The effectivity of using humic acid and fulvic acid from quickstick (*Gliricidia sepium*) compost to slow down lead (Pb) accumulation in red tilapia (*Oreochromis* sp.) cultivated in ex-tin mine waters

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Abstract. The global demand for tin (Sn) is partially fulfilled by Indonesia. In Indonesia, one of the locations for tin exploration is in the Bangka Belitung Islands Province. Mining in this province has been conducted on land, but because the tin ore supply on land has significantly decreased, the mining is beginning to be conducted on the coast. The effects of mining on the land are the formation of large and deep water-filled pits in the ground. These pits are locally known as *kolong*. Utilizing *kolong* water as a source of water for freshwater fish aquaculture is hindered by lead (Pb) contamination; therefore, there needs to be conducted depuration and prevention of accumulation in the fish during the time the fish are raised in the *kolong*. The purpose of this study was to test the ability and determine the best dose for the *G. sepium* compost to prevent the accumulation of Pb in red tilapia (*Oreochromis* sp.) cultivated in a *kolong*. The method employed was mixing the *G. sepium* leaf compost into the test feed for the fish (as a feed additive). The fish which had an average weight of 10±0.01 g were raised for three months in 20 cage units. Each cage unit was 1.5×1×1.5 m^3 in size and the stocking density was 50 fish $unit^{-1}$. A the end of the experiment, the *Gliricidia sepium* leaf compost mixed in the feed as a feed additive was able to prevent or slow down Pb accumulation in red tilapia raised in the ex-tin mine *kolong*. The 40 g kg^{-1} dose was the best in preventing or slowing down the accumulation of Pb in red tilapia cultivated in the Pb-contaminated waters.
Key Words: *kolong*, humic acid, fulvic acid, compost, depuration, tin mine.

Introduction. Currently, the pressure of heavy metal pollutants on aquatic environments in mineral ore-producing countries has increased, especially in developing countries which still practice the open mining system. One of the examples is in the Bangka Belitung (Babel) Islands Province, Indonesia. The open mining system in this province to obtain tin ore (Sn) has left behind a large number of mining pits. The pits from the Sn mining activities are in the form of large excavations, usually deep and water-filled, known by the locals as *kolongs*. On Bangka Island, the size of the *kolongs* left behind by Sn mining activities conducted by the state-owned company is 1,712.65 ha and on Belitung Island it is 1,035.51 ha (Robin et al 2017). The number of *kolongs* has increased uncontrollably after the people started to explore this natural resource *en masse*.

As the Sn ore reserves underground continue to be depleted, the need for alternative job opportunities that are not mine-related has increased. At the same time, the consumption of freshwater fish by the locals continues to increase annually; however, the demand for fish is not followed by a steady supply. Due to these facts, the Bangka Belitung Regional Government formulated a freshwater fish cultivation program by utilizing the *kolong*s as the medium. However, the *kolong* water and sediments contain the heavy metal Lead (Pb) above the acceptable standard (Henny & Susanti 2009). Lead (Pb) naturally occurs in the form of galena (PbS), gelesite ($PbSO_4$), and cerussite ($PbCO_3$) (Connel & Miller 1984). These are compounds associated with Sn in the Earth's crust. Therefore, when Sn is extracted or mined, Pb will be released to the surface. According to ATSDR (2007), Pb and its compounds are classified as group B, very toxic, and are most effective in bonding with the sulfhydryl group (–SH), can replace the position of the intermediary metal ion and together with the intermediary metal are soluble in fat, allowing them to penetrate and accumulate in cell membranes. In regard to the effect on human life, lead contamination cannot be lightly dismissed.

Robin et al (2017) found that cultivating red tilapia in new *kolongs* (< 20 years old) is still hazardous to the fish's lives and are dangerous for human consumption. In addition, the fish raised in these *kolongs* also demonstrated a very poor growth performance. This study supported the findings by Henny (2011) where the Pb content in flesh of restocked *kolong* red tilapia measuring 20-26 cm, reached 4 mg kg^{-1} (dry weight) and the Pb metal content in a small wild fish, *Puntius* sp., found in the restocked *kolong* was 73.27 mg kg^{-1} (dry weight). These Pb contents exceeded the maximum Pb contamination standards for fish. However, the accumulation of metals in living organisms can be minimized with help from ligands.

The use of natural materials from leafy greens has been proven to act as organic ligands. These organic ligands can help the fish excrete Pb from their systems (Robin et al 2017). One of the potential local plants is *Gliricidia sepium*, which is locally known as the Gamal plant. *G. sepium* can be easily grown and propagated and they are naturally occurring in the surrounding location. The organic materials from the leafy greens are also safe for both fish and humans. Another superiority of *G. sepium* is that this plant is suitable to and can thrive in ex-mine soil which tends to be acidic and the plant has leaf biomass which quickly and easily decomposes; therefore, it encourages the ex-mine land reclamation or replanting efforts. However, the utilization of leafy greens as an organic ligand must undergo a few processing stages. Another consideration is that the anti-nutrients contained by all leafy greens, making it necessary to further study the utilization of these greens as an organic ligand which is mixed with feed.

The results of a preliminary experiment in this study demonstrated 100 g of *G. sepium* leaves composted for 30 days contained 3.55% humic acid and 0.36% fulvic acid. Giannis et al (2009) stated that humic acid and fulvic acid can form complexes with metal ions. Humic acid and fulvic acid are hydrophilic colloids, causing them to have a high affinity to water and have a negative charge that can neutralize cations such as Ca^{2+} and Pb^{2+} (Nebbiosso & Piccolo 2012). The adsorption method for Pb is generally based on the interaction between the Pb ion and the functional group in the humic acid and fulvic acid through a complex-forming interaction (de Wet & Visagle 2010). Orsetti et al (2006) added that Pb ions interact with the organic materials in compost to form a complex and strongly-chelated complex compound.

The complex compound will be strongly bound if the coordination compound which has a Pb ion as the core is bound by a ligand with two or more bonds (Hermana & Nurhayati 2010; Orsetti et al 2013). If two or more organic functional groups (such as carboxylates) coordinate the metal ion, they can form a chelate structure, a form of complexation (Garces et al 2008). Aeschbacher et al (2012) calculated the constant stability condition (K*i*cond) in a bivalent ion metal complex which is strongly bonded to humic acid by cation exchange was $Pb^{2+} > Cd^{2+} > Cu^{2+} > Ni^{2+} > Fe^{2+} > Co^{2+} > Mn^{2+} > Zn^{2+} > VO^{2+}$ and the order of stability of the metals bonded with fulvic acid was $Fe^{3+} > Al^{3+} > Cu^{2+} > Ni^{2+} > Co^{2+} > Pb^{2+} > Ca^{2+} > Zn^{2+} > Mn^{2+} > Mg^{2+}$. Mandal et al (2003) added that fulvic acid had a tendency to be more easily chelated to Pb^{2+} than to essential bivalent metals. In regard to these facts, the utilization of *G. sepium* compost is considered safe and it will not interfere with the absorption of essential metals in the biotas' bodies.

There is a strong correlation between the amount of Pb accumulated in the bodies of cultivated fish and their growth performance. Moreover, there is also a strong correlation between the fish's ability to eliminate Pb from their systems and the availability of ligands in their bodies. Therefore, it was considered paramount to conduct a study regarding the ability and effectiveness of the utilization of humic acid and fulvic acid from a leafy green source and to determine the best dose to prevent the accumulation of Pb in red tilapia cultivated in Pb-contaminated waters, especially in *kolongs* under 20 years old.

Material and Method

Time and places. The cultivation was conducted for 90 days, from April to June 2017. The study location was *kolong* TB. 1.6 (Mine 1.6), located in Bangka Regency, Bangka Belitung Islands Province, Indonesia (Figure 1). The *kolong* was 4 years old, had a depth of ±12 m and located at the coordinates S01°59.902', E106°06.637'. At the beginning of the study the water pH was 4.6 and sediment pH was 4.1, and the Pb accumulated in the water was 0.51±0.05 mg L^{-1} and in the sediment 63.03±0.31 mg L^{-1}.

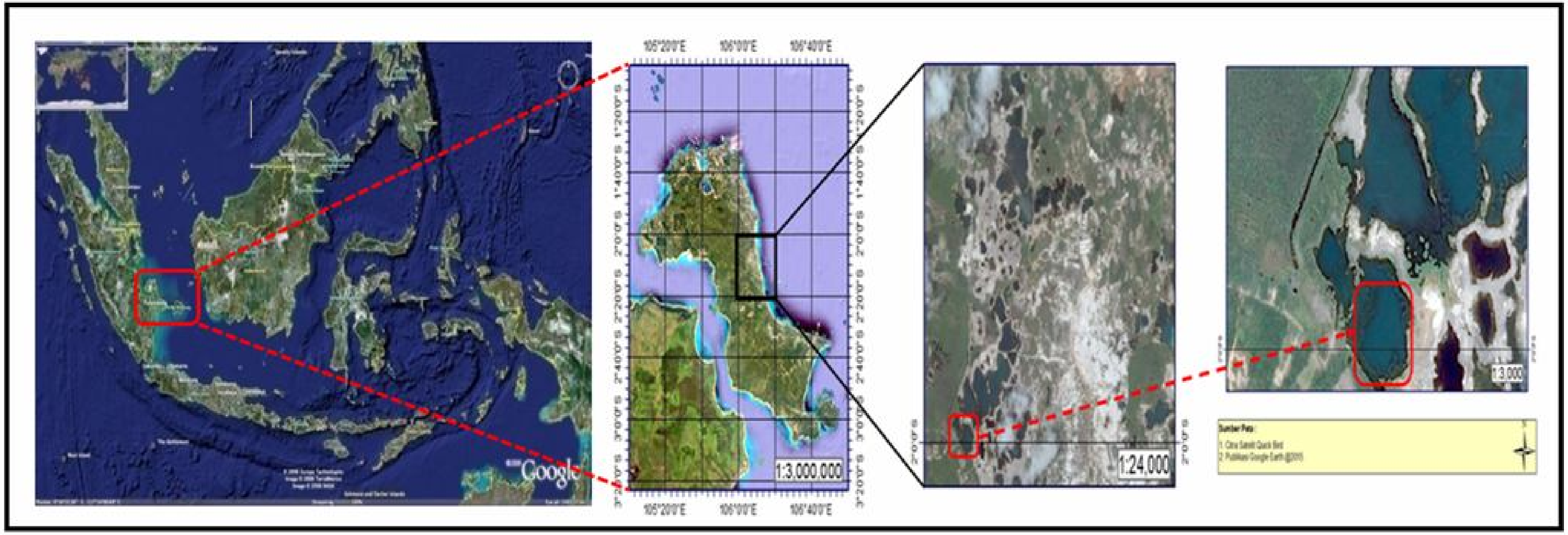

Figure 1. The map of the study location.

The chemical analyses included water and sediment quality analyses conducted at the Laboratory of Environment, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Bogor, Indonesia. The Pb analysis was conducted at the Integrated Laboratories, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University. The histological tests on the fish organs were conducted at the Laboratory of Aquacultural Fish Health, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University. The production of the feed which contained *G. sepium* leaf compost and the proximate analysis of the test fish's organs were conducted at the Laboratory of Fish Nutrition, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University. The humic acid and fulvic acid in feed were tested at the Indonesian Biotechnology Research Center for Estate Crops, Bogor.

Research design. The research design employed in the present study was a complete randomized design by applying four treatments and one control, each treatment repeated triplicate. The treatments applied were 0 g kg^{-1}, 10 g kg^{-1}, 20 g kg^{-1}, 30 g kg^{-1}, and 40 g kg^{-1}. The effectiveness parameters for the dose of the *G. sepium* leaf compost administered through the feed were the contents of Pb in the flesh, liver, kidneys, and gills of the fish on days 0, 30, 60, and 90.

The production of the G. sepium leaf compost. The first step in producing the compost was collecting the raw material, *G. sepium* leaves. The leaves that were collected were those which were green (still growing) and were then separated from the leaf stems. The leaves collected were a mixture of immature and mature leaves from *G. sepium* trees more than three years old. The *G. sepium* trees whose leaves were used in the study grew on yellowish brown podsolic-associated soil. The composting method followed that of Robin et al (2017). The proximate analysis to determine the compost maturity level, namely the C content, N content, C/N ratio, pH, moisture content, humic acid and fulvic acid content in the compost, referred to AOAC standards (1990).

Production of the feed mixed with G. sepium leaf compost. Production of the feed containing *G. sepium* leaf compost was done by mixing *G. sepium* leaf compost into commercial feed containing 31% crude protein (CP). The mixing was done by first grinding the commercial feed and *G. sepium* leaf compost. The grinding was done separately. The mixing of *G. sepium* leaf compost into the feed referred to the method in Robin et al (2017). Proximate analysis was then conducted on the feed to determine its nutrient contents which included protein, fat, and carbohydrate (Table 1). The final step was storing the feed mixed with *G. sepium* leaf compost into labeled containers so that the different feeds would not get mistakenly mixed up when being used.

Table 1

The results of the feed proximate analysis after being mixed with *G. sepium* leaf compost

*Dose of feed mixture (*g kg^{-1}*)*	*Moisture content*	*Ash content*	*Protein*	*Fat*	*Carbohydrate*	
					Crude fiber	*NFE*
0	4.9±0.20	9.0±0.08	31.7±0.11	6.0±0.03	6.8±0.36	41.8±0.41
10	4.7±0.22	9.4±0.05	30.2±0.14	6.3±0.09	6.3±0.20	38.1±0.46
20	5.7±0.31	8.6±0.06	30.0±0.10	5.8±0.03	7.2±0.39	38.7±0.30
30	5.2±0.28	9.0±0.08	30.7±0.10	5.9±0.03	7.0±0.15	42.0±0.42
40	5.2±0.33	9.2±0.02	30.3±0.16	6.1±0.07	5.8±0.27	42.5±0.43

The data presents the average value±SD (n = 3).

Raising the fish in the kolong. The total number of cages used for raising the fish was 20 units. The cages used for raising were floating net fish cages sized 1.5 × 1 × 1.5 m^3 with 0.5 cm mesh. Each fish cage unit was stocked with 50 test fish. The fish cages were classified into five treatment groups. There were four block units in each treatment group. These units consisted of three block units as growth analysis repeats and one block unit as a Pb content test. Before being placed in the cages, the total fish seed weight was recorded and 10 fish samples were collected for the Pb content in the flesh and 10 for the proximate analysis. The fish were kept for 90 days. During the rearing period, the fish were fed the commercial feed that had been mixed with the *G. sepium* leaf compost according to the treatment doses. The feed was given *at satiation* three times a day (08.00-09.00, 12.00-13.00, 16.00-17.00 Western Indonesia Meantime).

Water quality assessment. The assessment of the water quality was conducted three times, on days 0, 30, 60, and 90. The assessments were conducted triplicate directly at the *kolong* where the study was conducted. The water quality parameters assessed were the dissolved oxygen content (DO) and temperature of the water in the *kolong* using a digital Lutron DO 5510 DO-meter. The water and sediment acidity level (pH) were measured using a digital Hanna HI 98107pH-meter. The water turbidity was assessed using a Secchi disk. The Pb concentration in the water and sediment were measured using the APHA method (1976).

Test animals. The test animals used were red tilapia (*Oreochromis* sp.) with an initial average weight of 10±0.01 g fish^{-1}. The test fish were obtained from the Bangka Regency Local Fish Seed Center Bangka Belitung Islands Province, Indonesia. The fish seeds were produced by the same parents. Before being used, the fish seeds were adapted for one week in the fish cages and then selected to obtain a uniform size and sex. The fish used were all male.

Sampling and test parameters. During the study, sampling and measurements were conducted three times. Ten sample fish were collected randomly from each treatment fish cage. During each sampling, the dissolved oxygen, water pH, and water temperature were measured. On days 0, 30, 60, and 90, the gills, liver, kidneys, and flesh were collected for Pb analysis.

Data analysis. The data of the Pb concentration in the organs were presented based on the average±standard deviation (SD) and analyzed using one–way ANOVA followed by Fisher's test using Minitab 15. The difference was considered significant if $p < 0.05$. The Pb accumulation rate percentage in each organ observed in the test fish during the course of the 90-day rearing period was presented based on the average±standard deviation (SD) and analyzed using ANOVA followed by Duncan's test at a confidence interval of 95%.

Results

Water quality. The *kolong* average water temperature at the beginning of the rearing period was 29.3±0.23°C. On day 30, the average *kolong* water temperature was 31.2±0.26 °C. On day 60, the average *kolong* water temperature was 31.4±0.04°C and then slightly increased to 31.8±2.01°C on day 90. The turbidity was 1.8±0.004 m from the beginning to the end of the rearing period. At the beginning of the rearing period, the dissolved oxygen content in the *kolong* water 4.60±0.03 mg L^{-1} and on day 30 it dropped to 3.10±0.03 mg L^{-1} and on day 60 it was 3.43±0.13 mg L^{-1}, and then it experienced a slight decrease to 3.00±0.02 mg L^{-1} on day 90. The *kolong* water and sediment acidity level (pH) measurement results revealed that the *kolong* water and sediment were still acidic. During the study, the *kolong* water pH ranged between 4.8±0.04 and 5.00±0.01, whereas the results of the *kolong* sediment pH measurements were relatively stable from the beginning of the rearing period until the end, 4.1±0.07.

The Pb content in the organs of the test fish administered the 0 g kg^{-1} dose. The Pb content in the organs of the test fish fed the 0 g kg^{-1} dose (control) demonstrated an increase at each observation. Increases were found in all of the test fish's organs and accumulated as the rearing commenced. The greatest accumulation was found in the flesh at 5.88 mg kg^{-1} on the observation on day 90, while the lowest accumulation was found in the gills at 3.19 mg kg^{-1} on day 90 of the rearing period. On the other hand, the

Pb accumulation in the fish's liver and kidneys tended to be similar at the end of the rearing period, 3.88 mg kg^{-1} and 3.73 mg kg^{-1}, respectively.

The Pb content in the organs of the test fish administered the 10 g kg^{-1} dose. The Pb content in the organs of the test fish fed the 10 g kg^{-1} dose had a positive correlation with the observation time. The increasing trend of Pb accumulation demonstrated by all the test fish's organs was almost the same as the control treatment; however, the accumulation was slightly lower. The greatest accumulation was found in the flesh at 4.62 mg kg^{-1} on the day 90 observation, while the lowest accumulation was found in the liver at 2.42 mg kg^{-1} at the end of the rearing period. At this dose, the accumulation in the liver and kidneys of the test fish tended to be stagnant from day 30 to day 90 of the rearing period.

The Pb content in the organs of the test fish administered the 20 g kg^{-1} dose. The Pb content in the test fish's organs fed the 20 g kg^{-1} dose was proportionate to the observation time. The Pb accumulation pattern in all the test fish's organs was almost the same as exhibited by the control treatment, but the Pb accumulation value was lower. On observation day 90, the greatest accumulation was discovered in the flesh at 3.19 mg kg^{-1}. At the same time, the lowest accumulation was found in the kidneys at 2.14 mg kg^{-1}. At this dose, the accumulation in the fish's liver and kidneys tended to be stagnant. The stagnation started on day 30 and continued until the end of the rearing period.

The Pb content in the organs of the test fish administered the 30 g kg^{-1} dose. The observation results revealed that the fish fed feed mixed with *G. sepium* leaf compost at a dose of 30 g kg^{-1} experienced accumulation in all the observed organs. However, the Pb accumulation at this dose was lower than the accumulation in the 10 g kg^{-1} dose, 20 g kg^{-1} dose, and the control. At the end of the observation period, the greatest accumulation was discovered in the gills at 4.20 mg kg^{-1}. This was followed by the liver at 3.10 mg kg^{-1}, the flesh at 2.87 mg kg^{-1}, and kidneys at 2.38 mg kg^{-1}. In this dose, the accumulation that occurred in the liver, kidneys, and flesh of the fish tended to slow down. Stagnation was first observed on day 30 and continued until the end of the rearing period.

The Pb content in the organs of the test fish administered the 40 g kg^{-1} dose. The results of the observations in this treatment revealed that there was a decrease in the accumulation in the test fish's flesh. However, the Pb accumulation in the gills, liver, and kidneys was found to be greater than those of the other doses. At the end of the observation period, the greatest accumulation was found in the gills at 5.17 mg kg^{-1} followed by the liver at 3.22 mg kg^{-1} and the kidneys at 3.02 mg kg^{-1}. The lowest accumulation was found in the flesh at 1.81 mg kg^{-1} until the end of the observation period. In general, the amount of Pb accumulated in each of the test fish organs at different doses is presented in Figures 2 to 5.

The percentage of the accumulation rate of Pb into red tilapia organs fed the feed mixed with leaf forage compost. The percentage value of the accumulation of Pb into observation organs of the experimental fish during 90 days of the rearing period in *kolong* TB. 1.6, Bangka District, Bangka Belitung Islands Province, Indonesia was presented in Figure 7. Gills, liver and kidneys which were analyzed in this study were metabolism performance benchmarks of the experimental fish when it was given treatments. The flesh analyzed in this study was a benchmark for food safety. The smaller the accumulation rate value in the flesh, explained that the greater the value of the efficiency of the applied treatments. The greater the accumulation rate value in the metabolic organs (gills, liver, and kidneys), indicated the more effective treatments given to improve the performance of the metabolic organs in performing their functions. The results of this study proved that each treatment dose (except control) was able to prevent or retard the accumulation of Pb into the flesh. The dose of 40 g kg^{-1} was the best dose to prevent and retard the accumulation rate of Pb.

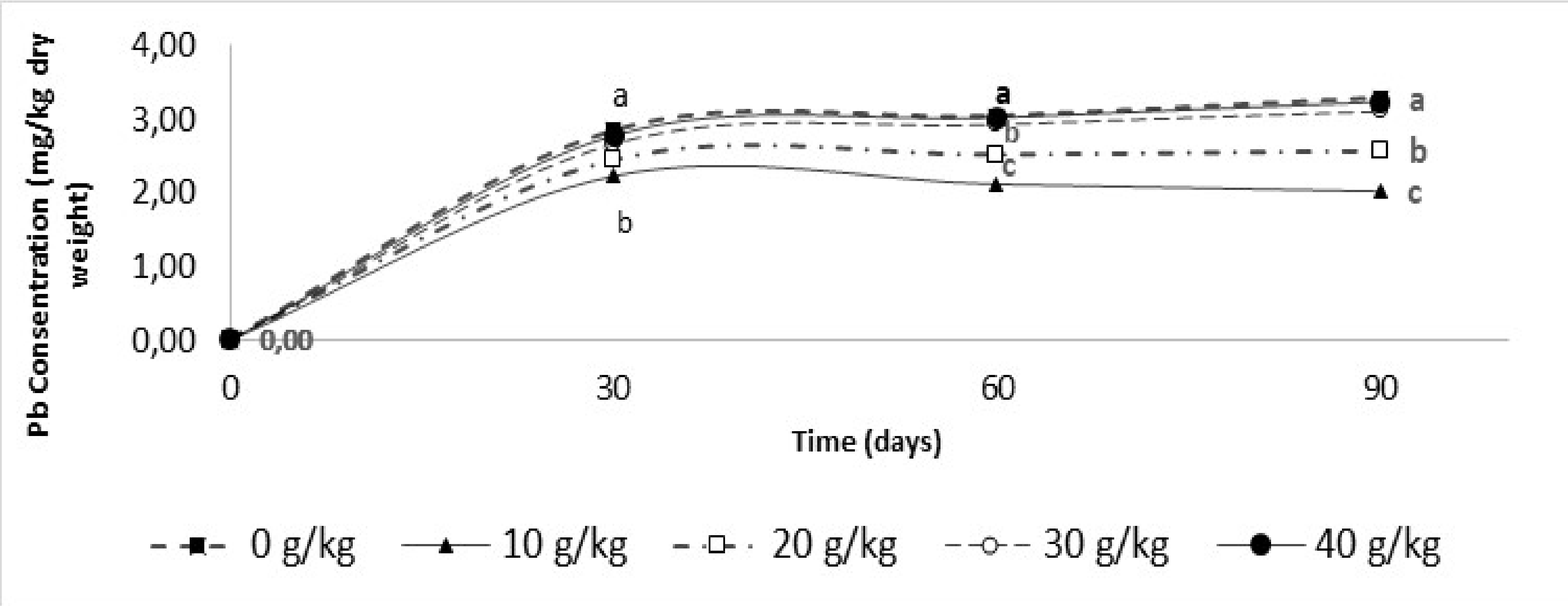

Figure 2. The Pb content in fish flesh. Different letters at each observation time indicate significant differences among treatments ($p < 0.05$).

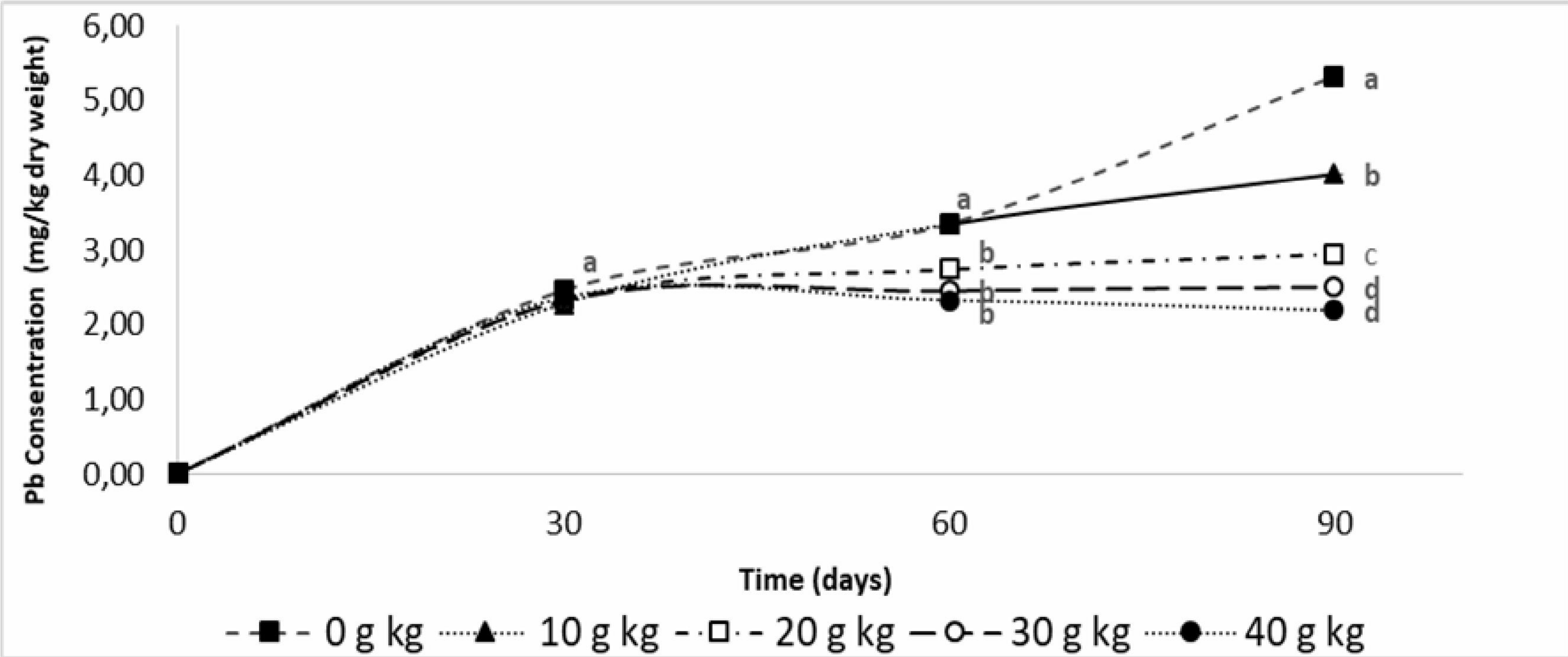

Figure 4. The content of Pb in fish's liver. Different letters in each observation time show significant differences among treatments ($p < 0.05$).

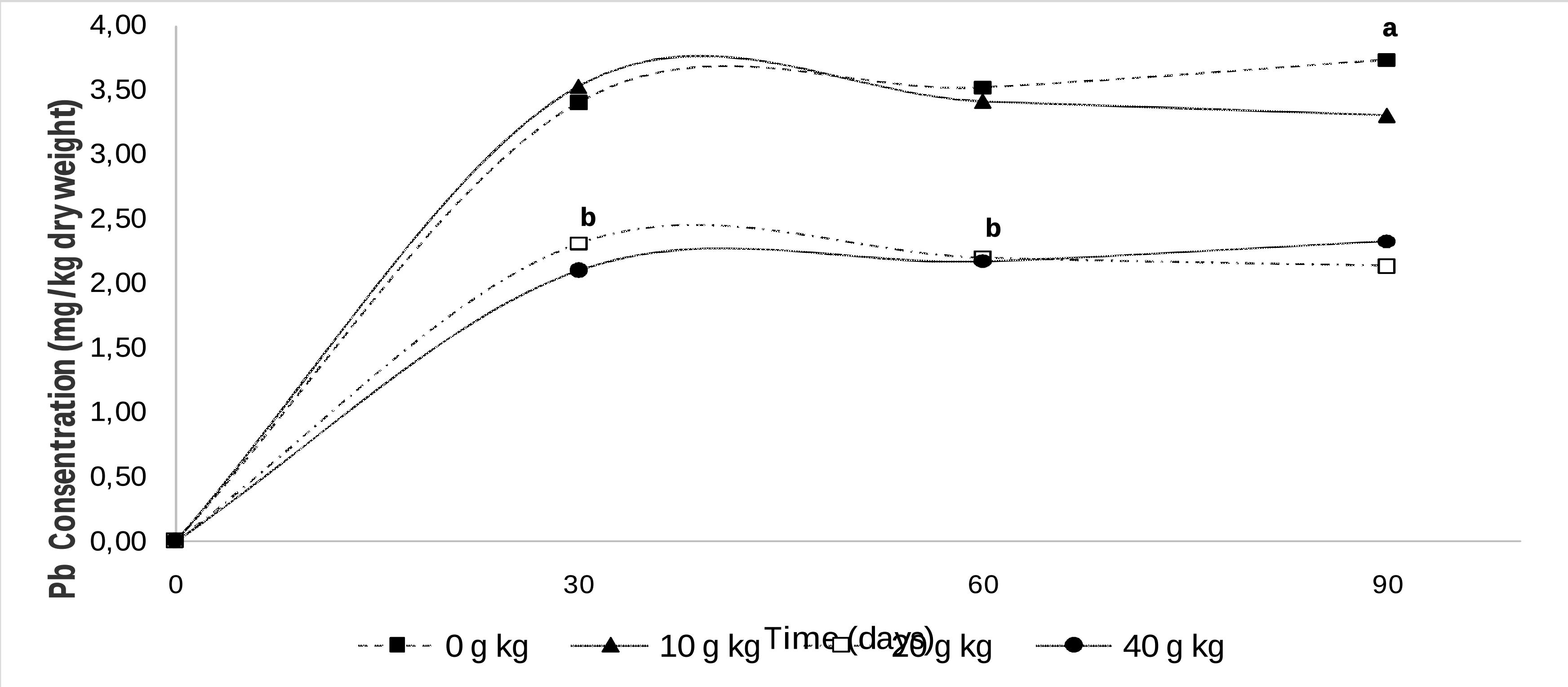

Figure 5. The content of Pb in fish kidney. Different letters in each observation time show significant differences among treatments ($p < 0.05$).

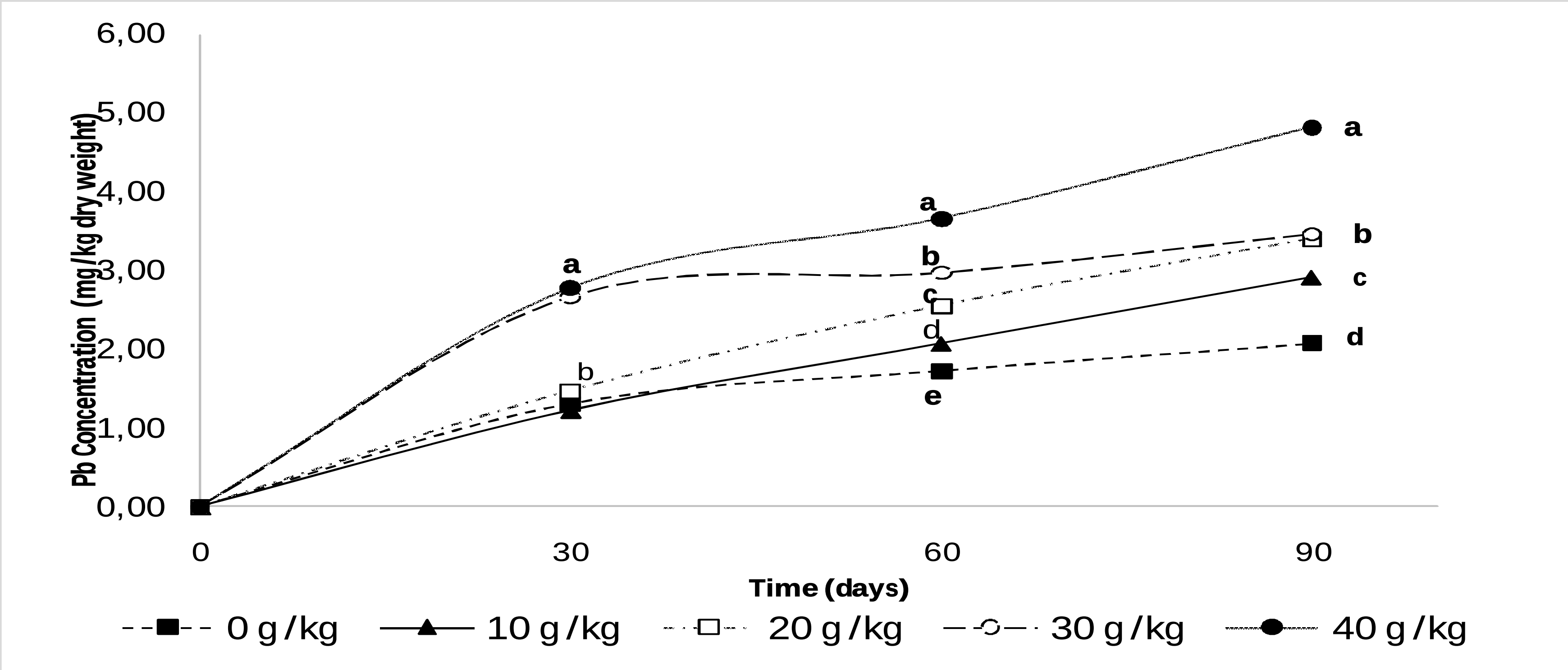

Figure 6. The content of Pb in fish gill. Different letters in each observation time show significant differences among treatments ($p < 0.05$).

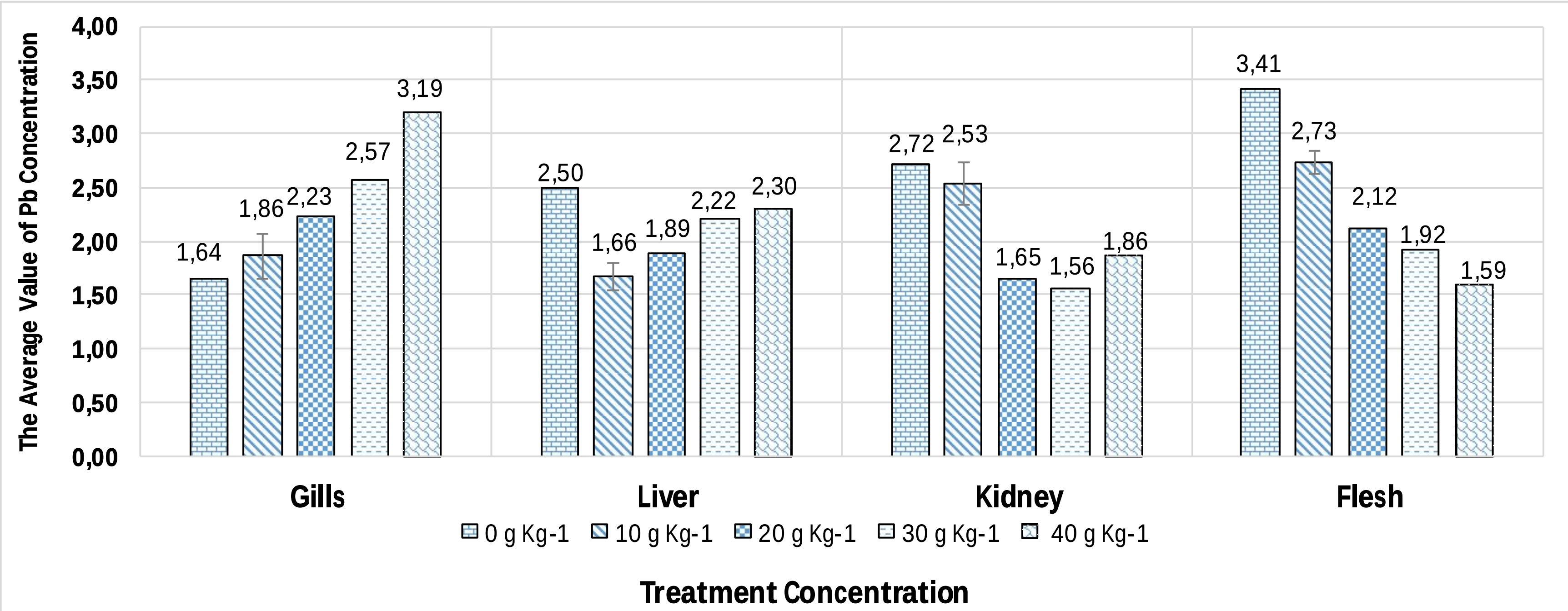

Figure 7. The average value of Pb concentration in each observation organ during 90 days of the observation.

Discussion. The Pb contamination in biota, positively correlates with the accumulation of Pb in the environment where that biota lives (Ahmed & Bibi 2010). The Pb contained in the water is found in the form of free ion or divalent ion (Pb^{2+}) and particulate ion or tetravalent ion (Pb^{4+}) (Fu & Wang 2011). Esfahani et al (2015) state that, Pb enters into the fish body through gills (respiratory pathway), mouth and the penetration on skin layer. The Pb that enters through respiratory pathway is 30%, it that enters through feeding pathway is 5-10%, while it that enters through diffusion on the skin is in very little amount and can be denied (Tierney et al 2014). From those absorbed amounts, it is only 15% that settles on the body tissues, the rest will be released with the metabolism rest such as urine and feces (Esfahani et al 2015). The Pb that enters through gills and mouth, enters and is bound in the blood, then following the bloodstream to organs passed by the blood such as liver and kidneys. The Pb that enters into the blood in the excessive amount, will be stored and accumulated in the body, especially in gills, liver, kidneys, flesh, intestine and blood. This process occurred in the flesh, liver, kidneys and gills of the experimental fish reared for 90 days in new *kolong* during this study conducted. This strengthened the statement of Esfahani et al (2015), in a particular condition (extreme), the absorption process of Pb from the environment was higher than the speed of the depuration process by biota, this condition caused the accumulation of Pb. This accumulation process was demonstrated by the Pb concentrations in all observation organs in control (0 g kg^{-1}), that continued to increase during the culture of red tilapia in *kolong*.

The administration of the feed mixed with leaf forage compost at doses of 10 g kg^{-1} and 20 g kg^{-1} showed the same tendency, there was the accumulation stagnation in metabolic organs and the increases in the flesh, but the increases were not as great as in control. It proved that both treatment doses influenced the accumulation rate of Pb into the body of the experimental fish. Different conditions were shown in doses of 30 g kg^{-1} and 40 g kg^{-1}. In both doses, the accumulations occurring within the metabolic organs increased, but the increases occurred in a little amount. In addition, the accumulations of Pb in the flesh of the experimental fish tended to retard. The higher the doses given, the higher accumulations of Pb in the metabolic organs (gills, liver, and kidneys) and that impacted on lower accumulation rates of Pb in the flesh.

These results proved that the leaf forage compost mixed in the feed was able to help and accelerate the natural depuration system of the experimental fish body to Pb that continued to enter from outside the body. These results indicated that the administration of the feed containing the leaf forage compost had a major effect in the prevention process of the accumulation of Pb in the experimental fish body reared in the Pb polluted *kolong*. This was in line with the statement from Kucasoy & Guvener (2009), the use of compost was very efficient to bind and clean heavy metals.

The slow absorption rate and the accumulation of Pb into the experimental fish body fed the feed mixed with the leaf forage compost, was caused by the presence of humic acid and fulvic acid contained in the leaf forage compost. This process was started from the feed containing the leaf forage compost eaten by the experimental fish, humic acid and fulvic acid would be in the digestive tract and entered into the bloodstream. The presence of humic acid and fulvic acid in the digestive tract would make the action of Pb in the digestive tract being different. In the fish stomach that has pH at a range of 4-5, 80% of the biological form is not active, so that Pb contained in the stomach will turn into divalent. The existence of humic acid and fulvic acid in the stomach prevents Pb in order to not in the form of divalent, it is very important to make Pb can not directly enter into the blood through the cell wall of the stomach. Humic acid and fulvic acid can form complexes with metal ions (Giannis et al 2009). Their active groups, ie carboxyl and phenolic, bind Pb in order not to be absorbed by the stomach cells, then sent into the intestine to be secreted with feces.

From the stomach and the digestive tract, fulvic acid will very easily enter into the blood. Fulvic acid is a substance that has a high chance to enter the experimental fish body and eliminates Pb from its organ. Fulvic acid existed in the blood, performs two action mechanisms (action inside cells and action outside cells). First, action within the blood cells, fulvic acid helps or together with metalloprotein (MT) to bind Pb in order to chemically inactivate, and then to bring Pb out of the cell. Second, action outside cells, fulvic acid performs chelation directly to the ionized Pb in the blood, then following the circulatory system to the osmoregulation organ (gills), the detoxifying organ (liver) and the secretion organ (kidneys).

Because fulvic acid had been available in the blood of the experimental fish, as an effect of the administration of the experimental feed mixed with the leaf forage compost, so the presence of fulvic acid in the blood around gills, would give a different response. Fulvic acid substance has a high amount of oxygen, but it has low carbon and nitrogen and also has charge that contains many oxygen functional groups that are carboxyl (–COOH) and hydroxyl (–COH), that is in line with the nature of Pb^{2+} that is very effective to bind with carboxyl and hydroxyl groups (Saito et al 2004; Fu & Wang 2011). El Deen et al (2010) add, Pb^{2+} ion can rapidly form complex link in the form of hard ligand (LH) with a ligand that has major carboxyl (–COOH) and phenolic (–OH) group. Thus, from gills of the experimental fish, fulvic acid with MT would bind Pb in the form of hard ligand (LH), then secrete it outside the body through gills. The running of this mechanism was strengthened with the results of the measurements of Pb concentrations in gills of the experimental fish in all applied doses of *G. sepium* leaf. The results of the measurements of Pb in gills of the experimental fish showed that the increases in Pb concentrations positively correlated with the increase of *G. sepium* leaf doses.

The same mechanism also occurred in liver and kidneys of the fish fed the feed mixed with the leaf forage compost. The addition of the leaf forage compost into the feed with various doses could improve the performance of liver as a detoxifying organ and kidneys as the secretion organ. The increase of these two organs performances, could be seen at the concentrations of Pb that tended to increase, fluctuate and even stagnant at every observation time. This condition was inversely related to liver and kidneys of the control fish, whose Pb concentrations always increased in the liver and kidneys. The fluctuation and the stagnation of Pb concentrations in liver and kidneys, in the fish treated the feed mixed with the leaf forage compost, indicated that there had been the taking and releasing process of Pb by those organs.

After gills, liver and kidneys, the next soft organ which is most vulnerable to be accumulated by Pb is the flesh. In the experimental fish body that was contaminated by Pb, as in liver, kidneys and especially Pb in the blood decreased significantly, then the experimental fish would clean another part of its body that was still accumulated by Pb (the flesh). This Pb cleaning by removing Pb and releasing it into the blood was performed by firstly synthesizing MT. The natural excretion ability of biota to localize and secrete Pb is highly dependent on the speed of each cell of the biota in producing MT (Miller et al 2013). The administration of treatments in the form of the addition of the leaf forage compost into the feed at different doses gave a positive effect on the depuration process of Pb from the experimental fish flesh. The higher the leaf forage compost doses added into the cultivation feed, caused the concentrations of Pb becoming lower, in the experimental fish flesh at each observation time. Significant differences in Pb concentration values in the experimental fish flesh, among control and treatments, indicated the increase of gills, liver and kidneys performances.

Conclusions. From the results obtained, it could be concluded that humic acid and fulvic acid derived from the leaf forage compost, were able to prevent or retard the accumulation of Pb into the body of red tilapia reared in the post-mining water or Pb polluted water. The method used was the leaf forage compost mixed into the feed as a feed additive. The dose of 40 g/kg was the best dose to prevent or retard the accumulation of Pb into the body of red tilapia.

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