Survivability of *Pterygoplichthys pardalis* from Ciliwung River, Jakarta, Indonesia based on metal toxicity test and bioaccumulation of cadmium, mercury, and lead

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Abstract. *Pterygoplichthys pardalis* is a Loricariidae species commonly found in extremely polluted environments such as the Ciliwung River. Previous research showed that the Ciliwung River waters contain heavy metals, such as Cd, Hg, and Pb originating from industrial, agricultural, and household wastes. The heavy metal content has been detected in the flesh, organs, and bones of *P. pardalis*. This exceeds the maximum limit value set by the Drug and Food Control Agency. The main reasons to conduct this research were based on the fact that there was no prior information or research regarding the survivability of *P. pardalis* in the Ciliwung River. Hence, this research aimed to determine the survivability of *P. pardalis* based on the threshold concentrations of heavy metal compounds (Cd, Hg, and Pb) causing lethal effects on *P. pardalis.* The preliminary and acute toxicity, mortality percentage, and bioaccumulation of Cd, Hg, and Pb in the body of *P. pardalis* were also determined. The results showed that *P. pardalis* have a higher survivability in toxic freshwater environment such as Ciliwung River.
Key Words: biomagnification, existing live, freshwater ecosystem, hazardous substances.

Introduction. *Pterygoplichthys pardalis*, Castelnau 1855*,* known as pleco in Indonesia, is one of the Loricariidae species originating from South and Central America. It features a flat dorso-ventral body coated in a tough skin, showing a geometric pattern of dark and light stripes on the head, presenting a subterminal mouth with a sucking filter type, living in a watery habitat, and the manifesting the ability to live in extreme environments. The adult species have large black spots on the ventral side of the body. Armbruster (2004) stated that *P. pardalis* is an invasive species with a wide distribution in tropical and subtropical freshwater environments. Furthermore, *P. pardalis* can live in a variety of extreme environmental conditions. This fish has a breathing apparatus like a labyrinth and presents modifications to the stomach and to different organs which adapted to the low dissolved oxygen conditions (Armbruster 1998). It has a relatively rapid growth without requiring intensive rearing (Pinem et al 2014).

Ciliwung River, which runs from Bogor to Jakarta, is one of *P. pardalis's* habitats in Indonesia. Based on research on the Ciliwung River in 2018-2019, three heavy metals, namely cadmium (Cd), mercury (Hg), and lead (Pb) were found in flesh and processed food products made from *P. pardalis* (Putri et al 2020; Elfidasari et al 2018). According to BSN (2009), the safe range for concentrations of three heavy metals (Cd, Hg, and Pb) is <1mg kg^{-1} for meat products and <0.3mg kg^{-1} for fishery products. Heavy metal concentration in *P. pardalis* from the Ciliwung River has exceeded the amount of the BSN normal range (Elfidasari et al 2019; Ismi et al 2019; Elfidasari et al 2020).

Cadmium is the most toxic and mobile elements in the environment (Berhorft 2013; Rahimzadeh et al 2017; Fatima et al 2019; Balali-Mood et al 2021). This metal is often used as the main or additional material in the battery, pigment, and plastic industries

(Jaishankar et al 2014; Turner 2019; Genchi et al 2020). Cd's entry into waters results in metal accumulation, causing toxicity to aquatic biota, including fish (Perera et al 2015; Uddin et al 2021; Prayoga et al 2022). Toxic effects caused by Cd on biota are determined from toxicity tests using animals. Furthermore, mercury may be found in nature or due to anthropogenic activities. Naturally, this heavy metal is found in the earth's rocks, specifically in the form of sulfides. Hg contamination of ecosystems has long been recognized as having a detrimental effect on aquatic biota at all levels of life, from the individual to the community structure (Driscoll et al 2013; Streets et al 2017; Gworek et al 2020; Li et al 2020). Lead is in the second position after arsenic (As) on the priority list of poisonous substances based on the combination of frequency, toxicity, and potential human exposure (Balali-Mood et al 2021; Renzetti et al 2021). Pb has been widely known for its long history in the human civilization, as it had been used since the last 6,000 years. The existence of Pb in the aquatic environment may cause poisoning and systemic disfunction (kidney filtration, bone hematogenesis, cardiovascular). It even may cause acute to chronical damage of human's peripheral and central nervous system. The form of Pb in the water may vary, such as hydroxide, carbonate, oxide, and sulfide compounds (Tchounwou et al 2012; Jaishankar et al 2014; Kim et al 2014; Andrade et al 2017; Balali-Mood et al 2021; Naidu et al 2021; Renzetti et al 2021)

The direct impact of metal accumulation on fish can be lethal or sub-lethal, which can cause genetic effects. The lethal effect is caused by disturbances in the central nervous system, causing death. Sublethal effects occur in body organs causing damage to the liver, decreased blood count, and reduced potential for reproduction, growth, and others (Heydarnejad et al 2013; Authman et al 2015; Garai et al 2021; Liu et al 2021; Yousif et al 2021). As a result of these diverse circumstances, further research is required to determine the survivability of *P. pardalis* by evaluating the toxicity and bioaccumulation of Cd, Hg, and Pb. The results of this research are expected to provide information regarding the survivability of *P. pardalis*, based on the toxicity and bioaccumulation of Cd, Hg and Pb, and on their effect on the water quality in the rearing aquarium.

Material and Method

Description of the study sites. The current study was an experiment research conducted at Jalan Haji Kelik, Kebon Jeruk, West Jakarta, and the National Nuclear Energy Agency (BATAN), Lebak Bulus, South Jakarta. The research was carried out from February to July 2022. The *P. pardalis* sample specimens from the Ciliwung River were kept in an aquarium and given Cd, Hg, and Pb with predetermined concentrations. The tools used include an acrylic aquarium, aerator, aquarium hose, digital camera, pH meter, digital thermometer, fish filter, TDS digital meter, coolbox, jerry can, X-ray fluorescence (XRF), oven, digital balance, surgical scissors, scalpel, funnel, sample vial, micropipette, measuring cup, petri dish, measuring flask, label paper, ziplock plastic, mortar, and pestle. The materials used include Cd, Hg, Pb metals, distilled water, pellets, concentrated HCl, and 70% alcohol.

Method

Test fish acclimatization. The rearing medium used was an acrylic aquarium with 30 x 30 x 20 cm. Before use, the aquarium was cleaned first using clean water and then dried in the sun, and the test fish were acclimatized for seven days. They were given adequate aeration and fed with pellets, with a feeding frequency of 2 times a day during acclimatization. The number of acclimatized fish was 180 heads measuring 10-13 cm, randomly selected based on health criteria. Acclimatization and toxicity tests were carried out in the preliminary phase.

Preliminary test. To the analysis of the highest and lowest concentration a recommendation from the Guidance Document No. 23 was used (OECD 2019). Cd, Hg, and Pb concentrations of 1.1; 11; 110; 1,100 and 11,000 mg L^{-1} were placed into an aquarium containing 6 L of water. Furthermore, 5 test fish heads/container were put into the aquarium. The mortality was observed at exposure periods of 24 and 48 hours. The percentage of *P. pardalis* deaths was observed every 24 hours for 48 hours of exposure. After the upper and the lower lethal threshold concentrations were obtained, the definitive concentration used in the test was determined using the logarithmic formula, according to the quantitative response method proposed by Finney (1971), as follows:

$$\text{Log}\ \frac{N}{n} = k\ (\log \frac{a}{n})$$

Where:
N - upper threshold concentration;
n - lower threshold concentration;
a - the smallest concentration in the series of concentrations used;
k - number of interval concentrations tested.

The definitive toxicity test aimed to determine the mercury toxicity (LC50) after 48 h of exposure, by observation every 24 for 48 h. The determination of LC50 value used the probit analysis. Probit analysis is used in toxicology to determine the relative toxicity of chemicals in living organisms. The probit analysis is done by looking at the response of the organism to various chemicals, then each concentration is compared to get the result.

Toxicity test. This stage was used to determine the toxicity levels of Cd, Hg, and Pb. The acute toxicity test was observed based on the Hg concentration in each test medium, on the upper threshold value (N), and on the lower threshold value (n) obtained from the preliminary test. *P. pardalis* were observed after 24, 48, 72, and 96 hours.

Fish mortality percentage. Observations of mortality were carried out on the first and final days of the toxicity test stage. The mortality is determined by the number of fish at the beginning minus the number of fish at the end of rearing. Furthermore, it was compared with the number of fish at the beginning of rearing (Effendi 1997):

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

Fish survivability. The estimated of survival is equal to the number of fish that survives the treatment divided by the total number of fish that passed (de Vis & Kemper 2013):

$$\text{Survivability (\%)} = \frac{\text{Number of survived fish}}{\text{total number of fish passed}} \text{ x } 100$$

Analysis of metal amount in organ and flesh. The determination of Pb concentration was carried out through X-ray fluorescence spectrometry (XRF). The sample of gill, liver, and flesh were separated based on how they were treated. The weight of each samples had to be measured before the drying process in the oven, at 105^{o}C, for 96 hours. Then, the samples were cooled down in a room temperature for 30 minutes and put into mortar for refining process. Soon after, the refined samples were injected to prepare the XRF.

Data analysis. The data obtained was inputted and grouped or tabulated into a table. The preliminary test results, acute toxicity test, and mortality percentage were shown in a tabular form, then analyzed descriptively and elaborated in a form of chart.

Results. A preliminary test was conducted to obtain a lower threshold concentration (LC0-48 hours), as well as the highest concentration. However, *P. pardalis* did not die within 48 hours of exposure. An upper threshold concentration (LC100-24 hours) was the lowest concentration that caused 100% mortality within 24 h of exposure. The results showed that *P. pardalis* mortality partially occurred for the Cd treatment with concentration above 11 mg L^{-1}. In comparison, 100% mortality occurred above 1,100 mg L^{-1} after 48 h of rearing (Table 1). Based on the results, the lower threshold concentration was 1.1 mg L^{-1}, where all the test fish were alive within 48 h of exposure. Meanwhile, the upper threshold concentration was 1,100 mg L^{-1} which caused death within 24 h of exposure. This indicates that this research has a threshold concentration between 1.1 L to 1,100 mg L^{-1}.

Table 1
The mortality percentage of *Pterygoplichthys pardalis* in Cd preliminary test for 48 hours

Concentration (mg L^{-1})	*Mortality time*	
	24 hours	*48 hours*
0	0%	0%
1.1	0%	0%
11	0%	20%
110	0%	40%
1.100	100%	100%
11.000	100%	100%

The concentrations of the Hg solution used in the preliminary test were 0.6; 6; 60; 600; and 6,000 mg L^{-1}. Based on the concentration given in each aquarium container, there were differences in behavior changes and mortality of each fish at each concentration. The number of *P. pardalis* deaths showed that the smallest concentration of Hg solution that can cause death in a 24 h exposure period (upper threshold) was 6,000 mg L^{-1}. Meanwhile, the highest concentration of Hg that can still allow test animals to live after 48 hours of exposure (lower threshold) was 6 mg L^{-1}. The way that Hg concentration affected the mortality rate of *P. pardalis* is presented in Table 2. The concentration of mercury solution is directly proportional to the mortality rate of fish. Water quality during the research did not affect the mortality rate of *P. pardalis* because it was still at the optimum value.

Table 2
The mortality percentage of *Pterygoplichthys pardalis* in Hg preliminary test for 48 hours

Concentration (mg L^{-1})	*Mortality percentage*	
	24 hours	*48 hours*
0	0%	0%
0.6	0%	20%
6	0%	60%
60	0%	80%
600	100%	100%
6,000	100%	100%

The results showed that the mortality rate of *P. pardalis* was influenced by the lead concentration. The lead concentration is directly proportional to the mortality rate of *P. pardalis* (Table 3). The various lead concentrations caused changes in *P. pardalis,* which were different from the control treatment. Based on the data obtained, the upper threshold was found at a lead concentration of 6,000 mg L^{-1}, which means the number of fish that died after 24 hours. Meanwhile, the lower threshold was found at a lead concentration of 0.6 mg L^{-1}, which means that all fish survived for 48 hours.

Table 3
The mortality rate of *Pterygoplichthys pardalis* in Pb preliminary test for 48 hours

Concentration (mg L^{-1})	*Mortality time*	
	24 hours	*48 hours*
0	-	0%
0.6	-	0%
6	-	20%
60	-	20%
600	-	60%
6,000	80%	80%

Metal toxicity test and fish survivability percentage. The preliminary tests determine the lower and upper threshold concentrations, which are the references for the concentrations selected in the toxicity test. Based on the calculations, the concentration series used in the Cd toxicity test was P1=4.378 mg L^{-1}; P2=17.42 mg L^{-1}; P3=69.15 mg L^{-1}; P4=273.83 mg L^{-1}; P5=1081.51 mg L^{-1} and the control of 0 mg L^{-1}. The toxicity test was conducted to determine the concentration that can kill 50% of the test animals within 96 hours. Observation on the survivability of *P. pardalis* in the Cd toxicity test showed no deaths in these fish, both in the control and treatment media (Figure 1). In the control media, there were no deaths because the test fish were not exposed to Cd. *P. pardalis* did not die in the treatment media because this fish adapts to a toxic aquatic environment. The toxicity test showed no death after a progressive exposure to Cd, while the preliminary test showed death (Figure 1).

Observations on the mortality percentage of *P. pardalis* were conducted by counting the number of individuals at the beginning and end of the research, in the acute toxicity test phase. Mortality percentage is the ratio between the number of survived test fish at the end and the fish number at the beginning of the investigation, in one population. The mortality percentage in the preliminary test showed that the control and treatment media with a concentration of Cd 1.1 mg L^{-1} did not cause death. Treatment media with concentrations of 11, 110, 1,100, and 11,000 mg L^{-1} caused 20, 40, 100, and 100% mortality, respectively (Figure 1).

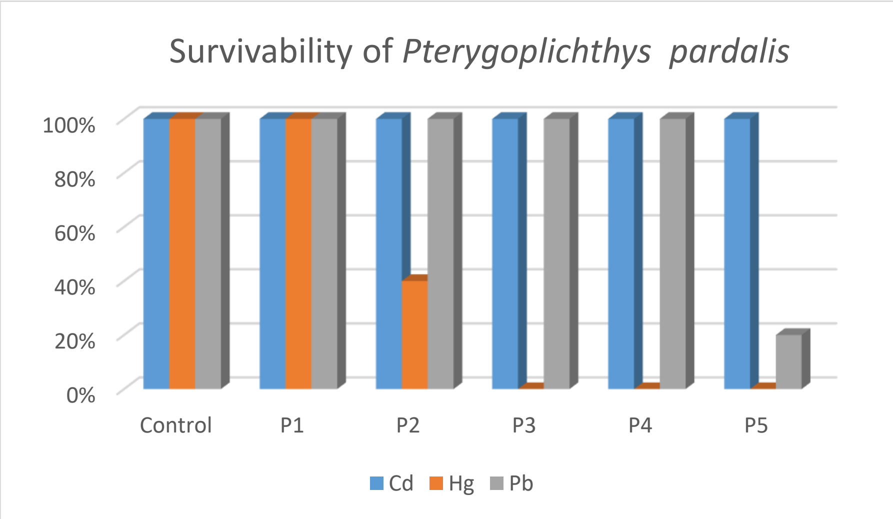

Figure 1. Cd, Hg, Pb toxicity test and survivability of *Pterygoplichthys pardalis.*

The acute toxicity test of Hg showed that most of the deaths of *P. pardalis* were found at concentrations of P3=37.44 mg L^{-1}, P4=148.49 mg L^{-1}, and P5=588.92 mg L^{-1}, which were lethal to all *P. pardalis*. Meanwhile, none of the test animals died at the lowest concentration of P1=2.38 mg L^{-1}, which is still tolerated by *P. pardalis* for 96 hours (Figure 1).

The survivability percentage of *P. pardalis* in the control concentration and the first treatment with Hg concentration of 2.38 mg L^{-1} was 100%. Furthermore, the survivability percentage was 40% in the second treatment with a concentration of 9.44 mg L^{-1}. The third, fourth, and fifth treatments had a survivability percentage of 0%, meaning that *P. pardalis* cannot adapt. At this concentration, fish have experienced severe stress and are more on the surface, hence, their ability to adapt decreases and causes death. The results showed that the survival rate of *P. pardalis* are inversely proportional to the concentrations of mercury solution.

In the acute toxicity test of Pb on *P. pardalis*, five different concentrations were obtained from a previous preliminary test using logarithm calculations. Acute toxicity test showed that death occurred at a concentration of 5,949 mg L^{-1} (Figure 1). Based on data on the number of deaths of *P. pardalis* in the acute toxicity test for 96 hours, no fish died at concentrations of P1=3.78 mg L^{-1}, P2=23.81 mg L^{-1}, P3=149.97 mg L^{-1}, P4=944.60 mg L^{-1}, and control. Meanwhile, 60% of the *P. pardalis* died at the highest concentration of

P5=5,949 mg L^{-1}. The death of *P. pardalis* in this acute toxicity test was caused by the toxic effect of the lead metal.

The result showed that the survivability percentage at the Pb concentrations of control, 3.78, 23.81, 149.97, and 944.60 mg L^{-1}, was 100%, which means there was no dead *P. pardalis*. Furthermore, the survivability percentage at a concentration of 5,949 mg L^{-1} was 40%. *P. pardalis* experienced one death per day on the first, second, and fourth days. The 60% mortality rate in the fifth treatment indicated that at Pb concentration of 5949 mg L^{-1}, *P. pardalis* growth rate declined, affecting the fish's normal function, fertility, and physiological processes.

Concentration of Cd, Hg, and Pb on organs and flesh. The analysis of the amount of heavy metal effect on the flesh, gill, and liver of *P. pardalis* examined in this research shows a positive result of the test on the toxicity of heavy metals Cd, Pb, and Hg. The amounts of Cd, Pb, and Hg found in the organs vary depending on each treatment (Figure 2). The highest concentration of Cd, Hg, and Pb were found on the gill, followed consecutively by the liver and the flesh. The highest amount of Cd was found on the gill sample of the treatment five, with the value of 70.4 μg g^{-1} and the lowest was found for the control, in the gill and liver samples. The highest amount of Hg was found on the gill sample of *P. pardalis*, for treatment five, with the value of 457.2 μg g^{-1}. On the other hand, the lowest amount of Hg was found on control, in the gill and liver samples, with a value <0.7 μg g^{-1}, which implies the amount of Hg on the organ was very little or none. The highest Pb amount was found on P5 gill sample, with the value of 527.8 μg g^{-1}, while the lowest were found on control, in the gill and liver, with the value of less than 0.7 μg g^{-1}, which indicates that the metal contamination on those organs were very small. The result of the analysis of various Cd, Hg, and Pb amounts' effect on P1 to P5 shows their proportionality with the concentration values: the higher the aquatic environment's exposure to Cd, Hg, and Pb, the higher the metal bioaccumulation on the gill, liver, and flesh of *P. pardalis*.

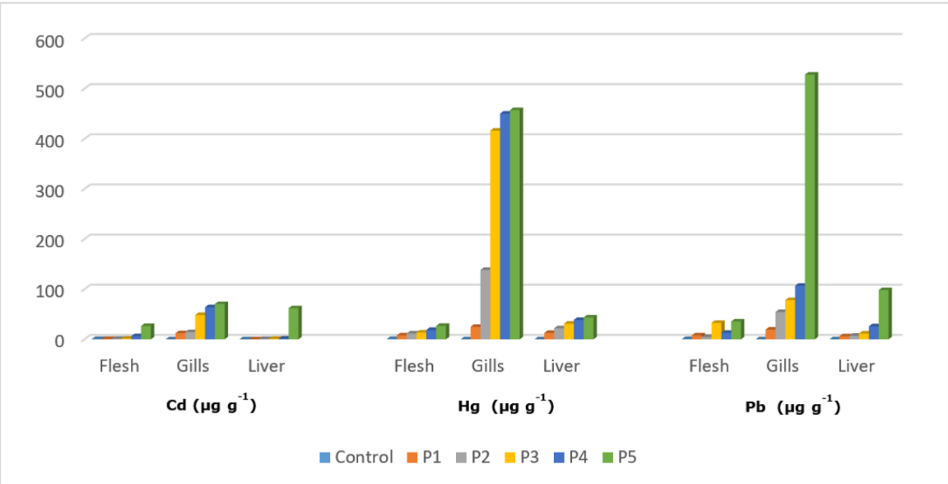

Figure 2. The concentration of Cd, Hg and Pb metals on flesh, gills, and liver of *Pterygoplichthys pardalis.*

Discussion. *P. pardalis* survivability is the ability of both individual or community of *P. pardalis* to survive in their habitat even when they are under extreme environment or situation. According to the result obtained on this research, *P. pardalis* have a high survival rate. This can be seen from the mortality rate of *P. pardalis* on aquatic environment contaminated with high concentration of heavy metal Cd, Hg, and Pb. *P. pardalis* that exposed to the heavy metals Cd, Hg, and Pb had shown a strong endurance and are able to survive even in an aquatic environment contaminated with high concentration of metal.

The survivability of *P. pardalis* is influenced by many factors, including the ability to survive in an extreme environment, physiological ability of the fish body to lower certain pollutants' concentration, the organ anatomy structure that are passed down genetically, and its own natural behavior Genetically inherited organ anatomy structure to all animals have similarities. Hence, the descendant will have a similar anatomy structure with its parents, including their ability to survive (de Vis & Kemper 2013; Palmer & Fedman 2012; Mobjerg et al 2011). During certain environmental circumstances, each animal performs a natural behavior called instinct or the innate behavior. This behavior is known as a congenital ability that continously developes and does not require any learning proccess. Apart from instinct, there is also learned behavior called ability to adapt. This behavior comes as a learning result from seeing and observing their parents' behavior (Mobjerg et al 2011; Wong & Candolin 2015). *P. pardalis,* which are bottom feeders search for food by sticking to rocks, punching holes in river walls and using its suction mouth at the river bottom. Several fish species have the capacity to survive in waters with low oxygen levels, thereby accumulating heavy metals in its system, including *P. pardalis*, which is a member of the Loricariidae Order (Rao & Sunchu 2017; Elfidasari et al 2020; Yousif et al 2021; Muneer et al 2022; Prayoga et al 2022; Tahity et al 2022). The behavioral adaptation of *P. pardalis* is supported by the adaptation of organ anatomy and physiological mechanism, promoting the high survivability rate of *P. pardalis* in an environment that had been extremely polluted with heavy metal.

The high amount of Cd, Hg, and Pb concentration in the gill and liver, compared with the flesh, are presumed to be related to the function of both organs. The gill of *P. pardalis* working as the first physiological organ linked directly with the aquatic environment, which potentially causes them to absorb more toxic substances from the water. The accumulation of Cd, Hg, and Pb on *P. pardalis* begins with the uptake process through the gill, later being absorbed to all body tissues and stored in the organs and flesh. The gills of *P. pardalis* are the main respiratory organ that uses a surface diffusion mechanism of respiratory gases (oxygen and carbon dioxide) between blood and water. Oxygen diluted in water will later be absorbed into the gill capillaries and get fixed by the hemoglobin before being distributed to all body parts. Meanwhile, the carbon dioxide emitted by the cells and tissues is released to the water around the gill (Tchounwou et al 2012; Authman et al 2015; Elbeshti et al 2018). Next, the high amount of Cd, Hg, and Pb concentration in the liver of *P. pardalis* is also associated with its function as detoxifying organ to filter toxic substances entering the body (Milanov et al 2016; Rajeshkumar & Li 2018; Alesci et al 2022; Al-Hasawi & Hassanine 2022). The factors that affect the mercury uptake and accumulation processes are the speed of metabolism, size and variety, the temperature, time, the sources and shapes of mercury, and also the stage of life of the organism (Rice et al 2014; Suhendrayatna et al 2019; Vieira et al 2021; Qu et al 2022).

Bioaccumulations in the body tissue of fish occurred after the absorption of metal from water or through contaminated food. The highest accumulation of heavy metal was found in the liver tissues. However, fish often absorbs heavy metal through its gill, which will later be transferred through the blood to the kidney. Bioaccumulation of heavy metal Cd, Hg, Pb in the aquatic organisms, such as fish, may happen physically or biologically (biochemical). The metals are proved to be simultaneously accumulated in all organs and tissues of the organism, which reflects a systemic uptake and subsequent distribution. The aquatic environment and fish habitats affect the elements' bioavailability (Owolabi & Awodele 2019; Moiseenko & Gashkina 2020; Yousif et al 2021; Muneer et al 2022;). Physically, absorption occurs when metal compounds get attached to the external body regions, to the gill, and also to other membrane holes. On the other hand, it also occurs through the food. The gill is directly associated with accumulation process through the water (Tchounwou et al 2012; Bose et al 2013; Jaishankar et al 2014; Gonzalez et al 2017; Rajeshkumar et Li 2018; Elfidasari et al 2020; Garai et al 2021).

Most of the heavy metals accumulated in aquatic water bodies originate from anthropogenic activities, such as agricultural cultivation, erosions of landfills, docking and embarking activities, sewage of industrial and domestic wastewater and some natural processes (Ali et al 2019; Sonone et al 2021; Taslima et al 2022). Heavy metals enter the fish body through two mechanisms known as direct and indirect. Direct mechanism occurs through the absorption of dissolved metals and nutrients in the fish body. These are absorbed during respiration through the gills. The Pb binds to the blood in the gills before circulating throughout the body (Tchounwou et al 2012; Authman et al 2015;

Raknuzzaman et al 2021; Yousif et al 2021; Zaynab et al 2022). Once absorbed in the body, they need a long time to be eliminated, thereby accumulating in the tissues or organs. The accumulation of heavy metals is usually through the gills, liver, kidneys, and flesh. Some of the metallic excreta are reabsorbed again by the biota, which leads to continuous metallic rotation in the aquatic environment (Ayotunde et al 2012; Mahboob et al 2016; Bawuro et al 2018; Ali et al 2019; Glencross et al 2020).

However, usually heavy metals enter the tissues of aquatic biota indirectly, through the food chain (Ayotunde et al 2012; Authman et al 2015; Rajeshkumar & Li 2018; Ali et al 2019; Elfidasari et al 2020; Sonone et al 2021; Yousif et al 2021; Tahity et al 2022; Zaynab et al 2022). *P. pardalis* is a bottom feeder and also the first consumer in the food chain, while plankton are the producers.

Conclusions. Based on the results of the current research, a lethal effect of heavy metals on *P. pardalis* was observed for a Hg concentration >9.44 mg L^{-1}. Furthermore, the Cd and Pb concentrations that can kill *P. pardalis* were >11 and >944.60 mg L^{-1}, respectively. *P. pardalis* have a higher survivability in toxic freshwater environments such as Ciliwung River. Their low mortality rate is due to a very high resistance to the toxicity of Cd, Hg and Pb.

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