Effect of lead (Pb) on seed germination of water spinach 
(*Ipomoea aquatica* Forsk)

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Abstract. In this research, the effect of lead (Pb) on seed germination rate (%) of water spinach (*Ipomoea aquatica* Forsk) have been investigated. The seeds were placed in 6 × 4 × 2 cm rockwool moistened with distilled water (control) and aqueous solutions of PbCl₂ (0, 50, 100, 150 and 200 μM). Germination test was done by sowing fifteen water spinach seeds with six replicates for each concentration Pb level for 4 days. Several parameters have been observed in the seed germination test. Such stress conditions revealed that by increasing the concentration of lead, the germination rate (%), the tolerance index (TI), and the seedling vigor index (SVI) decreased significantly, suggesting their toxic effect on water spinach. The obtained data were analyzed using one-way ANOVA. The results showed that for lead-treated seeds were significantly different compared to the controls and showed a gradual decrease towards an increase in lead concentration (Pb) in shoot and root fresh weight, shoot and root length, SVI, and, TI. Germination percentage and seedling dry weight of lead-treated germinated seeds were not significantly different compared to the controls. Due to limitations of the data, the maximum lead concentration thresholds for water spinach cannot be accurately determined, but it can be inferred that they would be greater than 100 and less than 200, respectively.

Keywords: Water spinach, *Ipomoea aquatica*, Pb, seed germination

1. Introduction

Freshwater, groundwater, and agricultural soil are among the important sources of heavy metal toxicity in crops. Contaminated irrigation water and heavy metal deposits in the topsoil can affect growth, quality, and quantity of plant yield [1]. The heavy metals with a density greater than 5 g / cm³ of essential and nonessential types are continuously increasing in soil due to the unmanaged municipal wastes, mining, fertilizer and excessive use of pesticides [2]. Lead (Pb) has attracted substantial concerns as a potential heavy metal pollutant because of the growing anthropogenic pressure on the environment [3]. Lead is not only a pollutant near the industry but also pollution from vehicle emissions. Plants display an outstanding ability to adapt against the various content of the growth medium and have promoted the uptake mechanism toward available nutrient in soils. Generally, plants are ready to take up the trace elements contained in the soil solution both in free ion and complex form [4].

Water spinach (*Ipomoea aquatica* Forsk) is an aquatic plant which is native in Southeastern Asia [5]. It is a popular herbaceous species cultivated across Indonesia and Asia for consumption. The propagation of water spinach can be achieved through cutting or generatively by seed. Commonly, this
species is cultivated in a water body or muddy swamp; it can easily grow without intensive labor, thus making it an affordable vegetable for the community [6].

The research’s aim was to examine the impact of lead (Pb) on seed germination of water spinach (Ipomoea aquatica Forsk) by using soilless media. The result of this study will be the basis for determining the maximum lead concentration threshold which still allows the water spinach to grow until it can be harvested.

2. Materials and method

Seeds of water spinach (Ipomoea aquatica Forsk cv Bangkok-LP 1) were obtained from PT East West Seed Indonesia. Seeds were surface sterilized in 0.5 % commercial sodium hypochlorite solution for 5 min and washed thoroughly using the running tap water. Germination was conducted in six replicates, each consisting of 15 seeds placed in a 6 cm × 4 cm × 2 cm rockwool moistened with varying concentrations of PbCl₂ solution (50, 100, 150 and 200/586 M) and arranged in a randomized block design. PbCl₂ concentration was determined between moderate concentration ranges compared to the high concentration used by previous researchers which was in a range of 0–20000 μM [7]. Distilled water was used as control. The research was conducted in a greenhouse in the Faculty of Mathematics and Natural Sciences, Universitas Indonesia. The seeds were enabled to grow under the natural lighting and temperature for 4 days. The mean of daily temperature and humidity were observed using a digital thermohygrometer. On the 4th day after germination, the shoot and root length, shoot and root fresh weight, shoot and root dry weight, vigor index, and tolerance index were measured and the germination percentage was recorded. The whole water spinach seedlings were harvested and washed thoroughly with tap water. The plants were separated into roots and shoots. All the samples were dried in an oven at 70 °C to a constant weight. According the germinated seeds number, vigor index (VI = GI x Hs, where Hs is shoot height) were calculated [8]. Afterward, the dry weight of seedling tissue was measured. The data obtained were analyzed using one-way ANOVA in IBM SPSS v. 25.

3. Results and discussion

The observations on fifteen seedlings for each replicate for the yield were used for calculating the mean performance. The results of each growth parameter after 4 days is presented in table 1. Table 1 shows most parameters measured exhibit significant differences compared to the control group. The germination percentage and seedling dry weight are not significantly different in all treatment groups. Lead has been reported to strongly affect the seed morphology and physiology [9].

The major impact of heavy metals on seeds are indicated by the overall abnormalities and decrease in germination [10], reduced root and shoot elongation [11] (as seen in table 1) all contributing to the inhibited growth of plants. Application of 50 μM PbCl₂ causes a negative influence on both shoot and root length. In Pb-treated plants, shoot length reduced 54.8–42.7 % as compared to the control.

| Table 1. Seed germination test results |
|---------------------------------------|
| Parameters                             | 0 μM | 50 μM | 100 μM | 150 μM | 200 μM |
|---------------------------------------|------|-------|--------|--------|--------|
| Total germination (%)                  | 96.67 a | 91.11 a | 92.22 a | 96.67 a | 95.56 a |
| Seedling vigor index                   | 704.6 a | 377.33 b | 357.78 bc | 306.16 bc | 265.69 c |
| Root tolerance index (%)               | 100 a | 58.03 b | 49.18 bc | 41.33 cd | 33.85 d |
| Shoot length (cm)                      | 3.63 a | 1.99 b | 2.07 b | 1.65 b | 1.55 b |
| Root length (cm)                       | 3.65 a | 2.12 b | 1.8b c | 1.51 cd | 1.24 d |

Values followed by the same letters are not significantly different at P < 0.05.
The shoot and root length reduction were due to the inhibitory effect of Pb. This is also supported by the results of research by Siddiqui et al. [12] on Brassica rapa var. turnip. Research by Titov et al. [4] on Hordeum vulgare L. cv. Otra and Triticum aestivum L. cv. Mironovskaya 808 shows that compared to root and shoot length parameters, germination percentage was the least sensitive to the increased lead concentration (Pb). Permeability of seed testa to lead (Pb) might influence the effect of lead on the germination percentage. In some plants, testa protects seeds from heavy metal stresses, especially at the beginning of imbibition [13].

Although the percentage of germination in all treatments studied were >90 %, the vigor of the seedlings was not the same. This is indicated by the decrease in the value of the vigor index as lead concentration increased. Besides the lower vigor results, seedlings treated with lead also showed a decreasing tolerance index (TI). Decreased seedling vigor and tolerance were also reported by Kabir et al. [14] in Thespesia populnea L. at lead concentrations of 10, 30, 50 and 70 μM. Besides T. populnea L., a gradual decrease in the vigor index and tolerance are also observed in Albizia lebbeck (L.) Benth. at concentrations of 10, 30, 50, 70 and 90 μM [15].

The tolerance index in the germination test uses the root length value for calculations. The root tolerance index shows the extent to which the ability of plants that treated in achieving certain parameter values, compared to the control plants. The tolerance index value obtained reveals the higher lead concentration, the more tolerance of the plant decreases. Seemingly, this suggests that among the available species of plant, the plants ability in absorbing the existing elements relies on the growth of plants, which is supported by the increase of plant transpiration (i.e. better growth leads to a better transpiration, hence a greater root uptake of water and solutes).

Shoot fresh and dry weight is the quantitative parameters to characterize the plant growth and development. Fresh weight is an indicator of photosynthesis that 90 % of fresh plant weight is the result of photosynthesis. Figure 1 and figure 2 show the shoot and root fresh weight decrease with increasing Pb concentration. This is caused by Pb which inhibits the chlorophyll synthesis by reducing the uptake of essential elements namely magnesium and iron by plants. Iron is essential for chlorophyll synthesis, thus decreasing the chlorophyll synthesis will affect the rate of photosynthesis. The consequent is a decrease in carbon supply to the shoot and roots further limits shoot and root growth. Meanwhile form figure 1, it can be seen that the dry weight was not significantly different between the Pb treatments. Shoot dry weight is the result of plant growth which shows the ability of plants to absorb nutrients. This is probably due to the Pb concentration does not interfere with the nutrient uptake so shoot dry weight between control and treated-plant was not significantly different.

![Figure 1. Effect of various concentrations of PbCl2 on shoot and dry weight of water spinach. Values followed by the same letters are not significantly different at P < 0.05.](image)
In conclusion, it can be said that lead is one of the heavy metals that has a harming effect on plants, lead can affect certain physiological processes that inhibit growth.

4. Conclusion
Our results from greenhouse studies reveal that lead at all concentration levels (50, 100, 150, 200 μM) significantly reduced the percentage of germination, seedling length, seedling fresh weight, seedling vigor index and seedling tolerance index of water spinach (Ipomoea aquatica Forsk). Germination percentage and seedling dry weight were not significantly affected. Due to limitations of the data, thresholds for water spinach cannot be accurately determined, but it can be inferred that they would be greater than 100 and less than 200, respectively. As for continuing this study, it is important to determine the spatial distribution and mobility of lead in soils and plants close to human activities.

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