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Heavy Metal Interactions during Accumulation and Elimination of Cadmium and Copper in the Liver of Juvenile Flounder, *Paralichthys olivaceus*

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Experiments were carried out to investigate the effects of metal interaction on the accumulation and elimination of Cd and Cu in the liver of flounder, *Paralichthys olivaceus*, exposed to sub-chronic Cd (0, 5, 10, 50, 100 μg/L)/Cu (10 μg/L) mixture. Cd exposure resulted in an increased Cd accumulation in the liver of flounder for exposure periods and concentration, and Cd accumulation increased linearly with exposure time. Cu accumulation profiles were similar to those of Cd. Cd concentration in the liver significantly decreased at the 10th depuration period and elimination rate was 66.20%, 86.22% in 50 and 100 μg/L at the end of depuration periods, respectively. Although, Cu elimination was similar to Cd elimination phase, Cd elimination rate was higher than that of Cu. Co-relationship of Cd and Cu have a positive correlation coefficient $r=0.8620$ ($P<0.01$) and support the strong relationship between Cd and Cu accumulation. As increase with the Cd exposure concentration, there were significant ($P<0.01$) differences between Cd and Cu accumulation.

Key words: Cadmium, Copper, *Paralichthys olivaceus*, Accumulation, Elimination, Interaction

Introduction

Heavy metals such as cadmium (Cd) and copper (Cu) are frequently present at elevated level of exposure in marine ecosystem. As a result, marine organisms including fish are increased to elevated level of these metals (Bryan, 1976).

Cd is a non-essential element that can have severe toxic effects on aquatic animals when present in excessive amounts (Sorensen, 1991). In fish, Cd has adverse effects on growth, reproduction and osmoregulation (Verbost et al., 1989; Sorensen, 1991; Lemaire and Lemaire, 1992; Soengas et al., 1996). Moreover, it affects respiratory functions and the composition of plasma by causing hypocalcemia, hypokalemia, and hyperglycemia (Sorensen, 1991). The activity of metabolic enzymes in the liver, kidney, muscles, and other tissues is disturbed following exposure to Cd (Sastry and Subhadra, 1982). Although adverse effects of Cd on various physiological functions of fish are well documented, metal mechanisms of these effects in marine organisms are poorly understood and investigated.

Cu is an essential metal for all organisms including fish, its plays an important role in organism metabolism and its concentration is well regulated (Cousins, 1985). However, Cu is one of the most toxic metals to fish and affects various blood parameter (Christensen and Tucker, 1976), growth (Langston, 1990), enzyme activity (Roesijadi and Robinson, 1994), and reproduction (Horning and Nieheisel, 1979).

Heavy metals are present as mixtures in sea water and the effects of mixtures of metals in aquatic organisms are complex (Hamilton et al., 1987). Since metal pollution is rarely limited to only a single metal, studies of interaction between metals are great significance in aquatic organisms. Many studies of metal interaction in aquatic organisms have concentrated on metal toxicity and accumulation

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(Sprague, 1964; Oakden et al., 1984; Elliott et al., 1986; Mathew and Menon, 1992; Pelgrom et al., 1994; 1995; Tao et al., 1999). However, studies on metal interaction during accumulation and elimination are rare (Kargin and Çoðun, 1999).

Generally, due to its central role in metabolism and its sensibility to metal, the liver of fish has been given study for accumulation and toxicological effect of metal in fish. In fish, the liver tends to concentrate metals and exhibits relatively high potential for bio-accumulation and detoxification (Roesijadi and Robinson, 1994).

The olive flounder, *Paralichthys olivaceus*, is an economically important food fish that commonly cultured in Korea and they have been cultured in land-based tanks (Jung et al., 2001). Olive flounder is particularly exposed to costal metal pollution, which its lives mainly on benthic area, from a few meters to about 150 m. Therefore, the aim of this study was to investigate the effects of metal interaction on the accumulation and elimination of Cd and Cu in the liver of flounder, *P. olivaceus*, during sub-lethal Cd/Cu mixture exposure.

Materials and Methods

Fish and control water conditions

Juvenile olive flounder (*P. olivaceus*) were obtained from a private flounder nursery in Jeju Island, Korea. Flounder were acclimated to the laboratory conditions (Table 1) for 3 weeks in three 1 ton of FRP (fiber glass reinforced plastics) tanks. Each tank received a flow of 7 L/min and was supplied with continuous aeration. Fish were fed olive flounder commercial diet daily at a rate of 3% body weight (as two 1.5% meals per day). After acclimatization, 240 fish (mean length, 17.10 ± 0.11 cm, body weight 52.50 ± 0.90 g; means ± S.E.) were selected for the experiments of Cd/Cu mixture exposure.

Exposure and depuration system

After 3 weeks in acclimating tanks, fish were randomly transferred to twelve 50 L of PVC exposure tanks (52×36×30 cm), which were renewal test with continuous aeration. Test seawater was renewed every three days and seawater temperature was maintained at 18.0 ± 0.2℃ in a constant temperature room. All olive flounders were maintained and tested under a 12 h light (09~21 h)/12 h dark illumination cycle. Seawater quality was measured every 10 days during the experiment periods, and fish were fed 3% body weight per day. Cd stock solution, with Cd added as $CdCl_2$ (Aldrich, USA) and Cu stock solution used $CuSO_4 \cdot 5H_2O$ (Aldrich, USA.). The exposure concentration of Cd were 0 (control), 5, 10, 50 and 100 μg/L and Cu were 10 μg/L. The experiment was designed first to exposure the fish to the Cd/Cu mixture for 30 days and then to transfer them into Cd/Cu-free seawater for another 20 days.

Table 1. Chemical components of seawater used in the sub-chronic cadmium exposure and depuration experiment. Values indicate means ± S.E.

Item	Value
Temperature (℃)	18.0 ± 0.2
pH	8.1 ± 0.2
salinity (‰)	32.7 ± 0.4
Ammonia (μg/L)	12.66 ± 1.25
Nitrite (μg/L)	1.37 ± 0.28
Nitrate (μg/L)	9.62 ± 1.01
Phosphate (μg/L)	5.05 ± 0.96
SS (mg/L)	5.62 ± 0.25
Dissolved oxygen (mg/L)	6.74 ± 0.84
COD (mg/L)	1.52 ± 0.08
Copper (μg/L)	2.32 ± 0.12
Cadmium (μg/L)	N.D.

*N.D.: Not detected.

Metal analysis

Liver tissue was sampled every 10 day for analysis of the liver metal concentration. Eight fish were removed each test concentration and control. Liver tissues were dried at 65℃ and kept in a desiccators until digestion. Dry tissue was digested with 1:1 HNO_3 (Suprapur grade, Merck, Germany), and samples were fumed to near dryness on a hot plate at 120℃ for overnight (APHA, 1992). After digestion, the residue was dissolved in 20 mL of 0.2 N HNO_3 and kept in a refrigerator until analysis for trace metal. Cd concentrations of the liver were measured using a graphite furnace atomic absorption spectrophotometer (AAS, Perkin-Elmer 3300, USA) and Cu were measured using a flameless AAS. Cd and Cu concentration in the liver of flounder were expressed as μg/g dry wt.

Statistics

Data are given as means ± standard error (S.E.). Statistics were using one-way analysis of variance (ANOVA) followed by the Duncan's multiple comparisons test of mean values if significant differences were found (P<0.05). For statistical analysis of relation between Cd and Cu were used correlation analysis followed by difference significance test for correlation coefficient (P<0.01).

Results

Cd and Cu accumulation

Cd accumulation in the liver of *P. olivaceus,* as a function of exposure time and concentration, are shown in Fig. 1. These curves show clearly that Cd

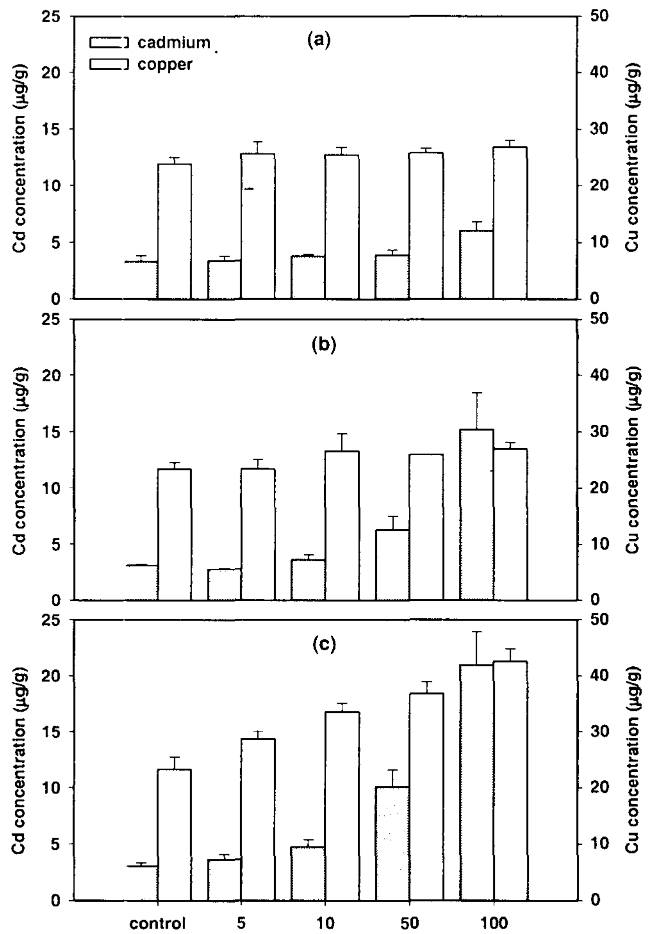

Fig. 1. Cadmium and copper concentration in liver of *P. olivaceus* during cadmium/copper mixed exposure (a: 10 days, b: 20 days, c: 30 days). Vertical bar indicate means ± S.E.

concentration in the liver significantly increased at 100 μg/L and 50 μg/L for 30 days of exposure (P< 0.05). During the first 10 days, Cd accumulation in the liver did not vary significant. After 30 days of exposure, Cd concentration values were 20.94 ± 4.3 μg/g and 10.19 ± 1.5 μg/g for exposed to 100 μg/L and 50 μg/L, respectively. On the other hand, at 5 μg/L and 10 μg/L exposure, Cd accumulation was not significantly different from that of the control. In addition to, Cd concentration in the liver was correlated with exposure periods. For fish exposed to 50 μg/L and 100 μg/L of Cd, the following linear relations were obtained:

Cd (50 μg/L) = 2.31 days + 0.12 (r = 0.9569, P<0.01)
Cd (100 μg/L) = 6.23 days − 4.24 (r = 0.9823, P<0.01)

Cu accumulation profiles were similar to Cd accumulation for 30 days exposure periods. After 20 days of exposure to 100 μg/L, the level of Cu accumulation had increased to 27.0 ± 1.10 μg/g. This increase continued and 42.6 ± 2.18 μg/g was measured after 30 days of exposure. At the 50 μg/L Cd exposure, a significant Cu accumulation in the liver appeared after 20 days (P<0.05). After 30 days of continuous exposure, Cu levels had increased to 36.8 ± 2.09 μg/g of liver tissue. No significant Cu accumulation occurred 20 days except exposed to 100 μg/L and 50 μg/L. Briefly, Cu accumulation was increased for Cd exposure concentrations and periods.

Cd and Cu elimination

Cd elimination in the liver of *P. olivaceus,* as a function of exposure time and concentration, are shown in Fig. 1. After 10 days of depuration to 100 μg/L, Cd concentration had decreased to 12.05 ± 3.73 μg/g (42.45%, elimination rate). This decrease continued and 2.89 ± 0.18 μg/g (86.24%) was measured after 20 days of depuration. During the 10 depuration days at 50 μg/L, Cd concentration was 8.89 ± 1.33 μg/g (11.89%). Cd concentration was similar to that of 100 μg/L Cd at 20 depuration days. At 5 μg/L and 10 μg/L Cd, the Cd concentration did not vary significant.

During the depuration periods, Cu elimination was similar to Cd elimination phase. After 10 days of depuration at 100 μg/L, Cu concentration decreased to 36.6 ± 1.53 μg/g (14.08%). This decrease continued and 28.0 ± 1.84 μg/g (34.27%) was measured after 20 days of depuration. During the 10 depuration days at 50 μg/L, Cu concentration was 31.16 ± 2.06 μg/g (15.33%). After 20 days of depuration at 50 μg/L and 100 μg/L, Cu concentration was higher than that of control, which was different from the profile of Cd elimination. At end of depuration days, Cu elimination rate was 24.18% and 34.27%, whereas Cd elimination rate was 66.20% and 86.24% at 50 μg/L and 100 μg/L Cd, respectively. Therefore, Cd elimination rate was higher than that of Cu in *P. olivaceus* (Fig. 1, 2).

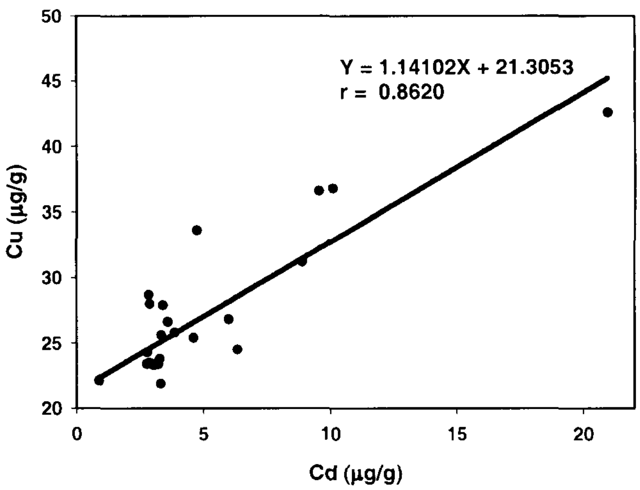

Fig. 3. Relationship between cadmium and copper concentration in liver of *P. olivaceus* reflecting significant (P<0.01).

Interaction between Cu and Cd

The correlation between Cd and Cu was plotted in Fig. 3. Cu concentration increased linearly with Cd concentration and regression equations can be expressed Cu=1.41Cd+21.30. This lead to a positive correlation coefficient r=0.8620 (P<0.01) and supports a strong relationship between Cd and Cu accumulation. For exposure concentration, correlation coefficient was 0.7928, 0.7969, 0.8501 and 0.8701, respectively. As increase with the Cd exposure concentration, there were significant (P<0.01) differences between Cd and Cu accumulation (Fig. 4).

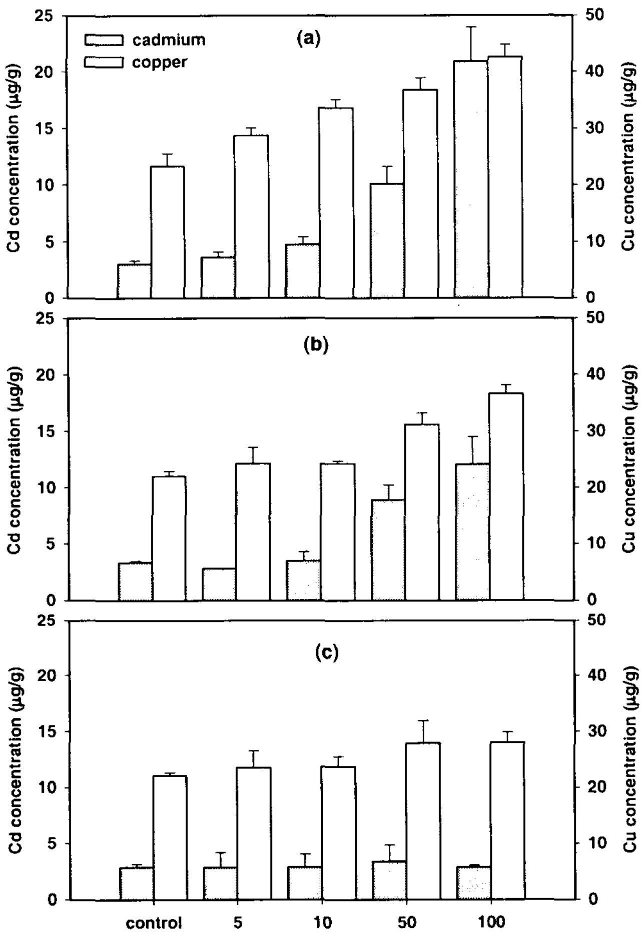

Fig. 2. Cadmium and copper concentration in liver of *P. olivaceus* during depuration periods (a: 30 days, b: 40 days, c: 50 days). Vertical bar indicate means ± S.E.

Discussion

Metal accumulation in tissues of fish is dependent upon exposure concentration and time as well as other factors such as temperature, age of fish, interaction with other metals, water chemistry and metabolic activity of fish (Pagenkopf, 1983; Heath, 1987; Goyer, 1991).

In this study, Cd exposure resulted in an increased Cd accumulation in the liver of flounder for exposure periods and time, and this agrees with other studies carried out with aquatic organisms (Gill et al., 1992; Pelgrom et al., 1994; 1995; McGeer et al., 2000). Liver plays a major role in detoxification and excretion of metals through the induction of metal -binding proteins such as metallothionein (MTs; Klaverkamp et al., 1984; Roesijadi, 1992). Cu accumulation in the liver of flounder was increased in the presence of Cd and this increase was strongly observed for Cd exposure higher. Elliot et al. (1986) studied metal interaction during accumulation by

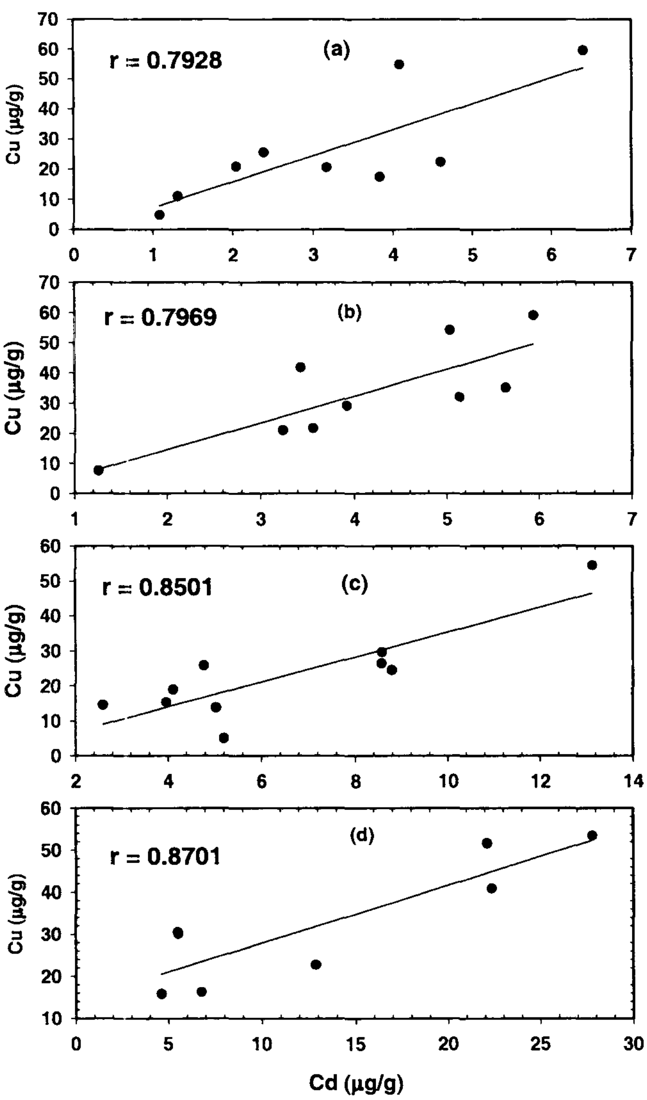

Fig. 4. Relationship between cadmium and copper concentration at exposure level in liver of *P. olivaceus* reflecting significant (P<0.01). (a: 5 µg/L, b: 10 µg/L, c: 50 µg/L, d: 100 µg/L)

the mussel (*Mytilus edulis planulatus*) and found that Cu accumulation was increased in the presence of Cd. Moreover, this situation was presence of a high level of Cd. In fish (*Oreochromis mossambicus*), Pergrom et al. (1995) reported that Cu/Cd co-exposure resulted in a significant increase of the liver Cu concentration compared to single Cu exposure. This suggests a disturbance of the Cu metabolism which might involve interactions between Cu and Cd during binding to MTs.

Several factors influence the elimination of metals from the tissues of fish. These include time, temperature, interacting agents, age of fish, metabolic activity of fish and biological half life of metal (Larson et al., 1985; Heath, 1987; Douben, 1989; Woo et al., 1993; Nielsen and Andersen, 1996). Kuroshima (1987) found that the Cd level in the liver of girella (*Girella punctata*) still increased during the clearance period in Cd-free seawater after the end of Cd exposure. Wicklund et al. (1988) showed that Cd accumulation in the liver and kidney of zebrafish (*Brachydanio rerio*) continued throughout the depuration period.

In this study, Cd elimination was started 10 day in depuration period after end of Cd/Cu exposure, we observed higher elimination rate at high concentration exposure. In tilapia (*Tilapia nilotica*), Cd elimination significantly increased both mixed concentration exposure concentration (Cd/Zn mixed exposure) compare to single exposure for Cd. This is due to interactions at sites binding both metals in tissues (Kargin and Çoðun, 1999). Wicklund et al. (1988) studied the effects of Zn on the elimination of Cd in the zebrafish (*B. rerio*) and found that Zn tended to increase the Cd elimination rate. This indicated that Cd elimination rate increased with Cu/Cd co-exposure compared to single Cu exposure and the reason is probably due to interaction Cd/Cu with MTs.

In this experiment, Cd elimination rate was higher than that of Cu in the olive flounder. Viarango et al. (1985) found that mussel (*M. galloprovincialis*) exposed to Cu and Cd showed different levels of metal elimination from its tissues, and that Cu is rapidly eliminated in the gill and digestive gland cells, showing a biological half life of 10 days, whereas Cd elimination is much slower. But, Kargin and Çoðun (1999) studied that Cd elimination rate increased in the liver of tilapia (*T. nilotica*) and this is due to interactions at sites binding both metals in tissues. Cd exposure raised hepatic Cubinding protein levels in bass, *Micropterus salmoides* (Weber et al., 1992). This is probably Cd elimination higher than Cu in juvenile olive flounder (*P. olivaceus*), because interaction both metal and increased Cubinding proteins.

Generally, metal accumulation by several species may be influenced by the presence of other metals and such interaction occurs at high concentration of the interacting metals (Elliott et al., 1986).

Ahsanullah et al. (1981) studied that Cd reduced Cu accumulation in shrimp (*Callianassa australiensis*). However, Elliot et al. (1986) observed that presence of a high level of Cd resulted in an increased Cu accumulation by mussel, *M. edulis planulatus*. In this study, Co-relationship of Cd and Cu have a positive correlation coefficient ($r=0.8620$, $p<0.01$), this supports the strong relationship between Cd and Cu accumulation. This seems to indicate that there is a significant synergistic effect between Cd and Cu on both accumulation and elimination. Moreover, such interaction is related to the metal exposure concentration, and Cu accumulation increased according to an increased metal exposure concentration.

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