Original Research Article

Ecophysiological Effects of Waterborne Zinc on the Grass Carp (*Ctenopharyngodon idellus*)

ABSTRACT

The juvenile grass carp (*Ctenopharyngodon idellus*) (19.68±0.17 g) were exposed to the solutions of zinc sulfate heptahydrate ($ZnSO_4.7H_2O$), to observe the toxic effects of waterborne Zn on this fish. The results showed that the median lethal concentration (LC_{50}) over 96 h of waterborne Zn^{2+} was 5.00 mg/L. After 8 weeks of chronic exposure, the final weight and the specific weight growth rate of grass carp decreased with the increasing Zn^{2+} concentration, and the differences were significant among the three groups ($P < 0.05$). The content of ash in the high exposure group was significantly higher than those in the other two groups ($P < 0.05$). The dry mass in the two exposure groups and the energy density in the low concentration group were significantly higher than those in the control group ($P < 0.05$). The contents of Zn in the hepatopancreas, gill, intestine, muscle, and whole body in the high concentration group were significantly higher than those in the control group ($P < 0.05$). The contents of Zn in hepatopancreas were significantly higher than those in other organs ($P < 0.05$). There was no significant difference for the contents of Zn in the intestine and gill in the two Zn exposure groups, but those were significantly higher than those in other organs except hepatopancreas ($P < 0.05$). The content of Zn in muscle was significantly lower than that in the other organs. It suggests that the pattern of energy allocation of the grass carp is changed by the Zn exposure. Fat was preferentially used to provide extra energy for the detoxification under the Zn exposure, and the rates of the protein and energy deposited in the body were reduced. Therefore, the growth of the fish was depressed. The grass carp mainly takes up Zn through the gill and distributes Zn to other tissues via blood circulation.

Keywords: waterborne zinc; zinc bioaccumulation of tissues; Ctenopharyngodon idellus

1. INTRODUCTION

Heavy metals are common pollutants in aquatic environments, and they mainly enter natural water through human activities, such as agriculture, industry, and domestic wastewater. With an increase in human activities, exogenous substances, including heavy metals, have been entering the aquatic environment, causing serious damage to the aquatic ecosystem. Heavy metal ions in water can directly or indirectly produce toxic effects by accumulating in aquatic organisms [1-2]. When humans consume algae, fish, and other aquatic organisms contaminated by heavy metals, heavy metals are absorbed by human bodies; they eventually accumulate, resulting in potential threats to human health [3].

Zinc is one of the common heavy metal pollutants and one of the biological essential elements. In recent years, with the rapid development of industry and agriculture, water pollution caused by release of Zn has gradually intensified. In the aquaculture industry, excessive Zn added in the artificial feed is not completely absorbed and released into the water body, polluting the aquatic environment and eventually causing toxic effects in fish [4]. In addition, studies have shown that zinc content in some water bodies of the upper Reachs of the Yangtze River reaches 4,390–15,150 μg/L, exceeding the concentration harmful to fish safety [5].

The growth performance, as the most intuitive indicator of fish health status, can directly reflect the impact of heavy metal exposure on fish health status [6]. Zn exposure can change the appetite and feeding behavior of fish and cause changes in the energy intake of fish [7]. Zn exposure can also change the basic metabolism of fish [8], making them consume extra

energy to cope with the physiological stress caused by the toxicity of Zn exposure, thus changing the proportion of energy used for growth [9]. Therefore, it can be considered that the growth performance and metabolic level of fish can be an important indicator of the effects of Zn exposure on fish.
Chemical compositions and energy density of fish can reflect the adaptability of fish to environmental factors [10]. Protein, fat, and ash are the main biochemical components of fish. Under certain conditions, they maintain a relatively stable proportion [10]. When the living environment of fish changes, there is a shift in its metabolism and energy distribution mode, resulting in changes in the biochemical composition of fish [9,11].
Heavy metals in the aquatic environment mainly enter fish through water bodies and food exposure pathways [1-2]. While zinc is a necessary micronutrient to maintain the growth of fish [12], if they are raised in water containing high levels of zinc, and the absorption of zinc in the tissues and organs is higher than its removal, it will accumulate within the fish [13-14]. This accumulation will harm the fish by interfering with the body's acid-base balance and ion adjustment. The gill function is impaired, resulting in the lack of oxygen supply to organs and tissue [15] and other toxicological stress effects. The accumulation rate of Zn in fish is mainly affected by abiotic factors, such as temperature, hardness, pH, salinity, exposure route, exposure time, exposure concentration, and biological factors, such as species, age, and life stage [13,16-18]. In addition, due to the differences in structure and function of various tissues and organs of fish, their affinity for heavy metals, operation and metabolism, and removal capacity are different, and differences in the cumulative distribution of tissues occur after exposure to heavy metals [13,18-19].
In this study, $ZnSO_4.7H_2O$ was used as a Zn ion source and grass carp were used as experimental subjects to observe the acute poisoning symptoms and semi-lethal concentration of grass carp within 96 h, and to measure the effects of exposure to different Zn concentrations in water on the growth index, resting metabolism, body biochemical components and Zn accumulation and distribution in each tissue of grass carp. These results provide basic data for discussing the ecotoxicological mechanism of zinc exposure in grass carp.

2. MATERIAL AND METHODS

2.1 Origin and domestication of experimental fish

The experimental fish were from the same batch of juvenile grass carps purchased from local fisheries. Before the experiment, 600 juvenile grass carps with body weights of about 15 g and no surface injury were selected for domestication under artificial breeding conditions in the laboratory. The domesticated fish were randomly assigned to 6 indoor recirculating aquaculture systems, with 10 breeding tanks (length × width × height = 42 cm × 29 cm × 25 cm) in each circulating system, and 10 fish in each tank. The acculturation temperature was 27.5 ±0.5 °C, water hardness was 25–30 mg $CaCO_3$/L, dissolved oxygen is greater than 6 mg/L, pH was 7.10 ±0.05, photoperiod was L:D = 12 h:12 h, the light was turned on at 08:00 and turned off at 20:00 (instantaneous switch) every day. After turning off the lights, feed the bait with commercial feed, and clean up the residual bait at 21:00. The experimental fish were domesticated for two weeks, water was changed every 2 d, and water volume was about 1/2 of the total volume.

2.2 Acute experiment

A total of 180 domesticated fish with similar body weight (19.68±0.17 g) were randomly divided into 6 groups with 30 fish in each group. Three breeding tanks were used in each group, and 30 L soft water was injected into each breeding tank. Temperature, hardness, pH, photoperiod, and dissolved oxygen were the same as those in domestication. Using $ZnSO_4.7H_2O$ as Zn ion source, the maximum survival and minimum total lethal concentrations of grass carp exposed to Zn in water were determined to be 2.0 mg/L and 8.0 mg/L by preliminary experimental results. According to the results of the pre-experiment, the Zn concentrations in the six treatment groups were 0 (control), 2.0, 3.5, 5.0, 6.5, and 8.0 mg/L, respectively. Each concentration group had three replicates, and each replicate consisted of one breeding tank with 10 fish. The experiment lasted for 96 h, during which no feeding was performed. The water was changed every 24 h, and the actual concentration of Zn in the water sample to be tested was taken before the water was changed. The zinc concentrations in the water bodies of each group were, as detected by flame atomic absorption spectrophotometry, were 0 (not detected), 2.03-0.07, 3.56-0.09, 5.03-0.06, 6.57-0.09, and 8.10-0.05 mg/L, respectively (Mean + S.E., n = 4). At the beginning of the experiment, the fish were observed every hour for 8 h, and then every 4 h. The symptoms of poisoning and death were recorded.

2.3 Chronic experiment

After being raised, fish with similar body weights (19.72 ±0.10 g) were randomly divided into three groups of 70 fish (see Table 1 for the initial body weight). Each group used a circulating aquaculture system (the specifications were the same as those of the domestic recirculating aquaculture system). Each system was composed of 10 replicates (10 aquaculture tanks), and 7 experimental fish were raised in each box. According to the acute experiment, the 96 h LC_{50} concentration

for Zn in the water body was determined to be 5.00 mg/L. The fish were exposed to low and high waterborne Zn concentrations of 1/60 and 1/30 of the 96 h LC_{50}, respectively. Another blank control, namely three experimental groups, was set. Zn concentrations in water were 0 (control), 83.41, and 166.82 μg/L, respectively. The experimental water dissolved oxygen, water temperature, water hardness, pH, photoperiod, water changing frequency and feeding time were the same as those in the acclimation period. Water samples were taken before each water change, and the concentration of Zn in the experimental water was determined by flame atomic absorption spectrophotometry. The actual concentration of Zn in each group during the culture period was 0 (not detected), 84.68 ±0.97 μg/L, and 169.39 ±1.97 μg/L, respectively (Mean ±S.E., n = 28).

After eight weeks of Zn exposure, the experimental fish were fasted for 24 h and then anesthetized with MS222. Thirty fish were randomly selected from each group; routine biological parameters of fish bodies were determined; and the liver, gill, kidney, intestine, muscle, and brain tissues were dissected and frozen with liquid nitrogen; and stored at −80 ℃ for testing. Eight fish from each group were randomly selected for measuring the resting metabolism of the fish, then dried to constant weight, and ground powder was used to determine the biochemical components and Zn content of the whole fish.

2.4 Calculation formulas of indicators related to the growth performance of grass carp

Specific weight gain rate (SGR_W, %d-1) = $100 \times (\ln W_t - W_0)/t$

Condition factor (CF, %) = $(W/L^3) \times 100$

Hepatosomatic index (HSI, %)=$100W_H/W_t$

Kidney index (KSI, %) = $100 \times W_K/W_t$

W_t and W_0 are the final and initial body weight (g), respectively. W_H is the hepatosomatic weight (g), W_K is the weight of the kidney, L is the length (cm) of experimental fish, and T is the experimental time (d).

2.5 Determination of resting metabolism

The oxygen consumption rate of experimental fish was measured by the fish flowing water respirator designed by the Institute of Aquatic Biology and Water Environment of Southwest University [20-22] and the water temperature was controlled at 27.5±0.5 °C. After eight weeks of Zn exposure in water, fish were fasted for 24 h and weighed. Eight fish were randomly selected from each treatment group and placed into the respirator, with one fish placed in each breathing chamber, and another breathing chamber without fish was used as a blank control. After acclimation for 24 h in the respiratory chamber, the oxygen consumption of resting metabolic respiration was measured every 2 h, and the average value of four times was used as the oxygen consumption of resting metabolic respiration.

The formula $R = (\Delta O^2 \times V)/m$ was used to calculate the resting metabolic rate of experimental fish.

Where, R represents the resting metabolic rate (mg $O^2 \cdot h^{-1} kg^{-1}$) of the experimental fish, ΔO^2 represents the difference of dissolved oxygen between the respiratory chamber containing the experimental fish and the blank respiratory chamber (mg O^2/h), V represents the flow rate of water in the respiratory chamber (L/h), and M represents the body weight of the experimental fish (kg).

2.6 Determination of fish components and estimation of energy density

The fish samples were dried at 70 °C to a constant weight to determine the dry matter content, and they were ground into a fine powder with a mortar to determine their composition. Protein content was determined by the Kjeldahl method (N × 6.25), fat content was determined by Soxhlet extraction method, ash content was determined by Muffle furnace after burning at 550 °C, and energy density was estimated by formula E(kJ/g) = protein content × 23.6 + fat content × 39.5 [23].

2.7 Determination of Zn content in the experimental water body and fish tissues

Fifty milliliters of experimental water samples were taken each time, and 0.25 mL of concentrated nitric acid was added to the samples after acidification and filtration. The Zn contents of the liver, kidney, gills, muscles, and brain of 8 fish were randomly selected from 30 fish that had been anatomically sampled in each group. The contents of Zn in whole fish were determined by fish meal. The Chinese national standard method was used for sample digestion and determination [24]. Samples weighing 0.1–1.0 g samples were placed in a 50 mL conical flask, 8 mL perchloric acid-nitric acid (1:4 v/v) was added to each sample, the acid was removed with an electric heating plate at 150 °C after digestion overnight, and finaly 0.05% nitric acid was added to a constant volume of 10 mL for testing. After treatment, the water and fish samples were determined by flame atomic absorption spectrophotometry (TAS-990, Beijing General Analysis Instrument Co., LTD.).

2.8 Data statistical analysis methods

Excel 2010 and SPSS 19.0 software were used for data collation and statistical analysis. The comparison between groups was performed by one-way ANOVA and LSD test. The significance level was set at $P < 0.05$, and the data were expressed as mean ± standard error.

3. RESULTS AND DISCUSSION

3.1 Toxic effects of acute exposure to waterborne Zn on grass carp

Following Zn treatment at various concentrations, all experimental fish showed the same progressive symptoms of toxicity. There was no significant difference in the behavior between the two groups during the whole experiment, while the other five Zn exposure groups gradually showed abnormal behavior with longer exposure times: some swam suddenly and stopped, some flipped up and down, sometimes swam around in the water, and collided with the walls of the water tank or other experimental fish. With longer exposure periods, the swimming ability of the exposed groups weakened. Some fish turned sideways or laid at the bottom of the tank, and their breathing rate first accelerated and then slowed down. Death in the lowest concentration group began after 60 h. In the highest concentration group, the first death occurred after 9.5 h, more than half had died at 20 h and all had died at 96 h. Most of the dead fish in the experiment opened their gill covers and breathed. Examination revealed that most of the dead fish exhibited hyperemia in their gill filaments, evidenced by their light red color. Compared with the control group, the fish exposed to Zn had increased mucus on their skin, and red spots appeared mostly in the decolorization pores and where the pectoral fin attaches to the body.

With increased waterborne Zn concentrations, the mortality of the experimental fish increased in a dose-dependent manner. With the exposure concentration of Zn as the independent variable and the percentage of fish death as the dependent variable, the regression equation was calculated respectively (Fig. 1).

$y = 13.845 x - 19.289$ ($N = 15$, $R^2 = 0.952$)

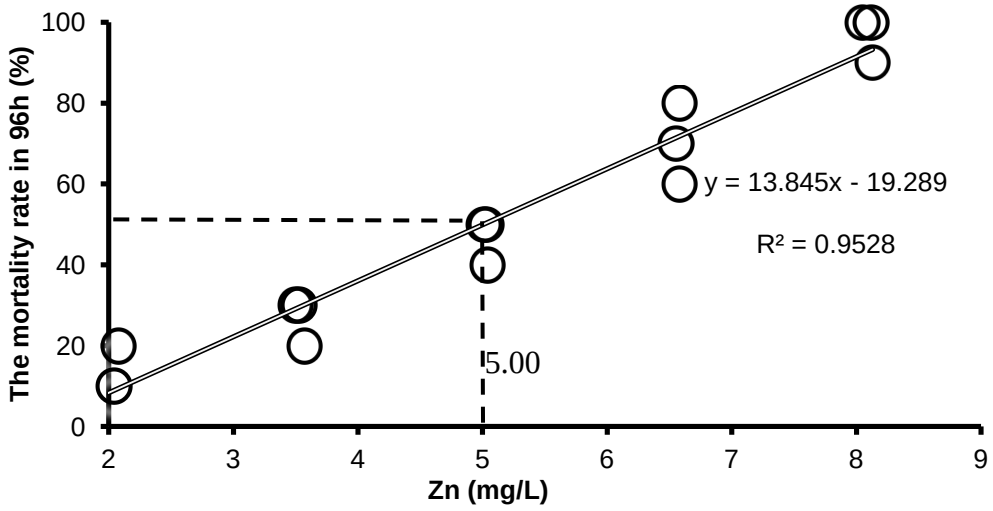

Fig. 1. The mortality rate of the grass carp in the waterborne Zn exposure for 96 h

Using the linear interpolation method, the LC_{50} of water Zn exposure to grass carp at 96 h was 5.00 mg/L (Fig. 1). Where x is the measured concentration of Zn in the experimental water (mg/L), and y is the mortality rate of the experimental fish (%).

3.2 Effects of chronic exposure to waterborne Zn on the growth performance of grass carp

After 8 weeks of exposure experiment, the final weight and specific weight growth rate of grass carp decreased with the increase of Zn exposure concentration (Table 1), and the differences of final weight and specific weight growth rate among the three treatment groups reached significant levels ($P < 0.05$). There was no significant difference in the condition factor among the three groups. The hepatosomatic index and kidney index of grass carp had no significant difference among the three treatment groups. With the increase of exposure concentration, the resting metabolic rate of grass carp tended to increase. The resting metabolic rate of the control group was slightly lower than that of the exposed group, while there was no significant difference in the resting metabolic rate among all treatment groups (Table 1).

Table 1. Effect of waterborne Zn exposure on growth performance in the

grass carp

Parameters of growth	Zn concentration(μg/L)		
	0	83.41	166.82
Initial weight (g) (n=70)	21.74±0.27	21.18±0.19	21.21±0.38
Final weight (g) (n=60)	61.67±1.18[a]	51.54±2.31[b]	44.61±2.35[c]
Specific growth rate (%/d) (n=60)	1.82±0.05[a]	1.53±0.07[b]	1.23±0.09[c]
Condition factor (%) (n=60)	1.74±0.01	1.75±0.02	1.71±0.02
Hepatosomatic indices (%) (n=30)	1.24±0.06	1.35±0.08	1.45±0.05
Kidney indices (%) (n=30)	0.31±0.02	0.30±0.02	0.29±0.02
Resting metabolic rate (mg O_2 $kg^{-1}.h^{-1}$) (n=8)	181.07±6.76	189.42±9.11	197.20±10.86

Note: The data are presented as mean±S.E.(n=8), a,b,c: different superscripts in each line indicate significant differences among treatments(P<0.05).

Although Zn is an essential metal element for animals, when the environmental concentration reaches a certain level, it will have adverse effects on the growth of animals [2,14,16]. The surface water quality standards of China stipulate that the content of Zn in class I water shall not exceed 50 μg/L [25]. In this study, the concentrations of Zn in the exposed group were 83.41 μg/L and 166.82 μg/L, respectively, which were 1.5–3 times higher than the Chinese standard for surface Class I water, suggesting that it should have a large toxic effect.

The growth state of fish can be used as an important indicator of the toxic stress of heavy metals [26]. Shukla and Pandey [27] found that the growth of *Channa punctatus* was significantly inhibited when zinc sulfate in water was 12 mg/L. Abdel-Tawwab et al. [11] found that tilapia (*Oreochromis niloticus*) significantly reduced the growth rate of specific body weight after 6 weeks of exposure to Zn (3.5 or 7.0 mg/L) in water. Liu et al. [28] found that the growth and survival of *Penaeus japonicus* larvae were inhibited when the concentration of Zn in seawater exceeded 40 μg/L. In this study, it was found that water Zn exposure inhibited the growth of experimental fish, and the final body weight and the specific growth rate of the exposed group were significantly lower than those of the control group ($P < 0.05$), indicating that the toxic effect of water Zn on grass carp was obvious at this concentration. Studies have shown that pollution stress can cause fish to spend extra energy for detoxification and anti-oxidation, and their metabolic rate increases, leading to changes in the energy distribution pattern and resulting in reduced energy for fish growth [29-30]. In this study, the resting metabolic rate of experimental fish increased with the increase of Zn exposure concentration, which showed an opposite trend to the change of growth rate, indicating that grass carp need to spend more energy to cope with Zn stress when the essential element is too much, which reduces the ratio of energy reserve and accumulation of the body, resulting in a decrease in the growth rate.

3.3 Effects of chronic exposure to Zn in water on body composition and energy density of the grass carp

With the increase of Zn concentration in water, the protein content and ash content of grass carp showed an increasing trend, while the fat content, dry matter content, and energy density showed an increasing trend firstly and then decreased (Table 2). Ash content of the fish body in the high Zn exposure group was significantly higher than that in the control group and low Zn exposure group ($P < 0.05$), and there was no significant difference between the control group and the low Zn exposure group. The dry matter content of fish in both exposure groups was significantly higher than that in the control group ($P < 0.05$), but there was no significant difference between the two exposure groups. The energy density of fish in the low concentration Zn exposure group was significantly higher than that in the control group ($P < 0.05$), but the energy density of fish in the high concentration Zn exposure group was not significantly different from that of the other two treatment groups. There were no significant differences in protein content and fat content among all groups (Table 2).

Table 2. Effects of waterborne Zn exposure on the body composition and energy density of grass fish (% wet mass)

Items	Zn concentration(μg/L)		
	0	83.41	166.82
Protein	13.40±0.31	13.84±0.25	14.05±0.33
Lipid	7.62±0.42	9.12±0.31	8.21±0.56

Ash	2.78±0.05^{b}	2.94±0.07^{b}	3.24±0.08^{a}
Dry mass	26.51±0.54^{b}	29.39±0.37^{a}	28.77±0.73^{a}
(KJ/g) Whole fish	6.16±0.16^{b}	7.17±0.13^{a}	6.76±0.31^{ab}

Note: The data are presented as mean±S.E.(n=8), a,b,c: different superscripts in each line indicate significant differences among treatments(P<0.05).

Malik et al. [31] and Zheng et al. [32] showed that exposure to zinc in water induced liver lipid deposition or increased lipoprotein lipase activity to increase fat content in fish. In this study, the fat content of grass carp in the exposed group was higher than that in the control group and the exposure was the highest at low concentrations. Abdel-Tawwab [11] found that Zn exposure in water increased the contents of water and ash, while reduced the contents of protein and fat in carp. Therefore, we believe that grass carp prefer fat as an energy source for detoxification under the exposure of Zn in water. Therefore, the energy density of grass carp decreased with the decrease of fat content, and its protein content was not significantly affected.

3.4 Effects of Zn exposure in water on Zn accumulation and tissue distribution in grass fish

With the increase of the concentration of Zn exposure in water, the accumulation of Zn in the liver, gills, kidneys, intestines, brain, muscles, and other organs and tissues of the experimental grass carp showed an increasing trend (Table 3). There were significant differences in Zn accumulation in the gills, intestines, and the whole body among all Zn concentration treatment groups ($P < 0.05$). Zn accumulation in the liver and muscle of the two exposure groups was significantly higher than that of the control group ($P < 0.05$), but there was no significant difference between the two exposure groups. The kidney Zn accumulation in the high Zn exposure group was significantly higher than that in the control group ($P < 0.05$), but the kidney Zn accumulation in the low Zn exposure group was not significantly different from that in the high Zn exposure group and the control group. There was no significant difference in brain Zn accumulation among all groups (Table 3).

The accumulation and distribution of Zn in fish tissues of the control group, low concentration exposure group, and high concentration exposure group were as follows: liver > kidney > gill > intestine > brain > whole fish > muscle, liver > kidney > gill > intestine > whole fish > brain > muscle and liver > intestine > gill > kidney > whole fish > brain > muscle. The Zn content in the liver of the two exposed groups was significantly higher than that in other tissues ($P < 0.05$), and the Zn accumulation in gills and intestines had no significant difference but was significantly higher than that in the whole fish, brain, and muscle of the two exposed groups (Table 3). Zn content in muscle of all treatment groups was significantly lower than that in other tissues ($P < 0.05$) (Table 3).

Table 3. Zn contents in different tissues of the grass fish after waterborne Zn exposure (μg/g wet-weight)

Organ	Zn concentration(μg/L)		
	0	83.41	166.82
Gill	23.69±0.99^{bz}	32.85±1.71^{cy}	57.97±4.01^{bx}
Hepatosomatic	42.82±3.60^{ay}	73.24±3.65^{ax}	83.45±4.23^{ax}
Kidney	39.85±3.10^{ay}	52.89±4.91^{bxy}	55.11±4.78^{bx}
Intestine	18.81±0.92^{cz}	31.52±2.33^{cdy}	67.06±2.52^{bx}
Muscle	3.90±0.16^{ey}	6.87±0.65^{fx}	7.34±0.25^{ex}
Brain	15.13±1.12^{d}	15.21±0.94^{e}	15.67±1.14^{d}
Whole fish	14.53±0.80^{dz}	25.32±1.12^{dy}	39.42±1.41^{cx}

Note: The data are presented as mean±S.E.(n=8). a,b,c,d,e: Different superscripts in the each row indicate significant differences among the various tissues in same treatments(P <0.05). x, y, z: Different superscripts in each row for the same tissue indicate significant differences among treatments(P<0.05).

Studies have shown that heavy metal content in fish tissues and organs is positively correlated with the metal concentration in the water environment [33-34]. In this study, Zn content in the liver, gills, kidneys, intestinal organs, and tissues of experimental fish increased with the increase of exposure concentration, showing a concentration-dose effect (Table 3).

In this study, the order of Zn content in all tissues of grass carps in the exposed group was liver > kidney > gill > intestine > whole fish > brain > muscle (low exposure group) and liver > intestine > gill > kidney > whole fish > brain > muscle (high exposure group). There was no significant difference in Zn accumulation in gills and intestines, which was significantly higher than that in the whole fish, brain, and muscle, and significantly lower than that in the liver ($P < 0.05$) (Table 3). Some studies have pointed out that the liver is important detoxification and excretory organ [35]. Zhou et al. [36] measured the MTs content of crucian carp under Zn^{2+} stress in the order of liver > kidney > gill filaments > muscle. We believe that when the concentration of Zn exposure in water exceeds the tolerance of the fish, the fish detoxifies through the combination of MTs and Zn. When the conjugate is transported to the liver and cannot be effectively excreted, it will accumulate in the liver to increase the content of Zn. Gills are the central part of ion regulation in the fish body and regulate ion balance inside and outside gills [12,37], in the exposure experiment, it can directly absorb dissolved metal ions in the exposed water and then enter the body through blood circulation [2]. The surface contains metal affinity proteins, resulting in high metal content on the gills of the fish body [18]. The accumulation of Zn in gills of grass carp exposed group increased, which was also caused by the fact that the excretion rate of Zn by gills could not offset the absorption rate of Zn, increasing the load of gills. This study found that Zn content in the intestine of the high concentration exposure group was only second to liver tissue but higher than all other tissues. Han et al. [38] pointed out that Zn in the intestine of fish exposed to Zn in water should be absorbed through gills and then circulated through the blood and accumulated in the intestine. As intestinal tissues have a high-affinity mechanism for Zn absorption, a higher cumulative content is formed. The results of this study support his view.

Previous studies have found that the muscle of fish has the lowest metal content [17,39-41]. Xu et al. [41] investigated the contents of eight metals in the skin, intestines, and muscles of *Misgurnus anguillicaudatus* and *Monopterus Albus* in the middle reaches of the Yangtze River and found that the contents of various heavy metals in muscles were the lowest. Yilmaz [42] found that the five heavy metals in the muscles of the European eel (*Anguilla Anguilla*) and tilapia were significantly lower than those in the liver and gills and thus proposed that fish muscle was an “inactive tissue” of metal accumulation. It was found in this study that Zn concentration in the muscle of fish in each treatment group was significantly lower than that in other tissues. Therefore, muscle experiences the lowest accumulation of heavy metals, and the influence of environmental heavy metal concentration on its content is uncertain. This suggests that if we only rely on the muscle content of the fish body to judge the pollution degree of water bodies in field investigation, there will be errors and thus be deemed unsuitable as environmental monitoring indicators.

4. CONCLUSION

The results showed that the pattern of energy allocation of the grass carp was changed on the Zn exposure. Fat was preferentially used to provide extra energy for the detoxification on the Zn exposure, and the rates of the protein and energy deposited in the body were reduced. Therefore, the growth of the fish was depressed. The grass carp mainly takes up Zn through the gill, which is then distributed to other tissues by blood circulation. Zn did not bioaccumulate in muscle tissue.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather, it was funded by the personal efforts of the authors.

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REFERENCES

1. Clearwater SJ, Farag AM, Meyer, JS. Bioavailability and toxicity of diet borne copper and zinc to fish. Comparative Biochemistry & Physiology Part C Toxicology & Pharmacology.2002; 132(3): 269-313.
2. Hogstrand C, VerbostPM, Bonga SW, Wood CM. Mechanisms of zinc uptake in gills of freshwater rainbow trout: interplay with calcium transport. American Journal of Physiology. 1996; 270(5): 1141-1147.
3. Viarengo A. Heavy metals in marine invertebrates: mechanisms of regulation and toxicity at the cellular level. CRC Crit. Rev. in Aquat. Sci. 1989;1: 295-317.
4. Wang YY. The contaminations of fish feed stuffs. Acta Hydrobiologica Sinica. 1991;15(2): 174-179. China.
5. Guo YY, Hua JH, Yang L, Zhou BS. Analysis of heavy metals in the surface water from the three gorges reservoir and its toxic effects on the embryonic development of the zebrafish. Acta Hydrobiologica Sinica ,2015; 39(5): 885-892. China.
6. Huang W, Liang C, Shan X, Xiao Z, Wang Q, Dou S. Toxic effects of zinc on the development, growth, and survival of red sea bream *Pagrus major* embryos and larvae. Archives of Environmental Contamination & Toxicology. 2010; 58(1): 140-150.
7. Gro KJ, Carvalho RN, Chipman JK, Denslow ND, Halder M, Murph CA et al. Development and application of the adverse outcome pathway framework for understanding and predicting chronic toxicity: ii. a focus on growth impairment in fish. Chemosphere. 2015; 120: 778-792.
8. Grobler E, Preez H, Vuren J. Toxic effects of zinc and iron on the routine oxygen consumption of *Tilapia sparrmanii* (Cichlidae). Comparative Biochemistry and Physiology Part C Comparative Pharmacology.1989; 94(1): 207-214.
9. Abdel-Tawwab M, Mousaad MN, Sharafeldin K M, Ismaiel N E. Changes in growth and biochemical status of common carp, *Cyprinus carpio* L. exposed to water-born zinc toxicity for different periods. International Aquatic Research. 20135(1): 11.
10. Jonsson N, Jonsson B. Body composition and energy allocation in life-history stages of brown trout. Journal of Fish Biology. 1998; 53(6): 1306-1316.
11. Abdel-Tawwab M, El-Sayed GO, Shady SH. Effects of dietary protein levels and environmental zinc exposure on the growth, feed utilization, and biochemical variables of Nile tilapia, *Oreochromis niloticus* (L.). Toxicological & Environmental Chemistry Reviews. 2012; 94(7): 1368-1382.
12. Watanabe T, Kiron V, Satoh S. Trace minerals in fish nutrition. Aquaculture.1997; 151(1): 185-207.
13. Javed M. Effects of zinc and lead toxicity on the growth and their bioaccumulation in fish. Pakistan Veterinary Journal. 2012a;32(3), 357-362.
14. Everall N C, Macfarlane NAA, Sedgwick RW. The interactions of water hardness and pH with the acute toxicity of zinc to the brown trout, *Salmo trutta* l. Journal of Fish Biology. 2010;35(1): 27-36.
15. Hogstrand C, Wilson RW, Polgar D, Wood C M. Effects of zinc on the kinetics of branchial calcium uptake in freshwater rainbow trout during adaptation to waterborne zinc. Journal of Experimental Biology. 1994; 186: 55-73.
16. Murugan SS, Karuppasamy R, Poongodi K, Puvaneswari S. Bioaccumulation pattern of zinc in freshwater fish *Channa punctatus* (bloch.) after chronic exposure. Turkish Journal of Fisheries & Aquatic Sciences. 2008;8(1): 55-59.
17. Adhikari S, Ghosh L, Giri BS, Ayyappan S. Distributions of metals in the food web of fishponds of Kolleru Lake, India. Ecotoxicology and Environmental Safety. 2008;72(4): 1242-1248.
18. Javed M. Growth responses of fish under chronic exposure of waterborne and dietary metals. International Journal of Agriculture & Biology. 2012; 14(2): 281-285.
19. Al-Balawi H, Al-Akel AS, Al-Misned F, Suliman E, Al-Ghanim KA, Mahboob S, et al. Effects of sub-lethal exposure of lead acetate on histopathology of gills, liver, kidney and muscle and its accumulation in these organs of *Clarias gariepinus*. Brazilian Archives of Biology & Technology. 2013; 56(2): 293-302.

20. Xie XJ, Sun RY. The Bioenergetics of the Southern Catfish (*Silurus meridionalis* Chen). 1. Resting Metabolic-rate as a Function of Body-weight and Temperature. Physiological Zoology. 1990;63(6): 1181-1195.
21. Fu SJ, Xie XJ, Cao ZD. Effect of meal size on postprandial metabolic response in southern catfish (*Silurus meridionalis*). Comparative Biochemistry & Physiology Part A Molecular & Integrative Physiology. 2005;140(4): 445-451.
22 Luo Y, Xie X. Effects of temperature on the specific dynamic action of the southern catfish, *Silurus meridionalis.* Comparative Biochemistry & Physiology Part A Molecular & Integrative Physiology. 2008; 149(2), 150-156.
23. Brett JR, Groves DD. Physiological energetics. Fish Physiology, Vol. VIII: Bioenergetics and Growth. New York: Academic Press, 1979.
24. Chinese Food Safety Standard (GB/T 5009.14-2003). Determination of zinc in food. Ministry of Health of the People's Republic of China.2003. China.
25. Chinese Environmental Protection Standard (GB 3838-2002). Surface water environmental quality. Ministry of Environmental Protection, PRC. 2002. China.
26. Mager EM, Grosell M. Effects of acute and chronic waterborne lead exposure on the swimming performance and aerobic scope of fathead minnows (*Pimephales promelas*). Comp Biochem Physiol C Toxicol Pharmacol. 2011; 154(1): 7-13.
27. Shukla J P, Pandey K. Effect of a sublethal concentration of zinc sulphate on the growth rate of fingerlings of *Channa punctatus* (Bloch), a freshwater murrel. CLEAN - Soil, Air, Water. 201014(6): 677-680.
28. Liu CQ, Wang AL, Wang WN. Influences of Zn^{2}+ and Mn^{2+} in seawater on the activity of alkaline [hosphatase (AKP) in postlarvae of Kuruma prawn *Penaeus japonicu*. Fisheries Science & Technology Information, 2002; 29(5): 195-197. China>
29. Rowe CL. Elevated standard metabolic rate in a freshwater shrimp (*Palaemonetes paludosus*) exposed to trace element-rich coal combustion waste. Comparative Biochemistry & Physiology Part A Molecular & Integrative Physiology. 1998; 121(4): 299-304.
30. Maltby L. Studying stress: The importance of organism level responses, Ecological Applications, 1999; 9: 431-440.
31. Malik DS, Sastry KV, Hamilton PD. Effects of zinc toxicity on biochemical composition of muscle and liver of murrel (*Channa punctatus*). Environment International. 1998; 24(4): 433-438.
32. Zheng JL, Luo Z, Liu CX, Chen QL, Tan XY, Zhu Q, et al. Differential effects of acute and chronic zinc (Zn) exposure on hepatic lipid deposition and metabolism in yellow catfish *Pelteobagrus fulvidraco*. Aquatic Toxicology. 2013; 132: 173-181.
33. Bervoets L, Blust R. Metal concentrations in water, sediment and gudgeon (*Gobio gobio*) from a pollution gradient: relationship with fish condition factor. Environmental Pollution. 2003; 126(1): 9-19.
34. Rajkowska M, Protasowicki M. Distribution of metals (Fe, Mn, Zn, Cu) in fish tissues in two lakes of different trophy in Northwestern Poland. Environmental Monitoring and Assessment. 2013;185(4): 3493-3502.
35. Hogstrand C, Haux C. Binding and detoxification of heavy metals in lower vertebrates with reference to metallothionein. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology. 1991; 100(1-2): 137-141.
36. Zhou YF, You Y, Wu W, Yang G, Chen JZ Dynamic changes of Metallothioneinn (MT) in different tissues of *Carassius auratus* exposed to different concentrations of Zinc. Jiangsu Journal of Agricultural Sciences.2009; 25(4): 818-823. China.
37. Qadir A, Malik RN. Heavy metals in eight edible fish species from two polluted tributaries (Aik and Palkhu) of the river Chenab, Pakistan. Biological Trace Element Research. 2011;143(3): 1524-1540.
38. Han Q, Liu T, Huang CH, Luo YS. Acute toxicity of Zinc to catfish and its accumulation and distribution in vivo. Heilongjiang Animal Science and Veterinary Medicine. 2016(1): 247-250. China.
39. Bustamante P, Bocher PYl, Miramand P, Caurant F. Distribution of trace elements in the tissues of benthic and pelagic fish from the Kerguelen Islands. Science of the Total Environment. 2003; 313(1-3): 25-39.
40. Rosemond SD, Xie Q, Liber K. Arsenic concentration and speciation in five freshwater fish species from Back Bay near Yellowknife, NT, Canada. Environmental Monitoring and Assessment, 2008; 147(1-3): 199-210.
41. Xu T, Huang Y, Chen J. Metal distribution in the tissues of two benthic fish from paddy fields in the middle reach of the Yangtze River. Bull Environ Contam Toxicol. 2014; 92(4): 446-450.
42. Yilmaz F. The comparison of heavy metal concentrations (Cd, Cu, Mn, Pb, and Zn) in tissues of three economically important fish (*Anguilla anguilla*, *Mugil cephalus* and *Oreochromis niloticus*) inhabiting Kycegiz Lake-Mugla (Turkey). Turkish Journal of Science & Technology. 2009; 4(1): 7-15.