

Cd and Se aqueous uptake and exposure of green mussels *Perna viridis*: influences of seston quantity

Wen-Xiong Wang*

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

ABSTRACT: Trace metals are available to marine bivalves through uptake from both the aqueous and dietary phases. In this study, I sought to determine if there is any interaction between these 2 exposure pathways. The uptake of Cd and Se by green mussels *Perna viridis* from the aqueous phase was measured at different phytoplankton concentrations (green alga *Chlorella autotrophica*). Controlled experiments were performed to ensure that, in the presence of algal particles, the majority of metal accumulation by the green mussels was due to uptake from the aqueous phase. The influx rate of metals was independent of the food concentration and the clearance rate of the mussels, indicating that metal uptake from the aqueous phase was not directly related to the food concentration. These results verify the assumption that metal uptake from different exposure pathways is additive and that there is no direct interaction between different routes of exposure. Metal uptake by green mussels was further tested after exposing the mussels to neurotransmitters (serotonin which stimulates ciliary pumping and dopamine which inhibits ciliary pumping). Complete inhibition of ciliary pumping was reached after addition of 100 μM of dopamine. Despite changes in the pumping activity due to exposure to neurotransmitters, there was no evidence showing the reduction or enhancement of metal uptake. A bioenergetic-based kinetic model was then employed to model the influence of seston quantity on the exposure pathways of metals in the mussels. Our calculations indicated that the seston quantity substantially influences the relative importance of aqueous versus dietary exposure, primarily due to the dependence of a green mussel's ingestion activity on seston quantity. At a higher seston concentration, the exposure pathways are not influenced by the change in seston concentration due to the maintenance of a maximum ingestion rate and pseudofeces production.

KEY WORDS: Metals · Mussels · Exposure pathway · Uptake · Cadmium · Selenium

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INTRODUCTION

Natural seston varies tremendously both in terms of quantity and quality in coastal and estuarine waters. One of the great challenges for marine suspension feeders such as bivalves is to adjust their physiological responses to variations in seston quantity and quality (Bayne 1993). A possible result of these physiological adjustments is the change in contaminant uptake by marine bivalves, but this has not yet been adequately studied. In marine bivalves such as mussels and oys-

ters, which have been employed as biomonitors for over 25 yr (Goldberg et al. 1978, O'Connor 1992), metals can be accumulated through both uptake from the dissolved phase and ingestion from the particulate phase. Over the past few years, extensive research has been conducted to study metal uptake from the dietary phases, including the measurements of metal assimilation efficiency under various food environments (Wang & Fisher 1999). These extensive studies have complemented earlier studies on metal uptake from the aqueous phase (e.g. Fowler & Benayoun 1974, 1976, Jackim et al. 1977, Fischer 1986) and have suggested that metal bioavailability is not only controlled by metal geochemistry (e.g. speciation), but also by bivalve

*E-mail: wwang@ust.hk

physiology (e.g. feeding and assimilation) and environmental quality (e.g. dissolved organic carbon concentration and salinity) (Wang & Fisher 1997, Wang 2001).

Recent studies have demonstrated that the bioenergetic-based kinetic model is a power tool for understanding metal bioaccumulation and bioavailability in marine herbivores such as bivalves and copepods (Wang & Fisher 1997). In the kinetic model, various physiological and geochemical parameters are identified to quantify the rates of metal intake from both the dissolved and particulate phases. One of the inherent assumptions in the kinetic model is that metal accumulation from both phases is additive, thus allowing for independent assessment of the metal influx from both uptake pathways (Luoma & Fisher 1997). Based on this assumption, metal influx from the dissolved phase has generally been quantified in the bivalves without the presence of food particles. However, the assumption that the metal accumulation from both phases is additive has never been tested on the bivalves. It remains unknown whether the influx rate measured without the presence of food particles is representative of the metal influx in a natural environment where food particles both are present and vary in quantity and quality in space and time.

It is well known that trace metal uptake is controlled by metal geochemistry (e.g. speciation). The influences of environmental quality such as food particle concentration on trace metal accumulation from the aqueous phase are undetermined. Among the many environmental variables, seston quantity and quality can affect several parameters described in the bioenergetic-based kinetic model, such as metal assimilation efficiencies, metal concentrations in ingested food particles, and ingestion rates of bivalves. Although previous studies have extensively focused on the influence of food environments on metal uptake from the particulate phase (reviewed by Wang & Fisher 1999), the issue of whether or not metal uptake from the aqueous phase can be affected by food particle concentration has not been studied. If there is any interaction between the 2 exposure pathways (aqueous vs dietary intake) as a result of change in food availability is not yet clear. Wang et al. (1996) showed that the bioaccumulation factor of metals in the mussels *Mytilus edulis*, calculated as the ratio of metal concentration in mussels to the total metal concentration in the ambient environment, was greatly dependent on the seston concentration.

In this study, we specifically examine the influence of food concentration on the aqueous uptake and exposure of metals by the green mussels *Perna viridis*, a biomonitor used in subtropical and tropical waters. However, technical difficulty exists because any metals added into the water may have been accumulated

by the food particles, which are then ingested by the mussels, thus complicating the interpretation of experimental results. I overcame this difficulty by choosing to working on 2 metals, Cd and Se. Cd accumulation by mussels has been shown to be dominated by uptake from the aqueous phase, primarily because of its low partition coefficient (e.g. less particle reactive) and its low assimilation efficiency from ingested food sources (Wang et al. 1996, Chong & Wang 2001). In contrast, most Se in marine mussels is obtained from ingestion of food particles as a result of the efficient assimilation and the low rate of uptake from the dissolved phase. Thus, these 2 metals represent 2 extremes of metal exposure in marine bivalves. I chose the green algae *Chlorella autotrophica* as the food particles to study the influence of food concentration on Cd and Se uptake. Previously, we have demonstrated negligible Se and Cd uptake by this green alga (Chong & Wang 2000, Wang & Dei 2001). Thus, I expected that the addition of green algae to mussels' filtering water will result in little Cd and Se accumulation in the food, eliminating the possibility of metal ingestion through dietary source. To test further whether differences in pumping activity will lead to changes in the metal influx of mussels, I used 2 neurotransmitters to stimulate or inhibit the mussels' ciliary pumping. This allowed me to quantify the influx of metals in the mussels. Finally, a bioenergetic-based kinetic model was used to predict the influences of seston quantity on the exposure pathways of metals in the green mussels.

MATERIALS AND METHODS

General experimental procedures. The green mussels *Perna viridis* (shell length 2.5 to 3.0 cm, with a dry tissue weight of 0.10 to 0.25 g) were collected from Tolo Harbor, Hong Kong. The mussels were maintained at 23°C in 29 psu seawater for about 1 wk before the experiments. They were fed with the diatom *Thalassiosira pseudonana* and green algae *Chlorella autotrophica* continuously during the acclimation period. All experiments described below were conducted at 23°C and 29 psu seawater.

The radiotracer technique was employed to quantify the uptake of Cd and Se by the mussels. Cd was traced by ^{109}Cd (dissolved in 0.1 N HCl, purchased from New England Nuclear, MA) and Se was traced by ^{75}Se (dissolved in 0.1 N HCl, purchased from Livermore National Laboratory, CA). The seawater was collected from Clear Water Bay, Hong Kong, and filtered through 0.22 μm Poretics filter before use. Radioisotope additions were 3.7 kBq l^{-1} (corresponding to 0.4 nM) for ^{109}Cd and 27.8 kBq l^{-1} (corresponding to 0.5 nM) for ^{75}Se . Metal uptake was measured at a fixed concentra-

tion of 17.8 nM for Cd and 25.3 nM for Se. Radioisotope and stable metals were equilibrated overnight before the uptake experiments. The radioactivity of mussels, soft tissues, and water was quantified by a gamma counter (Wallac 1480, EG&G, Turku, Finland). ^{109}Cd was detected at 88 keV, and ^{75}Se was detected at 264 keV. Counting times of the samples were adjusted to result in propagated errors <5%.

To measure the uptake rates of Cd and Se, the mussels were individually placed in 250 ml of 0.22 μm seawater containing both the stable metals and radiotracers. The mussels were exposed to the metals for 1 h. At 0.5 h of exposure, the water was gently shaken to homogenize the metals in the water. After 1 h of exposure, the mussels were removed and placed in clean nonradioactive water for 2 to 3 min, then dissected and the radioactivity of ^{109}Cd and ^{75}Se in the soft tissues was measured by the gamma counter. The tissues were subsequently dried at 80°C for 1 d before the dry weight was measurement. The influx rate of metals (I , $\text{ng g}^{-1} \text{h}^{-1}$) into the mussels was calculated as:

$$I = A / (SA \times t_1 \times W) \quad (1)$$

where A is the radioactivity in the mussel tissue (ccpm), SA is the specific activity of radioisotopes (ccpm ng^{-1}), t_1 (h) is the duration of exposure (1 h), and W is the tissue dry weight (g).

The clearance rate of the mussels was quantified by the indirect method (Widdows 1985). The mussels were individually placed in 2 l filtered seawater (<0.2 μm). At the exponential growth phase, the green algae *Chlorella autotrophica* were filtered from their growing medium to remove the algal metabolites and nutrients and then added into the feeding beakers at different cell densities (see below). Our unpublished data indicated that the retention efficiency of green mussels on *C. autotrophica* (2 to 5 μm) was close to 100%. The clearance rates on *C. autotrophica* were similar to those on bigger particles such as the diatom *Thalassiosira pseudonana* (4 to 6 μm) and the dinoflagellate *Prorocentrum minimum* (13 to 16 μm) (K. S. Chan & W. X. Wang unpubl. data). The cell suspension was gently homogenized by a magnetic stirrer. Every 20 min, a 10 ml water sample was taken for cell density measurement by a Coulter Counter (Beckman Coulter, Brea, CA). The clearance rate (CR) was calculated as:

$$\text{CR} = (\ln C_0 - \ln C_t) \times V / t_t \quad (2)$$

where C_0 and C_t are the cell densities measured at t_0 and time t_t , respectively, and t_t is the duration of feeding, and V is the volume of water. The mean clearance rate was calculated based on the 2 consecutive time point measurements.

Influences of food concentration on Cd and Se uptake. Two independent replicate experiments (Expt 1

and Expt 2) were carried out at different times. The clearance rates of the mussels were measured at different cell concentrations of *Chlorella autotrophica* for 40 min: 2000, 6000, 20 000, 150 000, and 600 000 cells ml^{-1} (corresponding to a dry biomass of 0.03, 0.10, 0.32, 2.4, and 9.6 mg l^{-1} , respectively). Pseudofeces were produced at the highest cell concentration (600 000 cells ml^{-1}). However, I did not measure the metal uptake at the highest cell concentration due to the appreciable accumulation of metals by the green algae (see below). There were 8 replicate individual mussels for each cell concentration treatment. After the clearance rate measurements, the mussels were immediately placed in 250 ml filtered seawater containing the metals and radiotracers and the same concentration of green algae for 1 h as described above. In this experiment, *C. autotrophica* cells were added into the exposure medium. After the exposure, the mussels were dissected and the radioactivity and dry weight of soft tissue were quantified.

With the addition of green algae into the exposure medium, metals may have been accumulated by the cells and subsequently ingested by the mussels. Thus, measurements of the radioactivity in the soft tissue may not only reflect metal uptake from the aqueous phase, but may also include metal accumulation by the green algae. To calibrate for this effect, the accumulation of Cd and Se by the green algae was experimentally quantified. The green algae were filtered from their growing medium and resuspended into 250 ml of 0.22 μm filtered seawater spiked with ^{109}Cd (3.7 kBq l^{-1}) and ^{75}Se (37.8 kBq l^{-1}). Cell concentrations were 2000, 6000, 20000, 150 000, and 600 000 cells ml^{-1} . There were 2 replicated bottles for each concentration treatment. At 0.25, 0.5, 0.75, 1, and 2 h of exposure, a 2 ml aliquot of water was taken for measurement of total radioactivity in the water (including cells and dissolved phase). A 40 ml water sample was filtered onto 1 μm polycarbonate membrane and the radioactivity was counted. A control treatment with no addition of green algae was used to monitor the sorption of radioisotopes onto the polycarbonate membrane (which was confirmed to be negligible). The radioactivity accumulated by the green algae was subsequently subtracted from the blank sample from the 'control' treatment. Possible metal accumulation by the mussels due to ingestion of 'radioactive' green algae was calculated as the geometric mean over the exposure period, and this amount was subtracted from the radioactivity in the soft tissue in calculating metal uptake from the aqueous phase only.

Influences of serotonin and dopamine on metal uptake. Two independent replicate experiments (Expt 1 and Expt 2) were carried out at different times for both serotonin and dopamine. Previous studies have

demonstrated that serotonin can stimulate the ciliary pumping of bivalve molluscs, whereas dopamine can inhibit the ciliary pumping of bivalves (Catapane et al. 1978, Jones & Richards 1993, Berias & Widdows 1995). To test whether metal uptake by the green mussels is dependent on the clearance rate, we also exposed the mussels to different concentrations of serotonin or dopamine and quantified the metal uptake rate. The clearance rates of mussels exposed to serotonin or dopamine were concurrently measured.

In the first experiment, mussels ($n = 8$) were exposed to serotonin or dopamine at different concentrations (0.01, 0.1, and 1 μM serotonin, and 1, 10, and 100 μM dopamine) for 2 h. The clearance rates were then quantified for 40 min (as described above) at a cell concentration of 20 000 cells ml^{-1} and immediately placed in a radioactive medium containing the same concentrations of dopamine or serotonin for 1 h. No food particles were provided during the radioactive uptake period. The clearance rate and metal uptake rate of 'control' mussels receiving no exposure to serotonin or dopamine were also quantified. Mussels were finally dissected and the radioactivity of the soft tissue was determined. This experiment was repeated twice.

In the second experiment, we examined the time course of metal uptake by the mussels by stepwise addition of different concentrations of dopamine or serotonin. Eight mussels were individually placed in 250 ml filtered seawater containing the stable metals and radiotracers. After 40 min of exposure, the mussels were removed and rinsed with filtered seawater, and the radioactivity of whole individual (including both

the shell and soft tissue) was counted non-destructively. The mussels were subsequently placed in 250 ml filtered seawater containing 1 μM dopamine or 0.01 μM serotonin and exposed to the same metal concentrations for 40 min, after which the radioactivity in the whole individual mussels was counted again. Later, the mussels were placed in 10 μM dopamine or 0.1 μM serotonin seawater for 40 min and the radioactivity of the whole individual mussels was counted. Finally, they were exposed to 100 μM dopamine or 1 μM serotonin for 40 min, and the radioactivity was quantified. In a concurrent experiment, the clearance rate of the mussels was measured as described above. The clearance rate was first measured without the addition of dopamine or serotonin for 40 min, after which dopamine or serotonin was added in a stepwise manner from 1 to 10 μM and finally to 100 μM for dopamine and from 0.01 to 0.1 μM and finally to 1 μM for serotonin. The mussels were exposed at each concentration for 40 min.

Because the radioactivity of the whole individual mussel (including both shell and soft tissue) was quantified in the second experiment, it was not possible to calculate the actual metal uptake into the soft tissue. Instead, we calculated the conditional concentration factor of metals (CCF) in the whole mussels using the following equation:

$$\text{CCF} = R_i/R_a \quad (3)$$

where R_i is the radioactivity in a whole individual mussel per kg of tissue dry weight, and R_a is the radioactivity in the ambient water. The decrease of radioactivity in the water due to the uptake by the green mussels was negligible.

RESULTS

Metal uptake at different concentrations of algal food

Over the 2 h exposure period, negligible amounts of dissolved ^{109}Cd and ^{75}Se were in fact accumulated by the green algae *Chlorella autotrophica*, especially at the low concentrations (Fig. 1). On average, <6.9% of Cd and <0.2% of Se were accumulated by the green algae over the 2 h exposure period. At the 3 low algal concentrations (<20 000 cells ml^{-1}), the accumulation of Cd and Se was essentially undetectable. Accumulation of Cd by the green algae was detectable at the 2 highest algal concentrations. Calibration for metal uptake by the mussels due to ingestion of 'radioactive' algae was therefore only necessary for the high food concentration treatment (150 000 cells ml^{-1}).

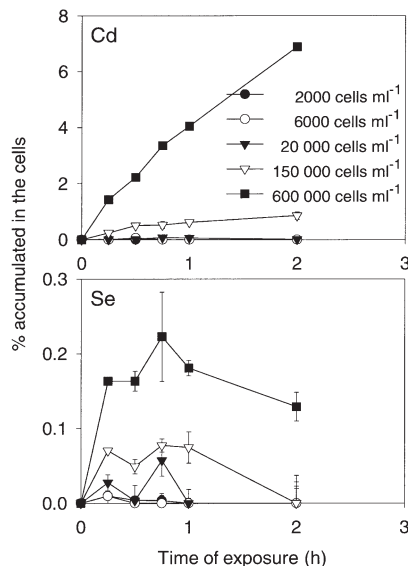


Fig. 1. *Chlorella autotrophica*. Accumulation of Cd and Se by green algae over a 2 h exposure period. Mean \pm semi-range ($n = 2$)

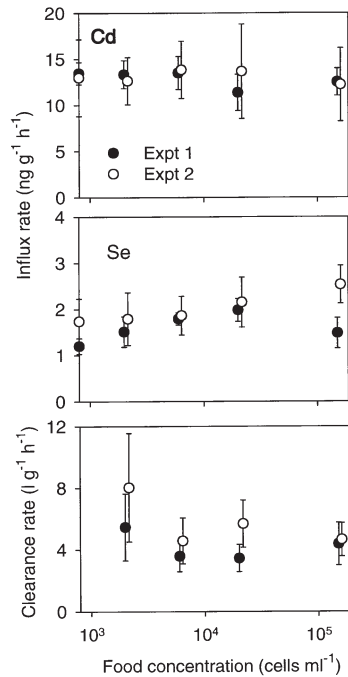


Fig. 2. *Perna viridis*. Clearance rate and influx rate of Cd and Se in mussels as a function of food concentration (green algae *Chlorella autotrophica*). Mean \pm SD (n = 8). Expt 1 and Expt 2 are 2 independent replicated experiments

The clearance rate (CR) and the influx rate of Cd and Se in the mussels exposed to different algal concentrations are shown in Fig. 2. The metal uptake rates were concurrently measured at 4 different food concentrations, thus minimizing the biological variability among different batches of experiments. The CR was somewhat higher at the lowest food concentration (2000 cells ml⁻¹), but was comparable among the other 3 food concentrations (6000 to 150 000 cells ml⁻¹). We also determined the CR at a much higher food concentration (600 000 cells ml⁻¹), at which the mussels produced pseudofeces, and found that the CR was reduced by about 25% compared with the CR measured at 20 000 cells ml⁻¹. However, the influx rate was not quantified at this cell concentration due to the detectable Cd (6.9% on the cells by the end of 2 h exposure) and Se (0.2% on the cells by the end of 2 h exposure) accumulation.

The influx rates of Cd and Se into the mussels were not dependent on the food concentration ($p > 0.05$, 1-way ANOVA). The influx rate of Cd, measured at the same ambient concentrations as Se, was about 4.8 to 11.2 \times higher than the influx rate of Se. Furthermore, Cd and Se influx measured in 2 repeated experiments using different batches of mussels were comparable. Because the CR of each experimental mussel was also concurrently quantified, we can calculate the absorption efficiency of the metals from the aqueous phase with the following equation:

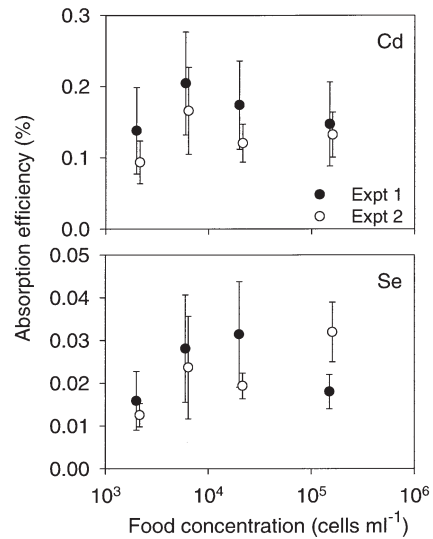


Fig. 3. *Perna viridis*. Absorption efficiency of Cd and Se from the dissolved phase in mussels as a function of food concentration (green algae *Chlorella autotrophica*). Mean \pm SD (n = 8). Expt 1 and Expt 2 are 2 independent replicated experiments

$$ABE = I_w / (CR \times C_w) \quad (4)$$

where ABE is the absorption efficiency from the dissolved phase, defined as the fraction of metals absorbed by the mussels from the volume of water filtered or pumped, I_w is the influx rate of metals into the mussels ($\mu\text{g g}^{-1} \text{h}^{-1}$), CR is the clearance rate ($\text{l g}^{-1} \text{h}^{-1}$), and C_w is the metal concentration in the dissolved phase ($\mu\text{g l}^{-1}$).

The calculated ABE of Cd and Se as a function of food concentrations is shown in Fig. 3. Food concentration significantly affected the Cd ABE in Expt 2 and the Se ABE in both replicated experiments ($p < 0.05$, 1-way ANOVA). In Expt 1, the ABE was the lowest at the lowest and highest food concentrations, and were higher at intermediate food concentrations (6000 to 20 000 cells ml⁻¹). Correlation between the metal influx rate and ABE with the CR indicated a negative relationship between the ABE and the CR (Fig. 4), whereas the influx rate did not correlate with the CR (Fig. 5). Thus, a mussel pumping at a higher rate tended to have a lower efficiency of absorption from the dissolved phase.

Metal uptake at different dopamine and serotonin concentrations

In 2 replicated experiments, additions of dopamine significantly inhibited the pumping activity of mussels, especially at the highest concentration (100 μM), at which the pumping rate was completely suppressed

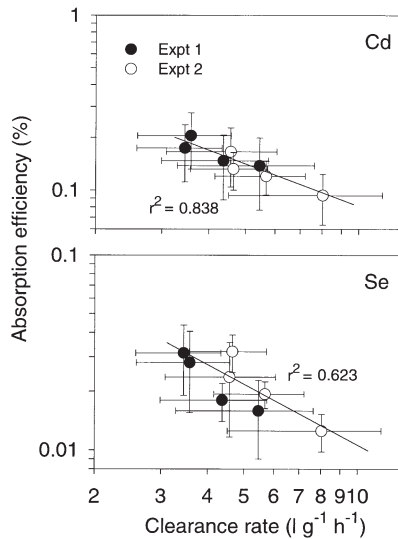


Fig. 4. *Perna viridis*. Absorption efficiency of Cd and Se from the dissolved phase as a function of the mussels' clearance rate affected by change in food concentration. Mean \pm SD ($n = 8$) Expt 1 and Expt 2 are 2 independent replicated experiments

(Fig. 6). The mussels maintained a comparable CR at the lowest dopamine concentration (1 μM), but the CR was reduced by 31 to 54% at 10 μM . The CR was enhanced by 25–57, 40–55, and 7–41% at serotonin concentrations of 0.01, 0.1, and 1 μM , respectively, in 2 replicated experiments. There were also considerable variations of the CR among the different experimental individuals.

In contrast to the variations in the CR at different concentrations of dopamine or serotonin, there was no

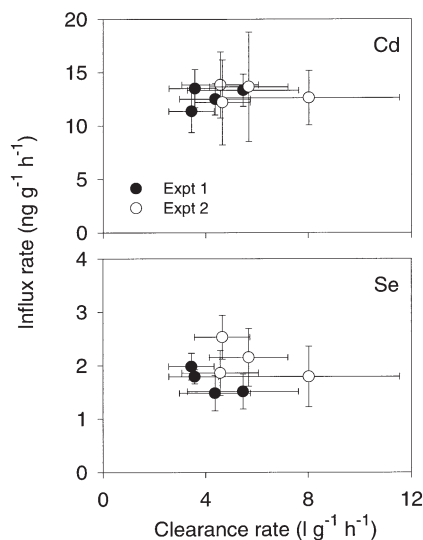


Fig. 5. *Perna viridis*. Influx rate of Cd and Se in the mussels as a function of the mussels' clearance rate. Mean \pm SD ($n = 8$) Expt 1 and Expt 2 are 2 independent replicated experiments

significant influence of dopamine and serotonin treatments on the uptake rate of Cd and Se ($p > 0.05$, 1-way ANOVA, Fig. 6). Only Se influx in Expt 1 was marginally affected by dopamine concentration ($p = 0.03$, 1-way ANOVA). The metal uptake rate varied between the 2 replicated experiments. For example, the influx rate of Se was 1.5 to 2.2 \times higher in Expt 2 than in Expt 1 in the dopamine experiment. When all experiments were considered together, there was a significant negative correlation between the mussel's ABE and the CR (Fig. 7).

Over the 160 min exposure period, the calculated conditional concentration factor (CCF) proceeded linearly over time (Fig. 8). The CCF was calculated as the ratio of metal concentration in both tissue and shell to metal concentration in the dissolved phase. With the stepwise addition of dopamine, the CR was gradually decreased and was totally arrested at addition of 100 μM dopamine. Nevertheless, there was no evidence that the metal uptake ceased at this concentration, as revealed by the linear pattern of CCF with time of exposure. The CR was maintained comparable with the addition of 0.01 μM serotonin. With a further stepwise addition of 0.1 and 1 μM of serotonin, the CR was then reduced. In this experiment, the CCF of Cd and Se also proceeded linearly with the time of exposure, indicating that metal uptake was not influenced by the variation in CR.

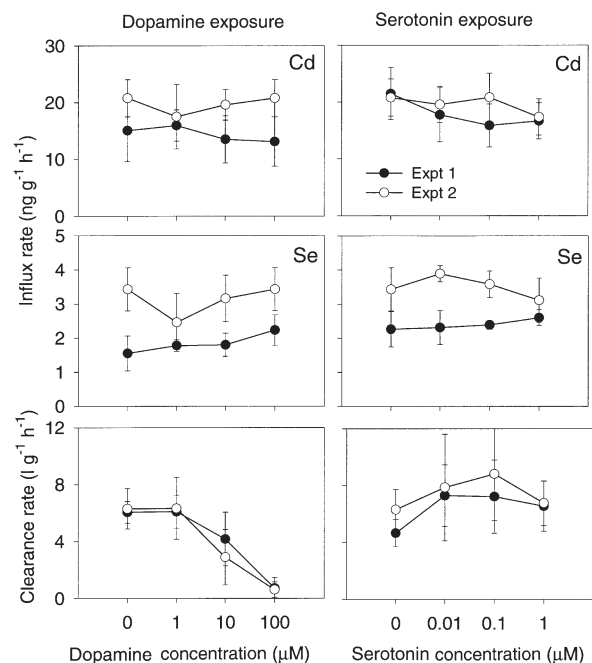


Fig. 6. *Perna viridis*. Clearance rate and influx rate of Cd and Se in the mussels exposed to dopamine and serotonin at different concentrations. Mean \pm SD ($n = 8$) Expt 1 and Expt 2 are 2 independent replicated experiments

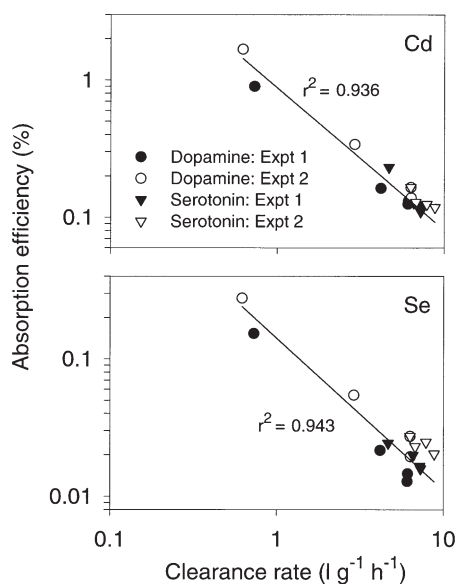


Fig. 7. *Perna viridis*. Absorption efficiency of Cd and Se from the dissolved phase as a function of the mussels' clearance rate affected by additions of different concentrations of dopamine and serotonin. Expt 1 and Expt 2 are 2 independent replicated experiments

DISCUSSION

Metal uptake influenced by food concentration and clearance rate

In this study, I opted to use the green alga *Chlorella autotrophica* as an algal food to specifically address the influence of food concentration on trace metal uptake from the dissolved phase, primarily because this alga has negligible accumulation of Cd and Se within the short-term exposure period. The green algae typically have a very low ability to accumulate Se (as selenite) from the ambient water (Chong & Wang 2000, Baines & Fisher 2001), and their uptake is further inhibited by a high concentration of phosphate (Wang & Dei 2001). The accumulation of Cd by the green algae was generally small, presumably due to the competitive inhibition by Zn in the ambient water (Sunda & Huntsman 1998). I believe that the choice of green algae may be the best available approach to study the influence of particle concentration on metal uptake from the dissolved phase. However, it is still difficult to study the uptake of particle reactive metals such as Ag, Zn, and Cr that may immediately absorb onto the particles following the introduction of particles into the water. In my study, a mussel's ingestion of 'radioactive' green algae was negligible at food concentrations between 2000 and 20 000 cells ml⁻¹. At the highest food concentration (150 000 cells ml⁻¹), about 21 to 27 % of ¹⁰⁹Cd and 10 to 16 % of ⁷⁵Se in the mussels may have come from the in-

gestion of 'radioactive' green algae; thus, a calibration for the possible algal uptake was necessary for a realistic interpretation of metal uptake by mussels.

Food concentration has been shown to greatly affect the pumping rate of bivalves (Bayne 1975, Widdows et al. 1979, Jørgensen 1990). I examined metal uptake over a wide range of food concentrations (2000 to 150 000 cells ml⁻¹ for green alga *Chlorella autotrophica*, corresponding to a biomass of 0.03 to 2.4 mg l⁻¹). I was not able to determine metal uptake at food concentration resulting in pseudofeces production because of measurable accumulation of Cd and Se by the green algae. In green mussels, the clearance rate (CR) was not greatly affected by the food concentration. At the highest food concentration (600 000 cells ml⁻¹ or 9.6 mg l⁻¹) with observable pseudofeces production, the CR was reduced by only 25 % compared with the CR measured at 10 000 cells ml⁻¹ (0.16 mg l⁻¹).

My study demonstrates that the uptake rate of metals was relatively unaffected by the difference in food concentration. The presence of food particles appears to have no direct influence on metal uptake from the dissolved phase. These results therefore validate the assumption that there is additive accumulation of metals from both the dissolved and particulate phases in the bioenergetic-based kinetic model (e.g. Wang et al. 1996, Luoma & Fisher 1997). In previous studies quan-

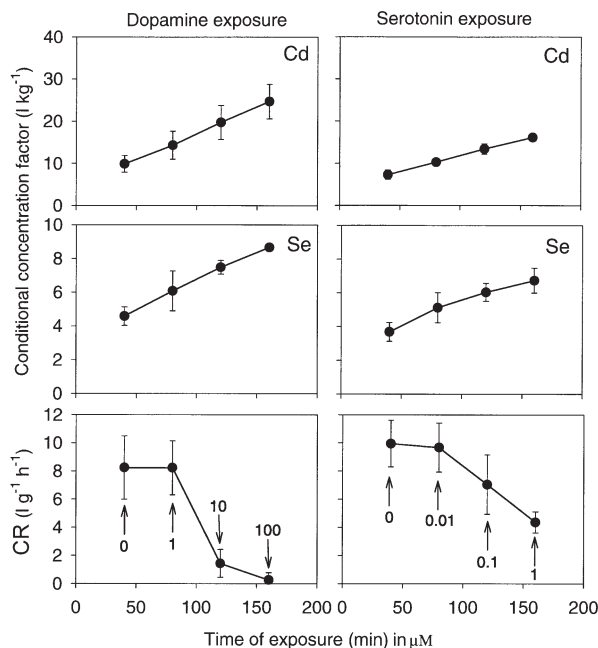


Fig. 8. *Perna viridis*. Clearance rate (CR) and conditional concentration factor of Cd and Se in the mussels exposed step wisely to different concentrations of dopamine and serotonin. Each arrow indicates the measurement after 40 min exposure to each concentration. Mean \pm SD (n = 8)

tifying the metal influx rate from the dissolved phase, bivalves were generally exposed to metals in the dissolved phase for a short exposure period to (1) minimize the decrease of pumping rate and (2) ensure the unidirectional flux with little influence of bi-directional flux (such as efflux) (Luoma & Fisher 1997). No food was added in measuring the influx of metals from the dissolved phase. My results imply that the influx rates measured under these conditions (e.g. without the presence of food particles) are representative of metal influx from the dissolved phase in the presence of food particles as is characteristic of natural waters.

Although the CR did not significantly affect the metal influx of a specific bivalve species, differences in CR did account for the difference among different species of marine bivalves. Wang (2001) recently showed that there is a linear positive relationship between the metal influx rate (or uptake rate constant) and the CR among the 8 species of marine bivalves studied (including mussels, oysters, and clams). One possible mechanism underlying the difference in metal uptake rate among different bivalve species may be related to the gill surface area controlling the CR of bivalves. This would require further investigation.

The absorption efficiency (ABE) of metals from the dissolved phase is influenced by the food concentration, but there is no consistent trend of metal ABE in response to food concentration in the 2 replicated experiments. In 1 experiment, metal ABE was higher at intermediate food concentrations but lower at both low and high food concentrations. The lower ABE at the low food concentration may be primarily caused by the higher CR of mussels. In a recent study, Wang (2001) showed that the metal ABE was negatively related to the CR among different individuals, thus an individual that pumped at a higher rate would reduce its ABE. Metal ABE is therefore limited by the pumping activity of the mussels (e.g. rate limiting). It should be pointed out that the metal ABE is calculated from the direct measurement of metal influx rate and a mussel's CR and, at best, should be considered as the indirect measurement. In contrast, metal assimilation from ingested food is not greatly dependent on the food concentration (Wang et al. 1995, Ke & Wang in press). For example, the metal assimilation efficiencies in mussel *Mytilus edulis* varied by <3× over a 15× variation in food concentration (ranging from low to high food concentration resulting in pseudofeces production) among the several metals examined (Am, Ag, Cd, Co, Se, and Zn) (Wang et al. 1995). Ke & Wang (in press) also showed that metal assimilation in the green mussel *Perna viridis* was relatively independent of food concentration.

Two neurotransmitter compounds were employed in this study to stimulate or inhibit the pumping activity of mussels. In earlier studies, Beiras & Widdows (1995) demonstrated that serotonin stimulated the pumping

of bivalve larvae at concentrations of 0.01 to 1 μM, similar to our experimental findings. Following a 2 h exposure to serotonin, the pumping rate of the green mussels increased by 7 to 57% over a serotonin concentration of 0.01 to 1 μM. Surprisingly, the CR was inhibited by exposing the mussels in a stepwise manner to different additions of serotonin for 40 min. Previous studies also found that different treatments with serotonin elicited contrasting pumping responses in bivalves (Gosselin 1961, Jørgensen et al. 1988, Jones & Richards 1993). The CR was inhibited by dopamine in a concentration-dependent manner. Complete inhibition of the mussel's CR was found at 100 μM, but there was no influence at a dopamine concentration of 1 μM. Catapane et al. (1978) and Jones & Richards (1993) found that dopamine at a concentration of 0.01 μM to 1 μM inhibited the beating of ctenidial lateral cilia.

The metal influx was not altered after exposing the mussels to either dopamine or serotonin, further indicating that the pumping activity probably did not affect metal uptake from the dissolved phase. Because Cd uptake was comparable at different additions of neurotransmitters, it is unlikely that these compounds altered the Cd speciation in the medium (e.g. by complexation). Passive diffusion through gills has been believed to be the major route for Cd uptake by mussels (Janssen & Scholz 1979, Carpene & George 1981, Roesijadi & Klerks 1989, Odžak et al. 1994).

Modeling metal exposure as a function of seston concentration

It is possible to model the exposure of Cd and Se as a function of seston concentration (TSS) using the following equation (Wang et al. 1996, Luoma & Fisher 1997):

$$C_{ss} = C_{ss,w} + C_{ss,f} \quad (5)$$

where C_{ss} is the metal concentration in bivalves under steady state conditions, $C_{ss,w}$ is the metal concentration in bivalves due to uptake from the dissolved phase, and $C_{ss,f}$ is the metal concentration in bivalves due to accumulation from the dietary phase. This equation assumes that the influx from the aqueous and dietary phases are additive (as demonstrated in this study). $C_{ss,w}$ and $C_{ss,f}$ can be calculated, respectively, by the following equations:

$$C_{ss,w} = k_u \times C_w / k_e \quad (6)$$

$$C_{ss,f} = AE \times IR \times C_f / k_e \quad (7)$$

where k_u is the metal uptake rate constant ($l \text{ g}^{-1} \text{ d}^{-1}$), C_w is the metal concentration in the dissolved phase ($\mu\text{g l}^{-1}$), AE is the metal assimilation efficiency from

ingested food particles, IR is the ingestion rate of the mussels ($\text{mg g}^{-1} \text{d}^{-1}$), C_f is the metal concentration in ingested food particles ($\mu\text{g mg}^{-1}$), and k_e is the efflux rate constant of metals in the mussels (d^{-1}). Thus, the fraction of metals (R) accumulated from the dietary phase is calculated as

$$R = \frac{C_{ss,f}}{C_{ss,w} + C_{ss,f}} = \frac{(AE \times IR \times C_f)}{[(k_u \times C_w) + (AE \times IR \times C_f)]} \quad (8)$$

assuming that the metal concentration in food particles can be predicted from the partition coefficient (k_d) multiplied by the metal concentration in the dissolved phase (C_w), Eq. (8) can be simplified as

$$R = \frac{(AE \times IR \times Kd)}{[k_u + (AE \times IR \times Kd)]} \quad (9)$$

Table 1 summarizes the parameters employed in kinetically modeling the exposure pathways of Cd and Se in the green mussels. The k_u , calculated by the influx rate divided by C_w , is $0.155 \text{ l g}^{-1} \text{ d}^{-1}$ for Cd and $0.022 \text{ l g}^{-1} \text{ d}^{-1}$ for Se. These values are similar to previous measurements of k_u in green mussels (Chong & Wang 2001, Wang 2001). In a previous study, Wang et al. (1995) demonstrated that food concentration had a relatively small effect on the AE of metals in blue mussels *Mytilus edulis*. For example, with an increase in a mussel's ingestion rate from 0.1 to 1.5 mg h^{-1} , the Cd AE decreased from 46 to 42%, and the Se AE decreased from 78 to 70%. In a more recent study, Ke & Wang (in press) also showed that the AE of Cd and Se in green mussels was not greatly influenced by the seston concentration. At a seston concentration of 3 mg l^{-1} , the AE of Cd was 14 and 48% in green mussels feeding on sediment and diatom, respectively, as compared with 18 and 39% at a higher seston load (12 mg l^{-1}). For Se, the AE was 26 and 59% in mussels feeding on sediment and diatom, respectively, at 3 mg l^{-1} , as compared with 18 and 59% at 12 mg l^{-1} . Thus, it is assumed that the AE of Cd and Se is independent of the seston load in our calculation. However the AE is greatly affected by the food quality. We used a range of AE for Cd (10 to 50%, with a median value of 30%) and Se (20 to 60%, with a median value of 40%) in the calculation. The IR is calculated as the CR multiplied by the seston concentration. Because the mussels were able to produce pseudofeces at the seston concentration of about 5 mg l^{-1} (Widdows et al. 1979, Wong & Cheung 1999), we assume that the IR of the green mussels was maintained constant at seston concentration $>5 \text{ mg l}^{-1}$. The mean CR of the mussels was calculated to be $5 \text{ l g}^{-1} \text{ h}^{-1}$ (based on the 2 replicated experiments in food concentration experiments) and was assumed to be somewhat comparable at seston concentrations below 5 mg l^{-1} . Only the experimental sizes of mus-

Table 1. *Perna viridis*. Numeric values of parameters used in modeling Cd and Se exposure in mussels

Parameters	Cd	Se
Uptake rate constant (k_u , $\text{l g}^{-1} \text{ d}^{-1}$)	0.155	0.022
Assimilation efficiency (AE, %)	10–50	20–60
Clearance rate (CR, $\text{l g}^{-1} \text{ d}^{-1}$)	5.0	5.0
Partition coefficient (k_d , l kg^{-1})	5000	5000
Seston concentration (TSS, mg l^{-1})	0.5–20	0.5–20

sels (shell length 2.5 to 3.0 cm) were considered in this study. Of the several parameters included in the model, there is uncertainty about k_d is dependent on seston load. Sung (1995) summarized that the Cd k_d was not significantly related to the seston load, but substantial variations of k_d were documented at similar seston loads. Luoma et al. (1998) demonstrated that the k_d is a function of the phytoplankton bloom. With increasing phytoplankton biomass (e.g. during the spring phytoplankton bloom period), metals are actively removed by the phytoplankton, resulting in a higher partitioning of metals in the algal cells (i.e. higher k_d). In our calculation, we assume that the k_d is independent of the seston load (but see Honeyman & Santschi 1988). Values of k_d (5000 l kg^{-1} for both Cd and Se) are taken from the literature (Fisher & Reinfelder 1995, Wang et al. 1996). Only means of Kd are considered, but it is recognized that k_d can often vary by >1 order of magnitude in natural waters (Luoma et al. 1998).

Thus, among the 4 parameters (k_u , AE, IR and k_d) required in kinetically modeling the exposure pathways of metals in mussels, k_u , AE, and k_d are independent of the seston concentration, whereas the IR increases linearly with increasing seston concentration until it reaches a maximum ($0.6 \text{ g g}^{-1} \text{ d}^{-1}$ at 5 mg l^{-1}). This model would predict that at the seston load $<5 \text{ mg l}^{-1}$, the quantitative significance of aqueous versus dietary exposure is greatly dependent on the seston concentration due to the dependence of IR on seston concentration (Fig. 9). If the k_d increases with increasing seston load, as has been observed in San Francisco Bay during the phytoplankton bloom period (Luoma et al. 1998), it is expected that the relative importance of dietary intake will increase more precipitously with increasing seston loads. The significance of dietary Cd exposure is greatly affected by the seston concentration and the Cd AE from ingested diets. With an increase in seston concentration from 0.5 to 5 mg l^{-1} , Cd accumulation shifts from its dominance by aqueous uptake to dietary uptake. Se accumulation in green mussels is overwhelmed by dietary intake, as has been observed in many previous studies (Luoma et al. 1992, Wang et al. 1996), but notable aqueous intake of Se is

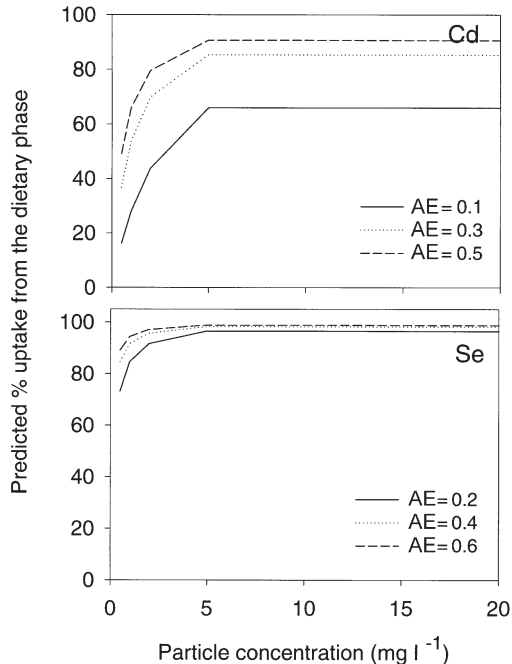


Fig. 9. *Perna viridis*. Model predicted percentage of Cd and Se accumulation from the dietary phase as a function of seston concentration. AE: metal assimilation efficiency

possible at a low seston concentration (e.g. 27% at 0.5 mg l^{-1} with an AE of 20%). At seston loads $>5 \text{ mg l}^{-1}$, the relative importance of dietary exposure remains constant as a result of the maintenance of a maximum IR and the independence of AE and k_d on seston concentration.

In summary, this study demonstrates that the metal uptake rate from the aqueous phase is relatively independent of the particle concentration and the clearance rate of the mussels. By varying the clearance rate of mussels due to the action of neurotransmitters, the metal uptake rates remain unchanged. These data validate previous approach in quantifying metal influx from the dissolved phase (e.g. without addition of food particles) and the assumption that metal accumulation is additive from different sources of exposure. Despite the seston concentration not affecting the uptake rate from the aqueous phase, a bioenergetic-based kinetic model predicts that seston concentration considerably affects the exposure pathways of metals in mussels, primarily due to the change in the mussels' ingestion rate as a function of seston concentration. At a much higher particle load (e.g. due to wind and tidal action), however, the exposure pathways are independent of seston concentration. Thus, variation in seston concentration should be considered in predicting the exposure pathways of metals in aquatic invertebrates. Modeling analysis allows incorporation of variability of

each parameter, which is often difficult to achieve by experimental manipulations either under the laboratory conditions or in the field.

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