Bio-elimination of lead (Pb) from the organs of red tilapia (*Oreochromis* sp.) using *Gliricidia sepium* compost as a feed additive

[1,3]Robin, [1]Eddy Supriyono, [1]Kukuh Nirmala, [1]Enang Harris, [2]Ridwan Affandi, [1]Dedi Jusadi

[1] Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia; [2] Department of Aquatic Resource Management, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia; [3] Department of Aquaculture, Faculty of Agriculture, Fisheries and Biology, Bangka Belitung University, Jl Balun Ijuk, Sungailiat, Bangka Belitung Province, Indonesia. Corresponding author: E. Supriyono, eddysupriyono@yahoo.com

Abstract. This study aimed to evaluate the effectiveness of *Gliricidia sepium* compost as a feed additive and the optimum dose to reduce the levels of lead (Pb) in the muscles of cultured red tilapia (depuration). *G. sepium* compost was added to the fish diet at the dose of 0 g kg^{-1} (control), 10 g kg^{-1}, 20 g kg^{-1}, 30 g kg^{-1} and 40 g kg^{-1}, fed three times daily. Experiments have been conducted on a laboratory scale. The fish with an average weight of 100 g were cultivated for seven days in an aquarium with the size of 50×33×30 cm^3 filled with 40 L water at a density of 20 fish per aquarium. The results showed that addition of *G. sepium* compost at a dose of 30 g kg^{-1} for seven days lowered the Pb concentration in red tilapia flesh to a level safe for human consumption (< 0.3 mg kg^{-1}). A corresponding concentration of Pb in the culture media suggested that Pb was released from fish's body into the water. Therefore, it could be concluded that *G. sepium* compost added to the diet could be used for bio–elimination of Pb in red tilapia.

Key Words: *kolong*, heavy metal, humic acid, fulvic acid.

Introduction. Until 2016, Bangka Belitung (Babel) Province, Indonesia, is still one of the world biggest producers of tin (Sn). The open-pit mining system has left behind many deep holes that are filled with water, locally known as "*kolong*" (lake) (Henny & Susanti 2009). The dimensions of these *kolongs* are usually approximately 75–200 m in length and width ranging from 2 to 50 m in depth. The current economic condition of the people of Babel is they are entering the post-mining era. As one alternative to drive the local economy in the post-mining era, the Babel provincial government is promoting the freshwater aquaculture sector by utilizing the *kolong*. At least 3,712.65 ha of *kolong* are potential for aquaculture. However, lead (Pb) is always found in the water and sediment of the *kolong* at the amounts exceeding the safe level for human consumption. The Pb content of tilapia flesh from fish sized 20-26 cm from *kolong* restocking reached up to 4 mg kg^{-1} (dry weight), while in the small wild fish *Puntius* sp. found in the same *kolong* was 73.27 mg kg^{-1} (dry weight) (Henny 2011). The preliminary study, stated that red tilapia (*Oreochromis* sp.) kept for three months in ten-year-old *kolongs* (> 10 years) and *kolongs* formed less than 10 years previously (< 10 years) had accumulated Pb in their flesh at concentrations of 0.188 mg kg^{-1} (> 10 years) and 24.33 mg kg^{-1} (< 10 years). However, the standard of maximum Pb content in processed fish is 0.3 mg kg^{-1}. Therefore, the depuration process is necessary to reduce Pb content before the fish are released to the market.

The utilization of local organic raw materials such as leaves as a heavy metal bio-elimination agent should be prioritized. *Gliricidia* leaves (*Gliricidia sepium*) are abundant in Babel Province, have great potential to be used due to the ease and speed in decomposing. Kocasoy & Guvener (2009) and Prasetiyono (2013) found that the results of decomposition of green leaves (compost) can be used to minimize heavy metals concentration in water. Compost minimizes heavy metals through ion exchange, adsorption, and chelating methods. The substances contained in compost (fulvic acid, humic acid, and humin) are able to adsorb heavy metal complexes through cation exchange, chelate formation and electrostatic binding (Hermana & Nurhayati 2010). Preliminary trials revealed that *G. sepium* composted for 30 days contained 3.55% humic acid and 0.36% fulvic acid.

The use of humic acid and fulvic acid as chelators in *G. sepium* compost have been mentioned by a number of previous studies. Pb ions will interact with organic materials in the compost to form complexes and chelates (Orsetti et al 2006). Humic acid and fulvic acid can form a complex with metal ions (Giannis et al 2009) and form complex bonds (Orsetti et al 2013b). The method for Pb adsorption is based on the interaction between Pb ions and the functional groups in the humic acid and fulvic acid (Fu & Wang 2011). Some studies on evaluating adsorption of metal cations by oxide particles have shown positive results (Nebbioso & Piccolo 2012; Orsetti et al 2013a; Esfahani et al 2015) and the ability of humic substances to bind with metal cations (Hermana & Nurhayati 2010; Xiong et al 2013; Qin et al 2015). Fulvic acid has a very complex composition, has small molecules, and can easily penetrate cells and spread throughout the living organism (Miller et al 2013).

Due to the aforementioned considerations, it is necessary to conduct a study with the inclusion of a biotechnological application on the effectiveness of *G. sepium* compost as a feed additive for rapid Pb depuration from red tilapia and the optimum dose of *G. sepium* compost as a feed additive to eliminate the heavy metal Pb from the body of the red tilapia until safe levels for human consumption.

Material and Method. This study was conducted for four weeks on April 2015. The first two weeks was used for the injection of Pb to the experimental fish, the next week was used for the natural depuration and adaptation, then the last week was used as the treatment period. The rearing of the experimental fish and water quality analysis were conducted at Environmental Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia. The analysis of Pb was carried out at Integrated Laboratory, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia. The histochemical analysis of fish organs was conducted at Aquatic Organisms Health Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia. The production of feed containing *Gliricidia* leaves compost was done at Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia. The analysis of humic acid and fulvic acid contained in the feed was conducted at the Research Center of Plantation Biotechnology Indonesia, Bogor, Indonesia.

***Composting Gliricidia leaves* (*G. sepium*)**. The process began with collecting raw materials, *G. sepium*. The leaves that were collected were ones that were still green (actively growing) and had been separated from the leaf stems. The leaves were a mix of young and old leaves from *Gliricidia* trees over three years of age. These trees grew in yellowish brown podzolic–association soil. A total of 230 kg of fresh *G. sepium* was chopped into ±1 cm pieces. The EM4 (effective microorganism 4) starter solution was prepared by mixing 250 g of sugar and 5 mL of EM4 solution in 10 L of water. A portion of the small pieces of *G. sepium* was then spread on the floor at a length of 1-1.5 m and thickness of ±2-3 cm, then covered with rice bran and EM4 starter solution was sprinkled on top as the first layer. The layering process was repeated on top of the first layer until all the materials were used. The compost materials were then put into 50 kg capacity bags. The temperature of the compost was monitored and the bags were flipped over daily. Water could be added if the temperature was too high and the humidity was low. The composting process took 30 days. Ripe compost was indicated by the color changing into black, a soil-like texture, a similar temperature as ground water, and a stable pH ranging between 6.5 and 7.5. The *G. sepium* compost was then sun-dried until the moisture level was less than 10% and ground into a powder and sieved using a 1 mm filter. Proximate analysis of compost was performed to confirm the ripeness of the compost by measuring C, N, humic acid and fulvic acid content, pH, and C/N ratio. The composition of *G. sepium* after being composted for 30 days is presented in Table 1.

Table 1
The results of proximate analysis of *G. sepium* before and after composting for 30 days

Composition	*G. sepium*	
	SM	*SD*
C (%)	54.06±1.09	37.92±1.65
N (%)	3.51±0.05	2.99±0.08
C/N ratio	15.40±2.33	12.68±0.47
pH	td	7.20±0.72
Moisture (%)	td	48.89±1.16
Humic acid (%)	td	3.55±0.35
Fulvic acid (%)	td	0.36±0.03

Data are presented as means ±SD (n = 3); td = not measured; SM = before composting; SD = after composting.

Preparation of feed containing G. sepium compost. Preparation of the experimental feed was conducted by mixing *G. sepium* compost with commercial feed containing 28% crude protein (CP). The commercial artificial feed and *G. sepium* compost were ground using a feed milling machine separately. The ground *G. sepium* compost was added to the commercial feed at the doses of 0 g kg^{-1} (control), 10 g kg^{-1}, 20 g kg^{-1}, 30 g kg^{-1} and 40 g kg^{-1} commercial feed. Water was also added to the mixtures up to 20%, and the separate mixtures were stirred thoroughly and re-pelleted using a pelleting machine. The feed was dried in an oven at a temperature of 60°C for 12 hours. The proximate analysis of the feed was performed to check the content of nutrients such as protein, fat, and carbohydrate. In addition, humic acid and fulvic acid contents of each experimental feed were also measured. The feed was stored in plastic containers with the appropriate labels so that they would not be confused during use. Table 2 presents the results of proximate analysis of feed mixed with *G. sepium* compost.

Table 2
The results of proximate analysis (% dry weight) of feed mixed with *G. sepium* compost

Dose (g kg^{-1})	*Moisture*	*Ash*	*Protein*	*Fat*	*Carbohydrate*	
					Crude fiber	*Nitrogen free extract*
0	4.86±0.20	8.95±0.08	31.71±0.11	5.92±0.03	6.77±0.36	41.79±0.41
10	4.65±0.22	9.36±0.05	30.24±0.14	6.35±0.09	6.28±0.20	38.12±0.46
20	5.65±0.31	8.60±0.06	30.04±0.10	5.82±0.03	7.20±0.39	38.69±0.30
30	5.19±0.28	8.93±0.08	30.93±0.10	5.95±0.03	6.99±0.15	42.01±0.42
40	5.16±0.33	9.16±0.02	30.26±0.16	6.09±0.07	5.80±0.27	43.53±0.43

Data are presented as means ±SD (n = 3).

Experimental fish. This study used red tilapia from the cultivation ponds of the Faculty of Fisheries and Marine Science, Bogor Agricultural University, as the experimental fish. A selection process was conducted to get a uniform size of fish. Fish with an average weight of 100 g were acclimatized for one week in a concrete tank at a size of 3×2x1.5 m with continuous water flow from the Regional Clean Water Company at a debit of 2 L s^{-1}. During the acclimation process, fish mortality must be less than 10% or acclimation must be extended for another week. During the acclimation process, the fish were fed three times a day (at 08.00-09.00, 12.00-13.00, 16.00-17.00 Indonesia Western Time) using feed that contained 28% crude protein (CP) at a rate of 3% of the body weight. After the acclimation process, all of the experimental fish (except the control) were injected with 1 mL of 10 ppm $Pb(NO_3)_2$. After injections were done eight times through the caudal vein at an interval of one injection every two days, the fish were then reared for 15 days in the same tank with a continuous flow of Pb-free water and the water debit was increased to 5 L s^{-1}. The Pb concentration in the fish flesh was analyzed as the initial Pb level (D–0 before the treatments). After the concentration of Pb could no longer be depurated by running water, the treatments were commenced. The fish were transferred to 15 aquariums sized 50×33×30 cm with a 40 L capacity and were stocked at a density of 20 fish per aquarium. The fish were adapted to the new aquariums for seven days with no treatments. The fish were still fed the 28% CP feed at a dose of 35% of the body weight. Aeration was provided to supply oxygen and the feces removed daily by siphoning. On the 8th day, the fish were fed the experimental feed containing *G. sepium* compost according to the treatment doses. Feed was fed three times a day (at 08.00–09.00, 12.00–13.00, 16.00–17.00 Indonesia Western Time) through *ad satiation* method for one week. Aeration to ensure an oxygen supply and feces removal was continued. At the end of the preparation, the experimental fish had contained 3 mg kg^{-1} Pb (dry weight) in the flesh, 0.039±0.09 mg kg^{-1} in the liver, and 0.312±0.23 mg kg^{-1} in the kidneys.

Assessment of water quality. The measurement of the water quality parameters was conducted five times, on day 0 (D-0), day 1 (D-1), day 3 (D-3), day 5 (D-5), and day 7 (D-7) of the treatments. The measurements were taken directly in each of the treatment aquariums, triplicate. The water quality parameters assessed were the dissolved oxygen level (DO) and the water temperature using a Lutron DO 5510 digital DO-meter, and the water acidity (pH) using a Hanna HI 98107 digital pH meter.

Experimental design. This study was conducted through the completely randomized design with four treatments and one control in triplicates. The treatments applied were different doses of *G. sepium* compost, namely 0 g kg^{-1}, 10 g kg^{-1}, 20 g kg^{-1}, 30 g kg^{-1} and 40 g kg^{-1} of feed. The effectiveness of *G. sepium* compost addition in the diet was determined by the level of Pb in the flesh, liver, kidney, feces, and rearing medium of the experimental fish.

Sampling and parameter evaluated. During the study, samples were collected five times, on day 0 (D-0), day 1 (D-1), day 3 (D-3), day 5 (D-5), and day 7 (D-7). On D-0, samples from the medium, feces, liver, kidney and flesh were collected to measure the Pb content, while on D-1, the medium, feces, and flesh were taken. On D-3, samples were collected from the medium, liver, kidney, flesh, and feces, while on D-5, samples were collected only from the medium, flesh, and kidney. On D-7, samples for Pb concentration were collected from the medium, feces, liver, flesh, and kidney. All Pb content analysis were conducted based on dry weight.

Organ and feces collection. Three fish were randomly taken from each aquarium for organ samples. The wet samples were collected separately based on the treatment and aquarium. In the laboratory, each organ was put in a 50 mL beaker glass and weighed using an analytical scale. The minimum weight for wet organ samples required for AAS analysis was 10 g. The samples were then put in a tray and spread out using a plastic spoon and dried in an oven at 105°C for 18 hours to make a dry-ash form. The samples were cooled down inside a desiccator for 30 minutes. This present study used a wet destruction method. The dried and pulverized samples were treated using the microwave digestion system. The amount of samples destroyed was 2 g for each treatment. A TFM vessel was placed on an analytical scale, the scale was set to zero. Approximately, 2 g of the sample was placed in the vessel and 8.0 mL of concentrated HNO_3 (65%) was slowly added to organ sample, homogenized and put in an HTC protective case and the lid was affixed. The vessel was placed in the microwave and the temperature sensor was set up and the microwave was operated at 180°C for 30 minutes. After the destruction process was completed, the vessel was cooled down to room temperature and the solution was filtered into a 10.0 mL volumetric flask using Whatman filter paper No. 41 and then the paper was washed with demineralized water. More demineralized water was added to the reach volume limit. Sample destruction was carried out in triplicates. Before the sample solutions were assessed, the atomic absorption spectrometer (AAS) was calibrated by measuring the standard metal solution and a blank solution. Each sample in 10 mL volumetric vessel was measured for its Pb content using the German-made PG 900 AAS at 283.3 nm wavelength. The metal standard was checked periodically between measurements to ensure a constant standard value.

***Water sampling and analysis**.* Water was taken from each aquarium and grouped based on the treatments. The collection was done using 500 mL metal-free polyethylene bottle, then filtered using 0.45 µm filter paper. The pH level was maintained at a range of 3.5-4 by adding 1 mL of concentrated HNO_3. The assessment of the Pb level in the samples was conducted in the laboratory with the addition of acetate buffer. Then 5 mL of ammonium pyrrolidine dithiocarbamate (APDC) was added, stirred for five minutes, then 10 mL of organic solvent methyl iso-butyl ketone (MIBK) was added, stirred for three minutes, and the solution was rested to allow the two phases to separate. The water part of the sample was collected and used for making the laboratory blank and standard solutions. Ten (10) mL of deionized double-distilled water was also added, stirred for five seconds, and set aside to allow the two phases to separate. The water part was then discarded. The final step was adding 1 mL concentrated HNO_3, stirring briefly and set aside for 15 minutes, then another 9 mL of deionized double-distilled water was added and stirred for two minutes. The Pb in the water samples was measured using the German-made PG 900 atomic absorption spectrometer (AAS) at 283.3 nm wavelength.

Data analysis. All data were presented based on the mean ± standard deviation (SD) and analyzed using one–way ANOVA, continued by Fisher's exact test using Minitab 16. The difference is considered significant when $p < 0.05$.

Results and Discussion. Bio-elimination of Pb from the body of red tilapia using *G. sepium* compost added to feed at different doses could influence the elimination rate in a number of organs observed. The proximate analysis of feed mixed with *G. sepium* compost is presented in Table 1. In general, the study results demonstrated that water quality parameters such as dissolved oxygen, water pH, and water temperature were within tolerable levels for red tilapia to thrive. The observations results of the behavior of the experimental fish showed that it was normal for all treatments. The average daily water temperature ranged from 25.0 to 26.8°C, while DO ranged from 4.00 to 5.76 mg L^{-1} and the pH ranged from 5.50 to 6.98.

Feeding the feed mixed with *G. sepium* compost decreased the Pb concentration in the flesh of the experimental fish in all the treatments from D-3 to D-7. The largest decreases were found at the 40 g kg^{-1} dose, followed by the 30 g kg^{-1} dose on D-7 at 0.275±0.02 mg kg^{-1} and 0.288±0.03 mg kg^{-1}, respectively. The reductions have brought them to the level safe for human consumption (< 0.3 mg kg^{-1}) (Figure 1), while fish in the control group showed no significant change.

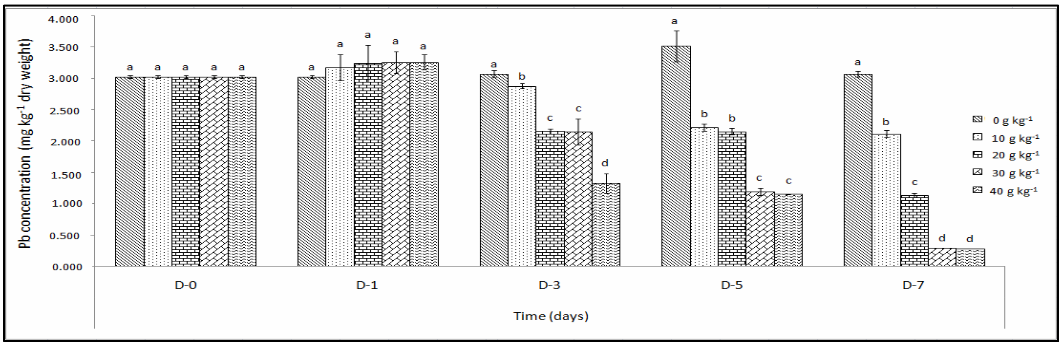

Figure 1. The concentration of Pb in fish flesh. Different letters in each observation time show significant differences among treatments ($p < 0.05$).

Before the treatments, the Pb concentration in the liver of the experimental fish was 0.039±0.09 mg kg^{-1}. On the D-3, the Pb concentration in the experimental fish livers showed a decrease. At the 10, 20, 30 and 40 g kg^{-1} doses, the concentration decreased to 0.022±0.001 mg kg^{-1}, while the 0 g kg^{-1} dose (control) decreased slightly to 0.035±0.002. This proved that feeding feed mixed with *G. sepium* compost had an influence on the concentration of Pb in the liver of the experimental fish. The lowest concentration of Pb was found on D-7 at the dose of 10 g kg^{-1} and 20 g kg^{-1} which had decreased to < 0.012 mg kg^{-1}, while at the 30 g kg^{-1} and 40 g kg^{-1} doses the Pb concentration increased slightly on D-3, which proved that there had been an increased activity in the liver of fish fed with feed mixed with *G. sepium* compost at doses of 30 g kg^{-1} and 40 g kg^{-1}. All treatments were significantly different from the control from D-3 and D-7 (Figure 2).

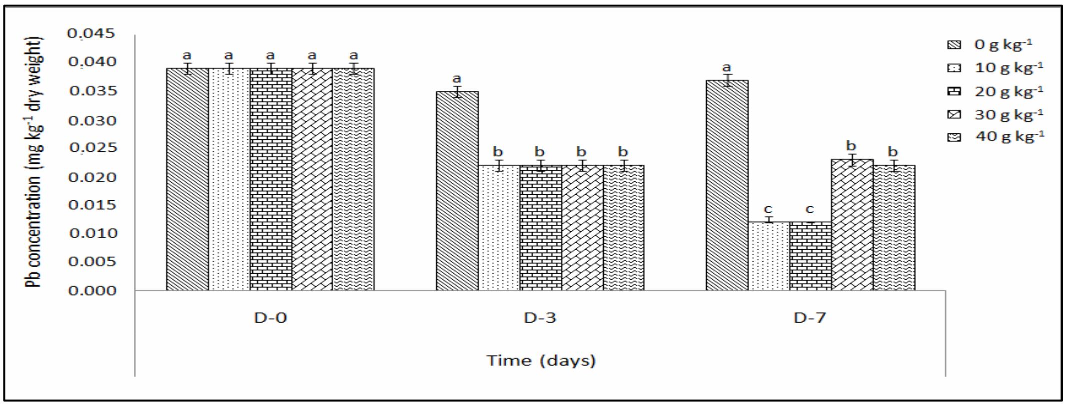

Figure 2. The concentration of Pb in fish liver. Different letters in each observation time show significant differences among treatments ($p < 0.05$).

At the beginning of rearing period (D-0), the concentration of Pb in the experimental fish's kidneys was 0.312±0.23 mg kg^{-1}. After the treatment was administered, there was a constant decrease in the concentration of Pb in the kidneys up to the final observations. The Pb concentration decreased to the limit safe for human consumption. At the doses of 10 g kg^{-1} g kg^{-1}, 30 and 40 g kg^{-1} in the final observations (D-7) demonstrated kidneys clean from Pb (Figure 3). At a dose of 0 g kg^{-1} (control), the concentration of Pb was almost the same from the beginning to the end of observation, while concentrations of Pb at doses of 10 g kg^{-1}, 20 g kg^{-1}, 30 g kg^{-1} and 40 g kg^{-1} increased sharply on D-3 and then from that day on declined until D-7. This indicates that feed mixed with *G. sepium* compost was able to assist the work of the kidneys in excreting Pb from the body of the red tilapia.

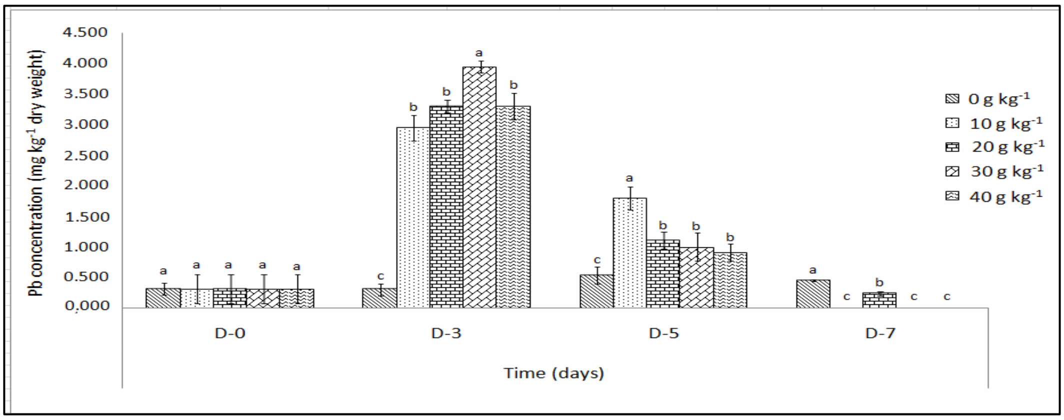

Figure 3. The concentration of Pb in the fish kidney. Different letters in each observation time show significant differences among treatments ($p < 0.05$).

On D-0, accumulation of Pb was not yet discovered in the feces of the experimental fish. After feeding the feed mixed compost with *Gliricidia* leaves, the average concentrations of Pb in the feces increased. The highest increase was found at the dose of 30 g kg^{-1} from D-3 to D-7 of the observation, while the content of Pb in the control fish feces was found to have not experienced a significant increase (Figure 4). Increased concentrations of Pb in the feces of experimental fish indicated that feed mixed compost *G. sepium* was able to help the digestive system to excrete Pb from the body of red tilapia through the intestines.

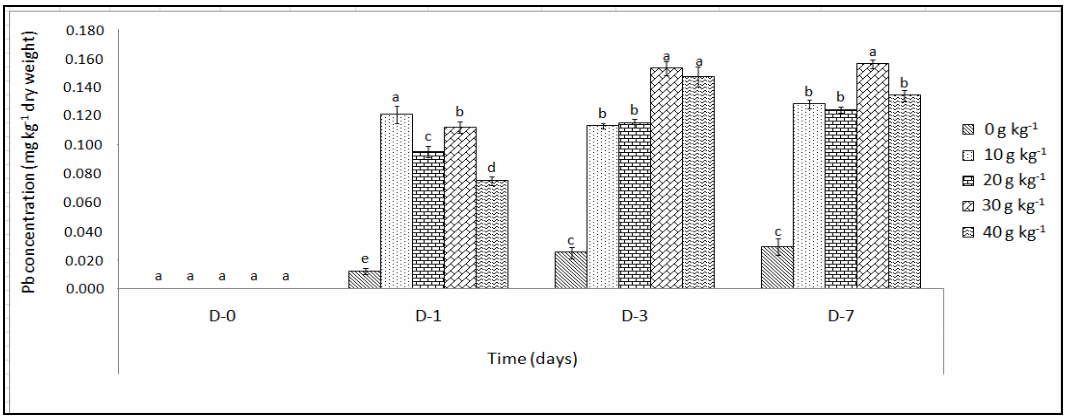

Figure 4. The concentration of Pb in feces of red tilapia (Mean±SD). Different letters in each observation time show significant differences among treatments ($p < 0.05$).

In general, the Pb concentration in all the treatments (except for the control) showed an increased level on the last day of observation. The highest concentration at D-7 was found at the 30 g kg^{-1} dose while the lowest was at 20 g kg^{-1}. This result suggested that there was a Pb bio-elimination which released Pb to the rearing medium (Figure 5).

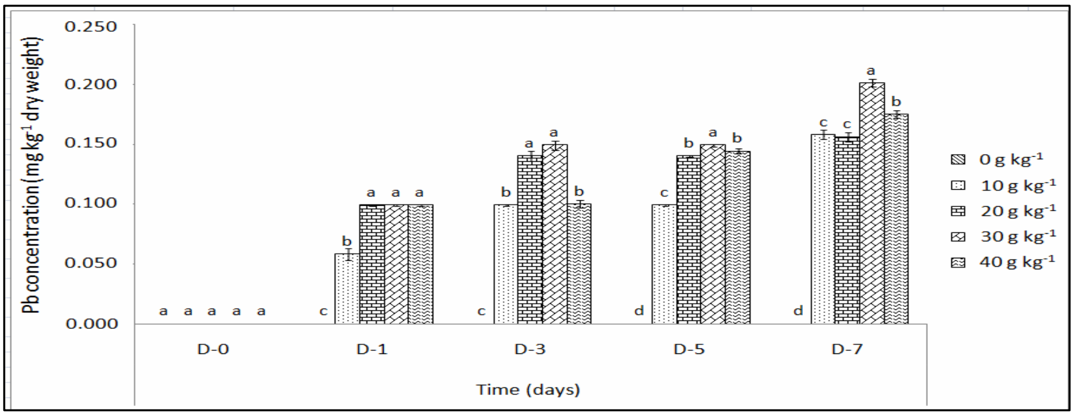

Figure 5. The concentration of Pb in rearing media (Mean±SD; n = 3). Different letters in each observation time show significant differences among treatments ($p < 0.05$).

In general, the results of this study indicate that the parameters of the water quality as dissolved oxygen, pH and water temperature of all treatment doses were within the red tilapia's range of tolerance and allow the fish to thrive. During the research, the fish were also in good condition, exhibited normal behavior, were healthy and there was no mortality.

The results of this research proved that feeding feed mixed with *G. sepium* compost could eliminate the heavy metal Pb from the flesh, liver, and kidney of the experimental fish. The content of humic acid and fulvic acid in composted *G. sepium* revealed that in every 100 g of *G. sepium* compost there was 1.814 mg of humic acid and 0.183 mg of fulvic acid.

It began with feeding feed containing *G. sepium* compost to the experimental fish that had Pb 3.026±1.43 mg kg^{-1} in their flesh, 0.039±0.09 mg kg^{-1} in the liver and

0.312±0.23 mg kg^{-1} in the kidneys. The active ingredients contained in the compost will enter the digestive system. In the stomach of fish which has a pH of 4-5, 80% of biological forms become inactive, so the Pb contained in the stomach change into a divalent form. The presence of humic acid and fulvic acid in the stomach will prevent Pb from changing into its divalent form. This is very important so that Pb could not directly enter the blood through the cells in the stomach wall. Humic acid and fulvic acid can form complexes with metal ions (Wu et al 2008; Giannis et al 2009). The active units, the carboxyl and phenolic units, bind Pb to prevent it from being absorbed by the stomach cells and send it along to the intestines. Because fulvic acid contains more oxygen functional groups i.e. carboxyls (–COOH) and hydroxyls (–COH) (Orsetti et al 2013b), fulvic acid is much more chemically reactive than humic acid. The capacity of fulvic acid exchange is more than double that of humic acid with a molecular weight (MW) between 275 and 2110 g mol^{-1} (Giannis et al 2009). This trait allows fulvic acid to easily penetrate cells and spread throughout the entire living organism (Miller et al 2013), making fulvic acid the most instrumental in eliminating Pb from organs.

Fulvic acid in a certain amount can go directly into the blood. The *port de entree* is from the stomach and intestines. In the stomach, the gateway for fulvic acid is the stomach cell walls through the diffusion mechanism, ion pumps and ion channels leading to the bloodstream and then into the blood. In the intestines, fulvic acid can directly enter the digestive cells, entering through microvilli cells by diffusion, through ion pumps and ion channels and then into the blood. In the blood, fulvic acid performs two mechanisms. First, outside of the cell, fulvic acid chelates directly with ionized Pb which is then carried to the secretory organ, the kidney. Second, in the cell, fulvic acid helps or collaborates with metalloprotein (MT) in binding Pb so it becomes chemically inactive and carries it out of the cell (Soto et al 2010). Fulvic acid follows the circulatory system, and blood cells carrying fulvic acid will deliver the acid to the detoxifying organ (liver) and secretory organ (kidney). Fulvic acid performs the same mechanism in the liver and kidney as in the blood cells. The result is the role of the liver as a detoxifying organ becomes more optimal. This is proven by the results of research that demonstrated liver activity on D-3, and that Pb concentrations in all treatment doses decreased more than the decline that occurred in the control treatment. Meanwhile, on D-7 the decrease in all treatment doses showed more fluctuations when compared to Pb concentrations in the liver of the control treatment which tended to be stagnant (Figure 2). Through the mechanism of bile liquid, fulvic acid will carry Pb that is chelated from the blood and liver to the intestines to be excreted through feces. In the intestines, humic acid will work more dominantly than fulvic acid due to the nature of humic acid which dissolves in alkaline pH, with the chelation mechanism strongly binding Pb to be excreted via feces. This mechanism is reinforced by the feces analysis results (Figure 4); there was an increase in the concentration of Pb in the feces of the experimental fish from D-1 to D-7 of the observation. On the other hand, the Pb content in the control fish feces was found to have not experienced a significant increase.

Observations on the kidney of the experimental fish also demonstrated a trend similar to that of the liver. After administration of the feed mixed with *G. sepium* compost, on average there was an increase in Pb concentrations in the kidney on D-3, and Pb concentrations decreased further on D-5 and Pb concentrations continued to decrease to D-7. This trend occurred in all the treatments (Figure 3) except for the control (0 g kg^{-1} dose). There were fluctuations in the concentration of Pb in the kidneys of the experimental fish; in some treatment doses at certain observation times, it was revealed that the addition of *Gliricidia* leaves compost into the maintenance medium could improve the function and performance of the kidney in eliminating Pb accumulated in the body of the tilapia. This phenomenon could also explain that by feeding feed mixed with *G. sepium* compost, Pb elimination through the kidney could also occur effectively. The proof for the elimination process through the kidneys was strengthened by results of the Pb concentration in the rearing medium (Figure 5). The Pb concentration decreased in the kidneys of the experimental fish from D-5 to D-7 of the observation, followed by an increased Pb concentration in the rearing medium.

In the condition of the decline in Pb contaminants in the body of the experimental fish, such as in the blood, liver, and kidney, the body of the experimental fish would naturally adjust. As a result of the formation of a gradient between the concentration of Pb inside and outside the muscle cells, then the law diffusion would work on the cells of the experimental fish's muscle. According to Di Giulio & Hinton (2008), the elimination rate of chemical residue will drop linearly a gradient is created between the intracellular environment and extracellular environment. The results of this study strengthened this statement as the administration of feed mixed with *G. sepium* compost was able to eliminate Pb in the flesh of the experimental fish from D-3 to D-7 of the treatments (Figure 1). The decline in Pb accumulation reached levels that were safe for human consumption (< 0.3 mg kg^{-1}) and was significantly different from the control. This proved that the use of *G. sepium* compost mixed into feed was able to eliminate the heavy metal Pb from the flesh of red tilapia. All treatment doses mixed with the feed could effectively eliminate Pb from the body of the experimental fish. Based on the consideration of efficiency in using *G. sepium* compost, 30 g kg^{-1} is the best dose that can be used to eliminate the accumulation of Pb in the flesh of the experimental fish.

Overall the results of this research demonstrated that the concentration of Pb in the liver, kidneys and flesh tended to decline proportional to the observation time, and inversely proportional to the Pb concentration in rearing medium and the experimental fish feces. This result indicates that mixing *G. sepium* compost with feed could chelate Pb ions from the organs of the fish and create stable complexes, reducing the opportunity for metal absorption by the body tissues. In addition, adding *G. sepium* compost as a feed additive could eliminate Pb from the body of the fish through urine. An increased concentration of Pb in water clearly represents the reduction of the metal burden in the tissues, which means mixing *G. sepium* compost into feed had a positive impact on the bio-elimination process in the body of the experimental fish.

Conclusions. The application of biotechnology through the addition of *G. sepium* compost into feed with 30 g kg^{-1} dose in seven days of administration could eliminate the heavy metal Pb from the body of the red tilapia until levels safe for human consumption. Field applications can be done by feeding the fish this feed additive before releasing fish from *kolong* aquaculture to the market.

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Authors:
Robin, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Jalan Agatis, Dramaga Campus, Bogor 11680, West Java, Indonesia; Department of Aquaculture, Faculty of Agriculture, Fisheries and Biology, Bangka Belitung University, Jl Balun Ijuk, Sungailiat, Bangka Belitung Province, Indonesia, e-mail: yumenku@ymail.com
Eddy Supriyono, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Jalan Agatis, Dramaga Campus, Bogor 11680, West Java, Indonesia, e-mail: eddysupriyono@yahoo.com
Kukuh Nirmala, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Jalan Agatis, Dramaga Campus, Bogor 11680, West Java, Indonesia, e-mail: kukuhnirmala@yahoo.com
Enang Harris, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Jalan Agatis, Dramaga Campus, Bogor 11680, West Java, Indonesia, e-mail: enangharris@yahoo.com
Ridwan Affandi, Department of Aquatic Resource Management, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Jalan Agatis, Dramaga Campus, Bogor 11680, West Java, Indonesia, e-mail: affandi_ridwan@yahoo.com
Dedi Jusadi, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Jalan Agatis, Dramaga Campus, Bogor 11680, West Java, Indonesia, e-mail: schenedesmus@gmail.com

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