Effectiveness of Waterchestnut (*Eleocharis dulcis* (Burm.f.) Trin. ex Henschel) in Phytoremediation of Coal Mine Acid Drainage in Constructed Wetlands

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***Abstract*- Open-pit coal mining causes the formation of acid mine drainage (AMD) which has a low pH and high metal solubility. Efforts to absorb heavy metals and neutralize the pH of AMD can be done by phytoremediation. This research aims to monitor the effectiveness of Water chestnut (Eleocharis dulcis (Burm.f.) Trin. ex Henschel) in the accumulation of iron (Fe) and manganese (Mn) in AMD phytoremediation and to determine the mechanism. E. dulcis was taken from the constructed wetlands of wastewater treatment pond Air Laya 02 PTBA Tanjung Enim, South Sumatra, Indonesia with a convenience sampling method. Measurement of iron (Fe) and manganese (Mn) content using (AAS)-flame. The results showed that E. dulcis was able to improve the quality of effluent from inlet, wetland to outlet by increasing the pH from 5.6 to 7.7 and reducing Fe content from 7.89 ppm to 0.25 ppm, Mn from 1.72 ppm to 0.76 ppm, and sulfate (SO4) from 531.33 ppm to 405 ppm. The accumulation of Fe and Mn by the roots of E. dulcis were 890.00 ppm and 63.37 ppm with Bio-Concentration Factor (BCF) values of 18.56 and 1.77, respectively, indicating that E. dulcis has potential as a hyperaccumulator plant because the BCF value is more than 1. The mechanism accumulation of Fe and Mn metals by E. dulcis is phytostabilization because of the value of Translocation Factor (TF) less than 1. Phytoremediation by E. dulcis in constructed wetland system can manage AMD waste so that it fully fills the quality standards of wastewater from coal mining activities.**

***Index Terms*- Acid mine drainage, waterchestnut, Eleocharis dulcis (Burm.f.) Trin. ex Henschel, phytoremediation, constructed wetlands, Bio-concentrastion Factor, Translocation Factor.**

I. INTRODUCTION

Indonesia has many coal mining locations, and generally uses an open mining system or is called open pit mining. One of the impacts of open pit coal mining is the formation of acid mine drainage (AMD). The reaction explaining pyrite occurred in the mining area and formed AMD [1]. AMD can be in the form of leachate, seepage, or drainage and arises as a result of mining activities in the form of acid water [2]. AMD is characterized by extreme conditions with characteristics, pH can reach less than 3 [3, 4, 5] and sulfate solubility [6], and high heavy metals [7,8] and are toxic in the environment [9], which are harmful to human health as well as aquatic flora and fauna [10]. The character of AMD has a low pH and contains high Fe, Mn, and sulfate (SO4) metals so that Al, Cu, Zn, Cd, Pb, As also dissolve in these acidic conditions [11]. The heavy metal toxicity and AMD acidity have destroyed the habitat of many aquatic species such as shrimp and fish [12].

One of the efforts that can be done is through phytoremediation [3,13]. Phytoremediation is carried out in polluted areas with low, medium or high pollution levels for the purposes of recovery, detoxification, repair and reclamation [14]. Phytoremediation is classified as an alternative in situ technology that utilizes plants and their rhizosphere to reduce contaminants in water [15]. Aquatic phytoremediation has been shown to be able to remediate heavy metals in AMD and maintain aquatic biodiversity [16]. The success of phytoremediation depends on the plants used because these plants must have the ability to accumulate large amounts of heavy metals (hyperaccumulation) [17].

Phytoremediation uses hyperaccumulator plants that have the ability to grow in acidic environments. Hyperaccumulator plants are defined as plants that have the ability to accumulate large amounts of heavy metals and large translocation factors [18]. In order to survive, plants activate various specific mechanisms for metal detoxification in which heavy metals can be chelated, transported, sequestered or detoxified in the plant vacuoles [19].

Several types of plants are classified as hyperaccumulators such as Fimbry (*Fimbristylis globulosa* Retz. Kunth), Water Chestnaut (*Eleocharis dulcis* (Burm.f.) Trin.ex Henschel), Cat Tail (*Typha latifolia* L.), Water Hyacinth (*Eichornia crassipies* Mart. Solm.) and others. etc. To estimate the potential of plants to accumulate heavy metals can be known by calculating the value of the bioconcentration factor (BCF) and translocation factor (TF) [20]. The BCF is used to determine the potential for accumulation of heavy metals from soil to plants and TF is used to determine the translocation of heavy metals from roots to shoots and as a ratio between heavy metal concentrations in the shoots and heavy metal concentrations in roots [21].

This study aims to explain how effective the roots and shoots of *E.dulcis* are in accumulating heavy metals iron (Fe) and Manganese (Mn) and to find out the mechanism used by *E.dulcis* in AMD phytoremediation in Constructed Wetlands (CWs) from Air laya 02 wastewater treatment pond PTBA Tanjung Enim, South Sumatra, Indonesia.

II. MATERIAL AND METHODS

Materials and Tools, The materials needed are concentrated HNO_3, $HClO_4$, deionized water and sulfate reagentVer4 Reagent Powder Pillows. The tools used are Atomic Absorption Spectroscopy (AAS), Spectrophotometer uv-vis.

Sampling of AMD and plants, Sampling of AMD at the inlet, wetland and outlet of the Tanjung Enim PTBA Air Laya 02 CWs, Tanjung Enim, South Sumatra Roots and shoots of *E dulcis* were collected in the wetland of wastewater treatment pond at three sampling points with 3 repetitions.

Sediment Preparation, Sediment preparation procedure based on SNI 06-6992.7-2004 [22]. The sediment was cleaned and dried in an oven for 2 x 24 hours at 40^0C. Grind until smooth and taken as much as 3 g. Added 25 ml of distilled water and then homogenized. The sample was added 5 mL of concentrated HNO_3 and then heated with a hotplate at 105^0C - 120^0C until the volume became 10 ml. The sample was added 5 mL of concentrated HNO_3 and 3 ml of concentrated $HClO_4$. and reheat then cool and filter. The filtrate of the test sample was added with distilled water until the volume reached 100 ml, and the solution was analyzed using AAS.

Plant preparation, The root and shoots preparation procedure for *E. dulcis* follows SNI 06-6992.7-2004[22]. Samples were cleaned and dried in an oven for 2 x 24 hours at 40^0C. Grind until smooth and taken as much as 3 g. Added 25 ml of distilled water and then homogenized. The sample was added 5 ml of concentrated HNO_3 and then heated with a hotplate at 105^0C - 120^0C until the volume became 10 ml. The sample was added 5 ml of concentrated HNO_3 and 3 ml of concentrated $HClO_4$. Reheat until white smoke appears and the test sample solution becomes clear. Cool then filtered, and the filtrate of the test sample was added with distilled water until the volume reached 100 ml, the solution was ready to be analyzed using AAS.

pH value in Mine Acid Drainage, The measurement of the AMD pH value was based on SNI 06-6989.11-2004 [23]. The test preparation was carried out by calibrating the pH-meter according to the tool's work instructions.

Content of Iron (Fe) in Acid Mine Drainage, The AMD sample was analyzed according to SNI 06.6989.4 -2009 [24]. The 50 ml sample was shaken until homogeneous and 2.5 ml nitric acid was added. Then heat it in an electric heater until the solution becomes 25 ml. Added 25 ml of distilled water, put it into a 50 ml volumetric flask through filter paper and determined 50 ml with distilled water. The test sample is ready to be measured for its absorption with AAS.

Content of Manganese (Mn) in Acid Mine Drainage, The AMD sample was analyzed according to SNI 06-6989.5-2009 [25]. The 50 ml sample was shaken until homogeneous and 2.5 ml nitric acid was added. Then heat it in an electric heater until the solution becomes 25 ml. Added 25 ml of distilled water, put it into a 50 ml volumetric flask through filter paper and determined 50 ml with distilled water. The test sample is ready to be measured for its absorption with AAS.

Content of Sulfate ($SO4^{2-}$) in Acid Mine Drainage, Calculation of sulfate contents ($SO4^{2-)}$ based on UPTD Environmental Laboratory Work Instructions DLHP No. 15.44/IK-LL/2021 [26]. Sulfate content ($SO4^{2-}$) is determined by the difference between the Sample and the Blank.

Bioconcentration Factor (BCF), Calculation of BCF to find out how much the concentration of heavy metals in roots comes from the environment. According to MacFarlane *et al*. [27], BCF can be calculated using the formula (Eq. 1) :

$$\text{BCF} = \frac{Concentration\ of\ heavy\ metal\ in\ plant}{Concentration\ of\ heavy\ metal\ in\ sediment} \quad \text{........ (1)}$$

According to Baker [28], a plant in accumulating heavy metals can be divided into 3 parts, are: BCF > 1 = accumulator, BCF < 1 = Excluder, BCF = 1 = Indicator.

Translocation Factor (TF), Calculation of TF is used to determine the transfer of heavy metal accumulation from roots to shoots. According to MacFarlane *et al*. [27], TF can be used to determine the status of plants as phytoextraction (TF>1) and phytostabilization (TF<1). TF can be calculated using the Eq. 2:

$$\text{TF} = \frac{Concentration\ of heavy\ \ metal\ in\ shoots}{Concentration\ of heavy\ \ metal\ in\ roots} \quad \text{........ (2)}$$

Data analysis, Quantitative data in the form of measurements of AMD pH, Fe and Mn metal content in AMD, and Fe and Mn content in the roots and shoots of *E. dulcis* and BCF, TF values by means of analysis and standard deviation (sd).

III. RESULT AND DISCUSSION

Based on research conducted on the effectiveness of *E. dulcis* in the accumulation of heavy metals Fe and Mn in AMD phytoremediation, the following results were obtained:

Consentration of Fe and Mn in Roots and Shoot of *E. dulcis*

Heavy metal levels in the roots and shoots of *E. dulcis* are used as an effort to monitor the success of phytoremediation. The concentration values of Fe and Mn metal levels accumulated in the roots and shoots of *E. dulcis* were used in the measurement of Translocation Factor (TF) and can be seen in Table 1.

Table 1. Fe and Mn content in the roots and shoots of *E. dulcis* in Air Laya 02 CWs PT. BA Tanjung Enim, South Sumatra.

Organs	Fe (ppm)	Mn (ppm)
Roots	890 ± 314,75	63,37 ± 72,09
Shoots	50,12 ± 24,95	46,98 ± 19,73

Note ± : standard deviation

Based on the results of the study it was known that the concentration of Fe accumulated in the roots of *E. dulcis* in Air Laya-02 wetland was much higher than the metal content of Fe in the crown. These results indicate that the accumulation of Fe by *E. dulcis* plants was more in the roots. The high level of Fe in the roots is because the roots are in direct contact with AMD and the roots also absorb metals dissolved in water.

These results indicate that *E. dulcis* plants absorb Fe ions with high concentrations. The cell walls of the roots of *E. dulcis* contain many phenolic compounds, making this plant highly tolerant to Fe. The results also show that the organ that plays a role in the absorption of Fe levels is the part of the root. *E. dulcis* stores excess Fe in its body in the roots and then the top of the plant or shoots. The high accumulation of heavy metal Fe in roots indicates that *E. dulcis* is capable of absorbing toxic materials in the environment. The trend of metal absorption in *E. dulcis* showed that the roots accumulated a greater concentration of elements compared to the shoots, and indicated that the absorption was higher from sediment than from water. The high levels of Fe metal in sediments and the demand by plants causes the value of absorbed Fe levels to be higher than Mn.

E. dulcis is a hyper accumulator and biofilter plant capable of accumulating toxic elements and heavy metals such as Fe and Mn metals. The ability of *E. dulcis* to absorb contaminants is called rhyzofiltration, meaning the ability of roots to absorb pollutant materials, especially metals from water in the soil and accumulate them in the roots. *E. dulcis* roots absorb iron in the form of ferrous ion Fe^{2+}. The oxidative process of Fe in the roots of *E. dulcis* is by sending oxygen to the roots so that Fe^{2+} dissolves in the soil to become Fe^{3+} which can settle in the roots. *E. dulcis* contains chelate compounds in the roots and stems, so they are able to tolerate excess Fe concentrations that enter the tissues.

The concentration of Mn metal in the roots was higher than the shoots. The uptake of Mn by the roots is higher because the roots are in direct contact with the sediment. Absorption by *E. dulcis* roots of Mn occurs more easily when Mn is in the form of Mn^{2+} ions. Mn deposition will occur when the sediment pH ranges from 7 to 9. The ability of *E. dulcis* to absorb Mn is much smaller than that of Fe. This is due to an imbalance in the needs of plants for micronutrients.

Bio-concentration Factor (BCF) and Translocation Factor (TF)

The ability of *E. dulcis* to accumulate Fe and Mn can be calculated using BCF and TF. BCF to find out how much heavy metals in roots come from the environment. While TF is used to determine the transfer of metal accumulation from roots to shoots. BCF and TF values are presented in Table 2. below:

Table 2. BCF and TF of Fe and Mn of *E. dulcis* in CWs Air Laya 02 CWs PT. BA Tanjung Enim, South Sumatra

Metal	**BCF**		**TF**
	Roots	***Shoot***	
Fe	18.56	1.04	0.05
Mn	1.77	1.31	0.74

Note: BCF < 1 = Excluder, BCF = 1 = Indicator, BCF > 1 = Accumulator/hyperaccumulator.
TF < 1 = Phytostabilization, TF > 1 = Phytoextraction

Based on the results in table 2, the BCF in the roots and shoots of *E. dulcis* showed significantly different amounts of adsorbed metal. This is presumably because the roots are in direct contact with sediment and acid mine drainage which contain high levels of heavy metals. The ability of E. dulcis roots and shoots to accumulate heavy metal Fe is classified as a hyperaccumulator because BCF > 1. *E. dulcis* is classified as a hyperaccumulator plant, which means that *E. dulcis* can accumulate Fe and Mn metals at high concentrations in its plant tissues.

The table shows that the TF for Fe and Mn is TF < 1 which means that the plant is classified as phytostabilization. The way phytostabilization works is to use the ability of the roots to change environmental conditions. Pollutants in polluted soil will be transformed by *E. dulcis* into non-toxic compounds without absorbing these pollutants into the plant body.

The translocation of metals from roots to shoots is low because Fe and Mn are essential metals. In general, the translocation of metals from roots to leaves for essential metals (Fe and Mn) is very low compared to non-essential metals. The low TF value of essential metals indicates that *E. dulcis* uses both metals for metabolic and growth activities.

Parameters of Acid Mine Drainage

The parameters for observing AMD quality are guided by Decree of the Minister of Environment and Forestry of the Republic of Indonesia No. 5 in 2022 (KepMen LHK RI No.5-2022) [29] The AMD parameters in this study can be seen in Table 3.

Table 3. AMD Parameters in Air Laya 02 CWs PT. BA Tanjung Enim, South Sumatra

Parameters	**quality standards**	**Inlet**	**Wetland**	**Outlet**
pH	6-9	5.6	5.9	7.7
Fe (ppm)	7	7.89 ± 0.36	0.38 ± 0.04	0.25 ± 0.06
Mn (ppm)	4	1.72 ± 0.08	1.38 ± 0.01	0.76 ± 0.21
SO_4^{2-}(ppm)	-	531.33 ± 37.85	430.00 ± 34.64	405.00 ± 25.00

Note. ± standart deviation

Table 3 shows that AMD in inlet and wetland locations has a low pH and this pH does not meet quality standards. The inlet and wetland pH values are included in the acidic pH. This is caused by the high level of acidity at the inlet and in the wetlands. The pH value has increased from inlet to wetland due to phytoremediation using *E. dulcis* which has the potential to increase pH. The pH value also increases at the outlet after being treated with lime and phytoremediation so that it meets quality standards and can be released into public waters. Accumulation by *E. dulcis* appears to be in line with the increase in pH. An increase in the pH value has proven that remediation of AMD by utilizing *E. dulcis* can increase the pH of AMD.

Based on Table 3, it is known that there was a decrease in Fe metal content from the Inlet location to Wetland and it can be interpreted that there was an increase in Fe absorption by *E. dulcis*. The decrease in Fe content from 7.89 ppm at the inlet location to 0.38 ppm at the wetland location indicated the high ability of *E. dulcis* to accumulate Fe metal. In addition to other factors such as the treatment of giving lime which of course also affects the decrease in Fe levels.

The decrease in Fe metal content also occurred from the wetland location to the outlet where the metal content in the wetland decreased from 0.38 ppm to 0.25 ppm. The reduction in metal content from the wetland to the outlet is smaller than the reduction in metal content from the inlet to the wetland. It is suspected that absorption by *E. dulcis* began to decrease because the plants were experiencing saturation.

Table 3 also shows the metal content of Mn below the quality standard. The Mn content decreased from the inlet location to the wetland by 1.72 ppm to 1.38 ppm and there was a potential absorption of Mn metal content by *E. dulcis*. The ability of *E. dulcis* to accumulate Mn was much smaller when compared to its accumulation of Fe. This is due to differences in plant requirements for micronutrients. *E. dulcis* need for Mn metal as a micro nutrient is not as big as *E. dulcis* need for Fe metal. When compared with the accumulation of Fe, *E. dulcis* only absorbed a small amount of Mn metal.

Based on the results obtained in Table 3, it can be seen that the high levels of sulfate in AMD. Sulfate levels are not regulated by quality standards and are tested to determine the correlation of high levels of acidity in AMD. Sulfate levels at the outlet location decreased from the wetland location of 430.00 ppm to 405.00 ppm. These results are in accordance with the decrease in Fe levels where it is suspected that the *E. dulcis* plant has experienced saturation at that location. High sulfate levels are one of the causes of low AMD pH.

IV. CONCLUTION

Based on the results of the study, it was concluded that there was a decrease in Fe levels at the AMD inlet to the outlet from 7.89 ppm to 0.25 ppm and Mn metal at the AMD inlet from 1.72 ppm to 0.76 ppm at the outlet. *E. dulcis* has the potential as a hyperaccumulator plant in which the BCF value shows BCF > 1. The phytoremediation mechanism by *E. dulcis* is phytostabilization with a TF value < 1. *E. dulcis* is known to be effective in reducing Fe and Mn levels so that it can meet the quality standards of coal mining waste water in constructed wetlands

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