Microelement Analysis In Edible Muscle Of Oreochromis Niloticus From Two Different Age Of Reclaimed Post Coal Mining Ponds East Kalimantan Using Sem-Edx

Sudrajat, Lariman, Rudi Kartika, Widha Prahastika

Abstract : This study aims to evaluate the ultrastructure of the muscle and bioaccumulation of heavy metal elements in Oreochromis niloticus muscle as a biomarker of environmental pollution in post-coal mining ponds. The fishes sample come from three different ponds, namely from the Control pond,Senong pit (5 years old), and Goldstar pit (10 years old). Heavy metal elements in fish meat were analyzed using Scanning Electron Microscopy combined with an Energy Dispersive tool X-ray (SEM-EDX). The results showed that the level of accumulation of heavy metals (K, Cu, Zn) in fish samples with the highest percentage of metal elements was Cu between 1.48-1.74%; Zn between 0,80-1.17% and K between 1.58-2.43%. Histological lesions in the muscle tissue of Tilapia, O.niloticus are in line with the accumulation of the metals. The levels of Cu, Cl, and K in muscle from Control are lower in longer ponds, highest Zn percentage in old ponds. These results provide evidence that microanalysis of metal elements in tissue with SEM-EDX and fish muscle ultrahistopathology as biomarkers can be used as an indicator of pollutant exposure in post-coal mine land waters. The findings of this study are expected to be used as consideration in the aspect of diagnostic methods in monitoring fish health status and quality of fish products originating from post-mining ponds.

Index Terms: Microelement Analysis, muscle, Oreochromis niloticus , Post Mining ponds

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1 INTRODUCTION

Open cut coal mining operations have become common practice over the last few decades in East Kalimantan Province Indonesia, as a method of extracting commercially useful coal found near the surface. Since backfilling is normally unfeasible practically or economically, an open-pit after completion of extraction operations is left. This is called a mine void after mine operations are discontinued and dewatering ceases. Most of those that extend below the natural groundwater table, fill by the inflow of groundwater, direct rainfall, and runoff from adjacent drainage basins and the void catchment. The natural filling may take many years to complete.

Subsidence ponds that have a water depth of <3 m and are suitable for aquaculture can be converted into aquaculture ponds. Fish is an important source of animal protein, which is cheap for humans that have very significant importance because of its economic value and sensitivity against pollutants. Aquaculture is defined as the production of aquatic organisms by the deliberate and controlled manipulation of their rates of growth, mortality, and reproduction, with the ultimate objective of harvesting products of commercial value. Aquaculture is one alternative for land reclamation and restoration after mining that could support the diversification of the economy in certain towns.

However, in its utilization activities as aquaculture ponds, the community is faced with health risks, namely the presence of high levels of heavy metals Fe, Al, Pb, Zu, Cd, and Zn in water and sediment [1]. Fish are at the top of the aquatic food chain and can accumulate high concentrations of heavy metals from water which can easily ascend through the food chain to higher organisms like human beings [2]. The persistence of heavy metals in the food chain and the difficulty of their elimination from the environment is the major problem [3] Some 'trace elements'e.g., Copper (Cu), selenium (Se), and zinc (Zn), are essential to maintain the body's metabolism, but they are toxic at higher concentrations. The heavy metals can enter the bodies to a small extent via food, drinking water, and water. The heavy metals concerned with environmental science chiefly include Pb, Hg, Cd, Cr, Cu, Zn, manganese (Mn), nickel (Ni), silver (Ag), etc. Further, heavy metals are metallic elements that have a relatively high density, and they are poisonous at a low quantity [4, 5]. Because fish are in an aquatic environment, they are very susceptible to pollution because they cannot escape the detrimental effects of pollutants in their environment. The success of fish to defend themselves is very much dependent on the proper regulation of absorption, intracellular compartments, and then the metal is translated to other parts of the organs [6]. The toxicity of these elements is because of its ability to oxidative stress and damage to living tissues in animals and humans furthermore, the accumulation of these elements can cause the intensive lesion to mucus tissues, affecting the intestine and skeletal [7]. The impact of heavy metal bioaccumulation in various fish organs with ultrastructural studies can play an important role in the diagnosis of fish health status caused by heavy metals [8,9]. Until now, there is very little information about the content of heavy metal elements in fish cultured from fish cages in post-mining ponds. According [10], the fish product can be dangerous to humans if it contains exceed heavy metals above the threshold of tolerance. Therefore, it is essential to assess heavy metals concentration in fishery products from Post Coal mine Ponds to guarantee that they

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are safe for human consumption. Owing to their capacity to store heavy metals and other organics, fish are believed to be a fascinating bio-indicator [11]. The SEM EDX microanalysis method can be used to analyze both semi-qualitative and semi-quantitative to detect environmental pollution and in the characterization of mineral bioaccumulated in the tissues. The advantage of SEM-EDX detection is its ability to morphologically and chemically analyze individual particles . This instrument can be considered as a useful tool in all works that require element determination, endogenous or exogenous, in the tissue, cell, or any other sample [12], enables to determine the elemental composition of the cell wall and to trace the distribution of metal ions on the surface of the cell [13]. This has prompted research on muscle ultra anatomy and to obtain information regarding the muscle of Nile tilapia in rearing in post-coal mining ponds and using electron microscopy in conjunction with X-ray microanalysis to determine the metal content of the muscle.

2. MATERIALS AND METHODS

2.1. Study Area

This research was carried out in two Post Coal Mine ponds of PT. Bukit Baiduri Energi is in the Pit Senong / TDSW C1C2 pond and the Goldstar Pit pond located in Bendang Village, Samarinda City, and Jongkang Village, Kutai Kartanegara Regency, East Kalimantan Province (Figure 1). This area is composed of the Balikpapan formation which consists of alternating quartz sandstones, silt clay, and shale with marl, limestone, and coal inserts, of Middle-Late Miocene age. The formation is covered inappropriately by the Kampung Baru Formation consisting of sandy claystone, quartz sandstone, siltstone, coal inserts, marl, limestone and lignite, late Miocene [14] .

2.2. Materials and Tools

The tools used are gill nets, surgical instruments, 50 ml measuring flask (Pyrex), oven (Memmert), filter paper, hot plate, desiccator, Erlenmeyer (Iwaki Pyrex), volumetric pipette (Pyrex), bulb, mortar, petri dish, and SEM combined with EDX.

2.3 .Sampling

The samples of tilapia O.niloticus cultivated in fish cages in post-coal mining ponds, included: Senong Pit Pond, GoldStar Pit, and Control. Fish samples measuring between 15-17 cm in total length were taken as many as 8 fish each from each cage. A particular weight from each sample had been taken of about 100 -150g and they were placed in the plastic box and clean and sterilized sacks for preventing the sample damage; particularly, the muscle samples which later placed in an icebox and transferred to a laboratory to be tested immediately. Take the muscles and then cut it into small pieces measuring 0.5cm x 0.5cm and dry it at 60^{O}C until the water content drops to 95%. Then the sample is powdered with a mortar and then stored in glass bottles.

2.4 Scanning Electron Microscopy (SEM-EDX)

Fish muscle powder was coated with a conductive material, namely a thin layer of palladium gold (20 nm thick). The specimens were studied under vacuum using a scanning electron microscope JED-2300 JEOL 6510 (LA) AT a voltage Acc. Voltage: 20.0 kV. The cleaned samples were placed between the glass slides and marked control slides as well as those from the aquaculture area in the former voids pond. The quantitative analysis of various elements present in the muscles of the control group as well as the affected fish were observed using the energy dispersive X-ray (EDX) microanalysis technique. The elemental composition and ultrastructural changes were determined by placing the "INCAx-act analyzer" scanner in the field of view of interest in the muscle. The X-ray spectrum of the specimen is then processed and analyzed to calculate the composition of the different elements in the sample.

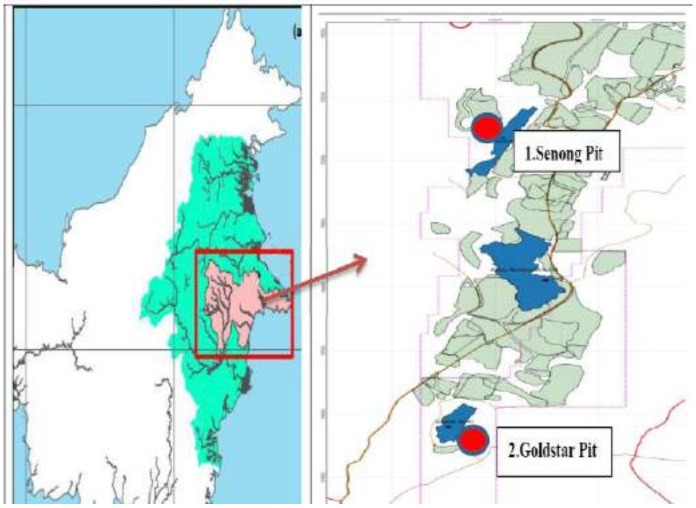

Fig. 1. *Location of the Study Area in East Kalimantan Province,Indonesia. Left. Borneo island, B. Red circle 1 Senong Pit 1 Post Mining Pond (5 years old) and red circle 2 is Goldstar Pit 2 Post Mining Pond (10 years old).*

3.1 Physical and chemical quality of water

The analysis results obtained from two post-mining ponds, namely, the Senong / TDSW C1C2 and Goldstar Pit are presented in Table 1.

Table 1. *Average Value of Water Quality Physics and Chemistry in Post Coal Mining Ponds in Samarinda,East Kalimantan Province*

No	Parameter	SI Unit	Location		Standard for Aquaculture*
			Pit Senong	Pit Goldstar	
1	Temperature	°C	32,0	32,7	Deviasi 3
2	Total Solid Dissolve	mg/L	218,7	337,3	1000
3	pH	-	8,24	7,39	6-9
4	Dissolve Oxygen	mg/L	4,72	4,73	4
5	NH_3-N	mg/L	0,44	0,35	
6	Cd	mg/L	<0,002	<0,002	0,01
7	Fe	mg/L	<0,003	<0,003	
8	Pb	mg/L	<0,003	<0,003	0,03

*Quality standard: East Kalimantan Provincial Regulations No. 02 of 2011 concerning Management of Water Quality and Water Pollution Control

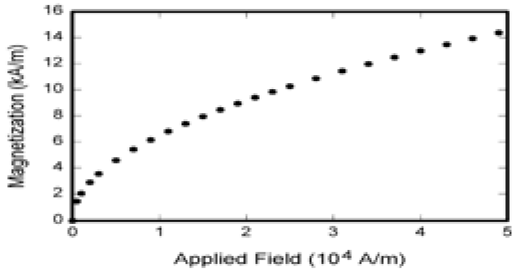

Fig. 1. Magnetization as a function of applied field. Note that "Fig." is abbreviated. There is a period after the figure number, followed by one space. It is good practice to briefly explain the significance of the figure in the caption.

3.2. Ultra fish muscle structure scan results by SEM

The SEM images of the samples are shown in Figure 2. The SEM image of the thin section shows a preparation layer with an average thickness of 50 µm. Based on Figures 2 a, b and c, we can see the difference in cell morphology in different fishes aquaculture at different ages of post-coal mine. As seen on Figure b and c cell looks shriveled if compared to control (Figure a) which look more hydrated and plump. This morphological change occurs due to the heavy ion adsorption process that happened in the cell wall during contact with their habitat. In habitat which older age, the morphological change become less shriveled.

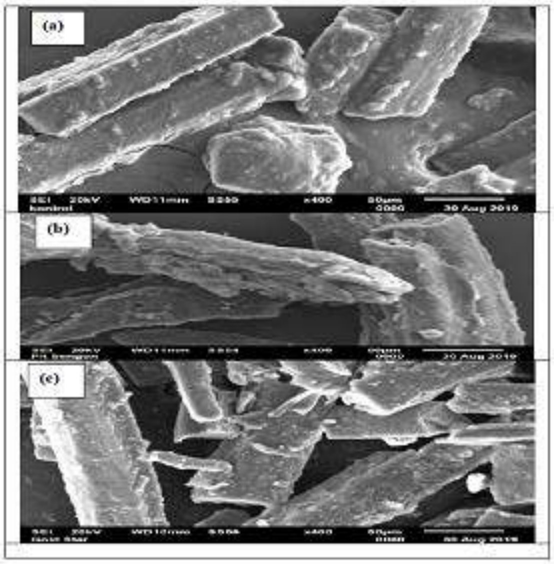

***Figure 2:** SEM Photo of Magnitude 400x*

Note: Control; b. Pit Senong is 5 years old Post-Mining and c.Pit Goldstar is 10 yeard old

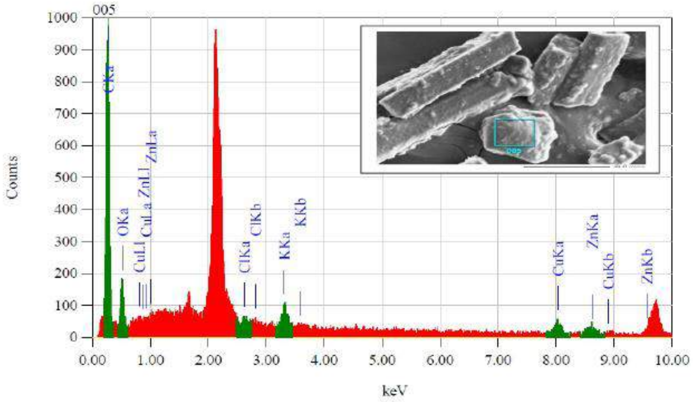

Figure 3. SEM and EDX microanalysis of the muscle tissue from *O. niloticus* Control.

3.3.Quantitative Evaluation of Minerals by Scanning Electron Microscopy

QEMSCAN is a scanning electron microscope (SEM) that provides rapid automated quantitative mineral analysis using Backscattered Electrons (BSE) and up to four energy dispersive X-ray (EDX) spectrometers. EDX devices that were integrated with SEM enabled qualitative morphology and chemical composition of samples containing ultrafine particles and minerals. EDX could be used to analyze quantitatively the percentage of content of each element. The results of EDX and mapping analysis on fish muscle (Figure 3, Fig. 4 and Fig.5). The results of the composition of muscle elements obtained from dispersion X-ray microanalysis (EDX) noted a slight difference in the weight percentage of Cu, Zn, and K metals in the sample(Figure 6). originating from post-mining pools with different post-mining ages compared to control samples. This indicates that metal is capable of accumulating on the surface of muscle tissue .

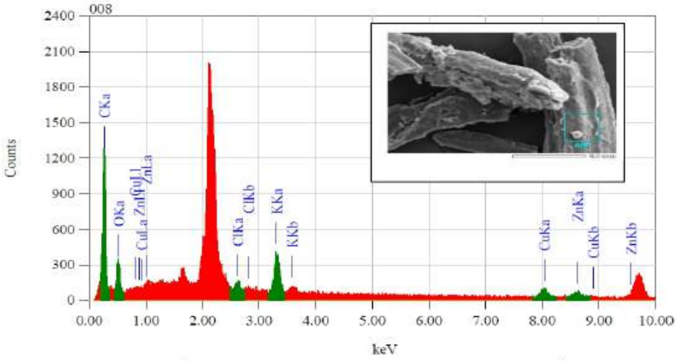

Figure 4. SEM and EDX microanalysis of the muscle tissue *O. niloticus* from Senong Post

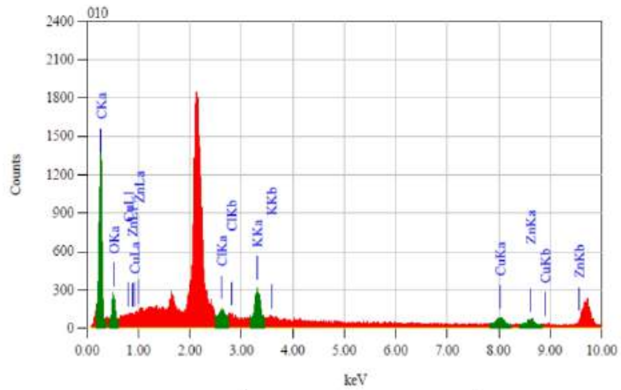

Figure 5. SEM and EDX microanalysis of the muscle tissue *O.niloticus* from Goldstar

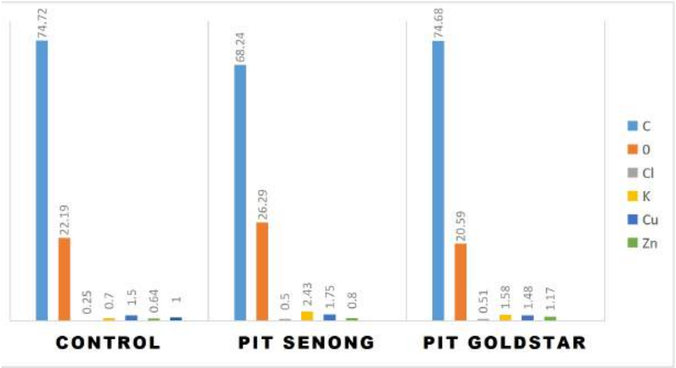

Figure 6: Atomic concentration of the elements (%) on the O.niloticus muscles of various ages of the Post Coal Mining poond

DISCUSSION

The results of this research have shown that the EDX-ray spectrum of the control group shows the essential elements commonly found in biological specimens, namely C = 74.72%, O = 22.19%, Cu = 1.50 as the dominant element and followed by K = 0.70%; Zn = 0.64%; Cl = 0.25% and not detecting of Cd, Pb and Hg. Elemental analysis spectrum shows appearance of Cu in weight percentage (1.50%) in muscle tissues following with low peaks of K and Zn. Fish from the pond 5 years after mining (Pit Senong) showed essential elements, namely C = 68.24%, O = 26.29%, K = 2.43%, Cu = 1.75, Zn = 0.80 and Cl = 0.50. Meanwhile, the 10 year old pool group (Pit GoldStars) was composed of C = 74.68%, O = 20.59%, K = 1.58%, Cu = 1.48, Zn = 1.17 and Cl = 0.51. Concerning the important role of muscle in nutrition and health can be selected as target organs. The present study of muscle as a target organ has shown the lowest concentration of metals. Being low in the concentration of heavy metals in the muscle by other researchers is reported that its reason is the presence of metallation in proteins responsible for removing and neutralizing the heavy elements and their toxic traces. Also, being low the concentration of heavy metals in the muscle rather than other tissues is because of this tissue's low metabolic activity [16]. This is in line with the results of research by[17] which reported that the highest levels of heavy metals were recorded in the intestine and the lowest was recorded in the muscles. The concentrations of Cu in the fish muscle were below the maximum permissible limit, however, Mn, Pb, and Zn exceeded the permissible limits.

All microelements can be classified into essential (biophilic) and non-essential. Biophilic microelements include a large group of metals (Fe, Co, Cu, Cr, Mn, Zn, etc.). They are functionally inherent in live organisms, where their concentrations can differ. However, if their concentration in the environment is high, they can be toxic for fish, and their accumulation in fish organisms can cause disturbances in some of their biochemical functions. Among Zinc (Zn) copper (Cu) and Iron (Fe) are essential for the body little, but the high amount of them create deleterious effects for creatures. Some trace elements that are known to perform functions essential to life include Mg, Ca, Mn, Co, Cu, Cr, and Zn. Others are extremely toxic (e.g. Cd, Pb, and Hg) and homeostatic mechanisms are necessary to control their levels within cells. The life of a living organism relies heavily on appropriate regulation of absorption, intracellular compartment, and then translocation of trace metals. Impact of the bioaccumulation of heavy metals in different organs of fish with ultrastructural studies can play an important role in the diagnosis of fish diseases caused by the heavy metals[8,18]. Based on the results of this study, it appears that the% w of the heavy metal atoms Cu, Zn, and K were detected in the fish meat molecules respectively for Cu between 1.48 to 1.75%; Zn 0.64 to 1.17% and K 0.70 to 2.43%. It appears that the highest Cu and K elements are found in fish muscles from Senong pond (5 years post-mining) and the lowest is in ponds over 10 years old, while Zn is mostly found in ponds 10 years post-mining. This confirms that heavy metals accumulated in fish muscles which is part of the public consumption. Zinc and copper are included in the group of essential trace elements required for maintaining cellular function and are integral components of numerous metal-containing enzymes. However, even essential metals depending on their concentration may exert beneficial or harmful effects on plant, animal, and human life. This is dangerous because unlike other pollutants (mainly organic), metals are not degraded or eliminated from the ecosystem. The metal ions, particularly the so-called heavy metal ions, such as mercury and lead, can be dangerous due to their toxic effects. The results of SEM EDX analysis showed that there were differences in the detection results, namely the elements of Pb, Cd, and Fe in tilapia meat were not detected. This is different from the AAS method which shows the accumulation of Cu, Zn[19] Pb, Cd, and Fe[20]. This difference in detection capacity occurs because in EDX it is only possible to detect elements. with an atomic number greater than 10. The minimum detectable elemental concentration, which requires several average signals, is about 0.1 mmol per kg of the dry specimen (ie, 10 ppm). The value given here for the detection limit refers to a biological sample whose average atomic number (which determines the intensity of the continuum) is generally quite low (e.g., C, H, N, Cl, P, K). Biological samples containing heavy metal elements provide a higher detection limit due to background. The distribution of heavy metals iron, manganese, zinc, and copper is distributed with different levels of accumulation in the tissues of fish living in lakes of Ińsko and Wisola, Northwestern Poland in the following ranges: Fe 0.8–240.6 ppm, Mn 0.2–8.4ppm, Zn 3.0–185.9 ppm, and Cu 0.14–7.76 ppm. The lowest level of metal is always detectable in muscle. The spleen, kidneys, and liver were found to accumulate the highest amount of Fe and other metals were found to have the highest levels as follows: Mn in the skin, gills, and gonads, Zn in the digestive tract and gills, Cu in the liver [21] This is closely related to the role of the heavy metals Mn, Fe, Cu, and Zn respectively, which are considered biologically essential [22]. Accumulation patterns of the heavy metals and trace elements in fish muscles followed the order: Fe> Zn> Cu>Mn>Pb>Cd (El-Bahri and Abdelghany, 2015). Microminerals (Cu, Fe, Mn, Se, and Zn) play an important role in the structural fraction of enzymes, in the formation of erythrocyte cells (Co, I, and Fe), in the regulation of glucose levels of activation of antioxidant enzymes (Mo), and maybe involved in the various processes of the immune system (Cu, Se, and Zn)[23]. Most of the trace elements present in fish are essential for human at low concentrations, however, high concentrations of these elements are toxic for human[4]. Based on the results of this study, we must be aware that the toxic effects of heavy metals in low levels are not immediately visible after several years because of their properties that tend to accumulate in living things. It is the nature of this accumulation that causes its effects to be even more dangerous for humans. When humans consume fish contaminated with metals continuously, there will be an accumulation or accumulation of these heavy metals in the body. Over time the levels of this heavy metal in the human body reach levels that cause poisoning that can endanger the health and even death.

4.Conclusions

In the tilapia O.niloticus muscles, originating from post-coal mining cage culture, heavy metal elements Cu was detected in the amount of 1.48 to 1.74%; Zn of 0.80 s.d. 1.17% and K of 1.58 s.d. 2.43%. The element composition of Cu and K was detected in a greater percentage in the 5 year old pond and on the other hand, the Zn element was greater in the 10 year old fish. The findings of this study have prospective as consideration in the aspect of monitoring fish health status and quality of fish products originating from post-mining ponds.

5.Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

6.Acknowledegements

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