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# Application of *Gliricidia sepium* leaves compost as depuration agent of leads (Pb) in the body organ of red tilapia *Oreochromis* sp.

# Penggunaan kompos daun gamal *Gliricidia sepium* sebagai pendepurasi timbal (Pb) pada organ nila merah *Oreochromis* sp.

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## ABSTRACT

This study was aimed to perform depuration of Pb contained in tilapia body. The experiments were conducted in aquarium using compost of *Gliricidia sepium* leaves at different concentrations i.e. 10g/L, 20 g/L, 30 g/L, 40 g/L, and 0 g/L (control). The result showed that Pb level in fish muscle immersed with compost of *Gliricidia* leaves at a dose of 30 g/L for five days successfully decreased to a safe limit for human consumption (<0.3 mg/kg). However, decrease of Pb level in fish liver and kidney to finally reach the safe limit required seven days. Decreasing level of lead in the organs of experimental fish along with the increasing level of Pb in compost and culture media indicated that Pb accumulated in fish were released into the culture media by compost through chelation process. To conclude, compost of *G. sepium* leaves can be used as the material for depuration of Pb in the body of tilapia

Keywords: humic acid, fulvic acid, depuration, *Gliricidia leaves*, lead, red tilapia

## ABSTRAK

Penelitian ini bertujuan untuk mendepurasi Pb yang terkandung di tubuh ikan nila. Percobaan dilakukan di dalam akuarium menggunakan kompos daun gamal pada konsentrasi 10 g/L, 20 g/L, 30 g/L, 40 g/L, dan 0 g/L (kontrol). Hasil penelitian menunjukkan bahwa, Pb di daging ikan yang direndam dengan kompos daun gamal pada konsentrasi 30 g/L selama lima hari, kadarnya menurun hingga batas aman untuk dikonsumsi manusia (<0,3 mg/kg). Penurunan Pb di hati dan ginjal untuk mencapai kadar aman membutuhkan waktu yang lebih lama, yakni tujuh hari. Seiring dengan menurunnya kadar Pb dalam organ ikan uji, kisaran Pb dalam kompos dan media budidaya meningkat, menunjukkan bahwa Pb dari tubuh ikan dilepaskan ke media budidaya dan terjadi proses khelat oleh kompos. Dengan demikian, kompos daun gamal bisa digunakan sebagai bahan pendepurasi Pb dari tubuh ikan nila.

Kata kunci: asam humat, asam fulvik, depurasi, daun gamal, timbal, nila merah

## INTRODUCTION

*Kolong* is a tin (Sn) post-mining lake in Bangka Belitung province (Babel). In general, the *kolong* depth ranges from 2–50 m in length and approximately of 75–200 m in width (Henny & Evi, 2009). Nowadays, Babel province has been in a period of post-mining. As one alternative to drive economic of community in the post-mining area, Babel provincial government promotes freshwater aquaculture sector in *kolong*. There are at least 3,712.65 hectares of potential *kolong* for aquaculture activities (Sujitno, 2007). However, heavy metals such as lead (Pb) are always found in the water and sediments of *kolong* in an amount exceeding safe level for human consumption.

The content of Pb in the muscle of tilapia with length of 20–26 cm in *kolong* restocking reached 4 mg/kg (dry weight) while the content of Pb in wild small fish *Punctius* sp. found in *kolong* restocking amounted to 73.27 mg/kg (dry weight) (Henny, 2011). The content of Pb was very high if compared to the content of Pb in tilapia found in Cirata Dam that was only about 0.003 to 0.065 mg/kg (Priyanto *et al*., 2008). According to the previous research, accumulation of Pb in red tilapia muscle maintained for three months in *kolong* which has been established for more than ten years (>10 years) and less than ten years were 0.188 mg/kg (>10 years) and 24.33 mg/kg (<10 years), respectively. Whereas, the quality standard of maximum Pb content in processed fish meat is 0.3 mg/kg (ISO, 2009). Therefore, it is necessary to conduct a depuration process to eliminate Pb before the fish is released into the market.

Availability of *Gliricidia sepium* leaves is relatively abundant in Babel province. Biomass of gamal leaves is quick and easy to decompose, making the leaves of this plant as a great potential material to be used. According to Kucasoy and Guvener (2009), decomposition product of green leaves (compost) is one of materials that can be used to minimize heavy metals in the water of culture media. Compost minimizes heavy metals through ion exchange, adsorption and chelate formation. The substance of compost (fulvic acid, humic acid, and humin) is able to adsorb heavy metal complex via cation exchange, chelate formation, and electrostatic bonding (Hermana & Nurhayati, 2010). Based on preliminary test results, the composition of *Gliricidia* leaves after composted for 30 days contain 3.55% humic acid and 0.36% fulvic acid. These results confirmed the findings by Prasetiyono (2013) which showed that compost of *Gliricidia* leaf contained 3.84% humic acid and 6.45% fulvic acid.

Humic acid is a macromolecular material with a molecular weight (MW) of 20,000–1,360,000 g/mol, which has functional groups such as phenolic ($OH^-$) or alcoholates ($OH^-$), carboxylic ($COOH^-$)and soluble at alkaline conditions as well as have cation exchange capacity amounted to 500–700 mEq/100 g (Piccolo, 2002). Fulvic acid is a mixture of weak aliphatic and aromatic organic matter that dissolves at all pH conditions (acidic, neutral, and alkaline) and classified as micromolecular with molecular weight of 275–2,110 g/mol (Nebbioso & Alessandro, 2012). Fulvic acid has oxygen content twice than that of humic acid, yet it has low carbon and nitrogen, making it more reactive in binding metal ions (Christopher *et al*., 2013).

Study of the use of humic acid and fulvic acid as heavy metal chelating agent have been done. However, the use of compost of *Gliricidia* leaf (CGL) for chelating and depurating heavy metals especially Pb from the fish body has never been done. It is necessary to conduct research on the ability of CGL and its optimum concentration to depurate the accumulation of Pb in red tilapia organ, through the method of mixing into the culture media.

## MATERIALS AND METHODS

### Compost of *Gliricidia* leaves (CGL)

Composting was done by collecting raw materials, *Gliricidia* leaves. The leaves collected were green leaves and had been separated from the petiole (leafstalk). The leaves used were a mix of young and old leaves collected from *Gliricidia* trees with age of more than three years. *Gliricidia* trees whose leaves were used in this study grow in yellowish-brown podzolic soils. As much as 230 kg of fresh *Gliricidia* leaves were chopped into smaller pieces of ±1 cm. Furthermore, EM4 (effective microorganism 4) starter solution was prepared by adding 250 g granulated sugar and 5 mL of EM4 solution into 10 L of water. The solution then was mixed. Half of compost material that had been cut into small pieces was spread over at a thickness of ±2–3 cm in an open container. Later, rice bran was spread over the raw material to cover the compost raw materials. Moreover, EM4 starter was sprinkled over the mixture. Subsequently, another half of compost was put on the top of the layer. EM4 starter was further sprinkled and bran was spread again. This process continued to perform until the compost raw materials was completely used.

After the mixing process was completed, the raw materials were put into a plastic bag with a capacity of 50 kg. Monitoring of compost temperature was carried out daily by turning over the compost. The addition of water could be done if the temperature was too high and the humidity decreased. Composting was conducted for 30 days. Compost color which had already been black with a soil-like texture, temperature was in accordance with the ground water temperature, and stable pH within the range of 6.5–7.5 were early indication of ripe compost. Furthermore, analysis was performed to determine the level of compost ripeness, C and N content, C/N ratio, pH, and moisture, as well as level of humic acid and fulvic acid within the compost. Composition of CGL after being composted for 30 days is presented in Table 1.

Each compost with a weight of 10 g/pack which was ready to use was milled finely and

Table 1. Composition of *Gliricidia* leaves compost before and after composted for 30 days

| Composition | *Gliricidia* leaves | |
|---|---|---|
| | BC | AC |
| C (%) | 54.06 | 37.92 |
| N (%) | 3.51 | 2.99 |
| C/N ratio | 15.40 | 12.68 |
| pH | nm | 7.20 |
| Moisture content (%) | nm | 48.89 |
| Humic acid (%) | nm | 3.55 |
| fulvic acid (%) | nm | 0.36 |
| [CEC](Cmol+/kg) | nm | 80.2 |

Note: BC: before composted; AC: after composted; nm: not measured CEC: cation exchange capacity.

then wrapped with 0.45 mm of thin porous cloth. Composts were immersed in the culture media according to the treatment concentration.

**Fish and rearing conditions**

Experimental fish used was red tilapia *Oreochromis* sp. obtained from fish rearing pond of Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor. The average weight of each fish was 100 g. Fish were acclimatized for one week in cement tank with the size of 2×3 m. and water continuously flowed at a flow rate of 2 L/s. During acclimatization, mortality of experimental fish should be less than 10%, if the mortality was more than 10%, then the acclimatization process was extended for another week. In this period, feed were given three times a day containing 28% crude protein (CP), as much as 3% of body weight. After a period of acclimation, the entire experimental fish (except control) were injected with 10 mg/L Pb $(NO_3)^2$. Injection was caried out eight times with time interval between injections every two days. As much as 1 mL $(NO_3)^2$ was injected into the blood through vena caudalis. After eight times of injections, maintenance was continued for fifteen days in the same tank for acclimatization. Water which did not contain Pb flowed continuously in a flow-through model. Water flow was increased to 5 L/s. Further, Pb contents in the experimental fish organ were analyzed (H-0; before treatments were given). At the end of preparation, Pb content in experimental fish used was 3.026 mg/kg (dry weight) in muscle, 0.039±0.09 mg/kg in heart and 0.312±0.23 mg/kg in kidney.

The treatment in this study was started by moving fish into 15 aquaria. Each aquarium had a size of 50×33×30 cm, a capacity of 40 L, and density of 20 fish/aquarium. Fish were adapted for seven days in an experimental aquarium without treatment. The fish were given feed containing 28% crude protein (CP), as much as 3% of body weight. Each aquarium had an aeration, fish feces were cleaned every day by siphoning. On the eighth day, CGL was put into each aquarium in accordance with the concentration of the treatment. Water exchange was conducted less than 1 L every day for all aquarium of treatments, shortly after feces were collected.

**Water quality measurement**

Water quality measurements were carried out five times, namely on d-0, d-1, d-3, d-5, and d-7 of maintenance. Measurements were carried out directly in each aquarium of treatment, with three replications. Water quality parameters measured were dissolved oxygen (DO) and water temperature, using Lutron DO-5510 Dissolved Oxygen meter, and water pH using Hanna HI-98107 pH meter.

**Experimental design**

The experimental design used was completely randomized design, with four treatments and one control. The treatment applied was an administration of compost of *Gliricidia* leaf on culture media with several concentrations namely 0 g/L (control), 10 g/L, 20 g/L, 30 g/L, 40 g/L. Each treatment was repeated three times.

**Sampling and test parameters**

Sampling was performed randomly five times in each aquarium. The number of red tilapia

*Oreochromis* sp. taken in each aquarium was three fish (n=3 fish). At d-0, media water, compost, fish liver, kidney, and muscle were taken. On d-1, only water, compost, and fish muscle were taken. On d-3, water, fish liver, kidney, and muscle were taken. On d-5, water and fish muscle were taken. On d-7, water, compost, fish liver, kidney, and muscle were taken. Test parameters measured from each sample was the concentration of Pb and all tests were calculated in dry weight.

Destruction method used was wet method with three replications. Each organ sample of experimental fish was put into 50 mL beaker glass and weighed using the analytical balance. A minimum wet organ taken was as much as 10 g. Later, dry ashing was conducted by placing the wet sample into ceramic cup using a plastic spoon then dried it in an oven for 18 hours at 105 °C. After the sample was dry, it was cooled in a desiccator for 30 min.

Prior to use, PG 990 atomic absorption spectrometry (AAS) made in Germany was calibrated according to manual instruction for the tool. Measurement of standard solution of metal and blanks as well as measurement of sample solution were then conducted. Measurement of Pb content in each organ solution placed in 10 mL volumetric flask was done in a wavelength of 283.3 nm. During the standard measurement of metal, performance were checked periodically to ensure constant standard value.

## Data analysis

All data were presented according to mean±standard deviation (SD) and analyzed using one-way ANOVA, followed by Fisher's exact test using Minitab 15. Result was considered significantly difference if P<0.05.

# RESULTS AND DISCUSSION

## Results

Depuration method through the immersion of CGL in the culture media successfully affected the accumulation of Pb in various organs of experimental fish. In general, the results in this study indicate that the parameter of water quality such as dissolved oxygen, pH, and temperature was still in the range of tolerance to ensure the maintenance of red tilapia fish. Observation on the behavior of experimental fish in all treatments showed a normal result. The study was conducted during the rainy season. The average water temperature during the study was fluctuated, the lowest was at 24.1 °C while the highest was at 25.8 °C. In average, the lowest dissolved oxygen content was 5.00 mg/L and the highest was 6.76 mg/L. The range of pH in vthe rearing media was 5.00–6.73.

*Depuration of Pb from muscle*

Pb content in the muscle of experimental fish before treatment was equal at 3.026±1.43 mg/kg (H-0). Along with the increasing CGL immersion period, Pb concentration in all experimental fish muscle decreased (except in control). The decrease in Pb concentration has occurred since d-3, that was observed at concentration of 30 g/L and 40 g/L and was significantly different from other treatments (Figure 1).

*Depuration of Pb from liver*

Before treatment, lead concentration in the liver of experimental fish was 0.039±0.09 mg/kg. Moreover, all concentrations showed significantly different result against control since d-3 and d-7. Increasing concentration of

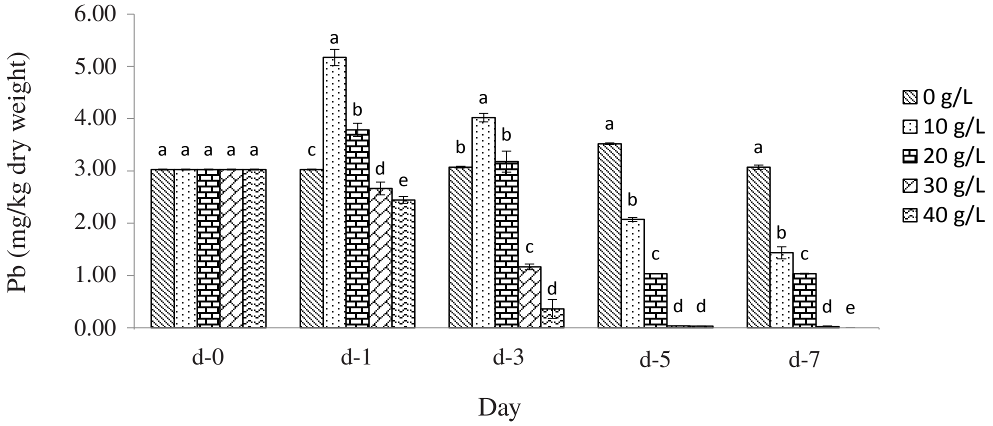


Figure 1. Concentration of Pb in the muscle of red tilapia *Oreochromis* sp. (mean±SD). Different letter above the bar chart denotes significantly different results (P<0.05).

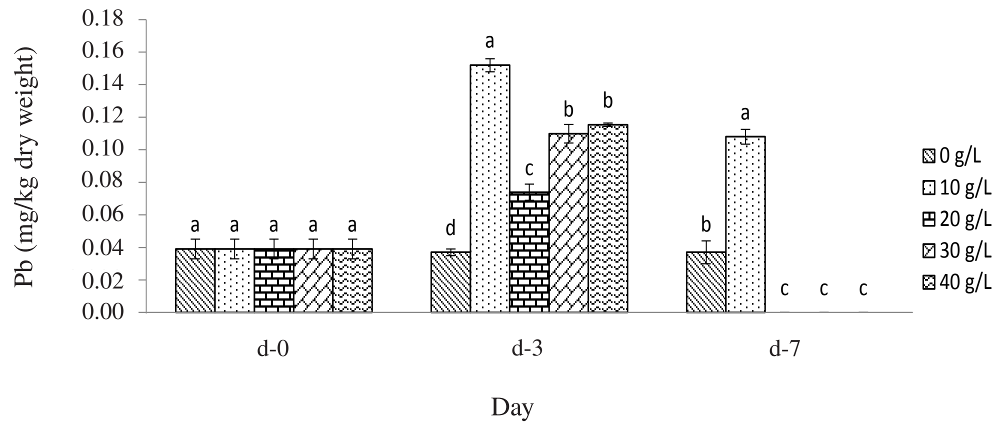


Figure 2. Concentration of Pb in the liver of red tilapia *Oreochromis* sp. (mean±SD). Different letter above the bar chart denotes significantly different results (P<0.05).

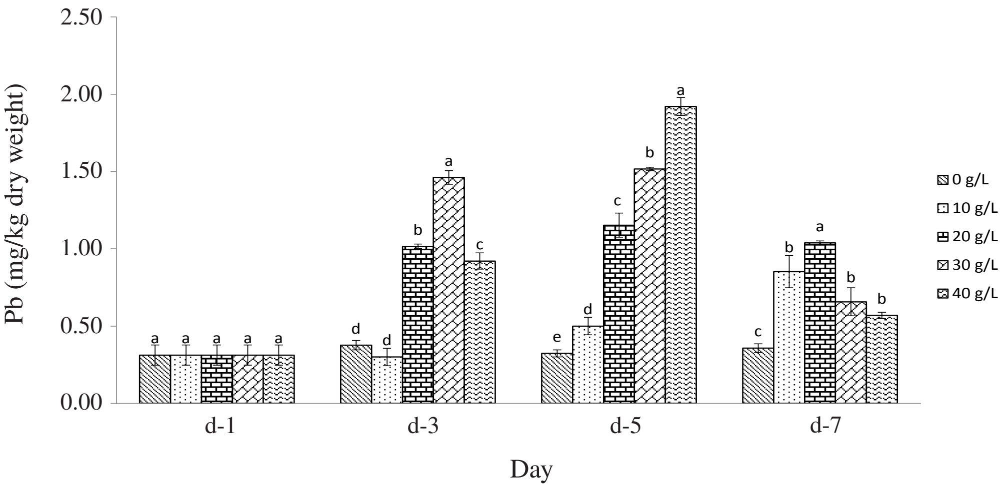


Figure 3. Concentration of Pb in the kidney of red tilapia *Oreochromis* sp. (mean±SD). Different letter above the bar chart denotes significantly different results (P<0.05).

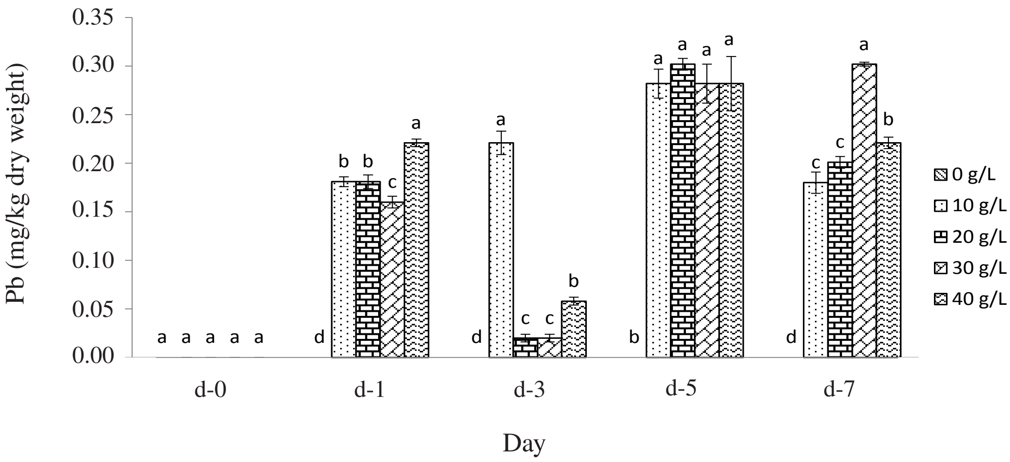


Figure 4. Concentration of Pb in the water culture media (mean±SD). Different letter above the bar chart denotes significantly different results (P<0.05).

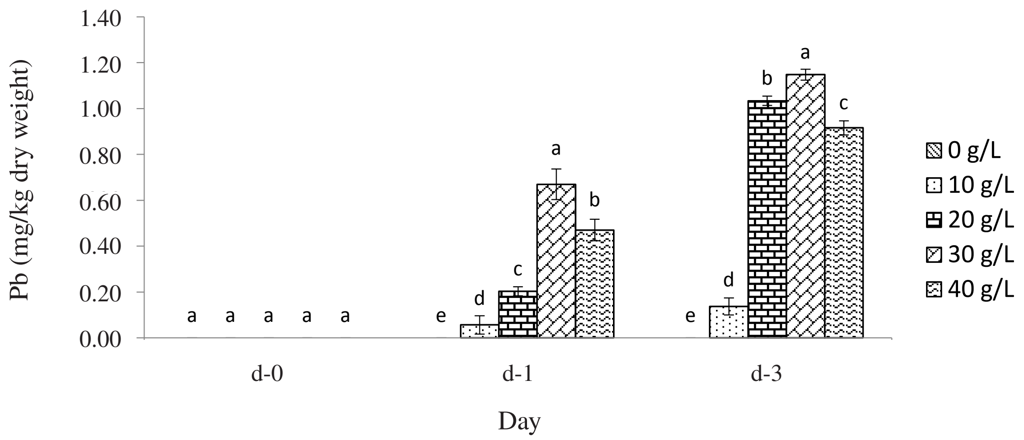


Figure 5. Concentration of Pb in compost (mean±SD). Different letter above the bar chart denotes significantly different results (P<0.05).

Pb in d-3 was observed which further decreased rapidly in d-7 to <0.012±0.00 mg/L (undetected), those were at concentration of 20, 30, and 40 g/L, proved that any concentrations of *G. sepium* leaves compost addition into the culture media resulted in significant effect in the liver of fish (Figure 2).

*Depuration of Pb from kidney*

At the beginning of culture period (d-0), concentration of Pb amounted of 0.312±0.23 mg/kg in the kidney of experimental fish. After the CGL addition in media, Pb concentration in the kidney increased since d-3 until d-5 of observation. Furthermore, a decline in concentration of Pb occurred in d-7. This trend was found in all concentrations of CGL treatment (Figure 3), except in control where the amount of accumulated Pb did not decrease during the observation period.

*Pb in water*

At the beginning of observation (d-0), Pb was not detected in the water used in all treatments Pb (<0.012±0.00 mg/L). However, the average concentration of Pb in all treatments (except controls) increased along with the culture period. At the end of the culture (d-7), the highest concentration of Pb was found in the media with CGL concentration of 30 g/L while the lowest was in 10 g/L (Figure 4).

*Pb in compost*

Observation of all concentration of treatment in d-0 resulted in the same value, that was <0.012±0.00 (not detected). Moreover, along with the increasing compost immersion time in water of treatment media, Pb in all concentration of treatment (except controls) have increased since d-3 and d-7. The highest increasing occurred in d-7. Above all, increase in concentration of Pb in compost showed significant difference between Pb concentration of 0 g/L and 10 g/L; and Pb concentration of 20 g/L, 30 g/L, and 40 g/L (Figure 5).

**Discussion**

According to the measurement result of humic acid and fulvic acid used in CGL, it is known that 1.814 mg of humic acid and 0.183 mg of fulvic acid were contained in every 100 g of CGL. Humic acid and fulvic acid have the ability to form complex with metal ions (Fu *et al*., 2011). Exchange capacity of fulvic acid which is more than twice higher than that of humic acid causes fulvic acid to be easier to penetrate cells and spread throughout the living organisms (Giannis *et al*., 2009). Due to the different nature of the two types of organic active matter, fulvic acid plays a greater role in the mechanism of depuration in organ cells through immersion method in the media, yet the role of humic acid outside the cell organ of experimental fish is also important. Fulvic acid enters the body of test fish through the water pumping process over the gills, through the water drunk by the test fish and direct penetration through the skin. Moreover, humic acid enters the fish while fish drinks the water.

As fulvic acid can easily enter through the gills, fulvic acid enters the gill cell by a mechanism of diffusion, ion pump and ion channel (Soto *et al*., 2010). The entrance of fulvic acid (by diffusion and ion pump) is in the form of respiratory cells contained in the secondary gill lamellae of fish and specific path in lamellar membrane, the path is directly connected to the blood stream gills and later into the blood.

Process of water intake directly from the fish mouth causes humic acid and fulvic acid enter the fish body. About 80% of organic compounds become inactive inside the fish stomach which has pH of 2–4; thus, Pb in the stomach will change into divalent form. The presence of humic acid and fulvic acid in the stomach will prevent Pb to change into divalent form. Its their active groups, those are carboxyl and phenolic, bind Pb and avoid from being absorbed by the stomach cells. In the intestines, fulvic acid can directly enter the digestive cells, that is entering through mikrovilli cells by diffusion, through ion pumps and through ion channels. Chelation mechanism of Pb by fulvic acid in mikrovilli cell of fish intestines is similar to the mechanism in gill cells (Soto *et al*., 2010). Pb chelated through the intestine is secreted back by mikrovilli cells out of the cell (in the intestines) to be excreted with feces and part of fulvic acids enter the blood. In the intestine, humic acid work more dominant than fulvic acid due to the nature of humic acid which is soluble in alkaline pH. Thus, Pb will be strongly bound through chelation mechanism then will be excreted through feces.

Similar to the statement of Orsetti *et al*. (2007), humic acids are capable to form a chelate with heavy metals with strong bond. The occurrence of this mechanism was proven by the increase in the Pb content in the water of culture media in all treatments. Increasing concentrations of Pb on the d-7 of treatment was found in concentration of 30 g/L (the highest) and 10 g/L (the lowest). This finding proved that the cation exchange capacity (CEC) of CGL has a maximum limit to bind Pb from water of culture media. In addition, CGL area that was in contact with culture media (such as compost layer that was too thick), also became the reason of decrease in Pb concentration in water of culture media at a concentration of 40 g/L on d-7.

Measurements of Pb in compost also showed significant results against control. Moreover, measurement result on CGL immersed in the culture media during the study indicated that all CGL contained Pb. This finding proved that CGL was able to bind Pb released into the maintenance water and later performed the chelation process; thus, Pb was not re-absorbed by the experimental fish. These results are consistent with the statement of Osman *et al*. (2009) and Deen *et al*. (2009) that the addition of fulvic acid and humic acid into the culture media will reduce the absorption and accumulation of heavy metals into the fish body. The highest concentration of Pb in CGL was 30 g/L, followed by concentration of 20 g/L, 40 g/L, and 10 g/L. The result of these measurements explains that organic ligand has a maximum capacity to bind metal. Wu *et al*. (2008) added that Pb ion and organic matter of compost will interact to form complex compound and chelate according to the amount of active ingredient of chelating agents.

Fluctuation of Pb contents in the culture media during the observation time indicated that process of which Pb was released from body of experimental fish into the media and later, chelation process of Pb from the water of culture media by CGL had occurred. Complex compounds will be strongly bound when the coordination compound, of which its Pb ion, is bound by ligand with two or more bonds (Orsetti *et al*., 2013). Adsorption method for Pb is generally based on the interaction between Pb ion and functional groups in humic acid and fulvic acid through the interaction of complex formation. Coordination complex is defined as cation compound which has empty orbital (central atom) with anion having one electron pairs (ligands) bind one another by sharing several lone electrons of the ligand (Orsetti *et al*., 2013). This mechanism occured in the water of culture media; thus, Pb was strongly bound in CGL and will not be released back into the water of culture media. Phenomenon of an increase of Pb in water of culture media and CGL during the research proved that immersion of CGL in the culture media of red tilapia *Oreochromis* sp. led to depuration process of Pb in the organs of experimentsl fish.

In the blood, fulvic acid will be more effective in binding Pb. There are two mechanisms of fulvic acid in the body. First, outside the cell, fulvic acid directly performs chelation which further will be transported to the secretory organ. Second, inside the cell, fulvic acid helps or together with methalloprotein (MT) in binding and carrying Pb out of the cells. Fulvic acid follows the circulatory system; thus, blood cells carries and transports fulvic acid to the detoxification organ (liver) and secretion organ (kidney). Similar mechanism is also performed by fulvic acid in the liver and kidney; therefore, the role of liver as detoxifying organ becomes more optimal (Soto *et al*., 2010). As proven in this research result, accumulation of Pb in the liver of experimental fish of all treatments on d-0 (the beginning of observation) resulted in the same concentration, that was 0.039±0.09 mg/kg. After the treatment of CGL immersed in water of culture media, there was increase of Pb in the liver on d-3, yet accumulation of Pb decreased on d-7. This trend was found in all treatments except control (concentration of 0 g/L) where the amount of Pb accumulation from the liver did not decrease. CGL immersion concentration of 20 g/L, 30 g/L, and 40 g/L was selected as effective concentration to depurate Pb in liver of red tilapia *Oreochromis* sp. through the media. It was observed that concentration of 20 g/L, 30 g/L, and 40 g/L was able to depurate Pb from 0.039±0.09 mg/kg to <0.012±0.00 mg/kg (not detected) on d-7 of observation. Increase and decrease in the concentration of Pb in fish liver during the study proves that immersion of CGL in the culture media can improve the liver performance and depurate Pb from the liver of red tilapia *Oreochromis* sp.

Result of measurement in the kidney also showed the same tendency toward the liver. On d-0, the ammount of accumulated Pb in fish kidney of all treatments was the same, equaled to 0.312±0.23 mg/kg. After treatment of CGL immersed in water of culture media, there was increase of Pb in kidney, in average, on d-3 and d-5 of observation, but the accumulation of Pb further decreased on d-7. This trend was found in all treatments (Figure 3), except in control (concentration of 0 g/L). Fluctuation of Pb concentration in fish kidney which occurred in several concentration treatment and at several time of observation showed that the addition of CGL into the culture media can improve the function and performance of kidney in eliminating Pb that has been accumulated in the body of red tilapia. This phenomenon also explained that the process of Pb elimination through the kidney became effective with CGL immersion method.

It was found from in the preliminary research that the amount of Pb remained in the muscle of experimental fish already contaminated with Pb and had been naturally depurated with continuous running water was 3.026±1.43 mg/kg (dry weight) of which this amount cannot be naturally re-depurated. Based on the quality standard, maximum Pb content in processed fish is 0.3 mg/kg (ISO 2009). On d-0 (the beginning of observation), Pb contained in the muscle of red tilapia amounted to 3.026±1.43 mg/kg.

Based on overall observations, the average accumulation of Pb in fish muscle in all treatment concentrations tended to decline. In general, decline occurred from d-5 until d-7. However, concentration of Pb in control during the observation relatively did not decrease. The best concentration of CGL in depurating Pb in the muscle of red tilapia through the medium was 30 g/L and 40 g/L. Based on the observation result, concentration of 30 g/L and 40 g/L was able to depurate Pb accumulated in the muscle of red tilapia, from being not safe to being safe for human consumption.

Decrease in Pb concentration in the muscle have occurred since d-3, but the concentration was still above the quality standard of safe for human consumption (<0.3 mg/kg). Significant declines continued until d-5 and d-7. Concentration of 30 g/L decreased to 0.035±0.01 mg/kg on d-5 of observation and further decreased to 0.023±0.06 mg/kg on d-7. Concentration of 40 g/L decreased to 0.032±0.02 mg/kg on d-5 and decreased again to <0.012±0.00 mg/kg (not detected) on d-7. Decrease in Pb concentration in muscle was due to increase in function of liver and kidney. Moreover, this decline also proves that there is activity of humic acid and fulvic acid as chelating agents of Pb in the body of red tilapia *Oreochromis* sp. Decrease in Pb concentration may be started since the rapid decline of Pb in blood of experimental fish due to elimination process of Pb which occurred effectively through the kidney. Furthermore, the occurrence of well chelation mechanism on Pb through the immersion of compost, so that Pb was released into the water of culture media and did not go back into the body of experimental fish, can also be the reason of declining concentration of Pb from the muscle of red tilapia *Oreochromis* sp.

In general, results of statistical analysis show that all test parameters observed during the observation period were significantly different from control. This condition proved that all concentrations of treatment (except control) affected the depuration process of Pb from the organs of experimental fish. Significantly difference result among the treatment concentration in each observation time indicated that different immersion concentrations of CGL will lead to different depuration effect on Pb accumulated in the organs of fish. Observation results described above proved that CGL was able to perform depuration of Pb from the body of red tilapia *Oreochromis* sp. through the media. Concentration of 30 g/L was found to be the best concentration of CGL in performing depuration of Pb from the body of red tilapia *Oreochromis* sp. through media.

## CONCLUSION

Based on the results, CGL immersed in the maintenance medium was able to depurate Pb in the muscle of tilapia *Oreochromis* sp. up to a safe limit for human consumption (<0.012 or not detected). The best concentration during five days of immersion was 30 g/L. The use of CGL with a five-day immersion method should be recommended for providing treatment of fish farmed in the post-mine of tin in *kolong*, Bangka Belitung Province, before the fish is released to the market.

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