Vol. 25(2):147-154 | Ocean and Polar Research | June 2003

Article

# Histological Responses of the Antarctic Bivalve *Laternula elliptica* to a Short-term Sublethal-level Cd Exposure

**Heeseon J. Choi*[1], In-Young Ahn[1], Yong-Suk Lee[2], Ko-Woon Kim[1], and Kye-Heon Jeong[2]**

[1]*Polar Sciences Laboratory, KORDI*
*Ansan P.O. Box 29, Seoul 425-600, Korea*
[2]*Department of Life Sciences, Soonchunhyang University*
*646 Eumnae-ri, Shinchang-myeon, Asan 336-190, Korea*

**Abstract :** To develop fast and sensitive biomarkers for metal exposures in Antarctic marine organisms, we examined histological alterations of an Antarctic sentinel bivalve species *Laternula elliptica* following a short-term exposure to a sublethal-level of Cd. Distinct histological alterations of tissues and cells of the gills, kidneys, and digestive glands were observed after 8- to 16- hours of exposure to Cd, while an increase of Cd concentrations in tissues was not detectable. Most alterations were highly localized in the epithelium of the three tissues; epithelia were found to be detached from the remaining tissue parts. In addition, ultrastructural changes such as cytosolic vacuolization, dilation of nucleus and rER membranes were detected in all three tissues, which suggested that the clams are subject to sublethal stresses. Thus, histological and ultrastructural changes on localized tissue parts were rapid and sensitive, suggesting that they may serve biomarkers for Cd exposures. Linkages between the shown ultrastructural changes and higher biological organization level responses are to be established by longer-term exposure experiments.

**Key words :** Biomarker, ultrastructure, histology, cadmium, *Laternula elliptica*.

## 1. Introduction

The Antarctica is the most pristine environment on earth. However, the pristine condition of the Antarctic marine environment has been vulnerable to input of anthropogenic pollutants of various sources with increasing human activities in Antarctica in recent years (Abbott & Benninghoff 1990; Suttie & Wolff 1993). Especially in coastal areas, elevations of various toxic contaminants in seawater, sediment, and organisms have often been reported (Lenihan *et al.* 1990; Kennicutt *et al.* 1995; Lohan *et al.* 2001). Signs of heavy metal pollution have also been reported in the coastal areas of King George Island where eight countries have been operating year-round stations (Lee *et al.* 1990; Hong *et al.* 1999; KORDI 1998). To prevent further deterioration and to enforce effective protection of the area, early detection of any deleterious impacts of heavy metal contamination by regular monitoring is essential. Various biomarkers that provide 'early warning' signs of heavy metal exposures and related stress in marine organisms have been developed and have been applied in temperate marine environmental monitoring since the 1980's (McCarthy & Shugart 1990; Huggett *et al.* 1992; Kramer *et al.* 1994). Adoption of such biomarkers, in addition to regular measurements of metal concentrations in the environment and organisms, would greatly improve the sensitivity of heavy metal pollution monitoring of Antarctic coastal waters.

Cadmium, one of the most toxic heavy metals (USEPA 1978; Langston 1990), is of particular importance in Antarctic marine ecosystem monitoring, since its level in Antarctic seawater is elevated by natural biogeochemical processes (Orren & Monteiro 1985; Fowler 1990). It is known that Cd readily accumulates through food webs (Fisher & Reinfelder 1995; Devi *et al.* 1996; Nott 1998). As a consequence, Cd levels in Antarctic marine herbivorous

*Corresponding author. E-mail : hschoi@kordi.re.kr

organisms are often highly elevated (Honda *et al.* 1987; Mauri *et al.* 1990; Berkman & Nigro 1992; Ahn *et al.* 1996; Bargagli *et al.* 1996; Moreno *et al.* 1997).

Cadmium is also highly elevated in the tissues of *Laternula elliptica* (Ahn *et al.* 1996; Ahn *et al.* 2001), a representative macro-benthic fauna in the shallow waters of King George Island and also widely distributed along the Antarctic coastal areas (Ahn 1994). This filter-feeding bivalve has been recognized as a sentinel organism for metal pollution monitoring in Antarctic shallow water by virtue of its high metal accumulating capacity, wide distribution, and high population density (Ahn *et al.* 1996; SCAR 1996).

Deleterious effects of metals can occur at different time scales depending on the biological organization levels that are affected. To develop sensitive biomarkers for monitoring heavy metal pollution in the Antarctic coastal environment, studies on biochemical, cellular and tissue level responses to Cd exposures at various time scales have been conducted using *L. elliptica* collected from King George Island. In this paper, we present preliminary results on histological alterations of the Antarctic bivalve *Laternula elliptica* resulting from a short-term Cd exposure. Accumulations of Cd were monitored in three organs, the gills, kidneys, and digestive glands, which previous studies had shown to have a strong tendency for metal accumulation (Ahn *et al.* 1996, 2001). Changes in the ultrastructure of the cells as well as tissue damage were monitored after exposing the clams to a sublethal concentration of Cd in the laboratory with light- and electron-microscopy. The possibility of using ultrastructural alterations as biomarkers of Cd exposures is also discussed.

## 2. Materials and methods

### Sample collection and preparation

*Laternula elliptica* were collected by SCUBA from 20-30 m depths in Marian Cove near King Sejong Station (62°13'S, 58°47'W) in December 2001. The collected clams were washed in natural seawater to remove surface debris and acclimated to the experimental condition for 2 days in a flow-through culture tank (Ahn & Shim 1998) prior to experimentation (ca. 1.0°C)

In the laboratory, *L. elliptica* of similar sizes (75-85 mm in shell length) were selected and 16 animals were put into each plastic container containing 40 liters of untreated (or natural) filtered (<0.2 μm) (control) or Cd-treated (50 μg Cd/liter) seawater (treatment). Seawater was aerated and water temperature was maintained at 1.0 ± 0.1°C throughout the experiment. The clams were not fed during the experiment. Three to four individual clams were removed at time intervals of 2, 4, 8, and 16 hours and the soft tissue parts were immediately dissected into muscles (siphon-mantle), gills, digestive glands, gonads, and kidneys, and additional parts. Subsamples of the tissue parts were fixed with Karnovsky solution buffered with 0.1 M cacodylate (pH 7.4) and kept at 4°C for histological examinations. The remaining tissues were frozen immediately in dry ice and kept at −70°C for metal analysis.

### Determination of Cd concentrations

Total Cd concentrations in tissues were determined by summing up the Cd concentrations in two different cell fractions: a soluble fraction and an insoluble particulate fraction. Cell fractionation and Cd concentration determination in each fraction were performed using the methods described in Choi *et al.* (2001). Cd concentrations were determined by inductively coupled plasma-mass spectrometry (Perkin Elmer, Elan 6100). The accuracy of the analytical method was tested using the standard reference materials for oysters (SRM 1566b, NIST, USA) and mussels (CRM278, IRMM, Belgium). The recovery rates of the oyster and mussel tissues were 104.5 and 102.2%, respectively.

### Light and electron microscopy

For light microscopic analysis, fixed tissue samples were washed with a 1 M phosphate buffer, dehydrated in ethanol series from 50 to 100%, replaced with 100% xylene, and then embedded in paraffin. The embedded samples were hardened at −20°C and sectioned to a 6 μm thickness. The sections were observed under a Nikon Optiphot-II microscope.

For transmission electron microscopic analysis, fixed tissue samples were cut into proper sizes in Karnovsky solution (pH 7.4) buffered with a 0.1 M cacodylate. The fixed tissues were fixed again with 1% $OsO_4$ for 2 hrs, dehydrated through an ethanol series to 100% ethanol and then replaced by acetone through a serial treatment. The tissue samples were processed for electron microscopy, embedded in Spurr mixture, incubated at 70°C for 3 days, cut to a thickness of 70-90 nm, and double stained with uranyl acetate for 20 min and lead acetate for 5 min. The sections were examined using a transmission electron microscope (JEM-1010).

For scanning electron microscopy, fixed tissue samples were washed with a fixing buffer for 24 hrs, cut into proper sizes, dehydrated through an ethanol series,

replaced with 100% isoamyl acetate, and dried with the aid of a critical point dryer. The dried samples were coated with gold to a thickness of 200 nm and examined under a scanning electron microscope (Hitachi, H-2500C) equipped with EDS (Kevex).

## 3. Results

### Cd accumulation

Fig. 1 shows Cd concentrations in each tissue part during the 16-hr Cd-exposure period. The mean Cd concentration in the treatment samples tended to be slightly higher than in the control samples after 8 to 16 hrs of Cd exposure, and the difference between the treatment and control seem to increase with exposure time in the gills and kidneys. However, the difference was not statistically significant apparently due to the small sample size (non-parametric Friedman test). Further exposure to Cd clearly showed a significant increase of Cd concentration in all three tissues after 2 days (Choi *et al.* unpublished).

### Histological responses

No distinct changes were detectable at either tissue or subcellular levels within 2 hours following the Cd exposure. However, after 8 to 16 hrs of Cd-exposures, histological alterations were clearly visible in all the three tissues examined. Following 16 hrs of Cd exposure, epithelial cell layers were observed to be detached from the remaining tissue parts in all three organs (Fig. 2). Scanning electron micrographs showed that the detachment of the epithelial layers was largely due to cell shrinkage (Fig. 2a,b). The inner tissue parts of the kidneys also became porous (Fig. 2a).

Added to this, in the kidney, vesicle-like spaces were observed between the inner and outer nuclear membranes (perinuclear space) of the epithelial cells (Fig. 3a). Electron-dense granules were observed both in control and Cd-exposed renal cells (Fig. 3b). There were, however, no distinguishable difference in the shape and quantity of

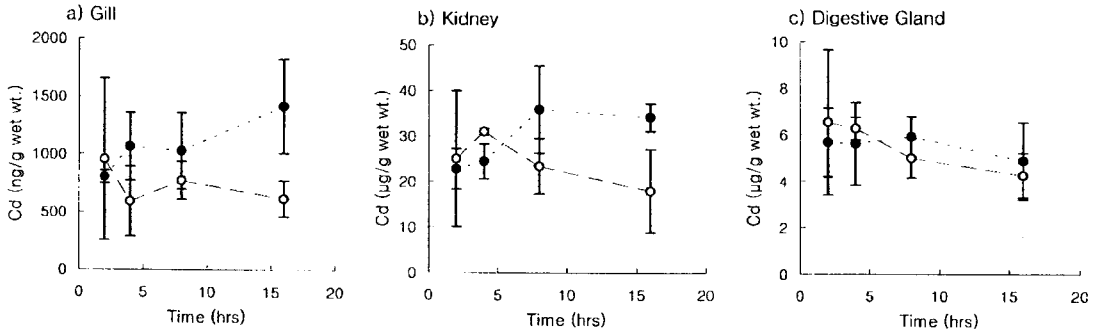


**Fig. 1. Cadmium concentrations in the gill, kidney, and digestive gland of *L. elliptica* from clean filtered seawater (in open circles) or exposed to 50 $\mu$g Cd · $L^{-1}$ (in closed circles) for 2, 4, 8, and 16 hours (Mean ± s.d., n=3 or 4 for each data point).**

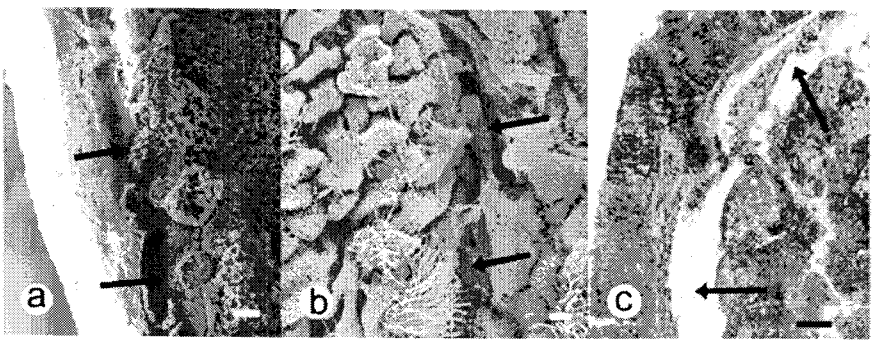


**Fig. 2. Scanning electron micrographs of *L. elliptica* (a) kidney and (b) gill epithelial parts. Digestive gland section is shown in light micrographs (c). In all three tissues, epithelium part was shrunken and detached from other tissue parts (shown in arrows). Scale bar 2 $\mu$m.**

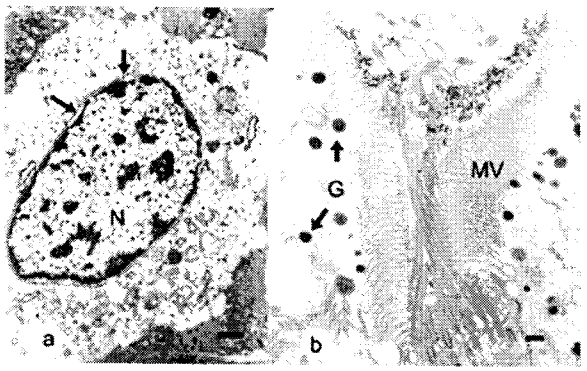


**Fig. 3. Transmission electron micrographs of the renal epithelial cells of *L. elliptica* exposed to 50 μg Cd · $L^{-1}$ for 16 hours. (a) Vesicle-like spaces (arrows) were forming in perinuclear space of the nucleus (N). (b) Electron dense granules (G) were detected in renal epithelial cells with microvilli (MV). Scale bar 1 μm.**

granules between the control and the Cd-exposed clams.

In the gills, the overall lamella structure was deformed after a 16-hr Cd exposure (Fig. 4a). Transmission electron micrographs showed that the lamella cells were shrunk and the nuclei were enlarged (Fig. 4b), and thus the ratio of nucleus to cytosolic fraction increased.

The epithelium of the digestive diverticula was mainly composed of columnar digestive and basophilic cells. TEM micrographs showed that normal digestive cells had large numbers of mitochondria encompassed by well-developed rough endoplasmic reticulum (rER) systems and an inclusion body in its nucleus (Fig. 5a). No changes in shape were observed in inclusion body even after the

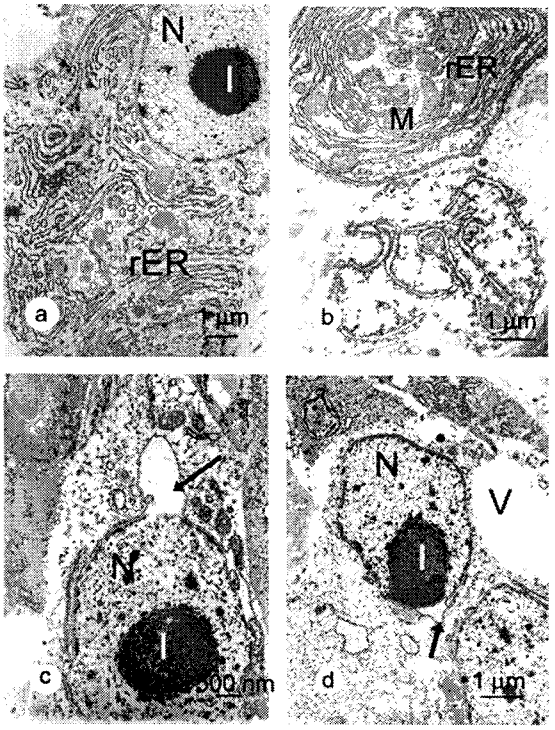


**Fig. 5. Transmission electron micrographs of the digestive cells in the epithelium of *L. elliptica* digestive gland. (a) Normal digestive cells had well developed rER. Nucleus contained an inclusion body (I). (b) After 8 hours of exposures to 50 μg Cd · $L^{-1}$, the rER was forming whirl-like structures containing many mitochondria in its structure. (c) Formation of a vacuole (arrow) was detected at the nucleus membrane after 8 hours. (d) Vesicle-like space (arrow) was shown in the perinuclear membrane after 16 hours of Cd exposure. A large vacuole (V) and inclusion body (I) were also detected.**

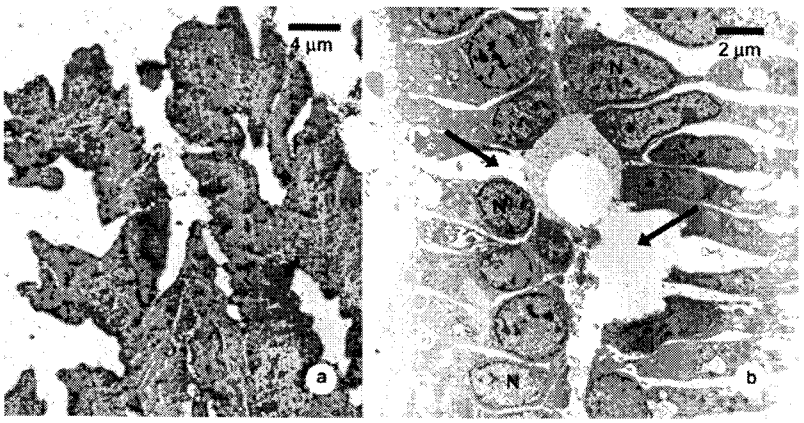


**Fig. 4. Gill sections of *L. elliptica* exposed to 50 μg Cd · $L^{-1}$ for 16 hours. (a) Distinct damages in lamella structure were detected by light microscopic observation. (b) TEM micrograph showed that epithelial cells were shrunken, leaving spaces between cells (arrows). Nuclei (N) were enlarged.**

16-hour Cd exposure (Fig. 5d). However, rER were forming whirl-like structures (Fig. 5b) and membranes of nucleus and rER were found to be dilated or ruptured in an 8- to 16- hour Cd exposed digestive gland (Fig. 5c,d). Vacuoles were forming near the nucleus with the increase in exposure time (Fig. 5c,d).

## 4. Discussion

Distinct damages (or alterations) in tissue and cell levels were observed in the three organs of *L. elliptica* following a short-term exposure to a sublethal concentration of Cd. The alterations were highly localized on the epithelium. Separation of the epithelium was evident in all three organs (Fig. 2). It was also the epithelial cells that showed significant changes in their ultrastructure, indicating that epithelial cells are the target sites for Cd toxicity. This finding is in concert with previous reports that epithelial tissues were the major sites for metal accumulation in mollusc tissues (George *et al.* 1986; Marigómez *et al.* 1990, 2002; Nigro *et al.* 1992; AbdAllah & Moustafa 2002) and that they were most susceptible for histological alterations resulting from the toxic effects of metals (Calabrese *et al.* 1984; Couch 1984; Moore 1985; Lowe & Clark 1989; Yevich & Yevich 1994; Najle *et al.* 2000). Epithelial cells play a crucial role in phyiological functions. Renal epithelial cells are involved in the excretion of nitrogenous waste and resorption of metaboliltes. Gill epithelial cells and cells of digestive glands play an important role in respiratory gas exchange and intracellular food digestion, respectively (Mason *et al.* 1984; Marigómez *et al.* 2002). Therefore, necrotic damage in the epithelium may result in serious dysfunction of the tissues, consequently leading to deleterious effects at higher biological organization levels.

It is not clear whether the observed ultrastructural changes are compensatory (potentially reversible) or pathological to the clams. However, such ultrastructural alterations indicate that at least the cells were under sublethal stress from Cd input. Similar ultrastructral changes were reported in the cells of animals exposed to toxic metals including Cd. Vacuolization of digestive cells of *L. elliptica* (Fig. 5) is most commonly observed in cellular responses of aquatic invertebrates, which are subjected to metal toxicity (Moore 1988; George *et al.* 1986; Hinton *et al.* 1992; Lawson *et al.* 1995; Najle *et al.* 2000; AbdAllah & Moustafa 2002); this was reported to be related to cellular detoxification processes (Krishnakumar *et al.* 1990; Rubio *et al.* 1993; Pawert *et al.* 1996). Nucleus size and membrane alteration (Figs. 3-5) is also a typical sublethal symptom of the toxic effects of metals (Hinton *et al.* 1992). No information on related intra-cellular processes of such ultrastructual changes is available for molluscs. Earlier studies on vertebrates, however, showed that Cd had an early effect on protein synthesis and thus Cd exposure induced changes in the ultrastructures of the nucleus and rER within a few hours (Sina & Chin 1978; Gamulin *et al.* 1982; Dudley *et al.* 1984). The same explanation may be applied to the dilatation of the perinuclear space and rER of the digestive cells that were detected in of this study.

Results from earlier studies made controversial predictions on the question of whether these short-term ultrastructural changes are reversible or irreversible. In many cases, the ultrastructural changes would often lead to different forms of histopathological conditions such as atrophy, hyperplasia, necrosis, and inflammation, which eventually results in dysfunction of tissues (Calabrese *et al.* 1984; Lowe 1988; Krishnakumar *et al.* 1990; Hinton *et al.* 1992). Long-term laboratory exposures to sublethal concentrations of Cd often developed various histopathological symptoms in mollusc tissues (Clark *et al.* 2000; Najle *et al.* 2000). On the other hand, intermittent doses of Cd for 6 months showed no signs of irreversible damage, as most of the cell ultrastructural alterations were reversible (Dudley *et al.* 1984). Such a discrepancy may be attributable to the inherent variability in the cellular detoxification ability among organisms and different levels of metal exposure. The scallop *Placopecten magellanicus* has developed an effective cellular Cd-detoxification system and showed no evidence of cell injuries despite a 6- to 7-fold increase in Cd content in the kidneys (Fowler & Gould 1988). Surviving in naturally Cd-elevated environment for a long period of time on a geological time scale (Berkman 1997), *L. elliptica* might have developed efficient adaptive strategies against Cd toxicity. The presence of biochemical Cd-detoxifying mechanisms of this species has been discussed previously (Choi *et al.* 2001). However, it is hard to make a prediction how fast and effectively those adaptive processes will respond if the clams are exposed to high concentrations of Cd over a relatively short period of time as in the case of anthropogenic contamination. Longer-term effects of Cd on ultrastructural changes should be monitored to elucidate the relevance of cellular- or subcellular-level responses to the histopathology, physiology, and survival of *L. elliptica*.

This study also showed that the histological response of *L. elliptica* gills, kidneys, and digestive gland cells to Cd exposure was rapid and sensitive. In fact, the response was

evident within 8 to 16 hours after Cd exposure, before a clear increase of Cd concentrations in tissues was detected by the conventional acid digestion method or SEM-EDS (Fig. 1). Rapid and sensitive alterations of cells and tissues of *L. elliptica* to Cd exposures indicate that they may be used as useful biomarkers for Cd exposures. Histological responses, either protective cellular adaptations or pathological changes, to metal exposure stresses occur in a relatively short period of time, and were suggested as suitable biomarkers for early and sensitive detection of heavy metal exposures and the resulting effects on organisms (Dudley *et al.* 1984; Moore 1985; Hinton *et al.* 1992; Yevich & Yevich 1994; Najle *et al.* 2000). Histological responses may also serve as ecotoxicologically meaningful biomarkers since they form an important link between effects at the biochemical level and those measured in whole organisms (Lowe 1988; Hinton *et al.* 1992). In addition, analysis of histological changes in target organs provides a valuable tool in understanding the role of specific cells and organelles in heavy metal metabolism (Moore 1985; Hinton *et al.* 1992; Rubio *et al.* 1993).

Previous attempts to use histological alterations for metal exposure monitoring in the marine environment have been focused on histopathology rather than ultrastructrual changes (Couch 1984; Hinton *et al.* 1992; Clark *et al.* 2000; Teh *et al.* 2000). However, as shown in this study, ultrastructural alterations may serve as better biomarkers for metal pollution monitoring. They respond to low concentrations of metal in very short time scales (within a few hours) and thus may provide earlier warning signs before they actually develop into pathological conditions, in which detection would take a longer time. Ultrastructural biomarkers developed so far are mostly based on lysosomes (membrane stability, lysosomal enlargement, and lysosomal lipofuscin content), which are prone to being damaged by stress (Moore 1988; Lowe & Pipe 1994). However, lysosomes respond to various chemical and non-chemical stressors and their alterations may be used as an integrative biomarker for multiple stressors rather than those specific to metals (Mayer *et al.* 1992). It is not clear if the ultrastructural changes detected in this study are specific to Cd or non-specific responses. Much work needs to be done for the shown ultrastructural alterations to be used as biomarkers for metal exposure monitoring in the Antarctic coastal environment. Linkages between ultrastructural changes and effects at higher biological organization levels such as pathological alterations should be determined. Linkages of specific alteration patterns to metal concentrations are also needed (Hinton *et al.* 1992; Wester *et al.* 2002; Ž nidaršič *et al.* 2003). In addition, methods for quantitative analysis of structural alterations should also be developed.

## Acknowledgements

The authors are indebted to the diver Seung Goo Ra for his great effort in collecting clams. We would like to express our gratitude to Professor Kwang-Sik Choi and one anonymous reviewer for their valuable comments on the manuscript. This work was supported by the Korea Science and Engineering Foundation (No. R03-2000-000-00004-0) and the Korea Ocean Research and Development Institute.

## References

Abbott, S.B. and W.S. Benninghoff. 1990. Orientation of environmental change studies to the conservation of Antarctic ecosystems. p. 394-403. In: *Antarctic ecosystems. Ecological change and conservation*. eds. by K.R. Kerry and G. Hempel. Springer-Verlag, Berlin Heidelberg.

AbdAllah, A.T. and M.A. Moustafa. 2002. Accumulation of lead and cadmium in the marine prosobranch *Nerita saxtilis*, chemical analysis, light and electron microscopy. *Environ. Poll.*, 116, 185-191.

Ahn, I.-Y. 1994. Ecology of the Antarctic bivalve *Laternula elliptica* (King and Broderip) in Collins Harbor, King George Island: Benthic environment and an adaptive strategy. *Mem. Natl. Inst. Res.*, 50, 1-10.

Ahn, I.-Y., S.H. Lee, K.T. Kim, J.H. Shim, and D.-Y. Kim. 1996. Baseline heavy metal concentrations in the Antarctic clam, *Laternula elliptica* in Maxwell Bay, King George Island, Antarctica. *Mar. Poll. Bull.*, 32, 592-598.

Ahn, I.-Y. and J.H. Shim. 1998. Summer metabolism of the Antarctic clam, *Laternula elliptica* (King and Broderip) in Maxwell Bay, King George Island and its implications. *J. Exp. Mar. Biol. Ecol.*, 224, 253-264.

Ahn, I.-Y., J. Kang, and K.-W. Kim. 2001. The effect of body size on metal accumulations in the bivalve *Laternula elliptica*. *Antarc. Sci.*, 13, 355-362.

Bargagli, R., L. Nelli, S. Ancora, and S. Focardi. 1996. Elevated cadmium accumulations in marine organisms from Terra Nova Bay (Antarctica). *Polar Biol.*, 16, 513-520.

Berkman, P.A. 1997. Ecological variability in Antarctic coastal environments: past and present. p. 349-357. In: *Antarctic communities. Species, structure and survival*. eds. by B. Battaglia, J. Valencia, and D.W.H. Walton. Cambridge University Press, Cambridge.

Berkman, P.A. and M. Nigro. 1992. Trace metal concentrations in scallops around Antarctica: extending the mussel watch programme to the Southern Ocean. *Mar. Poll. Bull.*, 24, 322-323.

Calabrese, A., J.R. MacInnes, D.A. Nelson, R.A. Greig, and

P.P. Yevich. 1984. Effects of long-term exposure to silver or copper on growth, bioaccumulation and histopathology in the blue mussel *Mytilus edulis*. *Mar. Environ. Res.*, 11, 253-274.

Choi, H.J., I.-Y. Ahn, S.-K. Ryu, Y.-S. Lee, I.-S. Lee, and K.-H. Jeong. 2001. Preliminary evidence for a metallothionein-like Cd-binding protein in the kidney of the Antarctic clam *Laternula elliptica*. *Ocean Polar Res.*, 23, 337-345.

Clark, S.L., S.J. The, and D.E. Hinton. 2000. Tissue and cellular alterations in Asian clams (*Potamocorbula amurensis*) from San Francisco Bay: Toxicological indicators of exposure and effects? *Mar. Environ. Res.*, 50, 301-305.

Couch, J.A. 1984. Atrophy of diverticular epithelium as an indicator of environmental irritants in the oyster, *Crassostrea virginica*. *Mar. Environ. Res.*, 14, 525-526.

Devi, M., D.A. Thomas, J.T. Barber, and M. Fingerman. 1996. Accumulation and physiological and biochemical effects of cadmium in a simple aquatic food chain. *Ecotoxicol. Environ. Saf.*, 33, 38-43.

Dudley, R.E., D.J. Svoboda, and C.D. Klaassen. 1984. Time course of cadmium-induced ultrastructural changes in rat liver. *Toxicol. Appl. Pharmacol.*, 76, 150-160.

Fisher, N.S. and J.R. Reinfelder. 1995. The trophic transfer of metals in marine systems. p. 363-406. In: *Metal speciation and bioavailability in aquatic systems*. eds. by A. Tessier and D.R. Turner. John Wiley & Sons, Chichester.

Fowler, B.A. and E. Gould. 1988. Ultrastructural and biochemical studies of intracellular metal-binding patterns in kidney tubule cells of the scallop *Placopecten magellanicus* following prolonged exposure to cadmium and copper. *Mar. Biol.*, 97, 207-216.

Fowler, S.W. 1990. Critical review of selected heavy metal and chlorinated hydrocarbon concentrations in the marine environment. *Mar. Environ. Res.*, 29, 1-64.

Gamulin, S., N. Car, and P. Narancsik. 1982. Effects of cadmium on polyribosome structure and function in mouse liver. *Experimentia*, 33, 1144-1145.

George, S.G., B.J.S. Pirie, A. Calabrese, and D.A. Nelson. 1986. Biochemical and ultrastructural observations of long-term silver accumulation in the mussel, *Mytilus edulis*. *Mar. Environ. Res.*, 18, 255-265.

Hinton, D.E., P.C. Baumann, G.R. Gardner, W.E. Hawkins, J.D. Hendricks, R.A. Murchelano, and M.S. Okihiro. 1992. Histopathological biomarkers. p. 155-209. In: *Biomarkers. Biochemical, physiological, and histological markers of anthropogenic stress*. eds. by R.J. Huggett, R.A. Kimerle, P.M. Mehrle, Jr., H.L. Bergman. Lewis Publishers, Boca Raton.

Honda, K., T. Yamamoto, and R. Tatsukawa. 1987. Distribution of heavy metals in Antarctic marine ecosystem. *Proceeding of NIPR Symposium on Polar Biology*, 1, 184-197.

Hong, S., C.Y. Kang, and J. Kang. 1999. Lichen biomonitoring for the detection of local heavy metal pollution around King Sejong Station, King George Island, Antarctica. *Korean J. Polar Res.*, 10, 17-24.

Huggett, R.J., R.A. Kimerle, P.M., Mehrle, Jr., H.L. Bergman. 1992. Biomarkers. Biochemical, physiological, and histological markers of anthropogenic stress. Lewis Publishers, Boca Raton. 347 p.

Kennicutt, M.C. II, S.J. McDonald, J.L. Sericano, P. Boothe, J. Oliver, S. Safe, B.J. Presley, H. Liu, D. Wolfe, T.L. Wade, A. Crockett, and D. Bockus. 1995. Human contamination of the marine environment-Arthur Harbor and McMurdo Sound, Antarctica. *Environ. Sci. Technol.*, 29, 1279-1287.

KORDI. 1998. Annual reports of environmental monitoring on human impacts at the King Sejong Station. Korea Ocean Research & Development Institute Report, BSPP 98001-02-1151-7. 407 p. (in Korean)

Kramer, K.J.M. 1994. Biomonitoring of coastal waters and estuaries. CRC Press, Boca Raton. 327 p.

Krishnakumar, P.K., P.K. Asokan, and V.K. Pillai. 1990. Physiological and cellular responses to copper and mercury in the green mussel *Perna viridis* (Linnaeus). *Aquat. Toxicol.*, 18, 163-174.

Langston, W.J. 1990. Toxic effects of metals and the incidence of metal pollution in marine ecosystems. p. 101-122. In: *Heavy metals in the marine environment*. eds. by R.W. Furness and P.S. Rainbow. CRC Press. Boca Raton.

Lawson, S.L., M.B. Jones, and R.M. Moate. 1995. Effects of copper on the ultrastructure on the gill epithelium of *Carcinus maenas* (Decapoda: Brachyura). *Mar. Poll. Bull.*, 31, 63-72.

Lee, S.J., K.T. Kim, and S.J. Kim. 1990. Trace metals in the surface waters of Maxwell Bay, King George Island, Antarctica. *Korean J. Polar Res.*, 1, 11-15.

Lenihan, H.S., J.S. Oliver, J.M. Okaden, and M.D. Stepheson. 1990. Intense and localized benthic marine pollution around McMurdo Station, Antarctica. *Mar. Poll. Bull.*, 21, 422-430.

Lohan, M.C., P.J. Statham, and L. Peck. 2001. Trace metals in the Antarctic soft-shelled clam *Laternula elliptica*: implications for metal pollution from Antarctic research stations. *Polar Biol.*, 24, 808-817.

Lowe, D.M. 1988. Alteration in cellular structure of *M. edulis* resulting from exposure to environmental contaminants under field and experimental conditions. *Mar. Ecol. Prog. Ser.*, 46, 91-100.

Lowe, D.M. and K.R. Clarke. 1989. Contaminant-induced changes in the structure of the digestive epithelium of *M. edulis*. *Aquat. Toxicol.*, 15, 345-358.

Lowe, D.M. and R.K. Pipe. 1994. Contaminant induced lysosomal membrane damage in marine mussel digestive cells: as in vitro study. *Aquat. Toxicol.*, 30, 357-365.

Marigómez, J.A., M.P. Cajaraville, and E. Angulo. 1990. Cellular cadmium distribution in the common winkle, *Littorina littorea* (L.) determined by X-ray microprobe analysis and histochemistry. *Histochem.*, 94, 191-199.

Marigómez, I., M. Soto, M.P. Cajaraville, E. Angulo, and L.

Giamberini. 2002. Cellular and subcellular distribution of metals in molluscs. *Microsc. Res. Tech.*, 56, 358-392.

Mason, A.Z., K. Simkiss, and K.P Ryan. 1984. The ultrastructural localization of metals in specimens of *Littorina littorea* collected from clean and polluted sites. *J. Mar. Biol. Ass. UK*, 64, 699-720.

Mauri, M., E. Orlando, M. Nigro, and F. Regoli. 1990. Heavy metals in the Antarctic scallop *Adamussium colbecki*. *Mar. Ecol. Prog. Ser.*, 67, 27-33.

Mayer, F.L., D.J. Versteeg, M.J. McKee, L.C. Folmar, R.L. Graney, D.C. McCumer, and B.A. Rattner. 1992. Physiological and nonspecific biomarkers. p. 5-85. In: *Biomarkers. Biochemical, physiological, and histological markers of anthropogenic stress*. eds. by R.J. Hugget, R.A. Kimerle, P.M. Mehrle, Jr., H.L. Bergman. Lewis Publishers, Boca Raton.

McCarthy, J.F. and S.T. Shugart. 1990. Biomarkers of environmental contamination. Lewis publishers, Boca Raton. 457 p.

Moore, M.N. 1985. Cellular responses to pollutants. *Mar. Poll. Bull.*, 16, 134-139.

Moore, M.N. 1988. Cellular- and histopathological effects of a pollutant gradient-summary. *Mar. Ecol. Prog. Ser.*, 46, 109-110.

Moreno, J.E.A. de, M.S. Gerpe, V.J. Moreno, and C. Vodopivez. 1997. Heavy metals in Antarctic organisms. *Polar Biol.*, 17, 131-140.

Najle, R., M. Elissondo, S. Gentile, M. Gentile, G. Vacarezza, and H. Solana. 2000. Histopathology of the digestive gland of an Antarctic limpet exposed to cadmium. *Sci. Total Environ.*, 247, 263-268.

Nigro, M., E. Orlando, and F. Regoli. 1992. Ultrastructural localization of metal binding sites in the kidneys of the Antarctic scallop *Adamussium colbecki*. *Mar. Biol.*, 113, 637-643.

Nott, J.A. 1998. Metals and marine food chains. p. 387-414. In: *Metal metabolism in aquatic environments*. eds. by W.J. Langston and M.J. Bebianno. Chapman & Hall, London.

Orren, M.J. and P.M.S. Monteiro. 1985. Trace element geochemistry in the Southern Ocean. p. 30-37. In: *Antarctic nutrient cycles and food webs*. eds. by W.R. Siegfreid, P.R. Condy & R.M. Laws. Springer-Verlag, Berlin.

Pawert, M., Triebskorn, R., S. Gräff, M. Berkus, J. Schulz, and H.-R. Köhler. 1996. Cellular alterations in collembolan midgut cells as a marker of heavy metal exposure: ultrastructure and intracellular metal distribution. *Sci. Total Environ.*, 181, 187-200.

Rubio, M.R., P. Tineo, J. Diaz, and A. Torreblanca. 1993. Effects of cadmium exposure on the ultrastructure of hepatopancreatic cells of *Thais haemastoma* (Gastropoda, Prosobranchia). *Mar. Environ. Res.*, 35, 47-51.

SCAR/COMNAP. 1996. Monitoring of environmental impacts from science and operations in Antarctica. A report for the Scientific Committee on Antarctic Research (SCAR) and the Council of Managers of National Antarctic Programs (COMNAP).

Sina, J.F. and B. Chin. 1978. Cadmium modification of nucleolar ultrastructure and RNA synthesis in physarum polycephalum. *Toxicol. Appl. Pharmacol.*, 43, 449-459.

Suttie, E.D. and E.W. Wolff. 1993. The local deposition of heavy metal emission from point sources in Antarctic. *Atm. Environ.*, 27A, 1833-1841.

Teh, S.J., I. Werner, and D.E. Hinton. 2000. Sublethal effects of chromium-VI in the Asian clam (*Potamocorbula amurensis*). *Mar. Environ. Res.*, 50, 295-300.

US EPA. 1978. Reviews of the environmental effects of pollutants: IV. Cadmium. U.S. Environmental Protection Agency, ORNL/EIS-106, EPA-600/1-78-026. 251 p.

Wester, P.W., L.T.M. van der Ven, A.D. Vethaak, G.C.M. Grinwis, and J.G. Vos. 2002. Aquatic toxicology: opportunities for enhancement through histopathology. *Environ. Toxicol. Pharmacol.*, 11, 289-295.

Yevich, P.P. and C.A. Yevich. 1994. Use of histopathology in biomonitoring marine invertebrates. p. 179-204. In: *Biomonitoring of coastal waters and estuaries*. eds. by K.J.M. Kramer. CRC Press, Boca Raton.

Žnidaršič, N., J. Štrus, and D. Drobne. 2003. Ultrastructural alterations of the hepatopancreas in *Porcellio scaber* under stress. *Environ. Toxicol. Pharmacol.*, 13, 161-174.

*Received Apr. 23, 2003*
*Accepted May 22, 2003*