Heavy metal (Hg, Cd, Pb, Cu) in the long whiskered catfish (*Mystus gulio* Hamilton, 1822) in Bojonegara Coastal Waters of Banten Bay, Indonesia

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Abstract. Anthropogenic activities such as industrial, domestic, and shipping are the main contributors to the entry of heavy metals into the Banten Bay and the surrounding areas. This research was conducted from August to October 2020 in the coastal waters of Bojonegara, Banten Bay. This study aims to estimate the accumulation of heavy metals (Hg, Cd, Pb and, Cu) contained in long whiskered catfish (*Mystus gulio* Hamilton, 1822) and to determine the tolerance limit for consumption the fish in the Bojonegara coastal waters of Banten Bay. Fish samples were taken using the purposive sampling once per month. Based on the analysis, the heavy metal content in the fish meat were <0.001 mg/kg (Hg), <0.005 mg/kg (Cd), <0.030 mg/kg (Pb) and 0.699-10.920 mg/kg (Cu); the values were less than NADFC of Indonesia (2018) and the Regulation of the Minister of Health Republic of Indonesia 382/1989 with a standard of 0.500 mg/kg (Hg), 0.100 mg/kg (Cd), 0.200 mg/kg (Pb), 20 mg/kg (Cu). The fish caught in Bojonegara Coastal Waters of Banten Bay have moderate to high accumulation levels of Copper (Cu). The safety level for *Mystus gulio* is 16.03 kg fish/week (for adults) and 4.81 kg fish/week (for children).

1. Introduction
The Banten Bay has water with an average depth ranging from 2-20 meters and can be categorized as a shallow water areas [1]. The bay is located in the northern part of the Java Island and the northeast of Cilegon City, which is one of the densely industrial areas. The Bojonegara coastal region is a strategic area as it has an industrial area, residential areas, and fisheries. An industrial area can contribute to heavy metals such as Co, As, Pb, Se, Cd, Cr, Cu, Fe, Zn, and Hg [2]. Domestic activities, fisheries, and shipping in Banten Bay are also some of the causes of the entry of heavy metal waste into the waters [3]. Heavy metals are compounds that have the nature to form complex bonds the so they can accumulate in organisms. Pb, Hg, Cd, and Cu in some measure can be categorized as pollutants and toxic material [4].

Fish is one of the organisms that are susceptible to heavy metal pollution in the waters. One species of fish that lives in the Bojonegara coastal waters is the long whiskered catfish (*Mystus gulio*). The fish live at the bottom of the waters that contains a minimum level of oxygen, therefore the long whiskered catfish are susceptible to exposure to heavy metals [5]. Several research activities were carried out in the Banten Bay areas, including studies on sediment transport [6], heavy metals in water
and sediment [7, 8], heavy metal content in mud crab [9], heavy metal content in blood cockle [10], heavy metal content in green mussels [11], and heavy metal content in blue swimming crab [12]. Research on long whiskered catfish that live in Indonesia was carried out by several research namely. The biological aspects of long whiskered catfish in Majakerta Waters, Indramayu [13]. The long whiskered catfish was found in the Serayu River, Banyumas [14]. Research on the content of heavy metals found in the long whiskered catfish in the Banten Bay has never been conducted. Therefore, this study aims to determine the level of accumulation of heavy metals (Hg, Cd, Pb, and Cu) contained in the flesh of long whiskered catfish (Mystus gulio Hamilton, 1822) and to determine the tolerance limit for consumption of long whiskered catfish in the waters of the Bojonegara, Banten Bay.

2. Methods

2.1. Time and Location

Sampling of long whiskered catfish (Mystus gulio) was conducted from August to October 2020 in the coastal waters of the Bojonegara, Banten Province (Figure 1). The process of dissection and morphometric measurements of the fish was conducted at the Macro Biology Laboratory, Faculty of Fisheries and Marine Sciences, IPB University. Wet destruction and analysis of heavy metal content of the fish was conducted at the Testing Laboratory, Department of Agroindustrial Technology, Faculty of Agricultural Technology, IPB University.

![Figure 1. Study location in coastal waters of Bojonegara, Banten Bay.](image)

The data was collected three times at intervals of once a month at two observation stations. Station 1 (Wadas River) which is close to residential areas and, Station 2 (Terate River) which is directly adjacent to the Java 7 steam-fired power plant (PLTU) complex.

2.2. Materials and equipment

The materials needed in this study were long whiskered catfish (Mystus gulio), aluminium foil, nitric acid, and perchloric acid. The equipment used in this study was a ruler, surgical instruments, freezers,
cool boxes, analytical scales, cast net (1.5 inches), Shimadzu atomic absorption spectrophotometer (AAS) type AA-6880 and, Perkin Elmer atomic absorption spectrophotometer (AAS) AAnalyst 100.

2.3. Research methods

Long whiskered catfish were captured using a cast net of 1.5 inches. The samples were packaged in a plastic bag and put into a cool box with ice cubes in it. The cool box with ice cubes was used in maintaining the quality of the samples to be analyzed. The samples were sorted into two size criteria: small (<10.1 cm) and large (≥10.1 cm). Morphometry was performed by measuring the fish length and total weight. The fish were then dissected and approximately 2-10 grams of its flesh were taken. The flesh was wrapped using aluminum foil and put in the freezer to maintain the durability of the samples. The samples taken were labeled and analyzed in a laboratory according to the standard method, APHA [15].

Heavy metals (Hg, Cd, Pb, Cu) in the fish flesh were analyzed in two stages, wet destruction and measurement of Hg, Cd, Pb, and Cu concentrations. The wet destruction was performed by adding nitric acid-perchloric acid to separate the organic compounds (fat) from the inorganic compounds (solution) [15]. The solution was then analyzed by the spectrophotometric method using Atomic Absorption Spectrophotometer (AAS). The wavelengths used for each analysis of the heavy metal content were 253.7 nm (Hg), 228.8 nm (Cd), 217.0 nm (Pb), 324.7 nm (Cu). The content of Hg, Cd, Pb and Cu in the water samples were also determined by the spectrophotometric method using AAS.

2.4. Data analysis

2.4.1. Analysis of water quality condition

Analysis of water quality conditions was used to determine the condition of the heavy metals of mercury, cadmium, lead, and copper obtained. The quality standard used to analyze heavy metals condition refers to the Government Regulation of the Republic of Indonesia No 22 (2021) for Sea Water Quality Standards for Biota.

2.4.2. Analysis heavy metal content of Hg, Cd, Pb, and Cu in flesh

The results that have been analyzed using AAS are then compared based on size. Fish samples were distinguished by two categories; large (≥10.1 cm) and small (< 10.1 cm). This size-based comparison was conducted to analyze the effect of heavy metal accumulation on the fish living around Bojonegara waters (Banten Bay) in different size categories of fish. The content of heavy metals in fish flesh is then compared with the quality standard according to National Agency of Drug and Food Control Republic of Indonesia Number 5 of 2018 Attachment VIII for heavy metals Hg, Cd, Pb (NADFC of Indonesia 2018) and the quality standard for copper (Cu) is according to the Directorate General of Drug and Food Control, Ministry of Health, Republic of Indonesia (Ditjen POM Depkes RI 1989 Number: 03725 / B / SK / VII / 89 (Table 1).

| Heavy metals content | Quality Standard |
|----------------------|------------------|
|                      | NADFC of Indonesia* 2018 (mg/kg) | Minister of Health Republic of Indonesia 382/1989 (mg/kg) |
| Mercury              | 0.50             | - |
| Cadmium              | 0.10             | - |
| Lead                 | 0.20             | - |
| Copper               | -                | 20 |

*National Agency of Drug and Food Control of Indonesia
2.4.3. Bioconcentration factors (BCF-o-w)
The bioconcentration factor is used to determine the ability of organisms to accumulate heavy metals in waters [16].

\[ BCF = \frac{C_t}{C_w} \]  

(1)

Where,
BCF : Bioconcentration factor
Ct  : Concentration of pollutants in organisms (mg kg\(^{-1}\))
Cw  : Concentration of pollutants in water (mg L\(^{-1}\))

The accumulation level category [17]:
Low Accumulation : BCF-o-w < 100
Medium Accumulation : 100 < BCF-o-w ≤ 1000
High Accumulation : BCF-o-w > 1000

2.4.4. Safety level
The safe limit for consumption is the maximum weight for consuming the organism every week for adults and children [18]. The safe limit for consumption can be determined from the smallest limit value of the type of heavy metal content so that there is no deposition of metals in the body that can cause death.

\[ MWI (mg) = \text{Weight}^* \times PTWI \]  

(2)

\[ MTI = \frac{MWI}{C_t} \]  

(3)

Where,
MWI  = Maximum Weekly Intake (mg)
PTWI = Provisional Tolerable Weekly Intake (mg/kg weight body)
MTI  = Maximum Tolerable Intake (mg/week)
Ct    = Concentration of heavy metals in fish meat (mg/kg)
*     = Average bodyweight of Indonesian adults is 50 kg and children are 15 kg (Indonesian Ministry of Health 2019) [18]

3. Results and Discussion

3.1. Water quality of Bojonegara coastal waters
Based on the study conducted between August-October 2020, the status of water quality at the two stations show that most of the parameters were not in accordance with the quality standards according to Government Regulation of the Republic of Indonesia 22/2021 [19] concerning Implementation of Environmental Protection and Management. Salinity at the two stations ranged from 4-34 psu. The degree of acidity (pH) of the waters during the observation ranged from 7-8 (Table 2). Based on the analysis result, the heavy metal content of mercury, cadmium and lead during the observation was below the detection limit, except for copper, which ranged from 0.0035-0.0060 mg/L (Table 3).
Table 2. Value of water quality parameters in coastal waters of Bojonegara.

| Parameters     | Unit | August        | September     | October       | Quality Standards a |
|----------------|------|---------------|---------------|---------------|---------------------|
| Turbidity      | NTU  | 31.7-72       | 62-113        | 80-146        | < 5                 |
|                |      | (51.5±28.90)  | (88±36.06)    | (113±46.67)   |                     |
| Temperature    | ºC   | 33.5-34       | 33.0-34.0     | 29.5-31       | 28-32               |
|                |      | (33.8±0.35)   | (33.5±0.71)   | (30.30±1.06)  |                     |
| Transparency   | M    | 0.325         | 0.2-2.3       | 0.175-0.225   | -                   |
|                |      | (0.33±0.00)   | (1.45±1.20)   | (0.2±0.04)    |                     |
| Salinity       | Psu  | 7-26          | 4-19          | 4-34          | Nature-34           |
|                |      | (16.50±13.44) | (11.5±10.61)  | (21.21±19.0)  |                     |
| TSS b          | mg/L | 103-216       | 174-329       | 214-415       | 80                  |
|                |      | (159.50±79.90)| (251.50±109.60)| (314.5±142.13)|                     |
| DO c           | mg/L | 4.7-4.8       | 5.4-5.7       | 6.1-6.2       | >5                  |
|                |      | (4.75±0.07)   | (5.55±0.21)   | (6.2±0.07)    |                     |
| pH d           |      | 8             | 7.8           | 7.3-8         | 7-8.5               |
|                |      | (8±0.00)      | (7.50±0.71)   | (7.75±0.64)   |                     |

aGovernment Regulation of the Republic of Indonesia 22/2021 [19]
bTotal suspended solid
cDissolved oxygen
dPotential hydrogen

Table 3. Heavy metal concentrations in coastal waters of Bojonegara.

| Heavy Metals | Unit | August     | September   | October    | Quality Standards a |
|--------------|------|------------|-------------|------------|---------------------|
| Hg           | mg/L | <0.0002 ± 0.0000 | <0.0002 ± 0.0000 | <0.0002 ± 0.0000 | 0.001               |
| Cd           | mg/L | <0.0010 ± 0.0000 | <0.0010 ± 0.0000 | <0.0010 ± 0.0000 | 0.001               |
| Pb           | mg/L | <0.0002 ± 0.0000 | <0.002 ± 0.0000 | <0.002 ± 0.0000 | 0.008               |
| Cu           | mg/L | 0.0035 ± 0.0021 | 0.0060 ± 0.0000 | 0.0060 ± 0.0000 | 0.008               |

aGovernment Regulation of the Republic of Indonesia 22/2021 [19]

3.2. The condition factor
The figure below is a graph showing the data condition factor of the fish that live in the Bojonegara coastal waters of Banten Bay. Based on the study, the value of the average condition factors for the fish has increased. The highest condition factor occurred in October with an average value of 1.0510 with the lowest value of 1.0160 in August (Figure 2).

3.3. Content of mercury (Hg) in the flesh of fish
The content of mercury (Hg) in the small and large fish taken in the Bojonegara coastal waters of Banten Bay was low as all of the monthly values were below (<0.001 mg/kg). It can be stated that the Hg content was still within the quality standard of National Agency of Drug and Food Control Republic of Indonesia (NADFC of Indonesia 2018) i.e., 0.5 mg/kg [20] (Figure 3).

3.4. Content of cadmium (Cd) in the flesh of fish
The content of cadmium (Cd) in the small and large fish taken from the Bojonegara coastal waters of Banten Bay was low as all of the monthly values were below (<0.005 mg/kg). It can be stated that the Cd content was still within the quality standard of National Agency of Drug and Food Control Republic of Indonesia (NADFC of Indonesia 2018) i.e., 0.1 mg/kg [20] (Figure 4).
Figure 2. Boxplot condition factors of *Mystus gulio* in the coastal waters of Bojonegara.

Figure 3. Content of Hg in both small and large *Mystus gulio* in the coastal waters of Bojonegara.

Figure 4. Content of Cd in both small and large *Mystus gulio* in the coastal waters of Bojonegara.
3.5. Content of lead (Pb) in the flesh of fish

The content of lead (Pb) in the small and large fish taken from the Bojonegara coastal waters of Banten Bay was low as all of the monthly values were below (<0.03 mg/kg). It can be stated that the Pb content was still within the quality standard of National Agency of Drug and Food Control Republic of Indonesia (NADFC of Indonesia 2018) i.e., 0.2 mg/kg [20] (Figure 5).

![Figure 5. Content of Pb in both small and large Mystus gulio in the coastal waters of Bojonegara.](image)

3.6. Content of copper (Cu) in the flesh of fish

The content of copper (Cu) in the small and large fish taken from the Bojonegara coastal waters of Banten Bay ranged from 0.699-10.920 mg/kg (Figure 6). It can be stated that the Cu content was still within the quality standard of Regulation of the Minister of Health Republic of Indonesia 382/1989 i.e., 20 mg/kg. The large fish had a higher range of Cu concentration values when compared to the small fish.

![Figure 6. Content of Cu in both small and large Mystus gulio in the coastal waters of Bojonegara.](image)
3.7. Bioconcentration factors (BCFo-w) in fish

The results of the analysis of the bioconcentration factors (BCFo-w) of heavy metals showed that the highest accumulation of heavy metals was copper (Cu) in September for the large fish (≥10.1 cm) with a value of 3120 (Table 4).

The bio-concentration factor value >1000 indicates that copper (Cu) is highly accumulative, this situation will be dangerous if there are organisms exposed to heavy metals for a long period of time. The value of the heavy metal bio-concentration factors of Hg, Cd, and, Pb in large and small fish flesh is worth 0, this is due to the heavy metals content in the flesh being below the detection limit of the AAS.

| Month     | Large (≥10.1 cm) | Small (<10.1 cm) |
|-----------|------------------|------------------|
|           | Hg   | Cd   | Pb   | Cu   | Hg   | Cd   | Pb   | Cu   |
| August    | 0*   | 0*   | 0*   | 800  | 0*   | 0*   | 0*   | 228  |
| September | 0*   | 0*   | 0*   | 3120 | 0*   | 0*   | 0*   | 199.71 |
| October   | 0*   | 0*   | 0*   | 754.29 | 0* | 0*   | 0*   | 237.43 |

*the value of heavy metals found in Mystus gulio meat and waters is below the detection limit of the AAS

3.8. Safety level of Mystus gulio

The fish is still safe for consumption in the condition that it does not exceed the specified safety level. The safety level for fish consumption for adults assuming a body weight of 50 kg is 16.03 kg of meat/week. The safety level for fish consumption for children assuming a body weight of 15 kg is 4.81 kg of meat/week (Table 5).

| Heavy metals | Safety Level (kg/week) |
|--------------|------------------------|
|              | Age*       | Large (≥10.1 cm) | Small (<10.1 cm) |
| Hg           | Adult      | 80.00            | 80.00            |
|              | Children   | 24.00            | 24.00            |
| Cd           | Adult      | 70.00            | 70.00            |
|              | Children   | 21.00            | 21.00            |
| Pb           | Adult      | 41.67            | 41.67            |
|              | Children   | 12.50            | 12.50            |
| Cu           | Adult      | 16.03            | 210.59           |
|              | Children   | 4.81             | 63.18            |

*Average bodyweight of Indonesian adult (50 kg) and children (15 kg) according to Indonesian Ministry of Health 2019 [18]

Bojonegara coastal waters of Banten Bay is a strategic area that is widely used by the surrounding community as fishing areas, residential areas to industrial areas. These anthropogenic activities will produce waste that can enter the waters. One of the detected wastes is heavy metals [8]. Heavy metals entering the waters will be dangerous for aquatics biota and those who use them, because of the characteristic of heavy metals which is they can settle in an environment for a long time and can enter the food chain system [21]. Heavy metals that enter the body of living organisms will easily form complex bonds that are harmful to the organism's body, and if organisms exposed to heavy metals enter the food chain, biomagnification of heavy metals will occur at a higher trophic level, including humans.
The long whiskered catfish (Mystus gulio) is a biota that belongs to the order Siluriformes forage on the bottom of the river bed and like shady waters overgrown with lots of plants so they can supply litter into the waters. These antennae of the fish also help long whiskered catfish to find food on the bottom of the river bed which tend to have little light input [22]. This fish lives in the downstream river (estuary), these fish live with various other fish species, such as snapper (Lutjanus argentimaculatus, L. Johnii), groupers (Epinephelus quoyonius), Plotosus lineatus, Oryzias javanicus, Ellochelon vaigiensis, Anabas testudineus, Oreochromis niloticus, Oreochromis mossambicus, Trichopodus trichopterus and, others [23]. This allows for formation of a food chain system between species that live in one ecosystem.

The water quality data shows that the coastal waters of Bojonegara, Banten Bay in the downstream river area has experienced waste input from the anthropogenic activities of the surrounding community, and is indicated by the measurement results of water quality parameters, most of which do not meet the quality standards according to Government Regulation of the Republic of Indonesia 22/2021, although the heavy metals content of Hg, Cd, Pb and, Cu in Bojonegara waters is still below the quality standards of Government Regulation of the Republic of Indonesia 22/2021. The environmental conditions of these waters tend to be tolerated by long whiskered catfish, and is indicated by the value of the condition factor of long whiskered catfish caught at the same time and location where the value of the condition factor of long whiskered catfish is more than one (> 1), i.e. 1.0160-1.0510 (Figure 2), and it means that the organism can still grow well [24]. From this statement, it means that the long whiskered catfish caught in the Bojonegara coastal waters of Banten Bay are not under excessive pressure from their environment and the availability of food in their habitat is still sufficient.

Large long whiskered catfish have a higher range of heavy metal concentration values when compared to small long whiskered catfish. Small sized/juvenile fish is a stage where the fish that is actively growing, and in the growth phase the fish need some minerals for body development. Copper (Cu) is one of the essential heavy metals needed by the body of organisms in certain quantities because it can function as a blood system hemocyanin and enzymatic formation for aquatic organisms [25]. In the growth stage, fish will actively absorb and use copper (Cu) obtained from food and in their environment.

Heavy metals are easily bound to fish tissue, if exposure to these heavy metals occurs for a long time, it will accumulate in the organism’s body which causes worse things such as heavy metal biomagnification. Biomagnification is a multilevel accumulation caused by the entry of biota exposed to heavy metals introduced to the food chain system. The presence of heavy metals in the body of organisms with lower trophic levels and move from one trophic level to the next. This biomagnification effect will be dangerous for organisms with higher trophic levels such as humans [26].

The highest accumulation of heavy metals occurred in copper (Cu) in September for the large long whiskered catfish (≥10.1 cm) with a value of 3120. BCFo-w value >1000 indicating that copper is very accumulative; the accumulation level will be very dangerous if organisms are exposed to heavy metals for long periods of time. The fish are benthic organisms that live and forage on the bottom of the river bed [5]. Benthic organisms are very vulnerable to exposure to pollutants, this is because the bottom of the water is a place for the deposition of the rest of life and pollutants from organisms that live in the water column, surface and above the water surface. The safe limit for consumption in one week of long whiskered catfish flesh for adults (50 kg) in Bojonegara coastal waters of Banten Bay is maximum of 16.03 kg of meat/week while for children (15 kg) can consume much as 4.81 kg of meat/week. This value is taken from the heavy metal copper (Cu) which has the lowest of MTI value.

4. Conclusion
This study concludes that the concentration of the heavy metals Hg, Cd, Pb and Cu in the flesh of long whiskered catfish is still within the quality standards of NADFC of Indonesia 2018 and Regulation of the Minister of Health Republic of Indonesia 382/1989. Heavy metal content in the large long
whiskered catfish (*Mystus gulio*) tends to be higher than found in the small one. The fish (*Mystus gulio*) caught in the Bojonegara coastal waters of Banten Bay have a low level accumulation of the heavy metals Hg, Cd and, Pb (BCFo-w <100); however, the bioconcentration factors of copper (Cu) in fish are classified as moderate (BCFo-w 100-1000) until high accumulation levels (BCFo-w>1000). The safety level for *Mystus gulio* consumption by humans is 16.03 kg fish/week (for adult) and 4.81 kg fish/week (for children).

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