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Comparative toxicity of mercury and cadmium to the juvenile freshwater snail, *Filopaludina martensi martensi*

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ABSTRACT: The toxicity bioassay of the juvenile freshwater snail, *Filopaludina (Siamopaludina) martensi martensi* exposed to mercury and cadmium was studied. The median lethal concentrations of mercury were lower than those of cadmium after 24, 48, 72, and 96 h of exposure. In metal bioaccumulation experiments, the mean residual concentrations of mercury and cadmium in the treated snail and the treatment water were assessed. However, more cadmium was found accumulated in its tissues.

KEYWORDS: LC_{50}, water pollution, probit analysis

INTRODUCTION

The freshwater snail, *Filopaludina (Siamopaludina) martensi martensi* is common in freshwater canals and ponds in Thailand. It is also a favourite food for Thais. In a freshwater ecosystem, *F. martensi* may be used as bioindicator of heavy metal contaminants in freshwater resources for several reasons. First, it lives in static water, and is therefore easy to use as an experimental animal. Second, it is a filter feeder and accumulates pollutants including heavy metals in its tissues. Third, it is abundant and plays important roles in the environment.

Mercury is one of the most toxic metals. It causes protein denaturation in the foot, gill, and digestive tract of freshwater mussels and snails[1,2]. Mercury also inhibits both alkaline phosphatase and acid phosphatase activities in *Macrobrachium rosenbergii*, while Cd inhibits only acid phosphatase[3]. Cadmium has been shown to be harmful to adult *Physa gyring*[4] and also adversely affects the development of the veliger of this species[5] and of *Lymnea stagnalis*[6,7]. The land snail *Helix pomatia*, when exposed to Cd, Cu, Zn, Cr, or Pb at low concentrations for 96 h, removed toxic metals continually by excretion and filtration[8]. In contrast, heavy metal residues in the shell and tissue accumulated more at lower concentration. In *Taphius glabratus*, accumulated Zn, Cd, and Cu was found in the protoplasmic tissue[9]. Consequently, heavy metal damaged cellular ionic exchange and biochemical reactions.

Since many rivers, canals, and ponds in Thailand are polluted with heavy metals, it is of interest to carry out assays to see whether *F. martensi* is a good bioindicator for them. Hence, comparative toxicity experiments for Hg and Cd together with metal bioaccumulation assays were conducted on juvenile *F. martensi*.

MATERIALS AND METHODS

The static bioassays were conducted according to described methods[10–13] including that of the American Public Health Association[14]. The median lethal concentrations (LC_{50} values) for *F. martensi* were determined by using 24-, 48-, 72-, and 96-h static bioassay experiments. Juvenile *F. martensi* were collected in plastic tanks from water inlets in Kasetsart University, Bangkok. To avoid experimental bias[15], the snails selected were of similar size, of the same age, and reared under the same conditions. The snails weight was 1–4 g and they had shells 0.7–1.0 cm in height and 0.5–0.7 cm in width. They were acclimatized to laboratory conditions (22–29 °C, DO 3–5 mg/l, pH 7–9) and were fed for at least 7 days before being used in the experiment[11]. They were neither fed nor aerated during the bioassays for 2 days before the start of the experiment[14].

The metals used in the toxicity experiments were mercuric acetate and cadmium nitrate, with respective purities of 98% and 99% (Fluka). Stock solutions (4.000 g/l) of the two metals were prepared using distilled water. The actual concentrations of metals were verified using a flame atomic absorption spectropho-

tometer (FAAS, GBC Avanta Ver 1.33). Aliquots of the stock solutions were added to each test container approximately 30 min before adding the snails. These experiments aimed at determining the LC_{50} values for juvenile freshwater snails when exposed to each metal at 24-, 48-, 72-, and 96-h periods. The acceptable control mortality was 5% representing an occasional weak organism in the group. If the control mortality exceeded 5% the test was then repeated under suitable conditions. The corrected mortality was calculated by Abbott's formula[15],

$$P = (P^* - C)/(1 - C)$$

where P and P^* are the corrected and observed proportions responding to the experimental stimulus, respectively. C is the proportion responding in the control test.

The regression equations of probit mortality (y) against x, the logarithm of the metal concentration, for juvenile snails exposed to metals for 24, 48, 72, and 96 h were estimated by probit analysis[15].

The behaviour, morphology, and general condition of treated snails were observed. The bioaccumulation capacity of Hg and Cd in *F. martensi* was analysed using the method described by FAO/SIDA[16]. First, the snail samples were prepared using the wet-ash or acid-digestion method. Then, the concentrations of Hg residue in the snail samples were determined using a hydride atomic absorption spectrophotometer (Perkin Elmer model FIAS 200). For the standard condition, the sensitivity is about 10 µg/l of Hg for absorbance. The concentrations of Cd in the snail samples were determined by using the FAAS. For the standard condition, the sensitivity was about 0.009 mg/ml of Cd for absorbance. A series of standard solutions of 3.0, 5.0, 10.0, 30.0, and 50.0 µg/l for Hg, and 0.1, 0.3, 0.5, 1.0, and 1.5 µg/l for Cd were used in order to cover the working range of the FAAS. The atomic absorbance of each concentration of the standard solution was recorded from the FAAS. The data obtained were used to construct a calibration curve of absorbance against the concentrations of the Hg and Cd standard solutions. For Cd determination, the FAAS was operated by installing a Cd hollow cathode lamp (lamp current 3.0 mA, 228.8 nm wavelength, 0.5 mm slit width, oxidizing air-acetylene flame). For the standard condition, the optimum range was 0.2–1.8 µg/ml and the typical sensitivity was 0.009 µg/ml. For Hg determination, the FAAS was operated by installing an electrodeless discharge lamp (current 8 mA, 253.7 nm wavelength, 0.70 mm slit width, 10 µg/l). For the standard condition, the standard curve was 3.50 µg/l and the typical sensitivity was 10 µg/l.

The experiments were conducted at the wet laboratory, Department of Zoology, Kasetsart University. The heavy metal residue analysis was done in cooperation with Thai Engineering Material Analysis Co., Ltd., Bangkok.

RESULTS

During the acclimation period, the snail mortality did not exceed 10%, and the control group showed no mortality during the test period. The LC_{50} results show that Hg was more toxic to *F. martensi* than Cd for all the exposure times tested (Table 1). The slope functions (b) indicate variations of the snail population responses. When exposed for 24 h the snails showed more variation in response to Cd (11.5) than to Hg (4.05). For longer exposure times, the variations in responses to the two metals were more similar (Table 1).

The behavioural and morphological changes in the juvenile snails subjected to different concentrations of Hg and Cd after 24-, 48-, 72- and 96-h were also observed. When they were exposed to low

Table 1 Regression equations of probit mortality (y) against x, the logarithm of the metal concentration, and LC_{50} values for *Filopaludina martensi* exposed to Hg and Cd.

Metal	Period of exposure (h)	Probit regression equation ($y = a + b(x - m)$)	LC_{50} (95% confidence limits) (mg/l)	Goodness of fit		
				df	χ^2	Probability
Hg	24	$y = 4.85 + 4.05(x - 10.94)$	9.67 (8.75,11.15)	5	0.94	0.0328
	48	$y = 4.88 + 1.48(x - 10.07)$	1.43 (1.06,2.26)	4	2.59	0.3722
	72	$y = 5.20 + 2.27(x - 10.04)$	0.89 (0.77,1.00)	4	6.19	0.8149
	96	$y = 5.59 + 3.42(x - 9.96)$	0.61 (0.49,0.70)	4	9.79	0.9558
Cd	24	$y = 4.86 + 11.50(x - 11.43)$	27.76 (25.87,33.67)	5	0.67	0.0156
	48	$y = 5.02 + 1.64(x - 10.71)$	5.01 (3.08,7.20)	4	0.31	0.0110
	72	$y = 4.95 + 2.04(x - 10.57)$	3.96 (3.34,4.81)	3	0.14	0.1487
	96	$y = 5.28 + 2.82(x - 10.46)$	2.33 (2.02,2.63)	4	2.13	0.2885

Table 2 Heavy metal concentrations (mg/kg dry weight) in *F. martensi* and water, after 96-h exposure ($n = 30$).

Heavy metal Concentrations (mg/l)	Hg (mg/kg bodywt.)	Cd (mg/kg bodywt.)
1.0	40.83	59.10
2.0	43.74	73.86
3.0	42.28	82.04
4.0	36.52	123.64

Table 3 Quality of water used in the experiments compared with standard water.

Parameter	Expt water	Standard water
Dissolved oxygen (mg/l)	3–5	>3–7
pH	7–9	5–9
Temperature (°C)	22–29	21–32

concentrations of heavy metals, the snail moved up and down, extended their bodies out of the shells, moved around the plastic jars, and secreted mucus. Snails in intermediate concentrations, increased mucus secretion, and moved slowly. They were inactive, unable to attach their feet or closed their opercula, and were incapable of retracting into their shells. At the highest metal concentrations, they produced large amounts of mucus and the bodies were extended from the shell but were unable to attach their feet. Finally, the snail sank down to the bottom, became motionless, and eventually died. Differences in behaviour of the snails towards the two metals were not apparent.

The concentrations of Hg and Cd found in the snail tissues and water after 96 h of exposure are shown in Table 2. The mean concentrations of Hg and Cd in the experimental water after 96 hr were 0.045 ± 0.0005 mg/l and 0.121 ± 0.0018 mg/l, respectively. The relative amounts of metal residues detected in snail tissue showed that Cd was higher than Hg (Table 2). The quality of the water used in the experiments is given in Table 3.

DISCUSSION

Mercury is more toxic to the clam *Donax faba* than Cd, Cu, or Zn[17]. The 96-h toxicity of Hg to the clam was 0.16 ppm[17]. The 24-h toxicity of Hg to *Donax faba* was found to be 1.8 ppm[18]. We also found that Hg is more toxic than Cd to *F. martensi*. In the freshwater snail *Physa acuta*, the Cd toxicity after 24-h and 48-h was 1.32 and 1.05 ppm, respectively[5]. The corresponding levels were 27.76 and 5.01 ppm, respectively, for *F. purpuratus*. The LC_{50} values of Hg and Cd after 48-h exposure to *Perna viridis* were 0.9 ppm and 6.24 ppm, respectively[19]. From our result, the 96-h LC_{50} of Hg for the freshwater snail, *F. martensi* was 0.61 ppm. This value showed a greater toxicity than that reported previously. Distinct results obtained in research reports may be caused by different biological factors such as snail health and physical factors such as temperature and water quality, owing to different habitats.

In our study, when *F. martensi* was exposed to high concentrations of heavy metals it secreted white slime. From our experiments, and those performed on the snails *Biomphalaria glabrata*[20] and *Taphius glabratus*[18], after 24 h of exposure the snails were more resistant to heavy metals as indicated by their behaviour and physiological responses. The snails sank to the bottom, closed their operculum tightly, exhaled bubbles and reduced their metabolic activities. In the presence of Cd and Cu at concentrations of 0.05–0.1 ppm, the snails were unable to attach their feet to the container surface[18]. Heavy metal deteriorates cell dynamics and damages cell membranes and tissues[9]. Furthermore, the exchange of intercellular and intracellular substances and fluid is perturbed. Consequently, there is more diffusion of heavy metals into cells causing cell necrosis. From our experiments, all juvenile snails exposed to 25–35 ppm of Cd, or 8–12 ppm of Hg died within 24 h, and the snails would have experienced the same processes of cell injuries.

Freshwater snail *Thiara tuberculata* exposed to $CuSO_4$ and $HgCl_2$ decrease their oxygen consumption[21] and their absorption of heavy metal from medium drops. Tantulvesn and Pornprapa[22] found that 0.05–0.100 ppm of Cd causes the freshwater snail *Taphius glabratus* to be immobile and to lower food and air consumption within 24 h exposure[22]. Correspondingly, our tests in which the snails were exposed to high heavy metal concentration, expressed little lower dissolved oxygen concentration, because the snail metabolism almost ceased. In the presence of lower metal concentrations and in the control, the level of dissolved oxygen was even lower since the snail still had their normal metabolic activities. Nevertheless, dissolved oxygen in every test was in the normal safe range for the snail.

We found that the snail accumulated more Cd than Hg. In addition, the accumulation will be different for each individual, species, season, and temperature. Heavy metals accumulated in the freshwater snail *Andara granosa* were highest in summer[23]. During the rainy season, less heavy metals were accumulated in animals because of better excretion and metal dilution in the environment[23]. In our experiments, the temperature range was 23–25 °C, the tested animals were

in static water, and the test times were short. Hence, temperature and test conditions had no effect on the juvenile freshwater snail uptake and accumulation of heavy metals. It can be concluded that the levels of heavy metal accumulation is high in the lower acute toxicity test. Less of the highly toxic metal Hg was accumulated in tissues than Cd because the animals died more quickly when exposed to Hg.

In conclusion, our results indicate that the chosen snail species may be used as a bioindicator for acute and subacute exposures to Hg and Cd.

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