Investigation of heavy metals release from sediment with bioturbation/bioirrigation

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HIGHLIGHTS

- DGT-Cu fluxes in pore water increased with the presence of chironomid larvae.
- Heavy metals were highly influenced by bioturbation/bioirrigation of loach.
- Organisms has significant impact on particulate heavy metals release from sediment.
- Fe/Mn hydrous oxides may sorb or coprecipitate with Cd, Cu, Zn and Pb.

GRAPHICAL ABSTRACT

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ABSTRACT

Bioturbation/bioirrigation can affect the remobilization of metals from sediments. In this study, experiments were performed to examine the effect of bioturbation/bioirrigation by different organisms on cadmium (Cd), copper (Cu), zinc (Zn) and lead (Pb) releasing from the spiked sediment. The diffusive gradient in thin films technique (DGT) revealed that at the end of exposure time, the labile heavy metals concentrations in the pore water for all metal and organisms combinations except Cu and chironomid larvae were much lower than that in the control group. However, the concentrations of heavy metals detected by the DGT were virtually indistinguishable among the treatments with tubificid, chironomid larvae and loach. The correlation analysis of heavy metals with iron (Fe) and manganese (Mn) suggested that Cd, Zn and Pb were most likely bound as Fe-Mn oxidation form in the pore water, but Cu was in other forms. After 28 d of exposure, bioturbation/bioirrigation produced a significant release of particulate heavy metals into the overlying water, especially in the treatment with loach. The bioturbation/bioirrigation impact on the Pb remobilization was less than the other three heavy metals. The effects of bioturbation/bioirrigation on the heavy metals remobilization in the sediment were complex that with studying the heavy metals remobilization in the sediment and water interface, the biological indicators should be recommended.

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1. Introduction

The sediment which buried heavy metals may act as a source of contaminants with certain disturbances such as bioturbation/bioirrigation by benthic organisms (Banta and Andersen, 2003; Thibodeaux and Bierman, 2003; Granberg et al., 2008; Josefsson

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et al., 2010). Several studies have demonstrated that bioturbation/bioirrigation can increase the transfer of heavy metals between sediment and water. Tubificid renewed the particles and adsorption sites for Cd at the interface of sediment and water, and also increased the Cd contents in the sediment layer (Ciutat et al., 2005). *Hexagenia rigida* nymphs lived in the contaminated sediment can also produce resuspension of large amounts of sediment particles rich in Cd and Zn into the water column (Ciutat and Boudou, 2003). There was strong Cd, Cu, and Zn mobilization by bioturbation/bioirrigation of the *Chironomus plumosus* as their activities of digging into the sediments, and afterwards strong decrease of the three heavy metals concentrations, but to higher levels compared to values of treatments without invertebrate impact (Schaller, 2014). The polychaete *Marenzelleria* spp (Hedman et al., 2008) and carp (Wall et al., 1996) caused the high remobilization of Cd from sediment to the overlying water with the bioturbation/bioirrigation activities. The oligochaete worm *Lumbriculus variegatus* can enhance Pb transfer across the interface of sediment and water and also enhance the availability of Pb (Blankson and Klerks, 2016). But some researchers found that bioturbation/bioirrigation may have little effect on the remobilization of heavy metals in the sediment. The presence of bivalve *Tellina deltoidealis* had little effect on the release of Zn and Pb from the sediment (Atkinson et al., 2007). The tubificid also provided little impact for the Pb remobilization from the sediment (Lv, 2009). The *Hediste diversicolor* did not influence the remobilization of dissolved mercury (Hg) from the sediment to the water since the sediment was rich in organic matter content, Fe/Mn total and hydrous oxides to retain the Hg in the sediment (Cardoso et al., 2008). And the bioturbation by *Monoporeia affinis* was also not sufficient to the release of Cu from the sediment enriched Cu (Ospina Alvarez et al., 2014). The different conclusions of the researches suggested that the influence of bioturbation/bioirrigation on the heavy metals fluxes in the sediment was very complex.

The bioturbation/bioirrigation effects on the heavy metals remobilization in the sediment were different with the different types of bioturbated organisms. The concentrations of dissolved Cu decreased and Mn increased with the presence of amphipod *Victoriopisa australiensis* compared with the presence only of bivalve *Tellina deltoidealis* for the different bioturbating intensity (Remaili et al., 2016). And the high bioturbation by *Victoriopisa australiensis* also produced significant release of DGT-labile Cd, Ni, Pb and Zn compared with the low bioturbation by *Tellina deltoidealis* in the pore and overlying water (Amato et al., 2016). The bioturbation tests with the burrowing *Hexagenia* sp. had consistently higher overlying water Ni concentrations compared with the amphipod (*Hyalella azteca* and *Gammarus pseudolimnaeus*) tests (Brumbaugh et al., 2013). Comparing the bioturbation by *Tubifex tubifex* and *Chironomus riparius* larvae on the release of uranium (U), the bioturbation by *T. tubifex* led to a high degree of U release from sediment into the overlying water for its effects on sediment reworking (Lagauzère et al., 2009). Until now, rare research exists evaluating the effect of the bioturbation/bioirrigation on heavy metals remobilization by different organism types, which can have great influence on the fate of heavy metals.

In fact, the toxicity and fate of metals in the sediments was obviously correlated with the partitioning of metals between the sediment particles and the pore water (Calmano et al., 1993; Chapman et al., 1998; Simpson, 2005). Bioturbation/bioirrigation can also change the concentrations of heavy metals in the sediment pore water. With the active burrowing by oligochaete *Lumbriculus variegatus*, concentrations of Cd in pore water from deeper horizons were always lower than those in the surficial sediments without bioturbation (Peterson et al., 1996). The pore water Fe(II) was oxidized by benthic organism bioturbation to adsorb Zn as the

formation of Fe hydroxide precipitates and result of lower Zn fluxes in pore water (Simpson and Batley, 2003). So it must pay attention to the concentrations of heavy metals in the pore water when study about the bioturbation/bioirrigation effects on the heavy metals remobilization in the sediment. The diffusive gradient in thin films (DGT) method can determine the average concentration in the interface of the sediment and solution within the placed time. With laboratory microcosm experiments, DGT may be a good method to investigate the bioturbation/bioirrigation effects on the changes of pore water heavy metal concentrations (Roulier et al., 2008; Simpson et al., 2012), and also it was a useful tool to assess the heavy metal bioavailability to benthic organisms in sediment (Amato et al., 2016). However, little work has been performed on the bioturbation/bioirrigation effect on the metal remobilization in the sediment pore water.

Three organisms were chosen for this study, including tubificid (*Limnodrilus hoffmeisteri*), chironomid larvae (*Chironomus plumosus* larvae), and loach (*Misgurnus bipartitus*), hereafter referred to as tubificid, chironomid larvae, and loach, respectively. Consequently, the present study aimed at the degree of bioturbation/bioirrigation effect on the heavy metals transfer processes by different types of riverine organisms. The heavy metals accumulated in the three organisms lived in the heavy metals spiked sediment was also investigated.

2. Materials and methods

2.1. Sediment and water collection and preparation

The sediment and water were sampled from the Ming Tombs Reservoir in the northwest of Beijing (China) in May 2013. The collected water were pre-aerated for 24 h and filtered through 0.45 μm cellulose acetate membrane filter. The percentage of the particle size below 60 μm of the sediment was 85.7%. The pH value of the sediment was 7.6. The sediment was rich in organic matter that the loss by ignition was 7.9% (He et al., 2015). Wet sediment was added to plastic mixing containers and then spiked with analytical reagent grade of $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$ and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ successively. During the first 2 months, the test sediment was stirred twice a week at room temperature in order to make it homogenized. The final Cd, Cu, Zn and Pb concentration of sediment after spiking in each experiment unit was $11.3 \pm 1.4 \text{ mg kg}^{-1}$, $881.1 \pm 68.9 \text{ mg kg}^{-1}$, $1132.8 \pm 86.3 \text{ mg kg}^{-1}$, and $875.8 \pm 70.4 \text{ mg kg}^{-1}$ (dry mass, $n = 16$), respectively.

2.2. Experimental design and setup

The experiments of the bioturbation/bioirrigation effects on the heavy metals releasing from the spiked sediment were conducted the same as previous study (He et al., 2015). The experiments were conducted for 29 d in glass beakers with diameter of 13 cm and height of 19 cm which were lined with high density polyethylene film. 600 mL water and about 2.0 kg contaminated sediment were added into each beaker. Four series of treatments with four replicates were conducted. The first treatment was a control group without organism adding, the other three series of treatments contained 2.0 g of different organism, respectively. The number of tubificid, chironomid larvae and loach as $7.5 \times 10^5 \text{ m}^{-2}$, $9.4 \times 10^4 \text{ m}^{-2}$, and $1.5 \times 10^2 \text{ m}^{-2}$ respectively. After 28 d of the exposure time, the survival rate of tubificid and chironomid larvae was about 70%–90%, and the loach were all survived. The main research contents were the dissolved and particulate heavy metals concentrations, pH value, and turbidity in the overlying water during the exposure time. In addition, the concentrations of heavy metals in the pore water were studied by the DGT (Lancaster

LA20QJ, UK) samplers which were deployed in the units on the 28th d after sampling in overlying water, following established procedures (Limited). As the DGT shape, the devices were insert vertically in the sediment about 10 cm depth, and the rest in the overlying water was about 4 cm. Concentrations determined by DGT were calculated by the well-documented procedures and equations according to literature (Denney et al., 1999; Buzier et al., 2006).

The experiment of bioaccumulation of the heavy metals by the three organism was similar as the method used before (He et al., 2015). The experiment was performed in plastic beakers with the diameter of 10 cm. Each beaker consisted of contaminated sediment (1 cm depth), water column (300 mL) and 1.0 g of organisms. Three bioaccumulation treatments (with tubificid, with chironomid larvae, and with loach) were conducted with four parallel groups. The surviving organisms were collected after 3, 5, 7, 10, 14, 21, and 28 d to measure the accumulation concentration of Cd, Cu, Zn and Pb. The collected organisms were cultured in the clean tap water for 6 h. Then rinsed with Milli-Q water at least three times and freeze-dried (FD-1A, Beijing Bo Kang Experimental Medical Instrument Co., Ltd.) before they were ready for microwave digestion with aqua regia.

Cd, Cu, Zn and Pb analyses were all conducted by inductively coupled plasma mass spectrometry (ICP-MS) (7500a). Fe and Mn were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Prodigy).

2.3. Data analysis

The data were prepared using Excel 2000. Statistical analyses of the data by using Origin 9.0 and SPSS Base 15.0 software. The correlation between Fe/Mn and Cd, Cu, Zn, and Pb was performed on plots of the concentrations of them in the pore water.

3. Results

3.1. DGT concentrations of heavy metals

The vertical distribution of heavy metals concentrations in the

pore water after 28 d detected by the DGT was shown in Fig. 1, the “0” represented the sediment-water interface.

The DGT concentration of Cd (Fig. 1a), Cu (Fig. 1b), Zn (Fig. 1c), and Pb (Fig. 1d) in the pore water ranged from 11.3 to 36.6 $\mu\text{g L}^{-1}$, 27.6–49.2 $\mu\text{g L}^{-1}$, 75.9–155.9 $\mu\text{g L}^{-1}$, and 12.0–40.0 $\mu\text{g L}^{-1}$ in the control group, respectively; from 7.8 to 28.6 $\mu\text{g L}^{-1}$, 22.7–54.5 $\mu\text{g L}^{-1}$, 50.8–163.5 $\mu\text{g L}^{-1}$, and 7.9–32.7 $\mu\text{g L}^{-1}$ in the tubificid group, respectively; from 6.8 to 29.8 $\mu\text{g L}^{-1}$, 42.2–69.4 $\mu\text{g L}^{-1}$, 98.3–183.4 $\mu\text{g L}^{-1}$, and 7.0–33.5 $\mu\text{g L}^{-1}$ in the chironomid larvae group, and from 8.1 to 29.2 $\mu\text{g L}^{-1}$, 21.7–37.8 $\mu\text{g L}^{-1}$, 41.3–165.3 $\mu\text{g L}^{-1}$, and 8.5–34.0 $\mu\text{g L}^{-1}$ in the loach group, respectively. DGT-Cd concentration in the control group had a peak at 6 cm depth, while concentrations detected by the DGT (C_{DGT}) of biological groups at this depth were lower. Only at 5 and 8 cm depth, DGT-Cd concentrations of biological groups were higher than that of the control group. The peak of DGT-Cu concentrations was at 1 cm depth in both the control and chironomid larvae group, at 8 cm depth in the tubificid group, and at 2 cm depth in the loach group. DGT-Zn concentrations in the control group had a peak at 3 cm depth, at 8 cm depth in the tubificid group, and at the 4 cm depth in both the chironomid larvae and loach group. DGT-Pb concentrations in the control, tubificid and chironomid larvae group had a peak at 6 cm, and at 5 cm depth in the loach group.

3.2. Heavy metals in the overlying water

The concentrations of dissolved and particulate heavy metals in the overlying water were shown in Fig. 2. Concentrations of dissolved Cd (Fig. 2a) and Zn (Fig. 2e) with the treatments of bio-turbators were higher than that in the control group at the beginning of the experiment, but at the end of the exposure time, only the dissolved Cd and Zn concentrations in the tubificid group, and the dissolved Zn concentrations in the chironomid larvae groups were higher than that in the control group. The dissolved Cu (Fig. 2c) concentrations changed gradually, except that in the loach group increased with time and was higher than that in the control group at the end of experiment. The concentrations of dissolved Pb (Fig. 2g) only in the loach group were higher than that in the control

Fig. 1. Vertical distribution of Cd (a), Cu (b), Zn (c) and Pb (d) in sediment pore water ($p = 0.05$): Control, no organism added to the sediment-water biotope; Tubificid, presence of tubificid; Chironomid larvae, presence of chironomid larvae; Loach, presence of loach.

Fig. 2. Evolution of dissolved Cd (a), Cu (c), Zn (e), and Pb (g), particulate Cd (b), Cu (d), Zn (f), and Pb (h) concentrations in the overlying water during the 28 days experiment for the four experimental conditions studied ($p = 0.05$): Control, no organism added to the sediment–water biotope; Tubificid, presence of tubificid; Chironomid larvae, presence of chironomid larvae; Loach, presence of loach.

group. Concentrations of particulate Cd (Fig. 2b), Cu (Fig. 2d), Zn (Fig. 2f), and Pb (Fig. 2h) in the loach group were much higher than that in the control group, but the other treatments with organisms had the similar change trend as that in the control group.

3.3. Heavy metals accumulated in the three organisms

Heavy metals accumulated in the three organisms with the exposure time was shown in Fig. 3. Tubificid had the best accumulated ability for Cd (Fig. 3a), Cu (Fig. 3b), Zn (Fig. 3c), and Pb (Fig. 3d), followed with chironomid larvae and loach. After 10 d, the heavy metals concentrations in the tubificid body reached the

equilibrium. While the accumulated Cd, Cu, and Zn concentrations in the chironomid larvae body needed only 3 d to reach the equilibrium, and the Pb concentration in the organism increased during the first 5 d, then decreased with the exposure time. Loach had the poor accumulation ability for the heavy metals, especially on the 7th d, the Zn concentration in the organism was lower than the original bioconcentration in loach at the beginning of the experiment.

4. Discussion

The peak of DGT concentrations of heavy metals in the pore

Fig. 3. The Cd (a), Cu (b), Zn (c), and Pb (d) accumulated in the organisms with the treatments of tubificid, chironomid larvae and loach during the exposure time (dry weight) ($p = 0.05$).

water of the biological groups were as follows: Cd, chironomid larvae > loach > tubificid; Cu, chironomid larvae > tubificid > loach; Zn, chironomid larvae > loach > tubificid; and Pb, loach > chironomid larvae > tubificid. And the Cd, Zn, Pb concentrations in the pore water of the biological groups were lower than that in the control group. Bioturbation/bioirrigation process can loosen the sediment, and increase the sediment surface with more sorption sites for heavy metals, resulting in the heavy metals bind onto the sediment, which tends to bring down the heavy metals levels in pore water. On the other hand, adsorption of continuous forming biofilm on the sediment particle and biological absorption (Schaller et al., 2010) for the heavy metals can be another reason for the lower concentrations of heavy metals in the pore water of biological groups compared to the control group. In addition, the respiratory and feeding purposes of the organisms caused open- or blind-ended burrows to enhance the transport of solutes out of burrows to the overlying water (Kristensen, 2001; Shull et al., 2009), and also introduced the overlying water to dilute the heavy metals concentrations in the pore water. DGT-Cd from the deeper horizons out of the burrowing zone were consistently lower than those in the surface sediments which was the same with the previous study (Peterson et al., 1996). The case that concentration of DGT-Cu in the chironomid larvae group was higher than it in the control group. This may be due to the bioturbation/bioirrigation by chironomid larvae which caused higher oxidation reduction potential (ORP) with strong oxidizing at the same depth of sediment, then the Cu existed as the dissolved forms for CuCl_2^{2+} , CuCl_3^{2+} (Ma and Wan, 1999). While the bioturbation/bioirrigation had little effect on Pb remobilization which was a stable element. Previous study also showed that the bioturbation effect was not significant on Pb in the deeper sediment (Qiao, 2011).

In the control group, the dissolved Cd, Cu, Zn and Pb in the overlying water were the result of the simple molecular diffusion from the sediment pore water. The strong increase of Cd, Cu, Zn and Pb in the overlying water with the treatments of organisms during the early of exposure time can be explained by the effects from burrowing (dig in) of the tubificid and chironomid larvae into the

sediment. DGT will measure all those species that are in labile equilibrium with the species that can bind to the binding agent. In this study, the correlation between heavy metals with Fe and Mn detected in DGT device (Fig. 4) showed that they had a strong positive correlation except Cu, indicating that Cd, Zn, and Pb were most likely bound as Fe-Mn oxidation form in sediment pore water, but Cu was in other forms. Previous literature demonstrated that Fe and Mn oxy-hydroxides were important parts for adsorbing heavy metals in freshwater oxic sediments (Tessier and Campbell, 1988; Luoma, 1989; Tessier et al., 1993). In this study, the correlation between heavy metals and Fe, Mn DGT concentrations stressed the importance of Fe and Mn (hydr)oxides for carrying heavy metals in the pore water.

Without the bioturbation/bioirrigation, the dissolved Cd, Cu, Zn and Pb in the overlying water were the result of the simple molecular diffusion from the sediment pore water. The strong increase of Cd, Cu, Zn and Pb in the overlying water with the treatments of organisms during the early of exposure time can be explained by the effects from burrowing (dig in) of the tubificid and chironomid larvae into the sediment, and the swimming of loach at the surficial sediment. This is in accordance with previous study by Schaller (2014). With bioturbation/bioirrigation, oxygen can penetrate deep into the sediment, the heavy metals presence in the form of acid-volatile sulfides (AVS) may resolve into the dissolved phase by the sediment oxidation, and then diffuse across the sediment-water interface (Ciutat and Boudou, 2003). Additionally, increased oxygen concentration of sediment can increase the solubility of Cd and Zn (Gambrell et al., 1991), and the stronger bound oxidizable fractions of Cd, Cu, Zn and Pb changed to weaker bound carbonate and exchangeable fractions of these heavy metals with the increased oxygen content of sediment (Calmano et al., 1993; Zoumis et al., 2001), so the dissolved Cd, Cu, Zn and Pb concentrations increased. And bioturbation/bioirrigation can enhance the heavy metals exchange rates between overlying water and pore water by increasing the contact zone of sediment and water (Lewandowski and Hupfer, 2005), resulting in the release of heavy metals into the water. In this study, the Cd, Cu, Zn and Pb migrated

Fig. 4. The linear correlation analysis between Fe, Mn and heavy metals in the sediment pore water detected by the DGT.

to the overlying water were dominated in particulate form (Rasmussen et al., 2000; Lv, 2009; Qiao, 2011), which was due to the resuspension of the sediment particles with the bioturbation/bioirrigation by the organisms.

However, the dissolved Cd, Cu, Zn and Pb concentrations in the overlying water decreased with the exposure time, this may be due to the precipitation and sorption processes to remove them from solution (Du Laing et al., 2009; Mostofa et al., 2013). The pH values of the overlying water (Table 1) in the bioturbation/bioirrigation groups increased after 5 d and achieved the maximum value (tubificid, 8.1 ± 0.0 ; chironomid larvae, 8.3 ± 0.2 ; loach, 9.1 ± 0.4)

after 21 d. The free Cd concentration decreased after 5 d and Zn decreased after 3 d may be due to the free Cd and Zn precipitated consequently with the high pH values in the overlying water. In some researcher's opinion, as the pH value increased, the first order of the hydrolysis of the heavy metals increased, so the metal hydroxyl complexes increased, the absorbed amount also increased (Boekhold et al., 1993). And there may be a better bond ability between the metal hydroxyl complexes and adsorption sites (Hodgson et al., 1964). The increase of pH value is propitious of combining of the points of double-proton and to increase the adsorption of heavy metals. Furthermore, the ionic-exchange

Table 1

The pH and turbidity values in the overlying water during the exposure time.

		Exposure time (d)				Treatment			
						Control	Tubificid	Chironomid larvae	Loach
pH	1					7.7 ± 0.0	7.5 ± 0.1	7.3 ± 0.0	7.5 ± 0.1
	2					7.8 ± 0.0	7.6 ± 0.1	7.4 ± 0.1	7.6 ± 0.1
	3					7.8 ± 0.0	7.6 ± 0.1	7.5 ± 0.1	7.7 ± 0.1
	5					7.9 ± 0.0	7.7 ± 0.1	7.6 ± 0.1	7.7 ± 0.0
	7					7.9 ± 0.0	7.8 ± 0.0	7.7 ± 0.0	7.7 ± 0.0
	9					8.0 ± 0.0	7.9 ± 0.0	7.8 ± 0.0	7.7 ± 0.0
	14					8.0 ± 0.0	7.9 ± 0.0	8.3 ± 0.4	7.8 ± 0.1
	21					8.0 ± 0.0	8.1 ± 0.0	8.3 ± 0.2	9.1 ± 0.4
	28					8.0 ± 0.1	8.1 ± 0.0	8.0 ± 0.1	9.0 ± 0.2
Turbidity (NTU)	1					4.0 ± 1.4	9.7 ± 2.1	108.2 ± 19.5	789 ± 76
	2					2.2 ± 0.7	6.1 ± 2.1	52.7 ± 4.1	464 ± 71
	3					2.0 ± 0.0	5.1 ± 0.9	29.5 ± 3.3	299 ± 47
	5					6.3 ± 3.9	6.8 ± 2.3	25.1 ± 3.9	251 ± 137
	7					2.9 ± 0.1	3.8 ± 1.3	12.1 ± 2.1	143 ± 3
	9					1.8 ± 0.7	2.2 ± 0.5	9.5 ± 1.2	25.6 ± 5.3
	14					1.2 ± 0.2	1.7 ± 0.3	4.3 ± 0.1	211 ± 68
	21					2.6 ± 0.9	6.9 ± 1.1	23.2 ± 3.1	870 ± 123
	28					2.0 ± 0.4	6.7 ± 1.5	6.6 ± 0.3	1286 ± 221

between Cd^{2+} and the layered silicate strengthen with the pH value increase (Inskeep and Baham, 1983). In addition, the Fe and Mn were well correlated with Cd, Cu, Zn and Pb in particulate form (Table 2). The newly formed Fe-Mn oxide in the suspended sediment particles tends to adsorb the free Cd, Cu, Zn and Pb (Turner et al., 2004), subsequently settled in sediment-water surface. The Fe oxides did the largest contribution to adsorb the Cd, and Mn oxides did the largest contribution to adsorb Pb (Dong et al., 2000). Therefore, large amounts of suspended particles caused by bio-turbation/bioirrigation provided much more absorptive surfaces for the heavy metals, so dissolved form concentrations of biological groups were lower than that of the control group and thereby reduce their mobility.

During the exposure time, the organisms were without food provisioning, they just lived with the organic detritus in the sediment. The acute lethal concentration of 50% (LC_{50}) of Cd, Cu, Zn and Pb for tubificid, chironomid larvae, and loach was shown in Table 3 (Wang et al., 2003; Bechard et al., 2008; Maestre et al., 2009; Mendez-Fernandez et al., 2013; Zhu et al., 2014; Jiang et al., 2016), the studies showed that the three organisms had higher tolerance of Cd, Cu, Zn and Pb. During the experiment, the concentrations of dissolved Cd, Cu, Zn and Pb were not beyond the LC_{50} values for the three organisms, so there would not turn up the acute death for the three organisms to avoid the phenomenon of no living organisms in the biological groups. According to the literature, the tubificid ingested sediment particles were the tiniest particles (Ciutat, 2003), which likely to have the highest heavy metals contents for their large surface-to-volume ratio, high organic carbon content, and high binding capacity of clay particles (Thorne and Nickless, 1981; Duquesne et al., 2006). Therefore, tubificid can

accumulate significant quantities of heavy metals after exposure to contaminated sediment. The heavy metals accumulated in the chironomid larvae was lower than in the tubificid may be for that they can clean their body to avoid high concentrations of heavy metals by excreting ingested them (Tessier et al., 1994; Alves et al., 2009; Schaller et al., 2011). Loach had the worst accumulation ability to heavy metals, this less bioaccumulation perhaps due to its filter feeding behavior. The laboratory studies of metal uptake showed that concentration factors were higher in invertebrates than in vertebrates (Taylor, 1983), and some researchers found that the levels of heavy metals in the predators were lower than those with other feeding habits in the same habitats (Timmermans et al., 1989; van Hattum et al., 1991). Heavy metals can enter fish through the gills by passive diffusion or by entering through the diet (Bosch et al., 2016). Therefore, although the maximum total heavy metals concentrations in the overlying water was in the loach group, the bioaccumulation seemed the minimum among the three organisms.

5. Conclusion

Bioturbation/bioirrigation by different organisms of the spiked Cd, Cu, Zn, and Pb sediments can enhance the transfer of soluble Cd, Cu, Zn and Pb from the pore water to the sediment or overlying water. The Cd, Zn and Pb in the sediment pore water were most likely bound as Fe-Mn oxidation form, and Cu was in other forms. The bioturbation/bioirrigation enhanced the quantity of suspended particles and lead a significant release of Cd, Cu, Zn and Pb to the overlying water, especially for the treatment with loach. The increased pH values in the overlying water by the bioturbation/

Table 2

Correlation analysis between Fe, Mn concentrations and concentrations of other heavy metals in the overlying water.

r		Dissolved Fe	Particulate Fe	Dissolved Mn	Particulate Mn
Dissolved	Cd	0.226**	—	0.150	—
	Cu	0.040	—	0.000	—
	Zn	0.167*	—	0.100	—
	Pb	0.017	—	0.131	—
Particulate	Cd	—	0.669**	—	0.621**
	Cu	—	0.921**	—	0.884**
	Zn	—	0.532**	—	0.476**
	Pb	—	0.988**	—	0.909**

**, $p < 0.01$; *, $p < 0.05$.

Table 3The LC₅₀ of Cd, Cu, Zn, and Pb for the three organisms.

	LC ₅₀		Reference		Reference		Reference	
	Cd		Cu		Zn		Pb	
Tubificid	11.8 ± 3.8 μM (96 h)	Mendez-Fernandez et al., 2013	0.002–0.158 mg L ⁻¹ (96 h)	Maestre et al., 2009	—	—	76.24 mg L ⁻¹ (96 h)	Jiang et al., 2016
Chironomid larvae	9.38 mg L ⁻¹ (24 h)	Bechard et al., 2008	2.09 mg L ⁻¹ (24 h)	Bechard et al., 2008	25 mg L ⁻¹ (24 h)	Bechard et al., 2008	0.61 mg L ⁻¹ (24 h)	Bechard et al., 2008
Loach	56.5 mg L ⁻¹ (96 h)	Wang et al., 2003	0.56 mg L ⁻¹ (96 h)	Zhu et al., 2014	2.86 mg L ⁻¹ (96 h)	Zhu et al., 2014	294.5 mg L ⁻¹ (96 h)	Wang et al., 2003

bioirrigation lead to the precipitation and adsorption of Cd, Zn and Pb may be the major reason for reducing dissolved form of these heavy metals concentrations in the biological groups after 28 d. Bioaccumulation of Cd, Cu, Zn and Pb by the three biological studies showed that the enrichment of tubificid was far greater than the other two organisms. Tubificid exposed to sediments accumulated significant quantities of Cd, Cu, Zn and Pb during the 28 d exposure, suggesting that tubificid inhabiting areas contaminated by Cd, Cu, Zn and Pb may provide a substantial dietary source of Cd, Cu, Zn and Pb for fish and other predators.

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