Hindawi
Journal of Applied Ichthyology
Volume 2024, Article ID 5716920, 13 pages
https://doi.org/10.1155/2024/5716920

Research Article

Regulation of *GeCu/Zn-SOD*, *GeMn-SOD*, *GeHsp90*, and *GeMT* in *Gymnocypris eckloni* in Response to Copper and Lead Ion Challenges

Jing Zhao,[1] **Zixuan Li**,[1] **Changzhong Li**,[1] **Rong Wang**,[1] **Zifeng Zhao**,[1] **Huayu Gou**,[1] **Lanying Li**,[1] **Anbin Xie**,[1] **Haotian Ren**,[1] **Bo Qiu**,[1] **Xiaodie Li**,[1] **Yanxia Chen**,[1] **Zhenji Wang**,[2] **Guojie Wang**,[2] **and Wenjie Jin**[1]

[1]*College of Eco-Environmental Engineering, Qinghai University, Xining 810016, China*
[2]*Qinghai Provincial Fishery Environmental Monitoring Center, Xining 810016, China*

Correspondence should be addressed to Wenjie Jin; kingsunny@126.com

Received 6 October 2023; Revised 13 February 2024; Accepted 21 February 2024; Published 7 March 2024

Academic Editor: Mohamed Abdelsalam

Copyright © 2024 Jing Zhao et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The contamination of aquatic environments by heavy metals can have detrimental effects on fish, impacting their growth and overall health, including the regulation of antioxidant genes. An investigation was carried out to assess the distribution and habitat of *Gymnocypris eckloni* in the Yellow River basin. Simultaneously, heavy metal concentrations in its habitat and in selected locations within the upper Yellow River were measured. In an effort to explore the potential roles of specific genes in antioxidant responses, *G. eckloni* was exposed to low concentrations of copper (Cu^{2+}) and lead (Pb^{2+}) for varying durations (12, 24, and 48 hours). The mRNA levels of *GeCu/Zn-SOD*, *GeMn-SOD*, *GeHsp90*, and *GeMT* were quantified in the gills, kidneys, and liver through qRT-PCR. The findings suggest that the habitat of *G. eckloni* is generally safe; however, occasional exceedances of safety standards could pose a potential threat to its growth. Importantly, the expression of *GeCu/Zn-SOD*, *GeMn-SOD*, *GeHsp90*, and *GeMT* exhibited responses to the low concentrations of copper-induced and lead-induced stress. Notably, *GeCu/Zn-SOD*, *GeMn-SOD*, and *GeMT* demonstrated heightened sensitivity to lead compared to copper. Furthermore, the expression of these genes displayed tissue-specific responses under identical metal stress conditions. These results indicate that *GeCu/Zn-SOD*, *GeMn-SOD*, *GeHsp90*, and *GeMT* genes have the potential to serve as early, sensitive biomarkers for the detection of metal toxicity induced by Cu^{2+} and Pb^{2+}. This study also provides valuable insights into the functioning of antioxidant genes under oxidative stress in fish.

1. Introduction

Gymnocypris eckloni Herzenstein, a freshwater fish, is a member of the *Gymnocypris* genus within the Schizothoracinae subfamily. These fish, native to the Qinghai-Tibet Plateau, are primarily found in the upper reaches of the Yellow River, including locations such as Zhaling Lake, Erling Lake, and the Naqi River basin in the Qaidam basin [1, 2]. Unfortunately, due to human activities and man-made hydroelectric projects, the habitat of *G. eckloni* has suffered significant damage, resulting in a reduction in its natural distribution [3]. In an effort to mitigate the decline in the wild population of *G. eckloni*, successful artificial reproduction of this species has been carried out in Qinghai province, China, since 2008.

Heavy metals, characterized by their high density, exceeding five times that of water [4], encompass elements such as lead (Pb), cadmium (Cd), zinc (Zn), copper (Cu), mercury (Hg), silver (Ag), chromium (Cr), ferrum (Fe), and more. Prolonged or sudden exposure to heavy metal pollution in water can significantly impact fish physiology, growth, reproduction, and even population dynamics [5, 6]. Fish can accumulate heavy metals within their bodies through predator-prey relationships, resulting in the degeneration of gill fibers and stem cells. X-ray

examinations have revealed that heavy metal stress induces slight curvature of the fish's spine and reduces calcium and phosphorus levels, leading to bone deformities [7]. Through the food chain, heavy metals can accumulate in fish, birds, and mammals and even pose risks to human health [8].

Biomarkers are more sensitive than general biological detection indexes. Thus, the key genes or gene clusters of algae, plankton, and fish can be tested to detect the health status of aquatic organisms and infer the true state of water quality. Metallothionein (MT), as a biomarker of heavy metal pollution in aquatic organisms, is a heavy metal binding protein [9, 10]. In general, the detoxification of MT depends to a large extent on its affinity with heavy metals in the following order: silver > copper > cadmium > lead > zinc > cobalt > iron [11, 12]. Superoxide dismutases (SODs) are a class of antioxidant enzymes, which can catalyze the disproportionation of superoxide anion free radicals to balance the oxygen free radicals in the body [13]. *Cu/Zn-SOD* and *Mn-SOD* are the important components in the antioxidant system of aquatic animals [14]. SOD activity was enhanced under heavy metal stress, which is a self-protecting mechanism of the body and can minimize oxidative stress injury [15]. The concentration of Cu ions in the feed affected not only the growth of fish but also its antioxidant capacity, which demonstrated that higher Cu ions in the feed of *Larimichthys croceus* reduced *Cu/Zn-SOD* activity and total antioxidant capacity in the livers [16]. When the content of copper in the feed decreased, the *Cu/Zn-SOD* activity in the *Ictalurus punctatus* liver decreased significantly [17]. Heat shock proteins (HSPs) are a family of protein molecules produced by cells under heat induction and other external stimuli [18, 19]. According to amino acid sequences, molecular weights, and functions, HSPs can be divided into several major families: HSP110, HSP90, HSP70, HSP60, and several small HSPs [20]. HSPs can not only respond to high-temperature stress but also be induced to express by environmental factors that may cause protein damage [21].

The heavy metals in the Yellow River basin are mainly accumulated in the sediments of lakes and rivers entering the lakes. Copper and lead are the most common heavy metals in the water, bottom mud, soil, and animals and plants in the basin. In this study, we conducted an assessment of key water quality parameters, dissolved oxygen, pH, and Cu^{2+} and Pb^{2+} at 11 monitoring sites situated in the upper reaches of the Yellow River within Qinghai province. Moreover, we selected concentrations of 0.01 mg/L of Cu^{2+} and 0.05 mg/L of Pb^{2+} in accordance with the China Water Quality Standard for Fisheries (GB 11607-1989) to expose *Gymnocypris eckloni* (*G. eckloni*) to these heavy metals. Subsequently, we examined the expression levels of *GeCu/Zn-SOD*, *GeMn-SOD*, *GeHsp90*, and *GeMT* in the gills, liver, and kidneys of these fish. The findings of this study indicate that the responses of fish to heavy metal stress, involving various regulatory mechanisms at different concentration levels, offer a more accurate representation of how aquatic organisms react to low-dose heavy metal pollutants. This approach holds a practical significance for early warning systems aimed at monitoring heavy metal pollution in water quality and the environment.

2. Materials and Methods

2.1. Detection of Heavy Metal Ions in Distribution Areas of G. eckloni. To understand the water quality status of the upper reaches of the Yellow River and its tributaries in Qinghai province, water environment monitoring was conducted in 11 monitoring sites in July every year for the following 4 years (Figure 1).

At the beginning of July, water quality parameters (dissolved oxygen and pH) were periodically measured using a CTD probe (Ocean Seven 310 CTD; Idronaut, Brugherio, Italy). At each sampling point, three samples were taken and placed in high-density polyethylene bottles, which were then taken back to the laboratory and stored in a refrigerator at 4°C. Determination of copper and lead ion concentration was performed by an atomic absorption spectrometer (ZEEnit 700P, Germany) within 24 h according to the China Water Quality Standard for Fisheries (GB 11607-1989). The Cu^{2+} standard solution (GNM-SCU-002-2013, 100 μg/ml with 5% HNO_3) (National Center for Analysis and Testing of Nonferrous Metals and Electronic Materials, China) was prepared with 2 mg/L mother solution and diluted in a concentration gradient of 0, 0.001, 0.002, 0.005, 0.010, and 0.020 mg/L, respectively. The Pb^{2+} standard solution (GNM-SPB-002-2013, 100 μg/ml with 5% HNO_3) (National Center of Analysis and Testing for Nonferrous Metals and Electronic Materials, China) was prepared with 2 mg/L mother solution and diluted in a concentration gradient of 0, 0.005, 0.010, 0.020, 0.050, and 0.100 mg/L, respectively.

2.2. Animals. In September, 120 three-year-old *Gymnocypris eckloni*, weighing 100 ± 2.5 g and 19 ± 3.4 cm in length, were obtained from the Qinghai Provincial Fishery Environmental Monitoring Center, Xining, China. The fish were kept in the tank (L: 48.0 cm, W: 25.0 cm, and H: 45.5 cm), and 40 L of 15 ± 0.5°C aerated dechlorinated tap water was put in, the water was changed every 12 hours, and the fish were fed once a day, and the light and dark cycle was maintained for 14 h/10 h.

2.3. Heavy Metal Stress. $CuSO_4{\cdot}5H_2O$ was purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). $Pb(NO_3)_2$ was supplied by Sinopharm Chemical Reagent Co. (Shanghai, China). 1000 mg/L Cu^{2+} or Pb^{2+} solution was prepared using double-distilled water, and the working liquid of 0.01 mg/L Cu^{2+} and 0.05 mg/L Pb^{2+} was prepared using 40 L aerated and dechlorinated tap water, respectively.

After seven days of acclimatization to the laboratory environment, 90 healthy fish were randomly assigned to nine groups. Three groups were selected as controls, while the other six groups suffered from heavy metal stress, 0.01 mg/L Cu^{2+} or 0.05 mg/L Pb^{2+}. Each pressure group had three repeats of 10 fish at a time. After heavy metals were treated for 12, 24, and 48 h, the gills, liver, and kidneys were dissected immediately following ether anesthesia (euthanasia) and stored at −80°C until use.

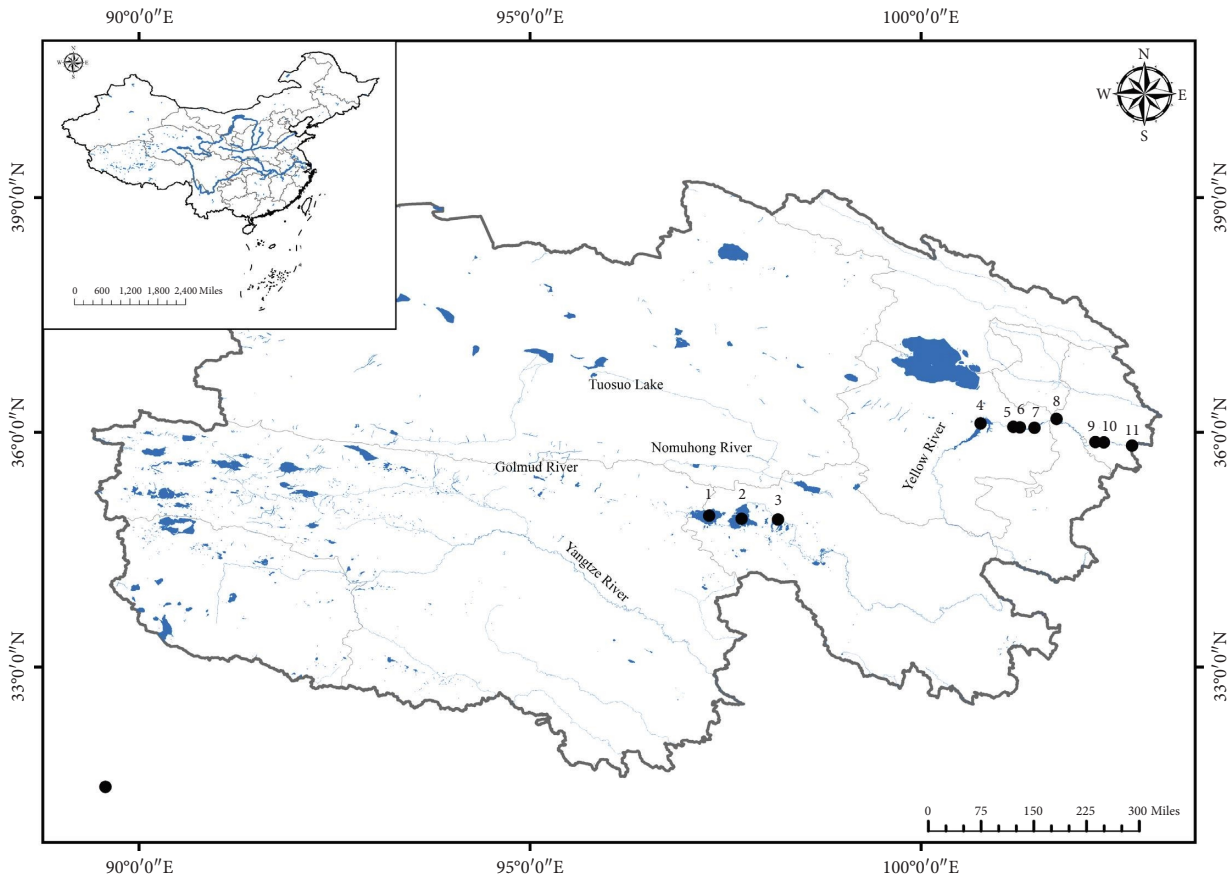

FIGURE 1: Distribution map of 11 water quality testing sites. 1–11 means Zhaling Lake, Erling Lake, Maduro Yellow River Bridge, Longyangxia Reservoir Center, Laxiwa Reservoir area, Laxiwa Reservoir tailwater, Guide Yellow River Bridge, Lijiaxia Reservoir area, Gongboxia Reservoir area, Sushi Reservoir area, and Jishixia Reservoir tailwater, respectively.

2.4. Heavy Metal Analysis. 0.2 g (accurate to 0.001 g) of frozen tissue samples was put into microwave digestion tubes, and 6 ml of HNO_3 and 2 ml of H_2O_2 were added to each tube. Put them into a microwave digestion system (MASTERr-40, China) for 10 min at 150°C and then for 25 min at 180°C and allow to cool to room temperature. After cooling, remove the digestion tubes and drive the acid to 1 ml at 150°C on the acid drive meter. The digestion liquid was transferred to a 25 ml volumetric bottle in which they were diluted using ultrapure water 2-3 times. Following acid digestion, all the samples were analyzed with AAS (AA-6880, China). The excitation wavelength of the lead ion was 283.3 nm, and the excitation wavelength of the copper ion was 324.8 nm.

2.5. Expression of GeCu/Zn-SOD, GeMn-SOD, GeHsp90, and GeMT. The GenBank accession numbers and reference of primers are given in Table 1. qPCR was conducted using the LightCycler® 96 (Roche, Basel, Switzerland) in a final volume of 25 μl containing 1 μl (10 μM) each of forward and reverse primer, 12.5 μl of SYBR Premix Ex Taq II (2×), 2 μl of cDNA, and 8.5 μl of ddH_2O. The relative abundance of genes was normalized by the expression of β-actin [22] and calculated using the $2^{-\Delta\Delta Ct}$ method.

2.6. Statistical Analyses. Expression levels of *GeCu/Zn-SOD*, *GeMn-SOD*, *GeHsp90*, and *GeMT* were assessed using one-way ANOVA followed by an LSD test with SPSS statistics 17.0 software. All data were represented as mean ± SD as shown in the figures. A probability value of 0.05 or below was considered significant.

3. Results

3.1. Detection of Copper and Lead Ions in the Distribution Area of G. eckloni. The detection data of multiple heavy metal ions from 11 monitoring stations in the distribution area of *G. eckloni* in Qinghai province (Table 2) showed that the measured values of Cu^{2+} and Pb^{2+} had little changes and were all within the maximum range of the China Water Quality Standard for Fisheries (GB 11607–1989, the permitted maximal value is $Cu^{2+} \leq 0.01$ mg/L and $Pb^{2+} \leq 0.05$ mg/L). In general, the dissolved oxygen and pH values of the 11 test sites meet the requirements of the national fishery and aquaculture standards, but the concentrations of copper and lead ions in a few test sections occasionally approach or exceed the requirements of the national fishery and aquaculture standards. For example, the maximum concentration of Pb^{2+} in Zhaling Lake is 0.051 mg/L, which is slightly higher than the GB 11607-1989 of no more than 0.05 mg/L. The Pb^{2+} is 0.044 mg/L in the Jishixia

Table 1: Oligonucleotide primers used for qPCR.

Genes	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Size (bp)	Sources and references
GeCu/Zn-SOD	GTCCGCACTTCAACCCTCA	ACCCAGGTCATCGTCTTTCTCA	165	KX826080
GeMn-SOD	TTCACCACAGCAAGCACCAT	AGCCGACATCTTCTCCTTCA	260	KY922773
GeMT	CGCCAAAACTGGAACGTG	CCGCAGGAATTGCCCTTG	147	[23]
GeHsp90	AGAAGCACCTAGAAATCAACC	TCTCGAACAGCAGGATCACCA	114	[23]
β-Actin	CCATGTACGTTGCCATCCAG	CGCAAGACTCCATACCCAA	426	[23]

Table 2: The maximal and minimal values of heavy metals concentration in the selected monitoring sections along the Yellow River.

National standard	DO (≥5 mg/L)		pH (6.5–8.5)		Cu^{2+} (≤0.01 mg/L)		Pb^{2+} (≤0.05 mg/L)	
Detecting section	Max	Min	Max	Min	Max	Min	Max	Min
Zhaling Lake	8.1	6.5	8.91	8.42	0.007	0.003	0.051	0.030
Erling Lake	7.4	5.95	8.66	8.30	0.008	0.004	0.027	0.020
Maduro Yellow River Bridge	7.0	6.2	8.61	8.52	0.008	0.007	0.030	0.020
Longyangxia Reservoir Center	8.4	7.9	8.60	8.32	0.006	0.001	0.030	0.016
Qunaihai, Laxiwa	9.1	7.0	8.51	8.22	0.006	0.001	0.024	0.010
Laxiwa Reservoir tailwater	9.4	9.1	8.52	8.22	0.010	0.003	0.025	0.012
Guide Yellow River Bridge	11.5	9.5	8.51	8.32	0.006	0.005	0.03	0.010
Lijiaxia Reservoir area	10.9	7.8	8.52	8.35	0.005	0.004	0.020	0.010
Gongboxia Reservoir area	11.5	9.4	8.51	8.31	0.007	0.002	0.030	0.020
Sushi Reservoir area	10.4	9.3	8.52	8.33	0.006	0.001	0.025	0.020
Jishixia Reservoir tailwater	10.9	7.8	8.42	8.28	0.003	0.002	0.044	0.008

Reservoir tailwater, which is close to the GB 11607-1989. In addition, the highest concentration of Cu^{2+} in the Laxiwa Reservoir tailwater reached 0.01 mg/L of the GB 11607-1989, while the concentration of Cu^{2+} in Zhaling Lake, Erling Lake, Maduro Yellow River Bridge, and Gongboxia Reservoir area was at risk of approaching the GB 11607-198.

3.2. Detection of Copper and Lead Ion Content in Experimental Water and Fish. In order to better understand the transcriptional response to Cu^{2+} stress and Pb^{2+} stress by *Gymnocypris eckloni*, we introduced Cu^{2+} and Pb^{2+} to the aerated and dechlorinated water at a theoretical Cu^{2+} concentration of 0.01 mg/L and Pb^{2+} concentration of 0.05 mg/L. Given that Cu^{2+} and Pb^{2+} may be present in water, resulting in an actual value that differs from the theoretical value, we employed an atomic absorption spectrometer to measure Cu^{2+} and Pb^{2+} concentrations in water of both the experimental and control groups. The results showed that the concentrations of Cu^{2+} and Pb^{2+} were 0.0085 ± 0.0001 mg/L and 0.0019 ± 0.0002 mg/L, respectively, in the blank group, whereas 0.01 mg/L of Cu^{2+} solution and 0.05 mg/L of Pb^{2+} solution contained an actual concentration of 0.0119 ± 0.0006 mg/L and 0.0501 ± 0.0001 mg/L (Figure 2).

Similarly, in order to further determine the content of copper and lead ions in *G. eckloni*, AAS results showed that the content of Cu and Pb ions in the gills, kidneys, and liver at 12 h, 24 h, and 48 h were close to 0.01 mg/kg and 0.05 mg/kg (Table 3).

3.3. Expression of GeCu/Zn-SOD after Cu^{2+} stress and Pb^{2+} Stress. In the gills, as shown in Figure 3, *GeCu/Zn-SOD*'s initial response was less intense after Cu^{2+} stress but increased significantly at 24 h ($n = 3$, $P < 0.05$). However, it increased rapidly and substantially under Pb^{2+} stress, especially at 12 h when the expression was 24.18-fold higher than that in the control group. In the kidney, the expression of *GeCu/Zn-SOD* under Cu^{2+} stress for 48 h and Pb^{2+} stress for 24 h was 70.65 and 11.74 times lower than that of the control group, respectively, and the expression of *GeCu/Zn-SOD* was significantly upregulated and reached the maximum under Pb^{2+} stress for 48 h ($n = 3$, $P < 0.05$). In the liver, the expression of *GeCu/Zn-SOD* decreased to varying degrees after Cu^{2+} stress, while under Pb^{2+} stress, *GeCu/Zn-SOD* expression increased first, then decreased rapidly, and then significantly upregulated to the maximum ($n = 3$, $P < 0.05$).

In general, the response rate of *GeCu/Zn-SOD* was the fastest in the liver and kidney, and the response intensity of *GeCu/Zn-SOD* to Pb^{2+} was stronger than that of Cu^{2+} stress in the gills, kidneys, and liver. Interestingly, compared with the control group, the expression of *GeCu/Zn-SOD* was sharply downregulated after Cu^{2+} stress for 48 h, but it was sharply downregulated earlier to 24 h under Pb^{2+} stress.

3.4. Expression of GeMn-SOD after Cu^{2+} stress and Pb^{2+} Stress. In the gills, as shown in Figure 4, the expression of *GeMn-SOD* was slow after Cu^{2+} stress, while it was upregulated rapidly and strongly after Pb^{2+} stress and reached a peak at 48 h ($n = 3$, $P < 0.05$). In the kidney, the expression of *GeMn-SOD* initially increased and then decreased after Pb^{2+} stress and Cu^{2+} stress, but it was an abnormally high expression and reached the highest value after 48 h of Pb^{2+} stress ($n = 3$, $P < 0.05$). In the liver, the response of *GeMn-SOD* was slow and significantly downregulated and reached the lowest

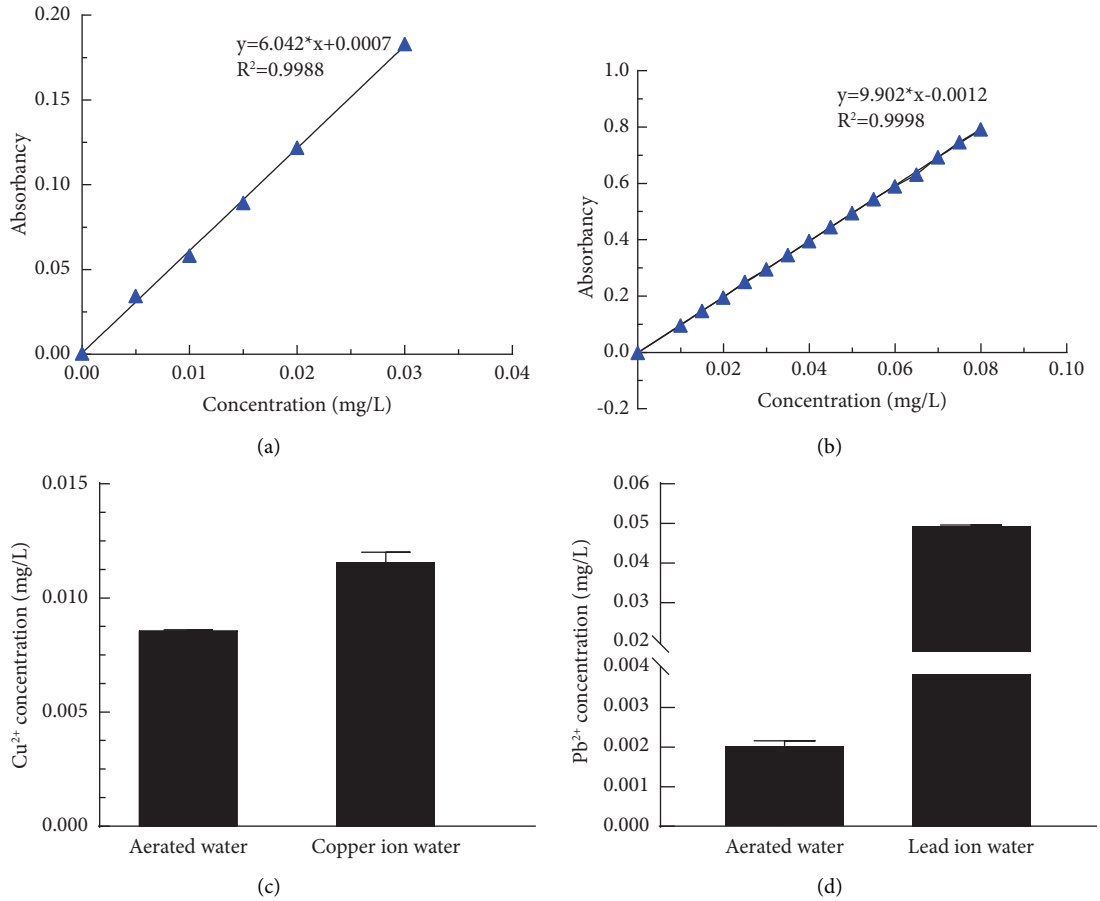

Figure 2: Determination of the copper and lead ion concentration in water. (a) Standard curve for determination of copper ion concentration in water. (b) Standard curve for determination of lead ion concentration in water. (c) Copper ion concentration in water of experimental and control groups. (d) Lead ion concentration in water of experimental and control groups.

Table 3: Mean concentrations of copper and lead in the gills, kidneys, and liver of *G. eckloni* (units mg/kg dry weight).

	Tissue	Cu	Pb
G. eckloni			
Blank group	Gills	—	—
	Kidney	—	—
	Liver	—	—
12 h	Gills	0.0094^{a}	0.1022^{a}
	Kidney	0.0118^{a}	0.0413^{a}
	Liver	0.0109^{a}	0.0568^{a}
24 h	Gills	0.0073^{c}	0.0227^{c}
	Kidney	0.0098^{b}	0.0340^{b}
	Liver	0.0084^{c}	0.0327^{b}
48 h	Gills	0.0090^{b}	0.0454^{b}
	Kidney	0.0080^{c}	0.0313^{c}
	Liver	0.0091^{b}	0.0227^{c}

The different letters in the same column indicate significant differences ($P < 0.05$) between different times (blank group, 12 h, 24 h, and 48 h) in the same tissue. $n = 3$ biologically independent replicates.

value after 48 h of Cu^{2+} stress ($n = 3$, $P < 0.05$), while the response was sharply downregulated after 24 h of Pb^{2+} stress and recovered to normal level after 48 h. In general, the response strength and speed of *GeMn-SOD* to Pb^{2+} stress were superior to that of Cu^{2+} stress.

3.5. Expression of GeHsp90 after Cu^{2+} stress and Pb^{2+} Stress. In the gills, as shown in Figure 5, the expression of *GeHsp90* was first significantly upregulated and significantly downregulated after Cu^{2+} stress ($n = 3$, $P < 0.05$) and then recovered to the normal level, while the expression of

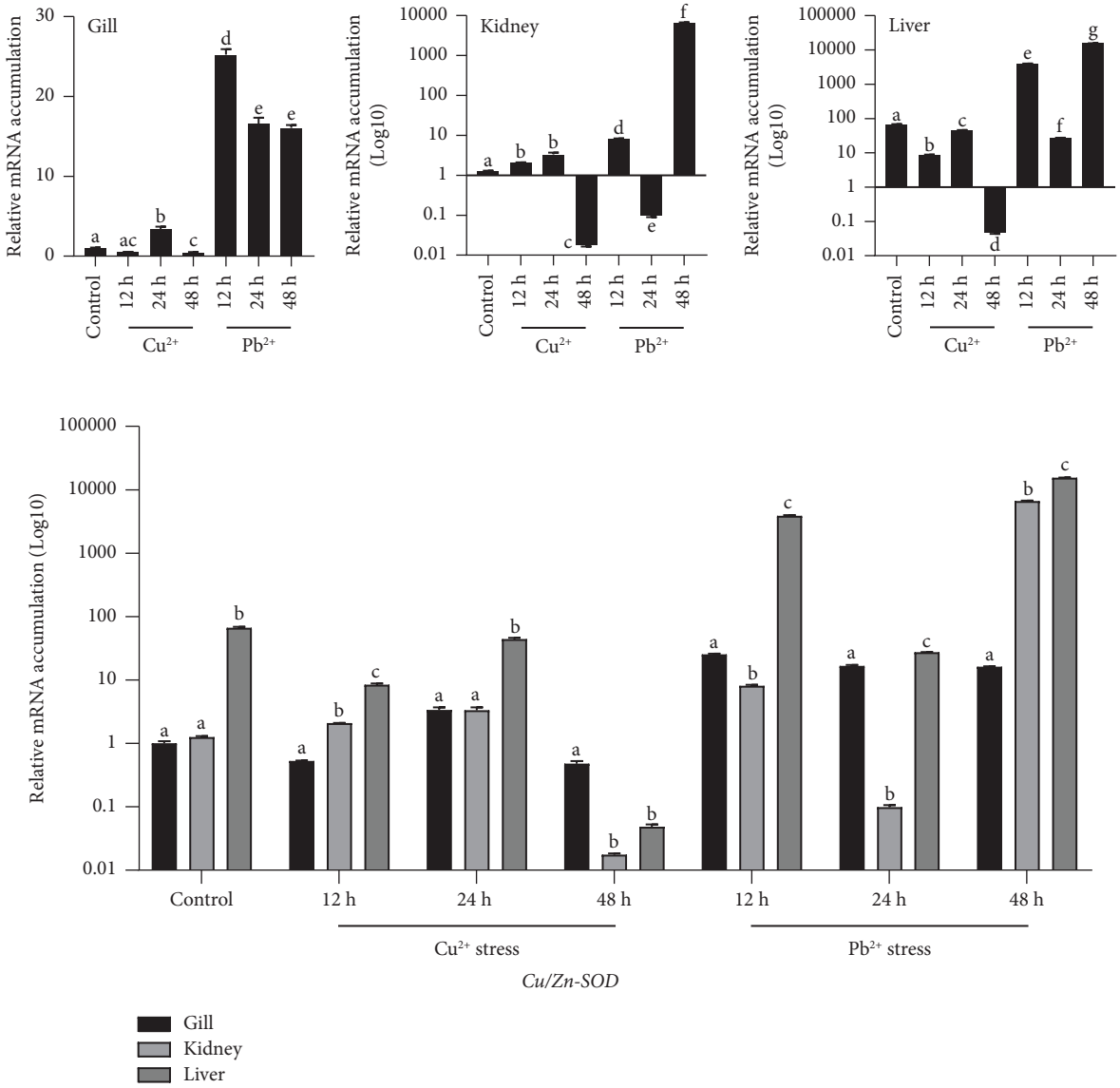

Figure 3: The accumulation of *GeCu/Zn-SOD* mRNA in the gills, kidneys, and liver of *G. eckloni* after copper (0.01 mg/L) and lead (0.05 mg/L) ions treatment, respectively. Data represent the means of relative mRNA accumulation for each indicated gene; error bars represent 1 SEM; $n = 3$ biologically independent replicates. Letters above each column indicate significant differences ($P < 0.05$) between tissues and time points.

GeHsp90 after Pb^{2+} stress was opposite to that after Cu^{2+} stress. In the kidney, the expression of *GeHsp90* increased first, then decreased, and finally upregulated after Pb^{2+} stress and Cu^{2+} stress. In the liver, the expression of *GeHsp90* was increased gradually and reached a peak at 48 h after Cu^{2+} stress, while the expression was first significantly downregulated and significantly upregulated after Pb^{2+} stress, and it was significantly lower than that in the blank control group ($n = 3$, $P < 0.05$). In general, *GeHsp90* has a strong response to both Cu^{2+} stress and Pb^{2+} stress. In addition, the expression line of *GeHsp90* in the gills and kidneys was upregulated and downregulated after Cu^{2+} stress, while it was continuously upregulated in the liver.

3.6. Expression of GeMT after Cu^{2+} stress and Pb^{2+} Stress. In the gills, as shown in Figure 6, the expression of *GeMT* was first significantly upregulated to reach the peak and significantly downregulated and then significantly upregulated to a state higher than that of the blank control group after Cu^{2+} stress ($n = 3$, $P < 0.05$). After Pb^{2+} stress, the expression of *GeMT* was mild, upregulated at 12 h, reached the peak at 24 h, and recovered to normal after 48 h. In the kidney, the expression of *GeMT* was upregulated significantly after Cu^{2+} stress and Pb^{2+} stress, respectively. In the liver, *GeMT* expression was significantly downregulated after Cu^{2+} stress but significantly upregulated after Pb^{2+} stress ($n = 3$, $P < 0.05$). In general, the response strength of *GeMT* in gills to Cu^{2+} stress was greater than that to Pb^{2+} stress. However,

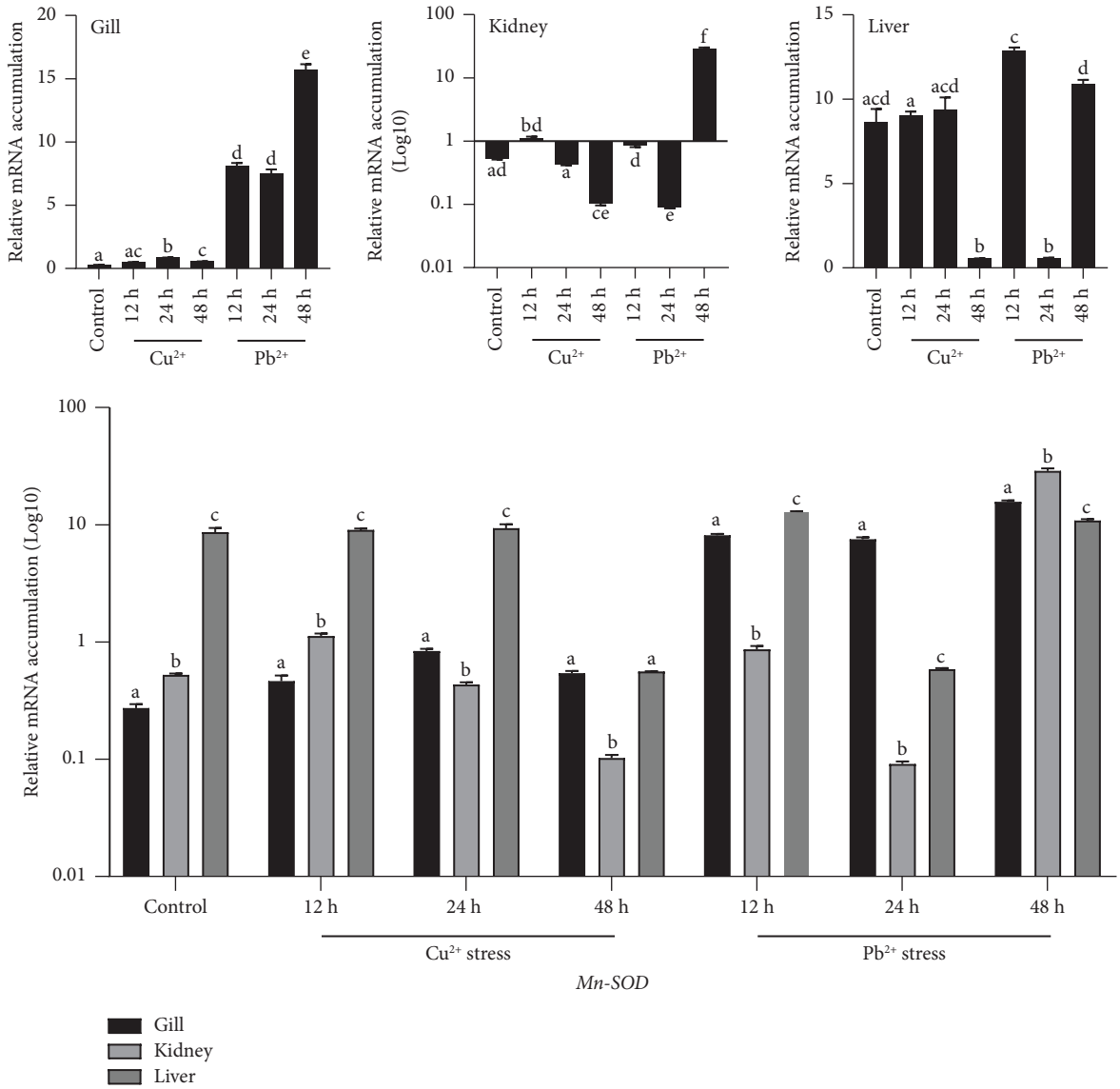

Figure 4: The accumulation of *GeMn-SOD* mRNA in the gills, kidneys, and liver of *G. eckloni* after copper (0.01 mg/L) and lead (0.05 mg/L) ions treatment, respectively. Data represent the means of relative mRNA accumulation for each indicated gene; error bars represent 1 SEM; $n = 3$ biologically independent replicates. Letters above each column indicate significant differences ($P < 0.05$) between tissues and time points.

the response intensity of *GeMT* to Cu^{2+} stress and Pb^{2+} stress in both the kidney and the liver was very high, but the response mode was different. For example, the expression of *GeMT* in the kidney was significantly upregulated after Cu^{2+} stress and Pb^{2+} stress, while the expression of *GeMT* in the liver was significantly downregulated after Cu^{2+} stress. In addition, the expression of *GeMT* after Pb^{2+} stress was opposite to that after Cu^{2+} stress.

3.7. Temporal and Spatial Expression Patterns of SODs, HSP90, and MT after Cu^{2+} stress and Pb^{2+} Stress. The expression of *GeCu/Zn-SOD* in the gills of the blank control group was taken as a reference value, and the expressions of *GeCu/Zn-SOD*, *GeMn-SOD*, *GeHsp90*, and *GeMT* in different tissues were analyzed (Figure 7). It was found that the response speed of *GeCu/Zn-SOD* in the liver was the fastest. The response speed and intensity of *GeCu/Zn-SOD* to Pb^{2+} in the gills, kidneys, and liver were superior to Cu^{2+} stress. The response intensity and response speed of *GeMn-SOD* to Pb^{2+} stress were superior to Cu^{2+} stress, except that the response speed of *GeMn-SOD* to Cu^{2+} was faster than that of Pb^{2+} in the kidney. The response intensity of *GeHsp90* to Cu^{2+} and Pb^{2+} was very strong. In addition, the expression of *GeHsp90* was upregulated and downregulated in the gills and kidneys after Cu^{2+} stress, while the expression of *GeHsp90* was continuously upregulated in the liver. The response of *GeMT* to Cu^{2+} stress was stronger than that to Pb^{2+} stress. In the kidney and liver, the response intensity of *GeMT* to Cu^{2+} stress and Pb^{2+} stress was very high, but the response mode was different.

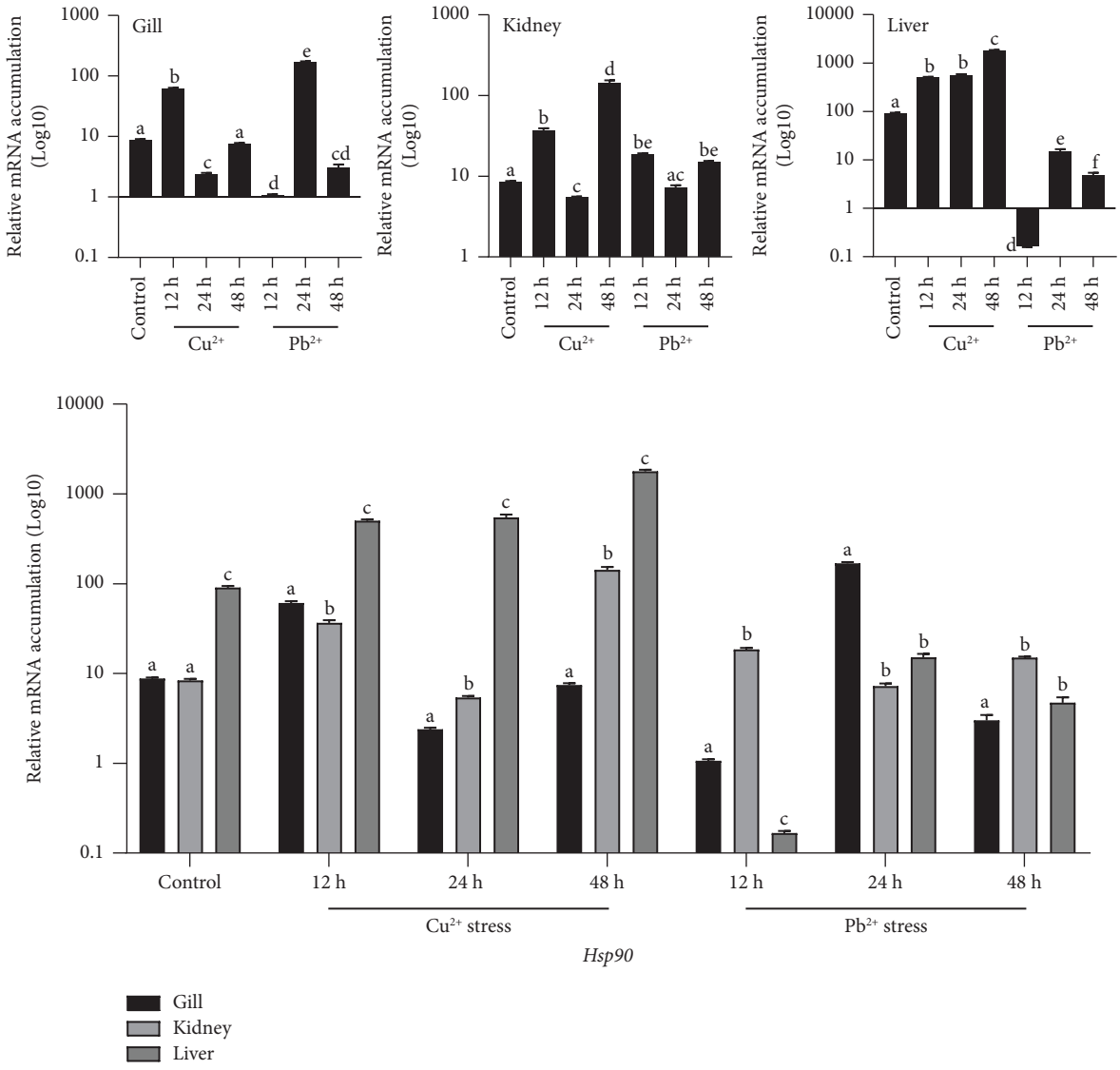

FIGURE 5: The accumulation of *GeHsp90* mRNA in the gills, kidneys, and liver of *G. eckloni* after copper (0.01 mg/L) and lead (0.05 mg/L) ions treatment, respectively. Data represent the means of relative mRNA accumulation for each indicated gene; error bars represent 1 SEM; $n = 3$ biologically independent replicates. Letters above each column indicate significant differences ($P < 0.05$) between tissues and time points.

4. Discussion

In this study, the 11 sampling sites in the Qinghai region of the upper reaches of the Yellow River involve the source segment with an average altitude of 4000 m and the gorge segment with an average altitude of 2000–4000 m. Monitoring sites no. 1–3 are located in the source section of the river, where there are few human activities, no industrial production activities, and the pollution of agricultural production to the water body is weak; thus, Cu^{2+} and Pb^{2+} mainly come from natural sources such as soil parent material. Starting from monitoring site no. 3, the surrounding terrain of the downstream monitoring site is dominated by valleys and cliffs, coupled with urbanization, reservoir construction, and weathering rock becoming a new source of pollution, and other factors that lead to dynamic changes of heavy metal ions in the river channel [25]. The dissolved oxygen level increases from upstream to downstream, and there may be no. 1–3 sample sites located in the source region of the Yellow River, with an average elevation of 4500 m, low temperature, slow water exchange, thin air, and relatively low dissolved oxygen level [26]. From no. 4 to no. 11 monitoring points, the altitude decreases, the oxygen content in the air increases, the water column drop is large, the water velocity is fast, and the dissolved oxygen level increases correspondingly. Overall, the detection data indicated that the measured values were all within the China Water Quality Standard for Fisheries (GB 11607-1989), which had little effect on aquatic organisms. However, some monitoring sites occasionally approach or exceed the standard. For example, the maximum concentration of Pb^{2+} in Zhaling Lake was 0.051 mg/L, and the content of Pb^{2+} in

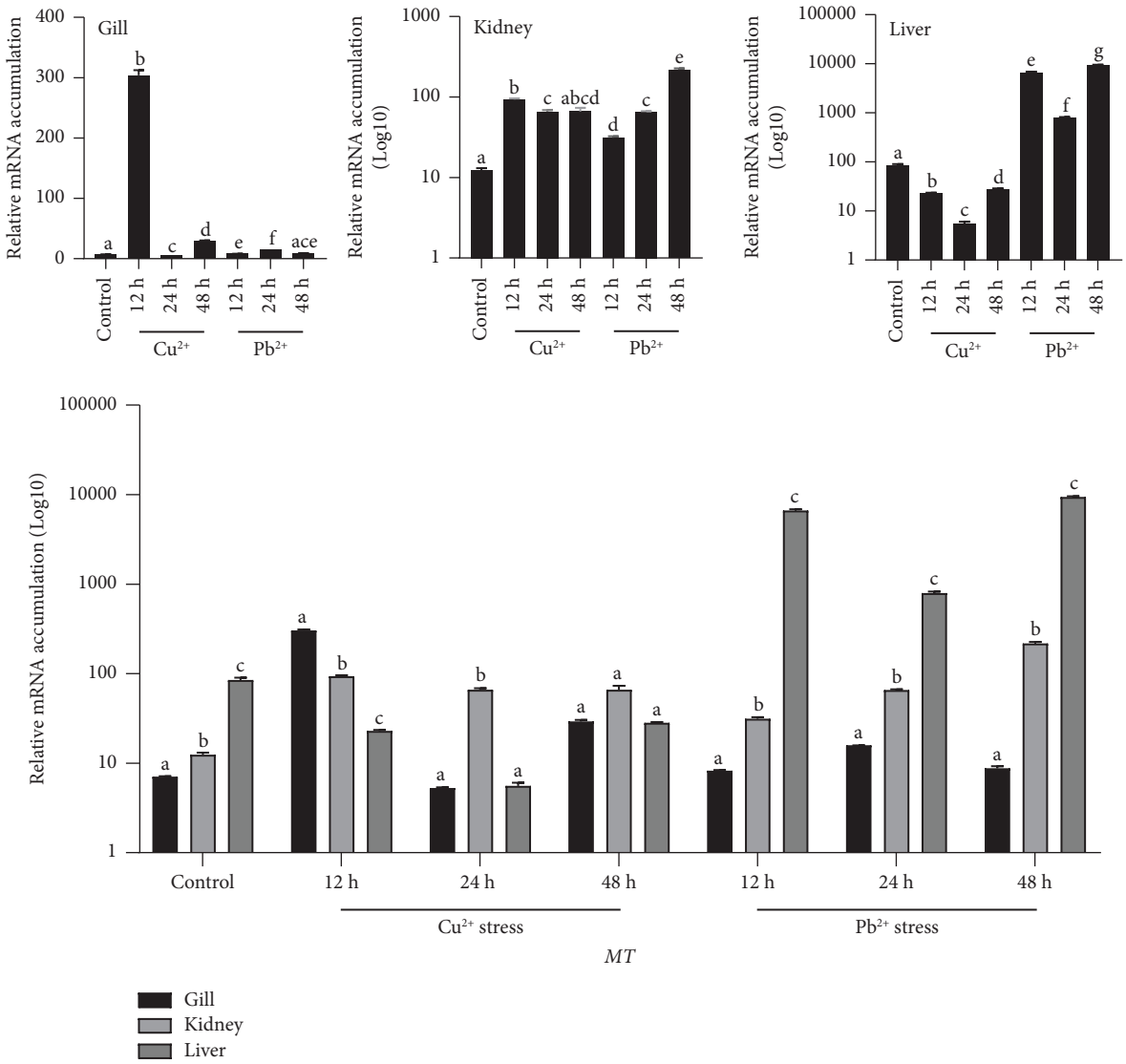

FIGURE 6: The accumulation of *GeMT* mRNA in the gills, kidneys, and liver of *G. eckloni* after copper (0.01 mg/L) and lead (0.05 mg/L) ions treatment, respectively. Data represent the means of relative mRNA accumulation for each indicated gene; error bars represent 1 SEM; $n = 3$ biologically independent replicates. Letters above each column indicate significant differences ($P < 0.05$) between tissues and time points.

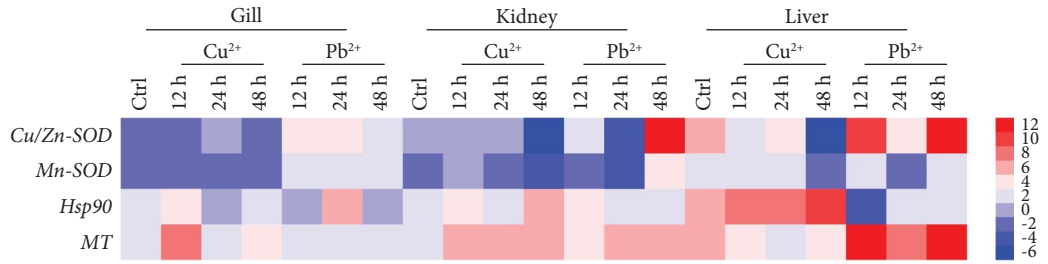

FIGURE 7: Temporal expression patterns of *GeCu/Zn-SOD*, *GeMn-SOD*, *GeHsp90*, and *GeMT* in response to copper and lead ions challenge in gills, kidneys, and liver of *G. eckloni*. Colors indicate Log2-fold differences in transcription relative to the β-actin internal reference, visualized using the HemI script [24], with negative values indicating downregulation and positive values indicating upregulation.

the tailwater of Jishixia Reservoir was 0.044 mg/L. In addition, the concentration of Cu^{2+} in the tailwater of Laxiwa Reservoir reaches up to 0.01 mg/L, and the concentration of Cu^{2+} in Zhaling Lake, Erling Lake, Maduro Yellow River Bridge, and Gongboxia Reservoir area was in danger of approaching GB 11607-198. This may be caused by

pollutants entering rivers through identifiable point sources such as urban and industrial wastewater and diffusion sources closely related to meteorological factors such as surface runoff, erosion, and atmospheric deposition [27, 28]. These results indicate that heavy metal pollution exists in the upper reaches of the Yellow River, and heavy metal concentrations must be measured regularly to reduce the risk of chronic poisoning in the future. In addition, as an indigenous fish living in water, the behavior and physiological changes of *G. eckloni* can also directly reflect whether heavy metals in the effluent environment exceed the standard. However, the adaptive regulation mechanism of *G. eckloni* to abnormal changes of heavy metal ions in the water environment and the screening of biomarker molecules that can be used for early warning still need further systematic studies.

Traditional toxicology often uses semilethal and lethal concentrations to determine heavy metal stress concentrations and explore the impact of heavy metals on fish. However, before the concentration of heavy metals reaches the semilethal concentration, genes such as the molecular partner, metal chelation, and immune response are already activated in the fish. When the lethal concentration is reached, the safety of the aquatic ecosystem and the growth of aquatic organisms are seriously affected, indicating that the signal molecules screened by traditional detection methods could not provide accurate warning signals for the protection of the aquatic ecosystem. Here, 0.01 mg/L of Cu^{2+} and 0.05 mg/L of Pb^{2+} concentrations were selected according to the GB 11607-1989 to treat *G. eckloni*, and the expression of genes related to antioxidant stress (*GeCu/Zn-SOD*, *GeMn-SOD*, and *GeHsp90*) and metal chelation (MT) was detected.

The activity of *Cu/Zn-SOD* in an organism tends to decrease under severe stress but increases under mild stress [29, 30]. The expression of *Cu/Zn-SOD* in the brain of fish was decreased after 0.60 mg/L of Cu^{2+} stress for 4 d [31]. On the contrary, the expression of *Cu/Zn-SOD* was increased in *Carassius auratus gibelio* after 0.005 mg/L or 0.050 mg/L of Cu^{2+} stress [32]. A similar phenomenon was observed in *Daphnia magna*, where *Cu/Zn-SOD* expression was increased when exposed to the mixture of copper, unionized ammonia, and low dissolved oxygen for 48 h [33]. Qiang et al. [31] found that *Cu/Zn-SOD* expression was increased rapidly and expressed intensively in the gills and liver and under Cu^{2+} stress. Similarly, we found that the expression of *GeCu/Zn-SOD* was the fastest and more intense in the liver. This is related to the liver as the main detoxification organ, carrying out heavy metal detoxification. Under 0.01 mg/L of Cu^{2+} and 0.05 mg/L of Pb^{2+} stress, the expression of *GeCu/Zn-SOD* was significantly increased, and the expression of *GeCu/Zn-SOD* under Pb^{2+} stress was significantly stronger than that under Cu^{2+} stress. Copper may serve as an important element in living organisms and is beneficial to living organisms at the right concentration [34]. However, lead is the nonessential and most toxic metal in the aquatic environment, and even very low concentrations of lead cause immune impairment in *Anguilla* [35]. Interestingly, after Cu^{2+} stress for 48 h and Pb^{2+} stress for 24 h, *GeCu/Zn-SOD* expression was significantly downregulated compared with the control group. Similar results were also observed in *Esomus detritus* [36]. These results may be related to Cu^{2+} promoting ROS overproduction and destroying SOD enzyme through the Fenton reaction [37]. Overall, Cu^{2+} stress and Pb^{2+} stress elicited oxidative stress, and the expression of *Cu/Zn-SOD* can protect organisms against future severe stress situations and it was more strongly under Pb^{2+} than Cu^{2+}. Also owing to the accumulation effect of heavy metals in fish being different, the expression of *Cu/Zn-SOD* shows certain tissue specificity.

Mn-SOD is one of the members of the first line of defense to remove ROS. Its expression and regulation play a crucial role in the maintenance of equilibrium redox homeostasis [38]. Studies have shown that the mRNA level of *Mn-SOD* generally increases in fish and mammals during metal ion or heat stress [39–41]. Under Cu^{2+}, Cd^{2+}, or Pb^{2+} stress, the expression of *Mn-SOD* was significantly upregulated in the gills, liver, and kidneys and showed a certain time dependence [14, 41, 42]. Similar results were found in this study, where both *GeCu/Zn-SOD* and *GeMn-SOD* were susceptible to Cu^{2+} and Pb^{2+}, and the response rate of *GeCu/Zn-SOD* and *GeMn-SOD* in the liver was faster than that in the gills and kidneys, which indicated that SODs could be useful as possible biomarkers for monitoring heavy metal contamination [43].

Heat shock proteins (HSPs) have been widely found to play an important role in protecting organisms from heat stress through combining heat shock factor 1 (HSF1) with heat shock elements (HSEs) to upregulate *HSP90* [44, 45]. Meanwhile, the expression of HSP90 is regulated by salinity, reduced oxygen level, food scarcity, and heavy metals [44–46]. Interestingly, according to earlier research, *HSP90* expression showed a time and concentration dependence under stress conditions. For example, after Cu^{2+} stress and Cd^{2+} stress for 30 days, Hsp90 mRNA approximately increased and reached maximum levels at 10 days and 15 days, respectively [47]. Similarly, previous studies indicate that the expression of *HSP90* under salt stress was upregulated from 0 to 40 ppt and reached a peak at 40 ppt in the muscle of *Huso dauricus* [44]. Here, *GeHsp90* expression was upregulated in the liver after 0.01 mg/L of Cu^{2+} stress, which consists of the previous study that significantly upregulated in the liver of fish after exposure to Cr^{6+} [48]. While, *GeHsp90* expression in the kidney was upregulated and downregulated after reaching the peak after 0.05 mg/L of Pb^{2+} stress. In addition, compared with Cu^{2+} stress, the expression of *GeHsp90* mRNA was more obvious in the gills, liver, and kidneys after Pb^{2+} stress. *HSP90* mRNA expression after heavy metal ion stress has certain tissue specificity and concentration dependence.

Metallothioneins (MTs) are small proteins rich in cysteine amino acids, which are responsible for the regulation and transportation of metal ions such as cadmium, copper, and zinc in living organisms [49]. Research has found that MT plays a crucial role in regulating essential metal levels within cells, preventing metal toxicity, and carrying out necessary physiological functions such as antioxidant activity and immune modulation [50]. In addition, MT

expression has been associated with different diseases, such as cancer, cardiovascular ailments, and neurodegenerative disorders [51]. Cadmium (Cd) is known to be one of the most potent inducers of metallothioneins (MTs) in various organisms including fish and has long been used as a model metal for studying MT induction [52]. Studies conducted in *Kryptolebias marmoratus*, a species of hermaphroditic fish, have shown that exposure to trace levels of cadmium can cause a strong induction of MT expression in a dose-dependent manner [53]. Here, the response strength of *GeMT* in the gills to Cu^{2+} stress was greater than that to Pb^{2+} stress. However, the response intensity of *GeMT* to Cu^{2+} stress and Pb^{2+} stress in both the kidney and the liver was very high, but the response mode was different. In general, the response of fish to heavy metal exposure varies depending on many different factors, including the species of fish, the duration and degree of exposure, as well as other environmental factors such as water temperature, pH, and dissolved oxygen levels [54]. Different species of fish may have different sensitivities to heavy metals and may respond differently to exposure, and the same species of fish may respond differently depending on the duration and degree of the exposure.

5. Conclusion

The objective of this research was to comprehensively evaluate water quality and heavy metal concentrations in the upper reaches of the Yellow River and its tributaries in Qinghai province. In addition, we sought to investigate the expression patterns of *GeCu/Zn-SOD*, *GeMn-SOD*, *GeHsp90*, and *GeMT* in response to exposure to 0.01 mg/L of Cu^{2+} and 0.05 mg/L of Pb^{2+} within the tissues of *Gymnocypris eckloni* (*G. eckloni*). Our findings reveal that, despite occasional instances of Cu^{2+} and Pb^{2+} levels exceeding recommended limits, the overall water quality of the Yellow River meets the Water Quality Standard. In response to Cu^{2+} and Pb^{2+} exposure, specific genes in *G. eckloni* were activated to combat oxidative stress. Notably, *GeCu/Zn-SOD*, *GeMn-SOD*, *GeHsp90*, and *GeMT* displayed heightened sensitivity to heavy metal stress in distinct tissues. Under Cu^{2+} stress, *GeCu/Zn-SOD*, *GeMn-SOD*, and *GeHsp90* exhibited more pronounced responses in the liver, while *GeMT* responded predominantly in the kidney. Conversely, under Pb^{2+} stress, *GeCu/Zn-SOD* and *GeMn-SOD* showed stronger reactions in the kidney, while *GeHsp90* and *GeMT* displayed greater sensitivity in the liver. Intriguingly, the response of *GeCu/Zn-SOD*, *GeMn-SOD*, and *GeMT* to Pb^{2+} stress exceeded their response to Cu^{2+} stress. These findings indicate that *GeCu/Zn-SOD*, *GeMn-SOD*, *GeHsp90*, and *GeMT* demonstrate tissue and heavy metal specificity, making them valuable biomarkers for monitoring pollution levels in the Yellow River.

Data Availability

The data used to support the findings of this study are included within the article.

Ethical Approval

All experiments involving *Gymnocypris eckloni* were conducted in compliance with the relevant regulations of the Science and Technology Ethics Committee of Qinghai University (Approval no. SL-2021029).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Wenjie Jin, Jing Zhao, Zixuan Li, and Changzhong Li carried out the experiment design, verification, and manuscript writing. Rong Wang, Zifeng Zhao, Huayu Gou, Lanying Li, and Anbin Xie were responsible for water sample monitoring and experimental fish farming. Haotian Ren, Bo Qiu, and Xiaodie Li carried out sample collection and experiment. Yanxia Chen, Zhenji Wang, and Guojie Wang provided experimental animals and provided technical support for the smooth development of the experiment. Jing Zhao, Zixuan Li, and Changzhong Li contributed equally to this paper and are the co-first authors. The authors agreed to data publication and informed about the publisher rules and terms.

Acknowledgments

The authors would like to thank Mr. Zhengang Wang and Mr. Baojun Lu from the Fisheries Environmental Monitoring Station (Xining, China) for their assistance in specimen collection and transportation. The teaching staff and graduate students from the College of Eco-Environmental Engineering, Qinghai University, who participated in this NSFC project, are also greatly appreciated. The research project received financial support from the National Natural Science Foundation of China (NSFC, 31460700) and the Youth Project of the Natural Science Foundation of Qinghai (2024-ZJ-994).

References

[1] Y. F. Wu and C. Z. Wu, *The Fishes of the Qinghai-Xizang Plateau*, Sichuan Science and Technology Publishing House, Chengdu, China, 1992.

[2] Y. F. Chen and W. X. Cao, *Schizothoracinae in Fauna Sinica, Osteichthyes, Cypriniformes III*, P. Q. Yue, Ed., Science Press, Beijing, China, 2000.

[3] Z. X. Shen, G. J. Wang, K. M. Li, S. L. Jian, Y. Q. Chen, and H. T. Guan, "The situation and protection measures of fish resources in upstream of longyang valley of the Yellow River," *Journal of Hydroecology*, vol. 35, pp. 70–76, 2014.

[4] N. Singh, V. K. Gupta, A. Kumar, and B. Sharma, "Synergistic effects of heavy metals and pesticides in living systems," *Frontiers in Chemistry*, vol. 5, p. 70, 2017.

[5] M. Shahjahan, K. Taslima, M. S. Rahman, M. Al-Emran, S. I. Alam, and C. Faggio, "Effects of heavy metals on fish physiology-A review," *Chemosphere*, vol. 300, Article ID 134519, 2022.

[6] M. Y. Elgendy, S. E. Ali, W. T. Abbas, A. M. Algammal, and M. Abdelsalam, "The role of marine pollution on the emergence of fish bacterial diseases," *Chemosphere*, vol. 344, Article ID 140366, 2023.

[7] J. Feng, X. Y. Yue, and Z. G. Feng, "Pathology of experimental Cd poisoning in Broiler chickens," *Chinese Journal of Animal and Veterinary Sciences*, vol. 5, pp. 468–475, 2001.

[8] J. Abah, S. T. Ubwa, D. I. Onyejefu, and S. A. Nomor, "Assessment of some trace metals content of oreochromis niloticus obtained from river okpokwu, apa benue state, Nigeria," *Research Journal of Chemical Sciences*, vol. 3, no. 3, pp. 70–75, 2013.

[9] S. Murakami, I. Miyazaki, N. Sogawa, K. Miyoshi, and M. Asanuma, "Neuroprotective effects of metallothionein against rotenone-induced myenteric neurodegeneration in parkinsonian mice," *Neurotoxicity Research*, vol. 26, no. 3, pp. 285–298, 2014.

[10] X. Xu, L. Duan, J. W. Yu et al., "Characterization analysis and heavy metal-binding properties of Cs MTL 3 in Escherichia coli," *FEBS Open Bio*, vol. 8, no. 11, pp. 1820–1829, 2018.

[11] M. Wang, Y. J. Xu, S. X. Pan et al., "Long-term heavy metal pollution and mortality in a Chinese population: an ecologic study," *Biological Trace Element Research*, vol. 142, no. 3, pp. 362–379, 2011.

[12] L. P. Chen, L. Ma, Q. Bai et al., "Heavy metal-induced metallothionein expression is regulated by specific protein phosphatase 2A complexes," *Journal of Biological Chemistry*, vol. 289, no. 32, pp. 22413–22426, 2014.

[13] Q. B. Lin, S. R. Liao, Y. H. Xiong, and L. E. Xue-Yi, "Progress in the study and application of superoxide dismutases," *Chemical World*, vol. 47, pp. 378–381, 2006.

[14] Y. S. Cho, S. Y. Lee, I. C. Bang, D. S. Kim, and Y. K. Nam, "Genomic organization and mRNA expression of manganese superoxide dismutase (Mn-SOD) from Hemibarbus mylodon (Teleostei, Cypriniformes)," *Fish and Shellfish Immunology*, vol. 27, no. 4, pp. 571–576, 2009.

[15] J. H. Kim and J. C. Kang, "Effects of sub-chronic exposure to lead (Pb) and ascorbic acid in juvenile rockfish: antioxidant responses, MT gene expression, and neurotransmitters," *Chemosphere*, vol. 171, pp. 520–527, 2017.

[16] J. J. Cao, X. Miao, W. Xu, J. Li, W. B. Zhang, and K. S. Mai, "Dietary copper requirements of juvenile large yellow croaker Larimichthys croceus," *Aquaculture*, vol. 432, pp. 346–350, 2014.

[17] D. M. GatlinIII and R. P. Wilson, "Dietary copper requirement of fingerling channel catfish," *Aquaculture*, vol. 54, no. 4, pp. 277–285, 1986.

[18] B. M. Dietz, D. Liu, G. K. Hagos et al., "Angelica sinensis and its alkylphthalides induce the detoxification enzyme NAD(P)H: quinone oxidoreductase 1 by alkylating Keap1," *Chemical Research in Toxicology*, vol. 21, no. 10, pp. 1939–1948, 2008.

[19] X. Jing, D. M. Ren, X. B. Wei et al., "Eriodictyol-7-O-glucoside activates Nrf2 and protects against cerebral ischemic injury," *Toxicology and Applied Pharmacology*, vol. 273, no. 3, pp. 672–679, 2013.

[20] J. W. Kaspar and A. K. Jaiswal, "An autoregulatory loop between Nrf2 and Cul3-Rbx1 controls their cellular abundance," *Journal of Biological Chemistry*, vol. 285, no. 28, pp. 21349–21358, 2010.

[21] F. Rusnak and P. Mertz, "Calcineurin: form and function," *Physiological Reviews*, vol. 80, no. 4, pp. 1483–1521, 2000.

[22] T. D. Schmittgen and K. J. Livak, "Analyzing real-time PCR data by the comparative C (T) method," *Nature Protocols*, vol. 3, no. 6, pp. 1101–1108, 2008.

[23] C. Z. Li, F. X. Ran, Z. X. Li et al., "Calcineurin immune signaling in response to zinc challenge in the naked carp Gymnocypris eckloni," *Bulletin of Environmental Contamination and Toxicology*, vol. 106, no. 5, pp. 792–798, 2021.

[24] W. Deng, Y. Wang, Z. Liu, H. Cheng, and Y. Xue, "HemI: a toolkit for illustrating heatmaps," *PLoS One*, vol. 9, no. 11, 2014.

[25] J. J. Liu, Z. J. Lai, F. Y. Deng, S. S. Zhou, L. Y. Zhang, and Y. Liu, "Heavy metals contents and speciation in surface sediments from gansu, ningxia and inner Mongolia sections of the Yellow River of China," *Spectroscopy and Spectral Analysis*, vol. 35, no. 10, pp. 2963–2969, 2015.

[26] X. P. Wang, X. J. Liu, L. Q. Wang, J. Yang, X. M. Wan, and T. Liang, "A holistic assessment of spatiotemporal variation, driving factors, and risks influencing river water quality in the northeastern Qinghai-Tibet Plateau," *Science of the Total Environment*, vol. 851, Article ID 157942, 2022.

[27] S. Salati and F. Moore, "Assessment of heavy metal concentration in the Khoshk River water and sediment, Shiraz, Southwest Iran," *Environmental Monitoring and Assessment*, vol. 164, no. 1-4, pp. 677–689, 2010.

[28] J. Ren, J. X. Hao, and L. Tao, "Concentrations, spatial distribution, and pollution assessment of heavy metals in surficial sediments from upstream of Yellow River, China," *Environmental Science and Pollution Research*, vol. 28, no. 3, pp. 2904–2913, 2021.

[29] J. L. Zheng, L. Zeng, M. Y. Xu, B. Shen, and C. W. Wu, "Different effects of low- and high-dose waterborne zinc on Zn accumulation, ROS levels, oxidative damage and antioxidant responses in the liver of large yellow croaker Pseudosciaena crocea," *Fish Physiology and Biochemistry*, vol. 43, no. 1, pp. 153–163, 2017.

[30] Q. L. Zhu, W. Y. Li, and J. L. Zheng, "Life-cycle exposure to cadmium induced compensatory responses towards oxidative stress in the liver of female zebrafish," *Chemosphere*, vol. 210, pp. 949–957, 2018.

[31] J. Qiang, F. Y. Tao, W. J. Bao et al., "miR-489-3p regulates the oxidative stress response in the liver and gill tissues of hybrid yellow catfish (Pelteobagrus fulvidraco♀ × P. vachelli♂) under Cu^{2+} exposure by targeting Cu/Zn-SOD," *Frontiers in Physiology*, vol. 10, p. 868, 2019.

[32] H. I. Falfushynska, L. L. Gnatyshyna, O. B. Stoliar, and Y. K. Nam, "Various responses to copper and manganese exposure of Carassius auratus gibelio from two populations," *Comparative Biochemistry and Physiology-Part C: Toxicology and Pharmacology*, vol. 154, no. 3, pp. 242–253, 2011.

[33] K. Lyu, X. Zhu, Q. Wang, Y. Chen, and Z. Yang, "Copper/zinc superoxide dismutase from the Cladoceran Daphnia magna: molecular cloning and expression in response to different acute environmental stressors," *Environmental Science and Technology*, vol. 47, no. 15, pp. 8887–8893, 2013.

[34] T. C. Pereira, M. M. Campos, and M. R. Bogo, "Copper toxicology, oxidative stress and inflammation using zebrafish as experimental model," *Journal of Applied Toxicology*, vol. 36, no. 7, pp. 876–885, 2016.

[35] N. Shah, A. Khan, R. Ali et al., "Monitoring bioaccumulation (in gills and muscle tissues), hematology, and genotoxic alteration in Ctenopharyngodon idella exposed to selected heavy metals," *BioMed Research International*, vol. 2020, Article ID 6185231, 16 pages, 2020.

[36] S. Vutukuru, S. Chintada, K. Radha Madhavi, J. Venkateswara Rao, and Y. Anjaneyulu, "Acute effects of copper on superoxide dismutase, catalase and lipid peroxidation in the

freshwater teleost fish, Esomus danricus," *Fish Physiology and Biochemistry*, vol. 32, no. 3, pp. 221–229, 2006.

[37] A. P. Francisco, P. H. R. Aride, A. B. de Souza, M. F. Polese, H. D. Lavander, and L. C. Gomes, "Toxicity of copper in Epinephelus marginatus (perciformes; serranidae) after ingestion of contaminated food," *Bulletin of Environmental Contamination and Toxicology*, vol. 105, no. 5, pp. 711–714, 2020.

[38] J. D. Zhang, H. J. Li, E. Amenyogbe, W. Z. Wang, J. S. Huang, and G. Chen, "Cloning of Mn-SOD gene and its mRNA expression difference and antioxidant enzyme activities under hypoxia stress of cobia Rachycentron canadum," *Molecular Biology Reports*, vol. 48, no. 10, pp. 6897–6909, 2021.

[39] K. Y. Kim, S. Y. Lee, Y. S. Cho et al., "Molecular characterization and mRNA expression during metal exposure and thermal stress of copper/zinc- and manganese-superoxide dismutases in disk abalone, Haliotis discus discus," *Fish and Shellfish Immunology*, vol. 23, no. 5, pp. 1043–1059, 2007.

[40] A. Elbaz, Y. Y. Wei, Q. Meng, Q. Zheng, and Z. M. Yang, "Mercury-induced oxidative stress and impact on antioxidant enzymes in Chlamydomonas reinhardtii," *Ecotoxicology*, vol. 19, no. 7, pp. 1285–1293, 2010.

[41] S. Somasundaram, J. S. Abraham, S. Maurya, R. Toteja, R. Gupta, and S. Makhija, "Expression and molecular characterization of stress-responsive genes (hsp70 and Mn-sod) and evaluation of antioxidant enzymes (CAT and GPx) in heavy metal exposed freshwater ciliate, Tetmemena sp," *Molecular Biology Reports*, vol. 46, no. 5, pp. 4921–4931, 2019.

[42] J. Wu, M. Bao, D. Ge et al., "The expression of superoxide dismutase in Mytilus coruscus under various stressors," *Fish and Shellfish Immunology*, vol. 70, pp. 361–371, 2017.

[43] K. Holovska, V. Lenartova, and M. Kicinkova, "SOD isoenzymes as possible biomarkers of enviromental pollution," *Chemical Papers-Slovak Academy of Sciences*, vol. 52, 1998.

[44] G. G. Peng, W. Zhao, Z. G. Shi et al., "Cloning HSP70 and HSP90 genes of kaluga (Huso dauricus) and the effects of temperature and salinity stress on their gene expression," *Cell Stress and Chaperones*, vol. 21, no. 2, pp. 349–359, 2016.

[45] H. T. Yu, Z. J. Yang, M. Y. Sui et al., "Identification and characterization of HSP90 gene family reveals involvement of HSP90, GRP94 and not TRAP1 in heat stress response in Chlamys farreri," *Genes*, vol. 12, no. 10, p. 1592, 2021.

[46] A. Ali, P. H. Krone, D. S. Pearson, and J. J. Heikkila, "Evaluation of stress-inducible hsp90 gene expression as a potential molecular biomarker in Xenopus laevis," *Cell Stress and Chaperones*, vol. 1, pp. 62–69, 1996.

[47] H. H. Liu, J. Wu, M. S. Xu, and J. Y. He, "A novel biomarker for marine environmental pollution of HSP90 from Mytilus coruscus," *Marine Pollution Bulletin*, vol. 111, no. 1-2, pp. 428–434, 2016.

[48] Z. Yu, S. F. Xu, J. L. Zhao, L. Zhao, A. Z. Zhang, and M. Y. Li, "Toxic effects of hexavalent chromium (Cr^{6+}) on bioaccumulation, apoptosis, oxidative damage and inflammatory response in Channa asiatica," *Environmental Toxicology and Pharmacology*, vol. 87, Article ID 103725, 2021.

[49] C. D. Klaassen, "Metallothionein and its role in toxic metal regulation," *Toxicology and Applied Pharmacology*, vol. 273, pp. 713–722, 2013.

[50] M. P. Waalkes, "Metallothionein and toxicology: a biologically conserved response to stress?" *Environmental Health Perspectives*, vol. 108, pp. 17–20, 2000.

[51] M. Si and J. Lang, "The roles of metallothioneins in carcinogenesis," *Journal of Hematology and Oncology*, vol. 11, no. 1, p. 107, 2018.

[52] B. Ruttkay-Nedecky, L. Nejdl, J. Gumulec et al., "The role of metallothionein in oxidative stress," *International Journal of Molecular Sciences*, vol. 14, no. 3, pp. 6044–6066, 2013.

[53] J. H. Lee, J. G. Kim, and J. S. Lee, "Cadmium-induced oxidative stress and metallothionein expression in the hermaphroditic fish Kryptolebias marmoratus," *Comparative Biochemistry and Physiology-Part C: Toxicology and Pharmacology*, vol. 143, pp. 322–329, 2006.

[54] D. Schlenk and W. H. Benson, "Target organ toxicity of trace metals in fish: effects of copper, cadmium, and zinc on the gill, liver, and kidney of the bluegill sunfish (Lepomis macrochirus)," *Environmental Research*, vol. 103, pp. 178–186, 2007.