Evaluation of phytoremediation in removing Pb, Cd and Zn from contaminated soil using Ipomoea Aquatica and Spinacia Oleracea

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Abstract. Phytoremediation is an innovative and profound method to clean heavy metals which have been released by human activities into the environment and where the metals had persisted over many years. Phytoremediation employs plants to remove the heavy metals. The aim of this study is to compare Ipomoea Aquatica and Spinacia Oleracea in effectiveness of phytoreduction capability and translocation capability in responds to lead, cadmium and zinc from synthetic soil within 35 days. The synthetic soil was prepared to contain 66 mg/kg Pb, 30 mg/kg of Cd and 66 mg/kg of Zn. I. Aquatica has shown higher removal percentage of Pb, Cd and Zn than S. Oleracea which are 27.7% of Pb, 29.0% of Cd and 32.8% of Zn. The translocation factor (TF) of root to shoot by I. Aquatica is 1.05 and it is higher than S. Oleracea, which is 0.79. Besides that, I. Aquatic has shown a higher combined bioaccumulation factor (BAC) of 0.78 while S. Oleracea has combined BAC of 0.68. The results show that I. Aquatica is more effective for phytoremediation. Furthermore, kinetic evaluation shows that heavy metals treated with I. Aquatica has shorter half-life compared to S. Oleracea which is 76 days.

1. Introduction
Soil contaminated by heavy metals has gained a considerable public awareness as it become a severe problem worldwide [1,2]. Rapid growth in industries, agricultural and disturbance of natural ecosystem make the heavy metals pollution to become a serious threat to the environment [3].

Heavy metals enter the ecosystem by natural as well as anthropogenic sources. Naturally, heavy metals are associated with the volcanic eruption, wind erosion and forest fire. The metals from the natural sources cause less harm to environment in general, whereas the metals from anthropogenic sources such as smelters, thermal power plants, mines, foundries and agricultural pose a threat to mankind [4,5].

Unlike organic pollutants, biodegradation of heavy metals is just out of question and hence are continuously accumulating in the environment. Accumulation of these heavy metals especially in agricultural soils and water resources poses a great threat to human health due to potential risk of their entry into food chain [3]. A number of physical, chemical and biological techniques can be used to remediate metal contaminated soils. However, phytoremediation has been recognized as cost effective method for remediation of metal contaminated soils [3,6].
Phytoremediation is a plant-based technology employed either raw or genetically modified plant species for restoring contaminated land and water sources [5] which the plant promotes the breakdown, immobilization, and removal of pollutants from the environment [6]. This is because, plants have a more direct effect on contaminant levels via phytoextraction, which concentrates contaminants (e.g., heavy metals) from the environment into plant tissues. Although this technology will give a little disturbance to the landscape, nevertheless, it reduces the cost of alternatively disposing hazardous wastes to a landfill or a storage facility located off-site [6]. Therefore, this study was carried out to evaluate phytoremediation capability of *Ipomoea Aquatica* and *Spinacia Oleracea* in removing lead, cadmium and zinc.

2. Material and methods

2.1. Preparation of unpolluted soils and seeds

The soil and seeds of *Ipomoea Aquatic* and *Spinacia Oleracea* were bought from the local market. The soil and seeds were preserved separately in containers which were tightly sealed to avoid contact with water and impurities.

2.2. Preparation of contaminated soil.

The unpolluted soil was beforehand tested for the concentration of heavy metals. Then, the synthetic contaminated soil was being prepared by adding metals in the nitrate solutions and spiked to the soil [7]. The concentrations of Zn(NO$_3$)$_2$, Pb(NO$_3$)$_2$, and Cd(NO$_3$)$_2$ added to the soil were 66 ppm, 66 ppm and 30 ppm, respectively. These concentrations were selected due to guidelines prepared by Department of Environment Malaysia [8] mentioning that the maximum natural occurring of Cd, Pb and Zn concentrations in soil are 14.4 mg/kg, 36 mg/kg and 54.3 mg/kg, respectively. More water is added and the soil was homogenized by mixing for a week.

2.3. Germination of plants and transferring to contaminated soil.

The seed was rinsed with clean water then sowed on the healthy soil and waited for at least 2 months for the plants to sprout and grow. All plants were watered daily. Observations were made visually to observe the growth of the plants, including roots’ length. After 2 months, it was transplanted to contaminated soil, without damaging the roots as much as possible in order to avoid transfer shock.

2.4. Phytoremediation study

Thirteen flowering pots with similar size were prepared with each pot was filled with 1 kg of contaminated soil. Then, the pot was planted with the prepared 2-month old of *Ipomoea Aquatic* and *Spinacia Oleracea*. This phytoremediation study was carried out for 35 days with interval time of 7 days.

2.5. Determination of heavy metals concentration in plants tissue and the soil

Harvested shoots and roots were washed thoroughly with tap water and then followed by distilled water. Later, both the roots and shoots were dried in the oven at 70 °C for 48 hours before being weighted and mashed by using the mortar and pestle [9]. Mashed dry plants tissue (0.4 g) were treated with 7 ml of HNO$_3$ and it was left to react for 2 days in a closed environment. The solution was then heated for one hour at 120°C on a hot-plate and several additions (2 ml) of H$_2$O$_2$ were added until digestion was colourless [10].

Distilled water was added until 50 ml. On the other hand, in order to digest 0.4 g of soil, the soil was added with 3 ml of H$_2$O$_2$, 3 ml of HNO$_3$ and 9 ml of HCl. The mixture is left alone for 48 hours in a closed environment and distilled water was added till 50 ml [9]. Then, the Atomic Adsorption Spectrometry (AAS) was used to obtain the heavy metal concentration for both plants tissue and the soil.
2.6. Data Analysis

The ability of plants in removing the heavy metals from the contaminated soil was expressed in term of percentage phytoreduction efficiency (PE) and calculated as in Equation 1.

\[ \%PE = \frac{C_0 - C_t}{C_0} \times 100 \]  

(1)

where \(C_0\) represents initial heavy metal concentration in soil while \(C_t\) represents the heavy metal concentration on \(t\)-day.

For Bioaccumulation factor (BAC), it can be calculated by using Equation 2.

\[ \text{BAC}_{metal} = \frac{\text{metal in plant (mg/kg)}}{\text{metal in soil (mg/kg)}} \]  

(2)

In order to calculate Translocation factor (TF) of heavy metals, Equations 3, 4 and 5 were used.

\[ TF_{root} = \frac{\text{metal in root (mg/kg)}}{\text{metal in soil (mg/kg)}} \]  

(3)

\[ TF_{shoot} = \frac{\text{metal in shoot (mg/kg)}}{\text{metal in soil (mg/kg)}} \]  

(4)

\[ TF_{rs} = \frac{\text{metal in shoot (mg/kg)}}{\text{metal in root (mg/kg)}} \]  

(5)

Meanwhile, the kinetic evaluation to assess the speed of heavy metal removal was calculated by using First-order kinetics equation expressed in Equation 6.

\[ C_t = C_0 e^{-kt} \]  

(6)

where \(C_t\) is the concentration (mg/kg) of residual metal at specific time and \(C_0\) is the initial concentration (mg/kg). A plot of \(\ln \left(\frac{C_t}{C_0}\right)\) vs. time to yield trend lines where slope \(k\) (day\(^{-1}\)), phytoextraction rate constant and half-life \((t_{1/2}) = \ln 2/k\), was also derived [9].

3. Results and discussion

3.1. Plant response to Pb, Cd and Zn concentration

Visual assessment of Ipomoea Aquatic and Spinacia Oleracea in response to the environmental stimuli at high concentration was monitored throughout the 35 days of the experiment. Throughout the study period, there was no plant death (necrosis) was recorded.

However, observations indicate that when the plants are transplanted to the contaminated soil, both plants show slower growth rate compared to the plants grown on healthy medium. This slower growth rate was confirmed as the plants’ height of both species was increasing slowly and the increment in height was only by 1 cm for the duration of 35 days, which also showed that their total dry mass did not increase much. The cause of the suppressed growth including reduced number of leaves and height is due to the high concentration of Pb [11]. Sytar et al. [12] reported that nitrogen supplement can minimize the inhibitory effect of heavy metals.

Furthermore, the leaf counts are different between each sample, in which Ipomoea Aquatic has between 6 – 8 leaves while Spinacia Oleracea has 4 – 6 leaves. Some of the plants also exhibited some signs of yellowing leaves (chlorosis). According to Alaribe and Agamuthu [9], these signs are not uncommon and it is concorded with their findings. Hence, they deduced that this trend implied that strong resistance potential of Ipomoea Aquatic and Spinacia Oleracea to Pb, Cd and Zn polluted soil.
Besides that, when the plants were harvested, the roots of both plants are found to be shorter than plants which has grown on unpolluted soil. This phenomenon could be caused by the high concentration of the Cd and Zn [11]. Furthermore, Laghlimi et al. [13] reported that the effects of root architecture, in this case when it is poorly grown will reduce the accumulation of the metals in plants.

3.2. Metal reduction in polluted soil

The percentage of phytoreduction process in reducing Pb, Cd and Zn is shown in Table 1. After 35 days, the reduction of Pb in the soil is as high as 27.72 % for I. Aquatica while the soil planted with S. Oleracea has lower reduction percentage of Pb which is 25.91 %. For the Cd in the soil, I. Aquatica has reduce 29.00 % in the soil which is higher compared to S. Oleracea planted on the contaminated soil, 25.27 %. On the other hand, I. Aquatica and S. Oleracea has close reduction percentage of Zn in the contaminated soil, which are 32.80 % and 30.85 %, respectively.

Table 1. Percentage phytoreduction efficiency of Pb, Cd and Zn in contaminated soil for 35 days with I. Aquatica and S. Oleracea

| Metals | Concentration (mg/kg) | Planted with | Planted with | Controls |
|--------|----------------------|--------------|--------------|----------|
|        |                      | I. aquatica  | S. oleracea  | w/o plants (%) |
| Pb     | 66                   | 27.72        | 25.91        | 4.73     |
| Cd     | 30                   | 29.00        | 25.27        | 2.27     |
| Zn     | 66                   | 32.80        | 30.85        | 6.80     |

High percentage of Zn reduction shows that both the plants are capable to tolerate with high concentration of zinc. Nonetheless, Zn is part of the essential micronutrients needed for vital physiological and biochemical functions of plant growth [13], besides this element also has higher mobility in the soil and better transfer into plants [12]. Thus, the plants tend to absorb more Zn than other contaminated ions.

Overall, I. Aquatica has higher removal percentage of all the metals compared to S. Oleracea. This could be due to the fact that I. Aquatica has a better root architecture (growth affected by contaminated ions) as compared to S. Oleracea [13]. As a result, the roots of I. Aquatica are more deeply and widely rooted which make absorption of metals easier and more effective.

3.3. Metal accumulation in plants, translocation and bioaccumulation factors

Accumulation of Pb, Cd and Zn in the roots and shoots was used to calculate the translocation factor (TF) and bioaccumulation factor (BAC). Marrugo-Negrete et al. [14] pointed out that TF and BAC are greater than 1 indicate that the plant has the potential to be used in phytoextraction. Therefore, both TF and BAC for each plant are shown in Table 2 and Figure 1, respectively.

Table 2 shows that both I.Aquatica and S. Oleracea have a translocation factor of Pb which is not more than 0.2 indicating that the plants have a low ability to translocate the Pb ions. However, higher TF$_rs$ value of I.Aquatica was recorded which suggesting that the ability of the plant to translocate the Pb ions from the roots to shoots are better as compared to S. Oleracea.

As of Cd, I. Aquatica has a higher translocation factor in the contaminated soil which tabulated in Table 2. The result reveals that the TF$_{root}$, TF$_{shoot}$ and TF$_{rs}$ of I. Aquatica are 0.20, 0.09 and 0.48, respectively while TF$_{root}$, TF$_{shoot}$ and TF$_{rs}$ of S. Oleracea are 0.17, 0.06 and 0.38, respectively. This implies that I. Aquatica is far better than S. Oleracea in translocating the Cd ion since the plant capable to reduce almost half of the absorbed Cd ions by the roots are translocated into the shoots.

In order to remove Zn, both of the plants exhibit high capability in translocation of Zn from roots to shoots. This shows that I. Aquatica has TF$_{root}$ of 0.13, TF$_{shoot}$ of 0.13 and TF$_{rs}$ of 1.05. On the other hand, S. Oleracea has TF$_{root}$ of 0.13, TF$_{shoot}$ of 0.11 and TF$_{rs}$ of 0.79. This indicates that I. Aquatica is
identified to have a better performance which removed a larger portion of the contaminants than *S. Oleracea*. Moreover, the result also shows that *I. Aquatica* has the potential to be used in phytoextraction to remove Zn since TF$_n$ of the plant is greater than 1 [14]. Overall, *I. Aquatica* is identified to have a better performance which removed a larger portion of the contaminants than *S. Oleracea*. This is due to higher tolerance to Zn as the plants require the Zn as essential nutrients, thus explains the high translocation factor. This is supported by Laghlimi et al. [13] who pointed out that Zn is an easily absorbed element and easily transported to shoots. However, a better removal percentage of contaminants can be achieved by mixing amendments into the contaminated soil [9].

**Table 2.** Translocation factor of metal uptakes for 35 days.

|                | 66 (mg/kg) Pb | 30 (mg/kg) Cd | 66 (mg/kg) Zn |
|----------------|---------------|---------------|---------------|
| *I. Aquatica*  | 0.16          | 0.20          | 0.17          |
| *S. Oleracea*  | 0.18          | 0.17          | 0.13          |
| TF$_{root}$    | 0.08          | 0.09          | 0.06          |
| TF$_{shoot}$   | 0.49          | 0.48          | 1.05          |
| TF$_{rs}$      | 0.23          | 0.33          | 0.79          |

Figure 1 shows a combined bioaccumulation factor of all the 3 metals for both the plants used in the experiment as it is necessary to determine which of the plants is superior in bioaccumulation for all the metals combined. During experimental period, both plants managed to accumulate around 20 % – 30 % from each of the contaminants.

![Figure 1](image.png)

**Figure 1** Comparison of combined BAC between *I. Aquatica* and *S. Oleracea*

Generally, the results show that BAC value of *I. Aquatica* and *S. Oleracea* in a multi-ions contaminated soil is 0.78 and 0.68, respectively indicating that *I. Aquatica* has absorbed more contaminants than *S. Oleracea*. Moreover, the ranking from highest to lowers ions adsorbed for *I. Aquatica* and *S. Oleracea* are Cd>Zn>Pb and Zn>Cd>Pb, respectively. The is because, *I. Aquatica* has a better growth rate than *S. Oleracea* when transferred the contaminated soil. Higher growth rate means more tissue and dry mass which can absorb more ions [15].
3.4. **Kinetic evaluation**

Kinetic evaluation is concerned with reaction rate to determine how fast or slow the response takes. In this study, a first order reaction is used to assess the removal rate of a plant to eliminate the contaminated ions (the reaction which occurred) which it is depending linearly on only one reactant concentration. Moreover, a half-life assessment is also determined in this study which represents the amount of time required for a reactant concentration to reduce by half of its beginning concentration. The half-life is useful in making prediction on the concentration of a substance over time. Table 3 shows the phytoreduction of *I. Aquatica* and *S. Oleracea* that best fitted to the first order kinetics model and the half-life for each element.

|           | I. Aquatica (mg/kg) | S. Oleracea (mg/kg) | Control   |
|-----------|---------------------|---------------------|-----------|
|           | k (day\(^{-1}\))   | ln(2)/k (days)      |           |
| Pb        | 0.009               | 75.13068647         |           |
| Cd        | 0.010               | 71.27807556         |           |
| Zn        | 0.011               | 61.54032294         |           |
| Pb        | 0.009               | 81.28850243         |           |
| Cd        | 0.008               | 83.42213263         |           |
| Zn        | 0.010               | 66.28533666         |           |
| Pb        | 0.001               | 501.0301928         |           |
| Cd        | 0.001               | 1058.154032         |           |
| Zn        | 0.002               | 344.4266769         |           |

The results display that *I. Aquatica* will be able to remove the heavy metals completely in 152 days or 5 months period. On the other hand, *S. Oleracea* needs another 168 days or almost 5.6 months to remove the heavy metals completely. Hence, the kinetic evaluation shows that *I. Aquatica* performed better than *S. Oleracea*. Similar findings also reported by Kitsteiner [16] who addressed that *I. Aquatica* has hollow stem which allows the heavy metals to transfer more freely. Hence, heavy metals can be absorbed more easily by *I. Aquatica*.

Besides, the result also reveals that contaminated soil without being treated with *I. Aquatica* or *S. Oleracea*, the heavy metals will stay in the soil for years before it might be gone according to first order of kinetics. However, heavy metals are not degradable which is why it will persist in the soil unless acted upon by natural abrasion such as wind and water or it is sometimes absorbed by microorganisms found in the soil [13].

3. **Conclusion.**

This study reveals the capability of *I. Aquatica* and *S. Oleracea* to act as a phytoremediator of heavy metals in soil. A better phytoreduction efficiency for Pb, Cd and Zn is achieved by *I. Aquatica* as compared to *S. Oleracea*. Visual observation shows that *I. Aquatica* has a better root architecture and bigger mass than *S. Oleracea* thus affecting the removal percentage of heavy metals. Among the heavy metals studied, Zn and Cd has more mobility in the soil and transfer easily compared to Pb. Based on study of translocation and bioaccumulation factor, both plants have TF and BAC value of not more than 0.5 which indicates that the plants are not qualified to be categorized as hyperaccumulator yet, unfortunately constrained by the period of experimentation. Both the plants have
shown potential to be effective in removing and storing Zn where TF of root to shoot of *I. Aquatica* and *S. Oleracea* are 1.05 and 0.79 respectively. The higher translocation factor of Zn is due to the properties of Zn as it is easily available and essential to plants. On the other hand, higher BAC is due the bigger mass and more tissues of *I. Aquatica* provide a higher bioaccumulation of heavy metals. Besides, the kinetic evaluation has shown the uptake rate of the metals. It is found that the plants have differ in their uptake rate of Cd, where *S. Oleracea* absorbs Cd slower than *I. Aquatica*. The half-life of the metals is the shortest when the contaminated soil is treated with *I. Aquatica*. The contributing factor to this finding is the hollow stem of the *I. Aquatica* which make it easier for metals’ transportation. Overall, the study shown that *I. Aquatica* provides a better result in phytoremediation when treating multi-ions contaminations.

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