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Indole-3-acetic Acid (IAA) Assisted Phyto-extraction Potential of *Ipomoea aquatica* Exposed to Lead (Pb) Stress

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Abstract

Agricultural lands are gradually being contaminated by heavy metals (HMs) obtained from urbanization and human activities. Lead (Pb) is one of the major heavy metals easily enters into food cycle and causes different health abnormalities. So elimination of this dangerous heavy metal from surface water is crucial and in this regard phyto-extraction based phytoremediation is an environmentally safe procedure. Again, this removal process can be amplified through the use of plant growth regulators exogenously. With this aim, an experiment was conducted to know the efficiency of the use of indole-3-acetic acid (IAA) on phyto-extraction of Pb by an aquatic hyper-accumulator plant *Ipomoea aquatica*. The plants were grown hydroponically with 200ppm Pb treated with 200ppm IAA exogenous spray or mixed with Hoagland solution (HS) or both. The control treatment was HS supplemented with Pb. From the experiment, it was observed that control treatment causes a great reduction in growth parameters as the plants suffered from Pb stress. Treatment with Control + IAA (spray+dissolve), produced tallest plants, longest roots and maximum dry weight. On the other hand, these parameters were got declined in case of Pb treated plants (control). Maximum Pb were accumulated on root, stem followed by leaf for Control + IAA (spray+dissolve). Control treatment caused less Pb accumulation on plant parts. So, maximum bioaccumulation factor (BCF) in root, shoot and leaf were 35.87, 15.33 and 9.15 respectively for Control + IAA (spray+dissolve) treatment. In case of root to shoot translocation, the maximum translocation factor (TF) value (0.5118) was found for Control + IAA (spray) than other treatments. Again for shoot to leaf, the maximum TF value (0.6051) was for HS + HM treatment (Control) and minimum TF value (0.4469) was observed for Control + IAA (spray). From the study, it is confirmed that, exogenous indole-3-acetic acid (IAA) successfully assist Pb phyto-extraction from aquatic bodies and *Ipomoea aquatica* is a potential heavy metal hyper-accumulating plant.

Keywords: Bioaccumulation factor; Heavy metal; *Ipomoea aquatica*; Indole-3-acetic acid; Hyper accumulator; Translocation factor.

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1. Introduction

The elements hazardous to living organisms are referred to as toxic metals and the majority of these metals have a higher atomic weight, which is why they are also called heavy metals (HM). Soil and water bodies are getting contaminated regularly by heavy metals. Heavy metal contamination is a widespread issue that threatens the ecosystem and causes substantial health risks to people. Heavy metal contamination is brought on by expanding industrialization, agricultural technologies and anthropogenic activities particularly in highly populated and emerging countries [1]. The atmosphere, irrigation with sewage, toxic inorganic herbicides, chemical fertilizers, livestock excrement and wastewater are some of the most frequent natural sources of heavy metals in soil and agriculture [2, 3]. Industrial wastes were dispersed both on land and in surface waters, where they eventually caused contamination from the deposition of hazardous metallic components and a number of well-recognized disorders in living organisms [4-6]. Since HMs like cadmium (Cd), lead (Pb), chromium (Cr), mercury (Hg), arsenic (As) etc. are non-biodegradable and can remain in the environment for a long time that make soil unusable for cultivation [7]. Yang, *et al.* [8], identified lead (Pb) as one of the major HM which is distributed extensively in the soil. Battery recycling, agro-chemicals, metal plating, smelting of ores are the most common sources of Pb and 0.1 mg/L is the permissible limit for ground and surface water [9, 10]. Heavy metals cause a great threat to crop production [11, 12]. High Pb concentrations in plants not only increase the synthesis of reactive oxygen species (ROS) but also disrupt the photosynthetic and chlorophyll metabolisms [13, 14], nutrient absorption [15], cellular metabolic activities [16], growth, DNA functioning [17] etc. When enters into food chain through vegetables and grains, Pb causes extra health hazards to human being including cancer, mental disorders, allergies, autism, dyslexia and kidney failure [5, 18].

Heavy metals constitute a long-term threat to the environment since they cannot be broken down by any biological or physical mechanism and remain present in the soil for a long time [19, 20]. Thus, heavy metal remediation is essential to protect the ecosystem from their harmful impacts and preserve it for future generations [21]. The removal of heavy metals has employed a number of physicochemical and biological procedures, which were described as a difficult task in terms of cost and methodologically sophistication [22, 23]. An innovative green method known as phyto-extraction removes significant amounts of heavy metals from soil and stores them in a harvestable component [24]. Phyto-extraction involves utilizing plants to restore a HM polluted environment, has been offered as a potential and environment friendly alternative to conventional physical and chemical approaches for this purpose [20]. Hyper-accumulation is the capacity of a plant to collect HMs in its above-ground components at concentrations up to 100–1,000 times higher than in non-hyper-accumulating species, without exhibiting phytotoxic symptoms [25]. According to Baker and Brooks [26], when grown in HM rich soils, hyper-accumulating plants acquire more than 1000 mg kg-1 dry weight of HMs in their shoots. For successful soil restoration, plants growing in contaminated areas need to be tolerant to HMs, be extremely competitive, have quick growth rates, and produce more aboveground biomass [21, 27]. A number of plants are associated with phyto-extraction processes. *Ipomoea aquatica* is an aquatic macrophyte, commonly used as heavy metal phyto-extractor [28, 29]. Again, in order to improve the efficacy of phyto-extraction in HM polluted soils, plant growth regulators (PGRs) assisted phyto-extraction has been examined as a promising phytoremediation approach [30-32]. Exogenous PGRs like auxin, gibberellin (GA_3), cytokinin (CKs), abscisic acid (ABA), ethylene (ETH), brassinosteroid (BR), salicylic acid (SA), strigolactones (SL) and jasmonic acid (JA) etc. were shown to be able to help phyto-extraction by positively influencing plant respiration, biomass yield and HM accumulation in above-ground plant tissues. Exogenous PGRs act in a variety of modes affecting plants in different ways and have powerful effects on plants physiology even in a low concentration [31-33]. Indole-3-acetic acid (IAA) belongs to cytokinin group of PGR that enhance chlorophyll synthesis, root-shoot initiation, cell differentiation and cell division. Exogenous cytokinin, such as IAA application increase transpiration rate and heavy metal absorption in different crop plants [34]. However, an experiment was done to evaluate the PGR assisted phyto-extraction potential of *Ipomoea aquatica* assisted by indole-3-acetic acid (IAA) exposed to lead stress in hydroponic culture.

2. Material and Method

2.1. Plant Material and Seedlings Preparation

Ipomoea aquatica (kalmi) seeds were collected from the local market. Seeds were surface sterilized with 0.1% $HgCl_2$ and grown on sand mixed with Hoagland nutrient media. A moderately acid regime (from pH 5.0 to 6.5) has been found to be suitable for most plants, that's why the pH of nutrient media was adjusted at a range of 5.8-6.2.

2.2. Plant Growth Condition, Treatments and Data Collection

After 25 days of seedling growth, healthy and equal sized seedlings were transferred to hydroponic pots (10L) containing with Hoagland solution (HS), 200 μM Pb (HM) as lead nitrate and 200 μM Indole-3-acetic acid (IAA). Control plants were grown only in HS supplemented with 200 μM Pb. Seedlings were carefully kept in the position so that the roots were in touch with solution. Oxygen was supplied with air bubbler into solution. All the pots were kept in a growth chamber (55 PPFD light, 70 % relative humidity, 25 °C). IAA (200 μM) were sprayed on the plants at five days' interval up to harvesting. The plants were grown for 75 days and pH of the nutrient solutions were maintained 5.8-6.2 and solutions were changed every after three days. The treatment combinations were as follows: HS + HM (200 μM Pb) = Control, HS + HM (200 μM Pb) + IAA (200 μM) (spray) = Control + IAA (spray), HS + HM (200 μM Pb) + IAA (200 μM) (dissolve) = Control + IAA (dissolve) and HS + HM (200 μM Pb) + IAA (200 μM) (spray+dissolve) = Control + IAA (spray+dissolve). In this experiment, three replications were used.

After 75 days of growing, leaf greenness was measured by leaf color chart. Harvesting was done carefully with no root damage. After harvesting plant height, root length, root dry weight, stem dry weight and leaf dry weight were measured.

2.3. Determination of Pb Accumulation

Lead (Pb) accumulation in plant parts namely stem, root, and leaves were analyzed as previously described [35]. The bioaccumulation factor (BCF) provides an index of the ability of the plant to accumulate the metal with respect to the metal concentration in the substrate. BCF and translocation factor (TF) of Pb were calculated as-

$$\text{Translocation factor (TF)} = \frac{\text{Pb concentration on shoot or leaf}}{\text{Pb concentration on root or shoot}}$$

$$\text{Bioaccumulation factor (BCF)} = \frac{\text{Content per gram dry plant tissue}}{\text{Initial concentration of Pb in the medium}}$$

2.4. Statistical Analysis

IBM SPSS Statistics V.25 was used to conduct the statistical analysis. One-way analysis of variance was used to find significant differences, which was followed by Tukey HSD ($p < 0.05$).

3. Results and Discussion

3.1. Effect of IAA on Growth Characteristics of *I. Aquatic* under Pb Stress

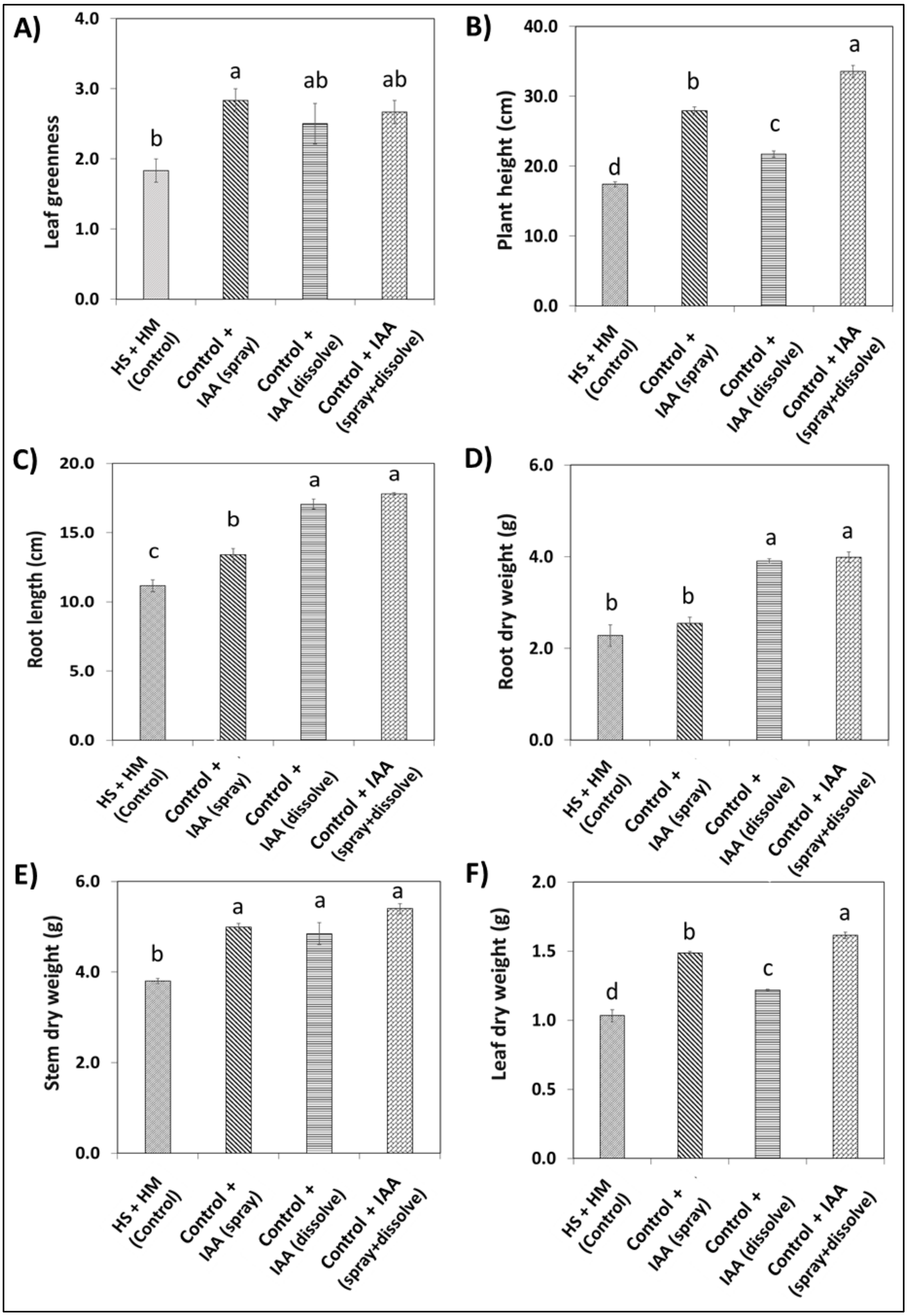
Growth characters such as leaf greenness, plant height, root length, root dry weight, stem dry weight and leaf dry weight of *I. aquatica* were significantly influenced by different level of treatments (Figure 1A-1F). Data revealed that application of control + IAA (spray) produced the maximum leaf greenness (2.8) of *I. aquatica* which was as good as control + IAA (dissolve) and control + IAA (spray + dissolve), and the minimum leaf greenness (1.8) was observed for HG + HM (control) (Figure 1A). Again, findings from this experiment revealed that the tallest plant (33.6 cm) of *I. aquatica* was found for control + IAA (spray + dissolve) compared to control + IAA (spray) and control + IAA (dissolve), while the shortest plant (17.40 cm) was observed for HG + HM (control) (Figure 1B). On the other hand, in case of root length and root dry weight, the longest root length (17.8 cm) and the maximum root dry weight (4.0 g) of *I. aquatica* was found for control + IAA (spray + dissolve) where they were statistically identical to control + IAA (dissolve). While, the shortest root length (11.20 cm) and the minimum root dry weight (2.3g) was observed for HG + HM (control) (Figure 1C, D). In case of stem dry weight, results revealed that application of control + IAA (spray + dissolve) produced the maximum stem dry weight (5.40 g) of *I. aquatica* which was at par with control + IAA (spray) and control + IAA (dissolve), and the minimum stem dry weight (3.8 g) was observed for HG + HM (control) (Figure 1E). Similarly, findings indicated that the maximum leaf dry weight (1.6 g) of *I. aquatica* was found for control + IAA (spray + dissolve) compared to control + IAA (spray) and control + IAA (dissolve), while the minimum leaf dry weight (1.0 g) was observed for HG + HM (control) (Figure 1F).

The results indicate that the application of IAA had a positive effect on the different morpho-physiological characteristic of *I. aquatica*. Evidence suggested that numerous plant development traits suffer when they are exposed to heavy metal stress, which lowers biomass production by interfering with various metabolic processes [36]. However, application of PGRs have been suggested as a way to reducing metal damage to plants and increase their metal tolerance [37, 38]. There have been numerous reports that IAA can increases root growth, shoot growth, and dry matter of plants that were stressed by heavy metals [39, 40]. Ji, *et al.* [41], found that IAA applied to *Solanum nigrum* plants grown on a heavy metal contaminated soil alleviated the toxic effects of pollution and increased biomass. This is most likely because PGR promoted cell division, increased photosynthetic activity, facilitated cell elongation, and promoted the build-up of dry matter [36].

3.2. Effect of IAA on Accumulation of Pb (mg g^{-1}dw) in different Tissues of *I. Aquatica*

Accumulation of Pb was observed to be significantly higher in root than that in stem, and leaf in all Pb-exposed concentrations (Table 1). In stem, the maximum accumulation of Pb (3066.00 mg g^{-1}dw) was found for control + IAA (spray + dissolve) compared to control + IAA (spray) and control + IAA (dissolve). While, the minimum accumulation of Pb (869.33 mg g^{-1}dw) was observed for HG + HM (control). In root, findings of the experiment revealed that the maximum accumulation of Pb (7173.67 mg g^{-1}dw) was found for control + IAA (spray + dissolve) compared to control + IAA (dissolve) and control + IAA (spray). While, the minimum accumulation of Pb (2107.33 mg g^{-1}dw) was observed for HG + HM (control). Similarly, in leaf, we can notice that the maximum accumulation of Pb (1829.67 mg g^{-1}dw) was found for control + IAA (spray + dissolve) compared to control + IAA (spray) and control + IAA (dissolve). While, the minimum accumulation of Pb (522.00 mg g^{-1}dw) was observed for HG + HM (control). On the other hand, in case of whole plant, results of the experiment revealed that the maximum accumulation of Pb (12069.33 mg g^{-1}dw) was found for control + IAA (spray + dissolve) compared to control + IAA (dissolve) and control + IAA (spray). While, the minimum accumulation of Pb (3498.67 mgg^{-1}dw) was observed for HG + HM (control). Again, in aerial part, by observing data we can easily state that the maximum accumulation of Pb (4895.67 mg g^{1} dw) was found for control + IAA (spray + dissolve) compared to control + IAA (spray) and control + IAA (dissolve). While, the minimum accumulation of Pb (1391.33 mg g^{-1}dw) was observed for HG + HM (control).

Figure-1. Effect of IAA on A) Leaf greenness; B) Plant height; C) Root length; D) Root dry weight; E) Stem dry weight; F) Leaf dry weight under Pb stress condition. The data are presented as means ±SE, with a sample size n = 3. By using Tukey HSD (p < 0.05), different letters between treatments were examined



The amount of Pb extracted by different parts such as stem, root and leaf of *I. aquatica* were significantly increased by the application IAA as dissolved and or exogenous spray, in respect to the control. The research by Hadi, *et al.* [42] and Saleem, *et al.* [43] reported that plants growing in contaminated soils absorb metals more readily when treated with different growth hormones. According to Ji, *et al.* [41], the addition of IAA treatment at 100 mg L^{-1} increased the Cd uptake by *S. nigrum* in roots and shoots. Similarly, in our study we observed that application of IAA as dissolved in combination with exogenous spray of found to be more effective than other treatments. On the other hand, Saad, *et al.* [29] and Laghlimi, *et al.* [44] suggested that strong root architecture of *I. aquatica* results into higher uptake of Pb from soil to the areal parts of the plant.

Table-1. Accumulation of Pb (mg g^{-1}dw) in different tissues of *I. aquatica*. The data are presented as means ±SE, with a sample size n = 3. By using Tukey HSD ($p < 0.05$), different letters between treatments were examined

Pb concentration (µM/L)	Mean Pb accumulation in different tissues				
	Stem	Root	Leaf	Whole plant	Aerial part
HS + HM (Control)	869.33 ± 38.12 d	2107.33 ± 65.41 d	522.00 ± 27.22 c	3498.67 ± 81.87 d	1391.33 ± 21.18 d
Control + IAA (spray)	2317.00 ± 31.88 b	4531.33 ± 221.95 c	1034.33 ± 31.05 b	7882.67 ± 340.69 c	3351.33 ± 118.88 b
Control + IAA (dissolve)	1958.00 ± 10.21 c	6231.33 ± 183.37 b	951.33 ± 56.96 b	9140.67 ± 148.49 b	2909.33 ± 57.04 c
Control + IAA (spray+dissolve)	3066.00 ± 61.25 a	7173.67 ± 85.08 a	1829.67 ± 71.92 a	12069.33 ± 198.32 a	4895.67 ± 113.62 a

3.3. Bioaccumulation Factor (BCF) and Translocation Factor (TF) of Pb in different Tissues of *I. Aquatica*

Bioaccumulation factor of Pb was observed to be significantly higher in root than that in stem and leaf in all the Pb-exposure concentrations (Table 2). In stem, the maximum bioaccumulation factor of Pb (15.33) was found for control + IAA (spray + dissolve) compared to control + IAA (spray) and control + IAA (dissolve). While, the minimum bioaccumulation factor of Pb (4.35) was observed for HG + HM (control). Again, in root, after examine the data it was noticed that the maximum bioaccumulation factor of Pb (35.87) was found for control + IAA (spray + dissolve) compared to control + IAA (dissolve) and control + IAA (spray). While, the minimum bioaccumulation factor of Pb (10.54) was observed for HG + HM (control). On the other hand, in leaf, data stated that the maximum bioaccumulation factor of Pb (9.15) was found for control + IAA (spray + dissolve) compared to by control + IAA (spray) and control + IAA (dissolve). While, the minimum bioaccumulation factor of Pb (2.61) was observed for HG + HM (control).

The root to shoot TF values were opposite to the shoot to leaves TF values (Figure 2A-2B). The maximum translocation factor was observed in shoot to leaf rather than root to shoot. In case of root to shoot, the maximum TF value (0.5118) was found for control + IAA (spray) compared to HG + HM (control) and control + IAA (spray + dissolve). While, the minimum TF value (0.3148) was observed for control + IAA (dissolve) (Figure 2A). On the other hand, in case of shoot to leaf, the ratio indicated that the maximum TF value (0.6051) was found for HG + HM (control) which was at par with control + IAA (dissolve) and control + IAA (spray + dissolve). While, the minimum TF value (0.4469) was observed for control + IAA (spray) (Figure 2B).

Figure-2. Effect of IAA on translocation factor A) Root to Shoot; B) Shoot to Leaves under Pb stress condition. The data are presented as means ±SE, with a sample size n = 3. By using Tukey HSD ($p < 0.05$), different letters between treatments were examined

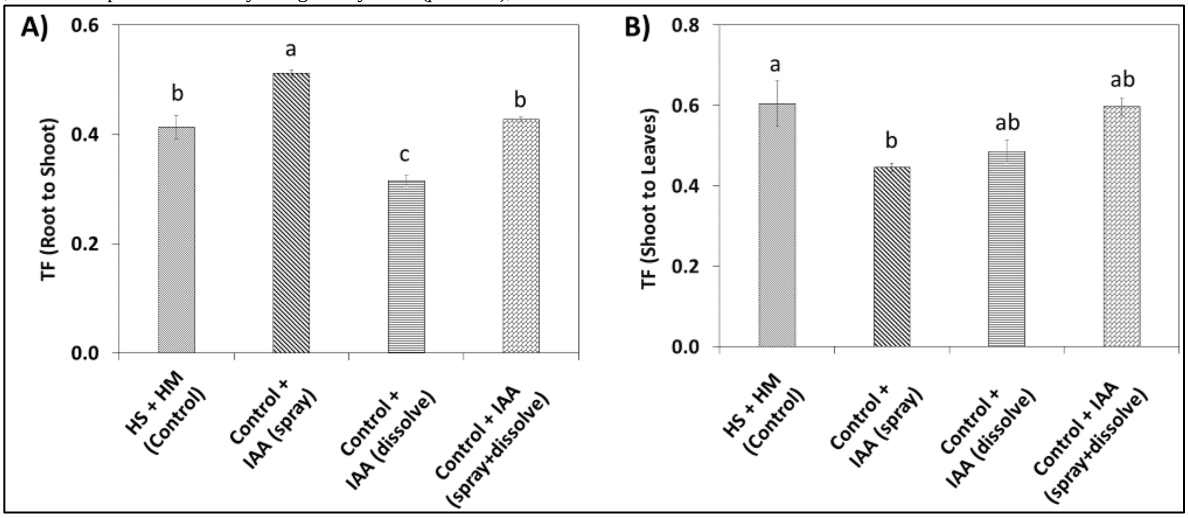


Table-2. Bioaccumulation factor of Pb in different tissues of *I. aquatica*. The data are presented as means ±SE, with a sample size n = 3. By using Tukey HSD ($p < 0.05$), different letters between treatments were examined

Pb concentration (µM/L)	Bioaccumulation factor		
	Stem	Root	Leaf
HS + HM (Control)	4.35±0.19 d	10.54±0.33 d	2.61±0.14 c
Control + IAA (spray)	11.59±0.46 b	22.67±1.11 c	5.17±0.16 b
Control + IAA (dissolve)	9.79±0.05 c	31.16±0.92 b	4.76±0.28 b
Control + IAA (spray+dissolve)	15.33±0.31 a	35.87±0.43 a	9.15±0.36 a

Wei, *et al.* [45], suggested that the phyto-extraction ability of plants depends largely on the bioaccumulation factor (BCF) and translocation factor (TF). Furthermore, Marrugo-Negrete, *et al.* [46] reported that, only plant species with BCF and TF values more than one have the potential to be exploited for phyto-extraction. Our results showed all the bioaccumulation factor of Pb were higher than one, which indicate that *I. aquatica* can be introduced as a Pb phyto-extraction plant. Chanu and Gupta [28] and Saad, *et al.* [29] also found high BCF and TF in *I. aquatica* and identifies it as a Pb hyper-accumulator plant. Again application of IAA in different mode such as dissolved and or sprays significantly increased different growth parameter and, BCF and TF as well. This outcome supports the hypothesis that phyto-extraction can be improved by increasing both the growth and, the metal accumulation in the upper parts of the plants assisted by plant growth regulators (IAA).

4. Conclusion

The above experiment revealed that, exogenous use of IAA is potential to enhance Pb-phyto-extraction by increasing Pb-accumulation rate in different plant parts of *Ipomoea aquatica*. Application of IAA improve different morpho-physiological characteristic including root growth, shoot growth, total biomass etc. and reduce toxic effects in plant. This is because PGRs helps in cell division, cell elongation, and photosynthetic performance which ultimately facilitate heavy metal accumulation. Our findings also demonstrate that all of the Pb-bioaccumulation factors are more than one, suggesting that *I. aquatica* might be used as a Pb phyto-extractor and IAA is very much helpful for this remediation process.

Data Availability

All the necessary data are included in the manuscript. If additional data are required, the corresponding author can be contacted.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

Conceptualization: Prosenjit Sarker and Shahin Imran; methodology: Prosenjit Sarker and Shahin Imran; experiment conductance: Prosenjit Sarker, Shahin Imran, Newton Chandra Paul and Md. Asif Mahamud formal analysis: Shahin Imran; writing—original draft preparation: Prosenjit Sarker, Newton Chandra Paul, Md. Asif Mahamud, Md. Assaduzzaman and Tusher Chakrobarty; writing—review and editing: Shahin Imran, Md. Assaduzzaman; visualization: Shahin Imran, Md. Assaduzzaman and Tusher Chakrobarty. All authors have read and agreed to the published version of the manuscript.

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