BIOASSAYS ON MARINE ORGANISMS Ⅱ. ACUTE TOXICITY TEST OF MERCURY, COPPER AND CADMIUM TO CLAM, *MERETRIX LUSORIA*

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海洋生物에 對한 生体實驗 Ⅱ. 백합에 대한 水銀, 구리 및 카드뮴의 毒性

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백합에 대한 水銀, 구리 및 카드뮴의 毒性程度를 究明하기 위하여 1978. 6. 28~7. 15間 靜水式으로 實驗을 實施하였다.

實驗結果 水銀 및 구리 용액에는 농도가 커질수록 점액을 분비하는 個体가 많아졌으나 카드뮴용액과 정상해수에 투입한 백합은 점액을 放出하지 않았다. 1*mg/l* 농도의 수조에서 15시간후에 분비한 점액은 水銀에서는 42.9%, 구리에서는 14.3% 나타냄으로서 수은이 구리보다 협기도가 더 컸다.

濃度와 死亡率과의 關係에서 96시간후에 半致死濃度(96*hr*—*LC*50)는 水銀 0.67*mg/l*, 구리 7.04*mg/l*, 카드뮴 7.10*mg/l*, 로서 毒性의 順位는 水銀—구리—카드뮴이었다.

또한 各水槽에서 50%의 斃死를 일으키는데 소요한 시간(Lethal Tim 50)은 일반적으로 농도가 클수록 *L. T.* 50값이 작아짐으로서 一定 濃度에서는 露出時間이 反應을 유발시키는 자극이 되고 있으며 실제 중금속의 4*mg/l*에서 *L. T.* 50값은 水銀 55.8시간, 구리 104.8시간, 카드뮴 111.9시간으로서 同一濃度에서는 水銀—구리—카드뮴順으로 死亡이 일어 났음을 밝혔다.

INTRODUCTION

The acute toxicity effects of mercury, copper and cadmium on arkshell and oyster which are very important species in commercial aspect were tested in the former study (Park and Kim, 1978) for water quality criteria.

Present study was performed to determine the toxicity effects of the heavy metals to the clam, *Meretrix lusoria*, chosen as the test animal because of its availability in Korean Waters. and commercial importance.

MATERIAL AND METHODS

Collection and Acclimation of Test Animals.

A thousand clams, *Meretrix lusoria*, were collected from Sacheon Bay on the south coast of Korea, and were acclimated for a week from 29 June to 5 July 1978 after they were accommodated in a large holding tank.

biometries of the clams, on the average, varied from 2.76 to 4.76*cm* and from 6.1 to 27.8 gram in shell length and weight, respectively.

The temperature in the holding tank was 21± 0.6°C and four dead specimens were found during the acclimation.

Testing Procedure

Five-series of concentrations of test solutions were made by diluting standard solutions of cadmium chloride ($CdCl_2$ 2½ H_2O), cupric sulfate ($CuSO_4$ $5H_2O$), and mercury bichloride ($HgCl_2$). In the copper test, sodium citrate($Na_3C_6H_6O_7$· $2H_2O$) was mixed to protection against precipit-

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ation of copper in the seawater.

A control test with no metal input was simultaneously established for data comparison. Each regular test container(22*W*×37L×38*cm H*) housed five or more healthy shellfish in a ten liter medium.

During the test, oxygen supply for all the time by continuous aeration and replacement of new test solution every 24 hours were performed to insure a constant metal concentration and to protection against degradation of excreted mucus substances. During the test no animals were fed.

Observation and Criteria for Death

The responses of the animals were checked at each fixed metal concentration by predetermined elapsed time. Mucus excreting rate was examined at 15, 20 & 24 hours after the starting point. When apparently the dead shellfish were found according to the criteria, both the dead and the survivers were counted.

Clams of wide-opened shellvalves were considered to be dead after being stimulated by prodding, and those with shells pressed together were transferred to natural seawater to judge whether they were capable of recovering. Only animals that failed to recover were considered as dead after 24 hours.

Data Presentation

The death rate of clams modified by the method of Tattersfield and Morris(1924). was calculated in a previously set span at fixed intervals, especially at 24, 48, 72, 96 and 120 hours.

The mortality and metal concentration were plotted on the logarithmic scale for the lethal median concentration after 96 hours (96*hr-LC* 50). The toxicity curve of mercury was plotted by logarithmic series of concentration and original mortality. However, both of the concentration and mortality were figured on the logarithmic scale for copper and cadmium test results.

The response of lethal time for half of the animals(*LT* 50) at each concentrated metal solution was derived from the logarithmic exposure time and their mortality, and then the 24, 48, 72 and 96*hr*—*LT* 50 were computed from the relation between the *LT* 50 and its concentration.

RESULTS

Excreting Rate of Mucus

Most of test shellfish insert their ex hale and in hale siphon into shellvalves immediately after exposure to a concentrated heavy metal solution. Mucus excreting individuals increased gradually in elapsed time and higher concentrations of mercury and copper test containers. However, no excreting shellfish were found in the cadmium and control tests. The rate of the excretion value in the 1*mg/l* test was 42.9% for the mercury solution and 14.3% for the copper after 15 hours. More individuals excrete mucus substances in the mercury than in copper test solution (Table 1).

Table 1. Rate of mucus secreted by the clam, *Meretrix lusoria*, for every concentration by time elapsed during the test (%)

Metal	Time elapsed	Conc. *mg/l* 0.2	0.4	0.6	0.8	1	2	3	4	5	6	8	10
Mercury	15hrs	0	—	28.6	—	42.9	—	—	85.7	—	—	85.7	—
	20hrs	0	0	20	40	60	—	—	—	—	—	—	—
	24hrs	0	—	28.6	—	71.5	—	—	100	—	—	85.7	—
Copper	15hrs	0	—	0	—	14.3	—	—	14.3	—	—	14.3	—
	20hrs	—	—	—	—	—	10	—	80	—	10	10	10
	24hrs	0	—	14.3	—	14.3	—	—	14.3	—	—	28.6	—
Cadmium	15hrs	—	—	—	—	0	0	0	0	0	—	—	—
	20hrs	—	—	—	—	—	0	—	0	—	0	0	0
	24hrs	—	—	—	—	0	0	0	0	0	—	—	—
Reference	Control: No animals secrete mucus substances.												

Mortality of Test Animals

When test shellfish were exposed to a concentrated high metal solution, they died in a short time span; the longer the exposure time, the higher the mortality.

The clams, generally speaking, revealed dull responses in the high cadmium test solution as shown in table 2.

Table 2. Mortailty (%) of clam *Meretrix lusoria*, for every concentration in the test with heavy metals

Metal	Conc. (*mg/l*)	Time elapsed 24*hr*	48*hr*	72*hr*	96*hr*	120*hr*	Water Temperature (°C)
Mercury	0.2	0	0	0	0	24.3	
	0.4	0	0	0	20	60	
	0.6	0	0	7.1	58.6	65.7	
	0.8	0	0	20	60	80	24±1
	1	0	7.1	28.6	80	90	
	4	0	28.6	57.1	100	100	
	8	0	28.6	100	100	100	
Copper	0.2	0	0	0	0	14.3	
	0.6	0	0	0	14.3	28.6	
	1	0	0	0	28.6	57.1	
	2	0	0	20	40	60	24±0.5
	4	0	0	7.1	37.2	68.6	
	6	0	0	20	40	80	
	8	0	0	17.2	51.5	82.8	
	10	0	0	20	60	80	
Cadmium	2	0	0	0	20	40	
	4	0	0	0	31.5	58.6	
	6	0	0	0	40	65.7	24±1
	8	0	0	0	72.9	82.9	
	10	0	0	0	58.9	92.9	
	20	0	0	28.6	100	100	
Control		0	0	0	0	0	24±1

In the *4mg/l* test container, mortality was found after 48 hours in mercury, 72 hours in copper and 96 hours in cadmium; their mortality rates after 96 hours were 100, 37.2 and 31.5 percent respectively.

The response and concentration relation in toxicity of heavy metals on clams was linear in copper and cadmium when plotted in natural logarithmic scale, but that of the mercury relation figured linear on semi-logarithmic scale(Fig. 1).

The *96hr-LC* 50 value was *0.67mg/l* for mercury, *7.04mg/l* for copper and *7.10mg/l* for cadmium. Consequently, it was concluded that the clams were most sensitive to mercury and least to cadmium.

The value of the median lethal time (*LT* 50) at each test concentration became lower in the case of higher metal concentrations. In the *0.2mg/l* test of heavy metals, the *LT* 50 was 152.1 hours in mercury and 209.9 hours in copper. It took a longer time to kill the clams in copper than in mercury.

Mean while, the *LT* 50 value in the *4mg/l* test concentration was 55.8 hours in mercury, 104.8 hours in copper and 111.9 hours in cadmium(Fig. 2). The *96hr-LT* 50 value was *0.64mg/l* for mercury, *5.30mg/l* for copper and *7.70mg/l* for cadmium.

DISCUSSION

The toxicity level of three heavy metals on clams in this study were mercury, copper and cadmium in order by showing the *96hr-LC* 50 values of *0.67mg/l*, *7.04mg/l* and *7.10mg/l* respectively,

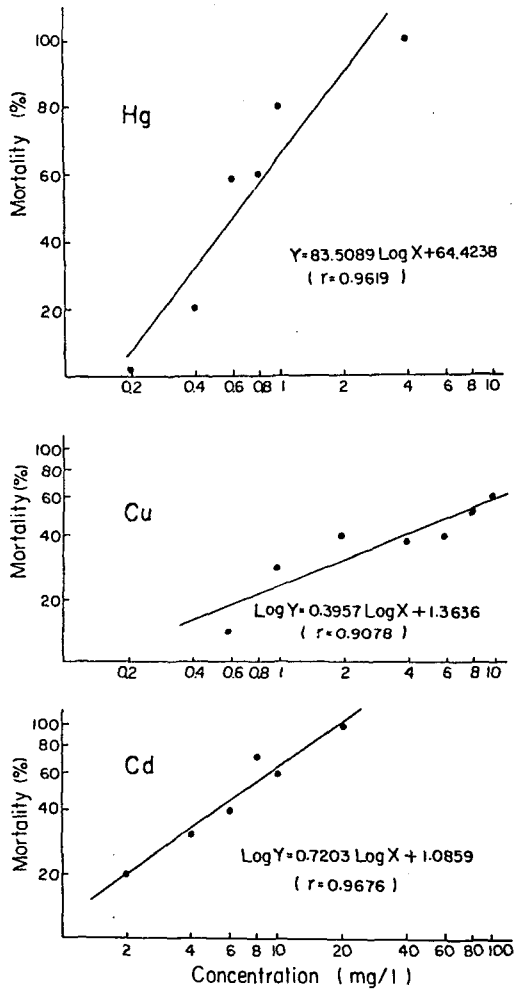

Fig. 1. Toxicity curve of mercury, copper and cadmium on *Meretrix lusoria* for 96 hours.

This level order between metals of relative toxicity was similar to that of the pacific oyster larvae whose 96*hr-LC* 50 values were 1.1*mg/l* for mercury, 2.54*mg/l* for copper and 19.5*mg/l* for cadmium (Park and Kim, 1978), and that of the american oyster embryos whose 48*hr-LC* 50 values were 0.0056ppm for mercury, 0.103ppm for copper and 3.80ppm for cadmium (Calabrease *et al* 1973,)

When compared with other shellfish for the *LC* 50 value, the 96*hr-LC* 50 value of mercury for clam was lower than that of *Anadara broughtonii*, which was 4.84*mg/l* as reported by Park and Kim (1978) and that of *Mytilus edulis*, 13*mg/l* as reported by Wisely and Blick (1967). In the copper test, the 96*hr-LC* 50 value of clam was much lower than the 48*hr-LC* 50 value of *Mytilus edulis*, 22.5*mg/l* by Wisely and Blick (1967), but higher than the 96*hr-LC* 50 value of *Anadara broughtonii*, 0.31*mg/l* by Park and Kim (1978) and that of *Crassostrea gigas*, 1.9*mg/l* by Fujiya (1960) according to water quality criteria (1972), National Academy of Sciences/ National Academy of Engineering in Washington D.C.

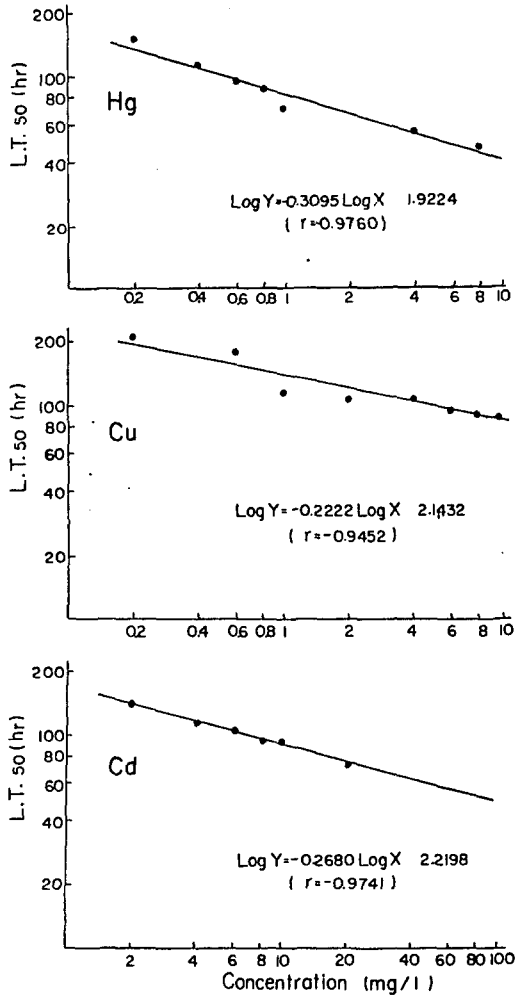

Fig. 2. Relation between *L.T.* 50 value and heavy metal concentration on *Meretrix lusoria* for five days.

While the 96*hr-LC* 50 value of cadmium for clams was much lower than that of the 25.0*mg/l* for *Mytilus edulis* reported by Eisler (1971). However it was higher than the 96*hr-LC* 50 value of 1.6*mg/l* for *Mytilus edulis planulatus* reporled by Ashanullah(1976) and that of 1.86*mg/l* for *Anadara broughtonii* reporfed by Park and Kim (1978).

The relation between the metal concentration and the *LT* 50 value was an inverse proportion; the higher the concentration, the lower the *LT*50 value in a concentrated fixed metal solution (Fig. 2).

For the *LT* 50 value in the test container of 1*mg/l* for mercury, it took 70.8 hours for clams and 151 hours for oysters to reach the median lethal time(Park and Kim 1978).

the tolerance of clams was considered to be smaller than oysters in mercury and cadmium. However, in the test with copper it was larger than oysters.

SUMMARY

The short-term acute toxicities of mercury, cadmium and copper for the clam, *Meretrix lusoria* were determined from 28 June to 15 July, 1978.

In the test with mercury and copper, the rate ot mucus excretion increased gradually at a higher concentration. But the clams did not excrete mucus in the solutions of cadmium and natural sea water. The rate of mucus excretion in mercury was 42.9%, and that in copper was 14.3% in a test solution of 1*mg/l*. mercury was more toxic than copper.

The median lethal concentration after 96 hours (96 *hr-LC* 50) was 0.67*mg/l* in mercury, 7.04*mg/l* in copper and 7.10*mg/l* in cadmium. Consequently it was found that mercury was the most toxic substance and cadmium was the least. meanwhile, it was considered that exposure time by stimulation in a fixed concentration caused the test animals to respond.

The *LT* 50 in each tank decreased gradually at a higher concentration. The *LT* 50 values of 4*mg/l* were 55.8 hours for mercury, 104.8 hours for copper and 111.9 hours for cadmium. In the same concentrated metal solution, the clams quickly responded to mercury.

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