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Metal accumulation in the polychaete *Hediste japonica* with emphasis on interaction between heavy metals and petroleum hydrocarbons

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The influences of petroleum hydrocarbons on Cd and Cu accumulation in H. japonica depend on their concentration combinations and exposure time.

Abstract

The accumulation of cadmium (Cd) and copper (Cu) in the polychaete *Hediste japonica* exposed to the mixture of Cd (or Cu) and petroleum hydrocarbons (PHCs) was investigated and compared with that exposed to single Cd (or Cu). The increased bioavailability of Cd or Cu with exposure concentrations resulted in an increase in the accumulation and net accumulation rate of Cd or Cu during single metal exposure. The net accumulation rate of Cd increased, but the net accumulation rate of Cu decreased with exposure time during single metal exposure, suggesting that *H. japonica* could actively regulate Cu burden in their body by inhibition of absolute uptake or promotion of excretion. The interactions between Cd (or Cu) and PHCs had complicated influences on the net accumulation rate of Cd and Cu in *H. japonica* under the condition of the binary mixture, which are dependent on their concentration combinations and exposure time.

Keywords: *Hediste japonica*; Bioconcentration factors (BCFs); Net accumulation rate; Heavy metal; Petroleum hydrocarbons

1. Introduction

Recent industrial activities along coastal regions in China have increased the discharge of industrial wastes into marine waters (Hu et al., 1996; Sun and Zhou, 2005; Zhou, 1995). As a result, the contamination of heavy metals such as cadmium (Cd) and copper (Cu) and petroleum hydrocarbons (PHCs) increasingly occurred in the coastal regions and their neighboring estuaries (Zhou et al., 2004). Cd is a non-essential element that has severe toxic effects on aquatic animals when it presents in excessive amounts (Sorensen, 1991). It is widely distributed in the environment since it coexists with zinc (Zn) in zinc ores. Although Cd is present in seawater or sediments at a trace level, it can be readily accumulated by marine invertebrates, such as the polychaete *Nereis diversicolor*, mussel *Mytilus edulis*, sea star *Asterias rubens*, clam *Scapharca inaequivalvis*, freshwater bivalve *Dreissena polymorpha* and amphipod *Hyalella azteca*. Cd can exert severe cellular damages to marine organisms through substitution of essential cations (Zn and Cu), which serve as co-factors in a number of enzymes (Erk et al., 2005). Therefore, Cd has been considered as one of the major potential heavy metal hazards with acute toxicity to aquatic organisms and humans.

Cu is one of the most common contaminants found at high concentrations in aquatic environment. That is to say, aquatic organisms are being exposed to elevated levels of Cu. In contrast to Cd, Cu, as an essential element for the subsistence of many animals, is a component of many metalloenzymes and respiratory pigments and plays an important role in the

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activities of cellular metabolism (Cousins, 1985). Both deficient and excessive amounts of Cu can cause adverse effects in all species. As one of the most important organic pollutants, the pollution of PHCs has been of great concern in estuarine ecosystems with the development of coastal petroleum industry. The pollution of PHCs originating from oil exploitation and abstraction, leakage of fuels by transportation, discharge and leakage of organic solvent from industrial sites could have toxic effects on the growth and development of plants and animals, and even human health.

The nereidid polychaete *Hediste japonica* (Izuka, 1908) is a common benthic invertebrate in brackish waters and has been considered to be an ecologically significant species (Sato and Nakashima, 2003). This species inhabits the intertidal flats in an estuarine environment. It can constitute an allopatric sibling species complex together with other two distinct species, that is, *Hediste* (*Nereis*) *diversicolor* and *Hediste limnicola* (Sato, 1999; Sato and Masuda, 1997; Sato and Nakashima, 2003). *H. japonica* is a deposit feeder, which can scavenge detritus and organic matters on the sediment surface. *H. japonica* is a key component in estuarine ecosystems functioning as an important food source for many migratory birds, and may therefore contribute to the transfer of contaminants to higher levels of food chains (Sato and Nakashima, 2003; Zhou et al., 2004). For aquatic organisms, bioaccumulation is the process that causes an increased contaminant concentration in the organism compared to that in its ambient environment. When a chemical is accumulated in the body of organisms, it would have potential to be transferred along food chains with toxic effects at higher trophic levels and may be subjected to biomagnification (Sun et al., 2001; Zhou, 1995; Zhou et al., 2004). Thus, it is of great importance to investigate the accumulation of metals in *H. japonica* under single and combined pollution in order to provide data for risk assessment (Zhou and Wang, 2006).

It has been a fact that organisms are generally exposed to a mixture of pollutants that can exert their toxicity simultaneously, in the natural environment. However, most of the literature and reports concerned with bioaccumulation of heavy metals in the organisms and potential toxicity have only resulted from the tests involving in individual pollutants in aquatic environment (Rainbow et al., 2000; Shuhaimi-Othmana and Pascoe, 2007). Little is acquired from the interaction among different contaminants, especially among heavy metals and organic pollutants. In the present study, *H. japonica* was exposed to heavy metals (Cd and Cu) and their combination with PHCs in a laboratory condition. The objective of this study is to investigate and compare the accumulation of Cd and Cu in *H. japonica* exposed to single and joint pollution of heavy metals and PHCs, and then to discuss the effects of PHCs on the accumulation of Cd and Cu.

2. Materials and methods

2.1. Sampling and pollutant-exposed experiments

H. japonica was collected by hand from the gulf of Haizhou (a relatively clean site) in the shore of the Yellow Sea, China, at low tide during June, in 2005. *H. japonica* was transferred from a field to our laboratory in cool boxes with sediments from the site of origin. In our laboratory, collected worms were initially rinsed using seawater from the site of origin and acclimated to seawater for 24 h. Seawater from the same site of origin was used in order to simulate the media of *H. japonica* habitats, eliminating disturbance factors that might affect uptake rate of heavy metals and hence potential toxicity or physiological responses. Then every 9 healthy worms with the similar fresh weight of 1.5 ± 0.5 g were put into a 250 mL acid washed glass beaker with 100 mL seawater containing various concentrations of the tested pollutants at 10 °C (±1 °C). All 100 mL exposure solutions were changed every other day. The worms were not given any food during the course of the experiment. All beakers used in the experiments had been earlier soaked using 5% HNO_3 to eliminate any adsorption of metals onto the beakers (Mouneyrac et al., 2003), and covered with glass utensils in order to reduce evaporation and heat dissipation. The reagents used in this study were of analytical grade. The tested form of Cd and Cu was $CdCl_2 \cdot 2.5H_2O$ and $CuSO_4 \cdot 5H_2O$, respectively. The tested PHCs were obtained from a gas station in Shenyang, Liaoning Province.

In order to ensure the survival of the worms during the experiment, the tested concentrations of Cd and Cu were lower than the values of LC_{50} (which was measured in our previous study and has not been published) and the highest concentration of PHCs was slightly higher than the value of LC_{50} (which was also measured in the previous work). The exposed concentrations in the single-factor pollution experiments were 0, 100, 500, 1000 and 3000 μg/L for Cd and 0, 100 and 150 μg/L for Cu. The concentrations in the combined pollution of Cd and PHCs were 0, 500 and 3000 μg/L for Cd and 0, 100 and 200 μl/L for PHCs. The concentrations in the combined pollution of Cu and PHCs were 0, 100 and 150 μg/L for Cu and 0, 100 and 200 μl/L for PHCs.

2.2. Determination of Cd and Cu accumulated in the worm

H. japonica exposed to various concentrations of the tested contaminants was removed from beakers to determine Cd and Cu contents on days 0, 3 and 6. Firstly, the surviving worms were frozen at −20 °C and then dried to the constant weight at 60 °C and later digested in concentrated HNO_3 and $HClO_4$ at 100 °C. The digested solutions were made up to the known volumes with distilled water. The concentrations of Cd and Cu in the digested acidic solutions were determined using the flame atomic absorption spectrophotometry (FAAS) (Zhou et al., 2003). The content of Cd and Cu was expressed as μg/g *dw*.

Bioconcentration factor (BCF) is often used to compare the level at which a given chemical is accumulated in an organism with the degree of contamination of its surrounding water or sediments (An et al., 2004; De Conto Cinier et al., 1999; Kim et al, 2004; Nguyen et al., 2005). Thus, BCF can be calculated by the following formula:

$$\mathrm{BCF} = \frac{C_{\mathrm{exposure}} - C_{\mathrm{control}}}{C_{\mathrm{water}}} \quad (1)$$

Where C_{exposure} and C_{water} are the concentration of Cd or Cu measured in exposed worms and in control groups (μg/g *dw*), respectively; and C_{water} is the concentration of Cd or Cu in exposure solutions (μg/L).

Moreover, net accumulation rates of Cd and Cu in *H. japonica* were also calculated according to the following expression (Zhou et al., 2003):

$$\text{Net accumulation rate} = \frac{C_{\mathrm{exposure}} - C_{\mathrm{initial}}}{T_{\mathrm{exposure}}} \quad (2)$$

Where C_{exposure} and C_{initial} are the concentration of Cd or Cu measured in exposed worms and in initial worms (μg/g *dw*), respectively; and T_{exposure} is the day of exposure (d). The net accumulation rate of Cd or Cu is expressed as μg/g/d.

2.3. Statistical analysis

All measurements were triplicately performed in each treatment. The experimental results were expressed as means ± standard deviations (SD). When significant differences were found by ANOVA as a function of exposed concentration of Cd, the multiple comparisons Duncan method after test for

homogeneity of variance was used to determine the differences between specific treatments. The values of BCF and net accumulation rate recorded under certain concentration of heavy metals were compared with those under combined pollution of heavy metals and PHCs (*t*-test). Statistical differences were significant at $P < 0.05$.

3. Results

3.1. Cd accumulation in H. japonica exposed to single Cd pollution

Only one worm exposed to 3000 μg/L Cd died at the end of the exposure. The time- and dose-effects on Cd accumulation in *H. japonica* exposed to a series of increasing concentrations of Cd were illustrated in Fig. 1. According to Fig. 1, Cd accumulation in *H. japonica* increased with the extension of exposure time and the rise of exposure concentrations. A highly significant ($P < 0.01$) effect of Cd concentrations in exposure solutions on Cd accumulation was found in *H. japonica* on days 3 and 6. Exposure to Cd on day 3 resulted in a significant increase in the accumulation of Cd in *H. japonica* at 500 μg/L Cd and at all higher Cd concentrations. On day 6, Cd content in *H. japonica* exposed to 3000 μg/L Cd was up to 581.50 μg/g *dw*, which was significantly ($P < 0.01$) higher than any other treatment. A significantly non-linear relationship between Cd accumulation in *H. japonica* and Cd concentrations in exposure solutions was found on days 3 and 6, as described in Fig. 1. The corresponding relationships could be expressed by following regression equations:

$$Y_1 = -6\times10^{-6}X^2 + 0.027X + 1.61 \quad \left(R^2 = 0.9959, p < 0.01\right) \quad (3)$$

and

$$Y_2 = 3\times10^{-5}X^2 + 0.095X + 6.70 \quad \left(R^2 = 0.9993, p < 0.01\right) \quad (4)$$

Where Y_1 and Y_2 were Cd accumulation in *H. japonica* after a 3- and 6-day exposure, respectively, μg/g *dw*; and X was the concentration of Cd in exposure solutions, μg/L.

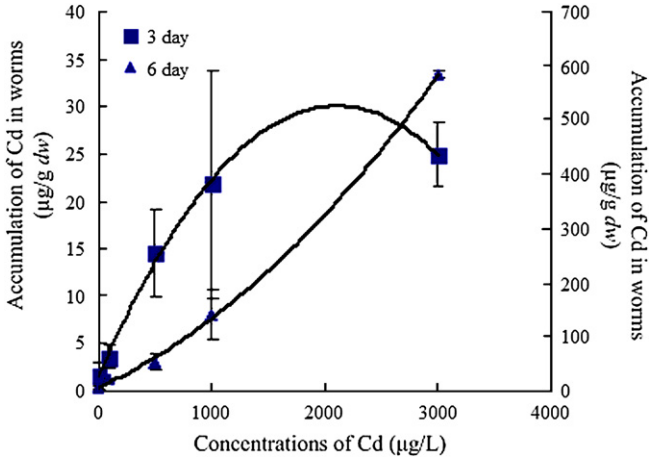

Fig. 1. Accumulation of Cd (μg/g *dw*) in *H. japonica* exposed to various concentrations of Cd after a 3- or 6-day exposure.

The BCFs of Cd for *H. japonica* at single exposure to Cd were shown in Table 1. Exposure concentrations of Cd did not have a significant effect on the BCFs of Cd on day 3, however, a significant effect ($P < 0.05$) was found on day 6. In our study, exposure concentrations did not show a consistent effect on the BCFs of Cd in *H. japonica*. Concretely speaking, on day 3, the highest value of Cd BCFs was found in *H. japonica* exposed to 500 μg/L Cd, while the lowest value of Cd BCFs was found at the exposure of 3000 μg/L Cd. On the contrary, the highest value of BCFs occurred in *H. japonica* exposed to 3000 μg/L Cd, while the lowest value occurred at 500 μg/L Cd after a 6-day exposure. Furthermore, the BCFs of Cd for *H. japonica* on day 6 were significantly higher ($P < 0.05$) than those on day 3 (Table 1). Therefore, the BCFs of Cd significantly increased with exposure periods.

The changes in the net accumulation rate of Cd in *H. japonica* exposed to single Cd were listed in Table 2. The rate of net accumulation of Cd significantly ($P < 0.05$) increased with exposure concentrations in single exposure experiment. The net accumulation rate of Cd was significantly enhanced at higher concentrations (1000 and 3000 μg/L) of Cd exposure for 3 and 6 days, compared with 100 μg/L Cd. The highest value for net accumulation rate of Cd was found in *H. japonica* after a 6-day exposure to 3000 μg/L Cd, which resulted in the highest accumulation of Cd. The rate of net accumulation of Cd also significantly ($P < 0.05$) increased with exposure time, excepting that exposed to 500 μg/L Cd.

3.2. Cd accumulation in H. japonica exposed to combined pollution of Cd and PHCs

Cd accumulation in *H. japonica* exposed to combined pollution of Cd and PHCs, as a function of exposure concentrations and exposure periods, was shown in Fig. 2. Exposure to combined pollution of Cd and PHCs resulted in higher Cd accumulation than the control. Cd accumulation in *H. japonica* under joint pollution of Cd and PHCs increased with exposure time. A highly significant ($P < 0.01$) increase of Cd accumulation on day 6 was found at joint exposure of 500 μg/L Cd and 200 μl/L PHCs, compared with that on day 3 (Fig. 2). As depicted in Fig. 2, exposure to higher concentration of

Table 1
Bioconcentration factors (BCFs) of Cd for *H. japonica* exposed to single Cd or combined pollution of Cd and PHCs after a 3- or 6-day exposure

Pollutant	Concentration	Exposure time (day)	
		Day 3	Day 6
Cd (μg/L)	100	18.90 ± 12.36 a	162.63 ± 4.44 b
	500	25.69 ± 9.32 a	92.42 ± 4.70 b
	1000	19.97 ± 9.95 a	133.27 ± 44.27 b
	3000	7.74 ± 1.13 a	191.65 ± 2.10 b
Cd (μg/L) + PHCs (μl/L)	500 + 100	81.63 ± 44.40	31.62 ± 10.28
	500 + 200	51.27 ± 13.31 a	133.83 ± 16.10 b
	3000 + 100	36.75 ± 9.79	90.68 ± 46.26
	3000 + 200	23.86 ± 9.31	97.25 ± 45.17

Means followed by different letters differ at $P < 0.05$ (*t*-test), and letters beside means refer to the differences between days of treatment.

Table 2
Rates of net accumulation of Cd (μg/g/d) in *H. japonica* exposed to single Cd or combined pollution of Cd and PHCs after a 3- or 6-day exposure

Pollutant	Concentration	Exposure time (day)	
		Day 3	Day 6
Cd (μg/L)	100	0.63 ± 0.41 a	2.59 ± 0.07 b
	500	4.28 ± 1.55	7.70 ± 2.50
	1000	6.66 ± 3.98 a	22.21 ± 7.38 b
	3000	7.74 ± 1.13 a	95.83 ± 1.05 b
Cd (μg/L) + PHCs (μl/L)	500 + 100	13.61 ± 7.40	3.95 ± 1.28
	500 + 200	8.54 ± 2.22 a	16.73 ± 2.01 b
	3000 + 100	36.75 ± 9.79	68.01 ± 34.69
	3000 + 200	23.86 ± 9.31	72.94 ± 33.88

Means followed by different letters differ at $P < 0.05$ (*t*-test), and letters beside means refer to the differences between days of treatment.

Cd (3000 μg/L) combined with PHCs (100 and 200 μl/L) resulted in the increase of Cd accumulation, compared with lower concentration of Cd (500 μg/L) combined with PHCs. Additionally, Cd accumulation in *H. japonica* exposed to the mixture of Cd and PHCs increased on day 3, compared with that exposed to Cd only. Contrarily, Cd accumulation in *H. japonica* exposed to combined pollution of Cd and PHCs on day 6 was lower than that exposed to single pollution of Cd.

The BCFs of Cd in *H. japonica* exposed to the mixture of Cd and PHCs were presented in Table 1. Exposure time had a significant ($P < 0.05$) effect on the BCFs of Cd in *H. japonica* exposed to combined pollution of 500 μg/L Cd and 200 μl/L PHCs, as shown in Table 1. The highest value of Cd BCFs was found in *H. japonica* exposed to the mixture of 500 μg/L Cd and 200 μl/L PHCs on day 6. The BCFs of Cd in *H. japonica* exposed to the mixture of 3000 μg/L Cd and PHCs (100 and 200 μl/L) increased with exposure time, although the difference was not significant (Table 1). The presence of PHCs increased the BCFs of Cd on day 3, compared with those in single Cd exposure. However, there were no such effects on day 6. After a 6-day exposure to the mixture of Cd and PHCs, both increased and decreased BCFs of Cd were observed in comparison to those exposed to Cd only.

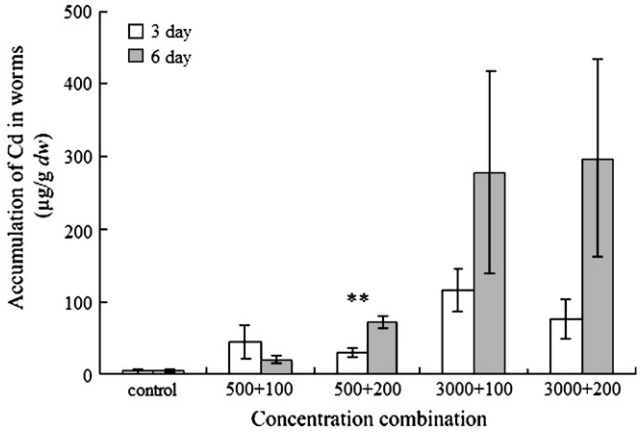

Fig. 2. Accumulation of Cd (μg/g *dw*) in *H. japonica* exposed to combined pollution of Cd and PHCs after a 3- or 6-day exposure. ** indicates significance at $P < 0.01$.

As shown in Table 2, net accumulation rate of Cd in *H. japonica* exposed to combined pollution of Cd and PHCs increased with exposure time, excepting that exposed to the mixture of 500 μg/L Cd and 100 μl/L PHCs. Particularly, net accumulation rate of Cd in *H. japonica* exposed to binary mixture of 500 μg/L Cd and 200 μl/L PHCs on day 6 was significantly ($P < 0.05$) higher than that on day 3. On day 3, exposure to the mixture of Cd and PHCs had higher net accumulation rate of Cd than single Cd exposure. However, on day 6, exposure to the mixture of higher concentration of Cd (3000 μg/L) and PHCs resulted in the decrease in the net accumulation rate of Cd, compared with single Cd exposure (Table 2).

3.3. Cu accumulation in *H. japonica* exposed to single Cu pollution

Exposure to 100 and 150 μg/L Cu resulted in the increase of Cu accumulation in *H. japonica* at the different time intervals, compared with the control. Cu accumulation in *H. japonica* after a 3-day exposure to 100 and 150 μg/L Cu was 18.94 and 19.90 μg/g *dw*, and 74.54% and 83.35% higher than that in the control, respectively. Similarly, Cu contents in *H. japonica* exposed to 100 and 150 μg/L Cu were 20.19 and 22.41 μg/g *dw* on day 6, and 70.43% and 89.15% higher than that in the control, respectively. The results also indicated that Cu accumulation increased with exposure time.

It was shown in Table 3 that Cu BCFs in *H. japonica* exposed to single Cd decreased with exposure concentration, but increased with exposure time. However, the difference was not significant. According to Table 4, net accumulation rate of Cu in *H. japonica* increased with exposure concentrations of Cu, but decreased with exposure time. On day 6, the net accumulation rate of Cu in *H. japonica* exposed at 100 and 150 μg/L Cu was 2.09 and 2.64 μg/g/d, and 48.40% and 41.59% lower than that on day 3, respectively.

3.4. Cu accumulation in *H. japonica* exposed to combined pollution of Cu and PHCs

Cu accumulation in *H. japonica* exposed to combined pollution of Cu and PHCs, as a function of exposure

Table 3
Bioconcentration factors (BCFs) of Cu for *H. japonica* exposed to single Cu or combined pollution of Cu and PHCs after a 3- or 6-day exposure

Pollutant	Concentration	Exposure time (day)	
		Day 3	Day 6
Cu (μg/L)	100	80.90 ± 15.90	83.46 ± 46.61
	150	60.31 ± 9.60	70.42 ± 15.46
Cu (μg/L) + PHCs (μl/L)	100 + 100	87.80 ± 2.67	133.86 ± 75.70
	100 + 200	47.53 ± 21.40	46.90 ± 11.24
	150 + 100	77.92 ± 16.75	67.81 ± 34.03
	150 + 200	22.30 ± 9.44	*

*The value was below zero because Cu accumulation was lower than the control.

Table 4
Rates of net accumulation of Cu (μg/g/d) in *H. japonica* exposed to single Cu or combined pollution of Cu and PHCs after a 3- or 6-day exposure

Pollutant	Concentration	Exposure time (day)	
		Day 3	Day 6
Cu (μg/L)	100	4.05 ± 0.54	2.09 ± 1.17
	150	4.52 ± 0.72	2.64 ± 0.58
Cu (μg/L) + PHCs (μl/L)	100 + 100	4.39 ± 0.13	4.46 ± 2.52
	100 + 200	2.38 ± 1.57	1.56 ± 0.37
	150 + 100	5.84 ± 2.76	3.39 ± 1.70
	150 + 200	1.67 ± 0.71	*

*The value was below zero because Cu accumulation was lower than the control.

concentrations and exposure periods, was shown in Fig. 3. Exposure to the binary mixture of Cu and PHCs resulted in the increase of Cu accumulation, compared with the control. Cu accumulation in *H. japonica* at each exposure concentration of combined pollution of Cu and PHCs increased with exposure time (Fig. 3). Exposure to combined pollution of Cu and PHCs for 3 days resulted in the decrease in Cu accumulation, compared with single pollution of Cu. And on day 6, Cu accumulation in *H. japonica* exposed to combined pollution of Cu and PHCs was not significantly higher than that exposed to single Cu.

Cu BCFs in *H. japonica* exposed to joint pollution of Cu and PHCs were shown in Table 3. Cu accumulation in *H. japonica* after a 6-day exposure to combined pollution of 150 μg/L Cu and 200 μl/L PHCs was lower than the control. Therefore, the BCFs of Cu cannot be calculated (Table 2). Exposure time did not have significant influences on the BCFs of Cu in *H. japonica* when exposed to the combined pollution. The addition of PHCs resulted in the raise or reduction in Cu BCFs at different time intervals, compared with single Cu exposure.

Table 4 indicated that net accumulation rate of Cu generally decreased with exposure time in Cu and PHCs mixture exposure experiment. The presence of lower concentration of PHCs (100 μl/L) could enhance the net accumulation rate of Cu in comparison to single Cu exposure. However, the addition of higher concentration of PHCs (200 μl/L) inhibited net accumulation rate of Cu, compared with single Cu exposure. Cu accumulation in *H. japonica* exposed to the mixture of 150 μg/L Cu and 200 μl/L PHCs was lower than that in the control, on day 6. Thus, the net accumulation rate could not be calculated.

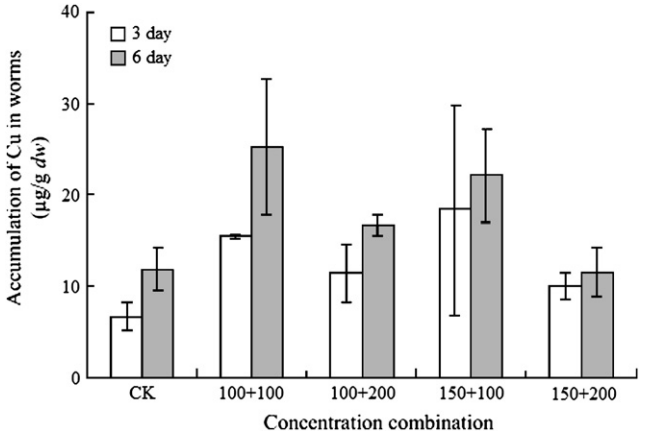

Fig. 3. Accumulation of Cu (μg/g *dw*) in *H. japonica* exposed to combined pollution of Cu and PHCs after a 3- or 6-day exposure.

4. Discussion

Most investigations on metal mixture interactions have considered the mortality as an indicator of toxicity, however, metal bioconcentration or bioaccumulation in the body of organisms are also related to the toxic effects of pollutants (Pelgrom et al., 1994, 1995) and the evolution of metal-resistance (Xie and Klerks, 2004). Our investigation showed that *H. japonica* could accumulate Cd and Cu from solutions with the amount increasing with the dissolved concentration and exposure duration in single metal exposure experiments. This result was in agreement with the previous observation for the polychaete *Nereis diversicolor* exposed to various concentrations of Cd, Cu and Zn for 21 days (Zhou et al., 2003). It was also shown in the present study that Cd and Cu accumulation in *H. japonica* exposed to the mixture of heavy metals and PHCs was enhanced with exposure time.

BCFs are generally used to measure how much a certain contaminant is accumulated in the tissues or organs of organisms with respect to aqueous exposure concentration. Our study indicated that the BCFs of Cd and Cu in *H. japonica* increased with exposure time under the stress of single pollutant. This was also the case of the BCFs of Cd in *H. japonica* exposed to the mixture of Cd and PHCs. These results were in accordance with the previous observations (De Conto Cinier et al., 1999; Kim et al., 2004; Shuhaimi-Othmana and Pascoe, 2007). It has been known that BCFs are concentration-dependent and an inverse relationship between BCFs and exposure concentrations usually occurs (De Conto Cinier et al., 1999; Kim et al., 2004; Nakhlé et al., 2006; Shuhaimi-Othmana and Pascoe, 2007). This is the case of the BCFs of Cu in *H. japonica* exposed to single Cu in the present work. However, there were no consistent effects of exposure levels on the Cd BCFs in single Cd exposure. Laboratory and field investigations (Meador et al., 2005) indicated that BCFs were usually highest at low exposure levels or at relatively clean sites and were lowest at high exposure levels, where adverse influences might have happened. Therefore, McGeer et al. (2003) thought that BCFs led to conflicting conclusions with ecotoxicological data and be of little value in hazard evaluation. Consequently, it is necessary to consider the accumulation rate of heavy metals to better expound bioaccumulation mechanisms of metals by organisms from aqueous solutions or to understand the dynamic changes of heavy metal accumulation. In the present study, the net accumulation rates of Cd and Cu in *H. japonica* were considered and compared with other studies.

The net accumulation rate of Cd or Cu has been investigated in *N. diversicolor* from different coastal sites (Bryan and Hummerstone, 1971, 1973; Zhou et al., 2003).

N. diversicolor from Avon and Restronguet Creek at 10,000 μg/L Cd exposure at 17.5 ppt salinity accumulated Cd at 55 and 18.5 μg/g/d, respectively (Bryan and Hummerstone, 1973). Additionally, net accumulation rate of Cd in *N. diversicolor* from Dulas Bay, Blackwater and West Thurrock was 16.4, 14.2 and 9.9 μg/g/d, respectively, when exposed to 1000 μg/L Cd at 16 ppt for 21 days (Zhou et al., 2003), comparable to 6.66 and 22.21 μg/g/d recorded in *H. japonica* after 3 and 6 days exposure. In the case of Cu, *N. diversicolor* from the Plym estuary and Restronguet Creek at 100 μg/L Cu exposure at 17.5 ppt can accumulate Cu at 7.6 and 14.8 μg/g/d, respectively (Bryan and Hummerstone, 1971). According to Zhou et al. (2003), *N. diversicolor* inhabiting in Dulus Bay, Blackwater and West Thurrock could accumulate Cu at 108, 19.7 and 20.6 μg/g/d when exposed to 100 μg/L Cu at 16 ppt for 21 days, respectively. However, net accumulation rate of Cu in *H. japonica*, after a 3- and 6-day exposure to 100 μg/L Cu, was only 4.05 and 2.09 μg/g/d, respectively. Therefore, the net accumulation rate of Cu in *H. japonica* is lower than that in *N. diversicolor* from other previous studies. It has been known that the factors influencing the accumulation of heavy metals in organisms are species, seasonal and physiological factors, size, sexual conditions, gametogenesis period, behaviors, and hydrodynamics of the environment (Boyden and Phillips, 1981; Chattopadhyay et al., 2002).

In our study, the increased bioavailability of Cd or Cu with the exposure concentrations resulted in the raise of net accumulation rate and consequently Cd or Cu accumulation in *H. japonica* at single-factor exposure. McGeer et al. (2000) found that a continuous increase in Cd accumulation occurred in the liver and kidney of rainbow trout exposed to sublethal levels of Cu, Cd and Zn over an extended exposure time and come to a conclusion that a passive regulation for Cd accumulation occurring in rainbow trout. Similarly, Cd accumulation in *H. japonica* increased with exposure time at single Cd exposure, either when net accumulation rate of Cd was considered or when the BCFs of Cd were evaluated. In the case of combined pollution, either increase or decrease in the accumulation rate of Cd or Cu was resulted in at different exposure concentration and exposure time, compared with that at single exposure, which suggested that both induction and inhibition of metal accumulation might occur in complex mixtures. In other words, different concentration combinations of Cd (or Cu) and PHCs and exposure time had inconsistent effects on the accumulation and net accumulation rate of heavy metals in *H. japonica*. Therefore, the presence of PHCs has extremely complex influences on the accumulation of heavy metals in *H. japonica*. However, a full understanding of the effects of interactions between heavy metals and PHCs on heavy metal accumulation in *H. japonica* will require further investigations.

The mechanisms of reducing metal accumulation in organisms include uptake inhibition, increased elimination and induction of detoxification enzymes. According to Rainbow et al. (2000), accumulation is equivalent to net uptake or net flux, which could be attained by subtracting excretion from absolute uptake. Thus, the rate of net accumulation is equivalent to that of absolute uptake minus excretion. It can be thus concluded that the reduced net accumulation rate of Cu in *H. japonica* with exposure time may be contributed to the decrease of absolute uptake or the increase of elimination through promoted excretion. The level of essential metals (Cu, and Zn) in organisms is prone to be highly metabolic regulated or eliminated in comparison to non-essential metals (Cd) (Fernandes et al., 2007; McGeer et al., 2000). Therefore, our investigation fits well with previous studies and indicates that *H. japonica* could actively regulate Cu burden in their body by either absolute uptake inhibition or enhanced excretion.

In the presence of higher concentration of PHCs (200 μl/L), the net accumulation rate of Cu at different time intervals was lower than that at single Cu exposure. This decrease could be presumed that the concentration of PHCs is high enough to induce the excretion of Cu from *H. japonica* or to reduce the bioavailability of Cu. On the other hand, several researchers put forward ''metal depletion hypothesis'' that concentrations of heavy metals in tissue of organisms are depleted when organic contaminants (such as PAHs and PCBs) are at elevated levels (Brown et al., 1987; Portman, 1972). However, the negative correlation between organic contaminants and metals is not always tenable for any metal, except for essential elements (Portman, 1972). In our present study, Cu accumulation in *H. japonica* exposed to the mixture of Cu and PHCs was not higher than that at single Cu exposure during the whole exposure period. It seems that our result supports metal depletion hypothesis. However, further studies still need to be conducted at other concentration combinations in order to prove inhibition of Cu accumulation in *H. japonica* by PHCs.

5. Conclusions

During single metal exposure, Cd exposure concentrations did not have a consistent effect on Cd BCFs while raised concentrations of Cu in exposure solutions resulted in the decrease of Cu BCFs in *H. japonica*. The BCFs of both Cd and Cu in *H. japonica* increased with exposure time. Cd accumulation in *H. japonica*, in terms of the amount and net accumulation rate, increased with exposure concentrations and exposure time, suggesting that a passive regulation for Cd accumulation occurring in *H. japonica*. The net accumulation rate of Cu decreased with exposure time, which suggested that *H. japonica* could actively regulate Cu burden in their body by the inhibition of absolute uptake or enhanced excretion. It could thus conclude that accumulation mechanism of non-essential metal Cd in *H. japonica* was different from that of essential metal Cu.

The presence of PHCs had complicated influences on the accumulation rate of Cd and Cu in *H. japonica* and thus on metal bioaccumulation. Under the binary mixture exposure, both increase and decrease in the BCFs and net accumulation rate of Cd and Cu was observed in comparison with single Cd or Cu exposure, which indicated that both induction and inhibition of metal accumulation might occur by the mixture. The interactive effects of heavy metals and PHCs on metal accumulation in *H. japonica* depend on their concentration combinations and exposure time.

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