



Responses of acid/alkaline phosphatase, lysozyme, and catalase activities and lipid peroxidation to mercury exposure during the embryonic development of goldfish *Carassius auratus*

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ABSTRACT

This study assessed the impact of mercury exposure on goldfish (*Carassius auratus*) embryos based on the dynamic characteristics of chemical parameters. Day-old embryos were exposed to different Hg^{2+} concentrations (0, 0.2, 1, 5, and 10 $\mu g/L$). Subsequently, the embryos were sampled every 24 h during embryonic development to measure acid phosphatase (ACP), alkaline phosphatase (AKP), lysozyme (LSZ), and catalase (CAT) activities, as well as malondialdehyde (MDA) content. The results revealed that the responses of ACP and AKP to mercury exposure presented in dose-dependent and time-dependent manners. The enzyme activities were significantly induced with increased concentrations and extended exposure (at 5 $\mu g/L$ after 72 h and 10 $\mu g/L$ after 48 h; $p < 0.05$ or $p < 0.01$). LSZ was not sensitive to lower Hg^{2+} concentrations, whereas LSZ significantly increased at higher concentrations and longer exposure (at 5 $\mu g/L$ at 120 h and 10 $\mu g/L$ after 72 h; $p < 0.05$ or $p < 0.01$). CAT activities were significantly inhibited at different periods of embryonic development, particularly at 5 and 10 $\mu g/L$ ($p < 0.05$ or $p < 0.01$). Reduced CAT activities were observed at 72, 96, and 120 h at 1 $\mu g/L$ ($p < 0.05$ or $p < 0.01$), whereas a decline at 0.2 $\mu g/L$ was evident at 96 h ($p < 0.01$). MDA content significantly increased at various stages of embryonic development, particularly at 10 $\mu g/L$ ($p < 0.05$ or $p < 0.01$), and increased further at 72, 96, and 120 h at 5 $\mu g/L$ ($p < 0.05$ or $p < 0.01$). At 96 h, MDA content was only increased by exposure to 0.2 and 1 $\mu g/L$ ($p < 0.01$). The activities of ACP, AKP, and LSZ remarkably increased at 120 h in contrast to 96 h ($p < 0.05$ or $p < 0.01$). Therefore, 96 h is an important shifting period of embryonic development because the activity of enzyme has been enhanced at this time. Thus, the increased ACP, AKP, and LSZ activities revealed an enhanced ability of the embryo to synthesize more enzymes and attenuate mercury damage. CAT activity negatively correlates with MDA accumulation. The enhanced enzyme activities after specific embryonic stages are used to strengthen the ability to cope with mercury stress and attenuate mercury damage. The biochemical parameters, except LSZ, exhibited sensitivity to mercury, suggesting that they may act as potential biomarkers in assessing the environmental mercury risk on *C. auratus* embryos.

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1. Introduction

In recent years, increasing amounts of heavy metals have been detected in aquaculture as the effluent from industrial and agricultural manufacturing is discharged into the rivers. Mercury is one of the most hazardous heavy metals, which comes from natural phenomena (e.g., crust erosion) and anthropogenic activities (e.g., released chemicals and pesticides) (Das et al., 2001; Nanda, 1993). Increased mercury levels in rivers mainly result from anthropogenic activities and potentially lead to environmental contamination and human health risk (Tchounwou et al.,

2003). Moreover, different forms of mercury, such as elemental, organic (e.g., MeHg), and inorganic (e.g., $HgCl_2$), can be found in the water and be modified by physicochemical factors. Waterborne $HgCl_2$ is the most common bioavailable form found in shore crab (*Carcinus maenas*) (Laporte et al., 1997). Waterborne mercury accumulates in different tissues of aquatic animals (Gilbertson and Carpenter, 2004) and affects the stability of aquatic ecosystems (Pelletier, 1995; St-Amand et al., 1999). Therefore, Elia et al. (2007) suggested that fish can be considered as an early indicator of environmental risk due to their sensitive responses to environmental fluctuations. Biochemical indices in fish have been proven to show the corresponding phosphorylation, antioxidation, and immune responses to mercury exposure, some of which have been proposed as biomarkers to assess the mercury contaminants in aquatic ecosystems (Martínez-Álvarez et al., 2005). These biomarkers indicate sensitively the impact of mercury pollutants on fish, and a

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battery of biomarkers is more effective to assess the influence of environmental pollutants (Cajaraville et al., 2000; Chevre et al., 2003; Dondero et al., 2006).

The increasing heavy metals in water can lead to serious effects on fish embryos that are particularly sensitive to intoxication during embryonic development (Jezierska et al., 2009). Waterborne mercury can directly affect the hatching process of embryos and larvae quality (Huang et al., 2010a,b). Therefore, high-quality water without mercury disturbance plays a significant role in maintaining the health of the embryos during embryonic development. Although mercury can penetrate the egg membrane and exert an adverse effect on fish embryos (Devlin, 2006; Huang et al., 2010b), toxic effects on larvae, fry, and juvenile fish have been the main focus of most previous studies (Berntssen et al., 2003; Huang et al., 2010a; Monteiro et al., 2010; Sastry and Gupta, 1978; Sastry and Sharma, 1980). However, the responses of biochemical indices, particularly phosphatase, lysozyme, and lipid peroxidation (LPO), to mercury exposure in fish embryos have not yet been fully elucidated; the biochemical mechanism used in coping with mercury stress remains unclear. On the other hand, understanding the influence of mercury on fish reproduction and assessing the potential risk on the health of the offspring are important.

The effects of waterborne mercury on acid phosphatase (EC 3.1.3.2, ACP) and alkaline phosphatase activities (EC 3.1.3.1, AKP) in grass fish, *Ctenopharyngodon idellus*, have been reported. The results indicate that ACP and AKP responses are sensitive to mercury exposure (Liu et al., 2007; Kong et al., 2007). Another study has suggested that ACP and AKP can be used as reliable biomarkers for the assessment of heavy metal pollution (Rajalakshmi and Mohandas, 2008). Antioxidant defense and antibacterial ability in fish under mercury stress generally decrease because of the effect of contamination (Kong et al., 2007; Wang, 2011). Interestingly, fishes, subjected to certain mercury intervention, can mobilize their own immune system to compensate for the decreased immunity by enhancing the activities of some immune factors (Wang, 2011), including lysozyme (EC 3.2.1.17, LSZ), an important innate immunity factor that can kill bacteria, thereby preventing bacterial infection. However, LSZ activity responses to mercury exposure in fish embryos have been less studied. Therefore, we hypothesized that inducing LSZ activity could improve the ability of eradicating harmful substances during mercury exposure in developmental embryos.

Catalase (EC 1.11.1.6, CAT) plays a vital role in reducing reactive oxygen free radicals and maintaining cellular homeostasis in organisms. The imbalance between producing and removing reactive oxygen species (ROS) may result in oxidative stress and subsequently lead to LPO (Sun et al., 2008). Malondialdehyde (MDA) is the final product of LPO, and its content reflects the levels of LPO to some degree. Therefore, MDA content is often used as a biomarker in evaluating the degree of oxidative stress resulting from xenobiotics; MDA accumulation is positively related to the level of oxidative stress (Shi et al., 2005a,b; Sun et al., 2008). Thus, MDA is also used as a detectable and valuable parameter in evaluating oxidative damage in the embryonic development of fish during mercury exposure.

The goldfish (*Carassius auratus*) is an important commercial fish popularly cultured in China. In recent years, the cultivation of *C. auratus* has been developed quickly because of its marketability. However, *C. auratus* is frequently affected by heavy metal pollution during breeding and growing, which can result in abortion during embryonic development, morphological abnormality, and an increased mortality rate of fish larvae. Observations at local farms also indicate a serious effect of mercury pollution on the embryonic development of *C. auratus* (Wang, 2011). Therefore, improving the embryo quality and preventing the effects of mercury on developmental embryos of *C. auratus* are important. However, only a few

studies on the influence of mercury exposure on fish embryonic development have been reported (Huang et al., 2010b). Studies on the changes of biochemical parameters during the embryonic development of *C. auratus* under mercury exposure can contribute to understanding the physiological responses to mercury stress in fish embryos. In the present study, fertilized eggs of *C. auratus* were exposed to different mercury concentrations, collected at different stages, and then used in analyzing biochemical parameters (ACP, AKP, LSZ, CAT, and MDA). The research aims to (1) assess the effects of mercury exposure on the physiological metabolism in developmental embryos of *C. auratus* based on the changes in biochemical indices, (2) investigate the validity of biochemical indices as biomarkers to indicate the biological response to mercury exposure, and (3) evaluate the potential risk on larvae health based on the impacts on the developing embryos under mercury exposure. This research further aims to achieve better understanding of the biological effects on *C. auratus* developing embryos under mercury exposure and to set guidelines for minimizing mercury damage for fish embryos, thereby improving fish larvae quality in aquaculture.

2. Materials and methods

2.1. Mercury exposure and sampling of fish embryos

Fertilized eggs of *C. auratus* were obtained by artificial insemination, subsequently collected, and randomly grouped to contain approximately 1000 to 1200 fertilized eggs per group. The eggs were incubated in 2 L polyethylene tanks with 1 L dechlorinated water at a light regime of 12 h L/12 h D. Water temperature was controlled at 18 ± 1 °C. After the developing eggs were differentiated between fertilized and non-fertilized (approximately 24 h after artificial insemination), the fertilized eggs were selected and transferred randomly in different tanks with mercury (Hg^{2+}) concentrations of 0.2, 1, 5, and 10 $\mu\text{g/L}$, respectively. The assigned mercury concentrations were prepared through diluting the stock solution of HgCl_2 (#83366, Sigma–Aldrich, US). Water without Hg^{2+} was considered as the control (0 $\mu\text{g/L}$). The nominal concentrations in the experiment were designated based on the observed Hg^{2+} LC₅₀ concentration of 50 $\mu\text{g/L}$ for 3-day-old embryos in the pre-experimental procedure and the mercury exposure regime previously reported (Huang et al., 2010a,b). All experiments were conducted in triplicate. Three fifths of the incubating solution in the tank was changed every 24 h using the designated mercury concentration solutions. Approximately 1 L of mercury solution was maintained in each tank by adding the solution with the same mercury concentration. After mercury exposure, the embryos were sampled at 24, 48, 72, 96, and 120 h (at 120 h, almost all embryos were hatched into larvae). During embryonic development, unfertilized or dead eggs were removed promptly from the tanks. The work described in this study has been carried out in accordance with the requirements for animal experiments.

2.2. Preparation of crude extract

Approximately 200 embryos were sampled from different tanks, placed on filter paper to absorb any surplus moisture, and then stored in Eppendorf tubes at -80 °C until biochemical assays were conducted. Embryos were homogenized in a ratio of 1:4 (1 g embryos: 4 mL 0.9% sodium chloride solution) to prepare the crude extract. Homogenization was carried out using 15 strokes to 20 strokes of a hand-driven Teflon Potter. All operations were performed on ice. The homogenate was centrifuged at $11,130 \times g$ at 4 °C for 10 min. The supernatant was divided into aliquots and stored at -20 °C to be used in measuring the activities of ACP, AKP, LSZ, and

CAT, as well as the MDA content. All measurements were conducted within one week.

2.3. Determination of biochemical parameters

The activities of ACP (King and Jagatheesan, 1959) and AKP (Sharma et al., 2005a; Varley et al., 1980) were measured according to the methods described. Production of 1 mg phenol in the reaction per gram protein per minute at 37 °C was defined as a unit of activity (U/g Pr).

Lysozyme activity was measured using the method described by Hultmark et al. (1980) and Liu et al. (2006). *Micrococcus lysodeikticus* cells were suspended by adding 0.1 mol/L ice-cold phosphate buffer (pH 6.4) to prepare the bacterial suspension ($OD_{570} = 0.3$). Crude extract (50 μ L) was mixed with 3 mL bacterial suspension in an ice-bath tube. The absorbance of the mixture was measured at 570 nm using a spectrophotometer before and after incubation at 37 °C for 30 min. A reduction of absorbance by 0.001 in 1 mg protein per minute in the reaction is defined as a unit of LSZ activity (U/mg Pr).

Catalase activity was determined using the molybdate colorimetric method (Cheng and Meng, 1994; Goth, 1991), with 1 μ mol H_2O_2 decomposed in 1 mg protein per minute in reaction solution defined as a unit of CAT activity (U/mg Pr). MDA content was determined according to the method described by Okhawa et al., 1979. The principle of this method is based on the chemical reaction between MDA and 2-thiobarbituric acid (TBA), yielding a red product with an absorption peak at 532 nm.

The protein concentration of the crude extract of *C. auratus* embryos was determined using the method described by Bradford, 1976, with bovine serum albumin (BSA, AMRESCO) as the standard.

2.4. Statistical analysis

Results for all determinations were presented as means $\pm$ standard deviation ($M \pm SD$) (To be concise, the data in figures just appear in $M \pm SD$). Statistical analyses were performed using one-way ANOVA and Student's *t*-test, implemented in the analysis software supplied by Microsoft Excel 2007. The level of statistical significance was set at $p = 0.05$ and $p = 0.01$.

3. Results

3.1. ACP activity responses to mercury exposure in embryos

Changes in ACP activity in developmental embryos of *C. auratus* during mercury exposure are shown in Fig. 1. ACP activity in different Hg^{2+} concentrations at the same exposure time showed no significant difference at 24 h ($p > 0.05$) compared with the control; however, ACP activity significantly increased during the following periods: 48 and 72 h at 10 μ g/L ($p < 0.05$); 96 h at 5 μ g/L ($p < 0.05$) and 10 μ g/L ($p < 0.01$); and 120 h at 1, 5, and 10 μ g/L ($p < 0.01$). ACP activities in embryos gradually increased under similar exposure concentrations, including the control at longer developmental time. ACP activities significantly increased at 96 and 120 h compared with 24 h exposure ($p < 0.01$). ACP activities were distinctly higher at 120 h than at 96 h exposures ($p < 0.01$).

3.2. AKP activity responses to mercury exposure in embryos

AKP activity responses of *C. auratus* developmental embryos to mercury exposure are shown in Fig. 2. AKP activities in fish embryos increased gradually with the increased mercury concentrations at the same exposure time. AKP activities at different mercury concentrations at 24 h showed no significant difference with the control ($p > 0.05$). However, AKP activities were significantly higher at 48 h only at 10 μ g/L ($p < 0.05$). AKP activities significantly increased at

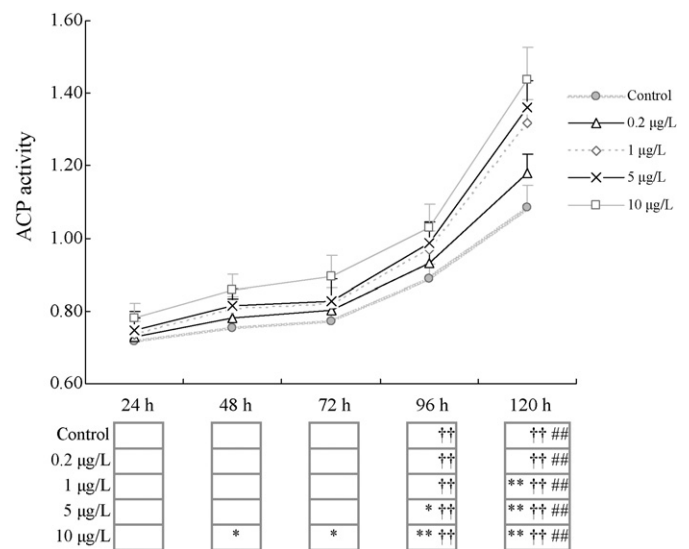


Fig. 1. Acid phosphatase (ACP) activity changes in developmental embryos of *C. auratus* exposed to different mercury concentrations. All data are presented as means $\pm$ standard deviation ($M \pm SD$). Enzyme activity unit is U/g Pr. Compared with the control, "*" represents significant difference ($p < 0.05$) and "**" represents extremely significant difference ($p < 0.01$). Compared with the 24 h exposure, "†" refers to significant difference ($p < 0.05$) and "††" refers to extremely significant difference ($p < 0.01$). For comparison between the 96 and 120 h exposure, "‡" stands for significant difference ($p < 0.05$) and "‡‡" stands for extremely significant difference ($p < 0.01$).

5 and 10 μ g/L at 72, 96, and 120 h exposures ($p < 0.05$ or $p < 0.01$). AKP activities increased gradually with the extension of embryonic development when AKP time-dependent changes in fish embryos at the same concentration were considered. AKP activities significantly increased at 72, 96, and 120 h compared with 24 h exposure at various mercury concentrations ($p < 0.05$ or $p < 0.01$), even with the control. In addition, statistical analysis showed that AKP activities were remarkably higher at 120 h than at 96 h ($p < 0.01$).

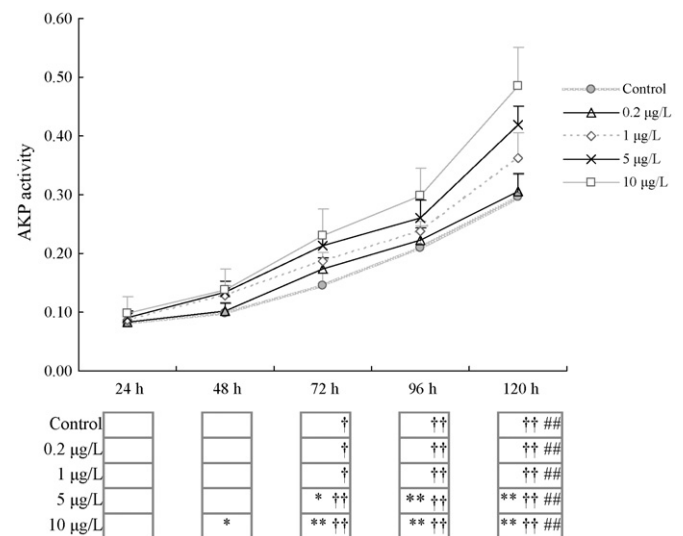


Fig. 2. Activity changes in AKP in developmental embryos of *C. auratus* exposed to different mercury concentrations. All data are presented as means $\pm$ standard deviation ($M \pm SD$). Enzyme activity unit is U/g Pr. Compared with the control, "*" represents significant difference ($p < 0.05$) and "**" represents extremely significant difference ($p < 0.01$). Compared with the 24 h exposure, "†" refers to significant difference ($p < 0.05$) and "††" refers to extremely significant difference ($p < 0.01$). For comparison between the 96 and 120 h exposure, "‡" stands for significant difference ($p < 0.05$) and "‡‡" stands for extremely significant difference ($p < 0.01$).

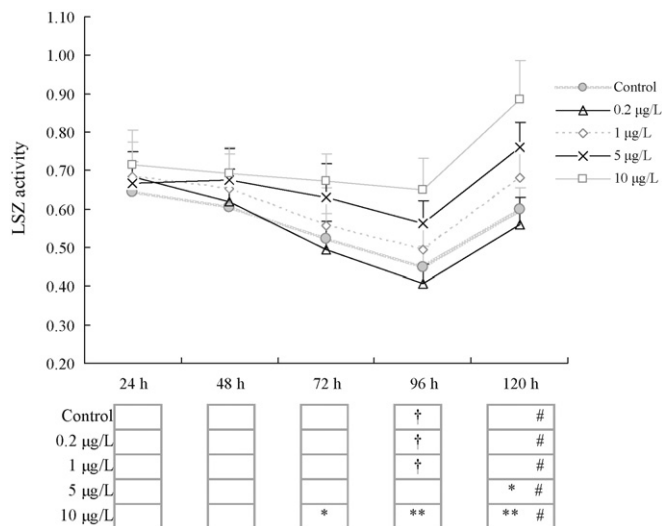


Fig. 3. Changes of LSZ activities in developmental embryos of *C. auratus* exposed to different mercury concentrations. All data are presented as means + standard deviation (M + SD). Enzyme activity unit is U/mg Pr. Compared with the control, “*” represents significant difference ($p < 0.05$) and “**” represents extremely significant difference ($p < 0.01$). Compared with the 24 h exposure, “†” refers to significant difference ($p < 0.05$) and “††” refers to extremely significant difference ($p < 0.01$). For comparison between the 96 and 120 h exposure, “#” stands for significant difference ($p < 0.05$) and “##” stands for extremely significant difference ($p < 0.01$).

3.3. LSZ activity responses to mercury exposure in embryos

The changes in LSZ activity in *C. auratus* embryos at various mercury concentrations indicated similar correlations with embryonic development to some extent (Fig. 3). LSZ activities at different concentrations during the same exposure time showed no significant effects on fish embryos at 24 and 48 h compared with the control ($p > 0.05$). A significant increase in LSZ activity was observed at 72 and 96 h exposures only at 10 µg/L ($p < 0.01$ or $p < 0.05$). On the other hand, LSZ activities significantly increased at 120 h at 5 and 10 µg/L ($p < 0.05$). LSZ activities exhibited gradually decreasing trend with embryonic development during the time-dependent effects at 0, 0.2, and 1 µg/L up until 96 h. At 96 h, LSZ activity reached the minimum; afterward, it increased. LSZ activities significantly decreased at 96 h ($p < 0.05$) at 0, 0.2, and 1 µg/L compared with 24 h exposure. However, LSZ activities showed no significant difference at 5 and 10 µg/L ($p > 0.05$). Moreover, LSZ activities at 120 h were significantly higher than at 96 h when exposed to any mercury concentration ($p < 0.05$).

3.4. CAT activity responses to mercury exposure in embryos

Changes in CAT activity at different stages of goldfish embryos after mercury exposure are shown in Fig. 4. CAT activities were significantly inhibited only at 5 µg/L at 24 and 48 h ($p < 0.05$) and 10 µg/L ($p < 0.01$). At 72 h, CAT activities were significantly inhibited only at 1, 5, and 10 µg/L ($p < 0.05$, $p < 0.01$). CAT activities declined remarkably at different mercury concentrations at 96 h, whereas CAT activities were inhibited significantly at 1, 5, and 10 µg/L at 120 h similar to 72 h exposure ($p < 0.05$, $p < 0.01$). CAT activities were significantly lower at 96 h ($p < 0.01$, $p < 0.05$), compared to 24 h, at all mercury concentrations as well as in controls. CAT activities significantly reduced at 72 h ($p < 0.05$) only at 1, 5, and 10 µg/L. At 120 h exposure, CAT activities decreased only at 5 and 10 µg/L. Furthermore, CAT activities at 120 h were significantly higher than at 96 h only at 0, 0.2, and 1 µg/L ($p < 0.05$). No significant differences were observed at 5 and 10 µg/L ($p > 0.05$).

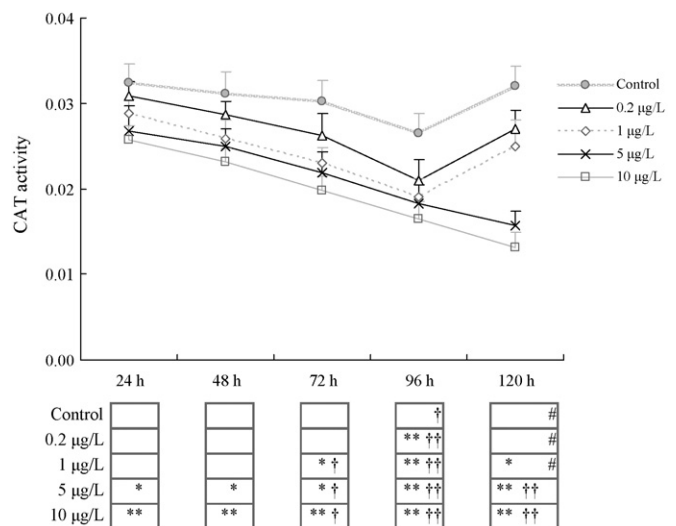


Fig. 4. Changes in CAT activity in developmental embryos of *C. auratus* exposed to different mercury concentrations. All data are presented as means + standard deviation (M + SD). Enzyme activity unit is U/mg Pr. Compared with the control, “*” represents significant difference ($p < 0.05$) and “**” represents extremely significant difference ($p < 0.01$). Compared with the 24 h exposure, “†” refers to significant difference ($p < 0.05$) and “††” refers to extremely significant difference ($p < 0.01$). For comparison between the 96 and 120 h exposure, “#” stands for significant difference ($p < 0.05$) and “##” stands for extremely significant difference ($p < 0.01$).

3.5. Changes of MDA content in embryos under mercury exposure

MDA content gradually increased in *C. auratus* embryos during the developmental stages until 96 h, in all mercury exposure groups as well as in the control (Fig. 5). A significant increase in MDA content was observed only at 10 µg/L ($p < 0.05$) during the 24 and 48 h exposures. Furthermore, MDA content increased at the 72 h exposure only at 5 µg/L ($p < 0.05$) and 10 µg/L ($p < 0.01$). MDA content significantly increased under mercury exposure at 96 h in contrast to the control ($p < 0.01$). MDA content

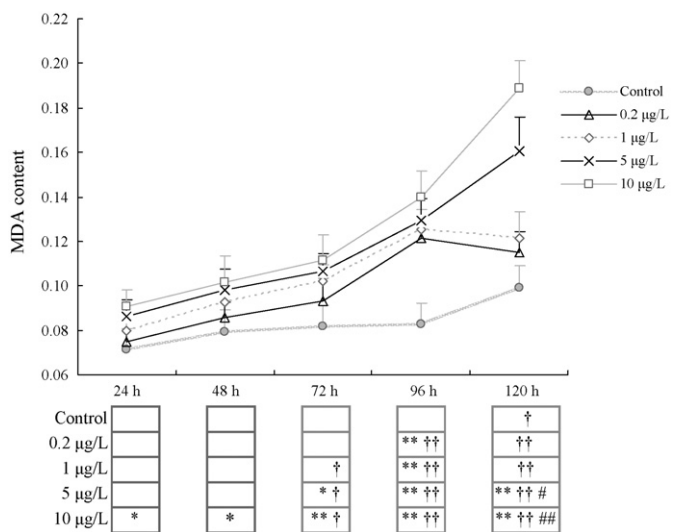


Fig. 5. Changes in MDA content in developmental embryos of *C. auratus* exposed to different mercury concentrations. All data are presented as means + standard deviation (M + SD). MDA content unit is nmol/mg Pr. Compared with the control, “*” represents significant difference ($p < 0.05$) and “**” represents extremely significant difference ($p < 0.01$). Compared with the 24 h exposure, “†” refers to significant difference ($p < 0.05$) and “††” refers to extremely significant difference ($p < 0.01$). For comparison between the 96 and 120 h exposure, “#” stands for significant difference ($p < 0.05$) and “##” stands for extremely significant difference ($p < 0.01$).

continuously increased at 5 and 10 $\mu\text{g/L}$ at 120 h exposure, indicating a significantly higher amount ($p < 0.01$); however, no significant difference occurred at 0.2 and 1 $\mu\text{g/L}$ ($p > 0.05$). MDA content in the control significantly increased only at 120 h compared with the 24 h exposure ($p < 0.01$). MDA content in the exposure groups was remarkably higher at 96 and 120 h at various mercury concentrations than at 24 h ($p < 0.01$), whereas MDA content was higher at 72 h only at 1, 5, and 10 $\mu\text{g/L}$. In addition, MDA content at 120 h was significantly higher than at 96 h only at 5 and 10 $\mu\text{g/L}$.

4. Discussions

4.1. ACP and AKP activity changes in *C. auratus* embryos

Phosphatase plays an important role in dephosphorylation reaction, particularly in performing signal transduction, physiological metabolism, and environmental adaptation. Embryonic development, as an important early life stage, is characterized by protein synthesis, organ growth, and various physiological activities. Mercury exposure potentially results in complicated physiological processes, exhibiting adverse effects on the physiological metabolism of embryos (Devlin, 2006; Huang et al., 2010b; Jezierska et al., 2009).

The majority of ACP is situated in cell lysosomes. Responses of cells to heavy metal pollution can be observed in lysosomal activities. For example, mercury exposure can greatly reduce the stability of the lysosomal membrane (Regoli et al., 1998). On the other hand, the enhanced peroxidation of lysosomal membranes can lead to membrane lysis and ACP release, as well as ACP activity increase (Mehra and Kanwar, 1986; Sharma et al., 2005b). Meanwhile, metallothionein synthesis and detoxification by robust metabolism are induced under mercury exposure. Thereby phosphorylation and dephosphorylation of proteins in signaling pathway regulated metabolism are enhanced, as well as ACP activity is promoted. Likewise, AKP activity increases in the blood serum and gills of *Cyprinus carpio* exposed to copper (Karan et al., 1998), which is attributed to the damage of cell membrane resulting from the elevated membrane permeability, with higher AKP synthesized in cells to meet the requirements of metabolism.

In this study, activities of ACP and AKP were stimulated under mercury exposure. ACP and AKP activities increased at various mercury concentrations in contrast to the results observed in the control. Significant difference occurred only at a higher mercury concentration or at longer exposure time. Therefore, the results revealed that the responses of ACP and AKP to mercury exposure presented in dose-dependent and time-dependent manners, which agree with the phosphatase activities induced by mercury exposure as reported in previous studies (Broeg, 2003; Rajalakshmi and Mohandas, 2008). ACP activity is also stimulated in the gill and hepatopancreas of the mussel *Lamellidens corrianus* under mercury stress (Rajalakshmi and Mohandas, 2008). In the flounder *Platichthys flesus*, a significant positive correlation between mercury concentration in the muscle and AKP activity in the liver is observed (Broeg, 2003). Sastry and Gupta (1978) demonstrated that ACP and AKP activities show a slight increase in the intestine and pyloric caeca of spotted snakehead *Channa punctatus* exposed to mercury for 96 h. In the current study, the activities of ACP and AKP in embryos were induced under mercury exposure, probably resulting from a higher enzyme concentration in embryos. ACP activities significantly increased at 96 and 120 h compared with the 24 h mercury exposure. Similarly, AKP activities remarkably increased at 72, 96, and 120 h. Thus, the ability to synthesize phosphatase in fish embryo increases with embryonic development.

4.2. Responses of CAT and MDA to mercury exposure in embryos

ROS resulting from oxidative stress can be affected by mercury exposure; they exhibit a close correlation with mercury toxicity (Huang et al., 2010a,b). Inorganic mercury likely increases H_2O_2 production by impairing the efficiency of oxidative phosphorylation and electron transport chain at the ubiquinone cytochrome b5 (Franco et al., 2009).

The changes in CAT activity in different aquatic animals vary after heavy metal exposure (Chandran et al., 2005; Farina et al., 2008; Huang et al., 2010b; Regoli et al., 1998), which may be attributed to the discrepancy of environmental factors, time, and intensity of exposure and structure of heavy metal compounds, among others. CAT activity in the flounder (*P. olivaceus*) is induced significantly at 5 and 10 $\mu\text{g/L}$ Hg^{2+} exposure for 10 days (Huang et al., 2010b). However, CAT activity is inhibited in the digestive gland of *Adamussium colbecki* at 5 $\mu\text{g/L}$ Hg^{2+} (Regoli et al., 1998). Similarly, CAT activity can be inhibited by Cd and Zn exposure in the digestive gland and kidney of gastropods (*Achatina fulica*) (Chandran et al., 2005). Moreover, CAT activity in *Porites astreoides* larvae remains unaltered after 96 h mercury exposure at 10 $\mu\text{g/L}$ (Farina et al., 2008). Atli and Canli (2010) provided a reasonable explanation on CAT activity response (whether it was induced, inhibited, or unaltered) in antioxidant defenses in *Oreochromis niloticus* to heavy metal exposures (Cd, Cu, Cr, Zn, and Fe); however, the complicated action involved in CAT activity makes it difficult to completely elucidate. Therefore, further studies on the response mechanism of CAT in early developmental stages of fish under heavy metal exposure are needed to provide additional evidence.

In the present study, CAT activity was inhibited under mercury exposure, indicating that the antioxidant enzyme CAT is sensitively influenced by mercury exposure. Thiol groups ($-\text{SH}$) are common in CAT, and mercury exhibits a high affinity for sulfhydryl compounds. Therefore, the combination of heavy metal with the $-\text{SH}$ groups can destroy the protein structure of CAT and inhibit its activity (Atli and Canli, 2010). The decrease in CAT activity may yield H_2O_2 from physiological metabolism. H_2O_2 may not be removed promptly, which can potentially damage the balance between production and elimination of ROS. Surplus H_2O_2 can induce oxidative stress, which initiates LPO, thereby leading to MDA accumulation. Therefore, MDA content can be used as a biomarker in evaluating the level of LPO. Furthermore, the products of LPO, such as MDA, have high affinity with the peptide, $-\text{SH}$ of the enzyme, amide, and nucleic acid. The cross-linkages in DNA and protein can be destroyed, thereby causing extreme cell toxicity (Spiteller, 1998; Viarengo, 1989). In this study, MDA gradually accumulated during the embryonic development at 5 and 10 $\mu\text{g/L}$, and MDA content significantly increased after 72 h in contrast with that of the control. ROS in cells was not eliminated on time, causing oxidative stress, LPO, and eventually, MDA accumulation, which is in agreement with the conclusions of previous studies (Bano and Hasan, 1989; Huang et al., 2010b). Bano and Hasan (1989) found that a significant increase in LPO occurs in the brain, liver, and muscle when the catfish *Heteropneustes fossilis* is exposed to mercury. MDA content in larvae of flounder *P. olivaceus* also increases significantly at Hg^{2+} concentrations of 5 and 10 $\mu\text{g/L}$ for 10 d (Huang et al., 2010b). Furthermore, MDA content increases in the gill of *Cyprinus carpio* exposed to mercury chloride solution (Arabi, 2004) as revealed in the current study, wherein the accumulation of MDA in embryos resulted from mercury exposure.

In the present study, CAT activities of fish embryos exposed to 0.2 and 1 $\mu\text{g/L}$ were significantly inhibited at 96 h. Elevated CAT activities were observed after 96 h, implying that the ability to synthesize CAT increases after the fish embryo has developed into a specific stage. MDA in fish embryos exposed to 0.2 and 1 $\mu\text{g/L}$ significantly accumulated at 96 h, with an observed decrease after

96 h, suggesting that the increased CAT activity can minimize MDA production and reduce the degree of oxidative stress that resulted from ROS. On the other hand, the enhanced synthesis of metabolic enzymes in fish embryo can improve the ability to maintain physiological homeostasis and ensure normal embryonic development. However, MDA accumulation was not reduced even after 96 h when mercury concentration was beyond the adjust critical value, particularly at 5 and 10 $\mu\text{g/L}$. Therefore, fish embryos cannot cope with oxygen stress caused by exposure to higher mercury concentration, thereby resulting in their abortion. Thus, a higher number of dead embryos were observed at higher Hg^{2+} concentrations, as described by Wang (2011).

4.3. Responses of LSZ activities to mercury exposure in embryos

The innate immunity in fish plays an important role in maintaining the immune defense system to prevent bacterial infections. The corresponding immune levels of fish are modulated to cope with adverse effects of pollution when they are subjected to heavy metal contaminants (Zelikoff, 1993). Therefore, investigating fish immunotoxicity under mercury exposure is important. One of the important innate immunity factors in fish is LSZ, which covers a wide antibacterial spectrum and destroys the peptidoglycan layer of the cell wall of predominant Gram-positive bacteria and some Gram-negative bacteria (Skouras et al., 2003). LSZ activity is regulated to improve the immune defense when the increasing pathogenic bacteria and other various stress factors attack the fish.

In this study, LSZ activities in fish embryos significantly increased at 72, 96, and 120 h at 10 $\mu\text{g/L}$ compared with the control. LSZ activities increased only at 120 h at 5 $\mu\text{g/L}$. It was indicated that LSZ activity can be induced only at higher Hg^{2+} concentrations with longer exposure time. Therefore, the responses of LSZ are not sensitive to mercury exposure in fish embryos, which may be attributed to the weak ability to synthesize LSZ in the embryos. However, LSZ can also be stimulated to adjust enzyme activity when mercury concentration further increases. Wang (2011) has addressed that LSZ activity can be induced to improve the weakened immunity defense under a certain mercury stress, which agrees with the previous proposal that LSZ activity can be induced by mercury exposure (Low and Sin, 1998). For example, LSZ activity can be induced in the kidney of blue gourami (*Trichogaster trichopterus*) with mercury exposure at 90 $\mu\text{g/L}$ for 2 weeks (Low and Sin, 1998). Moreover, LSZ activity is also enhanced in fish treated by a relatively low dosage of mercury (Low and Sin, 1998). In addition, LSZ activity can be significantly induced in the serum and kidney of tilapia (*Oreochromis aureus*) exposed to 0.6 mg/L mercury solution (Low and Sin, 1995a,b). Thus, LSZ activity can be induced by exposure to mercury at specific concentration. LSZ, as an important immunologic factor, plays an essential role in immune defense; the antibiotic activity can be modulated by self-adjustment under specific mercury exposure.

In the present study, the gradually decreased LSZ activities were observed up to 96 h (similarly observed in the control) as the exposure time was extended. Therefore, the weakening ability to synthesize LSZ is not sufficient to complement the gradually consumed LSZ, as described by Kong et al. (2011). However, LSZ activities in fish embryos are obviously higher at 120 h than at 96 h, implying that the synthesizing ability of LSZ can be enhanced after a specific period of embryonic development.

5. Conclusions

The activities of metabolic enzymes in fish embryos were affected by exposure to mercury in concentration-dependent and time-dependent manners despite the varying response patterns of

different metabolic enzymes to mercury. The activities of ACP and AKP were sensitively induced under mercury exposure, which is mainly used in enhancing the reactions of dephosphorylation. LSZ activity showed minimal responses to mercury exposure; however, LSZ activity can be modulated at higher concentration and longer time. The declined CAT activity induced by mercury damage can result in MDA accumulation, thereby causing LPO. At the same time, the strong oxidative stress occurred at higher mercury concentration. At the higher mercury concentrations, the activities of ACP, AKP, and CAT, as well as MDA content can be used as biomarkers in evaluating the impact of mercury exposure on *C. auratus* embryonic development and potential ecological risk on larval health. Moreover, the activities of ACP, AKP, and LSZ in fish embryos were enhanced after a specific period of embryonic development. The biological effects of mercury exposure on developmental fish embryo are complicated and may vary in different fish from various niches. Therefore, further studies are encouraged to obtain additional evidence that would support the proposed ideas in this study and to better understand the physiological and biochemical regulatory mechanism under mercury exposure. Studying gene regulation of metabolic enzymes is also necessary to illustrate the biological effects of mercury exposure on fish embryo at a molecular level.

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