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RESEARCH PAPER

# Differential Induction of Enzymes and Genes Involved in Oxidative Stress in Gill and Liver Tissues of Mudskipper *Boleophthalmus Pectinirostris* Exposed to Lead

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**Abstract**

A study was carried to test the response of mudskipper for 8 days under four lead concentrations. Fish exposed to 2 mg/L Pb had the lowest glutathione peroxidase (GPX) in liver and glutathione (GSH) activities in liver and gill. Liver superoxide dismutase (SOD) in fish exposed to 1 and 2 mg/L Pb was lower than that in fish exposed to 0 and 0.5 mg/L Pb. However, the higher content of malondialdehyde (MDA) in gill and liver was found in 2 mg/L Pb group. The lower SOD, glutathione s-transferase (GST), heat shock proteins 70 (HSP-70) and HSP-90 expression in gill and liver were found in 0 mg/L Pb group. During the 8-day period, catalase(CAT), GPX and GSH activates in gill were the lowest at 8d. The lowest CAT activity in liver and the highest MDA content in gill and liver were found at day 8. The highest values of SOD and HSP-90 expression in gill were found at day 2. but the highest values of HSP-70 expression in liver were found from days 6 to 8. This study indicated that Pb-induced ROS generation is not fully counteracted by antioxidant enzymes. Functional enzyme activity was affected by mRNA expression.

*Keywords*: Mudskipper; lead; oxidative stress; gene expression.

## Introduction

Lead (Pb) is a heavy metal element that widely exists in the environment, which is widely used in industrial processes and settings, among others in the cosmetic, food, and oil industries (Dahms, 2014). The Pb exposure in aquatic environment negatively affects growth, behavior and reproduction in fish (Burdena *et al*., 1998). Acute and chronic Pb exposure may result in tissue damage, central nervous system disorder, and blood deterioration (Palaniappan *et al*., 2008), induce muscular atrophy, lordoscoliosis, numbness, black tail, and caudal fin degeneration, in addition to hyperactivity, erratic swimming, and loss of equilibrium (Burdena *et al*., 1998). Zhang *et al*. (2007) found that lead toxicity is associated with oxidative stress in various tissues and cells of tadpoles. Reactive oxygen species(ROS), including the superoxide anion, hydrogen peroxide and hydroxyl radicals, have the potential to generate oxidative stress, impair cells by oxidizing membrane lipids and proteins. Li *et al*. (2016) found that oxidative stress of freshwater crab sperm induced by Pb was reflected insignificant up-regulation of ROS levels, a significant reduction of the total antioxidant capacity levels occurred after exposure to 14.7mg/L Pb and above at 7d compared to the control. To the author's best knowledge, the link between Pb accumulation and oxidative stress remains undefined in fish.

During the regulation of physiological homeostasis, several key enzymes and transcription factors are involved in these stress processes, such as superoxide dismutase (SOD), glutathione-s-transferase (GST), heat shock proteins 70 (HSP-70) and HSP-90. SOD is a key enzyme that can eliminate excess ROS caused by cellular oxidative metabolism into hydrogen peroxide (Fridovich, 1978). GSTs are involved in the detoxication of many chemical compounds including heavy metal, hydrocarbons, organochlorine insecticides and polychlorinated biphenyls (PCBs) (George and Buchanan, 1990). Heat shock proteins (HSP) are a subset of molecular chaperones, and play key role in the process of protein metabolism under stress conditions, including the refolding of denatured protein, maintenance of structure integrity and other regulatory processes (Feder and Hofmann, 1999). These proteins have been classified into several families according to their apparent molecular mass, such as HSP90(85-90 kDa) and HSP70 (68-73 kDa). HSP-90 play an important role in protecting organisms from damage, they are

needed even more after stress such heavy metals (Qin *et al*., 2013). HSP-70 proteins are highly conserved across all living organisms, not only in de novo protein folding, membrane translocation, degradation of misfolded proteins and other processes for the protection of cells, but also in responses to stress and inflammation (Jing *et al*., 2013).

The mudskipper *Boleophthalmus pectinirostris*, a burrow-dwelling fish inhabiting intertidal mudflats, is an exceptional model among fishes for their amphibious behavior and numerous physiological and morphological specializations adapted for amphibious life, which is a commercially important aquaculture species that inhabits the coastal areas of China. This study investigated the effects of different concentrations of lead and exposure time on antioxidant enzyme activities and expression of SOD, GST, HSP-70 and HSP-90 genes in the gill and liver of mudskipper. The aim of this study was to determine the link between Pb poisoning and oxidative damage in fish.

## Materials and Methods

### Experimental Design and Sampling

Three hundred mudskipper were purchased from a local fish farm (Ningbo, China) and transferred to indoor tanks (50 L in water volume) for two weeks of acclimatization. Afterwards, 240 uniform-sized fish (21.80±2.70 g) were stocked in 12 tanks, with 20 fish for each tank. They were exposed to four nominal Pb treatments at the concentrations of 0 (control), 0.5, 1 and 2 mg Pb /L, respectively, with triplicates for each treatment. The measured Pb concentrations for four treatments were 0, 0.6, 0.9 and 1.8 mg Pb/L, respectively. All tanks were supplied with seawater with a daily exchange rate of 1/2 tank volume. During the experiment, water quality variables were maintained at 20-25℃, salinity 12‰ and natural sunlight.

Experimental fish were sampled on 0, 2, 4, 6 and 8 days. For each exposure time, four fish from each tank were randomly collected, and then were anesthetized with tricaine methanesulfonate (MS-222) at 120 mg/L before weighing. gill and liver were quickly removed and freeze-clamped with liquid nitrogen-precooled aluminum tongs. Samples were stored at −80 °C until analyses, which were performed within a month.

### Antioxidant Enzyme Activity and Lipid Peroxidation Analysis

The frozen gill and liver were weighed and homogenized in ice-cold phosphate buffer (50 mM, pH 7.4). The homogenate was centrifuged at 2 000*g* for 15min at 4℃ and the supernatant was saved. The protein content was determined according the method of Bradford (1976), using bovine serum albumin as a standard. Superoxide dismutase activity was determined following the methods of Beauchamp and Fridovich (1971). Catalase activity was determined by measuring the decrease in $H_2O_2$ concentration (Aebi, 1984). Glutathione peroxidase activity was measured following the methods of Flohé and Günzler (1984). Glutathione activity was measured following the methods of Jollow *et al*. (1974). Malondialdehyde level was measured following the methods of Buege and Aust (1978). All assays were determined with commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) in accordance with the manufacturer's instructions.

### mRNA Expression Analysis

Total RNA extraction and first strand cDNA synthesis were performed with commercial assay kits (RNAiso Reagent kit and Prime Script™ PT reagent Kit with gDNA Eraser; Takara Bio, Dalian, China) in accordance with the instructions of the manufacturer. Real-time PCR reactions (20 μL) were performed in 96-well plates in a real-time PCR system (Eppendorf, German) with Taq™ DNA polymerase (Takara Bio, Dalian, China), containing 10μl GoTaq®qPCR Master mix, 2μl of cDNA, 0.8 μl of each primer and 6.4μl of $ddH_2O$. Primers used in the real-time PCR system were given in Supplementary (Table 1) . The thermal program included 5 min at 95℃, 40 cycles at 95℃ for 20s, 57℃ for 25s, and 72℃ for 25s. All reactions were performed in duplicate and each reaction was verified to contain a single product of the correct size by agarose gel electrophoresis. The relative mRNA expression of genes were calculated with the "delta–delta Ct" method (Schmittgen and Livak, 2008), where β- actin was amplified to confirm the steady-state level of the mRNA expression of the housekeeping gene and used as reference gene.

### Statistical Analysis

Values of parameters were used to detect interaction between lead levels and exposure time by two-way ANOVA. If a significant interaction was detected between the main effects, then the variable was analyzed using a one-factor ANOVA. If there was a significant *F*-test, subsequent comparisons of treatment means were performed using the Duncan's Multiple Range test. The level of significance was set at $P<0.05$. All analyses were performed using the SPSS 18.0.0 (Chicago, USA) for Windows.

## Results

### Antioxidant Enzyme Activity

In gill, glutathione peroxidase (GPX), glutathione (GSH) activities and malondialdehyde (MDA) content were affected by concentration of Pb and exposure time ($P<0.05$, Table 2). Fish exposed to

0 mg/L Pb had the highest GPX and GSH activities, and the lowest in 2 mg/L Pb (P<0.05). The higher content of MDA was found in 2 mg/L Pb group, followed by 1 and 2 mg/L Pb groups, and the lowest in 0 mg/L Pb group (P<0.05). During the 8-day period, catalase(CAT), GPX and GSH activates were the lowest at 8d. The significant lowest MDA content was found at hour 2, then gradually increased, and

**Table 1.** Primers used for sequencing and real-time PCR analysis of SOD, GST, HSP70 and HSP90 from mudskipper

| Primer | Forward primer (5′-3′) | Reverse primer (5′-3′) | Size (bp) |
|---|---|---|---|
| SOD | GGGCTGATAACATCGCTAA | GATGCCAATGACTCCACA | 185bp |
| GST | GTGATGATGGGCAGTGAA | TGTCTTGGAGGAAGTAGTTTT | 100bp |
| HSP70 | CCAGAGGAACAGGGTCAG | AGCAGTTTCTTGCGGTCA | 120bp |
| HSP90 | TATTGACACTGGGATTGGA | AGGTAGGCGGAGTAGAAAC | 150bp |
| β-actin | GAGCGTGGCTACTCTTTCA | GGAGGCAGCAGTGTTCAT | 200bp |

* SOD: superoxide dismutase; GST: glutathione s-transferase; HSP: heat shock proteins

**Table 2.** Effects of different concentrations of lead and exposure time on antioxidant system in mudskipper gill

| Concentration (mg/L) | Time (d) | SOD (U/mg prot) | CAT (U/mg prot) | GPX (U/mg prot) | GSH (U/mg prot) | MDA (nmol/mg) |
|---|---|---|---|---|---|---|
| 0 | 2 | 94.89±4.35 | $15.71±2.70^{b}$ | $164.77±25.07^{Cc}$ | $104.33±2.47^{Cc}$ | $129.65±6.49^{Aa}$ |
| | 4 | 92.64±8.24 | $15.46±1.92^{a}$ | $162.54±17.14^{Cb}$ | $106.47±3.43^{Cb}$ | $130.85±1.04^{Ab}$ |
| | 6 | 98.43±4.4 | $14.84±0.94^{a}$ | $164.77±6.95^{Cb}$ | $108.96±3.85^{Cb}$ | $129.65±1.80^{Ab}$ |
| | 8 | 98.75±2.01 | $15.21±1.31^{a}$ | $158.09±8.41^{Ca}$ | $109.32±2.69^{Ca}$ | $126.65±10.85^{Ac}$ |
| 0.5 | 2 | 118.84±2.85 | $18.45±1.88^{b}$ | $161.48±5.59^{Bc}$ | $100.22±5.47^{Bc}$ | $150.91±6.60^{Ba}$ |
| | 4 | 118.84±9.16 | $12.90±3.62^{a}$ | $152.87±20.76^{Bb}$ | $99.18±3.10^{Bb}$ | $160.77±18.29^{Bb}$ |
| | 6 | 103.60±4.07 | $13.51±1.04^{a}$ | $108.73±35.72^{Bb}$ | $98.15±2.07^{Bb}$ | $163.09±16.08^{Bb}$ |
| | 8 | 87.42±4.79 | $11.70±5.20^{a}$ | $93.66±11.18^{Ba}$ | $90.91±4.50^{Ba}$ | $181.09±12.19^{Bc}$ |
| 1 | 2 | 96.47±4.47 | $13.99±3.62^{b}$ | $100.03±9.22^{Ac}$ | $100.58±7.44^{Bc}$ | $156.79±5.06^{Ba}$ |
| | 4 | 131.69±6.04 | $10.67±2.09^{a}$ | $86..20±6.39^{Ab}$ | $95.00±3.97^{Bb}$ | $164.52±2.53^{Bb}$ |
| | 6 | 103.28±14.27 | $12.73±1.79^{a}$ | $75.56±27.89^{Ab}$ | $99.26±4.50^{Bb}$ | $170.59±9.22^{Bb}$ |
| | 8 | 78.13±6.40 | $12.78±0.79^{a}$ | $55.34±37.00^{Aa}$ | $80.92±4.95^{Ba}$ | $187.16±1.66^{Bc}$ |
| 2 | 2 | 99.65±6.90 | $20.02±3.74^{b}$ | $120.91±3.64^{Ac}$ | $106.27±10.60^{Ac}$ | $171.19±2.60^{Ca}$ |
| | 4 | 125.77±8.69 | $13.54±3.09^{a}$ | $84.11±38.03^{Ab}$ | $100.22±8.58^{Ab}$ | $176.29±7.85^{Cb}$ |
| | 6 | 116.35±11.40 | $11.54±1.59^{a}$ | $69.39±41.73^{Ab}$ | $85.76±2.02^{Ab}$ | $179.13±5.97^{Cb}$ |
| | 8 | 78.99±6.84 | $14.93±4.12^{a}$ | $32.59±20.52^{Aa}$ | $73.99±4.55^{Aa}$ | $193.30±5.20^{Cc}$ |
| Concentration×Time （P value） | | 0.135 | 0.848 | 0.202 | 0.001 | 0.097 |

* Different superscript numbers ($^{A, B, C}$) indicate a significant effect of concentration (P<0.05). Different lowercase letters ($^{a, b, c}$) indicate a significant effect of time (P<0.05). SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase; GSH: glutathione; MDA: malondialdehyde

**Table 3.** Effects of different concentrations of lead and exposure time on antioxidant system in mudskipper liver

| Concentration (mg/L) | Time (d) | SOD (U/mg prot) | CAT (U/mg prot) | GPX (U/mg prot) | GSH (U/mg prot) | MDA (nmol/mg) |
|---|---|---|---|---|---|---|
| 0 | 2 | $138.95±2.44^{Bc}$ | $55.36±2.53^{c}$ | $368.44±27.05^{b}$ | $149.89±3.10^{Bc}$ | $48.45±5.23^{Aa}$ |
| | 4 | $140.99±9.15^{Bb}$ | $53.37±4.61^{b}$ | $368.44±11.77^{b}$ | $149.48±5.82^{Bb}$ | $47.76±2.08^{Aa}$ |
| | 6 | $137.33±4.28^{Ba}$ | $51.19±2.17^{b}$ | $371.00±19.76^{a}$ | $149.48±12.46^{Ba}$ | $47.06±5.23^{Ab}$ |
| | 8 | $136.92±15.53^{Ba}$ | $52.92±1.09^{a}$ | $369.72±40.76^{a}$ | $151.13±6.08^{Ba}$ | $46.37±6.67^{Bc}$ |
| 0.5 | 2 | $140.28±10.37^{Bc}$ | $7.68±1.35^{c}$ | $293.80±27.92^{b}$ | $158.98±33.35^{Bc}$ | $53.73±1.22^{Ba}$ |
| | 4 | $151.07±5.18^{Bb}$ | $62.95±0.51^{b}$ | $390.86±32.76^{b}$ | $142.62±4.42^{Bb}$ | $57.97±1.22^{Ba}$ |
| | 6 | $142.77±1.44^{Ba}$ | $67.13±3.31^{b}$ | $344.96±36.56^{a}$ | $119.97±10.09^{Ba}$ | $65.74±2.12^{Bb}$ |
| | 8 | $119.53±8.72^{Ba}$ | $51.26±4.16^{a}$ | $314.79±58.76^{a}$ | $115.78±8.81^{Ba}$ | $70.70±3.24^{Bc}$ |
| 1 | 2 | $161.79±7.77^{Ac}$ | $86.55±30.02^{c}$ | $365.56±58.97^{b}$ | $180.17±11.87^{Bc}$ | $70.50±5.00^{Ca}$ |
| | 4 | $121.81±11.49^{Ab}$ | $60.92±3.00^{b}$ | $419.05±31.21^{b}$ | $161.15±8.56^{Bb}$ | $72.10±2.40^{Ca}$ |
| | 6 | $112.40±12.80^{Aa}$ | $78.73±1.61^{b}$ | $395.28±18.02^{a}$ | $142.61±6.54^{Ba}$ | $75.31±1.39^{Cb}$ |
| | 8 | $106.29±7.24^{Aa}$ | $55.26±1.26^{a}$ | $392.30±40.86^{a}$ | $131.20±5.14^{Ba}$ | $116.17±1.39^{Cc}$ |
| 2 | 2 | $146.41±11.16^{Ac}$ | $54.31±5.87^{c}$ | $597.99±10.55^{b}$ | $142.58±4.10^{Bc}$ | $71.56±2.61^{Ca}$ |
| | 4 | $134.09±17.57^{Ab}$ | $54.15±2.22^{b}$ | $417.76±8.73^{b}$ | $130.51±6.88^{Ab}$ | $72.31±2.26^{Ca}$ |
| | 6 | $123.33±23.28^{Aa}$ | $59.31±3.02^{b}$ | $403.78±79.79^{a}$ | $122.91±8.08^{Aa}$ | $75.33±3.45^{Cb}$ |
| | 8 | $116.24±16.45^{Aa}$ | $34.27±5.08^{a}$ | $375.84±49.12^{a}$ | $117.10±10.06^{Aa}$ | $118.26±3.45^{Cc}$ |
| Concentration×Time （P value） | | 0.005 | 0.058 | 0.066 | 0.032 | 0.001 |

* Different superscript numbers ($^{A, B, C}$) indicate a significant effect of concentration (P<0.05). Different lowercase letters ($^{a, b, c}$) indicate a significant effect of time (P<0.05). SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase; GSH: glutathione; MDA: malondialdehyde..

reached its highest level at day 8 (P<0.05). No significant differences were found in superoxide dismutase (SOD) activity at different times during 8-day exposure to different concentration of Pb (P>0.05). The interaction between concentration of Pb and exposure time was identified on GSH activity (P<0.001).

By comparison, Liver SOD, GSH activities and MDA content were affected by concentration of Pb and exposure time (P<0.05, Table 3). SOD in fish exposed to 1 and 2 mg/L Pb was lower than that in fish exposed to 0 and 0.5 mg/L Pb (P<0.05). The lower GSH activity was found in 2 mg/L Pb group (P<0.05). The lower content of MDA was found in 0 mg/L Pb group, followed by 0.5 mg/L Pb groups, and the highest in 1 and 2 mg/L Pb group (P<0.05). During the 8-day period, SOD, GPX and GSH activities decreased with the increasing exposure time (P<0.05), but was not significantly different from day 6 to day 8 (P>0.05). The significant the lowest CAT activity and the highest MDA content were found at day 8 (P>0.05). The interaction between concentration of Pb and exposure time was identified on SOD (P<0.005), GSH (P<0.032)activities and MDA (P<0.001) content.

**mRNA Expression**

The expression of SOD, glutathione-s-transferase (GST), heat shock proteins 70 (HSP-70) and HSP-90 in gill were affected by concentration of Pb and exposure time (P<0.05, Figure 1). The lower SOD GST, HSP-70 and HSP-90 expression were found in 0 mg/L Pb group (P<0.05), but no significant differences were found among 0.5, 1 and 2 mg/L Pb groups (P>0.05). During the 8-day period, SOD and HSP-90 expression decreased with the increasing exposure time (P<0.05), the highest values were found at day 2.

In liver, the expression of SOD, GST, HSP-70 and HSP-90 were affected by concentration of Pb and exposure time (P<0.05, Figure 2). The lower SOD, GST, HSP-70 and HSP-90 expression were found in 0 mg/L Pb group (P<0.05), but no significant differences were found among 0.5, 1 and 2 mg/L Pb groups (P>0.05). During the 8-day period, HSP-70 expression increased with the increasing exposure time, the highest values were found from days 6 to 8 (P<0.05).

## Discussion

Oxidative stress has been considered as one of the basic events involved in physiological disorder. Heavy metals create elevated levels of reactive oxygen species (ROS) and also deplete anti-oxidant enzymes (Company *et al*., 2004). Lead is known to bring about damage to tissues by generating ROS and inducing oxidative stress (Li *et al*., 2016). The ROS levels in mitochondria of mammal increased with the increase of lead doses, and result in DNA damage and oxidative damage. Under normal circumstances, the

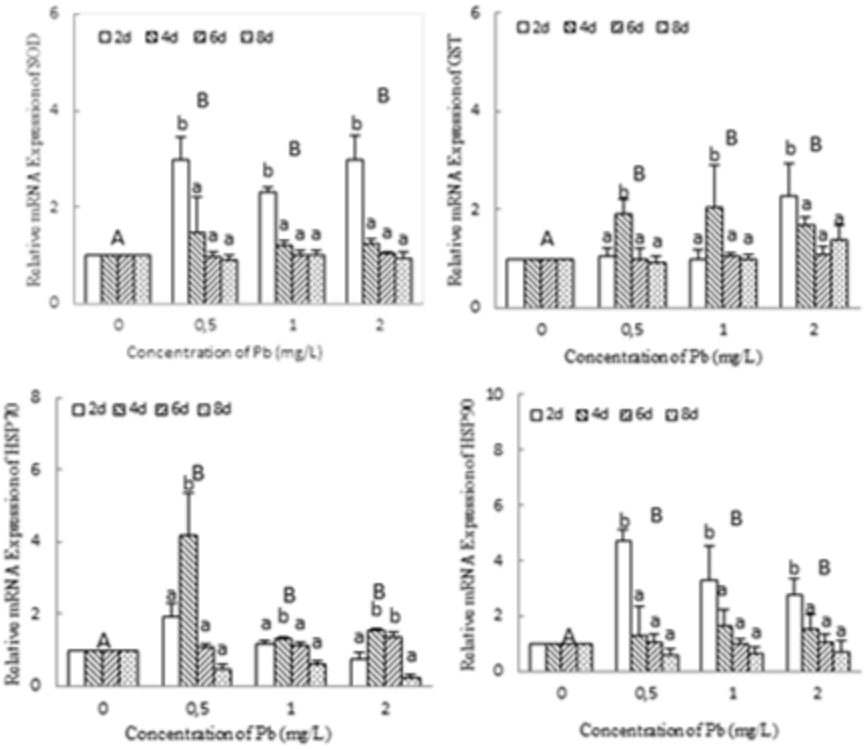


**Figure 1.** Effects of different concentrations of lead and exposure time on the expression of genes SOD, GST, HSP70 and HSP90 in mudskipper gill. Different superscript numbers ($^{A, B, C}$) indicate a significant effect of concentration (P<0.05). Different lowercase letters ($^{a, b, c}$) indicate a significant effect of time (P<0.05).

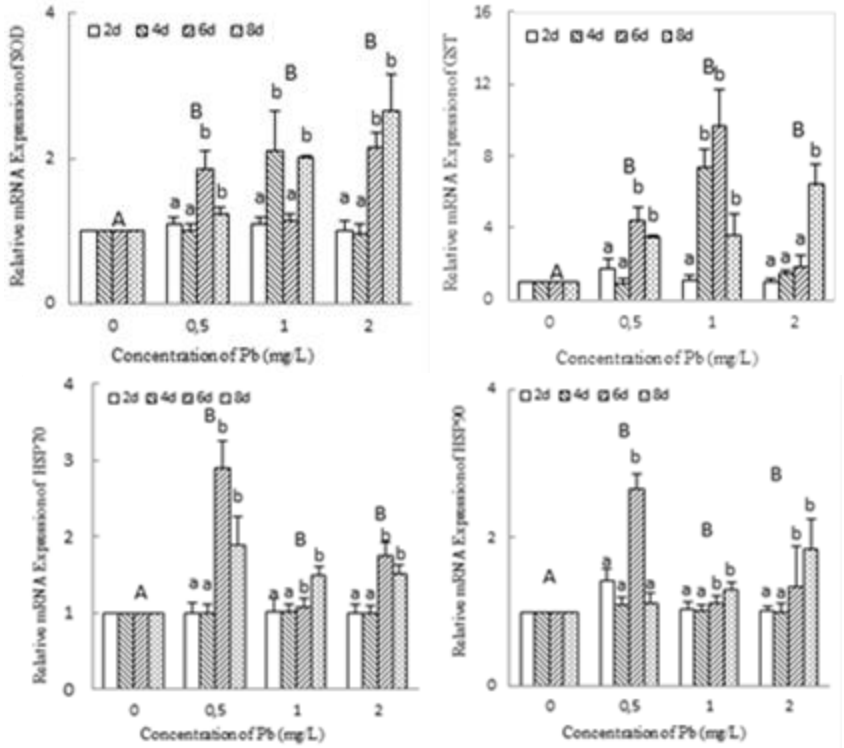


**Figure 2.** Effects of different concentrations of Pb and exposure time on the expression of genes SOD, GST, HSP-70 and HSP-90 in mudskipper liver. Different superscript numbers ($^{A, B, C}$) indicate a significant effect of concentration ($P<0.05$). Different lowercase letters ($^{a, b, c}$) indicate a significant effect of time ($P<0.05$).

antioxidant defenses of fish prevent the uncontrolled generation of ROS through enzymes, such as superoxide dismutase, catalase and glutathione peroxidase (Trenzado *et al*., 2009). In the present study, when fish were exposed to different concentrations of lead, antioxidant activity of gill and liver gradually decreased throughout the 8-day period, indicating that the scavenging function of antioxidant activities was impaired at higher concentrations and longer exposure time to Pb, which is in agreement with the finding in other fish. The effects of Pb stress on SOD in *Danio rerio* was induction effect, and stress on CAT was induction-inhibition effect, Pb toxicity was significantly. The minimum concentration for lactate dehydrogenase (LDH) is about 0.1mmol/L and the lethal concentration is 1 mmol/L, the CAT activity of *Carassius auratus* is less sensitive to Pb treatment (Li *et al*., 2016). The antioxidant enzymes activities may be saturated under a sustained compromised situation, which may lead to excessive accumulation of aldoketones. Aldehydes and ketones can cross link with nucleophilic groups of proteins, nucleic acids and amino phospholipids, and higher aldoketones levels lead to higher cell toxicity, accelerating the damage of cells and tissues (Buege and Aust, 1978). In the this study, MDA content of fish were exposed to lead gradually increased during the 8-day period. In addition, liver MDA content was higher than that in the gill throughout the 28-day period. Elevated liver MDA content may be considered one of the important factors in the pathogenesis of Pb poisoning in fish exposed to elevated environmental Pb, which coincided with regions of high antioxidant enzyme densities in the fish liver.

Exposure of vertebrate to stress conditions such as heat shock, oxidant injury and heavy metals pollution, results in the activation of heat shock genes and the synthesis of heat shock proteins (HSPs), which play an important role in protecting organisms from damage (Leppä and Sistonen, 1997). Qin *et al*. (2013) reported that the level of HSP-70 expression in tissues regulates the biological processes in fish that reduce the deleterious effects of environmental stress. Zhang and Zhang (2012) have shown that $Cu^{2+}$ and malachite green significantly up regulate hemocyte HSP-70 mRNA expression in the digestive glands and gills of oysters (*Crassostrea hongkongensis*). Similarly, increased HSP-70 levels were detected in various tissues in sea bream exposed to Vibrio alginolyticus (Deane *et al*., 2004). In the present study, the expression of HSP-70 in mudskipper gill and liver increased with the increasing concentrations of lead, which is in agreement with the expression of HSP-90. In aquaculture animals, HSP-90 was stress-inducible by hyperthermia treatment, pH, heavy metal exposure and pathogen challenge, and played an important role in the response to deleterious stress conditions (Chen *et al*., 2010). Several studies suggested that enzyme activity was affected by

mRNA and protein stability (Rigault *et al*., 2013). In the present study, the SOD enzyme activity was negatively correlated with mRNA expression of SOD gene in mudskipper gill and live with the increase of lead doses, which may be a regulation of physiological adaptation for response to high SOD enzyme requirement. Similarly, the expression of glutathione S-transferases in gill and liver increased with the increasing concentrations of lead. Hoarau *et al*. (2006) reported that the transcription of GST gene shows the lowest value in the digestive glands of mussels exposed to BaP, whereas the treatment with cadmium and the co-treatment with cadmium and BaP evoke GST gene expression higher than controls. GSTs are involved in the detoxication of many chemical compounds including heavy metal, hydrocarbons, organochlorine insecticides and polychlorinated biphenyls (PCBs) (George and Buchanan, 1990). In addition, the expression of HSP-70, HSP-90, SOD and GST genes in gill decreased with the increasing exposure time, but those in liver increased with the increasing exposure time. Because liver is one of the main detoxifying organs, it can be speculated that the increased expression of liver detoxification genes mRNA compensates for the decline of related enzyme or protein in liver tissues, thus ameliorating the pathogenic stress. However, this hypothesis requires further investigation.

In summary, the MDA accumulation in the gill and liver of mudskipper were exposed to different concentrations of lead show that Pb-induced ROS generation is not fully counteracted by antioxidant enzymes throughout the 8-day period. Functional enzyme activity was affected by mRNA expression and protein stability.

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