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Lead (Pb) absorption capacity in *Pterygoplichthys pardalis* (Castelnau, 1855)

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Abstract
Heavy metal pollution, such as lead (Pb), is widely found in several water bodies, impacting fish as aquatic organisms. The Amazon sailfin catfish (*Pterygoplichthys pardalis*) is a freshwater fish commonly found on the bottom and walls of rivers with high concentrations of heavy-metal deposits, such as the Ciliwung River. The Ciliwung River ecosystem is known to be contaminated with heavy metals, including cadmium (Cd), lead (Pb), and mercury (Hg). Based on the Government Regulation of the Republic of Indonesia No. 20 of 1990, the lead content in the Ciliwung River remains below the Threshold Limit Value (TLV). However, the high adaptability of *P. pardalis* to live in polluted waters increases metal accumulation within the fish's body. Heavy metals accumulate at the tissue and organ levels, such as the gills, muscles (flesh), liver, and bones. This study was conducted to calculate the absorption capacity of *P. pardalis* toward lead in water, as well as the mortality of *P. pardalis* at determined lead concentrations. The research methods included fish acclimatization, acute toxicity tests, water quality measurement, and analysis of lead content using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). The results showed that higher lead concentrations led to higher mortality in *P. pardalis*. The highest lead absorption was observed in P2 (11.49 mg/L) with 98% absorption, followed by P3 (139.76 mg/L) with 97% absorption, and P1 (1.95 mg/L) with 96% absorption.

Keywords: Pollution, lead, *Pterygoplichthys pardalis*, absorption, mortality

Introduction

The Amazon sailfin catfish (pleco) is an invasive freshwater species from the Amazon and belongs to the Loricariidae family [1]. Plecos belonging to the genus *Pterygoplichthys* of the Loricariidae family have been widely released into tropical and subtropical freshwater environments worldwide (Page, 1994). Generally, plecos are represented by several species, one of which is *Pterygoplichthys pardalis*. *P. pardalis* has a body pattern of separate black spots on its dorsal part [2]. This fish has a flat body shape, and its entire body is covered with hard scales except for the abdomen. A distinctive feature of this fish is its suctorial mouth, used for feeding, breathing, and attaching to the walls of water bodies. The pleco's adaptability is very high, allowing it to live in waters with high levels of pollution. The respiratory system, consisting of gills and a labyrinth organ, helps the pleco adapt to aquatic environments with high pollution levels [3].

P. pardalis can be found along rivers, lakes, and swamps. One of the habitats for *P. pardalis* is the Ciliwung River, which is known to be exposed to high concentrations of heavy metals. The presence of *P. pardalis* in the Ciliwung River has the potential to accumulate heavy metals into the fish's body tissues. According to SNI 7387:2009, fish and their processed products have maximum content limits for each type of heavy metal: Cd (< 0.1 mg/kg), Hg (< 0.5 mg/kg), and Pb (< 0.3 mg/kg) [4].

Lead (Pb) is one type of metal that can persist for a long time in aquatic ecosystems, and its presence is toxic in water, especially for fish. Pb is easily soluble in water and can bind with organic matter, making it easily absorbed by aquatic organisms. The industrial sector uses Pb as a raw material in the manufacturing processes of paint, batteries, pipes, ceramics, plastics, mining, and construction. Rapid development in this sector also increases Pb pollution in water bodies [5]. Pb entering the waters acts as a pollutant that can accumulate in water and sediment, then further accumulate in larger amounts (biomagnification) in living organisms through the food chain [6]. Environmental pollution of aquatic systems by heavy metals can disrupt ecosystem balance by interfering with organ functions, metabolism, and the cellular processes of living organisms [7].

The accumulation effect of Pb in aquatic biota is lethal when exposed to high concentrations. Low-concentration exposure is sublethal for fish, causing effects such as decreased gonad maturity that inhibits reproduction, closure of gill membranes, and metabolic inhibition [8]. The lethal and sublethal effects of Pb exposure on fish are due to its characteristics of being difficult to degrade and easily binding with organic matter. This allows Pb to persist in ecosystems and remain toxic to fish.

The concentration limit of Pb that can be absorbed by *P. pardalis* is quite high and can be lethal to other organisms. Research is needed to determine the concentration of metal exposure that is lethal to *P. pardalis*. Therefore, this study aims to obtain data on the absorption capacity of *P. pardalis* toward Pb and its relationship with the fish mortality rate. The benefit of this research is expected to serve as an informational reference regarding the Pb absorption capacity of *P. pardalis* and its relationship to fish mortality.

Materials and methods

1. Research Object and Location

This study used *P. pardalis* as the observation object, conditioned in aquariums and treated according to determined Pb concentrations. The research was conducted at the Greenhouse of Universitas Al Azhar Indonesia, Jl. Sisingamangaraja, and South Jakarta. The analysis of heavy metal absorption capacity in samples was performed at the National Research and Innovation Agency (BRIN), Lebak Bulus, South Jakarta.

2. Tools and Materials

Tools used in this study included acrylic aquariums, aerators, aquarium hoses, paranet nets, digital pH meters, digital Total Dissolved Solid (TDS) meters, digital thermometers, digital scales, measuring cylinders, Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), drying microwave, digestive microwave, desiccator, surgical tools, surgical wax boards, sample bottles, volumetric flasks, mortar, pestle, and measuring pipettes. Materials used included Amazon sailfin catfish (*P. pardalis*) sized 5-10 cm, pellets, PbCl2 metal, 0.3 N HCl solution, H2O2 solution, HNO3 solution, distilled water, 70% alcohol, markers, ziplock bags, tissue paper, labels, and gloves.

3. Research Stages

Acclimatization of Test Fish: Acclimatization was carried out in 12 units of 30 cm x 30 cm x 20 cm aquariums. Aquariums were cleaned and dried before use. Each was filled with 6 liters of water and aerated for 24 hours. After aeration, 5 healthy *P. pardalis* were placed in each aquarium. Acclimatization lasted 10 days with feeding three times a day.

Toxicity Test: Acute toxicity tests were conducted to determine toxicity levels, organ sensitivity, and hazard data. Concentrations used were control (K) 0 mg/L, Treatment 1 (P1) 3.8 mg/L, Treatment 2 (P2) 23.8 mg/L, and Treatment 3 (P3) 150 mg/L. Observations were conducted for 12 days, with sampling at 0, 3, 7, and 12 days. Dead fish were immediately removed to prevent water pollution.

Water Quality Measurement: Included pH, temperature, and TDS measurements using digital meters throughout the acclimatization and toxicity test stages.

Heavy Metal Absorption Analysis: Analyzed using ICP-OES. Samples were ground and digested with a mixture of 8 ml 65% HNO3 and 2 ml 30% H2O2 for 90 minutes at 180°C. The wavelength used for Pb analysis was 283.3 nm.

Fish Mortality Percentage: Mortality was recorded from day one to the last day. The percentage was calculated by comparing the number of dead fishes to the initial number of fishes.

Data Analysis: Data on metal absorption in the flesh of *P. pardalis* from ICP-OES were grouped by treatment and then analyzed descriptively.

Results & Discussion

Acclimatization is necessary to ensure that effects observed during testing are due to the treatment. It reduces physiological changes, maintains normal metabolic rates, and improves osmoregulation [9]. Aeration was maintained to dissolve oxygen and release dissolved gases. Water quality parameters during acclimatization were: temperature 29.4–32.4 °C, pH 7.75–8.15, and TDS 112–154 mg/L (Table 1).

Temperature affects fish metabolic activities, growth, and food intake. Optimal pH for fish is 6.5–9.0; values outside this range can inhibit growth or be lethal. The maximum TDS limit for aquaculture is generally less than 1000 ppm.

During the 12-day toxicity test, water quality was: temperature 29–32 °C, pH 7.15–8.3, and TDS 104–343 mg/L (Table 2).

Higher concentrations of heavy metals increased mortality. Previous research shows that high metal exposure increases fin movement and operculum opening as a stress response before metabolism declines and leads to death [10]. Pb concentration in water decreased by day 12 in all treatments (Figure 1), indicating metal transfer from water to the fish through absorption. The highest Pb absorption was in P2 (11.49 mg/L) at 98%, followed by P3 (139.76 mg/L) at 97%, and P1 (1.95 mg/L) at 96% (Figures 1 and 2).

Analysis of Pb absorption by the fish indicated that the high concentrations in each treatment were followed by an increase in the percentage of heavy metal absorption by the fish across all treatments (Figure 2). The changes in water quality during the research could influence the daily activities of *P. pardalis* in responding to and regulating its metabolism. Therefore, physical parameters of water, such as high temperatures, are capable of increasing the solubility of heavy metals, thereby supporting the absorption of heavy metals into the fish's body [11].

P. pardalis is a freshwater fish that possesses a different osmoregulation mechanism compared to marine fish. Salt concentrations in seawater are higher than in the fish's body, causing marine fish to excrete more water with dilute urine. Meanwhile, freshwater fish have higher salt levels than their environment, necessitating them to absorb more water from the surrounding aquatic environment. This osmoregulation challenge supports *P. pardalis* as a freshwater species in absorbing more water from its environment. Pollutants such as heavy metals dissolved in water also have the potential to be absorbed by *P. pardalis* through this osmoregulation mechanism [12].

The process of Pb absorption into the fish's body occurs through the food chain, gills, or diffusion through the skin [13]. The degree of Pb pollution in fish can be determined based on habitat, duration of exposure, and the fish's feeding habits. Heavy metals can enter the fish's body through two mechanisms: direct and indirect. Heavy metals can directly enter the fish's body simultaneously through the process of nutrient absorption. Both nutrients and heavy metals are absorbed through the gills, and Pb specifically binds to the blood, subsequently spreading throughout the body. Once absorbed, heavy metals require a long time to be degraded and, in certain amounts, will accumulate in several tissues and organs, such as the gills, liver, kidneys, and flesh.

Excreted products containing metals also have the potential to be reabsorbed by *P. pardalis*, considering its feeding habits, which focus on the bottom or walls of water bodies known to contain pollutant deposits, including heavy metals. Furthermore, heavy metals can also enter the fish's body indirectly through the food chain [14]. The biochemical mechanisms of mineral absorption and metabolism in fish are generally similar to land animals at the cellular level. Ion exchange occurring through the gills and skin increases the surface area for nutrient absorption into the fish's body, thus allowing other dissolved pollutants in the water to be absorbed as well. The gills cover more than 50% of the fish's surface area for mineral absorption and are considered the primary pathway for absorbing minerals dissolved in water [15].

The mortality percentage of *P. pardalis* obtained during the 12-day toxicity test showed a direct correlation with the percentage of heavy metal absorption in the water (Figure 2). The mortality percentage of *P. pardalis* was also directly proportional to the heavy metal concentration administered in each treatment. The higher the heavy metal concentration given—from P1 and P2 to P3—the higher the mortality percentage of *P. pardalis* (Figure 3). Mortality in *P. pardalis* was caused by the Pb metal absorbed by the fish's body and accumulated from the tissue to the organ level. Pb tends to undergo bioaccumulation and increases the toxicity level toward an organism; thus, the accumulation of heavy metals in high concentrations can increase the mortality of *P. pardalis* [16, 17].

Analysis of the fish mortality percentage during the 12-day toxicity test showed that the mortality rate from highest to lowest occurred in P3, P2, P1, and the control. The control treatment had the lowest number of deaths compared to P1, P2, and P3 because no metal exposure was administered, resulting in a minimal mortality rate. Fish in P2 and P3 exhibited the highest mortality percentages among all treatments. This is consistent with the different metal concentrations administered for each treatment (Figure 3).

A certain amount of Pb absorbed into the body binds with the blood and circulates throughout the system, leading *P. pardalis* to adapt to the presence of Pb in its body. High metal concentrations increase the potential for fish mortality. Fish deaths during the toxicity test were inseparable from the impact of Pb exposure. Pb exposure in fish can cause adverse effects such as edema, necrosis, secondary lamellar hyperplasia, lamellar fusion in the gills, and aneurysms [18]. Besides Pb, it is known that several heavy metals can cause damage to the gills and liver, leading to fish death. One such heavy metal that causes damage at the tissue level is cadmium (Cd). Cd can cause damage at the tissue and organ levels, particularly in the gills, liver, kidneys, and intestines [11].

The results obtained from the mortality diagram show that the fish mortality rate did not reach 100%. *P. pardalis* was able to survive during the Pb exposure because the water conditions used during acclimatization and toxicity testing were classified as safe for fish life (Table 2). *P. pardalis* is known for its ability to live in polluted waters with very low dissolved oxygen levels, heavy metal contamination, and various organic or inorganic waste pollution. The high adaptability of the Amazon sailfin catfish to its environment is a supporting factor for the fish's survival rate despite being in water conditions contaminated with Pb [19].

Fish exposed to heavy metals maintain their body condition in a state of homeostasis, which increases body metabolism and oxygen demand. The entry of heavy metals into fish occurs through the gills, body surface, osmoregulation mechanisms, and absorption through feeding. The effect of toxic substances in metals is determined by the toxic properties of Pb and the body's ability to perform detoxification and excretion processes, allowing the impact of Pb's toxic properties to be tolerated by the fish's body. However, if the amount of dissolved metal absorbed by the fish's body exceeds the determined threshold, it will result in death. Pb is known to cause severe toxic effects in many organisms because it can interfere with metabolism and trigger mutagenic responses [20, 21].

Tables and Figures

Table 1: Water quality measurements during the fish acclimatization test

Day	Temperature (ºC)	pH	TDS (mg/L)
1	30,37 ± 0,22	8,12 ± 0,07	149 ± 4,18
2	30,45 ± 0,41	8,08 ± 0,02	145,5 ± 4,15
3	30,2 ± 0,4	8,06 ± 0,11	151 ± 3,2
4	29,82 ± 0,3	8,03 ± 0,12	152,75 ± 2,38
5	29,8 ± 0,12	7,91 ± 0,05	143,25 ± 4,02
6	30,77 ± 0,46	7,9 ± 0,14	137,75 ± 4,6
7	31,32 ± 0,8	7,85 ± 0,08	133,25 ± 4,26
8	31,5 ± 0,33	7,85 ± 0,06	130,75 ± 2,58
9	30,47 ± 0,72	7,83 ± 0,08	126,25 ± 3,89
10	31,6 ± 0,24	7,78 ± 0,06	121,5 ± 5,76

Table 2: Water quality measurements during the 12-day acute toxicity test

Treatment	Parameter		
	Temperature (ºC)	pH	TDS (mg/L)
Control (0 mg/L)	31,97 ± 0,85	8,1 ± 0,14	228,82 ± 72,63
P1 (3,8 mg/L)	31,21 ± 0,96	8,11 ± 0,14	209,25 ± 56,8
P2 (23,8 mg/L)	30,58 ± 0,86	8,06 ± 0,16	203,53 ± 55,14
P3 (150 mg/L)	30,38 ± 0,98	7,59 ± 0,43	223,25 ± 65,05

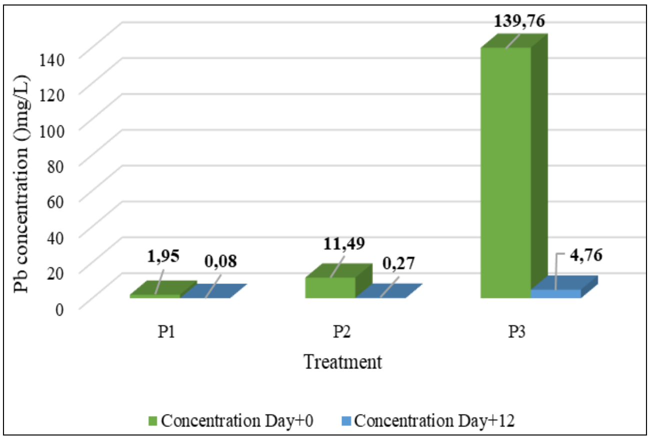

Fig 1: Diagram of the decrease in Lead (Pb) concentration in water over 12 days

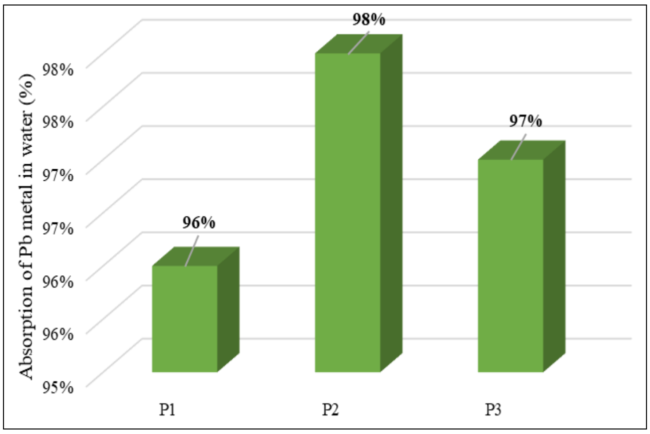

Fig 2: Diagram of the percentage of Lead (Pb) absorption by fish over 12 days

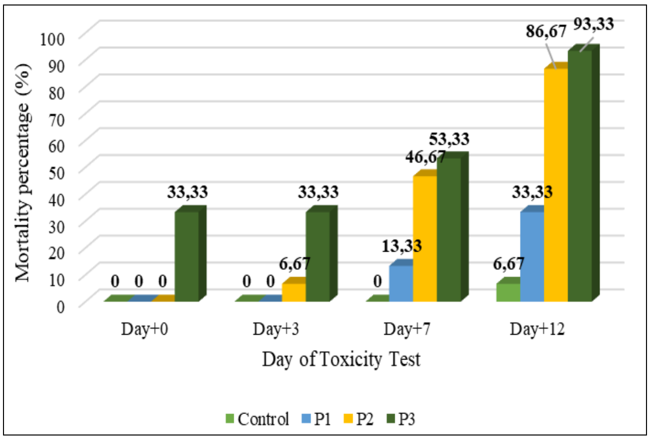

Fig 3: Diagram of the mortality percentage of *P. pardalis* during the toxicity test

Conclusions

Based on the analysis, the highest percentage of lead (Pb) absorption into the fish's body was observed in P2, with an absorption rate of 98% at a concentration of 11.49 mg/L. The fish mortality rate from highest to lowest was P3, P2, and P1. These results indicate a linear relationship between the concentration of lead exposure and the fish mortality rate; specifically, higher exposure concentrations result in higher fish mortality.

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