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Effect of Cadmium (Cd) on The Structure of Gill and Epipodite of Penaeid Shrimp, *Penaeus japonicus* Bate (Crustacea, Decapoda)

B. Yulinato *

Department of Marine Science, Faculty of Fisheries and Marine Science, University of Diponegoro, Semarang - Indonesia

Abstrak

Efek toksisitas sublethal cadmium terhadap struktur insang dan epipodite dari udang Penaeus japonicus dipelajari. Setelah pemaparan selama 4 hari, kadmium merubah struktur insang dari udang: akumulasi hemocyte dalam vena dan lamela, epithelium insang mengalami disorganisasi dan kanal hemolympha distal menjadi buntu. Struktur dari epipodite juga terganggu: cadmium menyebabkan nekrosis pada permukaan. Kerusakan struktur insang dan epipodit ini menjadi argumen penurunan kapasitas hypo- dan hyper-osmoregulasi udang ini.

Kata kunci: *kadmium, struktur, Penaeus japonicus, insang, epipodite.*

Abstract

The effect of sublethal toxicity of cadmium on the structure of gill and epipodite of the shrimp Penaeus japonicus was studied. After 4 days of exposure, cadmium affected the gill structure of shrimp: hemocyte accumulate in vessels/channels and lamellas, the gill epithelium is disorganised and the distal hemolymphatic lacuna is clogged. The structure of epipodites is also altered: cadmium causes superficial necrosis. The damage of gill and epipodite structure becomes a reason on the decrease of hypo- and hyper-osmoregulatory capacity in this shrimp.

Key words : *cadmium, structure, Penaeus japonicus, gill, epipodite.*

Introduction

Marine waters can function as a reservoir for various chemicals such as metals that can have an adverse effect on marine organisms. Metals may enter the marine environment as the result of human activities (Zabel, 1993). It is known that essential trace metals (Cu, Zn, Fe, etc.) are required by the aquatic biota for metabolisme, but the biological function of non-essential metals (Pb, Cd, Hg, etc.) are not known (Depledge and Rainbow, 1990).

Cadmium, a non-essential metal with a wide distribution, is a micropollutant with a wide range of ecological and physiological effects. It is accumulated by marine organisms, but as yet it has no known biological function and the internal body concentration is not regulated (White and Rainbow, 1985). It is considered a potential hazard to marine organisms (Rainbow, 1993).

The first time of cadmium presents on marine organism has been reported in the digestive gland of mollusca *Pecten maximus* (Sundelin, 1983). Cadmium also had been known as an agent of *"Itai-itai"* disease in Japan, that was noted as new era concerning the research of cadmium on consumable organisms (Kobayashi, 1970).

Marine invertebrates are known for their accumulation capacity of cadmium in the tissues. Crustaceans tend to accumulate cadmium in their body tissues, particularly in the gills and digestive gland in *Penaeus duorarum, Palaemonetes pugio,* and *P. vulgaris* (Nimmo et al. 1977); Astacus astacus (Meyer et al., 1991); Palaemon elegans (White and Rainbow, 1982). Finally, it seems that cadmium concentration in organisms can not be regulated by the crustacea (White and Rainbow, 1982). Cadmium toxicity has been reported attacking respiratory and osmoregulatory mechanism in aquatic invertebrates and fishes (Thurberg *et al.*, 1973; Lake and Thorp, 1974; Jones, 1975).

Cadmium is also able to modify the structure and ultrastructure of osmoregulatory and respiratory organs (gills and epipodhites) of *Astacus astacus* (Meyer *et al.*, 1991), *Crangon crangon* (Papathanassiou, 1985), *Penaeus duorarum* (Nimmo *et al.*, 1977; Couch, 1977), and *P. merguiensis*

* Corresponding Author

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(Darmono et al., 1990). Nimmo *et al.* (1977) and Couch (1977) reported a blackening gills and gill necrosis in pink shrimp *P. duorarum* during their exposure in cadmium. Degeneration of mitochondria in the gills of freshwater crayfish *A. astacus* has been reported by Meyer et al. (1977), causing the decrease of oxidative enzyme activities in the gills. Lake and Thorp (1974) reported early fine structural changes in the epithelial cells of gills of the freshwater shrimp *Paratya tasmaniensis.*

The purpose of this study is to report microscopical studies of gills and epipodites of normal and cadmium-exposed shrimp and to be used as a simple explanation for osmoregulatory dysfunction related to cadmium exposure in penaeid shrimp.

Materials and Methods

Animal

Shrimps, *Penaeus japonicus* (Bate, 1888), were obtained from Mari-Aude, a Mediterrannean shrimp farm located in Port-Leucate, Aude, France, in May 1992. After 2 h transport to the laboratory, shrimps were acclimatised during 7 days before the experiment, in seawater at 36‰ ± 1‰ and 25° C ± 1° C, in a 12 h light: 12 h dark cycle. The seawater being used in the laboratory was taken from Palavas coast, Herault, Montpellier. Shrimps were fed on frozen mussels during the pre-acclimatisation period. Test animals were adult stage (6 months old) with average weight of 15 ± 1 g.

Test Media, Exposure Technique and Test Container

Test containers used in the tests were aquariums with volume capacity 40 l. Test media used in the toxicity tests were natural seawater filtered with biological filter "Eheim" and aerated. Temperature, salinity and light cycle were set as same as that it was done during acclimatisation.
The exposure technique used in this study was a static renewable. Animals were exposed in still water and the test material was added to the test solutions to produce the desired test concentrations. Test solutions and control were renewed (every 48 h) during the test.

Test Materials

Stock solution (1000 mg Cd/l) was prepared from $CdCl_2.2H_2O$ (Fluka Chemika(, Buchs, Swiss). It was obtained by dissolution of 1951 mg of $CdCl_2.2H_2O$ in 1000 ml of distilled water. Selected experiment concentrations were made by addition of adequate volumes of the stock solution to seawater. The sublethal concentrations used for the test were 2 and 4 mg Cd/l [LC_{50}–96 h= ± 5.5 mg Cd/l (Bambang *et al.*, 1995)]. The cadmium concentrations were checked at the beginning and the end of each test on AAS (Atomic Absorption Spectrophotometer) "Varian" AA-1275.

Exposure and Histology

Groups of 10 test animals were exposed to 2 sublethal concentrations (2 and 4 mg Cd/l) of test material (Cadmium) and 1 control for 4 days. Animals were fed with the mussels during the test. After 4 days of exposure, the gills and the epipodites were lifted and immediately fixed in the solution of "Halmi" during 24 h. The composition of the "Halmi" solution (Martoja and Martoja-Pierson, 1967): Solution Picric Acid 10 ml + Susa of Heidenhain 90 ml (4.5 g mercuric chloride; 0.5 g sodium chloride; 80 ml distilled water; 2 g trichloracetic acid; 4 ml acetic acid; 10 ml formaldehyde). The samples were then embedded in TAAB embedding resin.

The samples (gills and epipodites) were dissected at about 7 µm of thickness with a mikrotome "Minot 1212". The colorants used for histology study were Hematoxylin, Orange-G molybdic and Light Green.

Results

Adult shrimps were exposed during 4 days, respectively in unpolluted media (Control) and two sublethal doses of 2 and 4 mg Cd/l (LC = Low Concentration and HC = High Concentration).

Control Animals

Gills

The gills of shrimp *P. japonicus* are dendrobranchiate, consisting of an axis bearing symmetrically numerous secondary lamellas which give rise, at right angles, to filaments that are divided into two branches near their termini (Figure 1). The gill filaments have the form of oval and along (Figure 2). A gill filament has an afferent vessel/channel and an efferent vessel/channel (V). Such filament is recovered by a fine cuticula (C). Both channels are separated by a longitudinal septum (S) (Figure 3). In certain filaments exist an distal hemolymphatic lacuna and a peripheral lacuna (LP) (Figure 3). The subjects of detailed study in the present report are the distal filaments of the gills, which are the smallest functional anatomical units of the gill system visible to the unaided eye. These are the structures that appear to be affected mainly by the blackening syndrome.

Epipodite

A transversal cutting demonstrates that epipodite is the form along (Figure 4). An extremity of the epipodite is occupied by a big hemolymphatic lacuna/sinus where in which found a tegumentary gland (GT). A cuticula (C) with a thickness of 0.6-1.5 µm covers the epipodite; under the cuticula presents a layer of epithelial cells (CE). A lacuna area separates

the two layers of epithelium, which is connected site by site by the pilaster cells (P) (Figure 4). The epithelial cells are higher (30-40 µm) than that is the gills. The lateral intercellular spaces (EIL) exist between the epithelial cells (Figure 5).

Exposed Animals

Gills

Cadmium modifies the structure of the gills of adult shrimp after 96 hours of exposure. The gills of the shrimps exposed to two concentrations of Cd (2 and 4 mg/lCd) demonstrated the black zones (Figure 6). But it is absent in the control animal (Figure 7). The intensity of blackening syndrome is depended on the concentration of Cd, where it is more important in the shrimps exposed to 4 mg/l Cd than in 2 mg/l Cd (Figure 8 and 9).

The hemocitary concentration (Hm) occurs in all part of the gills (Figure 10). The blood cells is more abundant in exposed shrimps than in control shrimp. The board of channel is thicker (EB). Some filaments (lamellas) are filled by the hemocytes (Figure 11). A disorganization of the cells exists in the level of the gill filaments, where in which the septum stretched (S) and the distal channel were clogged, which resulted the disappearance of the distal hemolymphatic lacuna (Figure 11).

Epipodite

A general observation demonstrated that the epipodites darken too under the effect of cadmium (2 and 4 mg/l Cd). The morphology of epipodites is modified after cadmium exposure (Figure 12). The superficial zone of melanisation are visible in the apical part of the epithelium. The vacuolization (Vc) occurs at the level of the epithelial cells. The lateral intercellular spaces are more important in the exposed shrimps than in the control shrimps. It results a rupture of the relation between the epithelial cells. The nucleus does not seem modified (Figure 13).

Discussion

Gill

Adults of *P. japonicus* exposed during 4 days on sublethal concentrations of cadmium (2 and 4 mg/l Cd) developed black gills, but no control shrimp developed black gills. The blackening syndrome is function of cadmium concentration. The structure of gill histology is also altered, at the level of the channels and the epithelium becomes thicker. Some filaments and gill lamellas are filled by the black materials. The development of the septum occurs in the level of gill filaments, resulting the disappearance of the distal hemolymphatic channels.

Some histology studies demonstrated that in the estuarian crustacea, exposure to sublethal concentrations of cadmium altered certain animal organs. The first organs suffering the structural alteration, are the gills and the digestive gland (Bubel, 1976; Nimmo *et al.*, 1977; Papathanassiou, 1985; Darmono *et al.*, 1990; Meyer *et al.*, 1991). The first areas to suffer from functional alterarions by any intoxicant, including heavy metals, are the gills. The large area for adsorption, the large volume of water passing over the gill surfaces, the adsorption on mucous sheaths secreted as a protective response, and the relatively small biomes of the gills compared to their surface area all result in high heavy metal concentrations, especially of cadmium, in this respiratory and/or osmoregulatory organ (Anderson and Brower, 1978). In general, toxic metals cause the structural modifications of crustacean gills, and the degree of damage depends on the metal concentration (Bubel, 1976; Papathanassiou, 1985; Darmono et al., 1990). The gills blackening caused by cadmium exposure is also reported by Nimmo *et al.* (1977) on the shrimp *P. duorarum*, exposed during 21 days to 1 mg/l Cd, by Couch (1977) on the same species exposed during 15 days to 0.7 mg/l Cd, and by Darmono *et al.* (1990) on *P. merguiensis* exposed during 15 days to 0.5 mg/l Cd. The gills of shrimps exposed to cadmium presented the blackening of the terminal filaments, continued by an autolyse, necrosis and depositing of opaque materials (Couch, 1977). In *P. merguiensis*, the gills demonstrated a hypertrophy of epithelial cells, aggregation of lamellar hemocytes and a melanisation of the gill lamellae accompanied by an necrosis after 15 days of exposure to 0.5 mg/l Cd (Darmono *et al.*, 1990). Lake and Thorp (1974) reported ultrastructural effects of cadmium on the gills of shrimp *Paratya australinesis* exposed to 3 – 5 mg/l Cd during 4 days. The alteration included accumulation of deposit in the mitochondria and degeneration of endoplasmic reticulum.

The reason for gill blackening on *P. japonicus* after exposure to two sublethal concentrations of cadmium can be varied. On the part of gill exterior, it is possible that the black spot came from cadmium absorbed by the gill surface in the moment of exposure to pollutant. Meanwhile, in the level of gill structure, the blackening could be origin from deposit of cadmium ion transported by hemolymph, or by crossing through the gill cuticula. Cadmium was strongly concentrated particularly in the gill and the digestive gland of *Astacus astacus* (Meyer *et al.*, 1991). The blackening could also be linked with the defensive mechanism. It can also be caused by the lesion that becomes black, as it was observed on P. duorarum (Nimmo *et al.*, 1977). They noted that the black filaments result an infiltration and an accumulation of hemocyte in the bottom of peripheral channel touched by the lession. But, it is not evident that the blackening of terminal gill

filaments was caused principally by the melanisation or by the response of hemocyte against the injury; it can also come from non-specific autolyse and/or necrosis that conduct to the deposit of dense material in the necrosed tissues (cadmium or metallic sulfite) (Couch, 1977). Two hypothesis were proposed by Nimmo *et al.* (1977) to explain the histological lesion observed on the gills of *P. duorarum* after exposure to cadmium. First, the soluble ion of cadmium has the toxic effects on the epithelial tissues of the gills. The lesion observed permits enter of bacteria, resulting the tissue lesion. Second, the gills react as a site of cadmium elimination. These metal will be collected in the hemolymph by the hemocyte, transported to the gills, accumulated in the gill lamellas, and finally eliminated the affected gills of lesion. In the shrimp *Crangon crangon*, alteration of the mitochondria membrane induces an increase of the permeability of gill cells exposed to cadmium, resulting an accumulation of metal that precipitates through the exterior of cells, in the form of microcristal (Bubel, 1976).

Epipodite

The studies concerning the epipodite are fewer than that is the gills (Bouaricha *et al.*, 1991; Lin, 1993). The function classically known for this organ is the cleaning of gills and the orientation of water current (Lockwood, 1968; Fisher, 1972; Dall *et al.*, 1990; Lin, 1993). But, it appears recently that this organ can also participate in the active ionic transport and play in role in the osmoregulation (Bouaricha *et al.*, 1991).

In our knowledge, previous studies of the effect of cadmium on the structure of epipodites are not exist. In this study, the structure of epipodites did not reveal important modification. But it is observed some vacuoles in the epithelial cells. It remained fine and simple, and it showed little deformation. It is possible that the exposure duration was insufficient to produce an important alteration on the epipodite.

Observed vacuoles in the epithelial cells may be caused by partial destruction of the epithelial cells of the epipodites. Lin (1993) noticed abundant vacuoles in the epithelial cells of the epipodite of *P. japonicus* after an exposure to a sublethal dose of ammoniac during 5 days. This author also observed a thickening of the epithelial cells, a disappearance of the lateral intercellular spaces and an increase of the number of mitochondria. In the shrimp *P. japonicus* exposed to cadmium, the vacuolization of the epithelial cells can be a first step of the metal effect on the epipodites. This modification might eventually conduct an alteration of the hemolymphatic circulation, contributing to the modification of permeability to the water and/or ions, resulting a decrease of osmoregulatory capacity of the shrimp.

Structural changes of gills and epipodites in relation to osmoregulatory disfunction

Our previous study demonstrated that sublethal cadmium exposure could significantly reduce hypo- and hyper-osmoregulatory capacity on penaeid shrimp *P. japonicus* (Bambang *et al.*, 1995). Jones (1975) has reported similar results. He reported that cadmium, zinc, and mercury significantly altered the osmoregulatory ability of isopod *Jaera albifrons*. Jones (1975) also stated that these toxic metals probably disrupt osmoregulation by affecting the structure of the gill tissue of the pleopods. This study showed that a disorganization of the cells exists in the level of the gill filaments, where in which the septum stretched, which result the disappearance of the distal hemolymphatic lacuna. Distal hemolymphatic lacuna is a channel where hemolymph of shrimp passes by this route to transport oxygen, Na^+, K^+, and Cl^- from the exterior media to internal body by passing firstly the gills and the epipodites. Oxygen, Na^+, K^+, and Cl^- are the important minerals needed by shrimp for ionic regulation, osmoregulation and respiration. When distal hemolymphatic lacuna is clogged, the transportation of oxygen and these minerals is disrupted, which will cause dysfunction of ionic regulation, osmoregulation and respiration. It is also postulated by certain authors that the distal gill filament tissue (epithelial cells, podocytes, and secretory cell) in decapod crustacea is an osmoregulatory, detoxyfying, respiratory, and secretory tissue. When they are disorganized and destructed by cadmium would lead to dysfunction and eventual organismic death, particularly if the crustacea were undergoing respiratory environmental stresses (Couch, 1977).

These histology results support our previous study on the decrease of the hypo- and hyper-osmoregulatory capacity in *P. japonicus* exposed to sublethal concentration of cadmium.

Conclusion

- Cadmium is considered as a toxic metal
- Exposure to sublethal concentrations of cadmium impairs histological structure of the gills and the epipodites of shrimp.
- Observed histological effects of cadmium are the gill blackening syndrome, the necrosis and the melanisation of the lamellas and gill filaments and the epipodites, the hypertrophy of the epithelial cells, the development of longitudinal septum of the gill filaments causing the disappearance of the distal hemolymphatic lacuna, disorganization of the epithelial cells, vacuolization of the epithelial cells.
- The damage of gill and epipodite structure cause the decrease of the hypo- and hyper-osmoregulatory capacity in this shrimp.

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References

Anderson, R.V., Brower, J.E. 1978. Patterns of trace metal accumulation in crayfish populations. *Bull. Environ. Contam. Toxicol.* 20: 120-127.

Bambang, Y., Charmantier, G., Thuet, P., Trilles, J-P. 1995. Effect of cadmium on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* (Crustacea: Decapoda). *Mar. Biol.*, 123: 443-5450

Bouaricha, N., Charmantier, G., Charmantier-Daures, M., Thuet, P., Trilles, J-P. 1991. Ontogenese de l'osmoregulation chez la crevette *Penaeus japonicus. Cah. Biol. Mar.*, 32: 149-158.

Bubel, A. 1976. Histological and electron microscopical observations on the effects of different salinities and heavy metal ions, on the gills of *Jaera nordmanni* (Rathke) (Crustacea, Isopoda). *Cell. Tiss. Res.*, 167:65-95.

Couch, J. A. 1977. Ultrastructure study of lesions in gills of a marine shrimp exposed to cadmium. *J. Invert. Pathol.*, 29: 267-288.

Darmono, Denton, G.R.W., Campbell, R.S.F. 1990. The pathology of cadmium and nickel toxicity in the Banana shrimp (*Penaeus merguiensis* de Man). *Asian Fish. Sci.*, 3: 47-62.

Depledge, M.H., Rainbow, P.S. 1990. Models of regulation and accumulation of trace metals in marine invertebrates. *Comp. Biochem. Physiol.*, 97: 1-7.

Dall, W., Hill, B.J., Rothlisberg, P.C., Sharples, D.J. 1990. The biology of the penaeidae. In: Advance in marine biology, Vol. 27. (Blaxter, J.H.S., Southward, A.J., Eds). Academic Press, New York: 203-349.

Fisher, J.M. 1972. Fine structural observations on the gill filaments of the freshwater crayfish *Astacus pallipes* Lereboullet. *Tissue Cell.*, 4: 287-299.

Jones, M.B. 1975. Synergistic effects of salinity, temperature and heavy metals on mortality and osmoregulation in marine and estuarine isopods (Crustacea). *Mar. Biol.* 30: 13-20.

Kobayashi, J. 1970. Relation between "itai-itai" disease and the pollution of river water by cadmium from a mine. In: *Proc. Int. Water Pollut. Res. Conf.*, 1: 25-52.

Lake, P.S., Thorp, V.J. 1974 The gill lamellae of the shrimp *Paratya tasmaniensis* (Atyidae: Crustacea). Normal ultrastructure and changes with low levels of cadmium. Proc. Eighth Int. Congr. Electron Microsc. Canberra, 2: 448-449.

Lin, H.P. 1993 Effets de deux facteurs de stress (azote ammoniacal et turbidite terrigene) sur le metabolisme hydromineral chez la crevette *Penaeus japonicus.* These de doctorat. Univ. Montpellier II, 200p.

Lockwood, A.P.M. 1968. Aspects of the physiology of crustacea. Oliver & Boyd, Edinburg, London, 328 p.

Martoja, R., Martoja-Pierson, M. 1967. Initiation aux techniques de l'histologie animale. Masson et Cie. Paris. 345 p.

Meyer, W., Kretschmer, M., Hoffman, A., Harisch, G. 1991. Biochemical and histochemical observations on effects of low-level heavy metal load (lead, cadmium) in different organ systems of the freshwater crayfish, *Astacus astacus* L. (Crsutacea: Decapoda). *Ecotoxico. Environ. Saf.*, 21: 137-156.

Nimmo, D.R., Lightner, D.V., Bahner, L.H. 1977. Effects of cadmium on the shrimps, *Penaeus duorarum, Palaemonetes pugio* and *Palaemonetes vulgaris.* In: Physiological respons of marine biota to pollutants (Vernberg, F.J., Calabrese, A., Thurberg, F.D., Vernberg, W.B., Eds). Academic Press, New York: 131-183.

Papathanassiou, E. 1985. Effects of cadmium ions on the ultrastructure of the gill cells of the Brown shrimp *Crangon crangon* (L.) (Decapoda, Caridea). *Crustaceana*, 48 (1): 6-17.

Rainbow, P.S., 1993. The significance of trace metal concentrations in marine invertebrates. In: Ecotoxicology of metals in invertebrates (Dallinger, R., Rainbow, P.S., Eds). Lewis Publishers. Boca Raton: 3-23.

Sundelin, B. 1983. Effects of cadmium on *Pontoporeia affinis* (Crustacea: Amphipoda) in laboratory soft-bottom microcosm. *Mar. Biol.*, 74: 203-212.

White, S.L., Rainbow, P.S. 1982. Regulation and accumulation of copper, zinc, cadmium by the shrimp *Palaemon elegans. Mar. Ecol. Prog. Ser.*, 8: 95-101.

White, S.L., Rainbow, P.S. 1985. On the metabolic requirements for copper and zinc in mollusc and crustaceans. *Mar. Environ. Res.*, 16: 215-229.

Zabel, T.F. 1993. Diffuse sources of pollution by heavy metals. *J. IWEM.*, 7: 513-520.

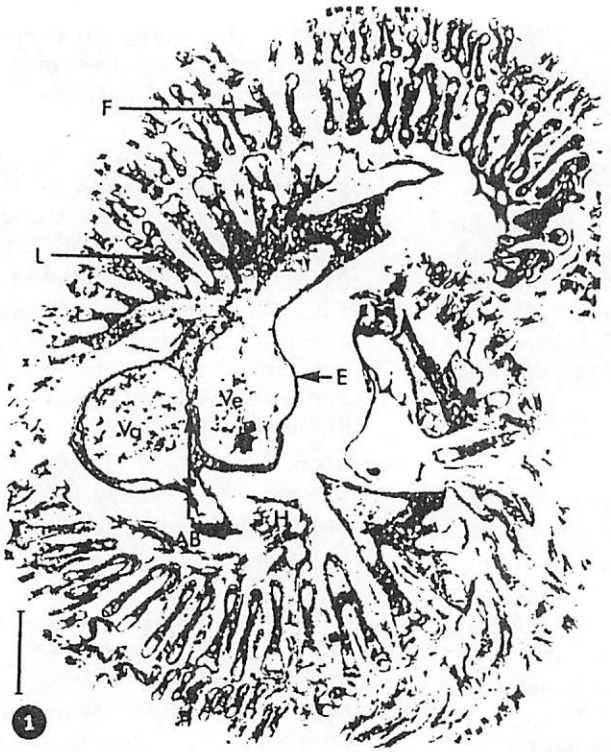

Figure 1. Gill of the control shrimp, Penaeus japonicus. Transversal section of a shrimp gill. Gill axis (AB), lamella (L), and epithelium (E). In the gill axis is observed the afferent channel (Va) and the efferent channel (Ve). F: filament; H: hemolymphe. Scale: the segment corresponds to 200 ! m.

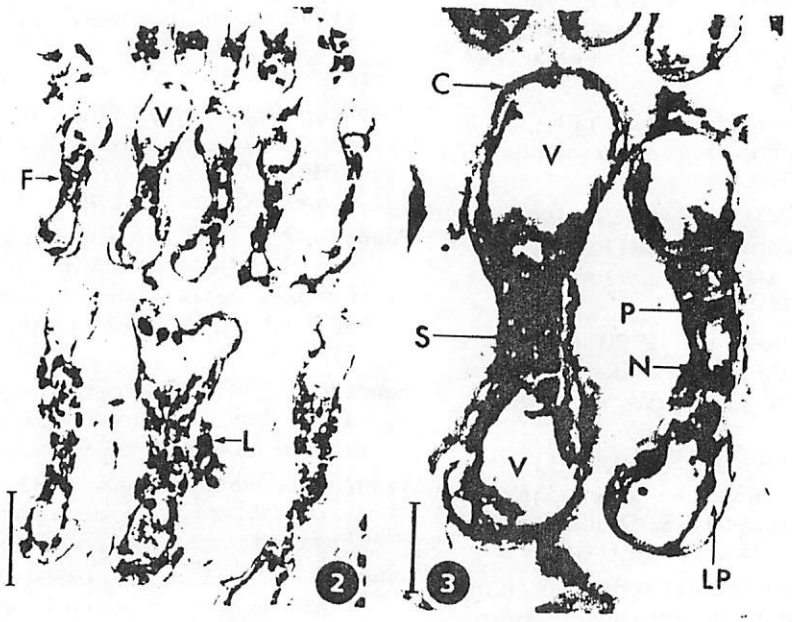

Figure 2. Gill of the control shrimp, Penaeus japonicus. Transversal section of the gill filament (F), showing its disposition compared by the lamella (L). V: channel/vessel. Scale: the segment corresponds to 50 ! m.

Figure 3. Gill of the control shrimp, Penaeus japonicus. Such filament is recovered by a fine cuticula (C). Two channels (V) are separated by a septum (S). Extension of septum in the channel forms the peripheral lacuna (LP). N: nucleus; P: pilaster. Scale: the segment corresponds to 20 ! m.

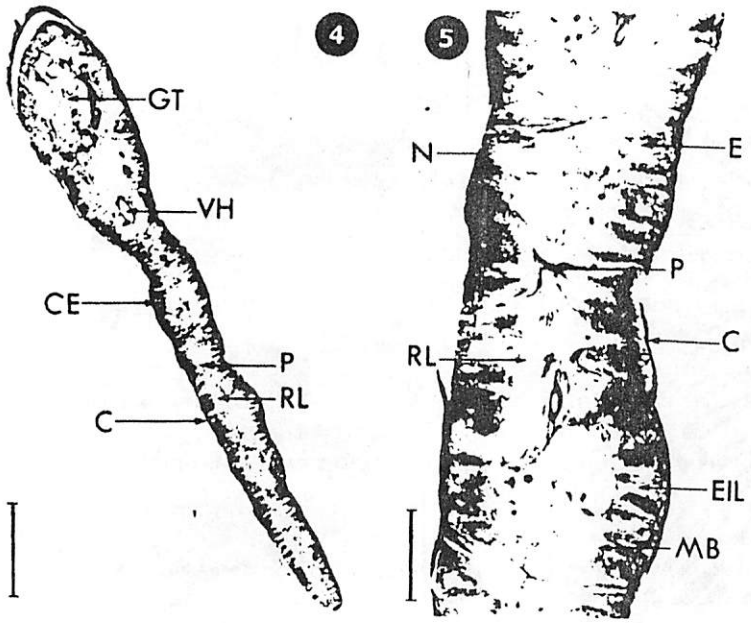

Figure 4. Epipodite of the control shrimp, Penaeus japonicus. Two epithelial sheets (CE) are separated by a lacuna area (RL). Pilaster cells (P) rejoin two epithelial sheets. A tegument gland (GT) is lodged in an extremity. C: cuticula; VH: hemolymphatic channel. Scale: the segment corresponds to 200 µm.

Figure 5. Transversal section of the epithelial cells of an epipodite from the control shrimp, Penaeus japonicus.. Pilaster cells (P) is in good visible. N: nucleus; MB: basal membrane; EIL: lateral intercellular space; C: cuticula; E: epithelium; RL: lacuna area. Scale: the segment corresponds to 50 µm.

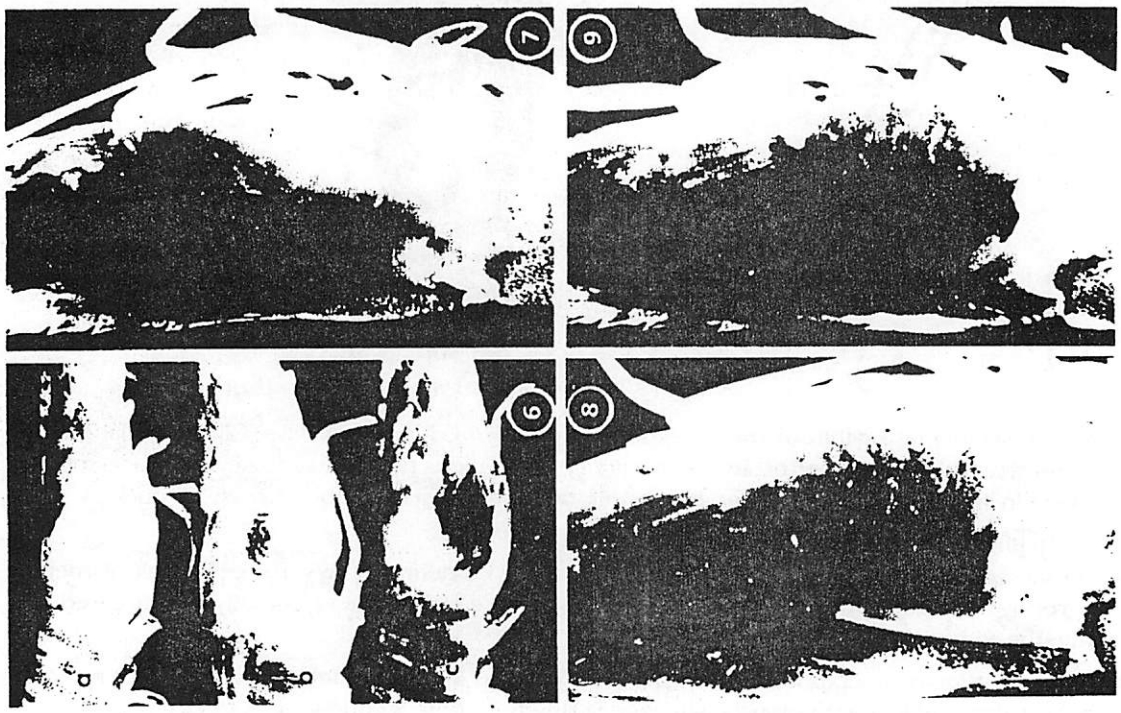

Figure 6. Gill of the control shrimp (a) and the cadmium exposed shrimp (a and b).
Figure 7. Gill of the control shrimp.
Figure 8. Gill of a shrimp exposed to 2 mg/l Cd during 4 days.
Figure 9. Gill of a shrimp exposed to 4 mg/l Cd during 4 days.

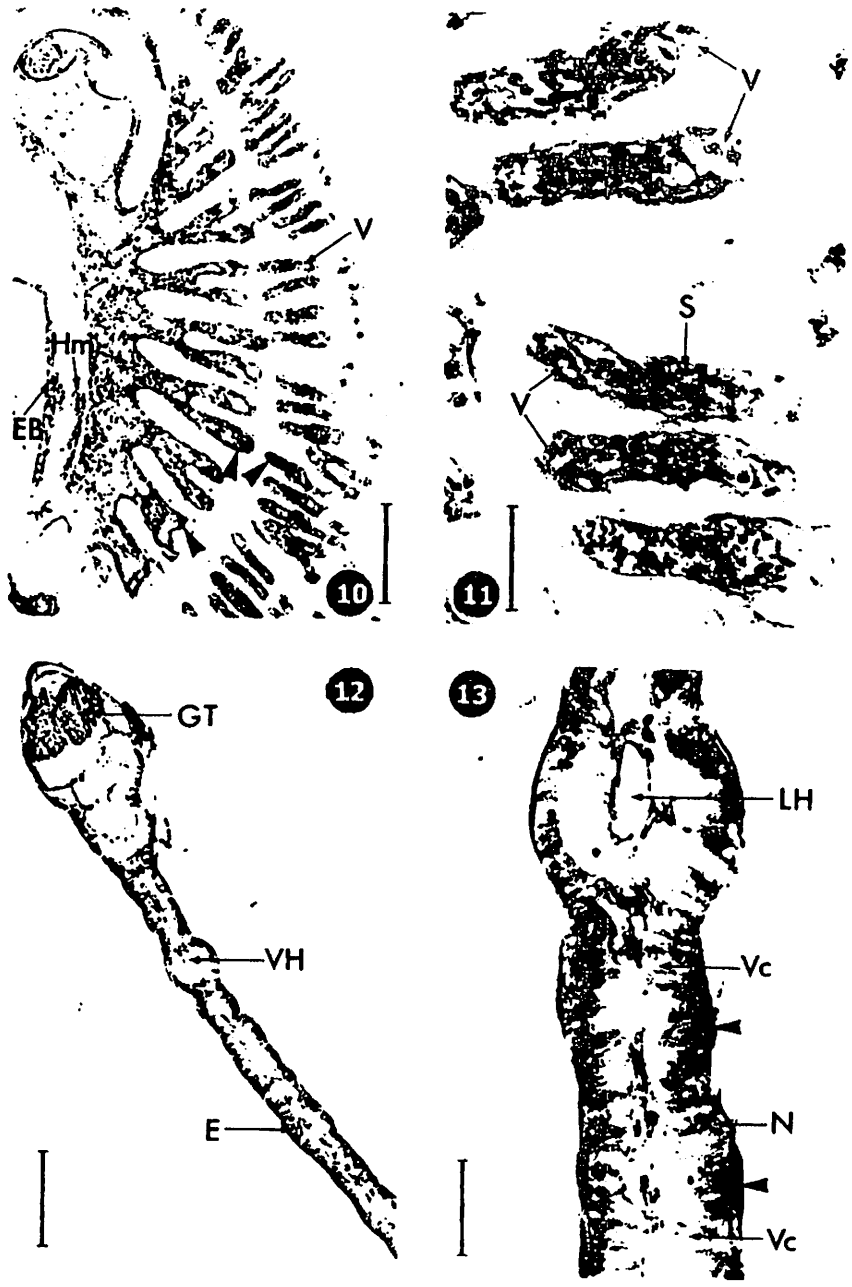

Figure 10. Transversal section of a gill from shrimp exposed to 4 mg/l Cd during 4 days. Gill epithelial (EB) is relatively thicker than that is the control. The hemocyte concentrations (Hm) occur in several places of the gills, particularly in the channels. The zones of melanisation are referred by the arrows. N: nucleus; VH: hemolymphatic channel. Scale: the segment corresponds to 200 µm.

Figure 11. Transversal section of a gill from shrimp exposed to 4 mg/l Cd during 4 days. The cells of gill filaments suffer hypertrophy. The hemolymphatic lacuna/channels (V) are clogged and partially disappeared. S: septum. Scale: the segment corresponds to 50 µm.

Figure 12. Transversal section of an epipodite from shrimp exposed to 4 mg/l Cd during 4 days. E: epithelium; GT: tegumentary gland; VH: hemolymphatic channel. Scale: the segment corresponds to 200 µm.

Figure 13. Transversal section of an epipodite from shrimp exposed to 4 mg/l Cd during 4 days. Vacuolization (Vc) exists in the epithelial cells. The zones of melanisation are referred by the arrows. N: nucleus; VH: hemolymphatic channel. Scale: the segment corresponds to 50 µm.