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Removal of pb in acid coal mine drainage using water hyacinth (*Eichhornia crassipes* Mart. Solms)

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Key words: AMD, Pb, Removal, Water hyacinth.

Abstract

An open-cut coal mining produces acid mine drainage (AMD). AMD has a pH <4 and contains Pb with concentrations exceeding the threshold allowed by the government. Several chemical methods have been done to reduce metals and neutralize pH. including the addition of lime industry. This method has weaknesses, which are expensive and produce inorganic sludge. This research was conducted by utilizing water hyacinth (*Eichhornia crassipes*) and local limestone. The goal is to know the growth and accumulation ability of water hyacinth in reducing Pb. Since the water hyacinth cannot grow at pH <4, it was previously treated with limestone to pH > 4. In the addition of limestone, the pH of AMD changes from 3.0 to 4.01; 4.36; 4.87. Furthermore, each planted with the same amount of water hyacinth for 21 days. The pH measurement results were obtained to 5.32; 5.95, 6.85. This condition gives the growth of water hyacinth (RG) is better and higher at pH 5.91. The result of Pb accumulation analysis with *Inductively Coupled Plasma Optical Emission Spectrometer* (ICP-OES) was obtained at part root+shoots of 1048,51 mg/kg dry weight with Bioconcentration factor (BCF) 102,89. In part leaves+stalk obtained 196.25 mg/kg dry weight with BCF 19.24. Thus water hyacinth can be utilized in the processing of AMD to reduce Pb and neutralize pH. The growth of water hyacinth is not only influenced by pH but also the accumulation rate of Pb, so it must be harvested periodically.

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Introduction

Acid mine drainage (AMD) is a sulfuric acid formed from the oxidation of pyrite (FeS_2) and other sulfide minerals are exposed to oxygen and water. AMD generally have a same level ($\text{pH} < 4$). In these conditions, AMD causes heavy metals more easily dissolved so that the concentration is higher with increasing time. Heavy metals are potential pollutants such as Lead (Pb).

Pb is one of the heavy metals are toxic. Humans are exposed to lead at a certain level may be causing brain damage and mental retardation (Cho-Ruk *et al.*, 2006). The surroundings were contaminated with Pb cause problems prolonged. This is due Pb cannot be degraded by microorganisms (Pehlivan *et al.*, 2009). Pb in plants directly found not bercun in plants, but can interfere with metabolic processes (Gupta *et al.*, 2012). The potential dangers of Pb are high, then the world health organization (WHO) set a threshold of exposure to lead in the drinking water of 0.15 ppb (WHO, 2011).

Some methods that can remove heavy metals in water such as coagulation, ion exchange, precipitation, electrolysis, and a reverse osmosis system (Balasubramanian *et al.*, 2009; Kim *et al.*, 2006; Kumari *et al.*, 2006). These methods have the drawback, besides the difficult and expensive so rarely used (Sharma, and Sohn, 2009). Currently, the coal mining industry uses lime to raise the pH and reduces heavy metals. Even this method also still expensive and can cause precipitation of inorganic sludge is high.

More recently, there is considerable interest in developing cost-effective technologies for the improvement of the quality of waste water contaminated with heavy metals using plants as an accumulator. This method is through the process of adsorption by the roots, localization and translocation that metal ions can be concentrated at the top until the leaves, so it's easy to be harvested and processed again (Jadia and Fulekar, 2009; Natarajan *et al.*, 2008; Gratao *et al.*, 2005).

Water hyacinth (*Eichhornia crassipes*) is one type of bog plants are abundant in southern Kalimantan and grow very fast, high production biomass and resistant to pests and diseases. This plant is able to grow on nutrient conditions, pH, temperature, and extreme toxic materials. In addition it has the potential to clean up wastewater (AMD) or hiperakumulator heavy metals Al, Pb, Cu, Fe, Mn, Ni, Cd, Cr, Co, Zn, Hg, and As (Skinner *et al.*, 2007; Michelle *et al.*, 2010).

Preliminary research has shown that the water hyacinth on AMD pH 3.0 cannot adapt and cause chlorosis on the leaves, stems, and roots fall out. At pH, 4 most aquatic plants die because they cannot adapt (Ratnaningsih *et al.*, 2010). Based on the above, the focus of this study is to demonstrate the potential of water hyacinth to reduce Pb at AMD. The hope is as an alternative that can be applied to the coal mining industry because it is cheap, easy, and environmentally friendly.

Materials and methods

Study area

This research was conducted at the Laboratory of Elementary Mathematics and Natural Sciences in Lambung Mangkurat University at Banjarbaru city. Acid mine water was collected from one of the locations of coal mining in Tanah Laut, South Kalimantan.

Procedure

Water hyacinth is taken from the surrounding rice fields Sungai Tabuk Kabupaten Banjar. Hyacinth fresh and flawless with a height between 15-20 cm, number of leaves between 4-6 strands. Plants were washed with tap water to remove any epiphytes and insect larvae that grow on plants. Furthermore, the acclimatization in artificial ponds filled with water wells. Water hyacinth is allowed to grow and develop by providing NPK fertilizer. Water hyacinth was selected to be sampled, hyacinth having 1 stolon or tillers on patiola with about 1-2 cm root length, leaf number 2 strands and leaves 2-3 cm diameter.

Limestone is obtained from miners in the area of the District Tarjun Pulau Laut, South Kalimantan. Limestone is broken down into a split with a uniform size of about 2 cm. AMD obtained from temporary one coal mine in South Kalimantan. AMD taken by draw then stored in plastic containers.

Data collection method

Besin arranged into 3 groups (A1, A2, A3). Each group, a basin filled with 7 L AMD and plus each 50 g limestone (A1), 100 g (A2), and 150 g (A3). Each group performed with 3 repetitions. All treatments performed pH measurements. The three treatment groups were left for 3 days, then measuring the pH. The three groups are then planted with each one clump of saplings hyacinth. pH measurement is done thirds for 21 days (0, 3, 6, 9, 12, 15, 18, and 21 days). Likewise harvesting water hyacinth and capture footage AMD each as much as 10 mL. AMD footage nitric acid are added preservatives and prepared with filtering system.

The clear solution is ready for testing. Hyacinth from each group were weighed to determine the total weight. Water hyacinth is separated into two parts, the first part (root + shoots) and the second part (leaves + stalks). The second part of each weighed, then cut into small pieces (0,5-1.0) cm. Then washed throughly with distilled water then dried with opened air. Each piece digested using standard methods APHA (1998). 1 g sample was then added HNO₃ HclO₄ as much as 10 mL and 3 mL, allowed to stand for 24 hours. Further heated to a temperature of 120°C for 2 hours, then cooled. Then added 25 mL of distilled water and heated at a temperature of 105°C for 2 hours, then cooled. Then added 10 mL HNO₃ and HClO₄ as much as 1.5 mL. Heated again until there is a solution to 15 mL. Diluted with 50 mL of distilled water, then filtered with Whatman paper prepared sample solution 42. Test, while AMD screened samples with membrane filter of 0.45 μm. Filtered water ready to be tested.

Data analysis

Relative growth (RG)

Data total weight of the water hyacinth is converted into value RG by the formula (Mohamad, 2010).

$$RG = \frac{\text{Final fresh weight (FFW)}}{\text{Initial fresh weight (IFW)}}$$

Accumulation Pb

Pb concentration on AMD an on water hyacinth measured by inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) ACTIVA S. Data changes in the concentration of Pb that accumulates in plants and then converted into value Bioconcentration factor (BCF) by the formula (Zayed *et al.*, 1998).

$$BCF = \frac{\text{Conc. of metal in plant tissue}}{\text{Initial Conc. of metal in external sol.}}$$

Statistical analysis

Data on average change in pH, total weight growth of water hyacinth, and BCF was analyzed by correlation test. Correlation test linear regression method (simple) porcessed with the help of the SPSS program (Statistical Product and Service Solutions) version 20.

Result

The average measurements of pH and the total weight of the water hyacinth growth, and RG calculation of the three variations of pH AMD during 21 days of treatment are presented in Table 1.

Table 1 shows that of the three different treatments for 21 days, also changes the pH different. Likewise, different pH changes followed the growth of the total weight of the water hyacinth different. At A1 pH increased by 1.31 or 32.67%, A2 1.59 (36.47%), and A3 1.98 (40.66%). Such conditions followed with an increase in the total weight of each A1 (14.92 to 84.60) g or 69.68 g (467.02%), A2 (15.62 to 116.50) g or 100.88 g (645.84%), and A3 (15.50 to 148.46) g or 132.96 g (857.81%). This phenomenon shows that there is a strong relationship (positive) between pH AMD with growth of water hyacinth.

Table 1. Total weight of Water hyacinth and AMD pH of the three treatments for 21 days.

Treatment	Parameter	Age (Days)							
		0	3	6	9	12	15	18	21
A1	pH	4,01	4,29	4,48	4,67	4,90	5,08	5,22	5,32
	TW (g)	14,92	17,62	21,21	30,90	43,51	60,72	75,90	84,60
	RG		1,18	1,20	1,46	1,41	1,40	1,25	1,11
A2	pH	4,36	4,85	5,07	5,28	5,48	5,67	5,83	5,95
	TW (g)	15,62	20,58	34,22	57,30	80,56	95,90	107,98	116,50
	RG		1,32	1,66	1,67	1,41	1,19	1,13	1,08
A3	pH	4,87	5,33	5,64	5,91	6,22	6,49	6,70	6,85
	TW (g)	15,50	24,83	41,80	72,62	98,50	122,80	139,66	148,46
	RG		1,60	1,68	1,74	1,36	1,25	1,14	1,06

Note: A1 = acid mine water add with 50 g of limestone, A2 = acid mine water add with 100 g of limestone; A3 = acid mine water add with 150 g of limestone; TW = total weight. RG = Relative growth.

This is evidenced by the results of correlation and linear regression of three treatments with a value of R (*correlation coefficient*) respectively 0,95 ; 0,96 ; and 0,98., as shown in Table 2.

The growth of water hyacinth

RG is used to view the condition of the growth of water hyacinth on the contaminated media.

The value also shows how plants respond to substances contaminants. That exist in the media, in this case is the As.

The higher the value of RG, then the plant can be categorized not give a negative reaction to the content of the media, on the contrary, the lower the RG, then the plant has given a negative response.

Table 2. The relationship of pH to total weight growth of water hyacinth.

pH	Independent variable	R	R Square	Adjusted R Square	Std. Error of the Estimate
A1	TW1	0.947	0.897	0.892	0.148
A2	TW2	0.962	0.926	0.923	0.143
A3	TW3	0.983	0.966	0.965	0.125

Note: A1 = 4.01 – 4.32, A2 = 4.36-5.95; A3 = 4.87-6.85; TW1 = total weight on A1, TW2 = total weight on A2, TW3 = total weight on A3, R = correlation coefficient.

RG hyacinth calculation result obtained from the three treatments for 21 days (Table 1), namely A1 (1.11 to 1.46), A2 (1.08 to 1.67), and A3 (1.06 to 1.74). RG respectively lows occurred on day 21 with each pH 5.32 (1.11) 5.95 (1.08) 6.85 (1.06). The highest RG each occurred on the 9th day with pH 4.67 (1.46) 5.28 (1.67), and 5.91 (1.74). Thus optimal growth occurs at pH 5.91. Increased RG occur until the 9th day and then decrease until day 21, though still an increase of up to nearly neutral pH (Fig. 1). This indicates that the water hyacinth gave negative responses after 9 days old.

Thus, unlike some previous studies where optimal growth of water hyacinth occurs at a pH close to neutral.

Lead reduction in AMD

Changes Pb concentration in AMD of three treatments for 21 days tended to decrease (Fig. 2), which each of A1 (5.79 mg/L or 56.76%), A2 (9.03 mg/L or 88.53 %), and A3 (10.20 mg/L or 100.00%). These results indicate that along with the increase in pH and water hyacinth age, the ability of water hyacinth in reducing Pb also increases or there is a strong relationship

between age hyacinths with Pb reduction capabilities on AMD. This is reinforced by the results of correlation and linear regression where $R = 0.85$.

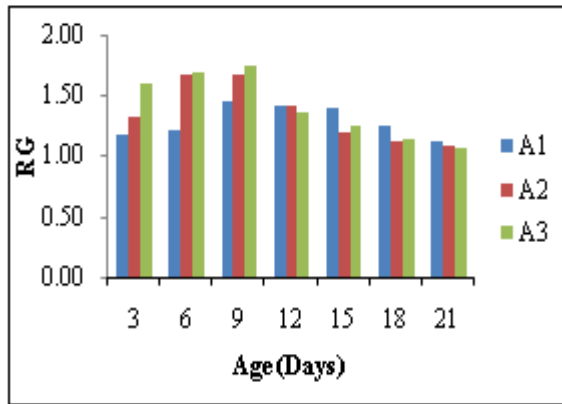


Fig. 1. Changes RG total weight of the water hyacinth on AMD.

Accumulation Pb

Pb accumulation of three treatments until the age of 6 days is still very low. It can be understood that the hyacinth is still in the stage adaptation. In addition to the low pH of the surface of the cell wall of root protonated or is positively charged, so the adsorption very little happened. With increasing age and pH increases significant accumulation, but by different amounts.

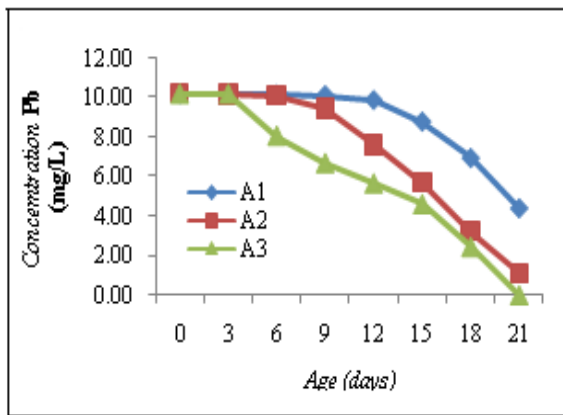


Fig. 2. Pb Concentration reduction in AMD for 21 days.

The highest accumulation in roots and shoots of the three treatments each A1 (pH 5.32) amounted to 505.75 mg/kg dry weight, A2 (pH 5.95) sebesar 678.30 mg/kg, and A3 (pH 6.85) for 1049.51 mg/kg. The same pattern also occurs in the leaves and stalks.

The highest accumulation in the A1 (106.45 mg/kg), A2 (157.32 mg/kg), and A3 (196.25 mg/kg). Based on the result of the accumulation of two different plant parts, then the value of BCF also different. BCF change of the second part of the plant shown in Fig. 3 and 4.

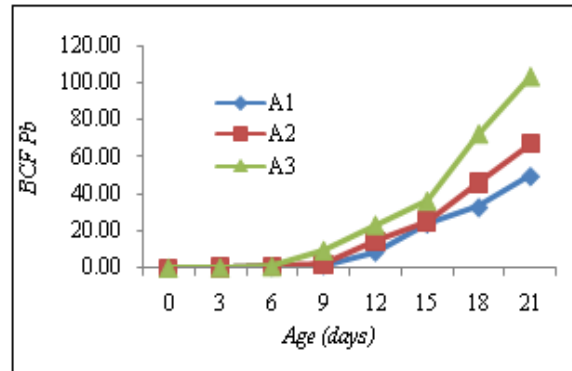
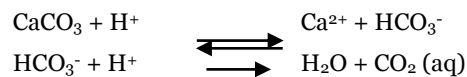


Fig. 3. Pb BCF changes in Root + shoot water hyacinth during the 21 days of treatment.

Discussion

Effect of pH on the growth of water hyacinth

Some factor what may increase the pH. In this study, using limestone in water hyacinth. Limestone generally contain calcium carbonate, and compound is capable of neutralize acidity of the water. AMD neutralization process the calcium carbonate is as follows:



Decomposition of calcium carbonate on AMD reacts with hydrogen as a carrier of acidic ions, producing neutral water. The more ionized calcium carbonate, the neutralization process faster and vice versa. At the root respiration produces carbon dioxide and water hyacinth with water produces bicarbonate ions as a nature alkinitas. Many researchers previously reported ability to increase the pH of the water hyacinth (if in acidic conditions) or decreasing the pH (in alkaline conditions) wastewater. Water hyacinth can raise the pH up to 80% of initial pH on coal AMD(Savitri, 2010).

In addition to the above factors, the presence of microorganisms can also increase the pH of the wastewater.

An increase in pH causes a decrease in the solubility of metals and trigger an increase in bacterial population (Fauziah, 2013). AMD teridentifikasi coal containing sulfate reducing bacteria thiobacillus sp and Pseudomonas sp (Ulfa *et al.*, 2013). Both bacterial species is sulfate oxidizing bacteria (BPS). The bacteria are aerobic.

The rise in $\text{pH} > 4$ causes the BPS doesn't develop. While the sulfate reducing bacteria (SRB) are anaerobic well developed, such as Desulfovibrio. Microbiological sulfate reduction is a process in which the sulfate is reduced to sulfide and bicarbonate. Sulfate reduction is a process in which the sulfate is reduced to sulfide reduction and toxic metals in waste water (Drury, 2006). In the process of sulfate reduction to form bicarbonate ions that generate alkalinity (Greben *et al.*, 2005).

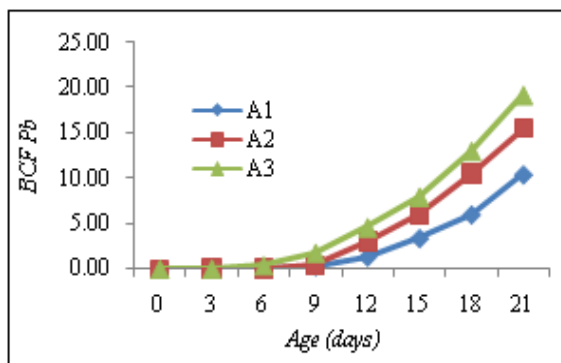


Fig. 4. Pb BCF changes in leaf + stalk water hyacinth during the 21 days of treatment.

Effect of pH on growth of water hyacinth

Some researchers have previously reported on the effect of pH on the growth of water hyacinth. pH effect on the growth of water hyacinth, where growth is best in a pH range of 5.5 to 7.0 (Lu, 2009). The optimum pH for growth of water hyacinth in the range of 5-8, although it can grow in media of pH 4-8 and grows optimally at pH 6-7 (Madkar and Kurniadie, 2003). Pertumbuhan plant at an optimal pH 7, at a pH of 3.2 to 4, two highly toxic to plants, pH 4.2-4.3 stunted growth, and 4.3 to 4.5 may be hampered (Télez *et al.*, 2008). The results showed not exactly the same as previous studies, but is still included in the pH range tolerated by plants. Thus these results indicate the presence of other factors came into effect in addition to the pH.

Another factor that may be caused by increasing concentration of metals adsorbed and accumulated in plants after the age of 9 days, then decreased until day 21. As we know that AMD is rich in heavy metals. High concentrations of heavy metals that can inhibit the growth of water hyacinth. Water hyacinth has a high rate of reduction for heavy metals (Fe, Zn, Cu, Cr, Cd, Mn, Ni, Hg, and As) without experiencing stunted growth (Liao and Chang, 2004). The high accumulation of heavy metals in the roots and shoots up to 10 times of the initial concentration, but did not reveal any signs of toxicity in plants (Mishra and Tripathi, 2008).

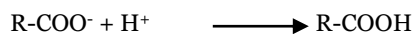
Lead reduction in AMD

Several factors can affect the ability of plants to reduce contaminants in the water, such as pH, temperature, and age. In general, Along with the growth, adsorbsi process and reduction of heavy metals continues. Plant growth affects the speed reduction of heavy metals (Sheoran and Sheoran, 2006). Accumulation of Zn, Pb and Cd by water hyacinth increased as rising pH (Aisen *et al.*, 2010).

Biosorption depends on the pH of the water media, because it is caused by the presence of functional groups in the cell wall and chemical configuration of the metal (Wierzba and Latala, 2010). The cell walls contain amine functional group (R-NH_2), amide (R-CO-NR_2), and a carboxylic acid (R-COOH), which can be protonated or deprotonated depending on the pH of the water medium. Increasing the pH will increase the negative charge on the surface of a cell until all the relevant functional groups deprotonated and can support the appeal of electrochemical and absorption of cations. This is consistent with the fact that with increasing age and the pH is then followed by a reduction in the concentration of Pb in AMD.

At the root of water hyacinth cells contain major functional groups, namely amines, hydroxyl and carboxyl, amine groups but very few (Lin *et al.*, 2012; Zhang *et al.*, 2010; Naja *et al.*, 2005).

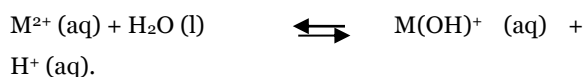
At low pH, the surface of the cell wall of water hyacinth roots protonated or is positively charged, so the adsorption of metal is happening is very small, because the carboxylic group tend to be in a neutral form, with the reaction:



At high pH surface functional groups protonated water hyacinth root cell so that the negatively charged. In this condition, the metal adsorption becomes larger. The higher the pH also will make more and more negatively charged functional groups, so that the adsorbent can act as ligands in the formation of the complex is also increasing, with the reaction:



Their negative charge will cause interactions between the positively charged metal with active sites on the surface of the root cell walls are negatively charged. At the same time, the surface will compete with OH⁻ ligands in the binding of metal cations, which will result in an increase in the adsorption of metals by plants. Factors that influence the load of active sites, also is a metal species in solution. Metal ions in solution before it is adsorbed by the adsorbent first undergo hydrolysis, produces protons and metal hydroxo complexes such as the following reaction:



Biosorption capacity cation dependent on physicochemical parameters (indexes most covalent and ionic radii or metal). According to Chojnacka (2013), covalent index higher are more likely to form a covalent bond with a biological ligand Thiel (R-SH), amino (R-NH₂), carboxyl (R-COOH) and hydroxyl (R-OH).

A decrease in the concentration of contaminants in the waste water, in addition to the adsorption of water hyacinth can also be caused by bacteria. The presence of bacteria in the root zone water hyacinth plays a major role in cleaning wastewater (Lingzhi *et al.*, 2007). Another factor that might influence is the occurrence of precipitation.

As rising pH and oxygen deprivation causes AMD transformed into anaerobic conditions. In these conditions the bacteria growing bacteria such as *Desulfovibrio preduksi sulfate*.

Accumulation Pb

Pb BCF value increased with age hyacinth and pH AMD. Changes in growth are most obvious initial reaction in plants exposed to heavy metals. The concentration of the metal/metalloid in the aquatic environment is the main factor affecting the absorption efficiency of metal/metalloid (Karimi *et al.*, 2009). In general, when the metal concentration in the water increases, the amount of metal accumulation in plants increased and values BCF also increased.

In this study, the BCF value obtained is still smaller than previous researchers who have found a range of time and pH are virtually identical, where BCF for Pb at pH 4.5; 6.8; and 8.5 on the roots of water hyacinth is greater than 1000 (Aisen *et al.*, 2010). According to Goswami *et al.* (2010), BCF roots and shoots of water hyacinth on the Pb at pH 5 within the period of 10 days, 349.8 and 11.53 of the initial concentration water media 15 mg/L. Roots of water hyacinth has a maximum efficiency of absorption of Pb by 90% or more after the 5th and 10th of exposure for different initial concentrations and pH. BCF hyacinth very high for Cd, Cu, Cr and Se at a concentration of external low, and decreased with the increased concentration of the external (Mohanty *et al.*, 2012). Thus, the low BCF Pb may be caused by their multilogam accumulated at the same time, as AMD contain some metals other than Pb.

Metals that accumulate in the roots translocated through simplast at high concentrations in a way that does not interfere with the function of the cytoplasm and eventually metal deposited at very high concentrations in shoots (Kumar, 2012). At the level of toxicity that can effect translocation will continue to the top, the stems and leaves. The low level of accumulation in the leaves and stalks more dependent on the limits of tolerance of plants to the toxic effects caused by metals.

It also may be caused by the nature of the water hyacinth as rizofiltration plant, so that the metal is absorbed tends to accumulate in the roots. Other possibilities due to the ability of tolerance to the level of accumulation of Pb in the roots is still not threatening his life, so that translocation to the leaf Pb still low. This can be seen in the growth rate of the water hyacinth, look no obstacles.

Conclusion

Based on the results and discussion above, this research can be concluded that the pH and the accumulation rate of Pb affect the relative growth of water hyacinth. The accumulation rate of Pb and the bioconcentration factor on the roots and shoots of water hyacinth is bigger than the leaves and stalks part. These results confirm that water hyacinth can be utilized as bio-accumulator so as to reduce/remove Pb concentration up to 100%. Utilization of water hyacinth in the processing of AMD should be harvested at certain period (about 9 days).

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