MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

DANG NGUYEN NHA KHANH

STUDY ON THE ACCUMULATION, DEPURATION AND EFFECTS OF LEAD (Pb) AND CADMIUM (Cd) ON THE LEVELS OF GLYCOGEN, INSULIN AND CORTISOL IN CLIMBING PERCH *(Anabas testudineus)*

SUMMARY OF DISSERTATION ON SCIENCES OF MATTER

Major: Inorganic Chemistry

Code: 9 44 01 13

Ho Chi Minh, 2025

The dissertation is completed at: Graduate University of Science and Technology, Vietnam Academy Science and Technology

Supervisor:

Nguyen Thi Kim Phuong, Assoc Prof, Dr, Graduate University of Science and Technology, Vietnam Academy Science and Technology.

Referee 1:

Referee 2: .....

Referee 3: .....

The dissertation is examined by Examination Board of Graduate University of Science and Technology, Vietnam Academy of Science and Technology at …… (time, date …)

The dissertation can be found at:

1. Graduate University of Science and Technology Library
2. National Library of Vietnam

INTRODUCTION

During the process of modernization and industrialization, along with the formation and development of export processing zones, industrial parks, and hundreds of thousands of chemical and processing facilities nationwide, the issue of environmental pollution has become increasingly severe. This is particularly true for heavy metal pollution such as lead, cadmium, chromium, nickel, etc., which not only affects soil, water, sediment, and air but also increasingly impacts aquatic organisms (shrimp, fish, mollusks). These toxic heavy metals, when present in water at levels exceeding permissible limits, penetrate and accumulate in the bodies of aquatic organisms, causing disorders and sometimes even disrupting normal physiological functions (respiration, movement, and metabolic regulation). This weakens their reproductive capacity and development, affecting their immunity, making them more susceptible to disease and mortality. For fish, the absorption of heavy metals from the environment occurs through organs like the gills, skin, and digestive system, and these metals can then accumulate in organs such as the gills, liver, kidney, and muscle. The consumption of fish products contaminated with heavy metals can pose potential dangers such as cancer, reduced immunity, and many other negative effects on consumers, especially children and pregnant women.

Lead (Pb) and Cadmium (Cd) are identified as belonging to the group of toxic heavy metals that seriously cause environmental problems and disrupt the balance of ecosystems. These heavy metals are non-biodegradable, only accumulate in living organisms, and most are known to be potential carcinogens. Consequently, the bioaccumulation and biomagnification of these toxic heavy metals along the food chain and food web pose health risks to food consumers. For fish, Pb and Cd have adverse effects on development and metabolic activities in the liver, kidney, muscle, and other tissues. The distribution and accumulation of Pb and Cd among different body parts (gills, intestines, liver, kidney, or muscle) depend on the source of contamination (food or water). Although numerous studies have documented that the accumulation of Pb and Cd is detrimental to fish health, the specific effects of Pb and Cd on many physiological functions have not yet been fully investigated.

Fish serve as valuable biomonitors for tracking aquatic ecosystems due to their rapid capacity to metabolize, detoxify, and accumulate heavy metals in their bodies. They are also highly sensitive to minor environmental changes, and fish are a vital food source, especially for the Vietnamese population. Therefore, assessing the impact of heavy metals in general and Pb, Cd in particular fish is extremely important. In addition, the results concerning the bioaccumulation of Pb, Cd in different fish tissues can be used as an effective index to evaluate Pb, Cd contamination in water. When fish encounter changes in their living environment (pollution, temperature changes, etc.), it increases stress. When stressed, the hypothalamus releases hormones that stimulate cells to produce cortisol. As cortisol levels rise, the fish's body needs to utilize a larger-than-normal amount of glucose to cope with these changes. This leads to the mobilization and decomposition of glycogen in the liver, causing a change in liver glycogen content. The disruption of glucose and glycogen levels, in turn, affects the insulin hormone in the pancreas. Therefore, determining the levels of cortisol in the plasma, insulin in the blood, and glycogen in the liver is considered an important tool for assessing the physiological impact of environmental pollution on fish health. Furthermore, studies on the elimination process of Pb and Cd are crucial for health protection, as they allow for the determination of the self-purification capacity of fish and other aquatic species contaminated with metals.

The climbing perch *(Anabas testudineus)* is a fish species that possesses both high nutritional and economic value for Vietnam's aquaculture industry. However, because they are often raised in rice fields, ponds, and stagnant water bodies (areas highly susceptible to heavy metal contamination from fertilizers, pesticides, industrial wastewater, or mineral exploitation in the region), they are very prone to heavy metal absorption. Despite the significant importance of climbing perch in Vietnam, there is relatively limited information regarding the effects of Pb and Cd, particularly concerning their accumulation, elimination, and physiological stress in this species. Therefore, the study titled **"Study on the accumulation, depuration, and effects of Lead (Pb) and Cadmium (Cd) on the levels of glycogen, insulin, and cortisol in climbing perch *(Anabas testudineus)*"** aims to address the following issues:

- To determine the acute toxicity threshold (LC_{50} in 96 hours) of individual metals, Pb and Cd, for the climbing perch..

- To determine the toxic effects of Pb and Cd on the changes in plasma cortisol, blood insulin, and liver glycogen levels, which are critical biomarkers for assessing the level of physiological stress when fish live in a heavy metal-contaminated environment.

- To investigate the accumulation rules of Pb and Cd in the tissues (liver, gills, and muscle) of the climbing perch. Furthermore, the self-purification capacity of climbing perch exposed to Pb and Cd will be monitored based on the elimination rates of these metals to provide information for aquaculture practices, ensure a safe food source, and mitigate risks to human health.

Research Content of the Dissertation:

1. Acute toxicity study (96-hour LC_{50}) of Pb and Cd on the climbing perch

2. Sub-chronic toxicity study of Pb and Cd on the accumulation and elimination process of Pb and Cd in the gills, liver, and muscle of the climbing perch.

3. Sub-chronic toxic effects of Pb and Cd on the changes in plasma cortisol, blood insulin, and liver glycogen levels in the climbing perch.

Scientific Significance: The research results of this dissertation will establish a foundation for studies in ecology and environmental toxicology, including the effects of pollutants on the climbing perch and the use of biomarkers to assess the potential impact of contaminants on human health.

Practical Significance: The research results of this dissertation can provide a scientific basis for managers in guiding the use of the food chain toward safety, while also utilizing these biomarkers as a useful tool for monitoring environmental pollution and warning against the risks of heavy metal transmission up the food chain.

Novel Contributions

- The acute toxicity thresholds of Pb and Cd to climbing perch were determined, with the 96-hour LC_{50} values being 120 mg/L for Pb and 38 mg/L for Cd;

- The accumulation rules of Pb and Cd in climbing perch were determined after 28 days of exposure, revealing that the accumulation order for Pb was Gill >> Liver > Muscle, and for Cd was Liver >> Gill > Muscle;

- The depuration rules of Pb and Cd in climbing perch were determined after 14 days in clean water, showing that the depuration order for Pb was Gill > Liver > Muscle, and for Cd was Liver >> Gill > Muscle;

- The effects of Pb and Cd on the activities of the Hypothalamic–Pituitary–Interrenal (HPI) axis, the pancreas, and liver function in climbing perch exposed to Pb and Cd were determined through the observed disturbances in the production of cortisol and insulin, and in hepatic glycogen breakdown.

Chapter 1. RESEARCH OVERVIEW

1.1. ENVIRONMENTAL TOXICOLOGY

1.1.1. Classification of Toxicity Tests

Toxicity tests are diverse, encompassing acute, sub-chronic, and chronic toxicity tests. Toxicity testing assesses how a chemical or product may negatively affect an organism's biological functions, including harmful and non-lethal impacts.

Acute Toxicity Tests: These involve a short exposure time, typically lasting from 24 to 96 hours, focusing primarily on immediate and severe effects such as mortality (measured by the 50% lethal dose, denoted as LD_{50}).

Sub-chronic Toxicity Tests: These involve repeated exposure over a moderate duration, usually from 28 to 90 days, aiming to identify potential toxic effects that may not be apparent in acute tests but become significant after multiple exposures.

Chronic Toxicity Tests: These focus on prolonged exposure, typically lasting 12 months or longer.

1.1.2. Toxicokinetics

After entering the body, toxicants in fish follow a pathway that includes distribution, biotransformation, and elimination, with significant accumulation in specific tissues and organs. The success of the detoxification process depends on the fish's ability to metabolize and excrete the toxicants effectively.

a. Transport, Distribution, and Accumulation: Toxicants are transported throughout the body via the blood and lymphatic systems to various organs such as the liver, kidneys, and muscle.

b. Detoxification and Depuration: In fish, detoxification and depuration are crucial processes involving multiple organs that work together to remove toxicants and waste products.

The liver is the primary detoxification organ in fish. The detoxification mechanism in the liver primarily involves biotransformation. During biotransformation, some toxicants can be converted into intermediate substances that are less toxic, more water-soluble, and can be more easily excreted.

Fish can eliminate the metabolized toxicants via the gills or kidneys. However, the efficiency of elimination through the gills or kidneys can vary depending on the nature of the toxicant and the fish species

1.2. OVERVIEW OF HEAVY METALS (LEAD, CADMIUM)

1.2.1. Lead (Pb)

Lead (Pb) is a metal belonging to group IVB of the periodic table. It is so heavy, soft, malleable, and has a bluish-gray color. It is a natural component of the Earth's crust and is often found in trace amounts. Natural Pb in surface water is estimated to be 0.02 µg/L and rarely exceeds a few µg/L. The toxic effects of Pb on organisms are diverse: they include histological changes such as signs of parenchymal cell necrosis, vascular collapse, fibrosis of the hepatic cords and connective tissue, loss of growth capacity and body weight, and damage to the central nervous system, peripheral nervous system, hematopoietic system, cardiovascular system, and organs like the liver and kidneys.

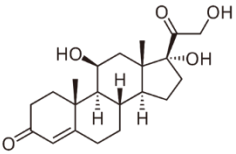
1.2.2. Cadimi (Cd)

Cadmium (Cd) is a soft, non-essential, and biologically toxic element belonging to group IIB of the periodic table, with an atomic mass of 112. The toxic effects of Cd on fish can manifest in various ways, including impacts on embryonic development, ion balance regulation, energy metabolism, growth, stress response, and immunity. Furthermore, Cd has been shown to cause dysfunction in several critical organs (gills, liver, and kidneys), affecting physiology, hindering fish development, and altering hematological indices. Cd accumulation in muscle is significantly lower compared to other body parts.

1.3. BIOCHEMICAL COMPONENTS

1.3.1. Hormone cortisol

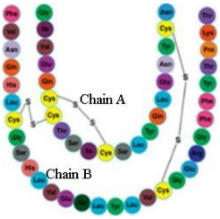
Cortisol is a steroid hormone that plays a key role in regulating metabolism, including the stress response. The molecular formula for cortisol is $C_{21}H_{30}O_5$, and its molecular weight is 362.465.



The physiological stress response in fish can be divided into three phases. *The first phase*, known as the primary response, involves the rapid release of stress hormones. *The second phase*, known as the secondary response, encompasses various biochemical and physiological effects, including changes in metabolism, hematological characteristics, cellular features, immune regulation, and osmoregulatory disturbance. Finally, *the third phase*, known as the tertiary response, involves changes in the overall performance of the fish. These changes are a result of chronically elevated levels of cortisol, which are caused by an exhausted negative feedback mechanism that prevents the hormones from returning to normal levels.

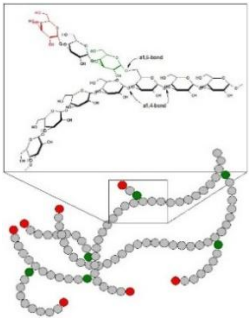
1.3.2. Hormone insulin

Insulin is a peptide hormone that is produced by the β cells of the islets of Langerhans in the pancreas. It is made up of two peptide chains, chain A (21 amino acids) and chain B (30 amino acids), which are connected by two disulfide bonds and an additional disulfide bond located within chain A.



Insulin controls the cellular energy supply and macronutrient balance, which is essential for the intracellular transport of glucose into tissues. Exposure to pollutants can cause metabolic alterations, disrupting insulin production and hindering the glucose transport process in fish.

1.3.3. Glycogen



Glycogen is a branched polymer of glucose. Glucose units are linked linearly by (1→4)-α glycosidic bonds, and every ten or so glucose units have a chain of glucose units branched by (1→6)-α glycosidic bonds – called β granules. These glycosidic bonds create a helical polymer structure with an average diameter of about 20 nm, with the protein glycogenin located at the core of each glycogen granule and involved in glycogen synthesis Glycogen is an important readily available energy source stored mainly in the liver and muscle of fish. Fish mobilizes liver glycogen during times of stress.

1.4. GENERAL INTRODUCTION TO THE CLIMBING PERCH *(Anabas testudineus)*

The Vietnamese name "Cá rô đồng" is known in English as the climbing perch, with the scientific name Anabas testudineus. The climbing perch is an amphibious freshwater fish belonging to the family Anabantidae of the order Perciformes, distributed throughout Asia. It has an elongated body, with the posterior (rear) part being laterally compressed, while the head and anterior (front) part is wide and gradually flatten towards the rear. The climbing perch can tolerate harsh water conditions (polluted and oxygen-depleted water) because it possesses an auxiliary air-breathing organ called accessory gill organ. Due to its omnivorous nature and good adaptability to poor-quality water environments that are oxygen-deficient and contain many toxic substances, the climbing perch is at high risk of accumulating pollutants in its body. The climbing perch is one of the commercially important fish species in Vietnam and other Southeast Asian countries.

Chapter 2. EXPERIMENTS

2.1. EXPERIMENTAL DESIGN

2.2.1. Acute toxicity test (LC_{50} in 96 hours) of Pb and Cd

- The experiments were conducted under static water conditions with no water change for 96 hours, and aeration was provided to ensure the necessary dissolved oxygen (DO) for the fish.

- The control water is clean water stored in a tank to reduce chlorine concentration; the content of Pb^{2+} and Cd^{2+} ions in the control water (analyzed according to TCVN 13092:2020) are 0.01 mg/L and 0.005 mg/L, respectively (these concentrations are within the permissible limits of the National Technical Regulation on Surface Water Quality – QCVN 08-MT:2015/BTNMT), and no additional Pb or Cd salts.

- Contaminated water is clean water spiked with Pb and Cd metals in the form of $Pb(CH_3COO)_2$ (Lead(II) acetate) and $CdCl_2$ (Cadmium chloride) salts.

+ Pb^{2+} concentrations: 30, 75, 90, 100, 150, 175, and 200 mg/L.

+ Cd^{2+} concentrations: 3, 5, 10, 20, 30, 40, 50, and 75 mg/L.

Each concentration was tested with four replications.

- Approximately 60 fishes individuals were randomly selected and cultured in four tanks for each concentration of Pb^{2+} or Cd^{2+}, with 15 fishes per tank. During this phase, the fishes were not fed. Dead fishes were removed immediately to prevent their decomposition from affecting the quality of the test water. The number of dead fishes were monitored at 24, 48, 72, and 96 hours.

2.2.2. Sub-chronic toxicity test of Pb and Cd

a. Fish rearing conditions during Pb and Cd exposure period

- Fish were raised in PP tanks placed in a temperature-controlled room (30 ± 1°C), illuminated 10 hours/day.

- Control water is the same as the acute toxicity test.

- Nước ô nhiễm là nước sạch được thêm các kim loại Pb, Cd dưới dạng muối $Pb(CH_3COO)_2$ và $CdCl_2$.

+ Pb^{2+} concentrations: 20, 30 và 40 mg/L.

+ Cd^{2+} concentrations: 3, 5 và 10mg/L.

Each concentration was tested with three replications (n = 3).

- The experimental fish had an average length of 9.0 ± 0.5 cm and weighed 13.0 ± 1.5 g. They were stocked at a density of 25 individuals per tank and were provided with aeration to ensure adequate dissolved oxygen for their survival. The fish were fed a mixed pellet feed at an estimated rate of 4% of their total body weight per feeding. They were acclimated to the environment for 12 days before being exposed to Pb^{2+} or Cd^{2+} contamination.

The control fish were kept under the same conditions but were not exposed to heavy metals.

- Water was changed every two days. After each water changes, the Pb and Cd concentrations were analyzed to ensure there was no deviation from the initial Pb and Cd concentrations.

b. Conditions for fish rearing during Pb and Cd depuration period

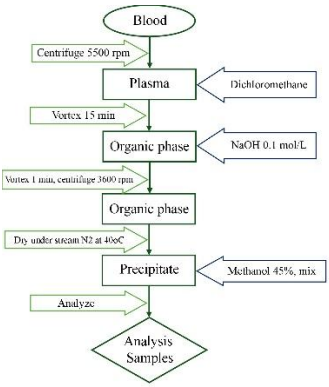
After the exposure period, the fish were transferred to PP tanks and kept in clean water for continuous culture. The culture conditions remained the same as during the exposure period, with clean water being replaced every two days.

c. Sampling for analysis

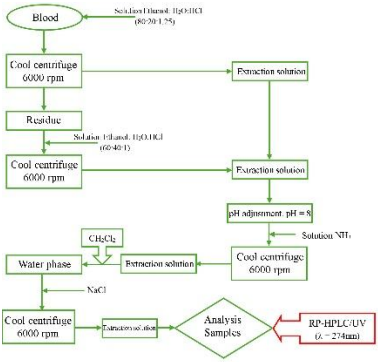
Samples were collected between 8:00 and 9:00 AM on days 7, 14, 21, and 28 after the fish began living in the Pb, or Cd contaminated water. This was done to study the accumulation and effects of these metals on changes in cortisol, insulin, and glycogen levels. Samples were also collected between 8:00 and 9:00 AM on day 14 after the contaminated fish were transferred to clean water, to study the depuration process.

2.2. CHEMICAL ANALYSIS METHODS

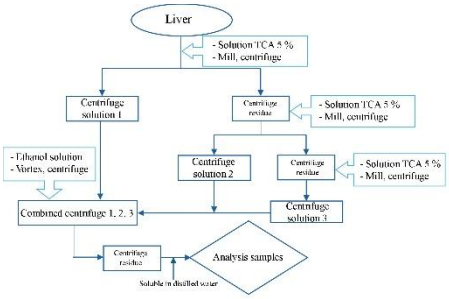
Process for the analysis of cortisol, insulin, glycogen, and total Cd and Pb in fish



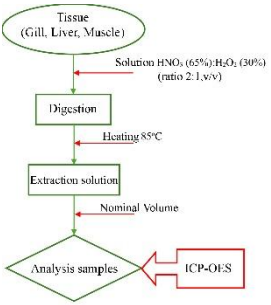
Blood sample processing protocol for cortisol analysis



Blood sample processing protocol for insulin analysis



Liver sample processing protocol for glycogen analysis



Sample processing protocol for total Cd and Pb analysis on ICP-OES instrument

Chương 3. RESULTS AND DISCUSSIONS

3.1. VALIDATION OF ANALYTICAL METHODS

To ensure accurate and reliable analytical results, analytical method validation is a crucial step. A validated analytical method is proven to be fit for its intended purpose, meaning the method consistently generates dependable results.

The results showed that the precision and recovery of the analytical methods for Cd, Pb, cortisol, insulin, and glycogen were all within the acceptable ranges according to the AOAC guidelines. A summary of the validation specifications for the analytical methods are presented in Table 3.1.

Bảng 3.1. *Specifications of the analytical method*

No.	***Specifications***	***Results***				
		Cortisol analysis method	***Insulin analysis method***	***Glycogen analysis method***	***Cd analysis method***	***Pb analysis method***
1	Retention time	7.442 min	5.188 min	-	-	-
2	Equation of the standard curve	$y = 0.05547\,x + 0.05246$	$y = 0.01017\,x - 0.03315$	$y = 0.00781\,x$	$y = 1864.9x + 44.7$	$y = 644.6x - 1388$
3	Correlation coefficient (r^2)	0.99989	0.99975	0.99836	0.9999	0.9998
4	LOD	0.87 ng/mL	0.011 ng/mL	1.187 mg/L	0.52 µg/L	1.20 µg/L
5	LOQ	2.65 ng/mL	0.032 ng/mL	3.598 mg/L	1.73 µg/L	3.90 µg/L

No.	Specifications	Results				
		Cortisol analysis method	*Insulin analysis method*	*Glycogen analysis method*	*Cd analysis method*	*Pb analysis method*
6	Daily precision (%RSD)	0.91 – 1.70 %	1.93 – 4.20%	2.82 – 5.06 %	-	-
7	Inter-day precision (%RSD)	1.08 – 1.74 %	1.57 – 4.17 %	1.89 – 4.05 %	-	-
8	Accuracy of the day (%RSD)	95.13 – 98.43 %	95.97 – 99.18 %	96.88 – 99.02%	-	-
9	Accuracy between dates (%RSD)	94.64 – 95.55 %	96.67 – 97.76 %	95.39 – 96.67%	-	-
10	Recovery efficiency	97.90 %	98.15 %	93.70 %	94.40 %	92.20 %

3.2. STUDY ON THE EFFECTS OF LEAD (Pb) ON CLIMBING PERCH

3.2.1. Acute toxicity limit study of Pb in climbing perch

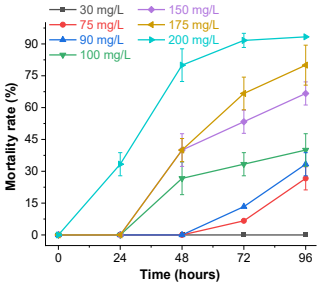


Fig 3.7. Mortality rate of climbing perch after 96 hours of Pb exposure

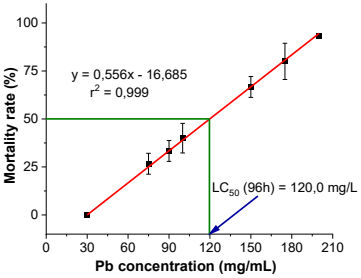


Fig 3.8. Acute toxicity threshold of Pb (LC_{50} 96 hours) for climbing perch

The results of the experiment revealed that fish exposed to Pb concentrations of 75 – 90 mg/L began to die after 72 hours of exposure. At higher concentrations of 100 – 175 mg/L, fish began to die after only 48 hours, and at 200 mg/L, fish began to die after just 24 hours. No mortality was observed at a Pb concentration of 30 mg/L or in the control group of fish. Based on these findings, a linear regression equation was developed to establish a correlation between the Pb concentration in the water and the mortality rate of the experimental fish. The equation, y = 0.556x - 16.685,

had a high correlation coefficient (r^2) of 0.999. The acute toxicity threshold of Pb (the LC_{50} at 96 hours) for the climbing perch was determined to be 120 mg/L.

3.2.2. Sub-chronic toxicity study of Pb in climbing perch

To investigate the sub-chronic toxicity of Pb in climbing perch, we selected exposure concentrations were $^1/_6$, $^1/_4$, $^1/_3$, respectively, which based on the LC_{50} 96h value. These concentrations were 20, 30, and 40 mg/L, and were labeled as 20Pb, 30Pb, and 40Pb, respectively.

3.2.2.1. Climbing perch under Pb exposure

a. Effects of sub-chronic Pb toxicity on the growth and development of climbing perch

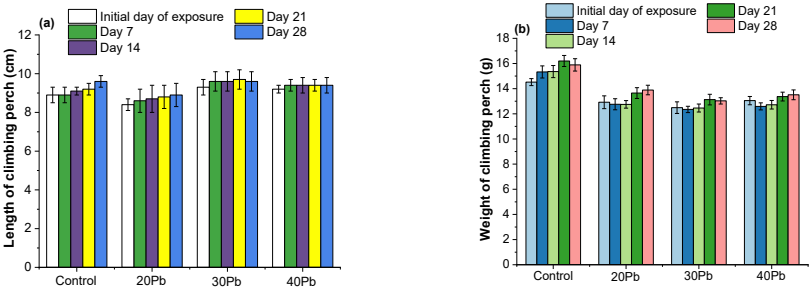


Fig 3.9. *Effect of Pb on changes in (a) length and (b) weight of climbing perch*

The presence of Pb had a significant impact on the growth of climbing perch. After 28 days of exposure, the length of Pb-exposed fish increased by 2.5% to 6.0% compared to their initial measurements (Fig 3.9a). Similarly, the weight of the exposed fish groups showed a modest increase of only 3.52% to 7.51% (Fig 3.9b). This limited growth could be attributed to the fish experiencing fatigue and loss of appetite, which may have hindered their development.

b. Sub-chronic toxic effect of Pb on the accumulation rules of Pb in climbing perch

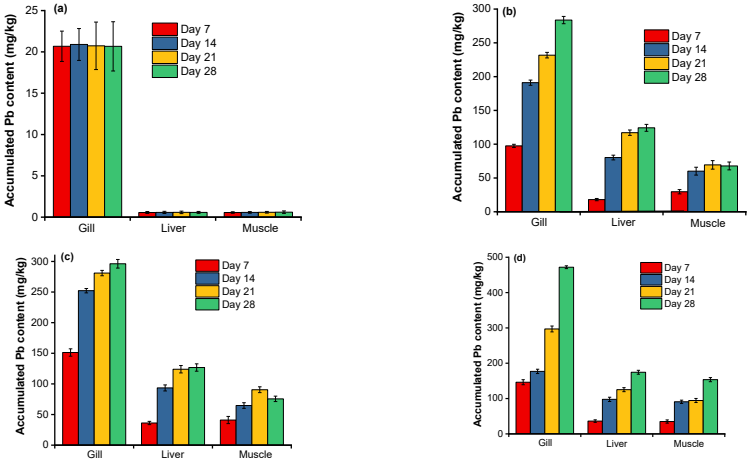


Fig 3.11. *Distribution and accumulation of Pb in gills, livers, and muscle of control fish (a) and fish exposed to 20Pb (b), 30Pb (c), and 40Pb (d)*

The results presented in Fig 3.11 indicate that the order of Pb distribution and accumulation in the fish's gills, liver, and muscle is as follows: Gills >> Liver > Muscle. This can be attributed to the fact that the gills are the primary organ in direct contact with the aquatic environment, and their negatively charged surface makes them particularly susceptible to metal cations, resulting in a higher accumulation of heavy metals compared to other organs in the body. Furthermore, the gills serve multiple functions, including underwater gas exchange, osmoregulation, ion regulation, acid-base balance, and nitrogenous waste excretion.

c. Sub-chronic toxic effect of Pb on the change in plasma cortisol in climbing perch

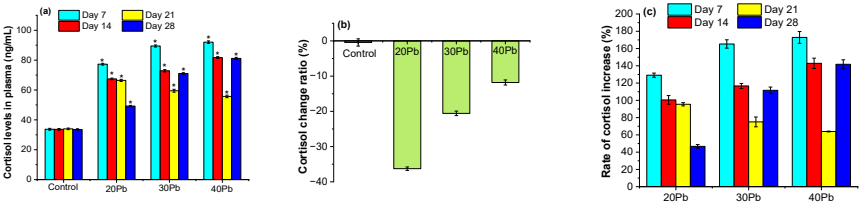


Fig 3.16. *(a) Changes in plasma cortisol concentration in control and Pb-exposed climbing perch; (b) Percentage increase of plasma cortisol in climbing perch between day 28 and day 7 from the onset of Pb exposure; and (c) Percentage increase of plasma cortisol in Pb-exposed climbing perch compared to the control fish (Mean value ± SD, n=3).*

**A significant difference ($p<0.05$) in plasma cortisol concentration between control and Pb-exposed fish*

Statistical analysis showed that the plasma cortisol levels in Pb-exposed climbing perch were significantly higher *($p < 0.05$)* than those in the control fish (Fig 3.16). These results indicate that prolonged exposure to high concentrations of Pb induces a stress response, negatively affecting the activity of the HPI axis and leading to a disruption in cortisol production. These findings confirm that Pb acts as a chemical stressor to climbing perch.

d. Sub-chronic toxic effect of Pb on the change in blood insulin in climbing perch

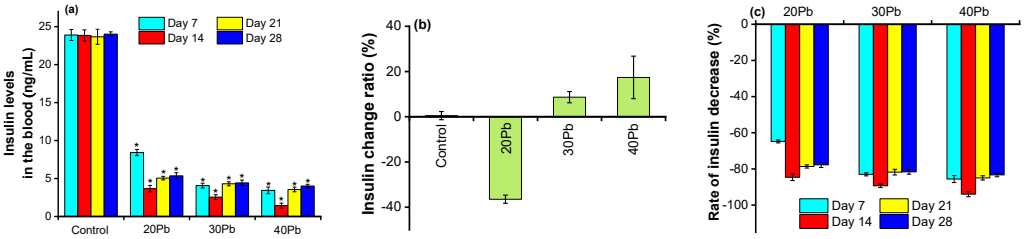


Fig 3.17. *(a) Changes in blood insulin concentration in control and Pb-exposed climbing perch; (b) Percentage change of blood insulin in climbing perch between day 28 and day 7 from the onset of Pb exposure; and (c) Percentage reduction of blood insulin in Pb-exposed climbing perch compared to the control fish (Mean value ± SD, n=3).*

**A significant difference ($p<0.05$) in blood insulin level between control and Pb-exposed fish.*

Fig 3.17 illustrates the concentration-dependent effect of Pb on insulin production activity in climbing perch. Notably, fish exposed to high concentrations of Pb exhibited lower blood insulin levels, which may be due to the anorexia experienced by these fish, resulting in a reduced capacity for insulin production. The results concerning the change in blood insulin in climbing perch indicate that prolonged exposure to high concentrations of Pb induces a stress response, negatively affecting the activity of the pancreas and leading to a disruption in insulin production.

e. Sub-chronic toxic effect of Pb on the change in liver glycogen in climbing perch

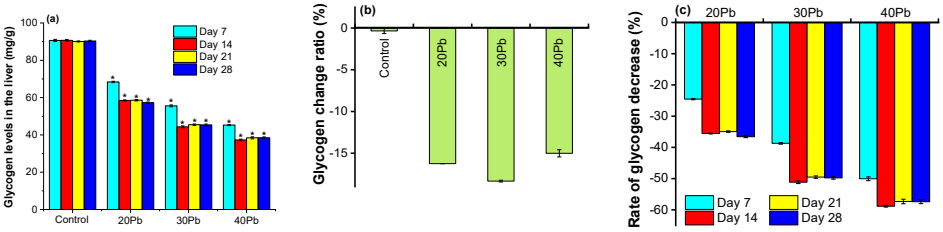


Fig 3.18. *(a) Changes in hepatic glycogen content in control and Pb-exposed climbing perch; (b) Percentage reduction of hepatic glycogen in climbing perch between day 28 and day 7 from the onset of Pb exposure; and (c) Percentage reduction of hepatic glycogen in Pb-exposed climbing perch compared to the control fish (Mean value ± SD, n=3).*

**A significant difference ($p<0.05$) in hepatic glycogen content between control and Pb-exposed fish*

Fig 3.18 shows a significant decrease in liver glycogen content in climbing perch exposed to Pb compared to the control fish. The reduction in liver glycogen content in the climbing perch indicates an increased mobilization of glucose from the stored form glycogen to meet the high energy demand caused by stress. The results of this study suggest that prolonged exposure to high concentrations of Pb induces a stress response, impairs liver function, reduces digestive enzyme activity, and affects food assimilation efficiency, leading to the breakdown of stored liver glycogen.

3.2.2.2. Climbing perch under Pb depuration

a. Changes in the weight and length of climbing perch during the depuration period (14 days)

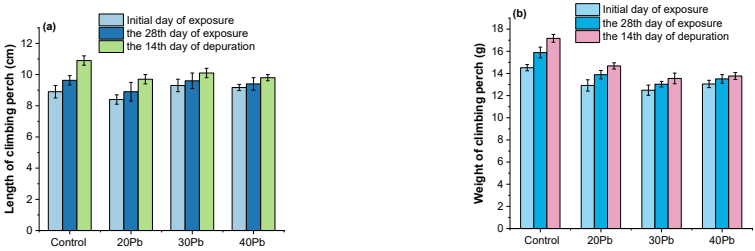
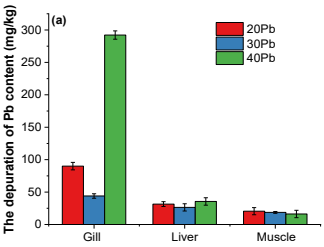


Fig 3.19. *Changes in (a) length and (b) weight of the climbing perch at the beginning of Pb exposure, the 28th day of exposure, and the 14th day of depuration*

The negative impact of Pb on climbing perch is clearly demonstrated by their slow recovery. The higher the concentration of Pb to which the fish were previously exposed, the slower their growth in both length and weight, even when subsequently kept in clean water.

b. The elimination of Pb from the gills, liver, and muscle tissue of climbing perch after 14 days of depuration



The Pb concentration eliminated from the fish tissues (gills, liver, and muscle) is illustrated in the adjacent graph. Although the rate of Pb removal from the gills, liver, and muscle of previously exposed climbing perch was relatively significant, it remains challenging to eliminate this toxic metal from the body once it has accumulated in the fish's tissues. The order of Pb elimination for the climbing perch is: Gills > Liver > Muscle.

3.3. STUDY ON THE EFFECTS OF CADMIUM (Cd) ON CLIMBING PERCH

3.3.1. Acute toxicity limit study of Cd in climbing perch

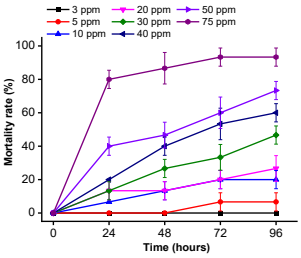


Fig 3.21. *Mortality rate of climbing perch after 96 hours of Cd exposure*

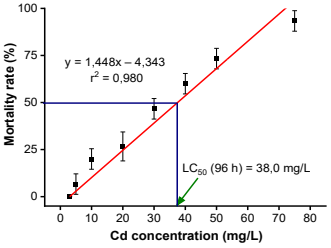


Fig 3.22. *Acute toxicity threshold of Cd (LC_{50} 96 hours) for climbing perch*

The results of the experiment revealed that fish exposed to Cd concentrations of 5 mg/L began to die after 48 hours of exposure. At higher concentrations of 10 – 50 mg/L, fish began to die after only 24 hours, and at 75 mg/L, fish began to die at first 24 hours. No mortality was observed at a Cd concentration of 3 mg/L or in the control group of fish. Based on these findings, a linear regression equation was developed to establish a correlation between the Cd concentration in the water and the mortality rate of the experimental fish. The equation, y = 1.448x – 4.343, had a high correlation coefficient (r^2) of 0.980. The acute toxicity threshold of Cd (the LC_{50} at 96 hours) for the climbing perch was determined to be 38 mg/L.

3.3.2. Sub-chronic toxicity study of Cd in climbing perch

To investigate the sub-chronic toxicity of Cd in climbing perch, we selected exposure concentrations were $^1/_{12}$, $^1/_8$, $^1/_4$, respectively, which based on the LC_{50} 96h value. These concentrations were 3, 5, and 10 mg/L, and were labeled as 3Cd, 5Cd, and 10Cd, respectively.

3.3.2.1. Climbing perch under Cd exposure

a. Effects of sub-chronic Cd toxicity on the growth and development of climbing perch

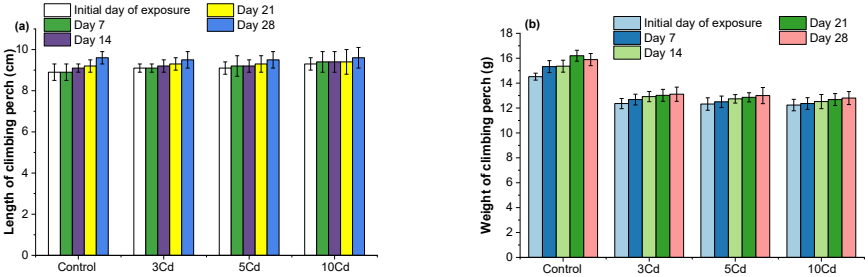
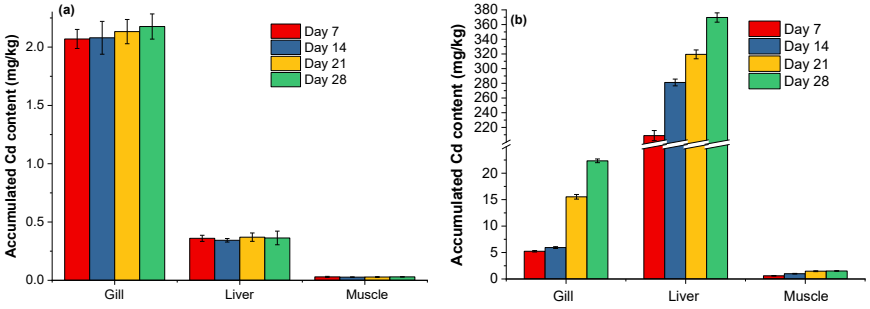


Fig 3.23. *Effect of Cd on changes in (a) length and (b) weight of climbing perch*

Cd significantly affects the growth in length of the climbing perch (Anabas testudineus). When compared to the baseline, the length of Cd-polluted fish after 28 days increased from 3.0% to 5.3% in the exposed group (Fig 3.23a). Similarly, the weight of the exposed fish group showed a slight increase, from 5.49% to 6.07% (Fig 3.23b). This could be attributed to the fish being fatigued and anorexic, resulting in poor development.

b. Sub-chronic toxic effect of Pb on the accumulation rules of Cd in climbing perch



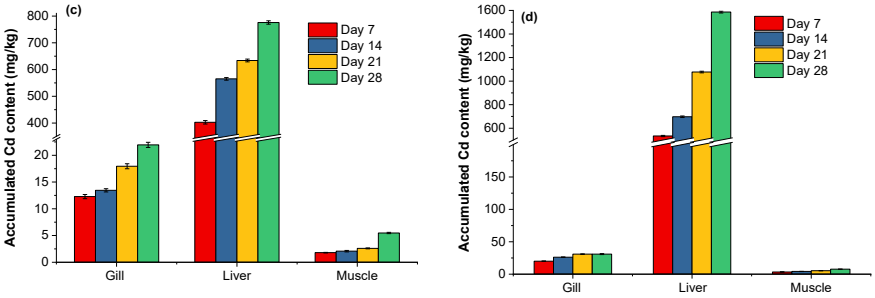
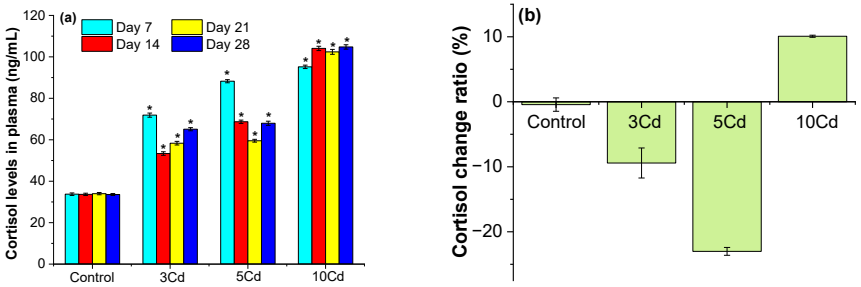


Fig 3.25. *Distribution and accumulation of Cd in gills, livers, and muscle of control fish (a) and fish exposed to 3Cd (b), 5Cd (c), and 10Cd (d)*

The results in Fig 3.25 show that the order of distribution and accumulation of Cd in the gills, liver, and fish muscle is as follows: Liver >> Gills > Muscle. Inorganic Cd tends to accumulate first in the liver through a binding mechanism involving detoxification proteins such as metallothionein (MTs). In the gills, however, Cd is absorbed via passive diffusion through calcium channels. Subsequently, Cd is actively transported into the bloodstream, passes through serum albumin, and travels to the storage organs.

c. Sub-chronic toxic effect of Cd on the change in plasma cortisol in climbing perch



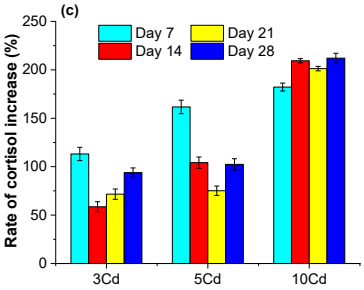


Fig 3.28. *(a) Changes in plasma cortisol concentration in control and Cd-exposed climbing perch; (b) Percentage increase of plasma cortisol in climbing perch between day 28 and day 7 from the onset of Cd exposure; and (c) Percentage increase of plasma cortisol in Cd-exposed climbing perch compared to the control fish (Mean value ± SD, n=3).*

**A significant difference ($p<0.05$) in plasma cortisol concentration between control and Cd-exposed fish*

The statistical analysis results showed that the plasma cortisol level in Cd-exposed climbing perch was significantly higher *($p<0.05$)* compared to the control fish (Fig 3.28). These findings suggest that the negative impact of Cd on the climbing perch is the induction of stress, resulting in increased cortisol production, and that a higher Cd concentration in the water leads to a greater increase in cortisol levels. Long-term Cd exposure disrupts cortisol secretion, resulting in abnormally altered plasma cortisol levels in the climbing perch.

d. Sub-chronic toxic effect of Cd on the change in blood insulin in climbing perch

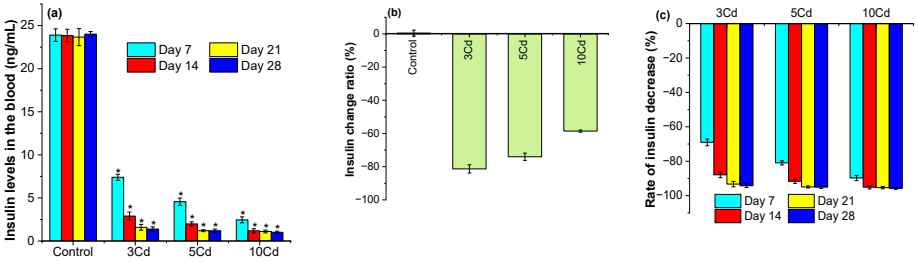


Fig 3.29. *(a) Changes in blood insulin concentration in control and Cd-exposed climbing perch; (b) Percentage change of blood insulin in climbing perch between day 28 and day 7 from the onset of Cd exposure; and (c) Percentage reduction of blood insulin in Cd-exposed climbing perch compared to the control fish (Mean value ± SD, n=3).*

**A significant difference ($p<0.05$) in blood insulin level between control and Cd-exposed fish.*

Fig 3.29 illustrates that the toxic stress caused by Cd in the climbing perch is a reduction in insulin production, and that prolonged exposure to higher Cd concentrations leads to lower blood insulin levels. The results concerning the changes in blood insulin in the climbing perch indicate that long-term exposure to high concentrations of Cd induces a stress response, negatively impacting the activity of the pancreas, which results in disrupted insulin production.

e. Sub-chronic toxic effect of Cd on the change in liver glycogen in climbing perch

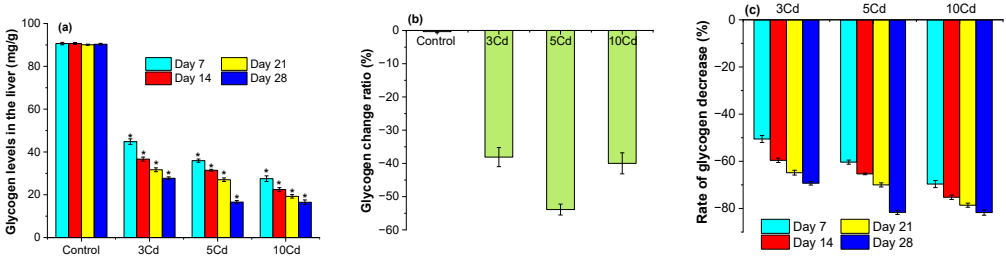


Fig 3.30. *(a) Changes in hepatic glycogen content in control and Cd-exposed climbing perch; (b) Percentage reduction of hepatic glycogen in climbing perch between day 28 and day 7 from the onset of Cd exposure; and (c) Percentage reduction of hepatic glycogen in Cd-exposed climbing perch compared to the control fish (Mean value ± SD, n=3).*

**A significant difference ($p<0.05$) in hepatic glycogen content between control and Cd-exposed fish*

Fig 3.30 illustrates that the glycogen content in the liver of Cd-exposed climbing perch was significantly reduced compared to that of the control fish. The longer the climbing perch was exposed to higher Cd concentrations, the lower the hepatic glycogen levels became. This reduction in liver glycogen content in the Cd-exposed fish suggests that the body is mobilizing glycogen reserves to meet the increased energy demand caused by stress. This research finding indicates that stress from Cd exposure leads to metabolic disruption, requiring the body to utilize glycogen (stored glucose) for energy production.

3.3.2.2. Climbing perch under Cd depuration

a. Changes in the weight and length of climbing perch during the depuration period (14 days)

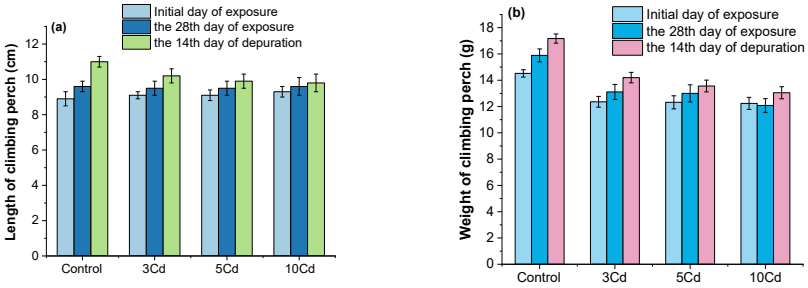
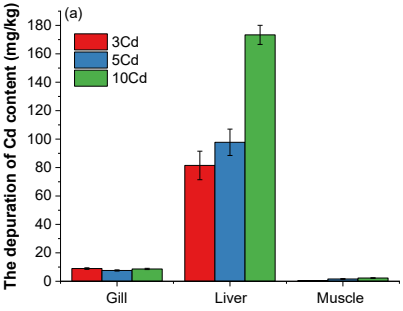


Fig 3.31. *Changes in (a) length and (b) weight of the climbing perch at the beginning of Cd exposure, the 28th day of exposure, and the 14th day of depuration*

The negative impact of Cd on the climbing perch is clearly demonstrated by the slow recovery status. Climbing perch previously exposed to higher Cd concentrations showed slower growth in both length and weight, even when living in clean water.

b. The elimination of Cd from the gills, liver, and muscle tissue of climbing perch after 14 days of depuration

The graph displays the amount of Cd eliminated from the fish tissues (gills, liver, muscle). It can be observed that the depuration of Cd from the gills, liver, and muscle of climbing perch previously exposed to Cd was minimal. The order of Cd elimination in climbing perch is as follows: Liver > Gills > Muscle



3.4. COMPARISON OF THE TOXIC EFFECTS OF Cd AND Pb ON THE CLIMBING PERCH *(Anabas testudineus)*

Table 3.7 summarizes and compares the toxic effects of Cd and Pb on the climbing perch during the exposure and depuration period.

Table 3.7. *Comparison of the toxic effects of Cd and Pb*

Toxic effects		**Pb**	**Cd**
Acute toxicity (LC_{50} 96 hours)		120.0 mg/L	38.0 mg/L
Sub-chronic toxicity (28	***The growth***	Long-term exposure (28 days) to Pb (20 - 40 mg/L) and Cd (3 - 10 mg/L) at sub-lethal concentrations resulted in the retarded growth of the climbing perch. Although the	

Toxic effects		Pb	Cd
days exposure)		length and weight of the Pb- and Cd-exposed climbing perch increased, the increase was insignificant compared to the control fish.	
	Similar accumulation	The accumulation of Pb and Cd in exposed fish increased with the duration of exposure and the concentration of Pb and Cd in the water.	
	Differential accumulation	- The order of Pb distribution and accumulation was as follows: Gills >> Liver > Muscle. - Pb accumulation in the gills ranged from 97.57 ± 2.33 to 471.84 ± 3.92 mg/kg of dry gill weight. - Pb accumulation in the liver ranged from 18.00 ± 1.65 to 174.45 ± 5.55 mg/kg of dry liver weight. - Pb accumulation in the muscle ranged from 9.67 ± 3.15 to 153.60 ± 5.78 mg/kg of dry muscle weight.	- The order of Cd distribution and accumulation was as follows: Liver >> Gills > Muscle. - Cd accumulation in the gills ranged from 5.24 ± 0.14 to 30.99 ± 0.73 mg/kg of dry gill weight. - Cd accumulation in the liver ranged from 208.84 ± 6.87 to 1586.10 ± 8.07 mg/kg of dry liver weight. - Pb accumulation in the muscle ranged from 0.60 ± 0.05 to 7.69 ± 0.13 mg/kg of dry muscle weight.
	Plasma cortisol	Long-term exposure to Pb and Cd disrupted the normal functioning of the hypothalamic–pituitary–interrenal (HPI) axis, leading to excessive cortisol production.	
	Impaired blood insulin levels	Long-term exposure to Pb and Cd impaired the function of pancreatic beta cells, resulting in reduced insulin production (insulin deficiency)	
	Hepatic glycogen breakdown	Long-term exposure to Pb and Cd impaired liver function, hindering digestion and disrupting metabolic pathways, which led to the breakdown of stored hepatic glycogen.	
Sub-chronic toxicity (14 days depuration)	***The growth***	Prolonged exposure to Pb and Cd induced toxic stress, which severely affected the recovery capacity of the climbing perch. Even when kept in clean water, the growth in weight and size of this fish group was significantly slower than the control group,	

Toxic effects		Pb	Cd
		demonstrating the long-term impact of Pb and Cd on the fish.	
	Similar depuration	There was a relatively high elimination of Pb and Cd from the gills and liver, but less from the muscle of the previously exposed climbing perch.	
	Differential depuration	- The order of Pb depuration was found to be Gills > Liver > Muscle. - The amount of Pb eliminated from the gills ranged from 89.99 ± 5.77 to 292.19 ± 6.40 mg/kg of dry gill weight. In comparison, the elimination of Pb from the liver ranged from 26.42 ± 5.59 to 35.53 ± 5.70 mg/kg of dry liver weight, while the elimination from the muscle ranged from 16.19 ± 5.68 to 20.53 ± 5.50 mg/kg of dry muscle weight	- The order of Cd depuration was found to be Liver > Gills > Muscle. - The amount of Cd eliminated from the gills ranged from 7.63 ± 0.42 to 8.95 ± 0.56 mg/kg of dry gill weight. In comparison, the elimination of Cd from the liver ranged from 81.48 ± 10.04 to 173.33 ± 6.71 mg/kg of dry liver weight, while the elimination from the muscle ranged from 0.41 ± 0.06 to 2.27 ± 0.22 mg/kg of dry muscle weight

Chapter 4. CONCLUSIONS AND RECOMMENDATIONS

4.1. CONCLUSIONS

Based on the study of the accumulation, depuration, and effects of Lead (Pb) and Cadmium (Cd) on three biochemical parameters—cortisol, insulin, and glycogen—in the climbing perch (Anabas testudineus), the dissertation draws the following conclusions:

1. The acute toxicity of Pb and Cd (LC50 96 hours) to the climbing perch was 120 mg/L and 38 mg/L, respectively.

2. The rules of distribution and accumulation of Pb and Cd in the climbing perch were determined. For Pb, the rule was: Gills >> Liver > Muscle, and for Cd, it was: Liver >> Gills > Muscle. The accumulated Pb and Cd content in the experimental fish increased with the concentration of

Pb and Cd in the water and the duration of exposure. 3. The rules of depuration of Pb and Cd from the climbing perch were found. The depuration rule for fish previously exposed to Pb was: Gills > Liver > Muscle, and for fish previously exposed to Cd was: Liver >> Gills > Muscle.

4. The toxic stress caused by Pb and Cd in the climbing perch involves disrupting the normal functioning of the HPI axis, leading to increased cortisol production, impairing pancreatic beta cell function, resulting in reduced insulin production, and weakening liver function, leading to the breakdown of stored hepatic glycogen.

5. The toxic stress induced by Pb and Cd caused the climbing perch to experience retarded growth during the exposure period (28 days) and slow recovery during the depuration period (14 days)

4.2. RECOMMENDATIONS

Based on the research findings, it is evident that heavy metals have a significant impact on the accumulation and depuration in fish and other aquatic organisms. This has a direct effect on the human food chain. Therefore, the dissertation proposes the following recommendations:

- Further investigation into the transformation mechanisms of heavy metals (such as Pb and Cd) within the tissues of the climbing perch.

- Study the synergistic effects of Pb and Cd on the tissues and biochemical parameters of the climbing perch.

- Investigate the levels of heavy metal accumulation in climbing perch living in their natural environment to raise awareness of the potential risk of heavy metal contamination in the food chain.

- Research the effects of heavy metal elimination processes (such as Pb and Cd) on the endocrine system of the climbing perch and other aquatic species that are of interest to local populations and the aquaculture industry.

- Study the accumulation and depuration of Pb and Cd in other aquatic species, particularly those that are frequently present in the food chain or have high commercial value and are relevant to the aquaculture industry.

- Conduct experimental studies on biological parameters (such as cortisol, insulin, glucagon, glucose, and glycogen) in various fish species as a means of assessing and monitoring environmental pollution levels.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Dang Nguyen Nha Khanh**, Ngo Thi Tuong Vy, Tran Ha Phuong, Pham Tuan Nhi, Nguyen Quoc Thang, Do Trung Sy, Nguyen Thi Kim Phuong, **"Effects of Cadmium and Lead on Muscle and Liver Glycogen Levels of Climbing Perch *(Anabas testudineus)*"**, *Bulletin of Environmental Contamination and Toxicology*, Vol.108, pp. 854 – 860, 2022 (H-Index 90, Q2).

2. **D. N. Nha Khanh**, N. T. Tuong Vy, N. Quoc Thang, D. Trung Sy, B. Quang Minh, N. The Anh, D. V. Bao Tran, L. Khue Tu & N. T. Kim Phuong, **"Accumulation and response to stress in climbing perch *(Anabas testudineus)* on exposure to high concentrations of lead and cadmium in water"**, *The European Zoological Journal*, Vol. 89(1), pp. 877 – 887, 2022 (H-Index 47, Q2).

3. **Dang Nguyen Nha Khanh**, Ngo Thi Tuong Vy, Doan Thi Minh Phuong, Nguyen Quoc Thang, Nguyen Thi Kim Phuong, **"A rapid HPLC combined with UV method for the determination of blood insulin and the evaluation of the effect of Pb on insulin secretion in climbing perch *(Anabas testudineus)*"**, *Conference Proceedings of analytica Vietnam 2025*, sesion 2: Pharmaceutical analysis and Health science, pp. 363 – 371, 2025