ISSN 1001-0742
CN 11-2629/X

2012
Volume 24
Number 10

JOURNAL OF ENVIRONMENTAL SCIENCES

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JOURNAL OF ENVIRONMENTAL SCIENCES

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Available online at www.sciencedirect.com

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Journal of Environmental Sciences 2012, 24(10) 1723–1730

JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Effect of periphyton community structure on heavy metal accumulation in mystery snail (*Cipangopaludina chinensis*): A case study of the Bai River, China

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Received 01 November 2011; revised 24 February 2012; accepted 23 April 2012

Abstract

The ratio of metal:P stoichiometry was used to identify the accumulation pathways of heavy metals (V, Cr, Co, Ni, Cu, Cd, and Pb) from periphyton to snails *Cipangopaludina chinensis* Gray (*C. chinensis*) in the Bai River watershed. The results showed that periphyton communities were mainly composed of two types of algae, filamentous green algae and unicellular diatoms. The proportion of unicellular diatoms in the periphyton community is a key factor that influences metal accumulation in *C. chinensis*. The V, Cr, Co, Ni, and Cd content of *C. chinensis* increased steadily as the corresponding metal content of periphyton increased, but Cu and Pb in the snail did not increase in the periphyton. Mechanisms of V, Cr, and Ni accumulation were found to be related to the proportion of diatoms, while Cd and Pb accumulation were dependent on the physiological characteristics of *C. chinensis*. The accumulation of Cu in *C. chinensis* was closely related to their grazing behavior. The metal:P stoichiometry revealed that Cr, Ni, and Cd can reduce the potential ecological risks associated with increased P inputs to the ecosystem. V and Co were considered to be relatively safe, regardless of the periphyton P content. Finally, Pb may not be prone to transfer to higher trophic levels, and may pose the lowest ecological risks of the studied heavy metals, but Cu can cause potential ecological risks when eutrophication has occurred.

Key words: heavy metal; metal:P; periphyton; Chinese mystery snails; *Cipangopaludina chinensis*

DOI: 10.1016/S1001-0742(11)60976-5

Introduction

Periphyton play an important role in fluvial food webs (Barbour et al., 1998). In river ecosystems, periphyton are usually the main source of primary production (Hauer and Lamberti, 2006), providing high-quality food for a diverse group of vertebrate and invertebrate grazers (Hill et al., 2010). The periphyton community composition is strongly influenced by river habitat factors, such as temperature (Munn et al., 1989; Butterwick et al., 2005), pH (DeNicola, 2000; Baffico et al., 2004), light (Mosisch et al., 2001; Greenwood and Rosemond, 2005; Tuchman et al., 2006), turbidity (Bilotta and Brazier, 2008; Kohler et al., 2010), and nutrient status (Hillebrand, 2002; Lange et al., 2011; Schneider and Lindstrom, 2011). Several studies have concluded that excessive nutrient inputs from anthropogenic activities can cause shifts in the periphyton community composition (Dodds et al., 2002; Bixby et al., 2008; Morin et al., 2008). Periphyton are sensitive to river habitat quality and can respond quickly to any changes in their habitats. For example, shifts in the periphyton community will affect benthic invertebrate grazers such as *Cipangopaludina chinensis Gray* (*C. chinensis*).

Existing research has indicated that periphyton can be an important accumulator of heavy metals (Newman and McIntosh, 1989), and that sorbed metals can then be transferred to consumers (Hill et al., 1996). This process can influence the food web structure and changes in snails will further affect members of higher trophic levels. Accordingly, research regarding the process by which metals are transmitted through the food web can effectively explain their toxicity toward higher trophic levels.

The Hai River Basin (HRB) is one of seven major river basins in China. The HRB is subject to anthropogenic interference and severe water pollution. Previous investigations have shown that both nitrogen and phosphorus (P) inputs in the region have increased (Li et al., 2004), and toxic substances such as heavy metals (Liu et al., 2006) have been widely detected due to land-use changes and the discharge of water pollutants through pesticide and fertilizer use, livestock and poultry culture, and municipal sewage discharge. These changes have led to the risk of eutrophication and heavy-metal pollution. The former causes shifts in the periphyton community structure (Kelly, 1998), while the latter results in toxicity to the benthos (Das and Khangarot, 2011). It is important to understand periphyton community changes and heavy metal accumulation in the benthos to enable effective river water quality management

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in the HRB.

A new method that uses the metal:P stoichiometry in periphyton and its grazer, *C. chinensis*, to identify the accumulation pathways of heavy metals in snails, was introduced in this study. Most ecological analyses have been constructed from single-currency descriptions but ecological stoichiometry is different. It is based on the idea that the ratios of elements available to organisms affect their transformations in ecosystems (Frost et al., 2002). Many traditional methods enabled a great deal of progress. We expect that this method could explain periphyton community changes caused by P inputs and the effects on heavy metal accumulation in the benthos. Based on the above considerations, we selected the Bai River, to investigate the heavy metal (V, Cr, Co, Ni, Cu, Cd and Pb) accumulation of snails.

1 Materials and methods

1.1 Study site

The Bai River is a first- to second-order stream in the Hai River Basin (35°N, 112°E–43°N, 120°E) that is a significant source of drinking water for Beijing (Wang et al., 2009). The river has a length of 250 km (Fig. 1), and the basin drains an area of 9072 km^2. The Bai River is the main water source of the Miyun Reservoir, which is the most important water source to Beijing (Guo et al., 2004). The major land use types in the basin are forest land, farm land, villages and small towns. No industrial sewage water discharges directly into the river due to strict environmental regulations. However, this area still has many environmental problems including water resources use, hydrological variations, and decreasing water levels. In recent years, the Bai River has turned into a shallow stream with a gravel/cobble riverbed owing to drought and water overuse. These habitat characteristics have stimulated benthic communities such as periphyton growth. In addition, the Bai River is a subalpine river. Cornfields lie on either side of the Bai River in this area. The excessive land reclamation generates agricultural non-point source pollution (Aji et al., 2008).

To investigate the relationships between periphyton community shifts and heavy metal accumulation in the benthos, we selected 22 sampling sites in the Bai River and its tributaries (Fig. 1). Many villages are located in this area, indicating potential anthropogenic interference. Sample sites were located in the river at an average of 8 km apart in areas characterized by similar physical conditions (depth, light, velocity, turbidity and riverbed). Field sampling was conducted during the summer of 2010.

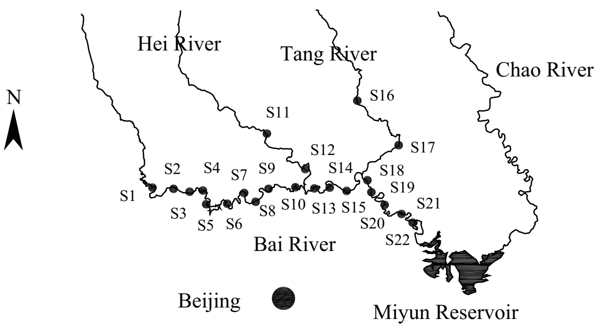

Fig. 1 Spatial distribution of 22 sampling sites (S1–S22) in the Bai River and its tributaries.

1.2 Analysis of periphyton and freshwater snails

Samples of periphyton and freshwater snails were collected from randomly selected rocks at depths ranging from 0.1 to 0.3 m. Lower depths were avoided so that a similar light intensity would be present because light can strongly influence the taxonomy of the periphyton community (Lange et al., 2011). Periphyton was removed from the surfaces of stones by vigorous brushing. Snails were collected using forceps regardless of their body size. Shell size was measured from the tip of the apex to the posterior edge of the aperture (Kenneth, 2001).

Samples of periphyton were partitioned into three subsamples for algal community structure identification, heavy metals analysis, and a backup. One subsample was preserved in 3%–5% glutaraldehyde solution (Barbour et al., 1998) and then transported to the laboratory, where it was analyzed using an Olympus BX51 microscope. The biovolume was calculated by applying average dimensions of up to 10 cells in each taxon to standard geometric shapes that best represented the shape of each taxon (Hillebrand et al., 1999). If the biovolume of some taxa was very low when compared with the dominant algae (biovolume < 5%), it was not considered in the study because it was assumed that its metals content would not significantly affect the overall community. Another subsample of periphyton together with a sample of snails was kept at lower than –16°C in a cooler (Mobicool) during transportation to the lab, where they were subsequently freeze-dried together. After drying, these samples (snails with shells) were ground into a fine powder with a glass mortar and pestle until the particles could pass through a lab sieve (pore size 150 μm). The sample powders were then digested using a CEM Microwave-Assisted Acid Digestion System and analyzed for P and heavy metals (V, Cr, Co, Ni, Cu, Cd, Pb) using ICP-MS (Plasma Quad 3).

1.3 Data analysis

Statistical analysis was conducted using the software PASW Statistics 18 for Windows. Mean analyses were used to determine the content of seven heavy metals in periphyton and snails. Both periphyton and the snail metal:P molar ratio were plotted with periphyton P content using the OriginPro 8 software for Windows. The metal:P ratios were fitted in linear or exponential mode based on their data distributions. The relationships between taxa and the elemental composition of the periphyton were explored by principal component analysis (PCA) using CANOCO for Windows 4.5 (Lepš and Šmilauer, 2003). We used the biovolume of each taxa of algae that comprised the total community to identify the relationships between the taxa of algae and their heavy metals content during PCA analysis.

2 Results

2.1 Periphyton community structure and snail taxa

Periphyton communities in the Bai River were mainly composed of two types of benthic algae, filamentous green algae and unicellular diatoms (Table 1). The former dominated most samples of the periphyton community. The major genus of filamentous algae in the sampling sites was *Cladophora*, but other taxa such as *Oedogonium* and *Spirogyra* were also present in the periphyton community. The unicellular algae (*Diatoma*, *Cymbella*, etc.) in this region were primarily epiphytic diatoms attached to the filamentous algae. Field sampling showed that all of the communities contained filamentous algae alone or filamentous algae with diatoms. The capacity of periphytic algae to accumulate metals has previously been reported and discussed (Duong et al., 2008). The relationships between periphyton metal contents and their taxa are of great help in identifying the conditions that lead to heavy metals accumulation by snails.

C. chinensis was widely distributed along the Bai River. This snail grazes on both algae (Lodge, 1986) and macrophytes (David, 1991). However, algae are the only food source available for *C. chinensis* in the investigated area. The substrate of the Bai River is gravel/stone; thus, few macrophytes such as weeds or cattails are present and the primary producers are periphyton.

Although the size of adult *C. chinensis* can reach 65 mm, sizes of snail samples in the Bai River ranged from 5 to 20 mm. *C. chinensis* can tolerate various ambient conditions from oligotrophic streams to hypertrophic lakes, and even occur in septic tanks (Perron and Probert, 1973). Large snails can survive for at least four weeks out of water under mesic conditions (Havel, 2011). These characteristics have resulted in the snails having a wider distribution in seasonal rivers or rivers with intermittent water resources such as the Bai River.

2.2 Metals and P content in periphyton and snails

The average P content of snails was 2.60±0.80 mg/g (mean±standard deviation), which was higher than that of periphyton (1.97±1.18 mg/g, Table 2). The larger deviation of periphyton indicated that snail P remained stable when

Table 1 Percentage (%) biovolume of algal taxa in the Bai River and its tributaries

Taxa	Sampling site																					
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22
Filamentous																						
Cladophora	0.8	0.5	0.4	0.2	0.4	0.2	0.7	0.4	0.6		1.0	0.1	0.7	0.8	0.9	1.0	1.0	0.8	1.0			0.1
Oedogonium	0.2											0.2										
Spirogyra								0.2				0.6								0.8		
Ulothrix																				0.1		
Rhizoclonium																					1.0	0.9
Oscillatoria										1.0												
Unicellular																						
Diatoma		0.5	0.6	0.8	0.6	0.8		0.1				0.1										
Cymbella							0.3	0.1												0.1		
Cocconeis								0.2	0.3				0.1	0.1	0.1							
Fragilaria									0.1													
Navicula													0.1									
Gomphonema													0.1	0.1				0.2				
Snail	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

✓ represents samples of *C. chinensis* were available in the sampling sites.

Table 2 Heavy metal contents in periphyton and snails in the Bai River and its tributaries

Site	P (mg/g)		V (μg/g)		Cr (μg/g)		Co (μg/g)		Ni (μg/g)		Cu (μg/g)		Cd (μg/g)		Pb (μg/g)	
	Periphyton	Snail	Periphyton	Snail	Periphyton	Snail	Periphyton	Snail	Periphyton	Snail	Periphyton	Snail	Periphyton	Snail	Periphyton	Snail
S1	3.10	3.46	75.46	45.87	6.90	5.31	15.62	7.65	21.18	11.01	46.07	35.85	0.19	0.24	7.18	5.20
S2	2.02	2.68	35.50	18.33	3.47	1.88	4.80	3.19	7.84	5.05	13.98	19.13	0.07	0.18	6.79	2.95
S3	1.83	2.67	32.17	10.86	4.30	1.20	4.23	3.21	7.20	4.66	29.37	16.70	0.06	0.15	12.03	2.14
S4	1.05	2.90	36.56	15.86	4.49	1.48	3.84	3.68	7.34	5.01	28.98	20.87	0.12	0.17	12.23	2.64
S5	1.27	3.42	40.22	13.81	5.00	3.03	5.10	3.08	8.58	4.88	38.21	82.07	0.08	0.18	10.67	13.32
S6	0.89	2.30	41.14	20.48	3.98	2.32	5.28	3.14	8.44	7.58	44.37	19.11	0.08	0.14	11.38	3.16
S7	1.87	0.61	8.65	12.00	45.98	9.64	1.94	2.31	15.59	16.78	35.03	8.14	0.05	0.05	6.42	10.55
S8	0.99	3.37	6.33	1.01	15.97	12.39	1.40	0.98	11.52	9.77	30.07	32.75	0.06	0.21	6.76	1.72
S9	0.96	3.12	14.96	2.08	31.31	11.77	3.98	1.22	19.29	7.76	16.45	29.17	0.10	0.23	11.54	2.08
S10	1.41	2.94	10.18	1.90	19.80	10.38	2.91	1.23	7.54	9.66	6.71	22.79	0.21	0.33	11.01	2.54
S11	2.15	3.78	6.89	2.32	13.26	7.81	2.15	1.98	6.89	4.79	7.43	19.32	0.21	0.56	7.77	2.42
S12	2.84	1.73	12.18	4.62	16.49	45.20	3.92	1.84	7.11	5.85	7.00	50.41	0.21	0.20	11.96	4.25
S13	2.17	3.65	39.04	24.70	40.26	68.54	16.65	9.40	20.22	20.69	89.61	99.56	0.16	0.22	8.81	3.88
S14	1.88	2.49	15.91	12.06	25.71	22.49	4.29	3.61	9.06	9.32	10.36	35.43	0.07	0.18	5.15	3.74
S15	2.59	2.76	14.45	5.89	13.60	20.38	4.07	2.71	7.08	8.78	8.9	43.35	0.09	0.24	5.44	2.02
S16	1.38	1.59	9.55	4.08	17.09	15.45	3.41	2.32	7.17	7.91	7.66	30.56	0.08	0.16	3.73	1.24
S17	1.23	1.66	14.75	5.75	29.61	38.88	4.44	2.64	10.68	12.68	10.8	34.20	0.08	0.15	5.19	1.74
S18	1.92	1.58	10.55	4.96	11.49	15.52	3.58	2.49	5.67	8.27	7.86	32.58	0.10	0.17	4.09	1.80
S19	1.38	2.41	18.45	11.36	19.10	30.62	5.17	3.71	10.38	19.26	16.65	57.72	0.10	0.15	6.96	4.15
S20	0.61	2.10	19.62	7.28	23.91	98.92	4.86	2.54	13.99	48.29	14.33	27.49	0.18	0.17	10.96	1.39
S21	4.04	2.91	20.97	4.37	29.93	33.26	4.12	1.79	18.59	20.05	13.04	46.21	0.09	0.13	11.87	1.77
S22	5.76	3.05	6.68	5.57	13.82	33.80	1.64	1.95	8.72	21.62	6.52	53.62	0.12	0.19	7.46	2.49
Mean	1.97	2.60	22.28	10.69	17.98	22.29	4.88	3.03	10.91	12.26	22.25	37.14	0.11	0.20	8.43	3.51
SD	1.18	0.80	16.86	10.19	11.97	24.20	3.81	1.96	4.91	9.79	19.83	21.66	0.05	0.10	2.88	2.95

SD: standard deviation.

compared with periphyton. Both snails and periphyton showed great variations in metal content. The levels of V and Pb in snails declined over time to a greater degree than the levels in periphyton, indicating low accumulation capabilities of the two metals. Conversely, Cr, Ni, Cu and Cd were prone to accumulation in *C. chinensis*. In other words, the accumulation capabilities of Cr, Ni, Cu and Cd in snails were stronger than those of V and Pb.

We selected the DCA method and by segments in the Detrending Method function of CANOCO in order to decide which ordination is better, the linear (PCA) or the unimodal (CCA). The results showed that the gradients (0.477) are not long (< 4) enough, so PCA was conducted to evaluate heavy metal variations. This analysis enabled the identification of the relationship between the periphyton metal content and the taxa. The eigenvalues of PCA axis 1 (0.527) and axis 2 (0.299) accounted for 82.6% of the cumulative variance in the heavy metals data. The species–environment correlations of PCA axis 1 (0.965) and 2 (0.889) tare high, and the first two axes account for 83.3% of the variance in the metal–taxa relationships. *Cymbella*, *Cocconeis*, and *Fragilaria* are major contributors of community Cr (Fig. 2). *Oscillatoria*, *Gomphonema*, and *Navicula* were positively correlated with periphyton P, while *Oedogonium* was negatively correlated. P was positively correlated with the numbers of diatom taxa. As P decreased, only the biovolume of *Oedogonium* in the periphyton community increased.

The V, Cr, Co, and Cd content of *C. chinensis* increased steadily as the corresponding metal content of periphyton increased (Fig. 3). Furthermore, the levels of Cr and Cd in snails tracked the periphyton Cr and Cd content almost exactly, and both contents were nearly equal. Cu and Pb in snails did not increase in the periphyton. As the Cu content of the periphyton increased, the abilities of the snails to accumulate metals declined. Overall, the Cu accumulation

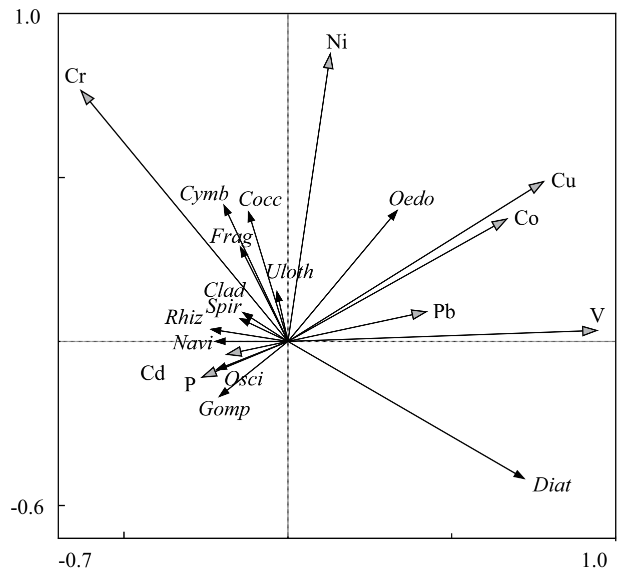

Fig. 2 PCA biplot of periphyton taxa and elemental composition. Taxonomic variables: *Clad*: *Cladophora*; *Oedo*: *Oedogonium*; *Spir*: *Spirogyra*; *Osci*: *Oscillatoria*; *Uloth*: *Ulothrix*; *Rhiz*: *Rhizoclonium*; *Diat*: *Diatoma*; *Cymb*: *Cymbella*; *Cocc*: *Cocconeis*; *Frag*: *Fragilaria*; *Navi*: *Navicula*; *Gomp*; *Gomphonema*.

in snails appeared to stabilize when the periphyton was in the range of 5–30 μg/g. The Pb accumulation of snails peaked when the periphyton levels were 9–11 μg/g. As the periphyton P increased from 0.5 to 6.5 mg/g, the level of P in the snails remained stable. This result is also in agreement with the existing ecological stoichiometry theory (Stelzer and Lamberti, 2002).

2.3 Metal:P stoichiometry of snails and periphyton

The periphyton metal:P ratios varied greatly and decreased as the periphyton P content increased (Fig. 4). All of the metal:P ratios of periphyton followed an exponential distribution. The Cu:P in snails increased as the periphyton P content increased, which was notably different from the other six metals. The Cr:P of the snail showed an exponential distribution and tracked the ratios of the periphyton they took in, indicating that snails maintain their body Cr:P based on the Cr:P in their periphyton intake. The Ni:P ratios in snails were found to be almost the same as those in periphyton, especially when P > 0.075 mmol/g. The slope of the Co:P linear fit equation was near zero (Fig. 4, $y = 0.061 - 0.001x$), indicating that the Co:P of snails was remarkably stable, similar to Pb.

For lead, the mechanism by which it was absorbed differed from that of Cu. The results clearly illustrate that the Pb:P ratios in snails were basically stable (Fig. 3, Pb:P linear fit: $y = 0.221 - 0.220x$). There is evidence that snails have strategies to excrete body lead (Everard and Denny, 1984), which explains why the snail Pb content did not increase with periphyton Pb.

3 Discussion

3.1 Relationships of metals accumulation between Periphyton and snails

Snails prefer grazing on diatoms to filamentous algae (Jokinen, 1982) and this grazing preference suggested that snails are prone to accumulate heavy metals present in diatoms. Accordingly, the proportion of unicellular diatoms in the periphyton community is a key factor that influences metals accumulation in snails. Nevertheless, physiological characteristics were also important for the accumulation process, indicating that the mechanism of metals accumulation is not fully dependent on the snails' grazing preference. For example, the Cr and Cd levels of the snail's body were similar to those of the periphyton they took in, while the levels of these metals were altered via different mechanisms.

The mechanism of Cr enrichment in these unicellular diatoms likely functions via the extracellular polymeric substances (EPS) they excrete. Studies have shown that algae can prevent Cr and Ni from reaching toxic levels inside cells (Wang and Wood, 1984) by excreting EPS that can remove metal ions from solution (Wood, 1983), which suggests that the Cr and Ni accumulation was highly influenced by the proportion of epiphytic unicellular diatoms in the periphyton community. Similarly, V was favorable to diatoms at ⩽ 20 mg/L, while filamentous algae

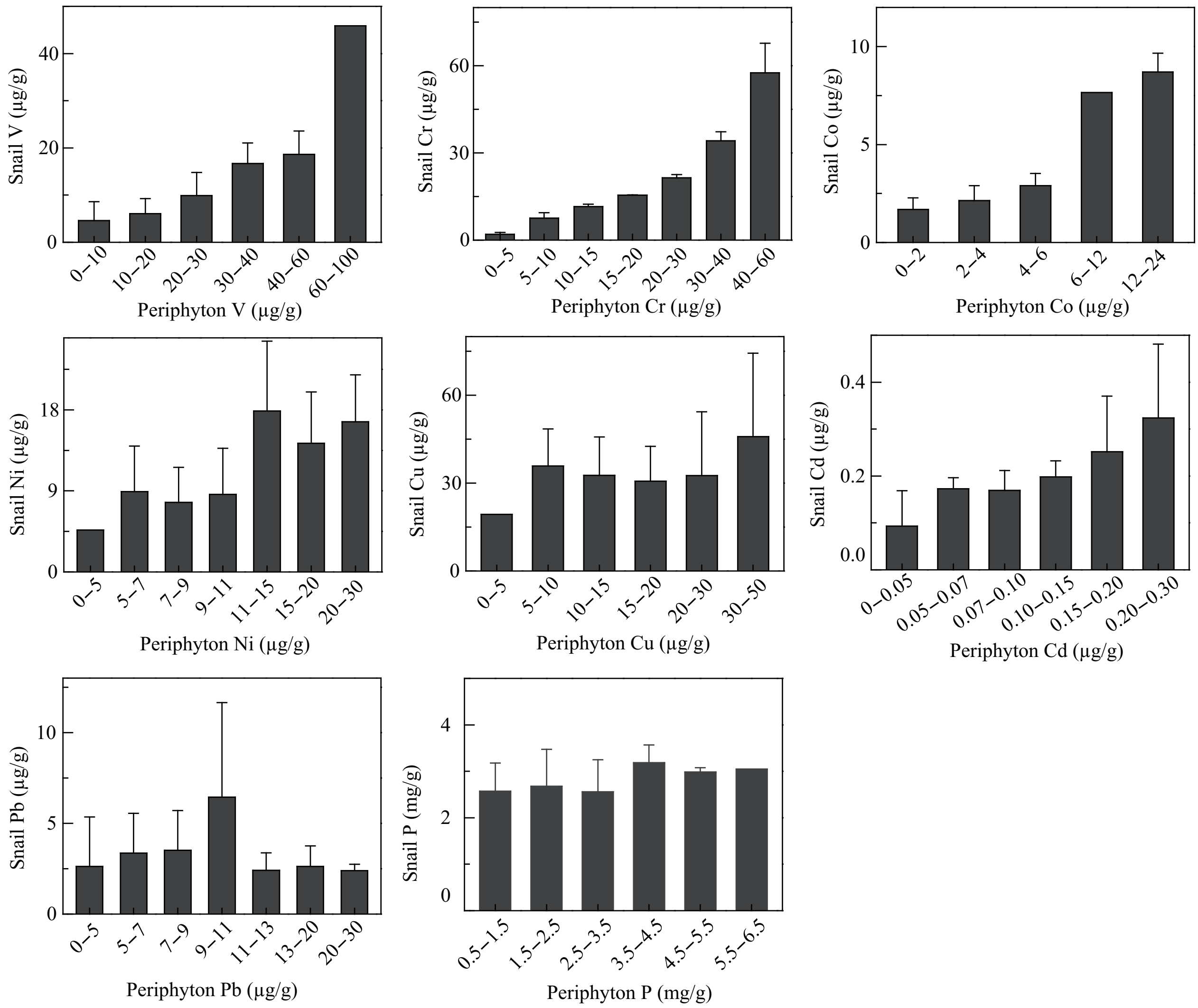

Fig. 3 Heavy metals and P content of snails and periphyton. The contents of the periphyton were grouped. Values are expressed as the mean ± standard deviation.

were favored at higher levels (Weitzel, 1979). For snails, Cr primarily accumulated in the digestive gland of their tissues (Cœurdassier et al., 2005; Kruatrachue et al., 2011). Cd was enriched in snail tissues in a dose-dependent manner (Das and Khangarot, 2010). Cd is considered to be a non-essential element for living organisms (Meador et al., 2005). Both filamentous algae such as *Rhizoclonium* and *Oscillatoria*, and unicellular diatoms like *Navicula* contributed to the periphyton Cd content (Fig. 2), indicating that Cd accumulation in snails may not be strongly related to the grazing preference. Overall, it is believed that V, Cr, and Ni accumulation relate to the proportion of diatoms in the community, while the Cd content is dependent on the physiological characteristics of the snails. For Pb, snails have strategies to excrete body lead as mentioned in the section above.

Earlier studies have shown that snails, especially adults, were tolerant to elevated Cu levels (Watton and Hawkes, 1984). Nielsen and Wium-Andersen (1971) found that small concentrations of Cu could influence the rate of photosynthesis considerably more in the diatom than in the green alga. Filamentous green algae increased with ambient Cu increase. This type of algae is not the first choice of the snails, which may explain why the Cu levels in snails did not increase with that in periphyton. PCA analysis also revealed strong negative correlations between periphyton P and Cu (Fig. 4). Moreover, increasing Cu only increased the filamentous algae *Oedogonium*, but decreased the proportion of diatoms. Taken together, these results verified that the accumulation of Cu in snails was closely related to their grazing behavior, but not their physiological characteristics.

3.2 Metal:P stoichiometry reveals the transfer process of heavy metals

It has been found that P in the runoff from agricultural land can accelerate the eutrophication of the Bai River (Daniel et al., 1998), and that the periphyton P increased with the P concentration of stream water (Stelzer et al., 2001). This process caused the metal:P ratios of periphyton to be strongly related to eutrophication. The metal:P ratios of periphyton decreased sharply with increasing periphyton P when $P < 0.075$ mmol/g (Fig. 4). In this stage, the Cr, Ni and Cd stoichiometry in periphyton and snails showed similar accumulation pathways. These findings indicated that the three heavy metals could reduce the potential

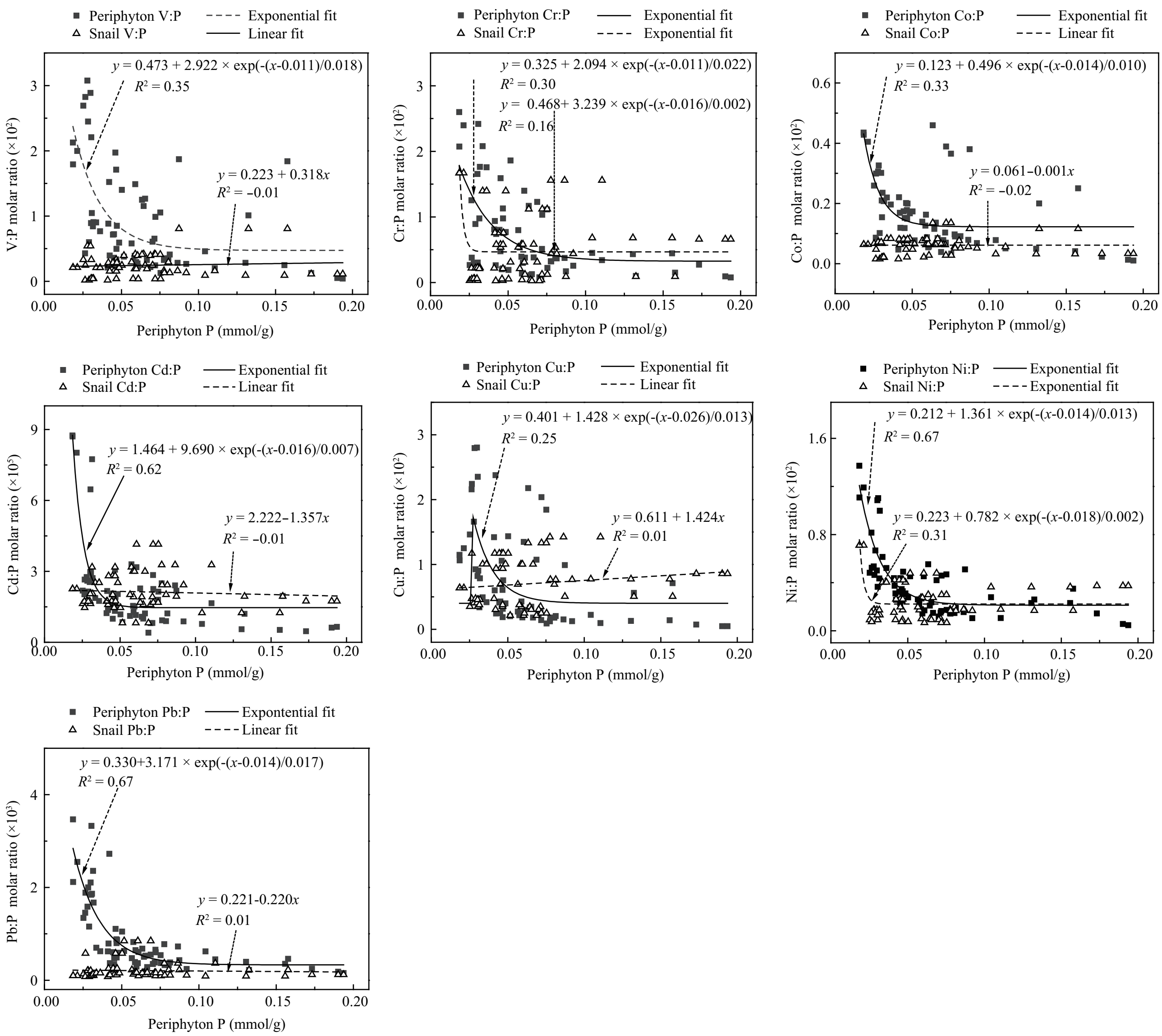

Fig. 4 Relationships of metal:P ratios (V, Cr, Co, Ni, Cu, Cd, Pb) between snails and periphyton.

ecological risks associated with increased P inputs to the ecosystems. Specifically, *C. chinensis* P remained stable, while the metal:P ratios in snails decreased, suggesting that their metals accumulation declined too.

The V, Co, and Pb content in snails remained stable regardless of the periphyton P content. The balanced characteristic of these metals differs from that of Cr, Ni and Cd, which were only maintained when the periphyton P > 0.075 mmol/g. V, Co, and Pb were considered to be relatively safe whenever P differed, and the risks of Cr, Ni and Cd will not continue to decline when periphyton P > 0.075 mmol/g.

The Cu:P stoichiometry indicated that Cu might pose a risk when eutrophication occurs. Cu has been classified as being extremely toxic for most algae, but it is an enzyme cofactor and essential component of the snail pigment, hemocyanin (Méndez et al., 2001). Previous experiments have shown that there is no significant difference in Cu accumulation among the digestive tract, digestive glands and gills, although the gills showed the highest concentration (Kruatrachue et al., 2011). These findings indicated that Cu may be prone to accumulation in snails and transferred to higher trophic levels.

4 Conclusions

Periphyton communities in the Bai River were mainly composed of two types of benthic algae, filamentous green algae and unicellular diatoms. The proportion of unicellular diatoms in the periphyton community is a key factor that influences metal accumulation in snails. Mechanisms of V, Cr, and Ni accumulation are related to the proportion of diatoms, while Cd and Pb accumulation are dependent on the physiological characteristics of *C. chinensis*. The accumulation of Cu in *C. chinensis* was closely related to their grazing behavior. P caused an increase in epiphytic unicellular diatoms in the periphyton community, and may also have enhanced metal accumulation in snails. Accordingly, P might also have potential ecological risks toward heavy metals.

The accumulation capabilities of Cr, Ni, Cu and Cd in snails were stronger than those of V and Co. The metal:P stoichiometry method indicated: (1) Cr, Ni, and Cd could have lower potential ecological risks when P inputs to the ecosystem increased. V and Co were considered relatively safe, regardless of the periphyton P content; (2) Cu can cause potential risks when eutrophication occurs; (3) Pb may not be prone to transfer to higher trophic levels and posed the lowest ecological risk among the heavy metals investigated herein.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 21107126), the Ministry of Water Resources' Special Funds for Scientific Research on Public Causes (No. 200901008) and the National Water Pollution Control Program (Special Water Project) (No. 2008ZX07209-010).

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JOURNAL OF ENVIRONMENTAL SCIENCES

Journal of Environmental Sciences (Established in 1989)
Vol. 24 No. 10 2012

Supervised by Chinese Academy of Sciences
Sponsored by Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences
Edited by Editorial Office of Journal of Environmental Sciences (JES)
P. O. Box 2871, Beijing 100085, China
Tel: 86-10-62920553; http://www.jesc.ac.cn
E-mail: jesc@263.net, jesc@rcees.ac.cn
Editor-in-chief Hongxiao Tang

Published by Science Press, Beijing, China
Elsevier Limited, The Netherlands
Distributed by
Domestic Science Press, 16 Donghuangchenggen North Street, Beijing 100717, China
Local Post Offices through China
Foreign Elsevier Limited
http://www.elsevier.com/locate/jes
Printed by Beijing Beilin Printing House, 100083, China

CN 11-2629/X **Domestic postcode: 2-580** **Domestic price per issue RMB ¥ 110.00**

ISSN 1001-0742