



Research article

Intestinal bacteria diversity of suckermouth catfish (*Pterygoplichthys pardalis*) in the Cd, Hg, and Pb contaminated Ciliwung River, Indonesia

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ABSTRACT

The contamination of aquatic environments with heavy metals poses a serious threat to fish, potentially leading to diseases or even death. Therefore, there is an urgent need for studies to investigate the adaptability of fish in heavy metal-contaminated environments. Several studies have explored the adaptability of suckermouth catfish (*P. pardalis*) to survive in the contaminated Ciliwung River. The findings obtained showed that the presence of intestinal bacteria helped these fish overcome the heavy metals in their intestines, thereby enabling the fish to survive. Analysis using the Next Generation Sequencing (NGS) technology has succeeded in identifying diversity of these bacteria in *P. pardalis* living in the Ciliwung River, which contaminated with Cd (0.3–1.6 ppm in the water & 0.9–1.6 ppm in the sediment), Hg (0.6–2 ppm in the water & 0.6–1.8 ppm in the sediment), and Pb (59.9–73.8 ppm in the water & 26.1–58.6 ppm in the sediment). Diversity index of intestinal bacteria in *P. pardalis* was relatively high, but it had a negative correlation with the presence of these contaminants. Actinobacteria, Firmicutes, and Proteobacteria were abundant in the intestines of *P. pardalis* from the upstream to downstream of the river, with an overall abundance range of 15–48%. Furthermore, *Mycobacterium* along with 6 other genera were identified as core intestinal bacteria. The presence of these bacterial communities in all the samples affected their survival in heavy metals-contaminated rivers. The fish's adaptability to live in this harsh environment indicated that it has the potential to be utilized as a bioremediator of heavy metals in river sediments.

1. Introduction

Heavy metals contamination in the aquatic environment has become a global issue due to its effect on inhabiting organisms [1]. This issue poses a serious threat to humans since contaminants can be accumulated in the body through food chains from the aquatic organisms consumed, such as fish [2]. Furthermore, the accumulation of heavy metals in fish, such as cadmium, chromium, nickel,

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arsenic, copper, mercury, lead, and zinc can cause severe toxicity. It can also weakens the immune system, causes organ and tissue damage, inhibit growth and decreases the reproductive ability [3] as well as cause mass mortality [4,5]. In humans, the high accumulation of these metals in the body through the consumption of contaminated fish can lead to the occurrence of neurological disorders, kidney damage, circulatory system problems, gastrointestinal disorders, and an increased risk of cancer [6]. Based on these findings, several studies have been carried out to assess the distribution of contaminants in the aquatic environment around the world, especially in fish due to their high consumption by humans (about 157 million tonnes of the world production for direct human consumption in 2020) [7]. Kamman et al. reported the occurrence of Hg, Cd, and Pb contamination in *European conger* along the coastal areas of Morocco [8]. Han et al. revealed contamination of As, Cd, Cr, Hg, and Pb in 15 species of marine fish in Zhejiang, China [9]. Furthermore, Nyantakyi et al. found Hg, Cd, Pb, Cu, As, Zn, and Cr contamination in the muscles of 10 species in River Tano, Ghana [10]. Hacon et al. reported high contamination by Hg in 45 traditionally consumed fish species in Amapá State, Brazil [11]. Several studies have also been carried out on other components in the aquatic environment, such as heavy metals in zooplankton along the Bay of Bengal, India [12], in the gastropods (*Pila globosa*) obtained from the east-coasts of Odisha state, India [13], and in the sediment, water, and plant from the Rosetta Branch of River Nile, Egypt [1].

Previous studies have explored heavy metals contamination in Indonesia, specifically in the Ciliwung River, an important river on Java Island, which flows from Mount Pangrango through three big cities, namely Bogor, Depok, and Jakarta [14–16]. Jakarta is popularly known as the second-largest urban agglomeration in the world [17]. The Ciliwung River has several important functions, such as a source of water for drinking, fisheries, livestock, agriculture, and urban businesses. Furthermore, there has been a rapid increase in human population and industrialization around this region, causing it to be very vulnerable to contamination, especially by heavy metals [16]. The Ciliwung River has experienced heavy metals contamination in its water and sediment, including Pb, Hg, Cd, Fe, Mn, Zn, Cr, Cu, and Ni [14,15,18]. The concentration of these contaminants, specifically Cd, Hg, and Pb tends to increase in the downstream region in Jakarta [16]. These metals are produced by industries which operate around the river, such as food, printing, timber, motorcycle, iron, fishery, livestock, and other industries [16].

The presence of these contaminants in the river has a toxic effect on the living organisms in the waterbody, such as the fish population. The high contamination of heavy metals in the river contributed to the decline in the number of fish. Previous studies showed that a total of 167 species disappeared between 1913 and 2009, and there are currently only 20 species in the waterbody [19]. Among those fish, the population of Loricariidae, namely suckermouth catfish (*P. pardalis*), has attracted more attention from several studies due to their high abundance in contaminated rivers [20,21]. Furthermore, the consumption of this species by humans has also become a concern because of its potential risk to health [16]. The people who live near the river often use suckermouth catfish as raw materials for processing food products, such as siomay (dumplings), otak-otak (grilled fish cake), empek-empek (savory fish cake), and fish chips [22,23].

Suckermouth catfish (*P. pardalis*) is an invasive species, which originates from Amazon River [24]. They were brought by aquarists and then entered the Ciliwung River intentionally or unintentionally. This fish has been reported to have the ability to adapt to heavy metals-contaminated environments as well as develop its population in the Ciliwung River. In 2016, its population density was 22 individuals/m² [20], but this number increased to 58 individuals/m² in 2019 [21]. The high abundance of this fish has led to its

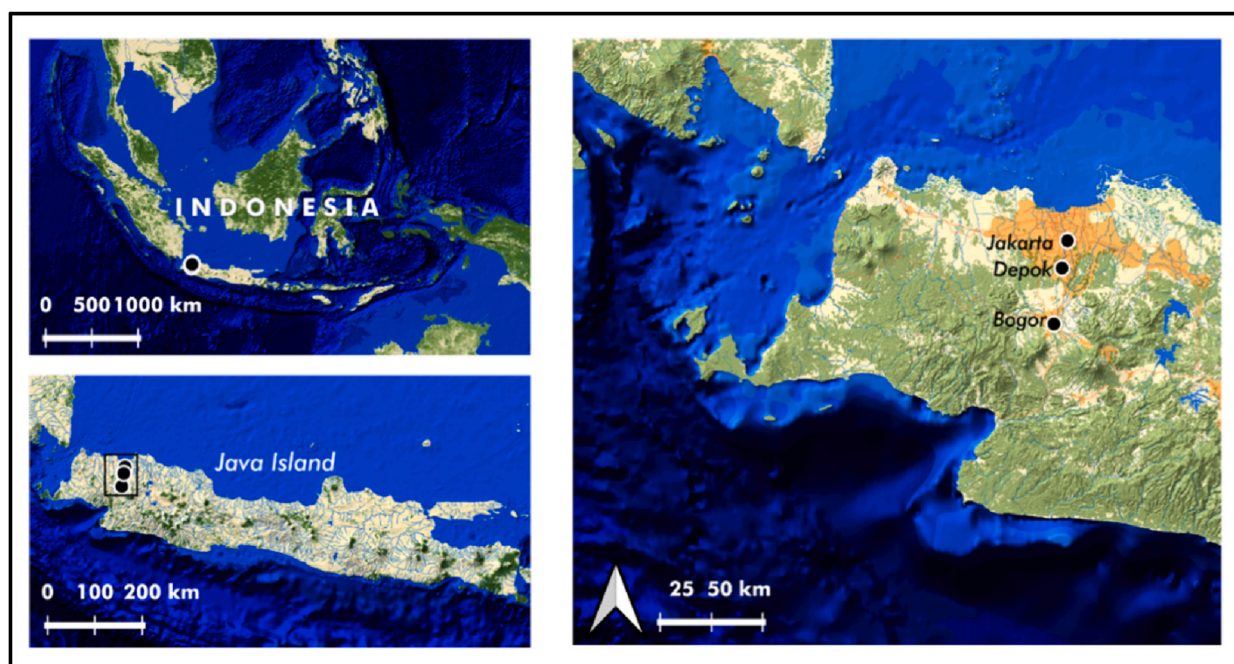


Fig. 1. Location of *P. pardalis* sampling and measurement of chemical-physical properties of Ciliwung River.

exploration by previous studies [25–32]. Elfidasari et al. revealed contamination of Cd, Hg, and Pb in the Ciliwung River and sediments [16]. These contaminants had also accumulated in the tissues of *P. pardalis* and their concentrations were higher than those previously measured by Ratmini in 2009 (Cd = < 0.03 mg/L, Hg = < 0.0005 mg/L, Pb = < 0.02 mg/L) and Ernawati in 2014 (Cd = < 0.005 mg/L, Hg = < 0.001 mg/L, Pb = ±2.88 mg/L) [25,33]. However, this high increase in the accumulation of heavy metals does not inhibit the growth of the fish population in the Ciliwung River [20,21].

The high population of *P. pardalis* in the heavy metals contaminated river is related to the presence of microbiota in their intestine, specifically bacteria. Intestinal bacteria help in the absorption of nutrients, immunity, gastrointestinal development, homeostasis, and xenobiotic metabolism [34]. Šyvokienė & Mickėnienė reported the presence of the community of hydrocarbon-degrading bacteria in several intestines of fish living in the Baltic Sea contaminated by oil spills [35]. These bacteria played an important role in the purification of contaminants. This indicated that the ability of *P. pardalis* to survive in waters contaminated by heavy metals in the Ciliwung River was supported by bacterial communities, thereby increasing their adaptability. The presence of heavy metals contamination could be affected the intestinal bacteria diversity. However, it also supports the growth of heavy metals resistant-bacteria which believed to have an important role in the fish adaptation in Ciliwung River. Therefore, this study aims to analyze the diversity of the intestinal bacterial community of *P. pardalis* and its correlation with the fish adaptability to survive in the Cd, Hg, and Pb contaminated Ciliwung River.

2. Materials and methods

2.1. *P. pardalis* sampling

Male *P. pardalis* fish samples (n = 3 fish in each site) were obtained using the purposive sampling method based on a certain habitat and size at three points of the Ciliwung River, namely the upstream, midstream, and downstream. Furthermore, the upstream, midstream, and downstream were located in Bogor (6°36'08.8" S and 106°48'08.7" E), Depok, (6°21'41.9" S and 106°50'18.3" E), and Jakarta (6°14'36.50" S and 106°51'45.03" E), respectively, as shown in Fig. 1. The upstream point was selected because it was the initial limit of the entry of the Ciliwung River water flow, while the middle point was used as a comparison between the other parts. The downstream point was selected because it was the final part of the river or place of accumulation of dirt and garbage contaminating the river. This indicated that this region had the highest contamination levels. A total of three *P. pardalis* measuring ±300 mm were taken from each sampling point, euthanized in water containing 1 g/L of tricaine methanesulfonate [36] and placed in a cooling box. The fish samples were then taken to the Microbiology Laboratory, National Research and Innovation Agency (BRIN), Pasar Jum'at, South Jakarta, Indonesia. The intestines of three fish measuring 0.5 cm from each habitat were taken and composted into a vial bottle for DNA extraction. This experiment was conducted in accordance to all local and national guidelines for the care of animals. The study's ethics approval was granted by Health Research Ethics Committee University of Pembangunan Nasional Veteran Jakarta (UPNVJ) with the number 2854/XII/2020/KEPK (date of Dec 31, 2020).

2.2. DNA extraction and sequencing of 16s rRNA

Intestinal DNA was extracted using the ZymoBIOMICS™ DNA kit based on the manufacturer's procedure. Furthermore, the isolates were amplified in the 16s rRNA gene, particularly in the hypervariable region V4 using a 515f-806r primer. PCR was then carried out by adding a Phusion® High Fidelity PCR Master Mix (Biolab New England) with several steps, namely 3 min of initial denaturation at 94 °C, 30 denaturation cycles for 1 min at 94 °C, annealing for 1.5 min at 55 °C, extension for 1.5 min at 72 °C, and final extension for 10 min at 72 °C. The amplicon quality was checked with a nanodrop spectrophotometer at wavelength ratios of 260/230 and 260/280 nm using electrophoresis of agarose gel. DNA extracts and PCR products were then sent to the Singapore Novogene Laboratory for sequencing with IonS5™XL (ThermoFisher).

2.3. Bioinformatics analysis

Raw single-end reads were obtained in fastq format and analyzed bioinformatically using Qiime 1.7.0 [37]. Sequence data were then filtered to obtain high-quality clean reads. Removal of barcode and primary sequences as well as truncation was performed using Cutadapt [38]. Subsequently, chimera sequences were removed using the UCHIME algorithm. The high-quality clean reads were then clustered into OTU using Uparse software (v7.0.1001) based on samples with similarities of ≥97% [39]. The representative sequence for each OTU was then screened for further annotation. For each of the representatives, the Silva Database [40] was used based on the Mothur algorithm to annotate taxonomic information, including classification from phylum to genus and their relative abundance. Diversity analysis was carried out on OTU to obtain alpha and beta data in the form of the Shannon diversity index and Principal Coordinate Analysis (PCoA), respectively. Core bacterial analysis was performed by detecting the abundance of each bacterium, which was more than 0.5% and can be found in all habitats [41]. Prediction of metagenome functions was carried out using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) [42].

2.4. Data analysis

Data analysis on the relative abundance of the phylum to the genus of bacteria was carried out descriptively. The process was also performed statistically using Pearson correlation in SPSS 22 software as well as Principle Component Analysis (PCA) biplot in R with

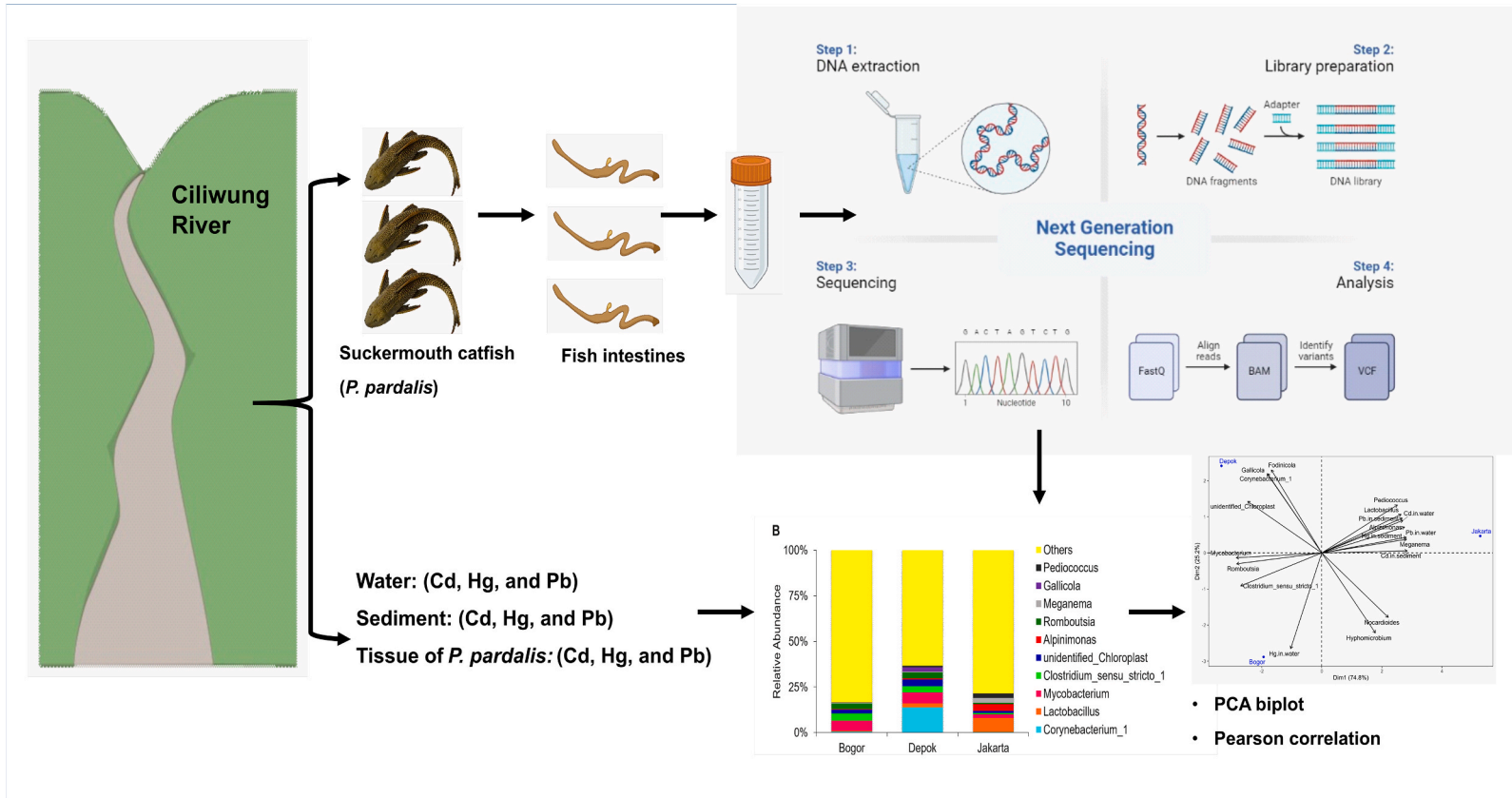


Fig. 2. Experimental design of intestinal bacteria diversity of *P. pardalis* and their correlation with Cd, Hg, and Pb contamination in the Ciliwung River, Indonesia.

factoextra and FactoMineR packages [43]. Furthermore, these analyses were performed to determine the correlation of the bacterial diversity to the concentration of heavy metals contamination of Cd, Hg, and Pb in water, sediment, and *P. pardalis* tissues from the Ciliwung River which measured by Elfidasari et al. [16]. The variables analyzed using PCA included diversity of phylum and genus of bacteria as well as the concentration of each heavy metals in water, sediment, and tissues from Bogor (upstream), Depok (midstream), and Jakarta (downstream). A detailed explanation of the experimental design is presented in Fig. 2.

3. Results

3.1. Intestinal bacterial communities of the *P. pardalis* in Ciliwung River

Identification of intestinal bacterial community of *P. pardalis* in the Ciliwung River showed that the fish intestinal was composed of 390 genera distributed in 24 phyla. The most dominant phyla included Actinobacteria, Proteobacteria, and Firmicutes. Furthermore, each of these phyla was abundant in certain parts of the river and their abundance changed in other parts. Actinobacteria were often found in the upstream region, followed by the midstream and downstream with percentages of 48.19%, 45.01%, and 29.2%, respectively. Proteobacteria were more dominant in the downstream at 39.4% compared to the midstream and upstream, namely 27.5% and 22.5%, respectively. Firmicutes were more abundant in the midstream and downstream with an abundance of 20.15% and 22.51%, respectively, compared to the upstream with 15.45%, as shown in Fig. 3A. At the genus level, *Mycobacterium* and *Corynebacterium_1* were often found in the intestines of *P. pardalis* living in the upstream and midstream with an abundance of 5.53% and 13.75%, respectively. However, *Mycobacterium* was also abundant with 5.99% in the midstream. *Lactobacillus* has a high percentage in the intestines of *P. pardalis* living in the downstream, as shown in Fig. 3B. The analysis showed that *Mycobacterium* along with six other genera, namely *Clostridium_sensu_stricto_1*, *unidentified_Chloroplast*, *Romboutsia*, *Fodinicola*, *Nocardioides*, and *Hyphomicrobium*, were indicated as the core bacteria of *P. pardalis* in the Ciliwung River. This was because they were abundant in each observation habitat, as shown in Fig. 4. The results of literature studies showed that each of them can purify contamination, specifically heavy metals.

3.2. Bacterial diversity versus heavy metal level

Metagenomic analysis of 16s rRNA showed that diversity index of bacteria living in the intestines of *P. pardalis* from the Ciliwung River was relatively high. Furthermore, those from the downstream had lower values compared to the upstream and midstream, as shown in Fig. 5. Based on the comparison of the structure of bacterial communities between each habitat using PCoA, the bacteria were

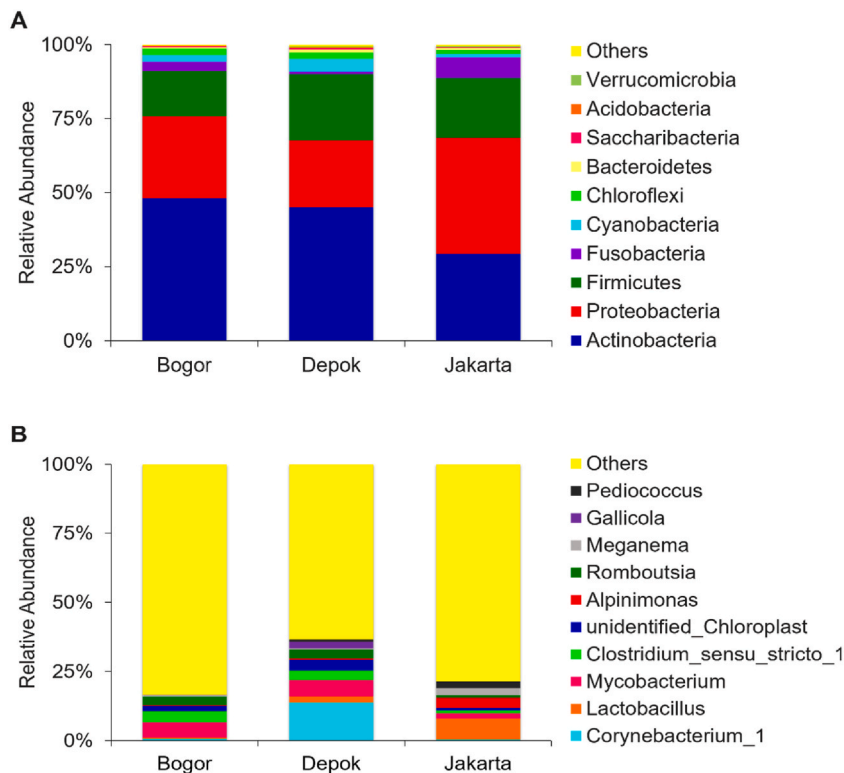


Fig. 3. Diversity of intestinal bacteria *P. pardalis* from Ciliwung River: A. Relative abundance at the level of the phylum. B. Relative abundance at the genus level.

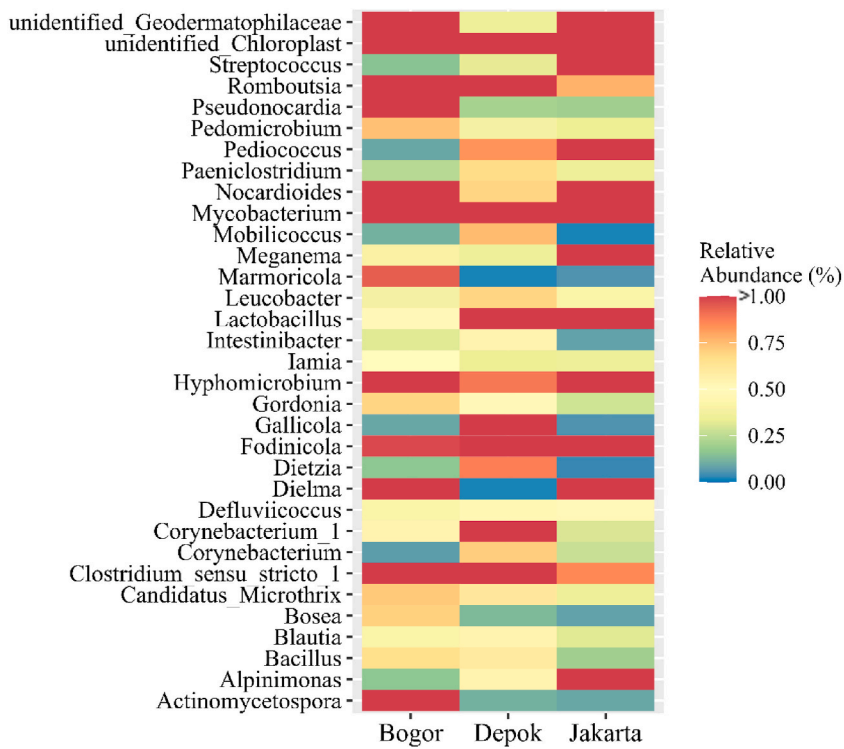
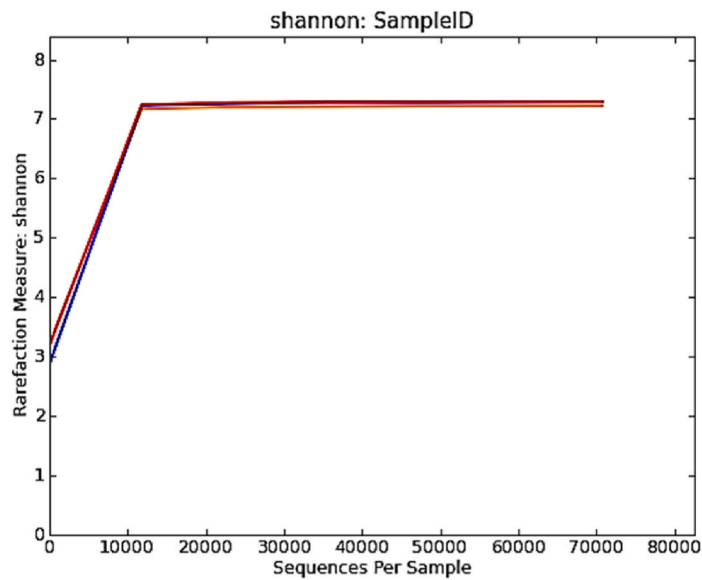


Fig. 4. Intestinal core bacteria *P. pardalis* the Ciliwung River.



Point	Shannon Index
Bogor (upstream)	7.291
Depok (midstream)	7.283
Jakarta (downstream)	7.219

Fig. 5. Shannon diversity index of intestinal bacteria *P. pardalis* the Ciliwung River.

grouped differently from each other, as shown in Fig. 6.

Regarded to the chemical-physical properties of water and sediment of Ciliwung River, Elfidasari et al. had previously measured the parameters such as pH, temperature, and dissolved oxygen (DO) (Table 1) and analyzed their correlation with Cd, Hg, and Pb concentrations [16]. The study showed that these environmental parameters affected the concentration of Cd, Hg, and Pb contained in water, sediment, and tissues of *P. pardalis*. Furthermore, the levels of heavy metals were generally higher in the downstream compared to the upstream region, as shown in Fig. 7. The downstream region in Jakarta experienced severe contamination of Pb in water (73.8 ± 1.5 mg/L), sediment (58.6 ± 1.1 , mg/L) and tissue of *P. pardalis* (2.25 ppm). The water and sediment were contaminated with Cd (6.3 ± 0.3 & 1.6 ± 0.2 mg/L respectively). Meanwhile Hg contaminated the sediment and tissue of *P. pardalis* (1.8 ± 0.9 mg/L & 1.3 ppm respectively). The study showed that the occurrence of these conditions was associated with a higher temperature in the downstream compared to other regions. The higher temperatures in the water led to the formation of heavy metals ions, as well as an increased rate of deposition in the sediment [44]. There was also a significant interaction between the heavy metals in the sediment, which accumulated in *P. pardalis* tissue, and its feeding behavior, namely a bottom feeder [16]. Meanwhile, in other regions, a higher amount of Hg and Cd was detected in the water and tissue from the upstream region, respectively. The study showed that these conditions correlated with the higher DO in the region. Several studies have reported that DO correlate with the solubility of metals in water. The results also showed that pH had no relationship with the concentrations of heavy metals in water, sediment, and tissue of *P. pardalis* from all habitats. This occurred due to the normal pH value conditions in the river [16].

The concentration of Cd, Hg, and Pb had an effect on the chemical-physical properties of the water and sediment of the river, and this affected intestinal bacterial community of the living organisms, specifically *P. pardalis*. Based on recent studies, increased concentrations of heavy metals, such as Cd, Hg, and Pb have a strong correlation with a decrease in bacterial diversity, which was indicated by the value of the Shannon diversity index. The most significant correlation was caused by Cd in water and Pb in sediment, as shown in Table 2.

The decrease in the Shannon index value strongly correlated with an increase in heavy metals accumulation in the tissue, specifically Hg and Pb, which experienced more increment in the downstream region. However, an increase in the amount of these contaminants has been reported to have a positive correlation with the abundance of some bacterial populations. Correlation analysis using biplot PCA showed that high concentrations of Cd, Hg, and Pb in the downstream have a positive correlation with the high abundance of the phyla Proteobacteria, Fusobacteria, and Verrucomicrobia, as shown in Fig. 8A. It also correlated with the genus *Lactobacillus*, *Pediococcus*, *Alpinimonas*, and *Meganema* in the intestines of *P. pardalis*, as shown in Fig. 8B. The high concentration of metals as well as the presence of the bacteria correlated with the accumulation of these contaminants in fish tissues, as shown in Fig. 8C and D.

Prediction of metagenome functions by PICRUST2 showed the existence of enzymes, which was assumed to be associated with Cd, Hg, and Pb accumulation in the tissues of *P. pardalis*, namely mercury (II) reductase (EC:1.16.1.1), alkylmercury lyase (EC:4.99.1.2), cadmium-exporting ATPase (EC:3.6.3.3), cystathionine gamma-synthase (EC:2.5.1.48), and nitrate reductase (EC:1.7.99.4). The abundance of each these enzymes is presented in Fig. 9.

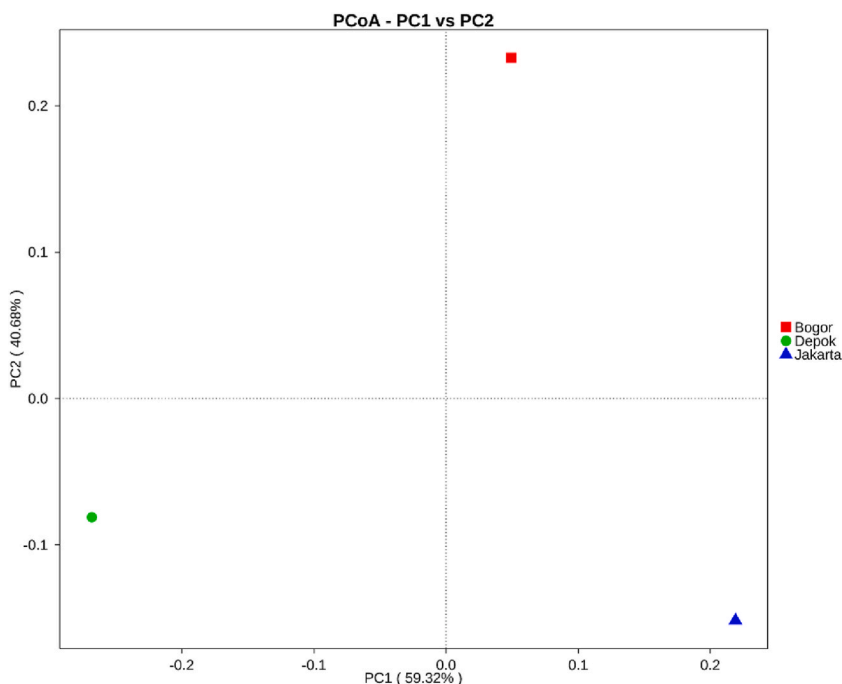


Fig. 6. Comparison of bacterial communities based on beta diversity analysis: Principle Coordinates Analysis (PCoA).

Table 1
Chemical-physical properties of the Ciliwung River.

Parameters	Bogor	Depok	Jakarta	Water quality standards class III according to GR No. 82/2001
pH in sediment	7.26 ± 0.55	7.23 ± 0.28	6.96 ± 0.17	6–9
pH in water	6.65 ± 0.15	6.79 ± 0.13	6.73 ± 0.5	6–9
Temperature (°C)	25.80 ± 0.86	25.53 ± 0.83	26.13 ± 0.83	–
DO (ppm)	5	4	3.4	3 (minimum)

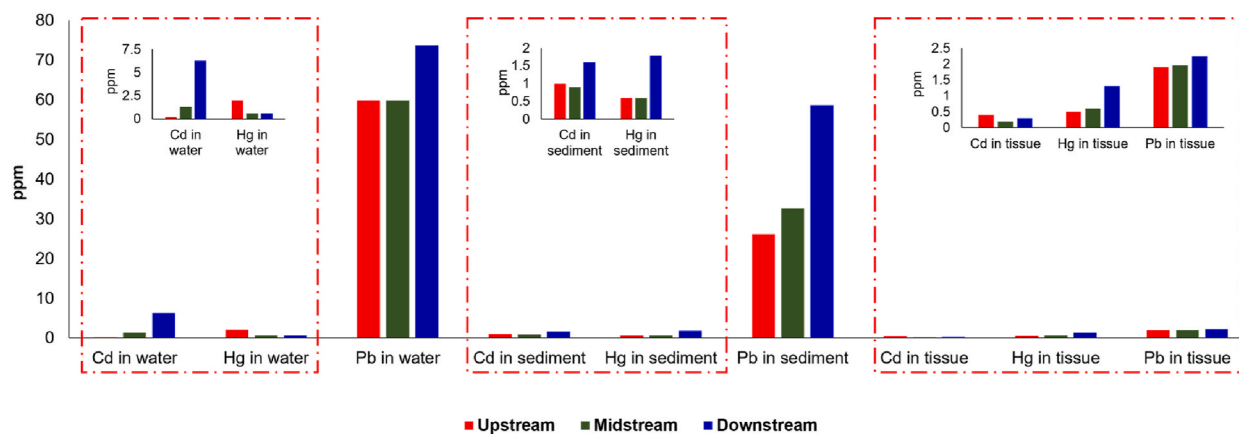


Fig. 7. The concentration of heavy metals (ppm) in water, sediment, and tissues of *P. pardalis* in the Ciliwung River [16].

Table 2
Correlation between heavy metals contamination with shannon index.

Heavy Metals Concentration (ppm)	Shannon Index			Pearson correlation (r)	Sig. (2-tailed)
	Bogor	Depok	Jakarta		
	7.29	7.28	7.21		
Cd in water	0.3	1.3	6.3	−0.999 ^a	0.026
Cd in sediment	1	0.9	1.6	−0.970	0.158
Hg in water	2	0.6	0.6	0.596	0.593
Hg in sediment	0.6	0.6	1.8	−0.993	0.073
Pb in water	59.9	59.9	73.8	−0.993	0.073
Pb in sediment	26.1	32.7	58.6	−0.997 ^a	0.050
Cd in tissue	0.4	0.2	0.3	0.115	0.927
Hg in tissue	0.5	0.6	1.3	−1.000 ^b	0.000
Pb in tissue	1.9	1.95	2.25	−1.000 ^a	0.011

^a Correlation is significant at the 0.05 level (2-tailed).

^b Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

Contamination of the aquatic environment by heavy metals has become a great concern because their accumulation in the waterbody can affect living organisms, such as fish. Several studies reported that the exposure of fish to these contaminants can weaken the immune systems [45], interfere with reproduction hormones and induce pathological changes [46], as well as inhibit growth and development [47]. It can also lead to mass mortality events, such as in the Czech Republic [4] and Yamaima River, Papua, Indonesia [5]. Therefore, the presence of heavy metals in the aquatic environment was harmful to the fish fitness and their population. Parab et al. revealed that Pb, Hg, and Cr caused a decline in the population of *Notopterus chitala* [48]. In the Ciliwung River, there was a high level of heavy metals contamination, such as Cd, Hg, and Pb in the water and sediment, as shown in Fig. 7. These contaminants also accumulated in aquatic organisms, such as *P. pardalis* [16]. However, compared to the disappearance of 167 fish species, which occurred between 1913 and 2009 in the Ciliwung River, the population of *P. pardalis* increased abundantly. A total of 22 individuals/m² was detected in 2016 [20], but increased to 58 individuals/m² in 2019 [21]. The high abundance in the population of *P. pardalis* was supported by their adaptability and the presence of microbiota, specifically bacteria, in the intestines.

Previous studies reported that diversity of bacterial communities in the intestines of fish can help in the absorption of nutrients, immunity, gastrointestinal development, homeostasis, and xenobiotic metabolism [34]. Furthermore, intestinal bacteria can help their host in homeostasis and the regulation of energy metabolism using secreted Short-Chain Fatty Acids (SCFAs), such as acetate,

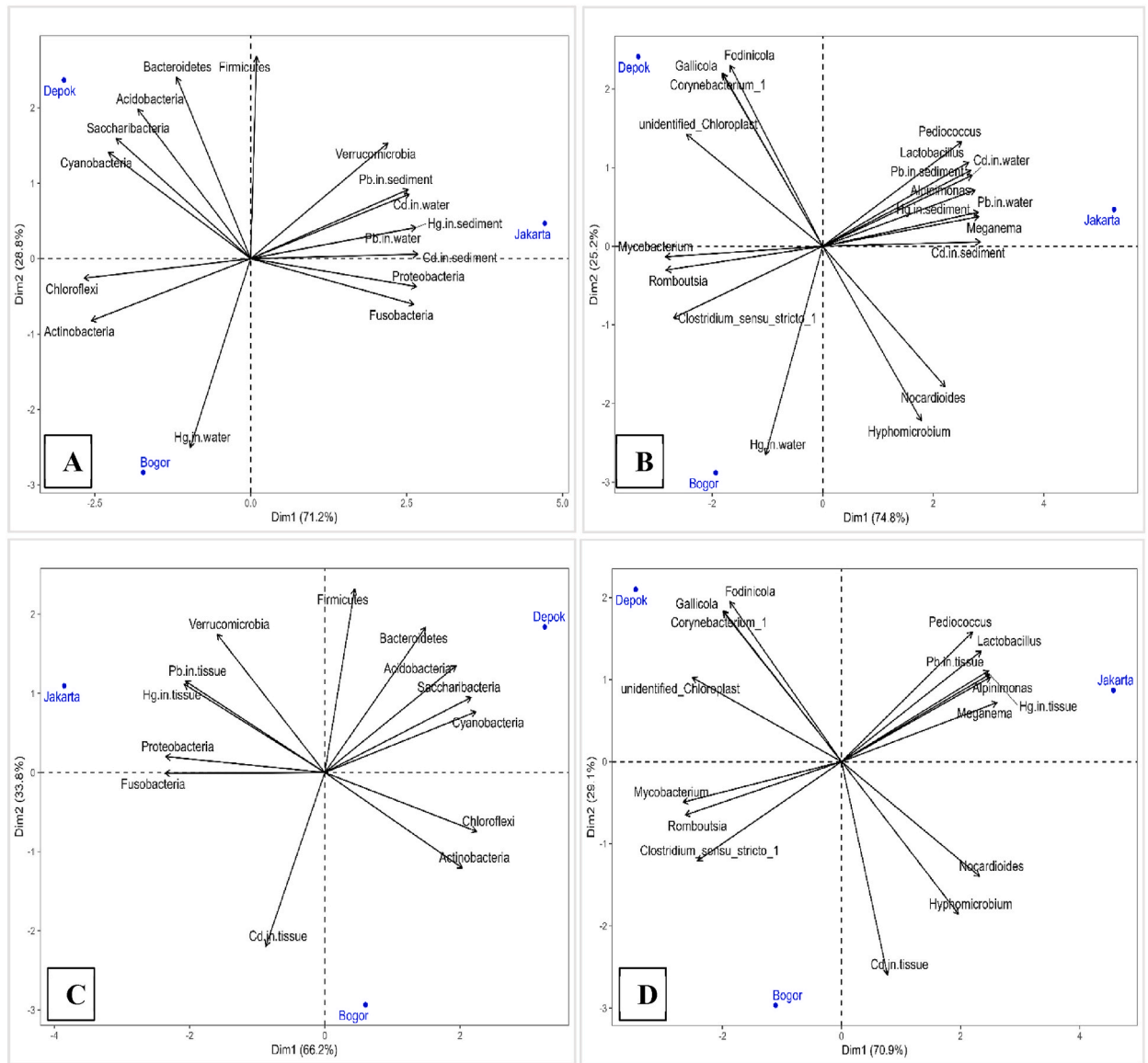


Fig. 8. Two figures above describe about the correlation of the (A) phylum and (B) genus of intestinal bacteria *P. pardalis* with the concentration of heavy metals in the water and sediments of the Ciliwung River. Meanwhile, the two figures below describe about the correlation of (C) phylum and (D) genus of bacteria with the concentration of heavy metals in the tissues of *P. pardalis*.

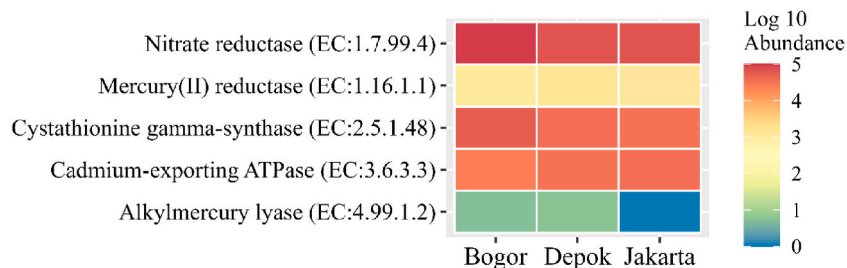


Fig. 9. Enzyme abundance in each habitat.

propionate, and butyrate [49]. This helped the host to adapt to their environments, but these functions were influenced by bacterial composition and abundance. A change in the bacterial composition and abundance coupled with a dominant population of pathogens bacteria can lead to microbial imbalance (dysbiosis) and disease [50]. A previous study reported that intestinal bacteria diversity was influenced by environmental factors, including salinity, season, geography, and diet, as well as genetic factors [51]. It was also affected by the presence of contamination in the water. The heavy metals in the water and sediment can enter the fish directly through the ingestion tract, thereby affecting the bacterial diversity in the intestine [52]. A previous study on the dusky millions fish (*Phalloceros caudimaculatus*) showed that diversity of intestinal bacteria in species living in contaminated water was lower compared to those in relatively uncontaminated areas [53]. Similar results were also found in the *P. pardalis* from the Ciliwung River. Diversity index of *P. pardalis* intestinal bacteria living in the downstream showed lower values compared to others in the upstream and midstream region, as shown in Fig. 5. This was believed to be associated with the level of the contaminated Ciliwung River, which was relatively high in the downstream, Jakarta [15]. Elfidasari et al. also stated that the level of heavy metals contamination, such as Cd, Hg, and Pb, tended to increase in this region [16]. The decline in diversity of the bacterial community was caused by the presence of bacteria that were sensitive to these contaminants [54]. However, contamination-resistant bacteria can grow more abundantly compared to other variants. They can also affect the physiological condition of fish to survive in contaminated water [35,55,56].

The intestine of *P. pardalis* from all parts of the Ciliwung River was abundantly inhabited by Actinobacteria, Proteobacteria, and Firmicutes. This result slightly different with the bacterial community of other Loricariidae, such as the fish from Amazon River which abundantly inhabited by Proteobacteria, Firmicutes, and Planctomycetes [36]. Kim et al. stated that the host habitat become a major determinant of shaped bacterial community than host taxonomy and trophic level factors [57]. The presence of contamination in the environment shaped the intestinal bacteria community of *P. pardalis* which help them to adapt in the heavy metals environments in the Ciliwung River.

Actinobacteria consisted of Gram-positive bacteria that were known to have an important role in the host's digestive tract, such as providing nutrients, detoxification, and protection against pathogenic bacteria [58]. They were also known to play a role in maintaining the host's homeostasis intestinal barrier through the production of SCFAs, including acetate, propionate, and butyrate. These compounds act as energy for intestinal epithelial cells to regenerate. The acetic acid formed can also protect the host from infection with enteropathogenic bacteria [59]. Among the genera in this study, Actinobacteria, such as *Mycobacterium*, *Fodinicola*, and *Nocardioideis* were dominant and considered core bacteria in the intestines of *P. pardalis*. *Corynebacterium_1* was also a member of this group, which was often found downstream and regarded as a transient microbe. Several studies reported that these genera were resistant and tolerant to contaminants. They can also transform metal contamination into a form of lower toxicity. Furthermore, *Mycobacterium* and *Corynebacterium* were members of Actinobacteria, which were resistant to toxic metals [60]. *Mycobacterium* had resistivity against Hg [61] and degraded various organic contaminants in the environment, such as Polycyclic Aromatic Hydrocarbons (PAHs) [62] whose concentrations were high in the Ciliwung River sediments [63]. Previous studies revealed that *Corynebacterium* can degrade aromatic compounds [64]. *Fodinicola* has been reported to participate in ferrous metal cycles [65] that can occur in water. *Nocardioideis* was also known to have high excitivity to extreme environmental conditions as well as the ability to transform and degrade complex compounds, including those that were environmentally toxic compounds, such as alkanes, crude oils, and their derivatives. It can also decompose herbicide compounds, including 2,4,5-trichlorophenoxyacetic, as well as aromatics [66].

The abundance of Firmicutes provided several benefits for fish, such as controlling energy homeostasis in humans and animals [67, 68]. They can also produce butyric compounds, which served as nutrients and energy for epithelial cells, increased the production of mucus, and acted as anti-carcinogen and anti-inflammatory agents. Therefore, an increase in the population of Firmicutes was often considered an indicator of fish trying to maintain their immune function [56]. Furthermore, a consortium between Firmicutes, Bacteroidetes, and Fusobacteria in fish intestines improved the stability of intestinal bacterial communities through the production of essential vitamins for detoxification [69,70]. Among other genera, *Clostridium_sensu_stricto_1* and *Romboutsia* were commonly found and considered core bacteria in the intestines of *P. pardalis*. Meanwhile, *Lactobacillus* and *Pediococcus* were found abundantly in the intestine of samples living in the Ciliwung River part of Jakarta and are considered transient bacteria. *Clostridium_sensu_stricto_1* and *Romboutsia* have a high tolerance to heavy metals contamination, such as Cd, Hg, and Pb [71–73]. A previous study also revealed that *Clostridium* was abundant as a cellulose degrader in herbivorous fish [74]. *Lactobacillus* was known to have the ability to reduce oxidative stress levels caused by exposure to toxic metals and helped to detoxify toxins in the host's digestive tract [75]. *Pediococcus* has also been classified as a lactic acid bacterium (BAL) and was widely used as a probiotic. A previous study revealed that it played a role in the bioremediation of heavy metals, including Cd and Pb [76].

Compared to Actinobacteria and Firmicutes, the high abundance of Proteobacteria was often considered an indicator of the declining condition of fish resistance. Its availability was also associated with the occurrence of dysbiosis and disease [77]. Chen et al. reported that the presence of silver nano contaminants (AgNP) supported the growth of bacteria groups with high tolerance to contaminants, such as Proteobacteria [70]. It also inhibited the proliferation of others that were sensitive to contamination. The high abundance of Proteobacteria in the intestines of *P. pardalis*, specifically those living in the downstream of the Ciliwung River, was an indicator of the occurrence of dysbiosis in intestinal bacterial community. It can also serve as an important indicator of this same condition in other fish, which affected their survival in the downstream. The high concentration of heavy metals, such as Cd, Hg, and Pb in the downstream correlated with the presence of Verrucomicrobia and Fusobacteria, as shown in Fig. 8A. The availability of Proteobacteria, Fusobacteria, and Verrucomicrobia was reported to have a correlation with the occurrence of dysbiosis in the host [78]. Among other genera, *Hyphomicrobium* was the most dominant in all habitats and was considered the core bacterium of *P. pardalis*. Meanwhile, *Meganema* was the most abundant genus in the intestines of samples from the downstream and was regarded as a transient bacterium. Based on its role, *Hyphomicrobium* was classified as an iron-oxidizing bacteria, which oxidized Fe^{2+} ions dissolved in water into water-insoluble Fe^{3+} and ferric hydroxide [79]. *Meganema* can convert carbon compounds into Polyhydroxyalkanoate (PHA) [80].

The results of previous studies stated that the existence of this genus correlated with the occurrence of dysbiosis in zebrafish exposed to Di-(2-ethylhexyl) phthalate (DEHP) [81]. This was also suspected to occur in *P. pardalis* living in the lower reaches of the Ciliwung River.

The occurrence of dysbiosis correlated with an increase in the accumulation of heavy metals in tissues [82]. The high abundance of Proteobacteria, such as *Meganema* in the intestines of *P. pardalis* living downstream was associated with higher contamination of heavy metals, such as Cd, Hg, and Pb in its tissues compared to those in other areas, as shown in Fig. 8D. The difference in the concentration of these contaminants in the tissues of samples from each of the habitats can also be influenced by the presence of enzymes that helped bacteria transform them into non-toxic forms with low absorptivity by the intestine. The low accumulation of Hg metal in the tissues of *P. pardalis* from the upstream was assumed to be related to the high abundance of mercury (II) reductase (MerA) and alkylmercury lyase (MerB). The existence of MerA can help bacteria in reducing toxic Hg^{2+} to a lower toxicity form that was volatile (Hg^0). Meanwhile, the presence of MerB helped these microbes to hydrolyze mercury-carbon bonds in organomercury compounds into Hg^{2+} forms, which were further reduced to Hg^0 by MerA [83]. Furthermore, organomercury compounds, such as methylmercury were more easily absorbed by the intestines compared to Hg^{2+} [84]. This indicated that the biotransformation of these molecules into the more difficult-to-absorb forms of Hg^{2+} and Hg^0 can minimize the accumulation of mercury metal in the tissues of *P. pardalis* living upstream. The lower availability of Pb in samples in the same habitat can also be obtained through a similar mechanism. The high percentage of nitrate reductase enzyme upstream played a role in reducing the accumulation of lead in tissues. The nitrate reductase enzyme was involved in the transformation of Pb(II) into a motivated compound, such as PbS, specifically in anaerobic conditions [85]. During anaerobic respiration, the bacteria needed other terminal electron acceptors apart from oxygen. Compounds, such as nitrates and Pb, have been used by bacteria as the last acceptor derived from the oxidation of NAD(P)H to NAD(P)⁺. Pb that has received electrons reacted with sulfur from the denitrification process through the nitrate reductase enzyme against the amino acids cysteine and methionine. The reaction between the Pb and sulfur formed a PbS compound that was subjected to precipitation [85] and then excreted through feces [55]. This causes lower accumulation of contaminants in the tissues of *P. pardalis* living in the upstream.

The amount of Cd, Hg, and Pb accumulated in the tissue was varied in each habitat. The ability of *P. pardalis* to survive in the heavy metals contaminated river was supported by its intestinal bacteria, showing the potential benefits of its adaptability to living through the accumulation of these toxic metals. This fish capability can be used for bioremediation of heavy metals in aquatic environments, specifically those in the river sediment due to its role as a bottom feeder.

5. Conclusion

The increase of Cd, Hg, and Pb contamination from upstream to downstream negatively correlated with diversity index of bacteria, where Cd in water and Pb in sediment had the most significant correlation. However, the bacterial diversity in the intestine of *P. pardalis* was still relatively high. Contamination-resistant bacteria from Actinobacteria, Firmicutes, and Proteobacteria were to be abundant in the intestine. Some of the genera from these phyla were detected as core bacteria and known to have resistance. They also have the ability to metabolize heavy metals into low-toxic forms using their enzymes. These bacteria helped *P. pardalis* to adapt and survive in heavy metals contaminated rivers, giving them the potential to be utilized as bioremediators of contaminants, specifically in the sediment.

Author contribution statement

Irawan Sugoro; Dewi Elfidasari; Mohammad Syamsul Rijal: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Syalwa Ersadiwi Shalsabilla; Diannisa Syahwa Rahma Fadila; Ade Cici; Devita Tetriana: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Megga Ratnasari Pikoli: Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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