Phytotoxicity Study of Native Plants in ex-mining lake water treatment

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Abstract. The use of phytoremediation in removing and degrade the contaminants gives great interest for researchers to explore further. This green technology is relatively eco-friendly and cost-effective. Before a full-scale system can be installed to ensure that the remedy is effective by selected plants using phytoremediation process, identification of these plants and its ability to resist toxicity of contaminants is crucial. In this study, the ability of native plants in Malaysia namely L. articulata, E. ochrostacs and E. dulcis to survive when exposed to acid mine drainage water (AMD) are investigated. This was done with free flow systems or free surface (FSF) for 30 days with water from ex mining lake, Tasik Puteri, Bukit Besi, Malaysia. The physical growth of those three plants such as height and withered leaves is monitored every seven day as indication of their survivability. Ferum (Fe) analysis is also carried out on the lower and upper part of the above three plants using Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). Results of the Fe analysis showed that E.ochrostacs has the highest accumulation ability compared to E.dulcis and L. articulata with a maximum accumulation of 123,584 mg Fe/kg at lower part and 55,151 mg Fe/kg at the upper part. Furthermore, E.ochrostacs was also able to tolerate and survive in the water of Tasik Puteri up to 80% survival rate during phytotoxicity study.

1. Introduction
Metal contaminations in ex-mining area are one of the most world environmental issues in which there are many countries all around the world are suffering from. In other words, ex-mining sites are known as one of the most sources of metal pollutant [1, 2]. Due to heavy metal wide sources, toxicity, accumulative behavior and lack of biodegradable properties, heavy metal are considered dangerous for the aquatic environment [3-5]. The type and level of ligands on which the metal could absorb, the water pH, the redox environment of the system and the oxidation state of the mineral components are among the factors that predominately control the solubility of the trace metals in surface waters [6]. When pyritic rocks exposed to air and water, it can produce acid mine drainage (AMD) which is characterized by pH as low as 2 and contain high concentration of metals that is toxic to aquatic organism [7].
Results reported from in situ analysis studies for Tasik Puteri, Bukit Besi shows that the pH is around 3 – 4\[8\]. According to Department of Environment (DOE) Water Quality Index Classification, the water quality of the lake is classified as class IV that is not suitable for recreational activities but more to irrigation. Therefore, treatment to the lake water is crucial for improving the water quality to achieve permissible Interim National Water Quality Standard (INWQS).

The main factor that possibly contributes to the acidic condition of the lake water is acid mine drainage (AMD). AMD are strong acidic streams rich in dissolved ferrous (Fe\(^{2+}\)) and non–ferrous metal sulfates occurring in mine working either operating or abandoned mining sites\[9\]. AMD is formed from the oxidation of sulphide bearing mineral by the addition of water and oxygen. Then, lead to the formation of an acidic drainage \[10\]. For Tasik Puteri case, AMD is produced because of the oxidation of pyrite (FeS\(_2\)) which is identified as one of the main iron ore in Bukit Besi mining operation.

Few efforts have been made in many conservative technologies (e.g. activated carbon adsorption, reverse-osmosis, nanofiltration, ion exchange, electrodialysis, chemical precipitation and disinfection) as treatment methods\[9, 11\]. However, those methods are generally expensive, labour and energy-intensive, metal specific and can generate secondary waste or sludge. Thus, an alternative method like phytoremediation should be highlighted since it is low cost, more secure and less destructive \[11\]. Phytoremediation is defined as the technologies that use selected living plants to clean up, remove, incapacitate or detoxify soil, air, water and sludge, contaminated with hazardous contaminants \[12\]. Furthermore, phytoremediation is an alternative solution for heavy metal remediation process since it is a cost-effective, long term applicability, environment- and eco-friendly ‘green’ technology \[13\]. In this study, three different submerged aquatic plants origin from the riverbank of Tasik Puteri, Bukit Besi are used. The plants are \textit{E. dulcis} (“Purun Tikus”), \textit{E. ochrostacys} (“Purun”) and \textit{L. articulata} (“Pu run Danau”) as shown in Figure 1.

Based on authors’ prior knowledge, limited study has been conducted using \textit{E. Dulcis} (“Purun Tikus”), \textit{E. Ochrostacys} (“Purun”) and \textit{L. Articulata} (“Purun Danau”) to treat contaminated ex mining lake water caused by AMD. Thus, these three native plants will be used in phytoxicity study to identify their survivability.

![Figure 1](image_url)

**Figure 1.** (a) \textit{E.dulcis} (“Purun Tikus”) (b) \textit{E.ochrostacys} (“Purun”) (c) \textit{L. articulata} (“Purun Danau”)

## 2. Materials and methods

### 2.1. Plant propagation
\textit{E. ochrostacys}, \textit{E. dulcis} dan \textit{L. articulata} are submerged aquatic plant. The reproduction of these three aquatic plants is through rhizomes. They have been collected from the riverbank of Tasik Puteri, Bukit Besi. The original habitat of the plants is in the same area around the mining lake. Therefore, it assumed that the growth stages of those plants were same. The plants then replanted in containers measuring 58 cm x 39 cm x 29.5 cm (l x h x w) containing in 3: 2: 1 soil (surface soil: organic...
material: sand). Since the plants are all aquatic plants, hence throughout the propagation period, the media of the garden soil is watered and the height of water is set on the sub surface so that plants can grow well.

2.2. Phytotoxicity study

Study of phytotoxicity is done for 28 days by using plastic bucket that act as batch reactor with a volume of 3L, a diameter of 27 cm and bucket depth of 27 cm. A total of 1 kg of sand is used and 3 L of water from Tasik Puteri is poured in each bucket and one bucket filled with tap water as control. Throughout the study period, no nutrient addition is done only a litre of water is added to each bucket every seven days to cover the loss of water by evaporation. Observation on the height and physical changes of the plant is recorded for every 7 days. This phytotoxicity study is done on March 2019.

3. Results and discussions

3.1. Plants propagation

Three different plants species are collected at the river bank of Tasik Puteri, Bukit Besi, namely E. dulcis (“Purun Tikus”), E. ochrostacys (“Purun”) and L. Articulata (“Purun Danau”). Each aquatic plant used for phytoremediation media taken from locations around the mine/original habitat shown in Figure 2. Meanwhile, Figure 3 shows the example of plant propagation.

Figure 2. Origin plant grown at Tasik Puteri

Figure 3. (a) Nursery soil blend (b) Planted parent plant (c) Planted plant aged 1 month

Height of the plant was recorded. Increment in height of the plant can be used to examine the survivability [14]. The physical plant growth is observed every seven days throughout the sampling days to examine the survivability in the new environment. From the graph in Figure 4, all the studied plants are survived in water of Tasik Puteri. However only E. ochrostacys show the highest increment in height indicate the highest of survivability.
3.2. Phytotoxicity study

Phytotoxicity study was conducted to determine the ability of studied plants to survive in the water of Tasik Puteri. 10 healthy plants of 42 days old were planted in each pail. After 28 days of exposure, the percentage of withered plants in each pail was determined relative to the total number of plants in the pail using the following equation (1) [15].

\[
\text{Percentage of withered plant} = \frac{\text{no of withered plant}}{\text{total plant}} \times 100
\]

The survival rate for *E. dulcis*, *E. ochrostacys* and *L. articulata* were 50%, 80% and 70%, respectively. It was observed that *E. ochrostacys* could survive longer than the other two species plants. Graph in Figure 5 showed the percentage of withered plant for each native plant.

3.3. Tasik Puteri water treatment

![Figure 4. Plant growth](image)

![Figure 5. Percentage of withered plant](image)
It is proposed that root absorbs the presence of contaminants in water for the studied plants (*E. dulcis, E. ochrostacys* and *L. articulata*). The mechanism of heavy metal removal occurs when an active adsorption process takes place. The existence of carboxyl groups at the root system also encourages a remarkable cation exchange through the cell membrane[16]. Figure 6 show results of heavy metal concentration in the root and stem of the studied plants. The level of iron uptake in lower part is the highest for *E. ochrostacys*. The finding correlated with the results reported by previous researchers [17, 18] that *Eleocharis*, which belongs to Cyperaceae Family, have a high adsorption capacity of toxic metals through the roots. Same pattern for iron uptake result as shown in Figure 6 for the upper part/stem of studied plants indicated that *E. ochrostacys* and *E. dulcis* become the best-studied plants for further phytoremediation study.

![Figure 6. Fe uptake of studied plants](image)

### 4. Conclusion

Based on heavy metal analysis and phytotoxicity test, it can be concluded that the most suitable plant to be used for phytoremediation is *E. ochrostacys* with the highest accumulation ability of Fe compared to *E. dulcis* and *L. articulata*. *E. ochrostacys* accumulate 123,584 mg Fe/kg at lower part and 55,151 mg Fe/kg at the upper part. Other than that, in phytotoxicity test *E. ochrostacys* was also able to tolerate and survive in the water of Tasi Puteri up to 80% survival rate based on percentage of withered plant and the highest increment based on physical growth. For the future planning, an integrated technique involves phytoremediation and adsorption process, namely as phyto-adsorption is proposed to enhance the phytoremediation process. It can be positively an innovative technique.

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