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RESEARCH ARTICLE

PATH ANALYSIS OF PLUMBUM (PB) ACCUMULATION IN THE REMIS (CORBICULA JAVANICA) IN THE MAROS RIVER, INDONESIA

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ABSTRACT

Heavy metals always can be found in the fresh waters, brackish waters or marine waters, although in the limited amount. One of highly toxic heavy metals to organism is Plumbum (Pb). The accumulation level of heavy metals in aquatic organism is quick especially that filter feeding in nature. Bivalves groups have been used as indicator organism in determining the pollution in waters. The research aimed at analyzing the heavy metal Pb bio-accumulation in *C. javanica* in Maros River. The Pb content average at the soft tissue of *C. javanica* based on the station location successively: Station A (Pappandangan) 6.051±0.064 ppm, Station B (Mannaungan) 2.067±0.024 ppm, Station C 2.326±0.105 ppm, and Station D 0.834±0.261 ppm. The obtained of bio-accumulation factor > 1 about 4.416-13.448. Based on β coefficient value, the most dominant influencer for Pb accumulation at gills is water, while at the stomach and organ total is sediment. *C. javanica* can accumulates the Pb in their body, so the clams can be used as bio-indicator for heavy metal pollution in the river.

INTRODUCTION

Background

Heavy metals are toxic and dangerous inorganic materials for aquatic organism, human or land creature life. Heavy metals are always found in fresh or marine waters, although in limited amount. Several heavy metals are used for various industries and agricultures for raw materials, additional materials or as catalyst (Darmono, 2001; Achmad, 2005; Asha *et al.*, 2010). Concentration of pollutant in waters is determined by several factors, such as: diluted and spread, concentrated through biological process by absorbing and physical and chemical process with absorption, precipitation, and ion exchange then finally will settle at the base of waters (Hutabarat and Evans, 1984; Sahara, 2009). The spread of heavy metals in general waters for certain period will follow the water spread and will be accumulated at the outfall area. If the heavy metal content is high exceeded normal limit, it produces complex danger for aquatic organism (Censi *et al*., 2006). The metal accumulation level at the aquatic organism is quick especially which filter feeding (Otchere *et al*., 2003; Waykar and Desmukh, 2012). Heavy metals accumulation at the sediment will influence to the accumulation at the macrozoobenthic that occupy the waters (Ordonez *et al*., 2011; Suwondo *et al*., 2004). If the heavy metals accumulation is out of control, then able to produce problems for life and environment (Siaka *et al*., 2000; Reboreda *et al*., 2010)

Maros River is one of coastal areas at the South Sulawesi that experiences various development activities (industries, city, settlement, agriculture, plantation, fisheries, mining, and transportation). The river has important role as catchment area in the hydrological cycle from around areas (Bapedalda of Maros Regency, 2003). Maros River has clams fisheries resources potentials that have important economic value for societies and animal protein sources that is Remis *(Corbicula javanica).* The Remis is abundant in Maros River throughout the year in the period from May to December

Bivalves group is aquatic organism that has low mobility and sediment is the habitat and as place to feed, so the heavy metal accumulated in the sediment will be accumulated at the bivalves (Zuykov *et al*., 2013). According to Goksu *et al*. (2005), that heavy metal that is accumulated at the clams in general come from waters, sediment, suspended solid and phytoplankton. The elements enter into organism body through food chain, gills and diffusion by skin surface. Clams have ability to accumulate heavy metals in their body so the heavy metal content in the clams will increase along with the period length the clams at the waters with heavy metals. Even the heavy metals content in the clams can be higher than the

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environment (Darmono, 2001). Nevertheless, mollusk has mechanism in their body to reduce the heavy metals, so very effective to be used as bio monitoring for the heavy metal pollution. Clams can survive at the environment with heavy metals because their ability to do detoxification by synthesizing metallothionein. As long as the heavy metal accumulation suitable with the *metallothionein* synthesis then the clams will survive, but if the accumulation increase then the *metallothinein* synthesis will reach the maximum level so there will be accumulation in the tissues, especially in the lever and kidney (Simkiss, 1981)

The heavy metals monitoring into waters is important to do because they have dangerous properties, that is long residence time, rapid accumulation, and high toxicity to organisms and aquatic environment (Darmono, 2001). Research that used several species either animal, plants, or microorganism in the recent decades have been done to monitor the pollution potentials and bivalves are made as popular choice to monitor the heavy metal monitoring in the waters (Huang *et al*., 2007). This research described the presence of significant influence between sediment to waters, sediment and water influence to gill, sediment and water influence to stomach and sediment and water influence to organ total of C. *javanica*. The research aimed at analyzing the bioaccumulation of Pb in *C. javanica* in Maros river of South Sulawesi

MATERIALS AND METHOD

Study Area

The research was conducted in Maros river of Maros Regency, its geographical coordinates are 5^o 0' 25" South, 119^o, 34' 20" East (Fig. 1) on in October 2013. Pb content analysis was done at Main Station Laboratory of Health Laboratory of Makasar. While the physical and chemical parameters have analyzed of water at the Oceanography Chemical Laboratory of Marine Science Department of Hassanudin University Makassar.

Sampling Procedure

Collection of Remis *C. javanica* used random sampling method at each sampling station (Fig. 2). Clams, waters, and sediment sample taking was done at nine points from each determined station. The collected clams sample amount from each sub station of 50 clams. Then the samples were cleaned then taken to the Breeding Laboratory of Marine Science and Fisheries Faculty of Hassanudin University Makassar then prepared for Pb content analyzed at the gill, stomach, and organ total each 10 clams.

Determination of Pb in Waters, Sediment, and Tissues of Remis in Maros River

Sample of gills, stomach, and organ total from each sampling unit were dried in oven with 70-80^o C temperature. After that from each dried sample was taken 500 mg, then be mashed then entered into beaker. Then be added 10 ml concentrated HNO_3 and then stirred slowly up to mixed. Then added $HClO_4$ 60%, then be heated on hot plate (increase the temperature slowly) until almost dry, add 10 ml HNO_3 and then be continued heating up to the white steam disappear. Then it was cooled and added by 10 ml HCl 1:1 and move to volumetric flask 50 ml squeezed with distilled water.

Water sample 500 ml from each sampling unit was filtered with the filter paper of nitrate cellulose with 0.45µm pores with diameter 47µm that previously cleaned with HNO_3. After that, be preserved with HNO_3 (pH < 2). Then the water sample of 250 ml entered into Teflon funnel, then extracted with HNO_3. The sediment sample is entered into Teflon beaker then be dried in oven at temperature 105^{o}C for 8 hours. After dry, then be rinsed off 3 times with distilled water free from heavy metals. Then be dried again and crushed up to homogenous. About 5 gr of sediment example was destructed in Teflon beaker with HNO_3 at 100^{o}C for 8 hours. The concentrations of Pb on waters and sediment, gills, stomach, organ total *C. javanica* were analyzed by atomic absorption spectrophotometry (APHA, 1998). The calculation of bioaccumulation factor value refer to Devagi *et al*. (2008)

Statistical Analysis

The mean and standard deviation of each study sites were calculated. Analysis of the station location influence to the metal accumulation in the soft tissues of clams and bioaccumulation factor were evaluated using one way analysis of variance (ANOVA) and Pos Hoc Duncan test ($p < 0.05$) according to Steel and Torrie (1995); Nazir (2009). Analysis of the relationship between Pb content at gills, stomach, and organ total of Remis C. *javanica* with the Pb contents in waters and sediment used path analysis. The analysis was used to know the influence level at causal relationship either directly or indirectly (Lleras, 2005). The calculation of path coefficient is used standardize regression analysis by looking at the simultaneous and partial influence at each equation. The used method is ordinary leas square (OLS), the least square calculated by SPSS version 15.0.

RESULTS AND DISCUSSION

Accumulation of Pb in Maros River

Fig. 3 is the results to represents the Pb content difference at soft tissues of C. *javanica*, waters and sediment at each station. Pb content average at the tissues of C. *javanica*, the highest in Pappandangan station of 6.051±0.064 µg g^{-1} DW and the lowest at Betang station of 0.834±0.261 µg g^{-1} DW. While Pb content in waters, the highest in Marusu station is 0.500±0.000 µg l^{-1} and the lowest in Batang station of 0.322±0.070 µg l^{-1}. While the Pb content on sediment, the highest in Mannaungan station of 5.268±0.455 µg g^{-1} and the lowest in Pappandangan station of 4.3220±0.175 µg g^{-1}. It showed that there is accumulation at the Maros River.

The station location influences to the Pb content at the soft tissues of *C. javania*. There was significant difference of Pb content in the soft tissues among station ($p < 0.05$). The high Pb content in the soft tissues at Pappandangan station was influenced by around environment, where there are activities that produced wasted that have potential to enter the river such as activities of agriculture (rice field and plantation), cold storage, ceramic plant and fishing port. While in Bentang station, the Pb content was the lowest that is caused by the less pollutant potentials around the station. Asha *et al.* (2010) explained that the heavy metal concentration in the bivalve tissues, waters, and sediment influenced by the increasing waste around the area that went into the waters. According Netpae and Phalaraksh (2009), research period also influence to the heavy metal content in clams, where in the research done in Bung Boraphet of Thailand at *Corbicula* sp (Asian Clams) contained heavy metal Pb, the highest in the tissues in August and February. According to Zuykov *et al.* (2013), heavy metal that went into water will experience sedimentation, dilution and dispersion then will be absorbed by aquatic organism.

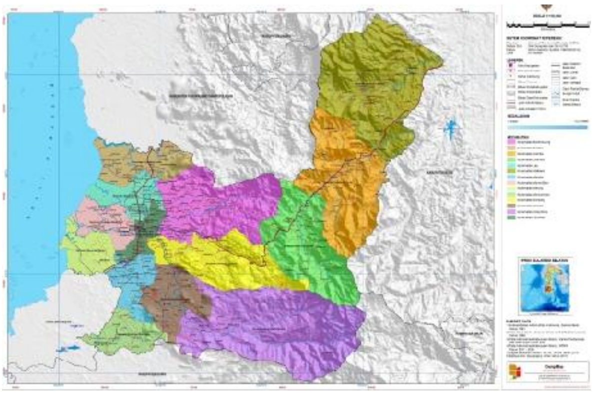

Fig. 1 Map of Maros Regency (Research Location)

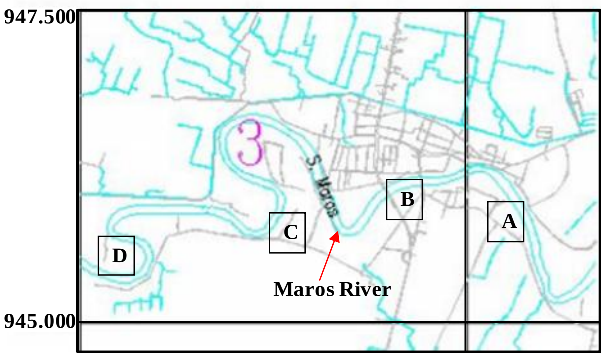

Fig 2 Position of Sampling Station in Maros River

Station A : -5,007569 S 119,590323 E
Station B : -5,008595 S 119,573200 E
Station C : -5,014153 S 119,555176 E
Station D : -5,007355 S 119,536937 E

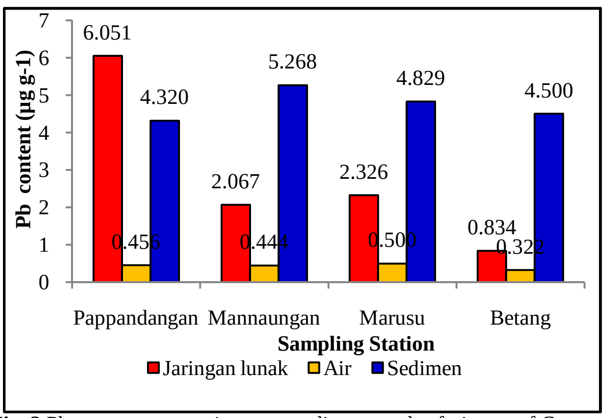

Fig. 3 Pb content average in water, sediment, and soft tissues of C. *javanica* in Maros River.

Path analysis

Path coefficient

Table 1 showed that the path coefficient (β) of waters and sediment influence to the Pb content in gill obtained R square of 0.221 or 22.1%. It means that the influence of waters and sediment variables to gill of 22.1% while the 77.9% influence by other variables. The water Pb influence significant to the Pb on gills ($t < 0.05$). While the sediment Pb did not influence significantly to the Pb on gills ($t > 0.05$).

The calculation of path coefficient (β) of the waters and sediment influence to the Pb content at the stomach (Table 1) obtained R square of 0.358 or 35.8%. It mean that the influence of waters and sediment variables to the Pb on stomach of 35.8% while the remain 64.2% influenced by other variables. The water Pb and Sedimen Pb influence significant to the Pb on stomach ($t < 0.05$). Then the calculation of path coefficient (β) of the waters and sediment influence to the Pb content at the organ total (Table 1) obtained R square of 0.321 or 32.1%. It mean that the influence of waters and sediment variables to the organ total lead of 32.1% while remain 67.9% influenced by other variables. The water Pb (X_1) and sediment Pb (X_2) influence significant to the Pb on stomach ($t < 0.05$). The results showed that the water Pb content influence to the Pb content of gill, stomach, and organ total in C. *javanica* (Y) in Maros River. While the Pb sediment only influence to the Pb of gill, but the Pb of stomach and organ total was not significant.
The accumulation of heavy metal compound and metal colloid through food collecting system such as gill at bivalves occur when the clams filter food in the form of microalgae, organic materials and other particles from the waters (Goksu *et al.*, 2005; Lazaro *et al.*, 2009).

Table 1 Path coefficient of water and sediment influence to Pb content in gill, stomach, and organ total of Remis C. *javanica*

Variable X	Variable Y	Beta (β)	$T_{calculation}$	Sig t	T_{table}	R square
Water	Gills	0.470	3.007	0.005	2.035	0.221
Sediment	Gills	0.005	0.033	0.974		
Water	Stomach	0.416	2.932	0.006	2.035	0.358
Sediment	Stomach	-0.511	-3.605	0.001		
Water	Organ total	0.353	2.420	0.021	2.035	0.321
Sediment	Organ total	-0.512	-3.511	0.001		

Model interpretation of path analysis

Fig. 4 explained that from the produced model showed in general there was influence of water Pb and sedimen Pb to the Pb content in C. *javanica of Maros River*. The highest positive β coefficient value by the relation between water Pb to the gilsl Pb, that is β = 0.470. It showed that the higher water Pb content will influence to the higher Pb content in gills of C. *javania* and vice versa. Gills were considered to represent the main target organ in terms of bioaccumulation to metal. According to Simon and Laplace (2004), when the uranium concentration in waters column reached the high exposure level, the gills seemed to be the main target organ. The highest negative β coefficient value is shown by the relation between Pb sediment content to organ total Pb of -0.512. It showed that the higher Pb content at sediment the lower Pb content at organ total of Remis C. *javanica* and vice versa. It showed that Pb at sediment bound by sediment so do not dissolved in waters. Sediment is the main storage of metal, in several cases more than 99% from total metal in waters system. The presence of Pb in sediment also caused the metal pollution in waters (Netpae and Pharalksh, 2010)

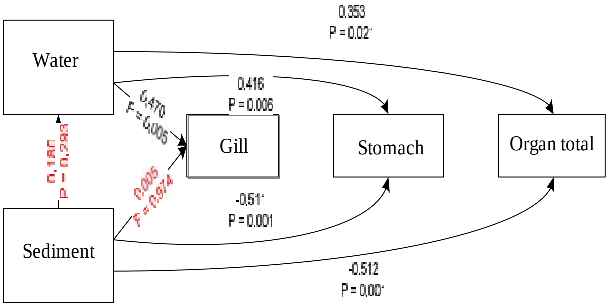

Fig. 4 Diagram of Path Analysis of Pb Content in Water and Sediment To Pb Content in C. *Javanica* in Maros River

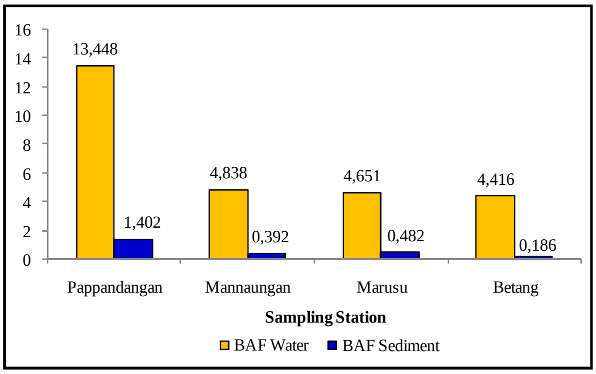

Fig. 5 Bioaccumulation factor of Pb in C.*javanicas*

Table 2 The direct and indirect influences in the path analysis

Independent variables	Dependent variables	Path coefficient	Significance	Explanation
Sediment	Water	0.180	0.293	Not Significant
Sediment	Gill	0.005	0.974	Not Significant
Water	Gill	0.470	0.005	Significant
Sediment	Stomach	-0.511	0.001	Significant
Water	Stomach	0.416	0.006	Significant
Sediment	Organ total	-0.512	0.001	Significant
Water	Organ total	0.353	0.021	Significant

The Goodness of Fit test used total determination coefficient. Data variance total that can be explained by the model measured with formulation: $R^2_m = 1 - P^2_{e1} P^2_{e2} \dots P^2_{ep}$, where:

$P^2_{e1} = 1 - R^2_1$
$P^2_{e2} = 1 - R^2_2$
$P^2_{e3} = 1 - R^2_3$
$P^2_{e4} = 1 - R^2_4$

Based on *R square* value from 4 equations so obtained values:

$P^2_{e1} = 1 - 0{,}172 = 0{,}828$
$P^2_{e2} = 1 - 0{,}221 = 0{,}779$
$P^2_{e3} = 1 - 0{,}358 = 0{,}642$
$P^2_{e4} = 1 - 0{,}321 = 0{,}624$

Total determination coefficient as follow:

$R^2_m = 1 - (0{,}828 \times 0{,}779 \times 0{,}642 \times 0{,}624) = 0{,}742$ or 74,2%
It indicated the data variance can be explained by the model of 74.2% or the information in data of 74.2% can be explained by the model. While remain 25.8% explained by other variable out of the model.

Table 3 Indirect influence hypotheses

Independent Variable	Intermediary Variable	Dependent Variable	Path Coefficient	Explanation
Sediment	Water	Gill	0.180 x 0.470 = 0.085	Not Significant
Sediment	Water	Stomach	0.180 x 0.416 = 0.075	Not Significant
Sediment	Water	Organ Total	0.180 x 0.353 = 0.064	Not Significant

The direct and indirect influences in the path analysis

Entirely, the model in the research was divided into 7 direct influences, and 3 indirect influences. From Table 4 can be known that there are two paths that did not influence significantly are the sediment Pb to the water Pb and the sediment Pb to the gill Pb of *C. javanica*. Based on the path coefficient value, the most dominant variable to the gill Pb is the water Pb content, while the stomach Pb and organ total Pb influenced mostly by sediment. Table 5, shown in the indirect influence between sediment Pb to the gill, stomach, and organ total Pb content through waters. Based on the path coefficient value, it was obtained that the indirect influence between sediment Pb content to gill, stomach, and organ total Pb content through waters was not significant. It was caused by direct influence between sediment to the waters did not influence significantly.

Table 4 Chemical and physical parameters average of water each station

Parameters	Unit	Station			
		A	B	C	D
DO	ppm	3.056	2.778	2.844	3.078
pH	-	6.994	5.721	5.630	5.338
CO_2	ppm	23.067	32.956	30.756	29.589
Salinity	ppt	0.000	0.000	0.000	0.00
Turbidity	JTU	121.889	67.000	80.556	91.444
Conductivity	mS/cm	20.911	30.944	38.922	60.333
Temperature	°C	30.644	31.089	31.322	31.789
Depth	Meter(s)	3.842	2.634	3.073	3.273
Current speed	cm/s	6.222	5.556	6.222	5.889

The polluted waters will accumulate the metal into sediment and organism through gravitation, bio-concentration, bio-accumulation, and bio magnification. The toxic substance contamination in aquatic organism occurs through three ways namely (1) organism surface (2) respiration or ingestion of water and (3) food intake (zooplankton, phytoplankton) that contained chemical pollutant (Bhosale *et al.*, 2011; Supriyanto *et al.*, 2007). The higher concentration of metal in the waters where the clams live will be followed by the accumulation of metal in the clams either through the food chain with intermediary of diatomae or direct contact with the gill tissues during food filtering (Huang *et al.*, 2007).

Bioaccumulation Factor

The bioaccumulation factor average of Pb in *C. javanica* at the Maros river obtained that, bioaccumulation of Pb in C.

javanica from waters between 4.416-13.448, while from sediment between 0.186-1.402. It showed that C.*javanica* accumulate the Pb more from the waters than from sediment (Fig. 5). The Pb from waters between stations, significantly influence to the bioaccumulation of Pb to the *C. javanica*s ($p < 0.05$), obtained that only one station that different significantly with other station, that is Pappadangan station. While among Betang, Marusu and Mannaungan stations are not different significantly. Bioaccumulation factor of Pb from sediment between the two stations that showed a significant influence of bioaccumulation Pb to the C *javanica* ($p < 0.05$), Betang station different significantly with Mannaungan, Marusu, and Pappandangan stations. If compared between BAF_w and BAF_s values, then bioaccumulation of Pb at C. *javanica* mostly come from waters and only little come from sediment. It showed at Pappandangan station, where the BAF_w value of 13.448, while BAF_s value of 1.402. The amount of bioaccumulation value of Pb at C. *javanica* that come from the water occurred when the clams filter food in the water that contain Pb then accumulated in the clam. Gadzala-Kopciuch *et al*. (2004), stated that mollusk grouped into water filter organism, because obtain their food by filtering water around their living place. Feed by filtering make possible the heavy metal accumulation in body of clam. The bivalves *Corbiculidae* is clams group that live in the waters base submerge their body into sediment, appear at the sediment surface and move by using legs while filtering food by filtering water that contain suspended solid. Mollusk in general have higher concentration factor than other aquatic organism.

Water Quality in Maros River

Tabel 4 is list the physicochemical parameter of the waters in Maros River. Parameter value average of water quality if compared with Water Quality Criteria based on Class II (Departement of Environmental RI, Number 51, 2004), where the pH value at Station A (Pappandangan) still suitable in the range 6-9, while at station B (Mannaungan): 5.721; Station C (Marusu): 5.630; and D (Betang): 5.338, which value under range that suitable for fishery. For optimum growth for waters biota the pH between 7-8.5 influence the chemical process in the waters, where low pH caused the metal toxicity will increase.

The DO (dissolved oxygen) at all stations does not suitable for class II allotment is less 4 mgl^{-1}. Aquatic to grow optimally needs the DO more than 6 mgl^{-1} to fulfill the biochemical process in their bodies. The DO in waters is influenced by waters temperature. If the waters temperature is high then oxygen dissolvability low. It shown that the waters temperature value of all station more than 30^oC. The low dissolved oxygen value will increase the heavy metal accumulation risk at the waters organism. Metal accumulation in waters and sediment are also influence by conductivity, where the metal binding capability in waters will increase with the increasing conductivity. The waters and sediment conductivity values at all stations is high more than $25mScm^{-1}$.

CO_2 value (carbon dioxide) at all station showed high value, more than for class II allotment less than 5 ppt. Aquatic organism tolerances for carbon dioxide (CO_2) content in waters is 10 mgl^{-1}. The high CO_2 content comes from respiration, decomposition process of organic materials in soils, rain, and diffusion from atmosphere. CO_2 in waters take role in the calcium bicarbonate balance in waters and the photosynthesis process of aquatic plants.

Water turbidity at all stations showed high value, are not suitable for class II allotment. High turbidity will influence the low DO in the waters cause the hindrance of sun light penetration so the photosynthesis process is disrupted. High turbidity also will disturb the respiration process for aquatic organism. Particles will cover gill surface so inhibit the oxygen diffusion dissolved at the gill surface. Bioaccumulation can be occurred if the pollutant taking by organism higher than the losing level of pollutant from the organism body. Pollutant will experiences absorption or adsorption then be transformed to all tissues, either in the initial form or other form after experience chemical transformation. It means mollusk able to accumulate more metal in their body compared with other aquatic organism, so they are often be used as bio-indicator of metal pollution in waters (Abdullah *et al*., 2007)

CONCLUSION

The results showed that Maros River has occurred accumulation of Pb in waters, sediment, and Remis *C. javanica*. Accumulation of Pb in gill, stomach, and organ total of Remis *C. javanica* is influenced by Pb content in waters, while the influence of sediment Pb only significant at the gills Pb content. The Goodness of Fit test obtained that 74.2% data can be explained by the model. The Bioaccumulation Factor Value of Pb in C. *javanica* from waters more than sediment, it was found that this C. *javanica* has the potential to be used as bio-indicator for the pollutant of Pb in Maros River.

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