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Cadmium (Cd) absorption capacity in *Pterygoplichthys pardalis* (Castelnau, 1855)

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Abstract
Pterygoplichthys pardalis (Suckermouth sailfin catfish or pleco fish) is included in the Loricariidae species capable of adapting to extremely polluted environments such as the Ciliwung River. Several studies have shown that the water of the Ciliwung River contains heavy metals such as cadmium (Cd), mercury (Hg), and lead (Pb) originating from industrial, agricultural, and household waste around the river. The content of these heavy metals including Cd has been detected in pleco fish's flesh, gills, liver, and bones, which exceeds the maximum limit value set by BPOM 2017. Therefore, this study aimed to calculate the absorption capacity of Cd and the correlation with mortality in pleco fish. The methods used were preliminary and acute toxicity tests, alongside mortality percentages. The results showed that fish could absorb Cd with various concentrations for 12 days of the test. The largest absorption percentage of 95% occurred in the third treatment. This showed a strong correlation of 99% with the percentage level of pleco fish mortality to the Cd content in water.

Keywords: Cadmium, mortality, pleco fish, *Pterygoplichthys pardalis*, toxicity

1. Introduction

Pterygoplichthys pardalis (*P. Pardalis*), or pleco fish or suckermouth fish, is a Loricariidae species originating in South America and Central America. This fish is characterized by a flat, dorso-ventrally body covered by hard skin, a head with a geometric dark and light line pattern, a subterminal mouth type of filter-sucking, and a freshwater habitat, showing potential to survive in extreme environments. Adult species have large black spots on the ventral part of the body. Pleco fish is an invasive species widely distributed in tropical and subtropical freshwater, including various extreme environmental conditions [1]. This fish has a labyrinth as a respiratory tract and modifications to the stomach as additional organs that adapt to low dissolved oxygen conditions [2]. Furthermore, it proliferates without requiring intensive maintenance [3].

One of the habitats of pleco fish in Indonesia is the Ciliwung River, which flows from Bogor to Jakarta. Based on previous studies conducted in 2018-2019, the content of three heavy metals including lead (Pb), mercury (Hg), and cadmium (Cd) was found in pleco fish flesh and processed food products [4, 5]. These metal concentrations were included in the> 1 mg/kg category as the safe limit for consuming flesh and > 0.3 for fishery products [6]. The Ciliwung River has been contaminated with heavy metals [7, 8].

Cd is among heavy metals with a high level of toxicity [9]. According to Awalina, until the end of the 20th century, 45% of total global pollution was caused by Cd [10]. This metal is often used as the main ingredient or additional material in the battery, pigment, and plastic industries [11]. The entry of Cd metal into waters can cause metal accumulation to cause toxicity to aquatic biota, including fish.

The direct impact of metal accumulation on fish is lethal and sublethal, which can cause genetic effects on the biota due to central nervous system disorders, leading to death. The sublethal effect occurs in body organs, which causes liver damage, decreased blood count, reduced potential for reproduction, growth, and chloride [12,13]. The influence of these conditions requires further investigations to determine the absorption capacity and mortality of

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pleco fish exposed to Cd. Therefore, this study aimed to calculate the absorption capacity of Cd and the correlation with mortality in pleco fish.

2. Methods
2.1 Study Object and Location
The sample of pleco fish was kept in an aquarium and given Cd at a predetermined concentration. The study was conducted at the University of Al-Azhar Indonesia Biology Laboratory in Kebayoran Baru District, South Jakarta City. The absorption capacity of heavy metals was analyzed at the National Research and Innovation Agency of the Republic of Indonesia (BRIN), Lebak Bulus, South Jakarta.

2.2 Tools and Materials
The tools used in this study included acrylic aquariums, aerators, aquarium hoses, digital cameras, pH meters, digital thermometers, fish filters, digital TDS meters, cool boxes, jerry cans, Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Digestive microwaves, and ovens. Other tools included digital scales, surgical scissors, scalpels, funnels, sample bottles, micropipettes, desiccators, measuring cups, crucibles, measuring flasks, label paper, ziplock plastic, paranet nets, mortars, and pestles. The materials used were pleco fish measuring 5-10cm, Cd metal, distilled water, pellets, concentrated HCl, H_2O_2, HNO_3, and 70% alcohol.

2.3 Study stage
2.3.1 Test Fish Acclimatization
The maintenance media used in this study was a 30cm x 30cm x 20cm acrylic aquarium cleaned with water and sun-dried before use. Furthermore, the test animals were acclimatized for 7 days with sufficient aeration and pellet feed provided 2 times a day. 180 healthy pleco fish measuring 10-13 cm were acclimated for preliminary and toxicity tests.

2.3.2 Toxicity Test
The test was used to determine the Cd toxicity level. An acute toxicity test was performed based on the concentration of Hg in each test media, where higher (N) and lower threshold values (n) were obtained from the preliminary test. The fish was observed according to the time series, namely 24, 48, 72, and 96 hours.

2.3.3 Aquarium Water Quality Measurement
Aquarium water quality was observed by measuring temperature, acidity level (pH), and Total dissolved solids (TDS) using a digital water thermometer, pH, and TDS meter, respectively. The study carried water quality parameter measurements at the acclimatization and toxicity test stages for 7 and 4 days, respectively.

2.3.4 Analysis of Heavy Metal Absorption Power
The absorption power of Cd in pleco fish was analyzed using the ICP-OES tool. Initially, the dry sample was destroyed using a microwave acid digestion tool to change the powder sample into a liquid form. This process was dissolved using 8 ml reagents of 65% HNO_3 and 2 ml of 30% H_2O_2 for 90 minutes at a temperature of 180°C. Subsequently, the sample passed through a heavy metal analysis test using the ICP tool at a wavelength of 214.438 mm for Cd. The working principle of the ICP-OES tool is to change the metal sample in the body of the fish and water into an aerosol form by argon gas. This method has multi-element analysis capabilities, a high level of selectivity, and accuracy, and a low detection limit, enabling the detection of all metal elements [14].

2.3.5 Fish Mortality Percentage
Mortality observation was conducted on the first and last day of the toxicity test. This included subtracting the number of fish at the beginning from the number at the end of maintenance. Subsequently, the results were compared with the number of fish at the beginning of maintenance [15]:

$$\text{Mortality \%} = \frac{\text{initial number of fish} - \text{final number of fish}}{\text{initial number of fish}} \times 100\%$$

2.4 Data Analysis
The data obtained in the study were inputted and grouped or tabulated into a table according to the objective and purpose. The results of preliminary test data, acute toxicity tests, and mortality percentages were tabulated in table form and analyzed descriptively.

3. Results and Discussion
3.1 Acclimatization
Acclimatization is the process of adapting to different environmental conditions, from breeding grounds to pond water, without causing stress. This process is conducted because all animals can regulate body morphology, thereby adapting to new environments [16]. Water quality parameters at temperature, pH, and TDS are calculated routinely for 10 days using available measuring instruments (Table 1).
The temperature measurements during the acclimatization phase showed results ranging between 30-32 °C (Table 1). Temperature is a factor that affects fish activity during the study because *P. pardalis* requires specific environmental conditions to survive. During acclimatization, this temperature range was highly supportive of the growth of *P. pardalis*. This range is still considered optimal for freshwater aquaculture, according to the quality standard set by Government Regulation 82 of 2001, which is 25-32 °C [17].
The pH values obtained during the 10-day acclimatization phase of *P. pardalis* were still within a normal range, between 7.3-8.22 (Table 1). The suitable pH range for freshwater fish farming is between 6 and 9. A lethal pH value for fish is below 4 or above 11, as not all aquatic organisms can survive changes in water pH. The impact of water pH on freshwater fish farming, if the pH changes, will disrupt the survival of the organisms as it affects fish growth and respiration processes [18].

Table 1: Water quality measurement during fish acclimatization for 10 days

Day	Temperature (°C)	pH	TDS (mg/L)
1	30,2	7,3	142
2	31	7,41	146
3	32	7,85	147
4	30,6	7,7	154
5	30	8	154
6	30	8,15	159
7	30	8,18	161
8	31	8,14	164
9	31	8,14	163
10	31,3	8,22	165

Normal temperature and pH conditions were also accompanied by safe total dissolved solids (TDS) values. The TDS values obtained during the 10-day acclimatization test

ranged from 142-165 mg/L (Table 1). The TDS values in each aquarium remained below <1000 ppm, which is categorized as suitable for fish farming activities. According to Government Regulation No. 82 of 2001, the maximum permissible TDS level for Class III aquatic fish farming is <1000 ppm. Low TDS values can be influenced by the presence of filtration media installed in the aquariums, while high TDS values can result from increased fish activity, such as residual feed and excretion from fish metabolism [19].

3.2 Metal Toxicity Test

The acute toxicity test was carried out for 12 days by administering heavy metal liquid Cd based on the predetermined treatment. For treatment concentrations 1, 2, and 3 1.435 mg/L, 2, 8.434 mg/L, and 3, 31.144 mg/L were dripped, respectively. These concentrations were determined based on the percentage of mortality levels carried out in a previous study [20]. Water quality parameters were measured at the toxicity test stage by measuring temperature, pH, and TDS (Table 2).

**Table 2:** Water quality measurement in acute fish toxicity test for 12 days

Concentration (mg/L)	Parameter		
	Temperature (°C)	pH	TDS (mg/L)
Control	29,8-32,8	7,4-8,3	154-266
P1 (1,435)	28,4-31,3	8,1-8,2	178-332
P2 (8,434)	28,6-30,4	8-8,3	181-242
P3 (31,144)	28,5-30,8	7,7-8,2	195-341

An acute toxicity test was conducted to evaluate the relative toxicity level of chemicals to certain aquatic organisms in short-term exposure time. Based on the data obtained for 12 days in each treatment, the water quality was still within acceptable limits or met the fish survival requirements with temperatures ranging from 28.5-32.8 °C. The safe temperature value for fish farming based on Government Regulation No. 82 of 2001 is 25-32 °C [17]. The pH value of aquarium water in the toxicity test ranged from 7.4 to 8.3. This value is still relatively safe for the freshwater fish's life, as the optimal pH range is approximately 7-8 [18]. Significant changes in pH towards acid or base can disrupt water quality. This is closely related to the solubility of heavy metals, where an increase in pH is correlated with a decrease in solubility. Generally, the ability of aquatic organisms to accumulate heavy metals depends on the type of organism, heavy metal, length of exposure time, and conditions of the aquatic environment. It was also reported that the concentration of heavy metals was directly proportional to pH value [21]. The measuring result of the TDS value parameters in the toxicity test ranged from 154-341 mg/L, showing the solubility level. In this study, TDS measurements were carried out to determine the distribution of TDS values in water [22].

3.3 Cd Metal Absorption in Pleco Fish

Based on the analysis, a higher concentration of Cd in water led to a greater percentage of absorption capacity in pleco fish (Figure 1). Changes in water quality such as temperature, pH, and TDS during the study significantly influenced the daily activities of pleco fish in responding to and regulating their body metabolism when toxic substances enter the environment (Table 1). Pleco fish are freshwater species that usually live in environmental conditions with lower salt concentrations than their bodies, thereby absorbing more

water to maintain osmotic balance. [8, 23, 24]. This is also related to the significantly high adaptability, which can live in water conditions polluted by various heavy metals as well as organic and inorganic waste [25, 26, 27].
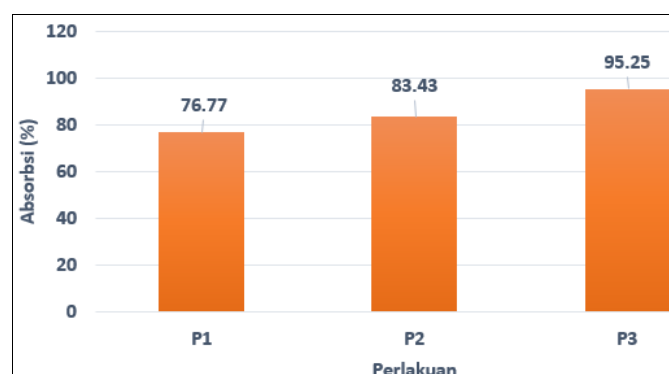
**Fig 1:** Percentage of Cd metal absorption for 12 days

Cd is absorbed by fish with water or food and transported through blood circulation to migrate and distribute to various tissues as well as organs of fish. After absorption, Cd will accumulate in detoxification organs such as the kidneys and liver. However, when carried by food intake, it will accumulate and be absorbed in the intestinal organs. [5, 8, 28]. Metal content absorbed by pleco fish in each treatment is distributed to all organs of the fish [4, 5, 8, 28, 29, 30]. Meanwhile, each organ has an absorption capacity and limits related to fish's respiratory system and metabolism.

3.4 Mortality Percentage of Pleco Fish

Based on the observation results, there was no death in the control treatment because the fish were not exposed to Cd metal. The results showed that the third treatment with the most fish deaths had a Cd metal concentration of 31.144 mg/L, followed by the second and first treatments with exposure of 1.435 and 8.434 mg/L, respectively. Pleco fish became disturbed and uncomfortable in a polluted environment, causing mortality at the metal concentration level given in each treatment. This was supported by data showing a decrease in Cd level, which contributed to the mortality rate in pleco fish, as presented in Figure 2.

The mortality percentage in pleco fish was directly proportional to Cd concentration in each aquarium (Figure 1). The results showed that a greater percentage of Cd absorption caused higher mortality. The mortality rate of *P. pardalis* in aquatic environments contaminated with high concentrations of heavy metals is influenced by the concentration of metals in the water [29, 30, 31]. *P. pardalis* exposed to the heavy metals Cd, Hg, and Pb had shown strong endurance and can survive even in an aquatic environment contaminated with high concentrations of metal. This highlights the unique characteristics of the pleco fish were their high adaptability by survival in extreme aquatic environments containing heavy metals and various wastes at high concentration [8, 32, 33, 34].

The mortality of pleco fish in the observation did not reach 100%. This was because, during the acclimatization process until the toxicity test, environmental parameters such as pH, TDS, and temperature were still relatively safe for freshwater fish cultivation, as shown in Table 2. Therefore, pleco fish can survive in conditions exposed to Cd, both with its high adaptability and environmental conditions that are relatively safe during the study [18, 29].
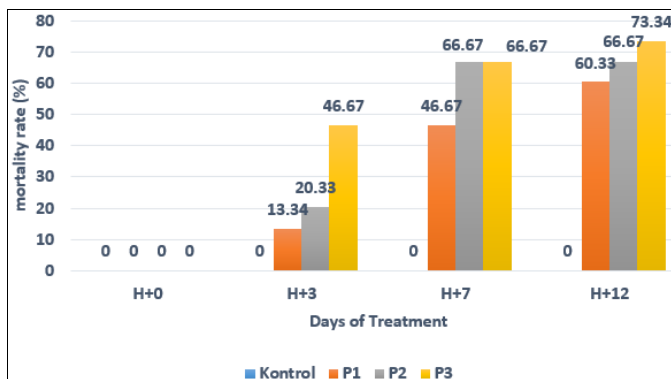
Fig 21: The mortality percentage of pleco fish

In fish, mortality is caused by disruption of the internal organ system due to the absorption of heavy metals through the respiratory and digestive systems. The effect of Cd accumulated on pleco fish causes damage to gills, intestines, and internal tissues such as the liver and kidneys. In a fish's body, metal is absorbed by the blood and binds to blood proteins, which are distributed throughout the tissues, thereby affecting the structure and function of the gills, liver, and gonads. Furthermore, Cd can affect physiological metabolism, the reproductive, and immune systems, causing metabolic, physical disorders, or death [30, 31, 35, 36, 37, 38, 39]. The heavy metal accumulation in fish is influenced by several factors, both from the external environment and the internal characteristics of the fish. These factors include the aquatic environment, fish biology, ecosystem interactions, anthropogenic, climate, and physiological activity [29, 32, 40, 41].

Cd is a heavy metal that is classified as non-essential and the most toxic at high concentrations. Exposure to Cd in fish can affect the reproductive rate, causing gradual extinction of generations in polluted water [35, 36, 38, 42]. The Cd metal could induce various epigenetic changes, causing variations in the chemical modification of DNA, histones, and chromatin. Therefore, fish have a greater risk of disease, which triggers the development of various cancer cells. This shows the need for various indicators to show the impact of Cd pollution on the aquatic environment to evaluate ecosystem safety [35, 36, 37, 38].

4. Conclusion
In conclusion, this study showed that pleco fish could absorb the administration of Cd with various concentrations for 12 days of the test. The largest absorption percentage of 95% occurred in the third treatment. This showed a strong correlation of 99% with the mortality percentage of pleco fish to Cd content in water.

5. Acknowledgment

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