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Histopathological Effects of Contaminated Sediments on Golden Apple Snail (*Pomacea canaliculata*, Lamarck 1822)

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Abstract *Pomacea canaliculata* were exposed experimentally to contaminated sediments from a tributary of the Mae Klong River, Thailand, for 3 months. The highest concentration of Cr, Zn and Fe accumulated in the digestive gland while the gill exhibited the highest concentration of Cu. In addition, histopathological changes (increased mucus vacuoles, loss of cilia, dilation of cells in the epithelial cells of digestive tract organs, and an increase in the number of dark granules in the digestive cells) were observed. The gill exhibited loss of cilia, wider hemolymph space, and degeneration of columnar epithelial cells.

Keywords *Pomacea canaliculata* · Histopathology · Contaminated sediments · Heavy metals

The pollution of aquatic ecosystems, by heavy metals and organic waste from domestic sewage, industrial and agricultural effluents, is an important environmental problem accompanying unregulated industrial and technological development, urbanization and agricultural intensification (Zhou et al. 1998). Polluted sediments, when deposited on land, also pose potential threats to terrestrial ecosystems. The Mae Klong River, Samut Songkhram Province, which flows into the Gulf of Thailand, south-west of Bangkok, is one of the largest and most important rivers in the country. The river and its tributaries are used both for irrigation and to provide water for aquaculture and industry. Several industries located near tributaries discharge effluents into the river, while effluents from agriculture, aquaculture and domestic use also affect water quality (Peebua et al. 2006). Elevated concentrations of heavy metals (Pb and Cr) have been detected in sediments in the Mae Klong River tributaries (Peebua et al. 2006).

Mollusks are known to bioconcentrate heavy metals in their soft tissues and shells (Jara-Marini et al. 2009, Li et al. 2009, Tapia et al. 2010). This, combined with their wide geographical distribution, high abundance in the benthic environment, selective absorption of certain ions, and sedentary nature makes them useful biomonitors of environmental pollution (Blackmore and Wang 2003). This study investigated the accumulation of heavy metals and associated histopathological manifestations in the soft tissues of the golden apple snail, *Pomacea canaliculata,* in the Mae Klong River and its tributaries. Although introduced to the country relatively recently, *P. canaliculata* is now the most widespread snail species in Thailand.

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Materials and Methods

Sediment was collected from a contaminated site along the Rang Tub Taeb tributary of the Mae Klong River (13°25′15.31″N, 99°59′53.15″E). Sediments were oven dried at 80°C for 3 days, then crushed into fine powder, sieved and weighed. Five grams of sediment was digested with 10 mL of HNO_3, filtered and diluted with Milli-Q water to 50 mL. Sediments were analyzed for heavy metal content using an inductively coupled plasma mass spectrometer (ICP-MS; Perkin Elmer SCIEX model ELAN 6000) for Cd, Cr, Cu, Pb, Ni and Zn concentrations and an inductively coupled plasma optical emission spectrometer (ICP-OES; Spectro Ciros CCB detector) for Fe concentration.

In order to test the effects of sediment exposure on snails, 1 kg of fine-powdered sediment was placed in glass bowls (diameter 24.5 cm, height 18 cm), 3L of declorinated tap water added, and left for at least 24 h. *P. canaliculata* collected from an uncontaminated site, reared and acclimatized for 3 months in tap water, were transferred to the bowls containing the sediments (20 snails/bowl, 3 replicates). The control group consisted of snails reared in bowls with only tap water. A non-renewal static system was utilized with constant volume of water and no aeration. Snails were fed once daily with commercial fish food. Water quality parameters were monitored weekly: temperature (25 ± 2°C), pH (6.8–7.3), and dissolved O_2 (7.5–7.8 mg L^{-1}). After 3 months exposure, ten and five snails from each replicate were sampled from each treatment group for heavy metal content and for histological analysis of lesions, respectively.

In order to determine metal accumulation in specific organs, soft tissues were separated from shells, and snail organs (digestive gland, digestive tract, and gill) were isolated. Tissues were dried at 80°C to a constant weight, then crushed and 0.5 g digested in HNO_3 (APHA 1998). Metal content was determined using a graphite atomic absorption spectrometer (GAAS; SpectrAA-50) for Cd, Cr, Pb and Ni concentrations, and a flame atomic absorption spectrophotometer (FAAS; SpectrAA-220FS) for Cu, Fe and Zn concentrations. For GAAS measurement, the snail sample solution was directly aspirated and atomized in a graphite furnace. For FAAS measurement, the snail sample solution was aspirated and atomized in flame.

For histopathological study, snail organs were dissected out and fixed in Bouin's solution for 24 h, washed with 70% ethanol, dehydrated through a graded series of ethanols, cleared in xylene for 6 h, infiltrated and embedded in paraplast. Tissues were sectioned on a rotary microtome at 5 μm thickness, stained with hematoxylin-eosin, and examined under a light microscope (Olympus CH40).

All data were presented as mean ± standard deviation (μg g^{-1} dry weight). Variations in heavy metal concentrations in golden apple snail and sediment were tested by one-way analysis of variance (ANOVA) and least significance difference (LSD) analysis. The level of significance was set at 0.05.

Results and Discussion

Sediments act as a reservoir for all contaminants and dead organic matter in the ecosystem (Hamed and Emara 2006).

Table 1 Heavy metal concentrations in sediment and organs of sediment-exposed golden apple snails

Metal	Sediment (μg g^{-1})	Probable-effects level[b] (μg g^{-1})	Organ	Metal concentration in snail (μg g^{-1})	
				Sediment-exposed snail	Control snail
Pb	7.7 ± 1.7	112	Digestive gland	0.09 ± 0.08	0
			Digestive tract	0.15 ± 0.15	0
			Gill	0.01 ± 0.03	0
Cr	5.5 ± 1.6	160	Digestive gland	1.15 ± 0.95[a]	0
			Digestive tract	0.00 ± 0.12	0
			Gill	0.00 ± 0.02	0
Ni	4.9 ± 1.2	42.8	Digestive gland	0.08 ± 0.04	0
			Digestive tract	0.08 ± 0.04	0
			Gill	0.18 ± 0.30	0
Cd	0.04 ± 0.01	4.12	Digestive gland	0.01 ± 0.02	0
			Digestive tract	0.00 ± 0.01	0
			Gill	0.01 ± 0.00	0
Zn	42.8 ± 9.9	271	Digestive gland	1,657.0 ± 458.3[a]	0
			Digestive tract	14.6 ± 12.2	0
			Gill	0.0 ± 4.8	0
Fe	5,049.3 ± 1,813.6	–	Digestive gland	2,439.1 ± 253.5[a]	0
			Digestive tract	1,101.3 ± 532.4	0
			Gill	344.9 ± 49.9	0
Cu	5.1 ± 1.4	108	Digestive gland	44.9 ± 17.9	0
			Digestive tract	70.9 ± 26.7	0
			Gill	94.3 ± 15.8	0

All data are presented as mean ± S.D

[a] Indicates the significant difference in the metal concentrations in the three organs using LSD test ($p < 0.05$)

[b] According to US EPA (1997)

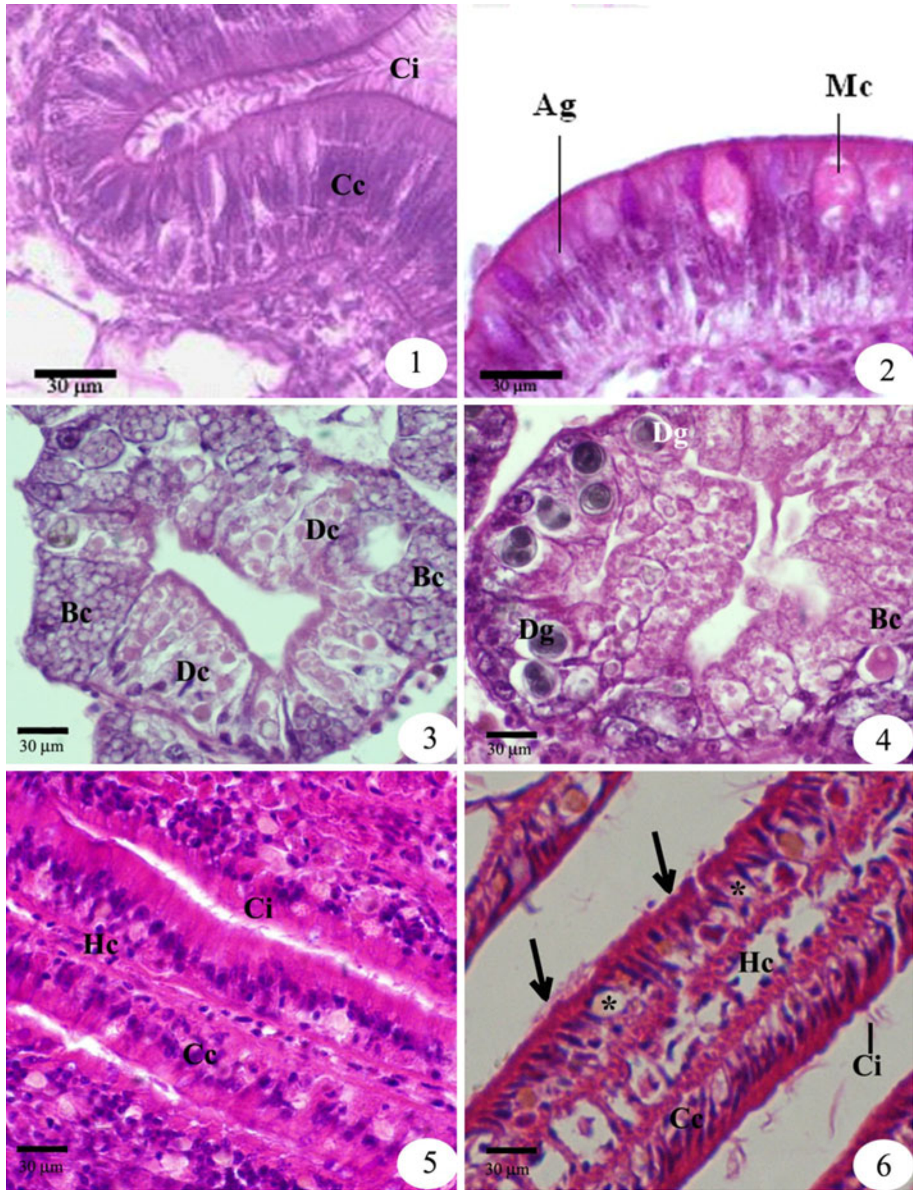

Fig. 1–6 **1** Digestive tract of control snail showing pseudostratified columnar epithelium (*Cc*) with long cilia (*Ci*) and basal nuclei. **2** Digestive tract of treated snail showing increase of mucus cells (*Mc*) and loss of cilia. *Ag* acidophilic granule. **3** Digestive gland tubule of control snail consists of digestive cells (*Dc*) and basophil cells (Bc). **4** Digestive gland tubule of treated snail showing numerous dark granules (*Dg*) in digestive cells. Most cells are dilated, and tubule lumen becomes smaller. *Bc* basophil cell. **5** Gill of control snail showing gill filaments with ciliated columnar cells (*Cc*) with cilia (*Ci*). Hemocytes (*Hc*) are present in hemolymph space.**6** Gill of treated snail showing loss of cilia (*arrows*) and numerous intercellular spaces (*) among columnar cells (*Cc*). Notice the wider hemolymph space and hemocytes (*Hc*). *Ci*, remnant of cilia

The heavy metal concentrations in sediments found during this study were ranked in the following order: Fe > Zn > Pb > Cr > Cu > Ni > Cd (Table 1). The highest concentrations of these metals found in the Mae Klong sediments did not exceed the probable-effects levels of the (US EPA 1997) (Table 1). There was no mortality of *P. canaliculata* after 3 months exposure. Tissue-specific metal accumulation showed that Zn, Cr and Fe were stored mainly in the digestive gland. There was no significant difference in the Cu accumulation among the different organs (digestive tract, digestive gland and gill; $p > 0.05$) although the gill showed the highest concentration (Table 1). Zn, Fe and Cu are essential for metabolism of mollusks while Cr is a non-essential element. Zn is known as an enzyme cofactor while Fe is the component of ferritin, the main Fe storage protein in cells (Goralska et al. 2000). Cu is an enzyme cofactor and essential component of the molluscan pigment, hemocyanin (Méndez et al. 2001). In gastropods and bivalves, the digestive gland is the major site of heavy metal storage and detoxification (Simkiss and Mason 1984). In addition, previous studies also reported the gill as a storage organ capable of the detoxification and bioconcentration of heavy metals in bivalves (Kraak et al. 1993).

Levels of some heavy metals were much greater in snails than in sediments (i.e., Zn increased 39 fold, while Cu increased 19 fold). Similar results were reported for freshwater and marine mollusks collected from contaminated water (Gundacker 2000; Madkour 2005; Hamed and Emara 2006). The solubility of heavy metals in the aquatic environment generally increases in the following order: Pb < Cd < Cr < Ni < Cu < Zn (Foster and Charlesworth 1996). Hence, Zn and Cu were clearly more available for snails than other metals. Pb probably had limited availability since it was strongly bound to humic acids in the sediment (Campbell and Evans 1987).

The digestive tract of control snails was lined by ciliated columnar epithelium, consisting of columnar cells and mucus-secreting goblet cells (Fig. 1). The ciliated columnar cells were long and slender in shape with a basal oval nucleus and acidophilic granules in the cytoplasm. In the sediment-exposed snails, the mucus vacuoles increased in number and size and loss of cilia was also observed (Fig. 2). The digestive route is most important for contamination by heavy metals (Viard et al. 2004), and the increase in mucus cells and mucus vacuoles implies that the digestive tract needs more mucus to dilute the toxins and may also contribute to the capacity for detoxification (Triebskorn 1989). The loss of cilia may be caused by the interaction of heavy metals with the cell membrane, which induces changes in composition, fluidity and stability of the membrane (Axiak et al. 1988). These results are similar to those reported by Riba et al. (2005), Supanopas et al. (2005) and Tanhan et al. (2005).

The digestive gland consisted of numerous blind-ending tubules that were composed of digestive and basophil cells. The most common digestive cells were columnar type with numerous vacuoles containing faintly stained inclusions. The basophil cells occurred singly or in groups and contained basophilic granules of various sizes (Fig. 3). They were secretory in nature while the digestive cells were involved in absorption and intracellular digestion (AbdAllah and Moustafa 2002). After sediment exposure, the digestive gland showed an increase of dark granules of variable size and dilation of digestive cells (Fig. 4). Similar dark granules were reported in the digestive gland of the slug *Derosceras reticulatum* (Luchtel et al. 1997), in the bivalve *Mytilus galloprovincialis* (Domouhsidou and Dimitriadis 2000), and the prosobranch snail *Littorina littorea* (Mason et al. 1984). These dark granules represented lysosomal residual bodies, suggesting the accumulation of toxicants such as heavy metals or molluscicides (Hamed et al. 2007).

The gill of the control snails consisted of a longitudinal central axis from which numerous slender, pointed gill filaments extended along one side. Gill filaments were ciliated columnar epithelial cells containing a dense oval nucleus and acidophilic granules (Fig. 5). Numerous hemocytes were found in the hemolymph space (Fig. 5). After sediment exposure, the gill exhibited a loss of cilia, and wider hemolymph space (Fig. 6). Columnar cells showed moderate to severe alterations such as cell swelling, cell degeneration and loss of cells creating large intercellular spaces (Fig. 6). The gills are important organs for respiration and they are primary sites of gas exchange, osmoregulation, ion transfer and ionic balance (Tkatcheva et al. 2004). These histopathological alterations of the gill by heavy metals produces respiratory problems, and disturbance of normal osmoregulatory and hemodynamic functions (Arellano et al. 1999).

This study demonstrated the use of both chemical (bioaccumulation of heavy metals) and biological (histopathological alterations) procedures to confirm the ecotoxicological effects of heavy metals in the environment. Although heavy metal concentrations in the Mae Klong sediments did not exceed permissible limits of US EPA (1997), Cu and Zn concentrations in snails exceeded the standard limits for consumption (20 μg g^{-1} for Cu and 100 μg g^{-1} for Zn; Ministry of Public Health, Thailand), and may therefore be of concern.

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