

Presence of trace elements in muscles of *Mugil cephalus* from Yogyakarta coast, Indonesia

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Abstract. Trace elements refer to any chemical elements including metals and minerals that naturally exist in the aquatic ecosystem. Fish can accumulate trace elements including heavy metals absorbed from water and through the food chain. It is necessary to explore the relationship between the level of trace elements (both essential and non-essential) and the physiological status of fish in order to maintain the wild fish stock and ecosystem sustainability in the coastal marine ecosystem. Mullet *Mugil cephalus* is detritus and filter feeder inhabiting the coastal estuaries. This species can be considered as a biological indicator for metal pollution because of its capability to accumulate heavy metals in its body. This study was undertaken to examine the presence of eight trace elements consisting of essential elements: iron (Fe), manganese (Mn), chromium (Cr), zinc (Zn) and copper (Cu), and non-essential elements: cadmium (Cd), lead (Pb), and mercury (Hg) in muscle tissues of *M. cephalus* taken from Opak and Bogowonto river estuaries, in coastal water of Southern Yogyakarta, Indonesia. Fish with total lengths of between 25 and 33 cm were selected. Fish muscle samples were analyzed for trace elements content by using Atomic Absorption Spectrophotometer and a Mercury Analyzer. According to the result obtained in this research, it may be concluded that the detected trace elements in *M. cephalus* muscle from two sampling sites were Cu, Fe, Zn, Hg. Other metals, namely, Cd, Cr, Mn, Pb, were not detected in fish muscle samples from both sites. The order of the mean concentration of trace elements in the muscle tissues of *M. cephalus* collected in both sampling sites was Fe > Zn > Cu > Hg. The information gained in this study is crucial to support the proper aquatic management and conservation of Yogyakarta coastal areas in Indonesia.

Key Words: bioaccumulation, coastal area, estuary, fish muscle, heavy metal, mineral.

Introduction. Trace elements are essential components in the freshwater and marine environment, that in general are found in small quantities. Trace elements refer to any chemical elements including metals and minerals, naturally existing in an ecosystem (Annabi et al 2017). In aquatic ecosystems, heavy metals are included as natural trace elements. The existence of heavy metals in the aquatic ecosystems has been mentioned in previous studies (Delgado-Alvarez et al 2017; Rosli et al 2018). In the aquatic ecosystems, trace elements could be accumulated in water and, under certain physicochemical circumstances, the chemical elements could be released and enter the food web (Adharini et al 2021; Bahhari et al 2017).

In general, trace elements can be categorized as biologically essential and non-essential. In the biological processes of an aquatic ecosystem, trace elements are important for fish metabolism (Rosli et al 2018), for example, copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn). On the other hand, the roles of metals such as lead (Pb), mercury (Hg), chromium (Cr) and cadmium (Cd) were still unknown in biological systems. These metals are even seen as non-essential elements and considered pollutants because of their toxicity in relatively small quantities (Rosli et al 2018).

As some trace elements play vital roles in fish metabolism, fish obtained the essential elements from diet, sediment and water (Helal et al 2018). The fish uptake the trace elements through specific ways, namely through the digestive tract, skin, or gills

(Ouali et al 2018). On the other hand, the process of non-essential elements resembles the essential elements as fish might uptake the non-essential elements and the elements are bioaccumulated in fish tissues. High-concentrated elements can be absorbed by fish from the diet and water. Nevertheless, bioaccumulation in aquatic organisms will primarily rely on quantities of trace elements and the period of exposure in the water despite other determinants such as pH, temperature, water hardness and salinity that also have a vital role in the process of trace elements' accumulation (Ouali et al 2018).

Heavy metals do not break down into less harmful compounds in an environment. The substance that contains metal may be changed, but the toxic metals remain to exist and their stabilities in water enable them to be transferable and bioaccumulated in fish or other aquatic species through food chain (Mwakalapa et al 2019). Moreover, the toxicity may lead to risk and adverse impact on health when they are consumed by human. The discussion on heavy metals' existence, toxicity at a certain amount of quantity and the persistence of the elements in the trophic chain should be advanced and developed. Therefore, considering the detrimental effects of heavy metals, it is essential to check and control their accumulation in aquatic species (Yousif et al 2021).

Mullet (*Mugil cephalus*) is a worldwide distributed fish and can be easily found in the coastal areas of tropical and subtropical climate zones. Included in the Mugilidae family, *M. cephalus* is a catadromous species that lives in temperate freshwater and estuary areas (Annabi et al 2017). As an omnivorous and pelagic species, *M. cephalus* feeding habits are closely related to sediment where the fish obtains its diet from crustaceans, algae, smaller fish, gastropods and polychaetes (Ouali et al 2018). For its feeding habits, juveniles get their diet from invertebrates, while adult fish obtain its diet from benthic algae, detritus, smaller species and plankton. *M. cephalus* generally lives in coastal, lagoon and estuaries, the area where the abundance of plankton is found (Unbekna et al 2020; Wijayanti et al 2020). Several studies are reported the feeding habit of *M. cephalus* as detritus and filter feeders in the coastal lagoon. Hence this species is susceptible to contamination from sediments and water. *M. cephalus* can be considered as a biological indicator for metal pollution because of its capability to accumulate heavy metals in its body (Ouali et al 2018).

The discussion about bioaccumulation and biomagnification of heavy metal contaminants in aquatic species is a topic of growing interest for scientists and environmental practitioners (Ouali et al 2018). For the past few years, the level of heavy metals as trace elements in fish have been widely explored worldwide. Several studies are focused on the concentration of trace elements in the edible organ fish muscles (El-Moselhy et al 2014; Annabi et al 2017; Bahhari et al 2017; Mwakalapa et al 2019; Ouali et al 2018). Heavy metals generated from the anthropogenic activity and natural sources are released to an aquatic environment and they may be trapped in sediments (Ouali et al 2018). The concentration and quantities of trace elements in upper parts of the food chain like fish can be higher than the elements found in sediments or water. Therefore, the level of contamination in an area is closely related to the health of aquatic organisms. The importance of tracing the health of aquatic environment should be highlighted through conducting regular investigation and monitoring of metal levels for the aquatic species, in order to maintain the wild fish stock and ecosystem sustainability in the marine ecosystem (Mwakalapa et al 2019).

M. cephalus is prevalent option in biomonitor studies in the coastal environments. For the purpose of understanding its health state and thereby tracking the suitable aquatic management and conservation of coastal areas, it is essential to explore the link between the level of trace elements (both essential and non-essential) and the physiological status of aquatic organisms. Therefore, this study was undertaken to examine the presence of eight trace elements consisting of essential metals (Fe, Mn, Cr, Zn and Cu) and non-essential (toxic) metals (Cd, Pb, and Hg) in muscle tissues of *M. cephalus* taken from Opak and Bogowonto river estuaries, in coastal water of Southern Yogyakarta Special Region Province, Indonesia.

Material and Method

Study area and sample collection. *M. cephalus* were collected from the Opak river estuary (latitude 8°00'45.6"S; longitude 110°17'10.7"E) and Bogowonto river estuary (latitude 7°53'57.2"S; longitude 110°01'54.3"E), the south coast of Yogyakarta Special Region Province, Indonesia (Figure 1). Both estuaries have a year-round association with the open ocean. The fish specimens were collected from local fishermen of each estuary, between July and September 2020. Considering that size and age may be significant determinants for trace element accumulation process, sample of similar body weight and length were selected. Fish with total lengths of between 25 and 33 cm were selected. This was aimed at getting the same length and weight per site area. After sampling, the 10 fish specimens from each site were transported in a cooler box to the laboratory. Initially, laboratory treatment was started by thawing the sample in a room temperature. Following this, biometric study was conducted for all the collected specimens. Fish total lengths were measured to the nearest millimeter using a tape measure on a flat surface, while fish weights were recorded to the nearest gram using digital balance (Ohaus NVT1601/3). The condition factor was calculated according to the following equation: $(\text{fish weight}/\text{fish length}^3) \times 100$. Samples were stored at -20°C for further analysis of trace elements.

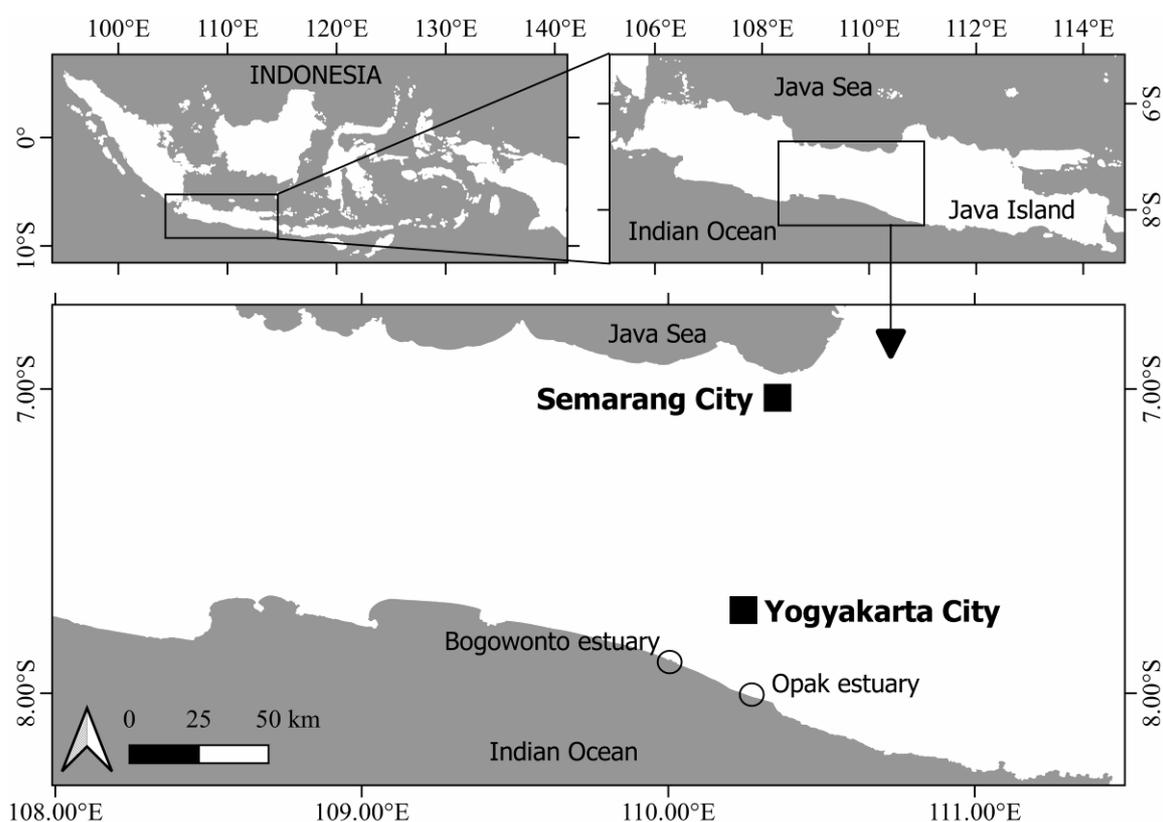


Figure 1. Geographical location of two estuarine sampling sites.

Sample preparation and trace element analysis. Fish muscle samples were selected for analysing the level of non-essential trace elements, such as cadmium (Cd), mercury (Hg), and lead (Pb) and also essential trace elements such as, copper (Cu), iron (Fe), chromium (Cr), zinc (Zn) and manganese (Mn). All the fish collected as samples were dissected using a sterile stainless knife and scissors. Approximately 100 g of fish muscle were separated. Subsequently, the muscles that were collected were macerated into small parts and combined in a blender until they were mixed homogeneously. After the mixing step, around 10 g of muscles from each sample were transferred into an Erlenmeyer flask. Throughout the study, all acids and chemicals used were analytical grade. Then, the sample was digested with addition of 15 mL of mixed reagent (HNO_3 :

HClO₄ in the ratio of 2:1) and heated on a hot plate until a color change was observed. The digested suspension was added 25 mL distilled water. After that, samples were filtered into 50 mL volumetric flask and distilled water was used to adjust the volume. Finally, the samples were analyzed for Pb, Cd, Cr, Cu, Zn, Fe and Mn using Atomic Absorption Spectrophotometer (ContraAA 300 Analytik Jena). Concentrations of analyzed elements were expressed on a wet weight basis (w.w), as mg kg⁻¹.

Hg analysis was quantified following the protocol: approximately 1 g of the muscle samples were weighed and transferred into a 100 mL Erlenmeyer flask. In this stage, the sample was digested with addition of 10 mL of mixed reagent (HNO₃ : HClO₄ in the ratio of 1:1) and heated on a hot plate until a color change was observed. Thereafter, the samples were filtered into 50 mL volumetric flask and distilled water was used to adjust the volume. Blanks were also prepared using the above procedure without adding fish samples. Hg was quantified with Mercury Analyzer (Lab Analyzer 254).

Statistical analysis. For the purpose of testing the concentration differences of heavy metals of fish muscle samples between study sites, the analysis of variance (ANOVA) was applied, followed by Student–Newman–Keuls (SNK) posthoc test. Therefore, the statistical analysis was utilized the statistical software package SAS OnDemand for Academics (2021 SAS Institute Inc., SAS Campus Drive, Cary, North Carolina, USA). P value < 0.05 was considered statistically different.

Results. Biological characteristics of *M. cephalus* collected from two sampling sites were recorded (Table 1). The mean condition factor of fish from both sites was comparable, 1.02 for mullet from Opak estuary and 1.07 for sample from the Bogowonto estuary.

Table 1

The recorded morphometric measures of fish samples from two sites

<i>Parameters</i>	<i>Opak estuary</i>		<i>Bogowonto estuary</i>	
	<i>Mean</i>	<i>Range</i>	<i>Mean</i>	<i>Range</i>
Total length (cm)	26.88	24.5-31.5	31.67	28.5-33.5
Weight (gr)	208.1	134.8-353.2	340.73	250.5-408.1
Condition factor	1.02	0.88-1.16	1.07	0.99-1.14

The concentrations of trace elements in fish tissues from different locations demonstrated considerable variations. Table 2 show the average concentrations of trace elements in the muscle of *M. cephalus* collected at Opak and Bogowonto river estuary. The elemental concentrations of *M. cephalus* are expressed in mg kg⁻¹ except for Hg, which is expressed in µg kg⁻¹ wet weight for greater accuracy. Among the eight trace elements investigated in two sampling sites, the elements namely, Cu, Fe, Zn, were detected in fish muscle. However, the level of Hg was very low. The rest of trace elements, such as Pb, Cd, Cr, Mn were below the level of quantification.

Among the trace elements, the highest level of Fe was found in the muscle of mullet collected from two sampling sites, then followed by Zn and Cu (Table 2). The non-essential elements as Hg in fish tissue presented significant differences (p < 0.05) between sites. The results showed the following bioaccumulative order of trace elements in *M. cephalus* tissue from Opak and Bogowonto river estuaries as Fe > Zn > Cu > Hg. Moreover, Fe, Zn and Cu concentrations in *M. cephalus* showed significant spatial variability. Statistically, fish samples from the Bogowonto river estuary showed a quite high level of Fe and Zn when it is compared to the Opak river estuary.

Table 2

Mean levels of trace elements of fish samples from two sites

Trace elements	Symbol	Opak estuary	Bogowonto estuary	P-value	MSE	PSE
Mercury ($\mu\text{g kg}^{-1}$)	Hg	23.52 ^a	20.23 ^b	0.0145	1.8802	0.4848
Lead (mg kg^{-1})	Pb	nd	nd	-	-	-
Copper (mg kg^{-1})	Cu	2.86 ^a	1.72 ^b	< 0.0001	0.0052	0.0254
Iron (mg kg^{-1})	Fe	4.52 ^b	6.60 ^a	< 0.0001	0.0317	0.0630
Zinc (mg kg^{-1})	Zn	3.60 ^b	3.75 ^a	< 0.0001	0.0029	0.0189
Cadmium (mg kg^{-1})	Cd	nd	nd	-	-	-
Chromium (mg kg^{-1})	Cr	nd	nd	-	-	-
Manganese (mg kg^{-1})	Mn	nd	nd	-	-	-

nd means not detected in muscle tissue, < LOD (limit of detection). LOD for Hg: 23.52 $\mu\text{g kg}^{-1}$; for Pb, Cd: 0.01 mg kg^{-1} ; for Cr: 0.015 mg kg^{-1} ; for Cu: 2.87 mg kg^{-1} ; for Zn: 3.63 mg kg^{-1} ; for Fe: 4.53 mg kg^{-1} ; for Mn: 0.025 mg kg^{-1} . Different letters within the same row indicate significant differences between tissues.

MSE: mean squared error. PSE: pooled standard error.

Discussion. *M. cephalus* is a benthic species that inhabits near the coast and shallow water. The fish species migrates between the brackish water in the mangroves and open sea (Mwakalapa et al 2019). *M. cephalus* is considered a zooplanktonivorous fish and has a high metabolic rate. According to their food habits, *M. cephalus* can be suitable as tool for description of environmental conditions of coastal waters (Stancheva et al 2013).

The mean condition factor values for fish analyzed in this study were in the same range between mullet from two sampling sites. Condition factor indicates general fish's health, physiological condition and environmental quality (Satriyo et al 2017; Rakocevic et al 2018).

Four trace elements (Cu, Fe, Zn, Hg) were detected in all fish muscle samples collected from two sites, demonstrating an ample distribution in the coastal area. Other trace elements (Cd, Cr, Mn, Pb) were not detected in samples from both sites. Compared to other studies from different geographical zones, Bahhari et al (2017) found lower levels of Cu (0.44 mg kg^{-1}) in the muscle of mullet from Jazan Coast, Saudi Arabia and Suryono et al (2018) also observed lower levels of Cu (0.6 mg kg^{-1}) in the muscle of mullet from Semarang waters, Indonesia. Cu concentrations in the muscle from mullet in this study were similar to the concentrations in the muscle of mullet from the estuary of Donan River, Indonesia, that maximal level of Cu was 2.6 mg kg^{-1} (Prastyo et al 2017). The Fe concentration in studied mullet muscles were higher than mullet from Tanzania coast, 1.79 mg kg^{-1} (Mwakalapa et al 2019). The concentration of Zn in this study was higher than in other studies. Bahhari et al (2017) observed lower levels of Zn (2.8 mg kg^{-1}) in the muscle of mullet from Jazan Coast, Saudi Arabia and Mwakalapa et al (2019) recorded lower levels of Zn (0.8 mg kg^{-1}) in the muscle of mullet from Tanzania coast. Hg concentrations in the mullet muscle in the present study were in the same range as mullet from Tanzania coast (Mwakalapa et al 2019). Zn, Fe and Cu are essential and have a specific role for organisms and their level on fish were mostly the highest. Essential trace elements such as Fe, Zn and Cu are deliberately managed by the physiological system in most organisms and their roles are vital as precursors in the enzymatic activities (Mwakalapa et al 2019).

The variation of concentration of trace elements found in our study may be credited to different rates of metabolism. Increasing metabolic demand facilitates absorption of metal. Organisms vary in food requirements, quantity of food intake and metabolic rates (Ayanda et al 2019). All these factors might determine the variation in trace element concentrations observed in the samples. Previous studies also stated the variation in metal bioaccumulation was species-specific (Rakocevic et al 2018) and might possibly depend on different rates of physiological activity, different metabolism rates, different ecological needs, different routes or rates of uptake and excretion of each trace elements (Sofoulaki et al 2018).

Regarding the spatial differences of trace elements found, the results showed no consistent increasing pattern of trace element levels in mullet in two sites, as seen in Table 2. Furthermore, mullets collected from the two locations were reported to accumulate different levels of trace elements (Cu, Fe, Zn). In this research, we reported the spatial differences of trace elements that the Bogowonto river estuary showed significantly highest concentrations of Fe and Zn, however, the Opak river estuary showed significantly highest concentrations of Cu and Hg compared to the other site. The level of trace elements found in fish organs represented the level of the trace elements in sediments or aquatic environments. The differences of trace element levels between the two locations that was found in the muscle of the fish might relate with the variation of chemical characteristics and trace elements level in sediments and aquatic environment where the fish were collected, as well as the season in which research took place (Suliaman & Suliman 2019). In addition, these spatial differences may have resulted from the geogenic sources or leaching of rocks in the water (Mwakalapa et al 2019). Moreover, the agriculture activities might also potentially contribute to the increasing level of trace elements even though it might not be the main contributor. Considering that our research locations were situated nearby the coastal farming areas, agricultural discharges were considered to be the source of Zn, Cu and Fe, as these mineral elements are linked with the production and manufacture of fertilizers (Ouali et al 2018). The province of Yogyakarta has rivers flowing from Mount Merapi to coastal waters and lagoons, and receive the surplus waters used for irrigation for intensive farming activities. Most of the farmers in the coastal area who have access to irrigation and drainage intensively cultivated their lands three times a year, growing rice in the rainy season and the early dry season while cassava, corn and sweet potato were produced in the dry season (Maulida & Subejo 2020).

In this research, the mean of trace element concentrations in mullet muscle tissues collected from Bogowonto and Opak river estuaries indicated spatial differences in trace element accumulation. However, the same trend of order of the mean concentration of trace elements in fish muscle collected in both sampling sites was observed as follows: Fe > Zn > Cu > Hg. The different results in the bioaccumulation patterns of heavy metals might be linked with the differences in assimilation capacities (Annabi et al 2017). The accumulation of trace elements in mullet might have resulted from potential factors, namely endogenous and exogenous factors. Endogenous factors include the size of the species, growth rate, feeding habits, metabolic status, species' specific factors, and also the variations in biokinetic uptake, depuration rate, and other physiological processes could lead to the differences in the concentration of trace elements found in fish tissues (Annabi et al 2017; El-Moselhy et al 2014; Ouali et al 2018). However, the exogenous factors include the bioavailability of the trace elements and geographical distribution (Annabi et al 2017; Suratno et al 2020). The information gained in this study is crucial to support the proper aquatic management and conservation of Yogyakarta coastal areas in Indonesia.

Conclusions. In this research, we assessed the levels of trace elements in *Mugil cephalus* muscle collected from Bogowonto river and Opak river estuaries in the southern coast of Yogyakarta, Indonesia. The results show that the detected elements were copper (Cu), iron (Fe), zinc (Zn), mercury (Hg). Other metals namely, cadmium (Cd), chromium (Cr), manganese (Mn), lead (Pb) were not detected in fish muscle samples from both sites. The order of the mean concentration of trace elements in the fish muscle from both sampling sites was Fe > Zn > Cu > Hg. In particular, Hg levels in muscle sample was negligible. Estuarine fish receive attention from fisheries scientists in evaluating the health of coastal marine habitats and further research should include routine monitoring of trace elements in wild fish for the welfare of fish stock in coastal waters.

Conflict of interest. The authors declare that there is no conflict of interest.

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