Doctoral Thesis

# Heavy Metal Removal from Mine Drainage by Using Pilot- and Lab-Scale Constructed Wetlands

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Doctoral Program in Advanced Architectural, Environmental

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Heavy Metal Removal from Mine Drainage by Using
Pilot- and Lab-Scale Constructed Wetlands
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廃水からの重金属の除去）

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Nguyen Thi Thuong
June, 2022
Kusatsu, Shiga, Japan

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# CHAPTER 1. GENERAL INTRODUCTION

## 1.1 Background

Mining industry plays an important role in the economic development of many countries over the world (Acheampong and Ansa, 2017; Dorin et al., 2014), however, these mining activities generates massive amounts of wastewater into the environment (Gunatilake, 2015). Mine drainage has been recognized as one of the most serious environmental problems faced by the mining industry (Joshua et al., 2022; Naidu et al., 2019; Skousen et al., 2019). It has generally high heavy metal concentration such as zin (Zn), cadmium (Cd), lead (Pd), copper (Cu), manganese (Mn), iron (Fe), arsenic (As), nickel (Ni), cobalt (Co), chromium (Cr) and an elevated sulfate level (Akcil and Koldas, 2006; Pat-Espadas et al, 2018; Skousen et al., 2019). In order to reduce or eliminate the formation and release of acid mine drainage (AMD), the law and regulations have been released since the 1970s (Environmental Protection Agency, 2017).

In Japan, since 1970, there have been about 7000 abandoned or closed mines due to economic reasons (Ueda and Masuda, 2005), with about 100 of them still being necessary for removing heavy metals from mine drainage (Koide et al.2012). To protect the environment from mine drainages, the Japanese government spends billions of yen on mine drainage treatment in each year (Ueda and Masuda, 2005). To date, several physicochemical technologies, such as neutralization, coagulation, and filtration, have been applied for mine drainage treatment; however, these methods have drawbacks in terms of the high capital, operation, and maintenance costs (Chen et al.,2018; Rodríguez-Galán et al., 2019). As well as this, generating sludge or waste that can be a risk to environmental health is also considered as a disadvantage of these methods (Fiset et al., 2003). According to the reports from Koide et al. (2012) and Otsuka et al. (2014), mine drainage treatment needs to continue to be treated for a long time, and it is predicted that large funds will be needed to address this. Therefore, to reduce the financial burden and achieve a sustainable treatment for the future, it is necessary to develop an alternative technology that consumes less energy, is easy to implement, and that is affordable in rural areas.

Constructed wetlands (CWs) are considered as a potential–green technology for mine drainage treatment. They have gained much attention all over the world, because of their effectiveness, simple operation, low cost, and high biodiversity value (Acheampong et al., 2017; Pat-Espadas et al., 2018; Yu et al., 2022).

The mechanism of heavy metals removal in constructed wetland is quite complex, may include many processes such precipitation, absorption, complexation, microbiological activity, and plant uptake (Matagi et al., 1998; Sheoran and Sheoran., 2006). In general, to achieve a high treatment performance, it is necessary to have effective strategies in the improvement of heavy metal removal. This is mostly aimed at improving the contribution of the three main components (substrate, plant, and microbial) in CWs. Additionally, the seasonal effects on the CW system are highly recommended (Pat-Espadas et al, 2018).

In the case of Japan, several small-scale CWs were applied for treating heavy metals from mine drainage. At Kaminokuni Dam, Hokkaido Prefecture, a CW planted with *Phragmites austlaris* was installed in 2000 for the Mn removal (Sasaki et al., 2003). A small-scale CW was installed in 2006 at Motokura Mine in Hokkaido Prefecture showing the high treatment performance for removing Zn, Pb, and As, which has been scaled up for a demonstration test to replace the existing neutralization process (Hokkaido Research Organization, 2013). However, the practice has not become widespread because the obtained knowledge is not always applicable to other cases. Notably, the chemical composition of mine drainage, soil and vegetation, terrain, and meteorological conditions differ among mines. Thus, systematic knowledge about the design and operation of the CWs should be fully accumulated and systematized for their widespread use. To design and operate the CWs in accordance with each mine wastewater, we also classified 100 mine drainages by multivariate analysis based on the water quality. Results showed that mine drainages can be classified into 8 types as shown in table 1.

Based on the characteristic of each type of mine drainage, a suitable CW would be employed. In our earlier studies, Cd removal from a neutral mine drainage in cluster II was studied using lab-scale CWs filled with loamy soil and planted with reeds or cattails (Soda et al., 2021). Lab-scale CWs filled with limestone or charcoal were also studied for removing Zn, Cu, Pb, and Cd removal from an actual acid mine drainage in cluster VIII (Hara et al., 2021).

In this study, a pilot-scale CW and a series of lab-scale CWs designed with accommodating substrates and plants to evaluate the applicability of CWs to treat mine drainages with various chemical characteristics. This is considered as a valuable tool to deeply understand the function of CWs. Additionally, the knowledge obtained from this research is expected to contribute to the design and operation of CWs at abandoned or closed mines in Japan.

Table 1.1 Classification of 100 mine drainages (No. 1–100) in Japan 2014–2016 based on the cluster analysis

| Cluster | Type | Mine drainages | Cd, mg/L | Pb, mg/L | As, mg/L | Cu, mg/L | Zn, mg/L | Fe, mg/L | Mn, mg/L | pH | Note |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | Neutral and low metal concentration type | 14 (No. 2, 10, 12, 30, 38, 49, 61, 67, 68, 84, 85, 87, 92, 96) | 0.00–0.04 | 0.00–0.24 | 0.00–0.082 | 0.00–4.00 | 0.00–4.20 | 0.00–34.93 | 0.00–0.97 | 5.8–8.0 | Partly JOGMEC type E (Neutral and Cd and Zn predominant type) |
| II | Weakly acidic and low metal concentration type | 24 (No. 6, 11, 13, 14, 15, 16, 22, 27, 34, 40, 43, 52, 62, 63, 70, 71, 72, 73, 80, 83, 86, 88, 99, 100) | 0.00–0.26 | 0.00–0.13 | 0.00–0.04 | 0.00–1.67 | 0.00–4.30 | 0.00–28.32 | 0.00–7.97 | 3.0–5.3 | |
| III | Weakly acidic and high Zn concentration type | 16 (No. 7, 8, 23, 24, 25, 29, 31, 32, 33, 37, 39, 47, 60, 64, 69, 98) | 0.00–0.14 | 0.00–0.21 | 0.00–0.05 | 0.00–6.01 | 7.42–19.67 | 0.00–41.41 | 0.00–8.83 | 2.9–6.7 | |
| IV | Weakly acidic and high Mn and Zn concentration type | 4 (No. 18, 20, 56, 66) | 0.00–0.03 | 0.00–0.10 | 0.00–0.03 | 0.02–1.53 | 2.57–11.46 | 0.00–11.80 | 34.54–75.93 | 4.6–6.3 | JOGMEC type C (Weakly acidic and Mn and base metals predominant type) |
| V | Acidic and high As concentration type | 3 (No. 35, 93, 90) | 0.00–0.01 | 0.00–0.32 | 0.98–1.87 | 0.00–0.84 | 0.00–2.98 | 0.00–59.00 | 0.00–0.00 | 3.1–7.4 | Partly JOGMEC types B (Strongly acidic and Fe and As predominant type) and D (Neutral and As predominant type) |
| VI | Acidic and high Fe concentration type | 9 (No. 4, 19, 21, 45, 74, 75, 76, 78, 89) | 0.01–0.04 | 0.13–0.68 | 0.08–0.34 | 4.55–18.10 | 2.38–7.97 | 88.74–120.50 | 6.06–45.50, | 2.4–5.1 | JOGMEC type A (Acidic and Fe and base metals predominant type) |
| VII | Acidic and extremely high Fe concentration type | 6 (1, 5, 17, 42, 57, 91) | 0.00–0.01 | 0.08–0.26 | 0.27–0.92 | 3.54–10.73 | 0.98–3.32 | 185.94–214.33 | 3.55–10.81 | 2.3–3.9 | Partly JOGMEC type B |
| VIII | Acidic and high Zn concentration type | 7 (No. 36, 50, 55, 58, 59, 65, 82) | 0.09–0.19 | 0.54–1.28 | 0.01–0.07, | 3.87–9.37 | 28.14–43.63 | 52.69–118.03 | 13.63–56.26 | 2.7–5.1 | Partly JOGMEC type A |

## 1.2 Research objectives

### 1.2.1 Broad objective

The overall objective of this study is to develop a green technology- CWs for heavy metal treatment from active or abandoned mine drainages in Japan, and subsequently to the world. In addition, this study proposes an innovative, cost-effective, ecological way for removing heavy metals from mine drainage using CWs. In that way, this study contributes to the widespread application of the CWs in wastewater treatment.

### 1.2.2 Specific objectives

(1) To evaluate the treatment performance of pilot-scale CWs for heavy metal removal from neutral mine drainage of a closed mine in Kyoto prefecture.

- To investigate the heavy metal removal efficiency under the different hydraulic loading rate.
- To evaluate the contribution of vegetation and bacteria in CWs on removing heavy metals from neutral mine drainage.

(2) To evaluate the feasibility of clam shells as a substrate in CWs for removing heavy metals from acid mine drainage (AMD).

- To understand physical and chemical properties of seashells
- To compare the effectiveness of CWs filled with by-products (clamshell, oyster shell) and those of CWs filled with conventional/commercial materials (gravel, limestone) in removing heavy metals from AMD.
- To clarify the heavy metal removal pathway in CWs

(3) To investigate the feasibility of oyster shells based CWs for treating AMD of an anonymous mine in Kyoto Prefecture.

- To compare the neutralizing ability of oyster shell and limestone.
- To evaluate the performance of CWs filled with oyster shells and limestone on heavy metal removal from both real and synthetic AMD.
- To clarify the heavy metal removal pathway in the lab scale CWs

**1.3 Research scope**

This study was implemented in both pilot and lab-scale CWs, under the mine drainage with various chemical characteristics.

- A pilot scale CWs was placed at a neutral mine drainage in Kyoto Prefecture, Japan.
- A series of lab-scale CWs was conducted in a greenhouse at Ritsumeikan University, Kusatsu, Shiga Prefecture, Japan. The wastewater used in the lab-scale CWs included both real and simulated AMD.

The plants used in this study included both typical wetland plants (*Typha orientalis, Typha latifolia , Typha domingensis*) and ornamental flowering plants (*Iris pseudacorus*).

The substrates of the CWs are commercial materials (pumice stone, limestone, gravel, loamy soil), and by-products (clam shells and oyster shells).

**1.4 Research significance**

*1.4.1 Scientific significance*

This study contributes to filling several research gaps regarding the removal of heavy metals from wastewater by using CWs, such as removal mechanisms, roles of the microbial community, accumulation of heavy metals in different parts of plants, etc. Therefore, it provides the solid scientific basis for improving the CWs system in future research.

This study identifies the treatment performance of heavy metals from mine drainage with various chemical characteristics. The effects of hydraulic loading rate and seasons, the roles of each component in CWs on removing metals are given. Additionally, this also demonstrates the applicability of both seashells as substrate in CWs for heavy metal decontamination. Therefore, it can provide essential information for designing and applying the CWs in the reality.

*1.4.2 Environmental and economic significance*

This study helps to meet the requirements of standard effluent regarding heavy metals. Thus, this study contributes to improving environmental quality.

In addition, this study provides an ecological way to reduce environmental burden caused by-products from seafood processing plant.

This study helps to reduce the cost of wastewater treatment, thus increasing the access of local people to the CW technology.

## 1.5 Thesis' outline

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┌─────────────────────────────────────────┐
│       Chapter 1. General introduction    │
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┌─────────────────────────────────────────┐
│       Chapter 2. Literature review       │
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| Chapter 3. Treatment performance of pilot-scale constructed wetlands on heavy metal removal from neutral mine drainage | Chapter 4. Heavy metal removal from simulated acid mine drainage using clamshell-filled and gravel-filled lab-scale constructed wetlands | Chapter 5. Removal of heavy metals from acid mine drainage by lab-scale constructed wetlands filled with oyster shells and limestone |
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│      Chapter 6. Summary and conclusion   │
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Figure 1.1. Research's outline

The research's outline is shown in the Figure 1. This research contains of 6 chapters. The main content of each chapter is presented as follows:

**Chapter 1: General introduction** provides the research background, identifies research objectives, research scope, main tasks, and research significance.

**Chapter 2: Literature review** provides information about mine wastewater, the heavy metal relevant regulations and treatment technologies. The focus is placed on using CWs for heavy metal removal from mine drainages. The heavy metal removal mechanisms in CWs are also described. Additionally, the necessity of recycling by-products as substrate in CWs and selecting plants for heavy metal removal are presented.

**Chapter 3** investigates the treatment performance of pilot-scale CWs with and without cattails on metal removal from actual neutral mine drainage of A-Mine in Kyoto prefecture. The use of CWs for heavy metal removal from mine drainage in Japan is also reviewed. This chapter also emphasis the role of vegetation and bacteria in removing heavy metal in CWs.

**Chapter 4** studies on recycling clamshell as substrate in lab-scale CWs for heavy metal removal from simulated AMD of a mine in Tohoku religion. The recycle of seashell as substrate

in CWs is reviewed. This also compares the use of by-products (clam shell) and conventional material (gravel) as filter media in CWs for metal removal from AMD. Additionally, the metal removal pathway in each CW is also clarified.

**Chapter 5** investigates the applicability of CWs employing oyster shells and limestone on heavy metal removal from AMD of a mine in Kyoto prefecture. Besides this, the AMD neutralizing ability of oyster shells and limestone is examined. This also shows the contribution of each component in removing heavy metal from AMD in CWs.

**Chapter 6** summarizes the main findings of this research, limitations, and recommendation for further studies.

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# CHAPTER 2. LITERATURE REVIEW

## 2.1 Mine wastewater characteristics and regulations

### 2.1.1. Mine wastewater characteristics

Mine drainage refers to any surface water or groundwater that drains from an active or abandoned mining operation. Mine drainage is formed by a chemical reaction between water and rocks containing sulfur-bearing minerals. As a result, waters become rich in sulfuric acid and dissolved iron. When the iron settles out of the water, it can form red, orange, or yellow sediments in the bottom of streams (USGS, 2019). The pH of mine drainage may range from 2 to 8, however, most of it is highly acidic with the pH in the 2.5–4 range, generally called AMD (Skousen et al., 2019). Mine drainage is characterized by very high concentrations of metals such as Fe, Zn, Cu, As, Mn, Cr, Cd, Ni, Co, and sulfate ions (as presented in Table 2.1) (Joshua et al., 2022). The conceptual model of mine drainage pollution is shown in Figure 2.1.

Mine drainage has been recognized as one of the most serious environmental problems faced by the mining industry. In general, the more acidic the water, the more likely it is to be harmful to living organisms (USGS, 2019).
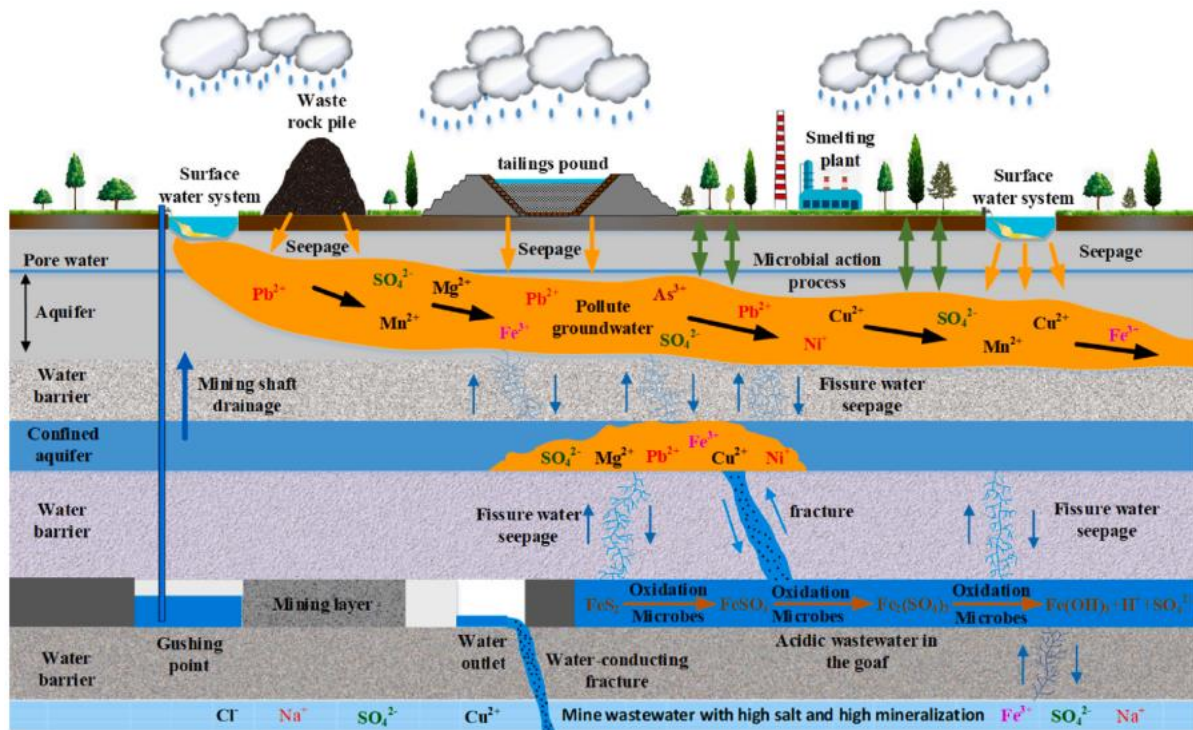


Figure 2.1 Pathway for the generation environmental contamination of mine drainage (Guan et al., 2021)

Many studies have been conducted to examine the ecotoxicology of mine drainage on the environment for the past decades. Generally, its negative impacts on the environment can be divided into four categories: (1) chemical impacts such as increasing metals and acidity in water and soil, (2) physical impacts such as increasing the turbidity of the water, reducing the penetration of light into the water, (3) biological impacts such as affecting living organisms in behavioral, respiratory, reproduction defects, and (4) ecological impacts (such as losing food source/prey, breaking the food chain, and modifying habitat (Chamorro et al., 2018; Joshua et al., 2022). The ecotoxicity of mine drainage was summarized in Figure 2.2
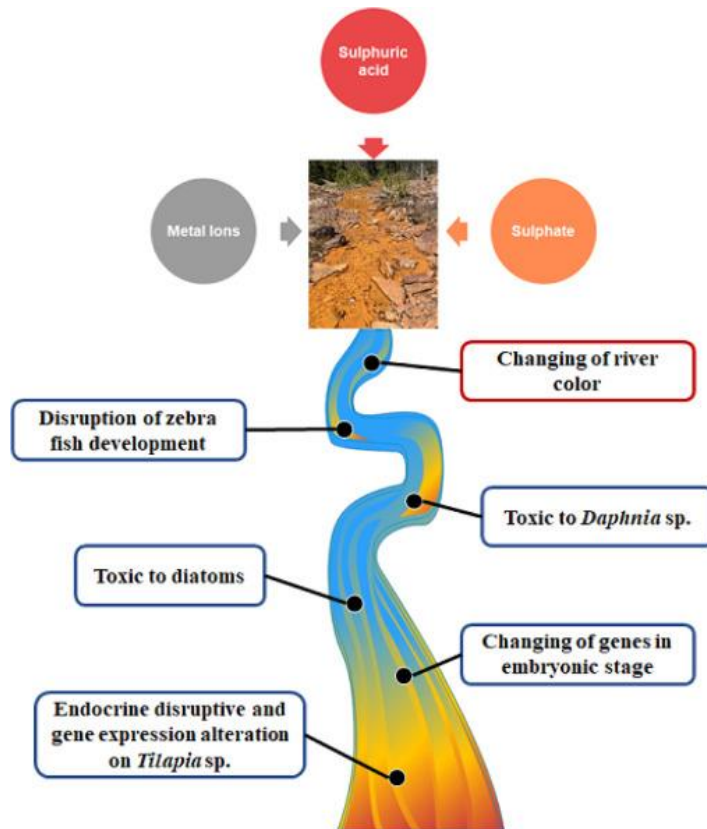


Figure 2.2 The effects of mine drainage on environment (Joshua et al., 2022)

In order to prevent the damages of mine drainage, improve water quality, and enhance ecological sustainability, a proper treatment for mine drainage has become a matter of urgency.

Table 2.1 Characteristic of mine drainage of several countries in the world

| Country | Temp (ºC) | pH | Fe (mg/L) | Zn (mg/L) | Cu (mg/L) | Mn (mg/L) | Pb (mg/L) | Ni (mg/L) | Cd (mg/L) | Co (mg/L) | As (mg/L) | $SO_4^{2-}$ (mg/L) | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Australia | 6 | 6.5 | 16.7 | 48.9 | 77.3 | 161.5 | - | 1.54 | - | - | - | - | Kaur et al., 2018 |
| Brazil | - | 1.3 | 457 | 82 | 95.7 | 2575 | | 102 | 347 | 20 | 14.9 | 18.2 | Amaral et al., 2018 |
| Chile | - | - | 1.1 | 44.7 | 531.2 | 72 | - | - | - | - | - | 4671 | Pino et al., 2018 |
| China | - | 3.2 | 106.9 | 19.56 | 26.2 | - | - | - | - | - | - | 13.7 | Wang et al., 2020 |
| China | - | - | 3150 | 4.9 | | 27.7 | - | - | - | - | - | 6883 | Chen et al., 2015 |
| Finland | - | - | 443 | 410 | 35.3 | 29.3 | - | - | - | - | - | - | Tolonen et al., 2014 |
| Germany | 11.1 | 4.7 | 20.6 | 4.4 | 0.7 | 210 | 0.001 | 21.6 | 0.1 | 4.6 | - | 6719 | Grawunder et al., 2014 |
| Japan | - | 2.1 | 583.5 | 15.4 | 12.6 | - | - | - | - | - | - | 2809 | S et al., 2007 |
| Japan | - | 4.1 | 4.7 | 20.3 | 19.6 | 0.51 | 1.03 | - | 0.27 | - | - | 172 | Hara et al., 2021 |
| Japan | - | 4 | 9 | 11.73 | 0.02 | 66.3 | 0.11 | - | 0.02 | - | - | - | Zhou et al., 2022 |
| Portugal | 14.5 | 2.5 | 2143 | 8.7 | 17.4 | 204 | - | - | - | - | - | 5880 | Valente et al., 2009 |
| South Africa | 25 | 2.4 | 6120 | - | - | 155 | - | - | - | - | - | 28.9 | Muliwaa et al., 2018 |
| South Africa | 3.1 | - | 2135 | 6.9 | - | 30 | - | 3.7 | - | 1.4 | - | - | Kefeni et al., 2018 |
| South Africa | 2.4 | - | 835 | 0.9 | - | 30 | - | 1.8 | - | - | - | - | Kefeni et al., 2018 |
| South Korea | - | 4.5 | 186 | - | - | 13 | - | - | - | - | - | 1950 | Seo et al., 2017 |
| Spain | - | 1–3 | 1141 | 78 | 82 | 115 | 0.001 | 0.2 | 0.4 | 1.4 | 2 | - | López et al., 2021 |
| Spain | 23 | 3.3 | 2040 | - | - | - | - | - | - | - | - | 5990 | Carrero et al., 2015 |
| Sweden | 20.1 | 3.2 | 6.3 | 0.068 | 0.003 | 5.2 | 1.45 | 69 | 0.45 | 20 | | 1278 | Grawunder et al., 2014 |
| USA | - | 2.8 | 38.2 | 16.8 | 149.7 | 8.3 | - | - | - | - | - | 1439 | Ruehl and Hiibel, 2020 |
| USA | 14 | 2.9 | 167 | - | - | 9.8 | - | - | - | - | - | - | Hammarstroma et al., 2003 |
| Vietnam | - | 7.3 | - | 0.8–1.3 | - | 2.6–3.8 | 0.4–0.7 | - | 0.3–0.6 | - | 0.2–0.3 | - | Nguyen et al., 2019 |

"-" Not Available

### 2.1.2. Heavy metal relevant regulations

The high concentration of heavy metals in water bodies can result in negative impacts on ecosystems (Chamorro et al., 2018; Joshua et al., 2022). Thus, to protect humans and organisms from heavy metal pollution, several guidelines and standards have been published/recommended for effluents discharges and potable waters.

Table 2.2 Permissible limits for industrial effluents discharge of several countries

| Metal (mg/L) | India | Bangladesh | Vietnam | Japan | WHO | USEPA | ANZECC | Oman |
|---|---|---|---|---|---|---|---|---|
| Zn | - | 5 | 3 | 2 | - | 2 | 2.4 | 5 |
| Cu | 3 | 0.5 | 2 | 3 | - | 0.2 | 1 | 1 |
| Pb | 0.1 | 0.1 | 0.5 | 0.1 | 0.05–1.5 | 5 | 1 | 0.2 |
| Cd | 0.2 | 0.5 | 0.1 | 0.1 | 0.1 | 0.01 | 0.06 | - |
| As | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | - | - | 0.1 |
| Fe | | 2 | 5 | 10 | 0.2 | 5 | 0.2 | 5 |
| Cr | 2 | 0.5 | 0.1 | - | - | - | - | 0.05 |
| Ni | 3 | 1 | 0.5 | - | 2 | 0.2 | 0.2 | 0.1 |
| Mn | - | 5 | 1 | 10 | 3 | 0.2 | 0.2 | - |
| Reference | Islam, 2017 | Islam, 2017 | TCVN, 2005 | MoEF, 1993 | Islam, 2017 | Islam, 2017 | ANZECC, 2000 | Al-Musharafi et al., 2013 |

"-" Not available

Table 2.3 Permissible limits for potable water several countries

| Metal (mg/L) | India | Bangladesh | Vietnam | Japan | WHO | USEPA | EU |
|---|---|---|---|---|---|---|---|
| Zn | - | - | 3 | 1 | - | - | - |
| Cu | 1.5 | 1 | 1 | 1.3 | 2 | 1.3 | 2 |
| Pb | 0.05 | 0.05 | 0.01 | 0.001 | 0.01 | 0.015 | 0.01 |
| Cd | 0.01 | 0.005 | 0.003 | 0.001 | 0.003 | 0.005 | 0.005 |
| As | 0.01 | 0.05 | 0.01 | 0.001 | 0.01 | 0.01 | 0.01 |
| Fe | - | - | 0.3 | 0.3 | - | - | - |
| Cr | 0.05 | 0.05 | 0.05 | 0.1 | 0.05 | 0.1 | 0.05 |
| Ni | 0.02 | 0.1 | 0.02 | 0.001 | 0.02 | 0.1 | 0.02 |
| Mn | - | - | 0.3 | 0.05 | - | - | - |
| Reference | Islam, 2017 | Islam, 2017 | TCVN 5502: 2003 | MoEF, 1993 | Islam, 2017 | Islam, 2017 | Islam, 2017 |

"-" Not available

In the world, to minimize the pollution burden on surface water as well as to control heavy metal pollution. Many countries have developed and applied national regulation of effluent discharge standards as shown in Table 2.2. Besides this, to avoid the health risks from heavy metals pollution, the potable water is also very strictly limited to very low concentrations of metals as illustrated in Table 2.3. For the same purpose, to reduce the formation and release of AMD, the law and regulations have been released since the 1970s (Environmental Protection Agency, 2017).

As illustrated in Table 2.2 & 2.3, the effluent discharge standards vary in different countries. This can be explained by the variation in the level of treatment technologies and background heavy metal concentrations in the water bodies in different countries.

In Japan, strict regulations related to heavy metals have been released to avoid health risks and negative impacts on the environment. The metal permissible limits for industrial effluents discharge are equal to those of levels that WHO recommended, except for Mn and Fe. Those permissible limits for potable water are even lower than those WHO recommended, except for Cr (Islam, 2017; MoEF, 1993)

Although each country has different standards for metal concentrations in water, the heavy metal levels in water generally should be kept far below the permissible limits for industrial effluents discharge. Therefore, in order to meet these stringent limits and improve water quality, the search for technologies treatment of heavy metals is required (Joshua et al., 2022).

## 2.2. Heavy metal removal technologies from mine drainage

Mine drainage has caused serious environmental pollution and significant economic losses in many countries over the world (Naidu et al., 2019; Wang et al., 2020). Thus, mine drainage treatment has become worldwide environmental concern.

Heavy metals in AMD have been treated by many methods including active treatments and passive treatments.

### 2.2.1. Active treatments

Mine drainages can be neutralized by using chemicals like limestone, soda ash briquettes, calcium carbonate, magna lime, hydrated lime, caustic soda, magnesium hydroxide (Sheoran and

Sheoran., 2006). The increase of pH in the solution to precipitate insoluble metals in the form of hydroxides or carbonates and for the rapid oxidation of ferrous iron is accomplished by providing enough alkalinity in the solution. In the range pH of 6–9, almost metals ions except $Fe^{3+}$, can be precipitated from wastewater. Thus, depending on the specific condition, the appropriate neutralizing agents are employed (Sukla and Alok, 2018). A conventional mine drainage treatment using lime for heavy metal removal is shown in Figure 2.3. Lime is used with the purpose of rising pH in acid mine wastewater, facilitating the precipitation of the metal ions in mine wastewater. Then flocculent is provided to the reactor to promote floc formation and enhance the settling rate of the precipitated metals in the form of larger particles. Then, the treated water is taken out from the flocculent reactor and sludge disposed of (Sukla and Alok, 2018).
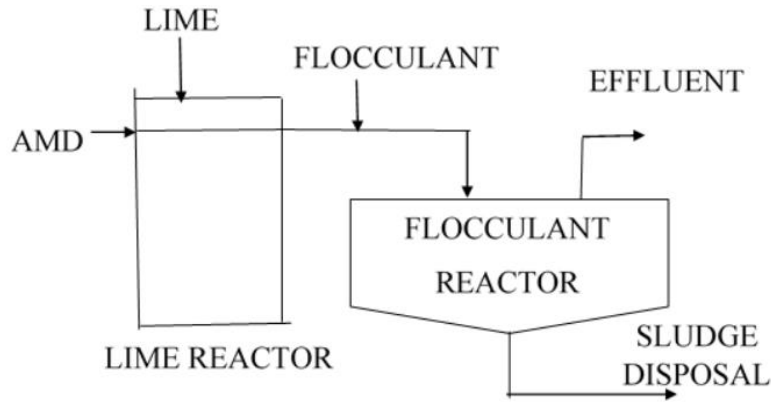


Figure 2.3 Conventional treatment plant for heavy metal removal from mine drainages
(Sukla and Alok, 2018)

Reverse osmosis is employed to treat mine drainages. This method using pressure to force water molecules through a semipermeable membrane. The treated water with high quality can be used potable or industrial use. Zhong et al (2007) reported that Ultra Pressure Reverse Osmosis and Nano filtration removed heavy metal effectively, representing 97% and 90%, respectively. However, this method has some drawbacks such as less water recovery efficiency, high cost, and difficulty maintenance. Furthermore, this method is less effective for mine drainges with high initial metal concentrations (Sukla and Alok, 2018).

Ion exchange is also applied for AMD treatment. In this method one or more undesirable ionic contaminants are removed from water by exchange with another non-objectionable, or less objectionable ionic substance. A gold wastewater in South Africa was treated by ion exchange

after precipitation process (Feng et al., 2000). Although this method can recover water well, the fee for the pretreatment is quite expensive.

Electrodialysis is also used for heavy metal removal from mine drainage. This is a membrane separation process based on the selective migration of aqueous ions through an ion-exchange membrane due to an electrical driving force. Electrodialysis has proven to be an efficient method for mine wastewater treatment. Nevertheless, the Fe ions in mine drainage can easily form the precipitates attached to the surface of cation exchange membranes. Consequently, it needs an increase of membrane voltage in a continuous manner. Thus, the Fe removal is considered as a pre-treatment for the electrodialysis process (Buzzi et al., 2013). This process requires a high cost.

In general, almost all active treatments are cost-ineffective and need further treatments of by-products such as sludge. Therefore, in an attempt to reduce the cost of treatment and reach sustainable treatment. Research efforts has been directed towards passive treatments, such as CWs.


### 2.2.2. Passive treatments

Passive treatment systems have been developed that do not require the ongoing addition of chemical reagents and that take advantage of naturally occurring chemical and biological processes to clean contaminated mine wastewater.

Constructed wetlands are currently the most widely-used passive mine wastewater treatment technology (Pat-Espadas et al, 2018). Pollutants are removed in CWs through the natural processes involving wetland vegetation, substrate, and microbial community. The mechanisms for heavy metal removal in CWs is described in the section 2.3 below. This method can be used the long-term remediation of mine drainages. However, to achieve an effective treatment, the consideration of the design criteria, conformational arrangements, and careful selection of each component is very necessary (Pat-Espadas et al, 2018; Skousen et al., 2018).

Limestone drains, is a passive treatment system for AMD, uses limestone gravel as a bed. The limestone drains generate alkalinity when the wastewater passes through the limestone bed. These drains are either the anoxic limestone drains or open limestone channels. The Fe and other dissolved metal ions can be precipitated/ co-precipitated when the pH increases in solution. An anoxic limestone drain was evaluated as an effective method for AMD treatment; however, this has a great potential for clogging (Watzlaf et al., 2000). The effectiveness of limestone drains has also been reviewed in the report of Skousen et al. (2017).

## 2.3. Constructed wetland for heavy metal removal from mine drainage

CW has been known as an effective passive technology for wastewater treatment. It has gained much attention from scientists all over the world, because of their effectiveness, simple operation, low cost, and high biodiversity value (Acheampong et al., 2017; Pat-Espadas et al., 2018; Yu et al., 2022). The use of CWs for mine drainage treatment has been early used in the USA from the early 90s, and spread rapidly in North America, Europe, and Australia (Johnson and Hallberg, 2005; Pat-Espadas et al, 2018; Rai, 2008).

### 2.3.1. *Heavy metal removal mechanism in CWs*

Heavy metals are mainly removed from mine drainage by CWs through three main components: (1) Substrates (mineral and/or organic) contribute to the removal mainly by adsorption and precipitation processes, (2) vegetation (emerging, floating, and submerged) contribute mainly by direct uptake mechanisms, and (3) microorganisms (bacteria and archaea) contribute by promoting reduction and subsequent precipitation of metals (Pat-Espadas et al, 2018; Sheoran and Sheoran., 2006). The whole process of metal removal in CWs is quite complex because of the supporting and interdepending of the components. In general, heavy metal removal in CWs can be explained by three main processes as follows: Physical, chemical, and biological processes.

**Physical removal processes:**

Settling and sedimentation have been recognized as the important processes in removing heavy metals from wastewater. Sedimentation is not a simple straightforward physical reaction. It generally occurs after other processes such complexation, precipitation, and co-precipitation. Sedimentation is a physical process after other mechanisms aggregate heavy metals into particles large enough to sink (Matagi and Mugabe, 1998). In this way, heavy metal can be removed from wastewater and retained in substrate or sediments. Many researchers have confirmed the effectiveness of the filtration of metal suspended on filter media from AMD in CWs (Council, 2003; Hares and Ward, 2004). Sinicrope et al., 1992 and Noller et al., 1994 also reported that Cd (75–99.7%), Pb (26%) Ag (75.9%), and Zn (66.7%) were removed through filtration process.

**Chemical removal process:**

<u>Sorption</u>

17

The most important chemical removal process in wetland soils is sorption, which results in short-term retention or long-term immobilization of several classes of contaminants. Sorption is the transfer of ions from water to the soil. Sorption actually describes a group of processes, which includes adsorption and precipitation reactions (Sheoran and Sheoran., 2006).

Adsorption

In sediments, heavy metals are adsorbed onto soil particles by cation exchange or chemisorption. Unlike organic pollutants which eventually decompose, after being absorbed on to humic colloids or clay, heavy metals will remain in the form of metal atoms. Their speciation may change over time as sediment conditions change (Sheoran and Sheoran., 2006).

The capacity of soils for retention of cations depends on many factors, including the properties of the metals involved (valence, radius, degree of hydration, and coordination with oxygen), the physicochemical environment (pH and redox state), the nature of the adsorbent medium (permanently and pH-dependently complexing ligands), the concentration and nature of the adsorbents, and other metals and soluble ligands are present (Matagi and Mugabe, 1998). It was reported that Cu and Pb generally tends to be adsorbed strongly than other metals like Zn, Mn, Ni and Cd (Sheoran and Sheoran, 2006). The pH value, contact time, and Fe concentration affected strongly to the adsorption capacity of Sb (Brix, 1993). According to Muller, 1988, more than 50% of heavy metals can be easily adsorbed onto particulate matter, and then removed from wastewater by sedimentation.

Wetland plants translocate oxygen from the shoots to the root rhizomes through their internal gas space aerenchyma. The roots and rhizomes in turn leak the oxygen to the reduced environment. It is these oxidised conditions that promote precipitation of oxyhydroxides of $Fe^{3+}$ and $Mn^{2-}$. The precipitated hydroxides also act as absorption sites for other phytotoxic heavy metals present in the water compartment of the wetland (Matagi and Mugabe, 1998).

Oxidation and hydrolysis of metals

In CWs, Fe, Al, Zn and Mn can form insoluble compounds through hydrolysis and/or oxidation processes resulting in formation of variety of oxides, oxyhydroxides and hydroxides (Hara et al., 2021; Sheoran and Sheoran., 2006).

The pH affected strongly to the removal of Al (Hedin et al., 1994). At pH 5, Al can be precipitated in hydroxides forms. Council (2003) reported that a CW Kentucky removed 33% Al from AMD through oxidation and hydrolysis processes.

The Mn removal is quite difficult, because its oxidation occurs around the pH value of 8. Mn removal can be promoted by bacteria by accelerating oxidation of $Mn^{2+}$ to $Mn^{4+}$ (Stumm and Morgan, 2012).

The Fe removal depends on pH, ORP and the presence of other anions (Council, 2003). Since pH rising to 3.5, $Fe^{3+}$ can be removed from wastewater. According to the report of Stark et al. (1994), a SIMCO wetland almost completely removed Fe after 8 years of operation.

Precipitation and co-precipitation

Precipitation is one of the principal mechanisms for removing heavy metals in wetland sediments. The bioavailability of heavy metals in aquatic ecosystems can be limited by the formation of metal precipitates. The metal precipitation depends on many factors such as the pH, solubility product Ksp, initial metal concentration and other anions. Precipitation occurs when the concentrations of cations and anions exceeding $K_{sp}$ values. Precipitation from a saturated solution of a sparingly soluble heavy metal salt may be represented by the dynamic equilibrium (Matagi and Mugabe, 1998). $MX^2_{(s)} \rightleftharpoons M^{2+}_{(aq)} + X_{(aq)}$, where the constant is $K_{sp} = [M^{2+}] [X^-]^2$.

Co-precipitation is also an important adsorptive mechanism in wetland sediments. Heavy metals can be co-precipitated with secondary minerals. Cu, Ni, Zn, and Mn are co-precipitated in Fe oxides, and Zn, Co, Ni, and Fe are co-precipitated in Mn oxides (Matagi and Mugabe, 1998). Otte et al. (1995) also reported that Zn and As were retained on Fe plaques at the surface of plant roots. Soluble Mn was also removed successful from AMD through oxidation of Mn(II) and precipitation of the resultant Mn(IV) (Hallberg and Johnson, 2005). While 83% soluble Cu was also removed by a CW in the report of Murray-Gulde et al. (2005).

Metal carbonates

The high concentration of bicarbonate in water can lead to the form of metal carbonates. Although carbonates are less stable than sulfides, they can still play an important role in the initial trapping of metal. There are very few cases of carbonate retention in CWs (Sobolewski, 1996). Carbonation formation can take place when bicarbonate alkalinity-producing bacteria in wetland sediments are significant or when limestone is present in the runoff of an acid mine drainage system (Council, 2003). Lin (1995) reported that Pb and Ni were removed effectively by carbonate precipitates. While 79% Mn and 25% Ni removed in form of metal carbonates was reported by Schiffer (1989). $M^{2+} + CO_3^{2-} \rightarrow MCO_3\downarrow$ , where M represents metal.

Metal sulfide

In CWs, sulfide can be generated from sulfate through SRB activities. Then, metals react with sulfide ions leading to the formation of highly insoluble metal sulfides as following equation (Sheoran and Sheoran., 2006).

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3$$

$$M^{2+} + H_2S + 2HCO_3 \rightarrow MS\downarrow + 2H_2O + 2CO_3$$
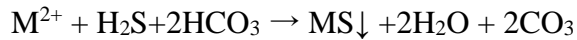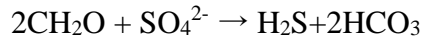
where $CH_2O$ and M represents for organic matter and metal, respectively.

According to Schiffer (1989), 83.3% Pb from wastewater was removed by precipitation as the insoluble sulfide. Similarly, the removal rate of Zn, Cu, and Pb to be 85%, 33%, and 79%, respectively, found in the report of Hawkins et al. (1997). While 93–99% of Fe and Zn were removed in the report of Collins et al. (2004). Many studies demonstrated that at the beginning of CWs, the adsorption of dissolved metals onto organic sites in the substrate material is an important process, however, sulfide precipitation will be a dominant process for heavy metal removal over time (Machemer and Wildeman, 1992). To enhance the heavy metal removal from wastewater in CWs by sulfide precipitation process, several researchers have made efforts to promote SRB activities by adding carbon sources (Chen et al., 2021a; Chen et al., 2021b).

**Biological removal processes**

Biological removal is also considered as an important pathway for heavy metal removal in wetlands (Sheoran and Sheoran, 2006).

Plant uptake is probably recognized as the main biological process for metal removal in CWs. The main heavy metal removal route in CWs is through roots in the case of emergent and surface-floating plants, and through leaves and roots in the case of either completely submerged leaves or both floating and submerged leaves or free-floating plants. The route of heavy metal removal by plant uptake is shown in Figure 2.4.

Microorganisms also contribute to heavy metal removal through their metabolic processes (Hallberg and Johnson, 2005; Russell et al., 2003). The removal of Cr, Cu, and Se was reported to be 40–84%, 36–88%, and more than 95%, respectively, by the reduction to non-mobile form through bacterial activities (Adriano, 2001; Nelson et al., 2002). Based on the processes biologically catalyzed by microorganisms, the Cr and U were also reduced to non-mobile forms, then, removed from the water (Fude et al., 1994). The sulfate reduction by SRB also facilitates the

increase of pH, which promote the precipitation in the metal hydroxides/sulfides (Sheoran and Sheoran, 2006).

## 2.4. Recycling materials as substrate in CWs for heavy metal removal

Among three main components in CWs, substrate is considered as an indispensable component in CWs, because it plays a very important role in supporting plant growth, intercepting pollutants, and providing reactive substances for pollutant transformation (Vohla et al., 2011). The surface microenvironment of the materials (such as elemental composition, specific surface area, pore-volume) can greatly affect the establishment of biofilm and microbial community structure, which has an important impact on the treatment performance and sustainability of CWs (Deng et al., 2021). In general, in order to optimize the treatment efficiency of CWs, the physicochemical properties of substrates should be carefully selected. The conventional materials such as gravel are often used in CWs, however, they have limitation in metal adsorption capacity, and low AMD neutralization potential. Thus, the finding alternative materials is very important (Wang et al., 2022). In addition, to achieve sustainable mine drainage treatment, and reduce the economic burden, the recycling of environmentally friendly materials with low/no cost in abundant available is very necessary.

Many researchers have investigated various materials as adsorbents for heavy metal treatment from mine drainage as shown in Table 2.4.

Table 2.4 Several by-products used as adsorbents for heavy metal removal from AMD

| Adsorbent | Experiment | pH | Removal efficiency (%) | Reference |
|---|---|---|---|---|
| Saw dust | Batch | 5.01 | Cu (80%), Fe (10%) | Stanković et al., 2009 |
| Compost manure | Batch | 4 | Cu (77.2%), Zn (47.5%), Pb (93%) | Zhang, 2011 |
| Shrimp shell | Batch | 3.04 | Fe (96%), Mn (78%) | Núñez-Gómez et al., 2020 |
| Mussel byssus | Batch | 3.04 | Fe (96%), Mn (78%) | Núñez-Gómez et al., 2021 |
| Seashells | Batch | 5.5-7.3 | Mn (54.4%), Al (100%), Fe (100%) | Masukume et al., 2014 |

| Adsorbent | Experiment | pH | Removal efficiency (%) | Reference |
|---|---|---|---|---|
| Rice husk | Batch | 4 | Fe (99%), Zn (98%), Cu (99%) | Chockalingam and Subramanian, 2006 |
| *Eucalyptus tereticornis* bark | Batch | 3.2 | Fe (96%), Zn (75%), Cu (92%) | Chockalingam and Subramanian, 2009 |
| Modified fly ash | Batch | 5 | Fe (99%), Zn (97%), Mn (92%), Co (95%), Cu (92%), Ni (94%) | Ileri and Sanliyuksel Yucel, 2020 |
| Modified fly ash | Batch | 2.8 | Fe (96%), Zn (92%), Mn (60%), Ni (89%), Pb (94%) | Sahoo et al., 2013 |
| Fly ash | Batch | 10.5–11.0 | Mg (100%), Cu (100%), Pb (100%) | Gitari et al., 2006 |
| Iron slags | Batch | 5.5 | Cu (88%), Pb (95%) | Feng et al., 2004 |
| Steel slag | Batch | 5.5 | Cu (16%), Pb (32%) | Feng et al., 2004 |

It is recognized that many materials have been recycled as adsorbents for removing heavy metals, however, there have not many studies investigating recycling materials as substrates in CWs. To the best of author's knowledge, only Bavandpour et al. (2018) reported the high applicability of oyster and mussel shells as a substrate in CWs for removing Cu, Fe, Mn, Pb, and Zn in synthetic AMD. Therefore, studies recycling by-products as filter media in CWs for mine drainage treatment are necessary.

## 2.5. Selection plants for wastewater treatment in constructed wetlands

The vegetation is one of the most conspicuous features of wetlands. It plays an important role in producing roots and rhizomes as substrates for attached bacteria. Additionally, the roots and rhizomes also oxygenate the areas adjacent to the roots and adsorb pollutants from the wastewater. (Sandoval et al., 2019).

The plants in wetlands can uptake metals through the mechanisms of phytostabilisation, phytovolatilization, phytoextraction, and rhizofiltration as shown in Figure 2.4.
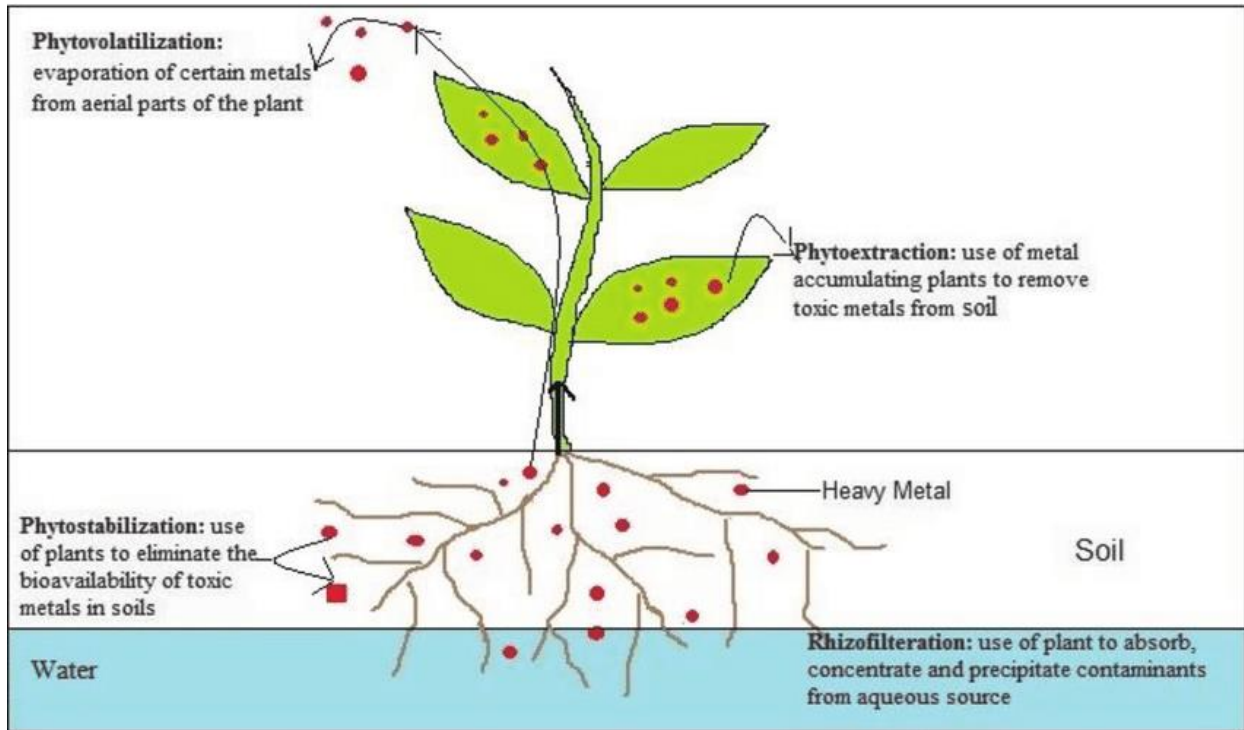


Figure 2.4 The mechanisms of heavy metals uptake by plant (Kushwaha et al., 2015)

The uptake of elements is affected by the characteristics of plants. Thus, to have a high uptake capacity, the plants should be screened to select those species having superior remediation properties (Hamad, 2020; Tangahu et al., 2011). Several plants have commonly been used in CWs for wastewater treatment such as cattails, common reeds, and Cyperus (Hamad, 2020). In USA and Europe, *T. latifolia* and *P. australis* are also used popularly in CWs (Scholz and Lee, 2005). Several studies have also been conducted to find the potential plants in CWs for heavy metal treatment. In the batch experiments, Khalid and Ganjo (2021) released that *Nasturtium officinale* can remove Zn through phytoextraction better than other plants *(Veronica anagallis-aquatica, Mentha longifolia, and Cyperus iria)*. Schück and Greger. (2020) concluded that *Carex pseudocyperus* and *C. riparia* are the most potential plants used in CWs for heavy metal removal from wastewater after screening 34 plant species.

In addition to the pollutant treatment, it is recognized that wetland plants probably play an important role in providing social and economic benefits such as creating landscapes and reducing

environmental stress and disease. Thus, ornamental flowering plants have been gaining much attention from many researchers in the world (Sandoval et al., 2019). Ornamental flowering plants have been used in CWs for treating various kind of wastewaters such as domestic, nursery, fishpond, municipal, storm and ground water (Sandoval et al., 2019). Several species like *Canna sp, Iris sibirica, Polianthus tuberosa L.* have been employed in CWs for heavy metal removal from wastewater. Gao et al. 2015 reported that 92% of Cd was removed from synthetic polluted river water by CWs planted with *Canna sp*. A wetland planted *Iris sibirica* removed 95% As from sewage wastewater. Singh and Srivastava (2016) also reported that metals from domestic wastewater were removed effectively by a CW planted *Polianthus tuberosa L.* The removal efficiency was 73–87% for Pb and Fe, 31–34% for Cu and Zn, and 20–26% for Al and Ni.

However, to the best of authors knowledge, there are very few studies on CWs using ornamental flowering plants in removing heavy metals from AMD. Moreover, the role of plants in CWs for the decontamination heavy metals from AMD is also exists many conflicting results. Some scientists totally agreed that the presence of plants can certainly promote heavy metal removal (Di Luca et al., 2016; Fritioff and Greger, 2006; Klink et al., 2013), whereas others argued that its removal efficiency was not enhanced or insignificant with the presence of plants (Lee and Scholz, 2007; Mays and Edwards, 2001).

Therefore, further studies should investigate using ornamental flowering plants in CWs for heavy metal removal from AMD. Additionally, comparative studies using CWs with and without plants, and evaluating the metal concentration in different parts of the plants are highly considered a valuable tool to deeply understand the role of plants in heavy metal elimination from AMD.

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# CHAPTER 3. TREATMENT PERFORMANCE OF PILOT-SCALE CONSTRUCTED WETLANDS ON HEAVY METAL REMOVAL FROM NEUTRAL MINE DRAINAGE

**Abstract**

*This chapter presents the heavy metal removal from neutral mine drainage of a closed mine in Kyoto prefecture in pilot-scale CWs planted with and without cattail. Two CW systems (length 63 cm, width 63 cm, height 98 cm, 300 L) were operated from September to December 2019, divided into four phases with the flowrate and hydraulic retention time (HRT) were 72 L/d and 3.8 days, 115 L/d and 2.4 days, 158 L/d and 1.7 days, 216 L/d and 1.2 days, respectively. After 3.5 months of operation, $13m^3$ mine wastewater was treated in each CW. The short HRT of 1.2 days in the CWs was sufficient to achieve the effluent standard for Cd (0.03 mg/L). The unplanted and the planted CWs reduced the average concentration of Cd from 0.031 to 0.01 and 0.005 mg/L, Cu from 0.09 to 0.04 and 0.03 mg/l, Zn from 0.52 to 0.14 and 0.08 mg/L, Fe from 0.06 to 0.03 and 0.02 mg/L, and As from 0.011 to 0.006 and 0.006 mg/L, respectively. Heavy metals were removed mainly by adsorption to the soil in both CWs. The biological concentration factors in cattails were over 2 for Cd, Zn, and Cu. The translocation factors of cattails for all metals were 0.5–0.81. Sulfate-reducing bacteria (SRB) belonging to Deltaproteobacteria were detected only from soil in the planted-CW, and its population increased in the warm weather. Although cattails were a minor sink, the plants contributed to metal removal by rhizofiltration and incubation of SRB, possibly producing sulfide precipitates in the rhizosphere. These results encourage the spreading of CWs for mine drainage treatment in Japan.*

A major part of Chapter 3 was published in the following paper:

## 3.1. Introduction

As introduced in chapter 1&2, the mining industry is the major producer of mine drainage containing toxic metals, which seriously threats to the ecosystem and human health. Thus, it is very important to remove heavy metal from mine wastewater. Several active treatments processes such as neutralization, coagulation, reverse osmosis, and ion exchanges have been used for removing heavy metals from mine drainage, however, these methods are mostly expensive in terms of capital and operation costs (Rodríguez-Galán et al., 2019).

In the case of Japan, more than 7000 mines have been abandoned or closed since the 1970s because of increasing labor costs and import liberalization of mineral resources (Ueda and Masuda, 2005). Among them, approximately 100 mines with absent owners must treat mine drainage continuously despite a lack of production, which has imposed large economic burdens on national and local governments for over a hundred years (Ueda and Masuda, 2005). A promising alternative as a passive technology for mine drainage treatment is CWs because of their simple operation, low cost, and moderate effectiveness (Acheampong and Ansa, 2017; Pat-Espadas et al., 2018; Sobolewski, 1999).

Mechanisms of heavy metal removal in CWs are quite complicated, involving various processes such as precipitation, absorption, filtration, complexation, microbiological activity, and plant uptake (Pat-Espadas et al., 2018; Sobolewski, 1999). Wetland plant species show considerable variations in metal uptake and translocation abilities though phytoextraction (Ai et al., 2013; Chen et al., 2014; Liu et al., 2010). Many contradictory results have been found regarding the use of plants in CWs. Mitsch and Wise. (1998) and Sobolewski. (1999) reported that plant uptake only plays a minor role in the overall remediation of metals. In contrast, other studies have found the opposite (Fritioff and Greger, 2006, Vardanyan and Ingole, 2006). Additionally, sulfate-reducing bacteria (SRB) in wetland soil decomposed organic matter to lower molecular weight acids and bicarbonate leading to increased alkalinity and formation of metal sulfide precipitates (Eger, 1994; Sasaki et al., 2009). The bacteria–plant interaction is regarded as important because it implies symbiotic mechanisms for heavy metal removal and tolerance.

CWs are considered as the most currently the most widely-used passive mine water treatment technology (Pat-Espadas et al., 2018). A few small-scale CWs have been applied for mine drainage treatment (Sasaki et al., 2003&2009; Kato et al., 2000). In a dozen of the 80 mines, drainage includes only a few toxic metals, slightly exceeding the effluent standard with neutral pH (5.8–

8.6) (Ueda and Masuda, 2005). Installation of the CW is expected to be prioritized for treating such neutral mine drainage (NMD). Our research group has studied lab-scale CWs for treating NMD of such a typical mine in Kyoto Prefecture (A-Mine) (Soda et al., 2021). Synthetic wastewater with cadmium (Cd) at 0.11 mg/L was de-signed to simulate the NMD of A-Mine. The CWs were filled with loamy soil and were planted with common reeds or cattails. The synthetic NMD was treated in a 1-week cycle sequencing batch mode in the CWs. Unplanted CWs removed Cd sufficiently to satisfy the effluent standard (0.03 mg/L) from the NMD, mainly by soil adsorption. The presence of emergent plants, especially cattails, enhanced metal removal, presumably by filtration with their elongated roots and by metal sulfide precipitation by SRB in the rhizosphere.

Based on the results obtained from the lab-scale CW (Soda et al., 2021), the present study investigated the treatment performance of pilot-scale CWs with and without cattails on metal removal from actual NMD of A-Mine. The NMD was treated in a continuous flow mode at the hydraulic retention time (HRT) of 3.8–1.2 days in autumn and winter. The accumulation of heavy metals in soil and the plant biomass was measured. Bacteria including SRB were also studied in both water and soil samples of the CWs.

## 3.2. Materials and methods

### 3.2.1. Pilot-scale CW setup and operation

The present study was conducted at a mine drainage treatment plant of A-Mine from September to December 2019. This mine had been developed since the 1910s, mainly for tungsten production, by a private company. It was finally closed in the 1980s. The plant is now managed by the local government to treat mine drainage of about 200 $m^3$/d through coagulation, sedimentation, and pH adjustment. In recent years, the pH values and the Cd and Zn concentrations of this mine drainage have been, respectively, 6.67–7.32 mg/L, 0.020–0.11 mg/L, and 0.63–2.00 mg/L (Soda et al., 2021). The effluent standard in Japan is 5.8–8.6 for pH, 0.03 mg/L for Cd, and 2.0 mg/L for Zn. A schematic of the pilot-scale CWs is illustrated in Figure 3.1. Two polyethylene tanks (63 cm length, 63 cm width, 98 cm height, 300 L) were packed from bottom to top with pumice stone (13.5 cm depth, bulk 54 L, Hyuga soil; Hyugatsuchi Hanbai Co., Miyazai, Japan), limestone (8 cm depth, 4 kg; Konan Shoji Co., Ltd., Tokyo, Japan), middle loamy soil (akadama)

(17 cm depth, bulk 70 L, 45.5 kg; Sowa Recycle Corp., Tokyo, Japan), fine loamy soil (akadama) (17 cm depth, bulk 70 L, 45.5 kg; Hirota Shokai, Co., Japan), and limestone (2 cm depth, 1 kg; Konan Shoji Co., Ltd.). The middle and fine loamy soils had cation-exchange capacity of 12.2 and 6.09 meq/100 g, respectively. The water level was set at 75 cm with an overflow pipe connected at 10 cm depth to the CW. A weather monitoring station (SenSu-1502; Data Technology Inc., Tokyo, Japan) was placed at the unplanted CW to record daily solar radiation, precipitation, and air temperature.
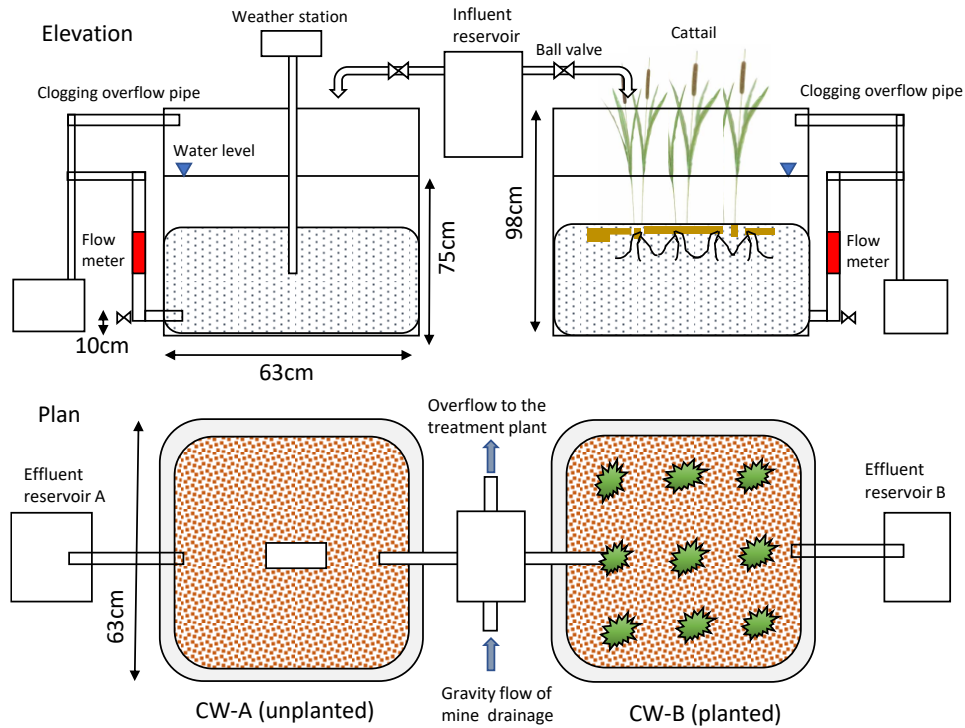


Figure 3. 1 Schematic of pilot-scale CWs: CW-A was unplanted, and CW-B was planted with cattails. Water samples were collected from the influent and effluent reservoir tanks.

Two CWs were produced in parallel: one was left unplanted (CW-A); the other was planted with cattails (CW-B). On 18 September 2019 (day 0), 9 young plants of cattail (*Typha latifolia L.*) were planted to the CW-B (75±10 cm shoots, 13±3 cm roots, 180±88 g-wet/plant). Because a typhoon damaged the cattails, four plants of Southern cattail (*Typha domingensis*) were added to the CW-B on 5 October (day 17) (41±1 cm shoots, 12±1 cm roots, 194±28 g-wet/plant). These emergent plants were purchased from Tojaku Engei Co. Ltd. (Kyoto, Japan). Before planting to the CWs, the roots were washed gently using river water to remove the original culture soil.

The water level in the influent reservoir tank was kept constant by gravity inflow and overflow of the NMD. The NMD was fed continuously through polyvinyl chloride pipes with ball valves to the CWs at hydraulic loading of 72 L/d for 31 days (phase I), 115 L/d for 21 days (phase II), 158 L/d for 1 days (phase III), then 216 L/d for 32 days (phase IV). If there was no evapotranspiration, then these conditions caused HRT of 3.8, 2.4, 1.7, and 1.2 days. The NMD penetrated downward into the soil layers in the CW and overflowed up-ward through the pipe. The effluent rate was recorded using a digital flowmeter (OF05-ZAWN; Aichi Tokei Denki Co., Ltd., Aichi, Japan).

### 3.2.2. Sampling and sample preparation

Influent and effluent samples of the CWs (each 100 mL) were collected from the reservoir tanks twice a week (Figure 3.1). The samples were transported to the laboratory at Ritsumeikan University for measuring heavy metal contents and other water parameters. After 79 days of operation, three water samples (influent, effluent of CW-A and CW-B) and two soil samples at about 14 cm depth (CW-A and CW-B) were collected for bacterial analyses.

Soil and plant samples were collected for analyzing the heavy metal content before and after CW operation for 3.5 months. The original soil and plant samples had not been polluted by any heavy metal. Samples of the loamy soil were collected at depths of about 3 cm (upper), 8 cm (middle), and 14 cm (lower) from the soil surface and were dried using an oven at 102°C to constant weight. They were then crushed to less than 1 mm. Plant samples were washed well with tap water to remove soil and stone. After the washed samples were separated into two parts, shoots and roots, they were dried in the oven at 80°C to constant weight. Subsequently, they were ground into powder and stored for additional analysis.

### 3.2.3. Analytical methods

The pH, dissolved oxygen (DO), oxygen reduction potential (ORP) and total dissolved solids (TDS) of water samples were measured respectively on site using a pH meter (F-21; Horiba Ltd., Tokyo, Japan), an ORP meter (ORP57; Milwaukee Instruments Inc., NC, USA), a DO meter (Ultrapen PT5; Myron L Co., CA, USA), and a TDS meter (ASTDS1; AS One Corp., Osaka, Japan). Heavy metal (Cd, Fe, Cu, Zn) concentrations were measured using inductively coupled plasma spectroscopy (ICP-OES 700 series; Agilent Technologies Japan, Ltd., Tokyo, Japan).

Suspended solids (SS), sulfate, T-N and T-P were measured according to a standard procedure (APHA, 2012). Average values were analyzed statistically using one-way analysis of variance (ANOVA) followed by post hoc Student–Newman–Keuls testing.

Soil samples were digested using a wet digestion method. A portion of 0.5 g of each dried sample was digested with 6 mL of solution of HCl/HNO$_3$ (3:1, v/v) and 1.5 mL H$_2$O$_2$. Then the mixture was heated at 180°C for 2 h using a digestive furnace. For plant samples, 0.1 mg of each dried sample was digested by 3 mL of HNO$_3$ and 0.6 mL of H$_2$O$_2$ using a furnace at 145°C for 2 h. After digestion, all soil and plant samples were cooled. Then the supernatants were filtered through 0.45 µm filter paper (Advantec Co. Ltd., Tokyo, Japan). Filtrates were used for metal content determination using ICP analysis.

Heterotrophic bacteria and SRB were enumerated using the plate-count technique with R2A agar medium (Merck KGaA, Germany) and Postgate's medium F: 10.0 g tryptone, 3.5 g sodium lactate, 0.5 g sodium sulfite, 2.0 g magnesium sulfate heptahydrate, 0.5 g iron (II) sulfate heptahydrate, 0.5 g ammonium ferric citrate, 15 g agar, and 1 L pure water, 7.1 pH.

The middle soil samples were suspended in 5 mg/L sterile sodium tripolyphosphate solution (tpp) (5 g-wet in 50 mL), mixed vigorously with a vortex mixer for 2–3 min, and treated with a sonicator (UD211: Tomy Seiko Co. Ltd., Tokyo, Japan) to disperse soil particles. The mixture was diluted with tpp and was used for pourplating. Agar plates for heterotrophs were incubated aerobically for 1 week at 28°C. Agar plates for SRB were incubated anaerobically for 3 weeks at 28°C in chambers containing Anero Pack Kenki (Mitsubishi Gas Chemical Co. Inc., Tokyo, Japan).

Water samples and middle soil samples were subjected to DNA extraction and Illumina Miseq 16S rRNA gene sequencing at Bioengineering Lab. Co. Ltd. (Kanagawa, Japan). Amplicon sequencing and data processing work were conducted there.

### 3.2.4. Calculating heavy metal removal and accumulation

The heavy metal removal efficiency (%) was calculated as following equation:

$$\text{Removal efficiency } (\%) = \frac{(Q_iC_i - Q_eC_e)}{Q_iC_i} \times 100\% \tag{Eq 3.1}$$

where, Qi (L/d) and Qe (L/d) respectively denote the inflow rate and the outflow rate. Ci (mg/L) and Ce (mg/L) respectively stand for the metal concentration in influent and effluent.

The amount of metal removed in the CWs, M (mg), was calculated using rectan-gular integration as:

$$M = \sum(Q_i C_i - Q_e C_e)\Delta t \qquad\qquad \text{(Eq 3.2)}$$

where, where $\Delta$t (days) represents the time interval of the measurement.

The amount of metal accumulated in the plant biomass $M_p$ (mg) was determined using the following equation:

$$M_p = C_{pf}X_f - C_{pi}X_i \qquad\qquad \text{(Eq 3.3)}$$

Therein, $C_p$ (mg/kg-dry) and $X$ (kg-dry) respectively represent the average metal contents in the plant biomass and the total plant biomass (shoots and roots). Subscripts i and f respectively signify initial and final conditions

In addition, the bioconcentration factor (BCF) and the translocation factor (TF), which are demonstrated the phytoaccumulation and translocation capability of cattail, respectively, were also calculated by the following equation (Eq.4) and (Eq.5):

$$\text{BCF} = \frac{C_p}{C_s} \qquad\qquad \text{(Eq 3.4)}$$

where, $C_p$ (mg/kg) and $C_s$ (mg/kg), stand for the heavy mental content in the whole plant and the soil sample in the upper layer, respectively.

$$\text{TF} = \frac{C_a}{C_r} \qquad\qquad \text{(Eq 3.5)}$$

in which, $C_a$ (mg/kg) is the heavy metal content in aboveground biomass, and $C_r$ (mg/kg) is the heavy metal content in the roots of cattail.


## 3.3. Results

### *3.3.1. Operational conditions of the CWs*

Figure 3.2 presents temperature, daily solar radiation, and precipitation at A-Mine during the experimental period. Phase I (days 0–29) started in September 2019 with the influent rate of 72L/d (HRT 3.8 days). Under warm conditions with maximum daily temperature of 20–35°C and solar radiation of 50–620 kLux·h/day, the effluent rate of the CWs sometimes decreased to 60% of the inflow rate because of evapotranspiration. The typhoon No. 18 (Mitaga) brought 14 L of rainwater to each CW on third and fourth October, resulting in temporal increases of the effluent rates on days 17 and 18. Because cattails were partly damaged by the typhoon, they were additionally planted on day 17, as described in the section 3.2.1. After the typhoon passed, the effluent rate of

the CWs decreased on days 20 and 21 because of temporary clogging by heavy rains. Typhoon No. 19 (Hagbis) again brought heavy rains of 42 L to each CW on 12 October 2019, resulting in increases of the effluent rate on days 24 and 25. Nevertheless, no marked damage was sustained by cattails in the CW-B.

For phase II (days 29–51), the inflow rate was set to 115 L/d to each CW (HRT 2.4 days). The effluent rate of the CWs was almost equal to the inflow rate under the moderate temperature and solar radiation of < 390 kLux·h /day. Temporary clogging occurred in the CW-A on days 49 and 50. The minimum temperature was sometimes below 10°C. Some of the cattail leaves in the CW-B turned brown.
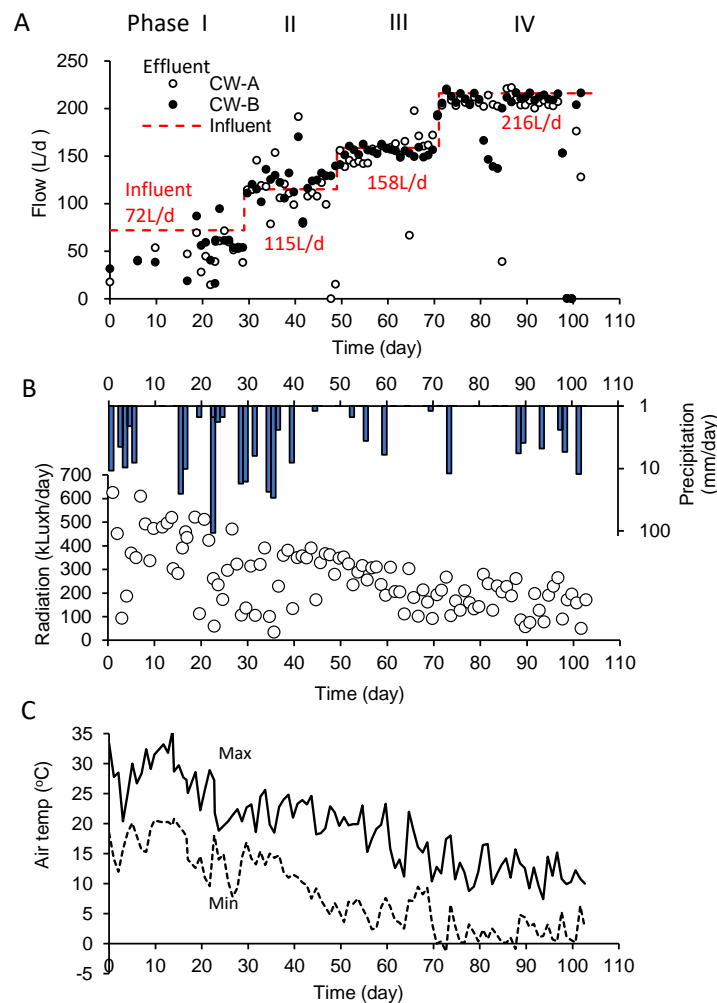


Figure 3.2 Experiment conditions for treating NMD by pilot-scale CWs at A-Mine. Flow rates of the CWs: (A) solar radiation and precipitation (B) and air temperature (C). Eighteenth September 2019 was defined as day 0.

In phase III (days 52–71), the inflow rate was set to 158 L/d (HRT 1.7 days). No clogging occurred in the CWs, except for day 66.

In phase IV (days 72–108), the inflow rate was finally set to 216 L/d (HRT 1.2 days). The daily minimum temperature was sometimes below 0°C. The solar radiation was below 300 kLux·h/day. The cattail shoots were almost withered. Although temporal clogging occurred a few times in both CWs, the effluent rate of the CWs was close to the inflow rate. Through the experiments, the pH values in effluent (6.3–7.2) were slightly higher than those in influent (6.2–6.9), probably because of the influence of limestone in the CWs, although no significant difference was found between the CWs (p < 0.05). The ORP values were positive at +50 – +150 mV in both influent and effluent. The DO concentrations in effluent of the CW-B tended to be lower than those in influent and effluent of the CW-A, suggesting oxygen consumption by root and microbial respiration. The TDS concentrations in effluent were lower than those in influent in phase I, indicating high removal of dissolved matters in the CWs. However, the TDS concentrations in effluent were almost identical with those in effluent in phases II–IV. Influent and effluent sulfate concentrations fluctuated at 40–120 mg/L through the experimental phases. The T-N and T-P concentrations were, respectively, <1.0 mg/L and <0.13 mg/L in both influent and effluent.

### 3.3.2. Heavy metal removal

The time courses of Cd, Zn, Cu, Fe, Mn, and As concentrations in the CWs are shown in Figure 3.3. Metal concentrations in the NMD were always lower than the Japan effluent standard, except for Cd. Both CWs well removed Cd, Zn, Cu, Fe, Mn, and As during the experimental period (p < 0.01).

The average influent Cd concentrations in phases III and IV were higher than those in phases I and II. The Cd concentration decreased to a level below 0.01 mg/L in both CWs, especially in the CW-B to below 0.005 mg/L. Although no significant difference was found between the CWs in phases I and II, effluent Cd concentrations of the CW-B were lower than those of the CW-A in phases III and IV. Shortening of HRT apparently had no marked influence on the effluent Cd concentrations.

Both CWs well removed Zn from the NMD with the stable influent concentration at 0.52±0.1 mg/L (Figure 3.3B). Although no significant difference was found, the effluent Zn concentrations of the CW-B tended to be lower than those of the CW-A, slightly higher than Japan's

environmental quality standard for Zn (0.03 mg/L) for the protection of aquatic life. Shortening of the HRT had no significant influence on effluent concentrations.

The average influent Cu concentrations were lower than 0.08 mg/L in phases I–III but increased to 0.11 mg/L in phase IV. The effluent Cu concentrations were also ranked as phases I and II < III < IV ($p < 0.05$). However, no significant difference was found in effluent concentrations between the CWs.
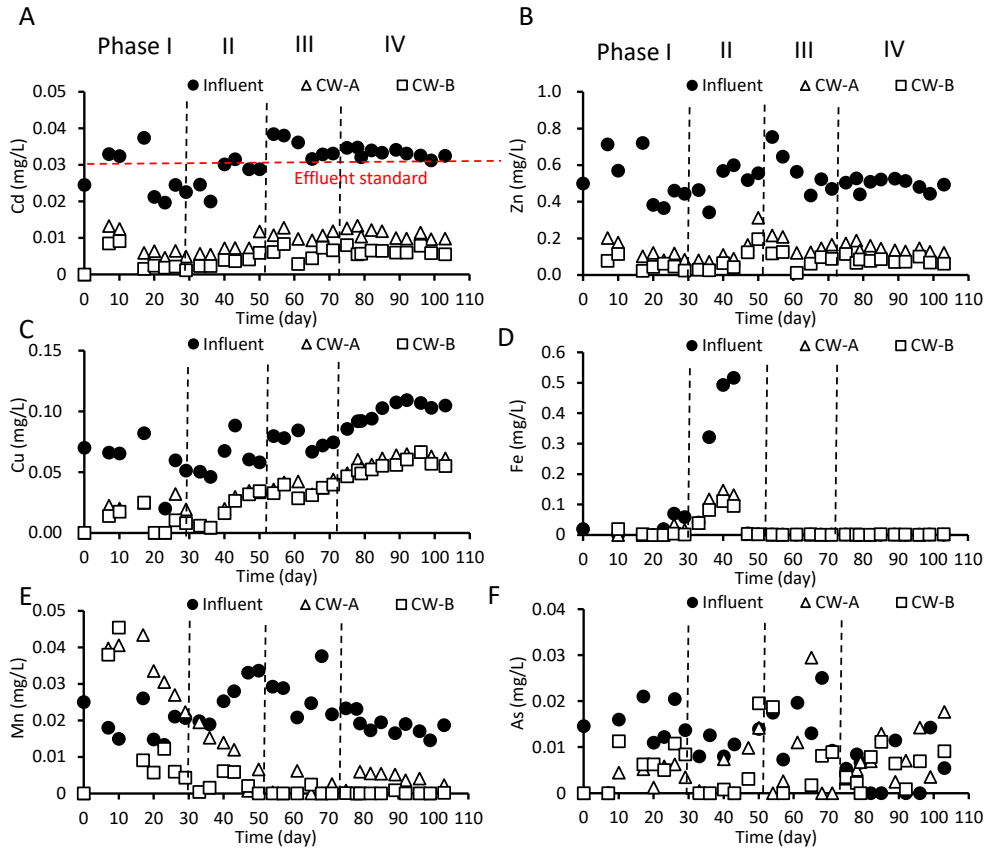


Figure 3.3 Time courses of metal concentrations in influent and effluent in unplanted CW (CW-A) and cattail-planted CW (CW-B) treating NMD: Cd (A), Zn (B), Cu (C), Fe (D), Mn (E), and As (F).

The influent and effluent Fe concentrations were less than 0.05 mg/L and 0.004 mg/L, respectively, in phases I, III, and IV, but were sometimes over 0.3 mg/L and 0.14 mg/L, respectively, in phase II.

The influent Mn concentration fluctuated at 0.01–0.04 mg/L. In phase I, the Mn concentrations in effluent of the CW-A were higher than that in influent, indicating release of Mn from the soil. In addition, the effluent Mn concentrations in the CW-B were sometimes higher than

the influent concentrations. Then, both CWs removed Mn well from the NMD in phases II–IV (p < 0.05).

The influent As concentration fluctuated at 0.01–0.04 mg/L, although the effluent As concentrations of both CWs showed no significant difference from one another. The average effluent As concentrations of both CWs were lower than the environmental quality standard for As (0.01 mg/L).

Table 3.1 Mass removal (%) for heavy metals from NMD by the CWs.

| Element | | Phase I (n=7) | Phase II (n=6) | Phase III (n=6) | Phase IV (n=10) | Total |
|---|---|---|---|---|---|---|
| Cd | CW-A | 81.1±8.2 | 77.3±5.5 | 72.4±10.9 | 69.4±6.3 | 74.3±8.9 |
| | CW-B | 91.2±5.2 | 87.5±3.6 | 81.8±5.9 | 82.9±3.7 | 84.5±5.8 |
| Zn | CW-A | 83.1±8.8 | 80.1±8.9 | 74.0±9.7 | 73.1±6.3 | 76.9±8.9 |
| | CW-B | 92.1±4.3 | 87.8±9.1 | 81.3±12.1 | 85.9±4.0 | 86.6±8.2 |
| Cu | CW-A | 80.0±18.1 | 74.9±22.4 | 54.9±18.1 | 45.6±8.7 | 61.2±21.3 |
| | CW-B | 90.2±6.9 | 75.9±22.7 | 50.5±11.9 | 50.2±8.3 | 64.3±21.2 |
| Fe | CW-A | 77.2±23.6 | 52.4± 43.0 | 22.7±56.8 | -10.0±36.6 | 22.1±51.6 |
| | CW-B | 97.3±3.1 | 52.0±36.5 | 7.4±48.5 | -64.0±99.1 | -1.4±89.2 |
| Mn | CW-A | -24.7±54.2 | 47.3±45.2 | 93.4±11.2 | 86.3±12.9 | 54.5±57.6 |
| | CW-B | 37.4±57.3 | 87.8±9.9 | 98.6±3.7 | 99.3±1.7 | 82.2±37.4 |
| As | CW-A | 83.0±6.8 | 75.4±37.3 | 51.7±85.7 | 32.6±64.4 | 60.6±57.9 |
| | CW-B | 60.8±17.0 | 95.9±5.7 | 9.6±81.7 | 18.5±62.6 | 43.3±60.9 |

The average metal removals in each phase of the CWs are presented in Table 3.1. Removals for all metals except for Mn in both CWs decreased with the increase in the influent rate. Results show that CW-B exhibited higher removals for Cd and Zn than CW-A throughout the experiments. The Fe removals in both CWs were negative values at the high influence rate in phase IV, indicating solubilization of the accumulated Fe in the CWs through phases I–III. By contrast, removals for Mn especially in the CW-A were low at the low influent rate in phase I.

The metal amounts removed in the CW-A and the CW-B were, respectively, $3.44 \times 10^2$ mg and $3.97 \times 10^2$ mg for Cd, $5.91 \times 10^3$ mg and $6.68 \times 10^3$ mg for Zn, $7.41 \times 10^2$ mg and $7.77 \times 10^2$ mg for Cu, $3.90 \times 10^2$ mg and $4.23 \times 10^2$ mg for Fe, $2.25 \times 10^2$ mg $2.88 \times 10^2$ mg for Mn, and 75.8 mg and 48.5 mg for As through 3.5 months of operation.

### 3.3.3. Accumulation of heavy metals in soil

The distribution of heavy metals in the loamy soil layer in the CWs is presented in Figure 3.4. Metal contents in soil increased in both CWs during the 3.5 months operation. The CW-B accumulated more metals in soil than the CW-A, possibly because of rhizofiltration. The metals were accumulated mainly in the upper soil layer, except for Fe and Mn. These findings indicate that soil adsorption Cd, Zn, Cu, and As were removed effectively. The uniform distribution of Mn and Fe in soil indicates that those were precipitated towards the bottom.
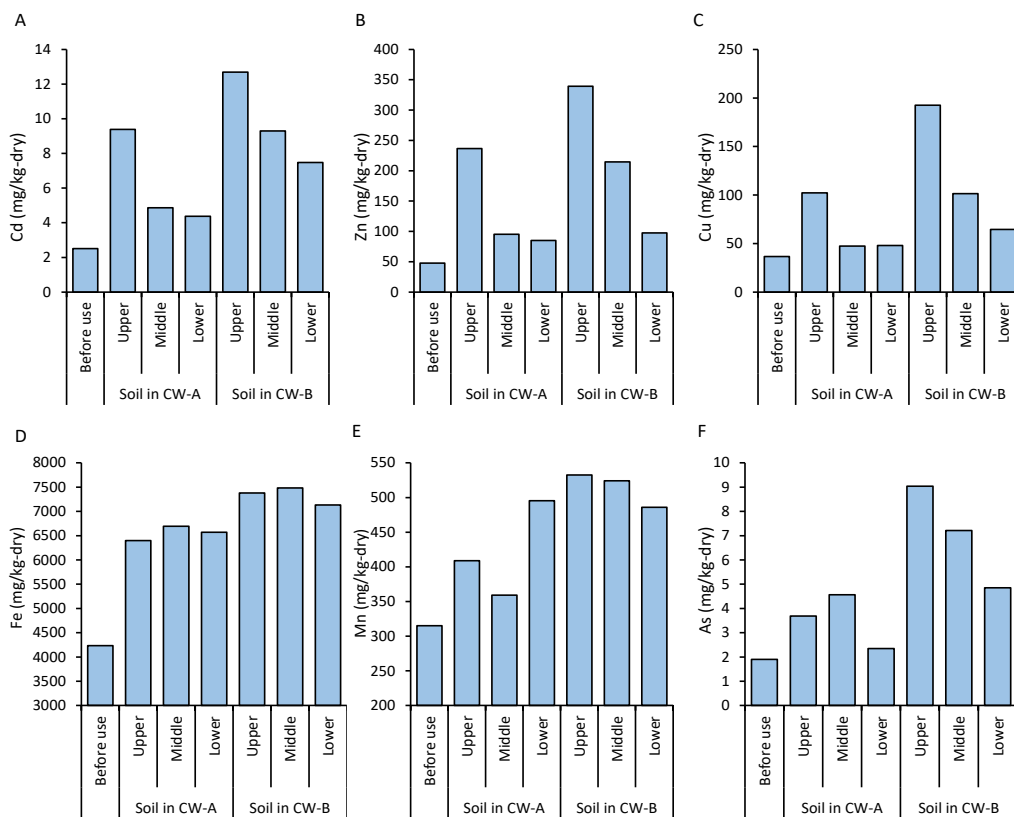


Figure 3.4 Metal contents in loamy soil in the unplanted CW (CW-A) and the cattail-planted CW (CW-B) before and after the NMD treatment experiment.

### 3.3.4. Accumulation of heavy metals in plant biomass

Cattails in the CW-B were harvested at the end of phase IV. Although the shoots of the plant withered, the roots extended well to the lower soil layers. The final plant biomass was 90.8 g-dry of shoots and 173.9 g-dry roots, 264.7 g-dry in total. The metal contents in the plant biomass are shown in Figure 3.5. Both shoots and roots of the plant in the CW-B accumulated metals at high

contents within a typical range (Klink et al., 2013). The contents of Cu, Zn, Fe, and Mn, as essential elements, exhibited higher concentrations than Cd in the plant biomass. The amounts of metals in the plant biomass were 7.0 mg for Cd, 189.5 mg for Zn, 163.8 mg for Cu, 935.9 mg for Fe, 49.4 mg for Mn, and 0.7 mg for As. The ratio of those to the amounts removed in the CW-B were 1.8% for Cd, 2.8% for Zn, 21.3% for Cu, 220% for Fe, 49.9% for Mn, and 0.7% for As. This contradictory value for Fe (220%), which is easily oxidized and precipitated, might be attributable to the underestimation of the influent concentration and biomass contents before use. The remaining amounts of the removed metals in the CWs are expected to be accumulated in the soil.
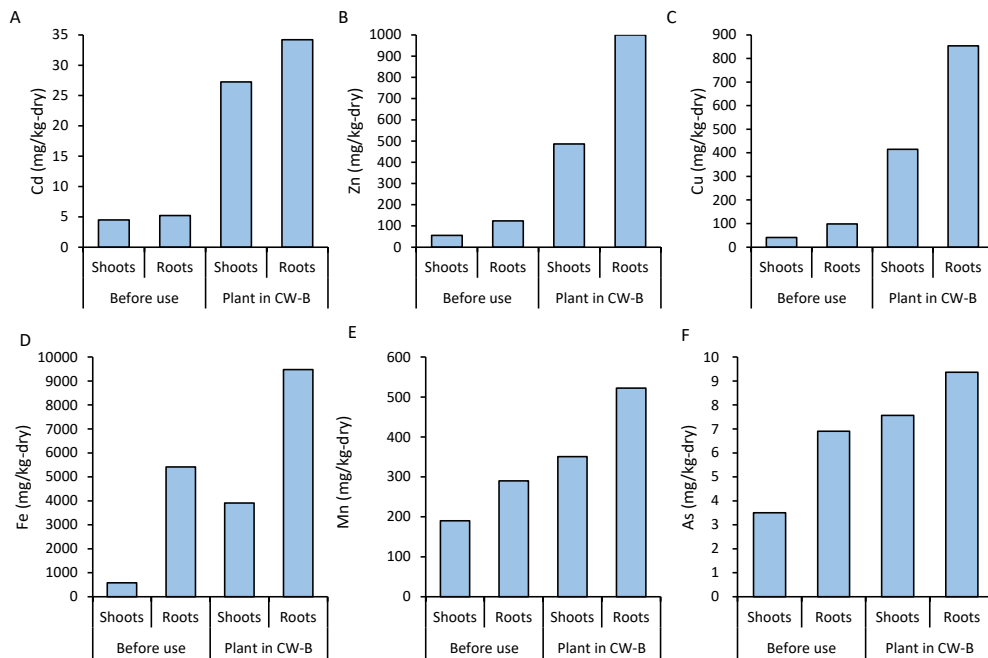


Figure 3.5  Metal contents in the cattail biomass in the planted CW (CW-B) before and after the NMD treatment experiment.

Table 3.2 Bioconcentration (BCF) and translation (TF) factors of cattail in the CW-B after NMD treatment.

|  | BCF | TF | |
|---|---|---|---|
|  |  | CW-B | Before use |
| Cd | 2.29 | 0.80 | 0.86 |
| Zn | 3.03 | 0.49 | 0.44 |
| Cu | 4.69 | 0.49 | 0.41 |
| Fe | 0.85 | 0.41 | 0.11 |
| Mn | 0.67 | 0.67 | 0.66 |
| As | 0.87 | 0.81 | 0.51 |

The BCF and TF values for the metals in the CWs are displayed in Table 3.2. The BCF values for Cd, Zn, and Cu were higher than 2, indicating high uptake to the plant biomass from the soil. However, the BCF values for Mn and Fe, as easily oxidizable metals and As as an adsorptive element to the insoluble metal oxides, were below 1. The TF values were 0.5–0.81 in a typical range (Soda et al., 2012), indicating that a certain amount of metals were translocated to the shoots from the roots.

### 3.3.5 Bacterial communities

Heterotrophs counted on the R2A medium were $10^3$–$10^5$ CFU/mL in influent and effluent of the CWs. Heterotrophs in the effluent of the CW-B were slightly higher than those of the CW-A. SRB were not quantitative but were detected only from the soil sample of the CW-B by the F medium.

Water and soil samples in the CWs on day 79 were served for bacterial 16S rRNA gene sequencing. The obtained numbers of operational taxonomic units (OTUs) for the samples were 16,500–25,800. Figure 3.6 shows the bacterial composition based on the 16SrRNA gene sequences in the water and soil samples. The influent sample showed the highest diversity index on the phylum level ($H'$) among the water samples. The soil sample of the CW-B showed higher diversity index than that of the CW-A. Uniquely, the phylum OD1 and the phylum OP3 often found in groundwater (Hiller et al., 2015) were predominant only in the influent (NMD) and effluent samples. At the phylum level, Proteobacteria and Bacteroidetes respectively accounted for 30–50% and 5–20% in all samples. Among the phylum Proteobacteria, the classes Alphaproteobacteria and Betaproteobacteria were predominant in all samples. Deltaproteobacteria including typical SRB also accounted for high ratios. Cyanobacteria, which obtain energy via photosynthesis, accounted for 4–12% in all samples, except for the soil sample of the CW-A. However, soil samples were characterized by Actinobacteria, which are Gram-positive bacteria and Firmicutes, most of which have a gram-positive cell wall structure. The phylum Thermi accounted for 15% of the soil sample of the CW-A. The phylum Chloroflexi accounted for 12% of the soil sample of the CW-B.
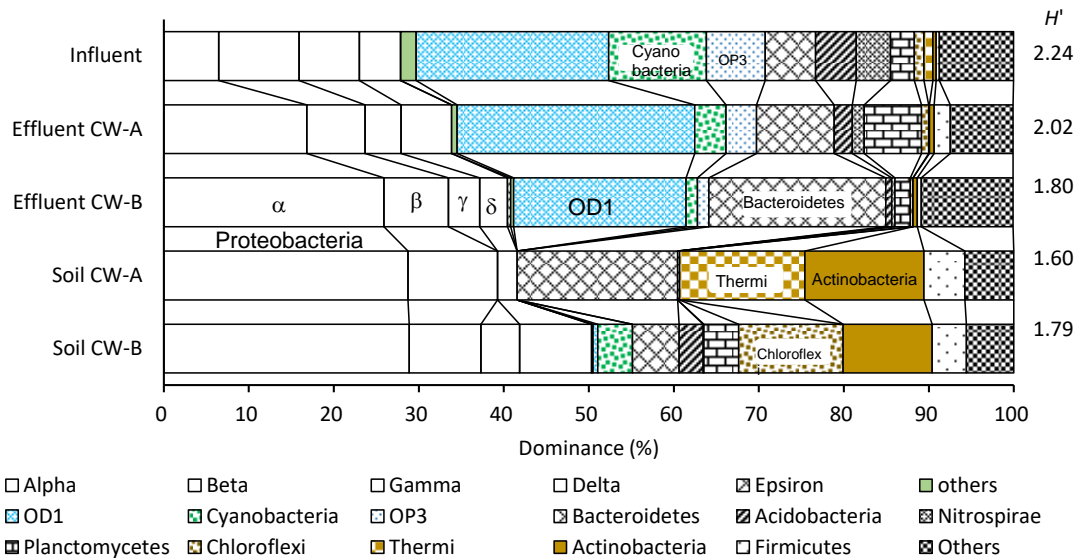
Figure 3.6 Bacteria community compositions at the phylum level in water and soil samples of the unplanted CW (CW-A) and the cattail-planted CW (CW-B) treating the NMD.

Table 3.3 shows the number of OTUs of typical SRB possibly producing metal sulfide precipitation in the CWs. Five families of SRB were found only in the CW-B soil sample: Desulfovibrionaceae (Castro et al., 2000), Syntrophaceae (Desulfomonile (Inaba et al., 2018), Desulfarculaceae (Kuever, 2014), Desulfobacteraceae and Desulfobulbaceae (Peng et al., 2018), in the class Deltaproteobacteria. Bacteria of the family Thermodesulfovibrionaceae (Medeiros et al., 2015) in the phylum Nitrospirae were found in all samples except for the CW-A soil sample. The ratio of those to the total OTUs was highest in the soil sample from the CW-B (0.93%).

Table 3.3 Typical SRB (%) detected in water and soil samples of CWs treating NMD.

| | OTU_No | Influent (NMD) | Effluent CW-A | Effluent CW-B | Soil CW-A | Soil CW-B |
|---|---|---|---|---|---|---|
| Desulfovibrionaceae | OTU754 | 0 | 0 | 0 | 0 | 0.19 |
| *Desulfovibrio mexicanus* | OTU_683, OTU 1685 | 0 | 0 | 0 | 0 | 0.29 |
| Syntrophaceae *Desulfomonile* | OTU1970 | 0 | 0 | 0 | 0 | 0.05 |
| Desulfarculaceae | OTU2672 | 0 | 0 | 0 | 0 | 0.03 |
| Desulfobacteraceae | OTU1430 | 0 | 0 | 0 | 0 | 0.09 |
| Desulfobulbaceae *Desulfobulbus* | OTU1330 | 0 | 0 | 0 | 0 | 0.1 |
| Desulfobulbaceae | OTU3199, OTU2681, OTU1812, OTU1826 | 0 | 0 | 0 | 0 | 0.17 |

| | OTU_No | Influent (NMD) | Effluent CW-A | Effluent CW-B | Soil CW-A | Soil CW-B |
|---|---|---|---|---|---|---|
| Thermodesulfovibrionaceae | OTU3078, OTU2773, OTU401, OTU 2368, OTU 2901, OTU 3191 | 0.24 | 0.14 | 0.05 | 0 | 0.01 |
| Total (%) | | 0.24 | 0.14 | 0.05 | 0 | 0.93 |

## 3.4. Discussion

The present study demonstrated high removals for Cd and other metals in the pilot-scale CWs, especially in that planted with cattails (CW-B), from the NMD of A-Mine. The HRT of CWs for mine drainage treatment is typically a few days to several weeks (Acheampong and Ansa, 2017; Sobolewski, 1999). However, in this study, HRT of 1.25 days was sufficient for Cd removal from the NMD without severe clogging. Further studies on evaluating the soil adsorption capacity of heavy metals, plant-bacteria contributions in warm seasons, and mathematical modeling will be needed for the scaling up of the CWs. At a dozen mines in Japan, the drainage includes only a few toxic metals, slightly exceeding the effluent standard in Japan with neutral pH values (5.8–8.6) like A-Mine (Ueda and Masuda, 2005). Installation of the CWs should be assigned priority for treating such NMD. This study using pilot-scale CWs was conducted only in autumn and winter because of limitations of site use at A-Mine. Feasibility studies for high seasons for plant growth and evapotranspiration in spring and summer will be necessary for additional design of the CW.

Even in the unplanted CW (CW-A), Cd, Zn, and Cu were well removed from the NMD, resulting in its accumulation, especially in the upper soil layer (Figure 3.4). Therefore, the main mechanism for metal removal in the CWs should be soil adsorption, as demonstrated by our lab-scale study (Soda et al., 2021). Loamy soils (akadama) have generally low organic carbon content (<0.1%) but high permeability, water retention, and metal sorption properties governed by its specific chemical constituents, especially of CaO, by synergistic effects of physicochemical sorption and ion exchange mechanisms (Bhakta and Munekage, 2012).

The amounts of metals extracted by the plants were small compared to the total amounts removed by the CWs, as reported earlier (Pat-Espadas et al., 2018; Soda et al., 2021). The phytoextraction efficiency is related to biomass, BCF, and TF (Table 3.2). A specific hyperaccumulator can concentrate more than 100 ppm Cd, 1000 ppm Co, Cr, Cu, or Pb, or 10,000 ppm Zn or Ni (Reeves, 2003). The cattails used for this study are non-hyperaccumulating but fast

growing indigenous plants. Cattails generally wither in winter. Therefore, the shoots should be harvested at the end of autumn for collecting the accumulated metals. The heavy metals in the harvested biomass can be disposed to an industrial waste landfill site as the sludge produced in the coagulation process of A-Mine. Young shoots of perennial plants can germinate from the remaining rhizomes in spring.

Furthermore, cattails contributed to rhizofiltration by their elongated roots and bacterial metabolisms in the rhizosphere (Table 3.3, Figure 3.6). Many researchers have reported heavy metal retention in planted CWs as significantly higher than in unplanted CWs (Choi et al., 2006; Sasaki et al., 2009). A soil layer that has accumulated heavy metals up to its adsorption capacity should be dredged for renewal of the metal-removing capability of the CW. Rhizofiltration by the plant is helpful for decreasing the dredging soil depth for CW renewal.

Diverse bacteria including SRB were detected from the CW-B soil sample (Table 3.3). This result suggests that the plant controlled rhizosphere bacteria suitable for metal removal. The plant rhizospheres generally become aerobic if oxygen is secreted to form the root sufficiently. However, oxygen consumption by root respiration becomes remarkable during nighttime or when the shoots withered. Under such conditions, an anaerobic environment can be formed in the rhizosphere in which sulfate is reduced by SRB (Chen et al., 2014). Although the chemical forms of the metals accumulated in the CWs were not determined in this study, precipitation of insoluble sulfides such as CdS, ZnS, and CuS can enhance metal removal in the CW (Pat-Espadas et al., 2018; Sobolewski, 1999; Eger, 1994). Metal removal by plant uptake, rhizofiltration, and bacterial sulfide formation will be enhanced in warm seasons.

### 3.5. Conclusion

Heavy metals were removed effectively from the NMD by the pilot-scale CWs at a closed mine in Kyoto prefecture in autumn and winter. Removals for Cd, Zn, Cu, Fe, and As in the CWs tended to decrease with HRT from 3.8 days to 1.2 days. Results show that HRT of 1.25 days was sufficient to achieve the effluent standard for Cd without severe clogging. The main mechanism for metal removal in the CWs was adsorption by loamy soil filled in the CWs. Additionally, cattails contributed to metal removal by rhizofiltration and phytoextraction and incubation of SRB, possibly producing sulfide precipitates in the rhizosphere. These results encourage the spreading

of CWs for mine drainage treatment in Japan. Further studies in warm seasons are needed for scaling up of CWs.

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# CHAPTER 4. HEAVY METAL REMOVAL FROM SIMULATED ACID MINE DRAINAGE USING CLAMSHELL-FILLED AND GRAVEL-FILLED LAB-SCALE CONSTRUCTED WETLANDS

**Abstract**

*This chapter describes the removal of heavy metals from synthesized acid mine drainage (AMD) using lab-scale constructed wetlands (CWs) in sequencing batch mode. Lab-scale column-type CWs (ID 12.5 cm, H 50 cm) filled with clamshells or gravel and with loamy soil were planted or not planted with cattails (Typha orientalis). Synthesized AMD containing 20 mg/L Zn, 0.3 mg/L Cd, 20 mg/L Cu, 1.1 mg/L Pb, 0.6 mg/L Mn, and other minerals (pH=4.1) was fed to CWs (1 L/column) with hydraulic retention times (HRT) of 7 days in Phase I, 4 days in Phase II, 2 days in Phase III, and 7 days in Phase IV. Results indicate that pH became neutral in all effluents, and that heavy metals were removed effectively in all CWs. The removal efficiencies were 85.3–92.6%, 84.1–98.3%, 96.5–99.7%, 98.3–99.1%, and 64.0–83.8%, respectively, for Zn, Cd, Cu, Pb, and Mn. During 6 months of operation, 790.7 mg of Zn, 10.6 mg of Cd, 762.4 mg of Cu, 40.1 mg of Pb, and 19.9 mg of Mn were fed to each CW. Of those, the amounts retained in the substrate contributed 67.0–84.3% for Zn, 71.0–75.8% for Cd, 81.5–92.3% for Cu, 45.4–84.5% for Pb, and 37.0–73.7% for Mn removal in all CWs. Analyses using SEM/EDX and BET showed that the clamshell and gravel surfaces were not changed greatly by treatment. However, the metal composition was more diverse. Their percentages were increased, although that of calcium was lower after treatment. Higher metal removal in clamshell-based CWs than in gravel-based CWs demonstrated clamshells as a potential wetland substrate for removal of heavy metals from AMD.*

A major part of Chapter 4 was published in the following paper:

## 4.1. Introduction

It is recognized that substrate plays a crucial role in the removal heavy metals from mine wastewater in CWs. Most metals are removed from AMD, mainly through interaction with substrates such as gravel, sand, and limestone (Wang et al., 2020; Yang et al., 2018). Therefore, selecting substrates with high filtration and adsorption capacities and high ecological activity is important. Furthermore, to minimize the wetland system cost, the substrate should comprise locally abundant materials available at low cost.

In recent years, along with intensive development of the marine shellfish industry, especially in Asian countries, seashells are generated as a by-product, creating challenges for their disposal. It is estimated that every 1 kg of clams produces up to 700 g of clam shells. Consequently, Vietnam, with the average production of 430,700 tons of clams per year, up to 301,490 tons of clamshells/year can be expected to be produced (Nguyen, 2019). In most countries, the seashells are mainly dumped in unsanitary landfills. In China alone, about 10 million tons of shell waste is disposed of in landfills each year. This shell waste has come to present severe difficulties (NBS, 2010). Actually, this non-hazardous solid waste should be recycled into a useful material, consequently reducing adverse effects on the environment. Seashells have been used as low-cost adsorbents for heavy metal removal from groundwater (Xu et al., 2019), storm water (Bremner et al., 2020; Xu et al., 2021), and industrial wastewater (Tudor et al., 2006). Masukume et al. (2014) first used seashells as an adsorbent for removing Fe and Mn from synthetic AMD. Bavandpour et al. (2018) also reported the high applicability of oyster and mussel shells as a substrate in CWs for removing Cu, Fe, Mn, Pb, and Zn in synthetic AMD.

Our research group has studied shells of white hard clams (*Meretrix lyrata*) as a substrate in CWs for removal of phosphorus from swine wastewater (Nguyen et al., 2020). No report of the relevant literature describes a study using clamshells as the wetland substrate for removing heavy metals from AMD. The $CaCO_3$ content of clamshells is comparable to that of limestone, a popular material for neutralizing the pH of AMD (Pat-Espadas et al., 2018; Yao et al., 2014).

In that context, the lab-scale experiment was designed to evaluate the feasibility of clamshells in being used as substrate in CWs for removing heavy metals from synthetic AMD. Gravel, the conventionally used material, was also used for comparison with clamshells for neutralizing pH and for removing heavy metals. Removal of heavy metals in the CWs was investigated under 2–7 days of hydraulic retention time (HRT) which is typically a few days to several weeks for mine

drainage treatment (Acheampong &Ansa, 2017; Sheoran & Sheoran, 2006). In addition, distributions of heavy metals that accumulated in the substrate and plant tissues in CWs were investigated. The main purposes of this chapter were to (1) evaluate the use of clamshell-based CW for heavy metal removal, (2) elucidate the physicochemical properties of substrates before and after treatment, and (3) clarify the pathways for heavy metal removal in CWs.

## 4.2 Materials and methods

### 4.2.1 Simulated acid mine drainage

Synthetic AMD was prepared to simulate the typical characteristics of the actual AMD in the Tohoku region of Japan (Hara et al., 2021). The wastewater presented in Table 4.1  was produced by dilution of $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$, $CdCl_2$, $MnSO_4.5H_2O$, $PbCl_2$, $FeSO_4.7H_2O$, $CaSO_4.2H_2O$, NaCl, $MgSO_4.7H_2O$, KCl, and $AlNa(SO_4)_2.12H_2O$ in tap water. In the simulated AMD, the Zn, Cd, Cu, and Pb concentrations were much higher than their Japanese effluent standards, which are, respectively, 2 mg/L, 0.03 mg/L, 3 mg/L, and 0.1 mg/L. However, the pH value was lower than its effluent standard (5.8–8.6).

Table 4.1 Chemical composition of simulated AMD (Avg. ±SD, n =39) and the effluent standard in Japan

|  | Concentration (mg/L) | Effluent standard (mg/L) |
| --- | --- | --- |
| Zn | 20.27±4.27 | < 2 |
| Cu | 19.60±1.09 | < 3 |
| Cd | 0.27±0.06 | < 0.03 |
| Mn | 0.51±0.11 | < 10 |
| Pb | 1.03±0.51 | < 0.1 |
| Fe | 4.70±0.43 | < 10 |
| Ca | 7.25±0.70 |  |
| Na | 7.25±0.70 |  |
| Mg | 10.00±1.00 |  |
| K | 1.50±0.20 |  |
| Al | 6.00±0.60 |  |
| pH | 4.10±0.40 | 5.8–8.6 |

### 4.2.2 Substrates

Clamshells (*Meretrix lyrata*) were collected from a clam farm in Thai Binh province, a large clam farming area in northern Vietnam, with 3,430 ha (MARD, 2014). After the clamshells were washed and dried under the sun, the dried shells were crushed and sieved to 4–6 mm particles. The basic physical properties of the sieved clamshells were 44% porosity and 1.16 g/cm$^3$ bulk density. The CaCO$_3$ content of the clamshells was 90% (Nguyen et al., 2020).

Gravel used for this study (GA Corp., Higashiosaka, Osaka, Japan) had particle size of 7–9 mm, porosity of 46%, and bulk density of 1.23 g/cm$^3$. The large pieces of gravel and loamy soil (Akadama; Hirota Shokai, Co., Japan) were used to avoid clogging and to cover the top layer of CWs.

### *4.2.3 Lab-scale CW setup and operation*

Lab-scale CWs were placed in a greenhouse at Ritsumeikan University, Shiga Prefecture, Japan and were operated for 6 months, from early July 2020 through early January 2021. A schematic diagram of the experiment setup is presented in Figure 4.1. Each CW consisted of a plastic container (ID 12.5 cm, H 50 cm) filled from bottom to top with coarse gravel (13–15 mm, 1.5 cm depth, 0.4 kg), clamshells (4–6 mm, 20 cm depth, 2.85 kg) or fine gravel (7–9 mm, 20 cm depth, 3 kg), and loamy soil (3–4 mm, 1 cm depth, 0.12 kg). The CWs were planted with cattails (*Typha orientalis*, 87±1 cm aboveground, 11±1 cm root, 186±12.3 g-wet/plant) (C-P; G-P), or were left unplanted (C-UP; G-UP) being used as control experiments. These mature plants were purchased from Tojaku Engei Co. Ltd., Joyo, Kyoto, Japan. Before being planted in the CWs, these plant roots were washed gently using tap water for removal of original culture soil. All CW tests were conducted in duplicate. A weather recorder (TR-7Ui; T&D Corp., Japan) was placed at the greenhouse to record the daily air temperature. Before supplying simulated AMD, all CWs were kept filled with tap water for 10 days to support the adaptation of plants.

For the sequencing batch experiment, one liter of simulated AMD was fed to each column. After the defined period, the treated water was fully discharged from the bottom. Then fresh AMD was fed again to each CW. Influent and effluent samples were collected to measure heavy metals and other water parameters. This operation was repeated for 6 months, divided into four phases with different hydraulic retention times (HRT): In Phase I (2 Jul. – 2 Sep. 2020) in summer, AMD was treated 9 times with HRT of 7 days. In Phase II (3 Sept. – 3 Nov.) in autumn, AMD was treated 15 times with HRT of 4 days. In Phase III (3–22 Nov.) in late autumn, AMD was treated 9 times

with HRT of 2 days. In Phase IV (23 Nov. – 3 Jan. 2021) in winter, AMD was treated 6 times with HRT of 7 days.
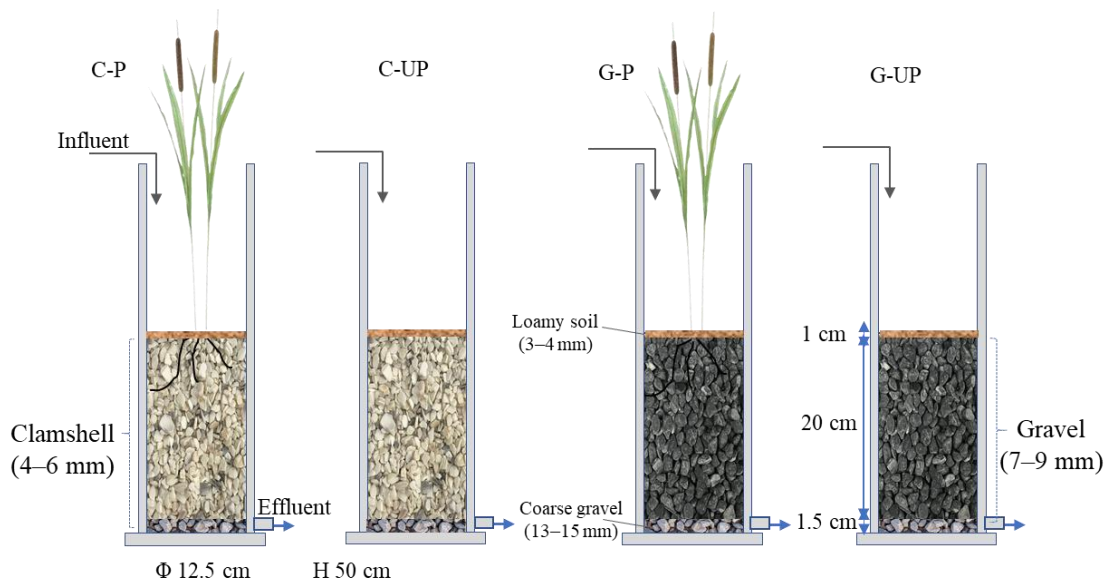


Figure 4.1 Diagram of lab-scale CWs for removing heavy metals from synthetic AMD

### *4.2.4 Sampling and analysis*

Water samples of the CWs were centrifuged for 10 min at 3000 rpm using a refrigerated centrifuge (Thermo Sorvall Legend XTR, Germany), followed by measurement of the concentrations of heavy metals (Zn, Cd, Fe, Cu, Mn, Fe) with inductively coupled plasma spectroscopy (ICP-OES 700 series; Agilent Technologies Japan, Ltd., Tokyo, Japan). Other water parameters (pH, SS, TDS, DO, sulfate…) were measured as described in the section 3.2.3.

Substrate and plant samples were collected before and after 6 months of operation for analyses of the heavy metal contents. The substrate samples were dried at 100 °C to constant weight and were then crushed to less than 1 mm. Plant samples were washed well with pure water to remove soil and stone. Then they were separated into belowground (roots) and aboveground biomass (shoots). They were dried at 80 °C to constant weight. Subsequently they were ground into powder.

The crushed substrate and plant samples were analyzed according to a modified aqua regia method (Ha et al., 2019). For the substrate, 0.5 g of the dried sample was digested in 3.75 mL of HCl (35–37%) and 1.25 mL of $HNO_3$ (65%) for 24 h. Then the mixture was heated to 95 °C for one hour. After cooling to 25°C, 5 mL of pure water was added. For the plant biomass, 0.1 g of the dried sample was digested in 1 mL of $HNO_3$ (65%) for 48 h. Then, 9 mL of pure water was

added. All the supernatant of digested substrate and plant samples were filtered through 0.45 µm filter paper (Advantec Toyo Kaisha Ltd., Japan). The filtrates were used for heavy metal content determination using ICP-OES. After all experiments were conducted in duplicate, the mean was recorded.

The unused and used substrate samples were observed using scanning electronic microscopy (SEM, FlexSEM 1000 II; Hitachi Ltd., Japan). The mineral composition was ascertained using energy-dispersive X-ray spectroscopy (EDX, ADX 8100; Shimadzu Corp., Japan). The surface area and pore size of samples were measured using a gas-adsorption system (Belsorp Max II; MicrotracBEL Corp., Japan).

Heterotrophic bacteria and sulfate-reducing bacteria (SRB) were enumerated respectively using the plate-count technique with R2A agar medium (Merck KGaA, Germany) and Postgate's medium F (as described in 3.2.3 section).

### 4.2.5 Calculation

Evapotranspiration (%) was calculated using the following equation.

$$\text{Evapotranspiration (\%)} = \frac{(V_i - V_e)}{V_i} \times 100, \qquad \text{(Eq 4.1)}$$

Heavy metal removal efficiency (%) was calculated as

$$\text{Removal efficiency (\%)} = \frac{(V_i C_i - V_e C_e)}{V_i C_i} \times 100, \qquad \text{(Eq 4.2)}$$

where $V_i$ (L) and $C_i$ (mg/L), and $V_e$ (L) and $C_e$ (mg/L) respectively stand for the water volumes and the concentrations of heavy metals in the influent and effluent.

The amount of a heavy metal removed by the substrate ($M_s$) was calculated as

$$M_s(\text{mg}) = (C_a - C_o) \times m, \qquad \text{(Eq 4.3)}$$

where $C_o$ (mg/kg) and $C_a$ (mg/kg) respectively represent the contents of heavy metals in a substrate before and after experimentation, and where $m$ (kg) is the total mass of the substrate.

The amount of heavy metal accumulated by cattail plant ($M_p$) was determined as

$$M_p \text{ (mg)} = C_{pa} m_a - C_{pi} m_i, \qquad \text{(Eq 4.4)}$$

where $C_{pi}$ (mg/kg) and $m_i$ (kg), and $C_{pa}$ (mg/kg) and $m_a$ (kg) respectively stand for the contents of heavy metals in plants and the total dried-biomass of cattails before and after experiments.

## 4.3  Results

### 4.3.1 Evapotranspiration and water quality parameters

Temperatures in the greenhouse storing the CWs are presented in Figure 4.2A. The average temperatures in Phases I, II, III, and IV were, respectively, 31.2±10.1, 23.8±9.5, 15.4±6.3, and 13.4±5.1°C. The cattails grew well from 83–85 cm to 98–99 cm in Phase I (Figure 4.6). Plant growth slowed in Phase II. Leaves of cattails began to wither in Phase III. Aboveground parts had almost died by the end of the experiment.

A
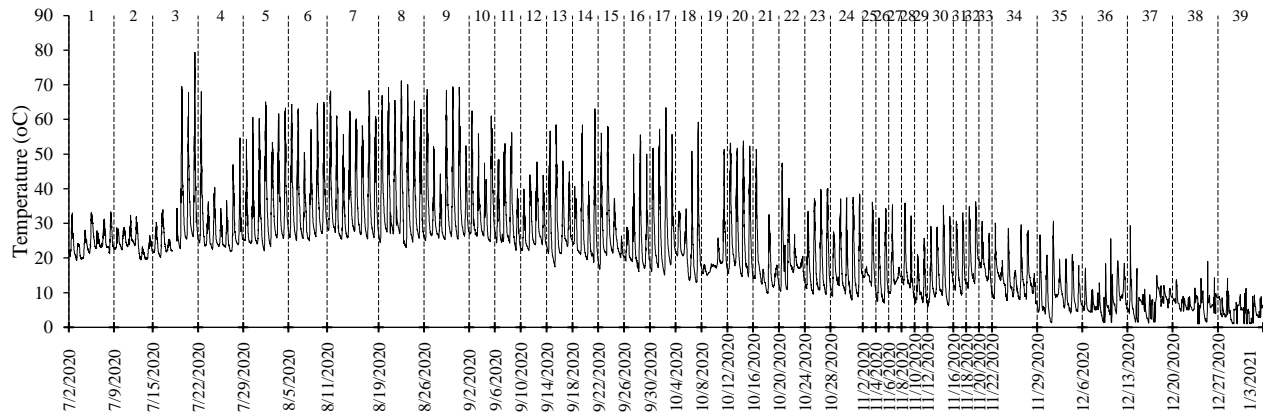


B
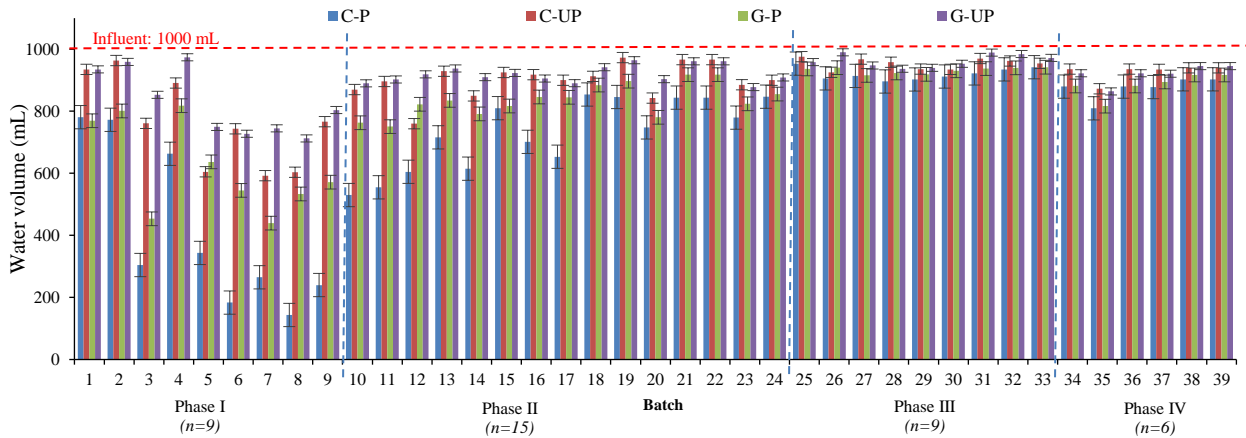


Figure 4.2 Temperature and water balance in CWs operated in sequencing batch mode during 6 months of operation. (A) Air temperature in the greenhouse storing the CWs. (B) Effluent water volume of the CWs.

The water balances in the CWs are also shown in Figure 4.2B. Table 4.2 presents the average evapotranspiration in each phase. The highest evapotranspiration (17.1– 58.9%) was achieved in Phase I as the rapid growth of cattails, high temperature, and long HRT. The high evapotranspiration strongly affects the heavy metal removal efficiency (as shown in Eq 4.1). Evapotranspiration in Phase IV was smaller than that in Phase I because of its lower temperature, although HRT was the same (7 days).

The average values of water parameters in each phase are also shown in Table 4.2. The effluent pH values reached 6.9–8.0 in all CWs in all phases. Its values in the clamshell-based CWs were slightly higher than those of the gravel-based CWs. The DO levels in effluent of the planted CWs were lower than those of the unplanted CWs, suggesting high oxygen consumption by the roots and microorganisms in the rhizosphere. The effluent TDS concentrations were higher than those of the influent. Its value was decreased with shorter HRT, implying that the dissolved matter was released from the substrate in the CWs. The ORP values were positive. They increased gradually during all phases. The SS concentrations were decreased gradually through the phases. The sulfate concentrations were decreased in the CWs effluents, suggesting that SRB reduced sulfate to sulfide.

Table 4.2 Evapotranspiration and water parameters of influents and effluents during four phases (Avg. ± SD)

| Phase I (HRT=7 days, *n*=9) | | Effluent | | | |
| --- | --- | --- | --- | --- | --- |
| | Influent | C-P | C-UP | G-P | G-UP |
| Evapotranspiration (%) | | 58.9±25.5 | 23.7±14.4 | 38.1±14.6 | 17.1±10.5 |
| pH | 4.6±0.3 | 7.0±0.1 | 7.5±0.2 | 6.7±0.1 | 6.9±0.2 |
| ORP (mV) | 205.0±5.8 | 56.7±81.5 | 47.2±80.0 | 101.2±48.6 | 96.5±36.2 |
| DO (mg/L) | 12.4±1.1 | 6.1±2.6 | 6.6±3.5 | 5.1±1.8 | 9.7±2.1 |
| SS (mg/L) | | 62.0±27.1 | 63.5±27.0 | 103.3±47.8 | 88.3±23.0 |
| TDS (mg/L) | 206.5±6.1 | 429.2±40.7 | 357.1±56.8 | 352.7±21.3 | 284.7±14.4 |
| $SO_4^{2-}$ (mg/L) | 164.2±23.9 | 102.5±21.0 | 114.8±17.1 | 101.8±15.8 | 118.1±23.8 |
| Phase II (HRT= 4 days, *n*=15) | | | | | |
| Evapotranspiration (%) | | 27.0±11.4 | 10.0±5.5 | 16.3±5.2 | 8.0±2.7 |
| pH | 3.8±0.2 | 6.9±0.2 | 7.2±0.1 | 6.8±0.1 | 7.1±0.1 |
| ORP (mV) | 202.5±11.2 | 119.1±19.2 | 111.0±20.9 | 121.7±19.2 | 110.2±24.7 |
| DO (mg/L) | 9.6±1.5 | 5.2±1.0 | 7.9±1.0 | 6.5±1.0 | 8.6±0.7 |
| SS (mg/L) | | 17.6±17.4 | 18.1±15.6 | 67.7±73.9 | 66.5±62.5 |
| TDS (mg/L) | 210.6±13.4 | 337.7±38.8 | 243.9±17.1 | 274.2±18.9 | 235.5±12.8 |
| $SO_4^{2-}$ (mg/L) | 171.6±26.1 | 137.6±24.9 | 141.3±29.9 | 142.0±29.5 | 150.3±17.7 |

| Phase III (HRT=2 days, $n$=9) | | Effluent | | | |
|---|---|---|---|---|---|
| | Effluent | C-P | C-UP | G-P | G-UP |
| Evapotranspiration (%) | | 9.7±1.9 | 5.7±1.8 | 8.7±1.0 | 5.3±2.1 |
| pH | 4.0±0.4 | 7.0±0.2 | 7.2±0.13 | 6.9±0.2 | 7.1±0.2 |
| ORP (mV) | 197.5±2.8 | 135.6±10.1 | 123.0±15.8 | 131.2±12.6 | 124.5±21.6 |
| DO (mg/L) | 8.8±0.3 | 6.7±0.7 | 8.8±0.4 | 7.5±0.8 | 9.2±0.3 |
| SS (mg/L) | | 5.1±0.6 | 5.7±1.6 | 7.5±3.0 | 12.8±4.1 |
| TDS (mg/L) | 199.4±5.1 | 252.5±20.1 | 222.4±6.5 | 228.8±8.8 | 221.3±7.7 |
| $SO_4^{2-}$ (mg/L) | 172.8±9.7 | 122.7±23.4 | 128.7±21.6 | 129.8±27.3 | 148.0±14.9 |
| Phase IV (HRT=7 days, $n$=6) | | | | | |
| Evapotranspiration (%) | | 12.4±3.4 | 7.3±2.7 | 11.5±3.7 | 7.9±2.9 |
| pH | 3.9±0.1 | 7.2±0.1 | 7.4±0.1 | 7.3±0.1 | 7.2±0.1 |
| ORP (mV) | 200.3±0.5 | 136.5±23.5 | 120.3±23.4 | 131.4±22.6 | 125.9±21.2 |
| DO (mg/L) | 9.0±0.1 | 7.4±1.1 | 9.9±0.5 | 8.0±0.7 | 9.8±0.1 |
| SS (mg/L) | | 3.0±0.7 | 3.9±1.3 | 3.9±1.1 | 5.5±0.6 |
| TDS (mg/L) | 200.5±4.8 | 274.4±7.7 | 227.3±8.6 | 240.0±8.6 | 226.4±8.3 |
| $SO_4^{2-}$ (mg/L) | 136.0±25.0 | 94.1±13.4 | 100.5±15.2 | 94.1±7.0 | 104.8±14.3 |

### 4.3.2  Heavy metal removal

The average concentrations of those metals in influent and effluent in each phase and their removal efficiencies are presented respectively in Figure 4.3 and Table 4.3. The average Zn concentration in influent (20.2 mg/L) was approximately ten-fold higher than the Japan effluent standard (2 mg/L).

Results show that Zn was well removed in all CWs. The average effluent Zn concentrations of the clamshell-based CWs (0.08–1.40 mg/L) were lower than the effluent standard in Phases I and II. They slightly exceeded it in Phases III and IV (2.68–2.95 mg/L). Its level in both planted and unplanted gravel-based CWs (2.27–5.03 mg/L) exceeded the standard in Phases II–IV.

The Cd was removed effectively from the influent (0.27 mg/L, in average) in all CWs. The effluent Cd concentrations (0.004–0.027 mg/L) in the clamshell-based CWs well satisfied the effluent standard (0.03 mg/L) in all phases, except for Phase III in the C-UP CW (0.034 mg/L), whereas those in the gravel-based CWs (0.035–0.059 mg/L) in Phases III and IV were higher than the standard.

Data for Cu and Pb removal showed mutually similar trends. The concentrations of Cu (0.006–1.024 mg/L) and Pb (0.006–0.024 mg/L) in effluent of all CWs were kept far below their respective

standards of 3 mg/L, and 0.1 mg/L during four phases. Their removal decreased with shortened HRT.

Results show that Mn and Fe concentrations in AMD were lower than the effluent standard of 10 mg/L for each. The Fe removal showed a similar trend to those of other metals. Its concentration in effluent tended to increase with shorter HRT. Also, Mn and Fe removal data in the planted-CWs were higher than in the unplanted-CWs. The CWs demonstrated a slightly different trend for Mn removal than for other metals. The Mn levels in effluent in the planted CWs were lower than those in the unplanted CWs in Phase III in clamshell-based CWs and in Phases II–IV in the gravel-based CWs. Actually, among the investigated metals, Mn showed the lowest removal in the CWs.
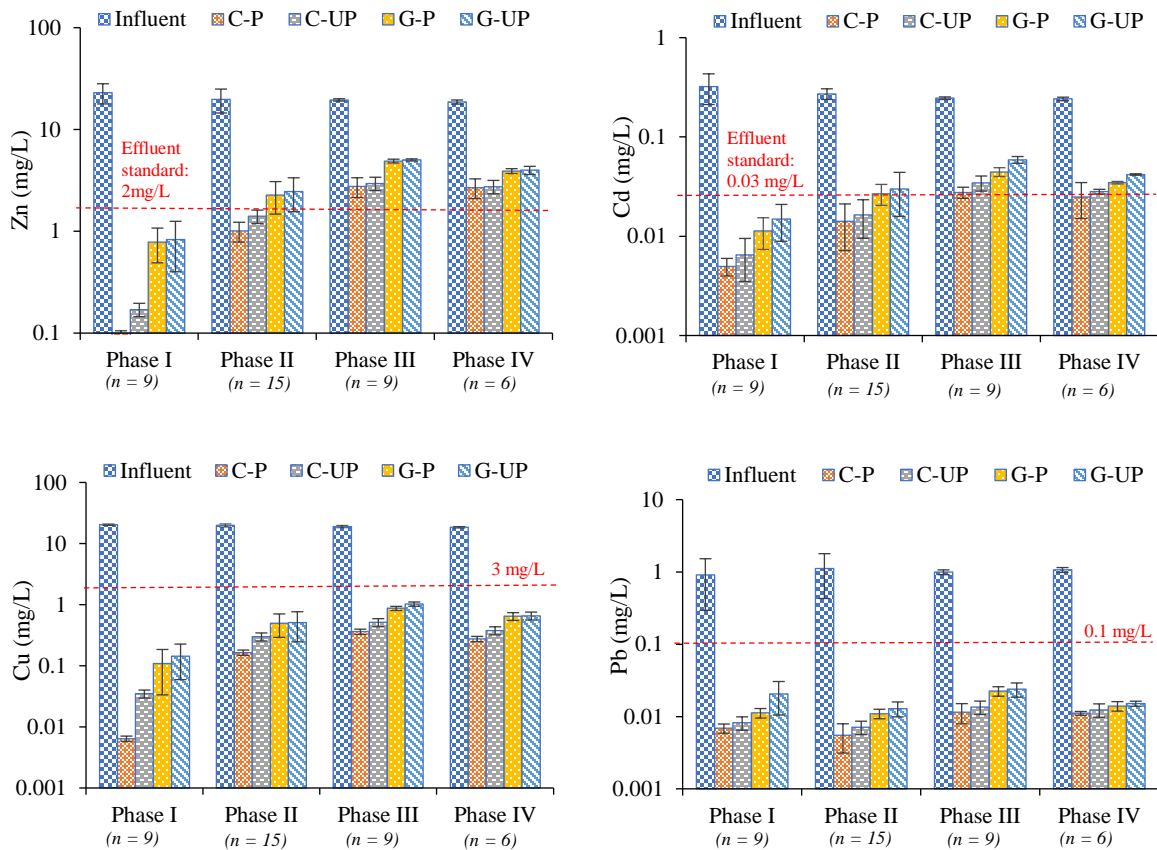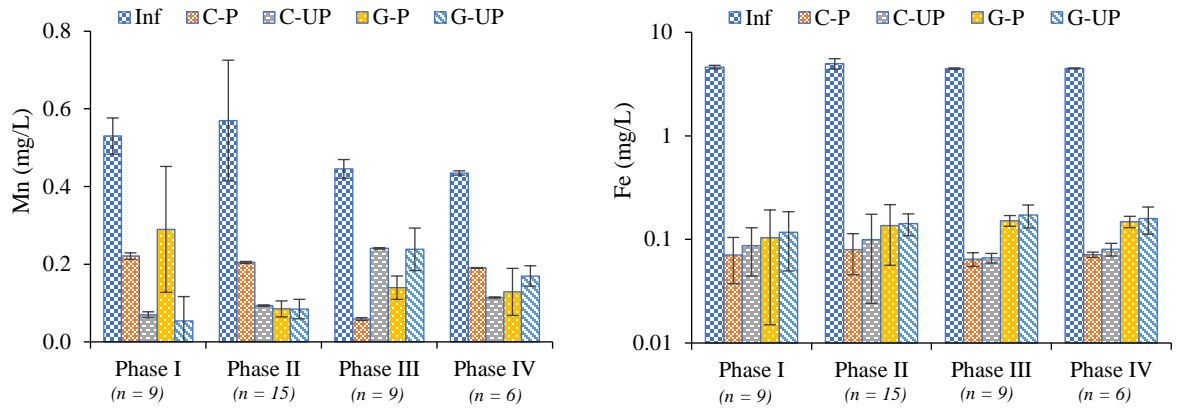
Figure 4.3 Heavy metal concentrations (avg. ± SD) in influent synthetic AMD and
clamshell-based and gravel-based CW effluents in four phases

Table 4.3  Removal efficiencies (Avg. ± SD) of heavy metals in CWs

|        |       | Phase I | Phase II | Phase III | Phase IV | Avg. ±SD |
|--------|-------|---------|----------|-----------|----------|----------|
| Zn (%) | C-P   | 99.9±0.1 | 96.2±3.1 | 87.0±1.0 | 87.3±2.1 | 92.6±6.5 |
|        | C-UP  | 99.5±0.4 | 93.8±2.7 | 85.6±1.8 | 86.3±2.1 | 91.3±6.6 |
|        | G-P   | 97.7±1.3 | 90.2±3.0 | 76.6±0.9 | 81.4±2.1 | 86.5±9.4 |
|        | G-UP  | 97.1±1.4 | 88.6±3.3 | 75.2±0.9 | 80.4±0.8 | 85.3±9.6 |
| Cd (%) | C-P   | 99.6±0.5 | 98.7±2.8 | 98.0±1.5 | 96.8±3.7 | 98.3±1.2 |
|        | C-UP  | 95.8±0.9 | 94.4±2.7 | 91.6±2.5 | 89.5±0.4 | 92.8±2.8 |
|        | G-P   | 89.6±0.9 | 86.6±2.4 | 83.1±1.7 | 76.9±0.7 | 84.1±5.5 |
|        | G-UP  | 90.9±2.6 | 89.2±5.2 | 87.3±2.0 | 84.0±1.2 | 87.9±3.0 |
| Cu (%) | C-P   | 100.0±0.1 | 99.9±0.6 | 99.7±0.3 | 99.4±0.6 | 99.7±0.2 |
|        | C-UP  | 99.3±0.1 | 98.5±1.5 | 97.9±0.8 | 97.6±1.5 | 98.3±0.8 |
|        | G-P   | 98.2±0.2 | 97.4±1.0 | 95.7±0.3 | 94.8±0.4 | 96.5±1.6 |
|        | G-UP  | 98.7±0.3 | 98.1±1.2 | 96.9±0.6 | 96.7±0.4 | 97.6±1.0 |
| Pb (%) | C-P   | 99.5±1.0 | 98.8±0.2 | 98.8±0.3 | 97.5±0.1 | 98.6±0.8 |
|        | C-UP  | 99.6±1.5 | 99.3±0.3 | 99.0±0.2 | 98.7±0.1 | 99.1±0.4 |
|        | G-P   | 98.9±1.3 | 98.7±0.5 | 97.9±0.5 | 97.7±0.2 | 98.3±0.6 |
|        | G-UP  | 99.1±0.3 | 98.9±0.5 | 98.8±0.6 | 98.7±0.2 | 98.9±0.7 |
| Mn (%) | C-P   | 81.0±19.5 | 88.8±17.8 | 64.7±6.1 | 91.5±7.3 | 81.5±12.0 |
|        | C-UP  | 78.1±12.5 | 84.4±7.5 | 87.3±8.3 | 85.5±7.8 | 83.8±4.0 |
|        | G-P   | 88.0±23.0 | 48.6±2.3 | 70.9±6.0 | 48.7±12.7 | 64.0±19.1 |
|        | G-UP  | 61.7±10.9 | 75.7±6.0 | 73.7±10.4 | 64.1±5.8 | 68.8±6.9 |
| Fe (%) | C-P   | 99.4±0.7 | 98.5±0.3 | 98.5±0.2 | 97.8±0.1 | 98.6±0.6 |
|        | C-UP  | 98.9±0.9 | 98.3±1.0 | 97.8±0.2 | 97.3±0.2 | 98.1±0.7 |
|        | G-P   | 98.7±1.5 | 98.6±1.1 | 96.8±0.4 | 96.3±0.4 | 97.6±1.2 |
|        | G-UP  | 98.6±1.4 | 98.3±0.6 | 97.1±1.0 | 96.7±1.0 | 97.7±0.9 |

### 4.3.3   Metals accumulated in substrates

Figure 4.4 shows the heavy metal content in the substrate before and after treatment. Through the experiment, the total amounts of metals accumulated in each substrate are shown in Table 4.7. The levels of all metals in the substrate had increased in all CWs by 6 months after the start of the experiment.

The Zn contents in the original clamshell and the gravel were, respectively, 7.4 and 8.5 mg/kg. After treatment, the Zn content increased 29–33 times in the clamshell-based CWs and 22–26 times in the gravel-based CWs.

Before treatment, the Pb, Mn, and Fe contents were 1.4, 12.0, and 209.0 mg/kg in the clamshells, respectively, and were 3.1, 7.3, and 152.0 mg/kg in the gravel. After treatment, those contents increased 1.2–5.8 times in the clamshells and 1.4–4.6 times in the gravel.

The highest accumulated rate in the substrates was shown by Cu. Its contents after treatment were 68–74-fold and 56–63-fold higher, respectively, than the original contents of 3.3 mg/kg in the clamshells and 3.8 mg/kg in the gravel.

Although Cd was not detected in the original clamshells, the contents after treatment had increased to 2.65–2.77 mg/kg. The Cd content in the original gravel was 0.03 mg/kg, but Cd had accumulated up to 2.63–2.72 mg/kg after 6 months of operations.
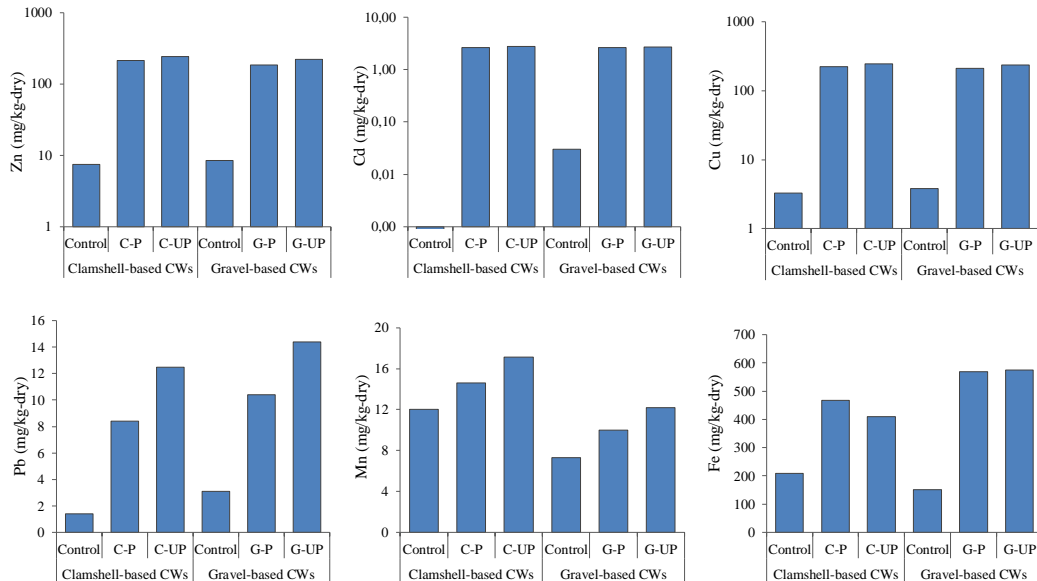


Figure 4.4 Heavy metal contents in clamshell and gravel as CWs substrates before and after AMD treatment

### 4.3.4 *Surface image and chemical compositions of substrates*

Figure 4.5 presents SEM images of the clamshell and the gravel samples in the CWs before and after experiments. The surfaces of both unused and used clamshells and gravels were smooth with few micropores. Results of BET surface area analyses are presented in Table 4.5.

The specific surface area of the unused clamshells and the gravels in this study were, respectively, 0.67 and 0.46 $m^2/g$, smaller than those of oysters (1.75 $m^2/g$, Yoon et al., 2009) and cockle shells (3.40 $m^2/g$, Kim et al., 2018).

The surface textures and shapes of the material appear to have remained largely unchanged during the experiment. Table 4.4 presents major elements of the clamshells and the gravels before and after the experiment. The percentage of Ca decreased, whereas those of other metals increased during treatment.
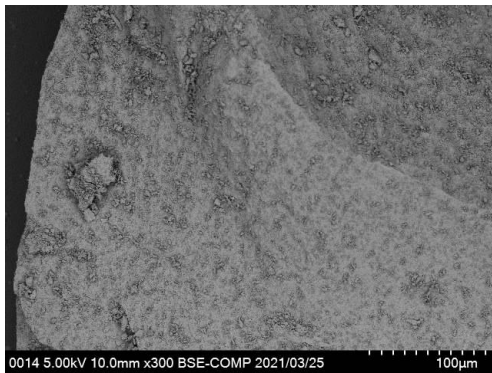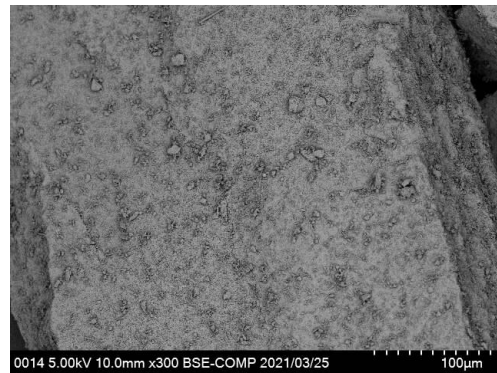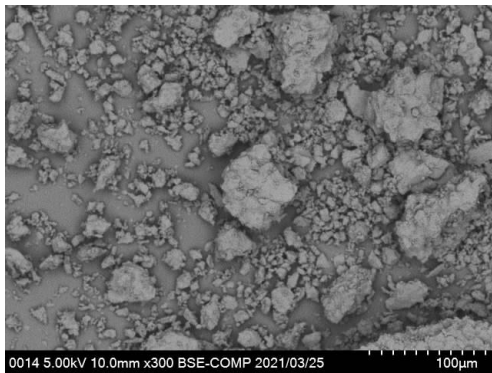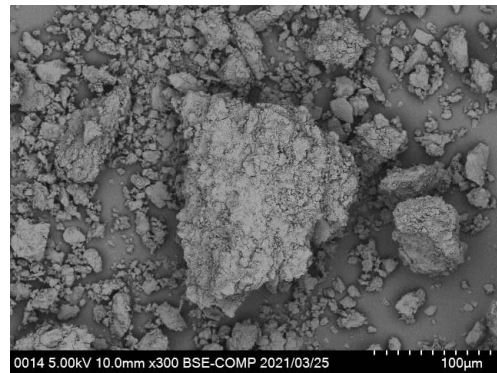


| Unused clamshell | Used clamshell |
|---|---|



| Unused gravel | Used gravel |
|---|---|

Figure 4.5 Surfaces of clamshell and gravel before and after being used as filter media in CWs

Table 4.4 Percentages of elements in unused and used filter materials (%)

| | Unused clamshell (%) | Used clamshell (%) | Unused gravel (%) | Used gravel (%) |
|---|---|---|---|---|
| Ca | 98.01 | 91.32 | 95.89 | 91.80 |
| Si | 0.44 | 2.90 | 1.27 | 2.30 |
| Al | 0.28 | 2.45 | 0.16 | 2.45 |
| Fe | 0.25 | 1.94 | 0.11 | 1.94 |
| Cu | 0.02 | 0.16 | 0.03 | 0.16 |
| Mn | 0.01 | 0.05 | 0.05 | 0.09 |
| K | 0.06 | 0.15 | 0.07 | 0.10 |
| Zn | 0.005 | 0.09 | 0.004 | 0.14 |
| Cd | ND | 0.003 | 0.03 | 0.033 |
| Pb | ND | 0.001 | ND | 0.007 |

Table 4.5 Surface area, pore volume, and pore size of unused and used clamshell and gravel, other seashells

| | Particle size (mm) | Specific surface area ($m^2/g$) | Pore volume ($cm^3/g$) | Pore size (nm) | Reference |
|---|---|---|---|---|---|
| Unused clamshell | 4–6 | 0.67 | $3.2 \times 10^{-3}$ | 1.9 | This study |
| Used clamshell | 4–6 | 1.10 | $5.6 \times 10^{-3}$ | 2.1 | This study |
| Unused gravel | 7–9 | 0.46 | $2.3 \times 10^{-3}$ | 2.0 | This study |
| Used gravel | 7–9 | 0.77 | $3.9 \times 10^{-3}$ | 2.0 | This study |
| Oyster shell | 1.65 | 1.75 | N/A | N/A | Yoon et al., 2009 |
| Cockle shell | Powder | 3.40 | $17 \times 10^{-3}$ | 19.0 | Kim et al., 2018 |

### 4.3.5. Plant uptake

During the experiment, the roots of cattails expanded $30 \pm 3$ cm in the clamshell-based CWs and $20 \pm 2$ cm in the gravel-based CWs (Figure 4.6). Cattails were harvested at the end of the experiment, amounting to 167.4 and 172.0 g of dry biomass, respectively, in the G-P and C-P CWs. In which, the G-P and C-P CWs respectively had 18.0 and 19.0 g of the aboveground part and 149.0 and 153.0 g of the belowground part.

The heavy metal contents in cattails before and after treatment are presented in Figure 4.7. The metal amounts in cattails increased considerably through four phases, especially in the belowground part. After 6 months of operation, the total amounts of Zn, Cd, Cu, Pb, Mn, and Fe accumulated in the cattail biomass were, respectively, 152.0, 2.2, 122.7, 19.4, 7.9, and 1655.8 mg

in the C-P CW. Those were, respectively, 156.9, 1.8, 111.9, 17.6, 7.2, and 1701.5 mg in the G-P CW.

Phase I      Phase II      Phase III      Phase IV



Clamshell-based CW after Phase IV      Gravel-based CWs after Phase IV
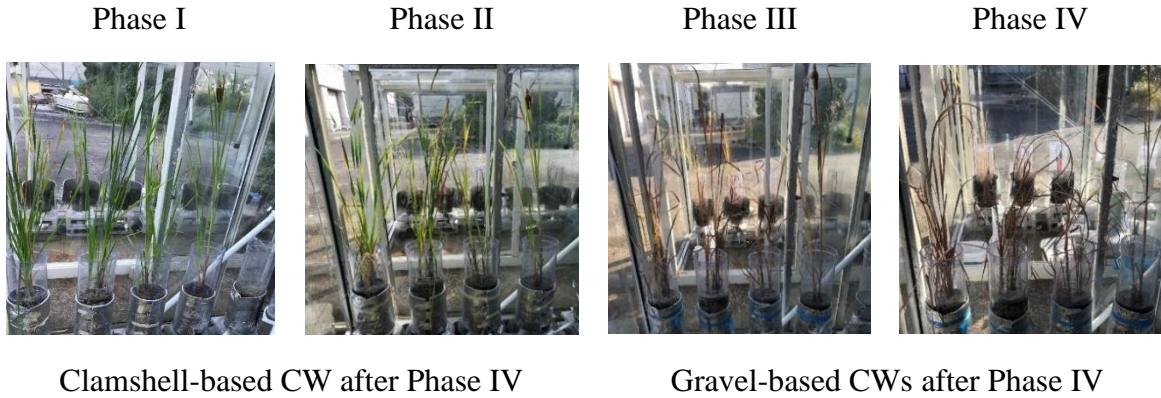


Figure 4.6 Aboveground and belowground parts of cattails after treatment.
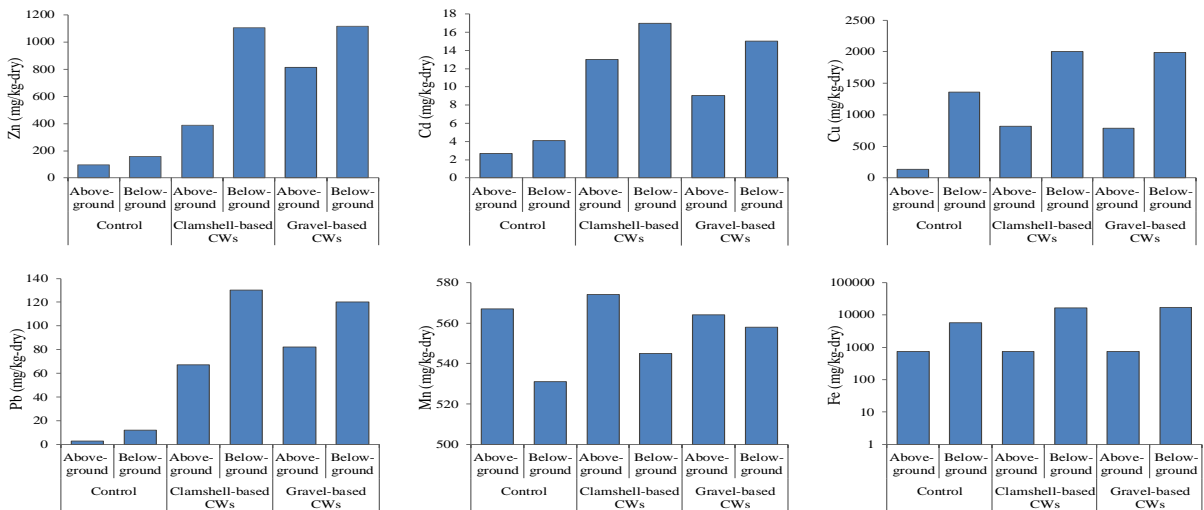


Figure 4.7 Contents of heavy metals in cattail biomass before and 6 months after of AMD treatment

### 4.1.6. Bacteria community

The numbers of heterotrophs in effluent heterotrophs were $5 \times 10^3 - 2 \times 10^4$ CFU/mL in effluent and $7 \times 10^7 - 5 \times 10^8$ CFU/dried-mg in the substrate. The unplanted CWs showed slightly higher numbers in effluent, whereas the planted CWs were found to have slightly higher numbers in the substrate.

A large population of SRB ($1.5 \times 10^2 - 3 \times 10^3$ CFU/g-dry) was detected in the substrate, and $5 \times 10^0 - 1.1 \times 10^2$ CFU/mL in effluent of the planted CWs. A few SRB ($5 - 10$ CFU/mL, $2 \times 10^1$ CFU/g-dry) were detected in the unplanted clamshell-based CWs, although those were not detected in unplanted gravel-based CWs (Figure 4.8, Table 4.6). The population of SRB in the clamshell-based CWs was higher than those of the gravel-based CWs.
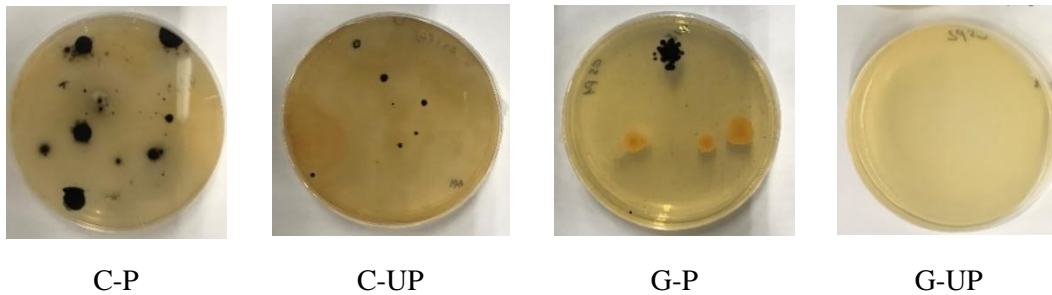


|  C-P  |  C-UP  |  G-P  |  G-UP  |

Figure 4.8 SRB was detected from water samples.

Table 4.6 Population of SRB in effluent during four phases and soil samples at the end of the Phase IV experiment

|  | C-P | C-UP | G-P | G-UP |
|---|---|---|---|---|
| **Effluent samples (CFU/mL)** |  |  |  |  |
| Phase I ($n=4$) | $10^1 - 10^2$ | $5 \times 10^0 - 2 \times 10^1$ | 0 | 0 |
| Phase II ($n=3$) | $3 \times 10^1 - 10^2$ | $5 \times 10^0 - 2.5 \times 10^1$ | $10^1 - 2 \times 10^1$ | 0 |
| Phase III ($n=2$) | $2 \times 10^1 - 10^2$ | $10^1 - 2 \times 10^1$ | $10^1 - 3 \times 10^1$ | 0 |
| Phase IV ($n=2$) | $3.5 \times 10^1 - 1.1 \times 10^2$ | $2 \times 10^1 - 3 \times 10^1$ | $2.5 \times 10^1 - 5 \times 10^1$ | 0 |
| **Soil samples (CFU/g-dry)** |  |  |  |  |
|  | $3 \times 10^3$ | $2 \times 10^1$ | $1.5 \times 10^2$ | 0 |

### 4.1.7. Mass balance

In this experiment, metals in synthetic AMD can be removed through adsorption by the substrate, plant uptake, precipitation, and other processes. After 6 months of operation, the total amounts of Zn, Cd, Cu, Pb, Mn, and Fe fed to each CW were, respectively, 790.7, 10.6, 762.4, 40.1, 19.9, and 183.3 mg. The amounts of metals found in the effluent, the substrate, and the plant biomass are presented in Table 4.7. Figure 4.9 presents the ratio of the metal removal routes for each. Accumulation in the substrate was a main pathway for heavy metal removal, representing 67.0–84.3% for Zn, 71.0–75.8% for Cd, 81.5–92.3% for Cu, 45.4–84.5% for Pb, and 37.0–73.7% for Mn of the inflow in all CWs. In the planted CWs, accumulation in the cattail biomass accounted for 19.2–20.5% for Zn, 16.6–20.8% for Cd, 14.6–16.1% for Cu, 43.9–48.3% for Pb, and 36.1–39.9% for Mn. The remaining minor amounts were attributed to influences such as measurement error and other, unknown processes.
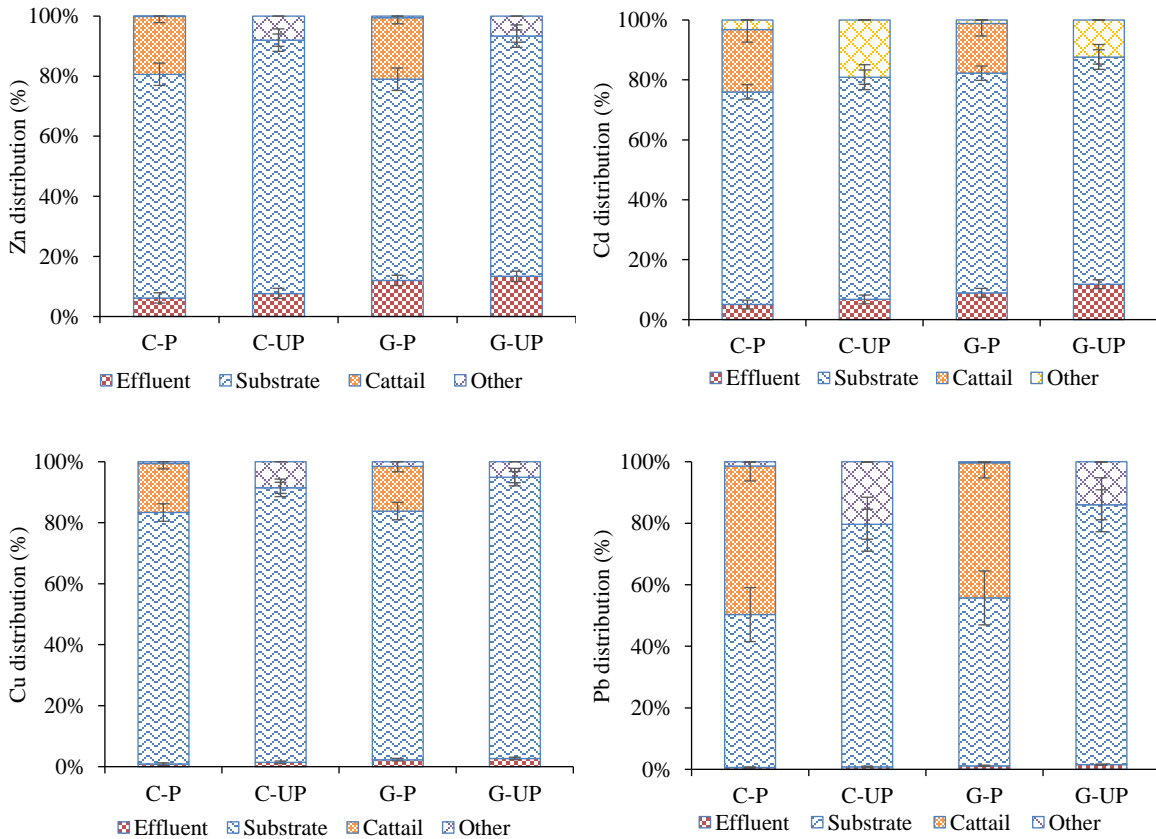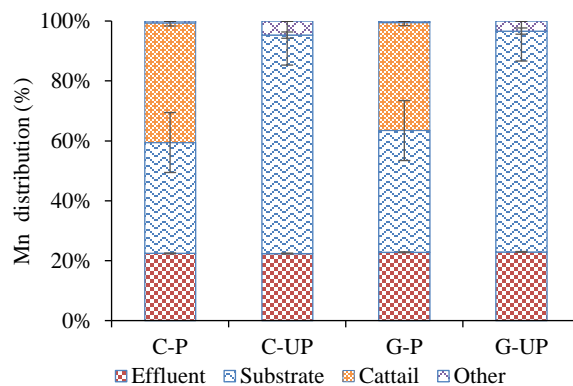
Figure 4.9 Zn, Cd, Cu, Pb, and Mn removal pathways in the CWs using clamshell-based media or gravel-based media, planted or unplanted with cattail

Table 4.7 Mass balance of heavy metals (mg) in the CWs

|  |  | Effluent | Substrate | Cattail | Other |
|---|---|---|---|---|---|
| Zn (mg) | C-P | 48.9 | 588.8 | 152.0 | 1.0 |
|  | C-UP | 60.8 | 666.6 |  | 63.3 |
|  | G-P | 95.0 | 529.5 | 157.0 | 9.1 |
|  | G-UP | 105.7 | 632.4 |  | 52.6 |
| Cd (mg) | C-P | 0.5 | 7.6 | 2.2 | 0.3 |
|  | C-UP | 0.7 | 7.9 |  | 2.0 |
|  | G-P | 1.0 | 7.8 | 1.8 | 0.1 |
|  | G-UP | 1.3 | 8.1 |  | 1.3 |
| Cu (mg) | C-P | 6.4 | 629.0 | 122.8 | 4.2 |
|  | C-UP | 10.9 | 686.3 |  | 65.2 |
|  | G-P | 17.6 | 621.6 | 112.0 | 11.2 |
|  | G-UP | 20.5 | 703.5 |  | 38.4 |
| Pb (mg) | C-P | 0.2 | 20.0 | 19.4 | 2.3 |
|  | C-UP | 0.3 | 31.6 |  | 20.4 |
|  | G-P | 0.5 | 21.9 | 17.6 | 0.2 |
|  | G-UP | 0.6 | 33.9 |  | 5.6 |
| Mn (mg) | C-P | 4.5 | 7.4 | 8.0 | 0.1 |
|  | C-UP | 4.5 | 14.5 |  | 0.9 |
|  | G-P | 4.5 | 8.1 | 7.2 | 0.1 |
|  | G-UP | 4.6 | 14.7 |  | 0.7 |

## 4.3  Discussion

During 6 months of operation, clamshell showed the high feasibility and effectiveness as being used as a substrate in CWs for removing heavy metals from AMD. The dominant $CaCO_3$ content

in clamshells has the benefit of neutralization of acidic wastewater. The crushed clamshells can generate ion hydroxides leading to increased pH as the following equation: $CaCO_3 + H_2O \rightarrow Ca^{2+} + CO_2 + 2OH^-$. With the increase of pH, metal hydroxides such as $Cu(OH)_2$, $Zn(OH)_2$, $Pb(OH)_2$, and $Cd(OH)_2$ can be precipitated in the CWs (Hara et al., 2021). According to the theory of solubility product at 20°C, pH for achieving the Japan effluent standard is over 6.77 for Cu (3 mg/L), 7.87 for Zn (2 mg/L), 9.47 for Pb (0.1 mg/L), and 10.3 for Cd (0.03 mg/L) by precipitation of those hydroxides, although the amphoteric metal can be dissolved at higher pH conditions (Hara et al., 2021). Also, Cu should be removed well by the neutralization process because the effluent pH reached 6.9–7.5 in all CWs (Table 4.2). Additional mechanisms for removing Zn, Pb, and Cd in the CWs are expected to exist, such as sedimentation, filtration, precipitation, adsorption, microbial reactions, and uptake by vegetation. By contrast, Fe and Mn can be removed easily by formation of their insoluble oxides such as $Fe(OH)_3$ and $MnO_2$.

Crushed clamshells can provide active sites for adsorbing divalent cations of those metals. The metal removal efficiency was found to be lower for shorter HRT, indicating adsorption as an important mechanism for removing heavy metals. The metal-removal capacity of the substrates saturated gradually according to the lower metal removals in Phase IV than in Phase I with the same HRT. Sludge or scale containing metals on the surface of the substrates possibly prevented the substrate–AMD interaction. The clamshells would be washed or replaced with new ones at a certain interval like conventional media. The clamshells present the benefit of easy handling because their density (1.16 $g/cm^3$) is much lower than that of the gravel (1.23 $g/cm^3$). Further studies will be needed for optimizing the particle size of the crushed clamshells because smaller particles increase the surface area but clogging probability.

In the planted CWs, cattails made a marked contribution (14.6–48.3%) to heavy metal removal, indicating phytofiltration, plant uptake, and oxidation–reduction in the rhizosphere. This result agrees completely with the previous reports (Hafeznezami et al., 2012; Klink et al., 2013; Ventura et al., 2021), confirming the presence of plants can certainly enhance heavy metal removal. Among the investigated heavy metals, the amount of accumulated Cd (1.8–2.2 mg) in cattails was lowest in comparison with other metals. This is true probably because of the lower concentration of Cd in simulated AMD than in other metals. Also, Cd is a toxic element, whereas other metals are essential elements for plants (Selinus et al., 2005). The metals accumulated in the CWs can be collected easily by harvesting the aboveground part of the plant biomass.

The clamshell-based CW extended the cattail root (Figure 4.6) and incubated SRB in a larger population (Table 4.6). The root exudate of cattails was a possible electron donor and carbon source for SRB. An anaerobic environment can be formed in the rhizosphere in which sulfate is reduced by SRB (Chen et al., 2014). Together with reduction of the sulfate concentration, the presence of SRB can contribute to heavy metal removal through the following process.

Electron donors + $SO_4^{2-} \rightarrow HS^-$, $S^{2-}$

$Cd^{2+}$, $Cu^{2+}$, $Fe^{2+}$, $Zn^{2+}$, + $S^{2-} \rightarrow CdS$, $CuS$, $FeS$, $ZnS$
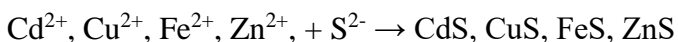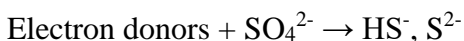
Many earlier studies have demonstrated metal sulfide precipitation as an effective method of removing heavy metals from CWs (Stein et al., 2007; Yeh, 2008). The SRB activities might also contribute to higher heavy metal removal efficiencies in planted CWs, even when all the aboveground parts of cattails died in Phase IV. This finding also implies that the roots of the plant played an important role in creating favorable conditions for the growth and development of microbial communities. These favorable conditions might be attributable largely to the fact that the clamshell particles (4–6 mm) were smaller than those of gravel (7–9 mm), creating better anaerobic conditions for SRB growth.

## 4.4 Conclusion

Lab-scale experiments demonstrated that CWs filled with clamshells have high capability for heavy metal removal from synthetic acid mine drainage. Zn, Cd, Pb and Fe were almost completely (> 93%) removed by clamshell-based CWs during four Phases. Accumulation in the substrates was a main pathway for heavy metal removal, contributing 49.7–82.5%, followed by plant uptake (16.1–39.0%), and other processes (0.6–3.2%). Subsequent SEM/EDS and BET analyses indicated the physicochemical properties of clamshell were changed only slightly after being used as substrates in CWs. Higher metal removal in clamshell-based CWs than in gravel-based CWs demonstrated clamshells as a potential wetland substrate for removal of heavy metals from AMD. This encourages recycling seashells for wastewater decontamination. By that way, it might contribute to reducing solid waste in a simple, cheap, and eco-friendly way.

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# CHAPTER 5. REMOVAL OF HEAVY METALS FROM ACID MINE DRAINAGE BY LAB-SCALE CONSTRUCTED WETLANDS FILLED WITH OYSTER SHELLS

**Abstract**

*This chapter clarifies the applicability of constructed wetlands (CWs) filled with oyster shells (OS) for heavy metal removal from acid mine drainage (AMD). Lab-scale CWs consisted of columns (ID 12.5cm, H 50cm) packed with oyster shell/limestone and loamy soil. Those were then unplanted or planted with cattail. Synthetic and actual AMD containing 7.3 mg/L of Zn, 38.0 mg/L of Fe and other minerals (pH=4.0) was 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. OS with high content of $CaCO_3$ had higher neutralizing ability for AMD than limestone. During the 7 months of operation, all CWs were highly effective for removing Zn (88.6–99.2 %), Fe (98.7–99.7 %), and other metals such as Cd, Cu, Pb, As, and Mn (48.2–98.9 %) from both the actual and synthetic AMD. The mass balance study indicated that accumulation in substrates was a main pathway in removing heavy metals, representing 44.8–99.3% in all metals, followed by the biomass (8.8–29.9%) in the planted CWs. Other processes only played a minor role in removing heavy metals in this study. The higher metal removal in the OS-based CWs showed the valuable potential of this aquaculture byproduct as a filter medium. These experiments demonstrated that the lab–scale CW is a useful tool for feasibility studies on installing CWs for mine drainage treatment.*

A major part of Chapter 5 was published in the following papers:

> **Nguyen, T. T**., Soda, S., Kanayama., A., 2022. Removal of heavy metals from acid mine drainage by lab-scale constructed wetlands filled with oyster shells and limestone. Water. (In preparation)

## 5.1 Introduction

As mentioned in the introduction of chapter 4, screening substrate in CWs is an effective strategy to enhance heavy metal removal from mine drainage. Moreover, to minimize the cost of

the wetlands, and achieve a sustainable AMD treatment as well, recycling of waste or by-products as filter media in wetlands has been gaining a lot of attention from researchers. In a similar approach in chapter 4, this study investigates oyster shell as filter media in CWs for removing heavy metals from both real and mine wastewater.

Oyster shells (OS) are generated as by-products from the seafood processing industry. The residue from these shells, often has residual meat, cause undesirable odors, and attracts flies and mosquitoes. As a result, most market oyster meat traders must pay for disposing of OS by landfilling. It often requires a large land area for landfilling (Yao et al., 2014). Therefore, an innovative way for recycling OS is necessary to reduce the environmental burden as well as to provide an economic value to seashells.

In fact, OS is composed mainly of $CaCO_3$ and are a non-hazardous waste material, which is abundantly available. Due to these advantages, OS has been recycled for many purposes, such as construction materials (Miyaji and Okamura, 2000; Yang et al., 2005; Yoon et al., 2004), catalysts (Nakatani et al., 2009), acid gas absorbents (Asaoka et al., 2009; Jung et al., 2007), and bactericidal agents (Choi et al., 2005).

For wastewater treatment, OS has been investigated as an adsorbent for removing phosphorus (Yuangsawad and Na-Ranong, 2011) and heavy metals (Masukume et al., 2014). Regarding the use OS as substates in CWs, Park and Polprasert (2008) reported the high applicability of OS as a substrate in CWs for removing phosphorus as Ca-P precipitation from swine wastewater. As far as we know, only Bavandpour et al. (2018) used the mixed seashells including OS and mussels as the substrate in CWs for heavy metal removal from synthetic AMD. OS can neutralize acids in AMD resulting in precipitation of metals in hydroxides forms such as $Fe(OH)_3$, $Zn(OH)_2$, and $Cu(OH)_2$. Also, arsenate can be precipitated in the presence of Ca and Fe. If OS can be used as a substrate in CWs for AMD treatment, costs for OS disposal, CW maintenance will be reduced, and high metal removal will be achieved. Nevertheless, CWs using OS as a substrate in CWs have not been fully studied for removing heavy metals from AMD. To fill this research gap, this study investigated a lab-scale CWs filled with OS for metal removal from both real and simulated AMD. Limestone, as a commercial material, was also used as a main substrate in CW columns for comparison with OS.

In this study, we evaluated feasibility of OS-based CWs for treating AMD of an anonymous mine in Kyoto Prefecture. The target AMD contains zinc (Zn) of 5-8 mg/L, iron (Fe) of 6-80 mg/L,

cadmium (Cd) of 0.01-0.06 mg/L, and arsenic (As) of 0.01-0.15 mg/L with pH of about 3. Now this AMD is treated by mechanical aeration for oxidizing Fe with pH neutralization by quicklime. Subsequently, chemical sludge is removed from the water phase in sedimentation tanks. The objectives of this study are (1) to compare the neutralizing ability of oyster shell and limestone, (2) to evaluate the performance of CWs filled with oyster shells and limestone on heavy metal removal from both real and synthetic AMD, and (3) to clarify the heavy metal removal pathway in the lab scale CWs.

## 5.2 Materials and Methods

### 5.2.1 Actual and simulated AMD

In this study, both actual and synthetic AMD were provided to wetland columns.

Actual AMD was collected twice directly at a mine in Kyoto prefecture, Japan (December 2020, July 2021). After being collected, AMD was transported to the laboratory at Ritsumeikan University, Shiga prefecture, Japan. To prevent metals from oxidizing, AMD was kept with the dissolved oxygen (DO) level less than 0.5 mg/L by purging nitrogen gas. Among these metals, the Zn and Fe concentrations were much higher than the Japanese effluent standard: 2 mg/L and 10 mg/L, respectively. Cd and Pb levels have also sometimes exceeded the standard 0.03 mg/L and 0.1 mg/L, respectively. The pH value was also lower than the effluent standard (5.8–8.6).

Synthetic AMD was prepared based on the characteristics of the actual AMD. The chemical composition of the synthetic AMD and Japan effluent standards for metals are shown in Table 5.1

Table 5.1 The chemical composition of AMD (Avg $\pm$ 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 |

| | Synthetic AMD | | Real AMD (mg/L) | Effluent standard (mg/L) |
|---|---|---|---|---|
| | Reagents | Concentration (mg/L) | | |
| 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 | Stock solution | 0.40±0.06 | 0.30±0.10 | < 100 |
| pH | | 3.70±0.40 | | 5.8-8.6 |

### 5.2.2 Substrates

Oyster shells for aquariums (O-O-Company Fukuoka, Japan) were crushed and sieved through a 6–10 mm screen (porosity 51%, density 0.71 g/cm$^3$). Limestone (Konan Shoji Co., Ltd., Tokyo, Japan) was used to compare with oyster shells (8–9 mm, porosity 44%, density of 1.42 g/cm$^3$). Loamy soil (akadama, Hirota Shokai, Co., Japan) was used to cover the top layer of CWs. This amount was insignificant and was ignored in the calculation.

### 5.2.3 Batch-experiments for neutralizing AMD using OS and limestone

In a 300 mL-Erlenmeyer flask, 200 mL of synthetic AMD and 200g pieces of limestone or oyster shell were placed. Then they were shaken on a rotary shaker at 70 rpm and 27°C for 24 hours. The change of pH was measured by using a pH meter with a data logger (CUSTOM, IWC-6SD, Taiwan). The water samples of 1.0 mL were collected at pH 3, 4, 5, 6, 7, and 8. The samples were filtered through 0.45 µm filter paper for measuring the metals.

### 5.2.4 Lab-scale CWs setup and operation

Lab-scale CWs were installed in a greenhouse at Ritsumeikan University in Kusatsu City, Shiga Prefecture, Japan in November 2020–July 2021. For two months from May to June 2021 in

the 7-month experiment, CWs were kept by being filled with tap water because the campus was under the State of Emergency for the COVID-19 pandemic.
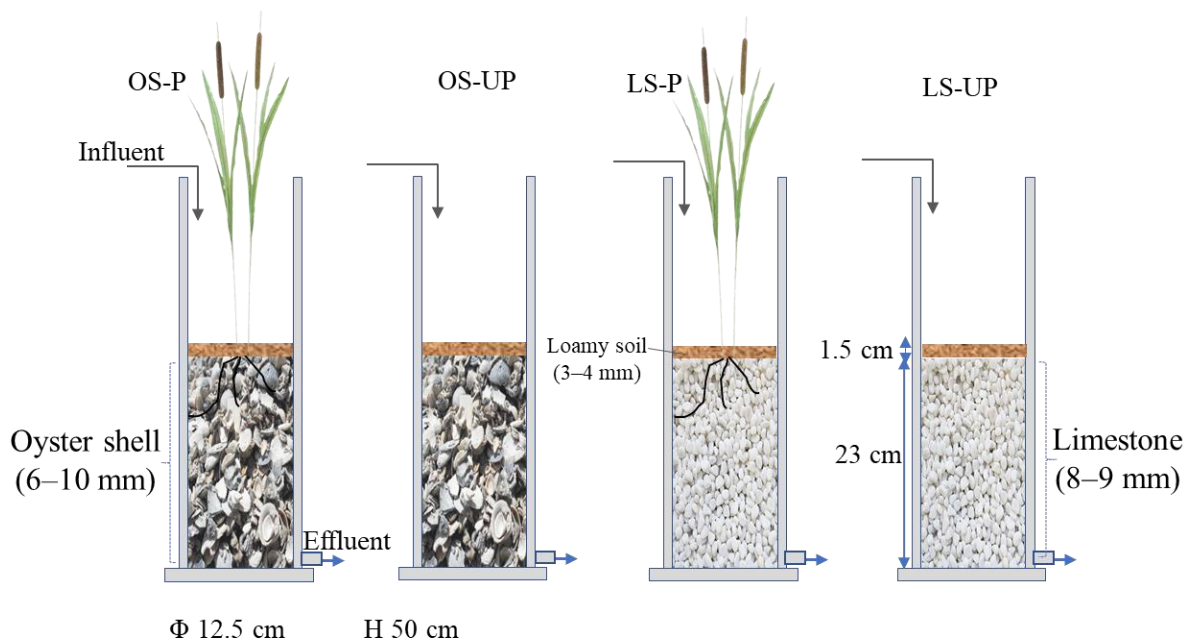


Figure 5.1 Diagram of lab-scale CWs

A schematic of the experiment setup is illustrated in Figure 5.1. Each CW consists of a plastic container (ID 12.5 cm, H 50 cm) packed with OS pieces (23 cm depth, 2 kg) or limestone (23 cm depth, 4 kg). On 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 cm shoot, 6 ± 1 cm root, 168 ± 4.4 g-wet/plant) were planted to CWs (OS-P; LS-P), or were left unplanted (OS-UP; LS-UP). These cattails were bought from Tojaku Engei Co. Ltd., Joyo, Kyoto, Japan. Before planting to the CWs, the roots were washed gently using tap water for removing original culture soil. All CWs were conducted in duplicate. A weather recorder (TR-7Ui, T&D Corp, Japan) was placed in the greenhouse to record daily radiation and air temperature. Before suppling AMD, all CWs were kept filled with tap water for one week.

In sequencing batch treatment for 27 times, one liter of actual or synthetic AMD was fed to each column. Actual AMD was supplied in the batch number 5, 6, 7, 25, 26 and 27. Other batch treatment was run with the synthetic AMD. After the defined hydraulic retention time (HRT), the

treated AMD was fully discharged from the bottom of the CWs. Then, fresh AMD was fed again to each CW. This sequencing batch treatment was operated with HRT of 7 days.

### 5.2.5 Sampling and analysis

Influent and effluent samples were collected 27 times, whereas substrate and plant samples were collected before and after 7 months of operation. In total, there were 243 water samples, 10 substrate and 10 plant samples. All water, substrate and plant samples were transferred to the civil and environmental engineering laboratory at Ritsumeikan University for analysis.

In the laboratory, the water samples were centrifuged for 10 minutes at 3000rpm for measuring the metals (Zn, Cd, Fe, Cu, Mn, Fe, As) using inductively coupled plasma spectroscopy (ICP-OES 700 series, Agilent Technologies Japan, Ltd., Tokyo, Japan). Other water parameters were measured as described in the 4.2.4 section.

Regarding the substrate and plant samples after being collected, they were treated and analyzed as in the 4.2.4 section. All experiments were implemented in double to record the mean. Heterotrophic bacteria and sulfate reducing bacteria (SRB) were enumerated using the plate-count technique with R2A agar medium and Postgate's medium F (as described in the 3.2.3 section).

## 5.3 Results

### 5.3.1 pH neutralization of materials in the batch experiment

The Figure 5.2 shows the pH changes and the metal concentrations against the pH values in the AMD neutralization test by oyster shell and limestone for 24 hours. The oyster shell pieces exhibited the higher neutralization rate than that of limestone. The pH value in the AMD shacked with oyster shell reached 7 for one hour, and 8 for about 3 hours, then kept stably for 24 hours. While pH value in the AMD shacked with limestone reached 7 for 9.5 hours and gradually 8 after 23 hours.

Concentrations for all soluble metals decreased with the increase of pH of the AMD shaken with oyster shell and limestone as expected from the solubility product of metal hydroxides. Over pH levels of 7.8–8.1, the Zn concentration in the AMD was lower than the Japan effluent standard (2 mg/L). The soluble Fe concentration (0.2–3 mg/L) in the AMD was far below the standard (10 mg/L) over pH levels of 5 with formation of orange precipitation. Removals for the other metals in the AMD were more than 90% over the pH level of 8, except for Mn (41–47%).

81
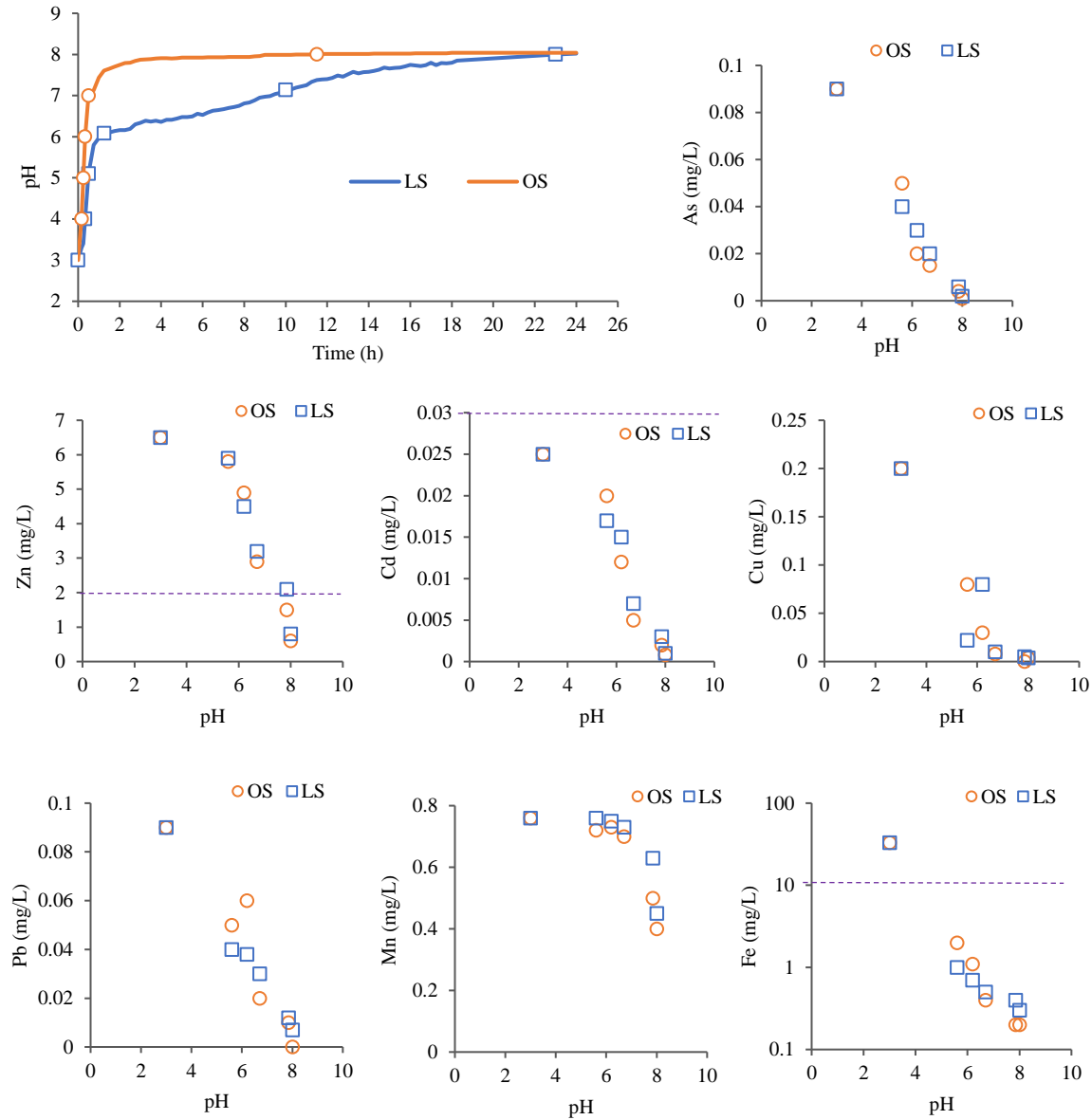
Figure 5.2 The neutralization rate of oyster shell and limestone for 24 hours, and the relationship between pH and metal concentrations.

### 5.3.2 Environmental condition in greenhouse and water parameters

The air temperature inside the greenhouse where the lab-scale CWs set up is displayed in Figure 5.3. The average temperature in the late autumn (Nov 2020), winter (Dec 2020–Feb 2021), spring (Mar–May 2021), and summer (Jun–Jul 2021) were 15.2±6.2, 8.3±7.6, 18.3±10.0, 27.0±7.5°C, respectively. In winter the aboveground part of cattails withered, while the roots were still alive. The young shoots started to grow in spring, and extended until summer.
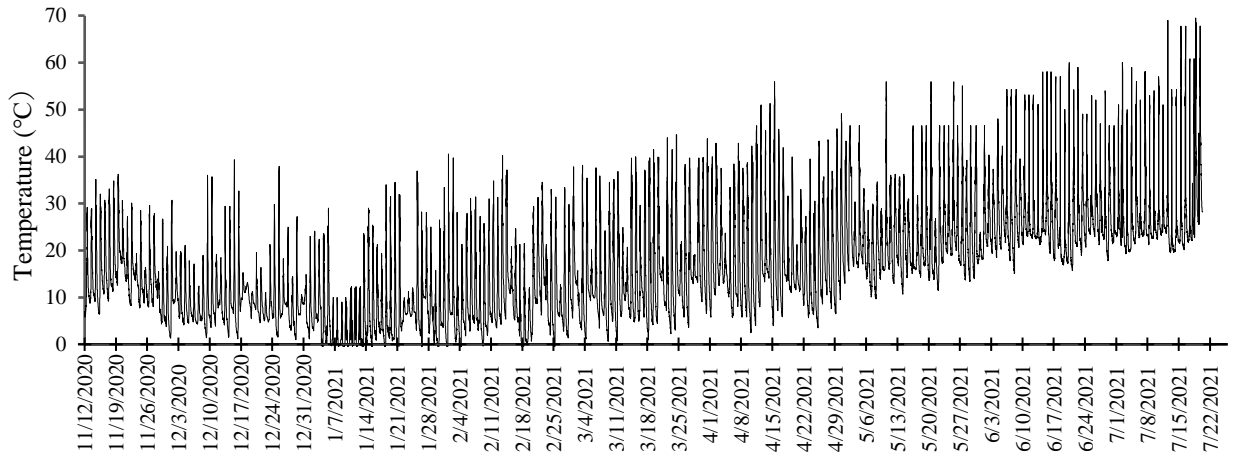
Figure 5.3 Temperature at green house during 7 months of operation
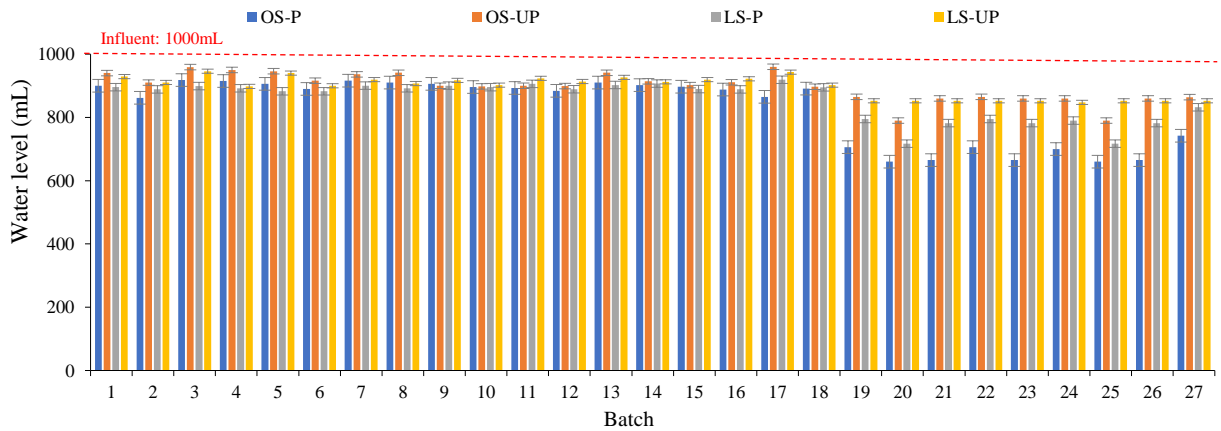


Figure 5.4 The difference between inlet and outlet of water volume during the experiment

Input and output of the water volume in the CWs in the sequencing batch treatment is shown in Figure 5.4. Although evapotranspiration estimated from difference of input and output was insignificant in late autumn and winter, high evapotranspiration in hot summer with rapid plant growth contributed significantly to the mass removal efficiency for heavy metals in summer, as shown in Eq 4.1. Table 5.2 shows the average evapotranspiration through the experiment. It reached 8.4–11.5% and 12.5–20.4% in the planted and the unplanted CWs, respectively.

Table 5.2 Evapotranspiration and water parameter of influent and effluent during the experiment (Avg $\pm$ SD)

| | 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) Evaporation (%) | 1 | 0.87±0.03 (14.0±5.5) | 0.92±0.03 (8.4±3.8) | 0.88±0.01 (12.5±2.6) | 0.91±0.01 (9.2±1.8) | 1 | 0.83±0.10 (20.4±15.2) | 0.85±0.10 (11.5±6.9) | 0.87±0.03 (16.7±7.8) | 0.89±0.03 (11.3±4.7) |
| PH | 3.70±0.40 | 7.21±0.29 | 7.30±0.28 | 7.26±0.27 | 7.35±0.35 | 3.20±0.20 | 7.02±0.29 | 7.10±0.27 | 7.08±0.38 | 7.07±0.52 |
| ORP (mV) | 185.0±9.9 | 75.5±38.2 | 79.0±31.9 | 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) | 9.23±0.28 | 8.2±2.7 | 9.8±2.9 | 7.6±1.4 | 9.5±0.9 | 0.4±0.2* | 9.3±3.42 | 13.1±4.2 | 8.9±2.9 | 10.9±2.42 |
| SS (mg/L) | | 29.1±19.6 | 28.6±17.7 | 30.4±17.6 | 24.9±118.2 | | 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 | 320.8±56.5 | 238.8±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) Removal (%) | 236.1±22.1 | 216.0±29.8 (8.5±11.5) | 206.2±32.5 (12.5±12.8) | 200.2±22.9 (15.2±9.3) | 218.6±18.7 (7.4±8.2) | 236.5±9.5 | 190.5±24.4 (19.4±12.7) | 193.5±42.2 (18.8±17.4) | 205.0±40.3 (13.1±18.9) | 228.0±36.0 (3.9±15.5) |

*DO was controlled less than 0.5 mg/L to avoid metal oxidization

The average values of pH value, ORP, DO, TDS, and sulfate concentrations before and after the treatment with the actual and simulated AMD are also shown in Table 5.2.

The influent pH values ranged between 3.2 and 4.1 for both real and synthetic AMD. All effluent pH values reached 6.9–8.3. Although the influent DO concentration of synthetic AMD was 8.3–9.7 mg/L, it of actual AMD was controlled less than 0.5 mg/L to avoid metal oxidization. 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. The low DO level was found in the planted CWs in late spring and early summer, implying the DO consumption of roots and rhizosphere. The TDS concentration in oyster shell-based CWs were higher than those of limestone-based CWs. The ORP values generally were positive and dropped in late spring and summer. The SS concentration was similar in all CWs for both actual and synthetic AMD treatment. The sulfate concentrations generally were reduced after treatment, especially in the planted CWs.

### 5.3.3 Overall performance of CWs for heavy metal removal

The concentration of heavy metals in influent and effluent from both actual and synthetic AMD, and the average mass heavy metal removal efficiencies are presented in Figure 5.5 and Table 5.3, respectively. The metal concentrations in real AMD collected 6 times were actually fluctuated. Overall, after treatment, heavy metals were removed effectively by all CWs from both actual and synthetic AMD. The removal trend was similar in both actual and synthetic AMD. These mass removal efficiency in oyster shell-based CWs were higher than limestone-based CWs, except for Cu.

Table 5. 3 Mass removal efficiency (%) for heavy metals from simulated and real AMD by using CWs filled with oyster shell and limestone during the experiment (Avg ± SD)

| | 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 |

Among the investigated metals, Fe showed the highest concentration during the experiment. The Fe level in both actual and synthetic AMD (38 mg/L, in average) was much higher the effluent standard (10 mg/L). Fe was removed successfully by all CWs with the removal efficiency of 99.2–99.9% in all CWs.

The average Zn concentration in synthetic and actual AMD were, respectively, 7.2 and 7.6 mg/L, which were approximately four-fold higher than the effluent standard (2 mg/L). Zn was well removed from both actual and synthetic AMD by all CWs. Its concentration in effluent was 0.00–0.25 mg/L in oyster-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. Cd and Pb in synthetic AMD were, respectively, 0.02–0.09 mg/L, and 0.06–0.41 mg/L. Those in actual 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, those accounted for 56.0–100% for both actual and synthetic AMD.

The average As, Cu and Mn concentration in actual and synthetic AMD were, respectively, 0.06, 0.20, and 0.99 mg/L, which were lower than the standard: 0.1, 3.0 and 10 mg/L, respectively. Those were also removed well by all CWs with the average mass removal of over 90 % for As, 97 % for Cu and 76 % for Mn in all CWs.
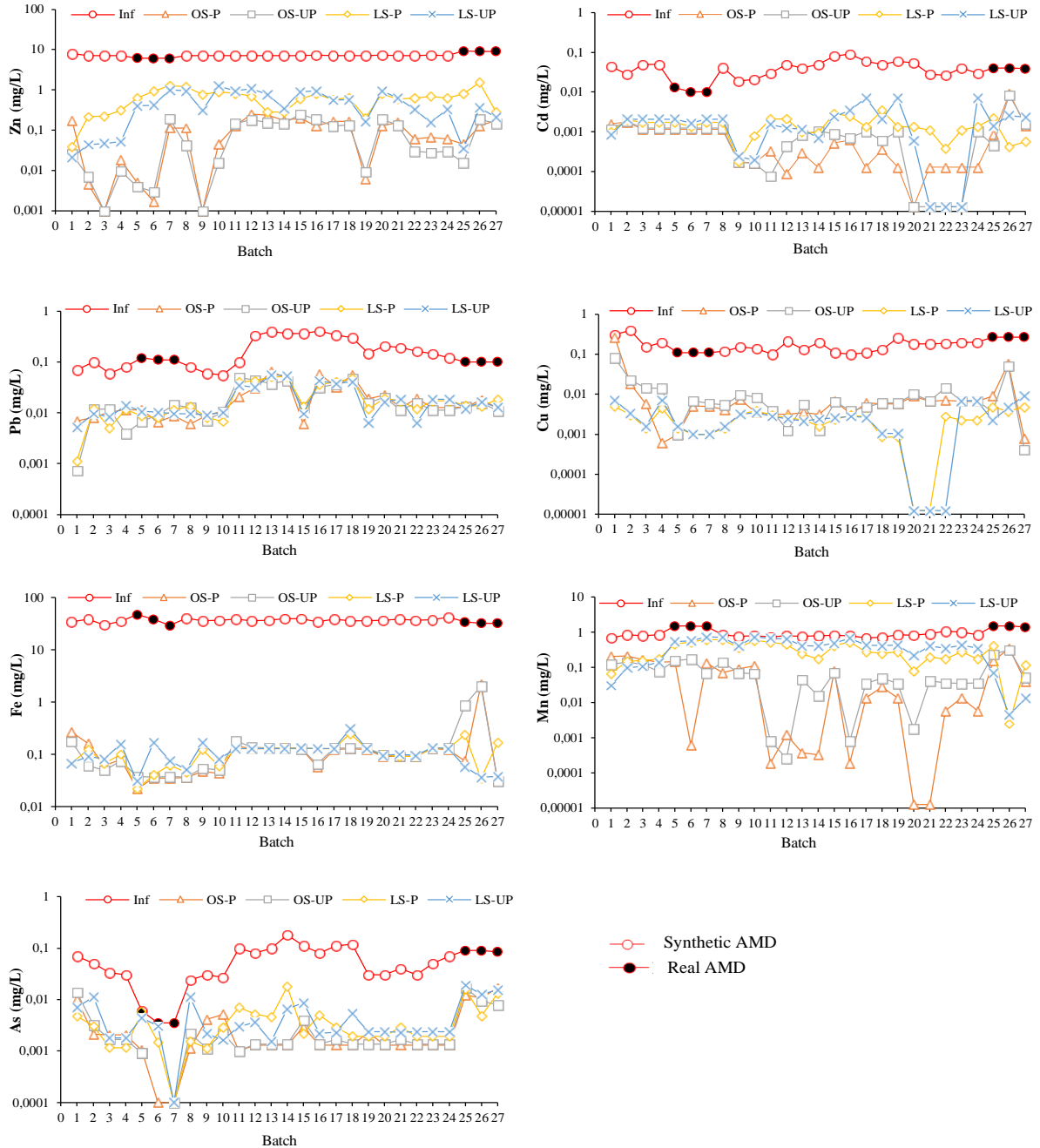


Figure 5.5 Heavy metal concentrations in influent and effluent from actual and synthetic AMD during the experiment

### 5.3.4 Accumulation of heavy metal in substrates

The metal contents in the substrates in the CWs before and after treatment are shown in Table 5.4. At the end of the experiment, the total amounts of heavy metals accumulated in each substrate is also shown in Table 5.6. In general, the contents for all heavy metals in the substrates increased in all CWs during 7 months of operation. Its accumulation in oyster shell was higher than that of limestone, except for Mn.

Table 5.4 Heavy metal concentrations in substrates and cattails before and after the experiment

| | Substrate | | | | | | Cattail | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Oyster shell-based CW | | | Limestone-based CW | | | Control | | Oyster shell-based CW | | Limestone-based CW | |
| | Control | OS-P | OS-UP | Control | LS-P | LS-UP | Above-ground | Below-ground | Above-ground | Below-ground | Above-ground | Below-ground |
| As (mg/kg) | 0.21 | 0.86 | 0.95 | 0.24 | 0.57 | 0.61 | 3.01 | 5.36 | 3.50 | 6.38 | 3.65 | 6.21 |
| Zn (mg/kg) | 150.00 | 221.00 | 244.50 | 23.20 | 57.30 | 69.00 | 102.20 | 158.00 | 247.60 | 463.40 | 178.50 | 456.90 |
| Cd (mg/kg) | 0.02 | 0.40 | 0.54 | 0.02 | 0.20 | 0.27 | 2.01 | 4.12 | 2.09 | 5.80 | 2.11 | 6.01 |
| Cu (mg/kg) | 14.00 | 15.10 | 16.30 | 2.09 | 2.78 | 3.20 | 86.23 | 104.80 | 87.10 | 106.70 | 87.20 | 106.20 |
| Pb (mg/kg) | 2.60 | 4.46 | 4.66 | 2.70 | 3.62 | 3.69 | 3.01 | 12.00 | 5.10 | 13.60 | 3.98 | 13.40 |
| Mn (mg/kg) | 87.00 | 96.70 | 98.85 | 25.40 | 28.90 | 29.45 | 236.20 | 211.50 | 238.00 | 214.60 | 240.20 | 217.90 |
| Fe (mg/kg) | 566.00 | 938.50 | 1050.20 | 346.50 | 516.05 | 591.25 | 734.00 | 5787.87 | 798.20 | 6887.10 | 801.20 | 7241.10 |

Among the investigated metals, Cd showed the highest accumulation in the substrates. Its content in both original oyster shell and limestone was 0.02 mg/kg. After treatment, Cd content increased 20–27 times in the oyster shells and 10–14 times in the limestone. Before use in the CWs, the Zn, Cu, Pb, Fe, Mn and As content were, respectively, 150.0, 14.0, 2.6, 566.0, 87.0 and 0.2 mg/kg in oyster shells, and were, 23.2, 2.1, 2.7, 346.5, 25.4 and 0.24 mg/kg in limestone. After use in the CWs, those content increased 1.1–4.5 times in oyster shells and 1.2–3.0 times in limestone.

### 5.3.5 Accumulation of heavy metals in cattail and bacteria community

During the experiment, the top height of cattails increased to 88–129 cm, and the roots expanded to 28–32 cm in the planted CWs. Cattails were harvested at the end of the experiment in all of about 163 g-dry from each planted CW, including about 23 g-dry of the aboveground part and 140 g-dry of the belowground part. The heavy metal concentrations in biomass before and

after being used in CWs were shown in Table 5.4. The metal amounts in cattails increased significantly during the experiment, especially in the belowground part. The Zn, Cu, Fe, and Mn concentrations, as essential elements, showed higher levels than As and Cd in the plant biomass.

After 7 months of operation, the amounts of Zn, Cd, Cu, Pb, Fe, Mn and As accumulated in the plant biomass were, respectively, 48.72; 0.30; 2.17; 0.44; 230.40; 4.74 and 0.24 mg in the OS-P CW. Those were, respectively, 45.00; 0.33; 2.03; 0.40; 286.94; 4.83 and 0.22 mg in the LS-P CW.

Table 5.5 BCF and TF values of cattail in the planted CWs after the experiment

| | 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 |

The BCF and TF values for the metals in the planted CWs are presented in Table 5.5. The BCFs values for all investigated heavy metals were in a range of 3.22–69.57 in all the planted CWs, implying the ability of cattails in the uptake of heavy metals from the substrate. The higher BCF values were obtained in the LS-P CW.

In this study, the contents of metals in the shoots were much lower than those of roots, except for Mn. This implies that the belowground parts of cattail were mainly responsible for heavy metal phytoextraction. The TF values were 0.11–1.11 in total metals.

The population of heterotrophic bacteria ($9\times10^6$–$8\times10^7$ CFU/dried-mg) found from the substrate in the planted CWs was higher than that of in the unplanted CWs ($4\times10^5$–$5\times10^6$ CFU/dried-mg), a large population of SRB ($2\times10^2$– $4\times10^3$ CFU/g-dry) was only detected in the

planted CWs. Those imply that the roots of cattail play an important role in creating a favorable condition for the organisms' growth.

### 5.3.6 Mass balance study

In the present study, heavy metal from the actual and synthetic AMD can be classified into losses through substrate, plant uptake and other processes. After 7 months of operation, the total amounts of metals fed to each CW were 197.6 mg for Zn, 1.09 mg for Cd, 4.91 mg for Cu, 4.62 mg for Pb, 986.1 for Fe, 26.2 mg for Mn, and 1.7 mg for As. The amounts of heavy metals distributed to each component are shown in Table 5.6.

As is illustrated in table 5.6, accumulation in the substrate was a principal pathway for heavy metal removal, 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 all CWs. In the planted CWs, In the planted CWs, accumulation in the cattail 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. Other processes were the smallest among all the heavy metal removal routes, it only accounted for 0.1–3.5% for the planted CWs and 0.4–8.1% for the unplanted CWs in all metals.

Table 5.6 Distribution of heavy metal (mg) in each part during the experiment

|  |  | Effluent | Substrate | Cattail | Other |
|---|---|---|---|---|---|
| As (mg) | OS-P | 0.07 | 1.30 | 0.24 | 0.06 |
|  | OS-UP | 0.07 | 1.48 |  | 0.12 |
|  | LS-P | 0.10 | 1.32 | 0.22 | 0.03 |
|  | LS-UP | 0.12 | 1.48 |  | 0.07 |
| Zn (mg) | OS-P | 2.35 | 142.00 | 48.72 | 4.54 |
|  | OS-UP | 2.12 | 189.00 |  | 6.48 |
|  | LS-P | 15.17 | 136.40 | 45.00 | 1.03 |
|  | LS-UP | 12.50 | 183.20 |  | 1.90 |
| Cd (mg) | OS-P | 0.02 | 0.76 | 0.30 | 0.01 |
|  | OS-UP | 0.02 | 1.03 |  | 0.04 |
|  | LS-P | 0.04 | 0.72 | 0.33 | 0.01 |
|  | LS-UP | 0.05 | 1.00 |  | 0.04 |
| Cu (mg) | OS-P | 0.40 | 2.20 | 2.17 | 0.13 |
|  | OS-UP | 0.27 | 4.60 |  | 0.04 |
|  | LS-P | 0.06 | 2.76 | 2.03 | 0.06 |
|  | LS-UP | 0.07 | 4.44 |  | 0.40 |

|  |  | Effluent | Substrate | Cattail | Other |
|---|---|---|---|---|---|
| Pb (mg) | OS-P | 0.47 | 3.70 | 0.44 | 0.01 |
|  | OS-UP | 0.47 | 4.11 |  | 0.04 |
|  | LS-P | 0.49 | 3.66 | 0.40 | 0.08 |
|  | LS-UP | 0.48 | 3.96 |  | 0.18 |
| Mn (mg) | OS-P | 1.70 | 19.40 | 4.74 | 0.34 |
|  | OS-UP | 1.86 | 23.70 |  | 0.62 |
|  | LS-P | 7.33 | 14.00 | 4.83 | 0.02 |
|  | LS-UP | 9.56 | 16.20 |  | 0.42 |
| Fe (mg) | OS-P | 3.81 | 745.00 | 230.40 | 6.83 |
|  | OS-UP | 4.21 | 968.40 |  | 13.44 |
|  | LS-P | 2.59 | 678.20 | 286.94 | 18.31 |
|  | LS-UP | 2.70 | 979.00 |  | 4.35 |

## 5.4 Discussion

### 5.4.1 The relationship between pH changes and metal removal efficiencies

Oyster shell demonstrated comparable or higher potentials to limestone for pH neutralization and metal removal from AMD. The greater heavy metal removal efficiency was observed at the higher pH.

Crushed oyster shell and limestone in CWs also showed a high neutralization and metal accumulation capabilities of AMD treating operations. With main component containing more than 90% calcium carbonate, limestone and oyster shell has a merit in generating ion hydroxides leading to neutralization of acidic wastewater (Yao et al., 2014). Beside this, crushed oyster shell could release to carbonate species, such as $HCO_3^-$ and $CO_3^{2-}$, these species were responsible for the formation of insoluble metal carbonates. $Ca^{2+}$ and $CO_3^{2-}$ released from the aragonite surface can also react with the fluid to form solid-solution nuclei.

Theoretically, the metal levels can be satisfied with the Japan effluent standard since the pH is over: 6.77, 7.87, 9.47, and 10.3 for Cu, Zn, Pb and Cd, respectively (Hara et al., 2021). The consistent result was obtained in this study. Except that Mn was less sensitive to the increase of pH, other heavy metals were successfully removed (>90%) at pH reached 8 in the batch experiment.

### 5.4.2 Heavy metal removal mechanism in constructed wetlands

Oyster shell and limestone in CWs showed the key role for heavy metal removal from both synthetic and actual AMD during the experiment period. The metal remained in substrate was a dominant heavy metals removal pathway (44.8–99.3%). Metals generally were trapped mainly in the substrates through absorption, precipitation, co-precipitation, and ion exchange (Yeh, 2008).

The high metal oxides content in oyster shell and limestone was advantage to support metals removal. The increase of pH leaded to the formation of metal oxides and hydroxides, and As might be removed via co-precipitation process (Allende et al., 2014).

In CWs, the plant is considered as an important design component for decontaminant (Nguyen et al., 2021). In the present study, the contribution of cattail was 8.8–44.3% to heavy metal removal. Cattail might accumulate heavy metal in tissues or release root exudates, which often control the mobility of metals (Saeed et al., 2021). In addition, formation of litter and biofilm layers contributes to heavy metal removal by adsorption mechanisms (Pat-Espadas et al., 2018). Besides this, the roots of cattail can provide additional surface areas for supporting microbial growth, metabolism, this was evident by the higher population of bacteria found in the planted CWs.

Together with the reduce sulfate concentration in effluent, the presence of SRB could contribute to heavy metal removal via the metal sulfide precipitation. The SRB activities might also contribute to higher heavy metal removal efficiencies in planted CWs, even when all the aboveground parts of cattails died in winter season. This finding also implies that the roots of the plant played an important role in creating favorable conditions for the growth and development of microbial communities.

### 5.4.3 Recommendations for further studies and utilizing alternative materials as filter media in CWs for heavy metal removal.

This study investigated the heavy metal removal in the CWs filled with oyster shells/limestone and planted with or without cattail from both real and synthetic AMD for 7 months. All CWs showed the high heavy metal removal, all effluent heavy metal concentrations were kept far below the Japan effluent standard during 4 seasons. The purified water can be rendered for further water reuse.

Results gained from this study demonstrated the substrate played a crucial role in removing heavy metals from both actual and simulated AMD, however, the optimal particle size has not investigated. In further studies, therefore, the particle size should be optimized to have a high performance and avoid clogging. The substrate might be gradually saturated, this was evidenced by the decrease of alkalinity in the outlet and removal efficiency during the operation. Thus, the adsorption capacity of material should be identified to estimate the CWs lifespan. Additionally, further research should examine the speciation of the pollutants in the solid phase, the change of physicochemical properties of material before and after treatment by using sequential extraction

procedures, XRD, SEM-EDX, and FTIR analysis. This information is important to fully understand the fate of the pollutants in the wetland matrix, consequently, it can strongly support designing effective CWs in removing contaminants.

The plant showed a significant contribution to heavy metal removal, especially in the case of Zn, Cu, Fe, Mn, which were essential elements for plant growth. These metals can be collected easily by harvesting aboveground part of plant biomass. In this study, SRB also was found in CWs. However, the SRB's class was not identified, and the contribution of sulfide precipitation to the overall performance was not calculated in this study. Further studies should focus on the role of SRB communities in the treatment performance of CWs. It is well known that metal sulfide precipitation is an effective way to remove heavy metals in CWs (Machemer and Wildeman, 1992; White and Gadd 1997; Wu et al., 2013). Consequently, heavy metal removal can be enhanced significantly through this mechanism by creating a favorable condition for SRB development.

Although oyster shells are by-product waste and have normally no cost, oyster shells need to be crushed before use. Further studies, therefore, should consider the real cost of media (including transport fees, crushing). Economic evaluation is also important in the operation and maintenance of CWs effectively.

In Japan, together with the long coastal, many oyster culturing areas, oyster shells are available in abundant (Hasegawa et al., 2015; Ventilla, 1984). Also, the local governments are doing efforts to reduce the cost of treatment of 80 mines drainage containing toxic metals (Ueda & Masuda, 2005) by using environmentally friendly green technologies. Thus, recycling oyster shells as filter media in CWs for mine drainage treatment can bring both economic and ecological benefits. Future real-scale CWs employing oyster shells should be installed to treat heavy metals from these mine drainages.

## 5.5 Conclusion

Both oyster shells and limestone showed a high AMD neutralization potential. Heavy metals from both actual and synthetic AMD were effectively removed by the Lab-scale CWs employing oyster shells and limestone during 7 months of operation. All effluent heavy metal concentrations were kept far below the Japan effluent standard during 4 seasons. The purified water can be rendered for further water reuse.

Accumulation in the substrates was the main pathway for heavy metal removal in CWs, followed by that in the cattail biomass in the planted CWs. The higher metal removal efficiency in the oyster shell-based CWs also demonstrated oyster shell as a promising material in CWs for heavy metal removal from AMD. The finding of this study may pave the way for recycling seashells as filter media in CWs for wastewater treatment and encourages the reduction in using natural stones such as limestone, and gravel.

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## CHAPTER 6. SUMMARY AND CONCLUSIONS

This thesis has been completed by 6 chapters.

**Chapter 1** started with the background of this study. This briefly introduced the mine polluted water and the necessity of mine drainage treatment. Additionally, this pointed out the drawback of conventional treatment methods and emphasized the advantages of using an eco-friendly technology (constructed wetland) for heavy metal removal from mine drainages as well. This chapter also gave an overview of the mine wastewater characteristics and the challenges in using CWs for heavy metal removal from mine drainages in Japan. The aim, scope, and significance of this research are also described in chapter 1. This chapter ended up with the layout of the thesis.

In **chapter 2**, the characteristic and toxicity of mine drainages were presented. The composition of mine wastewater in Japan and other countries was also reviewed. Mine drainage containing such toxic metals threatens ecosystems and human health. To protect the environment from mine polluted water, several guidelines and standards have been published/recommended for effluents discharges. Besides this, many methods have been applied for mine drainage treatment, including active and passive treatments. The active treatments such as neutralization, coagulation, sedimentation, filtration, reverse osmosis, ion exchange, and electrodialysis, have drawbacks in term of operation and maintenance cost, and need further treatments of by-products as well. Whereas passive treatments such as constructed wetlands can limit these disadvantages.

This chapter mainly focused on using CWs for heavy metal treatment from mine drainage. Metal removal mechanisms from mine drainage by using CWs were also described. In general, the whole process of metal removal in CWs is quite complex because of the supporting and interdepending of the components. To achieve a high treatment performance, it is necessary to have effective strategies in the improvement of heavy metal removal. This is mostly aimed at improving the contribution of the three main components (substrate, plant, and microbial) in CWs. For example, the substrate and vegetation should be screened carefully to enhance the adsorption and uptake capacity, respectively. Creating a favorable condition for the growth of plants and bacteria can be achieved by providing necessary compounds or inoculating specialized bacterial consortiums in CWs. Additionally, the seasonal effects on the CW system are highly recommended.

In addition, CWs are generally characterized as a cost-effective technology. To minimize the cost of the wetlands as well as achieve a sustainable mine drainage treatment, the recycling of waste or by-products as substrates in wetlands has been gaining a lot of attention from researchers.

Many materials such as sawdust, compost manure, rice husk, seashells, shrimp shell, steel slag, and fly ash, have been recycled as adsorbents for removing heavy metals, however, there have not many studies investigating recycling materials as substrates in CWs. Therefore, studies recycling by-products as filter media in CWs for mine drainage treatment are necessary.

In this chapter, the role of vegetation in CWs was reviewed. Together with the contaminant decontamination, CWs planted with the ornamental flowering plants for creating both economic value and landscapes, have tended to increase in recent years. According to research, microorganisms also play a very important role in removing heavy metals in CWs, especially in the case of substrate saturated. Thus, scientists have promoted the activity of microorganisms to enhance the treatment performance of CWs metals in many ways, such as providing other carbon sources, inoculating specialized bacteria etc.

In **chapter 3**, a pilot-scale CWs was firstly conducted at a mine drainage treatment plant of Otani mine. This mine drainage is in cluster II according to classification with weakly acidic and some kinds of metals slightly exceeding the effluent standard. This study was investigated with two kinds of CWs planted or unplanted with cattail for 3.5 months, with the hydraulic retention time at 3.8-1.2 days. There was $13m^3$ of mine wastewater was treated in each CW during the experiment. Results showed that the short HRT of 1.2 days in the CWs was sufficient to achieve the effluent standard for Cd (0.03 mg/L). The planted CW showed higher metal removal efficiency than unplanted CW. Heavy metals were mainly accumulated in upper soil layer in both systems, and these accumulation in planted CW was higher than unplanted CW. Cattail accumulated heavy metal through the uptake and the adsorption of roots process, all studied heavy metal concentration accumulated in belowground parts were higher than those in aboveground parts. Translocation factor of all heavy metal was less than 1, while bioconcentration factor was higher than 1. Bacteria detected in soil sample from planted CW was more diverse than unplanted CW, whereas it was lower in water samples. Together with the reduction of sulfate concentration, sulfate reducing bacteria was predominant in soil sample from the planted CW, also demonstrated the enhancement of heavy metal removal by anaerobic microorganisms surround the root of plants.

In brief, this present study showed that heavy metal can be removed effectively by constructed wetlands, the higher removal for heavy metal in the planted CW suggests important roles of vegetation in phytofiltration, plant uptake, and oxidation/reduction in the rhizosphere. These results encourage the spreading of CWs for mine drainage treatment in Japan.

In **chapter 4**, a lab-scale experiment was conducted to evaluate the feasibility of clamshells as a substrate in constructed wetlands (CWs) for removing heavy metals from AMD. Synthetic AMD was simulated according to the Obanazawa mine drainage which was classified to cluster VIII with low pH of 3-4 and high concentration of Zn and Pb. This study was investigated with two kinds of CWs planted with cattail, the one was filled with by-product (clamshell) and the other one was filled with conventional material (gravel). The experiment was operated under hydraulic retention times of 7 days in Phase I, 4 days in Phase II, 2 days in Phase II, and 7 days in Phase IV for 6 months. The results indicated that clamshell showed the high feasibility and effectiveness as being used as a substrate in CWs for removing heavy metals from AMD. The dominant $CaCO_3$ content in clamshells has the benefit of neutralization of acidic wastewater. The effluent pH values in clamshell-based CWs were higher than in gravel-based CWs. The Zn, Cd, Pb and Fe were almost completely (> 93%) removed by clamshell-based CWs during four Phases. The metal removal efficiency was found to be lower for shorter HRT, indicating adsorption as an important mechanism for removing heavy metals. The metal-removal capacity of the substrates saturated gradually according to the lower metal removals in Phase IV than in Phase I with the same HRT. This study also demonstrated the significant contribution of vegetation in heavy metal removal. Metals uptake by plants up to 16.1–39.0%. In addition, clamshell-based CWs planted with cattail showed a better favorable condition than gravel-based CWs in the growth of SRB which promoted metal removal through metal sulfide precipitation.

Higher metal removal in clamshell-based CWs than in gravel-based CWs demonstrated clamshells as a potential wetland substrate for removal of heavy metals from AMD. This encourages recycling seashells for wastewater decontamination. By that way, it might contribute to reducing solid waste in a simple, cheap, and eco-friendly way.

In **chapter 5**, another lab-scale experiment was investigated to evaluate the applicability of CWs employing oyster shell and limestone on heavy metal removal from Fukuchiyama mine drainage. This mine drainage was classified to cluster III with low pH around 4, has 2- and 3-times higher concentration for Zn and Fe, respectively, than effluent standard values. Synthetic and actual AMD containing 7.3 mg/L of Zn, 38.0 mg/L of Fe and other minerals (pH=4.0) which was fed to the CWs (1 L/column) under a hydraulic retention time of 7 days in a sequencing batch mode. Results showed that both oyster shell and limestone had the high AMD neutralization ability. During the 7 months of operation, all metal concentrations in effluents were satisfied well with

effluent standard. CWs demonstrated high efficiencies for removing metals from both the actual and synthetic AMD. Both oyster shell and limestone showed the key role for heavy metal removal during the experiment period. The metal remained in substrate was a dominant heavy metals removal pathway (44.8–99.3%). Metals generally were trapped mainly in the substrates through absorption, precipitation, co-precipitation, and ion exchange. The high metal oxides content in oyster shell and limestone was advantage to support metals removal. The increase of pH leaded to the formation of metal oxides and hydroxides, and As might be removed via co-precipitation process.

In the planted CWs, the plants also showed a significant contribution to heavy metal removal, especially in the case of Zn, Cu, Fe, Mn, which were essential elements for plant growth. Together with reduction of the sulfate concentration, the presence of SRB can contribute to heavy metal removal through metal sulfide precipitation processes.

The higher metal removal efficiency in the oyster shell-based CWs also demonstrated oyster shell as a promising material in CWs for heavy metal removal from AMD. The finding of this study may pave a way in recycling seashells as filter media in CWs for wastewater treatment and encourages the reduction in using natural stones such as limestone, gravel. Future real-scale CWs employing oyster shells should be installed to treat heavy metals from AMD.

In **chapter 6**, the major findings of this work were summarized. In this study, a pilot and lab-scale CW and two lab-scale CWs were investigated for heavy metal treatment from different mine drainages. All CWs showed high metal treatment performance during the experiment period. However, the lab-scale CWs have limitations in operational conditions such as only conducting in sequencing batch modes, having different buffer zone, and pre-existent microorganisms in wastewater. Besides this, to avoid clogging in CWs, the particle size of wetland substrates should be selected carefully. The life span of CWs should also be estimated.

The small-scale and lab-scale experiments are crucial steps for scaling up and designing practical CWs for heavy metal removal from mine drainages. Further studies should be investigated in the practical condition based on the valuable information obtained from this study.

**APPENDIX**

**RESEARCH OUTCOMES**

LIST OF PUBLICATIONS

1. Nguyen, T. T., Soda, S., Kanayama, A., Hamai, T., 2021. Effects of Cattails and Hydraulic Loading on Heavy Metal Removal from Closed Mine Drainage by Pilot-Scale Constructed Wetlands. Water, 13(14), 1937. https://doi.org/10.3390/w13141937.

2. Nguyen, T. T., Huang, H., Nguyen, T. A. H., and Soda, S., 2022. Recycling clamshell as substrate in lab-scale constructed wetlands for heavy metal removal from simulated acid mine drainage. Process Safety and Environmental Protection. https://doi.org/10.1016/j.psep.2022.04.026.

3. Nguyen, T. T., Soda, S., Kanayama., A., 2022. Removal of heavy metals from acid mine drainage by lab-scale constructed wetlands filled with oyster shells and limestone. Water. (In preparation).

4. Nguyen, T. A. H., Ngo, H. H., Guo, W. S., Nguyen, T. H. H., Soda, S., Vu, N. D., Nguyen. T. T., Pham, T. T., 2020. White hard clam (*Meretrix lyrata*) shells media to improve phosphorus removal in lab-scale horizontal sub-surface flow constructed wetlands: performance, removal pathways, and lifespan. Bioresource technology, 312, 123602.

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6. Soda, S., Sasaki, R., Nguyen, T.T., Hayashi, K., Kanayama, A., 2021. A Laboratory Experiment System for Developing Mine Drainage Treatment Technologies Using Constructed Wetlands—Sequencing Batch Treatment of Cd-Containing Neutral Mine Drainage—. Resources Processing, 67(3), 111–116.

7. Hara, T., Kawamoto, K., Fukuda, K., Nakagawa, I., Nguyen, T. T., and Soda, S., 2021. Trials on neutralization and metal removal for acid mine drainage utilizing lab-scale

constructed wetlands packed with limestone and charcoal. J. Water Waste, 63, 437–443. (in Japanese).

8.  Soda, S and Nguyen, T.T., 2022. Classification of mine drainages in Japan based on water quality for considering application of constructed wetland treatments (in preparation).

## CONTRIBUTIONS TO SCIENTIFIC FORUMS

1.  Nguyen. T. T., Nguyen, T. A. H., Sato. K., Vu. N. D., Nguyen. T. H. H., Nguyen. M. K., Le. V., 2019. Applicability of white hard clam (*Meretrix lyrata*) shell as filter media in constructed wetlands to enhance phosphorus removal from swine wastewater (Oral presentation). *Green Technologies for Sustainable Water (GTSW) 2019 conference*. Ho Chi Minh city, Vietnam. 1–5 December, 2019.

2.  Nguyen. T. T., Soda. S., Kanayama. A., Hamai. T., 2020. The Effect of Vegetation on Heavy Metal Treatment from Neutral Mine Drainage by Using Pilot-Scale Constructed Wetlands (Oral presentation). *The Water and Environment Technology Conference 2020.* Japan. 7–8 November, 2020.

3.  Nguyen. T. T., Soda. S., Kanayama. A., Hamai. T., 2020. Treatment performance of pilot-scale constructed wetlands on heavy metal removal from neutral mine drainage: The role of vegetation (Oral presentation). *The 57th Environmental Engineering Research conference*. Japan. 9–11 December, 2020.

4.  Nguyen. T. T., Huang. H., Nguyen. T. A. H., Soda. S., 2021. Heavy Metal Removal from Simulated Acid Mine Drainage Using Clamshell- and Gravel-Filled Lab-scale Constructed Wetlands (Oral presentation). *The Water and Environment Technology Conference Online 2021(WET 2021)*. Japan, 11–12 August, 2021.

5.  Nguyen. T. T., Soda. S., Kanayama. A., 2021.  Removal of heavy metals from acid mine drainage by lab-scale constructed wetlands filled with oyster shells and limestone (Oral presentation). *International Conference on Challenges in Environmental Science*

& *Engineering. The 14th CESE Conference 2021*. Melbourne, Australia. 6–7 November, 2021.

6. Nguyen. T. T., Huang. H., Soda. S., Kanayama. A., 2021. Removal of Zinc from Acid Mine Drainage by Lab-Scale Constructed Wetlands (Oral presentation). *The 58th Environmental Engineering Research Forum / Public Symposium.* 16–18 November, 2021.

7. Nguyen. T. T., Huang. H., Nguyen. T. A. H., Soda. S., 2021. Treatment of Heavy Metals in Simulated Acid Mine Drainage Using Lab-scale Constructed Wetlands Filled with Clamshell and Gravel (Oral presentation). *VANJ conference 2021*. 4–5 December, 2021.

8. Nguyen, T. T., Huang, H., Soda, S., 2022. Removal of multiple heavy metals from simulated acid mine drainage mixed with domestic wastewater using lab-scale constructed wetlands planted with iris and cattail. *The Water and Environment Technology Conference Online 2022 (WET 2022).* Japan, 9–10 July, 2022.

AWARDS

1. 2019 The first-place oral presentation award winner at Green Technologies for Sustainable Water (GTSW) 2019 conference. Dec 3, 2019.

2. 2020 The fighting-spirit award in research proposal contest 2020 at Risumeikan University.

3. 2021 The excellent presentation award at the Water and Environment Technology Conference. August 11, 2021.

4. 2021 The best poster presentation award at VANJ conference 2021. Dec 5, 2021.

5. 2022 Grant Kokusaiteki scholarship for one month doing oversea research in Vietnam (3 February - 3 March, 2022).