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Uptake of Aqueous and Dietary Metals by Mussel *Perna viridis* with Different Cd Exposure Histories

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The influences of different Cd pre-exposure regimes (route, concentration, and duration of Cd exposure) on the bioavailability of Cd, Ag, Hg, and Zn to the green mussels *Perna viridis* were quantified in this study. Following pre-exposing the mussels to Cd, we measured the mussel's tissue Cd concentration and clearance rate, as well as the metal dietary assimilation efficiency (AE) and the influx rate from the dissolved phase of the four studied metals. Differences in the route (aqueous and dietary pathways) and the history of pre-exposure (combined Cd concentration and duration) did not significantly affect the subsequent Cd dietary and aqueous uptake. The Cd dietary AEs increased following both the dissolved and dietary Cd pre-exposure. There was a significant correlation between the Cd AE and the accumulated Cd body concentration in the mussels. Dietary assimilation of Hg and Zn also increased slightly (but not significantly) after Cd pre-exposure, but the AEs of Ag remained constant. Except for the significant decrease in the dissolved uptake of Hg, Cd pre-exposure did not apparently affect the uptake of the other three metals from the solution. Metal−metal interactions are likely to be affected by the specificity of metallothionein induction. Our study demonstrated that the Cd body concentration as well as the environmental Cd concentration instead of the history of pre-exposure was more important in affecting the Cd accumulation in the mussels. Such factors need to be considered in interpreting metal body concentrations in biomonitors.

Introduction

The influences of past history of metal exposure (i.e., pre-exposure) on metal bioaccumulation in marine invertebrates have received increasing attention (*1*−*4*). Recent laboratory and field studies both suggest that biochemical or physiological processes induced by ambient metal stress may lead to variations in metal concentrations in these monitoring organisms. For example, the induction of sulfide by the pre-exposure of the green mussel *Perna viridis* to Ag resulted in an elevation of Ag dietary assimilation (*5*). A higher level of metallothionein (MT), a low molecular weight and heat-stable metal-binding protein (*6*, *7*), was correlated with a greater Cd tissue concentration in the marine clams *Mactra veneriformis* and *Ruditapes philippinarum* collected from a Cd-contaminated bay (*8*). Mouneyrac et al. (*9*) also observed a significant relationship between Cd and metallothionein-like protein (MTLP) concentrations in the clam *Macoma balthica* originating from estuaries with and without industrial influences (but the levels of contaminants in the water were not quantified). These studies strongly suggest that metal pre-exposure and the corresponding biological responses should be cautiously taken into account when using biomonitoring data to evaluate metal pollution in coastal seawater.

The relationships between Cd exposure and metal body concentration as well as the induction of MT have been extensively studied in marine invertebrates (*9*−*12*). Nevertheless, the link between Cd pre-exposure and the resulting body concentration to subsequent metal accumulation in aquatic organisms is just starting to be understood. In the green mussel *P. viridis*, Blackmore and Wang (*13*) tested the Cd dietary assimilation and influx from the dissolved phase by pre-exposure to different levels of dissolved Cd. Their results demonstrated that Cd pre-exposure significantly increased the Cd assimilation efficiency (AE) but not the dissolved uptake rate, accompanied by an increase in the Cd soft tissue concentration and an increasing percentage of Cd distributed in MTLP. Dietary Cd pre-exposure was not considered in that study, but a kinetic modeling study indicated that dissolved and dietary exposures were equally important in the overall Cd bioaccumulation in *P. viridis* (*14*). It is thus necessary to compare the influences of aqueous versus dietary Cd pre-exposure on subsequent metal accumulation.

In natural environments, it is possible that the same metal tissue concentrations are the result of different exposure regimes (different ambient metal concentrations and durations of exposure). Whether or not the history or regime of pre-exposure instead of the metal body concentration will lead to physiological and biochemical modifications and thus to changes in metal bioaccumulation is rather speculative. Furthermore, aquatic animals are simultaneously exposed to a variety of metals under natural conditions. Thus, the influence of pre-exposure to one or more metals on the biological uptake of other metal may occur. This aspect remains essentially unstudied at present (*15*). Roesijadi and Fellingham (*16*) found that pre-exposure of the mussel *Mytilus edulis* to Cd or Cu (but not Zn) resulted in an increased tolerance to subsequent Hg(II) exposure because of the significant induction of gill MT, which bound with Hg to decrease its toxicity. The bioavailability of Hg to the mussels following the metal pre-exposures was not quantified in that particular study.

The green mussel *P. viridis* is a widely distributed biomonitor of metal pollution in tropical and subtropical seawater (*17*). In the present study, a series of pre-exposure experiments were conducted to address (1) if Cd accumulation in the mussels was influenced by the route of Cd pre-exposure (dietary vs aqueous); (2) if different exposure regimes resulting in comparable Cd body concentrations affected subsequent Cd bioaccumulation; (3) if Cd pre-exposure affected the bioaccumulation of other metals (Zn, Ag, and Hg) to the mussels. The Cd concentrations used in this study ranged from relatively low (typically coastal) concentrations to high Cd concentrations. We considered Cd, Zn, Ag, and Hg in this study largely because of their similarity in preferentially binding with sulfur-containing ligands (except for Zn). Following different Cd pre-exposures, the metal AE, dissolved uptake rate, mussel's clearance rate, and Cd body concentration were determined to examine the influences of Cd pre-exposure on subsequent metal bioaccumulation.

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TABLE 1. Nominal Concentrations of Cd and Exposure Durations Used in Different Pre-Exposure Experiments on the Green Mussel *Perna viridis*

expt	duration (weeks)	Cd concentration (μg L^{-1})			
1. dissolved and dietary Cd pre-exposure	1, 3, 5	D10[a]	F100[b]		
2. different Cd pre-exposure regimes	5	D2			
	3	D3.3			
	1	D10			
3. influences of Cd pre-exposure on uptake of other metals	5	D0.1	D1	D5	D20

[a] D: dissolved exposure; the mussels were directly exposed to Cd in the seawater. [b] F: food exposure; the diatom food *Thalassiosira pseudonana* was exposed to Cd in the growth medium before being fed to the mussels.

Materials and Methods

Mussels and Experimental Designs. Green mussels *Perna viridis* (shell length 3.0−3.5 cm, tissue dry weight ~0.15 g) were collected from Wu Kai Sha, Tolo Harbor (experiments 1 and 3, see below) and the New Airport, Lantau Island (experiment 2) at different times. Mussels were collected from the New Airport (experiment 2) due to the unavailability of mussels from Tolo Harbor when the experiments were conducted. Typical salinity in Tolo Harbor was 27−30 psu (practical salinity units), whereas the salinity in the New Airport, which received a significant influence from the Pearl River Estuary, was 15 psu. A recent study found that the ambient dissolved Cd concentrations in the New Airport site were 50−73 ng/L, as compared to 16−35 ng/L in the Tolo Harbor (*18*). The mussels were transported to the laboratory immediately after collection. Throughout the whole period of the experiments, they were maintained in natural coastal seawater (Clear Water Bay) at 23 °C and 30 psu and fed the diatom *Thalassiosira pseudonana* (clone 3H) at a ratio 1−2% of tissue dry weight per day. Mussels collected from the New Airport site were acclimated in a stepwise manner to this salinity over a period of 2 weeks. Three independent experiments were then conducted by exposing the mussels separately to different levels of dissolved (as $CdCl_2$) or dietary Cd for different durations (Table 1) as described below.

Experiment 1: Dissolved versus Dietary Pre-Exposure. In this experiment, we compared the effects of dissolved versus dietary Cd pre-exposure on subsequent Cd accumulation by green mussels. For the dissolved pre-exposure, on a daily basis, mussels were maintained in 10 μg L^{-1} Cd-spiked seawater for 18 h and fed the diatom *T. pseudonana* in unspiked seawater for the remaining 6 h. In the dietary exposure, mussels were placed in clean seawater and fed with Cd-enriched diatoms (300 μg Cd g^{-1}) for 4 h each day. These diatom cells had been previously spiked with Cd (100 μg L^{-1}) for 4 days and then filtered from the growth medium, rinsed with filtered seawater, and finally resuspended in filtered water before being fed to the mussels. Our preliminary experiments indicated that within this short feeding period (4 h), most Cd (>80%) remained in the diatoms. Cd uptake by the mussels in the dietary exposure treatment was thus assumed to derive mainly from the food phase. Diatoms were spiked with a relatively high Cd concentration (100 μg L^{-1}) such that tissue Cd concentrations in the mussels were comparable between the aqueous and dietary treatments after 5 weeks of pre-exposure. Bioavailability measurements of Cd were made after exposing the mussels for 1, 3, and 5 weeks.

Experiment 2: Different Combined Concentration and Duration Pre-Exposure. We examined the influence of comparable Cd body concentrations derived from different exposure regimes on the assimilation of Cd. The mussels were exposed to dissolved Cd with combined concentration and duration, to end up with comparable Cd tissue body concentrations among the different treatments. Three treatments were achieved in this experiment, i.e., 10 μg L^{-1} × 1 week, 3.3 μg L^{-1} × 3 weeks, and 2 μg L^{-1} × 5 weeks. Exposure was conducted at different times so that the bioavailability measurements were carried out simultaneously.

Experiment 3: Influences of Dissolved Cd Pre-Exposure on Other Metals. This experiment was further designed to test whether Cd pre-exposure influenced uptake of Ag, Hg, and Zn. The mussels were exposed to dissolved Cd for 5 weeks at four different levels, i.e., 0.1, 1, 5, and 20 μg L^{-1}. Afterward, uptakes of Cd, Zn, Ag, and Hg by the mussels were determined simultaneously.

Besides the Cd treatments, each of the three independent experiments involved a control treatment, in which neither the seawater nor the food was Cd-enriched. Bioavailability measurements in the control were conducted concurrently with those performed in the Cd treatments. To keep the nominal metal concentrations relatively constant during the pre-exposure period and good water quality, the seawater in all exposure treatments was renewed every other day.

Cd Concentration in Soft Tissues. To determine the Cd concentration in soft tissues following pre-exposure, the tissues were dried in glass tubes at 80 °C until a constant weight was reached. There were five replicates for each group. All the dried samples were digested using concentrated Ultrapure HNO_3 (69%, Aristar grade BDH Ltd.). Afterward, the digests were analyzed by inductively coupled plasma-mass spectroscopy (ICP-MS) (Perkin-Elmer, Elan 6000). Standard oyster tissues were concurrently digested and quantified for Cd concentrations, and agreement was within 10%. The soft tissue Cd concentration was expressed as the μg g^{-1} dry weight.

Metal Assimilation Efficiency from Food. The pulse−chase feeding technique was employed to quantify the metal assimilation efficiency (AE), as described by Chong and Wang (*19*). Briefly, diatoms *T. pseudonana*, cultured in f/2 levels for N, P, Si, and vitamins, and f/20 levels for trace elements minus Zn, Cu, and EDTA, were radiolabeled with ^{109}Cd (for all experiments), ^{65}Zn + ^{110m}Ag + $^{203}Hg(II)$ (for experiment 3) for 4 days. These radiolabeled diatoms were collected by filtering through a 3 μm polycarbonate membrane followed by resuspension in 0.22 μm filtered seawater. To minimize desorption of loosely labeled radiotracers from diatoms to water during subsequent feeding periods, filtration and resuspension were repeated one more time. In each treatment, the mussels were placed in polypropylene beakers containing 500 mL of seawater and allowed to open and pump normally (within 10 min). Afterward, the radiolabeled diatoms were added into the water giving a cell density of 4−5 × 10^4 cells mL^{-1}, which was maintained relatively constant by further additions at 10 min intervals. The mussels were removed after 30 min of feeding and radioassayed after being rinsed with seawater. From each group, five individuals were transferred into individual polypropylene beakers (180 mL seawater) held in a 10 L enclosed recirculating flow-through aerated seawater aquarium, to depurate the ingested radiotracers for 72 h. During the depuration period, non-radioactive *T. pseudonana* was fed twice daily at a ratio of 1−2% dry weight per day, and the radioactivity retained in the mussels was assayed at intervals from 3 to 12 h. Feces were collected frequently (i.e., every 0.5−1.0 h within the first 12 h and then every 6−10 h afterward) throughout the depuration period. The metal AE was calculated as the

TABLE 2. Cd Body Concentration (μg g^{-1}, Mean ± SD; n = 5), Cd Dietary Assimilation Efficiency (AE, %, Mean ± SD; n = 5), Cd Influx Rate from the Dissolved Phase (ng g^{-1} h^{-1}, Mean ± SD; n = 8), and Clearance Rate (CR, L g^{-1} h^{-1}, Mean ± SD; n = 8) in the Green Mussel *Perna viridis* Following Cd Pre-Exposure (Experiments 1 and 2)[a]

expt	pre-exposure duration	pre-treatment[b]	Cd concentration	Cd dietary AE	Cd dissolved influx	CR
1	1	C	0.5 ± 0.2	18.6 ± 4.1	23.0 ± 6.6	14.6 ± 2.3
		D10	2.8 ± 0.5**	32.0 ± 5.5**	23.4 ± 8.1	16.2 ± 4.5
		F100	1.1 ± 0.2**	27.7 ± 8.1	22.8 ± 6.0	12.4 ± 4.7
	3	C	0.5 ± 0.1	20.4 ± 4.4	27.2 ± 7.7	14.6 ± 5.0
		D10	7.5 ± 2.5**	34.9 ± 3.2**	32.5 ± 7.7	15.9 ± 4.8
		F100	4.0 ± 0.5**	37.5 ± 11.7**	31.0 ± 5.4	13.2 ± 3.4
	5	C	0.4 ± 0.1	16.6 ± 11.7	32.1 ± 11.2	10.7 ± 3.7
		D10	10.8 ± 2.3**	42.1 ± 9.1**	31.7 ± 2.3	13.5 ± 5.2
		F100	14.0 ± 3.6**	45.0 ± 9.7**	25.2 ± 5.3	17.6 ± 5.5*
2	5	C	2.0 ± 0.4	14.7 ± 2.7	ND[c]	ND
	5	D2	5.2 ± 1.2**	33.1 ± 4.6**	ND	ND
	3	D3.3	5.7 ± 1.3**	30.2 ± 8.0**	ND	ND
	1	D10	4.6 ± 0.6**	27.5 ± 3.0**	ND	ND

[a] Significant difference from the control treatment is indicated by *, $p < 0.05$, and **, $p < 0.01$. [b] See Table 1 for details. [c] ND: not determined.

percentage of the initial ingested metals remaining in the mussels following 60 h of depuration.

Metal Influx Rate from the Dissolved Phase. Eight mussels from each group were carefully cleaned to remove any particles on the shells and then placed in filtered (0.22 μm) seawater. Filtered seawater was spiked with ^{109}Cd (3.7 kBq L^{-1}, corresponding to 0.3 nM, for all experiments), ^{65}Zn (9.25 kBq L^{-1}, corresponding to 0.06 nM) + ^{110m}Ag (3.7 kBq L^{-1}, 0.3 nM) + ^{203}Hg (1.48 kBq L^{-1}, 0.3 nM, for experiment 3), as well as stable metals Cd (as $CdCl_2$, 17.8 nM) and Zn (as $ZnCl_2$, 76.9 nM) and allowed to equilibrate for 24 h prior to experiments. Stable Cd and Zn were spiked to elevate background Cd and Zn concentrations by about 5−20 times, whereas spikes of radioactive ^{110m}Ag and ^{203}Hg elevated the total Ag and Hg concentrations by 10−30 times. Mussels were then put individually in 200 mL of the radioactive filtered seawater for 1 h. Following the 1 h 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 then calculated.

Mussel's Clearance Rate. From each group, eight mussels were placed individually in polypropylene beakers containing 1.5 L of glass-fiber-filtered (GF/ C) seawater. After the animals pumped water normally, *T. pseudonana* was added into each beaker to generate a concentration of 10^4 cell mL^{-1}. Immediately after this addition, 10 mL of water was sampled from each beaker and the cell density was measured using a particle analyzer. Further samplings of water and measurements of cell density were performed at 20 and 40 min. The algal suspension was stirred by a magnetic stirrer throughout the experimental period. After the experiments, the mussels were dissected, and their soft tissues were dried at 80 °C to derive the dry weights (g). The clearance rate of each individual mussel was calculated from the mean of the two consecutive measurements at 20 min intervals and expressed as L g^{-1} h^{-1}.

Radioassay and Statistical Analysis. The radioactivity of the experimental samples was assayed by a Wallac 1480 NaI (T1) gamma detector (Wallac Turku, Finland). All counts were corrected for radioisotope decay, and the counting time was adjusted to yield a propagated counting error of less than 5%. 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. All data were analyzed by *t*-test or analysis of variance (ANOVA) following appropriate transformation. Statistical significance was accepted at $p < 0.05$.

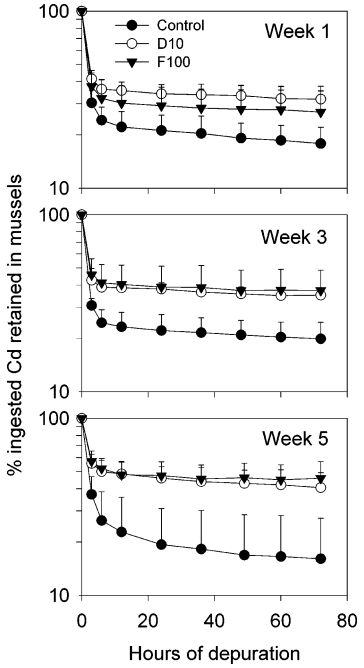

FIGURE 1. Cd retention in Cd pre-exposed mussels *Perna viridis* following a pulse ingestion of radiolabeled diatoms (*Thalassiosira pseudonana*) in experiment 1. Data are mean + SD (n = 5). Control: mussels received no Cd pre-exposure. D: dissolved exposure. F: food exposure. Numbers in the legends are the Cd concentrations used in the exposure treatment (see Table 1).

Results

Experiment 1: Dissolved versus Dietary Cd Pre-Exposure. This experiment tested the effects of dissolved versus dietary Cd pre-exposure on subsequent Cd uptake from both food and water by the green mussels. Following the pre-exposure, Cd soft tissue concentrations in both the dissolved and dietary pre-exposure treatments were significantly higher ($p < 0.01$) than those of the controls (Table 2). Comparable Cd body concentrations were achieved after 5 weeks of pre-exposure (11−14 μg g^{-1}), whereas aqueous Cd pre-exposure resulted in a higher Cd concentration than dietary Cd pre-exposure within the first 3 weeks. Cd AEs generally increased in the mussels pre-exposed to Cd, as a result of more efficient retention during the initial digestion period (within 12 h following feeding) (Figure 1 and Table 2). After 1 week of pre-exposure, Cd AE increased significantly ($p < 0.01$) only in the dissolved 10 μg L^{-1} group (32%) but not in the food

TABLE 3. Cd Body Concentration (μg g^{-1}, Mean ± SD; n = 5), Metal Assimilation Efficiency (AE, %, Mean ± SD; n = 5), Metal Influx Rate from the Dissolved Phase (ng g^{-1} h^{-1}, Mean ± SD; n = 8), and Clearance Rate (CR, L g^{-1} h^{-1}, Mean ± SD; n = 8) in the Green Mussel *Perna viridis* Following Cd Pre-Exposure (Experiment 3)[a]

		dietary AE				dissolved influx rate				
pretreatment	Cd concentration	Cd	Hg	Ag	Zn	Cd	Hg	Ag	Zn	CR
C	0.6 ± 0.1	22.7 ± 4.5	59.9 ± 5.3	15.6 ± 2.0	23.4 ± 4.8	29.7 ± 9.7	16.7 ± 3.5	8.6 ± 2.4	159.4 ± 49.5	7.9 ± 3.1
D0.1	0.7 ± 0.1	31.1 ± 6.2	67.3 ± 4.1	16.0 ± 5.3	29.7 ± 3.3	30.5 ± 4.2	14.4 ± 4.4	7.1 ± 0.7	159.8 ± 25.4	8.1 ± 2.8
D1	2.0 ± 0.3**	35.3 ± 5.3**	65.6 ± 3.6	12.5 ± 2.3	26.2 ± 2.2	33.1 ± 7.9	13.6 ± 5.0	8.6 ± 2.5	189.0 ± 47.9	7.6 ± 2.5
D5	7.4 ± 0.9**	44.2 ± 8.4**	68.7 ± 8.1	14.4 ± 6.1	29.2 ± 8.1	26.1 ± 6.4	9.2 ± 2.5**	8.0 ± 1.2	132.9 ± 54.2	8.2 ± 1.3
D20	22.5 ± 2.5**	58.4 ± 0.7**	69.7 ± 6.3	17.1 ± 6.3	29.9 ± 5.7	20.9 ± 4.7*	7.2 ± 3.2**	8.1 ± 3.7	129.8 ± 30.9	8.6 ± 3.3

[a] Significant difference from the control treatment is indicated by *, $p < 0.05$, and **, $p < 0.01$.

100 μg L^{-1} group (28%) as compared to the control (19%). After 3 and 5 weeks of the pre-exposure, Cd AEs in these two Cd-treated groups were significantly higher ($p < 0.01$) than those of the controls. Over the whole pre-exposure period from week 1 to week 5, there was no significant ($p > 0.05$) difference in the Cd dissolved uptake rate between the control and the dissolved or food exposure treatments. The clearance rates were generally unaffected by Cd pre-exposure, except that the food 100 μg L^{-1} group exhibited a significantly higher ($p < 0.05$) clearance rate than did the control following 5 weeks of pre-exposure (Table 2).

Experiment 2: Different Combined Concentration and Duration Pre-Exposure. The influences of comparable Cd body concentrations derived from different exposure regimes on the consequent assimilation of Cd were tested in this experiment. Although Cd levels and exposure durations were different among the treatments (i.e., 2 μg L^{-1} for 5 weeks, 3.3 μg L^{-1} for 3 weeks, and 10 μg L^{-1} for 1 week), the body Cd concentrations detected in the mussels were rather similar (4.6−5.7 μg g^{-1}, $p > 0.05$), and all were significantly higher ($p < 0.01$) than those in the control (Table 2). Mussels from all the exposure treatments assimilated Cd at a significantly higher (1.9−2.3×, $p < 0.01$) efficiency than did the control, but no significant difference ($p > 0.05$) was evident among the three Cd-exposed groups (Figure 2 and Table 2).

Experiment 3: Influences of Cd Pre-Exposure on Other Metals. We tested whether Cd pre-exposure affected subsequent dissolved and dietary uptake of Ag, Hg, and Zn. After 5 weeks of Cd pre-exposure, with one exception (dissolved 0.1 μg L^{-1} treatment), Cd tissue concentrations of all the exposed mussels were significantly greater ($p < 0.01$) than that of the control. This increase was the largest (37.5×) following the 5 week pre-exposure to 20 μg L^{-1}. The retention of the four metals in mussels during the 72 h depuration period is shown in Figure 2. Except for the dissolved 0.1 μg L^{-1} group, Cd assimilation in the other three exposure treatments was significantly more efficient ($p < 0.01$) than that of the control (Table 3). Given the characteristic depuration pattern of Cd, the first stage of digestion (within 12 h) played an important role in determining the Cd AE.

In contrast, AEs of Ag, Hg, and Zn in the Cd pre-exposed mussels were not obviously affected by the pre-exposure (Figure 2, Tables 3). Although there were slight increases in the assimilations of Hg (by 10−16%) and Zn (by 12−28%) by mussels in the Cd pre-exposed treatments, none of them was statistically significant ($p > 0.05$). It is also interesting to note the differences in the depuration pattern among the four metals (Figure 2). Cd was rapidly egested within 12 h, after which there was much less loss from the mussels. In contrast, Zn and Ag were continuously lost throughout the whole period after the initial egestion, although the Zn depuration was relatively slower than that of Ag during this period. Hg(II) exhibited a totally different depuration pattern from the other three metals and was characterized by a rapid loss within the first 3 h, followed by a second period of almost

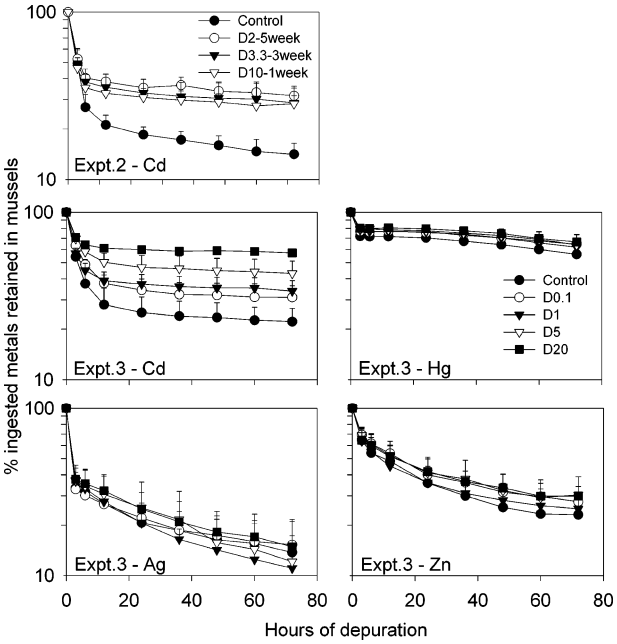

FIGURE 2. Cd retention in the Cd pre-exposed mussels *Perna viridis* following a pulse ingestion of radiolabeled diatoms (*Thalassiosira pseudonana*) in experiments 2 and 3. Data are mean + SD (n = 5). Control: mussels received no Cd pre-exposure. D: dissolved exposure. Numbers in the legends are the Cd concentrations used in the exposure treatment (see Table 1).

no loss during 3 to 24 h, and a third period of continuous but more gradual loss after 24 h.

Cd pre-exposure for 5 weeks did not significantly affect the dissolved influx rate of Cd, except a significantly ($p < 0.05$) lower influx rate in the 20 μg L^{-1} treatment (Table 3). Hg influx rate decreased by 14−57% in the Cd pre-exposed treatments, among which the dissolved 5 and 20 μg L^{-1} treatments had a significantly lower influx rate ($p < 0.01$) than the control had. For Ag and Zn, there was no significant difference in influx rate between the pre-exposed mussels and the controls. In addition, no significant difference in mussel's clearance rate between the exposed mussels and the control was observed ($p > 0.05$) (Table 3), but overall the clearance rates measured in this experiment were much less than those measured in experiment 1.

Correlation between Metal Bioavailability with Cd Tissue Concentration. In experiment 1, a control treatment (without Cd pre-exposure) was included in all measurements conducted at different periods of pre-exposure (1, 3, and 5 weeks). Since there were variations in the measurements of the control animals conducted at different times, we calculated the index of Cd assimilation (i.e., AE index) as the percentage of their respective control values during each measurement to examine the inter-relationships between Cd assimilation and Cd body concentration. For experiments 2 and 3, we

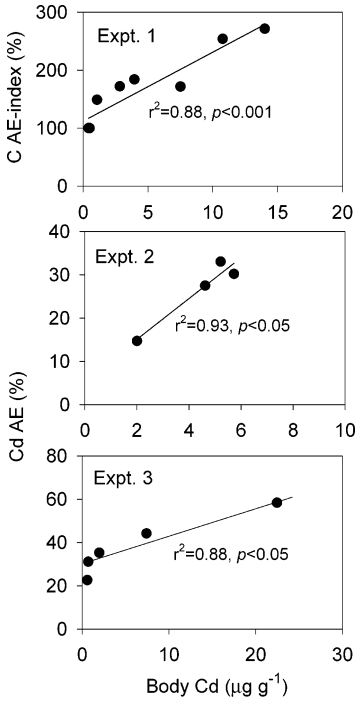

FIGURE 3. Relationship between the index of the metal assimilation efficiency (AE) or Cd AE and the Cd body concentration in the mussels *Perna viridis* following Cd pre-exposure in experiments 1, 2, and 3. The index was calculated as the percentage of their respective control values.

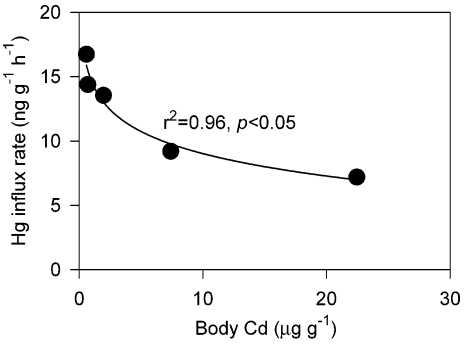

FIGURE 4. Relationship between the Hg influx rate from the dissolved phase and the Cd body concentration in the mussels *Perna viridis* following Cd pre-exposure in experiment 3.

used the direct measurements of Cd AE to correlate with the Cd body concentration. In all experiments, a significant ($p < 0.05$) linear relationship was evident when the Cd AE (or Cd AE index) was correlated with the Cd body concentration (Figure 3). No relationship was however found for the AEs of Ag, Hg, and Zn with the Cd body concentration. Moreover, dissolved influx rate (or index) of the metals did not have a clear relationship with Cd body concentration, except for Hg, which decreased with increasing Cd tissue concentration (Figure 4).

Discussion

Cd Body Concentration Following Cd Pre-Exposure. In the present study, the Cd body concentrations in the mussels collected from the New Airport area (2.0 μg g^{-1}) were higher than those collected from Tolo Harbor (0.5−0.6 μg g^{-1}). This difference in Cd body concentration was caused by the difference in Cd influx from the aqueous phase due to Cd speciation as well as dissolved Cd concentrations in these two sites (*18*). Specifically, the salinity at the New Airport site was much lower, which led to a higher Cd uptake from the dissolved phase as a result of the change in mussel physiology and more bioavailable free Cd ion (*20*). However, each independent experiment was used to address a specific question, and we made no attempt to draw comparisons among the experiments that employed different populations of mussels collected at different times and locations. The Cd body concentrations achieved following Cd pre-exposure were 0.7−22.5 μg g^{-1} with the highest concentrations observed in individuals pre-exposed to dissolved 20 μg L^{-1} Cd for 5 weeks (experiment 3). Such Cd body concentrations are environmentally realistic. For example, Chu et al. (*21*) reported that the accumulated Cd tissue concentration in *Perna viridis* collected from Tolo Harbor, Hong Kong was 9 μg g^{-1} in 1986. The Cd tissue concentration was up to 8.8 μg g^{-1} in the marine clam *Mactra veneriformis* collected from Cd-contaminated bay in Northern China (*8*, *22*), where the sediment and dissolved Cd concentrations were as high as 488 mg kg^{-1} and 10 μg L^{-1} (*23*).

The Cd body concentration was manipulated by exposing mussels under different regimes (dissolved vs dietary, history of exposure including different combinations of Cd dissolved concentration and exposure duration). In experiment 1, the Cd body concentrations were comparable after 5 weeks of pre-exposure between the aqueous and dietary treatments. However, a much higher (10×) Cd concentration was required to be spiked in the diatoms, mainly because we used a short period of feeding (4 h a day) during the pre-exposure period, with an aim to reduce the potential exposure of mussels to aqueous Cd (resulting from Cd desorption from the spiked diatoms) in this treatment. Chong and Wang (*14*) demonstrated that aqueous and dietary Cd were equally important for the Cd accumulation in green mussels, but the relative importance of both exposure pathways was dependent on the Cd partitioning in the ingested particles. In experiment 2, the exposures (concentration × duration) of the three treatments were targeted to be similar. The comparable Cd body concentrations also indicated that the uptake of dissolved Cd was directly proportional to the dissolved Cd concentration and the duration of exposure, consistent with numerous studies in marine mussels (*14*, *24*, *25*). Furthermore, no evidence suggested that the Cd accumulation reached steady-state conditions over the 5 weeks of pre-exposure.

Effects of Cd Pre-Exposure on Cd Uptake. Despite the importance of dietary Cd accumulation, research on whether dietary metal pre-exposure can modify subsequent metal bioavailability is extremely limited. Blackmore and Wang (*13*) earlier demonstrated that Cd assimilation increased significantly with increasing pre-exposure of green mussels to dissolved Cd, but they did not examine dietary Cd pre-exposure. The present study clearly showed that following pre-exposure to Cd via either aqueous or dietary pathway, Cd assimilation increased linearly with increasing Cd tissue concentration in green mussels. One possible mechanism is that a higher level of MT induced by Cd pre-exposure tends to sequester more Cd from the dietary phase, resulting in a more efficient Cd assimilation in the mussels (*13*). Consistently, we also found that the clams *M. veneriformis* and *Ruditapes philippinarum* collected from a Cd-contaminated bay had distinguishably higher Cd AE as compared with populations from less contaminated or relatively clean sites, in correlation with a higher MT concentration in the digestive glands of the clams (*8*).

The influences of Cd pre-exposure on Cd assimilation appear to be consistent in mussels with different exposure histories. Metal tissue concentration is a result of exposure under different regimes in the natural environment. We deliberately conducted experiment 2 to examine the influences of similar Cd body concentrations derived from different exposure regimes on Cd assimilation. Mussels under

different exposure regimes (i.e., dissolved 2 $\mu g\,L^{-1}$ for 5 weeks, 3.3 $\mu g\,L^{-1}$ for 3 weeks, and 10 $\mu g\,L^{-1}$ for 1 week in experiment 2) ended up with similar Cd body concentrations and meanwhile failed to show significant difference in Cd AEs, implying that Cd assimilation was mainly determined by the Cd body concentration instead of the pre-exposure regime. Thus, when evaluating the influences of Cd pre-exposure on subsequent Cd accumulation in mussels, the observed Cd body concentration can be solely taken into account regardless of the pre-exposure regime. It should be realized, however, that our experiments were performed under laboratory conditions within a relatively short-term (1−5 weeks) pre-exposure period. It may be possible that Cd binding in biological tissues does not entirely reflect Cd binding under field environments in which the animals are exposed to metals throughout their lifetimes.

In contrast to dietary Cd assimilation, Cd pre-exposure did not significantly affect the Cd uptake from the dissolved phase, similar to the results of Blackmore and Wang (*13*). It is thus likely that aqueous uptake and dietary assimilation of Cd in green mussels were controlled by different mechanisms. Recently, Ng and Wang (*26*) demonstrated that the subcellular distribution of Cd in green mussels after dietary exposure was different from that after dissolved exposure. Cd was evenly distributed among the different subcellular fractions after dietary exposure, which was in contrast to the dominant distribution in the insoluble fraction after dissolved exposure, suggesting that the individual fraction for storing Cd was dependent on the exposure route. A higher uptake of Cd from the dissolved phase was related to its increasing partitioning in the heat-sensitive protein fraction but not to the metallothionein-like protein fraction (*26*).

Given the significant influence of Cd pre-exposure on Cd assimilation, it is likely that the accumulated Cd concentration in the mussels may not entirely reflect the ambient bioavailable Cd concentration but may also reflect the physiological responses to the Cd exposure. This factor clearly needs to be considered in interpreting the Cd tissue concentrations in biomonitors collected from different locations. Furthermore, comparison of the Cd dietary assimilation among different populations of mussels also needs to incorporate the different exposure histories that the mussels may have experienced. Although it has been relatively well established that the environmental conditions (such as food quantity/quality) and digestive physiology can substantially affect Cd dietary assimilation, the history of Cd exposure and the resulting biochemical/physiological modification in the mussels is another factor requiring further attention.

Effects of Cd Pre-Exposure on the Uptake of other Metals. Among the four metals (i.e., Cd, Zn, Ag, and Hg) involved in this study, Cd and Hg generally showed a closer relationship in terms of their uptake mechanisms, in which the Cd body concentration and MT induction possibly play an important role. The assimilations of both Cd and Hg are clearly related to the induction of MT by Cd pre-exposure, as shown in other studies that Cd- and Hg-binding metalloproteins are very similar (*27*, *28*). Therefore, it is very likely that the observed increase of Hg AE (but statistically insignificant) was a result of the enhanced level of Hg binding with the Cd-induced MT. In contrast to the Hg dietary assimilation, Cd pre-exposure significantly decreases the influx of Hg from the dissolved phase, further suggesting that dietary assimilation and aqueous uptake of Hg were controlled by different mechanisms. The detailed mechanisms underlying the depression of Hg dissolved uptake following Cd pre-exposure remain undetermined at present. A recent study also showed a decrease in Hg dissolved uptake following pre-exposure to Cu (*15*), another metal that has been identified as an MT inducer in marine invertebrates (*7*, *29*). In a previous study, Roesijadi and Fellingham (*16*) found that the pre-exposure of the mussel *Mytilus edulis* to Cu or Cd in the aqueous phase resulted in an enhanced tolerance to subsequent Hg(II) exposure. One possible mechanism underlying the increasing tolerance to Hg is a result of the reduction in Hg uptake due to Cd or Cu pre-exposure. In green mussels, aqueous uptake of Hg(II) was more important than dietary uptake of Hg(II) (*30*). Thus, it is likely that the overall Hg(II) accumulation in the mussels may be depressed as a result of Cd pre-exposure.

Factors controlling Zn accumulation by mussels appeared to be different from those of Cd and Hg, although Zn and Cd are chemically similar. Both the Zn dietary assimilation and aqueous uptake were not considerably affected by the Cd pre-exposure. In *P. viridis*, Zn is not a strong MT inducer, even though it can potentially bind to available MT. It is mainly bound with metal-rich granules (MRGs) when accumulated by the mussels to a significantly high level (*13*, *15*). Since the binding affinity of Cd with MT is stronger than that of Zn with MT, it may be difficult to discern any disturbance of Zn uptake caused by Cd pre-exposure. Blackmore and Wang (*13*) found that when the Cd body concentration reached a very high level (i.e., 25.9 $\mu g\,g^{-1}$), the Zn AE increased by 1.4× in the green mussels.

The bioaccumulation of Ag by the green mussels was completely unaffected by elevated Cd body concentration, likely caused by the difference in their bindings. Earlier experimental studies demonstrated that the majority of Ag in marine bivalves is bound with sulfides with only a small fraction of Ag associated with the protein fraction (*15*, *31*, *32*). Our previous study suggested that the elevated Ag AE by the mussels after both aqueous and dietary Ag pre-exposure was a result of Ag binding with sulfide induced by Ag pre-exposure (*5*).

In conclusion, our data indicated that Cd exposure and resulting Cd body concentration can play an important role in the assimilations of Cd and the dissolved uptake of Hg but have an insignificant effect on dissolved Cd and Zn uptake and Ag intake from both food and water. The different bioavailabilities of the metals to the Cd pre-exposed mussels may be closely related to the different biochemical bindings and storages of metals in the animals. Differences in the route or history of Cd pre-exposure did not cause significant differences in the effects on Cd bioaccumulation in mussels. Thus, when using the measured metal body concentration as a criterion to measure metal contamination in seawater (e.g., with a biomonitor), the influence of metal exposure needs to be considered carefully.

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