Available online at www.sciencedirect.com
SCIENCE DIRECT®
Environmental Pollution 132 (2004) 265–277
ENVIRONMENTAL POLLUTION
www.elsevier.com/locate/envpol

Modification of trace metal accumulation in the green mussel *Perna viridis* by exposure to Ag, Cu, and Zn

Dalin Shi, Wen-Xiong Wang*

Department of Biology, The Hong Kong University of Science and Technology (HKUST), Clear Water Bay, Kowloon, Hong Kong

Received 9 February 2004; accepted 20 April 2004

"Capsule": *Prior exposure of mussels to one metal may alter the rates of bioaccumulation of other metals.*

Abstract

To examine the Cd, Hg, Ag, and Zn accumulation in the green mussel *Perna viridis* affected by previous exposure to Cu, Ag, or Zn, the dietary metal assimilation efficiency (AE) and the uptake rate from the dissolved phase were quantified. The mussel's filtration rate, metallothionein (MT) concentration, and metal tissue burden as well as the metal subcellular partitioning were also determined to illustrate the potential mechanisms underlying the influences caused by one metal pre-exposure on the bioaccumulation of the other metals. The green mussels were pre-exposed to Cu, Ag, or Zn for different periods (1–5 weeks) and the bioaccumulation of Cd, Hg, Ag, and Zn were concurrently determined. Pre-exposure to the three metals did not result in any significant increase in MT concentration in the green mussels. Ag concentration in the insoluble fraction increased with increasing Ag exposure period and Ag ambient concentration. Our data indicated that Cd assimilation were not influenced by the mussel's pre-exposure to the three metals (Cu, Ag, and Zn), but its dissolved uptake was depressed by Ag and Zn exposure. Although Hg assimilation from food was not affected by the metal pre-exposure, its influx rate from solution was generally inhibited by the exposure to Cu, Ag, and Zn. Ag bioaccumulation was affected the most obviously, in which its AE increased with increasing Ag tissue concentration, and its dissolved uptake decreased with increasing tissue concentrations of Ag and Cu. As an essential metal, Zn bioaccumulation remained relatively stable following the metal pre-exposure, suggesting the regulatory ability of Zn uptake in the mussels. Zn AE was not affected by metal pre-exposure, but its dissolved uptake was depressed by Ag and Zn pre-exposure. All these results indicated that the influences of one metal pre-exposure on the bioaccumulation of other metals were metal-specific due to the differential binding and toxicity of metals to the mussels. Such factors should be considered in using metal concentrations in mussel's soft tissues to evaluate the metal pollution in coastal waters.

Keywords: Pre-exposure; Bioaccumulation; Mussels; Copper; Silver; Zinc; Mercury; Cadmium

1. Introduction

In biomonitoring programs using marine bivalves as sentinel monitors of metal pollution, it is important to appropriately interpret the observed metal tissue concentrations such that the real contamination history can be accurately reflected by the monitoring data. Any factor potentially affecting metal bioaccumulation in marine bivalves thus need to be carefully examined, including the extrinsic conditions such as salinity (Blackmore and Wang, 2003) and food composition (Wang and Fisher, 1996), and the intrinsic conditions such as the biochemical or physiological changes as induced by the history of metal pre-exposure (Blackmore and Wang, 2002). Among the effects caused by various external and internal factors, the influences of metal pre-exposure on metal bioaccumulation have

* Corresponding author. Tel.: +852-2358-7346.
E-mail address: wwang@ust.hk (W.-X. Wang).

0269-7491/$ - see front matter
doi:10.1016/j.envpol.2004.04.023

received increasingly attentions in recent years (Boisson et al., 1998; Wang, 2002, 2003).

In natural environment, marine organisms are simultaneously exposed to a mixture of metals. Thus, it is very likely that exposure to one metal may generate influences on the bioaccumulation of other metals (Amiard-Triquet and Amiard, 1998). For example, Popham and D'Auria (1982) reported that the uptake of Zn and Pb by the blue mussel *Mytilus edulis* facilitated the Cu accumulation. Similarly, the concentration of Cu in the same mussel species increased with increasing Ag levels in the ambient seawater (Calabrese et al., 1984). Fraysse et al. (2002) showed that Zn and Cd + Zn mixture increased the Ag uptake by the Asiatic clam *Corbicula fluminea* and the zebra mussel *Dreissena polymorpha*. In a recent study, the green mussel *Perna viridis* reduced its Cd uptake from the dissolved phase following pre-exposure to Zn (Blackmore and Wang, 2002). These experimental evidences indicate that trace metals may interact in their bioaccumulation, but the physiological and biochemical mechanisms underlying such interaction remain less well understood.

The influences of one metal pre-exposure on the uptake of other metals may occur for those having similar propensity in binding with the same type of ligands. Such influences are metal-specific due to the different underlying uptake mechanisms. For example, we recently found that the assimilation efficiency (AE) of Hg(II) by the green mussels increased significantly following pre-exposure to Cd for 5 weeks, presumably as a result of Hg binding with the Cd-induced metallothionein (MT) (Shi and Wang, unpublished). In contrast, Ag uptake was not affected because it was mainly bound with the insoluble compounds, such as the sulfide complex in the mussels (George et al., 1986; Shi et al., 2003). The similar chemical characteristics of Cd and Zn lead to a close relationship in their assimilation and dissolved uptake by different species of marine bivalves (Chong and Wang, 2000; Wang, 2001).

The green mussel *P. viridis* has been used as a biological monitor of metal pollution in tropical and subtropical coastal waters (Phillips and Rainbow, 1988; Chong and Wang, 2001). In this study, we quantified the AE and the dissolved uptake rate of four metals (Cd, Hg, Ag, and Zn) in the green mussels following pre-exposure to three metals (Cu, Ag, or Zn) for different durations and at different ambient concentrations. In addition to examine the bioaccumulation of metals following the pre-exposure to the same metals, we also specifically investigated the interaction among the different metals in their bioaccumulation by the mussels. The mussel's filtration rate was quantified to monitor the possible toxic effects exerted by the metal exposure. The MT concentration as well as the stable metal subcellular distribution was determined to examine the potential biochemical or physiological modifications after metal pre-exposure. Meanwhile, the tissue metal concentrations in the mussels were measured to evaluate their effects on subsequent metal uptake. The four metals (Cd, Hg, Ag, and Zn) examined in this study generally share a similarity in binding with the sulfur ligands such as the MT. Cu, Cd, and Hg have been identified as MT-inducers in marine bivalves (Langston et al., 1998) and are among the most toxic metals. We did not quantify the Cu bioavailability in the mussels due to the lack of suitable radiotracer (e.g., ^{64}Cu which has a relatively short half-life, 12.71 h).

2. Material and methods

2.1. Mussel collection and metal pre-exposure

The green mussels *P. viridis* with a shell length of about 3.5 cm were collected from Yuan Shu Au, Tolo Harbour, Hong Kong. This collection site is uncontaminated by metals (Rainbow and Blackmore, 2001; Blackmore and Rainbow, 2001). The mussels were transported to the laboratory immediately after the collection. Throughout the whole experimental period, they were maintained in natural coastal seawater collected from the Clear Water Bay, Hong Kong, at 23 °C and 30 psu with continuous aeration, and fed with the diatom *Thalassiosira pseudonana* (clone 3H) at a ration 1–2% of tissue dry weight per day. All the mussels were acclimated to the laboratory conditions for one week before the experiments described below.

In two independent experiments described below, groups of mussels were exposed separately to different concentrations of dissolved Cu (as $CuSO_4$), Ag (as $AgNO_3$), or Zn (as $ZnCl_2$) for different durations (Table 1) in 60 l polypropylene containers. During the pre-exposure period (a total of 5 weeks), each day the mussels were exposed to metal spiked seawater for 18 h and fed the diatom *T. pseudonana* in unspiked seawater for the remaining 6 h. In the control treatments, the seawater was not spiked with the metals. In order to

Table 1
Perna viridis. Metal concentrations (μg L^{-1}) and exposure durations (weeks) used in two pre-exposure experiments with the green mussels

Expt.	Duration (wks)	Metal concentration (μg L^{-1})		
		Cu	Ag	Zn
1	1, 3, 5	1	–	–
		4	–	–
		15	–	–
		30	–	–
2	1, 3, 5	–	0.3	10
		–	3.0	100

For all the treatments, the mussels were directly exposed to the metals in the seawater.

keep the nominal metal concentrations relatively constant during the pre-exposure period, the seawater in all exposure treatments was renewed every other day. The mussels were then sampled after 1, 3, and 5 weeks of exposure, and the following measurements were conducted.

2.2. Metal concentration in soft tissues

For each treatment, five individual mussels were sampled following 1, 3 and 5 weeks of pre-exposure for quantification of metal concentration in the soft tissues. The mussels were dissected and the soft tissues were individually placed into acid-cleaned glass tubes. The dry weights of the tissues were first determined by drying them at 80 °C to constant weights. Afterward, concentrated Ultrapure HNO3 (69%, Aristar grade BDH Ltd) was added to digest the dry tissues. The digests were subsequently diluted with Nanopure water and the metal concentrations were analyzed by Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) (Perkin–Elmer, Elan 6000). Random checks were made with a standard reference material (1566A Oyster tissue, US Department of Commerce, Technology Administration, National Institute of Standards and Technology, Gaitherburg, MD, USA) and agreement was within 10%. The stable metal tissue concentrations were expressed as $\mu g\, g^{-1}$ dry weight.

2.3. Stable metal fractionation

The subcellular distribution of accumulated metals in the mussels pre-exposed to Ag or Zn was quantified using a modified method of Wallace et al. (2003). At the end of the pre-exposures, three replicated mussels from each group were dissected and the wet weights of the soft tissue were measured. Afterward, the soft tissue was homogenized in 4 mL Tris buffer solution, and the homogenate was centrifuged at 1450 *g*(15 min at 4 °C). The pellets included tissue fragments and other cellular debris (i.e., membranes and metal-rich granules—MRG). The supernatant was further centrifuged at 100,000 *g* for 1 h at 4 °C to separate the cytosol and proteins from the organelles (i.e., intracellular pellets containing nuclear, mitochondrial and microsomal fractions). Following heat treatment (10 min at 80 °C) and ice-cooling (1 h), the 100,000 *g* supernatant was centrifuged again at 50,000 *g* for 10 min at 4 °C. The supernatant contained the heat-stable proteins or metallothionein-like proteins (MTLPs) and the pellets contained the heat-sensitive proteins (HSPs) due to heat denaturation. The organelles and the 1450 g pellets were then combined as one fraction (as insoluble fraction, Ng and Wang, 2004). The metal concentrations in the insoluble fraction as well as the HSPs and MTLPs were determined using the method described above, and were expressed as $\mu g\, g^{-1}$ wet weight of the whole soft tissue. The subcellular fractionation of Cu was not quantified in Expt. 1 with the Cu pre-exposure.

2.4. Metallothionein (MT)

The MT concentration in the digestive gland of the mussels was determined using a modified silver saturation method (Scheuhammer and Cherian, 1986, 1991; Leung and Furness, 1999). Three digestive glands were separated from three individuals and the wet weights were determined. The replicated tissues were then homogenized and ultrasonicated individually in 4× volume of cold 0.25 M sucrose at 4 °C, followed by centrifugation at 20,000 *g* for 20 min at the same temperature. Subsequently, 0.5 mL of the supernatant was mixed with 0.3 mL of 0.5 M glycine buffer and 0.5 mL of 20 μg Ag mL^{-1} mixed with radiotracer ^{110m}Ag (3.7 kBq mL^{-1}). The mixture was incubated at room temperature for 10 min to saturate the MT with excessive Ag. The Ag not bound with MT was then removed by adding 0.1 mL rabbit red blood cell haemolysate, heating for 5 min at 100 °C and centrifuging for 5 min at 1200 *g*. The addition of haemolysate, heating and centrifugation were repeated twice. The supernatant obtained was further centrifuged for 20 min at 20,000 g. The amount of Ag remaining in the final supernatant was quantified for ^{110m}Ag. The MT concentration was calculated as 3.55 times the Ag concentration and expressed as the $\mu g\, g^{-1}$ wet weight of the digestive gland (Scheuhammer and Cherian, 1991).

2.5. Filtration rate (FR)

Eight mussels from each treatment were placed individually into 1.5 L glass fiber filtered (GF/C) seawater within a polypropylene beaker. After the mussels opened and pumped normally (usually within 10 min), the diatoms *T. pseudonana* (filtered from their growth medium) were added to each beaker at a concentration of 10^4 cells mL^{-1}. The algal suspension in each beaker was stirred by a magnetic stirrer. Immediately after adding the algae, a 10 mL aliquot water sample was removed and the cell density was counted using a Coulter Counter (Z1, Coulter). Further water samples were taken at 20 min and 40 min and the cell density was determined. After the FR measurements, the mussels were dissected and the soft tissues were dried at 80 °C overnight to measure the dry weights. The FR was finally calculated as described in Shi et al. (2003).

2.6. Metal assimilation efficiency (AE)

The metal AE was determined using the radioactive pulse-chase feeding technique (Wang and Fisher, 1999). The diatom *T. pseudonana*, grown in f/2 nutrients for N, P, Si and vitamins, and f/20 nutrients for trace elements minus Zn, Cu and EDTA, was radiolabeled with the

isotopes ^{110m}Ag, ^{109}Cd, ^{203}Hg(II), and ^{65}Zn, as a mixture for 4 days. After the radiolabeling, the uniformly radiolabeled diatom cells were harvested by filtering through a 3 μm polycarbonate membrane and resuspending in 0.22 μm filtered seawater. The resuspension was performed twice to minimize the desorption of radiotracers from the diatoms to the ambient waters during the radioactive feeding period.

Mussels were placed into 500 mL of GF/C seawater and the radiolabeled diatoms were added into each beaker giving a cell density of 4–5 $\times$ 10^4 cells mL^{-1} after the mussels opened and pumped normally. This cell density was maintained by further additions at 10 min intervals. Following 30 min of radioactive feeding, the mussels were rinsed with clean seawater and assayed for their radioactivity. In each group, five mussels were placed separately into polypropylene beakers (180 mL seawater) held in a 10 L enclosed recirculating flow-through aerated seawater aquarium. Nonradioactive *T. pseudonana* was fed twice daily at a ration of 1–2% dry weight per day to depurate the initially ingested radioisotopes. The radioactivity retained in the mussels was counted over a 72 h depuration period at time intervals from 3 to 12 h. Metal AE was determined as the percentage of initial radioactivity retained in the animals after 60 h of depuration. Feces were collected frequently throughout the depuration period.

2.7. Metal uptake from the dissolved phase

The 0.22 μm filtered seawater was spiked with the radiotracers ^{110m}Ag, ^{109}Cd, ^{203}Hg(II), and ^{65}Zn, as well as the stable metals Cd (as $CdCl_2$, 18 nM) and Zn (as $ZnCl_2$, 77 nM), and allowed to equilibrate for 24 h prior to the experiments. No stable Ag or Hg was added into the water. The radioisotope additions were 3.7 kBq L^{-1} (corresponding to 0.3 nM) for ^{109}Cd, 9.25 kBq L^{-1} (corresponding to 0.06 nM) for ^{65}Zn, 3.7 kBq L^{-1} (corresponding to 0.3 nM) for ^{110m}Ag, 1.48 kBq L^{-1} (corresponding to 0.3 nM) for ^{203}Hg(II). Eight mussels from each group were carefully cleaned and then placed individually in 200 mL of radioactive filtered seawater for 1 h. This short-term exposure avoided the possible decline of the mussels' ventilating activity due to the absence of food particles and minimized the decrease of ambient metal concentrations. Following the exposure, the mussels were dissected and the radioactivity of the soft tissues was assayed. The soft tissues were finally dried at 80 °C overnight and the dry weights were determined. The metal influx rate from the dissolved phase was expressed as ng g^{-1} h^{-1}.

2.8. Radioassay and statistical analysis

Radioactivity was measured using a Wallac 1480 NaI (T1) gamma detector (Wallac Turku, Finland). All counts were related to standards and radioactive decay was corrected. The gamma emission of ^{109}Cd was determined at 88 keV, ^{203}Hg at 258 keV, ^{110m}Ag at 658 keV, and ^{65}Zn at 1115 keV. Counting times were adjusted to yield a propagated counting error $<5\%$. Statistical analysis was carried out by *t*-test or analysis of variance (ANOVA). Statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Soft tissue metal concentration, MT concentration, and subcellular concentration

In general, the Cu and Ag concentrations in the mussel's soft tissue increased significantly with increasing durations and concentrations of Cu (Table 2) or Ag pre-exposures (Table 3). The Cu tissue concentrations in the metal pre-exposed mussels were 1.2–4.2 times higher than the controls. The increase in Cu tissue concentration was more significant in the two higher concentration treatments (15 and 30 μg L^{-1}, Table 2). In Expt. 2, except for the 0.3 μg Ag L^{-1} treatment after 1 week of pre-exposure, the Ag tissue concentrations of all the exposed mussels were significantly higher than those of the controls (Table 3). Such increase was however not obvious in mussels pre-exposed to Zn, except at the highest Zn concentration (100 μg L^{-1}) after 3 and 5 weeks of exposure. At this concentration, the Zn tissue concentration was elevated by 22–33% after 3–5 weeks of pre-exposure (Table 3).

The MT concentrations in the digestive gland of mussels pre-exposed to Cu were 1.53–2.92 μg g^{-1} wet weight, which was generally higher (but not statistically significant) than those of the controls (Table 2). There was no statistically significant difference in MT concentrations between the Ag- or Zn-exposed mussels and the controls (Table 3). No relationship between the MT concentration and the metal tissue concentration was observed following either Ag or Zn pre-exposure.

The stable metal concentrations in different subcellular fractions following Ag and Zn exposure are showed in Fig. 1. There was generally no significant difference in Zn partitioning among the different subcellular fractions. Most Zn was concentrated in the insoluble fractions (including cellular debris, organelles, and metal-rich granule), and only a small amount of Zn was found in the MTLP fraction. In contrast, the association of Ag with the insoluble fraction increased obviously following the Ag pre-exposure. The increase was significant ($p < 0.01$) in the 3.0 μg L^{-1} Ag treatment after 1 week of exposure, as well as in the 3.0 and 0.3 μg L^{-1} Ag treatments after 3 weeks of exposure. The majority of Ag was distributed in the insoluble fraction and negligible amount was found to in the HSP or MTLP fractions.

Table 2
Perna viridis. Metal concentrations (mean ± SD, $n = 5$) in the soft tissue, metallothionein (MT) concentration (mean ± SD, $n = 3$) in the digestive gland, filtration rate (FR, mean ± SD, $n = 8$), metal assimilation efficiency (AE, mean ± SD, $n = 5$), and metal influx rate from the dissolved phase (mean ± SD, $n = 8$) of the green mussels following Cu pre-exposures

Duration (wks)	Treatment	Cu ($\mu g\ g^{-1}$)	MT ($\mu g\ g^{-1}$ ww)	FR ($L\ g^{-1}\ h^{-1}$)	AE (%)				Influx rate ($ng\ g^{-1}\ h^{-1}$)			
					Cd	Hg	Ag	Zn	Cd	Hg	Ag	Zn
1	C	5.8 ± 0.4	1.03 ± 0.10	6.9 ± 1.5	19.5 ± 1.0	61.5 ± 9.2	14.1 ± 4.7	18.9 ± 5.3	27.1 ± 8.7	5.0 ± 1.0	23.6 ± 8.7	201.7 ± 48.9
	Cu1	7.1 ± 0.3	1.97 ± 1.25	6.9 ± 1.5	24.3 ± 4.9	59.6 ± 4.7	13.2 ± 6.7	15.6 ± 3.4	26.3 ± 6.0	4.5 ± 0.9	32.0 ± 2.7	205.7 ± 31.1
	Cu4	8.7 ± 1.4*	1.53 ± 1.07	4.5 ± 1.4**	20.0 ± 2.3	65.5 ± 2.7	17.7 ± 5.0	16.6 ± 4.7	30.8 ± 8.6	5.6 ± 1.6	16.4 ± 4.4*	231.5 ± 48.0
	Cu15	10.2 ± 1.3**	2.03 ± 0.93	7.1 ± 2.5	22.6 ± 9.0	62.7 ± 6.2	11.3 ± 5.3	15.2 ± 3.9	32.6 ± 7.6	4.2 ± 1.2	6.3 ± 1.0***	226.5 ± 49.6
	Cu30	16.4 ± 3.8***	1.97 ± 0.76	3.9 ± 1.6**	20.8 ± 2.5	66.5 ± 4.2	21.1 ± 8.6	16.0 ± 2.0	26.3 ± 4.9	4.9 ± 0.6	6.1 ± 2.1***	221.2 ± 30.3
3	C	5.7 ± 0.6	2.20 ± 0.47	9.3 ± 1.3	22.7 ± 7.7	59.7 ± 8.7	19.2 ± 6.3	25.9 ± 5.0	28.3 ± 8.7	6.2 ± 1.4	23.3 ± 3.3	356.5 ± 73.7
	Cu1	9.0 ± 1.6*	2.73 ± 0.50	10.0 ± 2.0	18.8 ± 5.3	60.0 ± 3.2	19.3 ± 8.6	26.0 ± 3.2	22.6 ± 6.0	4.6 ± 1.6	14.4 ± 3.4***	335.1 ± 63.6
	Cu4	8.5 ± 1.1*	2.09 ± 1.12	7.1 ± 2.1*	19.1 ± 8.4	62.7 ± 2.8	21.6 ± 6.0	22.5 ± 2.9	24.3 ± 3.0	4.9 ± 1.2	20.7 ± 6.5	330.9 ± 81.5
	Cu15	10.1 ± 1.0**	1.95 ± 0.56	9.5 ± 2.8	17.3 ± 6.2	61.3 ± 6.2	19.2 ± 6.0	21.9 ± 2.0	27.2 ± 8.5	4.7 ± 1.1	10.3 ± 2.0***	269.7 ± 88.4
	Cu30	22.4 ± 6.1***	2.92 ± 0.46	4.0 ± 1.2***	18.4 ± 3.4	56.1 ± 0.9	26.1 ± 2.2*	27.4 ± 4.4	24.1 ± 4.9	3.8 ± 0.9**	7.9 ± 2.4***	263.1 ± 71.3*
5	C	5.6 ± 0.3	1.82 ± 0.49	10.6 ± 2.4	27.9 ± 6.1	63.8 ± 3.1	20.6 ± 7.2	25.4 ± 2.5	30.0 ± 4.1	8.5 ± 2.0	9.5 ± 1.4	224.1 ± 67.4
	Cu1	8.8 ± 0.5**	1.99 ± 0.17	18.5 ± 1.4**	32.2 ± 6.9	58.4 ± 4.7	13.5 ± 3.9	23.5 ± 2.4	27.0 ± 5.2	5.9 ± 1.5*	9.1 ± 1.8	241.5 ± 108.8
	Cu4	7.2 ± 0.8	1.91 ± 0.17	15.8 ± 5.0	31.8 ± 9.5	61.8 ± 7.5	11.5 ± 1.9*	24.6 ± 6.0	30.3 ± 9.8	5.0 ± 0.6***	7.0 ± 1.6**	264.9 ± 82.0
	Cu15	13.5 ± 4.9**	1.98 ± 0.29	14.0 ± 3.3	27.8 ± 5.0	65.7 ± 9.7	13.0 ± 2.4	24.4 ± 6.6	21.0 ± 4.9	4.2 ± 0.5***	5.5 ± 1.5***	225.6 ± 70.3
	Cu30	23.7 ± 6.0***	2.79 ± 0.51	14.4 ± 2.9	26.4 ± 9.8	67.3 ± 12.9	33.5 ± 12.0	22.2 ± 5.4	20.8 ± 4.4	4.6 ± 0.8***	6.0 ± 1.8**	225.8 ± 74.2

Significant difference from the respective controls is indicated by $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. C: control. Numbers in the treatments are the Cu concentrations used in the exposure treatment (see Table 1).

Table 3
Perna viridis. Metal concentrations (mean ± SD, $n = 5$) in the soft tissue, metallothionein (MT) concentration (mean ± SD, $n = 3$) in the digestive gland, filtration rate (FR, mean ± SD, $n = 8$), metal assimilation efficiency (AE, mean ± SD, $n = 5$), and metal influx rate from the dissolved phase (mean ± SD, $n = 8$) of the green mussels following Ag or Zn pre-exposures

Duration (wks)	Treatment	Ag ($\mu g\ g^{-1}$)	Zn ($\mu g\ g^{-1}$)	MT ($\mu g\ g^{-1}$ ww)	FR ($L\ g^{-1}\ h^{-1}$)	AE (%)				Influx rate ($ng\ g^{-1}\ h^{-1}$)			
						Cd	Hg	Ag	Zn	Cd	Hg	Ag	Zn
1	C	0.4 ± 0.0	110.0 ± 30.2	2.28 ± 0.44	5.1 ± 1.5	24.0 ± 8.8	52.6 ± 6.4	19.4 ± 3.6	23.4 ± 6.4	32.3 ± 9.8	9.3 ± 1.2	9.8 ± 2.7	212.3 ± 43.2
	Ag0.3	0.6±0.2	–	3.26 ± 0.29	7.0 ± 1.4	19.6 ± 6.4	57.4 ± 8.4	27.8 ± 5.9*	27.3 ± 5.7	23.7 ± 5.7	8.7 ± 1.9	8.0 ± 2.3	190.2 ± 49.7
	Ag3	1.6 ± 0.6*	–	2.92 ± 0.11	4.0 ± 1.4	21.7 ± 4.2	59.8 ± 4.1	32.7 ± 3.5***	28.5 ± 3.5	20.0 ± 7.7*	7.7 ± 2.3	7.4 ± 3.1	177.9 ± 56.3
	Zn10	–	114.9 ± 15.1	3.05 ± 0.62	5.3 ± 1.5	24.3 ± 6.2	59.4 ± 6.0	31.0 ± 7.0*	28.2 ± 4.9	18.1 ± 2.9**	8.8 ± 1.8	6.6 ± 1.9*	178.1 ± 31.3
	Zn100	–	124.8 ± 33.9	2.42 ± 0.16	4.0 ± 1.2	21.3 ± 7.7	56.7 ± 14.5	24.6 ± 8.9	27.1 ± 3.7	12.2 ± 2.5***	8.1 ± 1.8	5.3 ± 0.8**	131.1 ± 52.6**
3	C	0.2 ± 0.1	92.9 ± 12.4	3.57 ± 0.23	5.9 ± 1.9	19.8 ± 5.4	56.0 ± 8.9	13.4 ± 4.7	33.9 ± 8.5	27.4 ± 6.4	9.3 ± 3.4	10.2 ± 3.7	216.1 ± 54.4
	Ag0.3	0.9 ± 0.4*	–	4.01 ± 0.15	6.2 ± 1.6	28.2 ± 6.8	55.5 ± 10.2	19.3 ± 4.7	26.7 ± 6.4	28.8 ± 8.3	9.6 ± 2.4	8.8 ± 2.6	222.3 ± 48.7
	Ag3	4.5 ± 0.7***	–	3.76 ± 0.95	3.4 ± 1.1	23.7 ± 7.3	57.9 ± 6.3	27.5 ±7.3*	28.2± 4.0	20.8 ± 8.5	7.8 ± 2.2	6.4 ± 1.7	187.2 ± 52.3
	Zn10	–	97.7 ± 21.6	4.09 ± 0.36	3.9 ± 0.6	26.2 ± 8.6	61.0 ± 7.9	18.9 ± 4.7	32.7 ± 9.0	21.5 ± 5.3	9.0 ± 2.9	6.3 ± 2.1	219.5 ± 71.6
	Zn100	–	124.0 ± 23.7*	4.20 ± 0.30	3.7 ± 0.9	24.4 ± 3.5	60.5 ± 6.0	20.1 ± 5.8	23.6 ± 3.7	12.6 ± 3.2***	7.2 ± 0.9	6.3 ± 0.9	119.9 ± 35.7**
5	C	0.1 ± 0.1	119.4 ± 13.4	1.95 ± 0.45	5.2 ± 1.3	18.8 ± 5.2	52.4 ± 8.5	11.7 ± 2.8	27.2 ± 4.8	26.5 ± 6.8	9.6 ± 1.2	8.3 ± 0.9	246.1 ± 60.6
	Ag0.3	1.2 ± 0.1***	–	2.37 ± 0.16	5.6 ± 1.6	16.0 ± 3.5	52.4 ± 11.7	17.9 ± 5.5	23.1 ± 2.5	31.1 ± 4.2	7.7 ± 1.8*	7.7 ± 2.3	272.3 ± 19.2
	Ag3	6.6 ± 1.1***	–	3.01 ± 0.39	5.1 ± 1.0	15.6 ± 4.7	49.2 ± 12.4	21.4 ± 3.3**	24.1 ± 7.9	19.2 ± 6.6	5.5 ± 1.2***	5.9 ± 2.2	181.6 ± 41.4*
	Zn10	–	100.6 ±25.2	4.57 ± 0.22	5.2 ± 2.0	16.5 ± 3.4	55.0 ± 6.2	13.1 ± 4.0	35.6 ± 7.5	23.7 ± 7.9	7.5 ± 0.9**	6.2 ± 1.7	225.7 ± 43.8
	Zn100	–	145.4 ± 18.5*	2.44 ± 0.70	3.9 ± 0.8	19.5 ± 2.6	52.0 ± 7.5	17.5 ± 3.2	18.8 ± 6.5	14.9 ± 1.3**	5.5 ± 1.0***	5.5 ± 1.4	172.9 ± 25.6*

Significant difference from the respective controls is indicated by $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. C: control. Numbers in the treatments are the Ag or Zn concentrations used in the exposure treatment (see Table 1).

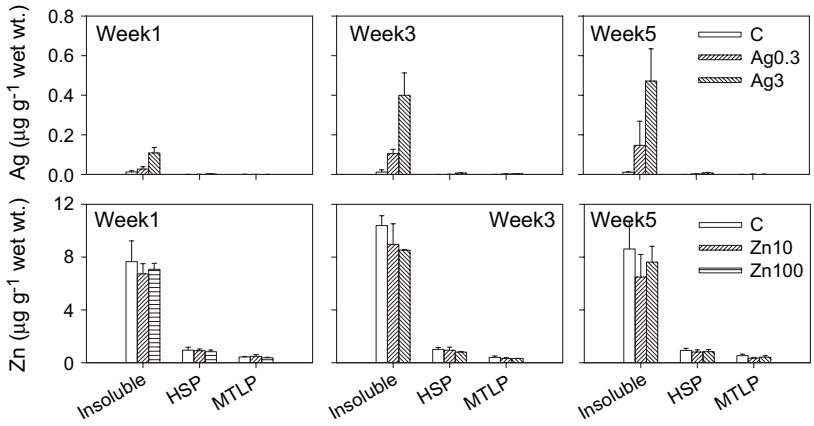

Fig. 1. *Perna viridis*. Concentrations of Ag and Zn in different subcellular fractions of mussels following Ag and Zn pre-exposure. HSP: heat-sensitive protein; MTLP: metallothionein-like protein; insoluble: insoluble fraction including cellular debris, organelles, and metal-rich granules. Data are mean ± SD ($n = 3$). C: control. Numbers in the legends are the Ag or Zn concentrations used in the exposure treatments (see Table 1).

3.2. Mussel's filtration rate (FR), metal assimilation efficiency (AE), and influx rate from the dissolved phase

In Expt. 1, the FR was significantly inhibited ($p < 0.01$) in mussels pre-exposed to 4 (1 week of exposure) and 30 μg Cu L^{-1} (1 and 3 weeks of exposure) (Table 2). Surprisingly, no significant difference in FR was detected in the 15 μg Cu L^{-1} treatment. The FRs of the mussels increased slightly with increasing duration of the pre-exposure. After 5 weeks of pre-exposure, there was no significant difference of the FRs with the controlled mussels (except for 1 μg Cu L^{-1}treatment), indicating that the feeding activity of the mussels had recovered. In Expt. 2, neither the Ag nor the Zn pre-exposure resulted in any significant effect on the mussel's FR over the 5-week exposure period (Table 3). The FRs in this experiment were generally lower than those measured in Expt. 1.

The retention of metals in the mussels following a pulse-chase radioactive feeding is shown in Figs. 2 and 3. The depuration of the unassimilated Cd, Ag and Zn was generally fast within the first 12 h, after which the Cd loss became much slower and somewhat constant while Ag and Zn were continuously lost during the remaining period. The depuration pattern of Hg was different from the other three metals. The initial rapid egestion of Hg occurred within the first 3 h following the radioactive feeding, presumably due to the rapid passage of unassimilated food materials. Subsequently, there was almost no Hg loss from the mussels during 3–24 h, which was immediately followed by a continuous but more gradual loss. The calculated AEs of the four metals are shown in Tables 2 and 3. Among the four metals, the AEs of Hg were the highest and were rather comparable among the different experiments. The AEs of the other 3 metals (Ag, Cd, and Zn) were similar. The AEs of Cd, Hg, or Zn in the mussels were not significantly affected by pre-exposure to the 3 metals (Cu, Ag, or Zn). The Ag AEs increased significantly in mussels pre-exposed to 30 μg Cu L^{-1} at week 3, but the difference with the control was relatively small (Table 2). For mussels pre-exposed to Ag, the AE increased significantly after 1 week of exposure (Table 3). In addition, pre-exposure to 10 μg Zn L^{-1} for 1 week resulted in a significant increase in Ag AE ($p < 0.05$).

The uptake rates of Cd and Zn from solution by the mussels were generally not affected by the Cu pre-exposure (Expt. 1), with one exception (Zn influx after 3 weeks of exposure to 30 μg Cu L^{-1}, Table 2). The influx rates of Hg and Ag were however, reduced by the Cu pre-exposure. Within the first 3 weeks of pre-exposure, the reduction in Hg influx rate was only significant ($p < 0.01$) in mussels in the 30 Cu μg L^{-1} treatment. On week 5, Hg uptake in all the Cu pre-exposed mussels was significantly lower ($p < 0.05$) than the controls. For Ag, its influx was also depressed significantly by the Cu pre-exposure, particularly in the two highest Cu treatments (15 and 30 Cu μg L^{-1}). Such influences were obvious after 1 week of Cu exposure.

When the mussels were pre-exposed to Ag, the Cd influx rate was only significantly ($p < 0.05$) reduced after 1-week exposure to 3 μg Ag L^{-1} (Table 3). The Hg influx rate was generally reduced in the two Ag treatments throughout the 5-week pre-exposure period, and the reduction was statistically significant on week 5. A similar trend was observed for Ag influx, but the difference from the control treatment was not significant. The Zn influx rate decreased significantly in mussels exposed to 3 μg Ag L^{-1} for 5 weeks ($p < 0.05$).

In general, the Cd influx was depressed by the Zn pre-exposure, and this effect was significant in the 10 μg L^{-1} Zn treatment (week 1, $p < 0.01$) and in the 100 μg L^{-1} Zn treatment ($p < 0.01$). Zn pre-exposure also significantly decreased the Hg influx rate on week 5 ($p < 0.001$). Significant influence of the Zn pre-exposure on Zn influx

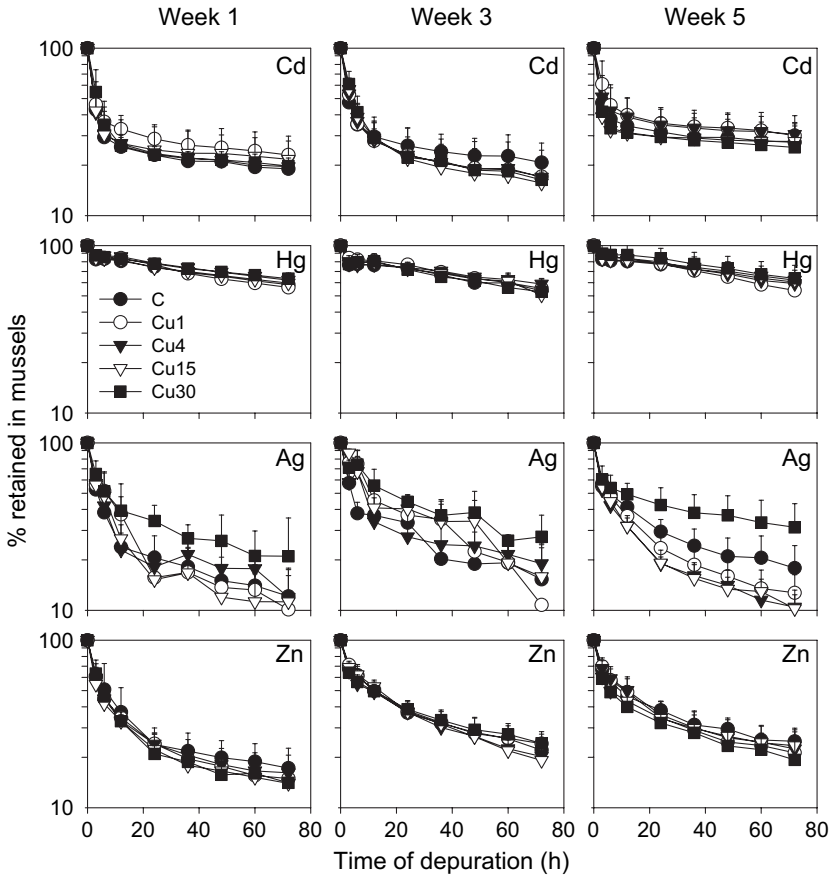

Fig. 2. *Perna viridis*. Retention of four metals in the Cu pre-exposed mussel following a pulse ingestion of the radiolabeled diatoms (*Thalassiosira pseudonana*). Data are mean ± SD ($n = 5$). C: control. Numbers in the legends are the Cu concentrations used in the exposure treatments (see Table 1).

was found in the higher Zn treatment (100 μg L^{-1}) throughout the 5 weeks exposure ($p < 0.05$).

3.3. Relationships between metal bioavailability and metal tissue burden

Since the metal AE and influx rate from the dissolved phase were determined simultaneously in the pre-exposed and the control treatments, the percentages of these values relative to their respective controls were calculated as the index of metal bioavailability (i.e., AE-index and influx rate-index). These indexes were then correlated with the metal concentration in the soft tissues. In Expt. 1, no significant relationship between the metal AE-index and the Cu tissue concentration was detected. In the meantime, the influx rate-index of Cd, Hg, and Ag decreased significantly with increasing Cu tissue concentration (Fig. 4), whereas the Zn influx was not affected by tissue Cu concentration. In Expt. 2, an increase in the AE-index of Ag was significantly correlated with the Ag tissue concentration (Fig. 5), whereas the AE-indexes of Cd, Hg and Zn were not correlated with the Ag tissue concentration. Similarly, the AE-indexes of all four metals were not dependent on the tissue Zn concentrations. The influx rate-index of Ag, Hg, or Zn also decreased significantly with an increase in Ag tissue concentration (Fig. 6). Following Zn pre-exposure, the influx rate-index of Hg and Zn decreased significantly with increasing Zn tissue concentration (Fig. 6). Cd uptake was not correlated with increasing Ag or Zn tissue concentration.

4. Discussion

4.1. Metal bioaccumulation and mussel's filtration activity

A few important parameters critical in determining the metal bioaccumulation in marine mussels were quantified following metal pre-exposure, including the metal assimilation efficiency from the dietary sources, metal influx rate from the dissolved phase, and the filtration rate of the mussels. Such measurements were coupled with the quantification of metal tissue concentration, metal subcellular fractionation for a better interpretation of the influence of metal pre-exposure on metal bioaccumulation. In our experimental exposure, the Cu concentrations encompassed from those typically

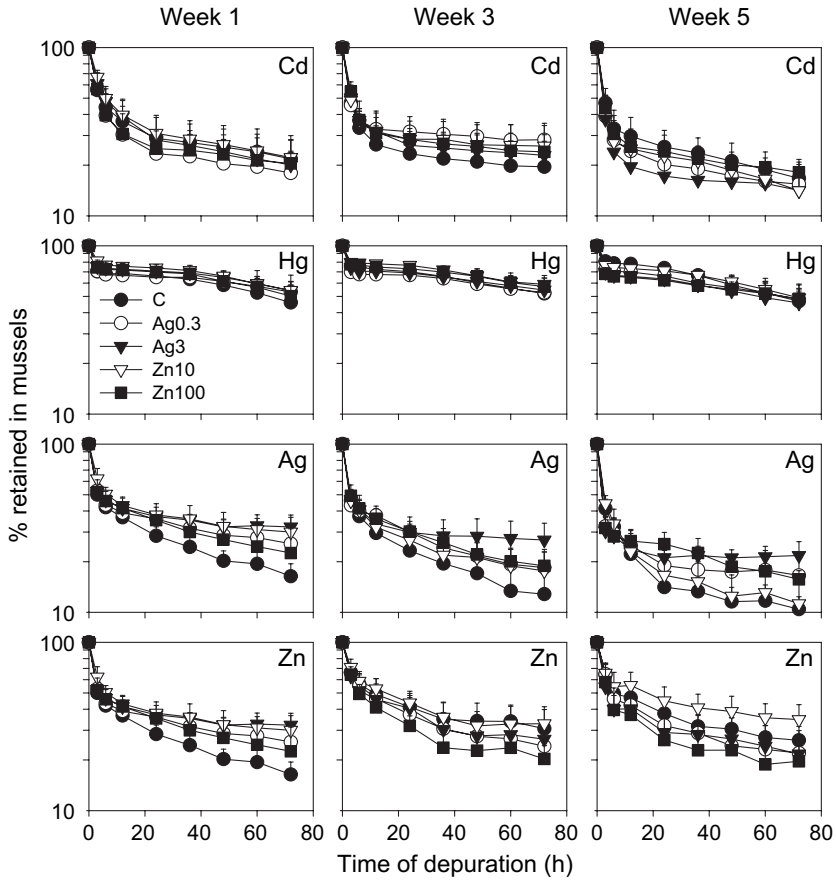

Fig. 3. *Perna viridis*. Retention of four metals in the Ag or Zn pre-exposed mussel following a pulse ingestion of the radiolabeled diatoms (*Thalassiosira pseudonana*). Data are mean ± SD ($n = 5$). C: control. Numbers in the legends are the Ag or Zn concentrations used in the exposure treatments (see Table 1).

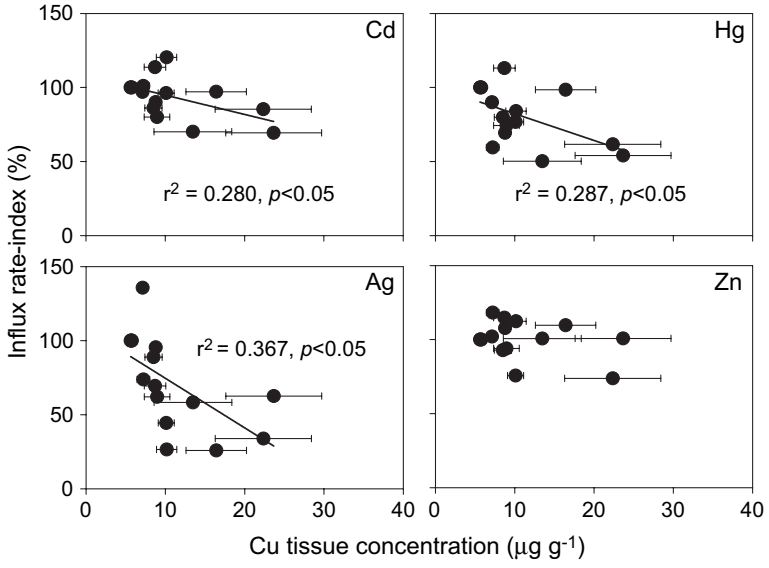

Fig. 4. *Perna viridis*. Relationships between the index of the metal influx rate and the Cu tissue concentration in the mussels following Cu pre-exposure. The index was calculated as the percentage of their respective control values at each week of measurements. Data are mean ± SD ($n = 5$).

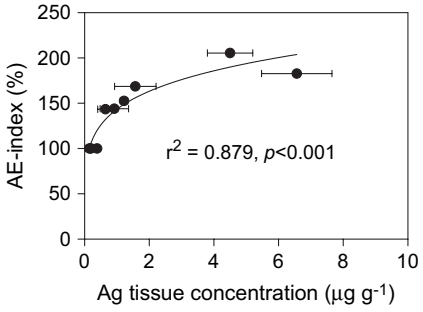

Fig. 5. *Perna viridis*. Relationship between the index of the Ag assimilation efficiency (AE) and the Ag tissue concentration in the mussels following Ag pre-exposure. The index was calculated as the percentage of their respective control values at each week of measurements. Data are mean ± SD (n = 5).

found in Hong Kong coastal waters (23 nM, Wang, W.-X., unpublished data) to relatively high concentrations. The lowest Ag and Zn concentrations used in our exposure were about 5× higher than their typical concentrations in Hong Kong coastal waters. The resulting concentrations of all the three metals in the mussel's tissues were also typical of those found in Hong Kong coastal waters (from relatively clean site to contaminated site, Rainbow and Blackmore, 2001).

Of the 3 metals considered in this study, the green mussels appeared to regulate the tissue Cu and Zn concentrations, consistent with several studies in this species of mussels (Chan, 1988; Blackmore and Wang 2002). Earlier experimental study demonstrated that the mussels were able to regulate the tissue Cu concentration at about 30 μg L^{-1} (Chan, 1988). In our study, there was essentially no increase in the tissue Cu concentrations at the 3 lower Cu ambient concentrations with increasing periods of exposure, suggesting that the mussels were indeed actively regulating their Cu tissue burdens at these dissolved concentrations. At the highest concentrations, there were some increases in the tissue Cu concentrations within the first 3 weeks of exposure, afterward the concentrations were maintained relatively constant. Similarly, the Zn tissue concentrations in the mussels were constant over the 5-week exposure period at 10 μg L^{-1}, but generally increased at 100 μg L^{-1}. These data suggest that both the exposure concentration and period should be considered in examining trace metal regulation in the mussels.

The filtration rates of the bivalves can be employed as an indicator of metal toxicity (Kraak et al., 1994; Naimo, 1995). In the present study, significant reduction in the green mussel's filtration rate was observed during the early stage of Cu pre-exposure (1–3 weeks), suggesting that Cu was toxic to the mussels at these dissolved concentrations. On the other hand, the recovery of the filtration rate following 5 weeks of

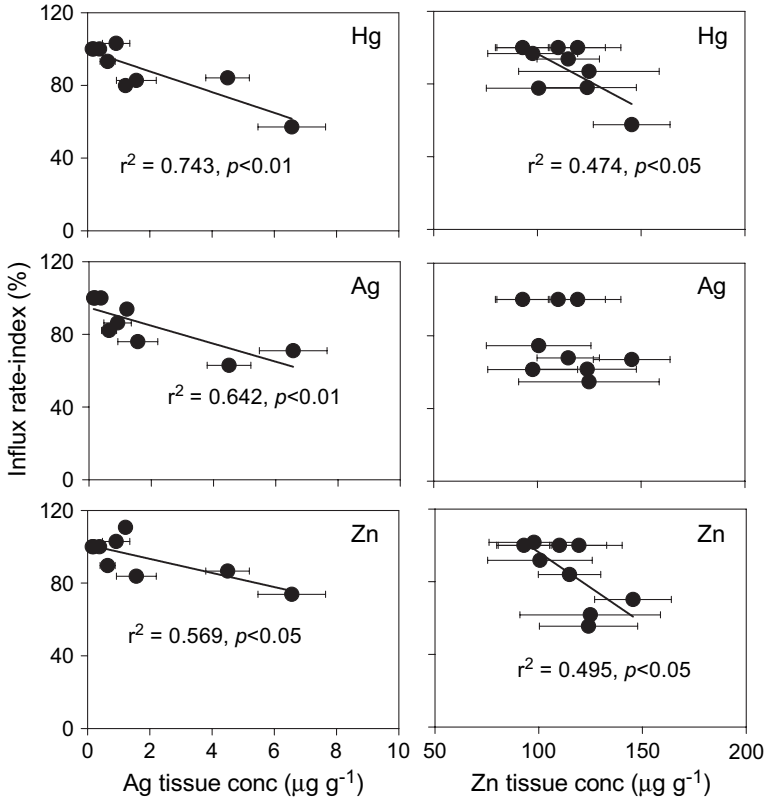

Fig. 6. *Perna viridis*. Relationships between the indexes of metal influx rate and the Ag or Zn tissue concentration in the mussels following Ag or Zn pre-exposure. The index was calculated as the percentage of their respective control values at each week of measurements. Data are mean ± SD (n = 5).

pre-exposure indicates that the mussels may have acclimated to Cu with extended periods of exposure. The filtration rates of all the pre-exposed mussels were indeed higher than the controlled mussels on week 5. We recently found that following Ag pre-exposure, the green mussel's filtration rate was initially depressed with Ag tissue burden $<2.5\ \mu g\ g^{-1}$, but it then recovered when the exposure period was longer (Shi et al., 2003). Similar phenomenon was also observed in mussels pre-exposed to $3\ \mu g\ Ag\ L^{-1}$ in the present study. Since the two exposure experiments (Cu, and Ag/Zn) were conducted at different seasons, the FRs measured in Expt. 2 (Ag/Zn) were also notably lower than those measured in Expt. 1 (Cu). Generally, metal uptake rate quantified after different periods of pre-exposure to different metals was not correlated with the change in FR, consistent with the recent conclusion by Wang (2002).

4.2. Cd accumulation following metal pre-exposure

Our experimental results indicated that the pre-exposure of mussels to Ag, Cu, and Zn typically had a greater influence on metal uptake from the dissolved phase, whereas it had a relatively small effect on metal assimilation from the dietary phase, with an exception of Ag AE after Ag pre-exposure (Table 4). The metal AEs were all comparable to the unexposed mussels over different periods of pre-exposure. In contrast, our recent experiment found a significant influence of Cd pre-exposure on the Cd assimilation. One of the possible explanations for the increase in Cd AE was the significant induction of MT by exposure to Cd, which results in a stronger Cd sequestration from the dietary source. Among the 3 metals (Ag, Cu, and Zn) examined in this study, only Cu was found to slightly increase the MT production, but such induction was much less notable than those found for Cd exposure.

In the present study, the Cd uptake from both solution and diet by the green mussels was generally unaffected by Cu pre-exposure, even though there was a slight induction of metallothionein (MT). However, there was a significant negative relationship between the dissolved Cd uptake and the Cu tissue concentration when all data were pooled together, suggesting that the pre-exposure history played an important role in metal accumulation. Although Cd and Cu can both induce MT in the marine mussels (George and Olsson, 1994; Langston et al., 1998), the forms of MT with which the two metals reacted (induction and binding) were rather different (Ivankovic et al., 2002). For example, the prevailing part of the total Cd-MT occurred in the dimeric form, i.e., MT-20 in the Cd-exposed mussel *M. edulis* (Frazier et al., 1985; Mackay et al., 1993). In contrast, the MT-10 component was predominantly expressed by Cu exposure in the mussel *Mytilus galloprovincialis* (Viarengo et al., 1984). The differential binding with different forms of MT may thus explain the lack of influences of Cu pre-exposure on Cd bioaccumulation in the mussels. In addition to Cu, Ag pre-exposure did not affect the dissolved (except at $3\ \mu g\ L^{-1}$ for 1 week) and dietary Cd uptake. It has been suggested that Ag may mainly induce and bind with the insoluble compounds such as the sulfide complex instead of the metallothionein-like protein (MTLP) in the mussels (Shi et al., 2003). Our present measurements further confirmed that the majority of Ag was indeed associated with the insoluble fraction and there was negligible Ag in the MTLP or HSP fractions. Therefore, differences in binding and storage resulted in the relative independent accumulation of Ag and Cd in the mussels. In contrast to Cu and Ag pre-exposure, pre-exposure to Zn resulted in a significant depression of Cd influx from solution, consistent with a recent study by Blackmore and Wang (2002). One possibility for the reduction in Cd influx was the regulation of Zn uptake by the mussels following Zn pre-exposure, leading to a simultaneous inhibition on Cd uptake due to the chemical similarity of these two metals. Our data also demonstrated that there was a significant coupling between the influxes of these two metals in the mussels (data not shown), thus their transport across the mussel tissues may share similar pathways. However, Cd uptake was not related to Zn body concentration, presumably because Zn was actively regulated by the mussels during the exposure period. Under such circumstance, the exposure concentration was more important in controlling Cd uptake than the body concentration.

4.3. Hg accumulation following metal pre-exposure

The assimilation of Hg in the mussels was generally little disturbed by the pre-exposure to all three metals (Ag, Cu, and Zn), whereas its dissolved uptake rate was negatively impacted by the metal pre-exposure. The

Table 4
Summary of the influences of metal pre-exposure on metal accumulation in marine green mussels *Perna viridis*

Pre-exposure	Assimilation	Dissolved uptake	References
Cd	Increase: Cd; no influence: Hg, Ag, Zn	Decrease: Hg; no influence: Cd, Ag, Zn	Blackmore and Wang (2002), Shi & Wang (unpublished)
Cu	Increase: Ag ; no influence: Cd, Hg, Zn	Decrease: Hg, Ag; no influence: Cd, Zn	This study
Ag	Increase: Ag; no influence: Cd, Hg, Zn	Decrease: Cd, Hg, Ag, Zn. Increase: Ag	This study, Shi et al. (2003)
Zn	No influence: Ag, Cd, Hg, Zn	Decrease: Cd, Hg, Ag, Zn	This study, Blackmore and Wang (2002)

digestive behavior of Hg appeared to be in strong contrast with other metals. Following the initial 3 h of digestion, the Hg was very efficiently retained in the mussels for one day before a notable depuration emerged again. In contrast, the digestion of the other metals was a more or less continuous process. A few previous studies have demonstrated that the exposure of the mussel *M. edulis* to Cu, Cd, and Zn resulted in an enhanced tolerance to Hg toxicity due to the increased levels of MT following the metal pre-exposure (Roesijadi et al., 1982; Roesijadi and Fellingham, 1987). It remains unknown from these previous studies whether or not the decrease in Hg uptake in addition to the detoxification by MT can serve as another potential mechanism for the decrease in Hg toxicity. In clam *Macoma balthica*, Boisson et al. (1998) reported that the lower Hg uptake rate from a metal (including Ag, Cd, Cu, and Zn) polluted estuary as compared to clams from a relatively uncontaminated estuary may be the result of adaptive trait for survival. In a recent study, we also found that the Hg dissolved influx rate of the green mussels decreased significantly following the pre-exposure to 0.1–20 $\mu g\,L^{-1}$ Cd for 5 weeks (Shi and Wang, unpublished dat). Therefore, it is likely that the enhanced tolerance to Hg toxicity following metal pre-exposure observed in previous studies may be partially caused by the reduction of Hg uptake. Nevertheless, the mechanisms underlying the significant reduction in Hg uptake need to be further illustrated. Any change in FR (such as the reduction by Cu exposure) did not account for the decrease in Hg uptake by the mussels.

4.4. Ag accumulation following metal pre-exposure

Ag uptake from the dissolved phase was substantially inhibited by Cu pre-exposure, e.g., a 4× decrease after 1 week exposure to 30 $\mu g\,L^{-1}$. The potential interaction between Ag and Cu has been previously investigated (e.g., Ettajani et al., 1992). For example, an increase of tissue Cu concentration was observed in the mussel *M. edulis* exposed to elevated levels of Ag, presumably because of the involvement of a (Ag, Cu)-binding protein (Calabrese et al., 1984; Geroge et al., 1986). One possible explanation for the presence of this protein is the induction by Cu exposure, followed by partial displacement of this metal by Ag (George et al., 1986). In the oyster *Crassostrea gigas*, it has been suggested that Cu may occupy the majority of available binding sites common to both Ag and Cu, thus only a few sites may be available for additional binding with Ag (Ettajani et al., 1992). Consequently, a decrease in Ag influx by Cu pre-exposed mussels may result from the saturation of binding sites by Cu.

The observed patterns of the variations in Ag AE and dissolved uptake following Ag pre-exposure in the present study agreed well with our previous study, which indicated that the induction of sulfide by Ag pre-exposure played an important role in subsequent Ag bioaccumulation from both dissolved and food pathways (Shi et al., 2003). Our present study further demonstrated that Ag concentration in the insoluble fraction of mussel's tissue increased significantly following Ag pre-exposure. The predominance of Ag binding with the insoluble fraction (e.g., sulfide) may account for the increase in Ag AE from the ingested diatoms. Shi et al (2003) indicated that the influx rate of Ag decreased at Ag tissue concentration of 2.5 $\mu g\,g^{-1}$, beyond which the dissolved uptake increased. Consistent with this previous study, we also observed that the Ag influx decreased at an Ag tissue concentration of 4 $\mu g\,g^{-1}$, beyond which the influx appeared to increase again (albeit only one tissue concentration was examined above 4 $\mu g\,g^{-1}$).

4.5. Zn accumulation following metal pre-exposure

The Zn AE in the green mussels was generally unaffected by pre-exposure to Cu, Ag and Zn, but its dissolved uptake decreased following Ag and Zn pre-exposure. The lack of influences of Cu pre-exposure on Zn bioaccumulation further indicates that the Zn uptake was probably not related to MT induction by Cd or Cu (Blackmore and Wang, 2002). However, it is also possible that the binding affinity of Zn with MT was weaker than those of Cd and Cu, thus its bioaccumulation was not affected by Cd- or Cu-induced MT. George and Olsson (1994) also found that the MT measurements may not be appropriately used as an indicator of Zn exposure in the common mussels. In our study, the majority of accumulated Zn was indeed distributed in the insoluble fraction, whereas only a small fraction was found in the HSP and MTLP fractions. A depression of Zn influx rate from solution has been previously reported by Blackmore and Wang (2002), which suggested that the mussels were able to regulate Zn by reducing its influx from the dissolved phase.

Based on the results obtained from this study, it appears that the potential biochemical or physiological changes caused by Cu, Ag, and Zn pre-exposure in the green mussel and their subsequent influences on the bioaccumulation of Cd, Hg, Ag, and Zn were less related to the induction of MT. Moreover, both Cu and Ag are one of the most toxic metals and reduced the filtration rates of mussels over 1–5 weeks of exposure. However, physiological processes such as the pumping activity of the mussels may not be responsible for the observed decrease in metal influx following Cu and Ag pre-exposure. Saturation of binding sites by metal pre-exposure resulting in less sites available for metal influx may be an important mechanism underlying the decrease in metal influx. Zn is not a strong MT inducer and most Zn were associated with the metal-rich

granules when it is accumulated (Blackmore and Wang, 2002). Therefore, the control of Zn pre-exposure on metal uptake (if any) may be principally related to the regulation of its uptake by the mussels.

Our study indicates that the pre-exposure of mussels to one specific metal may bring considerable influences to the bioaccumulation of other metals. Aqueous uptake was more influenced by metal pre-exposure than the dietary assimilation, thus it is likely that the relative significance of different exposure pathways of metals may be modified by pre-exposure to different metals. Therefore, the pre-exposure history should be fully taken into account when using the observed metal tissues to refer to metal pollution, particularly in the fields where multiple metal contaminations co-occur.

Acknowledgement

We thank the anonymous reviewers for their very constructive comments on this work. This study was supported by a Competitive Earmarked Research Grant from the Hong Kong Research Grants Council (HKUST6097/02M) to W.-X. Wang.

References

Amiard-Triquet, C., Amiard, J.C., 1998. Influence of ecological factors on accumulation of metal mixtures. In: Langston, W.J., Bebianno, M.J. (Eds.), Metal Metabolism in Aquatic Environments. Chapman and Hall, London, pp. 351–386.

Blackmore, G., Rainbow, P.S., 2001. Biomonitoring of spatial and temporal patterns of trace metal bioavailabilities. In: Rainbow, P.S., Hopkin, S.P., Crane, M., (Eds.), Forecasting the Environmental Fate and Effects of Chemicals. Wiley, London, pp. 191–209.

Blackmore, G., Wang, W.-X., 2002. Uptake and efflux of Cd and Zn by the green mussel *P. viridis* after metal pre-exposure. Environmental Science and Technology 36, 989–995.

Blackmore, G., Wang, W.-X., 2003. Inter-population differences in Cd, Cr, Se, and Zn accumulation by the green mussel *Perna viridis* acclimated at different salinities. Aquatic Toxicology 62, 205–218.

Boisson, F., Hartl, M.G.J., Fowler, S.W., Amiard-Triquet, C., 1998. Influence of chronic exposure to silver and mercury in the field on the bioaccumulation potential of the bivalve *Macoma balthica*. Marine Environmental Research 45, 325–340.

Calabrese, A., MacInnes, J.R., Nelson, D.A., Greig, R.A., Yevich, P.P., 1984. Effects of long-term exposure to silver or copper on growth, bioaccumulation and histopathology in the blue mussel *Mytilus edulis*. Marine Environmental Research 11, 253–274.

Chan, H.M., 1988. Accumulation and tolerance to cadmium, copper, lead and zinc by the green mussel *Perna viridis*. Marine Ecology Progress Series 48, 295–303.

Chong, K., Wang, W.-X., 2000. Assimilation of cadmium, chromium, and zinc by the green mussel *Perna viridis* and the clam *Ruditapes philippinarum*. Environmental Toxicology and Chemistry 19, 1660–1667.

Chong, K., Wang, W.-X., 2001. Comparative studies on the biokinetics of Cd, Cr, and Zn in the green mussel *Perna viridis* and the Manila clam *Ruditapes philippinarum*. Environmental Pollution 115, 107–121.

Ettajani, H., Amiard-Triquet, C., Amiard, J.-C., 1992. Etude expérimentale du transfer de deux elements traces (Ag, Cu) dans une chaîne trophique marine: eau—particules (sédiment natural, microalgue)—mollusques filtreurs (*Crassostrea gigas* Thunberg). Water Air Soil Pollution 65, 215–236.

Fraysse, B., Baudin, J.-P., Garnuer-Laplace, J., Adam, C., Boudou, A., 2002. Effects of Cd and Zn waterborne exposure on the uptake and depuration of ^{57}Co, ^{110m}Ag, and ^{134}Cs by the Asiatic clam (*Corbicula fluminea*) and the zebra mussel (*Dreissena polymorpha*)—whole organism study. Environmental Pollution 118, 297–306.

Frazier, J.M., George, S.G., Overnell, J., Coombs, T.L., Kägi, J.H.R., 1985. Characterization of two molecular weight classes of cadmium-binding proteins from the mussel *Mytilus edulis* (L). Comparative Biochemistry and Physiology 80C, 257–262.

George, S.G., Olsson, P.-E., 1994. Metallothioneins as indicators of trace metal pollution. In: Kramer, K.J.M. (Ed.), Biomonitoring of Coastal Waters and Estuaries. CRC Press, Boca Raton, FL, USA, pp. 151–178.

George, S.G., Pirie, B.J.S., Calabrese, A., Nelson, D.A., 1986. Biochemical and ultrastructural observations of long-term silver accumulation in the mussel, *Mytilus edulis*. Marine Environmental Research 18, 255–265.

Ivankovic, D., Pavicic, J., Kozar, S., Raspor, B., 2002. Multiple forms of metallothionein from the digestive gland of naturally occurring and cadmium-exposed mussels, *Mytilus galloprovincialis*. Helgoland Marine Research 56, 95–101.

Kraak, M.H.S., Wink, Y.A., Stuijfzand, S.C., Buckert-de Jong, M.C., de Groot, C.J., Admiraal, W., 1994. Chronic ecotoxicity of Zn and Pb to the zebra mussel *Dreissena polymorpha*. Aquatic Toxicology 30, 77–89.

Langston, W.J., Bebianno, M.J., Burt, G.R., 1998. Metal handling strategies in molluscs. In: Langston, W.J., Bebianno, M.J. (Eds.), Metal Metabolism in Aquatic Environments. Chapman and Hall, London, pp. 219–283.

Leung, K.M.Y., Furness, R.W., 1999. Induction of metallothionein in dogwhelk *Nucella lapillus* during and after exposure to cadmium. Ecotoxicology Environmental Safety 43, 156–164.

Mackay, E.A., Overnell, J., Dunbar, B., Davidson, I., Hunziker, P.E., Kägi, J.H.R., Fothergill, J.E., 1993. Complete amino acid sequences of five dimeric and four monomeric forms of metallothionein from the edible mussel *Mytilus edulis*. European Journal of Biochemistry 218, 183–194.

Naimo, T.J., 1995. A review of the effects of heavy metals on freshwater mussels. Ecotoxicology 4, 341–362.

Ng, T.Y.T., Wang, W.X., 2004. Detoxification and effects of Ag, Cd, and Zn pre-exposure on metal uptake kinetics in the clam *Ruditapes philippinarum*. Marine Ecology Progress Series 268, 161–172.

Phillips, D.J.H., Rainbow, P.S., 1988. Barnacles and mussels as biomonitors of trace elements: a comparative study. Marine Ecology Progress Series 49, 83–93.

Popham, J.D., D'Auria, J.M., 1982. Effects of season and seawater concentrations on trace metal concentrations in organs of *Mytilus edulis*. Archives of Environmental Contamination and Toxicology 11, 273–282.

Rainbow, P.S., Blackmore, G., 2001. Barnacles as biomonitors of trace metal availabilities in Hong Kong coastal waters: change in space and time. Marine Environmental Research 51, 441–463.

Roesijadi, G., Drum, A.S., Thomas, J.T., Fellingham, G.W., 1982. Enhanced mercury tolerance in marine mussels and relationship to low molecular weight, mercury-binding proteins. Marine Pollution Bulletin 13, 250–253.

Roesijadi, G., Fellingham, G.W., 1987. Influence of Cu, Cd, and Zn preexposure on Hg toxicity in the mussel *Mytilus edulis*. Canadian Journal of Fisheries and Aquatic Sciences 44, 680–684.

Scheuhammer, A.M., Cherain, M.G., 1986. Quantification of metallothioneins by a silver-saturation method. Toxicology and Applied Pharmacology 82, 417–425.

Scheuhammer, A.M., Cherain, M.G., 1991. Quantification of metallothionein by silver saturation. Methods in Enzymology 25, 78–83.

Shi, D., Blackmore, G., Wang, W.-X., 2003. Effects of aqueous and dietary pre-exposure and resulting tissue concentration on silver biokinetics in the green mussel *Perna viridis*. Environmental Science and Technology 37, 936–943.

Viarengo, A., Pertica, M., Mancinelli, G., Zanicchi, G., Bouqueganeau, J.M., Orunesu, M., 1984. Biochemical characterization of copper-thioneins isolated from the tissues of mussels exposed to the metal. Molecular Physiology 5, 41–52.

Wallace, W.G., Lee, B.G., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). Marine Ecology Progress Series 249, 183–197.

Wang, W.-X., 2001. Comparison of metal uptake rate and absorption efficiency in marine bivalves. Environmental Toxicology and Chemistry 20, 1367–1373.

Wang, W.-X., 2002. Interaction of trace metals and different marine food chains. Marine Ecology Progress Series 243, 295–309.

Wang, W.-X., 2003. Metal bioaccumulation in bivalve mollusks: recent progress. In: Villalba, A., Reguera, B., Romalde, J.L., Beiras, R. (Eds.), Molluscan Shellfish Safety, Intergovernmental Oceanographic Commission of UNESCO and Conselleria de Pesca e Asuntos Maritimos da Xunta de Galicia. Santiago de Compostela, Spain, pp. 503–520.

Wang, W.-X., Fisher, N.S., 1996. Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: effects of food composition. Limnology and Oceanography 41, 197–207.

Wang, W.-X., Fisher, N.S., 1999. Assimilation efficiency of chemical contamination in aquatic invertebrates: a synthesis. Environmental Toxicology and Chemistry 18, 2034–2045.