Relative Treatment Efficiency Index of *Eichhornia crassipes* in Removing Cd, Pb and Ni from Wastewater

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**Abstract.** Relative treatment efficiency index (RTEI) is used to identify the efficiency of the treatment by the plant itself. The higher the RTEI indicates the more efficient the treatment by the plant studied. In this research, *Eichhornia crassipes* were used to evaluate its RTEI value in removing heavy metals cadmium (Cd), nickel (Ni) and lead (Pb) from wastewater. *E. crassipes* were grown in Faculty of Science & Natural Resources’s (FSNR) lake water and spiked with 1 mg/L, 3 mg/L and 5 mg/L of Cd, Ni and Pb respectively. The experiment were conducted within 14 days period to evaluate its potential in phytoremediation of heavy metals from the wastewater. The results showed that the removal of Cd, Ni and Pb by *E. crassipes* were highest at 1 mg/L respectively. It was observed that *E. crassipes* were effective in removing Pb compared to Cd and Ni. Antagonistic effects between heavy metals were found in affecting the removal efficiency of each other except for metal Pb. *E. crassipes* were able to achieve highest RTEI = 0.91 with 98.2% removal of Pb followed by RTEI = 0.52 with 50.2% removal of Cd and RTEI = 0.62 with 44.7% removal of Ni. After 14 days treatment, *E. crassipes* show significant toxic effects on plant roots and leaves as the concentration of heavy metal increased. The accumulation of heavy metals in plant tissues were shown in ranged from 7.48 mg/kg to 2864.12 mg/kg (Cd), 46.18 mg/kg to 3628.21 mg/kg (Ni) and 2.69 mg/kg to 6012.68 mg/kg (Pb). The results also revealed that the accumulation of heavy metals was higher in the roots than stalks and leaves. It was found that bionconcentration factor (BCF) of Pb, Cd and Ni in root at 1 mg/L were exceed 1000. Study on translocation factor (TF) of all metals were recorded low in ranged of 0.01-0.79 respectively. This indicates that the phytoremediation mechanism uptakes of *E. crassipes* is rhizofiltration. This study give a better understanding on the potential of *E. crassipes* in removing heavy metals from wastewater especially industrial wastewater.

1. **Introduction**

Water pollution with heavy metal is non-biodegradable as they can exist in diverse oxidation states that can last long in the environment [1]. Heavy metals are mainly contributed by industrial activities such as electroplating, metal ore mining and textile manufacturing that release industrial wastewater with heavy metals [2-4]. The pollution of heavy metals in the environment is a major cause of concern for humans. The presence of heavy metals in living things can lead to the manifestation of deleterious effects such as kidney damage due to exposure to cadmium, neurological damage via exposure to mercury, and risks of getting skin cancer from consuming food that contains arsenic. One way to tackle this issue is via the process of phytoremediation. Phytoremediation can be defined as a low cost, energy-friendly, as well as aesthetic method of using green plants in order to restore, detoxify, and reclaim places that are polluted with varying levels of environmental pollutants [5]. Phytoremediation is currently being studied as it requires low cost, simple in terms of operation and maintenance, and at the same time, is efficient...
in the removal of various types of pollutants such as organic matter, nutrients, and heavy metals. There are many different types of plants that removes pollutants from the environment through differing pathways and mechanisms, which are phytoextraction, phytostabilisation, phytovolatilization, phytotransformation, and rhizofiltration [6]. Previous research showed that *E. crassipes* have high tolerance with heavy metals as well as they can accumulate those heavy metals through their roots and translocate then into shoots [7]. According to Fazal, et al. [7], treatment of constructed wetland with *E. crassipes* is more efficient in removing Pb – 1.19 mg/L with removal efficiency (83.4%) as compared to control. In this study, *E. crassipes* is chosen to remove heavy metals from wastewater. The special characterization of this plant with rapid growth rate, large root systems and ability to absorb a large range of metals make it suitable in phytoremediation of heavy metal. The main purpose of this study is to evaluate the potential of *E. crassipes* in removing heavy metal Cd, Pb and Ni (1 mg/L, 3 mg/L and 5 mg/L) from wastewater. The efficiency of this plant will be determine by relative treatment efficiency index (RTEI) which is used to compare the experiment with plant effect and unplanted control treatment. The toxicity symptoms of *E. crassipes* exposed to heavy metals were also recorded in this study.

2. Methods

2.1 Sampling of *E. crassipes*

Mature *E. crassipes* plants of uniform size were collected and rinsed with running tap water to remove dirt, debris as well as organic matter and eventually with deionized water. The washed plant specimens were acclimated in glass tank filled with FSNR’s lake water about two weeks at natural ventilation and temperature at 30 ±2°C. Plants were washed when aphids and bugs are present. Figure 1 shows the location where plant specimens were collected.

![Sampling area of *E. crassipes*](image)

**Figure 1.** Sampling area of *E. crassipes* located in Jalan Sumiling Mogon, Papar District with coordinate 115° 56’48.72” E, 5° 44’10.2” N.
2.2 Experimental Set up
The experiment was conducted by growing *E. crassipes* in experiment tanks (45.5 cm x 30 cm x 30.5 cm) with FSNR’s lake water. Simulated wastewater was created by adding analytical grade stock solution of heavy metals (Cd, Pb and, Ni) into the experiment tank. *E. crassipes* were arranged with total weight of 100 g in each experiment tank. Experiments with *E. crassipes* were carried out in single heavy metals (Cd, Pb, and Ni) added with different concentration levels (1, 3 and 5 mg/L) respectively. The concentrations of 1 and 3 mg/L were chosen based on the previous research that have been done and this study provide a new data with high concentration tested up to 5 mg/L. This is to observe the tolerance and potential of *E. crassipes* within 14 days treatment. Any toxicity effect was also observed by photo-documentation. Figure 2 demonstrated the experiment with plants at different heavy metals and different concentration levels studied.

![Figure 2. Schematic drawing of experiment with single and mixed Cd, Pb and Ni at different concentrations with *E. crassipes*](image)

2.3 Heavy Metal Analysis in Wastewater and *E. crassipes*
About 100 mL of wastewater were sampling throughout the 14 days experiment. The wastewater was filtered and preserved in HNO₃ and were stored in acid-rinsed polyethylene bottle at 4°C. Analysis of heavy metals in plants tissues were done by USEPA method 3050b. Plant samples *E. crassipes* were collected before and after 14 days of experiment. 2 g of plant parts (leaves, stalks and roots) in experiment tank were cut randomly for digestion process. The samples were filtered then transferred to 100 mL volumetric flask and diluted with distilled for heavy metals analysis using ICP-OES Perkin Elmer Optima 5300 DV.

2.4 Determination of Relative Treatment Index (RTEI), Distribution of Heavy Metals in Plant Parts, Bioconcentration Factor (BCF) and Translocation Factor (TF).
According to Marchand, et al. [8], RTEI is used to compare the experiment with plant effect subjected to control experiment. The index were calculated based on formula below:

\[
Relative \ Treatment \ Efficiency \ Index = \frac{(T - C)}{(T + C)} \tag{1}
\]

Where, 
- \(T\) = removal efficiency of treatment experiment
- \(C\) = removal efficiency of control experiment
For distribution of heavy metals in plant part, the sample (leaves, stalks and roots) of *E. crassipes* was calculated as suggested by Aktaruzzaman, et al. [9]:

\[
\text{Distribution of heavy metals in plant parts} \left( \frac{\text{mg}}{\text{kg}} \right) = A \times \left( \frac{B}{W} \right)
\] (2)

Where, 
- \( A \) = concentration of metal in digested solution (mg/L)
- \( B \) = final volume of digested solution, mL
- \( W \) = sample weight (g)

Based on Zayed, et al. [10], bioconcentration factor is used to assess the ratio of the concentration of metals in plant tissues to the concentration of metal in wastewater. While translocation factor is used to determine the ability of *E. crassipes* in translocation heavy metals from root to shoot. The formula used to calculate BCF and TF are shown below:

\[
BCF = \frac{\text{Metals in plant (mg/kg)}}{\text{Metals in water (mg/L)}}
\] (3)

Where, 
- Metals in plant = concentration of metals in shoots and roots
- Metals in water = concentration of metals in wastewater

\[
TF = \frac{\text{Metals in shoots (mg/kg)}}{\text{Metals in roots (mg/kg)}}
\] (4)

Where, 
- Metals in plant = concentration of metals in aerial parts
- Metals in water = concentration of metals in root

3. Results & Discussion

3.1 Removal Efficiency and Relative Treatment Efficiency Index (RTEI) by *E. crassipes*

Table 1 shows the removal efficiency of *E. crassipes* in treatment and control experiment with RTEI value. The results showed that the highest removal and RTEI value was recorded in experiment containing 1 mg/L of Pb with 98.2% and 0.91 (RTEI). The higher RTEI value indicates the efficiency of the treatment between the plant and control experiment. The potential of *E. crassipes* in treating the metals were observed decreased as the concentration of metals increased from 1 mg/L to 3 mg/L. As summary the removal efficiency of 1 mg/L heavy metals increased in the order of Pb>Cd>Ni, while the removal efficiency at 3 mg/L and 5 mg/L heavy metals increased in the order, Pb>Ni>Cd. The result also showed that the control data in experiments tank do not evidence significant changes during 14 days of experiment. Data on the removal efficiency of control experiments were ranged in 0.35%-22.5%. The loss of metal concentration in control experiment is probably due to interaction with the tank material along the experiments. The results revealed that even though Pb is not an essential element for plant, *E. crassipes* were able to tolerate and remove high concentration of Pb as compared to other metals. The removal pattern of Ni and Cd changed at 3 mg/L and 5 mg/L due to the toxicity of Cd is higher than Ni. Mohamad and Latif [11] stated that Cd at concentration more than 2 mg/L, the growth of *E. crassipes* was inhibit which indicates that the plant will induce the defense system to avoid metal toxicity. Meanwhile Gonzalez, et al. [12] stated that *E. crassipes* had oxidative damage when it is exposed to Ni more than 3 mg/L.
Table 1. Removal efficiency, RTEI value and toxicity symptoms of Cd, Ni and Pb by *E. crassipes*

| Heavy metals | Concentration (ppm) | Plant Experiment | Toxicity symptoms |
|--------------|---------------------|-----------------|-----------------|
|              | Initial             | Final           | Removal efficiency (%) | RTEI | Days | 0 | 7 | 14 |
| Cd           | 0.911 ± 0.067       | 0.454 ± 0.001   | 50.1             | 22.8 | 0.38 | H | H | UH |
|              | 3.136 ± 0.061       | 2.931 ± 0.155   | 6.54             | 2.60 | 0.43 | H | UP | -  |
|              | 5.150 ± 0.012       | 4.991 ± 0.018   | 3.09             | 0.35 | 0.80 | H | UR | -  |
| Ni           | 1.034 ± 0.004       | 0.572 ± 0.001   | 44.68            | 10.5 | 0.62 | H | UD | UH |
|              | 3.249 ± 0.050       | 3.014 ± 0.267   | 7.23             | 4.73 | 0.21 | H | UP | -  |
|              | 5.183 ± 0.061       | 4.874 ± 0.163   | 5.96             | 3.38 | 0.28 | H | UP | -  |
| Pb           | 1.180 ± 0.041       | 0.021 ± 0.005   | 98.2             | 4.47 | 0.91 | H | H | H  |
|              | 3.272 ± 0.023       | 1.880 ± 0.968   | 42.5             | 3.36 | 0.85 | H | H | H  |
|              | 5.240 ± 0.035       | 4.922 ± 0.049   | 6.07             | 3.48 | 0.27 | H | UH | UP |

H= Healthy; UH= Unhealthy with yellow leaves; UP= Unhealthy and partially wilting; UD= Unhealthy and deteriorated with delicate stolon; UC= Unhealthy and completely wilting; UR= Unhealthy and losing roots; (UC and UR indicates the plants were died).

The tolerance of *E. crassipes* exposed to heavy metals at different concentrations was photo-documented and the toxicity evidence was recorded within 14 days of experiment. *E. crassipes* exposed to 1 mg/L and 3 mg/L showed tolerance and were observed healthy along the experiment. Meanwhile *E. crassipes* exposed to 5 mg/L of Pb were showed toxicity symptoms with wilting and unhealthy yellow leaves. At 1 mg/L Ni, the plants was unhealthy with delicate stolon and the plant was died at day 10. The results of toxicity symptoms showed that as the concentration increased at 3 mg/L and 5 mg/L of Cd, the plants died with losing roots at day 10 and day 6 respectively. It was notify that *E. crassipes* died with losing roots at day 8 in both 3 mg/L and 5 mg/L of Ni. Figure 3 showed that, the toxicity symptom of *E. crassipes* exposed to high concentration of Cd, Ni and Pb within 14 days treatment.
3.2 Distribution of Cd, Ni and Pb in plant parts of E. crassipes

Figure 4 showed the accumulation and distribution of metals in plant part of E. crassipes (root, leaves and stalk). The results showed that accumulation of all heavy metals are varied at different concentration levels studied. E. crassipes exposed to 1 mg/L showed the accumulation were in range from 3.69 mg/kg to 6012.68 mg/kg. While at 3 mg/L the accumulation metals can be observed in range of 2.69 mg/kg to 2068.77 mg/kg and at 5 mg/L the metals were in range of 20.11 mg/kg to 3763.53 mg/kg. It can be showed that all metals were accumulated highly in root as compared to leaves and stalk at all concentration studied. The accumulation of Cd, Ni and Pb were higher in root than in stalks and leaves due to its characteristics of floating plant which mostly use its roots to concentrate, absorb and uptake heavy metals in the root or within the surface of the root [13]. The accumulation of Pb in root were highest with (60.12.68 mg/kg) at 1 mg/L followed by Ni (3628.22 mg/kg) and Cd with (2068.77 mg/kg). It is important to note that the distribution of all metals were in order of roots>leaves>stalks. This can be explained that roots exudates play an important role in the acquisition of several essential metals. According to Kanazawa, et al. [14], some species of plants have been documented to exude from roots a class of organic acid called sidephores (mugineic and avenic acids) which were shown to significantly enhance the bioavailability of water-bound ion. These processes were able to make the metal more available for plant uptake.

3.3 Bioconcentration Factor (BCF) and Translocation Factor (TF) of E. crassipes

BCF index was used to determine the ability of E. crassipes to accumulate and concentrate heavy metals in root and shoot with respect to different concentrations and different type of metals studied. Table 2 shows the bioconcentration factor of Cd, Ni and Pb at three different concentration treatment. The results showed that the maximum value of BCF was found in root at 1 mg/L of treatment with Pb (5095.49) >Cd (3143.92) >Ni (2782.38) respectively. According to Zayed, et al. [10], the higher BCF value with more than 1000 indicates that the plant is an ideal hyperaccumulator plant that can accumulate metals from contaminated medium. These findings support the idea that the heavy metal binding capabilities of root exudates may be an important mechanism for stabilizing heavy metals in the vicinity of the root thus making them available to the plant and lessening the experienced toxicity. This is important to show that E. crassipes in this study can be a good phytoaccumulator of Pb followed by Cd and Ni in roots at 1 mg/L of treatment studied. The data also showed that as the concentration of metal increased in the experiment, the BCF value of Cd, Ni and Pb in root were decreased. Ahlawat and Lata, [15] reported that the BCF of Cd decreased as the concentration increased above 2 mg/L which show similarity in the
present study. Gonzalez, et al. [16] explained that \textit{E. crassipes} exposed to Ni more than 2 mg/L can cause toxic effects to the plants.

Figure 4. Distribution of Cd, Ni and Pb) at (a) 1 mg/L, (b) 3 mg/L and (c) 5 mg/L in different plant parts of \textit{E. crassipes}. 
Table 2. Bioconcentration factor (BCF) and Translocation factor (TF) of Cd, Ni and Pb in roots and shoots of *E. crassipes*

| Concentration of metals studied | Heavy metal | BCF (root) | BCF (shoot) | TF |
|---------------------------------|-------------|------------|-------------|----|
| **1 mg/L**                      | Cd          | 3143.92    | 98.90       | 0.03 |
|                                 | Ni          | 2782.38    | 243.38      | 0.09 |
|                                 | Pb          | 5095.49    | 50.62       | 0.01 |
| **3 mg/L**                      | Cd          | 86.26      | 7.80        | 0.09 |
|                                 | Ni          | 48.58      | 57.73       | 1.19 |
|                                 | Pb          | 632.27     | 5.35        | 0.01 |
| **5 mg/L**                      | Cd          | 30.67      | 10.35       | 0.34 |
|                                 | Ni          | 33.26      | 45.31       | 1.36 |
|                                 | Pb          | 718.23     | 177.32      | 0.25 |

TF index was used to determine the ability of *E. crassipes* to accumulate and concentrate heavy metals with respect to different concentration and different type of metals studied. The TF value with more than 1 indicates that the metals mainly stored in shoot while TF value less than 1 means that more heavy metals are stored in roots of the plants [17]. Based on Table 2, the data showed that all metals studied were observed to have low TF value which is less than 1 except for treatment with metal Ni at 3 mg/L and 5 mg/L which were found higher in TF value with 1.19 and 1.36. This can be explained with the mobility and elevation rate of Ni is higher from roots to shoots compared to Cd and Pb. This finding is line Emamverdian, et al. [18] stated that heavy metal Ni is easier to translocate in to aerial parts due to its different distribution in plant tissue as compare to Pb and Cd. This study remark that *E. crassipes* prefer to translocate Ni in leaves and stalks as compared to roots part.

4. Conclusion

The results of the study showed that experiment contacting *E. crassipes* were able to tolerate and treat Pb with high RTEI (0.91) and high removal efficiency of 98.2% upon 14 days of treatment. The selectivity of heavy metal by *E. crassipes* was in descending order of Pb>Cd>Ni respectively. *E. crassipes* was observed with no toxicity symptoms while treated Pb at 1 and 3 mg/L experiment. It can be noted that the maximum quantity of metal contaminant was contained in roots as compared to shoot. The distribution pattern of all metals are shown in roots> leaves> stalks. This study proved that the ability of *E. crassipes* to remove heavy metal especially Pb with BCF factor of root is more than 1000 at 1 mg/L concentration studied. However, the plants must be harvested regularly to avoid the plants from wilting and releasing heavy metals back into the environment.

5. References

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