Toxic effects of heavy metal Cu^{2+} on the pacific oyster *Crassostrea gigas*

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Abstract. The effects of different concentrations of heavy metal ions on the survival of the Pacific oyster *Crassostrea gigas* were studied by using experimental ecology method in 96 h. The results showed that the LC50 of copper ion was 21.748mg/L and the safe concentration was 2.1748mg/L mg/L. Under the condition of laboratory, under laboratory conditions, the research of Cu^{2+} Stress on the *C. gigas* gill and digestive gland and adductor muscle tissue SOD, GPx and the induction of CAT activity. The results showed that the activities of SOD, GPx and CAT in the *C. gigas* were significantly changed by copper ion + stress. The results showed that in the low concentration Cu^{2+} treatment could induce the three kinds of enzymes, in the high concentration Cu^{2+} treatment group, SOD and CAT and GPx on the inhibition of the effect. The sensitivity of the three antioxidant enzymes to copper ion showed a certain difference. The sensitivity of the three kinds of tissue enzymes to Cu^{2+} treatment was digestive gland> fascia> gill. The experimental results show that the single factor for copper in water pollutants, the *C. gigas* digestive gland tissue SOD, GPX and CAT activity has certain significance to it, but will it as index applied to the actual water need further study.

1. Introduction

Crassostrea gigas, commonly known as oyster, is the world's largest aquaculture shellfish, is also one of China's well-known aquaculture octopus one of China's *C. gigas* farming in the world's first production [1]. *C. gigas* have a very high accumulation of heavy metals, which are 1 to 2 orders of magnitude higher than other aquatic organisms [2-3]. Under normal circumstances, living in heavy metal pollution in the sea area of *C. gigas*, the body will enrich the relatively high concentrations of heavy metals. As a result, many countries have adopted *C. gigas* as indicators of organisms, which are used to monitor marine heavy metal pollution [4]. Studies have shown that domestic *C. gigas* in the presence of heavy metals in varying degrees, reflecting the existence of heavy environmental pollution in the breeding environment [5-6]. Although Cu is a necessary nutrient in the body, but when the body absorbs excess Cu, the organism will cause poisoning [7]. In this study, gill and digestive gland tissues and adductor tissue of *C. gigas* were selected as the object of study. The gills were directly exposed to the tissues of the water bodies. The digestive glands were the main detoxification organs in the body. The effects of copper stress on the physiological status of the two tissues were and the possible mechanism was discussed. In order to screen out the biomarkers this could reflect the representative

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and high sensitivity of lead in the sea area of Jiaozhou Bay, and to explore the possible differences in the antioxidant responses of different tissues.

2. Materials and methods

2.1. Experimental materials

The experimental use of *C. gigas* was purchased from Yantai near the sea oyster farms freshly caught just for less than 12 hours. Before the experiment, *C. gigas* were washed thoroughly before the experiment, and the attachments were removed and placed in a large culture box of 0.8 × 0.5 × 0.8 (m) for about 3 days, domesticated during the feeding without feeding, day and night continuous oxygenation.

C. gigas will be starved for 3 days, the larvae were treated with semi-static water for 7 days, and the culture density was 50-60 individuals/m^3, continuous inflatable, the culture temperature was (20±1)℃, the ratio of light to dark was 12h: 12h, and control the light intensity is below 10 μmol·m^{-2}·s^{-1}, the salinity of the aquaculture water is 31, the pH is 8.0, and the water is changed at a fixed time every day. Daily feeding the right amount of bait, bait for the algae and algae Qinghai mixed. After holding for 7 days, the healthy individuals with the length of 7-8 cm and the body weight of 16-19 g were selected for the experiment.

Experimental contaminants were aqueous lead nitrate solution with a mother liquor concentration of 10 mg/ml. Dose into the set concentration.

2.2. Experimental methods

2.2.1. Acute toxicity test. After the pre-test to determine the experimental drug mass concentration range (96 h after the maximum concentration of live and the total lethal concentration limit), According to the logarithmic interval, five concentration groups were determined to be 15.00 mg/L, 20.25 mg/L, 27.34 mg/L, 36.91 mg/L, 50.00 mg/L, one control groups were set up, each group has three parallel samples.

The experimental containers were 3 L glass cylinders and each group was randomly placed in 50 domesticated *C. gigas* and cultured in a semi-static manner. During the experiment, the water was changed every 24h. After adding water, the concentration of Cu^{2+} solution was kept constant, and the dead individuals were removed at any time. For the long-time *C. gigas*, the individuals who did not respond within 5 min And the number of deaths in each group of *C. gigas* at 24 h, 48 h, 72 h and 96 h were recorded.

2.2.2. Determination of antioxidant enzyme activity. According to the results of acute toxicity test, two experimental gradients, namely 0.04 mg/L (low concentration treatment group) and 0.1 mg/L (high concentration treatment group) were designed. The natural seawater was used as blank control group. And the concentration of water in each concentration group was 3L. Thirty domesticated C. gigas were randomly distributed with the same method. The experimental time was 25 days. The samples were sampled at 0 d, 3 d, 7 d, 12 d, 18 d, and 25 d, respectively, and the samples were tested in parallel for the antioxidant enzyme activity of C. gigas. No oyster died during the experiment.

2.3. Determination of enzyme activity

2.3.1. Sampling and sample pretreatment. Two oysters were randomly selected from each of the two experimental groups. The capsules, gills and digestive gland tissues were preserved in liquid nitrogen. Before the measurement, approximately 0.5g of the samples were taken from the caudal muscle, the gill and the digestive gland, and 1: 3 (w/v) of the pre-cooled 0.86% saline was added to the pre-cooled glass homogenizer. Ice bath homogenate 6~8 min; centrifuge at 10 000rpm for 20 min at 4°C. Take the supernatant and store it in a refrigerator at -80°C for activity determination of SOD, GPx and CAT.

2.3.2. Determination of the activity of peroxidase GPX. Use the Nanjing Institute of Bioengineering Institute of the determination of the kit.

According to GPx can promote the reaction of H_2O_2 and GSH to produce H_2O and GSSG, so the activity of GPx can be expressed by the speed of its digestion, and the activity of GSH can be obtained by measuring the consumption of GSH in this enzymatic reaction. The activity unit of GPx in the tissue is defined as the tissue protein per milligram, and the non-enzymatic reaction is deducted per minute. The concentration of GSH in the reaction system is reduced by 1 μmo/L to one enzyme activity unit.

2.3.3. Catalytic activity of catalase CAT. Use the Nanjing Institute of Bioengineering Institute of the determination of the kit.

The reaction of catalase decomposition and H_2O_2 can be quickly stopped by the addition of ammonium moly date, and the remaining H_2O_2 reacts with ammonium moly date to produce a complex that defines the amount of H_2O_2 decomposed by 1umol per gram of tissue protein for 1 hour as a viable unit (U).

2.4. Data processing

The experimental data were analysed by spss 13.0 and plotted with sigma plot 10.0. Using spss13.0 statistical software, the probability of the concentration of Cu^{2+} as the abscissa and the probability of death of *C. gigas* was plotted as the ordinate, and the semi-lethal concentration and 95% confidence interval of 96h were calculated and the safety concentration and the calculation Half of the lethal concentration[8]. The results were expressed as mean ± standard error (mean ± SD); the data between groups were compared by double tailed test, $p < 0.05$, was considered significant difference; $p < 0.01$, was considered significant difference.

3. Experimental results

3.1. Acute Toxicity of Cu^{2+} to C. gigas

Table 1. Acute toxicity of $Cu^{2+}$to *C. gigas*

Cu^{2+} concentration (mg/L)	Stress time			
	24h	48h	72h	96h
	mortality rate %	mortality rate %	mortality rate %	mortality rate %
0	0.00	0.00	0.00	0.00
15.00	0.00	0.00	5.00	10.00
20.25	10.00	10.00	25.00	45.00
27.34	10.00	20.00	55.00	75.00
36.91	17.50	30.00	65.00	95.00
50.00	40.00	75.00	100.00	100.00

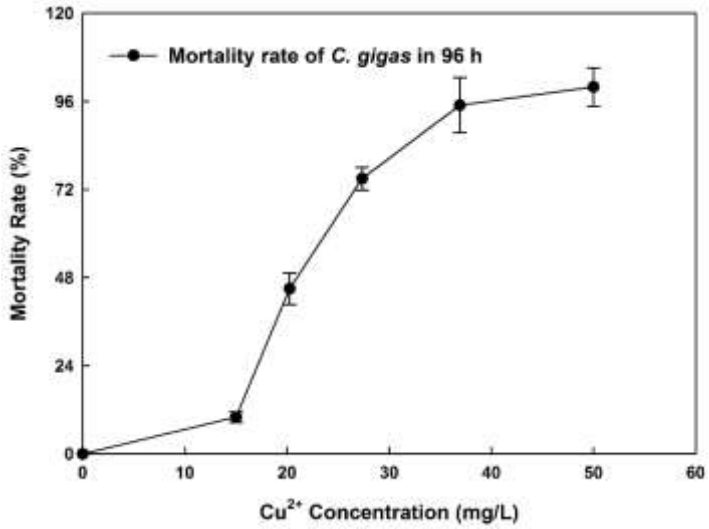


Fig. 1 Mortality rate of *C. gigas* under Cu^{2+} in 96 h

The mortality of *C. gigas* increased with the increase of Cu^{2+} concentration, the lowest concentration group (15.00 mg/L) within 72 h of the death of individuals, and the highest concentration group (50.00 mg/L) 24 h that is dead. Over time, the mortality rate increased by 50% at a dose of 50.00 mg/L at 96 h. The control group was normal and no death. Fig.1 shows the acute experimental toxicity curve of *C. gigas* 96 h. Linear regression analysis showed that the regression equation was Y = 7.434X - 9.942, where p <0.01 and half of the lethal concentration LC_{50} = 21.748 mg/L.

3.2. Effects of Copper on Antioxidant Enzyme Activities in Different Tissues of C. gigas

3.2.1. Effects on SOD activity

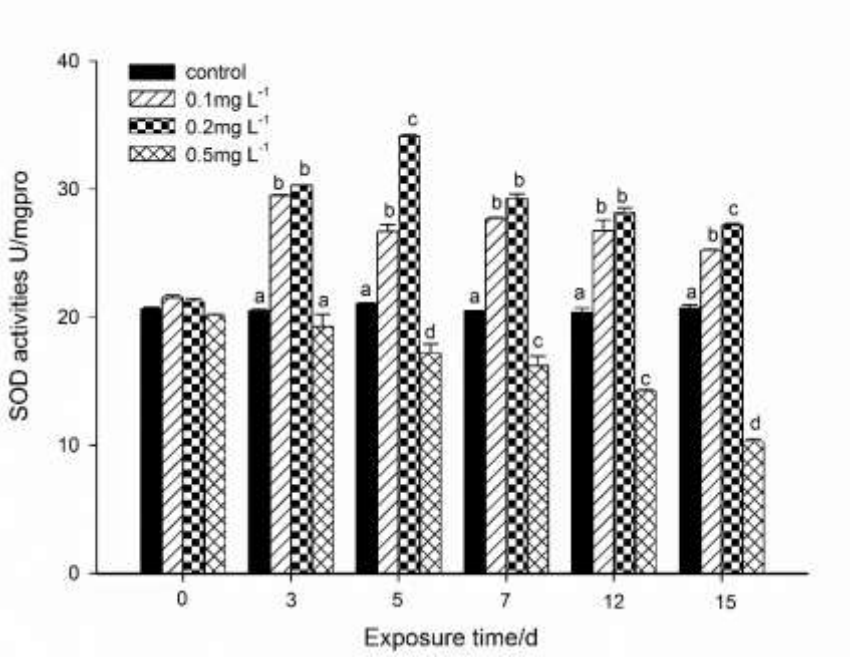


Fig. 2 Cu^{2+}effects on the SOD activity in the muscle tissue

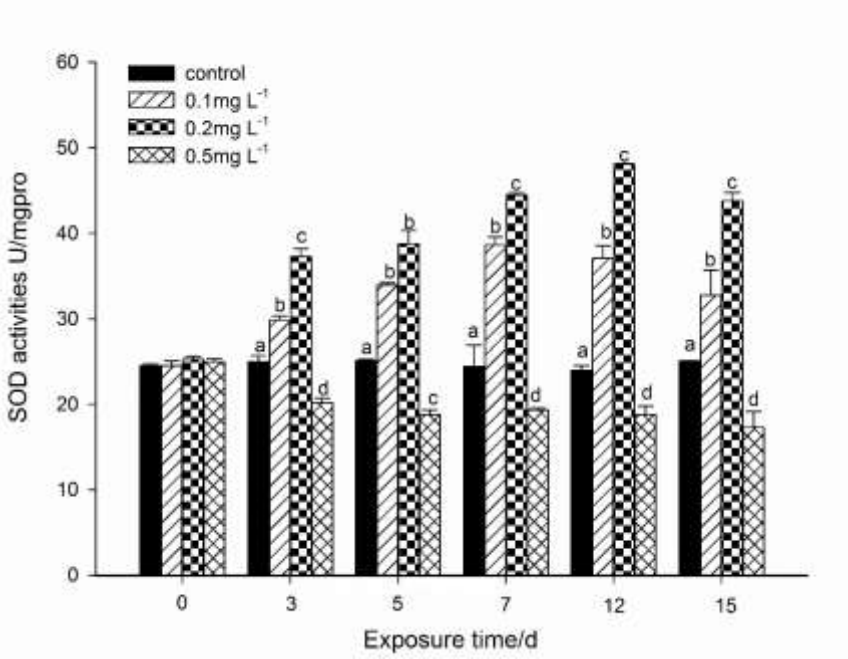


Fig. 3 Cu^{2+} effects on the SOD activity in the visceral mass

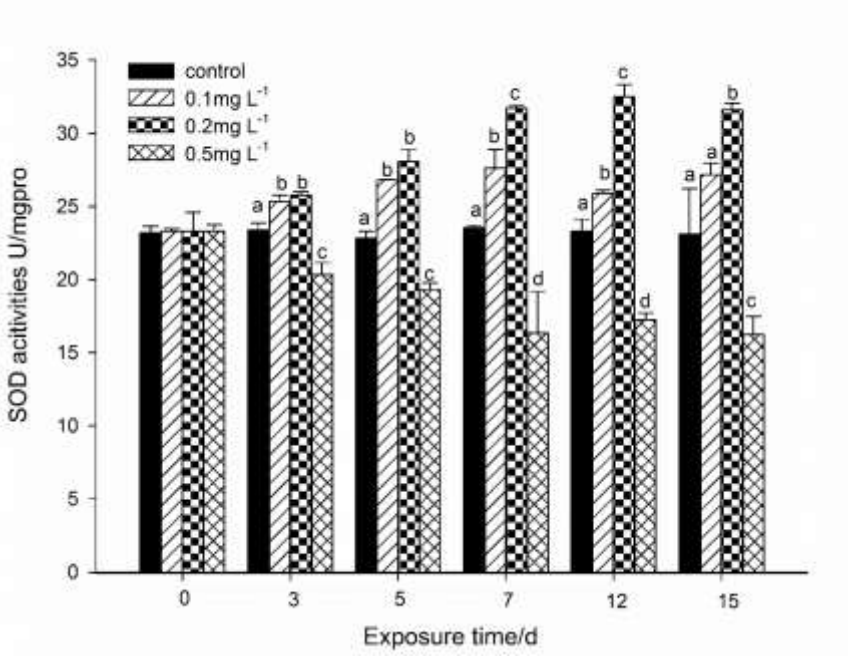


Fig. 4 Cu^{2+} effects on the SOD activity in the gill

It can be seen from Fig. 2-4 that, as time goes on, it can be seen from the above graph that the changes of SOD activity in the three tissues during the stress of Cu^{2+} against *C. gigas* are very different, Where the gill tissue is least sensitive to Cu^{2+}, digestive gland tissue sensitivity of the strongest, closed muscle tissue between the two. The activity of SOD in the digestive gland of *C. gigas* was significantly increased (p <0.05) under the low concentration (0.1 mg/L and 0.2 mg/L) Cu^{2+} stress, and the uptake was increasing in the first 25 days (p <0.05), but there was a trend of ascending and descending. The concentration of SOD in gills was changed, but the change was not as obvious as the above two tissues.

In the high concentration of Cu^{2+} (0.5 mg/L), the SOD activity of the three tissues decreased in different degrees.

3.2.2. Effects on GPX activity

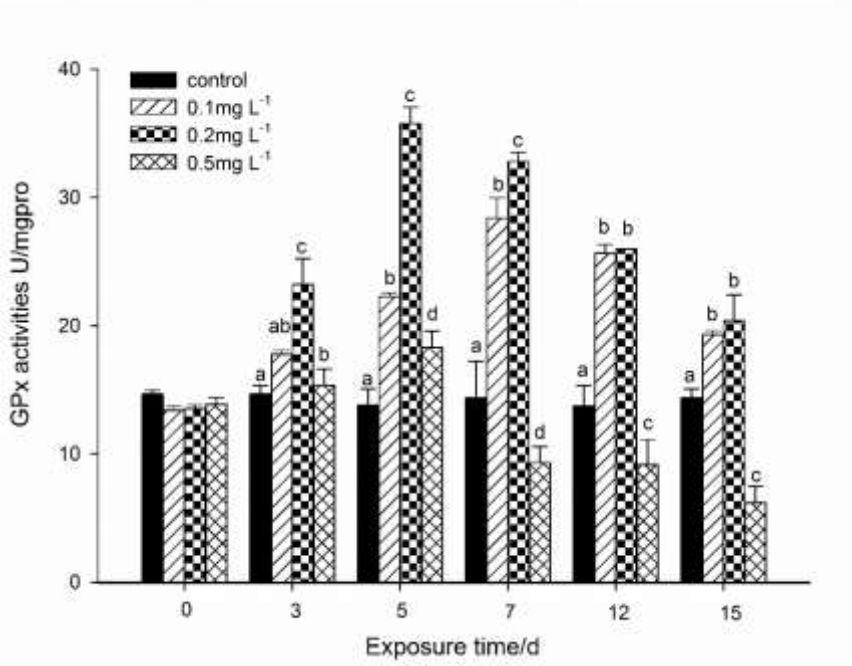


Fig. 5 Cu^{2+} effects on the GPx activity in the muscle tissue

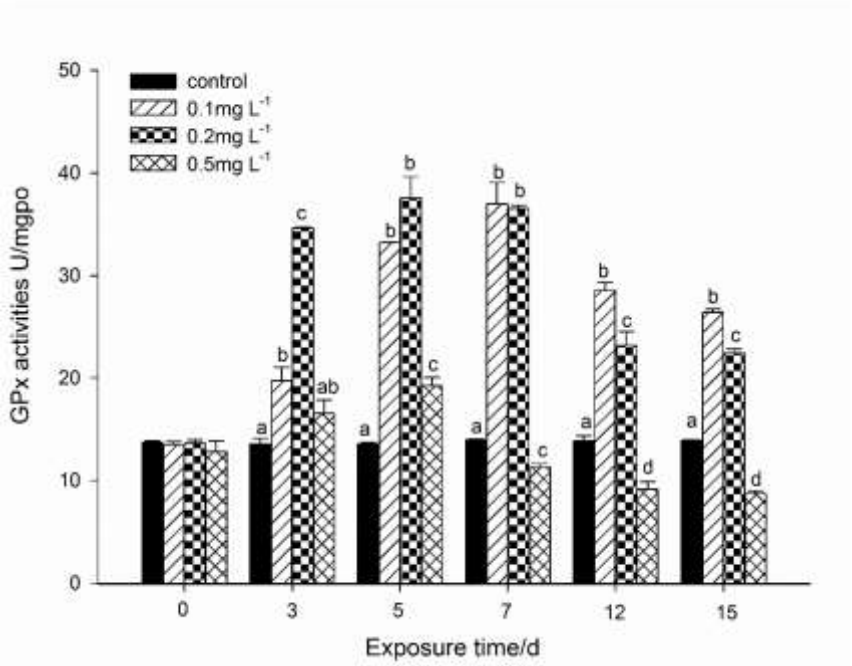


Fig. 6 Cu^{2+} effects on the GPx activity in the visceral mass

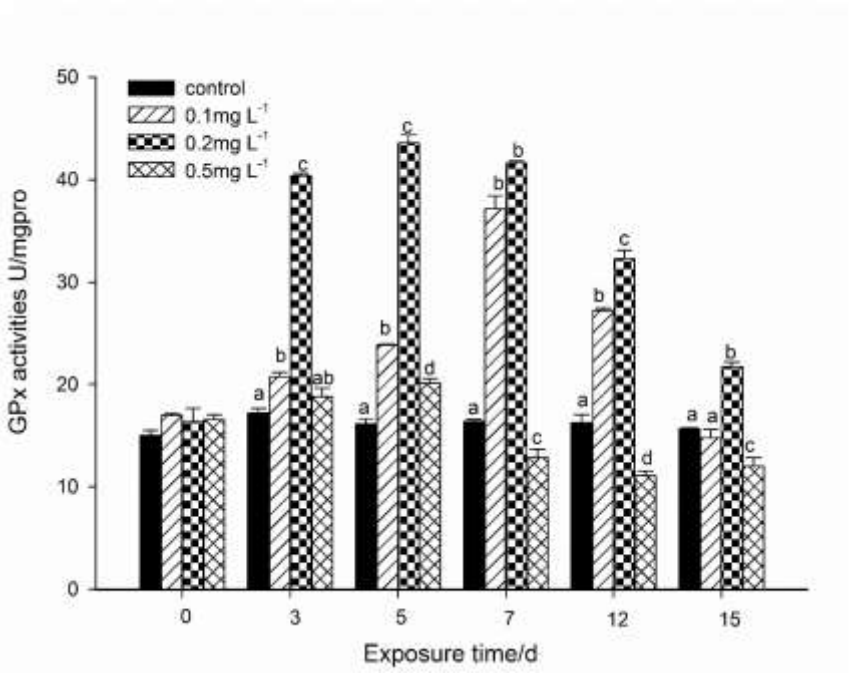


Fig. 7 Cu^{2+} effects on the GPx activity in the gill

The fluctuation of GPx activity in control organism of Fig. 5-7 was much higher than that of SOD and Cu^{2+} stress on the activity of GPx. The activity of GPx in digestive gland and cranial muscle and gill of *C. gigas* was significantly increased (p <0.05) under the stress of two low concentrations of Cu^{2+} (0.1 mg/L and 0.2 mg/L), and the initial stress was increased When the stress reaches a certain degree, it begins to fall again. In the high concentration (0.5 mg/L) Cu^{2+}, the GPx activity of the three tissues began to increase slightly. But soon fell below normal.

3.2.3. Effects on CAT activity

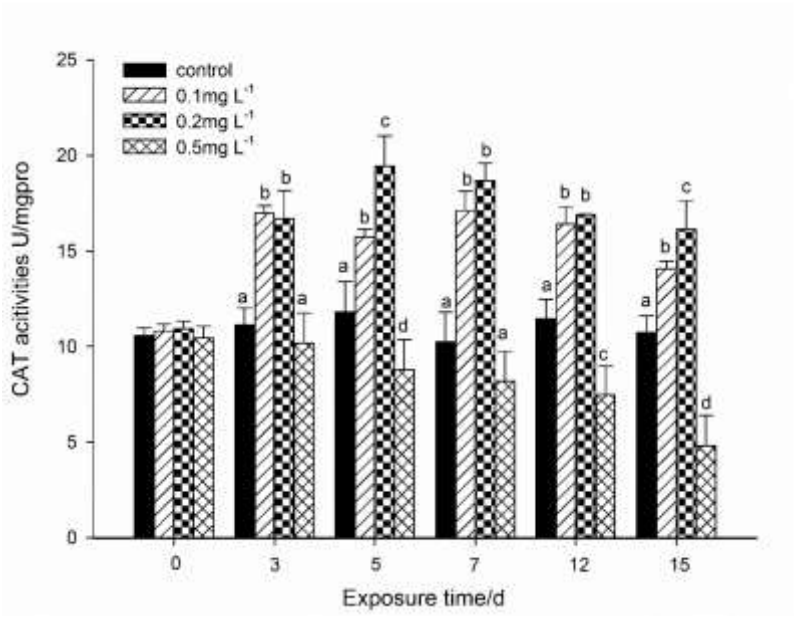


Fig. 8 Cu^{2+} effects on the CAT activity in the muscle tissue

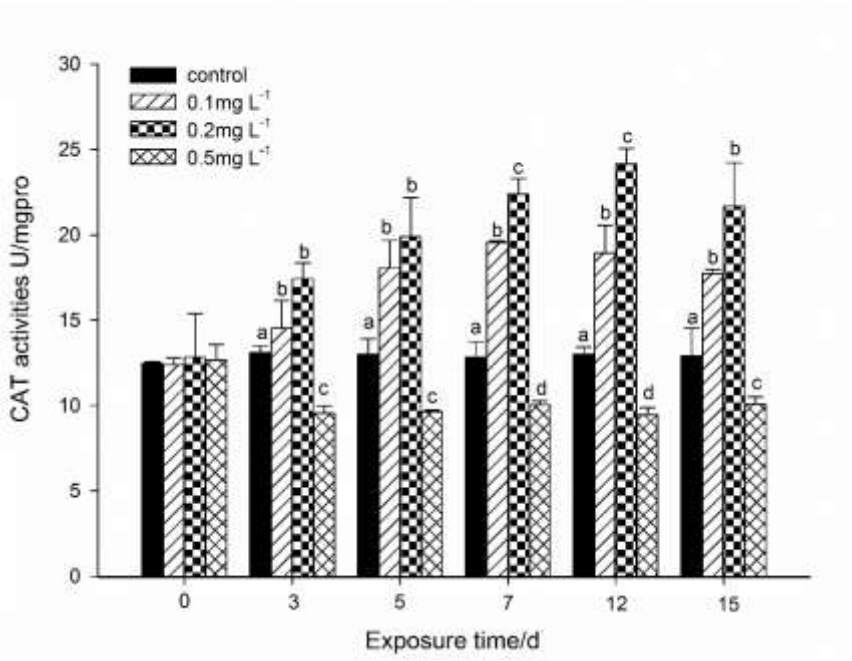


Fig. 9 Cu^{2+} effects on the CAT activity in the visceral mass

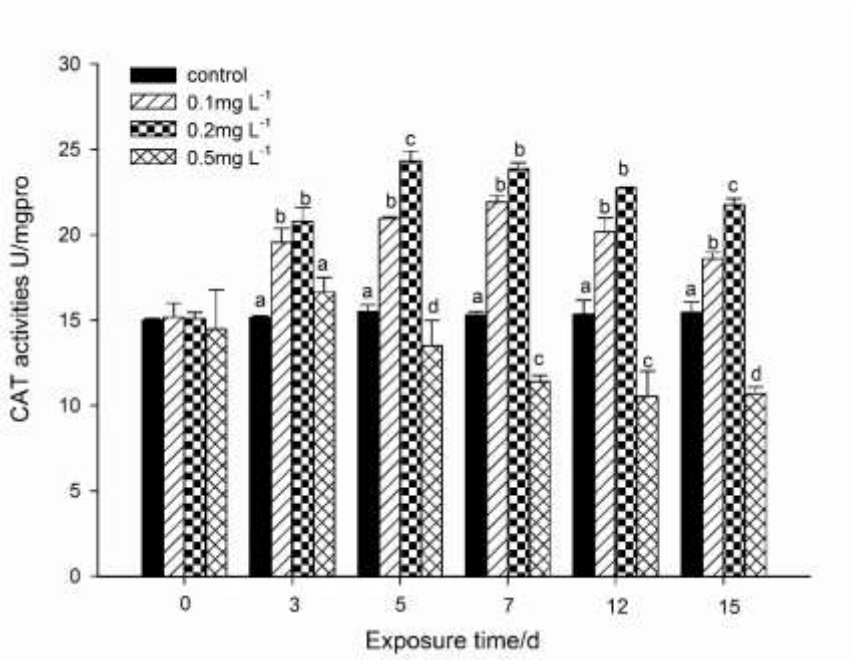


Fig. 10 Cu^{2+} effects on the CAT activity in the gill

It can be seen from Figure 8-10 that under Cu^{2+} stress, in the process of Cu^{2+} stress on *C. gigas*, the changes of CAT activity in the three tissues were very different, and the gill tissue is the least sensitive to Cu^{2+}, and the digestive gland the most sensitive, closed muscle tissue between the two. The activity of CAT in the digestive gland was significantly increased (p <0.05) under the stimulation of two low concentrations of Cu^{2+} (0.1 mg/L and 0.2 mg/L), in the first 25 days of metal accumulation stage has been on the rising trend, but there was a trend of ascending and descending (p <0.05). The concentration of CAT in the gills was changed, but the change was not as obvious as the above two

tissues. In the high concentration of Cu^{2+} (0.5 mg / L), the CAT activity of the three tissues decreased in different degrees.

4. Discussion

Studies have shown that dissolved oxygen, pH, temperature and other changes and heavy metal pollution will make the body of superoxide dismutase (SOD) activity significantly changed[9-10]. Therefore, for aquatic organisms, SOD is a class of sensitive molecular Eco toxicological indicators. In this experiment, Cu^{2+} can induce SOD activity to enhance the body's antioxidant capacity.

CAT catalyzes the formation of H2O, which has the same function as SOD, and cleanses the oxygen free radicals produced in the living body. Therefore, the activity of CAT is similar to that of SOD, which is related to the activity of SOD in the liver tissue CAT activity changes in the trend is the same [11].

Glutathione peroxidase (GPx) is one of the important active oxygen free radical scavengers in the body. When oysters are under Cu^{2+} stress, they produce a lot of peroxy radicals, and the peroxyl radicals are disrupted by superoxide (SOD), which is decomposed into H_2O_2, and the increase of H_2O_2 induces the increase of glutathione peroxidase (GPx) activity and also affects the activity of catalase (CAT) [12].

Oysters produce excess reactive oxygen species (ROS) in the body at the beginning of Cu^{2+} stress, and the amount of reactive oxygen species (ROS) produced at high concentrations is the largest and the lowest concentration is produced. Therefore, the levels of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activity in the gills, Glutathione peroxidase (GPX) activity levels are induced to increase due to increased activity of reactive oxygen species (ROS), which is consistent with the "toxic effects" between toxic contaminants and organisms. But the stress continues to proceed, with the passage of time, in the high copper concentration group, due to oyster body produced a large number of reactive oxygen species (ROS), when the production rate of these reactive oxygen species (ROS) exceeds the rate of clearance of antioxidant enzymes in oysters, the "toxic inhibitory effect" between toxic and stressed organisms is produced, reactive oxygen species (ROS) on the cells caused by the oxidation reaction, the cells of unsaturated fatty acids into saturated fatty acids, causing changes in cell structure and the destruction of some physiological life activities, causing cell aging and death, and then All three tissues and organs caused damage, so that the three antioxidants were so reduced and dropped below the control group level.

The antioxidant enzyme activities of different tissues showed a certain difference in the sensitivity of copper ions, and the sensitivity of the three tissues was digestive gland> hamster muscle> gills. Although the three kinds of tissues and organs of *C. gigas* have a certain "toxic excitatory effect", but "toxic excitatory effect" on the digestive gland tissue far more than the gill tissue. This has nothing to do with the gill tissue detoxification function.

The activity of SOD, GPX and CAT in the digestive gland of Pacific oyster has some indication for the single factor of copper pollutants in water, but it is necessary to apply it as an indicator to the actual aquaculture water.

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