Toxicity of heavy metals on survival and physiological responses of three bivalves molluscs

(*Crassostrea gigas*, *Tegillarca granosa*, *Ruditapes philippinarum*)

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Effects of heavy metals on marine bivalves

1. **Introduction and purpose**
2. A field study
3. Indoor bioassay
   - Acute toxicity
   - Chronic toxicity
4. Conculsion

◎ Introduction

- Heavy metals and chemicals in marine environment
  : survival, growth, physiological changes etc

- Trace metal : Cu, Zn, Fe, Mn – essential
  Cd, Hg, Cr, Pb – unessential and pollutant

- The majority of shellfish farms are located along seashores, thereby increasing the possibility of exposure to various pollutants including heavy metals

- From a long–term perspective, such an exposure may eventually reduce the production of shellfish

◎ Purpose

: to examine the effect of heavy metal on shellfishes through the following 2 processes

1. The accumulation of heavy metals in shellfish collected from the coaster areas around industrial estates located in the South Sea region

2. The toxic influence of Cu and Pb on survival, respiration and structure of organic system by the indoor experiment : acute and chronic toxicity

⇒ **Possibility of index for finding the level of heavy metals toxicity**

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Materials & Methods

- Species : *Crassostrea gigas*, *Mytilus edulis, Tegillarca granosa, Ruditapes philippinarum*
- Sampling location : South sea of Korea, - Items : Histological analysis

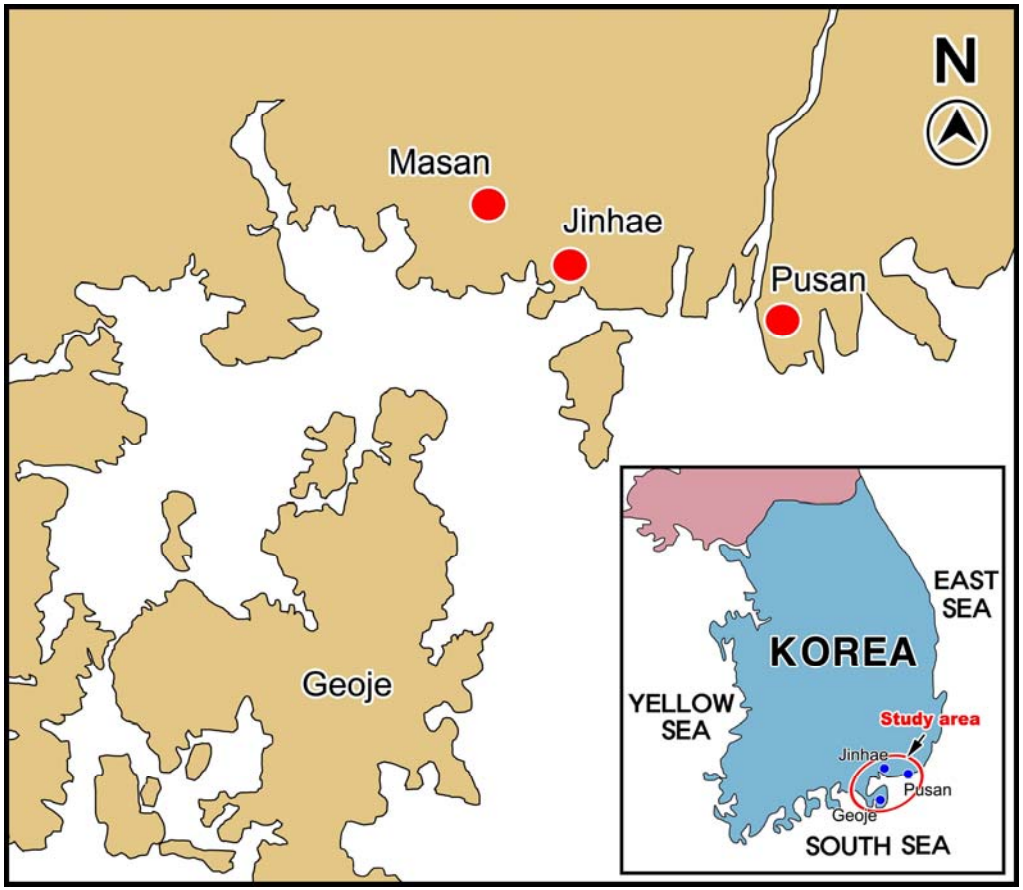

Fig. . Map of the study area.

Various species used in study
& cultured in the south sea

Mussel, *Mytilus edulis*

Oyster, *Crassostrea gigas*

Granular ark,
Tegillarca granosa

Manila clam,
Ruditapes philippinarum

Table. Concentration of heavy metals in sediment of study area, oyster and mussel

	pH	Sal.	Cr	Cd	Cu	Pb	Zn	Fe	Hg
Study area	**7.94**	**28.5**	**234.8**	**11.01**	**60.11**	**941.0**	**1,442**	**-**	**2.44**
Oyster	**-**	**-**	**8.15**	**8.97**	**39**	**17.93**	**132.8**	**-**	**1.71**
Mussel	**-**	**-**	**0.5-24**	**0.2- 60**	**4- 8**	**0.4-30**	**62-250**	**-**	**-**

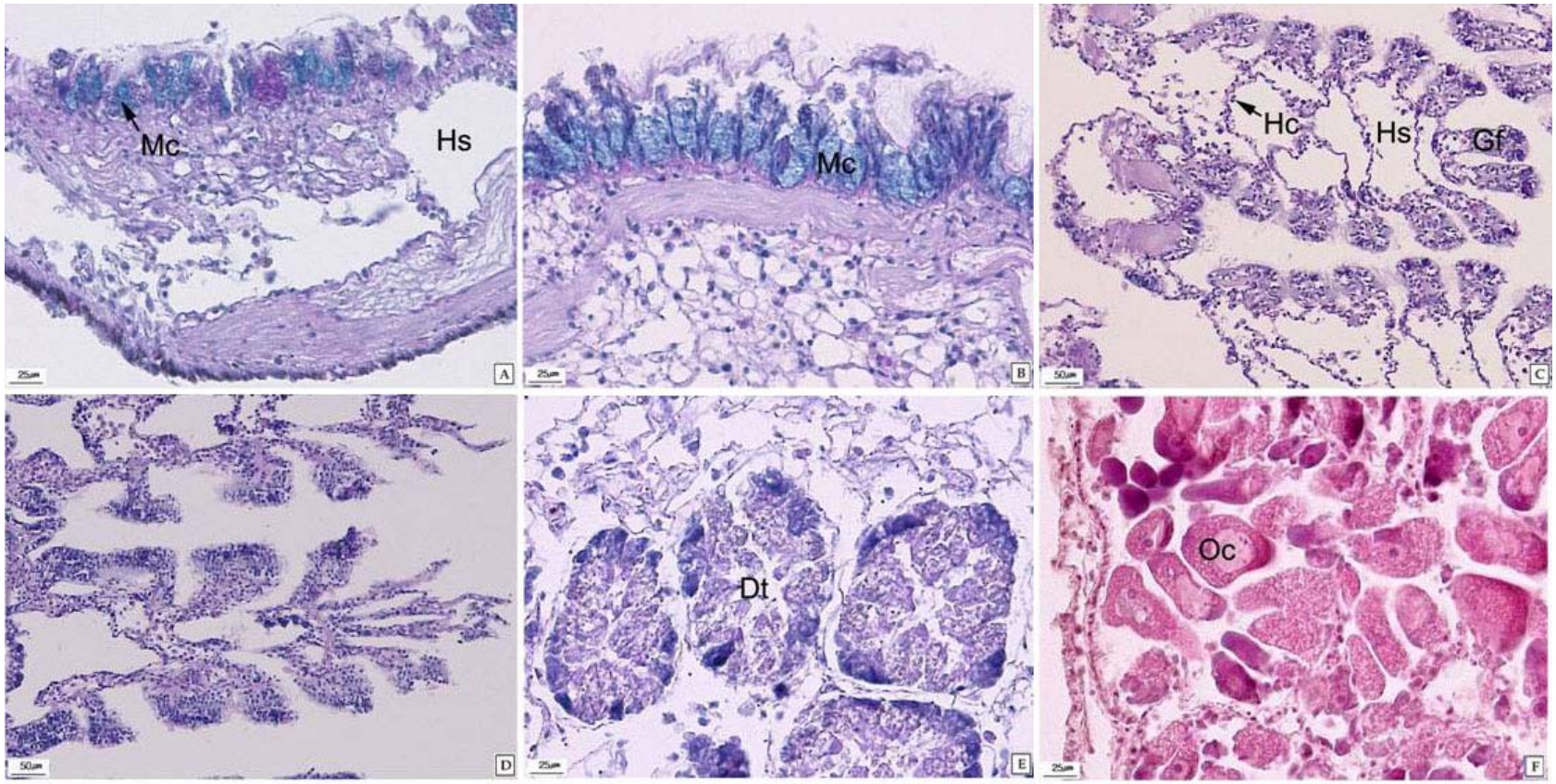

Fig. . Mantle cavity organs of the oyster, *Crassostrea gigas* collected from study area. A: Mantle. AB-PAS (pH 2.5) reaction. B: Mantle. AB-PAS (pH 2.5) reaction. C: Gill. AB-PAS (pH 2.5) reaction. D: Gill. AB-PAS (pH 2.5) reaction. E: Mid-gut. AB-PAS (pH 2.5) reaction. F: Ovary. H-E stain. Dt: digestive tubule, Gf: gill filament, Hc: hemocyte,Hs: hemolymph sinus, Mc: mucous cell, Oc: oocyte.

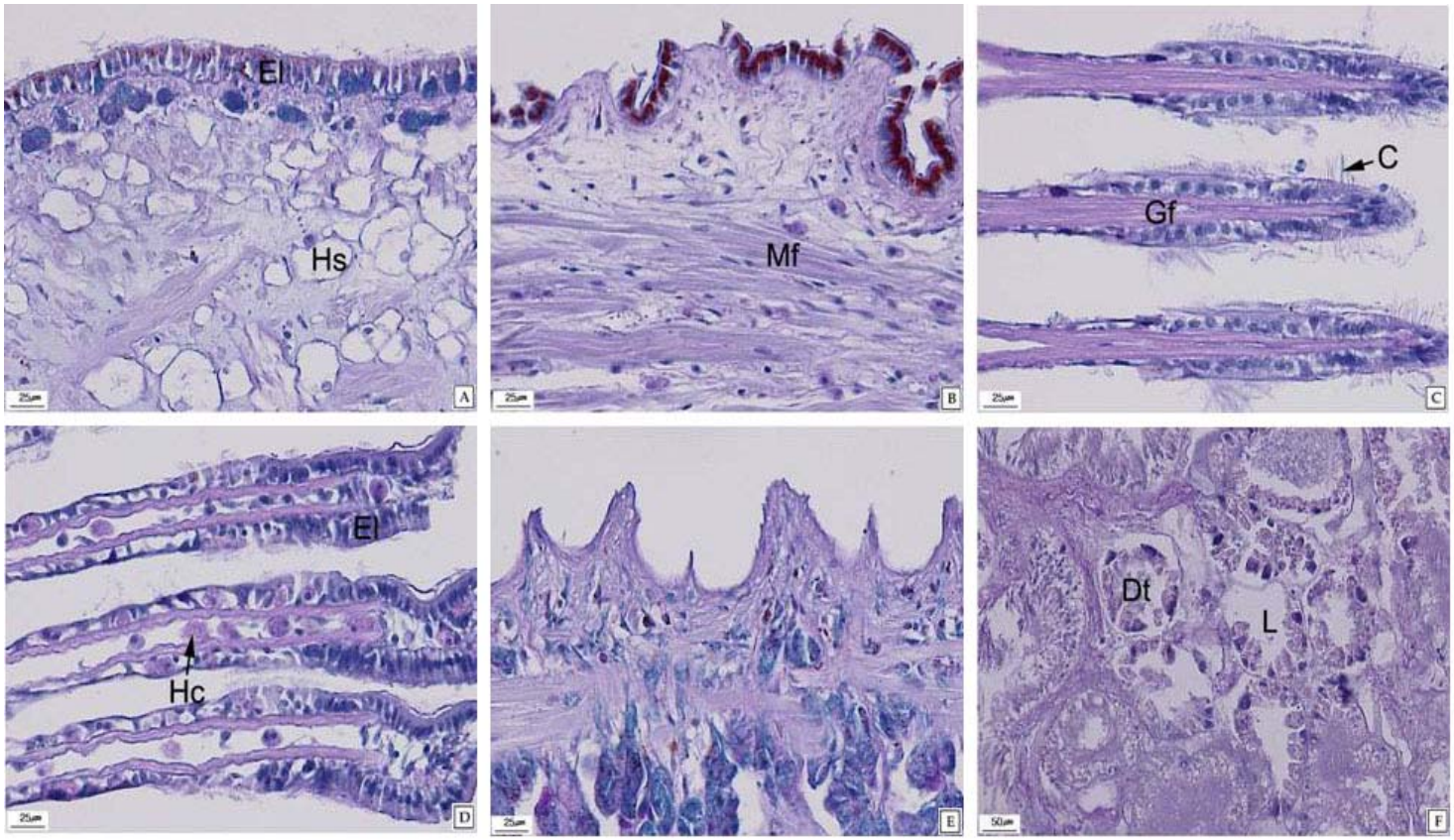

Fig. . Mantle cavity organs of the mussel, *Mytilius edulis* collected from study area. A: Mantle. AB-PAS (pH 2.5) reaction. B: Mantle. AB-PAS (pH 2.5) reaction. C: Gill. AB-PAS (pH 2.5) reaction. D: Gill. AB-PAS (pH 2.5) reaction. E: Foot. AB-PAS (pH 2.5) reaction. F: Mid-gut. AB-PAS (pH 2.5) reaction. Dt: digestive tubule, El: epithelial layer, Gf: gillfilament, Hc: hemocyte, Hs: hemolymph sinus

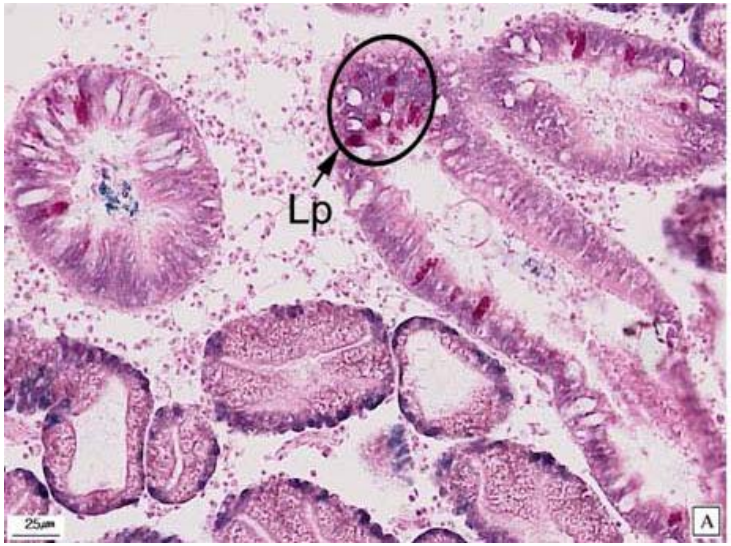

Fig. . Lipofuscin accumulation in mid-gut of oyster, *Crassostrea gigas*. collected from study area. Lp: lipofuscin.

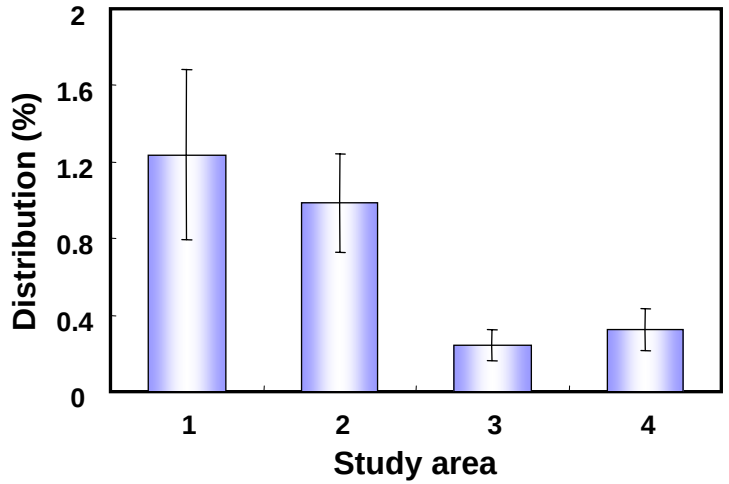

Fig. . Lipofuscin distribution in Mid-gut of the oyster, *Crassostrea gigas* collected from study area.

The distribution of lipofuscin decrease as the distance from the polluted area increases

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Copper

Acute Mortality

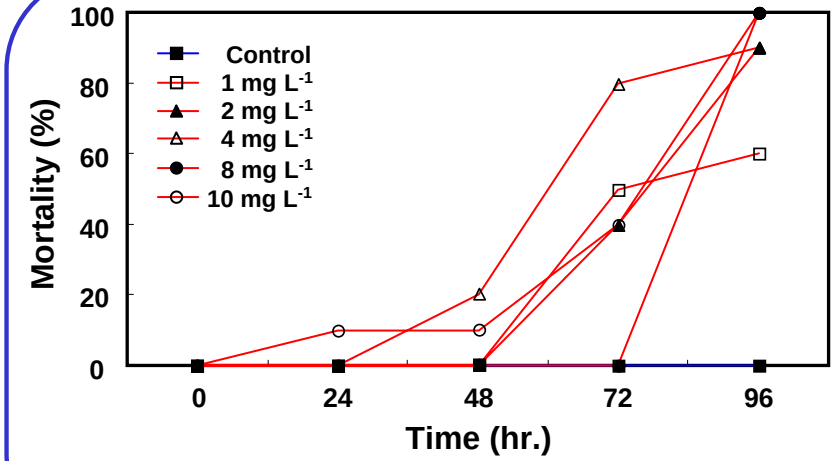

Fig. . Mortality of the oyster, *Crassostrea gigias* acute exposed to Cu.

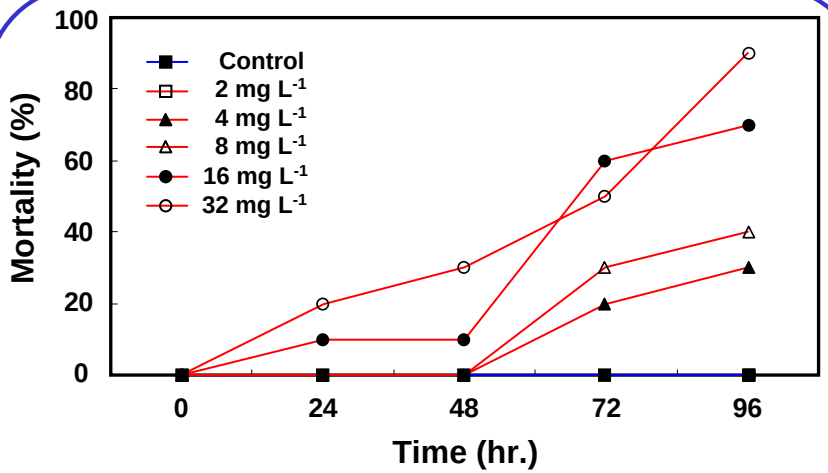

Fig. . Mortality of the granular ark, *Tegillarca granosa* acute exposed to Cu.

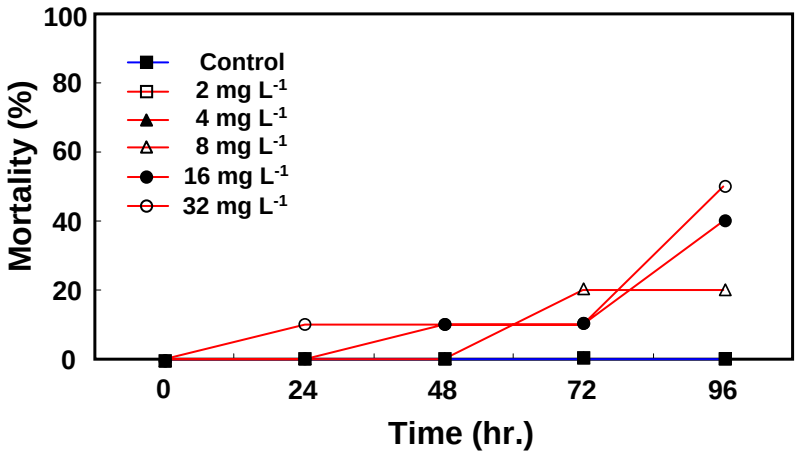

Fig. . Mortality of the manila clam, *Ruditapes philppinarum* acute exposed to Cu.

Lead

Acute Mortality

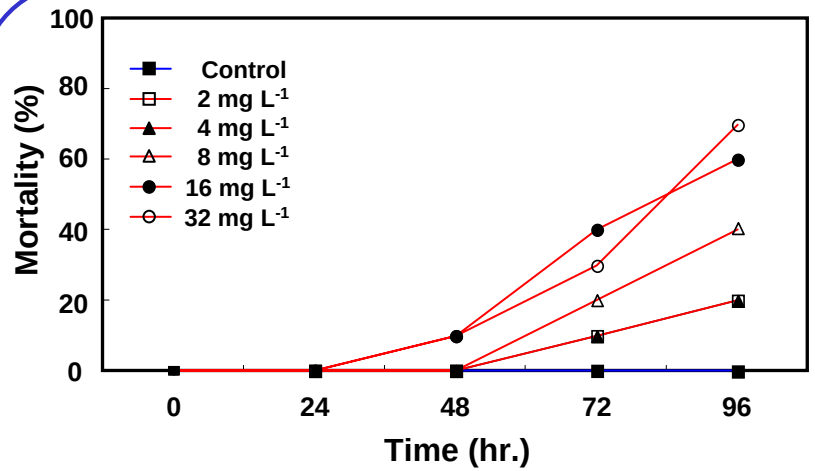

Fig. . Mortality of the oyster, *Crassostrea gigias* acute exposed to Pb.

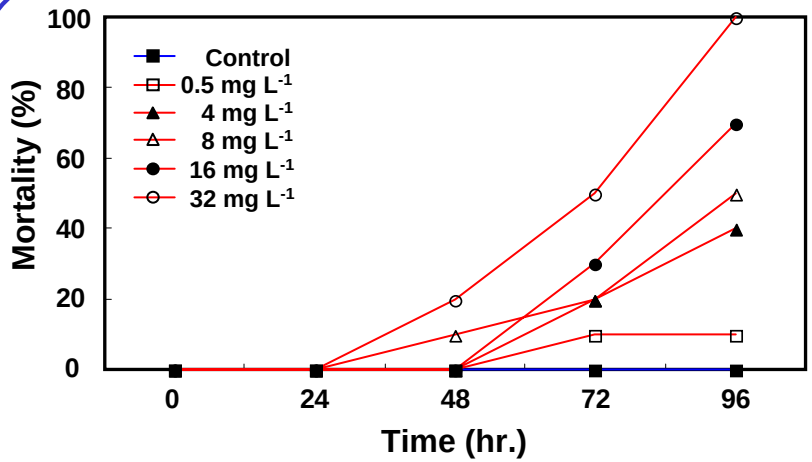

Fig. . Mortality of the granular ark, *Tegillarca granosa* acute exposed to Pb.

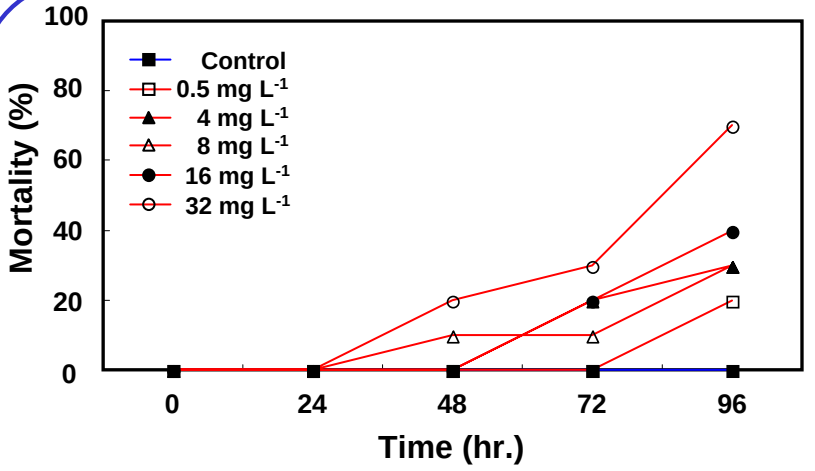

Fig. . Mortality of the manila clam, *Ruditapes philppinarum* acute exposed to Pb.

Copper

Acute Oxygen consumption

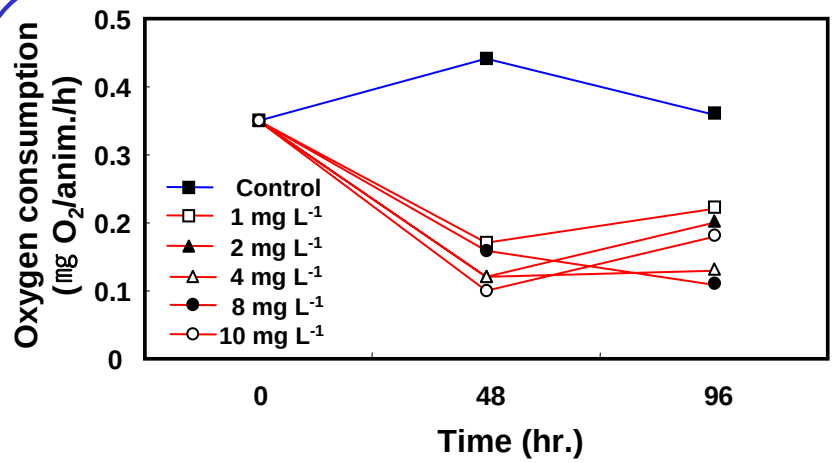

Fig. . Oxygen consumption of the oyster, *Crassostrea gigas* acute exposed to Cu.

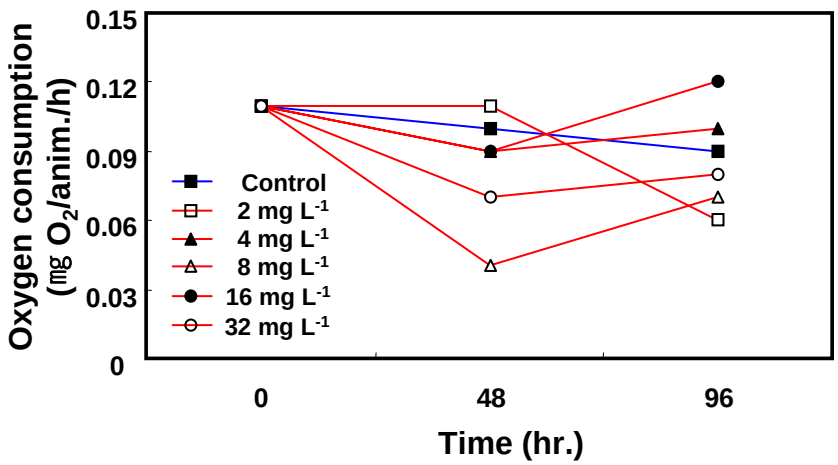

Fig. . Oxygen consumption of the granular ark, *Tegillarca granosa* acute exposed to Cu.

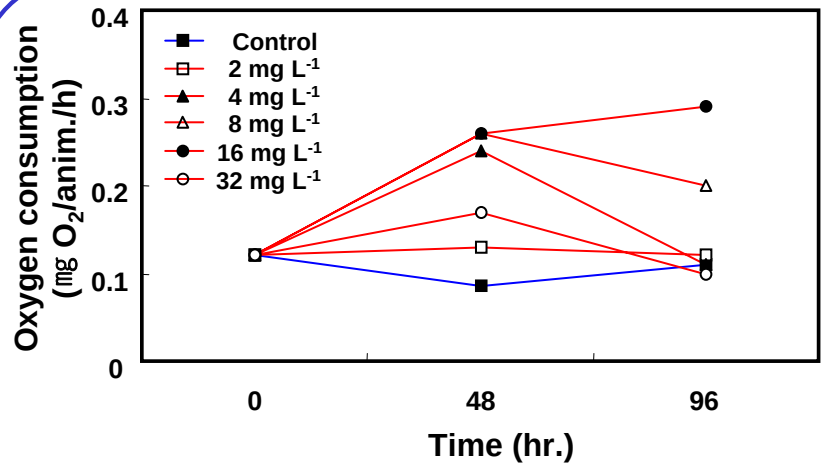

Fig. . Oxygen consumption of the manila clam, *Ruditapes philppinarum* acute exposed to Cu.

Lead

Acute Oxygen consumption

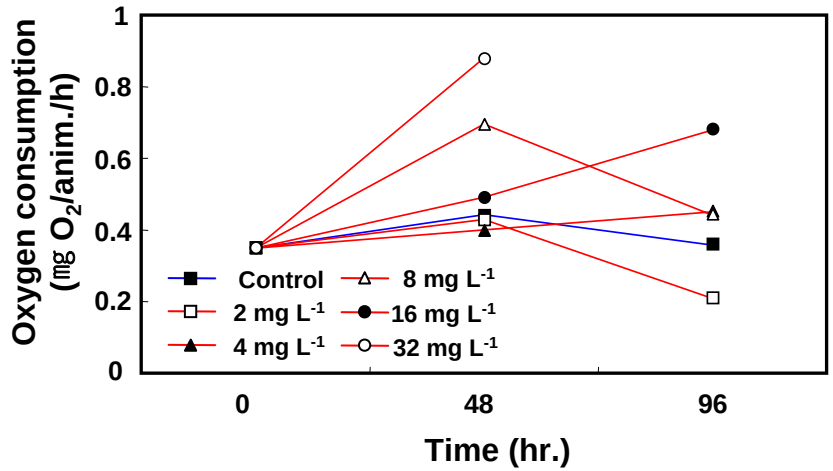

Fig. . Oxygen consumption of the oyster, C*rassostrea gigas* acute exposed to Pb.

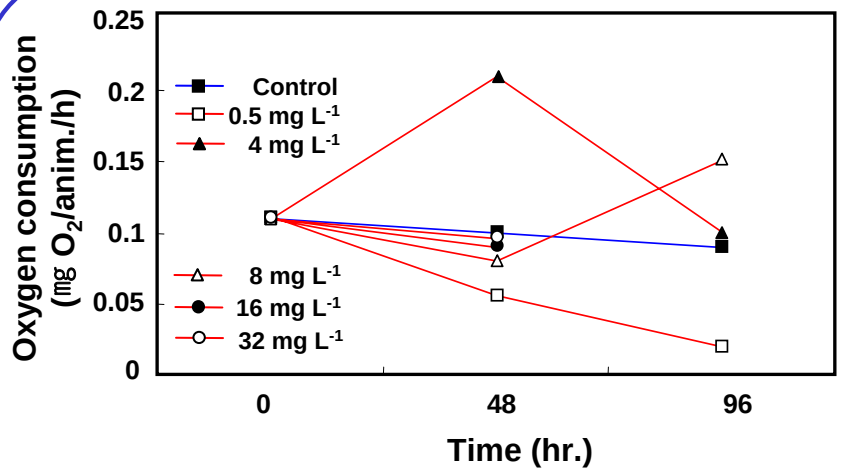

Fig. . Oxygen consumption of the granular ark, *Tegillarca granosa* acute exposed to Pb.

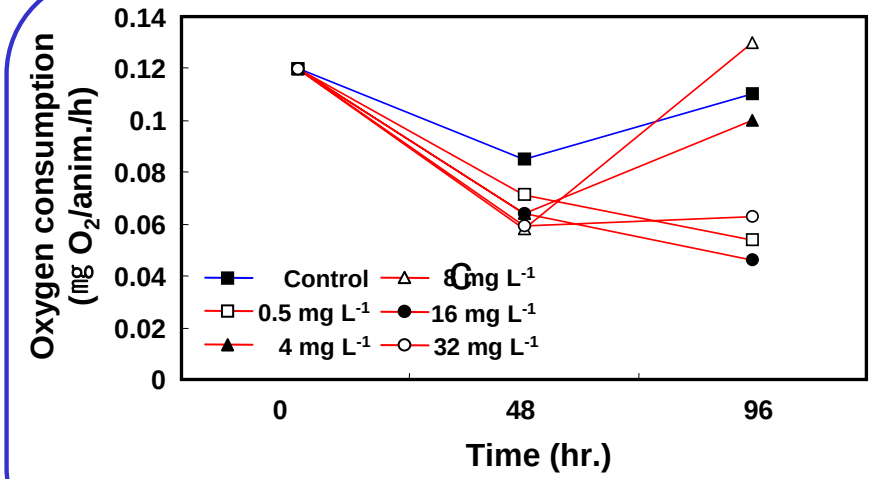

Fig. . Oxygen consumption of the manila clam, *Ruditapes philppinarum* acute exposed to Pb.

Table. LC_{50}-96hr of bivalves 3sp. exposed to Cu and Pb

Species	Environmental condition			LC_{50}-96hr. (㎎/ L)	
	Temp. (℃)	Sal. (psu)	pH	Cu	Pb
Crassostrea gigas	20± 1	33 ± 0.5	7.8-8.2	0.44	16.45
Tegillarca granosa	20 ± 1	33 ± 0.5	7.8-8.2	10.96	7.97
Ruditapes philippinarum	20 ± 1	33 ± 0.5	7.8-8.2	29.07	20.59

Copper

Chronic Mortality

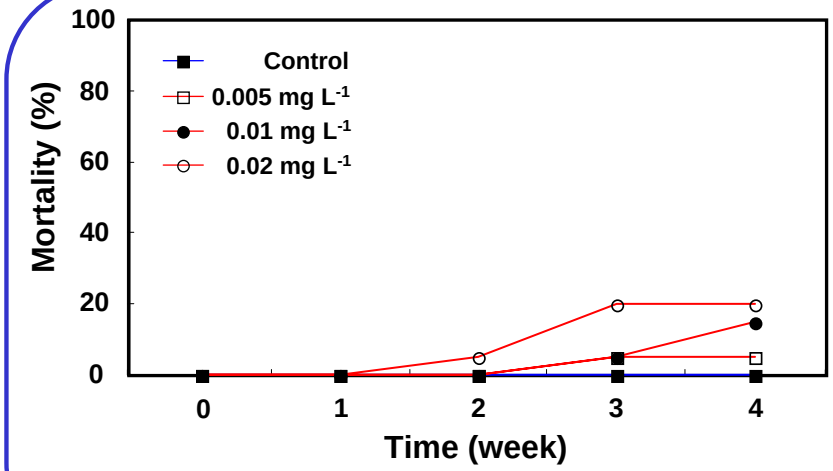

Fig. . Mortality of the oyster, *Crassostrea gigias* chronically exposed to Cu.

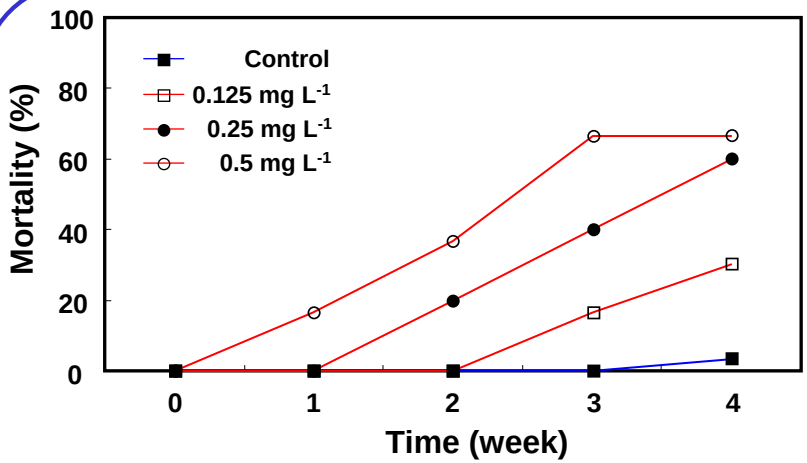

Fig. . Mortality of the granular ark, *Tegillarca granosa* chronically exposed to Cu.

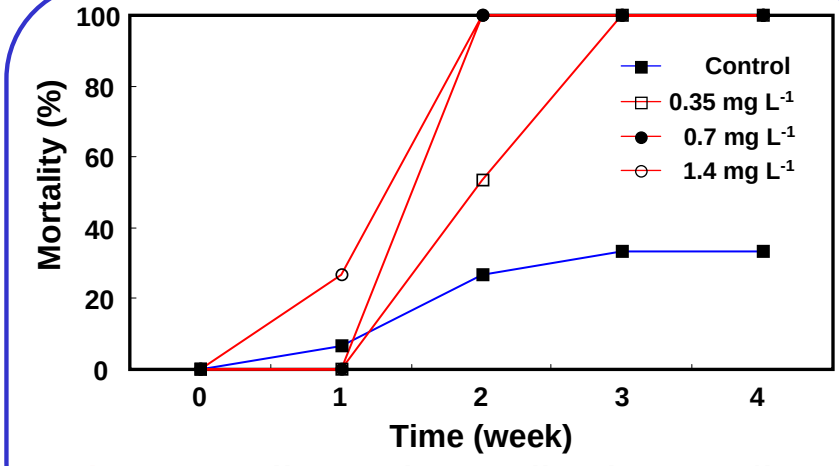

Fig. . Mortality of the manila clam, *Ruditapes philppinarum* chronically exposed to Cu.

Lead

Chronic Mortality

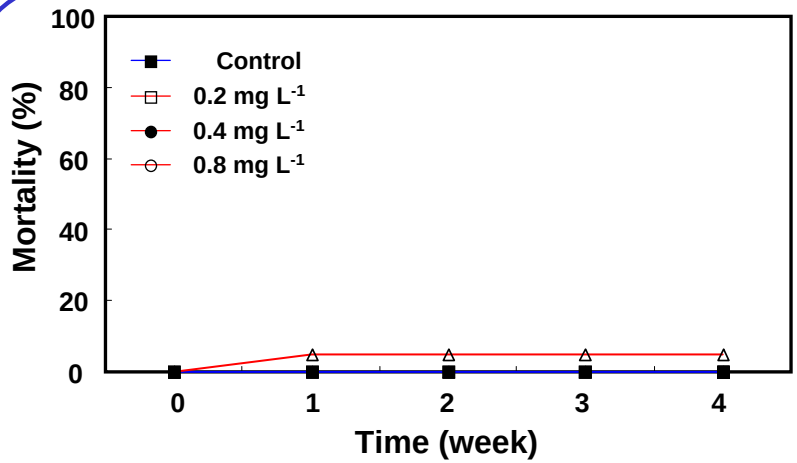

Fig. . Mortality of the oyster, *Crassostrea gigias* chronically exposed to Pb.

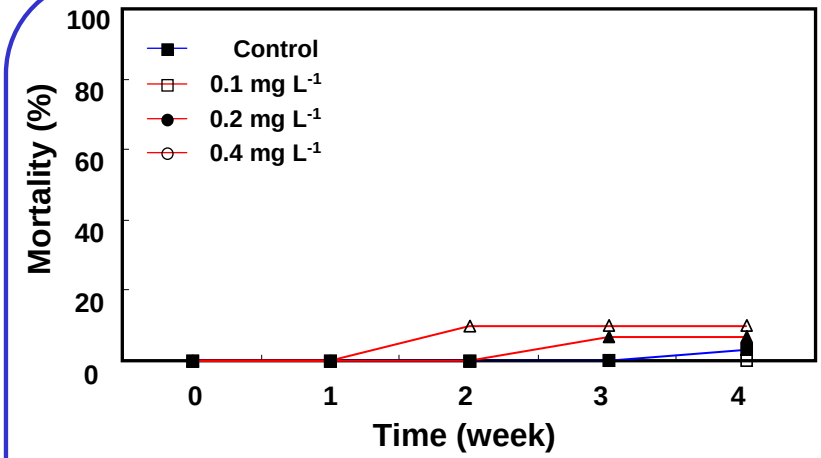

Fig. . Mortality of the granular ark, *Tegillarca granosa* chronically exposed to Pb.

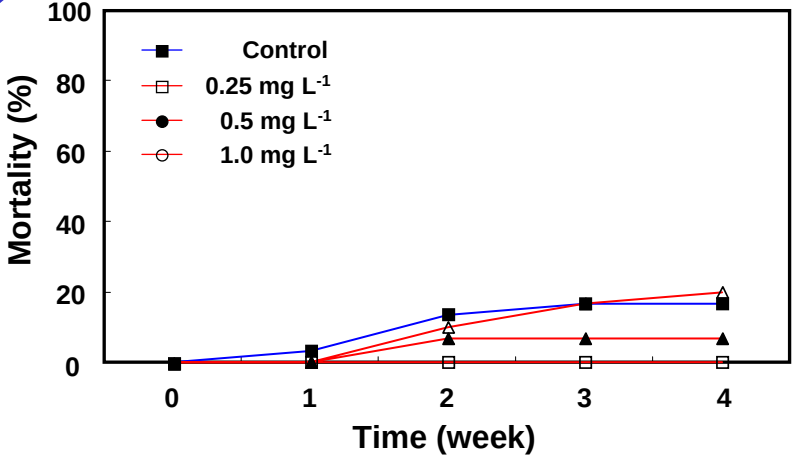

Fig. . Mortality of the manila clam, *Ruditapes philppinarum* chronically exposed to Pb.

Copper

Chronic Oxygen consumption

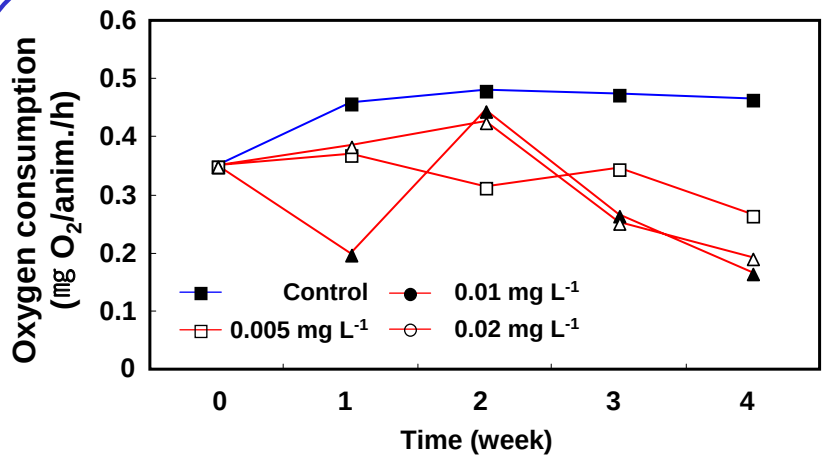

Fig. . Oxygen consumption of the oyster, *Crassostrea gigas* chronically exposed to Cu.

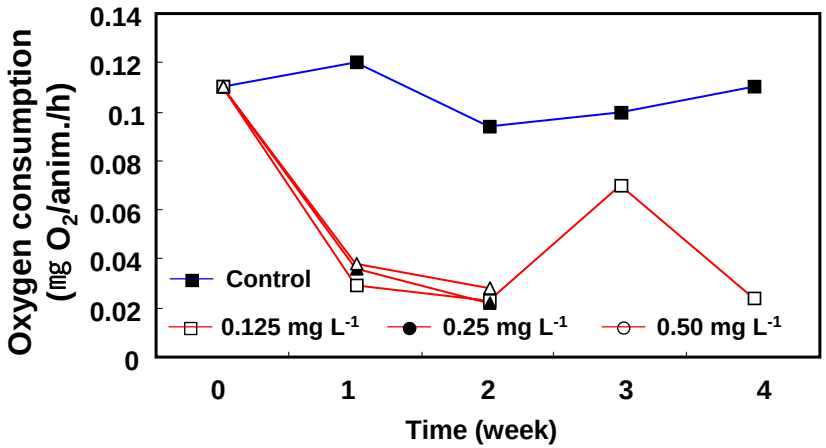

Fig. . Oxygen consumption of the granular ark, *Tegillarca granosa* chronically exposed to Cu.

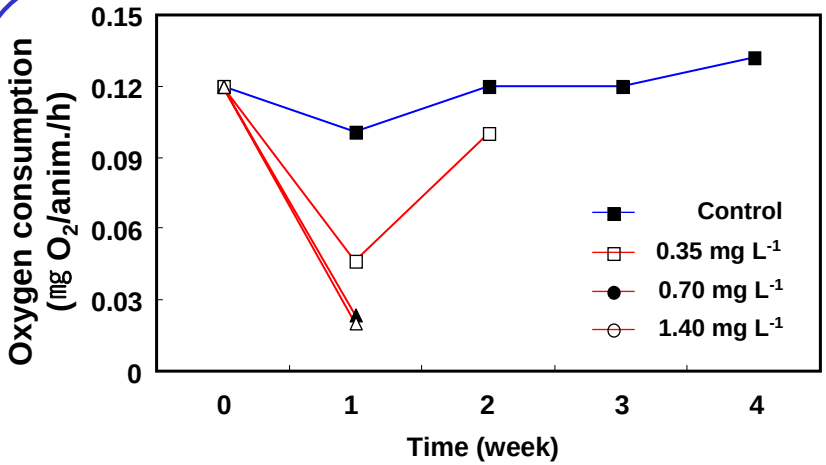

Fig. . Oxygen consumption of the manila clam, *Ruditapes philppinarum* chronically exposed to Cu.

Lead

Chronic Oxygen consumption

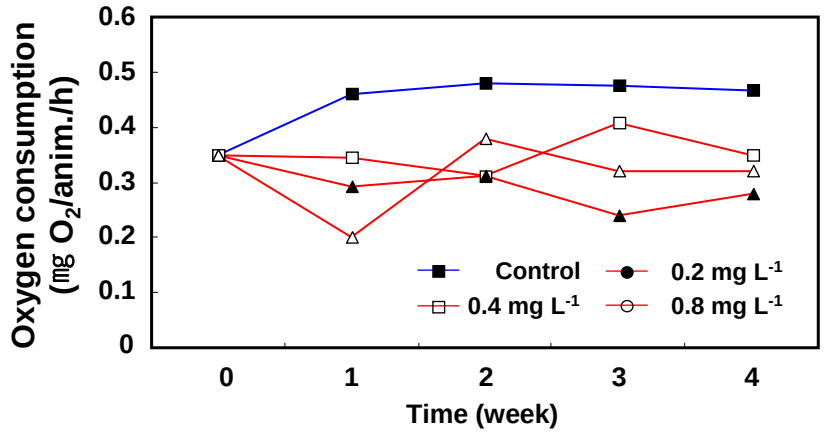

Fig. . Oxygen consumption of the oyster, *Crassostrea gigas* chronically exposed to Pb.

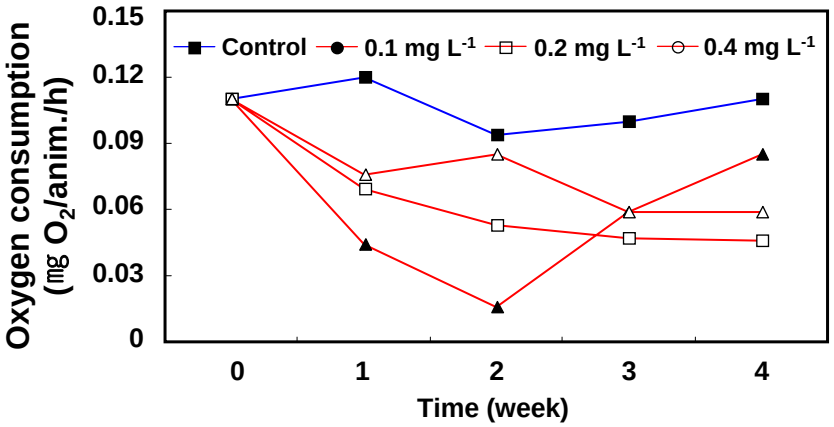

Fig. . Oxygen consumption of the granular ark, *Tegilarca granosa* chronically exposed to Pb.

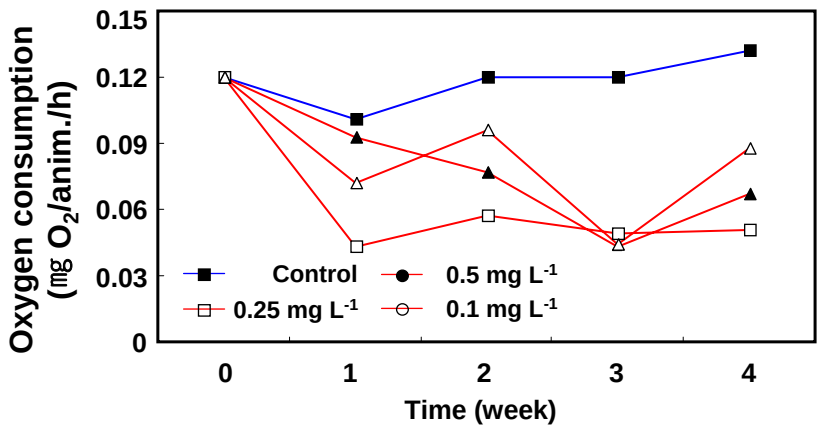

Fig. . Oxygen consumption of the manila clam, *Ruditapes philppinarum* chronically exposed to Pb.

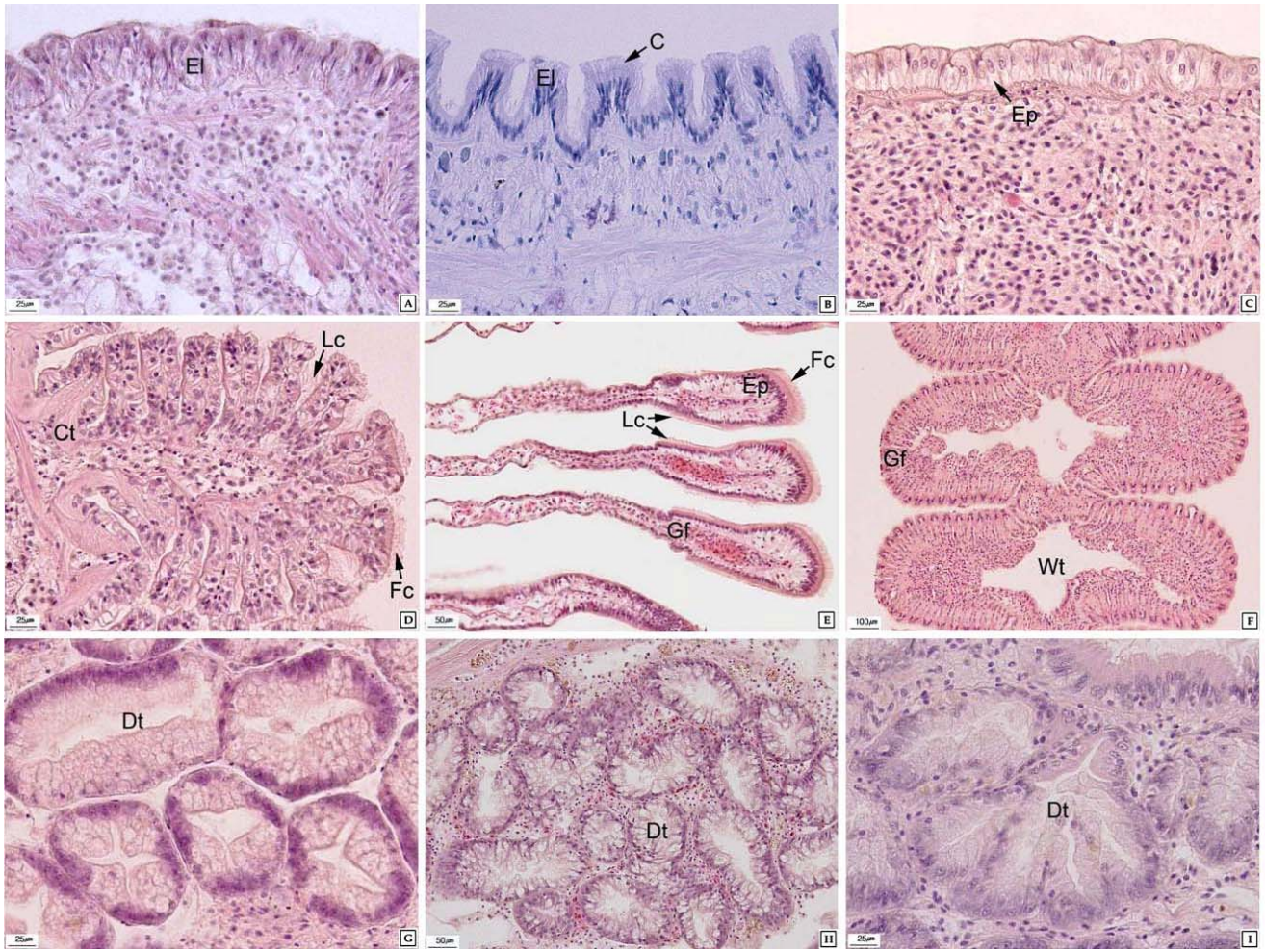

Fig. . **Histological structure of organs of control group *Crassostrea gigas*, *Tegillarca granosa* and *Ruditapes philppinarum*. A: Mantle of *Crassostrea gigas*. H-E stain. B: Mantle of *Tegillarca granosa*. H-E stain. C: Mantle of *Ruditapes philippinarum*. H-E stain. D: Gill of *Crassostrea gigas*. H-E stain. E: Gill of *Tegillarca granosa*. H-E stain. F: Gill of *Ruditapes philippinarum*. H-E stain. G: Mid-gut gland of *Crassostrea gigas*. H-E stain. H: Mid-gut of *Tegillarca granosa*. H-E stain. I: Mid-gut of *Ruditapes philippinarum*. H-E stain. C: cilia, Ct: connective tissue, Dt: digestive tubule, El: epithelial layer, Ep: epithelium, Fc: frontal cilia, Gf: gill filament, Lc: lateral cilia, Wt: water tubule.**

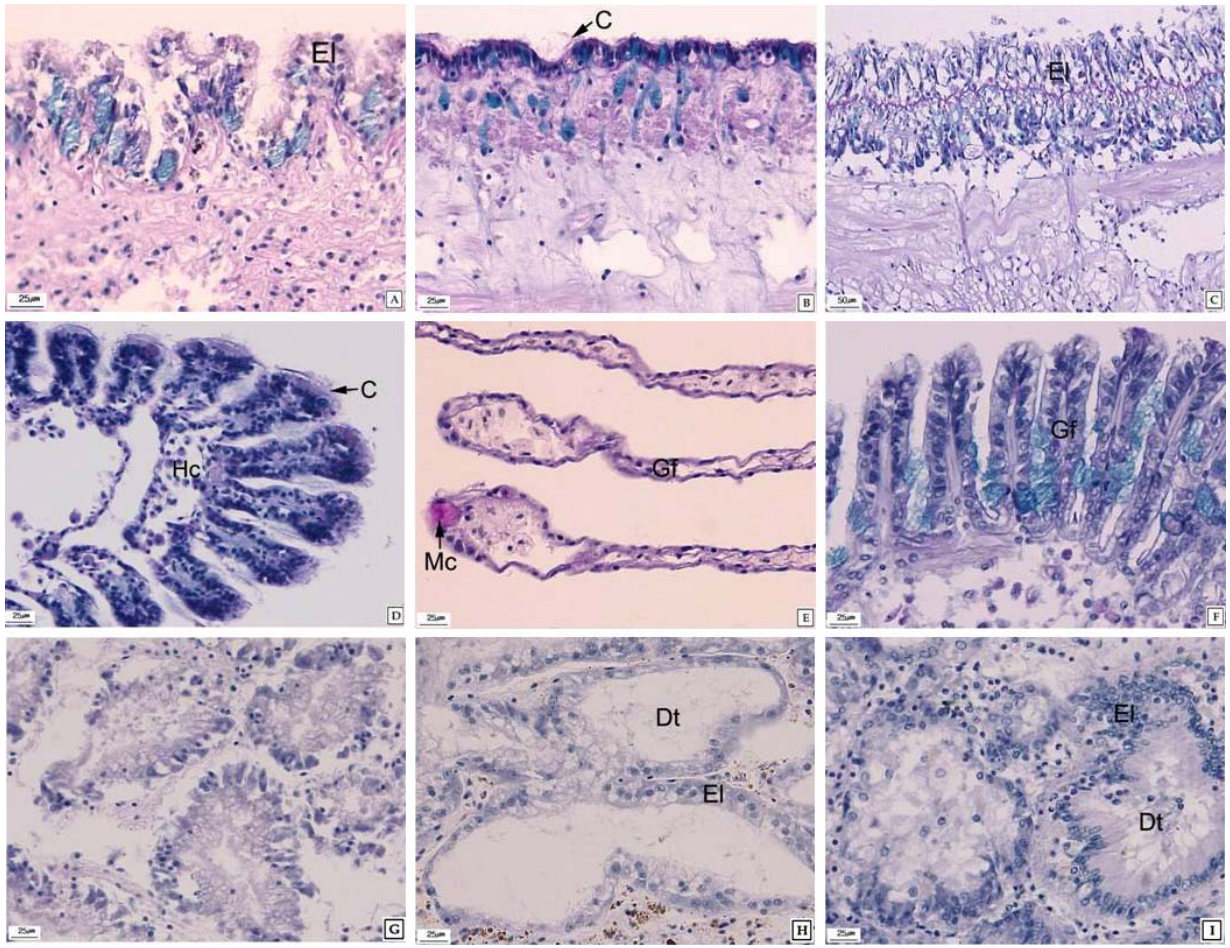

Fig. . Histopathological structure of organs of *Crassostrea gigas*, *Tegillarca granosa* and *Ruditapes philppinarum* chronic exposed to Cu. A: Mantle of *Crassostrea gigas*. 0.005 mg L^{-1}. AB-PAS (pH 2.5) reaction. B: Mantle of *Tegillarca granosa*. 0.125 mg L^{-1}. AB-PAS (pH 2.5). C: Mantle of *Ruditapes philippinarum*. 1.4 mg L^{-1}. AB-PAS (pH 2.5). D: Gill of *Crassostrea gigas*. 0.02 mg L^{-1}. AB-PAS (pH 2.5). E: Gill of *Tegillarca granosa*. 0.25 mg L^{-1}. AB-PAS (pH 2.5). F: Gill of *Ruditapes philippinarum*. 0.35 mg L^{-1}. AB-PAS (pH 2.5). G: Mid-gut gland of *Crassostrea gigas*. 0.02 mg L^{-1}. AB-PAS (pH 2.5). H: Mid-gut gland of *Tegillarca granosa*. 0.25 mg L^{-1}. AB-PAS (pH 2.5). I: Mid-gut gland of *Ruditapes philippinarum*. 0.35 mg L^{-1}. AB-PAS (pH 2.5). C: cilia, Dt: digestive tubule, El: epithelial layer, Gf: gill filament, Mc: mucous cell, Hc: hemocyte.

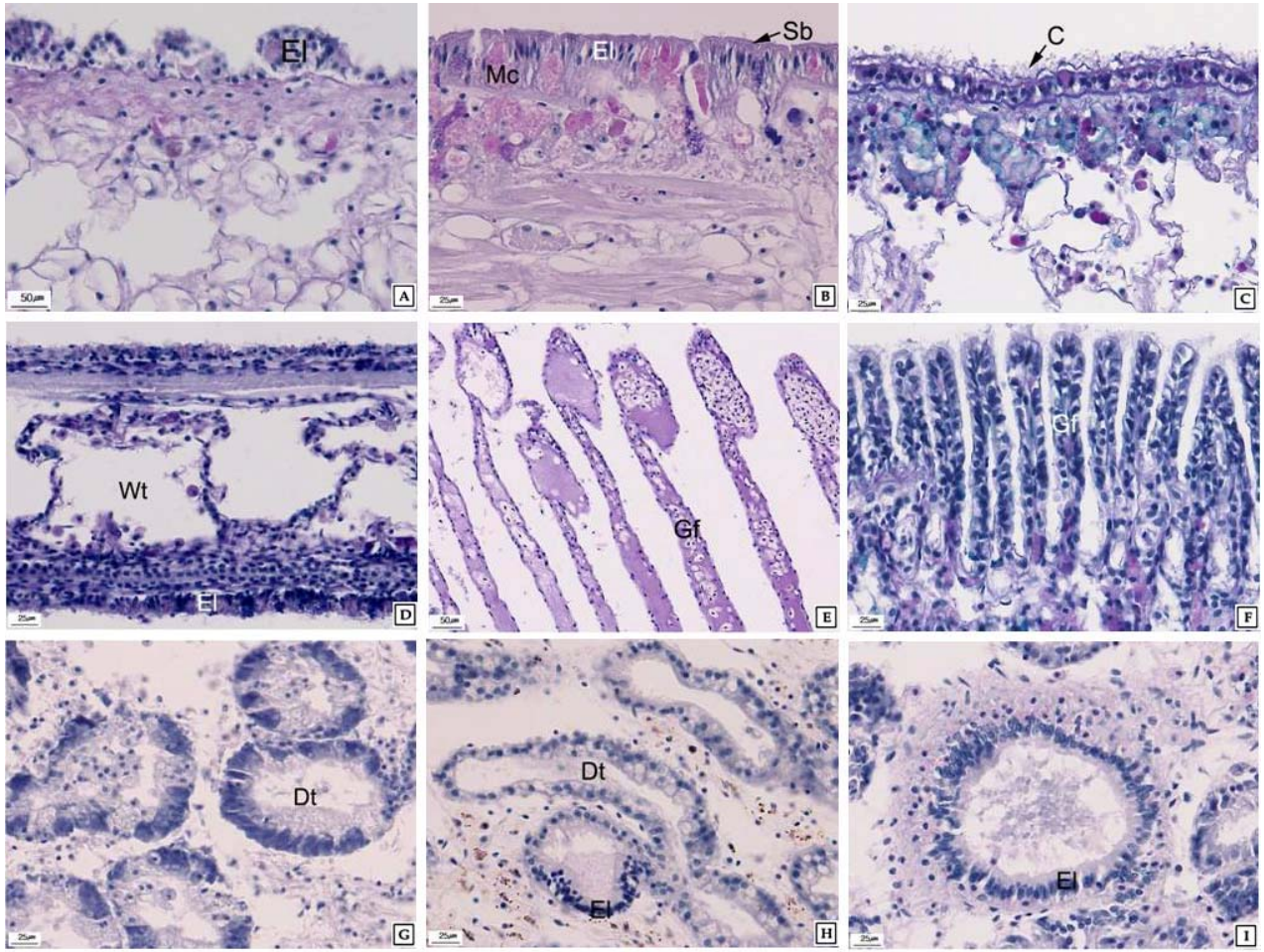

Fig. . **Histopathological structure of organs of *Crassostrea gigas*, *Tegillarca granosa* and *Ruditapes philppinarum* chronic exposed to Pb. A: Mantle of *Crassostrea gigas*. 0.2 mg L^{-1}. AB-PAS (pH 2.5) reaction. B: Mantle of *Tegillarca granosa*. 0.2 mg L^{-1}. AB-PAS (pH 2.5). C: Mantle of *Ruditapes philippinarum*. 0.25 mg L^{-1}. AB-PAS (pH 2.5). D: Gill of *Crassostrea gigas*. 0.2 mg L^{-1}. AB-PAS (pH 2.5). E: Gill of *Tegillarca granosa*. 0.2 mg L^{-1}. AB-PAS (pH 2.5). F: Gill of *Ruditapes philippinarum*. 0.25 mg L^{-1}. AB-PAS (pH 2.5). G: Mid-gut of *Crassostrea gigas*. 0.8 mg L^{-1}. AB-PAS (pH 2.5). H: Mid-gut of *Tegillarca granosa*. 0.4 mg L^{-1}. AB-PAS (pH 2.5). I: Mid-gut of *Ruditapes philippinarum*. 0.1 mg L^{-1}. AB-PAS (pH 2.5). C: cilia, Dt: digestive tubule, El: epithelial layer, Gf: gill filament, Mc: mucous cell, Sb: straited border, Wt: water tubule.**

Lipofuscin

Copper

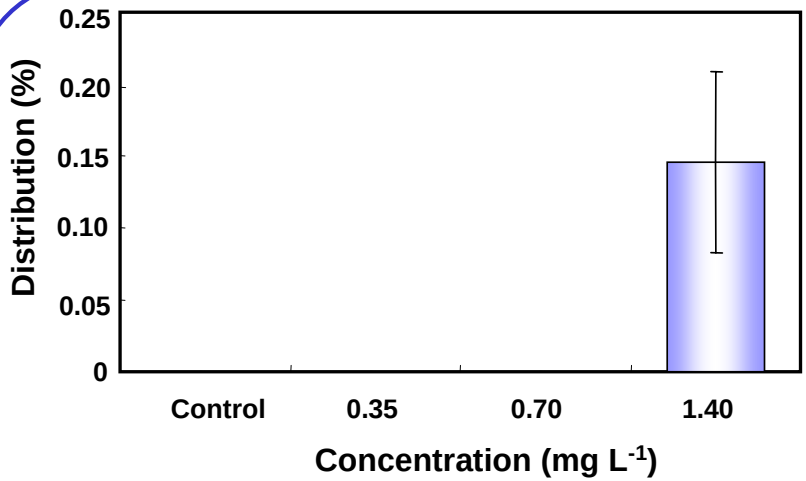

Fig. . Lipofuscin distribution in mid-gut of manila clam, *Ruditapes philippinarum* chronically exposed to Cu.

Lead

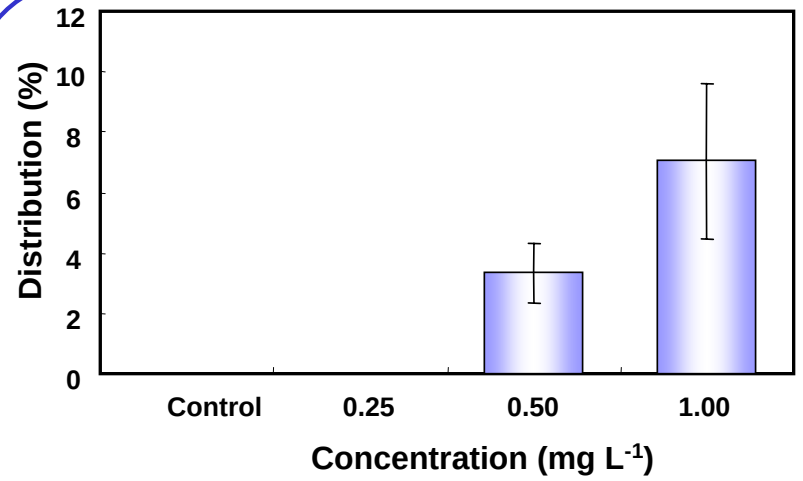

Fig. . Lipofuscin distribution in mid-gut of manila clam, *Ruditapes philippinarum* chronically exposed to Pb.

Conculsion

- Histopathological responses are obserbed in mantle, gill and mid-gut gland of 3 sp. sampled at study area

  ⇒ vanish of epithelium layer, increase of mucous cell, gill filament deformation, degeneration of oocyte etc.

- Distribution of Lipofucsine : differ with the species of shellfish and types of heavy metals

- Indoor bioassay : similar histopathological responses, lower metabolic rate or physiologically unstable

- Necessary of the continous management of areas surrounding the shellfish farms


Thank you