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Mass balance and transformation of heavy metals in an activated sludge reactor integrating aquatic worms

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ABSTRACT

Reactors containing aquatic worms can reduce waste sludge via biological predation by the worms; however, changes in the heavy metals in such reactors could influence the wastewater treatment. The objective of this study is to investigate the mass balance and the transformation of As, Cd, Cr, Cu, Ni, Pb, and Zn in a wastewater treatment system with aquatic worms. In the aquatic worm reactor, the concentrations of heavy metals in the waste sludge increased remarkably, while the total contents changed only slightly. The concentrations and total contents increased to a lesser extent in aquatic worms, while no significant change in these factors was observed in the effluent. As a result, in the activated sludge reactor integrating aquatic worms, the heavy metals in the sludge accounted for a large portion of the overall content (84.7–97.5%) and the residue of As, Cd, Cr, Cu, Pb, Ni, and Zn in the final sludge increased to 36.0, 60.4, 97.2, 94.8, 98.2, 46.9, and 85.5%, respectively, indicating that their bioavailability was reduced.

Keywords: Sludge; Aquatic worms; Heavy metals; Mass balance; Transformation

1. Introduction

Heavy metals in wastewater and sludge have received a great deal of attention because they impact on the environment and the health of fauna and flora in areas in which they are discharged [1,2]. During the traditional activated sludge process, adsorption and sedimentation are considered the dominant mechanisms of the immobilization of heavy metals [3]. Heavy metals in wastewater accumulate in waste sludge via sedimentation, absorption, and adsorption, after which they are removed from the treatment system with the discharged sludge.

A biological method to reduce the amount of waste sludge in the wastewater treatment system, and therefore sludge processing costs, is utilization of aquatic worms [4]. Indeed, addition of aquatic worms to a wastewater treatment system can reduce sludge production by 20–75% [5]. However, it is not clear how heavy metals that accumulate in the sludge are transformed by this process or what the metal

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bioavailability in waste sludge that has been treated with aquatic worms will be.

The benefits of application of sludge as a fertilizer are becoming increasingly restricted owing to heavy metals in the sludge [6]. Although aquatic worms have enabled efficient sludge reduction, the environmental risk associated with the heavy metals in sludge produced by aquatic worm reactors is not clear. Lucan-Bouché et al. concluded that the aquatic worm, *Tubifex tubifex*, may protect itself against the internal accumulation of toxic metals (Cu and Pb) [7]. Kaonga et al. reported that *T. tubifex* could accumulate some heavy metals in sewage sludge [8]. However, Hendrickx reported that aquatic worms do not specifically bioaccumulate metals from activated sludge [9]. Due to the efficient sludge reduction in the reactor integrating aquatic worms, the final distribution of heavy metals that used to accumulate in the initial sludge is unclear, and it could be analyzed by mass balance. Therefore, this study was conducted to investigate the variations of heavy metals in wastewater, activated sludge and aquatic worms in an aquatic worm reactor. To accomplish this, the transformation of As, Cd, Cr, Cu, Ni, Pb, and Zn were assessed in terms of their mass balance to determine the fate of metals associated with the sludge. To comprehensively assess the extent of metals accumulation and immobilization in sludge, the efficiency of accumulation and inactivation of heavy metals was also analyzed.

2. Methods

2.1. Materials

Wastewater, activated sludge, and aquatic worms were collected from the municipal wastewater treatment plant in Zhuji, China, and specifically, the aquatic worms were from the aerobic zone of the sequencing batch reactor. The aquatic worms (mainly *Limnodrilus hoffmeisteri*) were cultivated in tap water for 48 h to allow gut purging [10]. Worms were identified using an Olympus microscope and stereoscope. The mean length and weight of the worms were 1.92 ± 0.68 cm and 0.0019 ± 0.0008 g, respectively [11].

2.2. Experimental procedures

Wastewater samples were stirred for 1 h and then centrifuged at 4,000 rpm for 10 min [12], after which aliquots were collected to determine the initial concentration of heavy metals. The remaining water was used for the wastewater treatment experiments. The sludge was also stirred for 1 h and then centrifuged at 4,000 rpm for 10 min [12], after which aliquots were collected for extraction and determination of the initial concentrations of heavy metals. The remaining sludge was used for the wastewater treatment experiments. Tap water-cleaned aquatic worms were collected for determination of the initial concentration of heavy metals, while the remaining worms were used in the wastewater treatment experiment.

The length, width, and height of the glass reactor in the experiment were 0.30, 0.15, and 0.30 m, respectively. Each reactor was filled with 8 L of wastewater and activated sludge. In the reactor labeled as group A (an aquatic worm group), besides wastewater and activated sludge, 20 g of aquatic worms was added at the beginning of the experiment, this reactor also contained six elastic carriers (0.15 m height) in which the worms could be immobilized. The carriers were made of polyamide with high biological adhesion and low sludge accumulation. In the reactor labeled as group B (a blank group), there were only wastewater and activated sludge, and wastewater was treated without adding aquatic worms. In each reactor, the initial mixed liquor suspended solids (MLSS) concentration was 3,500 mg L^{-1}, the temperature of wastewater was maintained at 20 ± 1.16°C, and the wastewater was aerated to maintain the dissolved oxygen concentration at 2–5 mg L^{-1} to ensure sufficient oxygen [13]. Based on previous studies, the reactor with activated sludge and aquatic worms used in this experiment could work stably for 20–22 days [11]. The experiment was run for 20 days, at which time the effluent and waste sludge in both groups and the aquatic worms in group A were subjected to heavy metals analysis and the final MLSS was determined.

2.3. Analytical methods

For wastewater, suspended solids (SS) were determined by the gravimetric method, ammoniacal nitrogen (NH_4^+–N) was determined using Nessler's reagent, chemical oxygen demand (COD) was measured with a thermo reactor (Hach, USA) and total phosphorus (TP) was measured by spectrophotometry (Hach, USA) [14,15]. In addition, MLSS was determined according to the gravimetric method [15]. All reported data are based on five replicates to verify the reliability.

Heavy metal (As, Cd, Cr, Cu, Ni, Pb, and Zn) concentrations were determined by inductively coupled plasma mass spectrometry (ICP–MS) (Perkin Elmer, USA) [16]. In addition, sludge samples were fractionated by means of sequential extraction scheme, proposed by the Community Bureau of Reference protocol (BCR) [17,18]. The oven-dried samples were dissolved in HNO_3–$HClO_4$ before the total content was determined, or sequentially extracted via the BCR three-step method before determination of the chemical form of the heavy metals as described by Arain et al. [19]. All data reported are based on four replicates. The BCR procedure fractionated metals into the exchangeable, reducible, oxidizable, and residual phases.

2.4. Calculation

Several models have been proposed to describe the kinetic reactions of metals in the matrix. Most common is the first-order kinetic equation, which has been used to describe adsorption isotherms for a wide range of heavy metal species [20]:

$$\frac{dC_m}{dt} = k_1 C_w - k_2 C_m \tag{1}$$

where k_1 and k_2 are the forward and backward rate coefficients (nondimensional), respectively; C_w is the concentration of heavy metals in wastewater (mg kg^{-1}); C_m is concentration of heavy metals in biological matrix (mg kg^{-1}); and t is the experimental time (d).

Based on the kinetic model, retention and release reactions of heavy metals occurred during the wastewater treatment, resulting in the transport of heavy metals among wastewater, activated sludge and aquatic worms. Finally, they reached an equilibrium, and the mass balance was maintained. To investigate the accumulation changes of heavy metals on day 1 and day 20, mass balance was adopted to analyze the distributions of heavy metals in wastewater, sludge, and aquatic worms before and after wastewater treatment. The mass balance of heavy metals in the aquatic worm reactor was determined based on the total contents of heavy metals in wastewater, sludge, and aquatic worms, and 3–5% mass balance error is not exceeded [20,21]:

$$m_{w0} + m_{s0} + m_{a0} = m_{wt} + m_{st} + m_{at} \tag{2}$$

where m_{w0} is the initial total heavy metals in wastewater on day 1 (mg); m_{s0} is the initial total heavy metals in sludge on day 1 (mg); m_{a0} is the initial total heavy metals in aquatic worms on day 1 (mg); m_{wt} is the total heavy metals in wastewater on day t (mg); m_{st} is the total heavy metals in sludge on day t (mg); and m_{at} is the total heavy metals in aquatic worms on day t (mg).

m_w, m_s, and m_a can be calculated using the following equations:

$$m_w = C_w \times M_w \tag{3}$$

$$m_s = C_s \times M_s \tag{4}$$

$$m_a = C_a \times M_a \tag{5}$$

where C_w is the concentration of heavy metals in wastewater (mg kg^{-1}); M_w is the weight of wastewater (kg); C_s is the concentration of heavy metals in sludge (mg kg^{-1}); M_s is the weight of sludge (kg); C_a is the concentration of heavy metals in aquatic worms (mg kg^{-1}); and M_a is the weight of aquatic worms (kg).

The efficiency of accumulation and inactivation of heavy metals in sludge was assessed considering the accumulation level and the proportion of the residual form [18,22], which can be calculated using the following equation:

$$E = P_s \times P_r \times \frac{M_{s0}}{M_{st}} \tag{6}$$

where E is the efficiency of accumulation and inactivation of heavy metals in sludge (nondimensional); P_s is the percentage of heavy metals in sludge in the whole reactor (%); P_r is the percentage of residual heavy metals of the total content (%); m_{s0} is the initial weight of sludge on day 1 (kg); and M_{st} is the weight of sludge on day t (kg).

P_s and P_r can be calculated as follows:

$$P_s = \frac{m_s}{m_w + m_s + m_a} \tag{7}$$

$$P_r = \frac{m_{sr}}{m_s} \tag{8}$$

where m_{sr} is the weight of residual heavy metals in sludge (mg).

The concentrations of heavy metals in the sludge, aquatic worms, and wastewater on day 1 and 20 and the E values of group A and B were compared by a t-test (two-tailed test), which was conducted using SPSS 17.0 (a software for data analysis). P values < 0.05 were considered to indicate significance [23].

3. Results and discussion

3.1. Sludge reduction and effluent quality

The initial mass of the sludge in group A was 28.0 g and the final mass was 20.5 g, indicating a gravimetric reduction of 27%. The concentrations of heavy metals, SS, COD, NH_4^+–N, pH, and TP in the effluent in group A and B met the discharge standards [24]. The concentrations of As, Cd, Cr, Cu, Ni, Pb, and Zn in the effluent in group A were 0.00, 6.00×10^{-3}, 2.70, 0.538, 0.204, 0.514, and $6.00 \times 10^{-3}\,\mu g\,L^{-1}$, respectively, and those in the effluent in group B were 0.00, 9.00×10^{-3}, 2.77, 0.547, 0.205, 0.534, and $1.00 \times 10^{-3}\,\mu g\,L^{-1}$, respectively. The SS, COD, NH_4^+–N, pH, and TP in the effluent were 15, 48.2, 6.74, 6.77, $0.56\,mg\,L^{-1}$, respectively. These findings indicate that adding aquatic worms to the reactor can reduce sludge production without deteriorating the quality of the treated water.

3.2. Changes in heavy metal concentrations in activated sludge and wastewater with aquatic worms

The heavy metals concentrations in the initial and final sludge were determined (Fig. 1). When compared with the initial sludge (day 1), the concentrations of As, Cd, Cr, Cu, Ni, Pb, and Zn in group B on day 20 did not change significantly ($p > 0.05$), while those in group A had increased obviously on day 20 by 21.8, 30.1, 30.2, 26.5, 31.2, 32.2, and 26.4%, respectively. The accumulation of heavy metals in the sludge of group A was significant ($p < 0.05$), indicating that aquatic worms could promote the accumulation of metals into the sludge. This was likely because the final waste sludge in group A was actually worm excrement produced after sludge predation [25,26]. Aquatic worms feed on activated sludge for metabolism and excreted; therefore, when the readily biodegradable organic matter in sludge was consumed by aquatic worms, the heavy metals accumulated in the excrement with slowly degradable material discharged from the worms, forming the final waste sludge. As a result, the readily degradable material in the sludge was decomposed and the amount of sludge was reduced, leading to higher concentrations of heavy metals in the sludge.

The initial and final heavy metals concentrations in worms were compared and analyzed (Fig. 2). The concentrations of As, Cr, and Cu in aquatic worms differed significantly on day 1 and 20 ($p < 0.05$), while those of Cd, Ni, Pb, and Zn did not ($p > 0.05$). Therefore, aquatic worms could accumulate As, Cr, and Cu to some extent, as indicated by their concentrations increasing by 35.3, 32.4, and 13.5%, respectively. There was no significant accumulation of Cd, Ni, Pb, or Zn in aquatic worms. These results indicate that there was moderate metals bioaccumulation in aquatic worms and that this accumulation was selective of As, Cr, and Cu. Tubificidae are known to possess detoxification mechanisms that may involve excretion of metals, which would explain the limited bioaccumulation observed in this study [27].

3.3. Mass balance of heavy metals

The mass balance of heavy metals in the aquatic reactor was calculated according to Eq. (2). As shown in Fig. 3, the initial masses of As, Cd, Cr, Cu, Ni, Pb, and Zn in the aquatic worm reactor were 0.595, 0.054, 25.40, 3.74, 1.87, 2.17, and 51.66 mg, respectively, while the final masses were 0.580, 0.053, 24.48, 3.59, 1.83,

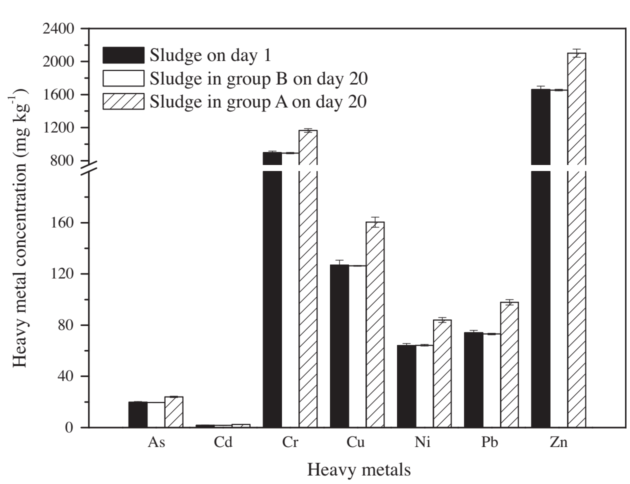

Fig. 1. Heavy metals concentrations in initial sludge (on day 1) and final sludge (on day 20) in group B and group A. Error bars show the standard deviations of the means ($n = 4$).

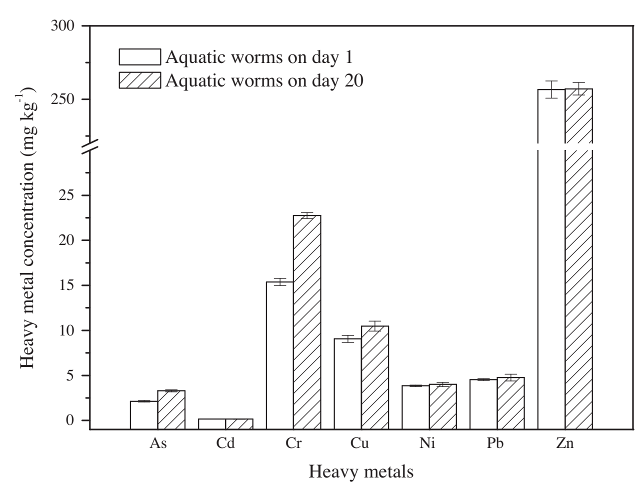

Fig. 2. Heavy metals concentrations in initial worms (on day 1) and final worms (on day 20). Error bars show the standard deviations of the means ($n = 4$).

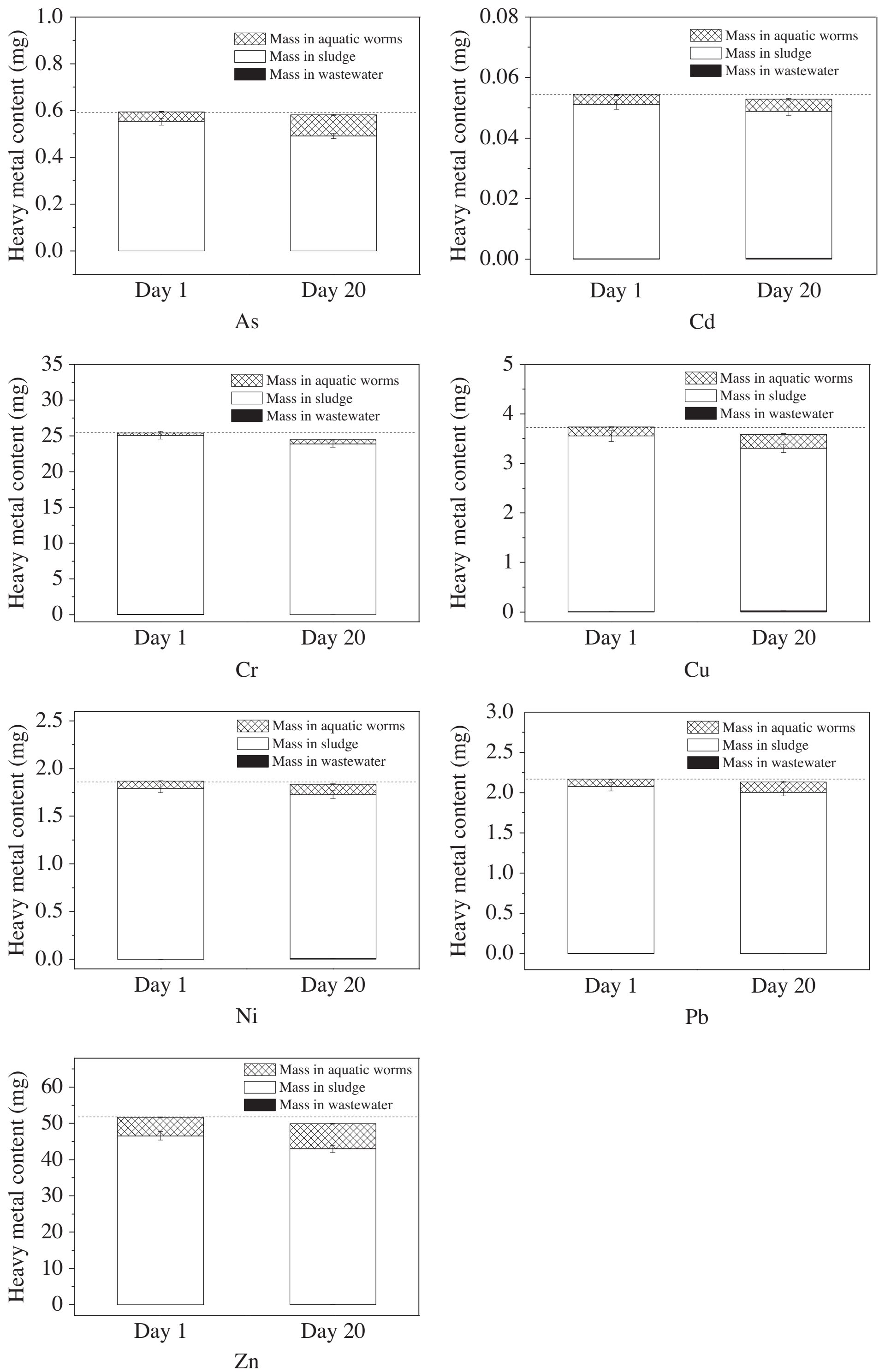

Fig. 3. Mass balance of heavy metals during activated sludge treatment of group A. Approximately > 80% of the total mass of each heavy metal analyzed accumulated in sludge (84.7–97.5%), while < 15% accumulated in aquatic worms (2.5–14.9%) and < 1% was discharged with the effluent (0–0.7%).

2.13, and 49.94 mg, respectively. For each heavy metal, the sum of the contents in wastewater, sludge and aquatic worms on day 1 was equal to that on day 20 (errors were within 4%).

As shown in Fig. 3, the heavy metals in the sludge accounted for a large portion of the overall content in the reactor (84.7–97.5%), less than 15% of the heavy metals (2.5–14.9%) were incorporated into the aquatic worm biomass, and less than 1% of the heavy metals (0–0.7%) were released to the effluent. Accordingly, sludge accumulated a large amount of heavy metals, aquatic worms contained relatively low amounts of heavy metals, and both contributed little heavy metals to the effluent.

The mass balance of each heavy metal was well maintained in the reactor, even though the locations of the heavy metals changed and some were transformed from the sludge to the worm biomass. Overall, heavy metals concentrations in the sludge increased significantly, but the contents changed only slightly, concentrations and contents in the aquatic worms increased slightly, and concentrations and contents in wastewater showed no significant variations ($p > 0.05$).

3.4. Accumulation and inactivation of heavy metals

Fractionation was conducted to analyze heavy metals in different chemical forms. Metals in the exchangeable, reducible, and oxidizable forms are labile so that they are easily utilized by plants, while metals in residual forms are not easily utilized by organisms and therefore have less toxic effects on the environment [18]. The residual proportions of Cr, Cu, Ni, and Zn in the initial and final sludge of group A and B were all higher than 80%. Compared with the initial sludge and sludge in group B, the residue of As, Cd, Cr, Cu, Pb, Ni, and Zn in group A increased to 36.0 ± 2.9, 60.4 ± 5.7, 97.2 ± 3.3, 94.8 ± 6.3, 98.2 ± 8.1, 46.9 ± 3.6, and $85.5 \pm 2.2\%$, respectively, reducing the risk of heavy metals accumulation by plants and animals [28].

As shown in Fig. 4, E was also calculated based on Eqs. (6–8). For group A, on day 20, the extent of accumulation and inactivation of heavy metals increased distinctly when compared with those on day 1. Accordingly, E increased by 32–69%, with the greatest increase being observed for Pb, indicating a notable accumulation and inactivation of this metal. Conversely, the values of E for As, Cd, Cr, Cu, Ni, Pb, and Zn in group A on day 20 increased by 40.2, 40.9, 38.9, 61.5, 35.1, 90.4, and 31.8%, respectively, showing higher accumulation and inactivation than in group B. Additionally, there was a significant difference in the accumulation and inactivation of heavy metals between the traditional activated sludge reactor (group B) and the aquatic worm reactor ($p < 0.05$). Specifically, E was drastically improved in response to the addition of aquatic worms, and most heavy metals showed reduced bioavailability after treatment.

4. Conclusions

In terms of a mass balance, heavy metals contents in an activated sludge reactor integrating aquatic worms were analyzed and the final redistribution was clarified. Metal concentrations increased greatly in the sludge, but only moderately in the worms. More than 80% of the total heavy metals accumulated in the final sludge, while less than 15% was transferred to worm biomass, and less than 1% was transferred to effluent. The addition of aquatic worms to an activated sludge reactor reduced sludge without deteriorating water quality and resulted in most heavy metals being accumulated in waste sludge and the metal bioavailability being reduced.

Heavy metals in the reactor can be removed via sludge discharge, and based on the sludge reduction, discharged sludge requiring further treated is obviously reduced. As for the final disposal, the sludge can be dried to produce solid recovered fuel or composted, depending on its characteristics.

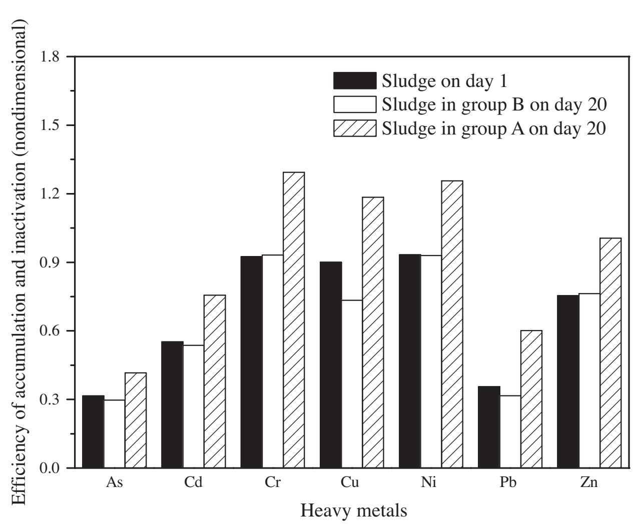

Fig. 4. Efficiency of accumulation and inactivation of heavy metals in initial sludge (on day 1) and final sludge (on day 20) in group B and group A.

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Nomenclature

k_1	forward rate coefficients (nondimensional)
k_2	backward rate coefficient (nondimensional)
t	experimental time (d)
m_{w0}	initial total heavy metals in wastewater on day 1 (mg)
m_{s0}	initial total heavy metals in sludge on day 1 (mg)
m_{a0}	initial total heavy metals in aquatic worms on day 1 (mg)
m_{wt}	total heavy metals in wastewater on day t (mg)
m_{st}	total heavy metals in sludge on day t (mg)
m_{at}	total heavy metals in aquatic worms on day t (mg)
C_w	concentration of heavy metals in wastewater ($mg\,kg^{-1}$)
C_m	concentration of heavy metals in biological matrix ($mg\,kg^{-1}$)
C_s	concentration of heavy metals in sludge ($mg\,kg^{-1}$)
C_a	concentration of heavy metals in aquatic worms ($mg\,kg^{-1}$)
M_w	weight of wastewater (kg)
M_s	weight of sludge (kg)
M_{s0}	initial weight of sludge on day 1 (kg)
M_{st}	weight of sludge on day t (kg)
M_a	weight of aquatic worms (kg)
E	efficiency of accumulation and inactivation of heavy metals in sludge (nondimensional)
P_s	percentage of heavy metals in sludge in the whole reactor (%)
P_r	percentage of residual heavy metals of the total content (%)
m_{sr}	weight of residual heavy metals in sludge (mg)

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