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Ability of Humic Acid in the Absorption of Heavy Metal Content of Lead and Iron in Fish Culture Media

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ABSTRACT

The young coal mining pits (young pits) found in West Aceh cause many problems. These pits that form ponds have the potential to be utilized for aquaculture activities. However, the main problem faced in the young pits is the high content of heavy metals. This makes the ponds dangerous for fish farming. Hazardous heavy metals in coal mines are Pb and Fe. Humic acid is one of the materials that can be used to minimize heavy metals and increase water pH. The use of humic acid is more efficient because this technology is easy and cheap and the raw materials are abundant. Humic acid acts as a substance of cation exchange ability found in compost. This research has a long-term goal of making humic acid contained in compost as an alternative material that can minimize heavy metals Pb and Fe, so that it can be used for fish farming activities. The specific objective is to determine the best capacity (dose of humic acid) in minimizing heavy metals and increasing the pH of young pond water in former mines and its effect on fish farming. This research method used a completely randomized design consisting of one factor, namely the dose of humic acid (0 g/L, 2.5 g/L, 5 g/L, 7.5 g/L) and three replications. The results showed that humic acid was able to minimize the content of heavy metals Pb and Fe in coal mine water with the best dose of 5 g/L. The percentage reduction produced was more than 90%. The results of fish rearing using ex-mining pond water treated with humic acid showed that the survival rate and growth rate of fish were higher.

Keywords: humic acid, heavy metals, fish farming media

INTRODUCTION

The former coal mining pits that form ponds are a problem to be developed for fish farming. Many people have utilized former coal mining ponds for fish farming using cages (Hafids & Pagoray, 2015; Kiswanto et al., 2022). Meanwhile, we know that the former coal mining ponds have changed both the condition and quality of the pond water due to the stripping of mining (Pertiwi, 2011; Setiawan et al., 2018). The impact of the new ex-coal mine pond still contains harmful heavy metal elements (Kiswanto et al., 2022; Kiswanto et al., 2018a; Kiswanto et al., 2020; Kiswanto et al., 2021).

The need for water as a fish farming medium for aquaculture activities is increasing along with technological advances. This is because the development of aquaculture continues to lead to the industrialization process. In addition to rivers, reservoirs, and lakes, there are still many sources of water that come from former mining ponds (Kiswanto, et al., 2020; Ira Triswiyana et al., 2019; Kurniawan, 2017; Yadav & Jamal, 2018). But not all water sources originating from mines can be utilized for fish farming. Basically, coal mine acidic water contains heavy metal elements such as Fe, Mn and Pb which make the water quality decrease. So that it cannot be used for fish farming because it will inhibit the growth or kill cultured organisms (Urbasa et al., 2019; Kiswanto et al., 2022; Maidie et al., 2010; Kiswanto, et al., 2020; Gautama, 2014; Kiswanto et al., 2018b).

Aceh province, West Aceh district is one of the areas where there are many coal mining pits in which there are water sources. However, most of the ex-mining water sources have low water quality. Water sources that have low quality are because the former mines are young. Young mines have high heavy metal content and low water pH ranging from 2.4-4.6 (Kiswanto et al., 2018b; Maidie et al., 2010; Ira Triswiyana et al., 2019). This condition causes young pits to not be utilized for aquaculture activities (Maidie et al., 2010; Kurniawan, 2017). Whereas in quantity, young pits are very abundant water sources, so they have the potential to be utilized for fish hatchery activities. Even now, many old pits are turning into young pits because the old pits are being re-mined by mining companies (Kiswanto, et al., 2020; Kiswanto et al., 2018a; Kiswanto et al., 2021).

(Kiswanto et al., 2018b) conducted research on the characteristics of coal mine acid water and found that there were several types of heavy metals in the former coal mining pit. Some of the heavy metals found are toxic essential minerals. One of the heavy metals found in the water is Pb and Fe. In the young pit, the heavy metal content in the water pool is quite high while the old pit has low heavy metal content in the water pool but still quite high in the sediment.

Water polluted with heavy metals at a certain concentration (lethal concentration) cannot be used for fish farming. At concentrations that are still tolerated by fish, water containing heavy metals is potentially harmful to fish and other organisms. In addition, heavy metals can accumulate in the body of fish, making it dangerous for human consumption from a health perspective. In water that has a very low pH content (below 5). Water cannot be used for the cultivation process because fish will be inhibited in growth and experience death (Hafids & Pagoray, 2015; Kiswanto et al., 2022).

In general, heavy metals can kill fish and accumulate in the fish body through the skin surface and biomagnification. Fish that accumulate heavy metals when consumed will inhibit enzymatic processes (Ismail & Moustafa, 2016; E..Prasetiyono & Syaputra, 2018), damage the central nervous system, kidneys, liver, and reproductive system (Noor et al., 2019). In water that has a low pH, fish will experience damage to the gills, skin and eyes (Becker et al., 2015).

Minimizing heavy metals can be done by membrane filtration, electrodialysis, chemical precipitation, exchange, ions, and adsorbs (Ahalya et al., 2003; Ismail & Moustafa, 2016; Kiswanto, Susanto, et al., 2020; Kiswanto et al., 2021). Heavy metal removal can also be done by coagulation, fluctuation, flotation, electrochemical treatment, ion chelating (Noor et al., 2019). In this study, heavy metal reduction using humic acid will be utilized. Humic acid has a very good role in binding harmful heavy metals (Adhiatma et al., 2014; Yuliyati et al., 2016). So that humic acid is able to minimize the metals present in the water source in the ex-mining pond. The binding pattern of heavy metals with humic acid is by adsorbing metal ions and also forming complex compounds and chelates so that the metal is difficult to free (Lasmi, 2016; Atik Rahmawati, 2012; Adhiatma et al., 2014). Humic acid is able to adsorb heavy metal complexes through cation exchange (Hermana & Nurhayati, 2010). Chelate formation and electrostatic bonding. In addition, humic acid, containing minerals in it, can exchange positions with ions in water when in contact. Besides being able to minimize heavy metals, humic acid can also be used to raise the pH of water (E. Prasetiyono, 2015; E. Prasetiyono & Syaputra, 2018; E. V. A. Prasetiyono, 2012). Water with a low pH can be raised due to the large content of negative functional groups contained in humic acid so that H^+ ions as the cause of acidity can be bound by humic acid (Yuliyati et al., 2016). Humic acid is widely found in compost and peat soil which are abundant and cheap materials. It also does not harm fish farming organisms (E. Prasetiyono, 2015).

Humic acid is usually contained in compost and can be used to minimize heavy metals and raise pH in aquaculture activities. The use of humic acid to minimize heavy metals in young water in aquaculture activities can be done by means of a separate treatment system (Meyzilia & Darsiharjo, 2017). The separate treatment system is that water is taken from the column and placed in a separate treatment container from the pit (Meyzilia, 2018; Pitulima, 2016). Then the treatment process is carried out using humic acid in the treatment container. Water that has been given humic acid treatment can be flowed and used into fish farming containers/ponds. Testing the effectiveness of the optimal dose of humic acid in minimizing heavy metals and increasing pH (Meyzilia, 2018). In addition, it is necessary to see the extent to which water that has been given humic acid treatment affects the growth and survival of fish (E. V. A. Prasetiyono, 2012).

Based on the above background, it is necessary to look at the essential heavy metal content, especially the heavy metals Lead (Pb) and Iron (Fe) in newly stocked fish in post-coal mine ponds in West Aceh. in West Aceh. Research in post-mining ponds basically aims to analyze the concentration levels of heavy metals lead (Pb) and iron (Fe) during one month of rearing in tilapia (Oreochromes). in tilapia (Oreochromis niloticus) cultivated in post-coal mine ponds in West Aceh. The benefits of this study are expected to provide information on the content of heavy metals lead (Pb) and iron (Fe) in fish meat cultivated in post-coal mine ponds.

MATERIALS AND METHODS

Tools and materials

The tools used in this study include: heavy metal treatment container (tarpaulin pond), fish farming container (tarpaulin pond), water filter, blower aerator, microwave, AAS (Atomic Absorption Spectrophotometer), UVVis Spectrophotometer, pH meter, DO meter and titration equipment, sample bottle, beaker, measuring cup, volumetric pipette, measuring flask, Erlenmeyer tube, digital balance, magnetic stirrer, thermometer. The materials used were fresh water, humic acid, 2-week-old sangkuriang tilapia (Length: 2 cm, weight: 0.053 gr). Fish feed (Frozen blood worm and commercial pellets), distilled water, reagent solution and standard solution for water quality measurement.

Research procedure

The research was divided into four stages. The first stage is the pre-treatment stage which consists of heavy metal activities and the pH of the water under the young.

The second stage was the treatment of water containing heavy metals lead (Pb) and iron (Fe) in the treatment container using humic acid. At this stage, humic acid was put into an aquarium container containing water containing Pb and Fe. This stage lasted for 24 hours. During the treatment process, aeration was carried out in the treatment container. Furthermore, the third stage is the post-treatment stage in the form of mechanical filtration of humic acid from the colonic water treatment container. The last stage is the fish rearing stage (fish farming). The fish reared were tilapia (Oreochromis niloticus) fry that were 2 weeks old. Fish maintenance is carried out for 30 days.

Observation parameters

Texture, color and pH

Humic acid analysis was done physically and chemically. Physically, the texture and color were like soft, black soil.

Humic acid content

The humic acid test aims to determine the level or amount of humic acid extracted with a mixture of strong base and sodium pyrophosphate solution. Humic acid dissolves in the extract of strong base solution and sodium pyrophosphate (Atik Rahmawati, 2012; Mindari et al., 2014).

Heavy metal analysis is carried out using an atomic absorption spectrophotometer (AAS). AAS is a spectro-analytical procedure for the qualitative and quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in gaseous form. The test of heavy metal content in water is carried out directly by measuring water samples using AAS, the test of heavy metal content in fish meat is carried out by first preparing fish meat samples by wet deconstruction using a microvave.

Physical and chemical water quality

Analysis of physical and chemical quality of water includes pH, DO (Dissolved Oxygen), temperature, TOM (Total Organic Matter), measured in the morning (at 08:00–09:00 a.m.). Measurements were taken at the beginning and end of humic acid treatment and at the beginning and end of fish rearing. The measuring instruments used to measure the physical and chemical parameters of water were pH meter to measure pH, DO meter to measure DO, thermometer, titration equipment and UVVis spectrophotometer.

Fish survival

Fish survival was calculated by observing the number of tilapia (Oreochromis niloticus) kept at the beginning of the observation and the number of tilapia (Oreochromis niloticus) kept at the end of the observation. Calculation of survival in fish using the formula (Nane, 2019):

$$SR = Nt/No \times 100\% \quad (1)$$

where: SR – survival rate (%);
Nt – number of fish at the end of observation (tail);
No – number of fish at the beginning of observation (fish)

Growth rate

Growth rate uses data obtained by taking tilapia (Oreochromis niloticus) at the beginning and end of the experiment and weighing its weight. Growth rate was calculated using the formula (Lambregts & Zonneveld, 2004):

$$a = \left(\sqrt[t]{\frac{Wt}{Wo}} - 1 \right) \times 100\% \quad (2)$$

where: Wt – average weight of fish on day t (gr);
Wo – average weight of fish on day 0 (gr);
t – time (day);
a – specific growth rate (% body weight/day).

Trial design

This study used a completely randomized design. This design refers to Matjik and Sumertajaya (2002). The treatment factor in this study was the dose of humic acid. After 24 hours, water and humic acid samples were taken and the heavy metal content (water and humic acid) and pH value in the water were measured. Each treatment refers to the results of Prasetiyono's research, namely:
- Treatment 1 – without humic acid (control);
- Treatment 2 – humic acid dose of 2.5 g/L;
- Treatment 3 – humic acid dose of 5 g/L;
- Treatment 4 – humic acid dose 7.5 g/L.

If there were differences between treatments, it was continued with Duncan's test. All data analysis was done quantitatively using the computerized SPSS program.

RESULTS AND DISCUSSION

Humic acid analysis

The substance contained in humic acid has the ability to adsorb heavy metals in water. This is mecause humic acid has a functional group to bind the heavy metal elements. Humic acid substances have the capacity to form complexes with metals through the formation of complex compounds and chelates (Hermana & Nurhayati, 2010). Humic acid is a substance that plays a role in the binding of heavy metals because humic acid contains heavy metal-binding functional groups.

Minimization of Heavy Metal Lead (Pb) and Iron (Fe) by Humic Acid. The initial concentrations of heavy metals Pb and Fe in water at the beginning of the treatment measured using an Atomic Absorbent Spectrophotometer (AAS) were 6.68 mg/L and 8.52 mg/L, respectively. The initial concentrations of Pb and Fe were the same in all experiments. The experimental results are shown in Table 1.

The minimization of heavy metal Pb in the experiment using humic acid based on Table 2. It was found that heavy metal Pb was able to be minimized by humic acid. Statistical tests showed that there was no significant difference in the use of 2.5 g/L, 5 g/L and 7 g/L doses of humic acid. This is because the optimal adsorption capacity of Pb heavy metal was maximized in being absorbed or bound by humic acid at a dose of 5 g/L so that the additional dose did not have much effect. Therefore, it can be concluded that the 5 g/L dose of humic acid is the best dose of humic acid in minimizing Pb heavy metals. Based on the research data obtained, the ability of humic acid to absorb heavy metals is very high, which is an average of more than 90%.

Minimization of heavy metal Fe in the experiment using humic acid based on Table 2. It was found that heavy metal Fe was able to be minimized by humic acid. The statistical test showed

Table 1. Average concentration of heavy metalz lead (Pb) remaining in water by minimization process humic acid by various doses

Humic acid dosage (g/L)	Average remaining heavy metal Pb concentration (mg/l)	The percentage of heavy metals absorbed by humic acid
0^a	6.68 ± 0.1068	-
2.5^b	0.68 ± 0.1782	90.08%
5^b	0.68 ± 0.0427	90.08%
7^b	0.68 ± 0.2060	90.54%

Note: The same superscript letter behind the number of doses indicates not significantly different.

Table 2. Average concentration of heavy metalz iron (Fe) remaining in water by minimization process humic acid by various doses

Humic acid dosage (g/L)	Average remaining Fe heavy metal concentration (mg/l)	The percentage of heavy metals absorbed by humic acid
0[a]	8.56 ± 0.1027	-
2.5[b]	0.56 ± 0.1344	95.15%
5[b]	0.56 ± 0.0424	95.15%
7[b]	0.52 ± 0.0374	95.65%

Note: The same superscript letters behind the number of doses show no significant difference.

that there was a significant difference in the use of humic acid doses of 2.5 g/L, 5 g/L and 7 g/L. This difference did not occur because the optimal capacity for absorption (adsorption) of heavy metal Pb was maximal in being absorbed or bound by humic acid at a dose of 2.5 g/L so that the increase in dose did not have much effect. Therefore, it can be concluded that the humic acid dose of 2.5 g/L is the best humic acid dose in minimizing heavy metal Fe. Based on the research data, it was found that the ability of humic acid to absorb heavy metals was very high, with an average of more than 90%.

Humic acid can be used to minimize lead (Pb) and iron (Fe) heavy metals because it has a functional group to bind heavy metals, which is able to adsorb and bind heavy metals by means of cation exchange, formation of electrostatic bonds, formation of complex bonds and chelates (Hermana & Nurhayati, 2010; Yuliyati et al., 2016). In addition, the positive mineral content in humic acid solids can also be exchanged for Pb metal cations (Hermana & Nurhayati, 2010). Humic acid substances have an important characteristic that is able to form soluble and insoluble complexes with metal ions. In addition, humic acid substances also contribute to the exchange of anions and cations, complexes or chelates and act as pH buffers. Therefore, in the process of adsorption of heavy metals with humic acid, the substance have the most role in the adsorption process.

This humic acid substance contains functional groups including: -COOH, -OH, -COH and C=O (Mindari et al., 2014). During the process, this functional group will undergo a deprotonation process so that H+ ions will be released from compounding and the functional group will be negatively charged. The negative charge on this functional group will play a role in binding heavy metals in water and binding H+ ions which will lower heavy metals in water and increase water pH.

In addition to the functional groups in humic acid, the reduction of heavy metals in water is also due to the positive mineral content of humic acid which can be exchange with heavy metal ions Pb (positively charged). This cation exchange can occur because P^{2+} cations which are divalent ions will easily be exchanged with positive monovalent ions contained in humic acid. The compound complex formed by this cation exchange humic acid is an outphere compound humic acid with weak bonding properties (Hermana & Nurhayati, 2010).

Adsorption of heavy metals by humic acid begins with physical adsorption of metal particles that approach the surface of humic acid through Van der Waals forces or through hydrogen bonds, then followed by chemical adsorption that occurs after physical adsorption. In chemical adsorption, the particles adhere to the surface by forming chemical bonds (usually covalent bonds), and tend to look for places that maximize the coordination number with the substrate (Septiani & Tasari, 2020).

Water quality during humic acid treatment

The water quality during the heavy metal adsorption process by humic acid at the beginning and at the end showed different results. This depends on the amount of humic acid used as a heavy metal adsorption agent. At the initial time before being treated with humic acid, the water quality in all types of humic acid treatment and the dose of humic acid was the same. The initial pH value of the water was low due to the use of an acidic standard Pb solution into the water. Table 3 shows the water quality before being treated with humic acid. After being given a dose of humic acid for 24 hours, the water quality of each type of humic acid and the dose showed different values (Table 4).

The comparison between the initial water quality and the end of the treatment showed that

Table 3. Water quality before humic acid treatment

pH	DO	TOM	Ammonia
3	3.1	0.51	0

Table 4. Water quality after humic acid treatment

Dosage	Humic acid (g/L)	Residual lead (Pb) in water	Residual iron (Fe) in water	pH	DO (mg/l)	TOM (mg/l)	Ammonia (mg/l)
0	0	6.86	8.56	3	3.8	0.50	0
2.5	0.0374	0.66	0.56	6.0	4.1	4.09	0.25
5	0.6735	0.66	0.56	6.8	4.0	7.45	1.5
7	1.1441	0.64	0.52	7.1	4.0	7.67	1.5

there was a change in water quality due to humic acid treatment. The pH value at the beginning and end of the humic acid treatment increased. This is because humic acid has the ability to raise the pH because humic acid has many negative functional groups that can bind H^+ ions (the cause of low pH) in water. On the other hand, the value of TOM (Total Organic Matter) also increased. This increase was due to the fact that at the end of the treatment the water was cloudier due to humic acid organic matter. In the ammonia component, almost all water quality at each dose of humic acid, the ammonia value increased. This increase occurred due to the influence of humic acid.

Research conducted proves that water treated with humic acid is able to produce water quality in accordance with the habitat of tilapia (*Oreochromis niloticus*). This can be seen from the pH value being above 5.

Pisciculture

The fish rearing process is carried out using water media whose heavy metals have been adsorbed by humic acid for 24 hours. The fish rearing process was carried out for 30 days resulting in fish conditions as shown in Table 5.

Fish maintenance in most treatments resulted in a fairly good survival rate and growth rate (above 90%). In treated water without humic acid (dose 0 g/L). survival rate and growth rate is the lowest (zero). Measurement of daily growth rate in each treatment showed high results which were quite good. The low growth rate was only found in controls with zero survival.

The high survival rate and growth rate of the fish that are kept indicate that the water quality is still suitable for fish farming. The survival and growth rate in the treatment showed that the fish could live in the treated water conditions (Kiswanto et al., 2022).

The remaining Pb and Fe metals in the water were minimized using humic acid, which was very low. Humic acid was able to reduce the concentration of heavy metals from 6.86 mg/L and 8.56 mg/L to the lowest values of 0.66 mg/L and 0.52 mg/L. This concentration is still above the water quality standard for fisheries, which is 0.03 set by the government. Even so, maintenance of fish at the concentration of heavy metals turned out to be very low in the amount of heavy metals that intruded into the body of fish in all treatment. The concentration of heavy metals in the fish's body is still far below the threshold set by BSNI, namely SNI 7387:2009.

CONCLUSION

Based on the results of the study, the following conclusions were obtained; Humic acid is able to minimize heavy metal Lead (Pb) and heavy metal

Table 5. Fish condition during 30 days cultivation

Humic acid dosage (g/L)	Average water pH	Pb Stored in water (mg/L)	Pb in fish body (mg/kg)	Fe stored in water (mg/l)	Fe in fish body (mg/kg)	Average daily growth (%)
0	3	6.86	-	8.56	-	0
2.5	6.2	0.66	0.0073	0.56	0.0050	10.27
5	6.9	0.66	0.0070	0.56	0.0080	10.67
7	7.2	0.64	0.0087	0.52	0.0046	10.16

iron (Fe) in fish culture media by more than 90%. Humic acid in addition to being able to minimize Pb and Fe metals, is also able to increase the pH value of the water in the pond so that the daily survival rate of fish is higher. Humic acid is very good for neutralizing water from coal mines by using organic materials contain humic acid.

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