Fish Aqua Sci 12(4), 324-330, 2009

KOSFAS FISHERIES AND AQUATIC SCIENCES
The Korean Society of Fisheries and Aquatic Science

Cadmium Accumulation and Elimination in the Tissues of the Manila Clam, *Ruditapes philippinarum*, after Sub-chronic Cadmium Exposure

Seok-Woo Jang, Seong-Gil Kim*, Ok-In Choi, Seong-Soo Kim and Ju-Chan Kang[1]

Marine Environmental Research Division, National Fisheries Research and Development Institute, Busan 619-705, Korea

[1]Department of Aquatic Life Medicine, Pukyong National University, Busan 608-737, Korea

Cadmium (Cd) accumulation and elimination were assessed in the tissues of the clam *R. philippinarum* at four experimental concentrations (control, 10, 20, 100, and 200 μg/L) over an exposure period of 2 weeks and an elimination period of 1 week. Cd accumulated in the digestive gland, gill, and residual clam tissues, and accumulation increased with time of exposure and concentration (100 and 200 μg/L). After 2 weeks of Cd exposure, the order of Cd accumulation in tissues was gill > digestive gland > residual tissues. An inverse relationship was observed between concentration factor (CF) and exposure level, but the CF showed an increase with exposure time. During the depuration period, Cd concentrations in the digestive gland, gill, and residual tissues decreased immediately on the cessation of exposure, except in individuals at the 200 μg/L concentration. The Cd elimination rate from tissues decreased in the order of digestive gland > gill > residual tissues during the depuration period.

Key words: Manila clam, *Ruditapes philippinarum*, Cadmium, Accumulation, Elimination

Introduction

Toxic heavy metal pollutants are increasingly being released into the environment as a result of industrialization. As a consequence, trace metals such as Cd have induced toxic effects in aquatic organisms. Cd is a nonessential metal that is widely distributed in the marine environment and that has severe toxic effects on aquatic animals when present in excessive amounts (Sorensen, 1991). Generally, Cd has adverse effects on the growth, reproduction, and osmoregulation of marine organisms. Moreover, Cd can cause various deleterious biochemical alterations in mollusk tissues, such as decreased activities of some enzymes and enhanced lipid peroxidation (Roesijadi and Robinson, 1994).

Marine animals can bioaccumulate metals from seawater, suspended particles, and sediments and through the food chain, and metal accumulation in marine organism tissues is dependent upon the rate of uptake, route, storage, and elimination (Blackmore, 2001). Metal accumulation and elimination studies are important from the point of view of health protection and the assessment of the toxicological effects of different metallic contaminants, allowing for the determination of the self-cleansing ability of contaminated organisms.

Bivalve mollusks are filter-feeding organisms that are known to accumulate high concentrations of trace metals in their tissues, and they are widely used as bioindicators for pollution in marine environments (Regoli and Orlando, 1994). They have a number of properties that make them useful sentinels for chemical pollution: they have wide geographical distribution, are stationary, and are normally the dominant species in their habitat. In addition, they are capable of withstanding baseline levels of pollution and are abundant in estuaries where much human contact with the aquatic environment occurs (Sheehan and Power, 1999). Moreover, because bivalves are an important food resource and a major ecosystem component, it is important to assess the effect of Cd on these shellfish.

The Manila clam *Ruditapes philippinarum* is an

*Corresponding author: cosmas@nfrdi.go.kr

economically important food shellfish in Korea that is commonly aquacultured throughout the Korean coastal region (Park et al., 2006). Despite its importance, relatively little information is available on the effect of metals on this species, particularly waterborne Cd exposure. Therefore, the aims of the present study were to investigate the Cd accumulation and elimination in clams that results from subchronic waterborne Cd exposure.

Materials and Methods

Experimental organisms and treatment

Manila clams *R. philippinarum* were collected in July 2005 from a clam farm in Go-heung, Korea. The clams were acclimatized for five days in a semi-static system. After acclimatization, clams (shell length: 35.81±2.51 mm, body weight: 10.40±2.16 g) were selected for the experiments. Fifty clams were separated into 50 L control and test tanks. The animals in the test tanks were exposed to different sublethal concentrations of cadmium sulfate at 10, 20, 100, and 200 μg/L ($CdCl_2$, Sigma Chemical, St. Louis, MO) for 2 weeks. After that, clams were transferred to clean seawater for 1 week. The experimental clams were maintained and tested under laboratory conditions in a 12:12 h light:dark cycle for further studies. Seawater was changed every 48 hours, and water quality was measured every 2 days during the experimental periods. The condition index (CI) of the clams was calculated as CI = fresh flesh weight (g)× 100/shell weight (g) according to Mann and Glomb (1978).

Cadmium analysis

The gill, digestive gland, and residual tissues were sampled every week for analysis of metal concentrations. Ten clams were removed from each test concentration and control tank. The weight and total length of each individual were recorded, and the gill, digestive gland, and residual tissues (adductor muscle, foot, and siphon) were sampled for Cd analysis. Gill and residual tissues were rinsed with 0.6% NaCl to remove any fine particulate matter, and the digestive gland was rinsed with 0.6% NaCl to remove any undigested food and feces. Tissue samples were freeze-dried at -80°C for 10 days using a freeze-dryer and were kept in a refrigerator until further analyses. Dry tissue was digested with 1:1 HNO_3 (Suprapur grade, Merck, Germany), and samples were fumed to near dryness on a hotplate at 120°C overnight. After digestion, the residue was dissolved in 20 mL of 0.2N HNO_3 and kept in a refrigerator until analysis for trace metal. Cd concentrations in clam tissues were measured using an ICP-MS (Elan 6000, Perkin-Elmer) and were expressed as μg/g dry wt. Quality controls were made using strand reference material (BCR-CE278) from the Institute for Reference Material and Measurements. These reference materials were treated and measured using the same procedures as the samples, and results showed good analytical efficiency within certified values (certified: 0.348± 0.13 μg/g; observed: 0.323±0.03 μg/g; recovery: 92.8%). Concentration factor (CF) is often used to compare the body burden of an organism with the degree of contamination of water. The following definition is used here: $CF = \{[Me]_{exp} - [Me]_{control}\} / [Me]_{seawater}$, where $[Me]_{exp}$, $[Me]_{control}$, $[Me]_{seawater}$ are the metal concentrations in the experimental, control, and exposure seawater groups, respectively. Elimination rate (%) is defined as the percentage decrease from initial value (after 2 weeks).

Statistical analysis

Data are expressed as means±standard error (SEM). All statistical analyses were performed using the SPSS/PC$^+$ statistical package. Prior to analysis, all data were tested for homogeneity of variances among groups using the Bartlett test. Comparisons of normalized data between control and treatment groups were made using one-way analysis of variance (ANOVA) followed by Duncan's multiple comparisons test of mean values if significant differences were found ($P<0.05$).

Results

Accumulation of cadmium in clams

No mortality occurred as a result of dietary Cd exposure throughout the experimental period. No differences were observed in the CI of exposed clams compared with controls (Fig. 1). Exposure to Cd induced significant Cd concentrations in the clam. Cd that accumulated in the digestive gland, gill, and residual tissues of *R. philippinarum* as a function of exposure time and exposure concentration are shown in Fig. 2. Cd accumulation in the digestive gland at high exposure concentrations (100 and 200 μg/L) was significantly increased with exposure period and concentration over the 2 week experimental period ($P<0.05$). Relatively low concentrations were observed in the digestive gland compared with those in other tissues, and Cd concentration values were 16.45±8.50 μg/g and 26.17±5.49 μg/g at 100 and 200 μg/L, respectively. No significant Cd accumulation occurred in the digestive gland of clams exposed

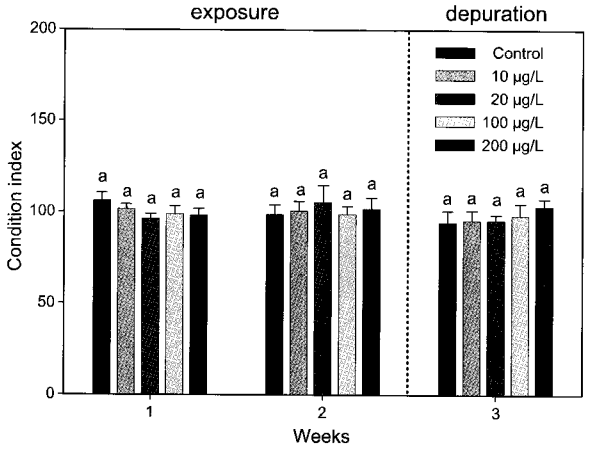

Fig. 1. Changes in the condition index (CI) of the clam *Ruditapes philippinarum* exposed to Cd for two weeks, followed by a depuration period of one week (mean±SE). Values in column with the same superscripts are not significantly different.

to 10 and 20 μg/L.

The Cd accumulation profiles of gill tissue depended on waterborne Cd concentration and exposure period (Fig. 2b). Although the profile of Cd accumulation in the gill was similar to the digestive gland profile, Cd accumulation in gill tissue was about an order of magnitude higher than that in digestive gland and residual tissues. During the first week, Cd concentration values increased sharply, reaching values of 41.82±26.99 μg/g and 88.93± 25.88 μg/g (6 and 13 fold increases compared with control) at 100 and 200 μg/L, respectively. At the end of the exposure, Cd concentration values were 103.21±26.25 μg/g and 186.78±106.08 μg/g at 100 and 200 μg/L Cd exposure, respectively, and were approximately 17 and 32 times higher than concentrations in the control group. No significant Cd accumulation occurred in the gill tissues of clams exposed to 10 and 20 μg/L Cd.

During Cd exposure periods, Cd accumulation in the residual tissues of clams at high exposure concentrations (above 100 μg/L) increased significantly after 1 week (Fig. 2c). After 2 weeks of exposure, the Cd concentrations in residual tissues were 30.27± 7.36 μg/g and 44.36±7.36 μg/g for clams exposed to 100 μg/L and 200 μg/L Cd, respectively. Conversely, Cd accumulation in clams exposed to 10 and 20 μg/L was not significantly different from that of the control during the 2 weeks of waterborne exposure. After 2 weeks of Cd exposure, the order of Cd accumulation in organs was gill > digestive gland > residual tissues. The accumulation factors are presented in Fig. 3 for the digestive gland, gill, and residual tissues at 10, 20,

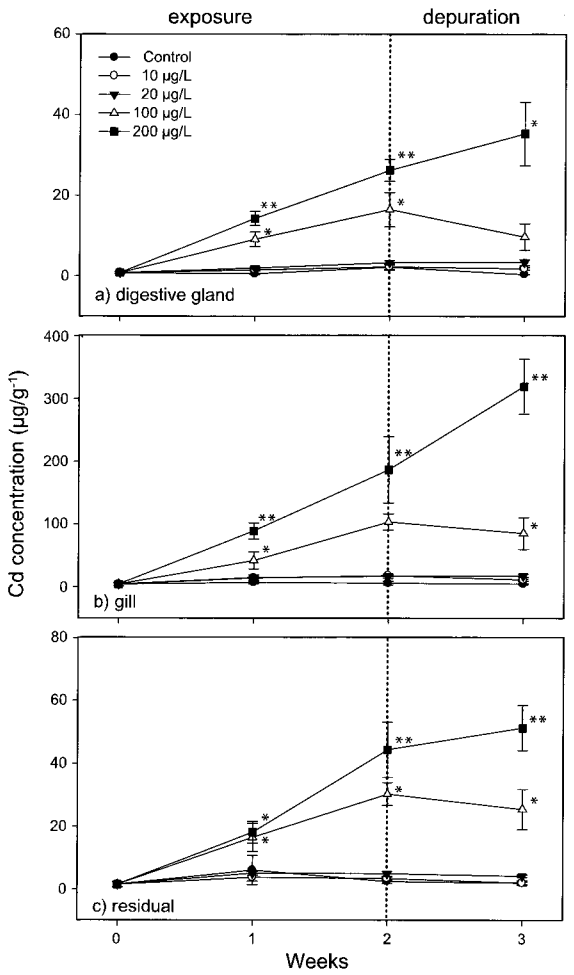

Fig. 2. Cd accumulation and elimination in the digestive gland, gill and other tissues of the clam *Ruditapes philippinarum*, exposed to Cd for two weeks, followed by a depuration period of one week (mean±SE). *and **indicate a significant differrence from control and *group ($P<0.05$).

100, and 200 μg/L Cd exposure. The accumulation factors were increased with exposure period in the digestive gland, gill, and residual tissues. An inverse relationship was observed between the accumulation factor and exposure concentration at 1 week. Although the accumulation factor in clams exposed to concentrations over 100 μg/L increased with time, it did not increase with exposure concentration.

Elimination of cadmium from clams

Cd elimination data from the digestive gland, gill, and residual tissues of *R. philippinarum* exposed to 10, 20, 100, and 200 μg/L Cd are presented in Fig. 2. During the depuration period, Cd concentration in the digestive gland, gill, and residual tissues decreased slowly on the cessation of exposure at 100 μg/L, and

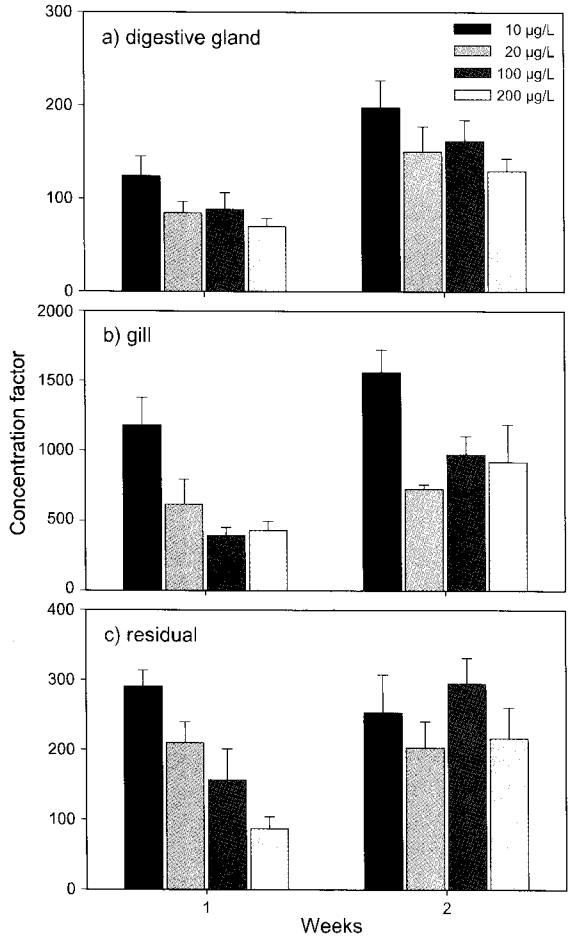

Fig. 3. Concentration factor (CF) over time in the digestive gland, gill and other tissues of the clam *Ruditapes philippinarum* exposed to 10, 20, 100 and 200 μg/L Cd, respectively (mean±SE).

the elimination rates from the digestive gland, gill, and residual tissues at the end of the depuration period were 41.22%, 17.39%, and 15.92%, respectively. However, the Cd concentration values in the digestive gland, gill, and residual tissues of clams exposed to 200 μg/L increased during depuration periods to 35.24±15.79 μg/g, 319.50±87.61 μg/g and 51.28±14.36 μg/g, representing increases of approximately 95, 66, and 27 times over the control, respectively. The order of Cd elimination from tissues during the depuration period was digestive gland > gill > residual tissues. Cd accumulation in the digestive gland, gill, and residual clam tissues increased with the time of exposure and with concentration over 100 μg/L, and the elimination rate decreased at exposures below 100 μg/L. In the 10 and 20μg/L Cd exposure groups, Cd concentration remained constant during the depuration period (Fig. 3) and did not vary significantly ($P<0.05$).

Discussion

Exposure to sublethal Cd induced significant differences in Cd concentration in clams. Metal concentrations in aquatic animal tissues are dependent upon exposure concentration and duration, as well as other factors such as salinity, temperature, interacting agents, and the metabolic activity of tissue (Roesijadi and Robinson, 1994). Moreover, it is known that metal accumulation in marine organism tissues depends on the rate of uptake, route, storage, and elimination (Blackmore, 2001). Cd accumulation in the gill, digestive gland, and residual clam tissues increased with exposure period and concentration during the 2 weeks of Cd exposure. Choi et al. (2007) found that Cd accumulation in the organs of the bivalve *Laternula elliptica* changed with exposure time and concentration. Similar results have been reported in other studies of Cd exposure in bivalves (Bebianno et al., 1994; Blasco and Puppo, 1999). Bivalve species including oysters, mussels, and clams are commonly used as biomonitors for the evaluation of trace metal pollution in the marine environment (Boening, 1999). Many bivalve mollusks have been utilized in the biomonitoring of metal pollution in aquatic ecosystems, and measuring accumulation is the most common method of using these species in biomonitoring (Zhou et al., 2008). Different bivalve species accumulate metals in different concentrations from a given environment because of their diverse accumulation and detoxification strategies (Blackmore, 1998). Therefore, it has been suggested that bioaccumulation is an important process through which trace metals can affect marine organisms, and more chemical-specific studies of Cd are necessary to understand the dynamic process of bioaccumulation and to critically consider the assessment of risk.

Generally, gills are physiologically complex and structurally delicate, and thus they are an important target for waterborne toxicants. This is possibly because the gill has direct contact with the aquatic environment. Moreover, the amount of mucus on the gill surface increases during metal exposure, which may contribute to higher metal concentrations on the gill surface (Reid and McDonald, 1991). In this study, the Cd accumulation profile of gill tissue depended on the waterborne Cd concentration and exposure period (Fig. 2b). Although the profile of Cd accumulation in gill tissue was similar to the digestive gland profile, Cd accumulation in gill tissues was approximately an order of magnitude higher than that in the digestive gland and residual tissues. Holwerda et al. (1989) proposed that gills are the major site for Cd

uptake from waterborne exposure in the freshwater mussel (*Anodonta anatina*) because of their large surface area and the immediate accumulation of any administered dose. As a possible mechanism, it is proposed that the large surface area of the gill and the gill mucus, which may act in ion exchange, contribute to the high metal concentrations found in gill tissues. Lekhi et al. (2008) suggested that the accumulation of dissolved Cd in oysters is a two-step process. Dissolved metals are taken up directly by shellfish and other aquatic organisms through exposed body surfaces, such as gills. Once in the gill, Cd can be redistributed to other internal organs before being eliminated by the kidneys and liver, or accumulated in association with metallothioneins (MTs). This perhaps explains why Cd levels in the gill tissue were significantly higher than were those in other clam tissues during the initial phase.

The digestive gland is where the majority of metal absorption occurred, and it showed a greater degree of Cd accumulation in mussels because of its large surface area in contact with Cd during waterborne exposure (Blasco and Puppo, 1999). However, the order of Cd accumulation in tissues in that study was gill > residual tissues > digestive gland after 2 weeks of Cd exposure. Amiard et al. (1989) proposed that the gills are a short-term storage organ, whereas absorption through the digestive gland leads to an accumulation of toxic metals for a longer time. A similar pattern of Cd transport from clam (*R. decussatus*) gills to digestive glands for storage after 14 days of Cd exposure has been reported (Bebianno et al., 1994). Moreover, Das and Jana (1999) proposed that Cd uptake by different tissues was not from a single source such as the hemolymph, implying that no common uptake pattern between organs exists, and gill uptake occurs through direct absorption from water. Nair and Robinson (2000) suggested that the primary mechanism of Cd transfer is from the blood plasma to various tissues. Also, lysosomes are important organelles in which metals are sequestered in mollusks, and digestive cells are considered one of the richest sources of lysosomes. The MTs and complex metals such as Cd are transported to the lysosomes, either for long-term storage or for subsequent elimination (Marigómez et al., 2002). Although little literature is available to explain the kinetics of these processes, it may be that Cd is regulated through the digestive gland and redistributed in the body by transport in the hemolymph, and that the target organs for Cd vary strongly with species and exposure condition (e.g., exposure concentration and duration).

In the present study, the CFs increased with exposure period in the digestive gland, gill, and residual tissues. An inverse relationship was observed between accumulation factor and exposure concentrations at 100 and 200 μg/L. Similar patterns in accumulation factor were also shown for amphipods (Shuhaimi-Othman and Pascoe, 2007) and freshwater mussels (Das and Jana, 1999) exposed to Cd. DeForest et al. (2007) reported statistically significant inverse relationships between animal bioaccumulation factors for a variety of metals in both saltwater and freshwater environments, and suggested that multiple metal and species specific mechanisms are potentially responsible for an inverse relationship between accumulation factor and exposure concentration. Thus, Cd accumulation in the clam was strongly influenced by exposure period and concentration, and the ability of clams to accumulate Cd agreed with the tissue accumulation order. Moreover, the determination of CF in various tissues suggested the highest accumulation in the gills, followed by residual tissues and digestive glands.

Factors such as time, temperature, interacting agents, age of organisms, metabolic activity, and biological half-life of the metal influence the elimination of metals from the tissues of organisms (Nielsen and Andersen, 1996). In this study, Cd concentrations in the digestive gland, gill, and residual tissues increased during the depuration period in the 200 μg/L exposure group and were approximately 34%, 71%, and 15% higher, respectively, than concentrations measured during the exposure period (Fig. 2). However, Cd concentration remained constant or slightly increased at exposures below 100 μg/L, and did not vary significantly ($P<0.05$). Das and Jana (1999) suggested that the rate of Cd elimination from mussels was strongly influenced by the amount of initial accumulation in the tissue, perhaps because Cd elimination was mediated through diffusion processes following a well defined potential gradient from tissue to water. Shuhaimi-Othman and Pascoe (2007) found that, in amphipods, essential metals (Cu and Zn) were eliminated rapidly, whereas no significant elimination of a nonessential metal (Cd) occurred during depuration periods. Kuroshima et al. (1993) suggested that once Cd is taken up by the body, it is difficult to excrete because of the slow elimination of Cd bound strongly to ligands. Viarengo et al. (1985) suggested that the biological half-lives of metals in mussels are related to the differential capacities of the cells to eliminate metals bound to the binding sites of MTs. Moreover, much higher Cd burdens in mussel tissues such as gill may be related to the slower cell

turnover and/or deposition of insoluble metal granules in the extracellular space and connective tissues of these organs (Zaldibar et al., 2004). Additionally, Yap et al. (2003) suggested two reasons why the levels of metals in different tissues of the mussel *Perna viridis* are not similar after depuration periods. First, different soft tissues have differential affinities or binding capacities of MTs. Second, different tissues have different physiological and regulatory functions. Thus, the rate of Cd elimination from the bivalve was strongly influenced by the initial Cd accumulation in the tissues and by the slow elimination of Cd that is strongly bound to ligands in clam tissues.

Acknowledgements

This work is funded by a grant from the National Fisheries Research and Development Institute (RP-2009-ME-029) of Korea.

References

Amiard JC, Amiard-Triquet C, Ballan-Dufrancais C, Berthet B, Jeantet AY, Martoja R and Truchet M. 1989. Study of the bioaccumulation at the molecular, cellular and organism levels of lead and copper transferred to the oyster *Crassostrea gigas* Thunberg directly from water or via food. Polish Acad Sci 34, 521-529.

Bebianno MJ, Serafim MA and Rita MF. 1994. Involvement of metallothionein in cadmium accumulation and elimination in the clam *Ruditapes decussata*. Bull Environ Contam Toxicol 53, 726-732.

Blackmore G. 1998. An overview of trace metal pollution in the coastal waters of Hong Kong. Sci Total Environ 214, 21-48.

Blackmore G. 2001. Interspecific variation in heavy metal body concentrations in Hong Kong marine invertebrates. Environ Pollut 114, 303-311.

Blasco J and Puppo J. 1999. Effect of heavy metals (Cu, Cd and Pb) on aspartate and alanine aminotransferase in *Ruditapes philippinarum* (Mollusca: Bivalvia). Comp Biochem Physiol 122C, 253-256.

Boening DW. 1999. An evaluation of bivalves as biomonitors of heavy metals pollution in marine waters. Environ Monit Assess 55, 459-470.

Choi HJ, Ji J, Chung KH and Ahn IY. 2007. Cadmium bioaccumulation and detoxification in the gill and digestive gland of the Antarctic bivalve *Laternula elliptica*. Comp Biochem Physiol 145C, 227-235.

Das S and Jana BB. 1999. Dose-dependent uptake and Eichhornia-induced elimination of cadmium in various organs of the freshwater mussel, *Lamellidens marginalis* (Linn.). Eco Eng 12, 207-229.

DeForest DK, Brix KV and Adams WJ. 2007. Assessing metal bioaccumulation in aquatic environments: The inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration. Aquatic Toxicol 84, 236-246.

Holwerda DA, de Knecht JA, Hemelraad J and Veenof PR. 1989. Cadmium kinetics in freshwater clams. Uptake of cadmium by the excised gill of *Anodonta anatina*. Bull Environ Contam Toxicol 42, 382-388.

Kuroshima R, Kimura S, Date K and Yamamoto Y. 1993. Kinetic analysis of cadmium toxicity to red sea bream, *Pagrus major*. Ecotoxicol Environ Saf 25, 300-314.

Lekhi P, Cassis D, Pearce CM, Ebell N, Maldonado MT and Orians KJ. 2008. Role of dissolved and particulate cadmium in the accumulation of cadmium in cultured oysters (*Crassostrea gigas*). Sci Total Environ 393, 309-325.

Mann R and Glomb SJ. 1978. The effect of temperature on growth and ammonia excretion of the Manila clam *Tapes japonica*. Estuar Coast Mar Sci 6, 335-339

Marigómez I, Soto M, Cajaraville PM, Angulo E and Giamberini L. 2002. Cellular and subcellular distribution of metals in molluscs. Microsc Res Tech 56, 358-392.

Nair PS and Robinson WE. 2000. Cadmium speciation and transport in the blood of the bivalve *Mytilus edulis*. Mar Environ Res 50, 99-102.

Nielsen JB and Anderson O. 1996. Elimination of recently absorbed methyl mercury depends on age and gender. Pharmacol Toxicol 79, 60-64.

Park KI, Park HS, Kim JM, Park YJ, Hong JS and Choi KS. 2006. Flow cytometric assessment of immune parameters of the Manila Calm (*Ruditapes philippinarum*). J Kor Fish Soc 39, 123-131.

Reid SD and McDonald DG. 1991. Metal binding activity of the gills of rainbow trout (*Oncorhynchus mykiss*). Can J Fish Aquatic Sci 48, 1061-1068.

Regoli F and Orlando E. 1994. Accumulation and subcellular distribution of metals (Cu, Fe, Mn, Pb and Zn) in the mediterranean mussel *Mytilus galloprovincialis* during a field transplant experiment. Mar Pollut Bull 28, 592-600.

Roesijadi G and Robinson WE. 1994. Metal regulation in aquatic animals: Mechanisms of uptake, accumulation and release. In: Aquatic toxicology, molecular, biochemical and cellular perspectives. Malins DC and Ostrander GK, ed. CRC Press, Boca Raton, 387-420.

Sheehan D and Power A. 1999. Effects of seasonality on xenobiotic and antioxidant defence mechanisms of bivalve mollusks. Comp Biochem Physiol 123C, 193-199.

Shuhaimi-Othman M and Pascoe D. 2007. Bioconcentration and depuration of copper, cadmium, and zinc mixtures by the freshwater amphipod *Hyalella azteca*. Ecotoxicol Environ Saf 66, 29-35.

Sorensen EM. 1991. Cadmium. In: Metal Poisoning in Fish, CRC press, Boca Raton, 175-234.

Viarengo A, Palmero S, Zanicchi G, Capelli R, Vassiere R and Orunesu M. 1985. Role of metallothioneins in Cu and Cd accumulation and elimination in the gill and digestive gland cells of *Mytilus galloprovincialis* Lam. Mar Environ Res 16, 25-36.

Yap CK, Ismail A, Tan SG and Omar H. 2003. Accumulation, depuration and distribution of cadmium and zinc in the green-lipped mussel *Perna viridis* (Linnaeus) under laboratory conditions. Hydrobiologia 498, 151-160.

Zaldibar B, Cancio I and Marigomez I. 2004. Circatidal variation in epithelial cell proliferation in the mussel digestive gland and stomach. Cell Tissues Res 318, 395-402.

Zhou Q, Zhang J, Fu J, Shi J and Jiang G. 2008. Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. Anal Chim Acta 606, 135-150.

(Received 2 August 2009; Revised 2 November 2009; Accepted 4 December 2009)