Thi Thuong Nguyen. 2025. Chapter 5: Constructed Wetlands Planted with Iris for Mine Drainage Treatment: Effects of Domestic Wastewater Feeding on the Removal of Multiple Heavy Metals. In Thi Thuong Nguyen (ed.), *New Challenges in Constructed Wetlands for Sustainable Wastewater Treatment: Intensification Strategies Based on Asian Experiences*. Osaka: Asia-Japan Research Institute, Ritsumeikan University. ISBN 978-4-910550-06-0

# Chapter 5
# Constructed Wetlands Planted with Iris for Mine Drainage Treatment: Effects of Domestic Wastewater Feeding on the Removal of Multiple Heavy Metals

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## 1. Introduction

In my presentation, I would like to introduce a case study on the utilization of constructed wetlands (CWs) for the removal of heavy metals from acid mine drainage (AMD) in Japan. First, I will provide a brief overview of the mining and metal pollution situation in Japanese mines. Japan boasts a rich mining heritage, having once been a premier global metal producer, contributing significantly to its wealth generation. However, this has also had negative impacts on the environment and human health. One of the prominent health risks associated with mining operations is the Itai-Itai disease, caused by exposure to cadmium (Cd). It is regarded as one of the top four pollution-related diseases in Japan. Since the 1970s, factors such as mineral reserve depletion, rising labor expenses, the liberalization of mineral resource imports, and environmental concerns have resulted in the closure or abandonment of the majority of mines in Japan. Nonetheless, the ongoing presence of mine drainage containing elevated levels of heavy metals, sulfates, and acidic pH remains a considerable hazard to both the ecosystem and human well-being. Presently, mine wastewater pollution is documented at 450 of the 7,000 nationwide. Among these, about 100 sites exhibit elevated levels of toxic heavy metals necessitating treatment before discharge (Nguyen et al. 2021; Ueda and Masuda 2005). The pH and ratio of metal concentrations to the effluent standards in Japan from 2014 to 2016 are presented in Figure 1.

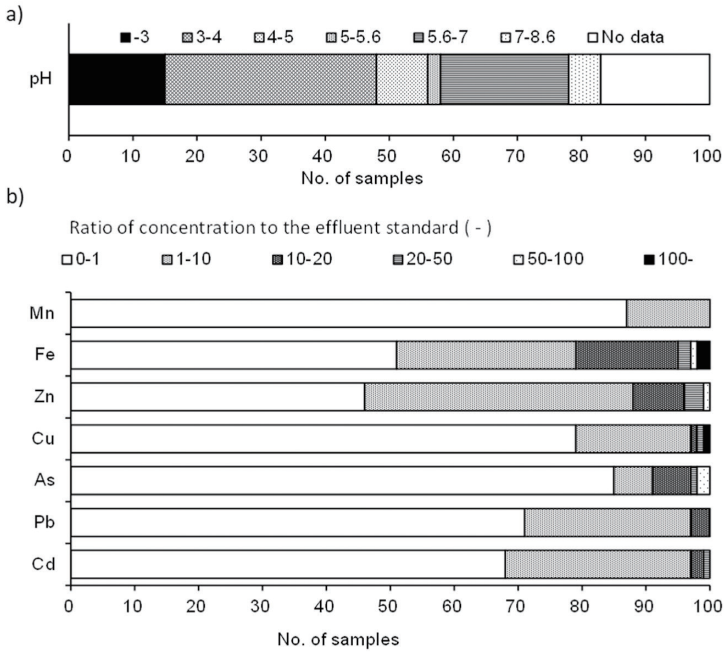


Figure 1: The average characteristics of 100 mine drainages in Japan, 2014–2016. (a) pH, and (b) ratio of metal level to the effluent standard (Cd 0.03 mg/L, Pb 0.1 mg/L, As 0.1 mg/L, Cu 3 mg/L, Zn 2 mg/L, Fe 10 mg/L, and Mn 10 mg/L). Source: Soda and Nguyen 2023.

In efforts to safeguard the environment and human health from mine wastewater pollution, the Japanese government annually allocates a substantial budget amounting to billions of yen for treatment purposes. Various physicochemical techniques such as precipitation, flocculation, and neutralization have been utilized for mine wastewater treatment. However, these methods often require high costs in maintenance, operation, and secondary waste management (Nguyen et al. 2022). Therefore, prioritizing the development of cost-effective and environmentally friendly technologies is crucial to minimize the treatment burden and ensure sustainable mine wastewater treatment practices. CWs present a promising passive solution for mine drainage treatment due to their simplicity in operation and maintenance, and cost-effectiveness. Metals removal in CWs primarily results from the collaborative interaction of three elements: plants, substrates, and microorganisms.

In our previous study (Nguyen et al. 2022), we found that CWs filled with limestone and planted with cattails exhibited a remarkable ability to remove heavy metals from AMD. The presence of sulfate-reducing bacteria (SRB) suggested metal removal occurred through sulfide precipitation. However, the contribution of SRB to metal removal was limited due to the low concentration of nutrients and carbon sources required for SRB activities. Hence, stimulating SRB activity by supplementing external carbon sources is feasible for sustainable AMD treatment. Domestic wastewater (DW) contains organic substances and nutrients that can serve as external carbon inputs to enhance bacterial activity. Additionally, these nutrients can greatly stimulate the growth of plants in constructed wetlands. Careful selection of plant species for CWs is crucial to attain optimal metal removal efficiency. Furthermore, integrating ornamental plants into wetlands is encouraged due to the potential for economic and social advantages, such as improved landscaping and decreased environmental pressure. Iris species, commonly grown for ornamental purposes in many countries, exhibit metal absorption capabilities, robust tolerance, and visual appeal. Nevertheless, there remains insufficient data on their efficacy in treating AMD.

In that context, in this study, we designed lab-scale CWs employed with iris (*Iris pseudacorus*) for AMD treatment. DW was introduced into AMD to stimulate the bioprocesses. The primary aims of the present study are to assess how effectively CWs remove heavy metals from AMD mixed with DW, and to examine the effectiveness of utilizing an ornamental flowering plant (iris) as a wetland plant for removing heavy metals from AMD.

## 2. Methodology

### (1) Synthetic Wastewater

AMD and DW used in this study were prepared based on the annual average chemical composition of real mine drainage in Kyoto and domestic wastewater in Shiga Prefecture, Japan, respectively. The heavy metal concentrations of stimulated AMD were 0.07 mg/L for cadmium (Cd), 0.22 mg/L for copper (Cu), 37.2 mg/L for iron (Fe), 0.89 mg/L for manganese (Mn), 0.09 mg/L for lead (Pb), and 7.61 mg/L for zinc (Zn). While DW contained 32.0 mg/L of total organic carbon (TOC), 10.1 mg/L of total nitrogen (TN), and 1.75 mg/L of total phosphorus (TP).

### (2) Setup and Operation of CWs

We conducted this experiment at the greenhouse on the BKC campus of Ritsumeikan University, Japan. Each CW system was filled with limestone and loamy soil and covered with aluminum foil to prevent light from penetrating the substrate. Cattails and irises were utilized as wetland plants. Cattail, a typical wetland plant, was employed for comparison with iris in heavy metal removal. Systems with cattails and irises were referred to as CW-cattail and CW-iris, respectively. Additionally, a control system (referred to as CW-UP) without plants was also prepared. The experiment was operated in four phases with different HRTs. Phases I to III exclusively utilized AMD. Prior to the commencement of phase IV, 400 mL of pond sediment and 100 mL of activated sludge were added to the CWs to inoculate microorganisms for 2 weeks. In phase IV, DW was supplied to the CWs along with AMD. The experimental diagram and time of operation are shown in Figure 2.

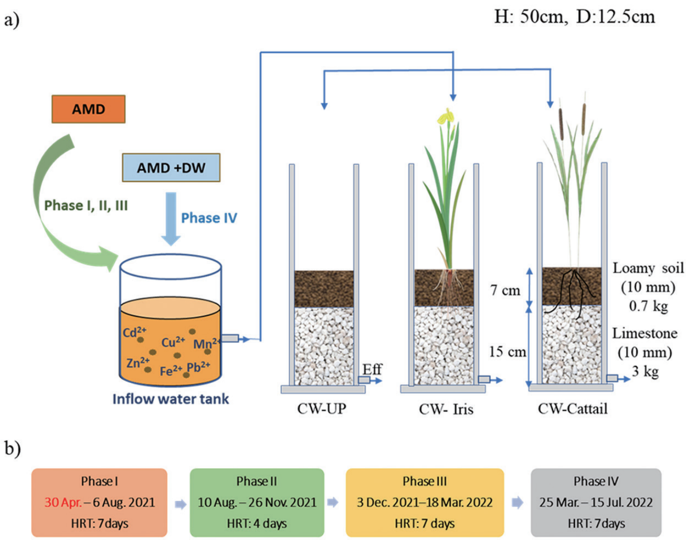


Figure 2: a) Diagram of CWs, b) Time of operation for four phases. Source: Author

We collected samples from both the inlet and outlet to analyze heavy metal concentrations and other water parameters such as pH, DO, ORP, sulfate ($SO_4^{2-}$), sulfide ($S^{2-}$), TOC, TN, and TP. At the end of the experiment, samples of plants and substrate were also collected to determine the accumulation of heavy metals.

## 3. Results and Discussion

### (1) Water Quality

During the experimental period, the pH levels of the effluent ranged from 6.5 to 8.3, with a slight increase observed in Phase IV, likely due to the buffering capacity of the supplied DW and the bicarbonate production from SRB activities. Effluents from planted CWs exhibited lower DO levels compared to unplanted ones, indicating enhanced oxygen consumption by roots and microorganisms in the rhizosphere. DO levels experienced a significant drop in Phase IV as a result of heightened oxygen requirements for organic matter decomposition. While ORP values were mostly positive in the first three Phases, they experienced a notable drop in the last Phase, ranging from -179 mV to -20 mV after one month. Sulfate concentrations decreased in CW effluents, with a marked reduction in Phase IV. Dissolved sulfide concentrations remained minimal (0–0.01 mg/L) in Phases I-III but gradually increased (0.037–0.21 mg/L) in Phase IV, indicating the reduction of sulfate to sulfide by SRB.

During Phase IV, all CWs demonstrated effective removal of organic matter and nutrients, achieving removal rates of 84.0–88.5% for TOC, 80.6–100% for TN, and 80.0–99.0% for TP. Nitrogen removal in CWs primarily occurred through microbial nitrification and denitrification processes, while phosphorus removal was mainly achieved through adsorption and precipitation processes. Additionally, plant presence enhanced nutrient uptake, evident from higher removal rates observed in planted CWs. These findings affirm the capability of CWs to effectively eliminate multiple contaminants from both AMD and DW simultaneously.

**(2) Heavy Metal Treatment**

The average concentration of heavy metals for each phase is presented in Figure 3. In Phases I, II, and III, there was a general decrease in heavy metal concentrations post-treatment. The most effective removal was observed in Phase I, with a 7-day hydraulic retention time (HRT). However, in Phase II, where the HRT was reduced to 4 days, removal efficiencies decreased. Despite maintaining a 7-day HRT in Phase III, removal efficiencies slightly decreased. These findings suggest that the substrate became increasingly saturated with metals in the absence of an external carbon source. Additionally, diminished plant vitality and reduced evaporation during late autumn and winter months adversely affected treatment performance in Phases II and III. Planted CWs exhibited superior removal efficiency for all investigated metals, except for Mn.

As observed in Figure 3, the influent concentrations of Cd and Zn exceeded the effluent standards. During Phase I, the Zn and Cd concentrations from outlet samples met the standards well. However, in Phase III, Zn concentrations in effluents exceeded the standard. The dominant process for Cd removal in CWs was identified as adsorption, and the decrease in HRT in Phase II resulted in lower Cd removal efficiency. Among the investigated heavy metals, Fe had the highest level in the influent, with an average concentration of 37.4 mg/L, approximately four times higher than the Japanese effluent standard of 10 mg/L. Hara et al. (2021) indicated that Fe can be easily removed through the formation of insoluble oxides, precipitation, and co-precipitation processes in CWs. In the current study, more than 99% of

Fe from AMD was removed by all CWs after treatment.

Cu, Mn, and Pb were effectively removed post-treatment. However, Mn removal efficiency (37.9–95.8%) varied significantly across all CWs during the three phases, possibly due to its lower sensitivity to pH changes and susceptibility to leaching with variations in ORP. Treatment performance for Mn notably declined in Phase III, particularly in planted CWs. Pb was effectively eliminated in Phase I but exhibited less than 50% removal efficiency in Phases II and III, except for those in the iris-CWs. Substrate saturation likely contributed to the reduced treatment performance of CWs. To achieve sustainable and effective AMD remediation, stimulating biogenic processes through supplementation with sludge and DW was deemed necessary.

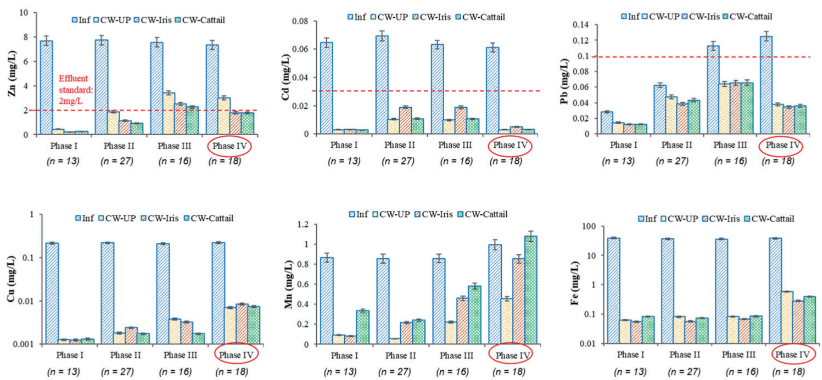


Figure 3: Heavy metal concentrations (avg. ± SD) in influent synthetic AMD and effluents in four phases. Source: Author

In Phase IV, we found that after adding sludge and DW, the effectiveness of heavy metal removal significantly enhanced, especially in the planted CWs. The stimulation of microbial activities facilitated the removal of heavy metals through their adsorption onto the mucus generated by microorganisms around plant roots. Additionally, the addition of DW as electron donors, particularly for SRB, encouraged the conversion of sulfate to sulfide. Subsequently, the sulfide reacted with soluble metals in the wastewater, precipitating them out. Nguyen et al. (2021) and Soda et al. (2021) also reported that metals can be removed in CWs through bacterial metabolism when they serve as trace elements. Furthermore, the supplementation of nutrients from DW promoted plant growth, leading to significant contributions to heavy metal removal from AMD. Throughout Phase IV, a gradual decrease in the Zn concentration was observed in the effluent, particularly noticeable in the planted CWs. The Zn concentration fell below the effluent standard, averaging approximately 1.7 mg/L. The efficiency of Zn removal increased from 57.0–72.2% in Phase III to 67.6–88.4% in Phase IV. Similar to Zn, there was a notable decline in the effluent Cd levels during Phase IV, reaching only 0.002–0.003 mg/L. Cd removal efficiency in Phase IV improved by 11.3–19.8% compared to Phase III. Unlike Cd and Zn, the effectiveness of Cu and Mn decreased after the addition of sludge and DW. Cu was likely predominantly removed in Phases I-III through binding sites on the media, which might have been diminished by the addition of sludge and microbial-produced mucus in Phase IV. Despite this, Cu removal efficiency remained high (97.5–98.5%) across all systems, whereas Mn removal ranged from 35.4–65.1% in Phase IV. Fe removal remained consistently high, with more than 99% removal in all systems during this phase.

**(3) Metal Accumulation in Substrate**

It is recognized that substrates play a crucial role in removing heavy metals from mine wastewater in CWs. Most metals are removed from AMD primarily through interaction with substrates such as gravel, soil, and limestone (Wang et al. 2020; Yang et al. 2018). Therefore, selecting substrates with high filtration and adsorption capacities, as well as high ecological activity, is important.

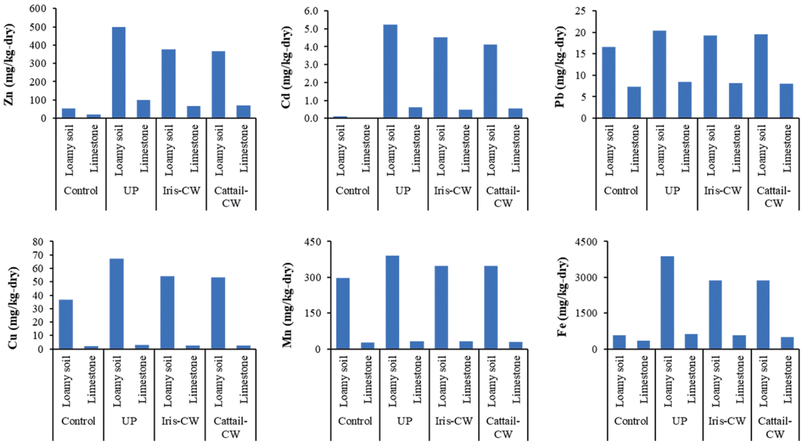


Figure 4: Heavy metal contents in loamy soil and limestone before and after the treatment. Source: Author

In the present study, loamy soil and limestone were used as substrates in CWs. Loamy soil has the characteristics of high permeability, porosity, and water retention. In addition, its chemical composition includes many metal oxides such as $SiO_2$, $Al_2O_3$, FeO, CuO, $K_2O$ and CaO, thus, it has the potential to adsorb metals and exchange ions. Soda et al. (2021) reported that soil adsorption was the main mechanism in CWs for removing Cd from neutral mine drainage. Limestone has been widely used as a substrate in CWs for mine drainage treatment because of its high durability and adsorption capacity. The main component of limestone is calcium carbonate which generates ion hydroxides leading to increased pH. With the increase of pH, metal can be removed through neutralization or precipitation as metal hydroxides. Figure 4 presents the heavy metal contents in the loamy soil and limestone before and after treatment. Overall, the concentrations of examined heavy metals in the substrates increased across all CWs during the experimental duration.

### (4) Plant Growth and Metal Uptake

Irises and cattails exhibited robust growth during Phase I and the early stages of Phase II. However, the aboveground parts started to wither towards the end of Phase II and continued through Phase III due to the cold winter weather. With the onset of warmer spring weather in Phase IV, the underground roots began to sprout again. Alongside nutrient supplementation, the plants thrived during Phase IV.

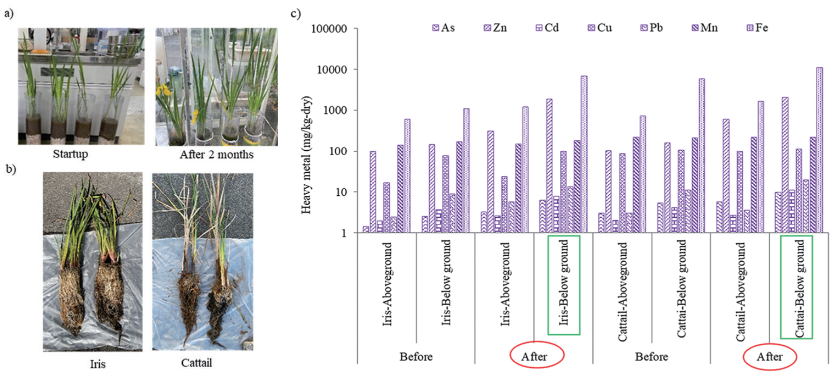


Figure 5: a) Irises and cattails on the first day and after two months of operation, b) the aboveground and belowground parts of plants after the experiment, c) heavy metal contents  in Iris and Cattail biomass before and 14.5 months after AMD treatment. Source: Author

At the end of Phase IV, irises and cattails were harvested. Each iris-CW yielded 250 g-dry of iris biomass, with 81 g-dry from the aboveground part and 169 g-dry from the belowground part. Similarly, each cattail-CW produced 208 g-dry of cattail biomass, including approximately 60 g-dry from the aboveground part and 148 g-dry from the belowground part. The heavy metal concentrations in iris and cattail biomass before and after treatment with AMD are depicted in Figure 5c. During the experiment, the metal levels in both irises and cattails notably increased, particularly in the belowground part. Essential elements such as Zn, Cu, Fe, and Mn exhibited higher concentrations than Pb and Cd in the plant biomass.

In this research, iris and cattail were likely to generate roots and rhizomes, serving as substrates for bacteria attachment. Moreover, these roots and rhizomes play a role in oxygenating the surrounding areas and absorbing pollutants from the wastewater. These plants possess the ability to absorb metals through mechanisms such as phytostabilization, phytovolatilization, phytoextraction, and rhizofiltration. The uptake of metals by plants was acknowledged as the primary biological process for metal removal in CWs (Sandoval et al. 2019). During the experiment, the amount of accumulated metals in iris was comparable to that of the typical wetland plant, cattail, suggesting that iris possesses a strong capability for heavy metal removal. Furthermore, the higher biomass of iris provides an advantage for metal accumulation.

### (5) Sulfate-Reducing Bacteria Population

Figure 6 illustrates the presence of SRB in effluent samples throughout the experiment. Initially, only a small number of SRB (ranging from 0 to 4×101 CFU/mL) were detected in effluent samples during Phases I-III. However, by Phase IV, the population increased to a range of 102 to 3×103 CFU/mL in the effluent. The introduction of sludge and DW acted as a stimulant for the growth of SRB. In CWs, SRB plays a critical role in removing heavy metals, as highlighted in previous study (Sheoran and Sheoran 2006).

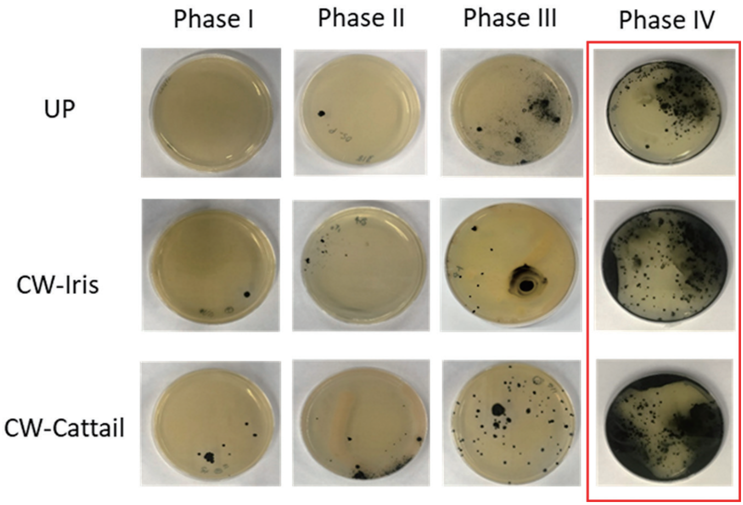


Figure 6: SRB population in effluent samples during four Phases. Source: Author

In this study, SRB was found to enhance the conversion of sulfate to sulfide, as indicated by a significant decrease in sulfate concentration and an increase in dissolved sulfide concentration during Phase IV. The produced sulfide reacted with dissolved metals, resulting in the formation of highly insoluble metal sulfides. Nguyen et al. (2021) also indicated that metal sulfide precipitation is recognized as an effective method for removing metals in CWs. Furthermore, the sulfate reduction by SRB also contributed to an increase in pH during Phase IV, promoting the precipitation of metal hydroxides and sulfides.

## 4. Conclusion

The laboratory-scale experiment aimed at co-treating AMD and DW effectively demonstrated the efficiency of the CW microcosm. It showcased improvements in effluent pH and the removal of heavy metals, sulfate, organic matter, and nutrients. Stimulating SRB activities by adding external carbon sources proved to be an effective method for enhancing heavy metal removal. Iris, when used as a wetland plant, showed significant efficacy in heavy metal removal. Additionally, incorporating ornamental flowering plants such as iris into CWs can enhance aesthetic appeal, creating visually pleasing landscapes and offering additional economic value.

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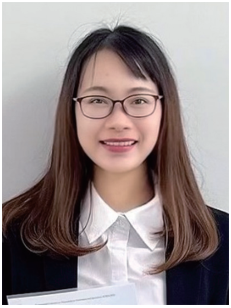
## 5. Dr. Thi Thuong NGUYEN



**Chapter 5.** Constructed Wetlands Planted with Iris for Mine Drainage Treatment: Effects of Domestic Wastewater Feeding on the Removal of Multiple Heavy Metals

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