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Distributions of and Correlations between Cd, Cr, and Hg Concentrations in Suspended Particles and Sediment in Aquaculture Ponds and in *Cirrhinus molitorella* Tissues

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Key words
***Cirrhinus molitorella*, Cr, Cd, Hg, Correlation**

ABSTRACT

A total of 204 *Cirrhinus molitorella* at a stocking density of 7 individuals/m^{3}were equally divided between three closed rectangular concrete ponds (4 m × 2 m × 1.6 m; water depth 1.2 m) with dissolved oxygen concentrations above 5.2 ± 0.3 mg/l, and pH 6.5–8.1. Water flow in the ponds was poor. The amount of feed increased from 0.81 ± 0.35 to 13.42 ± 1.89 g/fish-day throughout the experiment. Samples were collected between mid-March and October 2016, and we monitored the Cd, Cr, and Hg concentrations in gills, large intestines, small intestines, intestine contents, longitudinal muscles, and body wall, and in suspended particles and sediment in the ponds. The effect of fish growth on metal concentrations was determined. Simultaneously, the correlations between heavy metal concentrations in suspended particles, sediment, and fish body wall were assessed. The Cd, Cr, and Hg concentrations in large intestines, small intestines, and intestine contents increased over time as feed application increased, and were significantly higher in intestines than in other tissues. The Cd, Cr, and Hg concentrations in suspended particles and sediment increased significantly as time elapsed and feed increased. The Cd, Cr, and Hg concentrations in the intestinal systems increased as fish grew. Strong correlations were found between heavy metal concentrations in the intestine contents and suspended particles; intestine contents and sediment; and suspended particles and sediment, but correlations with concentrations in the body wall and other substances were weak. Positive relationships between feed provided and metal accumulation resulted from uncontrolled feeding. Poor water flow allowed unconsumed feed containing metals to supply suspended particles containing metals; these enriched the sediment and ultimately supplied metals to the fish.The results provide reference data for developing *C. molitorella* eco-aquaculture systems.

INTRODUCTION

A great deal of attention has been paid to *Cirrhinus molitorella* (Cyprinidae, Labeoninae, Crossocheilus) by researchers and consumers because the fish grows quickly, is very productive, is resistant to many diseases, has a high survival rate, has tender smooth flesh, and is considered delicious (Huang *et al*., 1986; Chen *et al*., 1990; Lin *et al*., 2011; Cao and Wang, 1991; Cheng *et al*., 2012). *C. molitorella* is farmed widely, particularly in China, India, and Southeast Asia. Numerous studies of *C. molitorella* have been performed to investigate its growth and physiological and biochemical characteristics (Zhang *et al*., 2006), nutritional needs and development (Mao *et al*., 1985; Yin *et al*., 2003), molecular genetics (Zhang *et al*., 2015; Yang *et al*., 2008; Cheng *et al*., 2007; Zhu *et al*., 1997), pathogens (Lang, 1978; Wang *et al*., 2008; Fang *et al*., 2015; Fu *et al*., 2016), and changes in water quality

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(Liu *et al*., 2015; Yao, 1988; Zhou and Li, 2005; Dai *et al*., 2013).

Improvements in trace analysis techniques have steadily increased concern about food safety, and it has been predicted that 'green' products will come to dominate the market (Akhane *et al*., 2015). Eco-aquaculture requires using an efficient feeding regime and a scientifically rigorous management regime to produce high quality products and to protect and improve the environment. Industrial, agricultural, and aquaculture activities currently cause considerable environmental pollution and have been responsible for deteriorating river and pond water quality (Ha and Huong, 2013). Aquatic organisms are frequently affected by heavy metal contamination (Dai *et al*., 2013; Xing, 2016). Cd, Cr, and Hg bioaccumulation has been detected in *C. molitorella*, such as in a large commercial *C. molitorella* farm in China (Ha and Huong, 2013); family farms in the Songhua River (Zhu *et al*., 2010) and Pearl River Deltas (Xie *et al*., 2010) in China; and fishery farms sharing the same river with electroplating plants in Southeast Asia, India, and southern China (Huang *et al*., 1986; Akhane *et al*., 2015). Not only is heavy metal accumulation in *C. molitorella* a serious problem in many aquaculture farms, but excessive levels of Cd, Cr, and Hg in *C. molitorella* sold in markets have also been detected (Xing, 2016). These fish are purchased and eaten by local consumers as they are a favourite species, which results into Cd, Cr, and Hg accumulation in the body, ultimately producing adverse health effects. For example, ingested Hg enters the liver before being distributed around the body, and can damage the brain, the nervous system in general, and vision (Xie *et al*., 2010). Cd can cause hypertension and cardiovascular and cerebrovascular diseases and negatively affect the bones, kidneys, and liver (Ha and Huong, 2013). Cr can negatively affect the upper respiratory tract, causing bronchitis, pharyngitis, laryngitis, and rhinitis (Zhu *et al*., 2010). Eco-aquaculture methods need to be used to manage *C. molitorella* farming activities in scientifically rigorous ways and to improve the quality (less heavy metal contamination) of the *C. molitorella* produced. Huang *et al*. (1986) found that heavy metals negatively affect *C. molitorella*, but the distributions of and correlations between heavy metal concentrations in *C. molitorella* tissues and in suspended particles and sediment in aquaculture ponds have not been studied.

Heavy metal concentrations are important water quality parameters affecting fish growth in eco-aquacultural systems (Akhane *et al*., 2015; Xing, 2016). *C. molitorella* are currently often cultured in ponds with little water movement and are generally provided with excess feed containing heavy metals with the aim of achieving high yields and profits. Commercial fish feed with an appropriate heavy metal content can promote fish growth under scientific feeding methods, proper water flow, and a certain amount of submerged vegetation which can absorb heavy metals (Yang *et al*., 2018; Huang *et al*., 2017). Overfeeding – when fish are provided with excess commercial feed containing heavy metals-causes environmental pollution and the accumulation of metals in fish tissues (Xing, 2016). Overfeeding can improve *C. molitorella* growth and development to some degree but causes the *C. molitorella* produced to become contaminated with heavy metals and pose risks to human health (Xie *et al*., 2010; Zhu and Zhang, 2010). The aim of this study was to monitor Cd, Cr, and Hg concentrations in *C. molitorella* aquaculture ponds, and to assess the correlations between the heavy metal concentrations in suspended particles, sediment, and fish tissues. These provide reference data for building *C. molitorella* eco-aquaculture systems to ensure environmental and food safety.

MATERIALS AND METHODS

Fish

Juvenile *C. molitorella* with significant differences in mean body weight of 6.50 ± 0.22 g, and total length of 2.66 ± 0.41 cm, were provided by Guangxi Nanning Heji Aquaculture (Nanning, China). The fish were then transported to the National Breeding Base (Nanning, China) (a journey of 30 min) in a specially prepared enclosed vehicle with the temperature kept at 22.1 ± 0.3 °C and the dissolved oxygen concentration kept at 5.2 ± 0.3 mg/l. The fish were allowed to acclimatize in a cement pond (6 m × 6 m × 2.5 m) for 2 weeks before the experiment. Moribund and dead fish were removed, then the experiment was performed in March–October2016.

Experiment setup

The experiment was performed at the National Breeding Base using three rectangular concrete ponds, each 4 m × 2 m × 1.6 m. The water in each pond was 1.2 m deep. Each pond was sterilized before use by allowing it to be exposed to sunlight. The stocking density was 7 individuals/m^3. The total volume of water in each pond was 9.6 m^3. Water flow was almost zero because each pond was enclosed on four sides. The dissolved oxygen concentration was kept at > 5.2 ± 0.3 mg/l, and each pond was kept between pH 6.5 and 8.1. A total of 68 fish (total length 3.85 ± 1.01 cm) were cultured in each pond. Juvenile fish were introduced to each pond in mid-March, and they were fed with basic special feed at a rate of 0.81 ± 0.35 g/fish-day. The feeding rates in April and May were 1.72 ± 0.85g/fish-day and 3.66 ± 1.02 g/fish-day. From June to

August the diet was supplemented with high protein feeds (such as fishmeal) to support enhanced metabolism at the high temperatures in these months. The feeding rates in June, July, and August were 6.37 ± 1.35g/fish-day with 20% high protein feeds, 9.24 ± 1.68 g/fish-day with 25% high protein feeds, and 11.81 ± 1.96g/fish-day with 30% high protein feeds, respectively. In September and October, fish were fed with 0.78 ± 0.14 g/fish-day with cooked corn powder supplementation. Metal-free supplements provided by Hongda Feed (Guangzhou, China) were added to the diet in June (1%), July (2%), August (4%), September (6%), and October (8%), supplying nutrients required for growth and development. The amount of basic feed given to the fish was increased as the fish grew, and the feeding rates in September and October were 12.37 ± 2.02 g/fish-day with 35% high protein feeds and 13.42 ± 1.89 g/fish-day with 40% high protein feeds, respectively. The background Cd, Cr, and Hg concentrations in the basic feed were 0.0125 ± 0.03, 0.015 ± 0.04, and 0.005 ± 0.02 µg/g, respectively. The fish were fed ad libitum at 08:00, 12:00, and 17:00 each day throughout the experiment; but, as mentioned above, the amount of feed consumed by the fish increased over time. The basic feed contained (with the content per 100 kg of feed in parentheses) casein (41.90 kg), fish meal (35.00 kg), α-potato starch (12.20 kg), wheat flour (7.00 kg), fish oil (6.40 kg), premixed vitamins (3.40 kg), premixed minerals (3.05 kg), cellulose (1.05 kg), and carboxymethyl cellulose (1.00 kg). (Table Ⅰ)

Sample collection

Suspended particles, sediment, and fish samples were collected at the same time from each pond in April, June, August, and October 2016. Each sample type was collected in triplicate at each sampling time. Sample information is given in Table Ⅱ. Each suspended particle sample was collected in 1 l of water, each sediment sample was 1 kg, and each fish sample was 10 individual fish. The fish were dissected and samples of the gills, large intestines, small intestines, intestine contents, longitudinal muscles, and body wall tissues were placed in labelled sterile sample containers. The intestines were inspected and separated at the abrupt change from the large intestine to the small intestine. The water samples and sediment samples were collected using a method published by Xing (2016). The samples were stored at −20 °C in an LTI I-201 low-temperature incubator (Shanghai Biology Technology, Shanghai, China) while being transported to the laboratory. For each water sample, a 500 mL aliquot was passed through 0.45 µm Whatman GF/F glass-fibre filter (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) in a PVD18968 vacuum filter system (Jieda Biochemistry, Guangzhou, China). The filter was then dried in an oven at 60 °C for 12 h. Each sediment sample was dried at the ambient temperature; then visible impurities were removed before the sample was dried in an oven at 60 °C. Each dry sediment sample was then ground and passed through a 100-mesh sieve, and the mass of the prepared sample was recorded. (Table II)

Microwave digestion and determination of heavy metal concentrations

The samples were digested and analysed following a method published by Xing (2016) with minor modifications. A 0.4 g aliquot of a prepared sediment sample or the equivalent of 2 mL of a suspended particle sample was placed in a JII15-631 PTFE digestion vessel (Shanghai Biology Technology, Shanghai, China), and 4 mL hydrofluoric acid and 6 mL pure nitric acid were added. The vessel was then placed in a microwave digestion systemMPds-667 (Jieda Biochemistry, Guangzhou, China); the temperature was gradually increased to 130 °C and then kept constant for 2 h. The sample was then cooled and the digest transferred to a 10 ml colorimetric tube for analysis.

A 0.5 g aliquot of the gills, large intestines, small intestines, intestine contents, longitudinal muscles, and body wall tissues was ground and placed in PTFE digestion tubes and 5 ml pure nitric acid was added. The next day, 1.25 ml pure nitric acid was added. The tubes were placed in a water bath at 90 °C for 1.5 h. We then added 20 mL H_2O_2 to each tube, then placed them in an MPds-667microwave digestion system (Jieda Biochemistry, Guangzhou, China), where they were digested following the method described in the previous paragraph. Subsequently 1 ml $KMnO_4$ was added. Finally, the volume was made up to 100 ml with deionized water.

Each sample digest was adjusted to 25 ml with deionized water in a volumetric flask. Blank samples were prepared using ultrapure water. The Cd and Cr concentrations in each sample digest were determined using an ABSM I-302 graphite furnace atomic absorption spectrophotometer (Beijing Chemical Analysis Instrument Co., Beijing, China) following methods GB/T5009.15-2003 and GB/T5009.12-2003, respectively. The Hg concentration in each sample digest was determined using an AFS02-117atomic fluorescence spectrophotometer (Jiangsu National Water Quality Equipment Manufacturing Company, Nanning, China) following method GB/T5009.16-2003. Quality control was achieved using Cd-, Cr-, and Hg-certified reference materials. The Cd certified reference material was RM-EC301 (Guangzhou Pein Co., Ltd. Guangzhou, China). The Cd and Cr certified reference material was RM-EC302 (National Standard Chemistry and Food Center, Beijing, China). The Hg certified

Table I. The feeding status,supplements, feeding rate of basic feed and the concentrations of Cd, Cr and Hg in each month. Fish were fed ad libitum at 08:00, 12:00, and 17:00 each day.

Months	Supplements	Feeding rate (g/fish-day)	Metal concentrations (µg/g)		
			Cd	Cr	Hg
Mid-March	No supplements	0.81 ± 0.35[a]	0.0101 ± 0.001[a]	0.012 ± 0.001 [a]	0.004 ± 0.000 [a]
April	No supplements	1.72 ± 0.85[b]	0.0215± 0.003 [b]	0.026 ± 0.001 [b]	0.009 ± 0.001 [b]
May	No supplements	3.66 ± 1.02 [c]	0.0457± 0.004 [c]	0.055 ± 0.003 [c]	0.018 ± 0.001 [c]
June	High protein feeds (20%), metal-free supplements(1%)	6.37 ± 1.35[d]	0.0796± 0.006 [d]	0.096 ± 0.007 [d]	0.032 ± 0.003 [d]
July	High protein feeds (25%), metal-free supplements(2%)	9.24 ± 1.68[e]	0.1155 ± 0.019 [e]	0.139 ± 0.026 [e]	0.046 ± 0.007 [e]
August	High protein feeds (30%), metal-free supplements(4%)	11.81 ± 1.96[f]	0.1476 ± 0.022 [f]	0.177 ± 0.031 [f]	0.059 ± 0.010 [f]
September	High protein feeds (35%), metal-free supplements(6%), cooked corn powder with 0.78 ± 0.14 g/fish-day	12.37 ± 2.02[g]	0.1546 ± 0.038 [g]	0.186 ± 0.049 [g]	0.062 ± 0.014 [g]
October	High protein feeds (35%), metal-free supplements(8%), cooked corn powder with 0.78 ± 0.14 g/fish-day	13.42 ± 1.89[h]	0.1677 ± 0.043 [h]	0.201 ± 0.053 [h]	0.067 ± 0.016 [h]

Differences were tested using one-way analysis of variance (ANOVA). Differences were considered significant at $P < 0.05$ and marked using different letters.

Table II. Sample collection and detection in the experiment.

Samples	Time	Number of samples	Location	Method	Detection method	Quality control standards
Fish	April 11, June 10, August 11, October 10	10 of fishes once	Sampled at equidistant three locations of two diagonal linesin the pond	Caught fish with net	Determination of Cd, Cr by graphite furnace atomic absorption spectrometer, determination of Hg by atomic fluorescence spectrometer	GB/T5009.15-2003(Cd) GB/T5009.12-2003(Cr) GB/T5009.16-2003(Hg)
Suspended particle		1 kg once		Pumped water at 0.5 and 1.2 m depth		
Sediment		1 kg once		Pumped the mud of bottom surface		

Table III. QA/QC (quality assurance/quality control) data for heavy metal analysis in fish standard reference materials.

Element	Measured mass ratio /µg/g^{-1}				RSD (%)	Accuracy (%)	Precision (%)
	Parallel 1	Parallel 2	Parallel 3	Average value			
Cd	0.01	0.01	0.02	0.013	9.43	96.32	98.91
Cr	0.45	0.47	0.41	0.44	5.63	98.07	96.54
Hg	0.18	0.19	0.20	0.19	4.30	96.28	98.34

RSD represents relative standard deviation.

reference material was RM-EC303 (National Standard Chemistry and Food Center, Beijing, China). A 1 g muscle sample was ground and placed in a Bunsen beaker, and 10 ml hydrofluoric acid and 8 ml pure nitric acid were added. The sample was microwave digested at 125 °C for 6 h. The Cd, Cr, and Hg concentrations were determined by making measurements at 214, 283, and 184 nm, respectively. The relative standard deviations (RSDs) of Cd, Cr, and Hg were < 9.43%, 5.63%, and 4.30%, respectively. The accuracy (Cd, 96.32%; Cr, 98.07%; Hg, 96.28%) and precision (Cd, 98.91%; Cr, 96.54%; Hg, 98.34%) of the method met our quality control requirements. (Table III).

Statistical analysis

The heavy metal concentration unit in fish and sediment was µg/g dry weight, and the concentration unit in suspended particles was µg/ml. Each result is shown below as the mean ± standard error of the mean. Correlation coefficient (r) in two samples was calculated based on linear-regression analysis. Differences between groups of samples were tested using one-way analyses of variance, and were considered significant at $P< 0.05$. All analyses were performed using SPSS v17.0 software (SPSS, Chicago, IL, USA).

RESULTS

Cd, Cr, and Hg concentrations in C. molitorella *tissues*

We found corresponding ranges of 0.010–0.102 µg/g, 0.010–0.210 µg/g, and 0.011–0.062 µg/g of Cd, Cr, and Hg concentrations in the fish tissue samples. The Cd, Cr, and Hg concentrations in the body wall, longitudinal muscle, and gill samples did not increase significantly as time elapsed and feed application increased ($P > 0.05$), but in the intestinal system they increased significantly ($P < 0.05$). The highest Cd, Cr, and Hg concentrations were detected in the intestine content samples, at 0.102 ± 0.005 µg/g, 0.210 ± 0.003 µg/g, and 0.062 ± 0.002 µg/g, respectively. The Cd, Cr, and Hg concentrations of the intestine content samples were significantly higher than those of the large intestine and small intestine samples. The various ranges of Cd concentrations in the intestine content, large intestine, and small intestine were 0.048–0.102, 0.041-0.08, and 0.038-0.102 µg/g, respectively. The Cr concentrations varied in ranges of 0.064-0.0.210, 0.053-0.127, and 0.044-0.103 µg/g, respectively. The various ranges of 0.064-0.0.210, 0.053-0.127, and 0.044-0.103 µg/g were detected in Hg concentrations of the intestine content, large intestine, and small intestine, respectively. The Cd, Cr, and Hg concentrations in the intestinal system increased by 2.43-4.03-fold, 2.15-2.67-fold, and 1.88-3.24-fold, respectively, as time elapsed and feed application increased. (Fig. 1).

Heavy metal concentrations in suspended particles and sediments

As time elapsed and feed application increased, the Cd, Cr, and Hg concentrations in suspended particles increased and reached their maximum values in October when they were 17.63 ± 1.86 µg/g, 12.36 ± 1.24 µg/g, and 0.25 ± 0.02 µg/g, respectively. In April, all heavy metals concentrations were at their lowest values, at 4.55 ± 0.21, 4.21 ± 0.16, and 0.02 ± 0.001 µg/g, respectively. In the suspended particle samples, the Cr concentrations were significantly higher than the Cd and Hg concentrations throughout the experimental period ($P < 0.05$). Finally, the Cd, Cr, and Hg concentrations in the suspended particle samples increased by 2.11-, 1.03- and 1.51-fold, respectively. The highest concentrations of Cd, Cr, and Hg in sediment samples were found in October, when their values were 2.64 ± 0.14, 4.35 ± 0.28, and 0.22 ± 0.06 µg/g, respectively. The concentrations of Cd, Cr, and Hg were lowest in April, when they were 1.57 ± 0.92, 3.06 ± 0.26, and 0.09 ± 0.001 µg/g, respectively. Compared with the Cd and Hg concentrations in sediment samples, the Cr concentrations in the same samples were significantly higher ($P < 0.05$). In the sediment samples, the Cd, Cr, and Hg concentrations had increased by 1.68-, 1.42-, and 1.44-fold at the end. (Fig. 2)

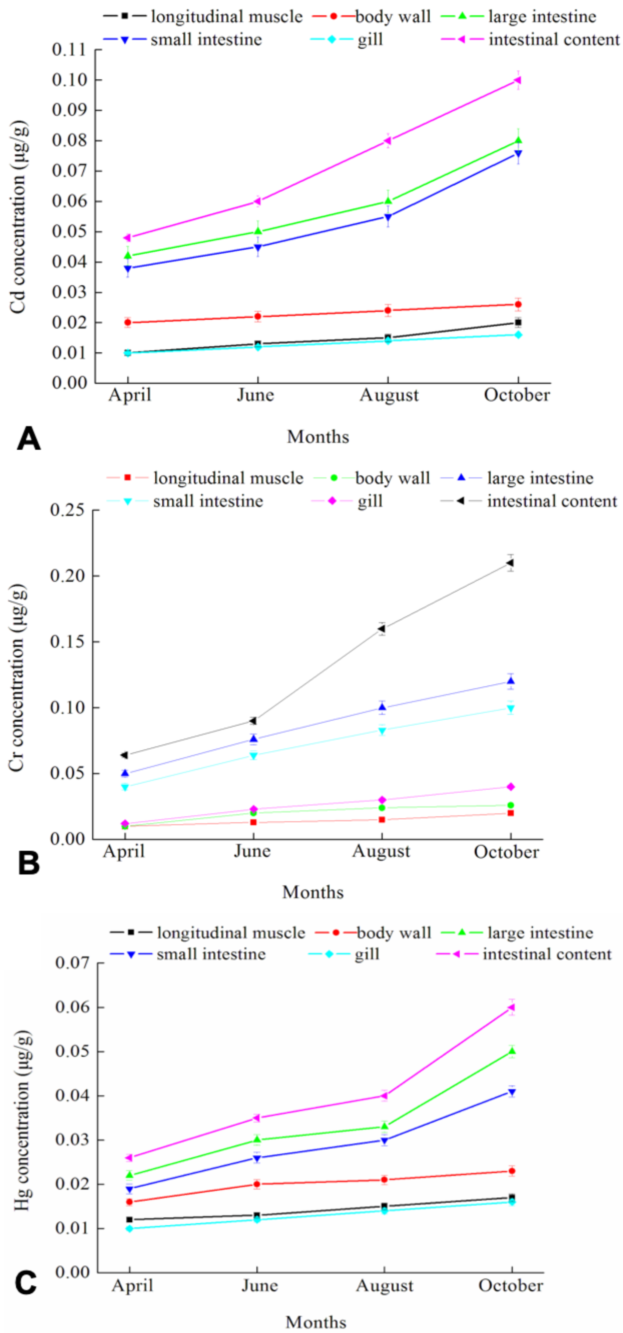

Fig. 1. Cd, Cr and Hg concentrations in different tissues of *Cirrhinus molitorella* (Crossocheilus) at different months. Data were expressed as means ± standard error of mean.

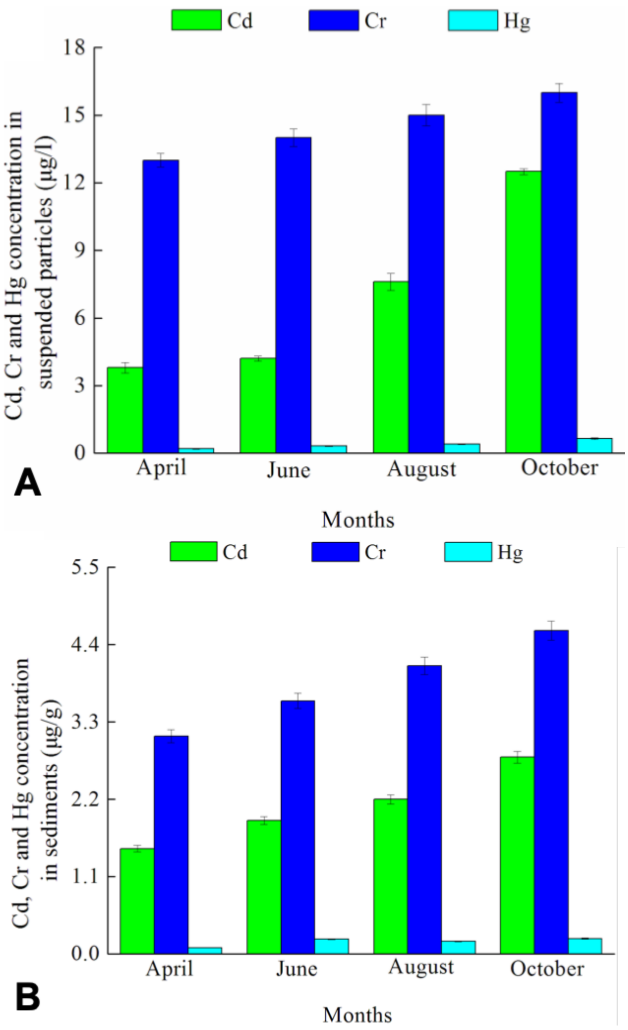

Fig. 2. The concentrations of Cd, Cr and Hg in suspended particles (A) and sediments (B) in pond with *Cirrhinus molitorella* (Crossocheilus) at different months. Data were expressed as means ± standard error of mean. Differences were considered significant at $P< 0.05$.

Total length

The initial total length of *C. molitorella* in mid-March was 3.85 ± 1.01 cm. The fish reached 5.67 ± 1.24 cm when first sampled in April. The fish body size increased significantly as time elapsed and feed application increased, and the maximum value in October was 16.74 ± 4.63 cm. The body size of fish increased 4.35-fold during the experimental period. (Table IV).

Correlation analysis

The relativities of Cd, Cr, and Hg concentrations in the intestine contents and suspended particles (r = 0.832, 0.890, and 0.902, respectively), intestine contents and sediment (r = 0.848, 0.851, and 0.879, respectively), and suspended particles and sediment (r = 0.852, 0.890, and 0.902, respectively) were higher than those in the body wall and other samples. The relativities of Cd, Cr, and Hg concentrations in the body wall and other samples were less than the correlation coefficient of 0.8. (Table V).

Table IV. Total length of sampled *Cirrhinus molitorella* (Crossocheilus) in each month. Data were expressed as means ± standard error of mean.

Months	Total length(cm)
Mid-March	3.85 ± 1.01 cm^{a}
April	5.67 ± 1.24 cm ^{b}
May	8.22 ± 0.76 cm ^{c}
June	10.37 ± 1.58 cm ^{d}
July	12.59 ± 2.13 cm ^{e}
August	14.07 ± 5.44 cm ^{f}
September	15.83 ± 3.28 cm ^{g}
October	16.74 ± 4.63 cm^{h}

Differences were tested using one-way analysis of variance (ANOVA). Differences were considered significant at $P < 0.05$ and marked using different letters.

DISCUSSION

Diet, lifestyle, and body structure influence the distribution of substances absorbed by aquacultured organisms (Cheng *et al*., 2012; Zhang *et al*., 2015). *C. molitorella* is a bottom-dwelling fish that consumes organisms and organic matter at the bottom of the water column (Mao *et al*., 1985; Yin *et al*., 2003; Huang *et al*., 2017). The Cd, Cr, and Hg concentrations were higher in the intestine content samples than in the other tissue samples; concentrations in the large and small intestine samples were the next highest; and concentrations in the body wall, gill, and longitudinal muscle samples were lowest. Similar distribution patterns were found by Xie *et al*. (2010) and Ha and Huong (2013). The Cd, Cr, and Hg concentrations in intestine contents and in the large and small intestines increased significantly as time elapsed and feed application increased. The results indicate that Cd, Cr, and Hg may have accumulated in some tissues of *C. molitorella* (a specialized demersal fish), according to the reports of Zhang *et al*. (2015), Xie *et al*. (2010), Zhu *et al*. (2010), Ha and Huong (2013), and Akhane *et al*. (2015). The Cd, Cr, and Hg concentrations in the intestinal system were different in different months, and this may have been related to larger amounts of Cd, Cr, and Hg being consumed when the fish were fed more, and to Cd, Cr, and

Table V. Correlation coefficient (*r*) of heavy metal concentrations in the body wall, suspended particles, sediments and intestinal contents.

Heavy metals	Intestinal contents & body wall	Suspended particles & body wall	Body wall & sediments	Intestinal contents & suspended particles	Intestinal contents & sediments	Suspended particles & sediments
Cd	0.727	0.681	0.656	0.832	0.848	0.852
Cr	0.689	0.702	0.683	0.890	0.851	0.911
Hg	0.684	0.666	0.701	0.902	0.879	0.896

r; correlation coefficient represents in two samples, which was calculated according to linear-regression analysis.

Hg being absorbed at different efficiencies in different seasons. Dai *et al*. (2013) found that heavy metal accumulation in a culture pond with *C. molitorella* resulted from poor water-flow conditions. Ha and Huong (2013) reported that Hg, Cd, and Cr concentrations in the *C. molitorella* intestinal system were lower than inthe water samples, as the running water in the open ponds diluted and transported away Cd, Cr, and Hg through water exchange with outside water, although the stocking density was high (15 individuals/m^3). Xing (2016) also found that, in *C. molitorella* at a lower stocking density of 6 individuals/m^3, the intestinal systems had higher Cd, Cr, and Hg concentrations than those of water samples. This was because the culture ponds had poor water flow and an unscientific feeding regime (1.75–13.6 g/fish-day, April–October). In this study, the increase in Cd, Cr, and Hg concentrations in the intestinal system of *C. molitorella* with stocking density of 7 individuals/$m^3$was higher than in suspended particles and sediment samples. The feeding regime of 1.72-13.42 g/fish-day in April-October was similar to that reported by Xing (2016). The results indicate that fish probably swallowed feed having excessive Cd, Cr, and Hg levels. The suspended particles and sediment formed from unconsumed feed containing heavy metals (due to the unscientific feeding regime and poor water-flow conditions in ponds) finally resulted in absorption and accumulation in the intestinal system and a decrease in Cd, Cr, and Hg concentrations in ponds.

In our study, the Cd, Cr, and Hg concentrations in the suspended particles, sediment, and intestinal system increased over time as feed increased from 0.81 to 13.42 g/fish-day, similar to the results reported by Xing (2016). These changes would have been affected by the conditions in the ponds, the amounts of feed provided, and the management regime (Huang *et al*., 1986; Xing, 2016; Xie *et al*., 2010; Zhu *et al*., 2010). There were no sources of pollution to the ponds other than the feed, but the Cd, Cr, and Hg concentrations were higher in the suspended particles and sediment in October than earlier in the year. We conclude that Cd, Cr, and Hg were supplied to the sediment in suspended particles derived from residual feed and faeces produced by the *C. molitorella*. It is important that the feeding method, feed composition, and amount of feed supplied are taken into account when developing ecologically appropriate aquaculture practices (Chen *et al*., 1990; Xie *et al*., 2010; Zhu *et al*., 2010). Particles suspended in water are very unstable, so the suspended particle concentration will vary temporally and therefore the sediment properties will also vary temporally (Xie *et al*., 2010; Zhu *et al*., 2010). The Cd, Cr, and Hg concentrations in the sediment and suspended particle samples were positively correlated. Water circulation in the ponds was limited, and the water rarely moved. Cd, Cr, and Hg in suspended particles will have sunk and enriched the sediment, indicating the importance of water movement to the eco-aquaculture of *C. molitorella*.

The total length of *C. molitorella* increased with feed amounts from 0.81 to 13.42 g/fish-day. Fish body size increased 0.47-4.35-fold, but the Cd, Cr, and Hg concentrations in the intestinal system increased by 2.43-4.03-fold, 2.15-2.67-fold, and 1.88-3.24 fold, respectively. The results indicate that the Cd, Cr, and Hg concentrations in the intestinal system increased as fish grew, similar to the results reported by Xing (2016). Excessive ingested Hg, Cr, and Cd can damage the brain and nervous system related to growing development, leading to a low growth rate (Ha and Huong, 2013; Xie *et al*., 2010; Zhu *et al*., 2010). After the experiment, we found that the growth in *C. molitorella* body size was similar to the result (4.28-fold) reported by Xing (2016); but it was lower than the data (5.11-fold) found by Ha and Huong (2013). The results may be attributed to the ponds with poor water-flow conditions causing Cd, Cr, and Hg accumulation in the body, ultimately affecting fish development.

Intestinal secretions can promote the dissolution of Cd, Cr, and Hg, facilitating the absorption of these metals into the body (Xing, 2016; Xie *et al*., 2010). Heavy metal transport to fish is influenced by specific physiological features, fish behaviour, food intake, metabolic processes, and excretion. These factors may cause inconsistencies in the correlations between the heavy metal concentrations in biological and non-biological matrices (Xing, 2016).

We found that the Cd, Cr, and Hg concentrations in the *C. molitorella* intestine contents correlated strongly with the concentrations in suspended particles and sediment. Strong correlations were also found in the concentrations between suspended particles and sediment. Only weak correlations were found with concentrations in the body wall and other substances. The results indicate that the Cd, Cr, and Hg concentrations in *C. molitorella* were mainly related to intestinal absorption. This further demonstrates that the intestines play important roles in Cd, Cr, and Hg absorption and processing, as reported by Ha and Huong (2013) and Zhu *et al*. (2010).

CONCLUSION

In summary, we monitored Cd, Cr and Hg concentrations in *C. molitorella* aquaculture ponds with a stocking density of 7 individuals/m^3 and poor water flow. Cd, Cr, and Hg accumulated in *C. molitorella* intestinal systems, and in suspended particles and sediment, as the amount of feed increased and time elapsed. Positive relationships were found between the amount of feed applied and accumulation of the heavy metals. The Cd, Cr, and Hg concentrations in the intestinal systems rose as the fish continued to grow. Strong correlations were found between heavy metal concentrations in intestinal contents and suspended particles; intestinal contents and sediment; and suspended particles and sediment. The results indicate that unscientific feeding regimes and poor water flow lead to unconsumed feed containing heavy metals to form suspended particles and sediment, ultimately supplying heavy metals to the *C. molitorella*. The data will help in the development of *C. molitorella* eco-aquaculture systems, but further study is needed to determine optimal feed composition. The construction of open ponds may also be an important step in transporting away Cd, Cr, and Hg through exchange with outside water.

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Statement of conflict of interest

The authors declare no conflict of interest.

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