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Research Article

Acute Toxicity Effects of Heavy Metals to Juvenile Climbing Perch, *Anabas testudineus* (Bloch, 1792) Within 96-Hour Exposure

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ABSTRACT

This study examines the acute toxicity effects of four heavy metals, arsenic (As), lead (Pb), cadmium (Cd), and zinc (Zn), on juvenile climbing perch, *Anabas testudineus*, under controlled laboratory conditions. Analytical-grade arsenic acid, cadmium sulfate, Lead(II) chloride, and zinc sulfate were used in preparing standard stock solutions. Fish mortality was recorded at 24-hr intervals over 96 hr. Median lethal concentrations (LC_{50}) and times (LT_{50}) for the juveniles were predicted using cumulative mortality, based on probit analysis run in Microsoft Excel. The water physical parameters, including pH (6.57), temperature (27.1–28.2℃), and dissolved oxygen (6.3–7.2 mg/L), were maintained within optimal ranges, ensuring that fish mortality and behavioural changes were mainly affected by metal toxicity. Arsenic recorded the highest toxicity (LC_{50}= 0.17 ppm), with 100% mortality at 1.0 ppm within 48 hr. Lead (Pb) demonstrated a narrow tolerance range, exhibiting no mortality at concentrations below 1.35 ppm but resulting in rapid 100% mortality at 3.41 ppm (LC_{50}= 2.47 ppm). Cadmium is toxic with sudden, lethal effects at higher concentrations (LC_{50}=2.5 ppm). Zinc became toxic at higher levels (100% mortality at 89 ppm, LC_{50}= 50.35 ppm). The fish toxicity was in the following order: As > Pb > Cd > Zn. An inverse relationship has been detected between LC_{50} values and metal concentration, highlighting the concentration- and time-dependent nature of toxicity. Unusual fish behaviour, including erratic swimming and excessive mucus production, indicated neurophysiological stress responses. This ranking (As > Pb > Cd > Zn) highlights arsenic as the most dangerous contaminant to *A. testudineus* in aquatic systems.

Key words: Arsenic, cadmium, lead, LC_{50}, LT_{50}, mortality, zinc

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INTRODUCTION

Acute toxicity refers to the immediate, harmful effects of hazardous substances on aquatic organisms and ecosystems, following short-term exposure of up to 96 hr (Shahjahan *et al.*, 2022). This acute toxicity test determines the toxicity levels of various pollutants, with findings commonly starting with behavioural abnormalities, followed by paralysis, and ultimately leading to mortality, using a rapid and efficient method (Abdulazeez *et al.*, 2024). Acute toxicity tests serve as a powerful predictor of chronic toxicity tests, which are demonstrated over prolonged exposures (days to months). Chronic toxicity tests can inhibit growth, affect the reproductive system, and change behaviour. In managing the environment, acute toxicity data are necessary to predict the ecological impacts of pollution cases, such as oil spills, and to establish authorised limits for poisonous substances in aquatic environments. Highlighting the environmental and economic importance of particular species in aquatic environments, choosing a hardy and ecologically relevant organism for toxicity assessments is vital to ensure meaningful environmental risk assessments.

In Malaysia, heavy metal pollution is primarily caused by various anthropogenic activities, including industrial discharge from manufacturing, agricultural runoff containing metal-rich pesticides and fertilisers, and vehicle engine emissions (exhaust, tread wear, brake pad wear & engine oil) (Alengebawy *et al.*, 2021; Jadaa & Mohammed, 2023). These pollutants have significant environmental effects that are uncertain, complex, and multifaceted for bioindicators. The study of heavy metal pollution in Malaysia primarily focuses on the physicochemical characteristics, measuring spatial and temporal concentrations in water and sediments. While these studies provide valuable information on pollution levels and dispersal over time, the effects and the risks on the biology and ecology of living organisms (bioindicators) are unclear. Furthermore, modelling aspects, such as the pollution load index (PLI), geo-accumulation index (Igeo), and ecological risk assessment models, are widely applied to forecast pollution and potential risks; however, the biological effects and interactions are often limited.

Toxic heavy metals, such as Arsenic (As), cadmium (Cd), zinc (Zn), and lead (Pb), severely impact fish health, even at minimal concentrations. Their toxicity exists from various mechanisms, including enzyme inhibition, disruption of metabolic processes, and interference with cellular functions. Metals such as Cd and Cu often accumulate in the liver and kidney tissues of fish, leading to structural organ deterioration, metabolic disruptions, and hindering growth and osmoregulation processes. Zinc accumulation in muscles, skin, and bones can deform skeletal structures, reduce growth, and impair immune function. Collectively, these metals affect the development, reproduction, and survival of fish, making them significant contaminants in aquatic ecosystems.

In aquatic environments, regulatory standards for heavy metals are set up to protect aquatic organisms and fish, which are a crucial dietary source of protein, from harmful exposure. Many countries, including Malaysia, establish permissible limits for essential and non-essential metals, such as cadmium, lead, copper, and zinc, based on their toxicity to freshwater species. Local authorities or agencies responsible for environmental regulations related to heavy metals in water, such as Malaysia's National Water Quality Standards (NWQS), the Department of Environment (DOE), and the Ministry of Health (MOH), specify maximum permissible concentrations to prevent severe and long-lasting effects on aquatic life. These standards evidence the importance of determining the tolerance levels of specific species, as different fish species respond differently to metal exposure. By establishing the acute toxicity levels for *Anabas testudineus* at 96 hr, this study provides comprehensive data that can help in evaluating the sufficiency of current regulatory limits for local freshwater ecosystems.

Anabas testudineus, commonly known as the climbing perch, is a resilient potamodromous species that is widely inhabited in the brackish and freshwater of tropical zones of Asia, including India, Bangladesh, China, and Southeast Asia, with the earliest findings having been recorded in Australia and Papua New Guinea (Froese & Pauly, 2023). Originating from the family of Anabantidae, this air-breathing fish inhabits water bodies such as rivers, reservoirs, swamps, flooded fields, rice paddies, estuaries, and canals, which are shallow, slow-moving and full of vegetation (Froese & Pauly, 2023).

In Southeast Asia, this fish poses significant commercial value due to its high delicacy, texture, and nutritional content, making it an affordable and vital protein source for humans and a valuable economic product in regional aquaculture (Froese & Pauly, 2023). The species is commonly cultured in aquaculture due to its high resilience and adaptability to extreme environmental conditions, facilitated by a labyrinth organ that enables an air-breathing mode in low-oxygen environments. However, despite the status of the "Least Concern" species of climbing perch, the natural populations and aquaculture endurance are pressured by anthropogenic activities, which cause habitat degradation and affect water quality, particularly through heavy metal pollution.

This study, which employed the juvenile climbing perch, *Anabas testudineus*, as the test organism, highlights the urgent need for organism-based ecotoxicological studies to determine metal toxicity and its biological responses. Studies focusing on the reaction of climbing perch to metal acute toxicity tests are vital to understanding bioaccumulation patterns, physiological stress responses, and species-specific sensitivities. That information is crucial in determining the degree of responsiveness of the fish, demonstrating its relevance as a bioindicator and establishing contaminant permissible limits that protect aquatic life. Although this fish has high resilience and adjusts well in extreme environmental conditions (anaerobic, low water levels, high temperature, contaminated water with concentrated heavy metals), its toleration is affected by food shortage (planktivorous for fry, carnivorous for adults) and intense competition (Froese & Pauly, 2023), triggering domestic movement. These biological traits, along with their non-migratory nature and ecological versatility, highlight their potential as a relevant bioindicator for assessing metal toxicity in Malaysian waters.

In addition, the species possesses well-characterised physiological and biochemical changes, including responses in oxidative stress biomarkers, gill and liver pathology, and enzyme activity, making it highly sensitive to both acute and sublethal metal exposure. Its abundance and widespread distribution, with its economic importance, manageability, and hardiness under controlled laboratory conditions, further evidence its reliability and relevance in toxicity studies. Collectively, these biological and ecological traits justify the selection of *A. testudineus* as a suitable bioindicator for assessing heavy-metal contamination in Malaysian freshwater ecosystems.

Following that, this study aims to assess the acute toxicity effects of selected heavy metals (arsenic, cadmium, lead and zinc) on juvenile climbing perch, *Anabas testudineus* (Bloch, 1792), within a 96-hr exposure period, to understand the immediate response of metal pollutants.

MATERIALS AND METHODS

Study area

A total of 400 juvenile climbing perch (*Anabas testudineus*) (2.2±0.5 g body weight; 3.0±0.5 cm total length) were collected from MIHA aquafarm, Setiu, Terengganu and brought back to the Toxicology Laboratory of Universiti Sultan Zainal Abidin (UniSZA) for further experiments.

Acclimatization

Upon arrival, the juveniles were acclimated for a week in the laboratory using aerated, dechlorinated tap water in three 5-L tanks. During acclimation, the juveniles were fed twice daily with commercial fish micropellets Aquadene®. Environmental conditions were maintained at 28 to 30℃ with a 12-hr light-dark photoperiod and room lighting of 144 to 164 lux, mimicking the natural habitat environment.

Metal stock solution preparation

Standard stock solutions of Arsenic (As), Cadmium (Cd), Lead (Pb) and Zinc (Zn) were prepared using analytical-grade Arsenic acid (H_3AsO_4), Cadmium sulphate ($CdSO_4$), Lead (II) chloride ($PbCl_2$), and Zinc sulphate ($ZnSO_4$), respectively. These solutions were diluted with deionised water in a 1-L volumetric flask to achieve three different concentrations (low, medium, and high) for each metal type. Final concentrations used for each metal were determined based on preliminary range-finding tests: As (0.10, 0.56, & 1.00 ppm), Cd (2.12, 3.32 & 5.47 ppm), Pb (0.93, 1.35, & 3.41 ppm), and Zn (39.50, 73.50 & 89.00 ppm), representing low, medium and high concentrations. A limitation of this study is that all test solutions were applied at nominal concentrations, with actual metal concentrations not being measured using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry).

Experimental design

The acute toxicity test was carried out under static conditions, with water renewal every 2 days (48 hr), ensuring the toxic NH_3 was removed, which formed from metabolic waste. The control and metal-treated groups each consisted of ten juveniles per 5 L tank, containing 2 L of the respective test solution. The fish juveniles were not fed throughout the toxicity test period to avoid large fluctuations in the metabolic wastes and fouling of the test solutions. The surrounding environment was maintained under constant conditions of temperature (28-30℃) and light (12-hr), using fluorescent lights (334-376 lux), which mimicked a natural environment, thereby minimising external stress factors. Water samples were taken after solution preparation for metal detection using ICP-OES. Water physical parameters of pH, temperature (℃), and dissolved oxygen (DO) (mg/L) in the control tank were recorded every two days using portable meters (model Hydrolab Quanta).

Acute toxicity testing

Fish mortality was recorded at 24-hr intervals (24, 48, 72 & 96 hr), and dead fish (indicated by the absence of response to gentle physical stimulation) were immediately removed (modified from Kumari & Chand, 2021). Throughout the metal exposure period, behavioural changes in juveniles were observed and documented, including erratic movements, rapid operculum movement, lateral swimming, and attempts to jump out of the water.

Statistical analysis

Median lethal concentrations (LC_{50}) and times (LT_{50}) for the juveniles of climbing perch exposed to metals were estimated using cumulative mortality data, analysed with probit analysis in Microsoft Excel. All reported LC_{50} values are expressed in parts per million (ppm), which correspond directly to milligrams per litre (mg/L). Probit graphs (also known as concentration-response curves) of different metals were plotted to determine the relationship between the concentration of a toxicant and the probability of a specific biological response (Mortality).

RESULTS AND DISCUSSION

Water physical parameters

The mean water quality parameters measured during the test were a pH of 6.57, a temperature range of 27.1 to 28.2℃, and a DO of 6.3 to 7.2 mg/L, aligning with the optimal conditions for freshwater fish (Mariu *et al.*, 2023). The physicochemical properties of the tank water remained relatively stable and within normal ranges across different treatments throughout the test period. The environmental parameters collectively play a crucial role in maintaining the physiological and ecological health of freshwater fish, thereby forming the fundamental basis of aquatic habitat quality. The optimal ranges of the parameters are essential for the growth, metabolism, immune response, reproduction, and behavioural stability of fish species (Mariu *et al.*, 2023).

In addition, environmental parameters can influence the bioavailability and toxicity of contaminants (including heavy metals), jeopardising the entire aquatic species depending on the environment. The study, which documented a standard range of water physical parameters, ensures that the observed effects on fish were primarily due to metal exposure instead of environmental stress. Studies have highlighted the importance of controlling environmental factors in toxicity experiments to obtain accurate and reliable results. Therefore, careful control of environmental parameters was crucial in this study to isolate the effects of metals on *Anabas testudineus.*

Cumulative mortality (%)

100% of the control juveniles of *Anabas testudineus* survived throughout the 96-hr experiment, indicating that the handling practices and laboratory conditions did not cause fish mortality and that the environmental conditions were suitable for the fish's survival. For arsenic (As), a precise dose- and time-dependent toxicity pattern is observed, whereby at the lowest concentration (0.1 ppm), mortality remained at 40% from 48 hr onward. In contrast, the medium dose (0.56 ppm) resulted in 60% mortality by 96 hr (Figure 1A). The highest dose (1.0 ppm) caused 100% mortality as early as 48 hr. These findings suggest a progressive toxic effect of As, likely due to cumulative physiological stress, oxidative damage, or interference with enzyme function, which intensifies with prolonged exposure (Ganie *et al.*, 2024).

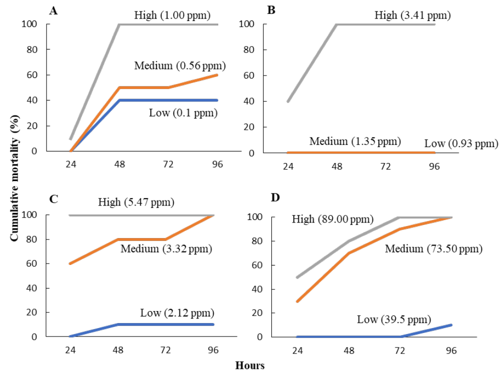

Fig. 1. Cumulative mortality (%) of juvenile climbing perch, *Anabas testudineus*, at low, medium and high metal concentrations. (A) Arsenic (As). (B) Lead (Pb). (C) Cadmium (Cd). (D) Zinc (Zn).

Lead (Pb) showed a more distinct threshold effect. No mortality was recorded at the low (0.93 ppm) and medium (1.35 ppm) concentrations throughout the 96 hr (Figure 1B), indicating that the juvenile *A. testudineus* has effective mechanisms in mitigating Pb accumulation within this range. This highly resilient species, which thrives in hypoxic and contaminated environments, likely reduces branchial metal uptake, sequesters Pb intracellularly through metallothioneins, and binds metals within lysosomal compartments to maintain Pb at sub-toxic internal concentrations.

However, at the high concentration (3.41 ppm), mortality spiked to 40% within 24 hr and reached total mortality (100%) by 48 hr, revealing protective mechanisms with finite capacity. Once this threshold is exceeded, the detoxification pathways of juvenile climbing perch appear to be rapidly overwhelmed, leading to systemic toxicity. The data also clearly showed that concentrations of Pb ranging from medium (1.35 ppm) to high (3.41 ppm) are the threshold for acute toxicity. The findings also indicate that lead's toxic effects may not be linear and only manifest significantly above a particular concentration, possibly due to saturation of the fish's detoxification mechanisms or acute gill damage occurring only at high doses (Shahjahan *et al.*, 2022).

In the case of Cd, the metal was found to be highly toxic, with rapid and complete mortality (100%) at the highest concentration (5.47 ppm) by 24 hr (Figure 1C). At the medium dose (3.32 ppm), mortality was already 60% at 24 hr, escalating to 100% by 96 hr. The lowest dose (2.12 ppm) also resulted in 10% mortality, starting at 48 hr. These results reflect cadmium's known potency in disrupting calcium and ion regulation, damaging gill tissue, and inducing oxidative stress, which together result in swift and severe physiological breakdown (Shahjahan *et al.*, 2022).

Zinc, although an essential trace element (Lall & Kaushik, 2021), demonstrated toxicity at elevated concentrations. The low dose in the present study (39.5 ppm) caused minimal mortality (10%) by 96 hr (Figure 1D). However, the medium (73.5 ppm) and high (89 ppm) doses resulted in significant mortality, reaching 100% by 96 hr, with effects appearing earlier at the highest dose. This pattern is consistent with zinc's dual role as both nutrient and toxicant, where high concentrations impair osmoregulation, enzyme activity, and cellular metabolism (Lall & Kaushik, 2021).

The exceptionally high toxicity of arsenic (As) compared to other elements can be attributed to its unique physicochemical characteristics and mode of action at the cellular level (Ganie *et al.*, 2024). The existence of As in multiple redox states and its ability to readily form inorganic species make it far more biologically reactive. These properties differ from those of Pb, Cd, and Zn, which are primarily heavy metals that exert toxicity through competitive ion substitution, oxidative stress, and bioaccumulation (Balali-Mood *et al.*, 2021). Arsenic (As) binds strongly to sulfhydryl-containing enzymes, leading to the sudden inhibition of essential metabolic pathways, disruption of mitochondrial ATP production, and impairment of gill ion-regulatory mechanisms (El-Ghiaty & El-Kadi, 2023). In contrast, other metals, such as Pb, Cd, and Zn, which exert slower rates of toxicity on juvenile climbing perch, are possibly due to their uptake and intracellular binding, which are more tightly regulated by metallothioneins and ion-transport barriers (Balali-Mood *et al.*, 2021).

The findings of cumulative mortality (%) illustrate that the acute toxicity of these metals varies in intensity and onset depending on their concentration and exposure duration. Cd and As showed the most distinct toxicity, while Pb exhibited a delayed but rapid, lethal effect at higher concentrations. Zinc's effects were time- and dose-dependent, with notable mortality at high doses. These results highlight the ecological risks posed by heavy metal contamination in aquatic environments, underscoring the need for ongoing monitoring and regulatory control to protect freshwater biodiversity.

Lethal Concentration (LC_{50}) values at 96 hours

Based on Table 1, Figure 2A and 3, As had the lowest LC_{50} (0.17 ppm), indicating that it is the most toxic of the four metals tested and required just a small amount to cause significant mortality in the juveniles, aligning with its known biochemical toxicity mechanisms of inhibiting critical enzymes, disrupting oxidative phosphorylation and respiration, and inducing cellular apoptosis (Ganie *et al.*, 2024). The less steep positive slope indicates a more spread-out response, with some individuals dying at low concentrations while others survive higher ones.

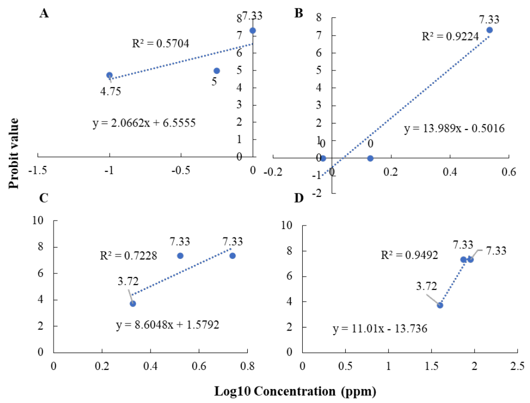

Fig. 2. Probit analysis for evaluation of acute LC_{50} values of heavy metals in juveniles of climbing perch, *Anabas testudineus*, at a 96-hr exposure period. (A) Arsenic (As). (B) Lead (Pb). (C) Cadmium (Cd). (D) Zinc (Zn).

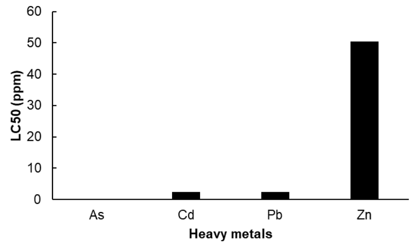

Fig. 3. LC_{50} of Arsenic (As), Cadmium (Cd), Lead (Pb) and Zinc (Zn) at 96 hr.

The moderate slope of the Pb probit graph suggests variability in response at lower concentrations, reflecting lower bioavailability and slower internal accumulation (Figure 2B). The Pb toxicity is considered slower to react compared to Cd and As, as Pb exerts sub-lethal effects over a more extended exposure period (such as accumulating gradually in soft tissues and bones) rather than interfering with calcium metabolism (in Cd) and impairing mitochondrial respiration and enzyme function acutely (in As) (Shahjahan *et al.*, 2022). The sub-lethal effects may not cause immediate mortality but can lead to chronic toxicity.

The probit line for Cd is steep and linear, reflecting a strong and consistent toxic response with increasing log10 metal concentration (Figure 2C). Cd and Pb had comparable LC_{50} values (2.50 ppm & 2.47 ppm, respectively), suggesting a moderate level of toxicity (Table 1, Figures 2B, 2C & 3). While Cd is well known to affect ion regulation, calcium metabolism and oxidative stress pathways, Pb primarily affects neurological and haematological systems (Shahjahan *et al.*, 2022). The almost similar LC_{50} values indicate both metals pose substantial toxic threats at relatively low concentrations.

Zn, with the steepest slope, had the highest LC_{50} (50.35 ppm), indicating that it is the least toxic among the tested metals and requires a considerable amount to cause significant mortality. It is presumed that almost all individuals die quickly and uniformly once the toxic levels are reached (Table 1, Figure 2D & 3). However, despite being an essential trace element and required in small amounts for normal biological function, Zn can be harmful at elevated levels, particularly when it interferes with enzyme systems and causes an ion imbalance in fish at the gill interface (Lall & Kaushik, 2021). The LC_{50} rankings confirm a descending order of acute toxicity: As > Pb > Cd > Zn.

Lethal Time (LT_{50}) values at different metal concentrations

The inverse relationship between heavy metal concentrations and LT_{50} values at low, medium, and high concentrations reflects the cumulative and dose-dependent nature of metals. The LT_{50} for all metals increased with a decrease in mean exposure concentration, indicating that lower metal concentrations require a longer time to achieve 50% mortality (Table 2). An increase in metal time exposure generally accelerates physiological stress and systemic failure (Shahjahan *et al.*, 2022). In the case of As, the steady decline in LT_{50} values reflects a clear concentration-dependent increase in acute toxicity. As's mechanism of action typically involves interference with energy metabolism and induction of oxidative damage, explaining the accelerated mortality at higher doses (Ganie *et al.*, 2024).

Table 1. LC_{50} of Arsenic (As), Cadmium (Cd), Lead (Pb) and Zinc (Zn) at 96 hr.

As	Cd	Pb	Zn
0.17	2.5	2.47	50.35

All values expressed as LC_{50} (ppm) at 96 hr.

Cadmium (Cd) exhibited a linear shift in LT_{50} across concentrations, whereby at low concentration (2.12 ppm), LT_{50} was at 53.28 hr, indicating minimal acute toxicity at this level (Table 2). However, the LT_{50} decreased to 47.63 hr at a medium concentration (3.32 ppm) and dropped to 0 hr at 5.47 ppm, indicating immediate mortality. This shift suggests a rapid and severe toxic effect beyond the threshold, in which Cd overwhelms the organism's defence systems, particularly by disrupting calcium ion channels and causing severe damage to gill and kidney tissues (Shahjahan *et al.*, 2022). The present study confirms the toxicity of Cd and its steep dose-response curve.

In contrast, Pb showed a threshold effect. At lower and medium concentrations (0.93 & 1.35 ppm), no LT_{50} values were recorded, indicating no significant mortality within the 96-hr observation period (Table 2). Only at a high concentration of 3.41 ppm was an LT_{50} of 27.11 hr observed, suggesting that Pb does not elicit acute toxicity until a critical internal concentration is reached. The present study highlights that Pb toxicity is less about gradual accumulation and more about surpassing a physiological limit. Pb toxicity is known to impair neurological and haematological functions. Still, its effects are often delayed or only apparent at higher doses (Wang *et al.*, 2022), which aligns with the current findings.

Zinc, although an essential micronutrient, showed acute toxicity at elevated concentrations. At low concentration (39.5 ppm), the LT_{50} was 7.11 hr, decreasing to 2.99 hr at medium (73.5 ppm) and 1.98 hr at high (89 ppm) concentrations (Table 2). The rapid decrease in LT_{50} highlights Zn's ability to induce toxic effects when regulatory mechanisms are overwhelmed quickly. Excessive Zn disrupts enzyme systems and impairs ion exchange at the gills, leading to swift osmoregulatory collapse and mortality (Lall & Kaushik, 2021).

In summary, all metals exhibited an inverse relationship between exposure concentration and LT_{50}, where an increase in metal concentration resulted in a faster onset of mortality. This relationship revealed the concentration-dependent nature of acute toxicity and the differing toxicodynamics of each metal.

Comparison of Lethal Concentration (LC_{50}) with previous studies

In this study, juvenile climbing perch documented higher response in LC_{50} values (As= 0.17 ppm, Cd= 2.5 ppm, Pb= 2.47 ppm) than adults in comparison with Akter *et al.* (2008) for As (18.211 mg/L), Khanh *et al.* (2022) for Cd (38 mg/L) and Pb (120 mg/L), and Parvin *et al.* (2011) for Cd (191.49 mg/L) (Table 3). The notable difference indicated higher juvenile sensitivity compared to adults, possibly due to immature physiological and detoxification mechanisms.

Among other juvenile species, carp of *Cyprinus carpio* reported higher response to Pb (0.44 mg/L) and Cu (0.30 mg/L) (Alam & Maughan, 1995), suggesting comparable or even higher sensitivity than juvenile climbing perch to specific metal subjection. In addition, a similar pattern of Cu > Cd > Zn was detected in juvenile red tilapia (*Oreochromis* sp.), with LC_{50} values of 0.45 mg/L (Cu), 0.7 mg/L (Cd), and 2.1 mg/L (Zn), indicating relative toxicity (Aldoghachi *et al.*, 2016).

Juvenile cyprinid species (*Labeo rohita* and *Catla catla*) documented higher LC_{50} values than juvenile climbing perch, for metals like Al (78.32 mg/L & 59.56 mg/L, respectively), Fe (49.75 mg/L & 91.68 mg/L), and Mn (64.13 mg/L & 55.26 mg/L), indicating a better endurance in these species (Abdullah & Javed, 2006; Abdullah *et al.*, 2007; Azmat *et al.*, 2012).

Furthermore, juvenile *Brachymystax lenok* were particularly responsive to Cu (0.134 mg/L), Zn (0.222 mg/L), and Pb (0.514 mg/L), with LC_{50} values that are lower than those documented in many tropical species, such as *A. testudineus* (Zheng *et al.*, 2023). This finding reveals the higher flexibility of juvenile climbing perch, which may be attributed to physiological differences in ion balance and metabolic direction. Blackfish (*Capoeta fusca*) exhibited a similar pattern, with LC_{50} values of 1.1 mg/L (Cu) and 13.7 mg/L (Zn), indicating higher endurance compared to salmonids. The range, however, is within tropical species, such as *Oreochromis mossambicus* and *Anabas testudineus* (Ebrahimpour *et al.*, 2010).

Tiwari *et al.* (2011) documented an LC_{50} of 14.95 mg/L for Cd in adult *Channa punctata* (snakehead fish), which is higher than the juvenile *A. testudineus*, suggesting an ontogenetic difference and species-specific resistance. A resilient species of snakehead, with higher tolerance to low-oxygen and polluted conditions, is documented to have higher LC_{50} values.

In addition to juvenile and adult comparisons, Nurulnadia *et al.* (2022) reported LC_{50} values of 14.8 mg/L for Zn and 0.67 mg/L for Cd (Table 3) for the embryonic stage of *Anabas testudineus*. The embryonic LC_{50} for Cd is closely linked to the present study, suggesting that Cd has a consistently severe toxic effect even at the beginning of life stages, impacting enzymatic functions and calcium metabolism. In contrast, the LC_{50} of Zn (14.8 mg/L) in the embryos is significantly higher than the typical juvenile fish species (e.g., *B. lenok*, 0.222 mg/L; *Labeo rohita*, 26.23 mg/L), showing a comparatively better tolerance to Zn during the embryonic stage. This finding may be attributed to essential biological roles of zinc in enzyme function and development, allowing embryos to mitigate moderate concentrations up to a threshold.

These comparisons revealed that the LC_{50} values in juvenile *A. testudineus* are in line with established patterns in aquatic toxicology, with Cu and Cd being generally more toxic to freshwater fish, with juveniles being more sensitive than adults. Moreover, different species likely reflect disparity in physiology, metabolic rate, and ecological change. These findings are significant for environmental risk evaluation, supporting the use of juvenile *A. testudineus* as a bioindicator subject for metal pollution in tropical freshwater systems.

Fish behavioural response

During the toxicity testing period, abnormal behaviour was detected in the juveniles of *A. testudineus* when they were exposed to different heavy metal concentrations, indicating a stress condition. The fish swam normally at the beginning of the metal exposure. However, as the metal exposure period and concentrations increased, the juveniles began to lose equilibrium, swimming

Table 2. LT_{50} values of Arsenic (As), Cadmium (Cd), Lead (Pb) and Zinc (Zn) at 96 hr.

As (ppm)	LT_{50} As (hr)	Cd (ppm)	LT_{50} Cd (hr)	Pb (ppm)	LT_{50} Pb (hr)	Zn (ppm)	LT_{50} Zn (hr)
0.10	91.96	2.12	53.28	0.93	Not determined	39.5	7.11
0.56	67.49	3.32	47.63	1.35	Not determined	73.5	2.99
1.00	32.57	5.47	Not determined	3.41	27.11	89.0	1.98

Nominal concentrations (ppm) with corresponding LT_{50} (hr) at 96 hr exposure.

erratically (including sudden lateral movements, darting, and looping), and surfacing or jumping repeatedly, showing clear signs of physiological and neurological stress. These behaviours indicated weakened and damaged sensory and motor control mechanisms (Shahjahan *et al.*, 2022).

Such behaviours are commonly associated with the neurotoxic effects of heavy metals, which affect neurotransmitter passage, ion channel function, and central nervous system control. Disruption to these pathways exhausts neural transmission, causing loss of motor coordination and abnormal behavioural responses (Shahjahan *et al.*, 2022). An extreme increase in epidermal mucus production was also detected in fish exposed to higher concentrations of metals and longer exposure times. Mucus, which is responsible for fighting against pathogenic, opportunistic, and resistant microbes in water, forms the frontline. However, extreme mucus secretion can accumulate and block the gills, causing suffocation and disturbing osmoregulation, as shown by the frequent opercular activity of surfacing and air gulping (Shahjahan *et al.*, 2022) (Figure 4).

Fig. 4. Dead juvenile climbing perch with red operculum due to heavy metal exposure.

These abnormal behaviours are in parallel with Shahjahan *et al.* (2022), who documented that fish are under stress conditions when exposed to sub-lethal to lethal concentrations of heavy metals. Fish exposed to different heavy metal concentrations portrayed irregular and continuous body and gill opercular movement, lateral swimming, loss of equilibrium, and were able to jump out of the tank. This study demonstrated the significant neurotoxic and physiological effects of heavy metals on aquatic organisms, specifically juvenile climbing perch. This study also confirmed the behavioural response of non-intrusive biomarkers in ecotoxicological evaluation. Based on the observed responses, it is recommended that strict regulations and monitoring of heavy metal concentrations in aquatic ecosystems be enforced to protect fish health and biodiversity.

Table 3. Comparison of LC_{50} values of different metals, fish species and life stages.

No.	Fish species	Stage	Heavy metals	LC_{50} (mg/L)	References
1.	Red Tilapia (*Oreochromis* sp.)	Juveniles	Cu / Cd / Zn	0.45 / 0.75 / 2.1	Aldoghachi *et al.* (2016)
2.	Carp (*Cyprinus carpio*)	Juvenile	Hg / Pb / Cu	0.16 / 0.44 / 0.30	Alam & Maughan (1995)
3.	Carp (*Labeo rohita*)	Juvenile	Fe / Zn / Pb / Ni / Mn / Al	49.75 / 26.23 / 22.11 / 22.01 / 64.13 / 78.32	Abdullah *et al.* (2007); Azmat *et al.* (2012)
4.	Carp (*Catla catla*)	Juvenile	Fe / Zn / Pb / Ni / Mn / Al	91.68 / 20.66 / 18.86 / 11.83 / 55.26 / 59.56	Abdullah & Javed (2006)
5.	Tilapia (*Oreochromis mossambicus*)	Fry and fingerlings	Cu / Pb / Fe	NA	Mashifane & Moyo (2014)
6.	Snakehead (*Channa punctata*)	Adult	Cd	14.95	Tiwari *et al.* (2011)
7.	Blackfish (*Capoeta fusca*)	Not mentioned	Cu / Zn	1.1 / 13.7	Ebrahimpour *et al.* (2010)
8.	Salmonid fish (*Brachymystax lenok*)	Juvenile	Cu / Zn / Pb / Cd	0.134 / 0.222 / 0.514 / 0.734	Zheng *et al.* (2023)
9.	Minnow (*Rasbora sumatrana*)	Not mentioned	Cu / Cd / Zn / Pb / Ni / Fe / Al / Mn	0.006 / 0.10 / 0.46 / 0.63 / 0.83 / 1.71 / 1.53 / 5.71	Shuhaimi-Othman *et al.* (2015)
10.	Guppy (*Poecilia reticulata*)	Not mentioned	Cu / Cd / Zn / Pb / Ni / Fe / Al	0.038 / 0.17 / 1.06 / 1.99 / 15.62 / 1.46 / 6.76	Shuhaimi-Othman *et al.* (2015)
11.	Climbing perch (*A. testudineus*)	Adult (11.70±0.29 cm; 19.5±0.35 g)	Pb / Cd	1.015 / 191.491	Parvin *et al.* (2011)
12.	Climbing perch (*A. testudineus*)	Adult (9±1 cm; 12.9±1 g)	Pb / Cd	120 / 38	Khanh *et al.* (2022)
13.	Climbing perch (*A. testudineus*)	Adult (12.3±2.9 cm; 38.24±1.9 g)	Pb	1.08	Saha *et al.* (2024)
14.	Climbing perch (*A. testudineus*)	Adult (12.01±0.21 cm; 19.05±0.35 g)	As / Hg	18.211 / 0.606	Akter *et al.* (2008)
15.	Climbing perch (*A. testudineus*)	Embryonic	Zn / Cd	14.8 / 0.67	Nurulnadia *et al.* (2022)
16.	Climbing perch (*A. testudineus*)	Juvenile	As / Cd / Pb / Zn	0.17 / 2.5 / 2.47 / 50.35	Present study

Above all the observed behaviours, heavy metal exposure commonly triggers additional stress-related responses that further cause neuromuscular deterioration and physiological dysfunction in fish (Shahjahan *et al.*, 2022). Juveniles may exhibit rapid ventilation rates, fin jerking, reduced physiological responses, and periods of stillness, indicating disturbed metabolic and homeostatic regulation resulting from exposure to heavy metals. Repeated and long-term exposure can also impact a fish's ability to avoid predators, forage, and school, which are necessary for survival in natural ecosystems. Even though prolonged metal exposure causes sub-lethal impacts, which the fish barely survive, their bodies, behaviour, growth, reproduction, and sensory systems are negatively affected (Shahjahan *et al.*, 2022). In severe cases, fish may exhibit unusually low energy and mental alertness, followed by sudden hyperactivity, which can lead to a final collapse of neural control before mortality.

Findings from this study also evidenced broader ecological and long-term consequences for freshwater ecosystems, particularly within the Malaysian waterbody and aquaculture sector, where heavy metal exposure is often intermittent yet extreme due to anthropogenic activities, including industrial discharge, mining runoff, and agricultural activities (Jadaa & Mohammed, 2023). In natural river systems, rapid behavioural dysfunction in fish, particularly juveniles, caused by prolonged heavy metal exposure reduces their ability to avoid predators, forage for food, or maintain position in flowing waters, leading to sudden declines in survival and recruitment. Repeated attacks of acute exposure can cumulatively weaken the population's strength, change species dominance, and disrupt trophic networks, ultimately reducing ecosystem productivity, stability, and biodiversity (Hamilton *et al.*, 2016). In the aquaculture sector, acute toxicity series cause mass mortalities, affect growth, reduce feed efficiency, and increase disease susceptibility, threatening the productivity and economic viability of freshwater fish farming.

The findings from this study also have a direct impact on environmental policy and regulatory frameworks that control water quality in Malaysia. The abnormal behaviour of juvenile climbing perch in this study evidenced the sensitivity to metal exposure that may fall below current permissible limits. Therefore, integrating acute behavioural biomarkers into national monitoring activities could strengthen forecasting systems for pollution events. The study also necessitates revisions to enhance the existing Malaysian water quality standards under the National Water Quality Index (WQI) and Department of Environment (DOE) guidelines, specifically for high-risk catchments and the aquaculture sector. Findings from the acute toxicity responses enable authorities to determine whether the current discharge limits are too lenient and need to be tightened, thereby cumulatively improving the policy and encouraging industries to adopt cleaner production technologies.

CONCLUSION

The present study demonstrated that the four heavy metals tested (As, Cd, Pb & Zn) have distinct effects on acute toxicity in juveniles of the climbing perch, *Anabas testudineus*, with As and Cd posing the most immediate and severe threats. The metal toxicity pattern are as follows: As > Pb > Cd > Zn, showed by smaller values of LC_{50} (As= 0.17 mg/L, Pb= 2.47 mg/L, Cd= 2.5 mg/L, and Zn= 50.35 mg/L). The LC_{50}/LT_{50} values and mortality patterns showed a concentration-dependent response, necessitating the strict monitoring and regulation of metal discharge into aquatic ecosystems. Abnormal behaviour due to metal toxicity and discomfort, as well as physiological responses, further prove the neurotoxic and osmoregulatory effects caused by these heavy metals.

The control group, which showed zero juvenile deaths, demonstrated that mortality and adverse effects in the metal-exposed fish are directly caused by the metals (As, Pb, Cd & Zn), rather than poor water quality or handling stress. This finding highlights the significance of maintaining environmental quality standards and emphasises the use of *A. testudineus* as a sensitive bioindicator in ecotoxicological research. Future research on the chronic and adverse effects of Cd at concentrations below its LC_{50}, specifically investigating its effects on the reproductive success and growth rate of adult *A. testudineus*, is highly recommended.

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ETHICAL STATEMENT

All experimental procedures were conducted in accordance with the institutional guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the UniSZA Animal and Plant Research Ethics Committee (UAPREC), with reference number UAPREC/008/051, ensuring compliance with relevant national legislation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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