water

MDPI

Article

Removal of Heavy Metals from Acid Mine Drainage with Lab-Scale Constructed Wetlands Filled with Oyster Shells

Thuong Thi Nguyen [1], Satoshi Soda [1,*] and Kengo Horiuchi [2]

[1] Graduate School of Science and Engineering, Ritsumeikan University, Biwako-Kusatsu Campus, Kusatsu 525-8577, Japan
[2] Japan Oil, Gas and Metals National Corp., Toranomon Twin Building 2-10-1 Toranomon, Minato-ku, Tokyo 105-0001, Japan
* Correspondence: soda@fc.ritsumei.ac.jp; Tel.: +81-(0)77-561-4836

Abstract: The present study investigated the applicability of constructed wetlands (CWs) filled with oyster shells (OSs) for removing heavy metals from acid mine drainage (AMD). Lab-scale CWs consisted of columns (ID 12.5 cm, H 50 cm) packed with OSs or limestone, which were left unplanted or planted with cattails. Synthetic and real AMD containing 7.3 mg/L of Zn, 38.0 mg/L of Fe, and other minerals (pH = 4.0) were fed to the CWs (1 L/column) under a hydraulic retention time of 7 days in a sequencing batch mode. The effluent pH values of the CWs reached 6.9–8.3. Results show that OSs with high $CaCO_3$ contents had higher neutralizing capability for AMD than limestone had. During 7 months of operation, all CWs were highly effective for removing Zn (88.6–99.2%); Fe (98.7–99.7%); and Cd, Cu, Pb, As, and Mn (48.2–98.9%) from both real and synthetic AMD. The mass balance in the CWs indicated accumulation in OSs or limestone as a main pathway for removing heavy metals, representing 44.8–99.3% of all metals, followed by biomass (8.8–29.9%) in the planted CWs. Other processes examined for this study only played a minor role in removing heavy metals. The higher metal treatment performance of OS CWs demonstrated the value of this aquaculture byproduct as a CW substrate.

Keywords: acid mine drainage; constructed wetland; heavy metal; oyster shell; limestone

check for updates

Citation: Nguyen, T.T.; Soda, S.; Horiuchi, K. Removal of Heavy Metals from Acid Mine Drainage with Lab-Scale Constructed Wetlands Filled with Oyster Shells. *Water* **2022**, *14*, 3325. https://doi.org/10.3390/w14203325

Academic Editor: Laura Bulgariu

Received: 16 September 2022
Accepted: 18 October 2022
Published: 20 October 2022

1. Introduction

Acid mine drainage (AMD) with high acid and metal concentrations is a significant challenge confronted by the mining industry [1–3]. About 7000 mines have been abandoned or closed since 1970 in Japan because of economic reasons [4]. About 100 of them are still obligated to remove heavy metals from mine drainage [5]. To date, several physicochemical technologies have been applied for AMD treatment, such as neutralization, coagulation, and filtration. Nevertheless, these methods have drawbacks in terms of the high capital, operation, and maintenance costs [6,7]. Therefore, reducing financial burdens and achieving a sustainable AMD treatment for the future necessitate development of an alternative technology that is affordable in rural areas.

Constructed wetlands (CWs) have been recognized as a potential green technology for mine drainage treatment. In fact, they have gained great attention worldwide because of their effectiveness, low cost, simple maintenance, and high biodiversity value [8–10]. The substrate serves an important role in heavy metal removal in CWs [10,11]. Consequently, a key strategy for heavy metal removal is the selection of substrates with high filtration, adsorption, and ecological capabilities. Furthermore, to reduce costs and to achieve sustainable AMD treatment, recycling of wastes or byproducts as filter media in CWs has been gaining attention.

Recently, large amounts of oyster shells (OSs) have been generated as byproducts from the intensive development of oyster cultivation [12]. Most market oyster meat traders must pay for OS disposal by landfilling [13]. Therefore, an innovative method for recycling OSs is necessary to reduce environmental burdens and to provide economic value to seashells. Because OSs are composed mainly of non-hazardous calcium carbonate ($CaCO_3$), OSs have been recycled for many applications such as construction materials [14–17], catalysts [18], acid gas absorbents [19,20], and bactericidal agents [21].

For wastewater treatment, seashells including OSs have been investigated as an adsorbent for removing phosphorus [22] and heavy metals [23]. Park and Polprasert [24] reported the high applicability of OSs as a wetland media for the removal of phosphorus as Ca-P precipitation from swine wastewater (98.3% removal). Bavandpour et al. [25] mixed seashells (including OSs and mussels) for use as a filter media in CWs for heavy metal removal from synthetic AMD. Results showed that OSs can neutralize acids in AMD, leading to over 99% removals of dissolved Cu, Mn, Fe, Pb, and Zn by metal precipitation in hydroxide forms. Additionally, arsenate can be precipitated in the presence of Ca and Fe [25]. If OSs were found to be useful as a substrate in CWs for AMD treatment, then costs for OS disposal and CW maintenance could be reduced and high metal removal could be achieved. Nevertheless, CWs using OSs as a substrate for removing heavy metals from AMD have not been studied adequately from the viewpoint of pH neutralization, the role of plants, and mass balance. Moreover, previous research [25] only focused on the investigation of heavy metal removal from synthetic wastewater. To fill this research gap and to assess metal removal from both real and simulated AMD, this study investigated lab-scale CWs filled with OSs. Limestone, as a commercial calcium carbonate material, was also used as a main substrate in CW columns for comparison with the OS substrate. Huge amounts of limestone are annually discharged in the world without sustainable management [26].

This study evaluated the feasibility of OS-based CWs for treating AMD of an anonymous mine in Kyoto prefecture. The target AMD contained zinc (Zn) at 5–8 mg/L, iron (Fe) at 6–80 mg/L, cadmium (Cd) at 0.01–0.06 mg/L, and arsenic (As) at 0.01–0.15 mg/L, with a pH of about 3. We treated this AMD using mechanical aeration for oxidizing Fe with pH neutralization by quicklime. Subsequently, chemical sludge was removed from the water phase in sedimentation tanks. The aims of this study are (1) to compare the neutralization capabilities of oyster shell and limestone, (2) to evaluate the performance of CWs filled with OSs and limestone for heavy metal removal from both real and synthetic AMD, and (3) to clarify the heavy metal removal pathway in lab-scale CWs.

2. Materials and Methods

2.1. Real and Synthetic AMD

Real AMD was collected twice directly at a mine drainage treatment plant in Kyoto prefecture, Japan (December 2020, July 2021). Among the metals, the Zn and Fe concentrations are much higher than the Japanese effluent standard: 2 mg/L and 10 mg/L, respectively. In addition, the Cd and Pb concentrations sometimes exceeded the respective standards of 0.03 mg/L and 0.1 mg/L. The pH value is also lower than the effluent standard (5.8–8.6). After being collected, AMD was transported to the civil and environmental engineering laboratory, Ritsumeikan University, Biwako-Kusatsu Campus, Shiga, Japan. To prevent metals from oxidization during storage, AMD was maintained to have a dissolved oxygen (DO) level of less than 0.5 mg/L by purging with nitrogen gas.

Synthetic AMD was prepared based on characteristics of the real AMD. The chemical composition of the synthetic AMD and Japan effluent standards for metals are presented in Table 1.

2.2. Substrates

Some OSs for aquaria (O-O-Company; Fukuoka, Japan) were crushed and sieved through a 6–10 mm screen (porosity 51%, density 0.71 g/cm^3). Limestone (Kohnan Shoji Co., Ltd., Tokyo, Japan) was used for comparison with OSs (8–9 mm, porosity 44%, density of 1.42 g/cm^3). Loamy soil (akadama; Hirota Shoten K.K., Kanuma, Japan) was used to cover the top layer of CWs. This amount was negligible; it was ignored in mass balance calculations.

Table 1. Chemical composition of AMD (avg ± SD, *n* = 27) and the effluent standard in Japan.

	Synthetic AMD		**Real AMD (mg/L)**	**Effluent Standard (mg/L)**
	Reagents	**Concentration (mg/L)**		
Zn	$ZnSO_4•7H_2O$	7.22 ± 0.20	7.63 ± 1.64	<2
Cu	$CuSO_4•5H_2O$	0.20 ± 0.07	0.20 ± 0.09	<3
Cd	$CdCl_2$	0.04 ± 0.02	0.03 ± 0.02	<0.03
Mn	$MnSO_4•5H_2O$	0.90 ± 0.29	1.40 ± 0.29	<10
Pb	$PbCl_2$	0.17 ± 0.10	0.11 ± 0.11	<0.1
Fe	$FeSO_4•7H_2O$	37.00 ± 2.53	35.40 ± 6.40	<10
As	$AsNaO_2$	0.06 ± 0.04	0.05 ± 0.05	<0.1
Ca	$CaSO_4•2H_2O$	30.00 ± 3.00	30.00 ± 4.50	
Na	NaCl	6.70 ± 0.60	6.80 ± 1.20	
Mg	$MgSO_4•7H_2O$	10.00 ± 1.00	10.10 ± 2.00	
K	KCl	1.50 ± 0.15	1.60 ± 0.20	
Al	$AlNa(SO_4)_2•12H_2O$	2.01 ± 0.21	2.60 ± 0.30	
N	KNO_3	0.40 ± 0.06	0.30 ± 0.10	<100
pH		3.70 ± 0.40	3.30 ± 0.16	5.8–8.6

2.3. Batch Experiments for Neutralizing AMD Using OSs and Limestone

Briefly, 200 mL of synthetic AMD with an initial pH of 3 and 200 g pieces of limestone or OSs were placed in a 300 mL Erlenmeyer flask. Then, they were shaken using an orbital shaker at 70 rpm and 27 °C for 24 h. The change in pH was recorded using a pH meter with a data logger (IWC-6SD; Custom Corp., Tokyo, Japan). Water samples of 10 mL were collected at pH 3, 4, 5, 6, 7, and 8. Then, they were filtered through 0.45 µm cellulose filter paper (Advantec Co. Ltd., Tokyo, Japan) to measure the metals.

2.4. Setting up and Operating Lab-Scale CWs

The lab-scale CWs were installed in a greenhouse at Biwako-Kusatsu Campus, Ritsumeikan University, in November 2020–July 2021. A schematic of the lab-scale experiment is shown in Figure 1. A typical cylindrical wetland system [25,27] was used in this study. Each column (ID 12.5 cm, H 50 cm) was packed with OS pieces (23 cm depth, 2 kg) or limestone (23 cm depth, 4 kg). The top of each column was covered by a layer of loamy soil (3–4 mm, 1.5 cm depth, 0.18 kg). Cattails (*Typha orientalis*, 72 ± 1.2 cm shoot, 6 ± 1.2 cm root, 168 ± 4.4 g-wet/plant) were planted in CWs (OS-P; LS-P), or were left unplanted (OS-UP; LS-UP). These cattails were purchased at Tojaku Engei Corp. (Joyo, Japan). Before planting in the CWs, the soil around the roots was removed gently by using tap water in the laboratory. All CW tests were carried out in duplicate. The radiation and air temperature in the greenhouse were also recorded by a recorder (TR-74Ui-S; T&D Corporation, Matsumoto, Japan). Before being supplied with AMD, all wetland columns were kept filled with tap water for one week.

In sequencing batch treatments for 27 times, one liter of real or synthetic AMD was supplied to each wetland column. Real AMD was fed in batch numbers 5, 6, 7, 25, 26, and 27. Another batch treatment was run with synthetic AMD. After the defined hydraulic retention time (HRT), the treated AMD was fully taken out of each CW. After that, fresh AMD was supplied to CWs again. This sequencing batch treatment was operated with an HRT of 7 days. HRT is usually a couple of days to several weeks for AMD treatment [8]. For two months, during May–June 2021 in the 7-month experiment, all wetland columns were filled only with tap water because the campus was complying with a State of Emergency for the COVID-19 pandemic.

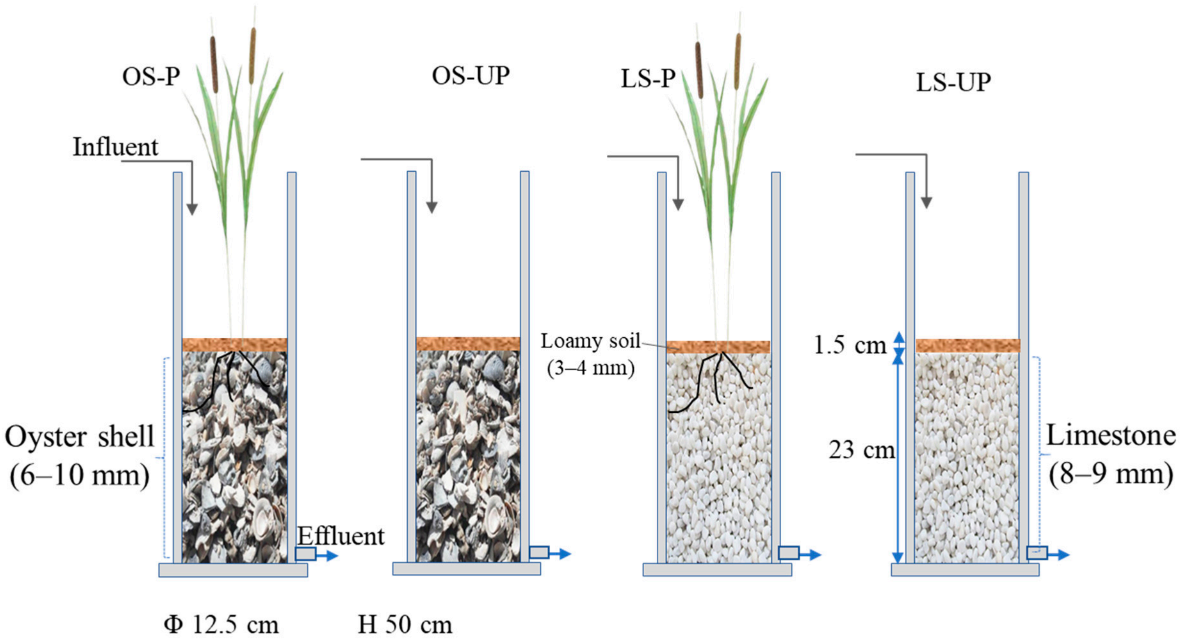

Figure 1. Diagram of lab-scale CWs filled with oyster shells (OSs) / limestone (LS) and unplanted (UP)/planted (P) with cattails.

2.5. Sampling and Analysis

The inlet and outlet water samples in the sequencing batch treatment were collected to measure heavy metals and other parameters. Suspended solids (SS) and sulfate (SO_4^{2-}) were analyzed according to the standard methods [28]. The total dissolved solids (TDS), oxidation–reduction potential (ORP), dissolved oxygen (DO), and pH value were measured by using a TDS meter (ASTDS1; AS One Corp., Osaka, Japan), an ORP meter (YK-23RP, Mother Tool Co. Ltd., Ueda, Japan), a DO meter (Hach HQ30D, CA, USA), and a pH meter (Laqua; Horiba Ltd., Kyoto, Japan), respectively. Water samples were centrifuged for 10 min at 3000 rpm to measure heavy metals (As, Cd, Cu, Fe, Mn, Pb, Zn) using inductively coupled plasma spectrometry (ICP-OES 700 series; Agilent Technologies Japan, Ltd., Tokyo, Japan).

Substrate and plant samples were collected before and after the 7-month sequencing batch operation. The unused and used OSs and limestone were dried at 98 ± 2 °C for 72 h; after that, they were crushed to a range size of 0.1–0.2 mm. After harvesting cattails, the plants were divided into aboveground (shoots) and belowground biomass (roots and rhizomes) parts, which were then washed well with pure water. These samples were dried at 82 ± 1 °C for 72 h. Then, they were ground to fine powder and were stored for additional analyses. The contents of heavy metals in prepared samples were determined according to a modified aqua regia method [29]. For the substrate, 500 mg of each sample was digested with 3.75 mL of HCl (35–37%) and 1.25 mL of HNO_3 (65%) for 24 h. After that, the mixture was heated at 96 ± 2 °C for 1 h. After being cooled to 25 °C, 5 mL of pure water was added to each sample. For plant samples, 100 mg of each sample was digested with 1 mL of HNO_3 (65%) for 48 h. Subsequently, 9 mL of pure water was added to each mixture. All supernatants of the digested samples were filtered through 0.45 µm cellulose filter paper (Advantec Co. Ltd., Tokyo, Japan). Then, the concentrations of heavy metals in the filtrates were measured by using ICP-OES.

Heterotroph and sulfate-reducing bacteria (SRB) were determined by using the plate count method with R2A agar medium and Postgate's medium F, respectively [30].

2.6. Calculation

Evapotranspiration (%) in the CWs was determined by the following equation:

$$\text{Water loss by evapotranspiration (\%)} = (\text{Vi} - \text{Ve}) / \text{Vi} \times 100 \tag{1}$$

Mass removal efficiency (%) for metals was calculated as

$$\text{Removal (\%)} = (\text{ViCi} - \text{VeCe}) / \text{ViCi} \times 100, \tag{2}$$

where Vi (L) and Ci (mg/L), and Ve (L) and Ce (mg/L) are the water volume and the concentration of heavy metals before and after the treatment, respectively.

The amount of heavy metals retained in CWs (Ms) by the substrate was determined using the following equation:

$$\text{M}_\text{s}(\text{mg}) = (\text{C}_\text{sa} - \text{C}_\text{sb}) \times \text{m} \tag{3}$$

where C_S (mg/kg) and m (kg) are the concentration of heavy metals and the total mass of the substrate, respectively. Subscripts b and a respectively stand for the before and after treatment.

The amount of heavy metals accumulated (Mp) in cattails was calculated as

$$\text{M}_\text{p}\ (\text{mg}) = \text{C}_\text{pa}\text{X}_\text{a} - \text{C}_\text{pb}\text{X}_\text{b} \tag{4}$$

where Cp (mg/kg) and X (kg) denote the metal concentration in cattails and the total dried biomass of plants before and after the experiment, respectively.

The bioconcentration factor (BCF) and the translocation factor (TF) were also obtained as follows [30]:

$$\text{BCF} = \text{C}_\text{p}/\text{C}_\text{s} \tag{5}$$

$$\text{TF} = \text{C}_\text{ps}/\text{C}_\text{pr} \tag{6}$$

where C_{ps} (mg/kg) is the concentration of metal in shoots and C_{pr} (mg/kg) is the concentration of metal in roots.

3. Results

3.1. pH Neutralization of AMD in the Batch Experiment

Figure 2a shows the metal concentrations against the pH values versus time in the AMD neutralization test with OSs and limestone for 24 h. The OS pieces had a higher neutralization rate than limestone. The pH value for the AMD shaken with OSs reached 7 after about 1 h and 8 after about 3 h, and then remained stable at pH 8 for 24 h. The pH value in the AMD shaken with limestone took about 9.5 h to reach pH 7 and then increased gradually to 8 after about 23 h.

Concentrations for all soluble metals decreased with the increase in the pH of the AMD shaken with OSs and limestone, as expected from the solubility product of metal hydroxides. At pH levels higher than 7.8–8.1, the Zn concentration (Figure 2b) in the AMD was far below the Japan effluent standard (2 mg/L). The soluble Fe concentration (0.2–3 mg/L) in the AMD (Figure 2f) was far below the standard (10 mg/L) at pH levels greater than 5, with formation of an orange precipitate. Removals of Cd, Pb, Cu, Mn, and As (Figure 2c–e,g,h) in the AMD were more than 90% over a pH level of 8, except for Mn (41–47%).

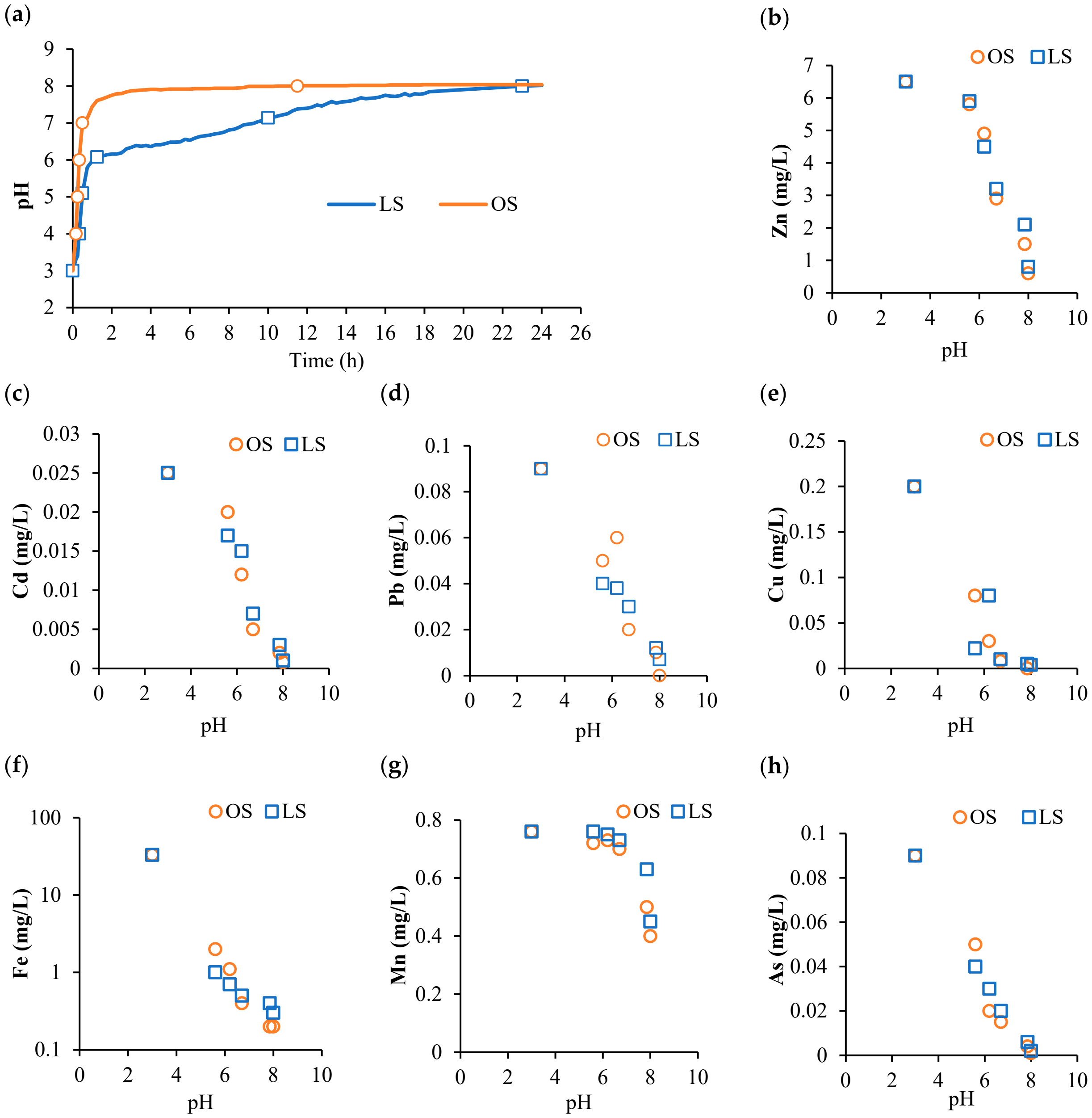

Figure 2. pH changes in AMD with oyster shells (OSs) and limestone (LS) for 24 h and relation between pH and metal concentrations: pH (**a**), Zn (**b**), Cd (**c**), Pb (**d**), Cu (**e**), Mn (**g**), Fe (**f**), and As (**h**).

3.2. Environmental Conditions in the Greenhouse and Water Parameters

The air temperature inside the greenhouse in which the lab-scale CWs were set up is displayed in Supplementary Figure S1. The average temperatures in late autumn (November 2020), winter (December 2020–February 2021), spring (March–May 2021), and summer (June–July 2021) were 15.2 ± 6.2, 8.3 ± 7.6, 18.3 ± 10.0, and 27.0 ± 7.5 °C, respectively. In winter, the aboveground parts of cattails withered, but the roots remained alive. The young shoots started to grow in spring, and continued to extend through summer (Supplementary Figure S2).

The input and output water volumes in the CWs in the sequencing batch treatment are shown in Supplementary Figure S3. Although evapotranspiration estimated from the difference of input and output was negligible in late autumn and winter, high evapotranspiration in hot summer with rapid plant growth contributed considerably to the mass removal efficiency of heavy metals, as shown in Equation (2). Table 2 shows the average evapotranspiration achieved during the experiment; it reached 8.4–11.5% and 12.5–20.4% in planted and unplanted CWs, respectively.

Supplementary Figure S4 presents the pH value, ORP, DO, TDS, and SO_4^{2-} concentrations in the inlet and outlet water samples during the 7-month operation. The average values of the AMD parameters before and after treatment are also presented in Table 2. The influent pH values were 3.2–4.1 for both real and synthetic AMD. After the treatment, the pH values reached 6.9–8.3 in all CWs. Although the influent DO concentration of synthetic AMD was 8.3–9.7 mg/L, it was controlled to less than 0.5 mg/L in the real AMD to avoid metal oxidization during storage. After treatment, the DO levels were 2.8–12.0 mg/L and 3.5–13.8 mg/L for the planted CWs and the unplanted CWs, respectively. A low DO level was found in the planted CWs in late spring and early summer, implying DO consumption of the roots and rhizosphere. The TDS concentrations in OS-based CWs were higher than those of limestone-based CWs. The ORP values were generally positive. They dropped in warm late spring and hot summer. The SS concentration was similar in all CWs for both real and synthetic AMD treatments. The sulfate concentrations were generally lower after treatment, especially in planted CWs.

3.3. Overall Performance of CWs for Heavy Metal Removal

The concentrations of heavy metals in influent and effluent for both real and synthetic AMD are displayed in Figure 3. The average mass removals in the CWs are presented in Table 3. The metal concentrations from water collected six times in the real AMD fluctuated. Overall, during treatment, heavy metals were removed effectively from AMD by all CWs. The removal trends were similar in both real and synthetic AMD. The mass removal efficiencies in CWs employing OSs were higher than those employing limestone, except for Cu.

Among seven target metals, Fe showed the highest concentrations during the experiment. The Fe concentration in both real and synthetic AMD (38 mg/L, on average) was much higher than the effluent standard (10 mg/L). From all CWs, Fe was removed with removal efficiency of 99.2–99.9%.

The average Zn concentrations in synthetic and real AMD were 7.2 and 7.6 mg/L, respectively, which were approximately four times higher than the Japan standard (2 mg/L). In fact, Zn was effectively eliminated by all wetland columns for both real and synthetic AMD. Its concentration in effluent was 0.00–0.25 mg/L in OS-based CWs and 0.02–1.51 mg/L in limestone-based CWs.

The Cd and Pb concentrations in influent sometimes exceeded the standard: 0.03 and 0.10 mg/L, respectively. The Cd and Pb concentrations in synthetic AMD were 0.02–0.09 mg/L and 0.06–0.41 mg/L, respectively. Those in real AMD were 0.01–0.04 mg/L and 0.10–0.12 mg/L, respectively. After treatment, Cd and Pb were eliminated effectively by all CWs: 56.0–100% for both real and synthetic AMD.

The average concentrations of As, Cu, and Mn in real and synthetic AMD were 0.06, 0.20, and 0.99 mg/L, respectively, which were lower than the standard. These were also removed well by all CWs, with an average mass removal of more than 90% for As, 97% for Cu, and 76% for Mn in all CWs.

Table 2. Evapotranspiration (Evapot) and water parameters of influent and effluent for CWs in 7-month AMD treatment (avg ± SD).

Parameters	**Simulated AMD (n = 21)**					**Real AMD (n = 6)**				
	Influent	**Effluent**				**Influent**	**Effluent**			
		OS-P	**OS-UP**	**LS-P**	**LS-UP**		**OS-P**	**OS-UP**	**LS-P**	**LS-UP**
Water (L/cycle)	1	0.8 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.0	0.9 ± 0.0
Evapot (%)	N/A	(14.1 ± 5.5)	(8.4 ± 3.4)	(12.5 ± 2.6)	(9.3 ± 1.8)	N/A	(20.4 ± 15.2)	(11.5 ± 6.6)	(16.7 ± 7.8)	(11.3 ± 4.7)
pH	3.7 ± 0.4	7.2 ± 0.3	7.3 ± 0.3	7.2 ± 0.2	7.3 ± 0.35	3.2 ± 0.2	7.0 ± 0.2	7.1 ± 0.2	7.0 ± 0.3	7.0 ± 0.5
ORP (mV)	185.0 ± 9.8	75.5 ± 38.2	79.0 ± 31.8	90.3 ± 34.2	88.6 ± 34.0	184.1 ± 6.6	69.6 ± 50.9	61.5 ± 50.0	65.4 ± 51.5	67.0 ± 52.0
DO (mg/L)	9.2 ± 0.3	8.2 ± 2.7	9.8 ± 2.9	7.6 ± 1.4	9.5 ± 0.9	0.5 ± 0.1 *	9.3 ± 3.4	13.1 ± 4.2	8.9 ± 2.9	10.9 ± 2.4
SS (mg/L)	N/A	29.1 ± 19.6	28.1 ± 17.7	30.4 ± 17.6	24.9 ± 118.2	N/A	15.1 ± 1.8	15.4 ± 4.8	17.9 ± 2.2	15.5 ± 0.9
TDS (mg/L)	228.8 ± 14.7	327.7 ± 69.7	320.4 ± 56.5	238.6 ± 8.5	232.3 ± 8.8	291.6 ± 1.5	308.6 ± 4.9	287.8 ± 1.4	255.2 ± 1.6	246.5 ± 7.7
SO_4^{2-}(mg/L)	236.1 ± 22.1	216.0 ± 29.9	206.4 ± 32.5	200.4 ± 22.9	218.6 ± 18.7	236.5 ± 9.5	190.5 ± 24.4	193.5 ± 42.2	205.0 ± 40.0	228.0 ± 36.0

Note: * DO was controlled to less than 0.5 mg/L to avoid metal oxidization. N/A—Not available.

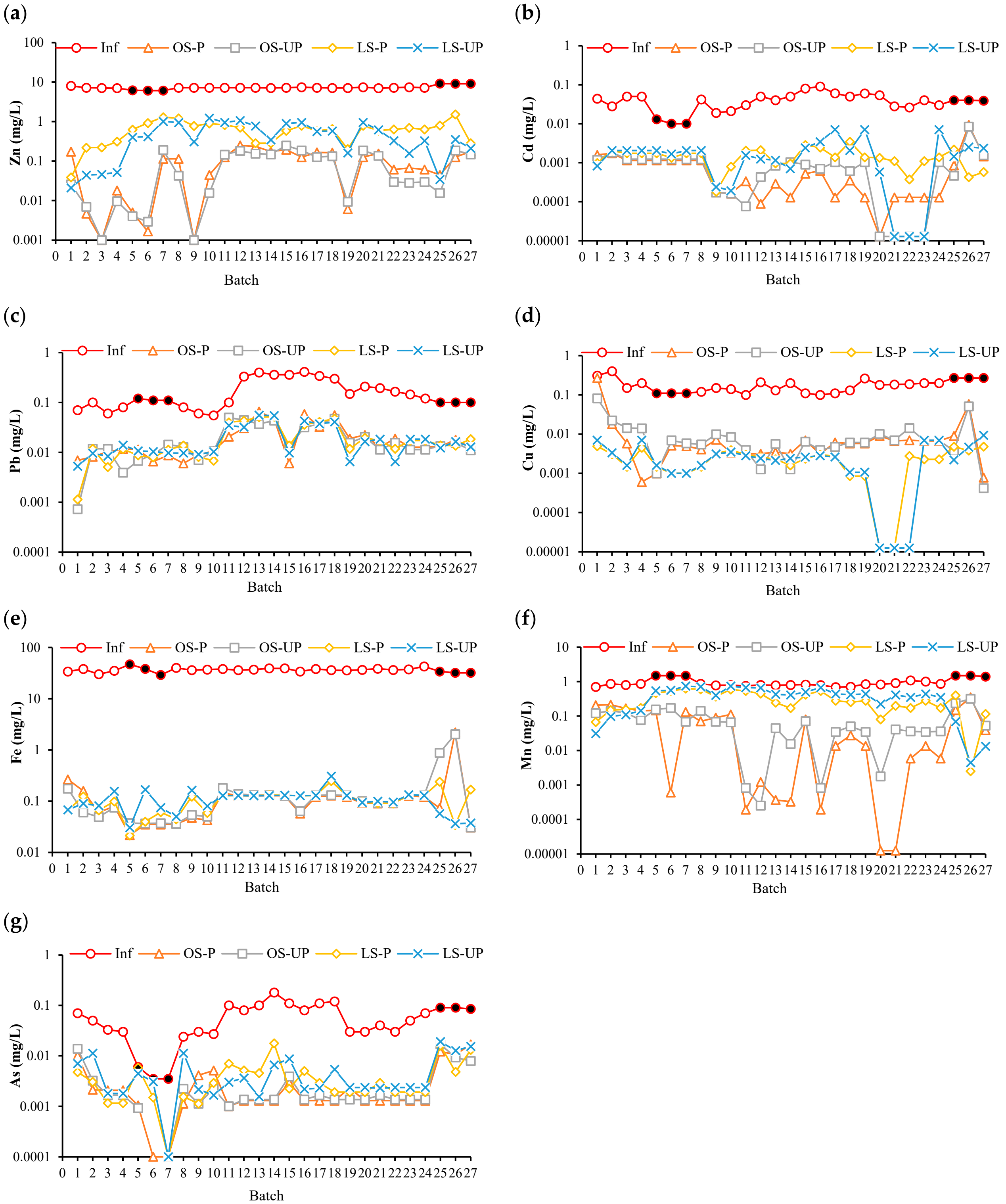

Figure 3. Heavy metal concentrations in influent and effluent for oyster shell (OS)/limestone (LS)-based planted (P)/unplanted (UP) CWs treating real and synthetic AMD in sequencing batch mode: Zn (**a**), Cd (**b**), Pb (**c**), Cu (**d**), Fe (**e**), Mn (**f**), and As (**g**).

Table 3. Mass removal efficiency (%) of heavy metals from simulated and real AMD using CWs filled with oyster shells (OS) or limestone (LS), unplanted (UP) or planted (P) with cattails (avg ± SD).

Metal Removal	**Simulated AMD (*n* = 21)**				**Real AMD (*n* = 6)**			
	OS-P	**OS-UP**	**LS-P**	**LS-UP**	**OS-P**	**OS-UP**	**LS-P**	**LS-UP**
Zn (%)	98.7 ± 1.0	98.9 ± 1.0	93.1 ± 3.5	93.2 ± 4.9	99.2 ± 0.7	99.0 ± 1.2	88.6 ± 7.2	91.6 ± 5.9
Cd (%)	98.9 ± 1.3	98.5 ± 1.4	96.9 ± 1.6	95.8 ± 4.8	91.5 ± 5.9	91.5 ± 5.3	89.9 ± 6.1	87.8 ± 7.8
Cu (%)	93.4 ± 16.5	94.8 ± 4.9	98.7 ± 0.8	98.4 ± 1.0	95.3 ± 5.9	95.6 ± 4.9	98.9 ± 0.9	98.7 ± 1.0
Pb (%)	89.4 ± 4.5	88.7 ± 8.8	89.1 ± 6.8	88.8 ± 6.1	91.0 ± 2.8	90.7 ± 2.5	88.3 ± 12.4	88.3 ± 10.3
As (%)	95.6 ± 4.5	95.9 ± 3.9	95.2 ± 2.2	92.6 ± 8.9	91.3 ± 6.3	92.7 ± 6.4	63.6 ± 40.5	48.2 ± 52.8
Mn (%)	93.8 ± 8.3	93.8 ± 5.3	67.9 ± 19.3	54.3 ± 24.4	92.6 ± 6.2	90.9 ± 4.4	65.8 ± 19.1	58.8 ± 25.9
Fe (%)	99.7 ± 0.1	99.7 ± 0.1	99.7 ± 0.1	99.7 ± 0.1	99.0 ± 2.1	98.9 ± 1.8	98.8 ± 0.2	98.7 ± 0.3

3.4. Heavy Metal Accumulation in Substrates

Figure 4 presents the metal contents in the OSs and limestone in the CWs before and after treating AMD. The total amounts of heavy metals retained in the substrates are displayed in Supplementary Table S1. The contents of all heavy metals in both OSs and limestone increased during the 7-month operation. Their accumulation in OSs was generally higher than that of limestone.

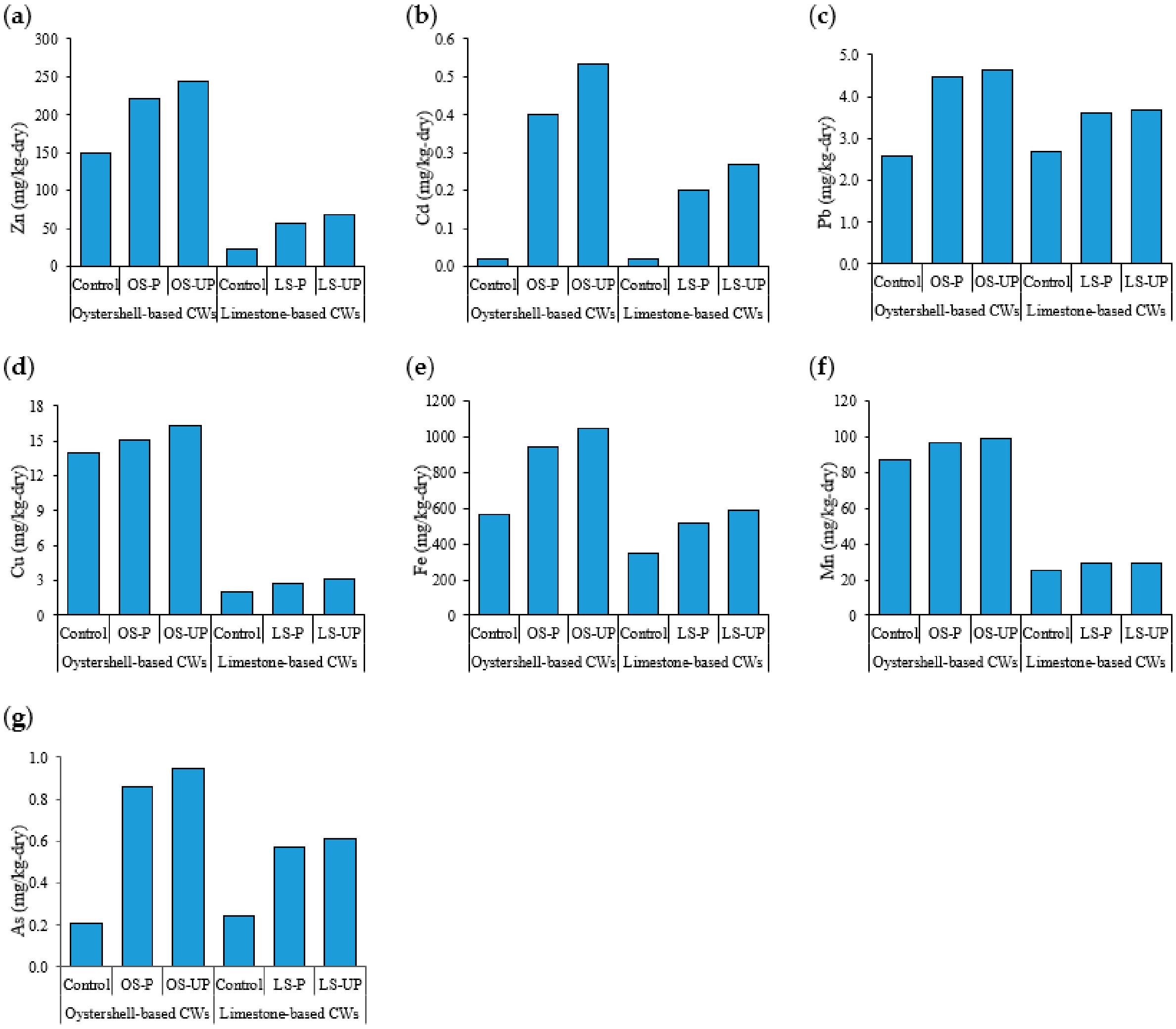

Figure 4. Heavy metal contents in oyster shells and limestone in CWs before and after 7-month AMD treatment: Zn (**a**), Cd (**b**), Pb (**c**), Cu (**d**), Fe (**e**), Mn (**f**), and As (**g**).

Among the studied metals, Cd demonstrated the highest accumulation in the substrates. Its content in both the original OSs and limestone was 0.02 mg/kg. After treating AMD for 7 months, the Cd contents increased 20–27 times in OSs and 10–14 times in the limestone. Before using the CWs, the Zn, Cu, Pb, Fe, Mn, and As contents were 150.0, 14.0, 2.6, 566.0, 87.0, and 0.2 mg/kg in OSs and 23.2, 2.1, 2.7, 346.5, 25.4, and 0.24 mg/kg in limestone, respectively. After using the CWs, those contents increased 1.1–4.5 times in OSs and 1.2–3.0 times in limestone.

3.5. Heavy Metal Accumulation in Cattails

During the experiment, the height of the cattail tops increased to 88–129 cm. In addition, the roots extended to 28–32 cm in the planted CWs. Cattails were collected after 7 months of the experimental operation as about 163 g-dry from each planted CW: about 23 g-dry of the aboveground part and about 140 g-dry of the belowground part. Supplementary Table S2 and Figure 5 show the concentrations of heavy metals in biomass before and after using the CWs. The contents of all metals in cattails increased significantly during the experiment, especially in the belowground biomass. The concentrations of Zn, Cu, Fe, and Mn, as essential elements, were found at higher concentrations than As and Cd in the cattail biomass.

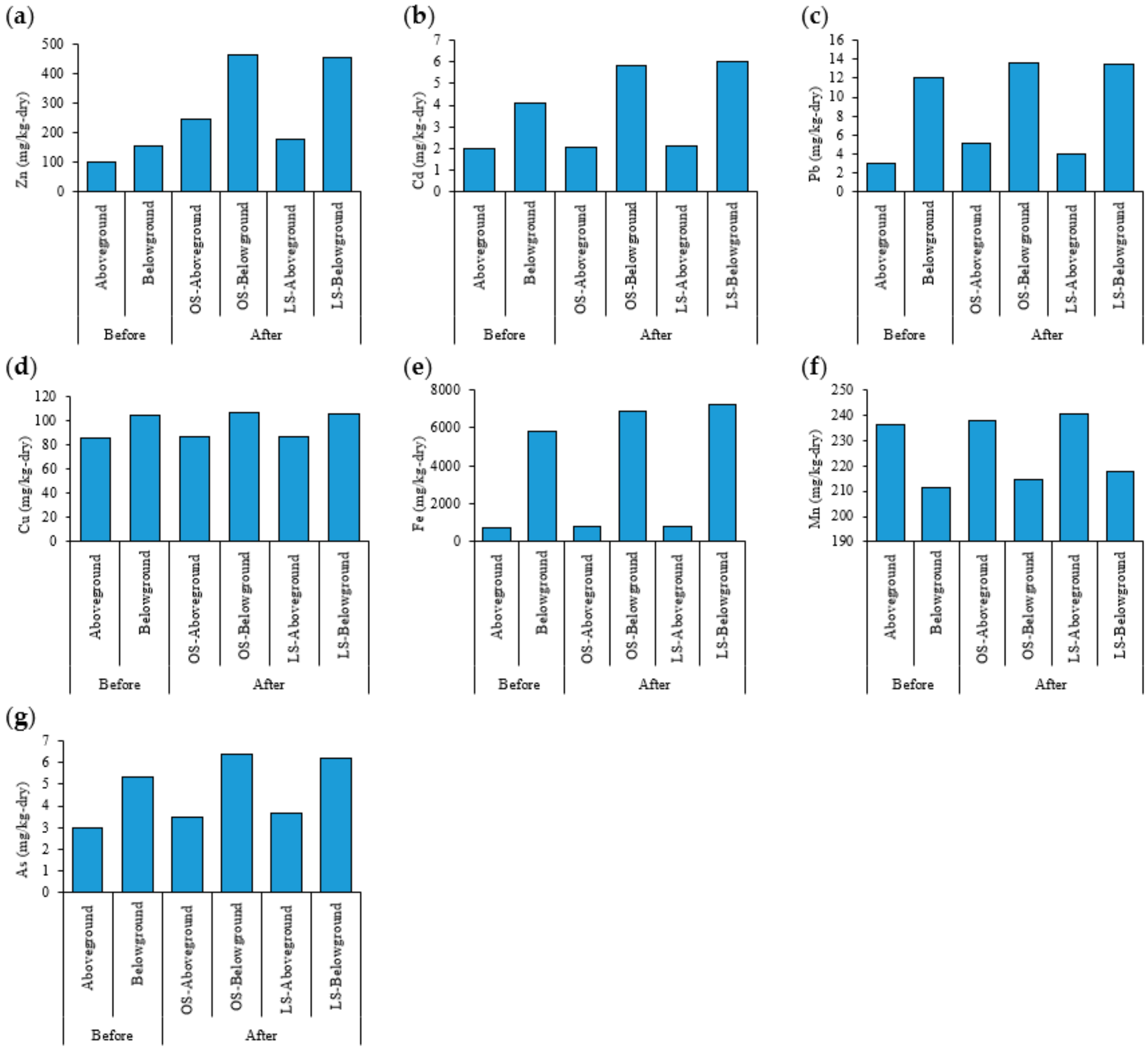

Figure 5. Accumulation of heavy metals in cattails in oyster shell (OS)/limestone (LS)-based CWs before and after 7-month AMD treatment: Zn (**a**), Cd (**b**), Pb (**c**), Cu (**d**), Fe (**e**), Mn (**f**), and As (**g**).

After the 7-month operation, the total Zn, Cd, Cu, Pb, Fe, Mn, and As accumulation values in the plant biomass were 48.72, 0.30, 2.17, 0.44, 230.40, 4.74, and 0.24 mg in the OS-P CW, respectively, and 45.00, 0.33, 2.03, 0.40, 286.94, 4.83, and 0.22 mg in the LS-P CW.

The BCF and TF values found for each metal in the planted CWs are presented in Table 4. The BCFs values for all investigated heavy metals were 3.22–69.57 in all the planted CWs, implying the ability of cattails for the uptake of heavy metals from the substrates. Higher BCF values were obtained in the LS-P CW. The metal concentrations in the shoots were much lower than those in the roots, except for Mn. The TF values were 0.11–1.11 for total metals.

Table 4. BCF and TF values of cattails in the planted CWs after 7-month AMD treatment.

CWs	Metal	TF		BCF
		Before Use	After Use	
OS-based CW	As	0.56	0.55	11.49
	Zn	0.65	0.53	3.22
	Cd	0.49	0.36	19.73
	Cu	0.82	0.82	12.83
	Pb	0.25	0.38	4.20
	Mn	1.12	1.11	4.68
	Fe	0.13	0.12	8.19
LS-based CW	As	0.56	0.59	17.30
	Zn	0.65	0.39	11.09
	Cd	0.49	0.35	40.60
	Cu	0.82	0.82	69.57
	Pb	0.25	0.30	4.81
	Mn	1.12	1.10	15.85
	Fe	0.13	0.11	15.58

3.6. Bacteria in CWs

The populations of heterotrophic bacteria ($9 \times 10^6 - 8 \times 10^7$ CFU/mg-dry) found in the substrates in the columns planted with cattails were larger than those in the columns without cattails ($4 \times 10^5 - 5 \times 10^6$ CFU/mg-dry). Large populations of SRB ($2 \times 10^2 - 4 \times 10^3$ CFU/mg-dry) were only detected in the planted CWs.

3.7. Mass Balance of Metals in CWs

Heavy metals in AMD fed to the CWs were discharged in effluent and accumulated in the substrates and the plant biomass. After the 7-month operation, the total amount of each metal fed to each CW was 197.6 mg for Zn, 1.09 mg for Cd, 4.91 mg for Cu, 4.62 mg for Pb, 986.1 mg for Fe, 26.2 mg for Mn, and 1.7 mg for As. The amounts of heavy metals distributed to each component are displayed in Supplementary Table S1.

As presented in Figure 6, heavy metals were mainly removed by the accumulation in the substrates in the CWs, representing 69.0–95.6% for Zn, 65.8–94.2% for Cd, 44.8–93.6% for Cu, 79.3–89.0% for Pb, 68.8–99.3% for Fe, 53.5–90.5% for Mn, and 77.8–88.5% for As of the inflow in each CW. In the planted CWs, accumulation in the plant biomass accounted for 22.8–24.7% for Zn, 27.5–29.9% for Cd, 41.3–44.3% for Cu, 8.8–9.5% for Pb, 23.4–29.1% for Fe, 18.1–18.5% for Mn, and 12.9–14.4% for As. Unknown processes including measurement errors accounted for 0.1–3.5% for the planted CWs and 0.4–8.1% for the unplanted CWs for all metals.

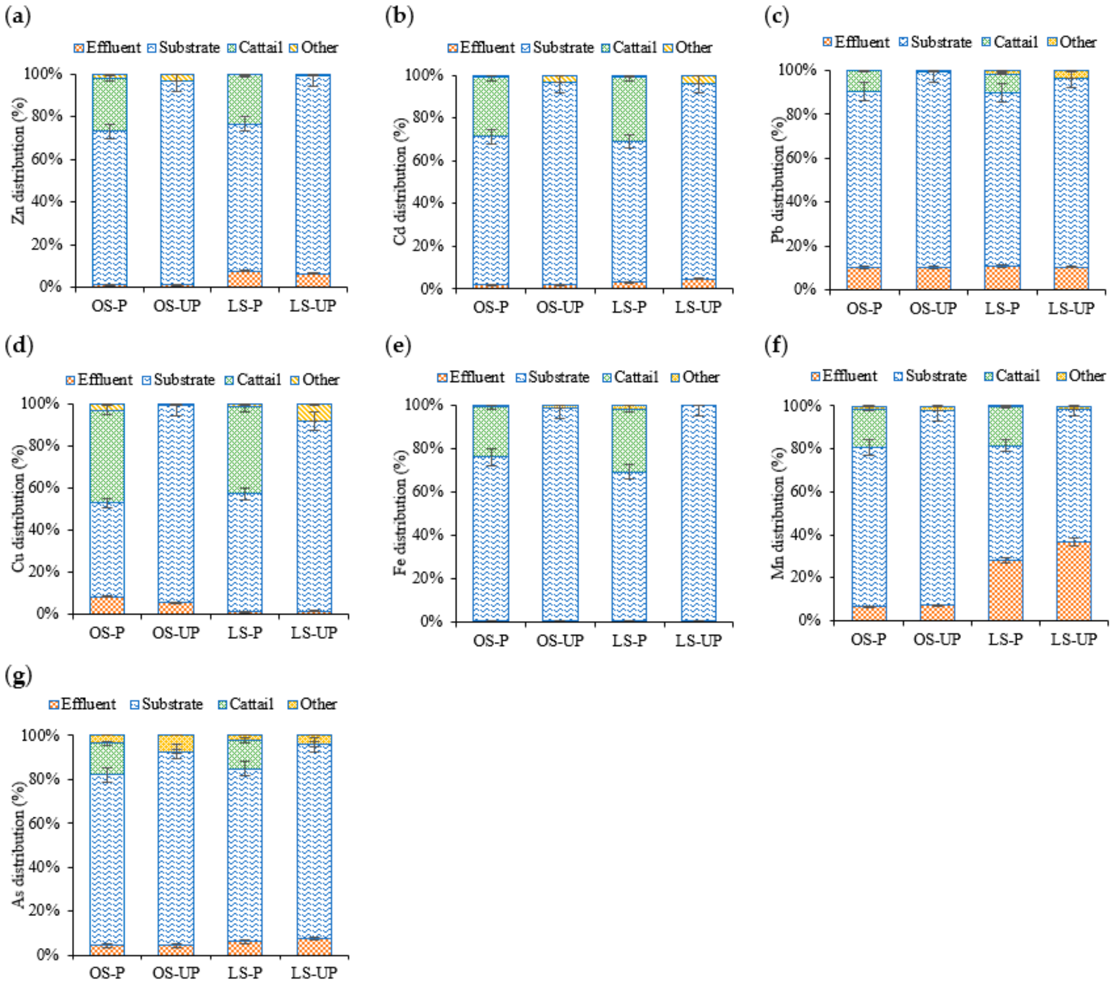

Figure 6. Heavy metal removal pathways in oyster shell (OS)/limestone (LS)-based planted (P)/unplanted (UP) CWs during 7-month AMD treatment: Zn (**a**), Cd (**b**), Pb (**c**), Cu (**d**), Fe (**e**), Mn (**f**), and As (**g**).

4. Discussion

4.1. Relation between pH Changes and Metal Removal Efficiencies

OSs demonstrated comparable or higher potential than limestone for pH neutralization and heavy metal removal from AMD (Figure 2 and Figure S6). Recycling OSs for CWs will decrease the consumption of virgin limestone [26]. A greater heavy metal removal efficiency was observed at higher pH. With the main component containing >90% $CaCO_3$, limestone and OSs have the merit of generating ion hydroxides, leading to the neutralization of acidic wastewater [13]. Moreover, crushed OSs can release carbonate species such as HCO_3^- and CO_3^{2-}, which are responsible for forming insoluble metal carbonates. The Ca^{2+} and CO_3^{2-} released from the aragonite surfaces can also react with the fluid to form solid–solution nuclei.

As inferred from the solubility products, the metal concentrations can theoretically satisfy the Japan effluent standard for pH for Cu, Zn, Pb, and Cd, which were 6.77, 7.87, 9.47, and 10.3, respectively [27]. These metals were removed consistently (>90%) at a pH as high as 8 in the batch experiment, except for Mn, which had less sensitivity to the increased pH.

4.2. Heavy Metal Removal Mechanism in Constructed Wetlands

Accumulation in OSs and limestone was a dominant metal removal pathway (44.8–99.3%). Metals were generally trapped mainly in the substrates through absorption, precipitation, co-precipitation, and ion exchange [31]. The high content of metal oxides in OSs and limestone was beneficial for metal removal. The increase in pH led to the formation of metal oxides and hydroxides. In addition, As might be removed via co-precipitation [32].

In CWs, plants are important design components for decontamination [30]. In the present study, the contribution to heavy metal removal by cattails was 8.8–44.3%. Zn, Cu, Fe, are Mn are essential elements for plant growth. These metals can be collected easily by harvesting the aboveground parts of plant biomass. Cattails might accumulate heavy metals in tissues and release root exudates that often control the mobility of metals [33]. Aside from this function, the cattail roots can provide additional surface area for supporting microbial growth and metabolism, as evidenced by the larger populations of bacteria found in the planted CWs. Additionally, the formation of litter and biofilm layers contributes to the removal of heavy metals by adsorption [9].

Together with the reduced sulfate concentration in effluent, the presence of SRB can contribute to heavy metal removal via metal sulfide precipitation. Metal sulfide precipitation is well known as an effective means for heavy metal removal from CWs [34–36]. The SRB activities might also contribute to higher heavy metal removal efficiencies in planted CWs, even when all aboveground parts of cattails die in winter. This finding also implies that plant roots played an important role in creating favorable conditions for the growth and development of microbial communities. Heavy metal removal by SRB can be enhanced considerably by providing carbon sources and electron donors such as acetate and lactate and inoculating specific bacteria such as *Desulfovivrio* and *Desulfobulbus* in CWs [30].

4.3. Recommendations for Further Studies in Using Alternative Filter Materials in CWs

OS-based CWs can effectively remove heavy metals from AMD. This result is consistent with the results reported by Bavandpour et al. [25], which showed the high treatment performance of CWs employing OSs mixed with mussel shells for removing Zn (75%), Cu (90%), Pb (98%), and Mn (77%). In our previous study [37], the CWs employing clamshells also showed a high capacity for removing heavy metals from synthetic AMD. The finding of this research further solidifies the recycling of bivalve shells as filter media in CWs.

Recycling OSs as filter media in CWs for mine drainage treatment can yield both economic and ecological benefits. As a waste byproduct, OSs are available in abundance from many oyster culturing areas [38,39]. The particle size of OSs should be optimized to ensure high performance and to avoid clogging. The substrate surface for adsorption might be saturated gradually. This saturation was evidenced by the decrease in alkalinity of the outlet and removal efficiency during the operation. Consequently, the adsorption capacity of materials should be identified to estimate the CW lifespan. Additionally, further research should be undertaken to examine the speciation of the pollutants in the solid phase, and the change of physicochemical properties of materials before and after treatment. Furthermore, local governments are undertaking efforts to reduce the costs of mine drainage treatment using environmentally friendly green technologies [4]. At more than 15 mines in Japan, the drainage contains Zn at high concentrations with low pH, similarly to the AMD in this study [4,37]. Knowledge obtained in this study will be helpful for installing pilot- and full-scale CWs at such mines. Future studies should include examinations of actual costs, including OS crushing, transport, and final disposal.

5. Conclusions

This study showed high AMD neutralization potential for both OSs and limestone. Heavy metals were removed effectively from both real and synthetic AMD using lab-scale CWs employing OSs and limestone during a 7-month operation. Accumulation in the substrates was a principal pathway for removing heavy metals in all CWs, with additional accumulation in the cattail biomass in the planted wetland columns. The higher metal removal efficiency in the OS-based CWs also demonstrated OSs as a potential material in CWs for removing heavy metals from AMD. The findings of this study might pave the way to the recycling of bivalve shells as substrates in CWs for wastewater treatment, and might encourage reduced usage of natural minerals such as limestone and gravel.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14203325/s1, Figure S1: Air temperatures in a greenhouse placed with CWs during 7-month AMD treatment, Figure S2: Cattail in CWs. Withered aboveground in autumn (a) and winter (b). Young shoots in spring (c). Matured (d) and harvesting biomass (e), Figure S3: Inlet and outlet water volume for oyster shell (OS)/limestone (LS)-based unplanted (P)/planted (P) CWs during 7-month AMD treatment in sequencing GA batch mode, Figure S4: pH, ORP, DO, and TDS concentrations in influent and effluent for oyster shell (OS)/limestone (LS)-based unplanted (P)/planted (P) CWs during 7-month AMD treatment in sequencing batch mode, Figure S5: Sulfate concentration in influent and effluent for oyster shell (OS)/limestone (LS)-based unplanted (P)/planted (P) CWs during 7-month AMD treatment in sequencing batch mode, Figure S6: Relation between pH and metal concentrations in effluents for oyster shell (OS)/limestone (LS)-based unplanted (P)/planted (P) CWs during 7-month AMD treatment, Table S1: Distribution of heavy metals (mg) in each part in oyster shell (OS)/limestone (LS)-based unplanted (P)/planted (P) CWs after 7-month AMD treatment, Table S2: Heavy metal concentrations in substrates and cattails in oyster shell (OS)/limestone (LS)-based unplanted (P)/planted (P) CWs before and after 7-month AMD treatment.

Author Contributions: Conceptualization, T.T.N. and S.S.; funding acquisition, K.H.; investigation, T.T.N. and S.S.; methodology, T.T.N. and S.S.; project administration, K.H. and S.S.; resources, T.T.N. and K.H.; supervision, S.S.; validation, S.S. and K.H.; visualization, T.T.N.; writing—original draft, T.T.N.; writing—review and editing, S.S. All authors have read and agreed to the published version of the manuscript.

Funding: This study was partly supported by the AY 2022 Program for Asia–Japan Research Development, Ritsumeikan University.

Data Availability Statement: The data used in this study are available from the first author upon reasonable request.

Acknowledgments: We are grateful to the editor and anonymous reviewers for their comments, which considerably improved this work.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Joshua, O.; Setyo, B.; Kingsley, O.; Chukwunonso, O.; Oluwaseun, J.; Stephen, N.; Felicitas, U.; Shabnam, A.; Chinenye, A. A review of treatment technologies for the mitigation of the toxic environmental effects of acid mine drainage (AMD). *Process Saf. Environ. Prot.* **2022**, *157*, 37–58. [CrossRef]
2. Naidu, G.; Ryu, S.; Thiruvenkatachari, R.; Choi, Y.; Jeong, S.; Vigneswaran, S. A critical review on remediation, reuse, and resource recovery from acid mine drainage. *Environ. Pollut.* **2019**, *247*, 1110–1124. [CrossRef] [PubMed]
3. Skousen, J.G.; Ziemkiewicz, P.F.; McDonald, L.M. Acid mine drainage formation, control and treatment: Approaches and strategies. *Extr. Ind. Soc.* **2019**, *6*, 241–249. [CrossRef]
4. Ueda, H.; Masuda, N. An analysis of mine drainage treatment cost and the technical development to prevent mine pollution. *Shigen-to-Sozai* **2005**, *121*, 323–329. [CrossRef]
5. Koide, R.; Tokoro, C.; Murakami, S.; Adachi, T.; Takahashi, A. A model for prediction of neutralizer usage and sludge generation in the treatment of acid mine drainage from abandoned mines: Case studies in Japan. *Mine Water Environ.* **2012**, *31*, 287–296. [CrossRef]
6. Chen, Q.; Yao, Y.; Li, X.; Lu, J.; Zhou, J.; Huang, Z. Comparison of heavy metal removals from aqueous solutions by chemical precipitation and characteristics of precipitates. *J. Water Process. Eng.* **2018**, *26*, 289–300. [CrossRef]
7. Rodríguez-Galán, M.; Moreno, F.M.B.; Vázquez, S.; Torralvo, F.A.; Vilches, L.F.; Zhang, Z. Remediation of acid mine drainage. *Environ. Chem. Lett.* **2019**, *17*, 1529–1538. [CrossRef]
8. Acheampong, M.A.; Ansa, E.D.O. Low-cost technologies for mining wastewater treatment. *J. Environ. Sci. Eng.* **2017**, *6*, 391–405. [CrossRef]
9. Pat-Espadas, A.; Loredo Portales, R.; Amabilis-Sosa, L.E.; Gómez, G.; Vidal, G. Review of constructed wetlands for acid mine drainage treatment. *Water* **2018**, *10*, 1685. [CrossRef]
10. Yu, G.; Wang, G.; Chi, T.; Du, C.; Wang, J.; Li, P.; Chen, H. Enhanced removal of heavy metals and metalloids by constructed wetlands: A review of approaches and mechanisms. *Sci. Total Environ.* **2022**, *821*, 153516. [CrossRef]
11. Wu, H.; Zhang, J.; Ngo, H.H.; Guo, W.; Hu, Z.; Liang, S.; Liu, H. A review on the sustainability of constructed wetlands for wastewater treatment: Design and operation. *Bioresour. Technol.* **2015**, *175*, 594–601. [CrossRef] [PubMed]

12. de Alvarenga, R.A.F.; Galindro, B.M.; de Fátima Helpa, C.; Soares, S.R. The recycling of oyster shells: An environmental analysis using Life Cycle Assessment. *J. Environ. Manag.* **2012**, *106*, 102–109. [CrossRef] [PubMed]
13. Yao, Z.; Xia, M.; Li, H.; Chen, T.; Ye, Y.; Zheng, H. Bivalve shell: Not an abundant useless waste but a functional and versatile biomaterial. *Crit. Rev. Env. Sci. Tech.* **2014**, *44*, 2502–2530. [CrossRef]
14. Yoon, G.L.; Yoon, Y.W.; Chae, K.S. Shear strength and compressibility of oyster shell–sand mixtures. *Environ. Earth Sci.* **2009**, *60*, 1701–1709. [CrossRef]
15. Miyaji, Y.; Okamura, T. Geo-material properties of wasted oyster shell sand mixture and its application as material for sand compaction pile. In *Coastal Geotechnical Engineering in Practice, Proceedings of the International Symposium IS-Yokohama 2000, Yokohama, Japan, 20–22 September 2000*; Balkema: Rotterdam, The Netherlands, 2000; pp. 675–680.
16. Yang, E.I.; Yi, S.T.; Leem, Y.M. Effect of oyster shell substituted for fine aggregate on concrete characteristics: Part I. Fundamental properties. *Cem. Concr. Res.* **2005**, *35*, 2175–2182. [CrossRef]
17. Yoon, H.; Park, S.; Lee, K.; Park, J. Oyster shell as substitute for aggregate in mortar. *Waste Manag. Res.* **2004**, *22*, 158–170. [CrossRef]
18. Nakatani, N.; Takamori, H.; Takeda, K.; Sakugawa, H. Transesterification of soybean oil using combusted oyster shell waste as a catalyst. *Bioresour. Technol.* **2009**, *100*, 1510–1513. [CrossRef] [PubMed]
19. Jung, J.H.; Yoo, K.S.; Kim, H.G.; Lee, H.K.; Shon, B.H. Reuse of waste oyster shells as a SO2/NOx removal absorbent. *J. Ind. Eng. Chem.* **2007**, *13*, 512–517.
20. Asaoka, S.; Yamamoto, T.; Kondo, S.; Hayakawa, S. Removal of hydrogen sulfide using crushed oyster shell from pore water to remediate organically enriched coastal marine sediments. *Bioresour. Technol.* **2009**, *100*, 4127–4132. [CrossRef]
21. Choi, Y.M.; Whang, J.H.; Kim, J.M.; Suh, H.J. The effect of oyster shell powder on the extension of the shelf-life of Kimchi. *Food Control.* **2006**, *17*, 695–699. [CrossRef]
22. Yuangsawad, R.; Na-Ranong, D. Recycling oyster shell as adsorbent for phosphate removal. In Proceedings of the 21st Thai Institute of Chemical Engineering and Applied Chemistry, Hat Yai, Thailand, 10–11 November 2011; pp. 1–5.
23. Masukume, M.; Onyango, M.O.; Maree, J.P. Seashell derived adsorbent and its potential for treating acid mine drainage. *Int. J. Miner. Process.* **2014**, *133*, 52–59. [CrossRef]
24. Park, W.H.; Polprasert, C. Roles of oyster shells in an integrated constructed wetland system designed for P removal. *Ecol. Eng.* **2008**, *34*, 50–56. [CrossRef]
25. Bavandpour, F.; Zou, Y.; He, Y.; Saeed, T.; Sun, Y.; Sun, G. Removal of dissolved metals in wetland columns filled with shell grits and plant biomass. *Chem. Eng. J.* **2018**, *331*, 234–241. [CrossRef]
26. Habert, G.; Bouzidi, Y.; Chen, C.; Jullien, A. Development of a depletion indicator for natural resources used in concrete. *Resour. Conserv. Recycl.* **2010**, *54*, 364–376. [CrossRef]
27. Hara, T.; Kawamoto, K.; Fukuda, K.; Nakagawa, I.; Nguyen, T.T.; Soda, S. Trials on neutralization and metal removal for acid mine drainage utilizing lab-scale constructed wetlands packed with limestone and charcoal. *J. Water Waste* **2021**, *63*, 437–443. (In Japanese)
28. APHA/AWWA/WEF. Standard Methods for the Examination of Water and Wastewater. *Standard Methods 541*; APHA: Washington, DC, USA, 2012; ISBN 9780875532356.
29. Ha, N.T.H.; Ha, N.T.; Nga, T.T.H.; Minh, N.N.; Anh, B.T.K.; Hang, N.T.A.; Duc, N.A.; Nhuan, M.T.; Kim, K.W. Uptake of arsenic and heavy metals by native plants growing near Nui Phao multi-metal mine, northern Vietnam. *Appl. Geochem.* **2019**, *108*, 104368. [CrossRef]
30. Nguyen, T.T.; Soda, S.; Kanayama, A.; Hamai, T. Effects of cattails and hydraulic loading on heavy metal removal from closed mine drainage by pilot-scale constructed wetlands. *Water* **2021**, *13*, 1937. [CrossRef]
31. Yeh, T.Y. Removal of metals in constructed wetlands: Review. *J. Hazard. Toxic Radioact. Waste* **2008**, *12*, 96–101. [CrossRef]
32. Allende, K.L.; McCarthy, D.T.; Fletcher, T.D. The influence of media type on removal of arsenic, iron and boron from acidic wastewater in horizontal flow wetland microcosms planted with Phragmites australis. *Chem. Eng. J.* **2014**, *246*, 217–228. [CrossRef]
33. Saeed, T.; Alam, M.K.; Miah, M.J.; Majed, N. Removal of heavy metals in subsurface flow constructed wetlands: Application of effluent recirculation. *Environ. Sustain. Indic.* **2021**, *12*, 100146. [CrossRef]
34. Machemer, S.D.; Wildeman, T.R. Adsorption compared with sulfide precipitation as metal removal processes from acid mine drainage in a constructed wetland. *J. Contam. Hydrol.* **1992**, *9*, 115–131. [CrossRef]
35. White, C.; Gadd, G.M. An internal sedimentation bioreactor for laboratory-scale removal of toxic metals from soil leachates using biogenic sulphide precipitation. *J. Ind. Microbiol. Biotechnol.* **1997**, *18*, 414–421. [CrossRef]
36. Wu, S.; Kuschk, P.; Wiessner, A.; Müller, J.; Saad, R.A.; Dong, R. Sulphur transformations in constructed wetlands for wastewater treatment: A review. *Ecol. Eng.* **2013**, *52*, 278–289. [CrossRef]
37. Nguyen, T.T.; Huang, H.; Nguyen, T.A.H.; Soda, S. Recycling clamshell as substrate in lab-scale constructed wetlands for heavy metal removal from simulated acid mine drainage. *Process Saf. Environ. Prot.* **2022**, *165*, 950–958. [CrossRef]
38. Hasegawa, N.; Onitsuka, T.; Takeyama, S.; Maekawa, K. Oyster Culture in Hokkaido, Japan. *Bull. Fish. Res. Agen.* **2015**, *40*, 173.
39. Ventilla, R.F. Recent developments in the Japanese oyster culture industry. *Adv. Mar. Biol.* **1984**, *21*, 1–57. [CrossRef]