Bull Environ Contam Toxicol (2007) 78:494–498
DOI 10.1007/s00128-007-9080-3

Field Assessment of Sediment Toxicities Within a Subtropical Estuarine Wetland in Hong Kong, Using a Local Gastropod (*Sermyla tornatella*)

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Published online: 9 June 2007

The area of Mai Po Marshes and Inner Deep Bay in Hong Kong (Fig. 1) was designated as a Ramsar site under *The Convention on Wetland of International Importance especially as Waterfowl Habitat* (Ramsar Convention) in 1995. Its value as a representative and unique estuarine wetland, especially as a key site for supporting some threatened bird species, has been well recognized. Pollutants such as metals in this region might pose adverse effects on water birds (Connell et al., 2002). Generally speaking, though the pollution level in this area has decreased gradually during the past decade (Blackmore, 1998), spatial distribution patterns (horizontal and vertical) of metal contamination in the sediments indicated some recent enrichment, especially at the coastal mudflat (Lau and Chu, 2000; Liang and Wong, 2003), whereas metals mobility was closely associated with organic matter, pH, Eh, salinity, and temperature in the aquatic environment (Yu et al., 2000; Liang and Wong, 2004). As a dynamic estuarine wetland with seasonal drainage and salinity change, Mai Po demonstrated that not all the sediments samples at all times showed severe pollution levels, judging from the chemistry data alone (Chapman and Wang, 2001). Thus, laboratory toxicity tests and field bioassessments appear to be the only ways to determine biological effects (Chapman et al., 2002). Elutriates of the sediments collected from Mai Po had low toxicities to alga (*Chlorella pyrenoidosa*) and amphipods (*Elasmopus rapax*) (Lau and Chu, 1999), and field toxicological studies using benthic animals have never been reported.

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In this study, a local gastropod species, *Sermyla tornatella*, was selected for the field toxicity experiment. *S. toratella* is a marshland snail commonly found at Mai Po Marshes (Morton and Morton, 1983), and it is the most dominant gastropod species contributing most of the total gastropod density and biomass in the mudflat (Cha, 1999). Its cosmopolitan distribution makes this species an appealing potential benthic organism for a future pollution monitoring program. By comparing the growth, mortality, and metal bioaccumulation in the snails among different sites with varied pollution levels, it is hoped that the roles of environmental factors in contributing to the sediment toxicities, such as metals levels or other aquatic physicochemical properties, can be inferred and can be helpful for future in-depth experiments on *S. toratella.*

Materials and Methods

Four sites were selected across the Mai Po Marshes, including mudflat (BWH), mangrove swamp (BW), landward side of the *gei wais* (LAND), and seaward side of the *gei wais* (SEA). Briefly, BWH represents locations close to the pollution source, Deep Bay, in general. Pollution in *gei wais* (including LAND and SEA) was low, and the metal concentrations in the sediments remained similar within the *gei wais* (Liang and Wong, 2003). LAND was characterized as stagnant water, high organic matter, and low pH in the sediments, whereas SEA underwent more dynamic water fluxes, greater sediment resuspension, and redox change. BW showed aquatic physicochemical dynamics that were similar to SEA, but that contained much higher levels of TOM and metals (Zn, Cu) in the sediments (Liang and Wong, 2003). However, there were no significant

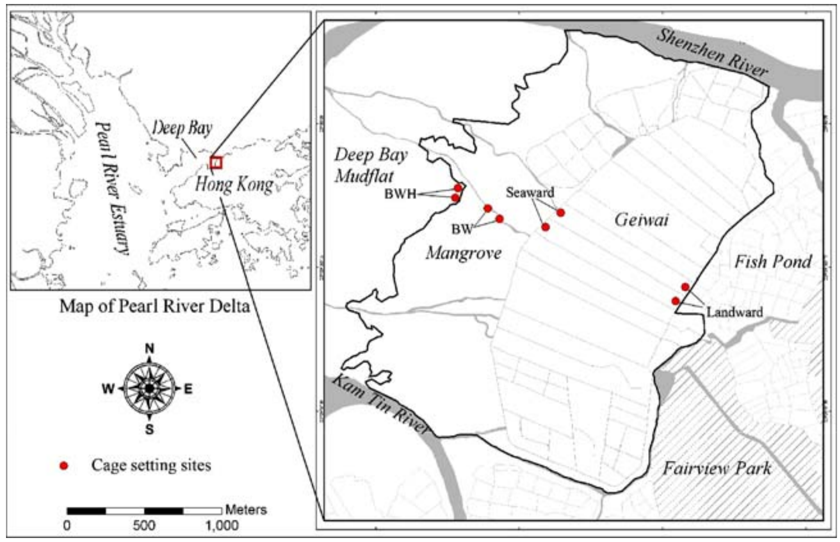

Fig. 1 Cage setting sites at Mai Po Marshes Nature Reserve

differences of Cd and Pb levels in the sediments among the four sites, possibly because of the high mobility of these two metals that associated with the dynamic changes of water salinity and organic matter in the sediments within the marshes (Liang and Wong, 2004).

Three hundred snails were collected in a relatively clean, shallow shrimp pond (local name *gei wai*) at Mai Po in October 1997 (Fig. 1), when *S. tornatella* was most abundant. Fifty of these snails were randomly chosen and brought back to the laboratory for the examination of body weight, length, and metal contents. Two cages, each containing 25 gastropods and 3-cm thick sediments (collected from the local site) at the bottom of the cage, were set at each site, with 2/3 of the cage immersed 10 to 20 cm under the water during high tide. All eight cages were left for 38 days (November 8–December 15, 1997). Weekly field visits were performed to check the condition of the cages. In mid-December, all cages containing the snails and sediments were removed out of the sediments and were brought back to the laboratory for further examination. Because of the short exposure time, control cages with the snails were not set at the site of collecting, and caging effects were not considered as a significant factor influencing the existence of the snails, but more efforts were put on the relative influences of different sites with varying pollution.

The sediment samples were collected from the four sites (LAND, SEA, BW, and BWH). Parameters, including pH, redox potential (Eh) (mV) (pH meter with oxidation-reduction potential probe), and electrical conductivity (EC) (mS cm^{-1}) (electrical conductivity probe) were measure in the laboratory immediately after sample collection. Total organic matter (TOM) (%) was determined by the percentage of loss of weight of oven-dried (103°C, 48 hr) sediments after combustion at 550°C. Total metals (Cd, Cr, Cu, Ni, Pb, and Zn) in the sediments (<63 μm) were determined by atomic absorption spectrophotometry (Varian Spectr AA-20 model) after acid digestion (mix acid, 5:1 conc. HNO_3 and conc. H_2SO_4). Sequential extraction for metal speciation analysis was performed according to the scheme proposed by Stover et al. (1976). Three replicates were performed for each sediment sample. The filtrates of each phase were analyzed for metals using Flame Atomic Absorption Spectrometry (Varian SpectrAA-20 model). The recovery rates of metals of the standard Reference Material (NBS 1646, estuarine sediment) were between 97%–117%.

Body size of the snail was determined by measuring the total length of shell. The growth of the snails was indicated by the percentage of change in body size at the end of the experiment as compared with that at the start (length %). A snail was considered to be alive if the gastropod was observed to emerge from the shell. At the end of the experiment, the dead snails were counted, and the mortality rate (mortality %) was calculated. Ten gastropods (alive at the end of the experiment) from each cage were randomly selected, were oven dried, and were crushed into a fine powder using a porcelain mortar and pestle. Metals in the powder were extracted by nitric acid digestion (120°C, 24 hr), and three replicates were conducted. Flame Atomic Absorption Spectrometry (Varian SpectrAA-20 model) was used for metal determination in the digested samples. The recoveries of metals using the certified standard reference material (NBS 1566a oyster tissue) were between 97%–115%.

Results and Discussion

Table 1 presents the physicochemical properties and trace metal contents in the sediments. The results were expected, based on the rational used in site selection. In general,

Table 1 Summary of data in the sediments at Mai Po Marshes (December 1997)

	Landward	Seaward	BW	BWH
pH	7.33 ± 0.41	7.76 ± 0.21	7.38 ± 0.07	7.63 ± 0.08
Eh (mV)	–149 ± 44.7	–111 ± 72.5	–191.5 ± 17.6	–182.5 ± 33.0
EC (mS cm^{-1})	7.30 ± 0.12	6.15 ± 1.37	8.28 ± 0.22	8.27 ± 0.36
TOM (%)	16.8 ± 4.99	7.64 ± 1.32	9.10 ± 1.61	10.1 ± 0.20
Cd				
KNO_3 (exchangeable, Cd1)	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
KF (adsorbed, Cd2)	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0
$Na_4P_2O_7$ (org matter, Cd3)	0.20 ± 0.1	0.15 ± 0.2	0.02 ± 0.0	0.31 ± 0.1
Na_2EDTA (carbonates, Cd4)	0.10 ± 0.1	0.11 ± 0.2	0.15 ± 0.1	0.19 ± 0.1
HNO_3 (sulfides, Cd5)	0.21 ± 0.0	0.05 ± 0.1	0.21 ± 0.2	0.36 ± 0.4
Total	0.65 ± 0.2	0.50 ± 0.2	0.53 ± 0.5	1.07 ± 1.0
Cr				
KNO_3 (exchangeable, Cr1)	0.70 ± 0.7	0.37 ± 0.2	0.00 ± 0.0	0.00 ± 0.0
KF (adsorbed, Cr2)	4.23 ± 1.0	3.23 ± 0.0	4.35 ± 3.0	5.31 ± 3.2
$Na_4P_2O_7$ (org matter, Cr3)	0.83 ± 0.3	0.92 ± 0.2	3.44 ± 1.5	2.96 ± 1.5
Na_2EDTA (carbonates, Cr4)	4.45 ± 1.3	3.30 ± 1.7	7.20 ± 3.2	10.8 ± 5.9
HNO_3 (sulfides, Cr5)	7.21 ± 2.8	8.35 ± 0.2	8.21 ± 2.1	8.65 ± 2.3
Total	19.9 ± 5.2	17.3 ± 2.6	24.4 ± 8.2	35.3 ± 3.5
Cu				
KNO_3 (exchangeable, Cu1)	0.71 ± 0.2	1.26 ± 0.1	0.99 ± 0.5	1.66 ± 0.1
KF (adsorbed, Cu2)	2.58 ± 0.6	2.49 ± 0.7	4.50 ± 3.5	2.35 ± 2.0
$Na_4P_2O_7$ (org matter, Cu3)	6.72 ± 1.8	5.31 ± 0.2	13.1 ± 2.3	14.2 ± 6.2
Na_2EDTA (carbonates, Cu4)	7.32 ± 3.1	6.43 ± 1.5	18.2 ± 6.8	15.6 ± 3.5
HNO_3 (sulfides, Cu5)	9.85 ± 2.6	6.68 ± 1.4	23.2 ± 3.6	17.9 ± 3.0
Total	30.8 ± 7.8	26.5 ± 0.3	83.9 ± 2.0	84.8 ± 1.3
Ni				
KNO_3 (exchangeable, Ni1)	2.33 ± 1.2	2.85 ± 0.5	1.74 ± 0.5	5.11 ± 1.5
KF (adsorbed, Ni2)	3.34 ± 0.2	2.10 ± 0.0	1.48 ± 1.2	1.86 ± 0.5
$Na_4P_2O_7$ (org matter, Ni3)	4.43 ± 1.7	2.27 ± 0.0	2.96 ± 1.5	3.26 ± 1.6
Na_2EDTA (carbonates, Ni4)	7.16 ± 0.8	6.05 ± 0.9	6.21 ± 4.7	5.26 ± 1.9
HNO_3 (sulfides, Ni5)	16.2 ± 1.2	14.8 ± 2.0	4.28 ± 3.2	4.45 ± 3.0
Total	39.2 ± 1.9	35.0 ± 1.3	45.0 ± 2.5	41.8 ± 0.9
Pb				
KNO_3 (exchangeable, Pb1)	1.49 ± 0.8	1.05 ± 0.4	1.70 ± 0.5	1.20 ± 1.5
KF (adsorbed, Pb2)	1.54 ± 0.5	0.82 ± 0.4	1.60 ± 0.6	1.24 ± 0.4
$Na_4P_2O_7$ (org matter, Pb3)	14.2 ± 8.0	10.3 ± 3.2	8.4 ± 1.5	10.9 ± 1.6
Na_2EDTA (carbonates, Pb4)	21.5 ± 6.8	26.5 ± 9.9	40.0 ± 14.7	37.2 ± 11.9
HNO_3 (sulfides, Pb5)	9.40 ± 4.1	11.2 ± 0.2	4.47 ± 3.2	5.12 ± 3.0
Total	59.5 ± 1.7	55.7 ± 4.6	62.4 ± 1.5	63.1 ± 1.1
Zn				
KNO_3 (exchangeable, Zn1)	2.34 ± 1.7	0.52 ± 0.4	1.82 ± 0.5	2.02 ± 1.5
KF (adsorbed, Zn2)	1.43 ± 1.2	1.43 ± 1.2	0.00 ± 0.0	0.15 ± 0.0
$Na_4P_2O_7$ (org matter, Zn3)	20.5 ± 7.1	12.5 ± 8	77.5 ± 1.5	76.3 ± 1.6
Na_2EDTA (carbonates, Zn4)	15.6 ± 0.3	21.6 ± 4.6	79.8 ± 14.7	87.3 ± 11.9
HNO_3 (sulfides, Zn5)	35.6 ± 6.3	37.2 ± 5.4	55.2 ± 3.2	72.8 ± 3.0
Total	125 ± 2.5	118 ± 7.3	303.7 ± 5.5	328.3 ± 5.2

TOM ranged between 7.64%–16.8%), with the highest result being detected at LAND, whereas TOM and pH were closely correlated because of acids derived from organic matter degradation. BWH and BW had significantly ($p < 0.05$) higher EC values (8.27–8.28 mS cm^{-1}) than those in *gei wais* (6.15–7.30 mS cm^{-1}), because of the salinity gradient that was high at coastal sites and low at inland sites. The values also indicated that BWH was the most polluted site, which contained the highest metal levels. However, the fractionation data showed that mobile phases, including exchangeable and adsorbed phases, accounted for small percentages (0%–26.1%) of the total metal concentrations among all the samples, whereas the less mobile phases, including organic matter complexed, carbonate, and sulfide bound, accounted for higher percentages (29.9%–86.1%).

The results on the growth and mortality of the caged *S. tornatella* are shown in Figure 2. Among the four sites, *S. tornatella* suffered the highest mortality rate (81.5%) and low growth (1.31%) at BWH, whereas SEA and BW supported better survival of *S. tornatella* (0%–3.22% mortality) and growth (2.07%–4.84%). Table 2 presents

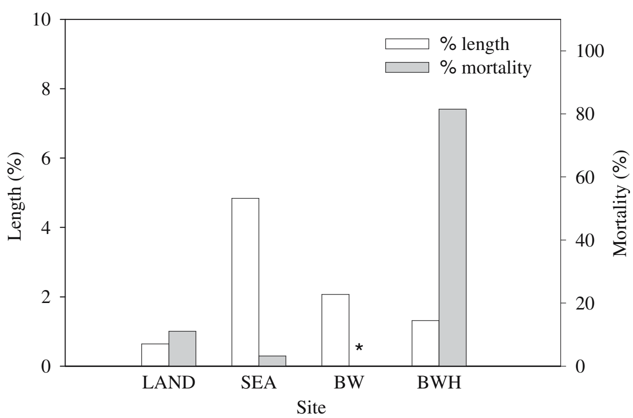

Fig. 2 Growth and mortality of caged *Sermylla tornatella* over 38 days of exposure at Mai Po Marshes (8 November–15 December 1997). *, 0% mortality; LAND, landward site in *gei wais*; SEA, seaward site in *gei wais*; BW, mangrove swamp; BWH, mudflat

metal accumulation in *S. tornatella*. Except for Cu, metal (Cd, Cr, Ni, Pb, and Zn) accumulation in *S. tornatella* showed no differences among the four sites. Though BWH was the most severely polluted, the survived *S. tornatella* at BWH did not contain significantly higher metals in the body ($p > 0.05$). *S. tornatella* at SEA accumulated the highest Cu in the body (22.1 $\mu g\ g^{-1}$), whereas at BWH had the lowest (12.0 $\mu g\ g^{-1}$). Severe pollution from Deep Bay, other than the metals in the sediments detected in the present study, appears to be responsible for the mortality of *S. tornatella* at Mai Po Marshes, because of the great difference in the mortality between BWH and BW where similar metal contents in the sediments were detected. Although BWH registered a higher Cd content (1.07 $\mu g\ g^{-1}$) than BW (0.53 $\mu g\ g^{-1}$) during the experiment, the concentration was lower than the probable effects level for sediments (3.5–4.2 $\mu g\ g^{-1}$) (CCME, 2002). Therefore, pollution from Deep Bay deemed further investigation in order to identify potential pollutants leading to the high death rate of *S. tornatella* at BWH.

The growth of the organisms not only reflected food (availability and quality) but also the environmental conditions. Depressed growth may be a result of starving or deteriorated food and degraded environment (such as pollution) as well as the interactions between food and the environment that lead to changed uptake and assimilation of nutrients for the organisms. Therefore, the pattern of a negative association between TOM and the growth (length %) (Table 1, Fig. 2) seems to have several explanations. On one hand, if TOM was supposed to be important food source for the snails, the compositions of TOM were different among the four sites, and high nitrogen-containing (N-containing) organic matter may be preferred by the gastropod for growth. Coastal sites (such as BWH, BW) suffered from domestic pollution that had high N-containing organic matter, whereas the TOM at the landward side in *gei wais* (LAND, SEA) was composed of plant litters and the degradation products with low N percentage in the organic matter (Li and Lee, 1998). On the other hand, organic matter is an important driving force for low pH and metal mobility in the sed-

Table 2 Metal bioaccumulation in *Sermylla tornatella* ($\mu g\ g^{-1}$ dw) (mean ±SD) at Mai Po Marshes. Within columns, means with the same superscript are not significantly different ($p > 0.05$) by the Student-Newman-Keuls test

	Cd	Cr	Cu	Ni	Pb	Zn
Start of exposure	4.30 ± 0.10^{a}	30.8 ± 0.14^{a}	16.8 ± 0.05^{b}	21.5 ± 0.03^{a}	40.6 ± 0.07^{a}	43.6 ± 0.13^{a}
End of exposure						
LAND	4.37 ± 0.39^{a}	26.2 ± 4.90^{a}	14.0 ± 1.22^{bc}	22.2 ± 1.56^{a}	39.9 ± 3.89^{a}	43.8 ± 3.66^{a}
SEA	4.33 ± 0.25^{a}	26.8 ± 2.11^{a}	22.1 ± 3.09^{a}	24.7 ± 3.64^{a}	44.8 ± 1.11^{a}	58.9 ± 0.44^{a}
BW	4.28 ± 0.04^{a}	18.2 ± 1.63^{a}	18.3 ± 1.63^{ab}	21.9 ± 0.67^{a}	39.6 ± 1.94^{a}	45.6 ± 2.90^{a}
BWH	4.08 ± 0.46^{a}	25.4 ± 0.74^{a}	12.0 ± 0.74^{c}	21.0 ± 1.45^{a}	37.1 ± 4.21^{a}	40.0 ± 3.92^{a}

iments (Förstner, 1995). Organic matter degradation may also cause unfavorable microenvironments (including low pH and high metal concentrations in pore water) that impair the growth of *S. tornatella*.

The results demonstrated that metal bioaccumulation might not necessarily inhibit the growth of the organisms (Table 2), implying that the depressed growth may have reduced the uptake and/or assimilation of metals (Rainbow, 2002). It seems that the slower-growing *S. tornatella* tended to contain less accumulated Cu, Pb, or Zn in the forms of metabolically available (excess readily excreted and/or detoxified, and/or metabolically required) and stored detoxified (such as metallothionein complexed) (Rainbow, 2002). On the other hand, metal concentrations in the sediments, including total and fractionation data, appeared not to be good predictors for bioaccumulation of metals in *S. tornatella*. Despite the broad range of metal levels in the sediments across the four sites (Table 1), metal body burdens in *S. tornatella* (except for Cu) showed no significant differences. As fractionation results indicated bioavailability via water, it is suggested that the dietary route played an important role in metal accumulation in the body at Mai Po Marshes.

In general, pollution and organic matter in the sediments are major toxicity factors in the survival and growth of *S. tornatella*. Metal bioaccumulation might not necessarily inhibit the growth of the organisms, whereas food quality and environmental factors seemed to play important roles. The seaward site in *gei wais*, which promotes the most growth and the least mortality of *S. tornatella*, could be used as a reference site for a toxicity study in *S. tornatella*. Further investigations are needed in order to develop *S. tornatella* as a bioindicator of metal and other pollutions at Mai Po Marshes.

Acknowledgments The author thanks Dr. L. Young, Dr. S. T. Chiu, Mr. K. H. Kok, and Miss P. L. Chiu for technical assistance. Financial support from the Academic Link sponsored by the British Council, and the Faculty Research Grant of Hong Kong Baptist University is gratefully acknowledged. The author is grateful to two anonymous reviewers.

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