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ACUTE TOXICITY AND BIOACCUMULATION OF LEAD IN THE SNAIL, *FILOPALUDINA (SIAMOPALUDINA) MARTENSI MARTENSI* (FRAUENFELDT)

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ABSTRACT

Acute lead toxicity studies were carried out in the snails, Filopaludina (Siamopaludina) martensi martensi *(Frauenfeldt). The 96-hour static bioassay was conducted in order to estimate the median lethal concentration (LC_{50}). The snails were exposed to lead nitrate [$Pb(No_3)_2$]. The LC_{50} for 24, 48, 72 and 96 hours were 319.47, 271.03, 235.35 and 191.69 mg/l, respectively. The percentage mortality of these snails increased with increasing concentration and exposure time. In the bioaccumulation experiment, the snails were exposed to 19.17 mg/l (10% 96-hour LC_{50}) of lead nitrate for 42 days exposure time and 30 days recovery time. During the 42-day exposure period, lead uptake occurred in different organs with the greatest uptake in the intestine, and less in the prostate gland, digestive gland, ovary and albumen gland, testis, stomach and cerebral ganglia. After exposure, lead concentration in all organs decreased during the 30 day recovery period.*

INTRODUCTION

Lead is a non-essential and accumulating metal. In vertebrates, up to 90% of the total body burden is bound to the skeleton, especially in areas of active bone formation[1]. In the microcrustacean *Daphnia magna*, about 90% of the total body burden is found in the exoskeleton[2]. In most aquatic arthropods and other invertebrates, lead accumulates in high levels not only in the exoskeleton, but also in the gills and the digestive gland[3-9]. Meyer *et al.*[10] reported that lead accumulated in different organ systems of the freshwater crayfish, *Astacus astacus*. High amounts accumulated in the digestive gland, carapace and gills, whereas the hindgut and musculature exhibited very low lead levels. Bolognani *et al.*[11] found in the freshwater gastropod, *Viviparus viviparus*, that most of the lead was stored in the mantle, while smaller amounts were stored in the digestive gland.

In the land snail, *Helix aspersa*, it was reported that the shell was a site of lead deposition[12]. The shell may be an important part of the snail's metal detoxification mechanism, perhaps helping to buffer the tissues in a similar way to the skeleton in vertebrates. Also in *H. aspersa* from contaminated sites[13],high amounts of lead have been found in the midgut gland. In aquatic mollusks, however, lead seems to be concentrated in the kidney rather than in the midgut gland[14,15]. The mechanisms involved in lead storage by *Helix pomatia* are not known but the metal seems to be associated with particulate structures[16]. Exposure in gastropods results in a high concentration being deposited in the digestive gland and alimentary

tracts[13,17-19]. Ireland[20] demonstrated that accumulation of lead in the tissues of the slug *Arion ater* was greater after acute exposure than after long-term exposure. Most of the lead was deposited in the intestine and least in the foot. Lead in digestive gland granules was high after acute lead treatment, but less than 45% of the lead was associated with the granules. Since, for most pollutants, uptake from water is the most important route, gills are a primary target organ and may constitute one of the first organs to exhibit symptoms of sublethal toxicity. Katalin[21] found that gill tissues of the mussel, *Unio pictorum,* were most effective at accumulation of all heavy metals. Sublethal concentrations of lead caused both oxygen uptake and gill morphological alterations in *Procambarus clarkii*[22,23].

Snails are the second largest group of the animal kingdom after insects, and they are important invertebrates of the world. Invertebrates may serve as helpful indicator organisms, as has already been shown for different artificial and natural microcosms[24], or for macroinvertebrate community structures, because they exhibit a predictable, graded response to heavy metal pollution[25-27]. It has been substantiated that invertebrates tend to concentrate lead more than other living aquatic organisms[10]. In the present study, the acute toxicity and bioaccumulation of lead were studied in the snail, *Filopaludina (Siamopaludina) martensi martensi* (Frauenfeldt) or "Hoi khom", one of the most common viviparíid snails which inhabit ponds and canals where there is considerable organic pollution in Thailand.

MATERIALS AND METHODS

1. Static bioassay experiment

Static bioassays were performed according to the methods of Parrish[28]. The LC_{50} values for lead were determined by using a four-day static bioassay experiment.

F. (S.) m. martensi is a native freshwater snail easily found in natural waters in Thailand. Adult snails with weights ranging between 6.5-7.5 g, a shell height of 2.4-3.5 cm and a shell width of 2.1-2.8 cm, were used in this study. The snails were acclimatized and reared under laboratory conditions for at least two weeks prior to any tests.

The lead solution used in the experiment was prepared by dissolving lead nitrate [$Pb(NO_3)_2$] of 99.5 % purity in distilled water at concentrations of : 10,000, 1,000 and 100 mg/l. Aliquots of a preformulated stock solution were added to each test container approximately 30 min before adding the experimental snails.

The lead concentrations used in the experiments were 50, 100, 150, 200, 250 and 300 mg/l. For each experiment, two liters of the test solution were placed in each container. Ten acclimatized snails were randomly selected and placed in each container. Three replicates were done for each experiment so that 30 snails were tested at each lead concentration. The snails were exposed for 96 h. The number of dead snails was recorded at 1.5, 3, 6, 12, 24, 48, 72 and 96 h. During this 96-h exposure period, the snails were not fed. The behavior and general condition of the snails were observed and dead snails were removed immediately upon being observed. The results were observed according to log time.

2. Analysis of bioassay results

In order to determine the median lethal concentration value (LC_{50}) of lead, the percentage mortality was plotted against doses on log-probability paper with doses on the logarithmic scale and percentage mortality on the probability scale. The median lethal concentration LC_{50}, confidence limits and slopes of probit lines were calculated using the method described by Finney[29].

Toxicity curves

The LC_{50} series in each experiment were used to construct a toxicity curve by plotting exposure times against median lethal concentrations (LC_{50}) in order to determine the threshold or incipient LC_{50} where the lethal threshold concentration was defined as the level of a toxicant which was lethal for 50% of individuals exposed for a sufficiently long period that acute lethal action has ceased[30]. The purpose of the toxicity curve was to give an overall picture of the progress of the test and to indicate when acute lethality had stopped. This curve indicates a lethal threshold concentration when it becomes parallel to the time axis[30].

Mortality of the controls

Control mortality should be virtually absent. It should not be greater than 5%, and should represent an occasional weak organism in a group. Anything more than this was regarded as unsatisfactory and resulted in a test being repeated under suitable conditions. Then, corrections for higher mortality controls was made by Abbottís formula[31].

$$P = \frac{P^* - C}{1-C}$$

Where P = corrected proportions responding to the experimental stimulus.

P^* = observed proportions responding to the experimental stimulus.

C = proportion responding in the control test.

Confidence limits of the LC_{50} values

Confidence limits of the LC_{50} values can be calculated by probit analysis[29]. This formal arithmatic method requires partial lethality at two concentrations.

3. Bioaccumulation of lead in living snails

For the bioaccumulation study, 10% of 96-h LC_{50} of lead was used with 100 female and 100 male snails subjected separately. The test solution in each container was replenished twice a week and aerated at all times. From each group, ten snails were collected randomly on days 4,7,14 and 42 during the exposure time and on days 7 and 30 of the recovery period. These samples were kept in water for 12-24 h before their organs were dissected out (i.e. intestine, stomach, digestive gland, prostate gland, testis, ovary with albumen gland and cerebral ganglia). They were weighed and kept frozen for residual analysis. The control consisted of 10 female and 10 male snails. They were acclimatized for two weeks prior to dissection as previously described.

Residual analysis

The analytical method used was that described by FAO/SIDA[32]. The concentration of lead in the snail samples was determined by simple flame atomic absorption spectrophotometry [Perkin-Elmer Model 3100]. Under standard conditions, the sensitivity was about 3.3 mg/l Pb for 0.001 absorption. A standard containing 10 mg/LPb typically gave an absorbance reading of about 0.003 units.

Construction of a calibration curve

A series of lead concentrations : 0, 5, 10, 20, 40, 60, 80 and 100 mg/l were used to construct a calibration curve of absorbance against the concentration of lead standard solution.

RESULTS

1. Acute toxicity of lead

On the 2nd - 4th days after the snails were put in the lead solution, they produced a very large amount of mucus and they closed their opercula. During the first hour of being exposed to the highest concentration of 300 mg/l, the snails did not move. Later they began to move their feet and lip musculature. After ten hours of exposure, the snails slowed down and gradually stopped eating. At 300 mg/l, the snails started to die within ten hours. The mortality rate increased significantly at 24 hours and then only slightly afterthat. Upon to 100 mg/l of lead solution, the snails started to die after 24 hr. The order of toxicological symptoms was production of a very large amount of mucus along the periphery of the mantle edge, followed by weakening of the adductor muscle, decreased tactile response, silt accumulation and finally dealth.

The percentage mortality, LC_{50} and LC_{80} values, 95% confidence limits, probit lines and slopes for snails exposed to lead at 24, 48, 72 and 96 hours are shown in Table 1. These key data allowed probit lines to be constructed (Figure 1). The toxicity curve relating median lethal concentration to exposure time is given in Figure 2. Figure 3 shows the percentage mortality of *F. (S.) m. martensi* exposed to lead at various concentrations for different times. It was found that lead was not acutely toxic to the snails *F. (S.) m. martensi*, because at the high dose of 300 mg/l of lead, only 50% mortality occurred within 24 hours.

TABLE 1. Acute toxicity of lead to *F. (S.) m. martensi* at different exposure times.

Lead conc (mg/l)	No. of experimental snails	No. and percentage mortality of snails							
		24 h		48 h		72 h		96 h	
		No.	%	No.	%	No.	%	No.	%
0	30	0	0	0	0	0	0	0	0
50	30	0	0	0	0	2	6.7	4	13.3
100	30	1	3.3	2	6.7	5	16.7	7	23.3
150	30	2	6.7	5	16.7	8	26.7	10	33.3
200	30	5	16.7	8	26.7	10	33.3	14	46.7
250	30	9	30	13	43.3	16	53.3	18	60
300	30	15	50	18	60	21	70	23	76.6
LC_{50} : Estimated by		319.47		271.03		235.35		191.69	
LC_{80} Probit analysis		503.64		450.42		496.12		444.41	
LC_{50} : Estimated by		315.00		270.00		235.00		190.00	
LC_{80} graph		518.00		455.00		495.00		385.00	
95% confidence limits :									
LC_{50}		272.12-447.74		334.61-343.33		195.80-308.56		157.41-244.30	
LC_{80}		382.60-989.07		352.61-749.37		360.98-946.44		324.17-831.30	
Slope of probit line		4.26		3.81		2.60		2.30	

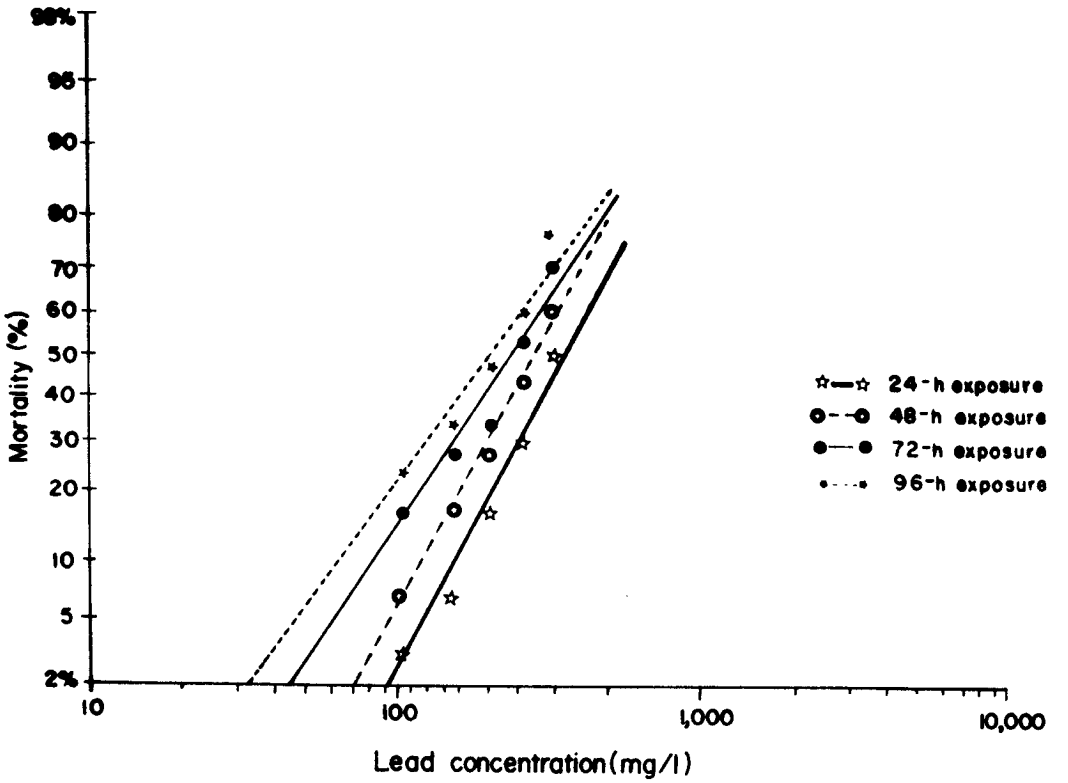

Fig. 1. Percentage mortality of *F.m. martensi* exposed to various concentrations of lead at 24, 48, 72 and 96 hours.

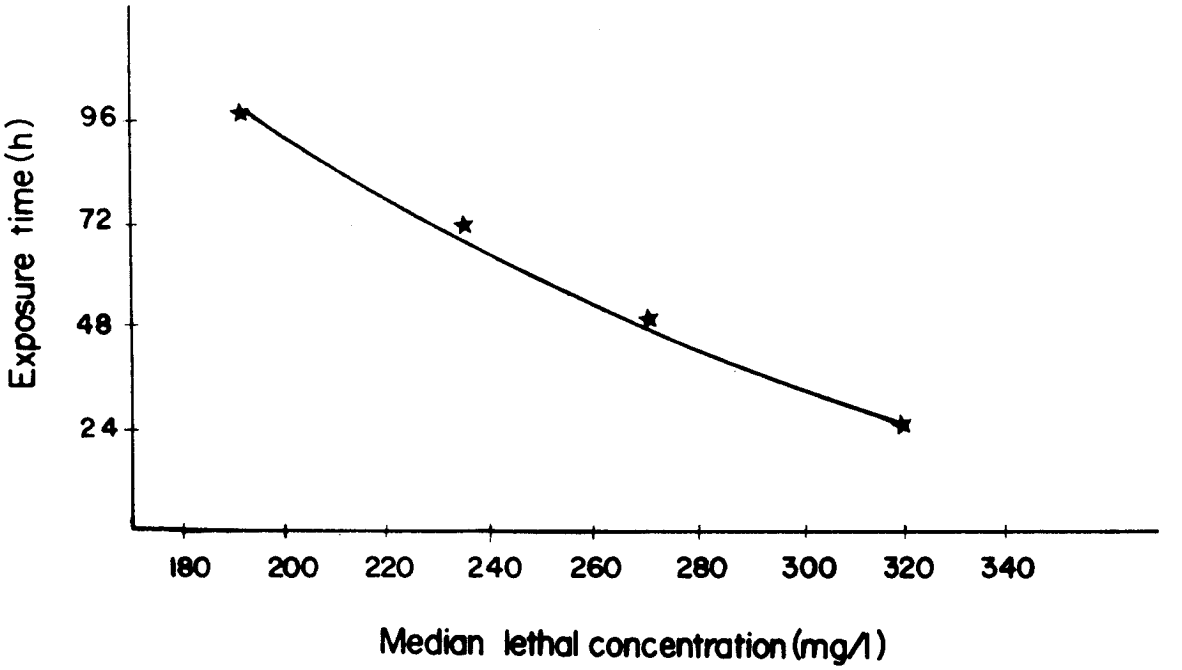

Fig. 2. Toxicity curve of *F.m. martensi* exposed to lead.

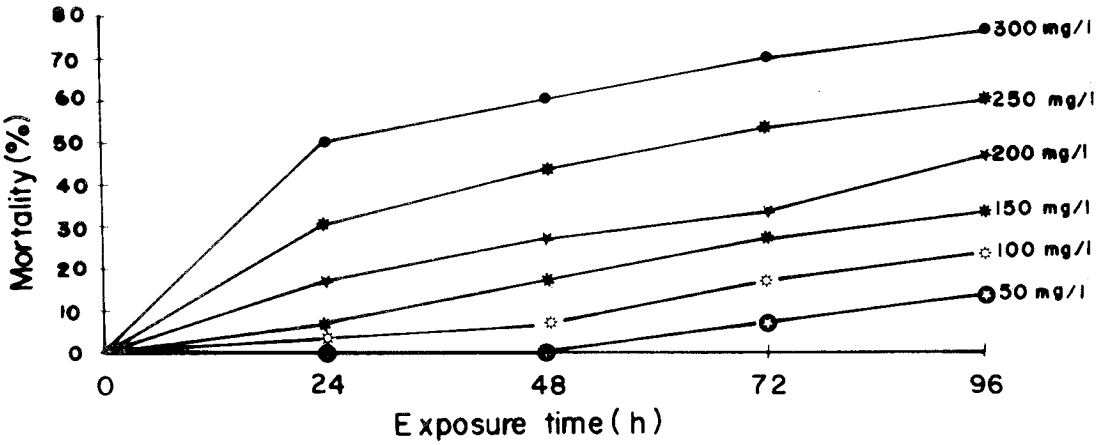

Fig. 3. Percentage mortality of *F. m. martensi* exposed to various concentrations of lead at different exposure times.

2. Bioaccumulation of lead metabolites in the experimental snails

The snails were exposed in the water contaminated with 19.17 mg/l (10% 96-h LC50) of lead. Lead accumulation in seven organs of the control and experimental snails at various time intervals are shown in Table 2. In the control snails little lead was found in the intestine (24.21 ppm), stomach (17.44 ppm), digestive gland (6.51 ppm), prostate gland (14.35 ppm), testis (2.72 ppm), ovary with albumen gland (13.43 ppm) and cerebral ganglia (26.86 ppm) (Table 2). In the experimental snails, the intestine accumulated lead rapidly at the levels of 203.36, 357.16, 515.61 and 1125.94 ppm, in 4,7,14 and 42 days, respectively, followed by prostate gland, digestive gland, ovary with albumen gland, testis, stomach and cerebral ganglia. It appears that most organs of the snails have a high affinity for lead accumulation. The main accumulation of lead took place in the intestine. By 42 days (6 weeks), lead concentration in the intestine was 60 times higher than that in the surrounding water (Table 2). The most rapid rate of lead uptake was 26.81 mg/g/d (Table 3). During the recovery period, a decrease in the lead concentration was observed. The intestine could excrete lead rapidly, followed by the digestive gland, prostate gland, testis, stomach, ovary and albumen gland, and cerebral ganglia. The most rapid rate of loss was 19.7/mg/g/d (Table 3).

TABLE 2. Lead concentration of individual organs at exposure times of 4, 7, 14 and 42 days and at recovery times of 7 and 30 days.

Organ	Lead conc. in control snails (ppm)	Lead conc. in experimental snails (ppm)					
		Exposure time				Rocovery time	
		Day 4	Day 7	Day 14	Day 42	Day 7	Day 30
Intestine	24.21	203.36	357.16	515.61	1125.94	800.00	591.44
Stomach	17.44	88.36	216.38	210.90	269.74	176.65	150.00
Digestive gland	6.51	187.49	191.83	376.57	498.44	432.87	290.64
Prostate gland	14.35	44.48	106.13	210.29	548.52	396.03	258.89
Testis	2.72	20.53	33.74	132.89	365.18	237.25	160.22
Ovary with albumen gland	13.43	125.44	168.63	192.54	478.65	305.35	136.29
Cerebral ganglia	26.86	42.58	96.99	114.18	234.19	113.78	49.06

TABLE 3. Rate of lead uptake and rate of lead loss by different organs.

Organ	Rate of uptake (mg/g/d)	Rate of loss (mg/g/d)
Intestine	26.81	19.71
Stomach	6.42	5.00
Digestive gland	11.87	9.69
Prostate gland	13.06	8.63
Testis	8.69	5.34
Ovary with albumen gland	11.40	4.54
Cerebral ganglia	5.58	1.63

DISCUSSION

Information concerning the acute toxicity of lead had been reported for many species of mollusks[11, 20, 33]. However, the LC_{50} values of *F. (S.) m. martensi* could not be compared with those of other species since various factors influencing bioassays such as temperature, D.O., pH, water hardness, the variability in bioassay techniques (static or continuous flow) were not provided[34-36]. *F.(S.) m. martensi* is relatively insensitive to lead when its LC_{50} value is compared to those of other organisms. These snails have the opercula to protect themselves when surrounding water becomes hazardous to them. This might be the cause of the high LC_{50} value. The insensitivity might also be attributed to the high turbidity from lead precipitation in the test water during the experiments. Although the effects of suspended solids on metal toxicity have not been studied extensively, Brown *et al.*[37] observed a direct relationship between concentrations of suspended organic solids and reduction in copper toxicity on rainbow trout, *Salmo gairdneri.*

From the bioaccumulation study, the lead residues in each organ of *F. (S.) m. martensi* increased as the exposure time increased. Among the organs tested the intestine displayed by far the highest rate of accumulation (more than 60 times the lead compared to the other organs). This result was similar to the study of Ireland[20] who studied the effect of chronic and acute lead treatment in the slug *A. ater.* He reported that most of the metal was deposited in the intestine and the least in the foot. In aquatic mollusks, lead seems to be concentrated in the kidney rather than in the midgut gland[14,15]. In the freshwater gastropod, *V. viviparus,* most lead was stored in the mantle, and smaller amounts in the digestive gland[12]. The exposure of gastropods to lead resulted in a high concentration being deposited in the digestive gland and alimentary tract[13,17-19].

An increase in lead accumulation in all organs studied in *F.(S.) m.martensi* was observed. Numerous mechanisms had been proposed for the accumulation of lead in the snails[38]. The following mechanisms of heavy metal accumulation may be summarized: (a) adsorption of ions at the membrane water interface, (b) absorption by active and/or passive diffusion of metal ions from the water across semi-permeable membranes into body fluids, and subsequent distribution to other organs, and (c) ingestion of ions with food or in combination with particulate matter or mucus and absorption through the gut wall[38]. According to the ingestion mechanism, lead would be taken up by the stomach, intestine and digestive gland. Uptake to

the other organs such as the testis and ovary would presumably take place by absorption mechanism. Uptake of lead into the body via permeable surfaces might occur all over the body, particularly in small and/or soft body invertebrates, mostly at sites of high permeability. Uptake from solution would also take place in the alimentary tract when any of the medium was swallowed during "drinking" or food ingestion[39]. Uptake of heavy metals from solution can take place in unexpected ways. Depledge and Phillips[40] showed that in large marine gastropods, *Hemifusius tuba*, seawater was taken into the foot as the animal expanded after retraction into the shell, and this seawater mixed freely with the blood. They pointed out its important implication as a route of heavy metal entry. It was observed that *F. (S.) m. martensi* could rapidly clear a portion of accumulated lead from their tissues when exposed to a relatively lead-free environment. Similar results have been reported for mollusks exposed to arsenic, mercury and lead[41-43]. Simpson[42] found that the mussel, *Mytilus edulis* transferred from a lead-contaminated site to a clear site rapidly lost lead in the first 30 days, but thereafter, concentrations remained at a level higher than those of mussels native to the site. Newman and McIntosh[44] found the change in lead concentration in soft tissues of the snail, *Physa integra* (from 32.2 ug Pb/g dry wt. to 12.4 ug Pb/g dry wt.) during the first four days of clearance. Thereafter, lead concentration in this species remained relatively constant. Williamson[19] reported that lead seemed to be excreted rather rapidly by the land snail, *Cepaea hortensis*. Rapid loss of lead has also been observed in several marine mollusks, such as *M. edulis*[25], *Crassostrea gigas*[45] and *Saccostrea echinata*[46].

Here, the intestine also displayed a relatively high rate of loss in the recovery period. The results elsewhere indicated that the rates of uptake were dependent on the concentration in the medium and the rates of loss were dependent on the internal lead concentration[20]. Pringle *et al.*[47] suggested that metal uptake by mussels, *Lamelli branchiata*, was proportional to the external concentration. It was to be expected, however, that the chemical form of lead in the medium influenced the rate of uptake. Comparisons are limited by virtue of the different experimental conditions employed.

Bryan[48] has proposed three mechanisms for the loss of metals from snail bodies : (a) excretion via the body surface or gills, (b) excretion via the gut, and (c) excretion via the urine. Metals may be excreted through permeable surfaces including the gills. The crabs, *Carcinus maenas* and *Cancer magister*, and the littoral prawn, *Palaemon elegans*, excreted zinc across the gills[49,50], whereas zinc loss in the urine from the antennary glands was more important for the lobster *Homrus gammarus*[49]. The cells lining the digestive tract of invertebrates may release metal-rich granules into the gut lumen as occur in the bivalve *Cerastodema edule*[51]. The cells of the bivalve kidneys also have the ability to excrete metal-rich granules[52]. The major excretory route of zinc from the mussel, *M. edulis* was in granular form from the kidney into the urine and they could accumulate metal-rich granules with the potential for excretion[53]. Lead was lost from all organs in *F. (S.) m. martensi*. The data did not indicate which mechanism (gill or body surface excretion, alimentary tract excretion and urinary excretion) was operative. It is possible that all three routes made a contribution.

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บทคัดย่อ

การศึกษาพิษการสะสมและผลกระทบของสารตะกั่วต่อเนื้อเยื่อในระบบต่าง ๆ ของหอยขม *Filopaludina (Siamopaludina) martensi martensi* (Frauenfeldt) ระบบที่ใช้ในการทดลองเป็นแบบ static bioassay ใช้เวลา 96 ชั่วโมง เพื่อหาค่าความเข้มข้นของสารตะกั่วที่ทำให้หอยขมตายเป็นจำนวน 50% ที่เวลา 24, 48, 72 และ 96 ชั่วโมง ซึ่งมีค่าเท่ากับ 319.47, 271.03, 235.35 และ 191.69 มิลลิกรัมต่อลิตรตามลำดับ เปอร์เซนต์การตายของหอยขมมีค่าเพิ่มขึ้นตามค่าความเข้มข้นของสารตะกั่วและเวลาที่เพิ่มขึ้น ส่วนการศึกษาเรื่องการสะสมสารตะกั่วในเนื้อเยื่อหอยขมนั้น การทดลองแบ่งเป็นสองกลุ่ม คือ กลุ่มเพศผู้ และกลุ่มเพศเมีย กลุ่มละ 100 ตัว หอยขมทั้งสองกลุ่มนี้ได้รับสารตะกั่วขนาด 19.17 มิลลิกรัมต่อลิตร (10% ของค่า 96-hour LC_{50}) เป็นเวลาต่อเนื่อง 42 วัน และพักต่ออีก 30 วันโดยไม่ได้รับสารตะกั่ว ทำการเก็บตัวอย่างหอยกลุ่มละ 10 ตัวที่เวลา 1, 7, 14, 42 วันในช่วงที่ได้รับสารตะกั่ว และ 7, 30 วัน ในช่วงไม่ได้รับสารตะกั่ว เพื่อหาปริมาณสารตะกั่วที่สะสมในอวัยวะต่าง ๆ ของหอยขม จากการทดลองพบว่าทุกอวัยวะมีการสะสมสารตะกั่วในเนื้อเยื่อเพิ่มขึ้นตามระยะ ที่เพิ่มขึ้นโดยที่ลำไส้มีสารตะกั่วสะสมมากที่สุด รองลงมาคือ ต่อมโพรสเทต ตับ รังไข่ และต่อมแอลบิวมิน เทสทิส กระเพาะอาหาร และปมประสาทเซรีบรัล และในช่วง 30 วันที่ปราศจากสารตะกั่ว พบว่าปริมาณสารตะกั่วที่สะสมมีค่าลดลงในทุกอวัยวะ