainage, the actual mine drainage exhibited higher TDS values, averaging 408 ± 70 mg/L in the influent, 409 ± 95 mg/L in the unplanted CW, and 464 ± 64 mg/L in the planted CW (Figure S3C). The influent pH averaged 6.4 ± 0.7 and increased to 7.9 ± 0.2 in the planted CW and 7.0 ± 0.1 in the unplanted CW (Figure 1A). The ORP decreased from 164 ± 9 mV in the influent to 126 ± 9 mV in the planted CW and 146 ± 13 mV in the unplanted CW (Figure S3A).

The influent Mn concentration averaged 63.5 ± 2.8 mg/L. The effluent Mn concentration averaged 48.1 ± 2.3 mg/L in the unplanted CW and 5.5 ± 2.8 mg/L in the planted CW (Figure 1B). The influent Zn concentration averaged 9.3 ± 2.2 mg/L. The effluent Zn concentration averaged 2.4 ± 1.4 mg/L in the unplanted CW and 0.21 ± 0.17 mg/L in the planted CW (Figure 1C).

The influent Fe concentration averaged 2.0 ± 1.6 mg/L, which was lower than expected, whereas the effluent Fe concentration averaged 0.04 mg/L in both CWs (Figure S4A). The effluent Cu concentration remained below 0.004 mg/L in both CWs (Figure S4B). The influent Pb concentration averaged 0.18 ± 0.07 mg/L, which was higher than expected, whereas the effluent Pb concentration remained below 0.04 mg/L in both CWs (Figure S4C). The influent Cd concentration averaged 0.014 ± 0.005 mg/L, whereas the effluent Cd concentrations averaged 0.013 ± 0.008 mg/L in the unplanted CW and 0.0089 ± 0.0084 mg/L in the planted CW (Figure S4D).

3.1.3. Phase III

During Phase III (Day 102–121), synthetic mine drainage was treated under moderate temperatures (24.9 ± 5.0 °C). The influent flow rate averaged 966 ± 53 mL/d (Figure S2), whereas the water losses through evapotranspiration and evaporation were 44.6 ± 4.0% and 38.0 ± 5.0% in the planted and unplanted CWs, respectively. The influent pH averaged

5.0 ± 0.5 and increased to 7.4 ± 0.2 in the planted CW and 6.7 ± 0.2 in the unplanted CW (Figure 1A). The ORP decreased from 255 ± 10 mV in the influent to 187 ± 16 mV in the planted CW and 223 ± 30 mV in the unplanted CW (Figure S3A). The reeds continued to grow, while brown precipitates accumulated on the surface of the limestone substrate in both CWs (Figure 2D).

The influent Mn concentration averaged 67.1 ± 3.0 mg/L. The effluent Mn concentration averaged 54.2 ± 2.8 mg/L in the unplanted CW and 14.5 ± 1.8 mg/L in the planted CW (Figure 1B). The influent Zn concentration averaged 15.3 ± 1.2 mg/L. The effluent Zn concentration averaged 8.9 ± 1.7 mg/L in the unplanted CW and 1.3 ± 0.4 mg/L in the planted CW (Figure 1C). The effluent Fe, Pb, Cu, and Cd concentrations remained consistently low, averaging less than 0.03, 0.008, 0.02 and 0.007 mg/L, respectively, in both CWs (Figure S4).

3.1.4. Phase IV

During Phase IV (Day 122–142), the influent flow rate of synthetic mine drainage was increased from 1 to 2 L/d, reducing the HRT from 2 d to 1 d. The influent flow rate averaged 1940 ± 26 mL/d (Figure S2), whereas the water losses through evapotranspiration and evaporation were 37.5 ± 6.4% and 30.7 ± 6.1% in the planted and unplanted CWs, respectively. The mean air temperature decreased to 20.8 ± 4.2 °C. The influent pH averaged 5.0 ± 0.7 and increased to 7.0 ± 0.3 in the planted CW and 6.5 ± 0.3 in the unplanted CW (Figure 1A). The ORP decreased from 236 ± 53 mV in the influent to 217 ± 47 mV in the planted CW and 218 ± 41 mV in the unplanted CW (Figure S3A). The DO concentrations ranged from 8.4 to 8.8 mg/L in both CW effluents (Figure S3B).

The influent Mn concentration averaged 66.3 ± 3.7 mg/L (Figure 1B). The effluent Mn concentration averaged 52.7 ± 3.2 mg/L in the unplanted CW and 20.0 ± 4.3 mg/L in the planted CW. The influent Zn concentration averaged 13.1 ± 2.0 mg/L. The effluent Zn concentration averaged 10.1 ± 0.5 mg/L in the unplanted CW and 3.0 ± 0.6 mg/L in the planted CW (Figure 1C). The effluent Fe, Cu, and Pb concentrations remained consistently low throughout Phase IV in both CWs (Figure S4). The effluent Cd concentrations averaged 0.016 ± 0.005 mg/L in the unplanted CW and below 0.006 mg/L in the planted CW.

3.1.5. Phase V

During Phase V (Day 143–170), the HRT was increased from 1 d to 2 d, and actual mine drainage was treated under relatively low-temperature conditions (15.1 ± 7.6 °C). The influent flow rate averaged 964 ± 32 mL/d (Figure S2), whereas the water losses through evapotranspiration and evaporation were 31.5 ± 5.1% and 28.6 ± 4.8% in the planted and unplanted CWs, respectively. The aboveground parts of the reeds had substantially senesced by day 170, likely because of the decrease in temperature (Figure 2E). The influent pH averaged 6.3 ± 0.4 and increased to 7.6 ± 0.3 in the planted CW and 7.1 ± 0.5 in the unplanted CW (Figure 1A). The ORP increased slightly from 209 ± 18 mV in the influent to 226 ± 28 mV in the planted CW and 225 ± 20 mV in the unplanted CW (Figure S3A).The DO concentrations increased to 9.7–9.8 mg/L in both CW effluents (Figure S3B).

The influent Mn concentration averaged 66.6 ± 3.6 mg/L (Figure 1B). The effluent Mn concentration averaged 46.1 ± 1.8 mg/L in the unplanted CW and 11.2 ± 4.0 mg/L in the planted CW. The influent Zn concentration averaged 10.7 ± 0.8 mg/L (Figure 1C). The effluent Zn concentration averaged 6.1 ± 1.1 mg/L in the unplanted CW and 1.9 ± 1.2 mg/L in the planted CW. The effluent Fe, Cu, Pb, and Cd concentrations remained consistently low throughout Phase V, with Cd concentrations below 0.01 mg/L in the unplanted CW and below 0.004 mg/L in the planted CW (Figure S4).

At the end of the experiment, abundant black precipitates were observed on the reed roots in the planted CW (Figure 2F).

3.2. Comparison of Treatment Performance Among Phases

Based on the phase-averaged effluent concentrations, the treatment performance was compared under different operational conditions. Mass-based removal efficiencies accounting for water loss through evapotranspiration and evaporation are also shown in Figure 3.

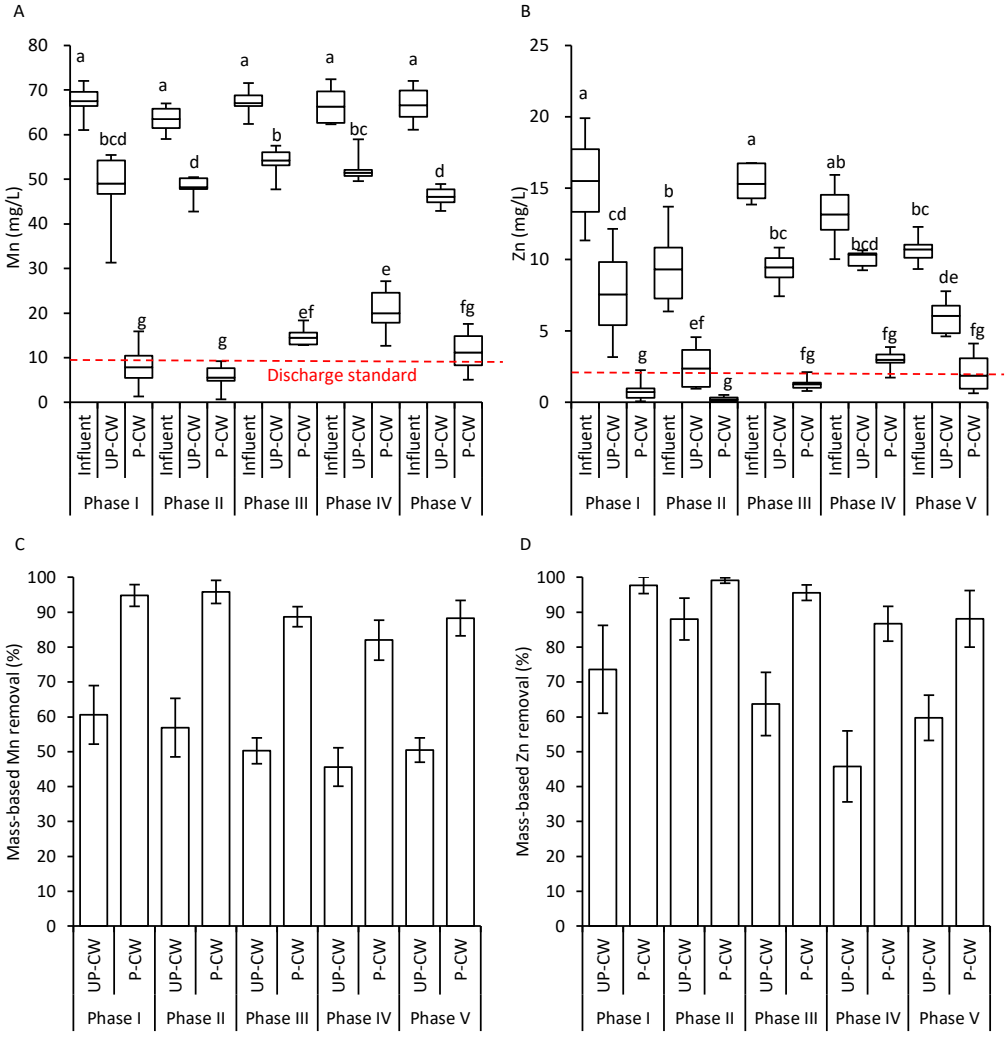


Figure 3. Box plots of (**A**) Mn and (**B**) Zn concentrations in the influent and effluents from the unplanted CW (UP-CW) and planted CW (P-CW) during each operational phase. Boxes represent the interquartile range, the horizontal line within each box indicates the median, and whiskers indicate the minimum and maximum values. Horizontal dashed lines indicate the Japanese discharge standards for Mn (10 mg/L) and Zn (2 mg/L). Different lowercase letters indicate significant differences among groups ($p < 0.05$, Tukey's HSD test). Mass-based removal efficiencies of Mn (**C**) and Zn (**D**) in the UP-CW and P-CW during each operational phase. Bars and error bars represent the mean and standard deviation, respectively. Removal efficiencies were calculated from influent and effluent metal loads using the measured influent and effluent flow rates.

The planted CW consistently produced significantly lower effluent Mn concentrations than the unplanted CW throughout the study ($p < 0.05$). The lowest Mn concentrations were observed during Phases I and II, when the HRT was 2 d, and the air temperatures were relatively high. In contrast, effluent Mn concentrations increased during Phase III

and reached the highest values during Phase IV, in which the HRT was reduced to 1 d. During Phase V, when the HRT was restored to 2 d under lower-temperature conditions, the effluent Mn concentration decreased compared with Phase IV but remained higher than those observed during Phases I and II. The mass-based Mn removal efficiency was consistently higher in the planted CW than in the unplanted CW, ranging from 82.0% to 95.8% in the planted CW and from 45.6% to 60.6% in the unplanted CW (Figure 3C). The removal efficiency in the planted CW was highest during Phases I and II (94.8–95.8%), decreased to 82.0% in Phase IV, and recovered to 88.3% in Phase V.

A similar trend was observed for Zn. The planted CW maintained significantly lower effluent Zn concentrations than the unplanted CW throughout the experiment ($p < 0.05$). Zn concentrations were lower during Phases I–III and V than during Phase IV, which exhibited the highest effluent Zn concentrations in both CWs. The mass-based Zn removal efficiency was also consistently higher in the planted CW, ranging from 86.7% to 99.1%, compared with 45.8% to 88.0% in the unplanted CW (Figure 3D). The lowest Zn removal efficiency in the planted CW was observed during Phase IV.

Overall, the unplanted CW did not meet the Japanese discharge standards for Mn (10 mg/L) or Zn (2 mg/L) during any operational phase. In the planted CW, the Mn concentrations were below the discharge standard during most of Phase I and throughout Phase II, but they exceeded the standard during Phases III–V. In contrast, the Zn concentrations in the planted CW met the discharge standard during Phases I–III and V but exceeded it during Phase IV.

The effluent Fe, Cu, Pb, and Cd concentrations remained consistently low throughout the study (Figure S4).

4. Discussion

4.1. Applicability of CWs to Actual Mine Drainage

This study demonstrated that planted CW was capable of treating high-strength Mn-rich mine drainage using both synthetic and actual conditions. Compared with synthetic mine drainage, actual mine drainage generally exhibits more complex and site-specific water chemistry, which may influence the treatment processes. During Phases II and V, in which actual mine drainage was treated, the planted CW maintained Mn and Zn removal despite higher TDS and variations in coexisting trace-metal concentrations (Figure S3).

Most previous studies have evaluated CWs using relatively low Mn concentrations, typically below 10 mg/L [9,11–15], as shown in Table 1. In contrast, the present study treated mine drainage containing over 65 mg/L Mn together with elevated Zn concentrations. Throughout the study, the planted CW consistently reduced the Mn and Zn concentrations to levels substantially lower than those achieved by the unplanted CW, and the effluent concentrations met the Japanese discharge standards during most operational phases. Notably, evapotranspiration and evaporation losses were not reported in the previous studies summarized in Table 1, making direct comparison of mass-based Mn and Zn removal efficiencies among studies difficult.

The treatment performance observed during the phases using actual mine drainage suggests that planted limestone-based CWs can retain treatment capacity under chemically more complex influent conditions. This is particularly relevant in Japan, where high-Mn mine drainage has been reported at numerous abandoned mine sites [2], and supports further evaluation of this system as a low-cost passive treatment option for high-strength mine drainage.

The actual mine drainage used in Phases II and V had higher TDS and more variable concentrations of coexisting trace metals than the synthetic influent. Such differences may affect ion activities, limestone dissolution, metal adsorption, and the saturation conditions

of carbonate- and hydroxide-containing mineral phases. However, these effects could not be quantitatively evaluated, because aqueous speciation and mineralogical analyses were not conducted. Moreover, because the influent type varied concurrently with HRT and environmental conditions among the operational phases, the independent effect of actual versus synthetic mine drainage could not be statistically separated in the present study. Therefore, the present results should be regarded as evidence of treatment applicability under actual mine drainage conditions rather than as a quantitative assessment of robustness against individual water-quality fluctuations.

4.2. Removal Mechanisms for Mn and Zn in CWs

Figure 4 shows the relationships between pH and Mn and Zn concentrations. The planted CW generally exhibited both higher pH and lower Mn and Zn concentrations than the unplanted CW. However, this relationship does not necessarily indicate that hydroxide precipitation was the dominant removal mechanism. The increase in pH and enhanced metal removal may instead have occurred concurrently as a consequence of limestone dissolution and the associated changes in carbonate chemistry.

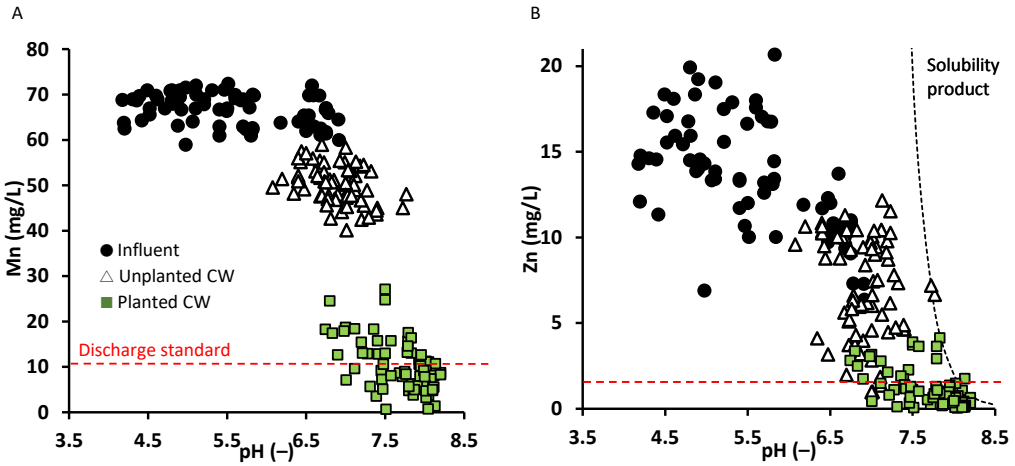


Figure 4. Relationship between pH and (**A**) Mn concentration and (**B**) Zn concentration. Horizontal dashed lines indicate the discharge standards for Mn (10 mg/L) and Zn (2 mg/L). The black dashed curve in (**B**) represents the theoretical solubility of Zn based on the solubility product of $Zn(OH)_2$.

For Mn, precipitation as $Mn(OH)_2$ is governed by the following equilibrium:

$$Mn^{2+} + 2OH^- \rightleftharpoons Mn(OH)_2(s). \quad (2)$$

The solubility product of $Mn(OH)_2$ ($K_{sp} \approx 1.6 \times 10^{-13}$ at 25 °C) indicates that a pH of approximately 9.5 would be required to reduce the Mn concentration to the Japanese discharge standard of 10 mg/L by hydroxide precipitation alone. In contrast, substantial Mn removal was observed at pH values of approximately 7–8, particularly in the planted CW (Figure 4A). Thus, $Mn(OH)_2$ precipitation alone cannot explain the observed Mn removal.

Similarly, precipitation of Zn as $Zn(OH)_2$ is governed by

$$Zn^{2+} + 2OH^- \rightleftharpoons Zn(OH)_2(s). \quad (3)$$

Based on the solubility product of $Zn(OH)_2$ ($K_{sp} \approx 3 \times 10^{-17}$ at 25 °C), a pH of approximately 7.8–8.0 would be required to reduce Zn to the Japanese discharge standard. Hydroxide precipitation may therefore have contributed to Zn removal at the higher pH values observed in the planted CW. However, the observed relationship between pH and Zn

concentration alone does not demonstrate that hydroxide precipitation was the dominant removal pathway.

Limestone dissolution may have played an important role in both pH elevation and metal immobilization. Dissolution of limestone ($CaCO_3$) consumes acidity and increases alkalinity and dissolved carbonate species, particularly bicarbonate, thereby creating conditions favorable for carbonate precipitation [14,16]. In the planted CW, CO_2 generated by root respiration may have further enhanced limestone dissolution and buffering capacity. The resulting conditions could favor the precipitation of Mn and Zn as carbonate minerals, such as $MnCO_3$ and $ZnCO_3$ [15]. Carbonate precipitation is particularly plausible for Mn in the present study because substantial Mn removal occurred at pH values considerably lower than those required for effective $Mn(OH)_2$ precipitation. However, mineralogical analyses were not conducted in the present study.

Another important pathway is the biological oxidation of Mn(II) to insoluble Mn oxides by MnOB. Biological Mn oxidation can occur under circumneutral conditions [4–6]. Mn oxides are effective sorbents for trace metals and may also contribute to Zn removal through adsorption, co-precipitation, and structural incorporation [17,18]. The relationship between Mn and Zn concentrations observed in the planted CW supports the possibility that Mn oxide formation contributed to Zn removal. In addition, abundant black precipitates were observed on the reed roots at the end of the experiment, which is consistent with, although not direct evidence of, Mn oxide formation. Because the precipitates were not mineralogically characterized, and microbial analyses were not conducted, the contribution of biologically mediated Mn oxidation could not be directly confirmed.

The relationships between pH and the concentrations of Fe, Cu, Pb, and Cd are shown in Figure S5. These metals generally decreased with increasing pH, although differences between the planted and unplanted CWs were relatively small for Fe and Cu. Throughout the study, the effluent concentrations of Fe, Cu, Pb, and Cd remained low in both CWs. Based on the solubility products of $Cu(OH)_2$ ($K_{sp} \approx 2.2 \times 10^{-20}$), $Pb(OH)_2$ ($K_{sp} \approx 1.2 \times 10^{-15}$), and $Cd(OH)_2$ ($K_{sp} \approx 2.5 \times 10^{-14}$) at 25 °C, the pH values required to achieve the Japanese discharge standards by hydroxide precipitation alone were estimated to be approximately 6.3, 9.7, and 10.5, respectively. Although Fe was introduced as Fe(II) in the synthetic mine drainage, oxidation to Fe(III) followed by precipitation, as poorly soluble Fe(III) (oxyhydr)oxides may have contributed to its removal. $Fe(OH)_3$ can theoretically precipitate at relatively low pH values of approximately 3–4 because of its extremely low solubility. These calculations indicate that hydroxide precipitation may have contributed to the removal of some metals; however, they do not establish hydroxide precipitation as the dominant removal mechanism in the CWs.

Such Fe precipitates may also have influenced the immobilization of other metals. Fe(III) (oxyhydr)oxides can provide sorption and co-precipitation sites for Zn and other trace metals and may therefore have contributed to their removal. The brown precipitates observed on the limestone surface (Figure 2) may have included Fe (oxyhydr)oxides as well as Mn-containing phases. Conversely, progressive accumulation of these mineral precipitates may have coated the limestone surface and partially reduced its dissolution through armoring or passivation. Such passivation could decrease alkalinity generation and thereby affect metal removal during prolonged operation. However, because mineralogical and surface analyses were not conducted, the composition of these coatings and the extent of limestone passivation could not be determined.

Overall, Mn and Zn removal in the limestone-based CWs was likely governed by multiple interacting processes, including limestone dissolution, carbonate precipitation, possible hydroxide precipitation, biologically mediated Mn oxidation and subsequent Mn oxide formation, and sorption or co-precipitation associated with Fe and Mn oxides. Previous

studies have associated Mn removal in CWs with both abiotic precipitation and biologically mediated Mn oxidation under circumneutral to alkaline and oxidizing conditions [10,15,19]. In the present study, the increase in pH favored conditions for Mn and Zn immobilization; however, the ORP and DO measurements did not show a clear vegetation-specific enhancement of oxidizing conditions. Therefore, the present water-quality data support the occurrence of favorable physicochemical conditions for Mn removal but do not allow biological Mn oxidation to be distinguished from abiotic precipitation processes. Because Ca concentrations, alkalinity, mineralogical composition, and microbial communities were not analyzed, the relative contributions of these mechanisms and the extent of limestone passivation could not be quantitatively distinguished.

4.3. Effects of Vegetation on Metal Removal

Species of the genus *Phragmites* are the most widely used emergent macrophytes in CWs for Mn-rich mine drainage treatment because of their high tolerance to metal-rich environments and their ability to modify rhizosphere conditions [11,12,15]. The planted CW consistently produced lower effluent Mn and Zn concentrations than the unplanted CW. This improvement was accompanied by consistently higher pH values, indicating that vegetation created physicochemical conditions favorable for metal removal. The higher pH observed in the planted CW was likely associated with rhizosphere processes. Carbon dioxide released through root respiration can enhance dissolution of the limestone substrate, thereby increasing the alkalinity and buffering capacity. Root-mediated ion exchange may also contribute to local increases in pH. These rhizosphere processes are consistent with the systematically higher pH and TDS observed in the planted CW and likely promoted Mn and Zn immobilization.

Although MnOB are generally heterotrophic and their activity is often limited by the low organic carbon availability in mine drainage, root exudates and decaying plant biomass may provide localized carbon sources that support MnOB activity. These endogenous organic compounds may include low-molecular-weight organic acids (e.g., acetate and lactate), sugars, and amino acids released from plant roots [20], all of which can serve as carbon sources for MnOB. Root surfaces also provide favorable sites for biofilm development and metal accumulation. Previous studies have shown that Mn oxides accumulate on root surfaces as coatings or plaques, which subsequently adsorb trace metals such as Zn [21,22]. At the end of the experiment, abundant black precipitates were observed on reed roots. Thus, the root-associated black precipitates observed in the present study are qualitatively consistent with possible Mn oxide accumulation on root surfaces, as reported in previous studies [21,22]; however, their mineralogical composition was not determined. In addition, because the DO concentrations were similar in the planted and unplanted CW effluents, and the oxygen conditions were not measured directly in the rhizosphere, enhanced oxygen availability due to vegetation could not be demonstrated in the present study.

This mechanism differs fundamentally from that of anaerobic sulfate-reducing systems used for mine drainage treatment. In such systems, external organic carbon is supplied to stimulate sulfate-reducing bacteria, resulting in the precipitation of metal sulfides such as ZnS and CdS. However, sulfide precipitation generally contributes less to Mn removal, because Mn sulfide is considerably less stable than the sulfides of Zn, Cd, Cu, and Pb [14]. In contrast, the planted CW may have supplied localized root-derived organic carbon and provided root surfaces favorable for microbial colonization and Mn accumulation. This combination of localized carbon supply and favorable root surfaces may represent a key advantage of vegetated limestone-based CWs for Mn-rich mine drainage treatment.

The superior performance of the planted CW was therefore likely attributable to the combined effects of enhanced limestone dissolution, elevated pH, localized carbon supply in the rhizosphere, favorable root surfaces for microbial colonization and metal accumulation, and possible biologically mediated Mn oxidation, which together promoted the removal of Mn and Zn. While no clear differences were observed for Fe and Cu, slightly lower Pb and Cd concentrations were obtained in the planted CW than in the unplanted CW (Figure S5), suggesting a modest contribution of vegetation to the removal of these trace metals.

The present study evaluated Mn and Zn removal primarily based on influent and effluent water quality and therefore did not directly determine the fate and distribution of metals retained within the CWs. Retained metals may have been adsorbed onto limestone, precipitated as secondary mineral phases, accumulated in deposited solids, or taken up by plant biomass. In addition, root development may have altered the pore structure and rhizosphere conditions, thereby indirectly affecting metal retention processes.

4.4. Effects of Temperature and HRT on Metal Removal

Temperature and HRT both influenced the treatment performance of the CWs. The lowest effluent Mn and Zn concentrations were observed during Phases I and II, when the HRT was maintained at 2 d, and the air temperatures were relatively high. The HRT of 2 d adopted in the present study is comparable to those reported for CWs treating Mn-containing wastewater [12,14], as shown in Table 1, whereas the results obtained at an HRT of 1 d demonstrate the feasibility and limitations of operating under shorter HRTs. In contrast, reducing the HRT from 2 d to 1 d (Phase IV) resulted in the highest effluent Mn and Zn concentrations, indicating that shorter HRTs limited metal removal. When the HRT was restored to 2 d (Phase V), the treatment performance improved despite the lower temperatures, although the Mn concentrations remained slightly higher than those observed during the warmer phases. These results suggest that HRT had a substantial influence on the treatment performance; however, the effects of HRT and seasonal temperature could not be fully separated under the present experimental design. In addition, influent type and illumination conditions also varied among the operational phases, and these concurrent changes may have contributed to the observed differences in Mn and Zn removal.

Nevertheless, temperature may also influence CW performance through its effects on vegetation and microbial activity. Although the water temperature was not monitored in the present study, the greenhouse air temperature ranged approximately from 15 to 30 °C, which is comparable to the water temperatures reported for CWs treating mine drainage [8,15]. As temperatures decrease, reed growth and physiological activity decline, potentially reducing oxygen release from the rhizosphere and consequently slowing biologically mediated Mn oxidation. Similar reductions in Mn removal under low-temperature conditions have been reported for planted CWs treating mine drainage [10]. In addition, mature vegetation can increase hydraulic resistance, thereby reducing the flow velocity and increasing the effective hydraulic retention time within CWs [23,24].

Future studies should include the continuous monitoring of water temperature to clarify its influence on Mn oxidation and seasonal treatment performance. Controlled experiments in which HRT and temperature are varied independently while maintaining the other operational conditions constant are also needed to quantitatively evaluate their individual and interactive effects on Mn and Zn removal. In addition, whole-system metal mass balances, including analyses of metals retained in the substrate, deposited solids, and plant biomass, together with mineralogical characterization, are needed to clarify the fate and long-term stability of retained metals. Overall, the higher Mn and Zn removal

observed in the planted CW should be interpreted as a vegetation-associated enhancement of aqueous-phase metal removal rather than as direct evidence of metal uptake by plants.

4.5. Implications for Practical Applications

Japan has a large number of abandoned and inactive mines that continue to generate mine drainage long after mine closure [1]. Because these sites no longer provide economic benefits, the long-term operation of conventional active treatment systems represents a considerable economic burden. Therefore, promoting the wider adoption of low-energy passive treatment technologies remains an important challenge for sustainable mine drainage management in Japan. In addition to Mn-rich mine drainage, CWs have also been investigated for the treatment of mine drainage containing elevated concentrations of Cd [25] as well as Fe and Zn [26], suggesting that the findings of this study may also contribute to the broader application of passive treatment technologies for various types of mine drainage.

The limestone loading in each CW was approximately 33 kg/m^2, and the hydraulic loading rates were 6.6 and 13.2 mm/d at HRTs of 2 and 1 d, respectively. The lower Mn and Zn removal observed at the 1 d HRT suggests that sufficient contact time should be considered in scale-up. Pilot- and field-scale systems should therefore be designed considering the mine drainage flow and chemistry, required HRT, and long-term limestone performance. Scale-up from the present 2 L systems should be based on hydraulic and metal loading conditions rather than a simple proportional scaling of reactor volume. The HRT of 2 d and hydraulic loading rate of 6.6 mm/d may serve as initial design references, although the effective HRT, hydraulic distribution, and metal loading should be verified under site-specific field conditions.

Several issues should nevertheless be addressed before pilot- and full-scale implementation. First, the role of biologically mediated Mn oxidation was inferred from the treatment performance but was not directly verified. Future studies incorporating microbial community analyses and functional gene characterization are needed to clarify the contribution of microorganisms to Mn oxidation in CWs. Second, the composition, stability, and long-term fate of the Mn-rich precipitates require further investigation. Mineralogical analyses are needed to distinguish the relative contributions of hydroxide precipitation, carbonate precipitation, and Mn oxide formation. The long-term accumulation of Mn-rich precipitates and suspended solids within the limestone bed may reduce the pore space and hydraulic conductivity, while progressive limestone dissolution and surface coating by precipitates may reduce alkalinity generation and reactive surface availability. Layered or graded substrate configurations may therefore be useful for maintaining hydraulic conductivity and facilitating maintenance or partial substrate replacement. In addition, the recovery, handling, and potential reuse of Mn-rich precipitates should be evaluated from the perspectives of resource recovery and long-term sustainability. Seasonal reed senescence, as observed during Phase V, and low winter temperatures may also reduce rhizosphere activity and Mn removal. For cold-season operation, sufficient bed depth, subsurface-flow configurations, and passive thermal insulation should be considered to moderate the temperature fluctuations, together with seasonal reed management based on the plant condition and metal accumulation. Periodic inspection and, where necessary, replenishment or partial replacement of limestone should also be considered based on changes in alkalinity generation, hydraulic conductivity, and substrate condition. Finally, these long-term operational requirements should be investigated under pilot- and full-scale field conditions.

5. Conclusions

Continuous-flow limestone-based CWs were successfully operated for 170 d to treat high-strength mine drainage containing over 65 mg/L Mn and 10–15 mg/L Zn. Effluent Mn concentrations in the planted CW ranged from 5.5 to 20.0 mg/L, compared with 46.1–54.2 mg/L in the unplanted CW. Likewise, effluent Zn concentrations ranged from 0.21 to 3.0 mg/L in the planted CW and from 2.4 to 10.1 mg/L in the unplanted CW. The planted CW consistently outperformed the unplanted CW under both synthetic and actual mine drainage conditions, demonstrating the applicability of the system to complex mine drainage.

Mn removal was governed by multiple interacting mechanisms, including hydroxide precipitation, possible carbonate precipitation, and biologically mediated Mn oxidation, whereas Zn removal was primarily controlled by hydroxide precipitation and adsorption onto Mn oxides. Vegetation enhanced the treatment performance by promoting limestone dissolution, increasing the pH, and creating favorable rhizosphere conditions for Mn oxidation. The treatment performance was also influenced by HRT, with the 2 d HRT providing more efficient Mn and Zn removal than the 1 d HRT, whereas seasonal conditions had comparatively smaller effects.

Overall, the present study demonstrates that planted limestone-based CWs provide a promising low-energy passive treatment technology for Mn-rich mine drainage. Given the large number of abandoned mine sites in Japan, where Mn-rich mine drainage remains an important environmental issue, these findings provide a scientific basis for the design and optimization of practical CW systems. Furthermore, the proposed approach may also be applicable to other types of mine drainage, including Cd- and Zn-rich mine drainage, thereby contributing to the broader implementation of passive mine water treatment systems.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/mining6030072/s1, Figure S1: Schematic diagram of the experimental constructed wetland (CW) setup. Synthetic or actual mine drainage was supplied from a common influent tank to the unplanted and planted CWs using a pump. Both CWs were constructed using plastic containers (W 655 × L 230 × D 180 mm), filled with 5 kg of limestone, and had a working volume of approximately 2 L. *Phragmites australis* plants were established in the planted CW. Influent was introduced to the bottom of each CW, and treated water overflowed into separate effluent tanks; Figure S2: Changes in the flow rate during the operation of the CWs. Black, orange, and green lines represent the influent, the effluent from the unplanted CW, and the effluent from the planted CW, respectively. Vertical dashed lines indicate transitions between operational phases (Phases I–V); Figure S3: Changes in (A) ORP, (B) DO, and (C) TDS during the operation of the CWs. Vertical dashed lines indicate transitions between operational phases (Phases I–V); Figure S4: Changes in metal concentrations during the operation of the CWs: (A) Fe, (B) Cu, (C) Pb, and (D) Cd. Vertical dashed lines indicate transitions between operational phases (Phases I–V). Horizontal dashed lines indicate the discharge standards for Fe (10 mg/L), Pb (0.1 mg/L), and Cd (0.03 mg/L); Figure S5: Relationships between pH and the concentrations of (A) Fe, (B) Cu, (C) Pb, and (D) Cd. Horizontal dashed lines indicate the discharge standards for Fe (10 mg/L), Cu (3 mg/L), Pb (0.1 mg/L), and Cd (0.03 mg/L). The black dashed curve in (B) represents the theoretical solubility of Cu based on the solubility product of $Cu(OH)_2$.

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Abbreviations

The following abbreviations are used in this manuscript:

CW	Constructed wetland
DO	Dissolved oxygen
HRT	Hydraulic retention time
MnOB	Manganese-oxidizing bacteria
ORP	Oxidation-reduction potential
TDS	Total dissolved solids

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