TISSUE-SPECIFIC ACCUMULATION AND DEPURATION OF CADMIUM IN TILAPIA: ROLE OF SALINITY AND CADMIUM CONCENTRATION

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Abstract. This study investigated the influence of salinity and Cd concentration on the accumulation and depuration of Cd in different tissues of tilapia. Following Cd exposure for 30 days, high Cd concentration significantly inhibited the fish growth. Cd accumulation in the fish was tissue-specific and dose-dependent with the greatest Cd accumulation in the liver, followed by the gill, scale and edible muscle except for 0.5 mg/L Cd in combination with high salinity. The bioaccumulation factors (BAFs) of Cd in the different tissues of the fish were influenced by three factors (time, toxicity levels and salinity of exposure), with the greatest BAFs in the liver in all cases. The depuration kinetics showed the ability of tilapia to eliminate Cd and the influence of salinity on the elimination varied according to tissues. Moderate salinity promoted the depuration of Cd in the tissues except for edible muscle. Among the four tissues, edible muscle showed the highest elimination rates (ER), ranging from 76.7% to 81.9% with varied salinities. These results suggest that Cd accumulation and depuration of tilapia are complex processes that are influenced by several factors. This knowledge may expedite more accurate risk assessment of heavy metals.
Keywords: *fish growth, different tissues, accumulation, bioaccumulation factors, elimination rates,*

Introduction

In the past few decades, heavy metals show sharp increase in aquatic ecosystems, especially close to industrial regions (Ruiz-Fernández et al., 2018; Samadani et al., 2018). Most of the metals are highly toxic and can result in serious damage to a variety of organ systems in exposed aquatic organisms (Asagba et al., 2008; Rebolledo et al., 2020). Compared with other toxic metal pollution, cadmium (Cd) is one of the most deleterious elements in water environments through industrial wastewater dumping (Ma et al., 2008). In recent years, Cd concentrations undulate from 0.011 to 25 μg/L in slightly polluted waters (Zheng et al., 2016) and even approach 1 mg/L in rivers near industrialized areas (Zhu et al., 2017) in China. Some investigation and research demonstrated Cd concentrations can reach and exceed 5 mg/L in extreme accidents, such as Cd mine accidental spill/release (Cui et al., 2012). Cd exposure results in serious damage to hydrobionts and threatens human health through the aqueous food chain (Chouvelon et al., 2019).

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In compassion, fish are easier to concentrate and accumulate heavy metals including Cd in large quantities both through water and food consumption because of their higher trophic level (Sana et al., 2009; Xue et al., 2018). The accumulated Cd in fish is distributed with various tissues including the liver, gill, edible muscle, heart and kidney, etc (Soares et al., 2008; Xue et al., 2018). The concentration enrichment of Cd in different tissues confirms that fish are capable to accumulate Cd in their bodies (Van der Oost et al., 2003; Bielmyer-Fraser et al., 2018; Mapenzi et al., 2020). However, the distribution pattern of Cd is rarely distributed uniformly within the tissues of aquatic organisms (Obasohan et al., 2008; Sani et al., 2011; Cao et al., 2012). Numerous studies revealed that it was determined by tissue-specific bioavailability and uptake mechanisms (Ranaldi et al., 2009; Bielmyer-Fraser et al., 2018) and regulated by a physicochemical factor such as salinity of water (Lin and Dunson, 1993; Dutton et al., 2011). Previous studies identified that salinity could be a major cause of the bioaccumulation of metal in fish (Bielmyer et at., 2006; Rebolledo et al., 2018). Karar et al. (2019) reported that Cd accumulated mostly in the gill tissues of Crab (*Portunus pelagicus*). Moreover, tissues including gill, muscle and hepatopancreas showed higher accumulation exposed to lower salinity than higher salinity. Previous studies indicated that increasing salinity resulted in decreasing metal toxicity to fish, due to ligand interactions and competing ions (Wood et al., 2004; Niyogi and Wood, 2004; Bielmyer et al., 2013).

Tilapia (*Oreochromis mossambicus*) is a kind of hardy and fast-growing fish that has been an essential source of protein and has recently been in high demand in many countries. It could live in both freshwater and saline water of up to 130‰, and grow and reproduce at salinities of up to 85‰ (Panfili et al., 2006). It has been reported that the fish easily concentrated and accumulated Cd in its tissues either through water or the food chain (Xue et al., 2018). Previous works indicated that bioaccumulated Cd in fish could be depurated in an uncontaminated environment (Kim et al., 2006; Palaniappan and Karthikeyan, 2009; Chen et al., 2018). The depuration process was influenced by environmental factors such as time, temperature, salinity and metabolic activity of fish. Amiard-Triquet et al. (1991) noted that salinity influenced the tissular redistribution of Zn in oysters in the depuration process. The influence of salinity of metal depuration studies is important for health protection, searching for a convenient and quick way for the self-purify of contaminated organisms.

The present study was designed to examine salinity effects on accumulation and depuration of Cd in different tissues of tilapia when exposed to a Cd concentration of 0.5 mg/L or 1.5 mg/L for 30 days in different salinities (0, 5‰, 15‰, 25‰). The depuration of Cd from the fish tissues was then tested over 30 days, following transfer to Cd-free water with different salinities (0, 5‰, 15‰, 25‰). This study has three main objectives to investigate: the kinetics of Cd accumulation and depuration in tilapia tissues in different Cd exposures and salinities; (2) the effects of salinity on Cd accumulation and depuration in different tissues of tilapia; (3) bioaccumulation factor (BAF) and Elimination rates (ER) of cadmium in different tissues of tilapia under the influence of salinity.

Materials and methods

Fish

Tilapia (*O. mossambicus*), (body weight 15.73 ± 0.41 g; total length 9.8 ± 1.0 cm) purchased from an unpolluted fishery farm in Nanjing, China (32°03’ N and 118°46’ E), was used as a representative fish in this study. Before Cd accumulation experiments, the

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fish were acclimated to laboratory conditions in aerated laboratory tanks, at optimal water temperatures of 20-25°C (pH 7.4-7.7) for a week under natural photoperiod conditions. During the acclimation period, the fish was fed with commercial, unpolluted fish food twice a day (morning and afternoon) at a daily rate of 5% body weight (fresh weight).

Accumulation experiment

After one week of acclimatization, fish that were in good health and uniform size were transferred to experimental aquariums (0.5 m × 0.5 m × 0.5 m) containing 75 L diluted (medium/distilled water, 1:25 v/v) MA medium [$Ca(NO_3)_2 \cdot 4H_2O$ 50 mg/L, KNO_3 100 mg/L, $NaNO_3$ 50 mg/L, Na_2EDTA 5 mg/L, bicine 500 mg/L, disodium β-glycerophosphate 100 mg/L, $CoCl_2 \cdot 6H_2O$ 5 mg/L, $FeC1_3 \cdot 6H_2O$ 0.5 mg/L, $Na_2MoO_4 \cdot 2H_2O$ 0.8 mg/L, $MgC1_2 \cdot 6H_2O$ 50 mg/L, $MnCl_2.4H_2O$ 5 mg/L, $ZnCl_2$ 0.5 mg/L, H_3BO_3 20 mg/L, and Na_2SO_4 40 mg/L] (Maeda et al., 1993) and different concentrations of Cd ($CdCl_2$) and salinity levels. Each aquarium accommodated 18 fish. To study the effect of salinity and Cd toxicity on Cd uptake of the fish, an orthogonal experimental design, (control +2 Cd-concentrations × 3 salinities × 3 replicates), was set up with salinity and Cd concentration as the two factors. The Cd and saline level were based on the pervious trial tests performed for 7 days. The results showed that 10 mg/L of Cd was median minimum lethal concentration for Cd (Xue et al., 2018) and 75‰ salinity was minimum lethal concentration for saline. The highest concentration for sub toxicity test experiments should not exceed one-third of the minimum lethal concentration. The fish were treated for 30 days, with nominal concentrations of Cd exposure levels of 0, 0.5 and 1.5 mg/L and salinity levels of 0, 5, 15 and 25‰, respectively. The exposure medium was replaced every two days and assuming that the elimination of the metal and salt is negligible during the exposure. There were 12 different treatments, and three replicates were used for each treatment. Two fish were removed from each test concentration every 10 days during the 30-day uptake experiment. The tilapia was harvested and washed with deionized water, and the liver, gut, edible muscle and scale were cut into small pieces and freeze-dried using a Freeze Dryers Vacuum-Concentrator (Model FD-1A-50, Nanjing, China). The dried samples were weighed and then ground into powder using a stainless mill, which was then used for analyses for total Cd concentration.

Depuration experiment (different salinity)

After being exposed to 1.5 mg/L Cd with free salinity for 30 days, the fish was investigated for Cd elimination. The fish were sequentially removed to tanks containing 75 L diluted (medium/distilled water, 1:25 v/v) MA medium with salinity levels of 0, 5, 15 and 25‰, respectively. The depuration period lasted for 30 days. During the period, the culture medium was renewed every 24 h. Two fish were removed from each aquarium every 10 days during the 30-day depuration experiment. After that, the gill, liver, edible muscle and scale of the fish were isolated, dried and digested. Cd concentrations were measured using the same method. For all the treatments with Cd, the toxicant water was renewed every 24 h to exclude the effects of remaining baits and feces.

Sample digestion

Samples of fish tissues were digested using the method of Yi et al. (2011) with minor modifications. In brief, 0.4 ± 0.01 g dry-powder samples of different fish tissues were put directly into acid–washed Teflon digestion vessels. 5 mL of concentrated HNO_3 was

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added to each vessel, which was then heated to 100°C using an XT-9800 pre-treatment heater until all HNO_3 was evaporated. Afterward, 5 mL of concentrated HNO_3:H_2O_2 (4:1 v/v) mixture solution was added into each vessel and digested in a microwave. To check the digestion efficiency, each digestion experiment was conducted in parallel with control treatments that included a reagent blank, a reference standard material (Yellow croaker, *Pseudosciaena crocea*, GBW08573, from the National Research Center for Standards, China), and a sample without addition of the mixture of concentrated acids. Specifically, microwave digestion included three steps: digestion at 0.5 MPa for 2 min, 1.0 MPa for 2 min, and 1.5 MPa for 3 min. After microwave digestion, the samples were cooled down at room temperature for at least 1 h before being transferred to plastic test tubes. DI water was added to each tube, reaching a final total volume of 100 mL before Cd concentration measurements (Yi et al., 2011).

Bioaccumulation factors

Bioaccumulation factors (BAFs) are often calculated as follows:

$$BAFs = C_{tissue}/C_{water} \quad \text{(Eq.1)}$$

where C_{tissue} is the Cd concentration in the tissue of exposed tilapia (expressed as mg/kg dry weight), and C_{water} is the Cd concentration in the solution (expressed as mg/L).

Elimination rates

During the depuration period, Cd in fish is eliminated from tissues to the aquatic environment. The elimination rates (ER) of Cd in fish tissue is defined as the quotient of

$$ER = (C_0\text{-}C_e)/C_0 \quad \text{(Eq.2)}$$

in which C_0 (expressed as mg/kg dry weight) is the Cd concentration in tissue at the beginning of the depuration experiment, and C_e (mg/kg, dry weight) is the end value of Cd concentration after a certain depuration period.

Statistical analyses

All experimental data were reported as mean values standard deviations. Bivariate correlation analyses were performed to determine the relationship among salinities and the kinetics of cadmium absorption and emission in different organs of tilapia under different cadmium concentrations using Excel 2016. Significant differences were identified by two-way ANOVA for the first phase (I) in *Table 1* and one- way ANOVA for in the second phase (II) in *Table 1* and for *Figures 1–4* using IBM SPSS Statistics 27.0.1 ($p < 0.05$)

Results

Fish growth

No mortality occurred throughout the experimental periods. The initial weight of the fish was 15.73 ± 0.41 g. After 30 days of exposure, i.e. in the first phase, the fish weight ranged from 25.33 g to 34.73 g (*Table 1*). Fish weight was not significantly affected in

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the low Cd (0.5 mg/L) group and significantly decreased in the high Cd (1.5 mg/L) group regardless of salinity in comparison with that of the control fish ($p < 0.001$, *Tables 1* and *2*). The influence of salinity on W_{t1} were no significant differences compared to the control except for a significant increase in the interaction of Cd-free and low salinity (5‰) group ($p < 0.001$, *Table 2*) and a decrease in the interaction of 1.5 mg/L Cd and high salinity group ($p < 0.001$, *Table 2*). The fish of average daily growth (AGT) showed the biggest value in the interaction of the Cd-free and low salinity group and the smallest value in the interaction of 1.5 mg/L Cd and high salinity group ($p < 0.001$, *Tables 1* and *2*). However, there were no significant differences in other groups from the control group.

After the additional 30 days' depuration period (the second phase), the final weight W_{t2} ranged from 48.53 g to 41.07 g. Because the fish came from the first phase of the interaction and 1.5 mg/L Cd and different salinities (0, 5‰,15‰, 25‰), which were significantly different in weight, it was hard to compare weight. In comparison, AGT was a better indicator of weight change in the depuration period. There were no significant differences in the salinity exposure group from the control group except in high salinity. It was 0.53 g/d, decreased in the high salinity group than that of the control fish, but the difference was not significant (*Tables 1* and *2*). In general, AGT was significantly influenced by high salinity in the depuration period.

Table 1. *The initial weight (W_0) and final weight 1 (W_{t1}) of tilapia in the first phase (I) and final weight 2 (W_{t2}) of tilapia in the second phase (II)*

Treatments		W_0 (g)	W_{t1} (g)	W_{t2} (g)	Average daily growth (AGT) (g/d)	Survival rate (%)
Cd (mg/L)	Salinity (‰)					
I						
Control		15.87 ± 0.51a	29.80 ± 1.98ad		0.46 ± 0.08a	100
Cd +	Salinity					
0	5	15.71 ± 0.36a	34.73 ± 1.19b		0.63 ± 0.03b	100
	15	15.50 ± 0.36a	30.83 ± 1.59a		0.51 ± 0.06a	100
	25	15.83 ± 0.32a	29.03 ± 1.70ad		0.44 ± 0.05a	100
0.5	0	15.73 ± 0.35a	27.40 ± 1.65ac		0.38 ± 0.03a	100
	5	15.90 ± 0.26a	28.47 ± 1.83ac		0.42 ± 0.06a	100
	15	15.70 + 0.78a	28.00 ± 2.86ace		0.41 ± 0.05a	100
	25	15.77 ± 0.45a	27.53 ± 0.83ac		0.39 ± 0.04a	100
1.5	0	15.77 ± 0.55a	25.33 ± 1.83ce		0.32 ± 0.07ac	100
	5	15.90 ± 0.20a	26.23 ± 0.42c		0.34 ± 0.01c	100
	15	15.57 ± 0.55a	26.37 ± 1.58cde		0.36 ± 0.06acd	100
	25	15.60 ± 0.62a	25.00 ± 0.17de		0.31 ± 0.03cd	100
II						
Control			29.80 ± 1.98a	48.53 ± 2.54a	0.63 ± 0.05a	100
	Salinity					
	0		25.33 ± 1.83a	42.50 ± 1.85a	0.57 ± 0.06a	100
	5		26.23 ± 0.42a	43.50 ± 2.98a	0.58 ± 0.11a	100
	15		26.37 ± 1.58a	44.33 ± 2.27a	0.60 ± 0.06a	100
	25		25.00 ± 0.17a	41.07 ± 0.91a	0.53 ± 0.03a	100

Values are means from each group where the means in each column with different letters are significantly different ($P < 0.05$) (separate analysis of variance for I and II)

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Table 2. *ANOVA results for the weight of tilapia in the first phase (I) and in the second phase (II)*

	Effect Cd			Effect of salinity			Interaction		
	Fstat.	**df**	**P value**	**Fstat.**	**df**	**P value**	**Fstat.**	**df**	**P value**
I									
W_{t1}	34.860	2	<0.001*	4.761	3	0.010*	1.869	6	0.128
W_{t2}	34.860	2	<0.001*	4.761	3	0.010*	1.868	6	0.128
II									
W_{t1}				1.268	3	0.333			
W_{t2}				0.629	3	0.611			

Two-way ANOVA for I; One-way ANOVA for II
*Statistically significant effects
df = ° of freedom, with effect degrees of freedom, followed by residual degrees of freedom
F stat. = F statistic

Cd accumulation

Figures 1 and *2* showed Cd concentration in the selected organ tissues of tilapia under different Cd exposure with kinds of salinities. The values and changes of Cd accumulation in different tissues in Cd-free with different salinities were nearly equal to the control, so they were represented by the control in *Figures 1* and *2*. Cd accumulation and variation was influenced by Cd content, salinity and exposure. On the whole, whatever Cd and salinity levels in the water expect for 0.5 mg/L Cd in combination with high salinity, the accumulation patterns of Cd are in the order: liver > gill > scale > edible muscle in the exposure period. Exposure to Cd resulted in a significant dose-dependent and time-dependent accumulation in the four tissues (liver, gill, edible muscle and scale) (*Figs. 1a* and *2a*, $P < 0.05$). The effects of salinity on Cd accumulation in the four tissues were different.

For the liver, Cd accumulation in the liver at both 0.5 mg/L Cd and 1.5 mg/L Cd exposure was significantly higher than that of the control (*Fig. 1a*, $P < 0.05$). Liver Cd accumulation was significantly increased after 10 days. After the end of Cd exposure, Cd accumulation values in the liver varied from 121.03 to 128.83 mg/kg at 1.5 mg/L with different salinities and were approximately 3-4-fold higher than that of 0.5 mg/L Cd exposure. Low salinity and moderate salinity (15‰) induced a slight increase in Cd content when tilapia was exposed to 0.5 mg/L Cd (*Fig. 1a*, $p > 0.05$). On the contrary, high salinity reduced a Cd slight decrease in the fish after 10 and 20 days (*Fig. 1a*, $p > 0.05$), and a significant decrease at the end of the exposure to 0.5 mg/L Cd (*Fig. 1a*, $P < 0.05$). The fish bioaccumulated Cd in the liver exposed to 1.5 mg/L Cd in combination with salinities was different from that of 0.5 mg/L Cd with the salinities. When exposed to 1.5 mg/L Cd, the highest Cd bioaccumulation resulted from low salinity and the lowest was from free salinity (0‰) (*Fig. 2a*, $p < 0.05$).

During Cd in combination with salinities exposure periods, Cd accumulation in gill sharply increased after 10 days (*Figs. 1b* and *2b*, $P < 0.05$). The gill Cd bioaccumulation increased with the increase of both exposure time and Cd content in the solution. At the end of the exposure, Cd accumulation values in gill were 31.57-41.08 mg/kg and 57.52-87.72 mg/kg for the fish exposed to 0.5 mg/L and 1.5 mg/L Cd in combination with different salinities, respectively. On the other hand, the

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accumulation of Cd in gill was influenced by the interaction between Cd and salinity. Low salinity resulted in a much higher increase of Cd in gill than that of 0‰ salinity exposed to 0.5 mg/L Cd during the whole exposure period (*Fig. 1b*, $P < 0.05$). Cd accumulation in the gill exposed to 0.5 mg/L Cd with moderate salinity was not significantly different from that in the 0.5 mg/L Cd with free-salinity (0‰) after 10 and 20 days. However, the value was significantly high at the end of exposure (*Fig. 1b*, $P < 0.05$). High salinity combined with high Cd resulted in a significantly lower Cd accumulation in gill than that in other treatments except after 20 days of exposure (*Fig. 2b*, $P < 0.05$).

Similarly, Cd accumulation in the edible muscle was significantly higher than control (0.089 mg/kg; *Figs. 1c* and *2c*, $P < 0.05$) for all of the treatments during the exposure period, with accumulation values of 1.27-1.45 mg/kg (14-16-fold) and 2.81-4.86 mg/kg (32-55-fold) for the fish exposed to 0.5 and 1.5 mg Cd /L concentrations with different salinities at the end of the exposure, respectively. When exposed to the two Cd concentrations (0.5 mg/L and 1.5 mg/L Cd) for 10 days and 20 days, salinity had no significant influence on edible muscle Cd accumulation, except that high salinity reduced significantly the accumulation (*Figs. 1c* and *2c*, $P < 0.05$). At the end of the exposure to 0.5 mgCd/L, there were no significant differences in Cd accumulation of edible muscle among the salinity treatments except that 15‰ salinity resulted in significantly higher accumulation than those of free salinity (0‰) and 25‰ salinity. However, the accumulation of Cd in the edible muscle was significantly the lowest in 5‰ salinity group and the highest in 25‰ salinity group exposed to 1.5 mg/L Cd for 30 days (*Fig. 2c*, $P < 0.05$).

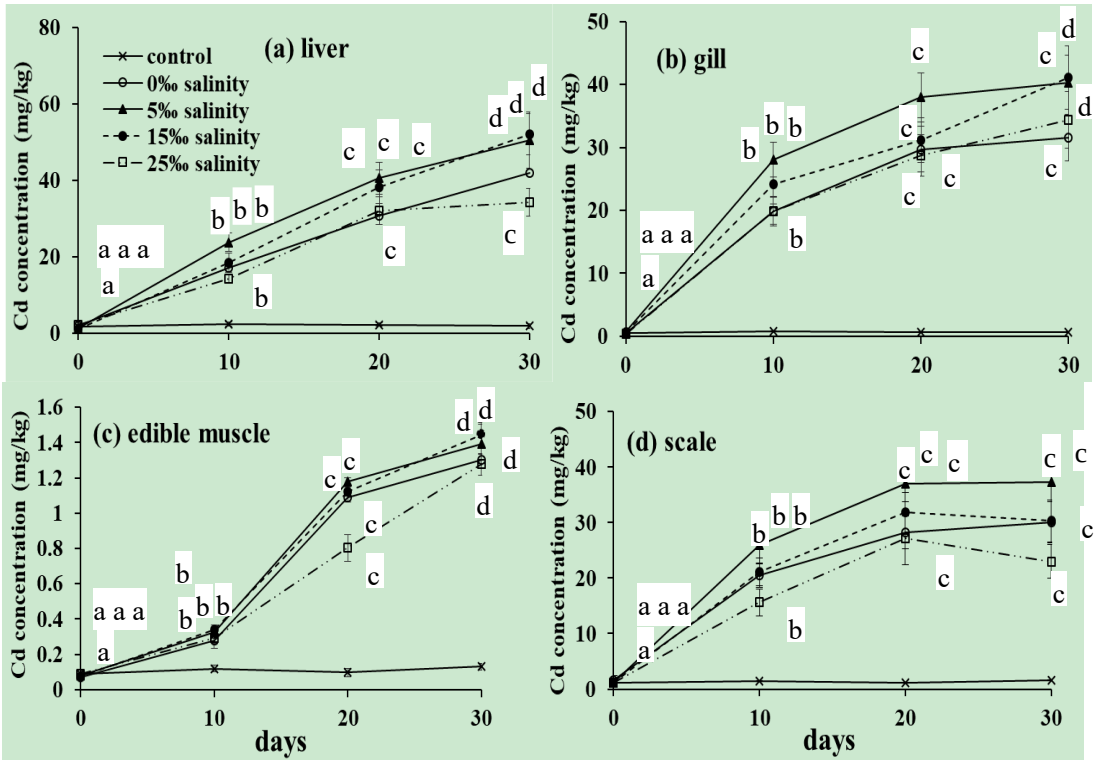

Figure 1. *Cd accumulation over time, in different tissues of tilapia exposed to 0.5 mg/L Cd with different salinities (0, 5‰, 15‰, 25‰) for 30 days. (a) liver, (b) gill, (c) edible muscle, (d) scale; (Mean ± SD; n = 12). Different lower-case letters at tops of bars indicate significant dissimilarities at p < 0.05*

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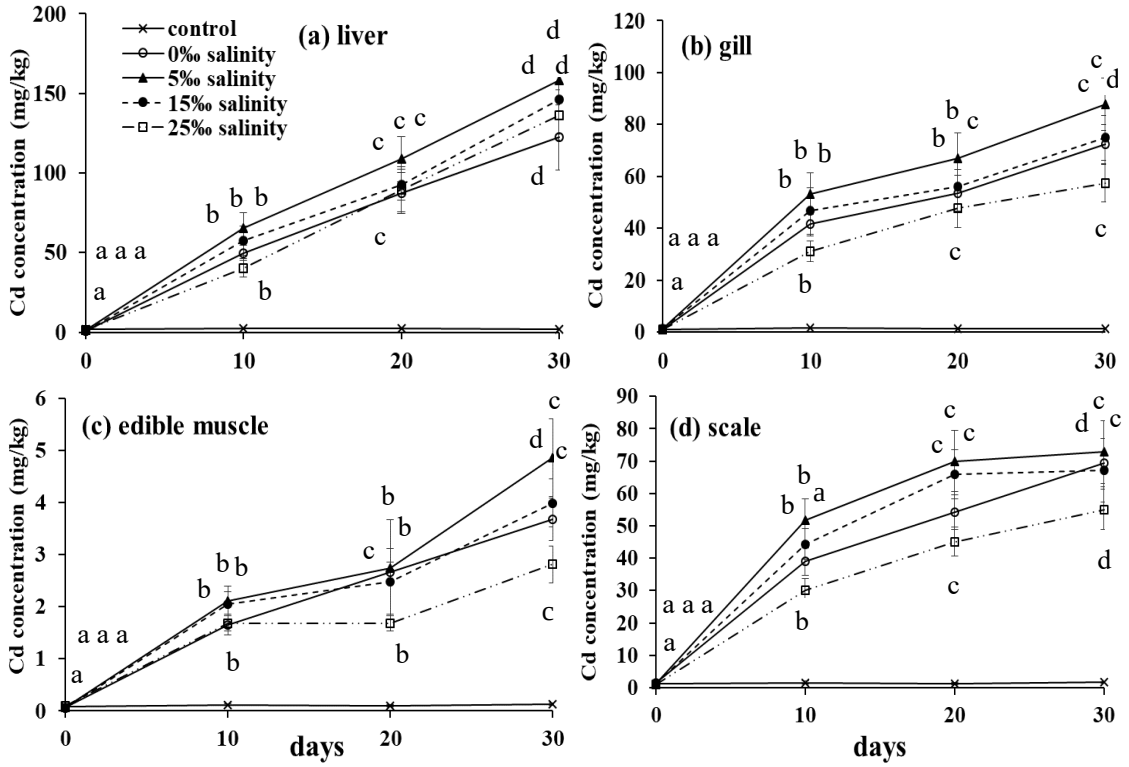

Figure 2. *Cd accumulation over time, in different tissues of tilapia exposed to 1.5 mg/L Cd with different salinities (0, 5‰, 15‰, 25‰) for 30 days. (a) liver, (b) gill, (c) edible muscle, (d) scale. (Mean ± SD; n = 12). Different lower-case letters at tops of bars indicate significant dissimilarities at p < 0.05*

During the exposure period, Cd accumulations in the scale were significantly higher than control (1.6 mg/kg, *Figs. 1d* and *2d*, $P < 0.05$) for all of the treatments, with accumulation values of 23-37.34 mg/kg (14-34-fold) and 55.08-72.82 mg/kg (34-45-fold) for the fish exposed to 0.5 and 1.5 mg Cd/L concentrations with different salinities at the end of the exposure, respectively (*Figs. 1d* and *2d*, $P < 0.05$). Compared to free salinity (‰), Cd accumulation in scale significantly increased exposure to 0.5 mg/L Cd in combination with low salinity during the whole exposure period (*Fig. 1d*, $P < 0.05$). There was no significant difference in scale Cd accumulation between the free salinity group and the moderate salinity group exposed to 0.5 mg/L Cd. However, high salinity significantly reduced the Cd accumulation in scale exposed to 0.5 mg/L Cd for both 10 days and 30 days. And in interaction 0.5 mg/L Cd with the high salinity group, the scale Cd accumulation exposed to 30 days was lower than that for 20 days. After being exposed to 1.5 mg/L Cd for 10 and 20 days, low salinity significantly increased the Cd accumulation in scale compared to free salinity (*Fig. 2d*, $P < 0.05$). At the end of the exposure to 1.5 mg/L Cd, Cd bioaccumulation in scale was no significant difference among different salinities except for high salinity. High salinity significantly decreased the scale Cd accumulation during the whole period of exposure to 1.5 mg/L Cd (*Fig. 2d*, $P < 0.05$).

Cd bioaccumulation factors (BAFs)

The bioaccumulation factors calculated according to *Equation 1* have been presented for liver, gill, edible muscle and scale at the two water toxicity levels with varied

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salinities (0, 5, 15, 25‰) for 30 days in *Figure 4*. The three factors (time, toxicity levels and salinity of exposure) all determined the BAFs of the four tissues.

BAFs in the liver increased with the exposure period with factors ranging from 26.94-105.66 (*Fig. 3a*). There was an inverse relationship between the bioaccumulation factors and the exposure Cd level with all salinities, during both the first 10 days and the second 10 days. However, the relationship was significantly affected by salinities during the last 10 days (*Fig. 3a*, $P < 0.5$). In the gill, the BAFs exposed to 0.5 mg/L Cd were significantly higher than those exposed to 1.5 mg/L Cd (*Fig. 3b*, $P < 0.5$). The BAFs were promoted by the low salinity at the same exposure time except exposed to 0.5 mg/L Cd during the last ten days (*Fig. 3b*). However, the BAFs in the edible muscle increased significantly with the exposure Cd increase during the first 10 days, then decreased significantly with the Cd increase during the second 10 days (*Fig. 3c*, $P < 0.5$). During the last 10 days, the BAFs were highest exposed to 1.5 mg/L Cd with 5‰ salinity (*Fig. 3c*, $P < 0.5$). For scale, Cd level had on significant effect on the BAFs with the same exposure time and salinity. High salinity significantly reduced the BAFs in the scale exposed to the same Cd level and time (*Fig. 3d*, $P < 0.5$).

Cd elimination

The depuration kinetics of Cd in the four tissues for 30 days after exposure to 1.5 mg Cd/L in combination with different salinities were shown in *Figure 4*. The ability of tilapia to eliminate Cd and the influence of salinity on the elimination varied according to tissues (*Fig. 4*).

For the liver, Cd concentrations decreased slightly during the depuration period. And salinity had no significant influence on the quantitative data of elimination during the depuration phase (*Fig. 4a*, $P > 0.05$). At the end of depuration, Cd concentrations were from 122.54 mg/kg to 120.27-113.85 mg/kg with different salinity. In terms of the elimination rates according to *Equation 2*, salinity, especially moderate salinity increased the ER of Cd in the liver. ER was 1.8% for free salinity, 3.3% for 5‰ salinity, 7.1 for 15‰ salinity and 4.6% for 25‰ salinity (*Fig. 4a*, $P > 0.05$).

In the gill, Cd concentration decreased significantly from 72.39 mg/kg at the end of 1.5 mg/L Cd exposure to 32.15-41.2 mg/kg at the end of the four salinities depuration (ER: 55.6-43.1%) (*Fig. 4b*, $P < 0.05$). Compared with free-salinity, salinities especially moderate salinity accelerated and increased significantly Cd elimination. During the first 10 days of depuration, Cd concentrations were 69.57 mg/kg (3.9%) for free salinity, 44.29 mg/kg (19%) for 5‰ salinity, 55.38 mg/kg (23.5%) for 15‰ salinity and 64.39 mg/kg (11.1%) for 25‰ salinity, respectively. After that, both low salinity and moderate salinity continued to promote significantly ($P < 0.05$) the Cd elimination depurated for the second 10 days. There was the lowest Cd concentration (41.56 mg/kg) and the highest ER (55.6%) for moderate salinity at the end of depuration (*Fig. 4b*, $P < 0.05$).

Similarly, Cd concentration in the edible muscle decreased immediately and continuously during the depuration phase (*Fig. 4c*, $P < 0.05$), following the end of 1.5 mg/L Cd exposure. During the first 10 days of depuration, low salinity significantly reduced Cd concentration in the edible muscle compared with the other salinities ($P < 0.05$) (0, 15, 25‰) (*Fig. 4c*, $P < 0.05$). The Cd concentrations were from 3.67 mg/kg to 0.77 mg/kg (79.1%) for free-salinity, to 0.66 mg/kg (81.9%) for 5‰ salinity, to 0.81 mg/kg (78%) for 15‰ salinity, to 0.87 (76.4%) for 25‰ salinity at the end of the experiment. Namely, in the edible muscle, there was no significant difference in edible muscle Cd concentration among the four salinities treatments at the end of depuration.

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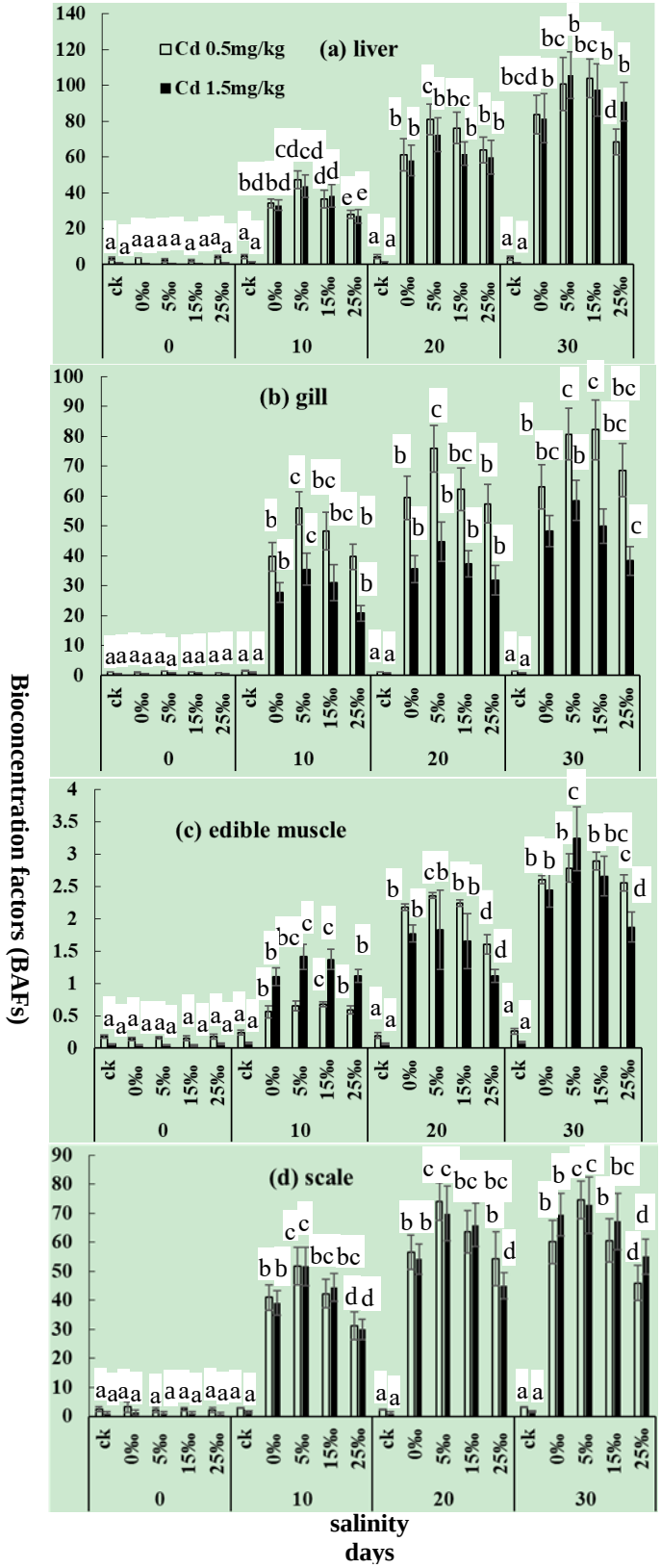

Figure 3. *Bioconcentration factors (BAFs) over time in liver, gill, edible muscle and scale of tilapia, exposed to different Cd (0.5 and 1.5 mg/L) in combination with different salinities (0, 5, 15, 25‰) for 30 days. (Mean ± SD; n = 12). Different lower-case letters at tops of bars indicate significant dissimilarities at p < 0.05*

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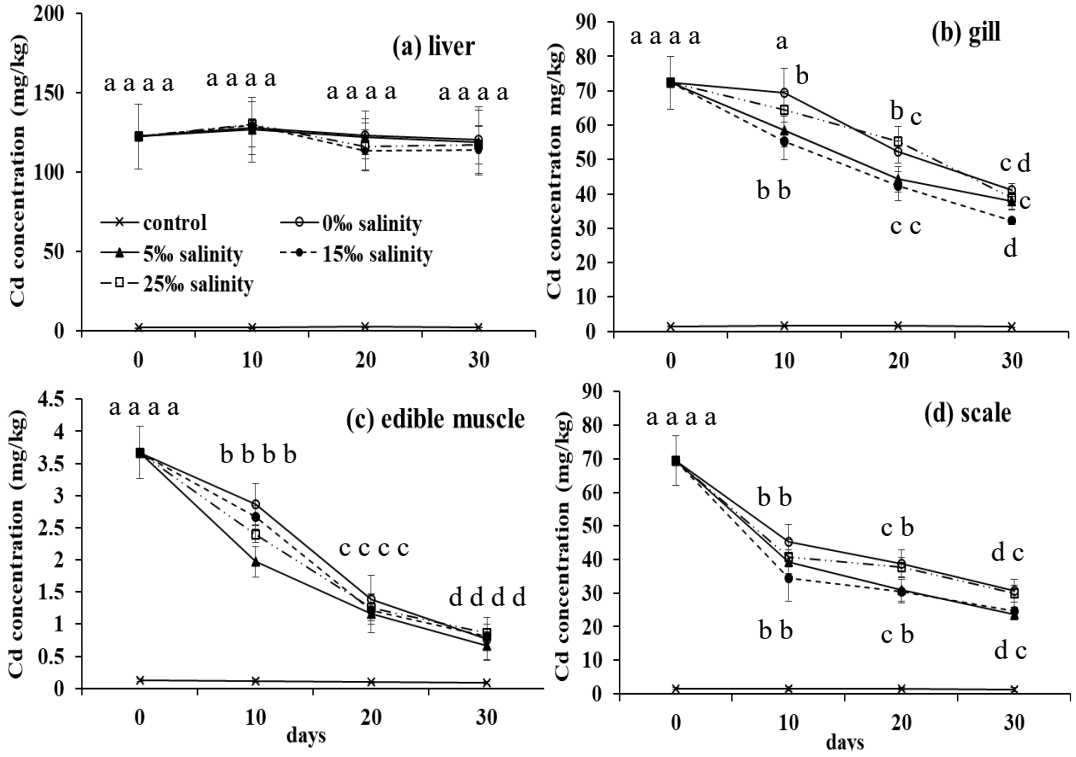

Figure 4. *Depuration of Cd from different tissues of tilapia for 30 days after exposed to 1.5 mg/L Cd in combination with different salinities (0, 5, 15, 25‰) for 30 days. (a) liver, (b) gill, (c) edible muscle, (d) scale. (Mean ± SD; n = 12). Different lower-case letters at tops of bars indicate significant dissimilarities at p < 0.05*

The Cd elimination of the scale showed two steps (*Fig. 4d*). During the first 10 days of depuration, Cd concentration in the scale decreased sharply from 69.51 mg/kg to 34.55-45.35 (34.7-50.3%) varied with salinities ($P < 0.05$). After that, Cd decreased slowly reaching 27.73-32.98 mg/kg (52.6-60.1%) at the end of the depuration. In comparison with free and high salinity, both low and moderate salinity significantly promoted Cd elimination of the scale (*Fig. 4d*, $P < 0.05$).

Discussion

The growth of fish is generally influenced by environmental factors, such as heavy metals, salinity, etc. (Kim et al., 2006; Tian et al., 2020). In the study, low Cd exposure had no significant decrease in tilapia growth rates, and high Cd exposure resulted in a reduction of the growth rates in the first phase. Studies found that significantly reduced growth rates in fish when exposed to different dietary Cd (Nogami et al., 2000; Kim et al., 2006). Foley et al. (2022) reported that young alewife exposed to long-term (3-5 years) heavy metals experienced negative growth impacts in the Tusket River (Nova Scotia, Canada), which is consistent with this study of low Cd exposure, probably due to the short exposure time (30 days). These studies showed that cadmium could inhibit the normal growth of tilapia. Fish are very susceptible to Cd due to Cd-induced disruption of physiological metabolism by generating highly reactive oxygen species (ROS), which leads to lipid peroxidation, and damage to proteins and DNA (Schieber

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and Chandel, 2014). Wu et al. (2019) did find a waterborne Cd-induced abundance of antioxidant enzyme activities and gene mRNA in fish different tissues (gills, liver and ovaries). Additionally, the physiological metabolic energy is consumed to detoxify and maintain normal metabolism in the body when exposed to heavy metals, which would slow down the growth rate (Marr et al., 1996). In the case of tilapia, the decreased growth rate is probably due to damage to the physiological metabolism and increased energy consumption to maintain normal metabolism, which may cause insufficient energy for growth.

Proper salinity usually mitigates acute metal toxicity in fish (Rebolledo et al., 2018). Bielmyer-Fraser et al. (2018) demonstrated that Cd toxicity decreased in both fish species (*Fundulus heteroclitus and Kryptolebias marmoratus*) with the increase of salinity up to 18 ppt. The results of this study are similar to those findings. Metal bioavailability to aquatic organisms in freshwaters varies with water chemistry, due to both ion competition and ligand interactions (Paquin et al., 2002; Niyogi and Wood, 2004). Bielmyer et al. (2018) demonstrated that complexation with chloride (Cl^-) reduced the toxic effects of Cd on *K. marmoratus*. Similar to the sodium ion (Na^+) findings. Na^+ reduced copper toxicity in salinity water in fish, resulting from the competition for Na channels (Blanchard and Grosell, 2006; Bielmyer et at., 2006). Additionally, environmental salinity could modulate growth in tilapia (Seale et al., 2020). It was found that the growth factor was greater in tilapia reared in seawater compared with those in freshwater (Seale et al., 2020). However, in this study, high salinity inhibited the AGT of tilapia, which agreed with previous research (Valentino-Alvarez et al., 2013), showing was more toxic at 33 psu than at 17-25 psu in white shrimp *Litopenaeus vannamei*. The reason probably is that the salinity is too high for these organisms to affect their physiological metabolic mechanism.

Metal accumulation and distribution are rarely uniform in the body tissues of fish, but are dependent upon tissue-specific bioavailability, exposure concentration and periods, etc. (Kim et al., 2006; Cao et al., 2012). Tilapia accumulated Cd in all measured tissues in a significant dose and time-dependent manner and the liver appeared to be the most important organ in Cd sequestration in the present study. Similar Cd accumulation patterns were also shown in other studies in aquatic animals (Berntssen et al., 2011; Yesilbudak et al., 2014; Ferro et al., 2021). Cao et al. (2012) also reported that Cd distribution indicated a significant decrease in the order of accumulation from liver > kidney > gill > muscle in flounder juveniles exposed to Cd concentration of 2, 4, 8 mg/L. For example, Cd accumulation in the liver exposed to 2 mg/L was 19 times higher than that in the control. Moreover, Cd accumulation and toxicity in aquatic animals are affected by salinity (Bielmyer-Fraser et al., 2018; Mozanzadeh et al., 2021). Zanders and Rojas (1996) reported Cd contents in the carapace, muscles, hemolymph, hepatopancreas and gills of crabs exposed to seawater (125, 75 or 25%) were significantly higher than those exposed to fresh water, especially in the diluted (25%) seawater treated crabs. It was indicated appropriate seawater would promote the rates of entry or absorption of Cd by crabs, probably related to Ca^{2+} transport mechanisms (Zanders and Rojas, 1996). In this study, the findings are in good agreement with those researchers. Although the influence of salinity on Cd accumulation was different in the four tissues, in general, the appropriate salinity enhanced the absorption of Cd and high salinity reduced Cd accumulation in varying degrees in the four tissues at the end of exposure (*Figs. 1* and *2*). Exceptionally, high salinity showed no influence on the Cd accumulation exposed 0.5 mg/L Cd (*Fig. 1b*).

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There were two purposes for determination of BAFs: one was to measure the cumulative amount of Cd relative to the aqueous exposure concentration; the second is to find out the difference in metal accumulation ability of different tissues of the fish. The BAFs of tilapia varied with different tissues, similar patterns of BAFs were also shown in rare minnows (Xiong et al., 2020). The liver had the highest BAFs among the observed tissues of the fish (*Fig. 3*). The same results were reported by Kim et al. (2004). Those results demonstrated the liver was easier to accumulate Cd than other tissues. Generally, the liver in fish plays a major role in the induction of metal-binding proteins such as metallothioneins (MTs), which could bind to metals to concentrate metals effectively in fish (Wang et al., 2014). In tilapia, BAFs in the liver, edible muscle and gill were increased more or less with exposure to Cd and periods for 30 days, which could help to monitor heavy metal pollution in an aquatic environment. In the same case, BAFs in scale increased for the first 20 days and then the rule was not obvious with different salinities. Salinity also influenced the BFAs of other tissues of the fish, resulting from the effect of salinity on Cd uptake by tilapia as discussed above.

During the 30 days of the depuration period, the Cd concentration in the liver of tilapia did not vary significantly (*Fig. 3a*). Harrison and Klaverkamp (1989) showed Cd concentration in the liver of rainbow trout and lake whitefish exposed to water remained the same constant during the depuration phase. It seems that the elimination of hepatic Cd is more difficult after the accumulation of Cd. There may be two reasons for this phenomenon. The first is the redistribution of Cd among tissues during the elimination period. As an organ with metal accumulation and detoxification function, the liver can transfer Cd from muscle to liver for excretion. The second is that Cd is closely bound to ligands in the liver, resulting in slower clearance than other tissues (Dutton and Fisher, 2014; Yin et al., 2021). In the present study, the Cd was rapidly eliminated in edible muscle and gill. Ghosh et al. (2020) demonstrated the bioaccumulation of metal in a specific tissue is temporary and may be redistributed and transported to other tissue as the burden of metals in them decreases during depuration (Yin et al., 2021). As the organ is in contact with the surrounding water most, Cd content in the scale is most affected by the solution. Cd in the scale was rapidly eliminated in the first 10 days of depuration and then the rate of depuration slowed down 10 days later.

The salinity also affected the depuration of heavy metals in aquatic animals (Chinnadurai et al., 2022). Chinnadurai et al. (2022) researched that compared with 15-psμ salinity, 25-psμ and 35-psμ salinity could effectively promote Pb, Fe, Zn and Cu emission in edible bivalve molluscs of India. The heart of *Mytilus edulis* was significantly reduced when they were moved from different ecological zones to 15 psμ salinity (Bakhmet et al., 2005). Similarly, it was found that appropriate salinity was conducive to the maintenance of normal physiological functions of the aquatic animals. Below, or above this range, the normal function of the animals might be disturbed, which may lead to metal depuration significantly (Rajesh et al., 2001). In our study, moderate salinity significantly promoted Cd elimination in both the liver and the gill. Besides, not only moderate salinity but also low salinity significantly increased Cd elimination of the scale. In contrast, salinity had no significant effect on Cd emissions from edible muscles (*Fig. 4*). Those results suggested that the effect of salinity on Cd elimination was related to tissues of tilapia.

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Conclusion

After 30 days of Cd with varied salinities exposure, AGT of the fish was noticeably decreased at the interaction of high Cd and high salinity concentrations. The biggest AGT in the interaction of the Cd-free and low salinity group and the smallest in the interaction of 1.5 mg/L Cd and high salinity group. In the depuration stage, the AGT showed a similar result. High salinity inhibited the fish growth. Accumulation of Cd in the fish was dose-dependent and time-dependent as follows: liver > gill > scale > edible muscle. Salinity showed different influence of the Cd accumulation in different tissues, and the influence was complex with exposure time. BAFs and ER indicated tissue-specific and exposure time-dependent and were by Cd and salinity. Among the four tissues, the live and the greatest BAFs, and the smallest ER, and the edible muscle showed the highest ER, ranging from 76.7% to 81.9% with varied salinities. Moderate salinity promoted the depuration of Cd and enhanced ER in the tissues except for edible muscle.

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