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Heavy Metal Accumulation in *Oxyloma hirasei* from the Upo Wetland

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ABSTRACT: Human activities have enhanced the influx of heavy metals to aquatic ecosystem and changed the abiotic environment such as the sediments supporting benthic organisms. The levels of heavy metals (Cd, Cr, Cu, Ni, Pb and Zn) in the benthic gastropod *Oxyloma hirasei* and the sediments around their habitat were investigated to examine heavy metal levels and the potential of the gastropod as a bioindicator. We detected different levels of heavy metals in the sediments of two wetland areas, Upo and Mokpo, in the relatively well-conserved Upo wetland, Ramsar Convention Area. *Oxyloma hirasei* had higher concentrations of heavy metals except Cr and Ni in their soft tissues than in their shells (Cd: 2.10~3.16, Cu: 19.73~28.66, Pb: 0.67~1.17, Zn: 216.1~285.7 μg/g dry weight in the soft tissues; Cr: 1.19~2.58, Ni: 0.47~1.16 μg/g dry weight in the shells). Differences in the Cd, Cu, Ni and Pb concentrations in *O. hirasei* soft tissues reflected differences in heavy metal concentrations in the sediments at the sampling sites. The coefficients of variation for Cd, Cu and Pb were lower than those for other metals in the soft tissues. Levels of Cd in the tissues of *O. hirasei* were the highest among the metals examined in this study. Therefore, the soft tissue of *O. hirasei* appears to be a promising bioindicator particularly for Cd.

Key words: Bioindicator, Cd, *Oxyloma hirasei,* Heavy metal accumulation, Upo wetland

INTRODUCTION

Rapid industrialization and urbanization since the Industrial Revolution has resulted in the dumping of pollutants into many ecosystems, including aquatic ecosystems. Anthropogenic activities, such as the release of domestic wastewater effluents, the use of coal-burning power plants and non-ferrous metal smelters, and the dumping of sewage sludge have greatly increased the influx of heavy metals to aquatic ecosystems (Nriagu and Pacyna 1988). The stability of heavy metals in sediments and their scarcity in natural environments make heavy metal concentrations in inorganic and organic elements of the ecosystem the most appropriate indicators of overall levels of aquatic pollution (Saeki and Okazaki 1993, Berto et al. 2006). Although the heavy metals Cu and Zn have essential roles in the biological activities of organisms, excessive quantities of heavy metals stunt the growth of organisms (Cœurdassier et al. 2005). In addition, heavy metals biomagnified through the food chain may threaten organisms at higher tropic levels, such as human and birds (AltIndag and Yigit 2005, Saha et al. 2006).

Materials from surrounding environments are carried into wetlands by the hydrologic inputs of precipitation, river flooding, and surface and groundwater inflows (Mitsch and Gosselink 2000). Therefore, pollutants flowing from anthropogenic sources into wetlands have accumulated in wetland sediments (Ahn et al. 2001, Kim and Rejmankova 2001, Kim 2005). These sediments are important in supporting biological organisms in wetland ecosystems. Heavy metals accumulating in the sediments can affect concentrations of heavy metals in the organisms that dwell in these sediments (Pourang 1996, Yap et al. 2002, Kim and Kim 2006). Benthic gastropods in wetlands have an especially close relationship with the sediments that comprise their habitat and feeding site. Gastropods, in turn, are used as food sources by birds and fish. Therefore, they are regarded as an important link for a transfer of heavy metals from the soils to organisms at a higher trophic level through aquatic food chains. In Korea, little information is available on heavy metal concentrations of gastropods in freshwater (Kim and Kim 2006).

The Upo wetland, which consists of four smaller wetlands, was designated as a Ramsar Convention Area, and has been relatively well protected under Korean environmental law. However, anthropogenic activities in the surrounding areas have affected the wetland. Although the Upo and Mokpo wetlands appear to have similar environments, the sources of anthropogenic disturbance for each are different, which means that they might contain different levels of heavy metals in their sediments. *Oxyloma hirasei*, a freshwater gastropod (Succineidae: Gastropoda), inhabits the mud around freshwater streams and rice fields (Kwon et al. 1993). This gastropod is widely distributed throughout the Upo wetland.

The purpose of this study was to assess the potential of *O. hirasei* as a bioindicator of heavy metal levels. This study will also

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provide information on the levels of heavy metals in sediments and biota from the Upo wetland, which is considered to be a relatively clean site.

MATERIALS AND METHODS

Study Area and Sampling

The Upo wetland is situated in Changnyeong-gun, Gyeongsang-nam-do province, southeastern Korea (N 35°33', E 128°25'). The wetland is the largest riverine wetland in Korea and contains high biodiversity (Ministry of Environment 1987). The wetland contains about 231.4 ha, consisting of four smaller wetlands including Upo, Mokpo (the northern part of the Upo), Sajipo (the northeastern part of the Upo) and Jjokjibeol (the southwestern part of the Upo) (Park et al. 2000). We sampled sediments and *O. hirasei* at Upo and Mokpo (Fig. 1). Upo is connected to the Nakdong River, about 5 km west of the wetland, through the Topyeong stream. Mokpo is separated from Upo by a dike and the water flows from Mokpo directly into the Topyeong stream without passing Upo.

In May 2006, samples of *O. hirasei* were collected from areas dominated by emergent plants such as *Zizania latifolia* and *Scirpus maritimus*, and stored in labeled polyethylene bags. The samples were then placed in containers with ice packs, and transported to the laboratory on the same day. Samples were stored in a refrigerator prior to sample processing. Ultimately, one hundred fifty specimens were selected from each population. The selected specimens were divided into five groups based on shell height. Shell heights of *O. hirasei* from Upo ranged from 3 to 6 mm with a mean of 4.1 mm and shell heights from Mokpo ranged from 3 to 5 mm with a mean of 3.9 mm. Sediment samples were taken at a depth of 0~5 cm from the surface around *O. hirasei* sampling sites using a hand grab sampler. Five replicate samples were collected at each site.

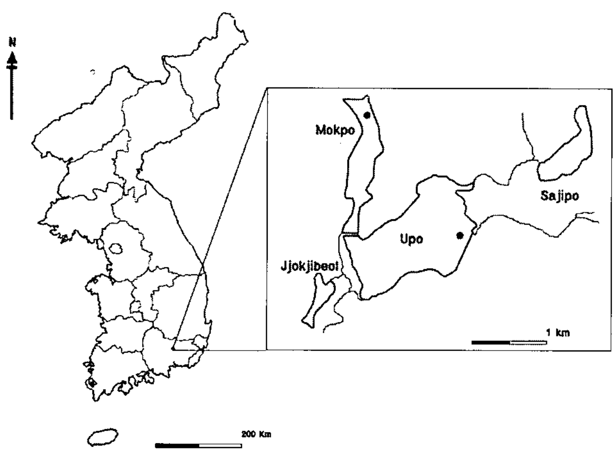

Fig. 1. The map of study area. Black dots mean the sampling sites.

Sediment samples were kept in a polyethylene bag and later air dried in the laboratory.

Sample Processing and Determination of Heavy Metal Levels

The gastropods were washed with distilled water and dissected into soft tissues and shells. The tissues were oven-dried at 105℃ for 48 hours. The dried tissues were then ground to a powder and stored in a zipped bag under refrigerated conditions for further analysis. 0.2 g samples were digested with 5 mL of concentrated nitric acid and 2 mL of 30% hydrogen peroxide. 1 mL of perchloric acid was then added to the digested samples, and the samples were digested again.

Dried sediment samples were passed through a 0.5 mm sieve and the fraction <0.5 mm was chosen for chemical analysis (Usero et al. 2005). Samples of exactly 0.5 g of sediment were taken from the sieved samples and placed in the digestion vessels. The first digestion of sediments was carried out with 5 mL of concentrated nitric acid and 2 mL of hydrofluoric acid. 3 mL of 4% boric acid was then added to the digested samples, and the samples were digested again.

Digestion was carried out in a microwave digester (MarsXpress, CEM, USA) using the microwave digestion programme of Kim and Kim (2006). Digested samples were diluted to a volume of 15 mL in volumetric plastic tubes with double distilled water. Heavy metal concentrations were then determined using an inductively coupled plasma mass spectrometer (ICP-MS) (ELAN 6100, Perkin-Elmer, USA).

Analytical Quality Control and Statistical Analysis

The accuracy of the microwave digester system was tested with Certified Reference Material: Buffalo River Sediment (RM8704) from National Institute of Standards & Technology (NIST) and Oyster Tissue (108-05-001) from Korea Research Institute of Standards and Science (KRISS). The comparison of measured metal concentrations in the CRM with certified values are shown in Table 1.

We used independent samples *t*-tests to test for significant differences in metal concentrations between the two study sites. Coefficient of variation (CV) values were used to determine the degree of variability in metal concentrations in the soft tissues and the shells of *O. hirasei* (Yap et al. 2003). The CV values were calculated by the formula shown below:

$$CV(\%) = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

The efficiency of heavy metal bioaccumulation by *O. hirasei* was

Table 1. Certified metal concentrations (CC) and analyzed concentrations (AC) in Certified Reference Materials (mean ± SD, μg/g dry weight)

	Cd		Cr		Cu	
	CC	AC	CC	AC	CC	AC
RM8704	2.94 ± 0.29	2.87 ± 0.06	121.9 ± 3.8	96.3 ± 2.1	-	-
108-04-001	7.40 ± 0.22	6.71 ± 0.59	0.45 ± 0.07	2.25 ± 0.26	330.1 ± 4.9	299.5 ± 27.7
	Ni		Pb		Zn	
	CC	AC	CC	AC	CC	AC
RM8704	42.9 ± 3.7	43.8 ± 0.7	150 ± 17	141 ± 6	408 ± 15	383 ± 11
108-04-001	-	-	1.52 ± 0.11	1.49 ± 0.30	835.4 ± 10.0	786.3 ± 84.5

studied based on concentration factors. The concentration factors (CF) were calculated according to the formula (Dobrowolski and Skowrońska 2002):

$$CF = \frac{C_g}{C_s} \times 100$$

Where C_g is average content of the element in the gastropod and C_s is average content of the element in the sediment.

RESULTS

Heavy Metal Concentrations in the Gastropods and the Sediments

The concentrations of Cd, Cr, Cu, Ni, Pb, Zn in the soft tissues and shells of *O. hirasei* are presented in Figs. 2 and 3. Concentrations of Zn in the soft tissues were higher than those of other heavy metals (range of 216.1~285.7 μg/g dry weight), whereas Ni concentration was the lowest in the soft tissues (0.14~0.92 μg/g dry weight). The concentrations of heavy metals in the soft tissues of *O. hirasei* from the Upo wetland were in the order of Zn >> Cu >> Cd > Pb > Cr > Ni. In the shells, concentrations of Zn were also higher than those of other metals (3.39~11.84 μg/g dry weight). However, in contrast to the soft tissues, Cd was the heavy metal with the lowest concentration in the shells. The metal concentrations in the shells decreased in the order of Zn > Cu = Cr > Ni > Pb > Cd.

The heavy metal concentrations in the sediments at *O. hirasei* sampling sites are shown in Fig. 4. In both sites, Zn showed the highest concentration and Cd exhibited the lowest concentration. These results are similar to those of other studies although heavy metal concentrations vary according to the lithological source and anthropogenic influx (Lau et al. 1998, Fang 2006). However, for the heavy metals analyzed, the concentrations in the sediments showed significant differences between sites (p<0.01), with higher metal

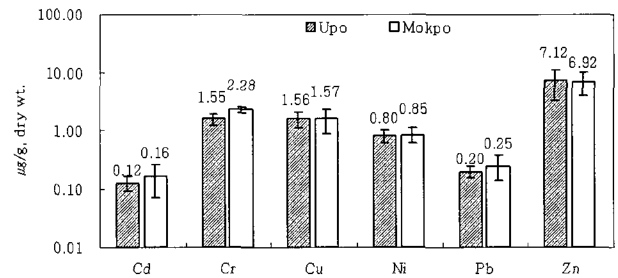

Fig. 3. Metal concentrations in the shells of *O. hirasei*. (mean ± SD, N=5)

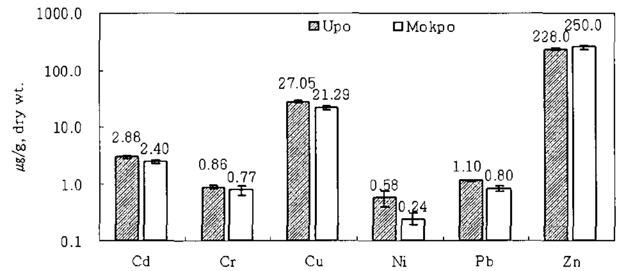

Fig. 2. Metal concentrations in the soft tissues of *O. hirasei*. (mean ± SD, N=5)

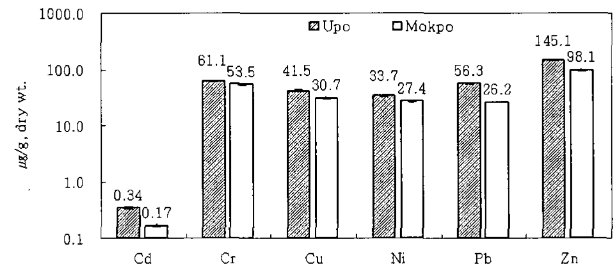

Fig. 4. Metal concentratons in the sediments of Upo wetland (mean ± SD, N=5)

levels at Upo than at Mokpo.

Comparison of Metal Concentrations between the Soft Tissues and the Shells

The partitioning factor (PF), defined as the ratio between the mean metal concentrations in the soft tissue and shells from each site (Cravo et al. 2004) is shown in Fig. 5. Cd, Cu, and Zn showed relatively high PF values, with averages of 19.1, 15.5, and 34.1, respectively while the values of Cr and Ni were relatively lower than other metals (0.4 and 0.5, respectively). These results might indicate that Cd, Cu and Zn are more likely to be incorporated into the soft tissues while Cr and Ni are more easily incorporated into the shells. Although the PF value of Pb was lower than those of Cd, Cu, and Zn, the average value of 4.4 suggests that Pb concentrations were consistently higher in the soft tissues than in the shells. Overall, the concentrations of Cd, Cu, Pb and Zn were much higher in the soft tissues than in the shells of *O. hirasei* while levels of Cr and Ni were much higher in the shells.

DISCUSSION

The Level of Heavy Metal Concentrations in *O. hirasei*

Although all gastropods are on the same trophic levels and have similar feeding habits, they display different capacities for bioaccumulation of heavy metals (Liang et al. 2004). The concentrations of Cd and Zn in soft tissues of *O. hirasei* were much higher than those in soft tissues of *Cipangopaludina chinensis malleata* from the Upo wetland, whereas levels of other metals (Cr, Cu, Ni, Pb) were much lower in *O. hirasei* than in *C. chinensis malleata* (Kim and Kim 2006). The shells of *O. hirasei* generally contained lower levels of heavy metals than *C. chinensis malleata* except for Cu and Zn. Zn concentration in the shells of *O. hirasei* was higher than those of other metals (Cd, Cr, Cu, Ni, Pb) while the element with highest concentration in *C. chinensis malleata* was Ni. This result might indicate that *O. hirasei* possessed higher bioaccumulation capacities for Cd and Zn than *C. chinensis malleata*.

Heavy metal accumulations of gastropods are generally dependent on habitat, metal, and species (Gundacker 2000, Liang et al. 2004). In this study, the concentrations of Cd, Cu, Ni and Pb in the soft tissues of *O. hirasei* were significantly different between Upo and Mokpo (Cd, Cu, Pb: $p<0.01$, Ni: $p<0.05$). Levels of heavy metals in gastropod tissues at Upo were much higher than those Mokpo. Considering that the heavy metals in Upo sediments were also much higher than those at Mokpo, we conclude that the different levels of heavy metals (Cd, Cu, Ni, Pb) in the soft tissues accurately reflect differences in the heavy metal levels of the environments inhabited by the gastropods. However, heavy metal concentrations in the shells exhibited no significant differences between the sites. This indicates that *O hirasei* shells do not reflect the environmental bioavailability of heavy metals as do the soft tissues. Therefore, the soft tissues of *O. hirasei* could be considered as a more useful bioindicator for Cd, Cu, Ni and Pb.

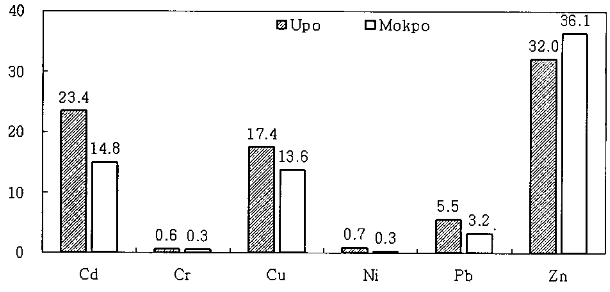

Fig. 5. Partitioning factors of *O. hirasei* from the sites.

Bioaccumulation of Heavy Metals in the Soft Tissues and the Shells of *O. hirasei*

The bioaccumulation of heavy metals in gastropod tissues seems to vary depending on the species, the physiological condition, and the habitat environment (Cubadda et al. 2001, Conti and Cecchetti 2003, Cravo and Bebianno 2005). The present study also showed that heavy metal levels in *O. hirasei* tissues were different from reported values from *C. chinensis malleata* at the same site (Upo). However, this study showed similar partitioning of Cu and Zn between the soft tissues and shells of *O. hirasei* to those reported from other gastropods at other sites. Regardless of species and habitat, gastropods usually showed much higher concentrations of Cu and Zn in their soft tissues than in their shells (Carral et al. 1995, Gundacker 1999, De Wolf et al. 2001). This pattern results from the fact that Cu and Zn are essential components of metabolically important biomolecules such as enzymes and the respiratory pigment hemocyanin (Langston and Spence 1995). While the distribution of Ni and Pb in the soft tissues and shells of *O. hirasei* were similar to that of *C. chinensis malleata*, *O. hirasei* seemed to accumulate more Cd into their soft tissues than *C. chinensis malleata*.

The efficiency of metal bioaccumulation in a gastropod can be

Table 2. Concentration factors (CF) of heavy metals in *O. hirasei* from the sites

	Sites	Cd	Cr	Cu	Ni	Pb	Zn
Soft tissues	Upo	8.43	0.01	0.65	0.02	0.02	1.57
	Mokpo	14.28	0.01	0.69	0.01	0.03	2.55
Shells	Upo	0.36	0.03	0.04	0.02	0.00	0.05
	Mokpo	0.97	0.04	0.05	0.03	0.01	0.07

Table 3. Concentration factors (CF) of heavy metals in *Cipangopaludina chinensis malleata* from Upo (from Kim and Kim (2006))

Tissues	Cd	Cr	Cu	Ni	Pb	Zn
Soft tissues	0.39	0.70	1.49	0.16	0.03	1.61
Shells	–	0.09	0.04	0.78	0.01	0.03

Table 4. Coefficient of variance CV of heavy metals in *O. hirasei* from the sites

	Sites	Cd	Cr	Cu	Ni	Pb	Zn
Soft tissues	Upo	5.9	7.7	3.3	30.4	3.9	4.6
	Mokpo	8.4	18.5	5.9	22.0	12.4	7.7
Shells	Upo	28.6	21.7	31.1	23.5	22.7	53.4
	Mokpo	57.6	11.2	44.5	27.4	46.4	43.0

represented by the CF value. This value could provide the basis for a more objective standard for comparisons between different species in their pattern of bioaccumulation of metals and for evaluation of their suitability as bioindicators for specific metal (Lau et al. 1998). Also, CV value presents the criteria for assessing a precision of each metal for spatial resolutions.

The concentration factors of the heavy metals in the tissues of *O. hirasei* were presented in Table 2. The CF values of the soft tissue were usually higher than the shell. The results suggest that the soft tissues of *O. hirasei* would be a more useful bioindicator rather than the shells although the potential of the shell of *C. chinensis malleata* as a bioindicator was suggested (Kim and Kim 2006). The CF value of Cd in the soft tissues was much higher than other metals and the value of Cd in the soft tissue of *C. chinensis malleata* (Table 3). This indicates that Cd was more efficiently bioaccumulated into the soft tissues than other metals and the soft tissue of *O. hirasei* could also be more useful bioindicator for Cd rather than *C. chinensis malleata*. From the above results, Cd, Cu, Ni and Pb of the soft tissues might be considered to reflect the heavy metal levels of sediments. However, while Ni concentrations in the soft tissues of *O. hirasei* might reflect the heavy metal level of sediments, the CV value was much higher than Cd, Cu and Pb (Table 4). The lower CV values of Cd, Cu and Pb strongly support that the heavy metals would have higher precision than Ni. Therefore, the soft tissues of *O. hirasei* can be more useful to biomonitor a change of Cd, Cu and Pb concentrations. Therefore, the soft tissue of *O. hirasei* appears to be a promising bioindicator particularly for Cd because Cd has good magnification capabilities with lower variability than other metals.

CONCLUSION

There were significant differences between Upo and Mokp in the Cd, Cu, Ni and Pb concentrations in the soft tissues of benthic gastropod, *O. hirasei*. These differences accurately reflect differences of the levels of heavy metals in their habitats. Furthermore, the low variability of Cd, Cu and Pb levels in the soft tissues suggest that *O. hirasei* may be useful for biomonitoring of levels of these heavy metals in Korean wetlands. Cd was accumulated into the soft tissues of *O. hirasei* at higher rates than the other metals. The good magnification of Cd, coupled with its lower variability in *O. hirasei* tissues suggest that the soft tissues of *O. hirasei* have potential for use as a bioindicator of heavy metal levels, particularly Cd, in the Upo wetland.

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