MINISTRY OF EDUCATION AND TRAINING

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"RESEARCH ON THE INFLUENCE OF LEAD (PB) ON THE DEVELOPMENT OF ZEBRAFISH *(DANIO RERIO)*"

Specialization: Ecology

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SUMMARY OF THE DOCTORS OF ECOLOGY

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Scientific instructor 2: Associate Professor, Dr.Nguyen Thi Phuong Thao

Reviewer 1:……
Reviewer 2: ……
Reviewer 3: ……

The thesis will be defended at the Academy-level Doctoral Thesis Evaluation Council, meeting at the Academy of Science and Technology - Vietnam Academy of Science and Technology at ... o'clock,……..,2023

The thesis can be found at:
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INTRODUCTION

1. The urgency of the thesis

Currently, population growth along with the rapid development of industry increases waste into the environment and they can have a harmful impact on the health of organisms. In particular, heavy metal pollution is considered an urgent issue that requires public attention.

Heavy metal pollution can cause worrying effects on ecological balance and the diversity of aquatic organisms. Among aquatic animals, fish are the unavoidable object of adverse effects of these heavy metal pollutants. The impact of metals, as well as other pollutants, on aquatic organisms can be assessed by toxicity testing, which is used to detect and evaluate the potential toxic effects of chemicals on aquatic organisms.

The question arises as to why zebrafish are used as a model for studying environmental toxins? In fact, the zebrafish model has been applied in many areas of research in the field of environmental toxicology and the use of this model is increasingly widely used.

To better understand the mechanism of action of Lead (Pb^{2+}) on zebrafish, it is necessary to study its impact on all developmental processes from embryo to adult fish, changes in gene structure, changes in liver, circulatory system, heart rate... from which the mechanism of action of lead (Pb^{2+}) on the human body is drawn. Therefore, the author proposes the research topic "Research on the influence of lead (Pb) on the development of zebrafish (*Danio rerio*)" which is very urgent, scientifically and practically significant to provide the scientific basis for assessing the effects of heavy metals on aquatic animals and vertebrates, especially humans. At the same

time, it is also a premise to use zebrafish as a model organism for testing, toxicology assessment, drug resistance in medicine, and research use in medicine.

2. Research objectives of the thesis

Determine the effect of lead (Pb^{2+}) on the development of zebrafish *(Danio rerio)* through changes in morphology, histology, gene expression changes. Contributing to the basis for further studies on the effects of lead (Pb^{2+}).

3. The main research contents of the thesis

Evaluate the impact of lead concentration on the development of zebrafish embryos and simultaneously determine the accumulation of lead (Pb^{2+}) in the developing organs of zebrafish (Danio rerio).

Assess the effect of lead (Pb^{2+}) on the structure of the intestinal and ovarian tissue of zebrafish (Danio rerio) using tissue sampling and sectioning methods.

Evaluate changes in the expression of genes responsive to lead (Pb^{2+}) and genes that control damage in zebrafish (Danio rerio).

4. The scientific and practical significance

The research results of the thesis delve into the study of the toxicity of lead metal ions on the development process of zebrafish. The research results provide a scientific basis for assessing the risk of heavy metals on aquatic animals, vertebrates, especially humans. At the same time, it is also a premise for using zebrafish as a model organism for testing toxicity, drug resistance in medicine, and research in medical fields.

CHAPTER 1. OVERVIEW

1.1. Lead overview (Pb^{2+})

Lead (Pb^{2+}) is a heavy metal that is persistent in both organic and inorganic compounds. However, exposure to an environment with lead (Pb^{2+}) can cause toxicity to humans and organisms. Lead (Pb^{2+}) can affect the nervous, digestive, circulatory, and immune systems of humans and other organisms. Lead (Pb^{2+}) accumulates in fish through respiration, digestion, skin contact, and through damaged organs. Lead (Pb^{2+}) enters the body mainly through active absorption and passive diffusion. The bioaccumulation in aquatic animals depends on species, age, and toxin form. The ability to excrete lead (Pb^{2+}) depends on the metal mixture, species, and biological characteristics of the tissues. Toxins can escape the bloodstream and invade tissues, where they are biologically transformed, excreted, and accumulated.

1.2. Overview of zebrafish

Fish are classified as a major class of aquatic vertebrates and are an integral component of toxicity testing strategies. Zebrafish are organisms that have been commonly used in many laboratories around the world for a long time and the genome sequence has been fully decoded. Analysis of the zebrafish genome revealed that there are more than 12,000 genes similar to the human genome, and about 70% of human genes have at least one gene similarity in zebrafish. Zebrafish are therefore promising alternative models for toxicology research, using different stages of fish life for toxicology testing. Moreover, zebrafish have a fast breeding cycle, the fish embryo lasts only 4-5 days, during this time, the fish is not able to feed itself, so it

is not considered an animal body. Therefore, the use of the combination is not bound by strict regulations for laboratory animals. Furthermore, a female can lay 50-200 eggs per graft. Therefore, a large number of embryos can be produced actively in the laboratory, so it is possible to use embryos to evaluate a large number of experimental substances. During the development of zebrafish, embryos form early and some organs in zebrafish are quite similar to other vertebrates but grow rapidly.

CHAPTER 2. MATERIALS AND RESEARCH METHODS

2.1. Time and place of study

Time: January 2018 to January 2021. The author conducts the selection of breeds, conducts breeding and induction of pollution concentrations, analyzes the results

Research location: Department of Animal Biotechnology - Institute of Tropical Biology.

2.2. Materials

The zebrafish (Danio rerio), in the adult stage has a length of 3.62 ± 0.04 cm and 1.00 ± 0.48 g of weight.

2.3. Contents and methods

2.1.1. Fish farming methods

Zebrafish are raised in a glass tank, with a diameter of 30cm, the number of fish raised is 30, according to the ratio ...., Select adult fish with a length of 3.62 ± 0.04 cm and 1.00 ± 0, 48 g weight, uniform morphology. Fish are reared in tanks with aeration to ensure the amount of dissolved oxygen 6.5 ± 0.7mg/L, pH=6.5 – 7.5, temperature 32^0C, fish feed is algae and plankton.

The control fish was fish raised in a water tank with no lead metal ion concentration.

Zebrafish is a species of fish that was raised in a Pb-free water tank. After the fish laid their eggs, the author collected the embryos to conduct experiments.

The repeatability of each experiment was 3 (n=3).

2.1.2. The influence of lead (Pb^{2+}) on the rate of fish embryo development at different stages

Count the number of live hatched embryos in the total number of embryos, observe some abnormalities of the embryos and determine the number of hatched embryos, find out the percentage of live embryos at different concentrations, each concentration using 40 zebrafish embryos at stages 1 – 2 cells, observe morphology, vitality and monitor embryo the survival rate of the embryos after each stage of division under a microscope, each experiment is repeated 3 times. Live embryos are healthy embryos and are capable of developing in subsequent stages, without deformity, without death.

2.1.3. Effect of lead (Pb^{2+}) on fish embryonic heart rate at different stages

To determine the heart rate at different concentrations, each concentration of the experiment used 40 zebrafish embryos in stages 1 – 2 cells, observing a healthy embryonic heart rate, no malformations after each stage of division under a microscope, each experiment repeated 3 times. Live embryos are healthy embryos and are capable of developing in subsequent stages, without deformity, without death.

2.1.4. Determination of lead accumulation (Pb^{2+}) in the evelopment organ of fish

An average of 50 embryos will be grown in a glass tank with a culture solution. Using a plastic straw, gently suck the embryo into the feeding tank. Observe the morphology, vitality and survival of the embryos after each stage of division. The fish embryos were collected and cultured in Hank solution (0.137M NaCl, 5.4mM KCl, 0.25mM Na2HPO4, 0.44mMKH2PO4, 1.3mMCaCl2, 1.0mMMgSO4, 4.2mMNaHCO3). The collection and embryo culture media were kept at 4^0 C until analysis.

2.1.5. Evaluation of the effect of lead (Pb^{2+}) on the structure of intestinal tissue and ovarian tissue

To assess the effect of lead (Pb^{2+}) on the intestinal and ovarian tissue structures of zebrafish, the fish will be grown in an environment containing Pb^{2+} concentrations from the embryonic stage to 90 days of age. Then collect the fish, anesthetize the fish with Lidocaine 2%, dissect the fish to remove the intestinal and ovarian samples, each experiment repeated 3 times.

Hematoxylin-eosin (HE) staining was used.

2.1.6. RNA extraction method

After egg collection, selecting embryos without deformities, uniform in morphology, placing 50 embryos in each glass tank containing raised tissue with pollution at different concentrations, after 90 days of starting to collect fish, selecting individuals without deformities in light, fish collected and preserving tissue samples in 70 degree alcohol solution, 40 degree negative cabinet.

Real-time RT-PCR

Gene expression was quantified by the Real-time qRT-PCR method with a PCRBIO 1-Step RT-PCR Kit).

- Relative quantitative analysis of gene expression :

Method 2 $^{-\Delta\Delta Ct}$ (Livak) (Real-time PCR Applications Guide – Biorads)

The $2^{-\Delta\Delta Ct}$ method was applied to evaluate the relative level of expression of gene expression. The method assumes that both the target and reference genes are amplified with near 100% efficiency and within 5% of each gene. To determine the relative differences in

the expression levels of target genes in different samples, the $2^{-\Delta\Delta Ct}$ method was performed according to the following steps:

- Standardize the CT value of the target gene with the reference gene for both the test sample and the calibration sample:

$\Delta C_{T(sample)} = C_{T(target, sample)} - C_{T(reference, sample)}$

$\Delta C_{T(normal)} = C_{T(target, standard)} - C_{T (reference, standard)}$

- Normalize the value of ΔC_T of the test sample with the C_T of the standard sample: $\Delta\Delta C_T = \Delta C_{T(sample)} - \Delta C_{T(standard)}$

- Finally, the expression rate was calculated according to the formula: Expression rate = $2^{-\Delta\Delta Ct}$

Evaluation Criteria:

- Changes in gene expression in response to heavy metals.
- Changes in gene expression control DNA damage.

The result was a proportional increase (or decrease) of the target gene in the test sample correlated with the reference sample and normalized to the expression of the reference gene.

2.1.7. Statistical methods

The data were processed and analyzed using Sigmaplot 11.0 software to compare the differences across all performance indicators on the survey groups. Data are presented as $\bar{x} \pm$ SD. The analyzed fish samples were repeated three times and assessed the difference between the experimental concentrations using ANOVA to statistically calculate the mean and the corresponding variance. After comparison by ANOVA, the results were considered to have significantly different values when $P < 0.05$.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Effect of lead (Pb^{2+}) on the rate of fish embryo development at different stages

In the process of breeding and mating zebrafish with the ratio of 2 female and 1 male fish in the aquarium, we obtain a good amount of embryos, the embryo structure is transparent. After the embryo collection process, the embryo will be grown in a suitable lead containing aqueous medium (Pb^{2+}) at concentrations of 0.1 µg/L, 1 µg/L, 10 µg/L, 20 µg/L, 100 µg/L. Each batch contained 50 fish embryos and was repeated three times with a 14 a.m. and 10 p.m. Dead embryos were counted discarded daily during observation.

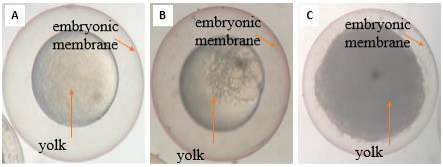

Figure 3.1. Morphology of zebrafish embryos; A – normal; B, C – abnormal

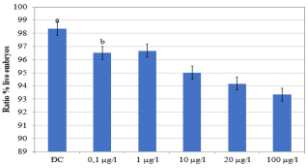

Figure 3.2. Show the percentage of live embryos in the stage of formation of 26 vertebrae (22h)

Results of analysis of the percentage (%) of live embryos at stages of lead concentration (Pb^{2+}) infection. Based on the results of Figure 3.2, we can see that the average survival rate in experimental batches tends to decrease with the gradual increase of Pb^{2+} experimental concentration. Fluctuations range from 98.33% - 4.17% ($P<0.05$). In zebrafish embryo development concentrations, embryo survival in control plots was higher than in experimental plots. Stage of formation of 26 vertebrae (22h): the percentage of live embryos at

concentrations of 0.1 µg/L and 1 µg/L decreased slightly compared to the control batch, by 1.83% -1.67% (P<0.05); the percentage of live embryos at concentrations of 10 µg/L - 100 µg/L compared to the control batch decreased by 3.33% - 5%

Pharyngeal stage (48h): the percentage of embryos alive in the control batch was the highest (95.83%) and the percentage of embryos alive at the concentration of lead (Pb^{2+}) 100µg/L was the lowest (88.33%), the percentage of embryos alive at the concentration of 0.1 µg/L and 1 µg/L compared to the control batch decreased by 1.67% – 3.33% (P<0.05), while the percentage of embryos alive at the concentration of 10µg/L and 20µg/L compared to the control batch decreased by 5% – 7.5% (P<0.05).

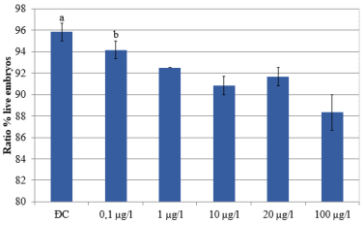

Figure 3.3. Shows the percentage of embryos alive at 48h stage

Extracapsular phase (72h): survival rate at 0.1 µg/L– 1 µg/L compared to control batch decreased slightly from 4.17% – 5%, survival rate at 10 µg/L – 20 µg/L compared to control batch decreased by 13.33 – 21.67, survival rate at 100 µg/L compared to control batch decreased by 43.33% (P<0.05).

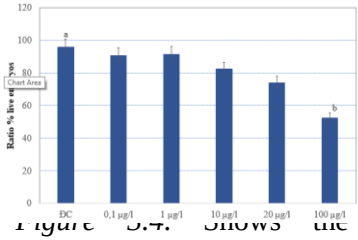

Figure 3.4. Shows the percentage of embryos alive at 72h stage

The last stage is the yolk digestion stage (168h) with the most obvious embryo survival rate change: the highest survival rate in the control batch (90.83%), the percentage of live embryos at 0.1 µg/L and 1 µg/L compared to the control batch decreased from 13.33% – 20.83% (P<0.05), the percentage of live embryos at 10 µg/L – 100 µg/L compared to the control batch decreased sharply from 84.17% -86.67% (P<0.05) reached the LC50% lethal threshold.

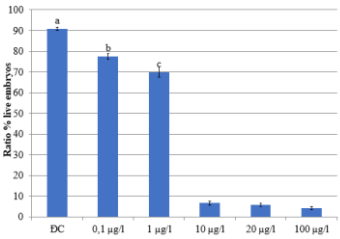

Figure 3.5. Shows the percentage of embryos alive at 168h stage

3.2. Effect of lead (Pb^{2+}) on fish embryo heart rate at different stages

At the exocyst stage, the heart rate of the lead-induced embryo (Pb^{2+}) at concentrations of 0.1, 1, 10, 20, 100 µg/L gradually increased from 8÷21 beats/min compared to the heart rate of the embryo in the control batch (P≤ 0.05). For the pharyngeal stage, the heart rate of the embryo at 0.1 µg/L increased insignificantly compared to the control batch, while the heart rate of the embryos at concentrations 1, 10, 20, 100 µg/L compared to the control batch increased from 11÷26 beats/ minute.

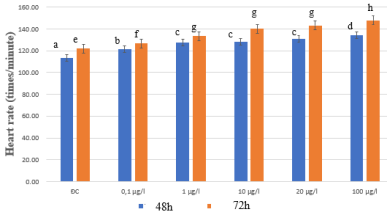

Figure 3.6. A graph showing the heart rate of zebrafish embryos at lead concentrations (Pb^{2+}) was tested at 48h and 72h.

3.3. Determination of lead accumulation (Pb^{2+}) in the developing organ of the fish

Based on the results, the amount of Pb^{2+} accumulated in the zebrafish's internal organs at the concentration of 100 µg/L accounted for the highest proportion (12.31 µg/g) and was 12.31 times higher than the concentration of 10 µg/L, 25.12 times higher than the concentration of 1 µg/L, 32.39 times higher than the concentration of 0.1 µg/L. For the control batch, the rate is 0%, proving that the amount of Pb^{2+} does not accumulate in the fish body.

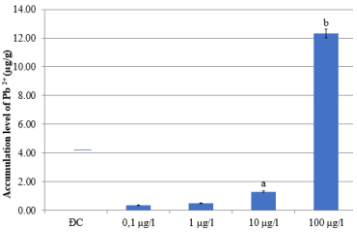

Figure 3.7. Pb^{2+} accumulation in zebrafish

The amount of Pb^{2+} accumulation in zebrafish muscles and bones shown in (Figure 3.8) shows that the concentration of 100 µg/L accounts for 2.22 µg/g and is 3.1 times higher than the concentration of 10 µg/L, 8.2 times higher than the concentration of 1 µg/L and the concentration of 0.1 µg/L. For the control batch, the presence of lead in the control group was not observed.

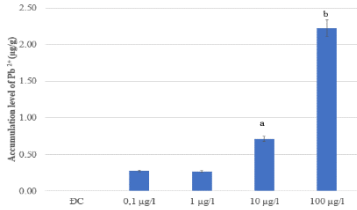

Figure 3.8. Pb^{2+} buildup in zebrafish muscles and bones

3.4. Effect of lead (Pb^{2+}) on intestinal tissue structure

The intestinal tissue structure of zebrafish has increased changes in the plumage when the fish is exposed to correspondingly high concentrations of Pb^{2+}. At concentrations of 0.1 µg/L to 100 µg/L there was an increase in the ratio of villi to control plots and there was a statistical difference ($P<0.05$), however the difference was not very large between concentrations.

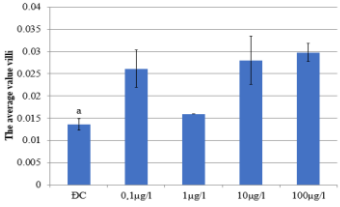

Figure 3.9. Demonstrates the influence of induced Pb $^{2+}$ concentrations on the gut of zebrafish.

The average value of velvet feathers at the concentration of 10 µg/L, 100 µg/L was 2 times that of the control lot. The ovarian tissue structure of zebrafish changes as the fish is exposed to higher levels of Pb^{2+}, the proportion of lipid particles decreases. At concentrations of 0.1 µg/L to 100 µg/L there was a statistically significant reduction in the ratio of lipid particles compared to the control batch, with a statistical difference ($P<0.05$), however the difference was not as large between the concentrations. The 100 µg/L concentration decreased the most, by 2.2-fold compared to the control batch, followed by a concentration of 10 µg/L, a concentration of 1 µg/L, a concentration of 0.1 µg/L. This shows that the concentration of lead ions increases, the number of lipid particles in the intestine decreases.

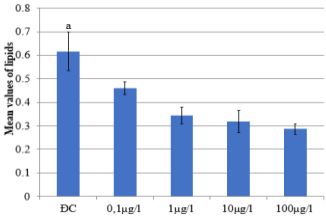

Figure 3.10. Presenting the effect of Pb^{2+} induction temperature on zebrafish eggs

3.5 Evaluation of changes in expression of lead-responsive genes (pb2 +) and zebrafish damage control genes.

3.5.1. Evaluation of GADD45A gene expression changes and lesion control

The results of real time RT-PCR evaluation of GADD45A gene phase 24h between experimental groups showed that all experimental concentrations showed a change in gene expression level, group 0.1 µg/L and group 1 µg/L gene expression level decreased compared to the control group, group 0.1 µg/L decreased nearly 1.6 times, group 1 µg/L decreased 11 times compared to the control group. But the 10 µg/L group had an increase in gene expression and a 1.6-fold increase compared to the 1 µg/L group.

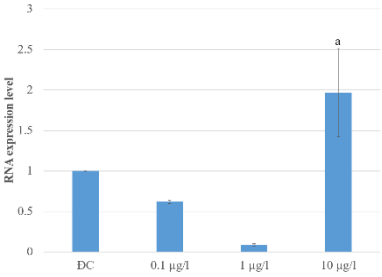

Figure 3.11. The mRNA changes of the 24-hour GADD45A gene between the experimental groups.

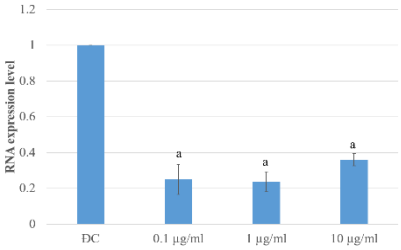

Figure 3.12. The mRNA changes of the GADD45A gene phase 168h between the experimental groups.

Real-time RT-PCR evaluation of the GADD45A gene stage 168h between experimental groups showed that all experimental groups had reduced gene expression compared to the control group, the 0.1 µg/L group and the 1 µg/L group had almost the same gene expression level and a 4-fold decrease compared to the control group, the 1 µg/L group decreased by 3 times compared to the control group.

However, the 10 µg/L group had increased gene expression again compared to the 0.1 µg/L group and the 1 µg/L group and increased 1.5-fold.

3.5.2. Evaluation of GADD45G gene expression changes and lesion control

The real time RT-PCR evaluation of the 24-hour GADD45G gene between the experimental groups showed that: the 0.1 µg/L, 1 µg/L, 10 µg/L group had increased gene expression levels with higher concentrations, leading to greater levels of altered gene expression, not changing much compared to the control group. A concentration of 1 µg/L had an increase in gene expression and a 2.3-fold increase compared to the control group. At a concentration of 10 µg/L, there was a sharp increase in gene expression and a 17-fold increase compared to the control groups.

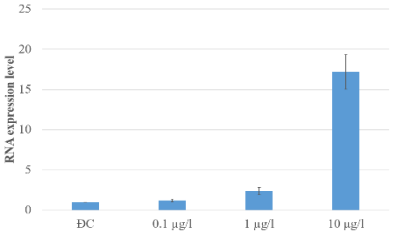

Figure 3.13. The mRNA changes of the 24-hour GADD45G gene between the experimental groups.

The real time RT-PCR evaluation of the GADD45G gene in the 168h period between the experimental groups showed that the 0.1 µg/L group and the 10 µg/L group had almost the same level of gene expression and a 10-fold

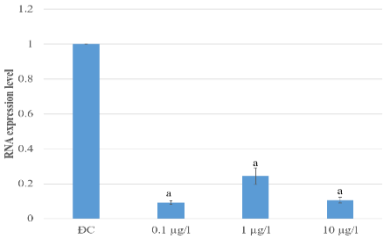

Figure 3.14. The mRNA changes of the GADD45G gene phase 168h between the experimental groups.

decrease compared to the control group. However, the 1 µg/L group had increased gene expression compared to 0.1 µg/L and the 10 µg/L group and increased 2.4-fold**.**

3.5.3. Evaluation of SOD1 gene expression changes and lesion control

The results of real time RT-PCR evaluation of the sod1 gene phase 24h between the experimental groups showed that: 0.1 µg/L, 1 µg/L and 10 µg/L had increased gene expression compared to the control group, 0.1 µg/L, 1 µg/L had slightly increased gene expression compared to the control group.

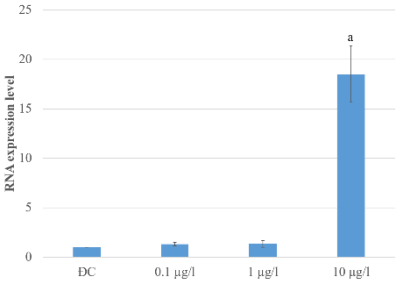

Figure 3.15. The mRNA changes of the 24-hour SOD1 gene between the experimental groups.

However, the 10 µg/L group began to have increased gene expression and increased more than 14-fold compared to the other groups. This may be because the time and concentration of 0.1 µg/L, 1 µg/L are not sufficient to alter the level of mRNA expression. The 00concentration of 10 µg/L began to change drastically compared to the control group. The real time RT-PCR of the SOD1 gene stage 168h between the experimental groups showed that the 0.1 µg/L and 1 µg/L groups had almost the same level of gene expression and a 2.5-fold

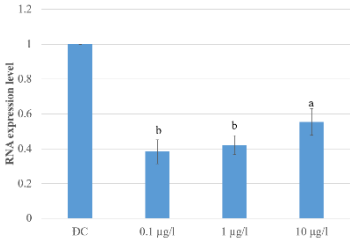

Figure 3.16. The mRNA changes of the SOD1 gene phase 168h between the experimental

decrease compared to the control group. However, the 10 µg/L group had increased gene expression compared to the 0.1 µg/L group and the 1 µg/L group and increased 1.5-fold. The expression level in the control group was 2-fold that of 10 µg/L.

3.5.4. *Evaluation of SOD2 gene expression changes and lesion control*

The results of the real time RT-PCR evaluation of the 24h SOD2 gene between the experimental groups showed that 0.1 µg/L, 1 µg/L had substantially the same and equivalent gene expression level compared to the control group, the concentration of 0.1 µg/L had a slight decrease compared to the control groupHowever, the 10 µg/L group had an increase in gene expression and a 14-fold increase compared to the control group, a possible increase in lead ion toxicity at a concentration of 10 µg/L with sufficient dose to alter the SOD2 gene in the 24h period.

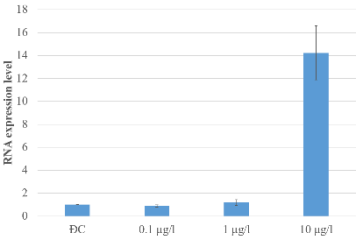

Figure 3.17. The mRNA changes of the 24-hour SOD2 gene between the experimental

The real time RT-PCR of the SOD2 gene stage 168h between the experimental groups showed that the 0.1 µg/L group, the 1 µg/L group and the 10 µg/L group were almost the same and decreased about 2.3 times compared to the

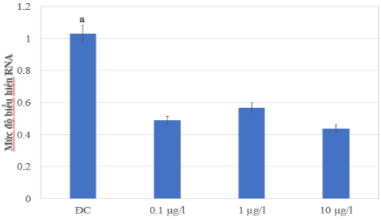

Figure 3.18. The mRNA changes of the SOD2 gene phase 168h between the experimental groups.

control group. However, the 1 µg/L group had a high level of gene expression compared to the 0.1 µg/L and 10 µg/L groups.

3.5.5. Evaluation of MT2 gene expression changes and lesion control

The results of the real time RT-PCR evaluation of the MT2 gene phase 24h between the experimental groups showed that the test lead ion concentrations both affected the MT2 gene phase 24h, 0.1 µg/L group and 1 µg/L group with almost the same gene expression level, not much changed compared to the control group. However, the 10 µg/L group significantly increased gene expression and was 9-fold higher than the control group and 8-fold higher than the 0.1 µg/L, 1 µg/L concentration.

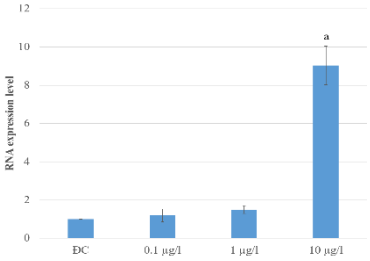

Figure 3.19. The mRNA change of the MT2 gene stage 24h between the experimental

The real time RT-PCR assessment of the MT2 gene in the 168h period between the experimental groups showed that the 0.1 µg/L group and the 1 µg/L group had almost the same level of gene expression and a 5-fold decrease compared to the control group. However, the 10 µg/L group had an increase in gene expression compared to the 0.1 µg/L and 1

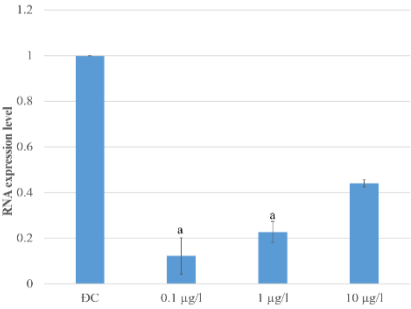

Figure 3.20. The mRNA changes of the MT2 gene in the 168h period between the experimental groups.

μg/L groups and a 2-fold increase. In the 100 μg/L group, gene expression levels increased sharply compared to concentrations of 0.1 μg/L, 1 μg/L, 10 μg/L and increased 6.5-fold compared to the 0.1 μg/L and 1 μg/L groups, 3.2-fold higher than the 10 μg/L group.

CONCLUSIONS AND RECOMMENDATIONS

1. Conclusion

Thesis evaluating the impact of lead (Pb^{2+}) on the development of seahorse embryos, including the percentage of surviving embryos, changes in heart rate, determination of lead (Pb^{2+}) levels in the body, assessment of the effects of lead (Pb^{2+}) on intestinal and ovarian tissue, evaluation of changes in genes and control of damage to GADD45A, SOD1, SOD2, GADD45G, MT2.

Lead (Pb^{2+}) reduces the survival rate of seahorse embryos and larvae through the increasing concentration stages. Furthermore, lead (Pb^{2+}) affects the physiological activity of fish: reducing the hatching rate of embryos, inhibiting the development of embryos and larvae, and causing deformities such as spinal curvature, pericardial edema, abdominal edema, eye edema, and loss of pigmentation. The heart rate also increases with each stage of concentration.

The presence of lead (Pb^{2+}) and its impact on humans and organisms cannot be predicted. The absorption capacity through the respiratory and digestive systems distributes harmful substances throughout the body, affecting the health of humans and organisms. This study identified the presence of heavy metals and negative effects in seahorse bodies, demonstrating a serious threat to both organisms and human health. The results obtained from this study will contribute to future research aimed at improving the environment to protect the health of humans and organisms.

Exposure to lead (Pb^{2+}) alters the expression levels of the genes GADD45A, GADD45G, SOD1, SOD2, MT2. Real-time RT-PCR showed gene deviation in the lead (Pb^{2+}) absorption process of seahorses at 24h and 168h stages.

The lead (Pb^{2+}) content accumulates throughout the entire body of adult seahorses in a concentration-dependent manner...

2. Recommendations

Further evaluation of the effect of lead on gene expression in relation to the development of fish embryos continues.

Evaluate the structure of organs involved in the metabolism of the liver and kidneys.

NEW CONTRIBUTIONS FROM THESIS

A systematic and comprehensive study on the effects of lead (Pb^{2+}) on some biological characteristics during the developmental stage of zebrafish was conducted. This is the first study to investigate the molecular-level impact of Pb^{2+}) on zebrafish.

The study contributes new insights into the effects of Pb^{2+} on the development of zebrafish, a widely used species in biological research. It provides new information on the biological reaction mechanisms and impacts of Pb^{2+} on different developmental stages of fish, and serves as a basis for developing risk assessment methods for the development of fish and other species in Pb^{2+}polluted environments.

LIST OF PUBLISHED RESEARCH PAPERS

1. Tuan Ngo Van , Thuan Tran Thi , Nga Nguyen Thi , Chi Nguyen Quynh Ho , Tram Le Ngoc Vo , Le Thanh Long , Thao Nguyen Thi Phuong , Effects of lead on structures of zebrafish intestines and oocytes , Ournal of Entomology and Zoology Studies , E - ISSN: 2320 – 7078, JEZS 2021, 9(2): 127 -129.

2. Tuan Van Ngo, Le Thi Lam, Cang Ngoc Ly, Tram Thi Bich Tran, Huy Nghia Quang Hoang, Chi Nguyen Quynh Ho, Mai Thi Phuong Nguyen, Thuan Tran Thi, Nga Nguyen Thi, Le Thanh Long, Thao Nguyen Thi Phuong. Effects of lead on the development of zebrafish embryo. European Journal of Molecular & Clinical Medicine, ISSN: 2515 -8260, V8, I3, 2021.