The pattern of Cu accumulation in milkfish (*Chanos chanos*) during growth period in fishpond in Dukuh Tapak Tugurejo Semarang, Indonesia

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Abstract. The environmental dreadful condition in the coastal area of Semarang is caused by an abrasion and mangrove ecosystem damage. Environmental degradation will affect the aquaculture ponds in coastal areas, which can also influence the quality of milkfish (*Chanos chanos*) reared in ponds. The purpose of this study is to determine Cu concentration in water and sediments on milkfish ponds and also distinguish the pattern of Cu accumulation in fish during their growth period. The samples used in this study were Cu in water, sediment and milkfish collected during 3 months research period. This study was an ecological approach. Data of Cu bioaccumulation in sediment, water and fish were analyzed based on the concentration factor (CF) formula and concentration ratio (CR). Research results show that the Cu content in water was between 0.2-0.3 mg L^{-1} and in sediment was between 18.09 to 22.81 mg L^{-1}. According to this result, water Cu content was above the threshold. Based on the rule no. 51 2004 on sea water quality standard by the Ministry of Environment - Indonesia, it states that the threshold of Cu level in marine biota is at 0.008 mg L^{-1}. The presence of Cu pollution in milkfish needs an attention related to the ability of fish to accumulate metals from the environment. Interestingly, Cu in fish was between 1.165-3.396 mg L^{-1}; bellow the threshold permitted by BPOM No. 03725/B/SK/VII/89 1989 amounted to 20 mg L^{-1}. People must be aware of the presence of this metal in fish since it could be harmful for human's health.
Key Words: accumulation pattern, milkfish, Cu, Dukuh Tapak.

Introduction. Metal pollution is a distressing issue in aquatic environments all around the world (Aiman et al 2016). Heavy metals can either affect the living things directly or indirectly. Therefore, heavy metals presence in water must be detected as early possible to anticipate the danger. Moreover, the heavy metals can accumulate in the body of an organism, including farmed fish commodities (Kamaruzzaman et al 2011). The fish product can be dangerous to human if it contains exceed heavy metals above the threshold of tolerance (Singh et al 2011).

The level of heavy metals in the river is relatively small; however, plant and animals can absorb and accumulate it biologically. Also they probably enter the food web (Govind & Madhuri 2014). Heavy metal pollution in the aquatic environment can be monitored by measuring its concentration in water, sediments and biota (Monroy et al 2014). Biotas accumulate the contaminants from the environment with enormous concentration (US EPA 2004). Among aquatic organisms, fish can be considered as one of the most significant bio-indicators of aquatic habitats condition (Findik & Cicek 2011). Fish are highly vulnerable to the presence of pollutants, and it considered as a key species with a high metal concentration exposure (Mahboob et al 2014).

Bioaccumulation is often defined as the accumulation of a chemical in an organism body with higher density comparing with the environment. This phenomenon is

associated with one of the most important properties of chemicals which include as a biological effect of the bioaccumulated chemicals. After entering the water body, metals are directly adsorbed in solid (sediments); remain soluble in water, or being absorbed by water biotas. The important point is the relation to biodiversity due to the tendency of the organism's ability to accumulate metals from the environment (Shukla et al 2007).
Copper (Cu) is included as a micro mineral because of its little amount of presence in the body. However, this metal enrolls in the physiological processes. In nature, it is found in the form of Cu sulfide (CuS) compounds. Although the human body needs it in small amounts, the excessive quantities lead to the health problem and toxicity (Fraga 2005). Milkfish (*Chanos chanos*) is a product of common fish farming in coastal areas. This fish is maintained in ponds near the coast in the tidal land. Its rearing requires clean and uncontaminated water. The quality of healthy environment will affect the quality of milkfish product.

Fishponds in Dukuh Tapak Tugurejo, Semarang are still using traditional farming systems employing the river and the sea water as the source of the aquatic environment. Therefore, there is a chance of heavy metal contamination from the water resources. Also, it can be accumulated in the water body and sediment in ponds. Martuti & Irsadi (2014) state that Cu content in the pond water in Dukuh Tapak was 0.001-0.007 mg L^{-1}, while the Tapak River, it was 0.013 to 0.037 mg L^{-1}.

Based on the problem identification explained, this study was conducted to determine the Cu content in water and sediments on milkfish rearing as well as the patterns of Cu accumulation in fish over a period of 60 days of growth in ponds.

Material and Method. This research was conducted during February to April 2015 in milkfish ponds in Dukuh Tapak, Tugurejo, Semarang, Indonesia. The site of study is shown in Figure 1. The sample in this study consisted of water, sediment, and milkfish body. To analyze Cu concentration, the samples were taken in time series (each time period was 15 days) within 60 days of research.

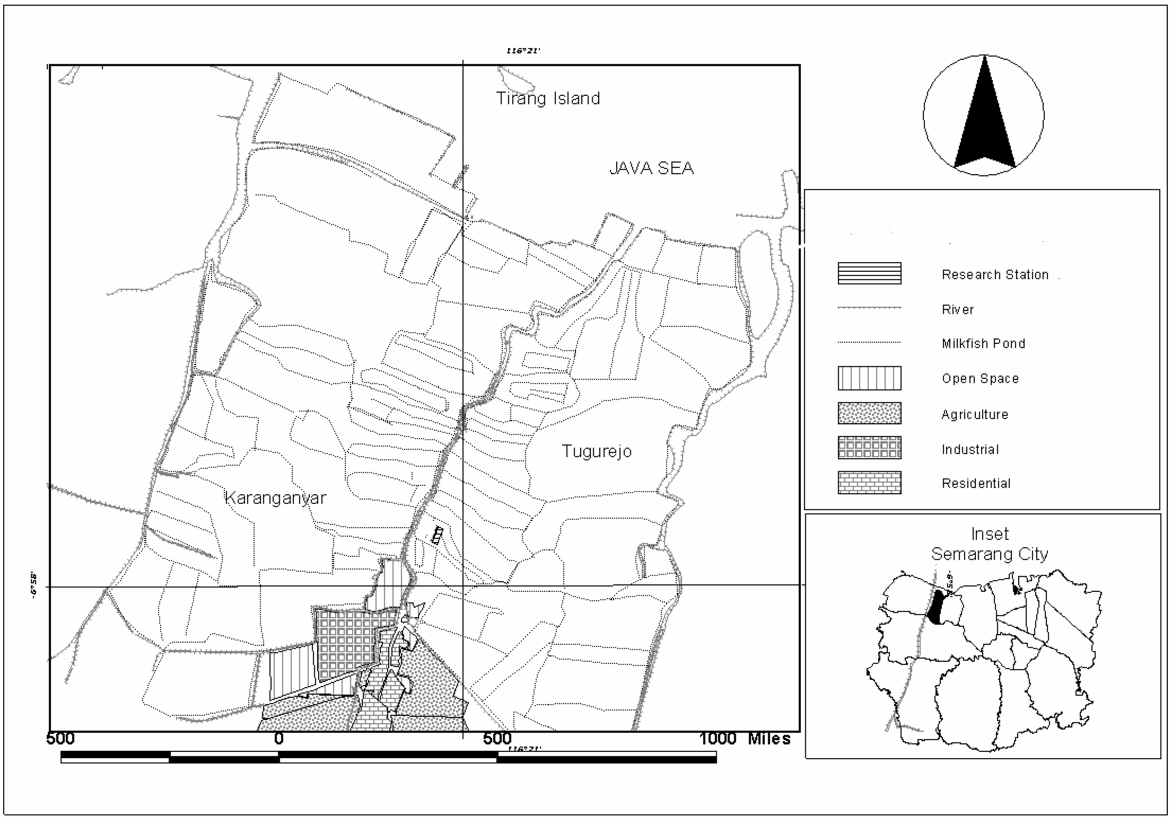

Figure 1. The Map of Tapak Region of Tugurejo, Semarang, Indonesia.

The study was started with a preliminary survey to ensure water quality and environmental parameters, and also the condition of the pond to be used as a place for fish rearing. Sampling was carried out on aquaculture ponds based on the designation for milkfish.

Sampling was done by random sampling to represent the condition of the pond. Water sampling was performed by taking 50 mL pond water for Cu analysis in the laboratory. Water samples were filtered with 0.45 mm Whatman GF/C filters to separate the suspended particulate matter. Subsequently, 0.5% (v/v) of HNO_3 was added to the filtered water for the sample precipitation. Next, sampling of pond sediment (50 g each in every time series) was conducted using a trowel in eight times of replication. Sediments taken were the part of the pond bottom to a depth of 10 cm. The sediment samples were dried at room temperature for at least 3 days. The dried solid sample then pulverized and sieved through a 1 mm stainless steel mesh. Milkfish samples were determined based on their size (±7 cm), and their age (±1.5 months). The milkfish was spread in the pond with a density of 10 fish m^{-2}. To analyze Cu concentration, 50 g of fish flesh were taken as the samples. Total of samples were 40. Then, the milkfish samples were rinsed repeatedly with de-ionized water to remove the dirt, and then cut into smaller pieces and dried at 110°C for 24 h. The dried samples were then grinded into powder form. Principally, a mixture of strong acids is used as media to oxidize the organic matter and sediments, which will release the metallic elements (Blekeer 2007). About 100 mg of dry sample of grinded roots was put in a destruction tube to which 2 mL of 4:1 (v/v) of 65% HNO_3:37% HCl concentrate were added. The mixture was hold for 15 minutes before further intense digestion using oven at 140°C for 7 h. Eight (8) mL de-ionized water was added into the digested sample in the destruction and then, it was mixed repeatedly and then poured into the polystyrene tube to keep at 4 °C prior the measurement. All samples of waters, sediments and milkfish were analyzed by atomic absorption spectrophotometer (Plus 932, Australia) to measure the concentration of Cu.

The obtained data were then measured for its Cu concentration factor (CF) in sediments and the concentration ratio (CR) as the ratio between the Cu level in milkfish with Cu in water and sediment. Based on the study results, the effect of Cu concentration in water and sediment and fish during the growth period (60 days) can be observed.

Results and Discussion. The concentration of Cu in pond water around Dukuh Tapak ranged between 0.2-0.3 mg L^{-1} as shown in Table 1. The results indicate that water-Cu concentration was above the threshold that has been determined by the Government. According to the Decree of the Minister of Environment-Indonesia No. 51 the year 2004 on Sea Water Quality Standard, it is stated that Cu for marine biota is at 0.008 mg L^{-1}. The result obtained in this study was higher than the results of research of Martuti & Irsadi (2014). They investigated that Cu in water of fishponds in Dukuh Tapak ranged between 0.001 to 0.007 mg L^{-1} and in Tapak River was between 0.005 to 0.037 mg L^{-1}.

Since the location of study site lies in the estuary region of Tapak River, Cu pollution in the pond water is possible. As proposed by Chaiyara et al (2013), the concentration of metals in water and sediments of an area depends on the presence of human activity on the upstream, such as mining, industrial, and residential activities. Dukuh Tapak is a region which is dominated by the settlements, agriculture and industries causing a potential risk of hazardous materials contamination including metals. The development of industry in the upstream part of Dukuh Tapak also has led to an increasing amount or volume of liquid waste and decreased the quality of river water. This condition causes the river water bodies contamination by wastewater from industrial activities. As presented by Semarang Environmental Division (Martuti & Irsadi 2014), Semarang, there are 14 industries located on the banks of Tapak River, which had a stake in determining the quality of the river. Those industries are engaged in the food, fish processing, ceramics, furniture, pharmaceuticals, wood processing, workshop and detergent The river was polluted by the industrial wastes; which give a negative impact to the aquaculture productivity in Dukuh Tapak.

During the study, it is revealed that Cu concentration in the sediment at the research site ranged from 18.09 to 22.81 mg Kg^{-1} (Table 1). According to the data in

Table 1, it can be seen that there is a declining trend of Cu concentration in the sediment since the first day of observation until the 60th day, i.e. 22.81 to 18.09 mg Kg^{-1}. This result proves that the sediment can absorb and accumulate Cu. The presence of metal in the sediment is highly dependent on water resources quality. According to Saifullah et al (2004), the concentration of heavy metal in mangrove ecosystem is concluded as consecutive decreases in the following order: sediments > root > stem > leaf > fruit > seawater. The concentration of heavy metals is higher in the smaller sediment particle than the sediment fraction; this is indicated by the high Mn, Cu, Zn and organic carbon content in the fine grain size fraction (< 63 µm). The presence of metal contamination in the sediments is limited to the total concentration of metals (Tam & Wong 2000).

The calculation concentration factor (CF) based on the metal content in sediments with metal content in pond water during the study period ranged from 623.72 to 966.85 (Table 1). It shows the ability of sediments to accumulate pollutants in the water body. Such results can occur through a process of accumulation of materials that do not dissolve in water, but it is absorbed in sediment hereinafter. Besides that, ponds are also stagnant pools, thus allowing the metal deposited under these conditions. Therefore, the sediment ability to accumulate metals from water lead to a filtration capacity of sediment which acts as a filter of pollutants in the pond water that comes from Kali Tapak River. As stated by Sany et al (2012), there is a close relationship between the metal in sediments with which dissolved in water. The presence of Cu concentrations in sediment was higher than in water because of the effect of metal deposition from water to sediment (Chaiyara et al 2013).

Table 1

Cu level in ponds water and sediment, and CF calculation of sediment in pond water

	Day				
	1	*15*	*30*	*45*	*60*
Sediment (mg Kg^{-1})	22.81	19.24	19.34	20.316	18.09
Water (mg L^{-1})	0.03	0.03	0.02	0.03	0.03
CF	760.33	663.31	966.85	677.2	623.72

The result of Cu in fish in ponds during the study period for 60 days shows that it tends to increase over time (Table 2). The concentration of Cu in fish ranged in between 1.17–3.40 mg Kg^{-1} (Table 2), it shows that the Cu content in fish flesh in this study was smaller than the threshold specified by BPOM No. 03 725/B/SK/VII/1989 of 20 mg Kg^{-1}. It means that the result of Cu concentration in fish is still safe for human consumption. However, the possibility of Cu accumulation nature in fish needs awareness since it can be dangerous to human. It is related to the process of biomagnification in human tissue to deposit the heavy metals. Also, human occupy the highest trophic levels of the food cycle, and it can be admitted that human will accumulate Cu higher compared to most fish (Gall et al 2015).

The process of bioaccumulation of heavy metals in fish could occur in the physical and biological or biochemical aspects. For example, the physical processes are initiated by the attachment of heavy metal compounds in the body parts, the surface of the body, gills and other membranes. Next, the biological process occurs through the food chain; it is possible for heavy metals absorption through water and food. It is automatically happened if the water is polluted, then it is suspected that the fish live in this aquatic environment will also be contaminated (Yi et al 2008). Metals will be carried away by the blood system and then it will be distributed into the tissues. As presented by Sole et al (2010), most of the aquatic organisms accumulate minerals through the process of eating and metabolic processes can lead to increased metal accumulation in body tissues. Furthermore, Venkatarmana et al (2012) expressed the accumulation of heavy metals in fish body occurs through the gills and from food. This is due to their metabolic activity, eating habits, ecological needs, pattern of life that makes the difference chemical element content of heavy metal that accumulates in fish.

Calculation of Concentration Ratio (CR) between fish and water media during the growing period was resulted in the range from 40 to 113, while CR between fish and sediments was in between of 0.06 to 0.19 (Table 2). These results indicate that the pattern of Cu accumulation is originated from water media to the body of fish. It is related to fish tend to live in the water column, and then it leads to a frequent contact with water. As can be seen in Figure 2, the chart shows elevated levels of Cu from time to time of the study, while the level of Cu in water is relatively constant. The figure clarifies the metal accumulation in fish. Accumulation of heavy metals may occur as direct bioconcentration from the water over the process of biomagnification through the food chain in aquatic animals (Cardwell et al 2013). It is important to be concerned that the metal in waters is associated with biodiversity, due to the ability of an organism to accumulate metals from the environment (Shukla et al 2007). Fish tend to have a great Cu concentration by accumulating it from water. The greater the value of Cu concentration in water, then the accumulation capacity in fish will increase. Total Cu concentration in the fish body is affected by the concentration of Cu in the organs of fish. Most Cu is accumulated in the muscles, skin and gonads (Yilmaz 2003).

Table 2

Cu concentration in water, sediment and fish body with their CR during the study period

	Day				
	1	*15*	*30*	*45*	*60*
Milkfish (mg Kg^{-1})	1.81	1.17	1.62	3.12	3.40
Water (mg L^{-1})	0.03	0.03	0.02	0.03	0.03
CR	60	40	81	104	113
Sediment (mg Kg^{-1})	22.81	19.24	19.34	20.32	18.09
CR	0.08	0.06	0.08	0.15	0.19

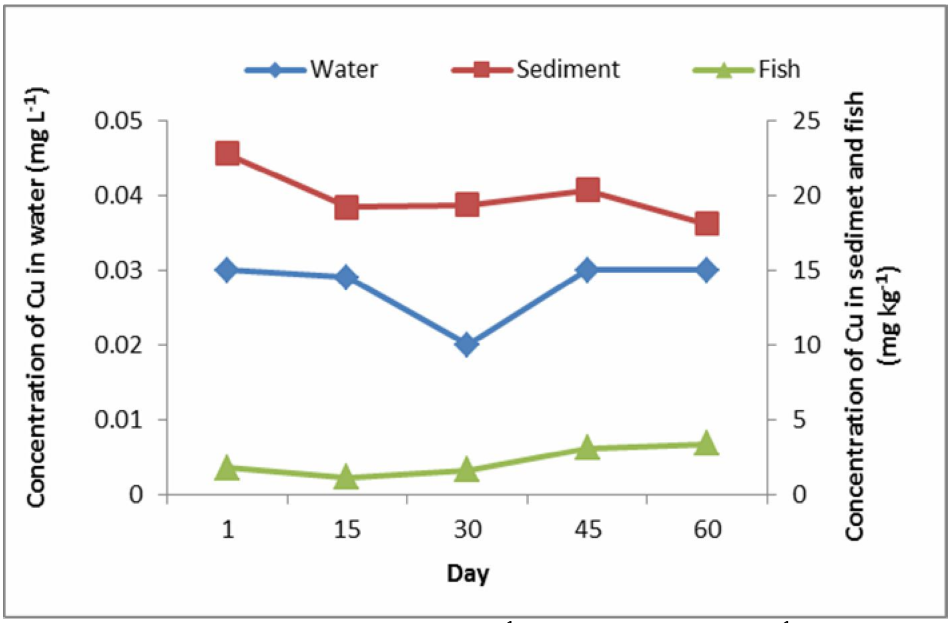

Figure 2. Concentration of Cu in water (mg L^{-1}), sediment (mg Kg^{-1}) and milkfish flesh (mg Kg^{-1}) in ponds.

Gill is the first organ to come in contact in the process of accumulation through the waterway (Shukla et al 2007). Acute toxicity of metals in fish is often characterised by damage to the gills and hyper secretion of mucus; subsequent mortality is associated with secondary physiological respiratory disorders that may interfere with ion balance and acid-alkaline state. The level of the physiological disorder depends on the absorption and accumulation in living organisms (Shukla et al 2007). The presence of anomalous behavior and subsequent death of the fish which exposed to toxic heavy metals (copper sulphate) can explain that the toxic effects are mediated through the nervous system is impaired. Moreover, it can affect almost all the vital activities of the organism, the dopaminergic pathway and related functions (Ezeonyejiaku et al 2011).

The fish body's ability to accumulate heavy metals in each tissue shows the significant differences (Chi et al 2007). The level of Cu accumulation in fish tissue showed five following order: gills > kidney > blood > liver > muscle (Shukla et al 2007). The bioaccumulation of heavy metals in fish may pose health risks to humans who consume it.

In fact, Cu is a micro mineral because of its small presence in the body. However, it is required for the physiological processes (Fraga 2005). In nature, Cu is found in the form of CuS compounds. Although the body needs a slight amount, the excess amount of Cu can be harmful to human health. Ashraf et al (2012) conveys that despite heavy metals analyzed in fish do not pose a health risk directly, but it is necessary to pay attention to their bioaccumulation process in humans who consume it. According to Newman & Mc Intosh (1991), the existence of Cu in fish is due to the metals in water, it does not cause any evasive action by fish. It is said that once the heavy metals flow into the body of the fish, then it will be transported to the whole part of the body via the bloodstream. However, it takes the long exposure of period to be accumulated in the tissues.

Conclusions. According to the study result, it can be concluded that Cu concentration in ponds water was ranged between 0.2-0.3 ppm, while in sediment was in between of 18.09-22.81 ppm. The fact also shows that the Cu in water was already above the threshold that has been stated by the Ministry of Environment No. 51 of 2004 on Sea Water Quality Standard. Therefore, a big attention on this issue is urgently needed to prevent a dreadful contamination of Cu in milkfish reared in Dukuh Tapak. Even though the concentration of Cu in milkfish tissues (1.17 to 3.40 ppm) is below the threshold permitted BPOM Indonesia No. 03 725/B/SK/VII/89, attention is still needed since the metal bioaccumulation ability in the human who consume it can be harmful to health.

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