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Toxicity and severe stress of lead (Pb) to hematology responses of java barb (*Barbonymus gonionotus*)

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Abstract
The aims of the research are to determine the hematological response of Java Barb which is exposed to heavy metal lead (Pb) at different doses. Water pollution occurs due to industrial activities that contain toxic materials which are increasing with the development of industrialization. One of the heavy metals that cause pollution in Sidoarjo, East Java Indonesia waters is lead (Pb). Heavy metal in waters at can affect the life of aquatic biota, especially fish. Lead (Pb) enter the body of an organism through the food chain, gills or diffusion through the surface of the skin.
Lead enters the fish cause toxic effect that increase in oxidative stress, organ damage and changes in the biochemical profile of the blood which indicates metabolism in the body is disturbed. Metabolic disorders will affect the growth of cultivated fish Java Barb (*Barbonymus gonionotus*), is one type of freshwater fish that is widely cultivated in Indonesia but is very sensitive to changes of water quality.
The research was an experimental using a completely randomized design consisting of 5 treatments and 4 replications. The treatment are differences in lead doses, namely A (0 ppm), B (0.66 ppm), C (1.32 ppm), D (1.98 ppm) and E (2.65 ppm). The parameters of observation were total erythrocytes, hemoglobin, total leukocytes and leukocytes differential, blood glucose and cortisol. Data obtained were analyzed using Anova to determine the effect of treatment. Differences between treatments were analyzed using Duncan's multiple distance test.
The results showed that distribution of different lead doses increase the number of leukocytes and reduce the total erythrocytes, hemoglobin hematocrit, leukocytes differential, blood glucose and cortisol.

Keywords: Java barb, lead, hematological, leukocytes differential, corticol

Introduction
Based on the Indonesian Ministry of Industry's performance report, the metal industries increased by 2.94% in 2015. The heavy metals cause pollution in in waters is lead (Pb), for example Minamata's disease in Japan's. Fish found in Minamata Bay have lumps such as tumors and paralysis [19]. While the mass mortalities due to Pb contamination, this case also have occurred in the Center of Java Indonesia [31]. Lead enter the body through the food chain, gills or diffusion and the surface of the skin. Lead have a toxic effect cause an increase in oxidative stress, organ damage and changes in the biochemical profile of blood which indicates metabolism in the body is disturbed. Metabolic disorders will affect the growth of cultivated fish [12].
Java Barb (*Barbonymus gonionous*) is a freshwater fish that is widely cultivated and economically consumed. Based on aquaculture production data in 2005, production amounted to 32,575 MT and continued to increase 132,600 MT in 2009. Fish very sensitive to changes in water quality. Physiologically, fish exposed to heavy metals will uptake through the food chain as well as passively diffuse through outside organs membranes [15]. Several studies have been conducted on lead content in fish, it was found that in Brantas River in Mojokerto Region (East Java), the lead in fish reached 0.268 ppm [5]. The results of Sahetapy and Tuhumury [25], lead pollution also occurred in the water of Ambon Bay, the range on Baronang (*Siganus canaliculatus*) and Kuweh (*Caranx sexfasciatus*) are 0.007 - 0.254 ppm. The presence of lead in the waters will have an impact on the disruption of fish and human health. In addition, a research around the Taloja industry in Mumbai India by Lokhande *et al.* [8], showed that the levels of heavy metals Pb, Cr and Zn were 31.4 mg/L, 35.2 mg/L and 33.1 mg/L respectively.
The usual parameters for index in determining fish health are total leukocytes, total

erythrocytes, hemoglobin, hematocrit, leukocytes differential, blood glucose and cortisol.

This study was conducted to determine hematology responses of Java Barb (*Barbonymus gonionotus*) which were exposed to heavy metal lead (Pb) at different doses. The benefit of this study is to provide information about the effect of heavy metal lead (Pb) exposure in Java Barb (*Barbonymus gonionotus*) through hematology, leukocytes differential and cortisol.

Materials and Methods

Preparation of fish

Aquarium will be used in this experiment is cleand, sterilized, and filled with 40 L of fresh water. The aquarium is aerated and 10 Java Barb werw put in each aquarium. The length of the fish are 10-12 cm, and aquarium covered to prevent of jumping fish when they in severe stress condition. The fish were observed for 14 days and recorded daily the mortality.

Master Solutions

Lead (Pb) solution was created by dissolving 1000 mg/L lead (Pb $(NO_3)_2$) made from 1.831 g dissolved in 100 ml of aquades, poured into a 1000 ml measuring tube and then diluted with distilled water to the boundary mark. The solution was taken in a certain volume and diluted to obtain the required concentration.

Determination of *Lethal Concentration* (LC_{50}) value

The LC_{50} obtained from several test and continued with acute toxicity. The upper threshold (LC_{100}-24 h) 100 ppm and lower (LC_0-48 h) 10 ppm were obtained. The range were used as the basis for determining the acute toxicity with doses of 0 ppm, 15.8 ppm, 24.9 ppm, 39.2 ppm, 61.7 ppm, and 97.1 ppm. Acute toxicity test was carried out for 96 hours with three replications. The 96-hour mortality data were analyzed using *probity analysis* to obtain the LC_{50} value. Based on the probity analysis, the LC_{50} were 33.11 ppm. The LC_{50} used to determine the dose in the sub chronic test. Sub chronic test were P0 (control), P1 (2% from LC_{50}-96 hours), P2 (4% from LC_{50}-96 hours), P3 (6%) LC_{50}-96 hours), and P4 (8%) LC_{50}-96 hours).

Blood samples

Blood samples were taken by 1-2 cc syringes as much as ± 1ml that has been rinsed before with Ethylene Diamine Tetra Acetic acid (EDTA) 10% as an anticoagulant. Blood taken from caudal peduncle then inserted to the tube immediately for next experiment.

Total leukocytes

The procedure for calculating leukocyte according to Blaxhall and Daisley [4]. The white blood cell or total leukocyte is calculated with the aid of a microscope at 400X magnification. The total leukocyte is calculated by counting cells in 4 small squares, according to the formula: ∑ Leukocyte = ∑ counted leukocyte cells x 50 cell/mm³.

Total erythrocytes

The calculation of erythrocytes according to Blaxhall dan Daisley [4]. The total erythrocyte was calculated by counting cells in 5 small *hemocytometer* with light microscope at 400X magnification, according to the formula: t = (A/N) x (1/V) x Fp.

Hemoglobin

Hemoglobin is a metalloprotein in red blood cells that functions as a carrier of oxygen from the gills throughout the body, as well as carriers of carbon dioxide back to the gills to be discharged out of the body. The calculation procedure for hemoglobin refers to the Srivastava *et al*. [29] hemoglobin is expressed in gram per deciliter (g/dl). To be sure the tissue oxygenation a sufficient hemoglobin level must be maintenance.

Hematocrit

blood samples were taken by 1 cc syringes. Blood was taken from the heart, immediately were inserted into micro hematocrit tubes of up to 3/4 parts, then corked with *crytoceals* about 1 mm deep. Blood samples were centrifuged at 5,000 rpm for 5 minutes. The length of blood settles, and total length of blood volume contained in the tube is measured using a ruler. Determination of hematocrit levels using *a microhematocrit reader*. Hematocrit levels are expressed as % of the volume of solid blood cells, Anderson and Siwicki [1].

Leukocyte differential

Leukocyte differential calculation according to Amlacher [26], briefly, starting coloring the specimen using Giemsa to facilitate easy observation. Specimens were observed with a microscope at magnification 400 times. The percentage of leukocyte cells is calculated by observing as many as 10 fields. The leucocyte grouped according to type (basophils, lymphocytes, monocytes, neutrophils, and eosinophils). The calculation of the lymphocyte, neutrophil, monocyte, and platelet cells number in mathematics as follows:

$$\sum \text{Total leukocyte (\%)} = \frac{Leukocyte\ cell\ component}{100} x100\%$$

$$(\%)\text{Basophil} = \frac{Basophil}{100} x100\%$$

$$(\%)\text{Neutrophil} = \frac{Neutrophil}{100} x100\%$$

$$(\%)\text{Lymphocyte} = \frac{Lymphocyte}{100} x100\%$$

$$(\%)\text{Eosinophils} = \frac{Eosinophil}{100} x100\%$$

$$(\%)\text{Monocytes} = \frac{Monocytes}{100} x100\%$$

Cortisol

Plasma cortisol were measured by the radioimmunoassay (RIA) kit technique. The kit consists of 1 vial tracer (cortisol labeled with iodine), 6 standards, 1 antiserum, serum control, and 2 tube boxes for serum samples. Cortisol measured by the Enzyme-Linked Immunosorbent Assay (ELISA) method competitive type of ELISA Sink *et al*., [27]

Blood glucose

Glucossure kit is used to measure blood glucose (AGM-2100 Glucosure kit, Easy Touch Glucosure). Fish blood was dropped on the glucose strip test, wait for 10 second, and glucose blood content was read [12].

Results and Discussion

The results showed that distribution of heavy metal lead (Pb) in Java Barb were different on each parameter compared to the control. The results were summarized in the Table 1.

Table 1 stated that the average lowest leukocyte in P1 (0 ppm) is 3.09 x10^4 cell/mm^3 and the highest leukocyte found in P5 (2.65 ppm) which is 7, 69 x10^4 cell/mm^3. The results showed that the average number of leukocytes is not increase but in normal range. According to Sasongko [14]. The normal leukocyte Java Barb ranges between 20,000-150,000 cells /mm^3. The leukocytes are in normal condition indicates that the process of hematopoiesis still occurs even though it has been exposed to lead chloride. The number of leukocytes in fish influenced by several factors, namely type or species, age and muscle activity. According to Sugito *et al*. [16], leukocytes will decrease if fish in a severe stress condition. Leukocytes will increase in infected fish as a form of the body's immune response against microorganisms. The increase of white blood cells indicates that fish immune is responding to the presence of foreign objects that enter their body.

The average results of the lowest number of erythrocytes were obtained in P5 (2.65 ppm), which was 1.04 x10^6 cell/mm^3 and the highest number of erythrocytes in P1 (0 ppm) was 2.98 x10^6 cells/mm^3. The Analysis of Variance (ANOVA) and followed by Duncan test are 95% confidence, the interval showed there is a significant difference. The results showed that the number of erytocytes was in the normal range except in P5 (2.65 ppm) erythrocytes were below the normal.

Table 1: Hematology of fish exposed to Lead

Lead Concentration (ppm)	Parameter			
	Leukocytes (x10^4 cell/mm^3)	Erythrocyte (x10^6 cell/mm^3)	Hemoglobin (g %)	Hematocrit (%)
P1 (0 ppm)	$3.09^{a} \pm 0,040$	$2.98^{d} \pm 0,039$	$6.23^{b} \pm 1.59$	$22.15^{c} \pm 0.55$
P2 (0.66 ppm)	$4.75^{b} \pm 0,194$	$1.99^{c} \pm 0,030$	$6.35^{b} \pm 0.33$	$21.95^{c} \pm 1.17$
P3 (1.32 ppm)	$5.63^{c} \pm 0,023$	$1.18^{b} \pm 0,029$	$6.67^{b} \pm 0.57$	$17.80^{b} \pm 0.42$
P4 (1.98 ppm)	$7.25^{d} \pm 0,074$	$1.12^{ab} \pm 0,025$	$5.83^{ab} \pm 0.30$	$12.67^{a} \pm 0.45$
P5 (2.65 ppm)	$7.69^{d} \pm 0,012$	$1.04^{a} \pm 0,009$	$4.77^{a} \pm 0.47$	$12.67^{a} \pm 0.74$

Note: Notations shown with different superscript letters in the same column indicate significant differences ($p<0.05$).

The red blood cells (erythrocytes) in the normal state of teleost is 1.05x10^6-3,0x10^6 cell/mm^3 but varies depending on species, stress and ambient temperature conditions, Mulyani [10]. The number of erythrocytes below the normal indicates that fish in an anemic condition caused by free ions lead due to toxicity of which can damage the erythrocytes. According to Wahyuni, [52], heavy metal lead can cause erythrocyte damage and the reduce the of erythrocyte solid volume.

The hemoglobin fish exposed to Pb is 4-7.6 gr/dl. The Hb is lower than reported by Bastiawan *et al*. [2]., 12.0 gr/dl – 14 gr/dl. The results of exposure to lead have a significant effect to Java Barb <0.05 which identified that there is influence between heavy lead (Pb) on hemoglobin levels.

Table 2: Leukocytes Differential on Lead Exposed Java Barb

Lead Concentration (ppm)	Parameter				
	Basophils (%)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)
P1 (0 ppm)	$0.25 \pm 0,00^{a}$	$53.50 \pm 0,01^{a}$	$44.00 \pm 0,02^{a}$	$1.75 \pm 0,00^{a}$	$0.50 \pm 0,00^{a}$
P2 (0.66 ppm)	$1.00 \pm 0,00^{a}$	$49.25 \pm 0,01^{b}$	$46.50 \pm 0,03^{a}$	$2.00 \pm 0,00^{a}$	$1.25 \pm 0,01^{a}$
P3 (1.32 ppm)	$1.75 \pm 0,00^{a}$	$47.00 \pm 0,04^{b}$	$45.50 \pm 0,05^{a}$	$3.50 \pm 0,01^{b}$	$2.25 \pm 0,01^{b}$
P4 (1.98 ppm)	$3.25 \pm 0,01^{b}$	$43.25 \pm 0,02^{c}$	$47.50 \pm 0,04^{a}$	$3.25 \pm 0,01^{b}$	$2.75 \pm 0,02^{b}$
P5 (2.65 ppm)	$4.50 \pm 0,01^{c}$	$41.00 \pm 0,02^{c}$	$43.25 \pm 0,03^{a}$	$7.50 \pm 0,01^{c}$	$3.75 \pm 0,00^{c}$

Note: Notations shown with different superscript letters in the same column indicate significant differences ($p<0.05$).

The presence lead in fish body will interfere their hemoglobin synthesis. Other than biosynthesis disruption, hemoglobin also caused by fish stress due to inappropriate environment.

The lowest hematocrit after Pb exposure was found in P5 (2.65 ppm), which was 12.67% and the highest hematocrit in P1 treatment (0 ppm) were 22.15%. According to Royan *et al*. [13], normal hematocrit values ranged between 21.00% - 22.67%. Hematocrit above the normal indicate that the process of hematopoiesis begins to be disrupted due to lead chloride exposure. According to Mazur and Iwana [9], stress conditions in animals can cause an increase in hematocrit values. Hematocrit values are closely related to the number of red blood cells. Hematocrit values in teleost range from 20-30% and some marine fish species 42%. Hematocrit values are also influenced by gender, body size and spawning period Jawad *et al*., [7].

The leukocyte differential of eosinophil and basophil cells is presented in Table 2, treated fish increase P1 to P4. This showed that an increase in eosinophil and basophil as an immune response to toxins is revealed. Eosinophils and basophils play a role in parasitic infections and allergic responses, associated with acute diseases [11]. Therefore, fish in normal conditions or not severe health conditions, both eosinophils and basophil are rarely found in P1 (0 ppm). The highest monocyte cell percentage increase occurs in P4 (1.89 ppm), this is related monocytes role to destroy foreign objects that enter the blood. Monocytes are cells in the bloodstream and develop into macrophages. An increase in the number of monocytes occurs during tissue requirements for the process of macromolecular Phagocytosis and can be found in the healing phase of infection [3]. The lowest monocyte cells percentage decrease occurs in P5 (2.65 ppm) this occurs because in addition to acting as an anti-inflammatory, corticosteroid hormone also plays a role in suppressing the immune response.

The average results of glucosa and cortisol is presented on Tabel 3. The highest cortisol levels showed in P5 (2.65 ppm) is 41.69 µg/dL were not significantly different from the P4 (1.98 ppm), 38.47 µg/dL (Table 3). P1 (control) showed the lowest cortisol, 9.36 µg/dL. The high level caused by hormonal response to stress that stimulates the hypothalamus to produce CRH. CRH release will stimulate the release of

ACTH by the anterior pituitary, then ACTH stimulates the adrenal cortex to release cortisol. When stressed, fish will release cortisol to the bloodstream as an effort to recover from stress [23]. Normal cortisol of fish is 0.7449-3.7246 µg/d [22], and the results indicated fish in stress condition.

Table 3: The average results of blood glucose and cortisol

Lead Concentration (ppm)	Parameter	
	Cortisol Level (µg/dL) ± SD	Glucose Level (mg/dL) ± SD
P1 (0 ppm)	9.36^{a}±3.4s	60.00^{a} ±3.36
P2 (0.66 ppm)	23.53^{b}±2.75	93.25^{b} ±4.92
P3 (1.32 ppm)	30.45^{c}±0.92	129.25^{c} ±6.99
P4 (1.98 ppm)	38.47^{d}±1.28	150.50^{d} ±1.73
P5 (2.65 ppm)	41.69^{d}±1.48	159.50^{e} ±2.88

Hyperglycemia is one of the secondary effects of stress caused by the primary response from excessive cortisol release [28]. Cortisol mobilizes and increases glucose production through the pathway of glucogenesis and glycogenolysis [24]. Glucose production is mediated by cortisol which in turn stimulates liver gluconeogenesis and at the same time ceases the absorption of sugar [17]. The results of the average blood glucose levels analysis during the study showed a significant difference from each treatment. The P5 (2.65 ppm) shows the highest blood glucose level of 159.50 mg/dL. The lowest blood glucose level was seen in P1 or control at 60 mg/dL in accordance with the normal blood glucose level limit according to Bartonkova [30s of 40-90 mg/dL.

References

1. Anderson DP, Siwicki AK. Injection or Immertion Delevery Of Selected Immunostimulan To Trout Demonstrate Enhancement Of Non Spesific Defenden Mechannism And Protective Immunity. *In*: Sharriff, M. J.R. Arthur, R. P. Subangshingle (Eds), Disasc In Asian Aquaculture Ii. Fish Health Section Asian Sociaty, 1993, 413-426.
2. Bastiawan D, Wahid A, Alifudin M, dan I. Agustiawan. Gambaran Darah Lele Dumbo (*Clarias spp.)* yang diinfeksi cendawan *Aphanomyces sp* pada pH yang berbeda. Jurnal penelitian Indonesia. 2001 ; 7(2):44-47.
3. Bijanti R. Hematologi ikan (Teknik Pengambilan Darah dan Pemeriksaan Hematologi Ikan). Edisi 2. Fakultas Kedokteran Hewan. Universitas Airlangga. Surabaya. Revkapetra Media, 2010, 105.
4. Blaxhall, Daisley KW. Routine Haemotological Methods For Use With Fish Blood. J Fish Biol, 1973; 5:577-581.
5. Enni Priatna DEBY. Kadar logam berat timbal (Pb) pada air dan ikan bader (Barbonymus gonionotus) di sungai Brantas wilayah Mojokerto. Lentera Bio, 2016; 5(1).
6. Irianto A. Probiotik Akuakultur. Gajahmada University Press. Yogyakarta, 2003.
7. Jawad LA, Al-Mukhtar MA, Ahmed HK. The relationship between haematocrit and some biological parameters of the indian shad, Tenualosailisha (Family Clupeidae). Anim. Biod Conserv. 2004; 27(2):47-52.
8. Lokhande RS, Singare PU, Pimple DS. Toxicity Study of Heavy Metal Pollutans in Wate Water Effluent Sample Collected from Taloja Industrial Estate of Mumbai India. Resources and Envirinment. 2011; 1(1):13-19.
9. Mazur CF, dan Iwana GK. Effect of handling and Stocking Density on Hematocrit, PlasmaCortisol, and Survival in Wild and Hatchery-reared Chinok Salmon. *Onchynchustshawytscha.* Aquaculture. 1993; 112:291-299.
10. Mulyani. Studi Pendahuluan Pengaruh Hormon Steroid terhadap Keragaan Hematologi Induk Ikan Kerapu Bebek Cromileptes Altivelis. Tesis. Makassar: Program Pascasarjana Universitas Hasanuddin, 2006.
11. Patrick TKW, Bruno DW, Susan Lim LH. Diseases and Disorders of Fish in Cage Culture. CABI Publishing. London, 2002, 126-127.
12. Permatasari S. Evaluasi penambahan vitamin E pada pakan terhadap status kesehatan dan kinerja pertumbuhan ikan nila Oreochromis niloticus yang terpapar timbal (pb). Sekolah Pasca sarjana Institut Pertanian Bogor, 2015.
13. Royan F, Rezeki dan S, Haditomo AHC. Pengaruh salinitas yang berbeda terhadap profil darah ikan nila (Oreochromisniloticus). J Aquac. Manage. Tech. 2014; 3(2):109-117.
14. Sasongko A. Biomassa bakteri nitrifikasi pada berbagai bahan filter dalam sistem resirkulasi aliran tertutup dan pengaruhnya terhadap kondisi ikan: gambaran darah. Program Pasca sarjana, Institut Pertanian Bogor. Bogor, 2001.
15. Siregar YI, Zamri A, dan Putra H. Penyerapan Timbal (Pb) pada Sistim Organ Ikan Mas (*Oreochromis niloticus*). Jurnal Ilmu Lingkungan. Universitas Riau. Riau. 2012; 6(1).
16. Sugito Nurliana, Aliza D, danSamadi. Efek Suplementasi Tepung Daun Jaloh Dalam Pakan Terhadap Diferensial Leukosit Dan Ketahanan Hidup Pada Uji Tantang Aeromonas Hydrophila Ikan Nila Yang Diberi Stress Panas. Seminar Nasional Teknologi Peternakan dan Veteriner, 2001, 509-518.
17. Wedemeyer GA, dan Yasutake. Clinical Methods for The Assessment on The Effect of Enviromental Stress on Fish Health. Technical Paper of The US Departement of The Interior Fish ang the Wildlife Service. 1997; 89:1-17.
18. Kementerian Perindustrian Indonesia. Berita Industri: Sektor Industri Logam Dasar Dipacu. 2015. https://kemenperin.go.id/artikel/17638/Sektor-Industri-Logam-Dasar-Dipacu Diakses pada 29 November 2016
19. Irukayama K. The pollution of Minamata Bay and Minamata Disease. Dalam: River Pollution II (KLEIN edt), 1969, 153-165.
20. Dinas Perikanan dan Kelautan [DKP]. Perikanan dan Kelautan dalam Angka: Dinas Perikanan dan Kelautan Provinsi Jawa Timur, 2009, 120.
21. Wahyuni M. Analisis Hubungan Konsentrasi Nitrogen Dioksida Di Udara Ambien Roadside Dengan Karakteristik Lalu Lintas Jaringan Jalan Primer Kota Padang (skripsi). Universitas Andalas. Padang, 2016.
22. Raoult V, Trompf L, Williamson JE, Brown C. Stress profile influences learning approach in a marine

fish. Peer J. 2017; *(5):34-45.*
23. Affandi R, dan Tang UM. Fisiologi hewan Air. UNRI-Press, Pekanbaru, 2002.
24. Iwama GK, Vijayan MM, Forsyth RB, Ackerman PA. Heat shoch proteins and phsycological stress in fish. American Zoology. 1969; (39):901-909.
25. Sahetapy JMF, Tuhumury N. Akumulasi dan Kamsungan Logam Berat Timbal (Pb) dan Cadmium (Cd) Dalam Tubuh Beberapa Speies Ikan Laut di Teluk Ambon. Prosiding Konas Pesisir dan Pulau Kecil DKP, Jakarta, 2008.
26. Alamcher E. Texbook of Fish Disease, T.F. H. Pubication, 1970.
27. Sink TD, Lochman T, Fectteau A. Validation, use, and disadvantage enzyme immunosorbent assay kit for detection of cortisol in Channel catfish, largemouth bass red pacu, and golden shiner. Fiah Physiol. Biochem, 2008, 95-101.
28. Reid S, Bernier NJ, Perry SF. The adrenergic stress response in fish: control of catecholamine storage and release. Comparative Biochem and Phyisiol. Toxici and Pharmaco. 1998; 120(1):1-27.
29. Srivastava T, Negandhi H, Neogi SB, Sharma J, Saxena R. Methods for Hemoglobin Estimation: A Review of What Works. Journal of Hematology & Transfusion. 2014.
30. Bartoňková J, Hyršl P, Vojtek L. Glucose determination in plasma by two different moderate methods. Acta Vet. Brno. 2016; 85:349-353.
31. Tjahyono A, Djoko Suwarno. The Spatial Distribution of Heavy Metal Lead and Cadmium Pollution and Coliform Abundance of Waters and Surface Sediment in Demak. J Ecol. Eng. 2018; 19(4):43-54.