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Water Quality and Histopathology of Climbing Perch (*Anabas testudineus* Bloch) at Cempaka Mining, South Kalimantan, Indonesia

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Abstract

The study on water quality and hispathology of climbing perch (*Anabas testudineus* Bloch) was conducted to analyze water condition, water quality determine the pollution index and to analyze the liver tissue of the fish in the area of diamond and gold mining areas. Samples were taken from three observation site. The analysis of water quality and fish's liver tissue were conducted in laboratory. Histology observation using the microtechnique method was conducted by preparing histological preparat of climbing perch. Based on classification of water quality, the parameter of alkalinity, mercury (Hg) in liver, Total Suspended Solid(TSS), Hg in worms, the depth level of site, ammonia (NH₃), Hg in snails, Dissolved Oxygen (DO), and pH were classified into Class B. The pollution index was between $1.0 < IP \leq 5.0$. Therefore, the area of Cempaka diamond and gold mining was mildly polluted. Tissue damage was around 50-80 %. There were severe level types such as inflammation, cloudy swelling, focal necrosis, karyolitic, hemorrhage, and hepatoma.

Keywords: Mercury pollution, Hispathology, Climbing perch

1. Background

The diamond and gold mining activities in Cempaka District have been started long time ago and became a legacy. The most popular mining activities can be found at Murung and Pumpung Village. Those places are tourism objects of diamond mining. Besides diamond, gold has been found in the area and refined using mercury (Hg). Hg was used to separate gold from fine sands and stones. The mixing process was used in water body or in the house. It was estimated that 5-10% of Hg through to the water during the process of mining activities (Lasut, *et al.*, 2010). The use of Hg has been applied at the mining area of North California USA, separating gold from alluvial soil (Saiki, *et al.*, 2010) and Buyat Bay Indonesia (Lasut, *et al.*, 2010).

Rising gold prices led the gold mining activities continue. The impact to the environment shown by the increase of trees cutting for land opening. Land opening in public area or conservation area threatens the biodiversity. The rehabilitation takes very long time (Howard, *et al.*, 2011; Hammond, *et al.*, 2007; Hilson & Vieira, 2007; Paterson & Heemskerk, 2001).

The mining process begins by digging hole which later turns into a lagoon mining pit. When it rains, the water will overflow and enter the lower area, enter the lagoon and swamp around the area. Local fish such as climbing perch that live in the swamp wandering into the lagoon to feed. When the change of seasons, conditions become dry and swamp water began to recede, fish were still looking for a place flooded and headed into the lagoon. Fish trapped in the lagoon during dry season, water temperature ranged from 32.40 to 33.80 °C. No fish were caught in June until September 2012. Climbing perch stress during the dry season as water temperature increased, to make climbing perch exposed to water containing Hg. Hg was found in the liver of climbing perch from 0.038 to 0.110 mgL⁻¹, either in the sediment, the worms, and snails. During the dry season, diamond and gold mining process at Cempaka have an influence on climbing perch and aquatic environments. Results of research on Argentina mining threatens biodiversity, one of the observation station showed toxicological effects on local fish because no fish Brown trout were caught in April 2002 and May 2006 (Carrola, *et al.*, 2009).

Water becomes yellowish dark caused by mining practices. If the water contains Hg, contaminated living things in the water will be consumed by human. The Hg concentration increases from time to time. It is because Hg is bioaccumulative in nature, therefore harms the environment and human (Shreadah, *et al.*, 2012; Mendil, *et al.*, 2010; Lasut, *et al.*, 2010; Iyabu, 2008; Shastri & Diwekar, 2008; Scheuhammer, *et al.*, 2007). Water quality with measurement in physics, chemical, and biological parameters has been a standard to determine water quality of an area. It is needed to know water condition, the change of water resource in the quality management and water pollution control. (Ahmed, 2004).

Histopathology analysis can be used as biomarker to investigate fish's health condition through change of liver structure which is the main target of the pollutant (Risjani, *et al.*, 2012; Reddy *et al.*, 2011; Olojo, 2005; Karadede, *et al.*, 2004). Fishes organ can accumulate heavy metal and contribute sensitive indicator from the aquatic pollution. Therefore, it is good to use fish to monitor a water environment. (Risjani, *et al.*, 2012; Reddy, *et al.*, 2011; Akter, *et al.*, 2008;

Belger & Rider Forsberg, 2006; Suchcharon & Lodenius, 1980). Organs which have been successfully used as the hispathology biomarker in the environment observation with Hg as the main target pollutant are gill, liver, and fish kidney (Campbell, 2010; Fernandez, *et al.*, 2008; Drevnick, *et al.*, 2008). Those organs are responsible for functionalizing vital activities such as respiration, excretion, accumulation, and poison biotransformation in fish. Based on score of semi quantitative scale, there are four categories of damage level: none, mild, moderate, and severe (Safahieh, *et al.*, 2012; Di Giulio & Hinton, 2008).

Liver is a vital organ which functions to detoxification and secrete the chemical material used in the digesting process, as well as in the metabolism and transformation of pollutant from the environment. Therefore, liver is an organ which mostly accumulate toxic and easy to be toxically contaminated. Some of the toxic which get into the body after being absorbed by cell will be carried through the liver porta vein to create liver damaged. The existence of Hg toxic can infect the structure of heart histology of yellowfin seabream (*Acanthopagrus latus*). It may cause liver pathology such as nuclear degeneration and vacuolation, oncotic, apoptic, focal, massive, cloudy swelling, and other necrosis order (Safahieh *et al.* 2012). The impact of heavy metal Cd to goldfish's liver (*Cyprinus carpio*) indicates degenerative change and necrosis during 30 days (Reddy, 2012). Morphology studies on liver bleak fishes (*Alburnus alburnus* L), rudd (*Scardinius erythrophthalmus* L), and perch (*Perca fluviatilis* L) in 'Student Kladenets' lake, Bulgaria, shows degenerative change, necrosis, and hyperemia (Velcheva, *et al.*, 2010). Liver histology of Ostariophysi, Characidae (*Oligosarcus jenynsii*) at Los Padres lake, Argentina, shows the same hepatocyte in teleost freshwater and seawater fish (Petcoff, *et al.*, 2006). The hispathology observation on the brown trout heart (*Salmo trutta f. fario*) in Tinhela river Portugal shows there is no pathology change (S₀), the parenchyma shape and stroma is clear. The change of toxipathic on S₂ and S₃ such as hyperplasia ductus bile, light dysplasia ephitel and adventitial fibrosis arm, the focus of hepatocyte is smaller and more basophilic and necrosis hepatocellular (Carrola, *et al.*, 2009).

Water quality and hispathology as a biomarker can be used to monitor water environment (Risjani, *et al.*, 2012; Kolawole, *et al.*, 2011). The study on inner organ such as fish's liver has an important function in body metabolism which later can be used as prediagnosis to the diagnosis of how damage on fish health starts. (Reddy, *et al.*, 2011; Fernandez, *et al.*, 2008). As the most popular fish in the area, therefore, it is important to monitor the water quality and climbing perch hispathology as biomarker in water environment at diamond and gold mining area at Cempaka District South Kalimantan as well as investigate the fish health through the organ (hispathology) and water quality in the living environment.

2. Materials and Methods

2.1 Study Site

The determination of sampling place was based on the areas which represent water environment around the mining area. The samples were taken from three site (Figure 1). Site selection was purposive, that certain places were considered important and represent the area sites study conditions. These sites were determined based on the following considerations:

- represents the mouth of the river/lagoon/swamp/pond in the area of artisanal

miners/traditional and human settlements, b) represents the mouth of the river/lagoon/swamp/pond around former PT Galuh Cempaka diamond and gold mine.

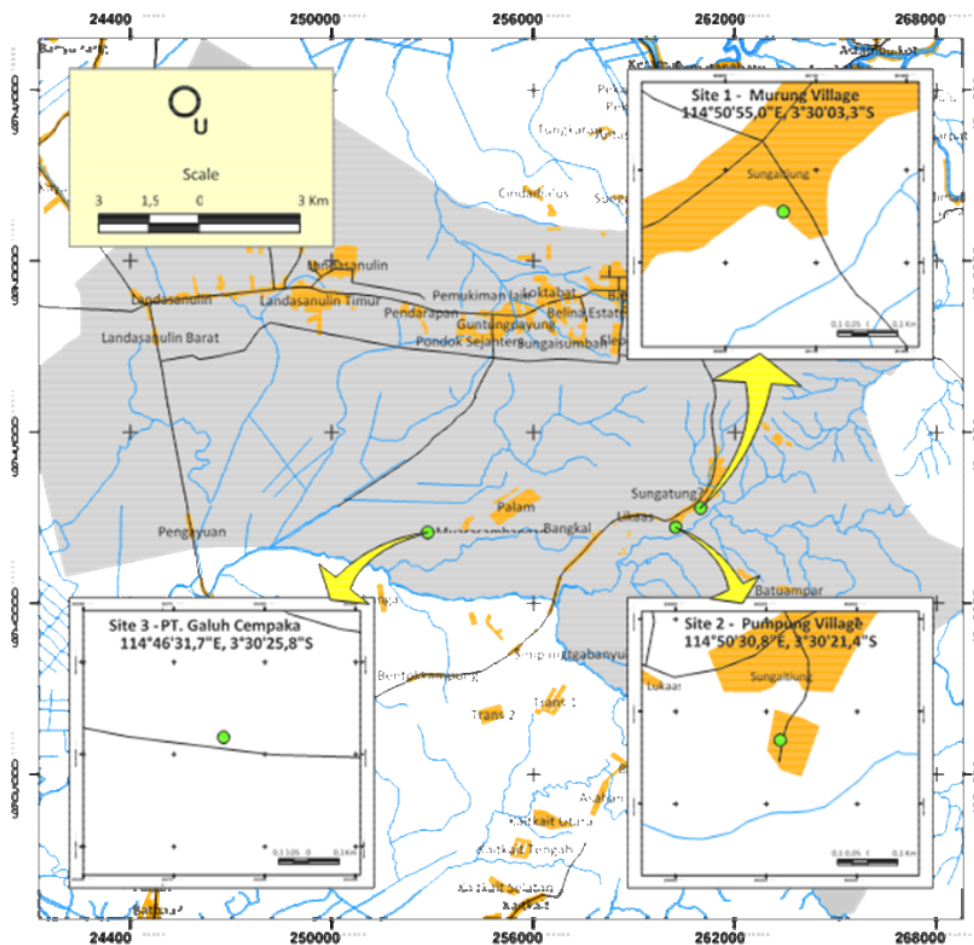


Figure 1. Sampling sites in Cempaka area, Site 1 located at Murung Village, Site 2 at Pumpung Village a traditional diamond and gold mining, Site 3 at PT Galuh Cempaka, a legal inactive diamond and gold mining

2.2 Physicochemical Parameters

Physical and chemical parameters were measured, such as temperature, TSS, total dissolved solid (TDS), transparency, depth, pH, DO, NH₃, alkalinity, biological oxygen demand (BOD), and Hg in water. Water samples were taken from three different spots. The sampling was done following the criteria of standard and water sampling method (APHA, 1998; Ademoroti,

1996). The sample was then brought to laboratory for further analyses. TDS and TSS were determined by filtration and evaporation method, transparency using secchi disk and depth was noted directly using roll-meter, while BOD was determined by Winkler titration method (Ademoroti, 1996; APHA 5210 B 2005).

2.3 Pollution Index

In order to determine water quality and pollution index, GRME Republic of Indonesia No. 115/2003 was used. The grading system was based on US EPA. Water quality status was determined by using Storet's method by comparing water quality data to water absolute quality matched with the result. The result of analysis on field was compared to the SKGR No. 04/2007 on the absolute quality of liquid waste, SKGR No. 05/2007 on the absolute water quality of river. MHR No. 416/Men.Kes/PER/IX/1990 on water quality criteria and monitoring, and GRR No. 85/2001 on water quality control and water pollution management.

Pollution index was used to determine level of water quality parameter towards water quality parameter allowed. The pollution index is around $0 \leq IP \leq 10$ with a criteria matched with the quality standard or good condition up to heavy polluted (GRME, No 115/2003). The determination of water quality status and pollutant index was as stated in GRME No. 115/1003 (in Appendix 2 on the criteria of water quality status determination). To determine the pollution index, mean of all amount was needed C_i/L_{ij} as the pollution standard point.

Pollution Index of water is the function of C_i/L_{ij} , which is:

$$IP_j = \sqrt{\frac{(C_i/L_{ij})M^2 + (C_i/L_{ij})R^2}{2}}$$

Where:

L_{ij} : Concentration of physicochemical parameters put on the standard of water denomination (j).

j : Standard of water denomination.

i : Parameter of water quality obtained from water sampling at certain location of sampling on certain river parameter.

C_i : Concentration of water quality parameter

$(C_i/L_{ij})R$: C_i/L_{ij} mean $(C_i/L_{ij})M$: (C_i/L_{ij}) maximum

2.4 Fish Collection and Mercury Analyzer

Samples of 100 of breeds climbing perch, 100 worms (*Lumbricus terrestris*), and 100 snails (*Pila globosa*) were obtained from river or swamp near the mining area. During sampling, the fishermen and local people helped. The tools used were pole line, cast net, hoe, and spade. Abdominal part of the fish was cut and its liver was taken. The liver, snails and worms were then analyzed in laboratory in order to investigate their Hg content. Determination of Hg found in fish liver, worms and snails used DMA – 80 Mercury Analyzer (US EPA method 7473).

2.5 Histology Procedure

Climbing perch which was considered as being polluted by Hg was taken and compiled. The cutting was then done to take out the liver. Histology observation using the microtechnique method was conducted by preparing histological preparat of the fish. In order to present the overall liver organ, each sample of liver organ was divided into three frontal parts, median, and distal. The procedure in creating the histological preparat was from Takashima and Hibiya (1995); Haschek *et al.*, (2010). They are fixation, dehydration, clearing, infiltration, embedding, sectioning, affixing, defarafinisation, staining, mounting, and labeling. Observation and calculation of the amount of liver organ cell in tissue was done with binocular microscope Olympus BX41 with 400 times of zooming in and photographing using digital camera Olympus CX21FS1.

There are four categories of damage level based on semi quantitative scale (Di Giulio & Hinton, 2008; Afshar, *et al.*, 2008; Safahieh, *et al.*, 2012). They are: (-) = none; (+) = mild (achieving 25% in 1 visual field); (++) = moderate (achieving 50 % in 1 visual field); and (+++) = severe (achieving 75% - 100% in 1 visual field). The percentage of organ damage was calculated based on the method used by Kim (2006) :

$$\text{Damage percentage} = \frac{\text{amount of cell damaged}}{\text{total amount of analysed cell}} \times 100 \%$$

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3. Results and Discussion

3.1 Water Quality

The measurement results of water quality in the area of Cempaka diamond and gold mining at three observation site are shown in Table 1.

From the approximate and average amount of parameter of physicochemical water quality, it is seen that there were differences pictured by the water condition near Cempaka diamond and gold mining area. The explanation of each water quality parameter as follows:

Temperature. Result of temperature measurement showed average temperature of 25.10-33.80 °C at the average of 29.12 °C (错误! 未找到引用源。). It is considered to be in the limit of absolute water quality based on SKGR No. 04/2007 on the absolute quality of liquid waste, SKGR No.5/2007 on absolute water quality of river and MHR No.416/1990 on

criteria of clean water quality.

Table 1. The average amount of physicochemical parameter and the status of liver tissue of climbing perch

Parameter	Min – Max	Mean SD	Tissue status		
			fish liver	worm	snail
Temperature (°C)	25.10 - 33.80	29.12 ± 4.42	visible	damaged	damaged
TSS (mgL ⁻¹)	62 – 143	107.67 ± 41.47	visible	damaged	damaged
TDS (mgL ⁻¹)	14 – 35	25 ± 10.53	visible	damaged	damaged
Transparency (cm)	3 – 50	21.33 ± 22.37	visible	damaged	damaged
Depth (m)	0.05 – 30	5	visible	damaged	damaged
pH	5.18 - 6.40	5.73 ± 0.53	visible	damaged	damaged
DO (mgL ⁻¹)	0.74 - 5.10	2.82 ± 2.11	visible	damaged	damaged
NH ₃ (mgL ⁻¹)	0.06 - 3.5	1.75 ± 1.65	visible	damaged	damaged
Alkalinity (mgL ⁻¹)	14.89 - 65.29	43.15 ± 25.75	visible	damaged	damaged
BOD (mgL ⁻¹)	5.50 - 16.00	9.97 ± 5.42	visible	damaged	damaged
Hg of water (µgL ⁻¹)	0.001 - 0.020	0.0005 ± 0.0820	visible	damaged	damaged
Hg of fish liver (mgL ⁻¹)	0.002 - 0.110	0.035 ± 0.039	visible	damaged	damaged
Hg of sediment (mgL ⁻¹)	0.001 - 0.005	0.003 ± 0.002	-	damaged	damaged
Hg of worm (mgL ⁻¹)	0.002 - 0.160	0.058 ± 0.063	damaged	damaged	damaged
Hg of snail (mgL ⁻¹)	0.0002 - 0.1759	0.091 ± 0.075	damaged	damaged	damaged

The optimum water temperature for tropical fish to grow is between 25-32 °C (Boyd & Lichkoppler, 1991). The first observation was conducted on dry season with high water temperature between 32.40-33.80 °C. Water was low and fish difficult to get because field and swamp were dry. The only water flows was brown and yellowish from the river. It was predicted that if fish swam to pit, they would be trapped for the whole dry season. However, climbing perch are able to adapt to living environment with high temperature and low O₂ content. As a result, they grow slowly. Mostly, the length of the fish body is 5.50-10.50 cm, and they weight 13.00-20.60 g. The size does not show balance between body length and weight.

In rainy season, where the temperature back to normal, fish could be found easily near the mining area. They were about 5.20- 14.20 cm and around 10.90-40.45 g in weight. It seemed that climbing perch grew during the beginning of rainy season. The world climate change will influence the temperature fluctuation in water and will affect growth, reproduction, age, and size of fish (Kuparinen, *et al.*, 2011). Temperature fluctuation may cause stress to goldfish (*Cyprinus carpio*) and increase cortisol secretion (Takahara, *et al.*, 2011).

Total Suspended Solid (TSS). Result showed that the TSS estimation was around 62-143 mg·L⁻¹ with an average of 107.67 mg·L⁻¹ (错误! 未找到引用源。). It reached the limit of water absolute quality based on SKGR No. 04/2007 on absolute quality of liquid waste and SKGR No. 05/2007 on absolute water quality of river.

TSS is needed for fish growth. The TSS needed is between 25-80 mg·L⁻¹ (Alabaster & Lloyd, 1980). It means that TSS in gold mining area is considered to be bad to support climbing perch growth and other fish's type's cultivation. If fish depend on vision to look for their food in water, the 1-100 mm colloid particles will block their sight. High turbidity may disturb respiration system, aquatic organism's vision, and osmoregulation (Effendi, 2003). Sun light penetration will be blocked if there are too many colloid particles in the water. Photosynthesis system will be slow which may decrease the O₂ content in water.

Total Dissolved Solid (TDS). TDS obtained from the laboratory analysis was between 42-35 mg·L⁻¹ with the average of 25 mg·L⁻¹ (错误!未找到引用源。). The result matched the limit of water absolute quality based on the SKGR No. 04/2007 on absolute quality of liquid waste, SKGR No.05/2007 on absolute water quality of river and MHR No. 416/1990 on the criteria of clean water quality.

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Water was yellowish brown and the bottom area could not be seen. It indicated that the level of TSS was higher than TDS. The high turbidity indicated that TSS, with colloidal solidity of 1-10 nm, was found more than TDS with colloidal <10 nm. The amount of TDS in water was influenced by rocks weathering, runoff, and anthropogenic such as domestic and industrial waste (Effendi, 2003). Dissolved solid consists of inorganic and organic substance, minerals, and also dissolved salts (Alabaster & Lloyd, 1980). The low amount of TDS indicated that the area of Cempaka gold mining lacked of organic and inorganic substance, minerals, and dissolved salts. However, TDS is not poisonous. The amount of TDS is needed by the living organism in the water and support climbing perch growth. If there is abundance of TDS amount, fish which depend fully on their vision to look for food will be disturbed, blocked by colloidal particles which are >10 nm in size. The sun light penetration will be blocked which slow down the photosynthesis process and decrease the O₂ content in the water.

Transparency. Transparency measurement predict water depth which allows the sunlight penetrated. The high level of turbidity will influence the level of water transparency. Transparency measurement used secchi disk, reading secchi disk in water and is influenced by phytoplankton and suspended solid in water. The more the amount of phytoplankton in water, the low the water transparency (Gianni, *et al*, 2012). Result of water transparency measurement and turbidity could be used to find out the assimilation process and the photosynthesis process. The result of measurement on field showed the transparency around 3-50 cm with 21.33 cm at average. The good water ecosystem for fish is a water which has ±45 cm of transparency (Boyd & Lichkoppler, 1991). Climbing perch could live at transparency measured at Cempaka diamond and gold mining area.

Depth. Depth measurement describes transparency level and temperature stratification level in water. There was no difference of temperature level at diamond and gold mining area because the measured depth was still reachable. It was about ± 0.05-30 m at average 5 m (错误!未找到引用源。). Depth of a water influences its temperature. The higher the water depth, the more the temperature level fluctuates (Alabaster & Llyod, 1980; Michael, 1984). Differences in water depth may cause thermal stratification under water. There were temperature stratification on top layer (epilimnion) of water surface with temperature of ±

30 °C in depth of 1-2.5 m, thermocline on temperature of 22-28 °C in depth of 1-1.5 m, hypolimnion in depth of 1.5-2.5 m on temperature of 20-21 °C (Effendi, 2003). At depth of 5-30 m there was probably temperature stratification. It was because water in pit was similar to lake water where there was water stratification in lake, and temperature difference between water surface and under water.

pH. Result of field measurement at Cempaka diamond and gold mining showed that pH was around 5.18-6.40 at the average of 5.73 (错误! 未找到引用源。). The result was considered not to match the limit of absolute quality of liquid waste based on SKGR No. 04/2007 on absolute quality of liquid waste and SKGR No. 05/2007 on absolute water quality of river and MHR No. 416/1990 on the criteria of clean water.

The result of filed measurement estimated that pH under water was acid. If pH is low or acid in a water, O₂ will decrease. Climbing perch can adapt to live in low content of O₂. Condition of pH may affect fish's appetite. At pH of 5.0-5.5, there will be a decrease of variability and composition of plankton types, periphyton, total abundance, zooplankton biomass, resistance of nitrification process, much benthos, and more filament of green alga (Effendi, 2003). Appropriate pH for living things under water is around 6.3-9.0. Usually, the average of 6.5-8.6 is good for fish to grow (Alabaster & Lloyd, 1980).

Dissolved Oxygen (DO). Result of dissolved O₂ measurement in water near Cempaka diamond and gold mining area was around 0.74 – 5.10 mg·L⁻¹ with average of 2.82 mg·L⁻¹ (错误! 未找到引用源。). The result was considered to be not appropriate with the limit of absolute water quality based on SKGR No.05/2007 on river water absolute quality.

O₂ content is defined as DO in a water needed by living organisms in the water. There was not much soluble O₂. The result of phytoplankton respiration and other water plants produced high O₂ and pH. Fish do not like water which lacks of O₂. Therefore, it was difficult to get fish because they have left the area to get a better habitat. In the end of the research (February-April), DO was around 4.40-5.10 mg·L⁻¹ meaning that it is 3 mg·L⁻¹, the water condition was getting better. Fish got in the water area of mining gold and they were starting to grow. Small fish was found. The size was around 5.50-10.50 cm and weighed about 13-20.60 g. This fact indicated that in the end of the research more fish and plants lived in the water near diamond and gold mining area. DO in water needed for fishes growth is around 3 mg·L⁻¹ (Boyd & Lichkoppler, 1991). Soluble O₂ content to support the life of aquatic biota should be about ±5 mg·L⁻¹ (Alabaster & Lloyd, 1980; Effendi, 2003). DO content in water which is considered good to support life of tropical fish should be at least 6 mg·L⁻¹ (OATA, 2008). In minimum, DO concentration in water is noted at 7 am, while the maximum content is noted at 5.30 pm (You, *et al.*, 2007; Cui, *et al.*, 2008).

Ammonia (NH₃). Result of NH₃ measurement in the area of gold mining was between 0.06-3.5 mg·L⁻¹ with an average of 1.75 mg·L⁻¹ (错误! 未找到引用源。). The result indicated that the content did not achieve the absolute limit of water quality based on SKGR No. 04/2007 on the absolute quality of liquid waste, SKGR No.05/2007 on the absolute water quality of river.

NH₃ and its salt are soluble in water. NH₃ comes from un-compounding of the organic- and inorganic-N. Organic-N is obtained from protein and urea, while inorganic-N found in soil and water is made of decomposition of organic material such as dead plants and aquatic biota. Rehabilitating process is called ammonification (Effendi, 2003). Measured NH₃ in water is total NH₃ (NH₃ and NH₄). Free NH₃ cannot be ionized, while ammonium (NH₄⁺) can be ionized. Free NH₃ which cannot be ionized tends to be toxic to aquatic organism. Level of poison of NH₃ to the aquatic organism will increase if DO decreases, pH, and temperature (Michael, 1984; Alabaster & Lloyd, 1980).

Tropical fish needs healthy water to live and should have NH₃ content around 0.02 mg·L⁻¹ (OATA, 2008). NH₃ content around 0.4-3.1 mg·L⁻¹ may cause death to fish (Boyd & Lickoppler, 1991). Death may happen when NH₃ concentration is about ±0.5 mg·L⁻¹ and the DO content is around 6 mg·L⁻¹. If DO content is ±0.2 mg·L⁻¹ and NH₃ content is around ±0.2 mg·L⁻¹, the environment will harm the fish and can cause death. Death on fish species varies, acute level of death is around 0.2-2.0 mg·L⁻¹ (Alabaster & Lloyd, 1980). Good environment for fish should contain 0.02 mg·L⁻¹ of free NH₃, 0.2 mg·L⁻¹ of NO₂ and 50 mg·L⁻¹ NO₃ in maximum (OATA, 2008).

Alkalinity. Result of alkalinity measurement in gold mining area was around 14.89-65.29 mg·L⁻¹ with an average of 43.15 mg·L⁻¹ (错误! 未找到引用源。). It meant that the alkalinity suited the criteria of good media which have 2-300 mg·L⁻¹ of alkalinity and hardness (Boyd, 1988). The optimum alkalinity in intensive fish cultivation is around 100-150 mg·L⁻¹ (Wedenmeyer, 1996). Much research report that there were negative correlations between alkalinity and Hg in fish tissue (Driscoll, *et al.*, 2007).

Alkalinity is considered to be a buffer capacity towards pH change in water (Davis & Masten, 2004). The higher the alkalinity, the higher the ability of water to buffer which therefore makes the pH fluctuation decrease. Mostly, in fresh water, alkalinity is formed by bicarbonate and carbonate which are around 72.4% (Effendi, 2003). Alkalinity is usually mentioned in mg·L⁻¹, while CaCO₃ is usually mentioned in CaCO₃. CaCO₃ is defined as substance which gives biggest contribution to the alkalinity and hardness in water. Big amount of CaCO₃ is found in soil. Therefore, the content in freshwater is considered to be high. The absorbance of CaCO₃ decreases as temperature and CO₂ increases (Effendi, 2003). Appropriate content of alkalinity in water should be around 30-500 mg·L⁻¹ CaCO₃. Alkalinity in natural water is ±40 mg·L⁻¹ (Boyd, 1988). Water which has alkalinity of >40 mg·L⁻¹ CaCO₃ is defined as hard water, while water which has <40 mg·L⁻¹ CaCO₃ of alkalinity is defined as soft water.

Biochemical Oxygen Demand (BOD). Result of Cempaka BOD was around 5.50-16.00 mg·L⁻¹ at average 9.97 mg·L⁻¹ (错误! 未找到引用源。). It was considered to match the limit of absolute quality material based on the SKGR No.04/2007 on absolute quality of liquid waste and SKGR No. 05/2007 on absolute water quality of river. BOD at Site 2 and Site 3 was under the limit of absolute water quality and suited the criteria of absolute river water quality. BOD at Site 1 was found to be above the limit of absolute quality of water and did not achieve the criteria of absolute water quality of river. BOD is some amount of O₂ needed by microorganism to decompose organic material. BOD measurement is needed to monitor

organic material which cannot be easily decomposed. Therefore, BOD is defined as organic pollution indicator (Mandal, *et al.*, 2010).

Mercury (Hg) of water. Result of Hg measurement was around 0.001-0.02 $\mu\text{g}\cdot\text{L}^{-1}$ with average of 0.0005 $\mu\text{g}\cdot\text{L}^{-1}$ (错误! 未找到引用源。) which was on the limit of absolute quality based on GRR No. 82/2001 on the management of water quality and water pollution control. Concentration of Hg at some gold mines in Canada showed about 0.002-0.00008 $\text{mg}\cdot\text{L}^{-1}$ of Hg which was still under the absolute quality of (NRC, 1996; 1998).

In majority, Hg in water is inorganic form around $\pm 95-99\%$ (Krabbenhoft, 1996). The form of ion from organic and inorganic salt is much found in the water. In the water, organic and inorganic ion will react with other substance or be absorbed by water plants and animal. Inorganic Hg is converted to methyl mercury (CH_3Hg^+) by microorganism. CH_3Hg^+ is a substance which is very poisonous and very absorbable to tissue.

Mercury (Hg) of sediment. Result of Hg sediment was around 0.001- 0.005 $\text{mg}\cdot\text{L}^{-1}$ with average 0.003 $\text{mg}\cdot\text{L}^{-1}$ (错误! 未找到引用源。). The amount of Hg in sediment, in order to support growth and life of living things, should not be more than 0.4 $\text{mg}\cdot\text{L}^{-1}$ (Turekian & Wedepohl, 1961). The amount of Hg in sediment at Cempaka gold mining area was still under the absolute quality of water. Generally, Hg in sediment does not have direct implication to human health as long as the sediment is not directly consumed by human. However, there is serious consequence if Hg enters the food chain (Nartey, *et al.*, 2011). Hg sediment in soil is around 40 – 200 $\text{ng}\cdot\text{g}^{-1}$, in form of Cinnabar (HgS). It is very reactive to environment (Davies, *et al.*, 1997). Hg measurement in the sediment was conducted to find out the existence of Hg naturally. It was considered that gold mining activity at Cempaka increased Hg content.

Mercury (Hg) of climbing perch. Bioaccumulation of Hg through food chain plays important part in deciding Hg capacity in fish body. It is because of the accumulative characteristics of Hg (Hall, *et al.*, 1997). The total of Hg in internal organ such as liver and the whole body of fish may vary among fish. Perhaps it represents physiological differences between different tropical groups (Altun, *et al.*, 2009). Generally, fishes was defined as bad environment contaminant and was being studied for the sake of food safety. Level of Hg on fresh water and sea water must be conducted as an intensive monitoring in order to protect human from Hg poisoning (Sary & Maryam, 2012).

The absorbed Hg from food enters tissue through blood. In soft tissue such as fish's liver and kidney, Hg will be detected (Hall, *et al.*, 1997). Hg would increase continuously because there was not much Hg metal secreted by system excretion of fish (Kalay dan Camli, 2000). The measurement of Hg content in climbing perch liver is around 0.002-0.110 $\text{mg}\cdot\text{L}^{-1}$ at average 0.035 $\text{mg}\cdot\text{L}^{-1}$ (错误! 未找到引用源。). Hg content in liver tissue of climbing perch Site 1 at Murung Village was 0.038 $\text{mg}\cdot\text{L}^{-1}$, while Hg content at Site 3 PT. Galuh Cempaka was 0.110 $\text{mg}\cdot\text{L}^{-1}$. The Hg content in fish and other aquatic organisms will be not more than 0.033 $\text{mg}\cdot\text{L}^{-1}$ (CCME, 2000). The measured Hg content lately has passed the allowed Hg content in aquatic biota. In other words, it is more than 0.033 $\text{mg}\cdot\text{L}^{-1}$.

Mercury (Hg) of worm. Hg content in worm was measured to see difference of Hg amount in vertebrate and invertebrate at gold mining area. The result of measurement at field showed that Hg content in worm was around 0.002-0.160 mg·L⁻¹ at average 0.058 mg·L⁻¹ (错误!未找到引用源。). Hg content in fish and other aquatic organisms will be appropriate if it is not more than 0.033 mg·L⁻¹ (CCME, 2000). Some of Hg content in worm, at Site 2, were under the limit of quality, while the rest, at Site 1, did not suit the allowed criteria. At Murung Village or Site 1, the Hg content in worm was about 0.057 and 0.160 mg·L⁻¹. It had passed the allowed amount which should not more than 0.033 mg·L⁻¹ (CCME, 2000). The monitoring of Hg content in worm body tissue is needed to be done. It is because there have been detected Hg content in worm's tissue lately. Worm was not found at Site 3 (PT Galuh Cempaka). Probably, water contained Hg or other heavy metal content which harmed the worms. As a consequence, there was not any worm found in soil. The only type of plant found around mining pit was *Fimbristylis globulosa*.

Mercury (Hg) of snail. Hg content in snail was measured to see the difference of Hg content in vertebrate (fish) and invertebrate (worm, snail) at gold mining area. The result of field measurement showed that Hg content in snail was around 0.013-0.176 mg·L⁻¹ at average 0.091 mg·L⁻¹ (错误!未找到引用源。). Hg content in fish and other aquatic organism should not more than 0.033 mg·L⁻¹ (CCME, 2000). Some of Hg content in snail at Site 2 were under the limit of absolute quality, while some at Site 1 and 3 did not suit the allowed criteria. Hg content in snail at Site 1 at Murung Village was about 0.079 mg·L⁻¹ and 0.169 mg·L⁻¹, while at Site 3, it was around 0.111-0.177 mg·L⁻¹. The amount was considered to be more than the allowed amount, which was more than 0.033 mg·L⁻¹. Hg monitoring in snail's tissue is needed to be done because there have been detected that Hg exists in worm's and snail's tissue.

3.2 Determination Pollution Index

Determination of water quality status can be done by discovering the pollution index towards physicochemical parameters. Pollution index from the measured quality at each site at field can be seen in Table 2.

Table 2. Pollution index at Cempaka mining area

No	Parameter	(C _i /L _{ij}) ² at Mean	(C _i /L _{ij}) ² Maximum	IP _{ij}
1.	Temperature (°C)	0,59	0,62	0,77
2	TDS (mg·L ⁻¹)	0,00	0,00	0,50 x 10 ⁻²
3	Hg of water (µg·L ⁻¹)	0,00	0,00	0,00
4	Hg of sediment (mg·L ⁻¹)	0,50 x 10 ⁻²	0,00	0,70 x 10 ⁻²
5	Transparency (cm)	0,09	0,25	0,42
6	BOD (mg·L ⁻¹)	0,69	1,96	1,30
7	Alkalinity (mg·L ⁻¹)	1,44	2,66	1,78
8	Hg of liver (mg·L ⁻¹)	2,34	6,11	2,04
9	TSS (mg·L ⁻¹)	2,22	6,15	2,55
10	Hg of worm (mg·L ⁻¹)	4,45	13,27	2,86
11	Depth (m)	39,78	76,88	3,19
12	NH ₃ (mg·L ⁻¹)	85,03	98,70	3,81

13	Hg of snail (mg·L ⁻¹)	11,97	27,37	4,24
14	DO (mg·L ⁻¹)	242,63	382,13	5,88
15	pH	129,95	180.47	6,42

BOD, alkalinity, and Hg in climbing perch liver, TSS, Hg in worm's body, depth level, NH₃, Hg in snail's body, DO, and pH based on classification of water quality status were included in class B in rate between $1.0 < IP \leq 5.0$ (错误! 未找到引用源。). The status of water quality at Cempaka gold mining area was mildly polluted. DO and pH in the range $5.0 < IP \leq 10$ with the criteria being moderately polluted. In the mining area, the water quality status of Cempaka diamond and gold IP, based on eight parameters measured, was mildly polluted.

3.3 Damage of Climbing Perch Liver Tissue

Observation result of liver hispathology on climbing perch showed that there were some damage such as inflammation (Figure 3), cloudy swelling (Figure 4), hemorrhage (Figure 2), focal necrosis and hepatoma (Figure 5). The identification result and damage level of climbing perch liver tissue can be seen in Table 3. Hispathology observation can be seen at Figure 2 to Figure 5.

Table 3. Identification and percentage of tissue damage on liver Climbing perch

No	Type of damage	Observation		
		1	2	3
1	Inflammation	+++	++	+
2	Cloudy swelling	+	++	+++
3	Focal necrosis	++	+	+
4	Karyolitic	+	+	+
5	Hemorrhage	-	+	-
6	Hepatoma	+++	-	-
Cell total		317	177	117
Percentage (%)		72,55	46,89	88,03

Notes: None (-), mild (+), moderate (++), severe (+++).

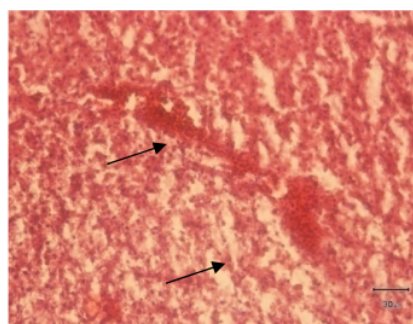
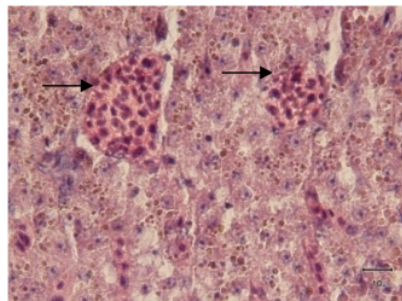
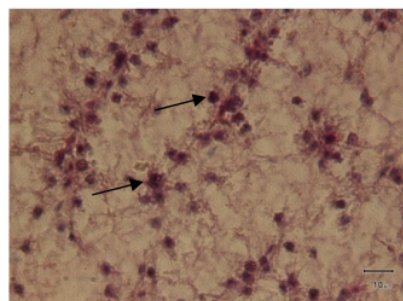


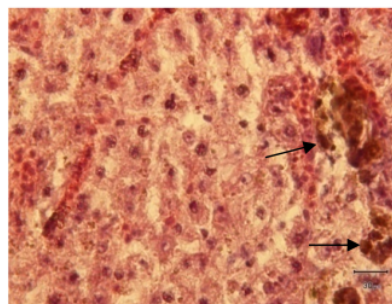
Figure 2. Section of the liver of climbing perch, showing hemorrhage (arrows). HE 400x



5
Figure 3. Section of the liver of climbing perch, showing inflammation (arrows). HE 400x



5
Figure 4. Section of the liver of climbing perch, showing cloudy swelling (arrows). HE 400x



5
Figure 5. Section of the liver of climbing perch, showing hepatoma (arrows). HE 400x

Hg content in climbing perch liver at Site 3 PT Galuh Cempaka was about 0.110 mgL^{-1} . It was measured without hispathology observation. Hg at Site 1, Murung Village, was about $0.038 \text{ mg}\cdot\text{L}^{-1}$. The content showed that Hg passed the allowed level. This was proved through hispathology observation by noting damage such as inflammation (Figure 3), cloudy swelling (Figure 4), focal necrosis, and karyolitic. Damage percentage in liver tissue of climbing perch was about 88.03% (at Site 3), while there was bad damage of 72.55% at Site 1. Damage on

climbing perch liver tissue can be seen as an indicator of water pollution which is caused by the increase of Hg in water, fishes live, and fishes liver tissue. This is the main reason of why liver, instead of other fishes organs, is always being an indicator of water (Reddy, *et al.*, 2012; Reddy, *et al.*, 2011; Pugazhvendan, 2009; Nicula, 2009).

Through hispathology observation, low concentration of Hg and high concentration in climbing perch liver showed physiological change which later may harms the tissue of fishes liver. Based on pollution index, Cempaka gold mining activity indicated mildly polluted. Damage of tissue was around 50-80% (Table 3). Generally, types of damage found were inflammation (Figure 3), cloudy swelling (Figure 4), focal necrosis, karyolytic, hemorrhagic (

Figure 2), and hepatoma (Figure 5) with severe of damage. Therefore, climbing perch liver could be applied as biomarker and an indicator of water pollution at Cempaka mining.

4. Conclusion

The finding showed that the water quality in Cempaka diamond and gold mining area was classified in Class B described by GRME Republic of Indonesia, No 115/2003. Status with level of pollution index around $0 \leq IP \leq 5.0$. Based on the pollution index status, the water quality at Cempaka diamond and gold mining area was mildly polluted with the tissue damage around 50-80%, which was considered as hard damage level.

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ORIGINALITY REPORT

11 %
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