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Assessing Impacts of Metallic Contamination along the Tidal Gradient of a Riverine Mangrove: Multi-Metal Bioaccumulation and Biomagnification of Filter-Feeding Bivalves

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Abstract: Most riverine mangroves (characterized by salinity fluctuations and tidal inundations), are seriously threatened by metallic pollution. Whether differences in salinity and tidal effects along the river continuum can affect metallic bioaccumulation and the biomagnification of species is still unknown. Bivalves are representative sessile inhabitants in mangrove ecosystems, with a high capacity to bioaccumulate metallic contaminants. The present study used two bivalves, *Meretrix lusoria* and *Mytilopsis sallei*, to monitor inter-site changes in metallic contamination and assess the associated ecological impacts along the tidal gradients of riverine mangroves. The concentrations of a total of six metals (Cr, Ni, Cu, Zn, Cd and Pb) in *M. lusoria* and *M. sallei*, collected at three different sites along Danshuei Riverine Mangrove, were investigated. The metallic concentrations of the whole soft body of the studied bivalves, and the associated surface sediment from each site, were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) to determine the inter-site effects on the bioaccumulation and biomagnification of metallic contaminants in bivalves. There are increasing concentrations of four metallic contaminants, Zn, Cr, Cd and Cu, in the seaward direction of the bivalves. The increasing mean metallic concentrations along the seaward direction may be the effect of salinity, further decreasing the rate of the elimination of these metals, thus resulting in a net increase in metallic contaminants. Our results clearly show prominent inter-site changes in the metallic burdens of bivalves in our study on riverine mangrove ecosystems associated with different levels of bioaccumulation and biomagnification of metallic contaminants. Thus, it is important to monitor multiple sites along the dynamic environment of riverine mangroves in order to gain a good understanding of the ecological impact of metallic pollution risks. The present findings provide important evidence of the use of simple indices to assess the ecological impacts of metallic pollution in riverine mangroves.

Keywords: metal burden; bioindicator; bioaccumulation; biomagnification; urbanized estuary; mangroves

1. Introduction

Mangroves are important, unique estuarine and coastal ecosystems that possess high bio-productivity, waste purification capacity and carbon sequestration potential, serving as important nursing grounds for fish and providing many other ecological functions and recreational services [1,2]. However, the ecological environments in mangroves can seriously deteriorate due to pollution and habitat degradation from the proliferation of human activities [2–5]. In particular, impacts due to the removal of riparian zones and the shrinkage of river volume, associated with habitat modifications to increase urban land use, could commonly result in a reduction in their buffering capacity for pollution and hydrological changes [6–9]. Moreover, expanding industrial, agricultural and municipal development leads to the production and discharge of waste rich in metallic contaminants into adjacent mangrove ecosystems. Mangroves, as the natural physicochemical sinks of materials drained from the surrounding landscape, are continuously exposed to the metallic contaminants from various types of point- and non-point source pollution [2,10,11]. Metallic contaminants are generally considered to be highly toxic and hazardous because of their stable persistence in aquatic environments, easy bioaccumulation in biotic species and biomagnification along food chains [12–14]. In the face of extensive urbanization and ever-increasing metallic pollution due to economic development, mangrove biota could be exposed to unprecedented effects due to toxicity from metallic contaminants [15,16]. However, metallic concentrations, which are influenced by the distance from the coast, sediment characteristics, hydrology, types of vegetation and man-made habitat modification, can vary within a mangrove site [12,17,18]. Findings from previous field and laboratory studies indicated that metal accumulation in the body tissues of biota varied over a wide range and the assimilation efficiencies of metals for different organisms were not directly related to the ambient contamination levels in mangrove habitats [19–21]. Hence, it would be essential to determine the change in the concentrations of metallic contaminants between the ambient environment and the body tissues of organisms in mangrove ecosystems in order to better understand the pollution risk to ecosystem health.

Biological responses to metallic pollution in mangrove ecosystems remain highly complex because contaminant bioavailability is influenced by a combination of multiple environmental factors including river discharge, tidal effects, temperature, salinity, concentrations of organic matter, nutrients and other contaminants [19,20,22]. In addition, the uptake of metallic contaminants by organisms and biomagnification extent in the field varies widely due to their physiological functions, food sources, and environmental exposure. In particular, the influence of these factors could be variable depending on their location along the tidal gradient in the highly dynamic environment of riverine mangroves [23–25]. However, previous studies of the ecotoxicological effects on mangrove fauna from metallic pollution mainly focused on coastal sites [20,21,26,27]. Only a few recent studies have suggested that the inter-site variations in these factors could play an important role in influencing the levels of bioaccumulation and biomagnification of metallic contaminants by affecting their bioavailability and the physiological conditions of organisms in estuarine ecosystems such as mangroves [25,26]. However, there are limited field investigations that evaluate inter-site changes in the metallic bioaccumulation and biomagnification in the faunal species of riverine mangrove ecosystems.

Bivalves, including mussels, oysters, and clams, are common benthic filter feeders in coastal and estuarine regions worldwide. Due to their high capacity to bioaccumulate chemical contaminants, bivalves are well-established bioindicators for monitoring and assessing the levels of metallic pollution in aquatic ecosystems [21,27–30]. As they are sessile animals, analyzing the tissues of exposed bivalves could reflect the bioavailability of metallic contaminants in the local environment [30,31]. Therefore, this enables us to monitor the inter-site changes in metallic contamination and assess their corresponding ecological impacts along the tidal gradients of riverine mangrove ecosystems.

The main objective of the present study was to investigate the metallic bioaccumulation and biomagnification extent of two dominant filter-feeding bivalves, including *Meretrix lusoria* (Veneridae) and *Mytilopsis sallei* (Dreissenidae) in three different sites along the Danshuei Riverine Mangrove in the highly urbanized northern area of Taiwan. We hypothesized that the differences in salinity and tidal inundation would cause differences in the metallic concentrations of sediment and their bioavailability, leading to inter-site differences in the metallic bioaccumulation and biomagnification capacity of the studied bivalves. The concentrations of six metallic contaminants, including zinc (Zn), copper (Cu), nickel (Ni), chromium (Cr), lead (Pb) and cadmium (Cd) in the whole soft body of the two studied bivalves were analyzed and compared with the environmental concentrations based on the associated surface sediment from each site in order to (1) evaluate the inter-site effects of the bioaccumulation and biomagnification of metallic contaminants in bivalves along the riverine mangrove and (2) to determine the inter-site variations in major uptake pathways between the ambient environment and the metallic contamination in bivalves in the riverine mangrove.

2. Materials and Methods

2.1. Study Sites

This study was conducted at three sites, including Waziwei (WZW) (25°09′ N, 121°25′ E), Wugu (WG) (25°06′ N, 121°27′ E) and Jiangzicui (JZC) (25°02′ N, 121°29′ E), located longitudinally along the 23.7 km-long, tidally influenced Danshuei Riverine Mangrove, situated at the downstream end of the Danshuei River (Figure 1). The Danshuei River is the largest river in northern Taiwan; it is highly urbanized, with a population of seven million. It drains 2726 km^2 of watershed area, and the water quality is generally polluted, primarily due to industrial and municipal discharges. With the rapid economic development of Taipei in recent decades, the increasing metallic pollution in Danshuei River, due to treated and untreated sewage, and various non-point source discharges, has become a matter of public concern [32]. The tidally influenced downstream estuary of the Danshuei River is lined by the extensive growth of mangrove plants, i.e., *Kandelia obovata* Sheue, Liu and Young (Rhizophoraceae), which form the Danshuei Riverine Mangrove. Regular water quality monitoring for metallic pollution in the Danshuei River has been carried out since the early 1990s, but a systematic sediment assessment of the river environment in Taiwan did not begin until 2014. Moreover, biomonitoring of the impact of sediment metallic pollution is not currently carried out in the ongoing government monitoring programs [33]. Such a paucity of long-term baseline knowledge about the metallic contamination of biota has resulted in difficulties regarding our understanding of the pollution risk to the mangrove ecosystem.

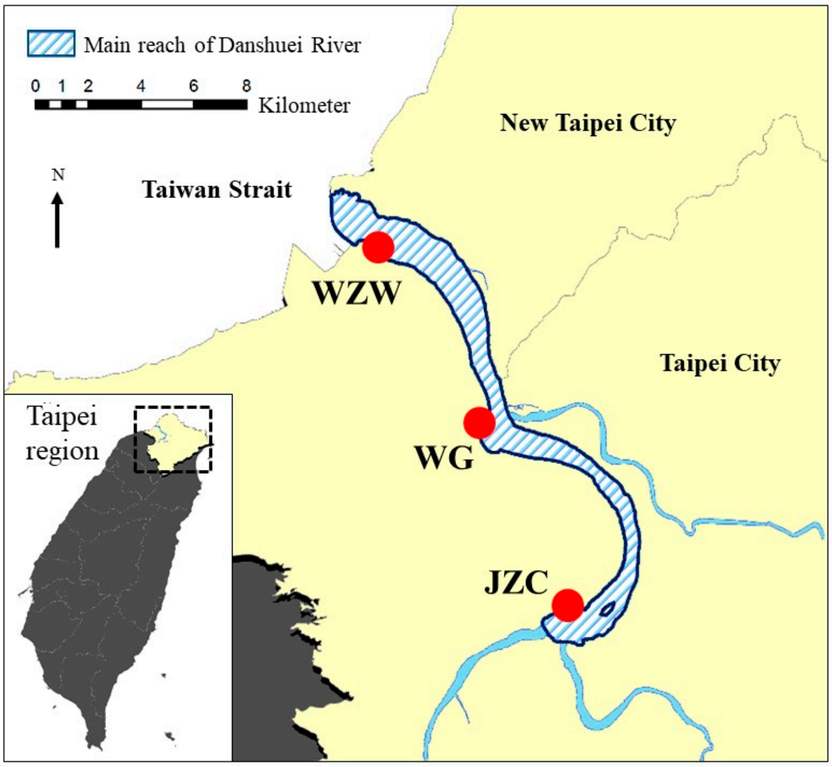

Figure 1. Map showing the three study sites, Waziwei (WZW), Wugu (WG) and Jiangzicui (JZC), along Danshuei Riverine Mangrove in Northern Taiwan.

During the study period of 2015–2016, the annual mean water temperature ranged between 13.1 °C (February) and 31.9 °C (July), and the annual total precipitation was 2405.1 mm [34]. The mean river width increased gradually from the upper reach, JZC (335 m), to the river mouth, WZW (1050 m), but the river depth was highest at WG (4.28 ± 0.69 m), followed by WZW (3.50 ± 0.23 m) and JZC (2.68 ± 0.37 m). The water quality of Danshuei River was classified as "moderately polluted" based on the River Pollution Index [32]. Along the studied riverine mangrove, the dissolved oxygen concentration (DO) was relatively low, but hypoxia was not observed during the study period. Both salinity (sal) and dissolved oxygen concentration exhibited a decreasing gradient from the seaward WZW (Sal = 18.69 ± 2.75; DO = 4.76 ± 0.38 mg/L) to the inland JZC (Sal = 1.17 ± 0.66; DO = 2.98 ± 1.47 mg/L) (Table 1). The mean pH values were consistent (7.18–7.76) among the three study sites. In this study, the mean ammonium–nitrogen, nitrate–nitrogen and phosphate concentrations ranged between 0.92–1.47 mg/L, 0.21–0.39 mg/L, 0.12–0.43 mg/L respectively. The mean turbidity was 13.0 to 94.5 NTU. As influenced by the tidal flushing, a clear decreasing gradient from JZC to WZW was observed for turbidity, nutrient levels and chlorophyll a concentration.

Table 1. Physio-chemical parameters (mean ± SD) of the studied Danshuei Riverine Mangrove measured during the study period.

	Study Site		
	WZW	**WG**	**JZC**
Geographical location	25°09′ N, 121°25′ E	25°06′ N, 121°27′ E	25°02′ N, 121°29′ E
Distance from sea (km)	1.11	8.51	19.02
Salinity	18.69 ± 2.75	6.48 ± 2.77	1.17 ± 0.66
pH	7.76 ± 0.03	7.42 ± 0.09	7.18 ± 0.14
Dissolved oxygen (mg/L)	4.76 ± 0.38	3.30 ± 0.47	2.98 ± 1.47
Ammonium–nitrogen (mg/L)	0.92 ± 0.05	1.90 ± 0.06	1.47 ± 0.35
Nitrate–nitrogen (mg/L)	0.21 ± 0.01	0.38 ± 0.04	0.39 ± 0.01
Phosphate–phosphorus (mg/L)	0.12 ± 0.04	0.20 ± 0.12	0.43 ± 0.22
Chlorophyll a (μg/L)	10.96 ± 0.42	39.39 ± 15.50	81.15 ± 6.70
Turbidity (NTU)	13.00 ± 5.66	82.00 ± 22.63	94.5 ± 17.68

Site codes: Waziwei (WZW), Wugu (WG) and Jiangzicui (JZC).

2.2. Field Collection and Sample Preparation

The field collection of sediment and bivalve samples was carried out at WZW, WG and JZC during 2015–2016. Three composite surface sediment samples (0–5 cm), each created from five sediment grab samples spaced 1 m apart, were collected from the mudflats at each study site. Two dominant bivalve species, *Meretrix lusoria* and *Mytilopsis sallei*, were collected by hand from each site. All sampling was undertaken during an ebbing tide. All sediment and bivalve samples were stored in labelled, sealed plastic vials and transported to the laboratory at 4 °C.

In the laboratory, the sediment samples were sieved (mesh size = 0.5 mm) to remove macroinvertebrates and other large organic debris, and then weighed after being oven dried at 44 °C to a constant weight. The sediment samples were powdered, ashed at 105 °C for three hours and then at 900 °C for six hours, and their final weights (to the nearest 0.01 g) were recorded after cooling for two hours. All sediment samples were stored in a desiccator prior to chemical analysis.

All bivalve samples were first cleaned and their species were identified, if needed, under a 10–40× dissecting microscope. Individual shell length and wet weight were measured to nearest 0.1 mm and 0.1 mg, respectively. The whole soft body tissue of the individual bivalves was separated from the shells for chemical analysis. In this study, the whole soft body tissue was used for the determination of metallic concentrations without being separated into different body tissues for chemical analysis, because the whole soft body would be consumed by higher predators in the natural environment and, thus, the metallic contamination of the whole soft body would actually reflect the true ecological impact. Three individual samples were grouped to form a single pooled sample. All biological samples were oven dried at 44 °C to a constant weight (0.1 mg), homogenized, and stored in a desiccator prior to chemical analysis.

2.3. Determination of Metallic Concentrations

Digestion was carried out for the sediment and bivalve samples prior to a chemical analysis of their metallic concentrations. An individual sediment sample was dissolved and fused with lithium borates and lithium bromide using a Claisse M4 Fluxer to produce glass discs for major element analysis by wavelength dispersive x-ray fluorescence (WDXRF) with the AXIOS mAX-Advanced WDXRF spectrometer (Malvern Panalytical, UK). The glass discs were then re-dissolved into a liquid state using nitric acid and hydrogen fluoride for chemical analysis. The dried bivalve samples were digested with 65% nitric acid (DigiPrep Jr, 50 mL) at 100 °C for three hours. Any undissolved remains from each sample after digestion were removed by passing the sample through 0.45 μm membrane filters.

The concentrations of the six studied metallic contaminants (Cr, Ni, Cu, Zn, Cd and Pb) in the bivalve body tissues and sediment samples were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700×, Santa Clara, CA, USA). The concentrations of all measured samples were compared with the calibration standards prepared by diluting the multi-element standard stock solution with 1% nitric acid (ICP multi-element standard solution IV, AccuStandard Inc., New Haven, CT, USA). The correlation coefficients of the calibration curves of all metal elements were >0.999. The validity of the analytical method was checked with Certified Reference Materials including TOLT-2 (lobster hepatopancreas from National Research Council Canada, Ottawa, Canada, NCR-CNRC,) for the bivalve tissues, and GSR-5 (shale from Institute of Geophysical and Geochemical Prospecting, China); BCR-2 (basalt from United States Geological Survey, USA, USGS); GSP-2 (Granodiorite from USGS); AGV-2 (Andesite from USGS) and PACS-3 (marine sediment from NRC-CNRC) for the sediment samples. The average recovery rates of biological and sediment reference materials for all six studied metals were higher than 86%.

2.4. Stable Nitrogen Isotope Analysis of Bivalves

The $\delta^{15}N$ isotopic ratios of the bivalve samples were determined for the calculation of the Trophic Magnification Factor (TMF), in order to evaluate the metallic biomagnification (see details in Section 2.5 below). The $\delta^{15}N$ isotopic values of the bivalve samples were measured using a continuous-flow elemental analyzer (Fison NA 1500) coupled to an isotope ratio mass spectrometer (Finnigan Mat Delta S) from the Plant Physiology and Ecology Laboratory, National Taiwan University. The $\delta^{15}N$ isotopic ratios were calculated according to Equation (1):

$$\delta^{15}N = \left(\frac{{}^{15}N/{}^{15}N_{sample}}{{}^{15}N/{}^{15}N_{standard}} - 1 \right) \times 100‰ \quad (1)$$

Atmospheric nitrogen was used as the reference standard. The measurement analytical error of $\delta^{15}N$ was ± 0.18 ‰.

2.5. Data Analysis

Bioaccumulation is defined as the net increase in contaminant concentrations in organisms from the environment. Metallic concentrations of organisms are often attributed to and positively correlated with ambient metallic concentrations through diffusion, respiration, or the consumption of prey associated with that environment. Hence, bioaccumulation is useful for assessing the combination of direct and indirect effects of ambient concentrations on biotic metallic concentrations. The biota–sediment accumulation factor (BSAF) was used to calculate the capacity of the bivalves to bioaccumulate the six studied metals from the associated sediment based on Equation (2) [35]:

$$\mathrm{BSAF}_{biota} = \frac{C_{biota}}{C_s} \quad (2)$$

where C_{biota} is the metallic concentration in the organism, C_S is the metallic concentration in the sediment and BSAF > 1 represents a net increase in the metallic concentration in the bivalves compared to the environment, i.e., the sediment.

Biomagnification or biodilution of toxic elements is defined as the net increase or decrease in contaminant concentrations in the body tissue up to trophic levels, due to trophic transfer. In this study, the Trophic Magnification Factor (TMF) was used to determine the biomagnification/biodilution levels of metallic contaminants through the mangrove food chain [36]. TMF is calculated as the slope (b) of regression, representing the change in contaminant concentrations within the target organism, i.e., C_{biota}, per unit change in δ^{15}N through the food chain, i.e., $\delta^{15}\mathrm{N}_{\mathrm{biota}}$, and the constant (a) refers to the background metal concentrations. The TMF was calculated following Equations (3) and (4) [36]:

$$Log(C_{biota}) = a + b \times \delta^{15}\mathrm{N}_{biota} \quad (3)$$

$$\mathrm{TMF} = 10^{b} \quad (4)$$

where TMP > 1 suggests the biomagnification of the contaminant, with increasing trophic levels through the food chain and TMP < 1 indicates the biodilution of contaminants through the food chain.

The inter-site differences in the metallic concentrations of surface sediment, bivalves, and BSAF values were compared using a one-way ANOVA. Tukey's post-hoc comparison test was performed for any significant difference detected ($p < 0.05$). All statistical analyses were performed with the Minitab 17.0 Package.

3. Results

3.1. Inter-Site Variation of Metallic Concentrations of Surface Sediment along Riverine Mangrove

Clear inter-site trends were observed for the metallic concentrations of sediments along the Danshuei Riverine Mangrove, where all studied metal contaminants, except Ni, showed the highest concentrations at WG, and sediment metallic concentrations at JZC were slightly lower than WZW (Figure 2). The results of the one-way ANOVA followed by a post-hoc Tukey's comparison revealed significant inter-site variation for all studied metallic contaminants except for Ni: Zn ($p < 0.001$; WG = WZW > JZC), Cu ($p < 0.05$; WG ≥ WZW ≥ JZC), Cr ($p < 0.001$; WG = WZW > JZC), Pb ($p < 0.01$; WG ≥ WZW ≥ JZC) and Cd ($p < 0.001$; WG ≥ WZW ≥ JZC). Zn concentration in sediment was 149.13 ± 4.45 µg/g-dw at WZW, and increased by 28% to 191.03 ± 7.73 µg/g-dw at WG, but it was reduced by 57% to 82.40 ± 1.68 µg/g-dw at JZC. Cu was 37.71 ± 0.81 µg/g-dw at WZW, and it experienced a 66% increase at WG to 62.56 ± 0.75 µg/g, but decreased by >50% to 29.67 ± 4.86 µg/g-dw at JZC. Cr was 73.58 ± 7.73 µg/g-dw at WZW, and it increased by 16% to 85.71 ± 5.09 µg/g-dw at WG, but then decreased by 46% to 46.33 ± 0.05 µg/g-dw at JZC. Pb, as with all studied metals except Ni, reached the highest values at WG with 113.63 µg/g-dw, which was 23% higher than its concentration at WZW and 41% higher than its concentration at JZC. Cd experienced a 24% increase from the 0.43 ± 0.01 µg/g-dw at WZW to 0.54 ± 0.02 µg/g-dw at WG. It decreased to 0.35 ± 0.02 µg/g-dw at JZC. In contrast to the other studied metals, Ni showed a different trend, with its concentration peaking at WZW (55.98 ± 23.02 µg/g-dw) and slightly decreasing towards the inland upstream direction. Ni was reduced by 29% and 24% at WG (39.86 ± 2.38 µg/g-dw) and JZC (30.15 ± 1.05 µg/g-dw), respectively.

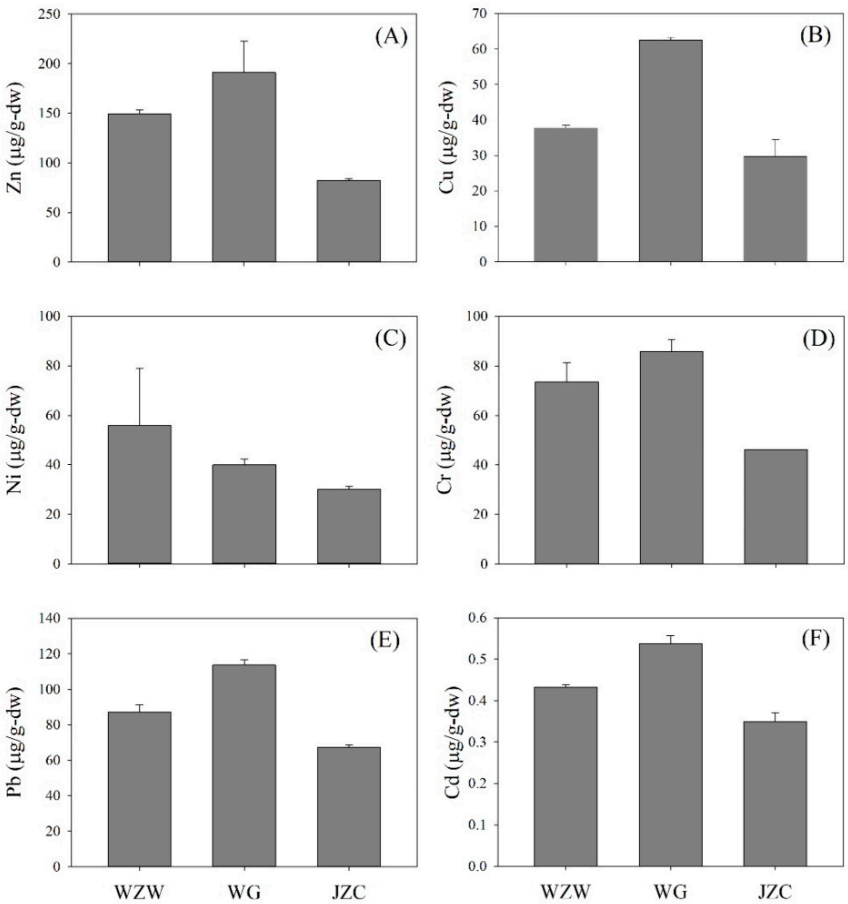

Figure 2. Metallic concentrations (mean ± SD) of (**A**) Zn, (**B**) Cu, (**C**) Ni, (**D**) Cr, (**E**) Pb, (**F**) Cd in surface sediment of the studied Danshuei Riverine Mangrove measured during the study period.

3.2. Inter-Site Variation of Metallic Concentrations of the Bivalves along Riverine Mangrove

A consistent trend of increasing concentrations for four metallic contaminants, Zn, Cu, Cr and Cd, in the seaward direction was detected for the bivalves (Figure 3). The concentrations of all metals in the bivalves were generally higher in WZW or WG, but were lowest in JZC. The results of the one-way ANOVA on the metallic concentrations of the bivalves demonstrated that all metallic contaminant concentrations, except that of Ni ($p > 0.05$), differed significantly among sites. According to Tukey's post-hoc tests, levels of Zn, Cr and Cd burdens in bivalves were similarly high at WZW and WG, but those at JZC were significantly lower ($p < 0.001$; WZW = WG > JZC). Cu burdens in bivalves were significantly higher at WZW compared to WG and JZC ($p < 0.001$; WZW > WG = JZC), whereas Pb burdens were significantly higher at WG than WZW and JZC ($p < 0.001$; WG > WZW > JZC).

Zn concentration in the bivalves was highest at 1523.57 ± 288.22 μg/g-dw in WZW, and it decreased by 15% and 97% at WG and JZC, respectively. Cr concentration in the bivalves was around 30 μg/g-dw in WZW and WG, but it decreased to 0.18 ± 0.07 μg/g-dw at JZC. The mean Cr concentration in the bivalves had similar trend to Zn, but its magnitude was ~0.02× compared to Zn concentrations. Cd also showed a similar trend to Zn and Cr; the mean Cd burden was higher at WZW (0.18 ± 0.04 μg/g-dw) and WG (0.17 ± 0.03 μg/g-dw), but it fell to only 0.08 ± 0.03 μg/g-dw at JZC. There was a decreasing trend in Cu concentration towards the upstream direction, from 93.55 ± 18.24 μg/g-dw in WZW, and it decreased by 38% to 58.21 ± 9.68 μg/g-dw in WG, then further decreased by 6% to 54.56 ± 15.66 μg/g-dw at JZC. Both mean Pb and Ni concentrations in the bivalves were lowest at WZW (Pb = 3.16 ± 0.88 μg/g-dw; Ni = 14.23 ± 2.74 μg/g-dw), and increased by 0.35× and 1.58× at WG, respectively. Ni remained at similar level at JZC, but Pb was not detected at JZC.

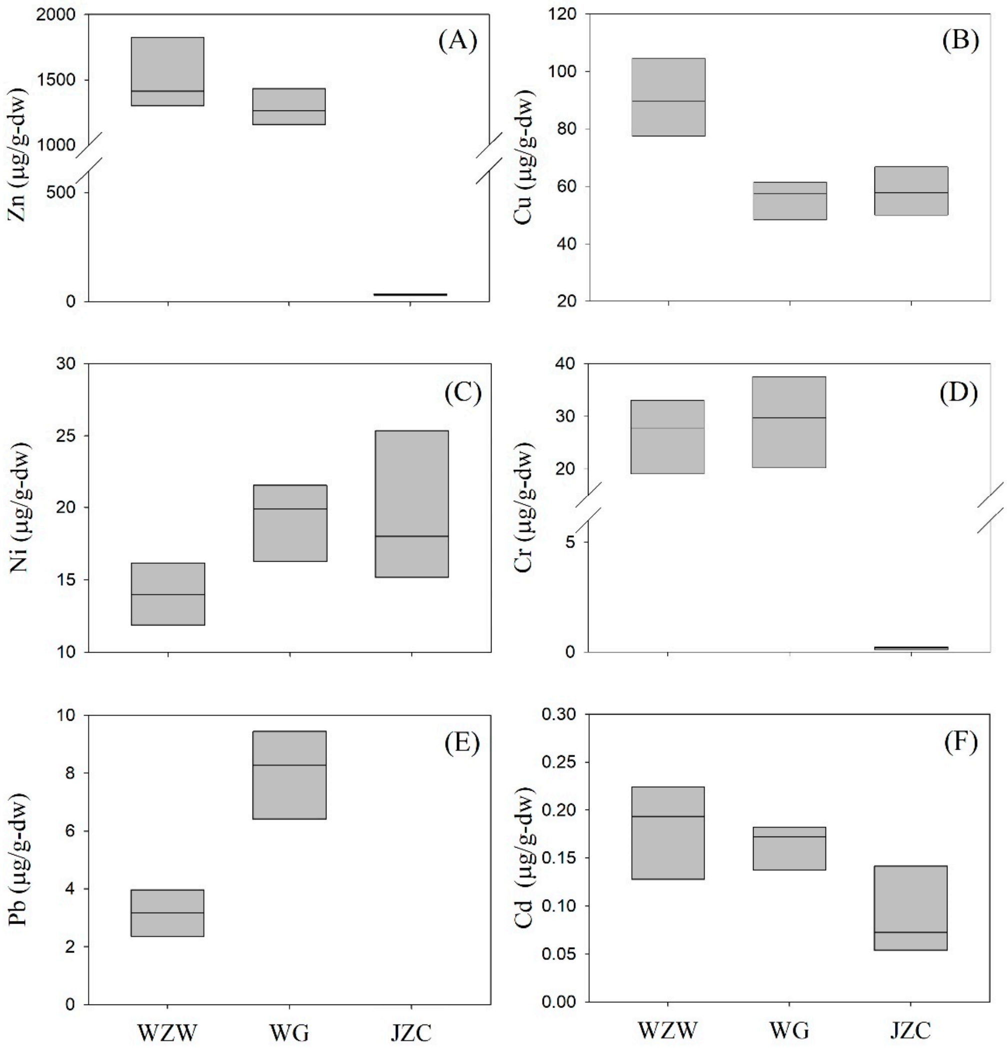

Figure 3. Boxplots showing the mean metallic burdens of (**A**) Zn, (**B**) Cu, (**C**) Ni, (**D**) Cr, (**E**) Pb, (**F**) Cd for the bivalves in the three study sites, i.e., WZW, WG and JZC, along Danshuei Riverine Mangrove.

3.3. BSAF of Metallic Contaminants in the Bivalves

The capacity of bivalves to accumulate all metals from the sediment showed significant inter-site differences for all metals (Table 2). Only Zn (at WZW and WG) and Cu (at WZW and JZC) demonstrated a significant net increase in their metallic concentration in the bivalves compared to the sediment, i.e., BSAF > 1. Among the six studied metals, the bivalves at WZW had the highest accumulation capacity for Zn (BSAF = 8.81–13.33) and Cu (BSAF = 1.87–3.05). Moreover, bivalves demonstrated the highest capacity to accumulate Zn at WG (BSAF = 5.93–11.21), and to accumulate Cu from sediment at JZC (BSAF = 1.19–2.52). Thus, bivalves tend to actively accumulate Zn in Danshuei Riverine Mangrove. However, our results revealed that the bivalves did not bioaccumulate Ni, Cr, Pb and Cd from the sediment.

Table 2. Inter-site comparison of mean values of biota–sediment accumulation factors (BSAF) of bivalves among the three study sites, i.e., WZW, WG and JZC, along Danshuei Riverine Mangrove.

Site	Studied Metallic Contaminants					
	Zn	**Cu**	**Ni**	**Cr**	**Pb**	**Cd**
WZW	10.30	2.40	0.25	0.36	0.04	0.42
WG	8.10	0.89	0.50	0.34	0.07	0.31
JZC	0.39	1.9	0.66	0.01	0	0.26
F	65.97 ***	20.56 ***	14.67 ***	18.48 ***	86.60 ***	3.86 *

* $p < 0.05$, *** $p < 0.005$.

3.4. Metallic TMF of the Bivalves

A high risk of trophic magnification in the bivalves for the studied metals was generally observed in the sediment, i.e., a benthic environment, at WZW (TMF = 0.77_{Pb}–1.38_{Zn}) and WG (TMF = 1.12_{Cr}–2.03_{Pb}) from Danshuei Riverine Mangrove (Figure 4). However, the metallic biomagnification of bivalves was not observed at JZC (TMF < 1). Strong inter-site differences were detected for the biomagnification capabilities of bivalves for different metallic contaminants, but the TMFs of all metallic contaminants peaked at WG. The trophic magnification capacities of bivalves for Zn, Cd and Pb showed high fluctuations among the three study sites, where the TMF values at WG were about 20%–50% higher than WZW and JZC for Zn and Cd, and 100%–170% higher than the other two sites for Pb. In contrast, the trophic magnification capacity of the bivalves was relatively low for Ni and Cr along the Danshuei Riverine Mangrove.

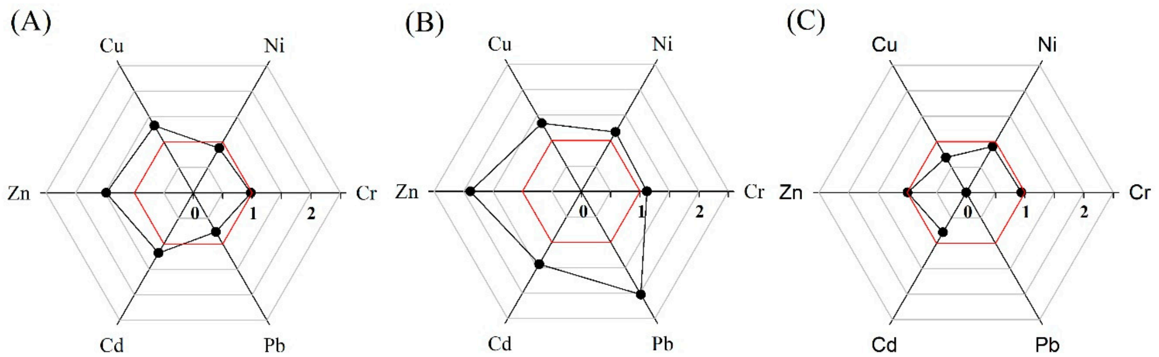

Figure 4. Trophic Magnification Factor (TMF) of the filter-feeding bivalves for the six studied metals including Zn, Cu, Ni, Cr, Pb and Cd in the three study sites, (**A**) WZW, (**B**) WG and (**C**) JZC, along Danshuei Riverine Mangrove.

4. Discussion

4.1. Metal Concentrations of Sediment along the Tidal Gradient of Riverine Mangrove

The concentrations of all metals in the sediment from Danshuei Riverine Mangrove lay within the intermediate ranges of contamination levels compared to other mangrove sites [20,21,37]. This revealed that the benthic environment of this major mangrove site from northern Taiwan was generally impacted by the contamination of the six studied metals, especially Zn, Ni and Pb. As a typical urbanized tidal river, the high pollution in Danshuei Riverine Mangrove is primarily due to waste discharge from industrial activities and municipal waste [32,33]. According to the Environmental Protection Administration of the Taiwanese Government, Danshuei Riverine Mangrove is moderately polluted due to industrial and domestic pollution [33]. Dahan River, as the major upstream tributary flowing into Danshuei River at JZC, has long been recognized as one of the most polluted rivers in Taiwan due to the extensive distribution of manufacturing industries in the region [32,33]. There are 615 registered "pollution control sources" in the Dahan River drainage basin in total, including

111 electroplating factories, 93 metal-finishing plants and 55 circuit board printing factories [38,39]. Moreover, the illegal dumping of industrial waste rich in metallic contaminants has been commonly reported, and up to 64% of factories have been prosecuted for illegal waste disposal.

Our results show that the distribution of ambient metallic concentrations in Danshuei Riverine Mangrove is largely caused by anthropogenic inputs from the surrounding urbanized area. However, inter-site factors, such as the strength of estuarine mixing, could play a crucial role in controlling the concentrations and the bioavailability of metallic contaminants. Previous studies on the hydrodynamic processes in estuarine systems have emphasized that the bioavailability and distribution of metallic contaminants and other toxic materials could affect water quality through dilution, residence time, mixing and erosion processes. The hydrodynamic processes associated with daily variations in tidal range, increasing dilution effects for contaminants near the river mouth by tidal flows and downstream flushing effects, particularly for high discharge during heavy rainfall, could be important for characterizing the longitudinal change in the metallic concentrations in sediment along the tidal gradient of Danshuei Riverine Mangrove [20,40,41]. Moreover, flow patterns influenced by river morphology could be highly important in shaping inter-site changes in sediment metallic concentration. In the present study, WG is a natural sediment sink due to its location at the confluence of tributaries and the main reach of the Danshuei River (Figure 1). The metallic contaminants could be absorbed on the surface of the sediment and become bioavailable via resuspension by tidal flows [42]. Thus, this could explain the observed higher concentrations of most metallic contaminants (Zn, Cu, Cr, Pb, Cd) in sediment compared to WZW and JZC. Therefore, ambient concentrations of metallic contaminants in sediment were spatially heterogeneous, because they were affected by the complicated interactions between river morphology, hydrodynamic processes and anthropogenic activities.

4.2. Metal Concentrations of the Bivalves along the Tidal Gradient of Riverine Mangrove

In Danshuei Riverine Mangrove, the metallic concentrations in the whole soft body tissue of bivalves were found to be at low to medium levels with respect to bivalves from other mangroves, except for Cr (see [20,37]). Compared to bivalves from the U.S., India, Singapore and Senegal, concentrations of the two highly toxic metals, i.e., Cd and Pb, in the bivalves were relatively low at all study sites, whereas concentrations of Zn, Cu and Ni measured in our study were in the intermediate range [18,43,44]. However, the Cr concentration detected in the bivalves was ~100 times higher than in bivalves from mangroves in different parts of the world. The main source of Cr in Danshuei Riverine Mangrove could primarily be associated with the intensive industrial activities, such as textile, steel and chemical manufacturing, electroplating and metal finishing, occurring in the drainage basin and the highly urbanized Taipei region [33,39]. Since the foraging environment and feeding habits of biotic components were closely associated to the level of metallic burden [15,45], bivalves as filter feeders, foraging in the benthic environment of the mangrove, were directly exposed to metallic concentrations in the sediment and accumulated considerable levels of Zn, Cu, Cr and Cd in Danshuei Riverine Mangrove.

In the present study, the increase in metallic concentrations in the seaward direction could largely be attributed to the bioaccumulation of the bivalves. Many previous studies indicated that elevated metallic concentrations from various anthropogenic sources contributed to the high ambient metallic concentrations in mangroves, this could increase the bioavailability of metallic contaminants and worsen bioaccumulation impacts [25,28,37]. This was consistent with our findings that Zn, Cu, Cr and Cd concentrations in the sediment and bivalves increased, with similar trends seen from JZC upstream to WG and WZW downstream along Danshuei Riverine Mangrove. In addition, the metallic burden on the bivalves due to Zn, Cu, Cr and Cd was significantly affected by inter-site variations, and showed a general trend of an increasing metallic burden towards the river mouth.

In order to determine the major contamination pathways of different metals in relation to the bivalves, the results of the BSAFs and TMFs were compared in order to evaluate the relative importance of environmental exposure and dietary uptake in the potential contribution to the metallic burdens on bivalves in urbanized mangrove ecosystems. Results from the BSAFs indicated that only Zn and Cu were effectively accumulated from the benthic environment, i.e., sediment, to bivalves at the downstream end of Danshuei Riverine Mangrove. These results were consistent with the TMFs, which showed that Zn and Cu represented two important metals capable of accumulating in the body tissue of bivalves through environmental exposure to sediment and dietary uptake. The levels of Zn and Cu in the bivalves were approximately 7.2× and 1.6× higher than those in the background sediment, and levels of Cd in the bivalves were constant with respect to the ambient sediment along the studied riverine mangrove. However, other metallic contaminants did not show any clear association between the body tissue of bivalves and the background sediment. Similar findings were reported in other ecotoxicological studies that showed a linear relationship between metallic levels (e.g., of Zn, Cu and Cd) in the bivalves and sediment [20,21,23].

A high risk of the trophic magnification of bivalves for the studied metals was obviously observed in the sediment, i.e., the benthic environment, at WZW (TMF = 0.77_{Pb}–1.38_{Zn}) and WG (TMF = 1.12_{Cr}–2.03_{Pb}) in Danshuei Riverine Mangrove. In particular, several metals, including Zn, Cu, Pb and Cd showed a high tendency for biomagnification in the bivalves. Although the metallic biomagnification of the bivalves was generally not observed at JZC (TMF < 1), the TMF values for Zn, Ni and Cr being close to critical levels, i.e., TMF > 0.9, have attracted concern in terms of the potential ecological risk. Strong inter-site differences were detected for the biomagnification capabilities of the bivalves for different metallic contaminants, but the TMFs of all metallic contaminants peaked at WG. The trophic magnification capacities of bivalves for Zn, Cd and Pb showed high fluctuations among the three study sites, where TMF values at WG were about 20–50% higher than WZW and JZC for Zn and Cd, and 100–170% higher than the other two sites for Pb. Thus, this has confirmed that dietary uptake represents an important source for determining the metallic burdens and characterizing the metal accumulation of all metallic contaminants in the bivalves at all study sites along Danshuei Riverine Mangrove.

In fact, the observed increase in the metallic burdens on bivalves in the field might not only be caused by extrinsic factors such as environmental exposure, diet characteristics and metallic bioavailability, but also by intrinsic factors, such as physiological functions that affect the metallic accumulation/elimination of the organisms [20,21,37]. A number of previous studies suggested that the elimination rates for metallic contaminants were consistently low for most aquatic species, including bivalves, crustaceans, and fishes [46,47]. In addition, bivalves, as filter feeders, have been widely reported as possessing an effective capacity for bioaccumulating metallic contaminants [18]. The increase in the metallic burdens (Zn, Cu, Cr, Cd) on bivalves in the seaward direction in this study could be reinforced by the effect of the increasing salinity along the tidal gradient; this could reduce the rate of metallic elimination and thus result in a net uptake of metallic contaminants by the bivalves [46,48,49]. Moreover, our results highlight the risk of trophic magnification in these benthic communities due to the consumption of a metal-enriched diet and through exposure to the contaminated sediment in the benthic environment [30,50]. Therefore, in comparison to bioaccumulation from background sediment, trophic magnification could be the dominant pathway of metallic accumulation in the bivalves from Danshuei Riverine Mangrove. This is in agreement with [23,51], where the authors suggested that the trophic magnification of Cu occurred in the benthic mollusk–crab community of the highly urbanized Pearl River Estuary in China. Other studies from California and Hong Kong have also revealed the high extent of trophic magnification of Cu and Cd in the benthic communities of mangrove ecosystems, in a similar manner to the findings in the present study, e.g., [17,44,52].

5. Conclusions

Though long-term data about the changes in the faunal community structure, the displacement of stress-sensitive native species by pollution-tolerant exotic species and the ecotoxicological responses of aquatic biota in the Danshuei Riverine Mangrove ecosystem have not been well documented due to a lack of systematic biological monitoring, the pollution risk associated with the metallic pollution of

Danshuei Riverine Mangrove has aroused public concern in recent decades [38,39]. Therefore, there is an urgent need for regular biomonitoring with appropriate bioindicators and assessment methods, which are essential for addressing the ecological impact and even human health risks of metallic pollution in Danshuei Riverine Mangrove, as well as other mangroves and estuarine habitats in Taiwan.

In summary, our results clearly show that prominent inter-site changes in the metallic burdens on bivalves in the studied riverine mangrove ecosystems are associated with different levels of bioaccumulation and biomagnification of metallic contaminants. Such patterns could be a consequence of the interactive effects of ambient concentrations and bioavailability of metallic contaminants, environmental exposure, diet characteristics and physiological functions of the bivalves. Thus, it is important to monitor multiple sites along the dynamic environment of riverine mangroves in order to gain a good understanding of the ecological impact of metallic pollution risks. The resulting bioaccumulation and biomagnification extent of metallic contaminants from sediment in Danshuei Riverine Mangrove highlighted that bivalves have been subjected to medium to high level of ecological impacts. The usefulness of BSAFs and TMFs as valuable predictive assessment tools for the evaluation of the ecological impact on bivalves and their potential to accumulate or magnify metallic contaminants from their ambient environment or trophic diets was clearly demonstrated [42,53]. The present findings provide important evidence regarding the use of simple indices for assessing the ecological impacts of metallic pollution in riverine mangroves.

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