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# ANATOMICAL AND PHYSIOLOGICAL RESPONSES OF ARSENIC HYPERACCUMULATING WATER SPINACH (*IPOMOEA AQUATICA*)

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**Abstract**

Phytoremediation is reported to be a highly efficient and environmentally friendly method for cleaning up soils contaminated with metals and metalloids. The potential of water spinach (*Ipomoea aquatica*) as a hyperaccumulator plant has been investigated through physiological and anatomical adaptations during this study. Three concentrations of arsenic (As), namely 50, 100, and 150 µM, along with a mock treatment, were tested at 15 and 30 days of treatment. Due to As application, the plants exhibited stunted growth, characterized by reduced plant height and weight compared to the controls. Additionally, As toxicity led to a decrease in chlorophyll content in the leaves of water spinach. Although the total protein content increased in roots treated with 50 and 100 µM As, it declined with the highest As treatment. As exposure caused a significant linear increase in proline content in the roots at both 15 and 30 days of treatment. The roots altered their internal structure by reducing diameter, cortical cell layers, lacunae in the cortex, and vascular tissue in response to As. Stem and leaf anatomy revealed that the primary site of As effect is the root, although it exhibited altered anatomy, particularly in vascular tissues, at the highest As concentration (150 µM).

## Introduction

The Bengal Delta Plain, formed by sedimentation from rivers and their tributaries and distributaries, contains significant amounts of arsenic (As) in its groundwater and sediments due to hydrogeochemical, geological, and biological factors that promote As mobilization. Human activities such as burning fossil fuels for energy, improper disposal of municipal waste, widespread use of fertilizers and pesticides, and irrigating with effluent contribute to increased levels of heavy metals, including As (Angon *et al.* 2024), effects on rice field weed diversity (Aziz and Ahmed 2010) in a country like Bangladesh that depends heavily on agriculture. Areas with high As concentrations ($\geq$0.01 mg/l) pose a serious issue in terms of As transfer from soil to water and plants. The target hazard quotient (THQ) for As was above the safety level (THQ>1), and the target cancer risk (TR) exceeded acceptable levels (10-4) for most vegetables studied in Bangladesh (Nowar *et al.* 2024). About 20 million people in Bangladesh use tube wells with As levels above the permissible limit, putting them at potential risk of As contamination. Long-term exposure to As can lead to serious health issues, including internal cancers of the skin, lungs, bladder, and kidneys, which can threaten life (Bashir *et al.* 2021). Therefore, it is urgent to monitor and control the entry of heavy metals into food chain (Shetty *et al.* 2025).

The study of As uptake in plants is important for two reasons: first, it indicates the fraction of As in the soil that is readily available for plant absorption, providing valuable toxicological information; second, it shows the potential of certain plant species for phytoremediation and cleanup of As from contaminated sites (Zhao *et al.* 2009). Phytoremediation is an efficient and eco-friendly method that can be up to 20 times more affordable than traditional techniques like oxidation, reduction, precipitation, ion exchange, adsorption, lime treatment, solid-liquid separation, physical exclusion, and reverse osmosis (Mocek-Płóciniak *et al.* 2023, Sahoo *et al.* 2025). If effectively applied to contaminated soil and water, this technology could be both economically feasible and socially acceptable in Bangladesh and other countries.

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*Ipomea aquatica*, commonly known as kolmi shak in Bangladesh, is an affordable source of green leafy vegetables for rural communities living in wetlands, valued for its distinctive taste and medicinal properties. It is also cultivated commercially in dry lands and widely consumed by urban populations. Studies have reported higher As accumulation in plants grown in wetlands and increased As concentrations in different parts of *I. aquatica* (Sultana *et al.* 2015). However, no research has yet examined the anatomical and physiological responses of *I. aquatica* to toxic As exposure, which could help explain what contributes to its metalloid tolerance. Therefore, this study aims to investigate the effects of As on the growth and physiology of *I. aquatica* and to explore the role of metabolites and anatomical structures in conferring As tolerance in this species.

**Materials and Methods**

Seeds of *I. aquatica* named BARI Gimakalmi were collected from Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh and stored in a refrigerator at 4°C. Seeds were thoroughly washed in running tap water, after being kept in 10% sodium hypochlorite solution for 5 min. The experiments were conducted in the Department of Botany, University of Dhaka.

Plants were grown in sand culture, and the earthen pots were filled with purified sand (≤80% silicate) according to Hewitt (1953). Surface-sterilized seeds were sown in each pot filled with purified sand. The pots were kept in a net house at a day/night temperature of 30 ± 2ºC / 25 ± 2ºC. To analyse the effect of As, sodium arsenite (LobaChemie, India) was utilized. Half-strength Hoagland solution was only applied to mock plants and 50, 100 and 150µM sodium arsenite solutions were added with half-strength Hoagland solution to treated plants at alternate day up to 30 days. Physio-biochemical experimental data were collected at $15^{th}$ and $30^{th}$ day of As treatment using the root samples only. Length was measured in cm with a scale and fresh weight (FW) with an electric balance (Scaltec, India) at $15^{th}$ and $30^{th}$ days after As treatment. Chlorophyll estimation of leaves was done according to the method of Arnon (1949). Determination of protein and proline was done according to the method of Lowry *et al.* (1951) and Bates *et al.* (1973), respectively. A Shimadzu UV Spectrophotometer UV-1800 was used to determine the absorbance of all the studied factors. Anatomical study was conducted using $21^{st}$ day old samples from root, stem and leaves. Temporary slides were prepared and studied under microscope (Carl Zeiss Lab A1 microscope) fitted with a digital camera (AxiocamERc 5s). Micrographs were taken through the Axio Vision Release 4.8.2 software. Statistical analysis of collected data was conducted with statistical software (GraphPad Prism 9.4.1). ANOVA was used to test for differences between different concentrations of As treatments on the same days of treatment against the control treatment.

**Results and Discussion**

A visible morphological symptom of As toxicity was found to be stunted growth. Total plant height, including the individual length of root and shoot, was observed to be decreased. Moreover, density of root system was reduced with increasing concentrations of As compared to the mock (Fig. 1a). The height of the As-treated plant root was decreased by 56.25 to 15.5% in the studied concentrations of As at 15 days of treatment. The height of the shoot decreased by 84.37 to 53.12% and the whole plant height declined gradually by 78 to 45.6% from 50 to 150 µM of As concentration at 15 days post-treatment. As reduced the height of the root by 61.43 to 15.35% at 30 days of treatment. Similarly, As decreased the height of the shoot by 80 to 41% and the whole plant height by 76.74 to 36.57% at 30 days of treatment (Fig. 1b). Unlike height measurements, the fresh weight (FW) of the whole plant did not change significantly at 50µM of As exposure compared to the control at 15- and 30-days post-treatment. Treatment with 100 and 150 µM of As decreased the FW of plants by 40 and 48% at 15 days post-treatment, while the decline was 57 and

69% at 30 days post-treatment for 100 µM and 150 µM of As, respectively (Fig. 1c). The effect of As toxicity on chlorophyll content in the leaves of *I. aquatica* plants has been declined compared to mock-treated leaves. Exposure to different concentrations of As showed a progressive reduction of total chlorophyll pigment of leaves (Fig. 2a).

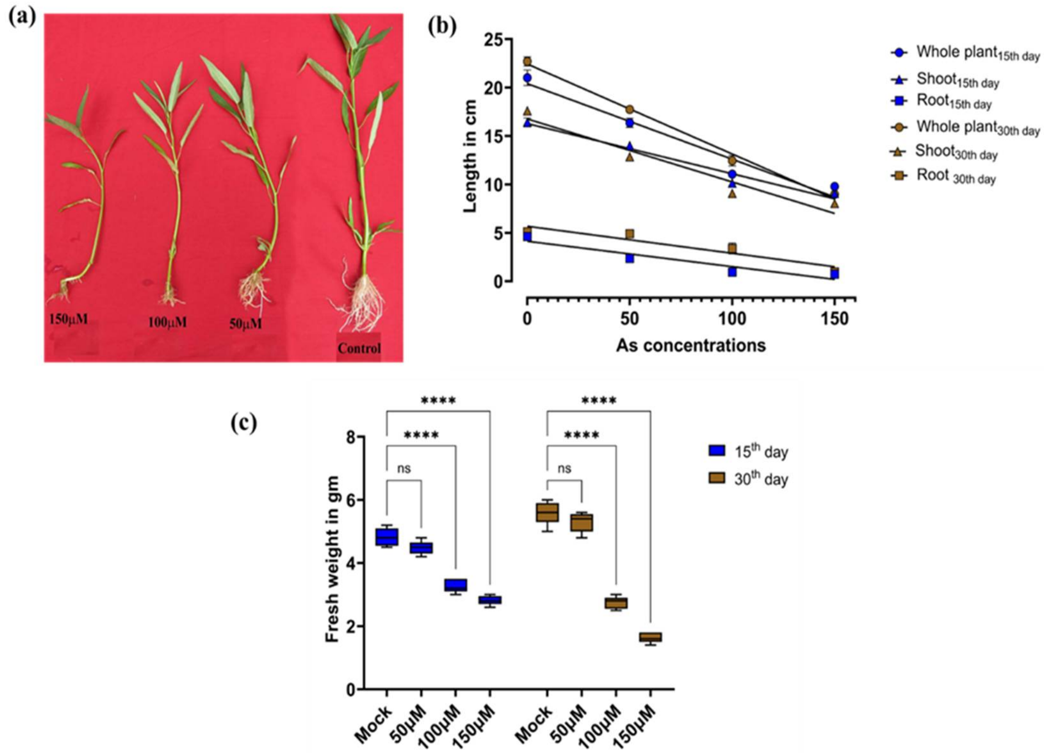


Fig. 1. Effects of different concentrations of As on growth of *I. aquatica* plants. (a): plants from each As concentration, (b): length of root, shoot and whole plant. (c): weight of whole plant. P value is significant when $P < 0.0001$ and insignificant when $P > 0.9999$.

As concentrations of 50 and 100µM showed a significant increment in protein content in the root of water spinach irrespective of days of treatment during the study. However, at the highest As treatment, though there was no significant increase but similar protein content as the mock was observed (Fig. 2b). The protein content decreased to 47 and 68% at 150µM from 100 µM at 15$^{th}$ and 30$^{th}$ days of treatment, respectively. The decrease at 150µM denotes *I. aquatica* may be intolerant to As or unable to stabilize the metalloid at this concentration. Interestingly, the amount of protein in the examined samples, irrespective of As concentrations, decreased with the ageing of plants, denoting a down regulation of age-related gene expression. The present experiment demonstrated a significant increase in proline content exposed to As stress compared to the mock at the two different time-treated samples. Plants exposed to As showed a progressive increase in free proline content compared to mock after 15 days of treatment, and the increase was almost fourfold at 150µM compared to mock. Proline content was the highest at 150µM of As treatment on the 15$^{th}$ day of treatment. There was a decrease in proline content at 30 days of treatment with the highest As concentration compared to the similar As at 15 days of treatment (Fig. 2c).

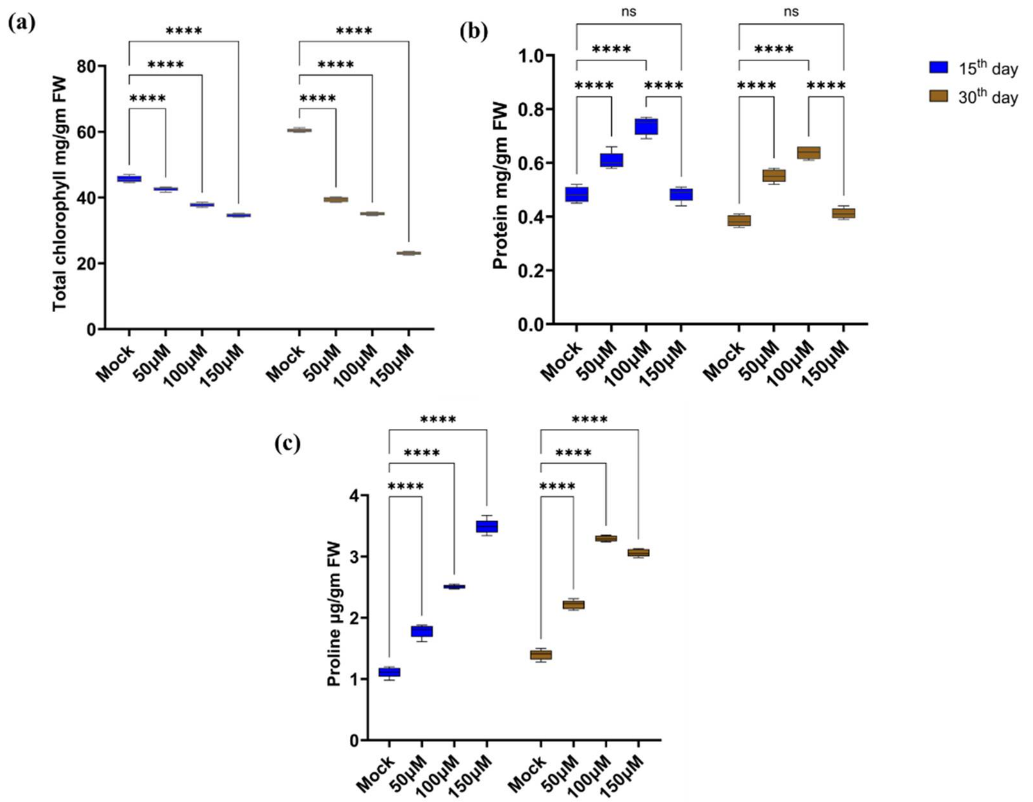


Fig. 2. Effects of different concentrations of As on growth of *I. aquatica* plants. (a): total chlorophyll content, (b): protein content in roots, and (c): same as (b) but with proline content. P value is significant when P <0.0001 and insignificant when P >0.9999.

In comparison to the mock, the root diameter decreased in the As-treated plants. Both in mock and As-treated roots, the epidermis was single-layered with thin-walled parenchymatous cell. The cortex is composed of thin-walled parenchymatous cells just beneath the epidermis. In the mock-treated root, 12-14 layers of cortical cells were present, whereas in the As-treated plant root, it was 8-10. Anatomical studies of the root also revealed a reduction of lacunae or air spaces in the cortex with progressing As exposure. Remarkable distortion of the vascular region was induced in root tissue by exposure to As, resulting in an indeterminate arch. Many smaller-sized metaxylem vessels were also found in 50 and 100μM As-treated roots, (Figs 3a-B and C). Besides, periderm formation was initiated at 100μM treated roots (Figs 3a-C) which was more definitely observed at 150 μM As-treated root samples (Figs 3a-D). The diameter of the stem was decreased in the As-treated stem. The stem is circular in outline in mock, whereas it is slightly wavy in As-treated stems. In the stem of mock and As-treated stem epidermis were monoseriate. However, the shape of the epidermal cells varied from rectangular to ovular in mock and As-treated samples, respectively. Between the epidermis and endodermis, cortical cells were present. The cortical cells of the As-treated stem occupied a smaller area than those of the mock stem. Vascular bundles were radially arranged, and secondary growth was observed in the stem of both mock and As-treated stems. In As-treated stem cambium ring was thin, and the development of xylem vessels was poor, resulting in reduced size and number compared to mock samples. Pith was also reduced in As-treated stem samples (Figs 3b A-D). Fewer anatomical alterations were found in As-treated leaves compared to other vegetative organs studied. Leaves of the As-treated plant were decreased in thickness. *I. aquatica* leaves have a collateral vascular bundle in the center of the midrib and are surrounded by a parenchymatous bundle sheath. Vascular areas in treated leaf became smaller as compared to mock leaf samples (Figs 3c A-D).

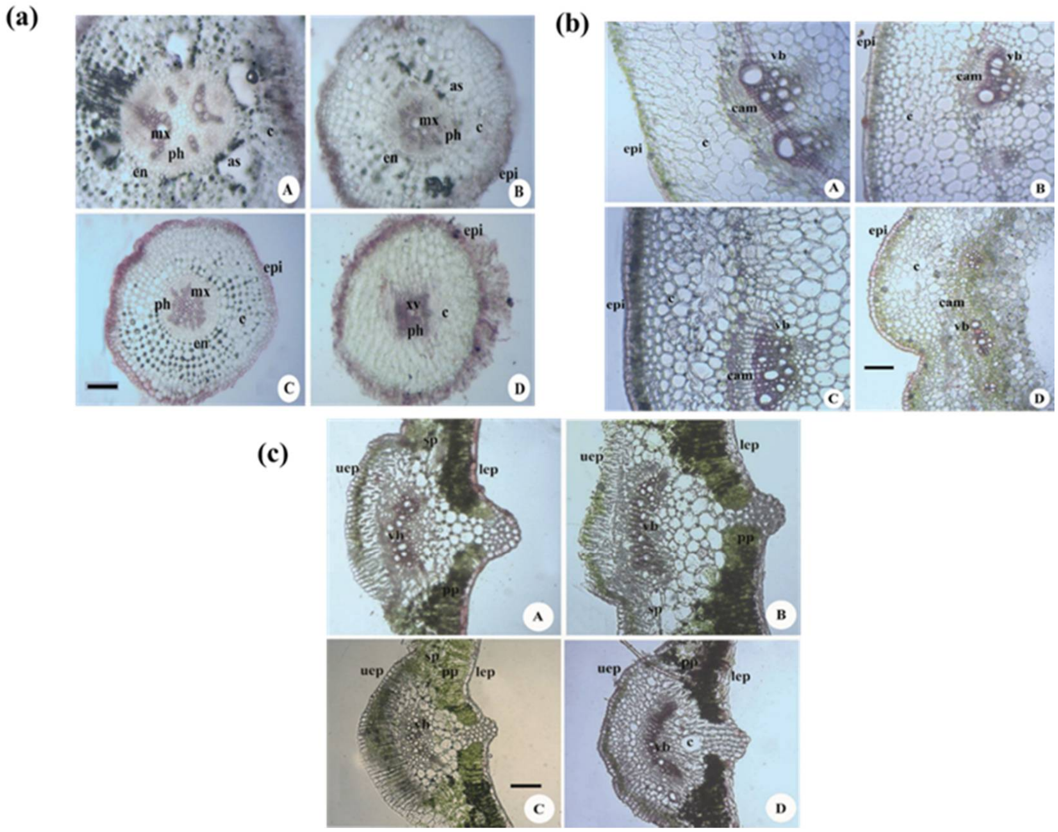

Fig. 3. Anatomy of vegetative organs of *I. aquatica* treated with different concentrations of As. (a) Transverse section of root. (b) Transverse section of stem. (c) Longitudinal section of stem. A: control, B: 50 µM, C: 100 µM, D: 150 µM As. epi: epidermis, c: cortex, en: endodermis, vb: vascular bundle, as: air space, ph: phloem, cam: cambium, mv: meta vessel, xv: xylem vessel, uep: upper epidermis, lep: lower epidermis, palisade parenchyma (pp) and sp: spongy parenchyma. Bar = 50 µm.

Several studies have examined how plants respond to As toxicity, highlighting their morphophysiological, metabolic, and molecular features (Abbas *et al.* 2018, Chandrakar *et al.* 2018). The present study has attempted to identify some of the diverse effects and implications of As on biochemical properties and anatomical structures in water spinach, since the mechanism of action of As in planta is poorly understood. Stunted growth with reduction of shoot and root length, alongside reduced FW of the whole plant, was documented under higher (100 and 150µM) concentrations of As-treated plants with increasing days of treatment. Such reduction was also reported from other plant species treated with As (Malik *et al.* 2011, Vromman *et al.* 2013). The present study found a decline in total chlorophyll content in As-treated leaves compared to mock up to the 15$^{th}$ day and 30$^{th}$ day of treatment, similar to findings with other plants like *Lemna minor* and *Hydrilla verticillata* (Duman *et al.* 2010, Srivastava *et al.* 2013, Abbas *et al.* 2018). However, toxicity symptoms in the appearance, such as wilting and violet coloration of leaves as reported in many crop plants with As treatment (Musil *et al*. 2014, Chandrakar *et al*. 2016), were not found in the studied sample (Fig. 1a), and exposure to As with our studied concentrations and days of treatment thus appeared not lethal.

The major sampling material chosen for the biochemical study was the roots, which was quite justified because the roots easily uptake the As from the sand. The studied concentrations of As increased protein content in the roots with variable levels compared to the mock, up to 100 µM of As. This was true for both time treatments, denoting the invariance of the age of the plants. However, exposure to As has been reported a decrease in total plant protein when As concentrations exceeded 300 µM (Singh *et al.* 2006, Gupta and Ahmad 2014). No significant changes were found between the mock and the highest As concentration (150µM) during the present study, indicating a dose-specific response of As. Proline is an important amino acid and is involved in plant growth regulation through signaling processes and is known to detoxify As-induced toxicity. Moreover, As-regulated oxidative stress could be successfully reversed in plants by exogenous supply of proline (Singh *et al.* 2015). The present study showed that proline increased significantly in response to different As treatments at both times of application. However, the exact mechanism through which proline can reduce the toxicity of As for the current study was not determined. There might be varieties of approaches as quenching of As-induced ROS generation, increasing activities of various antioxidants, altering stress-related gene expressions, and synthesis of phytochelatins (PCs), thereby sequestering As and improving plant tolerance against As (Chandrakar *et al*. 2017).

An interesting anatomical adaptation of the As-treated samples revealed transformation from aquatic to xerophytic, with a decrease of lacunae/air space in the cortex region. Moreover, deposition of phenolic compounds in the cortical region of the treated plant samples directly affects the phytostabilizing potential of *I. aquatica* (Figs 3a A-C). Smaller metaxylem vessels and reduced cortical layers similar to present findings have been reported in As-treated soybean and Cd-treated roots (Armendariz *et al*. 2016, Liza *et al*. 2020). Under waterlogged conditions, aerenchyma and Casparian bands were reported to develop in water spinach roots. These structural alterations improved anaerobic respiration enzyme activities and increased adventitious root activity, leading to better root system adaptation and function (Peng *et al.* 2024). However, under the present system, we did not find such alterations. Not only the root, but above-ground portions were affected by As, as observed from reduced shoot and whole plant length, aligning with the observed anatomical alteration in stem and leaf epidermal, vascular, and cortical tissues. Such alterations were also reported in chickpea treated with Cd (Liza *et al*. 2020). The anatomical alteration showed a gradual effect as root>shoot>leaf, determining the primary area of effect.

Anatomical adaptation alongside the alteration of plant metabolism might be reasons behind the As-treated reduced growth in *I. aquatica*. Thus, examination of varying As concentrations on *I. aquatica* appears to be growth-limiting, but no visible lethal effect denotes its potential as an As accumulator with a limit of 150µM concentration. Further analysis on enzymatic and non-enzymatic antioxidant analysis, gene expression of As transporters, and measurement of accumulated As in *I. aquatica* will widen better understanding.

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