Eichornia crassipes Potency as Hyperaccumulator Macrophyte in Phytoremediation of Acid Mine Drainage Containing Zn

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Abstract. Under present investigation, Eichornia crassipes (water hyacinth) has been tested in knowing the applicability of this macrophyte as phytoremediator of Acid Mine Drainage (AMD). Atomic Absorption Spectrometry (AAS) is utilized in analysing the accumulation of important heavy metal zinc (Zn) within plant tissues parallel with adaptive responses due to physiological and biochemical matters during exposure of actual AMD and artificial AMD having different concentrations (10, 20, and 30 mg/L of Zn) and extreme pH (3.0). There is slow-but-steady significant in increase in pH along with no morphological symptoms in exposure of artificial AMD. Conversely, in 2-weeks exposure of actual AMD there is critical morphological symptoms due to its toxicity in exposure of multi-metals along with immediate increase in the first 3-days and slow decrease in 11-days after for pH value. The decreasing in Zn concentration for both actual and artificial AMD is occurred even in high level concentration. The final concentration of Zn did not meet the quality standard, so it needs to be a serial treatment with each treatment has 3-days in retention time. Overall this methodology is applicable for the removal of Zn in AMD that has single-metal or various-metals in any amounts that is negligible or under its lethal dosage.

1. Introduction
Mining industry considered as industrial sector that dominantly affecting world’s economic growth in positive way. According to [1], production rate of mining industry has an inclined to be increased as in 2000, total production reach 11.3 billion metric tons and experiencing improvement in 2016 that total production reaches 16.7 billion metric tons. The main contributors of global total production for mining industry is Asia continental countries for 58.2% among other countries that 60% of them is developing countries [1]. Indonesia is one of the contributors as this country has potential geological resources that according to Ministry of Energy, Resources, and Minerals, Indonesia still have abundant of coal for 29.9 billion tonnes, nickel for 3.2 billion tonnes, bauxite for 1.3 billion tonnes, and tin for 1.9 billion tonnes [2].

On the other side, this industry plays a deadly role for environment especially aquatic life as it generates acid mine drainage (AMD) as its by-product that has a value of pH as low as it can leach heavy metals from soil or bedrock thus it has high concentration of heavy metals. Heavy metals are considered a dangerous threat to the environment due to three key criteria, i.e. persistence, bioaccumulation and toxicity. Heavy metals are non-degradable chemical residues which possess long
persistence times in the environment and gradually enter food chains and accumulate within higher trophic levels, endangering both animal and human life [3].

Heavy metals from aquatic environments can be removed by a variety of techniques which include chemical, physical and biological techniques such as precipitation, ion exchange, chemical reduction and oxidation, membrane filtration, solvent extraction, reverse osmosis and activated carbon adsorption. However, the applications of these advanced remediation strategies are limited especially in developing countries as they are not economically feasible and require high sophisticated equipment which can be expensive to acquire. As an example, the application of affixing limestone to AMD at Argo mine in Colorado in order to increase the pH expensing high planning cost for 6 million dollars and high operational cost for 1 million dollars per year [4]. Other than that, generation of secondary wastes, metal specificity and suitability only in a narrow range of concentrations, are the other major limitations of the previous methods [3].

One of alternative option in treating heavy-metals-contaminated water that is included to low-cost technology is phytoremediation which involved hyperaccumulator plants, for instance *Eichornia crassipes*, in eliminating certain heavy metals contaminant by accumulating, translocating, and concentrating them in significant amounts within plant tissues [3]. Nevertheless, certain plants known as hyper accumulators are capable of absorbing heavy metals which have no importance for plant metabolic processes but not all of them could survive the extreme condition that provided by AMD in the form of low pH and high concentration of heavy metals simultaneously [5].

In this study, the phytoremediation potential of *E. crassipes* was assessed in an ex-situ tank-based experimental system, in knowing its ability to accumulate zinc (Zn) as this heavy metal is likely found at high concentration in AMD along with its adaptive responses to the AMD extreme condition. The data generated in this study showed that *E. crassipes* is a good phytoremediation candidate for the sequestration of Zn and able to increase the pH until the value reach where it is ideal for this macrophyte to live.

2. Methodology

2.1. Plants acquisition and acclimatization

*E. crassipes* was obtained from water hyacinth cultivations in one of aquaculture store in Bogor, West Java. Healthy mature plants were selected for the experiment and rinsed with tap water in order to remove adhering mud particles and epiphytes. In order to adapt to the experimental conditions and to obtain substantial biomass, those plants were grown in plastic containers containing tap water without any additional nutrient for 7 days in the greenhouse of Environmental Engineering Laboratory, Universitas Indonesia.

2.2. Preparation of treatment/experiment series

One plant and two litres of certain contaminated water placed in glass container which has 16-cm in diameter and 24-cm in height. There would no addition of any nutrition for *E. crassipes* along the experiment periods. There will be two experimental set that each of them has different way to obtain the contaminated water and so does the objectives.

2.2.1. Experiment 1. The objective of this experiment was to determine the capability of *E. crassipes* in accumulating Zn in unfavourable growth condition. Zn solution (prepared using ZnSO$_4$.7H$_2$O) was added to obtain concentration of 10, 20, and 30 mg/L. Water pH was set to be ±3.0 to represent AMD extreme condition in artificial way. The study was conducted for 14-days, sampling on 0$^{th}$, 1$^{st}$, 2$^{nd}$, 3$^{rd}$, 4$^{th}$, 5$^{th}$, 6$^{th}$, 7$^{th}$, and 14$^{th}$ day for water samples and on 0$^{th}$, 7$^{th}$, and 14$^{th}$ day for plant samples. Control (in the absence of Zn and neutral pH) also performed in this experimental set.

2.2.2. Experiment 2. The objective of this experiment was to determine the influence of the interactive/competitive effects of other heavy metals that found in AMD on Zn removal capacities by
E. crassipes and knowing the applicability of this macrophyte in treating actual AMD. To model an actual condition in mining site, effluent sample of AMD in gold mine industry named J Resources Bolaang Mongondow is taken from the mining site. These AMD characteristics could be seen in Table 1 based on laboratory assessment. The study was conducted for 14-days, sampling on 0th, 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, and 14th day for water samples and on 0th, 7th, and 14th day for plant samples. Control (in the absence of any heavy metals and neutral pH) also performed in this experimental set.

### Table 1. AMD of gold mining site characteristics.

| Parameters | Value       |
|------------|-------------|
| Zn         | 7790 mg mg/L|
| Mn         | 13800 mg/L  |
| Fe         | 1000 mg/L   |
| Cu         | 400 mg/L    |
| pH         | 2.4         |

2.3. Atomic absorption spectrometry analysis
Water samples were filtered with Whatmann No.1 filter papers and analysed by Atomic Absorption Spectrophotometer (AAS, Perkin Elmer-USA Analyst 800) for Zn using SNI 06-6989.43:2005 as procedural standard. Dried plant biomass digested by wet digestion method according to SNI 6989.7:2009, were also analysed by AAS (Shimadzu AA6300) to determine the metal concentrations in E. crassipes tissues.

2.4. Measurement of fresh and dried biomass
On the 0th, 7th, and 14th day of the experiment, E. crassipes plants in each experimental set were harvested, rinsed with distilled water to remove any ions adhering to plant’s surface and dried out by blotting on filter papers separately. Then the fresh biomass was weighed, dried for 48 hours in an oven at 80°C and the dry weights subsequently measured.

2.5. Calculations
The following parameters were calculated using the values of the data obtained from the experiments.

2.5.1. Removal Efficiency. The removal percentage of metal ions by E. crassipes was determined by using the initial metal concentrations of the treatment and the final concentrations at the end of the experiment [6].

\[
\text{\%Removal} = \left( \frac{\text{Initial Concentration} - \text{File Concentration}}{\text{Initial Concentration}} \right) \times 100\
\]

2.5.2. Metal Uptake Capacity. The accumulation of metal ions in E. crassipes tissues was calculated by using the dried weights. The metal concentrations of digested biomass were calculated as follows [3].

\[
\text{Metal Uptake (mg/kg dw)} = \frac{\text{Metal Concentration of Dried Biomass} \times \text{Total Diluted Volume}}{\text{Dry Weight}}
\]

3. Results and Discussion

3.1. Phytoremediation by E. crassipes to artificial and actual AMD
In the first three-days of observation, removal efficiencies of both actual and artificial AMD experimental set have represented 98-99% of total removal that is executed by E. crassipes along experiment periods. The same results have been found in the former studies which indicates removal
efficiencies of Cu, Cd, Pb, and Zn have been reach the optimum value at the range of 48-72 hours in retention time by *E. crassipes* [7] [8] [9]. Removal of Zn is also detected in all experimental sets, yet the value is counted to low as the optimum of removal efficiency still didn’t make AMD comply to Minister of Environment Decree No. 202 Year 2004 Regarding Wastewater Standard for Gold and Copper Mine Activities and/or Businesses as the standard for Zn parameter is 5 mg/L. From recent study, variance in Zn removal efficiencies are obtained for each of AMD experimental sets. For actual and artificial AMD experimental sets in loading 10, 20, and 30 mg/L are having data distribution in which 50% of data obtained due to *E. crassipes* removal efficiencies have a higher value than 17.5%, 26.2%, 12.1%, and 14.2%, respectively. The reason in decreasing Zn removal ability of *E. crassipes* is because of acidic condition provided by both artificial and actual AMD. Metal movement into the plant tissue can be inhibited by hydrogen ions around the roots at a low pH, since they compete with the metal ions for pathway sites on the root surface. For instance, Mn uptake by *Phragmites australis* was lower at a pH of 3.5 than at 6.0 [10].

### Table 2. Range of removal efficiency and values of metal uptake by *E. crassipes* in treating AMD samples

| Parameter       | Unit | 10 mg/L | 20 mg/L | 30 mg/L | 7790 mg/L |
|-----------------|------|---------|---------|---------|-----------|
| Removal Efficiency | %    | 0-19.4  | 7.1-27.8| 8.3-18.2| 0-21.6    |
| Zn Uptake mg/kg dw |      | 1581.16 | 3165.31 | 3967.92 | 1190.02   |

*a* Artificial AMD  
*b* Actual AMD

Even though low removal efficiencies were observed in all experimental set, *E. crassipes* could accumulated considerable amount of heavy metals (Zn) within its tissues at the range of 1000-4000 mg/kg dw. Likewise, Mandakini et. al. found that *E. crassipes* could accumulate Cr, Ni, Pb, and Cd at the range of 1000-4500 mg/kg dw within its tissues [3]. Thus, *E. crassipes* could be an ideal hyperaccumulator plant in phytoremediation if the treatment is configured to a serial treatment because of high potency in uptake ability though it is exposed by an extreme growth condition. From Table 2, there is an anomaly for the metal uptake in actual AMD experimental set that it has lower value than in artificial one as even though Zn is existed in higher concentration than artificial one. It happened because of the presence in various heavy metals in actual AMD that triggered a cationic competition between heavy metals. The cationic competition between heavy metals and other nutrients for pathways into the root tissues is an important factor that affects the uptake and removal of heavy metals in AMD. Competition for sites of uptake is often associated with similarities in chemical properties such as ionic size, and the microscopic size of the aperture in the root surface through which these elements pass during the process of the uptake [11]. Several studies on macrophytes have shown the interaction of heavy metals and their competition for the site of uptake by plants. The uptake of cadmium was inhibited by the presence of Cu, Hg and Pb in a solution with *E. crassipes* [12]. The uptake of arsenic (As) was inhibited by the presence of phosphate in a solution with *Spirodela polyrhiza* since as uses the same channel of uptake as the phosphates [13], [14] found that U could enhance the uptake of Ca while inhibiting the uptake of magnesium by the roots of *Azolla filiculoides* exposed to a mixture of 10 ppm of CuSO$_4$, Cd(NO$_3$)$_2$, or UO$_2$(NO$_3$)$_2$ solution [14]. Similarly, Uranyl ions were found to compete for binding sites in the uptake of both Ca and Mg by *Cladonia rangiferina* [15].

3.2. Adaptive Responses by *E. crassipes* to artificial and actual AMD

There is fluctuation in both artificial and actual AMD that have the same tendencies to maintain the pH of medium to a higher value (more alkaline) as the final pH for artificial AMD in loading 10, 20, and 30 mg/L Zn respectively reach value of 6.0, 6.4, and 7.0 from the initial value is ±3.0 and for actual one reach value of 5.1 from the initial value is 2.4. According to data obtained along the study, variance in pH value for
actual and artificial AMD experimental sets in loading 10, 20, and 30 mg/L are having data distribution in which 50% of data-obtained due to pH fluctuation have a higher value than 4.7, 4.4, 4.5, and 4.1, respectively. This phenomenon considers as an adaptive response from *E. crassipes* in facing acidic growth condition that provided by both artificial and actual AMD thus bioavailability of heavy metals is facing an increase that potentially harmful to macrophyte than an extreme-alkaline pH. Bioavailability of heavy metals is dependent to pH value of medium that is decreasing in higher pH value so that when accumulation of heavy metals potentially interferes metabolism activities of macrophyte as an adaptive response, macrophyte could increase pH value surrounds root zone to suppress heavy metal bioavailability [16]. The alteration of pH value is occurred by excretion of buffer agent (OH\(^{-}\), CO\(_{3}\)^{2-}) from root zone until the pH of medium is favourable enough for macrophyte [17]. This adaptive response is capable to increase pH value so that the treated-industrial-wastewater comply to Minister of Environment Decree No. 202 Year 2004 Regarding Wastewater Standard for Gold and Copper Mine Activities and/or Businesses since all Zn loading variations in AMD artificial experimental sets have meet its quality standard (for pH parameter is 6.0-9.0) while actual AMD experimental set almost fulfil the standard.

**Figure 1.** pH fluctuation of actual AMD when it was treated by *E. crassipes*

**Figure 2.** pH fluctuation of artificial AMD when it was treated by *E. crassipes*

**Figure 3.** Variance of pH value in artificial AMD experimental set for 10 mg/L, 20 mg/L, 30 mg/L and actual AMD experimental set
Along the experiment periods, toxicity symptoms are more obvious from day to day and the critical one achieve at the end of the periods for actual AMD experimental set but for artificial one, the critical point is not to be found along the periods (see figure 3). Actual AMD provide more extreme growth medium by the presence of various heavy metals in high concentration and acidic pH thus *E. crassipes* could not survive and experiencing more severe toxicity symptoms. This is because excess heavy metals disrupt photosynthetic and metabolic processes through the inhibition of electron transport at the redox sites in the photosystem I and II. This generates reactive oxyradicals, leading to “oxidative stress”, that react and decompose membrane lipid peroxides [18]. Symptoms of heavy metal phytotoxicity in most aquatic plants are more conspicuous in the aerial plant tissues, specifically the plant leaves. From all toxicity symptoms in macrophytes, chlorosis, necrosis, and inhibited-growth are likely to be found that its severe level depend on characteristics and concentration of involved heavy metals [19]. While artificial AMD provide an acidic growth condition but in the absence of various heavy metals (single heavy metal) and in lower concentration so that *E. crassipes* could adapt the growth condition well. The adaptive response of heavy metals toxicity in many macrophytes (*E. crassipes* is included) is that its root have ‘iron-plaques’ as a thin-root coating layer of iron (oxyhydro-) oxides, which act as a barrier to some metal uptake by roots, and appear to be a characteristic adaptation of plants used to avoid metal phytotoxicity by adsorb and hinder the uptake of some metals [10] so that *E. crassipes* could continue to grow and proliferated in extreme growth condition that is provided by artificial AMD.

![Figure 4](image)

*Figure 4.* Obvious morphological symptoms captured at the end of experiment periods in artificial AMD experimental set for 10 mg/L (left), 20 mg/L (centre), and actual AMD experimental set (right).

4. **Conclusion**
The present study proved *E. crassipes* as a potential accumulator of Zn in treating AMD. This macrophyte has accumulated Zn up to 3967.92 mg/kg dw in unfavorable growth condition that is provided by AMD after 14-days of exposure and reach optimum removal efficiencies in 3-days of exposure though the water quality of AMD still did not meet the standard. So, this treatment needs to be managed as a serial treatment with each has 72-hours in retention time and *E. crassipes* should be harvested after 14-days of exposure. Survival ability of *E. crassipes* is impressive as this macrophyte could adapt with the acidic condition and still do its role to accumulated heavy metals. Moreover, *E. crassipes* is still found to have proliferation after 14-days of artificial AMD exposure and continue growing whereas *E. crassipes* reaches its critical condition in adequately long time which is after 14-days of actual AMD exposure. Based on accumulation capability and tolerances towards toxicity, *E. crassipes* can be recommended for the removal of Zn from acid mine drainage.

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