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Heavy Metals Contamination and Their Impacts on Fish Responses in Porong River, East Java, Indonesia

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Abstract: The presence of heavy metals and other contaminants may indicate the presence of a biological response in aquatic animals. This research investigates the heavy metal contamination and water quality on the immune response (IL-1β) of wader fish (*Barbonymus gonionotus*) in response to the presence of contaminants in the waters of the Porong River. The location area was selected because of its diverse utilization surrounding the river, including residential areas, industrial zones, and agricultural practices. The research procedure includes water quality measurements, plankton analysis, heavy metals exposure, and immune response (IL-1β) detection by PCR. In general, the waters of the Porong River were found to be polluted based on the assessment of water quality, as indicated by the measurement of CO_2, TDS, TSS, and salinity parameters surpassing the established threshold values. In the waters of the Porong River, the metals Cu, Hg, Cd, and Pb exhibited a descending order of penetrating power as follows: Cu > Hg > Pb > Cd. Conversely, in the sediment samples, the order of penetrating power was Cu > Pb > Cd > Hg. The PCR amplification results indicated the presence of IL-1β response in all examined fish, suggesting that it could be attributed to water contamination throughout the entire sampling site. In addition, the heavy metals contamination in the waters of the Porong River resulted in high expression of IL-1β in the fish sample. The findings lead to the conclusion that the waters of the Porong River are classified as polluted based on the measurements of water quality parameters, namely CO_2, TDS, TSS, and salinity, which surpass the threshold values established according to Government Regulation (PP) of the Republic of Indonesia Number 22 of 2021.

Keywords: *water quality, heavy metals, IL-1β, PCR, polluted waters*

1. INTRODUCTION

The Porong River is a downstream area of the Brantas River which has a high potential for heavy metal pollution, among others, due to polluting activities in Tarik, Prambon, Krembung, Porong, and Jabon districts[1)]. In addition, the Porong River is an example of a river that has experienced environmental pollution caused by the Lapindo mud overflow[2)]. Serious pollution problems can occur, including industrial wastewater containing heavy metals, sometimes entering the environment accidentally or illegally, and sedimentation of land, rivers, and groundwater[3)]. Pollution often affects the pace of development, in particular in the industrial sector, since its creation produces waste. Sediments from the Porong River include levels of lead (Pb) and cadmium (Cd), which are two to three times passed the permissible limit at least[4)]. Changes in land use or physical characteristics have affected the soil's ability to retain more water during rainfall before the direct runoff. The ability to make

hydrological forecasts has also become an important part of the sustainable management of water resources, water quality, and water-related natural threats[5]. In fact, water spaces such as streams, rivers, and lakes in particular, have an important role in creating urban cooling because water spaces have a higher evaporation rate than green open spaces[6]. Besides, aquatic biota is an excellent indicator of metal contamination in the aquatic environment[7]. Metabolic activity can accumulate heavy metals. Thus, they can get into the tissues of fish organs and cause cell swelling, necrosis, fibrosis, and cirrhosis[8]. Heavy metals are a core group of water pollutants because they have bioaccumulating properties and are non-degradable. Heavy metals can be found in aquatic environments through human activities such as the mining industry[9]. Methods, such as desalination[10], membrane[11], and bioremediation technology for sustainable wastewater treatment[12], have been developed by researchers to get clean water. Other researchers also focus on wastewater monitoring, especially detecting the heavy metals contamination in water is very important[13].

Currently, high heavy metal levels in the river waters are generally caused by the high input of industrial, mining, agricultural, and domestic waste[14]. In addition, heavy metals can accumulate in the fish environment. During the last 100 years, Pb, Cr, and Cd have been used in particular in paints, preservatives, toy manufacture, pesticides, lead bullets, and as gas additives[15]. Concentrations of dissolved Cr, Cd, and Pb in freshwater are generally between 10 and 500 ng/l, but concentrations of more than 1 mg/l have been observed in some industrial areas[16]. In heavily polluted areas, Cr, Cd, and Pb contents in sediments and soil can reach more than 1 mg/kg[17]. The presence of heavy metals and other contaminants may indicate the presence of a biological response in aquatic animals. Fish that live in contaminated water conditions in rivers, one of which is indicated by the emergence of an infant immune response namely IL-1β[18], is also shown in the type of Teleostei fish with the presence of IL-1β[19]. The presence of IL-1β also showed a response due to heavy metal pollution[14].

The Porong River in Sidoarjo Regency, East Java Province, has reported unexpected fish deaths in 2019[20]. The majority of the dead species were unique freshwater fish biota in the Porong River such as Keting, Kuthuk, and Bader fish [20]. One of the responses to fish mortality, because of the high expression of IL-1β caused by the contamination of heavy metals and organic matter in the water[19]. Research by El-Boshy and Taha[21] showed that heavy metals could impair the cellular and humoral immune responses of fish. Among cell immunological reactions, one of which can be seen by the presence of macrophage phagocytosis activity. Macrophages and monocytes produce and secrete IL-1β. It is produced as an inactive 31 kDa precursor called Pro-IL-1β in response to a molecular motif that acts on macrophages via pattern recognition receptors (PRR) to regulate pathways that regulate gene expression[22].

The activity of interferon-alpha cytokines (IFN-α) was the first to be studied. However, in the early 1980s, the first molecular cloning, such cytokines (IFNα, IL-1β, IL-2, and TNFα), was discovered. Research on the regulation of fish immunity began in the early 1990s because of the importance of fish in the aquaculture industry and the need to understand the issues related to immunity and disease. The first cloned cytokine in fish was TGF-β, followed by IL-1β and a few years later by TNF-α[23]. This specific cytokine was also the best and most studied property of the 11 IL-1β family members. Although most research are focused on the production of innate immune cells such as monocytes and macrophages, it is produced and secreted by a wide variety of cell types. IL-1β is a potent pro-inflammatory cytokine produced by cells of the innate immune system. It is produced without a signal sequence and does not follow conventional protein secretion pathways but instead uses one or more unconventional secretory pathways. This research aims to investigate the heavy metal contamination and water quality on the immune response (IL-1 β) of wader fish (*Barbonymus gonionotus*) in response to the presence of contaminants in the waters of the Porong River.

2. MATERIALS AND METHODS

2.1 Study Area

The location area of this study is presented in Fig. 1. The Porong watershed in Sidoarjo District in East Java Province was selected as the study site. The upstream area of the river is represented by the city of Batu, and the downstream part of the river is represented by Sidoarjo Regency. The consideration of choosing the location is due to varied utilization of land around the river, such as residential areas, industrial sites, and agricultural activities that affect pollution to the river. In these two locations, we determined five (5) points selected for the sampling location in this study.

Table 1. The Map of coordinates and elevation of the sampling site in the Porong River

Site	Latitude	Longitude	Altitude (AMSL)
1	7°32'46.0"S	112°41'55.1"E	8
2	7°32'33.7"S	112°46'26.5"E	3
3	7°33'57.0"S	112°52'11.1"E	3
4	7°45'14.0"S	112°31'35.6"E	1642

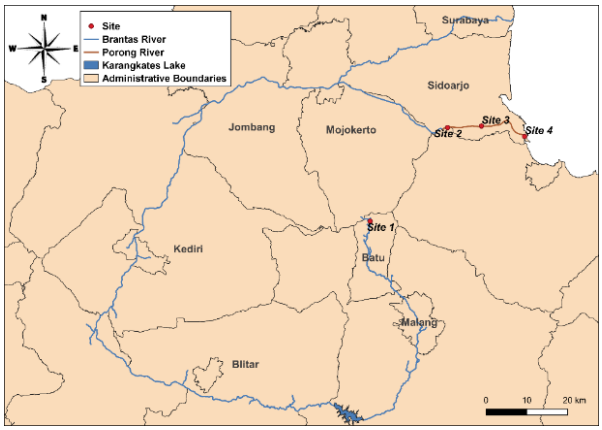

Fig. 1: Research Location in the Porong River.

2.2 Water Quality Measurements

Porong River water quality analysis with the presence of *B. gonionotus* fish consists of physical parameters (Total Dissolved Solid/TDS, Total Suspended Solid/TSS, temperature), and chemical parameters (pH, Dissolved Oxygen/DO, CO_2, nitrate, orthophosphate, Total Organic Matter/TOM, ammonia, salinity). The tools used consist of TSS meters, thermometers, pH meters, DO meters, Erlenmeyer, hot plates, cuvets, spectrophotometers, and porcelain cups. The materials used consist of Lugol, $KMnO_4$, H_2SO_4, na-oxalate, distillate water, Nessler, phenolphthalein indicator, Na_2CO_3, Disulfonic phenol acid, NH_4OH, Ammonium molybdate, and $SnCl_2$.

2.3 Plankton Analysis

Based on Nurruhwati et al.[24], plankton samples were taken by filtering 10 liters of water through a plankton net equipped with a storage bottle with a mesh size of 200 µm. The accommodated plankton was preserved with a 4% formalin solution of 3 drops. Calculation and identification of plankton were carried out at the Fisheries Center of the Faculty of Fisheries and Marine Sciences, University of Brawijaya, using a microscope and a plankton identification book[25] and The Marine and Fresh Water Plankton[26]. Observations were made by means of a sample bottle containing the sample being shaken slowly until homogeneous. The sample was taken using a drip pipette and dripped on a preparate glass. Next, it was observed using a microscope with an enlargement of 100x and 400x. The obtained sample was identified using a plankton identification book, and the sample was observed as many as three repetitions. Plankton sampling was carried out at a single point, where the measurements were carried out weekly. The plankton sampling procedure is as follows: 1) Take 25 liters of pool water at five different points by 5 liters at each point; 2) Filtering pool water using plankton net number 25; 3) Precipitating plankton at the end of the planktonnet using a film bottle; 4) Adding 3-4 drops of lugol solution; 5) Plankton samples are stored and taken to the laboratory for identification.

2.4 Heavy Metals Exposure

The method of Heavy Metal Examination in the water was in accordance with the American Public Health Association (APHA), American Water Works Association (AWWA), & Water Environment Federation (WEF)[27]. Identification analysis of the heavy metal's type was used as one of the parameters of water quality. Heavy metal type analysis was carried out using the Inductively Coupled Plasma Mass Spectrometer (ICP-MS) test method. The tools used consist of electronic scales and baking sheets, microwave digestion, desiccator, and drying oven. The materials used consist of internal standard indium, HNO_3, internal standard bismuth, and pure water. The samples used were in the form of water and sediment from each sampling point. Observed heavy metal were Pb, Cd, Hg, and Cu concentrations.

2.5 Detection of Immune response (IL-1β) by PCR

DNA was extracted from tissues preserved in absolute ethanol using a silica extraction kit (Gene). Each sample of fish kidney tissue was put into a 1.5 ml microtube, added 900 µl GL buffer, grounded with a pestle grinder, and centrifuged at 12,000 rpm (3 min). As much as 600 µL of the coating solution obtained was transferred to a new 1.5 mL microfuge tube, 40 µL silica was added, vortexed until homogeneous, and washed at 12,000 rpm (15 sec). After centrifugation, the supernantant was discarded, the silica pellet was washed with 500 µl GT buffer, vortex until the silica pellet formed a suspension and centrifuged at 12,000 rpm (15 sec). Then, the supernantant was discarded again, added by 1 ml of 70% ethanol wash flint and mixed with pellets until the flint pellets formed a suspension. The solution was centrifuged again at 12,000 rpm (15 sec), the ethanol was discarded, while the pellet was collected using a micropipette and added by 1 mL of ddH_2O to suspend the silica pellet. Finally, the silica pellets was put into vortex to create a suspension.

Specific primers of IL-1β used as follows F: ACGCCACCAAGAGCCTTTTA; R: GCAGCCCATATTTGGTCAGA with a gene target of 206 bp. PCR amplification was performed using a 25 µl volume PCR reaction mixture consisting of a master mix (KAPA Biosystems, KK510) 2 µl forward primer, 2 µl reverse primer, 2 µl template DNA, and 19 µl nuclease-free water. Amplification was carried out by setting the predenaturation temperature to 95 °C (3 minutes), followed by denaturation to 95 °C (30 seconds), annealing, extension 72ºC (1 min, 34 cycles) and the final stage of 72 °C (5 min). Temperature optimization was carried out to obtain the best annealing temperature by adjusting the thermal cycler gradient (T 100 Bio-Rad) at 4 different temperature levels, namely 58.0ºC, 56.1ºC, 53.8ºC, and 51.9ºC.

3. RESULTS

Based on the water quality measurement data in Table 2, the results of the water quality measurement for point 1 (upstream of the Porong River) were quite good with the parameters of TDS 87.33 mg/L, DO 16.13 mg/L, and pH 7.29, which in accordance with river water quality standards (PP. No. 22 of 2021).

The CO_2 levels at point 2 were higher above the recommended range for river water quality, at 27.72 mg/L. Point 3 had a salinity of 9.05 ppt and CO_2 concentration of 35.54 mg/L, which was extremely high for the waters. More metrics such as TDS 6792.50 mg/L, TSS 107.95 mg/L, CO2 23.76 mg/L, and salinity 8.60 ppt, were above the river water quality level (PP No. 22 of 2021) at point 4.

The standard quality value for suspended residue or suspended solids (TSS) concentration in river water is not more than 50 mg/L[28]. The optimal TDS level that complies with water quality standards in river water is not more than 1000 mg/L. Inorganic substances such as minerals, metals, salts, and soluble cations in water are included in the TDS content in the water[29]. The standard for carbon dioxide concentration in water is approximately 12 mg/L. Elevated levels of carbon dioxide in water can convert it into a toxic gas for fish and fish embryos[30].

Table 2. Results of water quality measurements in the Porong River.

Parameter	Standard	Site of Sampling			
		1	2	3	4
TDS (mg/L)	**1000**	87.33	305.50	672.00	**6792.50***
TSS (mg/L)	**50-100**	0.67	4.10	16.30	**107.95***
Temperature (°C)	**22-28**	19.33	27.00	27.50	27.50
pH	**6-9**	7.29	6.20	6.05	5.85
DO (mg/L)	**4-3**	**16.13***	3.25	4.40	3.85
CO_2 (mg/L)	**10-15**	9.24	**27.72***	**35.64***	**23.76***
Nitrate (mg/L)	**10-20**	0.02	4.00	4.14	2.77
Orthophoospate (mg/L)	**0,2-1**	0.11	0.17	0.17	0.14
TOM (mg/L)	**69.5 – 28.4**	6.74	10.39	7.58	6.32
Ammonia (mg/L)	**0.2-0.5**	0.13	0.22	0.07	0.29
Salinity (ppt)	**0–5**	0.04	0.04	**9.05***	**8.60***

3.1 Plankton Analysis

The data from the test results of the identification of plankton species in the Porong River basin is attached in Appendix 1. There were several species that indicate light to heavily polluted waters. The plankton species found in the Porong watershed were *Mycrocystis* sp., *Anabaena* sp., *Lygnbya* sp., *Botrycoccus brauni* sp., *Raphidiopsis* sp., *Lepocinclis* sp., *Polyartha trigla*. The types that were found are the anabaena sp. and *Mycrocystis* sp.

The measurement of the plankton density index is shown in Fig. 2. The result indicates that the highest plankton density level was at the first point of 6 cells/mL, while the lowest plankton density level was at the fourth point of 1 cell/mL. The plankton densities of the second and third sample were 3 and 2 cells/mL, respectively.

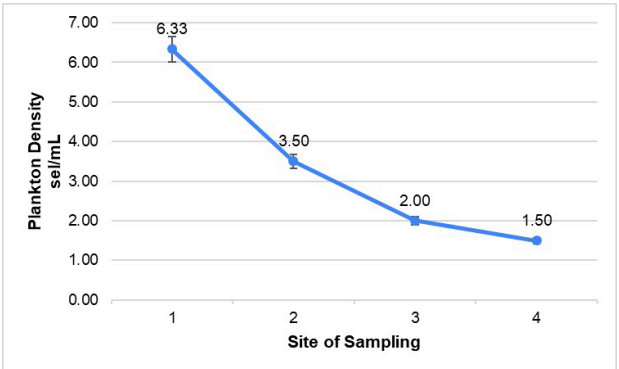

Fig. 2: Graph of the calculation of the plankton density index in the waters of the Porong River.

The Plankton Dominance Index, as shown in Fig. 3, indicates that the value of the dominance index (C'index) ranged from 0.11 to 0.63. The highest dominance index value at the 4th sampling point was 0.63, and the lowest dominance index value at the 1st sampling point was only 0.11. The dominance index values at the second and third sampling points were 0.15 and 0.56, respectively.

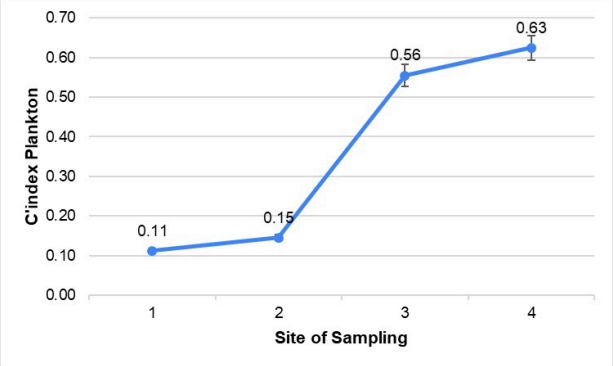

Fig. 3: The graph of the calculation of the plankton dominance index in the waters of the Porong River.

3.2 Heavy Metals of Porong River

The result of heavy metal analysis found in the Porong River basin, which are Pb, Hg, Cd, and Cu, is presented in Table 3. Analysis of heavy metals in Porong River water indicated that Pb heavy metals have the highest value at point 3, at 0.0006 mg/L. The highest Hg concentration was obtained at point 4, at 0.0009 mg/L. The Cd were not detected in all sampling points. The highest Cu concentration was found at point 4, at 0.0064 mg/L.

Table 3. Measurement results of heavy metals in water samples in the Porong River.

Parameter	Site of Sampling			
	1	2	3	4
Pb (mg/L)	ND	ND	0.0006	0.0004
Hg (mg/L)	ND	0.0005	0.0005	0.0009
Cd (mg/L)	ND	ND	ND	ND
Cu (mg/L)	ND	0.0027	0.0043	0.0064

Note: ND (Not detected).

The analysis result of heavy metal types found in Porong River sediments, including Pb, Hg, Cd, and Cu, is presented in Table 4. The highest level Pb contamination was found in sediment point 3 of 15.2 mg/L. The highest level of Hg was found in sediment point 4 of 0.109 mg/L. The highest Cd was found in the sediment point 4 of 0.203 mg/L. The highest Cu contamination was found in sediment point 4 of 67.5 mg/L. In this study, every sampling location obtained high concentrations of heavy metals contamination which exceeds river water quality standards (PP Number 22 of 2021).

Table 4. Measurement results of heavy metals in sediment samples in the Porong River.

Parameter	Site of Sampling			
	1	2	3	4
Pb (mg/L)	ND	10.3	15.2	13.6
Hg (mg/L)	ND	0.060	0.053	0.109
Cd (mg/L)	ND	0.183	0.162	0.203
Cu (mg/L)	ND	46.2	53.5	67.5

Note: ND (Not detected).

3.3 Immune response (Interleukin-1β)

Temperature optimization was carried out to obtain the best annealing temperature. From 4 temperature gradients obtained the best temperature of 51.9ºC. In addition, the amplification and electrophoresis were conducted. The electrophoresis results of IL-1β expression appeared in the band of 206 bp, as shown in Fig. 4 below.

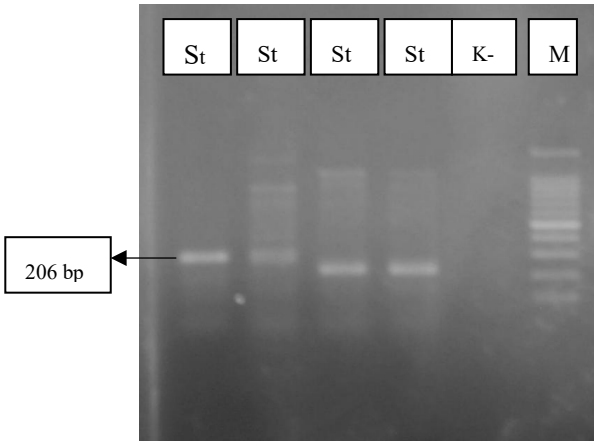

Fig. 4: IL-1β immune response detection results using PCR. St0) fish samples from upstream; St1) fish samples from Downstream location point 1; St2) fish samples from downstream location point 2; St3) fish samples from downstream location point 3; K-) negative control fish samples; M) Markers.

Based on the results of PCR amplification, it is shown that there was an IL-1β response in all fish tested. This was thought to be due to water contamination at the entire water sampling site. Various immunological cytokines were evaluated against several pathogens, indicating that the immune status of the fish was against infection. Among these cytokines, IL-1β and TNFα were considered pro-inflammatory cytokines that are produced in the early stages of infection[31].

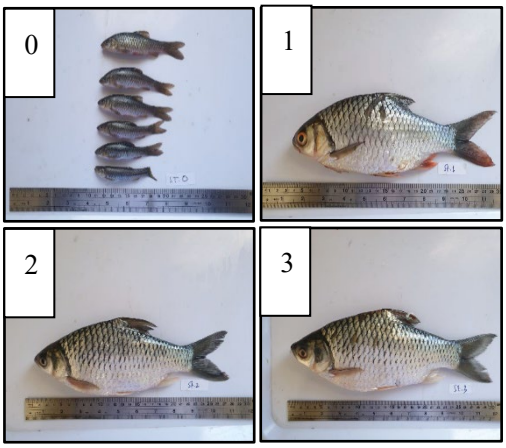

Fig. 5: Fish samples used for IL-1β by PCR assay

4. DISCUSSION

In general, the waters of the Porong River were categorized as polluted waters based on the water quality test where the results of CO_2, TDS, TSS, and salinity exceeded the threshold values that had been set based on the Government Regulation (PP) of the Republic of Indonesia Number 22 of 2021. According to Astuti and Indriatmoko[32], dissolved oxygen was an indicator of pollution of the aquatic environment. High oxygen indicates good waters, while low oxygen indicates water pollution. According to Adamczyk et al.[33], processes that affect primary productivity could affect the rate of absorption of biological CO_2 by autotrophs. Carbon dioxide levels that are too high in water is considered as toxic to the water environment. The waters that were located nearby human activity tend to have higher levels of CO_2 compared to those facing the ocean areas[34]. According to Pratama, et al.[35], the salinity concentration obtained through the CTD electrode sensor and the TDS concentration resulting from the water type ratio equation system had a very strong correlation relationship because both parameters were measured through the conductivity characteristics of the waters. The high salinity in the water had a major influence on the tolerance of plankton that was able to survive in that location[36]. Several researchers have analyzed the abundance and diversity of phytoplankton as biological indicators to evaluate water quality and the level of fertility in aquatic environments[37,38]. Plankton is used as an indicator of water quality in various ways. The quality of water is essentially determined by the abundance of plankton to determine the fertility level of the water. Plankton has a short life cycle and reacts quickly to environmental changes, making it an indicator that provides signs in determining water quality[39]. The abundance of plankton is also used to monitor the fertility level of the water[24].

Among the mentioned heavy metals (Cu, Hg, Cd, and Pb), the successive penetrating power from large to small in the waters of the Porong River was: Cu > Hg > Pb > Cd. On the other hand, in successive sediments, it was Cu > Pb > Cd > Hg. However, the content of heavy metals in the water was still below the threshold value determined by the Government Regulation (PP) of the Republic of Indonesia No. 22 (2021). In those heavy metal-containing waters, even if the concentrations are relatively low, the heavy metals can be absorbed biologically by aquatic biota and will be involved in the immune system. This causes a process called bioaccumulation. According to its chemical-physical properties, Pb metal is not needed at all in the life process of aquatic biota. It is classified as a chemical with high toxic potential when accumulated because it has a large affinity for the protein groups of living organisms. The result of the toxicity of this metal can be physical damage (erosion, degeneration, necrosis) and can be physiological disorders (impaired enzyme function and metabolic disorders). At a higher level of biological organization, heavy metals are capable of causing biochemical, metabolic, and physiological changes in the body and inhibiting the synthesis of proteins[14]. Previous studies have shown that metals can also cause oxidative stress by generating free radical ions. Fish tissues such as muscles, gills, liver, kidneys, and intestines have an antioxidant defense system that protects against metal-induced oxidative stress. One of the abilities is, the body can respond to heavy metal bioaccumulation in aquatic organisms with an immune response that includes IL-1β. It is involved in cell proliferation, differentiation, inflammation, apoptosis, also stimulates various environmental challenges in the fish immune system. Although more and more studies are focusing on heavy metal-induced immunotoxicity, fish reports are still very limited. These data indicate that the four metals individually may affect expression or function of IL-1β, thereby reducing the level of immunotoxicity in fish. IL-1β is a key early inflammatory cytokine, which enables organisms to respond to stress caused by the microbial invasion, tissue damage, or xenobiotics. It can activate macrophages, natural-killer cells (NK cells), lymphocytes and produce various effects that cause inflammation in various fish species[40]. The results of our study showed the expression of IL-1β in the kidney organs of fish due to exposure to heavy metals of water and sediments. IL-1β play crucial roles as pro-inflammatory cytokines in regulating both innate and adaptive immunity, as well as modulating the inflammatory response. IL-1β, being the first identified cytokine, is secreted by various immunological cells, including endothelial cells, macrophages, T lymphocytes, and other immune cells, upon stimulation by different pathogens[31,41].

5. CONCLUSION

Based on the water quality and heavy metals measurement results, the parameters of CO_2, TDS, TSS, and salinity in the waters of the Porong River exceeded the threshold values set by the Government Regulation (PP) of the Republic of Indonesia Number 22 of 2021, indicating a high level of water pollution. Among the analyzed metals, Cu, Hg, Cd, and Pb exhibited a

successive penetrating power in the following order in the waters of the Porong River: Cu > Hg > Pb > Cd. In sediments, the order was Cu > Pb > Cd > Hg. The contamination of these heavy metals has significantly increased the expression of IL-1β in fish inhabiting the Porong River. In the future, this finding can be adopted for the consideration of stakeholders in order to make regulations to reduce water pollution in the Porong River.

Acknowledgments

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Nomenclature

IL-1β	Interleukin-1β
DO	Dissolved Oxygen
COD	Chemical Oxygen Demand
BOD	Biological Oxygen Demand
ND	Not Detected

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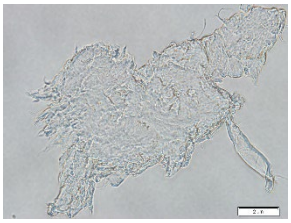
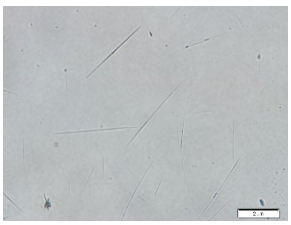
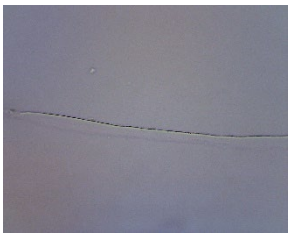
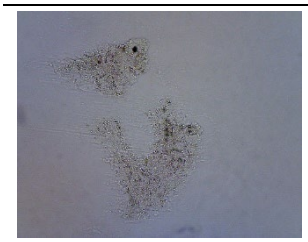
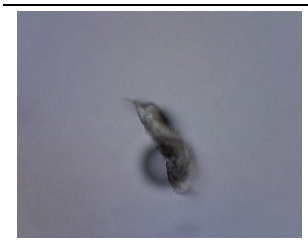
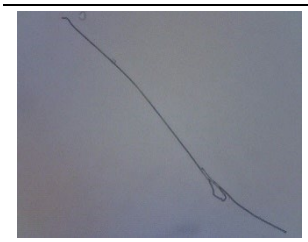
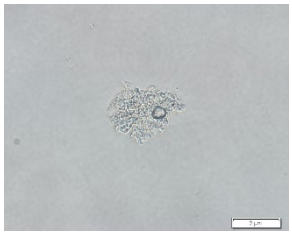
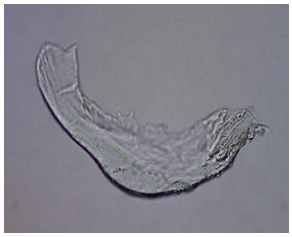
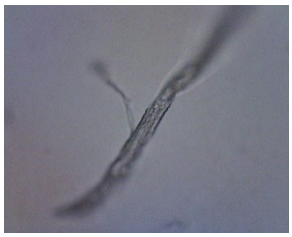
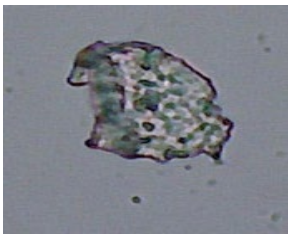
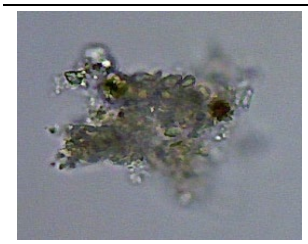
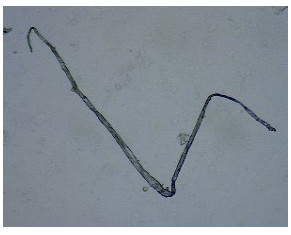
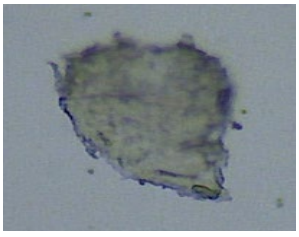
Appendix

Appendix 1. Plankton Identification Results.

LOCATION	PICTURE	SPECIES	DESCRIPTION
THE UPSTREAM			
		Anabaena sp.	*Anabaena* sp. flos aquae as biosensor heavy metal[42].
		*Botryococcus brauni*	Microalgae are unique that they grow well in wastewater and can be used to remove toxic metals and unwanted nutrients from wastewater. *Botryococcus brauni* around domestic wastewater treatment, *Botryococcus brauni* is able to decompose and eliminate COD, BOD, electrical conductivity (EC) and ammonia up to more than 70% of domestic wastewater, especially in the Adyar River (AR) domestic wastewater when compared to other wastewater, research in India [43]
		Chrococcus sp.	*Chrococcus* sp. is commonly found in freshwater. Usually living in a slightly acidic to alkaline environment, this plant causes the water to turn green. They can form dense and sometimes toxic blooms The toxic can threatening ecosystem function and degrading the quality of water for recreational purposes, drinking water, fisheries, and human health [44]
		Microcystis sp.	*Microcystis* sp. are oxygenated photosynthetic bacteria found in nutrient-rich, warm, low-turbulence fresh water [45].
		*Gyrosigma acumination*	*Gyrosigma acumination* was a toxicant that associated with water anthropogenically impacted by industrial pollutants[46].

	*Anabaena* sp.	*Anabaena* sp. flos aquae as biosensor heavy metal[42].
	*Aphanothece clathrata*	Domination of blue-green algae, *Aphanothece clathrate* might occur by the influence of wastewaters from urban mining, metallurgical and domestic activities[47].
	Cerataulina sp.	The epifloral community found by *Cerataulina* sp. associated with heavy metals (Cu, Zn, Ni, Pb, and Cd) in sediments at several river locations[48].
	*Trichocerca* sp.	In the context of zooplankton, Rotifers exhibit Cd tolerance. *Trichocerca* sp. proliferated and became dominant at the species level after Cd application, therefore LC50 values of the Rotifera species ranged from 90 to 39,000 g/L[49].
	Pleurosigma sp.	*Pleurosigma* sp., was reported which are tolerating the heavy metal pollution and also indicated the organic pollution found in water[50].
	*Oscillatoria tenuis*	The species *Oscillatoria tenuis* mostly grows in the surface waters of the Ganges River, which are polluted by fly ash leaching[51].
	Lyngbya	One of the Cyanobacteria species, *Lygbya* sp can increase the accumulation of starch and carbohydrates in the presence of metals (copper or cobalt)[52].

		Botryococcus brauni	*B. braunii* can grow in contaminated leachate obtained from an anaerobic process in decomposing sediments from polluted rivers while successfully remediating the presence of zinc, copper, nickel, iron, and nitrate[53].
		*Microcystis aeruginosa*	*M. aeruginosa* has the potential to survive in highly metal polluted environments, become a significant source of toxic metals in the food chain, and thereby contribute to increasing heavy metal toxicity to living organisms, including humans[54].
		Polyarthra trigla	The suburban waters mainly found communities similar to those found in the oligo-infested waters, namely *Polyarthra. Polyarthra* belongs to a very closely related and highly supported group of copepods[55].
		*Sulculeolaria monoica*	There are 17 phytoplankton genera in two separate classes, namely Diatom (*Sulculeolaria monoica*) and Dinoflagellates, and three zooplankton genera in the zooplankton class that have been identified in plankton samples from rivers[56].
THE DOWNSTREAM			
First Location		*Lepocinclis* sp	*Lepocinclis* can thrive in water bodies with high concentrations of organic matter, such as municipal sewage, sewage ponds and dairy runoff [57].
		Mycrocystis sp.	*M. aeruginosa* has the potential to survive in highly metal-contaminated environments, become a significant source of toxic metals in the food chain, and cause increased toxicity of heavy metals to live organisms, including humans[54].
		*Lyngbya limnetica*	One of the Cyanobacteria species, Lygbya sp can increase the accumulation of starch and carbohydrates in the presence of metals (copper or cobalt)[52].

		Anabaena sp.	*Anabaena* flos aquae as biosensor heavy metal[42].
		Raphidiopsis sp.	The emergence and presence of potentially toxic blue-green algae (*Raphidiopsis* sp) combined with high levels of TSS indicate a potential health risk to the local population[58].
Second Location		*Aphanocapsa elachista*	Filamentous of cyanobacteria colonial *Aphanocapsa* sp. is the most dominant genus in the waste stream, surpassing the green algae genus [59].
		*Botrycoccus braunii*	*Botryococcus* sp. grows in textile industry waste contaminated with heavy metals because of its ability to biologically remove Cr, Cu, As, and Cd[53].
		Lyngbya limnetica	Cyanobacteria species *Lyngbya* sp increased accumulation of starch and carbohydrates in presence of metal (copper or cobalt)[52].
Third Location		*Lyngbya limnetica*	One of Cyanobacteria species *Lyngbya* sp increased accumulation of starch and carbohydrates in presence of metal (copper or cobalt)[52].
		*Mycrocystis* sp.	*Mycrocystis* sp. has the potential to survive in highly metal-polluted environments as it is a significant source of toxic metals in the food chain, thereby increasing the toxicity of heavy metals to living organisms, including humans[54].