Biosaintifika 10 (3) (2018) 597-604

Biosaintifika

Journal of Biology & Biology Education

http://journal.unnes.ac.id/nju/index.php/biosaintifika

The Correlation Between Heavy Metal and Nutrient Content in Plecostomus (*Pterygoplichthys pardalis*) from Ciliwung River in Jakarta

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DOI: http://dx.doi.org/10.15294/biosaintifika.v10i3.16248

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History Article

Received 5 October 2018
Approved 19 November 2018
Published 31 December 2018

Keywords

Plecostomus; Fat; Protein; Ciliwung River; X-Ray Fluoresence (XRF) Spectrometer

Abstract

Most of the rivers' quality in Indonesia is in highly-polluted status including Ciliwung River in Jakarta. The pollution can affect river ecosystem especially the rivers biota such as plecostomus species that is mostly found in Ciliwung River. The information about correlation between heavy metal and nutrition content in Plecostomus flesh still limited, so the result from this research can give the new information about that. The aims of this research is identifying the effect of the heavy metals towards the contents of protein and fat of plecostomus flesh from Ciliwung River. The analyses methods use X-Ray Fluoresence (XRF) Spectrometer, the analysis of protein using kjeldahl method, and fat analysis using extraction method. The result of this research showed that small-sized plecostomus contained the highest fat and protein compared to medium-sized and big-sized plecostomus. The assumption was the contents of heavy metal which can impede protein and fat absorption. Meanwhile, the content of carbohydrate in small-sized plecostomus was the lowest. The identified type of heavy metal using XRF method was Pb, Hg and Cd with the concentration was higher than the maximum standard (SNI) so that the flesh of plecostomus from Ciliwung River in Jakarta is categorized unsuitable to consumption

How to Cite

Elfidasari, D., Ismi, L. N., Shabira, A. P., & Sugoro, I. (2018). The Correlation Between Heavy Metal and Nutrient Content in Plecostomus (*Pterygoplichthys pardalis*) from Ciliwung River in Jakarta. *Biosaintifika: Journal of Biology & Biology Education*, 10(3), 597-604.

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p-ISSN 2085-191X
e-ISSN 2338-7610

INTRODUCTION

Ciliwung River is one of the streaming rivers in DKI Jakarta province. The length of the revier is from upstream to downstream is 117 km. Its stream is from Pangrango Mountain's sring, in West Java. The quality of Ciliwung River currently is heavily polluted at all segments, from the upstream (Puncak, Kab. Bogor) up to downstream (DKI Jakarta) so that Ciliwung River is not good for doing activities. Along Ciliwung River, there are developing industries such as food industries, publishing houses, textile industries, transportation industries, metal, glasses, trades, fishery, animal husbandries, and farming. The wastes from the industries that are not processed properly will be the pollutants for Ciliwung River. The pollution can affect ecosystem especially for the river biota such as fish population in Ciliwung River. The pollution is dominated by domestic wastes marked by the high concentration level of BOD-COD, ammonia, phosphat, detergent, and E Coli bacteria (Yudo, 2010).

There are at least 20 species of fresh water fish live in Ciliwung River, Jakarta. It is dominated by plecostomus. Plecostomus is a fish originated from South America. This kind of species, spread to all areas including Indonesia. Plecostomus has an arrow-like body and layered by hard scales in black colour (Armbruster & Page, 2006). Plecostomus can adapt well to extreme environment so that plecostomus becomes dominant in Ciliwung River, Jakarta (Ploeg, 2008).

Currently, plecostomus from Ciliwung River is mostly used as the main material for food products such as dimsum, *otak-otak, empek-empek*, and fish chips for economical reason (Nurjannah, Nitibaskara, & Madiah, 2005). However, until now, there is limited information about the nutrition facts inside plecostomus. Generally, fish is categorized as a source of animal nutritions which have better nutrition values compared to other sources of animal nutritions. That is because the total amount of saturated fat within the fish is lower than animals. Besides, fish has higher protein. Protein inside fish is made of much essential amino acid such as leucine, which is good for our body.

Since there is no nutrition facts data in plecostomus has become the fundamental issue to conduct the research to identify the contents of protein, fat, and carbohydrate in plecostomus from Ciliwung River. Additionally, until now there is no information telling the effect of metal contents towards protein, fat, and carbohydrate in plecostomus. Therefore, there needs to be research and analysis that are able to identify the effect of metal level in protein, fat, and carbohydrate in plecostomus. It is expected that this research is significant as the source of information in relation to the feasibility of plecostomus from Ciliwung River as the source of food for people.

METHODS

Samples Collection Location

The plecostomus fish were collected from Ciliwung River, Jakarta, exactly at Jln. Inpeksi Ciliwung Letjen MT. Haryono Gg. Ciliwung, Cawang, East Jakarta (Figure 1). The samples collection were conducted from 26 August-22 September 2017. Plecostomus were caught using nets and were put into containers.

Figure 1. The samples collection location with the coordinate of 6^{0}14'36.50"S and 106^{0}51'45.03"E

Preparation of Plecostomus Samples

This research was conducted on 21 February-16 March 2018 at the Biology Laboratory and PTBGN Laboratory, Nuclear Energy National Agency (BATAN). The morphometric measurement was conducted including the total length and weight of plecostomus.

The Analysis of Metal Contents in Plecostomus Flesh

The dried flesh samples were filtered ± 100 mesh and scaled for 5 grams. The analysed samples were in the form of press powder. After that, metal content analysis was conducted to the samples using *X-Ray Fluoresence* (XRF) *Spectrometer* employing GeoChemPellet *2* method. The data were gained from the test analysis result using XRF both in qualitative and quantitative analyses.

Protein Contents Analysis

The protein contents in plecostomus samples from Ciliwung River were tested using kjeldahl method as in the following: for about 0.5 g was put into kjeldahl and added with concentrated H2SO4 and selenium. Then, it was heated to create colour change into greenish clear. After the extract was cooled, it was homogenized using distilled water, put into distillation flask, and distilled using NaOH 15%. The distilled was put into Erlenmeyer containing HCl and red methyl, and titrated using. The final point of titration was shown as yellow colour.

Fat Contents Analysis

Plecostomus flesh from Ciliwung River was tested its fat contents using extraction method as in the following: filtration paper to cover the samples was weighed (w). About 0.2 g (w2) of samples were wrapped using filtration paper and extracted using soxhlet for 3 hours using petroleum ether solvent. The extraction result was weighed using analytical scale (w1). The protein content was identified using the following calculation:

Fat content (%) = × 100%

Analysis of Carbohydrate Content

Plecostomus from Ciliwung River was tested its carbohydrate content using *by difference* method. Before calculating its carbohydrate content, the ash content inside the fish were analysed beforehand. The ash content analysis was conducted using dry-ash method as in the following: porcelain cup was put into microwave with the temperature of 105^{0}C for 30 minutes, then weighed (w1). About 0.2 g (w) samples were put into porcelain cups that had been weighed on to furnace burner around 600^{0}C for five hours. Furnace was turned off and waited for its temperature to reduce up to < 250^{0}C, then the porcelain cups on the furnace was weighed using analytical scale (w2). The ashes content was identified using the following calculation:

Ashes content (%) = × 100%

Carbohydrate content on the samples was calculated by adding the total amount of protein, fat, and ashes. The carbohydrate content was calculated using the following formula:

Carbohydrate content (%) = 100% - (protein + fat + ashes) %

Data Analysis

The obtained data were analysed using *ANOVA* test using SPSS version 17 and SPSS version 19 *correlation two nails*.

RESULTS AND DISCUSSIONS

The Concentration of Heavy Metal in Plecostomus

The correlation analysis results between the average weight of plecostomus based on the different size of its body and the concentration of heavy metal show negative correlation value (Table 1). It means the smaller Plecostomus have the higher heavy metal concentration (Pb, Hg and Cd). The safe limit of Pb in fishery and meat products according to the National Standard of Indonesia (SNI) is ≤ 0.3 mg/kg and ≤ 1 mg/kg. Meanwhile, the safe limit of Hg in fishery and meat products is ≤ 0.5 mg/kg and ≤ 0.03 mg/kg. The safe limit of Cd in fishery and meat products is ≤ 0.1 mg/kg and ≤ 0.3 mg/kg. The heavy metal concentration value of Pb, Hg, and Cd obtained from the three categories of plecostomus flesh in this research are beyond the safe limit to consume. Therefore, Plecostomus from Ciliwung's river is unsuitable to consumption (BSN 2009).

Table 1. Correlation value between average weight of plecostomus flesh and heavy metal concentration of Pb, Hg and Cd.

Size	Heavy Metal Concentration (ppm)		
	Pb	Hg	Cd
Small	3.6± 0.3	1.4 ± 0.3	0.6 ± 0.1
Medium	2.7 ± 0.3	0.8 ± 0.3	<0.5 ± -0.2
Large	2.2 ± 0.3	0.3 ± 0.3	<0.5 ± -0.2
Correlation	-1.000	-1.000	-0.816

Physiologically, flesh is not the appropriate part to accumulate heavy metal. Heavy metal which goes inside the fish body will undergo dilution process during growth process. This causes the increase of heavy metal in fish body will reduce as the body grows (Canli & Atli, 2003).The metabolism speed will decrease as the body grows, so that the addition of heavy metal is smaller compared to the increase of size and weight of the fish (Nurrachmi & Amin, 2010). The high and low concentration level of heavy metal within an organism is caused by some factors such as the difference between growth speed, metabolism speed, body sensitivity level towards certain heavy metal insertion, and the physiological need of metals (Leung & Soter, 2011).

The existence of metals inside plecostomus occurs due to accumulation of metals inside the body. The metal goes inside the body through direct and indirect mechanisms. The direct mechanism occurs through the metal absorption when fish absorb water and nutrients inside their body. Indirect mechanism occurs through food chain. Along the food chain, the transfer between material and energy from the hunting organisms occurred (Puspasari, 2006). The existence of heavy metal in the ocean triggers the accumulation inside organisms' bodies. The accumulation of heavy metal is hydrophobic inside organisms' body can occur through the water and food or this process is called bioaccumulation. Bioaccumulation of heavy metal is an example of negative effect of pollutant in an ecosystem. Plecostomus food containing negative effects is the organisms inside Ciliwung River, such as phytoplankton and zooplankton, or small fish (Van der Oost, Beyer, & Vermeulen, 2003).

The main pollutants in river are from organic and inorganic wastes. Organic waste is from houses, while inorganic waste is from industrial process. Inorganic waste contains poisonous materials and can endanger the life of river biota. Poisonous materials are such as whitening materials, textile colouring materials, and heavy metals (Aksari, Perwitasari, & Butet, 2015). Textile, metal, and glass industries located in Jakarta have become the main sources of Lead in Ciliwung River and have been affected by social and economic conditions of the people living along the river stream area of Ciliwung River (Eneji, 'Ato, & Annune, 2011).

According to (Fadhillah, Rinidar, & Armansyah, 2017), heavy metal concentration of Lead can be affected by the fish body size. The larger and heavier the body is, the higher concentration of Lead will be. Heavy metals can move from one level of trophic to another. This shows the increase of the thickness of creatures based on their trophic level which is called bio magnification. This causes that the larger size of the fish is can have more contents of heavy metal than smaller-sized fish. Therefore, the heavy metal concentration in fish flesh which is based on size cannot become the measurement of determining the high and low of heavy metal concentration, including plecostomus (Simbolon, Simage, Maxwel, & Jusadi, 2010).

Protein, Fat, and Carbohydrate Contents of Plecostomus Flesh

The ANOVA test conducted shows significant value of $p = 0.619$ meaning that the protein content inside smaller-sized fish is not far different from protein content inside medium-sized and larger-sized fish (Table 2). Plecostomus belongs to fresh water fish with high protein since the protein is > 20% (Nurjannah, Nitibaskara, & Madiah, 2005). The longer the body is, the higher protein inside the body will be. This is because the bigger the body is the more ability the fish to optimally synthesize protein inside its body. Nevertheless, in this research, plecostomus decreased its ability of protein content as the body grew longer which was caused by heavy metal contents of pollutants in Ciliwung River. The presence of heavy metals, especially Lead can disturb the function of liver of the fish since liver is the susceptible towards chemical contents (toxics) (Aksari, Perwitasari, & Butet, 2015). Based on the ANOVA test using *Duncan* examination, it was gained a significant value of $p = 0.974$ showing that fat contents inside smaller-sozed, medium-sized, and larger-sized plecostomus was not far different (Table 2). The indifferent result was caused since the significant value of $p > 0.05$ meaning the length of the body does not influence the fat contents inside the flesh. Plecostomus from Ciliwung River is categorized as low-fat fish for < 5%.. Plecostomus in this research had the tendency of decreasing amount of fat content compared to its body growth.

Table 2. The Result of ANOVA test of Nutrition Contents in Plecostomus.

Nutrition Contents	Significant Value
Protein	0.619
Fat	0.974
Ash	0.349
Carbohydrate	0.544

The result of ANOVA test of ash and carbohydrate contents in plecostomus shows significant value $p = 0.349$ dan 0.544 (**Table 2**). It means that there is no difference in ash and carbohydrate contents between small-sized, medium-sized, larger-sized plecostomus. The significant value of ANOVA analysis < 0.05 shows that the size of plecostomus body does not affect the ash and carbohydrate contents inside the flesh. In this research, ash content decreased respectively from smaller-sized to larger-sized plecostomus. The decrease is related to the growth phase in smaller-sized plecostomus.

The Correlation between Metal Contents and Protein, Fat, and Carbohydrate Values in Plecostomus

The correlation analysis result of metal contents towards protein, fat, and carbohydrate contents in plecostomus shows that the heavy metals of Pb, Cd and Hg have high correlation value towards protein value (Figure 1). The correlation of heavy metals of Pb, Cd and Hg towards carbohydrate value shows the lowest correlation value compared to protein and fat. This shows that the protein synthesis inside the fish body is influenced by the presence of heavy metals. Meanwhile, the fat and carbohydrate syntheses are less influenced by the heavy metal contents of Pb, Cd and Hg. Therefore, the presence of heavy metals will influence protein content within the fish body due to the impediment of nutrients absorption inside the fish body (Aksari, Perwitasari, & Butet, 2015).

The statistic result shows that Pb is the heavy metal that most influences nutrients in Plecostomus (Figure 1). Besides that, from the results obtained, Lead is the mostly found inside the body than other heavy metals (Table 1). Lead can influence organic materials inside the waste water and create complex compounds. The complex compounds of Lead are generally absorbed by the fish through its *tractus digestivus*. It is caused by the accumulation process of heavy metals through the food. The soft epithelium cell in small intestines absorb Lead before the Lead settles in the liver through liver porta vein. One of the toxicity mechanisms of metal ion is holding back the biological function groups which are essential in biomolecule, such as protein and enzymes (Fadhillah, Rinidar, & Armansyah, 2017). Plecostomus that are exposed by heavy metal of Pb are not dead, but having sub lethal effects. Sub lethal effects can influence the metabolisms of a compound. Pb is a cumulative toxic. It means that the toxicity characteristic of Pb will be present if it is accumulated in the bigger amount inside an organism (Hou, *et al.*, 2013).

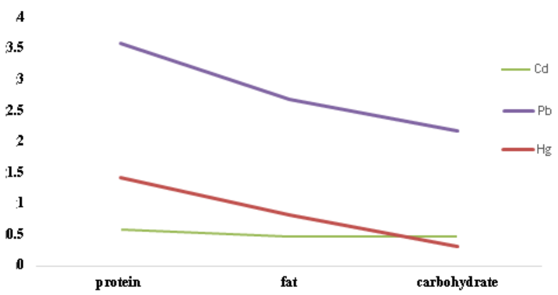

Figure 2. The Correlation between Heavy Metals of Pb, Cd and Hg towards Protein, Fat, and Carbohydrate.

The insertion of heavy metals into plecostomus body can be started from its gills, and spread to all over the body through blood, and are accumulated in liver. Liver detoxifies heavy metals by synthesizing *metallotionein*. Metallotionein is an additive protein which takes a role in homeostatic process to tolerate the presence of heavy metals and is formed by the result of binding of thionein protein (Dewi, 2014). The study was conducted on goldfish and parrot fish that the ability of each fish will accumulate different heavy metals. However, in the results of the research (Dewi, 2014), showed that the heavy metals contained in fish liver had undergone bioconsentration and bioaccumulation. The combination between heavy metals such as Lead and protein tissues can create *metallotionein*. Later, Lead that is combined with protein and create *metallotionein* will be sent by blood. However, the heavy metal high concentration makes Lead uneasy to create *metallotionein*. The high value of heavy metals also make them uneasy to be detoxified by liver. The time needed by the liver for detoxification process of heavy metals is quite longer, thus, the damage of tissues in livers will be much bigger than other organs (Odiko & Obirenfoju, 2017) (Santos, Gomes, Passos , Rocha, Salaroli , & Ngan, 2011).

Besides Pb, Hg is correlated towards fat contents in the fish. Hg will slowly methylate into Hg^{2+}, then enters the blood stream and oxidised into bivalence ion. Together with blood steam, Hg^{2+} that enters the liver can get metabolisms, degraded, and release Hg^{2+}. The accumulation of Hg within liver tissue can affect fat storing. Lead comes inside the body through food. The metal will enter the liver through porta vein assisted by blood. The high concentration level of Lead inside the liver can disturb the function of liver and increase the number of necrosis in liver tissues. Necrosis is liver cell death begun with sore. The damage of liver cells show the toxic effect of heavy metal of Lead constantly exposed to several fishes in rivers (Aksari, Perwitasari, & Butet, 2015), (Renika, Aunurohim, & Abdulgani, 2013)

Pb is the most dangerous compared to Hg and Cd since Pb is easy grouped with protein inside animals' body. Pb can disturb the enzyme activities that are involved in creating protein such as haemoglobin (Hb). Consequently, the creation of haemoglobin is blocked and create anaemia. Lead blocks the synthesis protein system of Hb by blocking the enzyme of aminolevulinate dehydratase acid (ALAD). The grouping of Fe into ALAD enzyme is blocked by Pb making Hb uneasy to create (Fadhillah, Rinidar, & Armansyah, 2017).

Liver organ that is accumulated by Pb can block the activity of existing enzymes so that it affects the protein contents in the fish. The existence of heavy metals in liver organ caused by the accumulation process is related to the disturbance of enzymes works in the liver cells. Those enzymes are phosphatase alkaline, phosphatase acid, catalase, dehydrogenase (Fadhillah, Rinidar, & Armansyah, 2017). The disturbance of enzymes works cause the tissues cell in liver organ to unable remove heavy metals, store glucose, produce cholesterol and bile. The correlation of fat and carbohydrate contents in plecostomus is not significant as in the correlation of protein contents. This causes the correlation between Pb, Hg and Cd towards fat and carbohydaret is less (Kaoud & El-Dahshan, 2010).

The correlation of heavy metals towards protein can be in the form of immunity system. Heavy metals can trigger immunity towards fish, so that when the body is inserted strange compound such as metals, the immune system will directly work and produce much protein. Besides, the correlation between heavy metals and protein can be in the form of physiological response of plecostomus to adjust itself to Ciliwung River condition. The way the fish adapts, can help the fish survive. The cells in their body respond to great threats. The main threats are toxic wastes in river such as Pb. Plecostomus can produce protein induced by threats (*stressed induced protein*).

The Factors of Concentration Increase of Lead (Pb), Mercury (Hg) and Cadmium (Cd)

Based on the research at the sampling location, there are houses, many industrial activities, and road construction projects surrounding Ciliwung River. Until now, there are still many industrial activities occurring and disposing their wastes on to the river without processing. Like the phytochemical analysis carried out by Dewi (2014), in Kaligarang waters in Semarang, the waters polluted by heavy metals Pb, Cd and Hg will cause fish that live in these waters to experience accumulation of heavy metals.

Kinds of industrial activities found along the riverbank are textile, transportation, metals, food, medicines, and hospitality. Wastes from them are BOD, COD, oils and fats, colouring, ammoniac, heavy metals, phosphor, sulphur, cyanide, phenol, and nitrogen. Heavy metals that are mostly found in the wastes are Pb, Hg, and Cd. The polluted water not only changes its colour but also changes smell, increases *E. Coli*, contains heavy metals and toxic materials, changes temperature and pH (Aksari, Perwitasari, & Butet, 2015).

The observation result conducted along the riverbank of Ciliwung River shows many transportations running along the riverbank. The smoke from them indirectly pollutes the river ecosystem through the air. Smoke from chemical burning also belongs to heavy metals. The smoke will group in the air with other molecules and create clounds. When rain comes down, the cloud emits acid water or famously known as acid rain (Eneji, 'Ato, & Annune, 2011).

The mixture between Hg and sulphur, chloral and oxygen will create salt used in antiseptic, diuretic medicine, and tooth patches in medical industries. In the area of Ciliwung River, there are medical industries that produce improperly processed wastes. High level of heavy metals are still in the wastes which can increase the concentration of heavy metals in the river. The heavy metal of Hg or mercury on Ciliwung River besides coming from medical industries is also from metal industries surrounding the river. Hg is used in the process of amalgams metal processing. Faded metals are cleaned and washed using mercury. The waste causes the increasing of Hg level in the river (Widhiasari, 2008).

As the number of population increases, the development along Ciliwung River Streamline Area, upstream and downstream become more intensive. The pollution in the upstream can potentially increase the heavy metal concentration in downstream. One of the industrial activities surrounding Ciliwung River which produces much waste such as suspended solid, settle able solid, TDS, and turbidity with the heavy metal high concentration of Cd is publishing industries. Cd in the atmosphere is from colouring materials used (Yudo, 2010).

The metal industrial activities also produce metal substance of Cd to the environment through the air. The process of metal processing, melting, and galvanisation produce high heavy metal pollutant. This activity does not get proper processing so that heavy metal such as Cd is disposed altogether and causing the increase of Cd concentration in the water. Besides through the air, Cd produced through metal layering process. In metal industries, the process of layering uses Cd as the primary material. It is thought that the industrial processing still contains Cd and disposed onto the water. The metal waste of Cd is also from housing activities. High level of Cd concentration is in metabolic wastes, water pipe corrosion, and detergent. Many houses along the riverbank also affects the increase of heavy metal concentration of Cd in the water (Aksari, Perwitasari, & Butet, 2015) (Yudo, 2010).

Ciliwung River in Jakarta is the downstream part has become a place of many wastes coming from the upstream. Compared to the upstream, the downstream part of Ciliwung River has more polluted materials. This causes heavy metal value in the downstream to become higher than other parts of the river (Aksari, Perwitasari, & Butet, 2015). The increase of alternating land use as industrial places every year has become the main factor in the increase of heavy metal in Ciliwung River. Another factor is road construction projects. The growth of housing complex along the riverbank has become the supporting factor of the increase of heavy metal concentration in the river. The pollution in Ciliwung River Jakarta consists of many wastes containing heavy metal. Most of them are Pb, Hg and Cd. Many transportations running along the river area also support the increase of heavy metal concentration.

CONCLUSIONS

The conclusions from this research are smaller-sized plecostomus has the highest protein and fat contents (50.0517% and 1.1261%). Meanwhile, the carbohydrate content in smaller-sized plecostomus is the lowest (47.,3753%). The correlation of heavy metal towards protein, fat, and, carbohydrate values in plecostomus show that Pb, Cd and Hg have the correlation towards protein value. Plecostomus flesh is identified contain three kinds of heavy metal such as Pb, Hg, and Cd. The highest heavy metal concentration in smaller-sized fish is Pb 3.6 ± 0.3 mg/kg, Hg 1.4 ± 0.3 mg/kg and Cd 0.6 ± 0.1 mg/kg. The result of heavy metal contents of Pb, Hg and Cd show the values are beyond the maximum value status of Indonesian National Standard (SNI) to consume in terms of meat and fishery products, so that the flesh of plecostomus from Jakarta Ciliwung River is unsuitable for consumption.

ACKNOWLEDGEMENT

Gratitude is expressed to the Ministry of Research, Technology and Higher Education as the funding of University Flagship Research (PUPT) year 2017 and Grant LP2M UAI year 2018 which has funded the research. Gratitude is upon Mr Udin who helped the sampling activity and Mr Dinardi as the laboratory attendant at the Biology Laboratory of Nuclear Energy National Agency (BATAN) of Nuclear Area in Pasar Pasar Juma'at, South Jakarta.

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