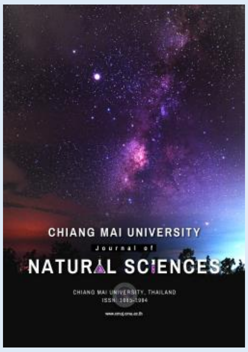

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Research article

Azolla Pinnata as Phytoremediation Agent of Iron (Fe) in Ex Sand Mining Waters

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Abstract Phytoremediation is one effective method used for reducing the iron (Fe) from waters. *Azolla pinnata* is a plant that has potential as an agent of phytoremediation of Fe in waters. This study aims to verify the ability of *Azolla pinnata* to reduce Fe from sand excavated water in Pasir Sakti District, East Lampung Regency, Indonesia. The study was conducted with three treatments. The treatments of 25% cover area, 50% cover area, and 75% cover of *Azolla pinnata*, with three replications each. The experiments were carried out until the water quality became suitable for aquaculture. The results showed that the area cover of *Azolla pinnata* had a different effect (P <0.05) on the decrease in iron concentration in the water. The treatment with 75% cover area of *Azolla pinnata*, showed a reduction percentage of iron concentration of 98.10%. It is the most significant reduction percentage of iron. Whereas the treatment with 25% and 50% *Azolla pinnata* cover area of succeeded in reducing the concentration of Fe in water 92.5% and 93.3% respectively.

Keywords: Aquaculture, Iron, Phytoremediation, Removal efficiency

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INTRODUCTION

Large-scale sand mining activities for industrial and building needs often do not heed the ecological aspects so that they can adversely affect to environmental conditions and ecosystems (Rahmadian and Dharmawan, 2014; Rizqan et al., 2016). The impact can be in the form of physical, chemical and biological damages (Siswanto et al., 2012). The Physical impact of sand mining can result in erosion and changes in soil structure (Asabonga et al., 2017). Large scale erosion of topsoil can form large holes. Furthermore, it is filled with rainwater and groundwater recharge to create large lakes (Saviour and Stalin, 2012; Asabonga et al., 2017; Gavriletea, 2017).

Identification of chemical compounds in the sand in Aceh and Cilacap-Indonesia- by Darmayanti et al., (2000), showed that the highest metal content was iron (Fe) reaching 30%. Fe minerals that dominate the sand content are Fe_2O_3 and Fe_3O_4, causing sand to be black coloured (Rusianto et al., 2012). According to Schumann et al., (2015), some sulfide minerals generally interact significantly with pyrite (FeS_2) Robertson et al., (2015); Schumann et al., (2015). Iron (Fe) and Sulfur (S) are metals and acids that are indicators of mining areas. The mining area can producing iron-based iron acids including pyrite (FeS_2), pyrhotite (FeS), chalcopyrite (CuFeS) and arsenopyrite (FeAsS) (Commonwealt of Australia, 2016; Runtti et al., 2017). The high metal content of Fe hurts aquatic organisms such as low growth fish and can cause the death of aquatic organisms (Baby et al., 2010; Rajeshkumar and Li. 2018).

Pasir Sakti Subdistrict (Lampung Province, Indonesia) is a vast sand mining area that forms a large basin. The process of rain and groundwater input causes the holes filled with water and form large lakes (Malik, 2017). The ex-sand dugouts are thought to contain high concentrations of iron, so they cannot be used for aquaculture activities. Therefore, some efforts are needed to reduce the Fe concentration in waters on ex sand mining.

Physical reduction of Fe from water can be made with activated carbon (Syauqiah et al., 2011; Abdi et al., 2016), filtration (Giwa et al., 2017), zeolites (Runtti et al., 2017; Nur'Aini and Wilopo, 2017). Chemically, it can be done using chitosan (Burke et al., 2002; Lapo et al., 2019), and Chelating Agent (EDTA) (Aziz et al., 2016). Biological treatment can use bacterial isolates (Leung et al., 2010; Irawati et al., 2017) or phytoremediation by aquatic plants (Bharti and Banerjee, 2012; Singh et al., 2012; Ajibade et al., 2013). Phytoremediation has economic advantages, is environmentally friendly, and is applicable so that it is easily implemented by the community (Lee, 2013; Ajibade et al., 2013).

Some species of aquatic plants are known as phytoremediation agents. According to Arimby (2014), *A. pinnata* can grow fast, can adapt to acidity, infertile soil, temperature and pollutants. *A. pinnata* can be found on the surface of rice fields, lakes, ponds and swamps (Hidayati, 2013). Fast biomass production in a short time causes *A. pinnata* to be an ideal plant as a phytoremediation agent (Anjuli et al., 2004; Sood et al., 2012). *A. pinnat*a is a weed in rice plants that can be used as a phytoremediation agent to reduce iron (Fe) (Thayaparan et al., 2013); and other metal such as Hg and Cd (Sood et al., 2012), Cu, Cr, Cd, and Zn (Shafi et al., 2015; Noorjahan and Jamuna, 2015).

Based on research by Vaceem and Banerjee (2012), *Azolla pinnata* has reduced Fe concentrations in water by up to 70%. Other studies have verified that Azolla pinnata can reduce Fe to 95.4% (Bharti and Banerjee, 2012). Akinbile et al., (2016) have stated that *Azolla pinnata* has been proven effective in improving the quality of domestic wastewater, reaching the highest value of 99.55%. This study tries to verify the ability of *A. pinnata* to reduce iron in ex-sand mining water in Pasir Sakti District, East Lampung, Indonesia. Furthermore, ex-sand mining waters can be used for aquaculture activities.

MATERIALS AND METHODS

Research design

This study uses *A. pinnata* as a phytoremediation agent to reduce iron in the water of ex-sand mining area of Pasir Sakti District, East Lampung Regency, Indonesia. The treatment has been constructed using different percentages of *A. pinnata* cover area in the experimental tanks, i.e.:

K: *Azolla pinnata* with 0% covered area (Control)
A: *Azolla pinnata* with 25% covered area
B: *Azolla pinnata* with 50% covered area
C: *Azolla pinnata* with 75% covered area

Experimental procedure

Preparation of Experimental Tanks

The experimental tanks is a semi-outdoor tarpaulin pond with a size of 2.5 x 1.5 x 0.75 m^3. The experimental containers, clean and rinse using fresh water and then dried, before used. Sand excavated water is put into each experimental tanks with a height of 50 cm. The initial Fe concentration was measured before the experiment was carried out, while the final Fe concentration was measured after the experiments had ended.

Preparation of *Azolla pinnata*

Before use, *A. pinnata* is cleaned using clean water to remove impurities, eggs of other organisms, and insect larvae that may be attached to plants. After cleaning, the *A. pinnata* was then put into the phytoremediation experimental tanks according to treatments.

Phytoremediation Process

The remediation process takes place until the Fe concentration is in accordance with the water quality standard, that is 0.03 mg/L (WHO). During the remediation process, no water was changed. Measurement of Fe in water is carried out every seven days.

Response Assessment

Fe concentration

The response of Azolla pinnata's ability to reduce iron (Fe) in water is measured in terms of: percentage of decrease in Fe in water (Sidek et al., 2018), translocation factor (TF) (Mellem et al., 2012), and bioconcentration factor (BCF) (Ghosh and Singh, 2005). As supporting data, dissolved oxygen (DO), temperature and pH of the water are also measured. Water samples were tested at the Integrated Laboratory and Technology Innovation Center of the Lampung University (referring to the EPA 200.7 Rev. 5 method).

The percentage reduction in Fe concentration was calculated by the equation of Sidek et al., (2018) as follows:

$$\text{Reduction of Fe}(\%) = \frac{\text{Initial Fe Concentration } (C_o) - \text{Final Fe Concentration } (C_f)}{\text{Initial Fe Concentration } (C_o)} x \ 100\%$$

Bioconcentration factors (BCF) calculated by the equation, according to Ghosh and Singh (2005); Kamar et al. (2009).

$$\text{Bioconcentration Factors} = \frac{\text{Fe concentrations in roots (ppm)}}{\text{Fe concentration in water (ppm)}}$$

The higher the value of BCF, the more suitable the plant as a phytoremediation agent (Ndimele and Jimoh, 2011). According to Testi et al., (2019), the value of bioconcentration factor classified as: Low ability (<250); Medium ability (250-1000); and high ability (>1000).

The translocation factor (TF) indicates the ability of plants to transfer metals from roots to stems and leaves. TF is calculated by the following formula (According to Mellem et al., (2012):

$$\text{Translocation factor} = \frac{\text{Fe concentrations in plant parts (ppm)}}{\text{Fe concentrations in the roots (ppm)}}$$

Water Quality Measurement

Water quality measurements include temperature, pH and dissolved oxygen DO. Measurements were taken at each unit of the experiment twice every day during the phytoremediation experiment.

RESULTS

Water Quality

Measurement of the physical and chemical water quality was conducted in the morning and evening. Average dissolved oxygen (DO) reaches 5.1 - 6.6 mg/L in the morning and 6.3 - 7.1 mg/L in the afternoon (Table 1). DO is determined by the balance of oxygen production and consumption in the waters. Oxygen is produced by autotrophic organisms through photosynthesis during the day. *A. pinnata* releases oxygen in the roots so that oxygen in the water will increase (Haberl and Langergraber, 2002). Whereas at night, oxygen is consumed by all organisms through respiration and for the decomposition of organic matter and produce carbon dioxide (CO_2). The concentration of CO_2 in the water will affect the pH value, the higher the level of CO_2, the lower the pH of the waters and vice versa.

Table 1. The concentration of dissolved oxygen (mg/L) based on the treatments.

Treatment	1st day		7th day		14th day		21st day	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
A	6.0 – 6.8	6.5 – 7.7	4.6 - 8.7	5.5 - 8.6	6.4 - 8.4	6.6 - 8.8	6.3 - 7.2	6.7 - 8.0
B	5.1 – 5.4	6.1 – 7.2	5.1 -7.6	6.1 - 8.6	5.7 - 7	6.4 - 8.5	6.9 - 6.8	6.5 - 8.1
C	4.7 – 5.4	6.1 – 7.2	3.4 - 6.5	5.4 - 8.2	5.1 - 7	6.7 - 7.6	5.7 - 7	6 - 7.4
D	4.7 – 5.6	6.4 – 8.2	4.2- 6.4	3.8 - 7	5.8 - 7	6.3 - 7.4	5.6 - 6.3	5.6 - 7
Averages	5.2	6.3	6.1	7.1	6.6	7.3	6.5	6.9

Table 2. Range of pH based on the treatments.

Treatment	1st day		7th day		14th day		21st day	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
A	5.3 – 5.9	5.4 – 6.0	5.5 - 6.4	5.3 - 6.5	5.4 - 6,6	6.0 – 6.1	6.2 - 6.1	5.6 - 6
B	5.7 – 5.9	5.8 – 6.0	5.4 6.9	5.8 - 6.4	5.1 - 6.3	5.2 - 6.5	5.4 - 6.2	5.8 - 6.5
C	5.6 – 5.8	5.6 – 5.9	5.5 - 6.2	5.6 - 6.3	4.8 - 6.3	4.8 - 6.5	5.4 - 6.2	5.6 - 6.4
D	5.7 – 5.8	5.6 – 5.9	5 - 6.3	5.6 - 6.4	5.1 - 6.4	5.2 - 6.4	5.8 - 6.2	5.8 - 6.4
Averages	5.7	5.8	5.9	6.0	6.0	6.2	6.2	6.3

The pH is a parameter associated with the concentration of CO_2 in water. The balance between photosynthesis and respiration also determinate by CO_2. Photosynthesis is a process that requires CO_2 so that it can increase the pH of the waters, while respiration produces CO_2 into the waters so that the pH of the waters decreases. The pH value in the study increases every week because A. pinnata is able to bind acids through the mechanism of decomposition of organic matter by associated microorganisms in the roots (Arimby, 2014). The process of decomposition of organic matter by microorganisms will produce OH- ions (Bwapwa et al., 2017). Increased pH can also occur by the photosynthesis (Gerloff-Elias et al., 2005). Intake of H+ ions in water for photosynthesis causes a decrease in H + ions in water so that the pH of the water increases. In this study, the pH values in all treatments ranged from 4.8 to 6.9 (Table 2). this value tends to be low, causing iron to tend to be in a dissolved form (Fe^{2+}) which is more easily absorbed by the plants (Gonzales and Guo, 2018). This condition causes Fe to be absorbed well by A. pinnata. The optimal pH for plants to absorb metals or Fe is 4.6 -7.4 (Colombo et al., 2014; Ahmadpour et al., 2015).

A. pinnata has a high-temperature tolerance of 5 - 35°C (Mentari et al., 2016). In this study, the temperature of each treatment was stable, ranging from 26.1 - 30.5°C (Table 3), thus allowing *A. pinnata* to live optimally. According to Mentari et al., (2016), optimal temperature for Azolla's growth is in the range 18-28°C. The increase of temperature can increase the diffusion of ions to the roots, so that it can accelerate the absorption of metal ions (Fritioff et al., 2005).

Table 3. Temperature range (°C) of ex-sand mining water by remediation treatment by *A. pinnata*.

Treatment	1st day		7th day		14th day		21st day	
	morning	Afternoon	morning	Afternoon	morning	Afternoon	morning	Afternoon
A	26.7 – 27.4	28.9 – 29.1	26.5 - 27.9	28 - 30.8	26.1 - 28.1	26.4 - 27.5	26.1 - 27.5	27.5 - 30
B	27.4 - 27.8	28.6 – 29.6	27 - 27.4	28 - 29.6	26.3 - 27.2	28 - 29	26 - 27.6	28 - 29
C	27.1 – 27.7	29 – 29.1	26.8 - 27.7	27.7 - 29.5	26.1 - 27.6	27.4 - 28.7	26.1 - 27.6	27.4 - 29.1
D	27 – 27.9	28.7 – 30.7	26.7 - 27.9	27.8 30.7	26.4 - 27.4	27.5 - 28.9	26.4 - 27.7	26.4 - 28.9
Averages	27.4	29.2	27.2	29.0	27.0	28.7	26.9	28.7

Concentration of Fe in the water

All of the phytoremediation treatments showed a significant decrease in Fe concentration. The highest reduction was shown by treatment D (98.104%). Furthermore, treatment C (93.474%), treatment B (93.319%) and lowest in treatment A (64.134%) (Table 4).

Table 4. Concentration (mg /L) and percentage of Fe decrease in water after treatment by *A. pinnata*

Day To	Treatment							
	A (0%)		B (25%)		C (50%)		D (75%)	
	Fe	%	Fe	%	Fe	%	Fe	%
0	0.987		0.943		0.702		1.002	
7	0.689	30.193	0.354	62.460	0.508	27.635	0.427	57.385
14	0.413	58.156	0.145	84.624	0.212	69.801	0.120	88.024
21	0.354	64.134	0.063	93.319	0.046	93.447	0.019	98.104

The results of this study indicate that the coverage area of *A. pinnata* has a significant effect ($P < 0.05$) on the decrease in Fe concentration in the sand mining water. Least Significance Different (LSD) tests showed that treatment A was significantly different from treatment B, C, and D. While the treatments for B, C, and D were not significantly different (Figure 1).

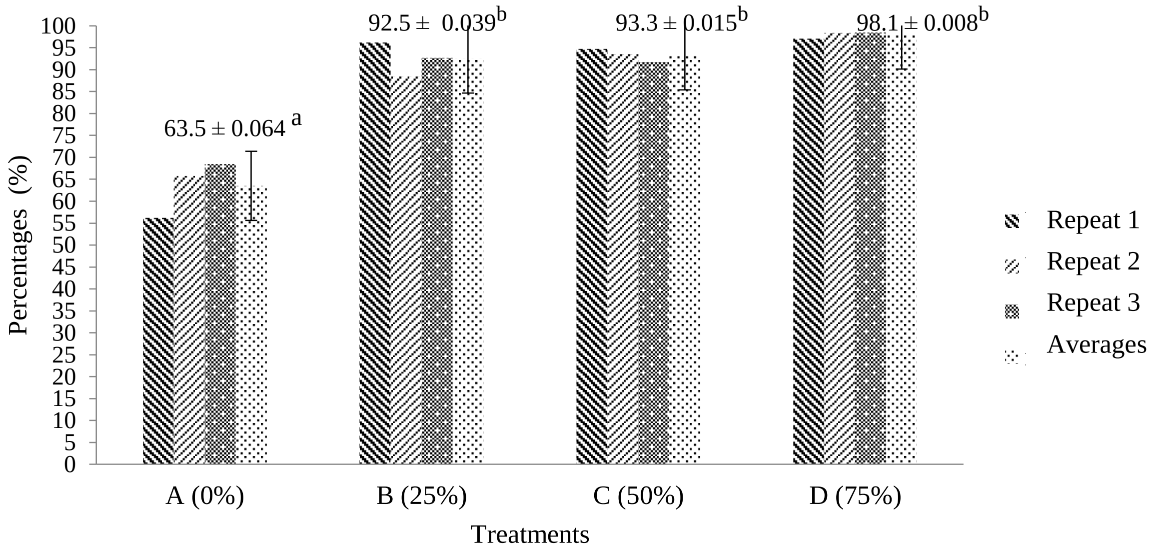

Figure 1. Graph of percentage of Fe concentration reduction by *A. pinnata*

Note: * The same letter notation shows results that are not significantly different from the 95 % confidence interval
* Different letter notation shows significantly different results with confidence interval 95 %

Concentration of Fe in Azolla pinnata plants

Fe in the water is absorbed by the epidermal cells of the root. Partly of Fe stored in the root and then put into the xylem through the simpas and apoplas to be transported from the root to the canopy. After xylem transport, heavy metals are transplanted and stored in leaf cells and redistributed again through phloems transport (Irawati, et al., 2017; Khatri et al., 2017). The lowest Fe and root concentrations of Fe occurred in treatment C because Fe concentrations in water were also low at 0.702 ppm (Table 4). Concentrations of Fe in the roots and leaves of each tretment are presented in Table 5, Figure 2 and Figure 3.

Table 5. Concentrations of Fe in roots and leaves of *A. pinnata*.

Treatments	roots (mg/kg)		leaves (mg/kg)	
	Day 0	Day 21	Day 0	Day 21
B	70.946	1210.10	76.089	1237.13
C	67.504	774.84	72.260	816.21
D	65.481	1060.16	70.125	1078.30

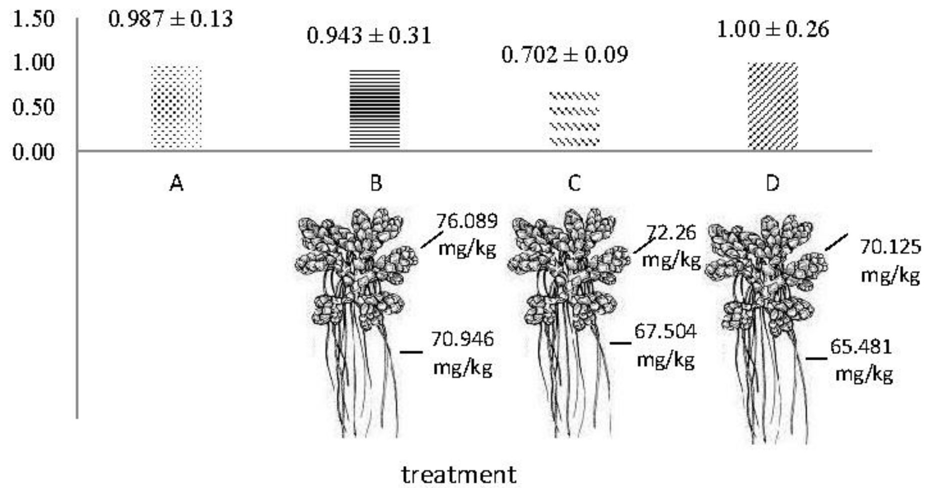

Figure 2. The concentration of Fe in water and plants before the experiment.

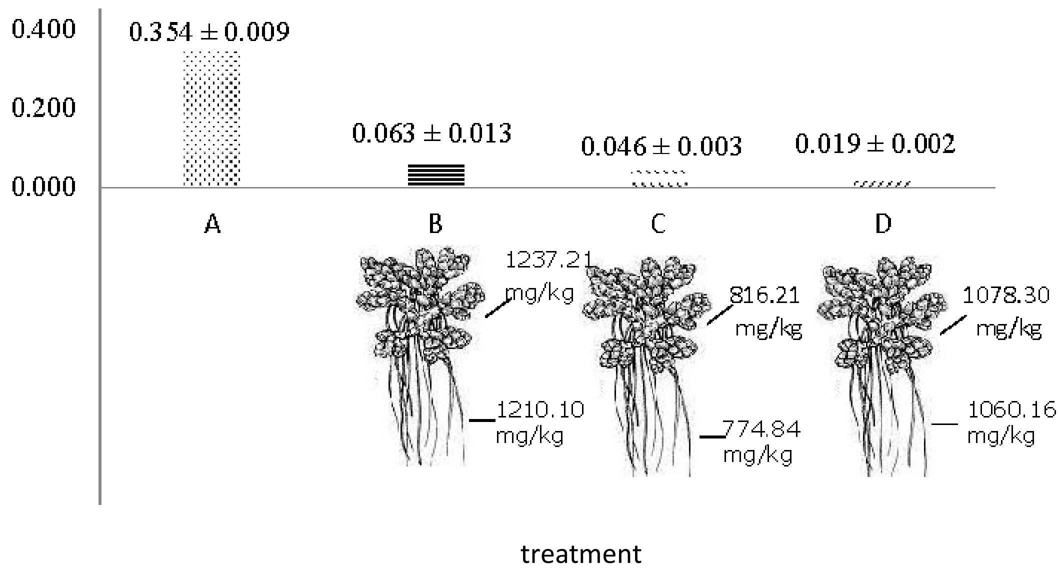

Figure 3. The concentration of Fe in water and plants after the experiment.

Bioconcentration factor (BCF) and translocation factor (TF) function to evaluate the feasibility of *A. pinnata* as a metal phytoremediation agent. In this study, the BCF value showed a high value reaching 1283.83 and the lowest being 1058.57 (Table 6). According to Testi et al., (2019) if the BCF value > 1000 then the plant has a high ability to absorb metals. Thus *A. pinnata* belongs to the accumulator plant against Fe. Accumulator plants are the ability of plants to absorb pollutants into their tissues (Sood et al., 2012; Thayaparan et al., 2013).

Table 6. Bioconcentration Factor and Fe Translocation Factors in *A. pinnata* roots.

Treatments	Bioconcentration	Translocation
B	1283.85	1.02
C	1104.46	1.05
D	1058.57	1.02

The value of Fe translocation from roots to leaves averaged 1.02. This value shows that Azolla pinnata has the ability of Metal Fe phytoextraction mechanism because

TF> 1 (Testi et al., 2019). Phyto-extraction is the process of absorbing heavy metals by plant roots which are then translated into the stems and leaves. Fe is absorbed by root epidermal cells. Some Fe is stored in the roots, then put into the xylem through a simplas and apoplasts to be transported from the roots to the canopy. After xylem transport, heavy metals are transplanted and stored in leaf cells and redistributed again through phloem transport (Irawati et al., 2017; Khatri et al., 2017).

DISCUSSION

The phytoremediation treatments of the water from ex-sand mining land showed a significant decrease in Fe concentration. This condition is thought to be because *A. pinnata* has rapid growth thereby increasing Fe absorption. Increased *A. pinnata* growth causes the amount of Azolla that can absorb Fe also increases properly. Although under specific conditions, differences in the number of plants can show the opposite response (Santoso et al., 2010; Mutmainah et al., 2015). This condition occurs because of the influence of bacteria and microalgae that can affect the availability of nutrient in the waters (Purwaningsih, 2009), which causes changes in the form of Fe in the waters (Syahputra, 2005).

The ability of *A. pinnata* to reduce metals in water has been conveyed by Shafi et al., (2015), that after ten days A. pinnata has accumulated Cu (0.90ppm), Pb (0.42ppm), Cr (0.27ppm), Cd (0.042ppm) and Zn (2.1ppm). Other studies have shown that *A. pinnata* can reduce the concentration of Pb and Cd in water by 70-94% (Soodet al., 2012). *A. pinnata* was also able to reduce Pb metal concentration by 83% during the four days of treatment (Thayaparan et al., 2013). The study by Noorjahan and Jamuna (2015) showed that *A. microphylla* after 96 hours of treatment was able to reduce n heavy metals such as Cr (Biountreated 84.74% and Biotreated 100%), Cu (Biountreated 87.21% and Biotreated 89.43%), Zn (Biountreated 99.74% and Biotreated 100%). 07% and Biotreated 100%). Therefore, it can be concluded that *A. pinnata* is a potential candidate to remove Fe in the ex sand mining water.

Fe in the waters have several forms, including Fe^{2+} and Fe^{3+} (Khatri et al., 2017; Runtti et al., 2018). Bacteria are microorganisms that help reduce Fe^{3+} to Fe^{2+} that can be absorbed by plants (Gonzalez and Lin Guo, 2018). This process occurs because organic materials that contain groups such as carboxylic acids, nitrogen, hydroxides, sulfates, phosphates that can form complex compounds with iron. Organic material at the beginning of its decomposition will increase the pH if it produces ammonia. However, ammonia which is converted to nitrate, will reduce pH. This decrease in pH will increase iron content because Fe^{3+} will be reduced to Fe^{2+} (Kathri et al., 2017; Gonzalez and Lin Guo, 2018).

The highest concentration of Fe in the roots and leaves was found in treatment B because *A. pinnata* cover area was lower than treatment C and D. It was possible for individuals of *A. pinnata* to absorb Fe more than treatments C and D. Concentrations of Fe accumulated in roots and leaves increased at the end of the study because the process of absorption of Fe by *A. pinnata* continued. High concentrations of Fe in plants cause a negative impact, which is a change in leaf colour *A. pinnata* turns yellowish. According to Deval et al., (2012), Fe concentrations can affect root length, leaf and biomass production. This condition can occur due to the inhibition of food hydrolysis and translocation, so that root growth becomes inhibited. In some cases, it can cause a decrease in the content of carbohydrates (Valavanidis et al., 2005). However, chlorophyll can be increased through an osmotic adjustment mechanism developed by experimental species. Similar to the results of this study, Sood et al., (2012) state that *A. pinnata* can reduce the concentration of heavy metals (Hg and Cd) with concentrations ranging between 310 and 740 mg/kg dry mass in Azolla's tissue. Thayaparan et al., (2013) state that the efficiency of lead (Pb) removal by plants depends on the duration of exposure. The maximum absorption of lead is 1383 mg/kg dry weight of *A. pinnata*.

CONCLUSION

The difference in the coverage area of *A. Pinnata* affects the percentage of Fe reduction in water taken from ex-sand mining land. The results of measurement of Fe content in roots and stems, as well as the bioconcentration factor values, indicate that the *A. pinnata* plant has a high ability to absorb Fe, so that *A. pinnata* can be classified as a strong accumulator plant for Fe. This study provides evidence that *A. pinnata* is a strong candidate as a phytoremediation agent for the reduction of Fe in water. The success of Fe reduction provides opportunities for the management and use of ex-sand mining sites. Furthermore, the land can be used for aquaculture. Aquaculture activities can provide benefits as a source of income and welfare for the community around the ex-sand mining sites in Pasir Sakti Regency, Lampung. Indonesia.

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