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Evaluation of deleterious impacts of arsenic oxide on morphology and biochemistry of water lily plant

*Mirza Naseer Ahmad[1], Rashida Sultana[2], Maleeha Umber[*2] and Abdul Wadood[2]*

[1]*Department of Geography, Faculty of Earth Sciences, Nusrat Jahan College Rabwah, Chenab Nagar, Pakistan and
[2]Department of Botany, Faculty of Life Sciences, Nusrat Jahan College Rabwah, Chenab Nagar, Pakistan*

Abstract

The experiment was performed to use water lily plant as bioremediation source against water dissolved arsenic oxide. Different concentrations of arsenic oxide (0, 100 and 200 mg/L) were dissolved in water. Arsenic dissolved water was placed in steel trays and lily plants were placed in arsenic contaminated water. On daily basis water was tested for arsenic levels using arsenic kit method. Up till four days lily plant was in good morphological order and dissolved arsenic concentration was decreasing because of absorption by lily plant. After one week arsenic was very much less in water which showed that it was effectively absorbed by water lily but plants' itself condition was adverse. Plant parts were subjected to various biochemical tests whose results reveled that arsenic oxide has disturbed plant growth. Hence, it has been concluded from this study that lily plant is non-resistant to arsenic stress after a certain concentration level.

Keywords: water lily, arsenic, stress, biochemistry, morphology

Full length article **Corresponding Author*, e-mail: maleeha.umber@njc.edu.pk

1. Introduction

Arsenic is one of the heavy metals found throughout the earth's crust as well as hydrosphere and is increasing day by day due to various natural and anthropogenic activities [1]. Arsenic in its different salt forms is polluting soils and water, in particular ground water by means of innate geological leaching from host sediments and rocks [2]. Irrigation of agricultural lands by such arsenic polluted water cause multiple types of defects in physiological, chemical, morphological, and anatomical attributes of crops. Even soil microbes are also affected by arsenic pollution. Damage to soil microbiology also indirectly contributes to retarded plant growth by decreasing soil fertility. Regular consumption of water containing high concentrations of arsenic can have adverse health effects, including skin disorders, lung cancer, and cardiovascular diseases [3]. In actuality, arsenic-contaminated water is one of the most serious global health threats, with currently estimated 150 million people relying on arsenic-contaminated groundwater [4]. The permissible concentration of arsenic in drinking water set by the World Health Organization (WHO) is 10 µg/liter whereas current rate of arsenic in Pakistan is 50 µg/liter [5].

At present time most common way to clean heavy metal contaminated water is a coagulation/filtration method that involves removing pollutants by chemically conditioning particles to agglomerate into larger particles that can be separated and settled, followed by running the contaminated water through various filters that trap the pollutants and hold them for disposal. One of the major problems with this method is the sludge-like by-product that is produced as a result of the settled and trapped contaminants [6]. Use of these methods for cleanup/disposal of contaminated water is very expensive and disruptive to the habitats that surround the water [7]. Scientists are searching for new and economic ways to alleviate this contamination problem. New and promising method that has been drawing interest for many years is called phytoremediation or phytoextraction. Phytoextraction is the use of plants to remove contaminants from water by pulling the contaminants out of the water through the root system and into the plant body [7]. This makes disposal easier and much less expensive by properly destroying the plants and the contaminants that are carcinogenic metals such as copper, chromium and mercury etc [8].

Water lilies are gaining foremost attention as phytoremediating aquatic plant. Water lilies are beautiful aquatic flowering plants that are distributed worldwide. The plant has wide-round leaves that float flat on the water surface. They are greenish in colour thus able to carry out

photosynthesis. Several Nymphaea species have also been used to purify heavy metal-contaminated water and soap-polluted wastewater [8]. This current research paper also describes phytoremediation working of water lily, which went good for few days. After one week plants were badly affected by absorbing arsenic oxide from water. Considering morphological status of water lilies, they were subjected to biochemical tests.

2. Materials and methods

An experiment was arranged at Department of Botany, Nusrat Jahan College Rabwah Pakistan, to check if water lily plants can successfully purify water from dissolved arsenic oxide without themselves being harmed and for how long, specific lily plants can show resistance against arsenic stress. Three steel trays were filled with equal amounts of water having dissolved arsenic oxide in each of them at following rates of concentrations: 0, 100 and 200 mg/L, respectively. Water lily plant was placed in each tray and left for 2 weeks. On daily bases water was tested using arsenic kit for calculating arsenic decline levels. After one week period, white crystals of arsenic were exuded out on stems and plant was wilted and leaves were burnt blackish colored.

2.1 Preservation and grinding

Water lily plants were removed from trays and stem, roots, leaves were separated. These plant parts were grinded in 50 mM potassium phosphate buffer and were then centrifuged at 14000 rpm for 15 minutes. After centrifugation supernatants were preserved in above mentioned buffer and various biochemical tests were carried out.

2.2 Total soluble proteins

Concentration of total soluble proteins was examined using previous method [9] with few amendments. The 1ml supernatant was reacted with 2ml Bradford Reagent and incubated for 15-20 min then reading was measured at 595 nm.

2.3 APX activity

The APX working was measured using the standard method [10]. The reaction solution (1600 µl) was comprised of 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 400 µl of enzyme extract. The absorbance was taken at 290 nm against the blank and the enzyme activity was represented in Umg^{-1} protein (U=change in 0.1 absorbance min-1 mg^{-1} protein).

2.4 H_2O_2 concentration

H_2O_2 concentration was determined according to the protocol described previously [11]. 0.1 ml of supernatant was added to 0.1 ml of 10 Mm potassium phosphate buffer (PH 7.0) and 1M IKI. The absorbance was taken at 390 nm.

2.5 Total phenolics

Total phenolics were evaluated with the help of Folin-Ciocalteu protocol [12] with few amendments. Samples were mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were shacked for fifteen seconds and were permitted to stand for 30 min at 40 ˚C so that the color develops. Then absorbance was taken at 765 nm on spectrophotometer.

2.6 MDA content

Malondialdehyde (MDA) was determined in accordance to method given in literature [13]. In the 2 ml TCA, added 2 ml of 0.6% thiobarbituric acid. It was heated at 100 ˚C for 20 minutes in water bath. After heating immediately cooled for 20 minutes and then centrifuged at 10000 rpm for 10 minutes. The resulting color was measured at 532 nm on spectrophotometer.

3. Results and discussions

3.1 Total soluble proteins

It is apparent from figure 1 that concentration of soluble proteins in all parts (roots, stem, leaves) of water lily decreased at both levels of arsenic oxide stress. However, comparing both stress levels, 100 mg/L and 200mg/L the second stress level showed more reduction in proteins in entire plant.

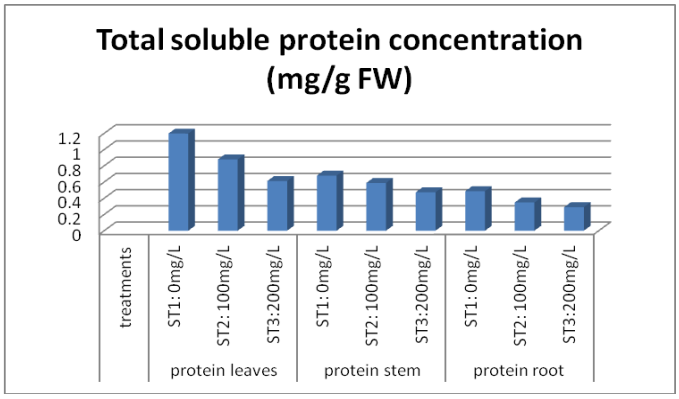

Figure 1. Figure represents decline in total soluble proteins at both levels of AsO

3.2 APX activity

APX activity has been decreased in all three plant parts of water lily in response to arsenic oxide stress. Among S2 and S3, S3 stress level has more drastically reduced APX activity (Figure 2).

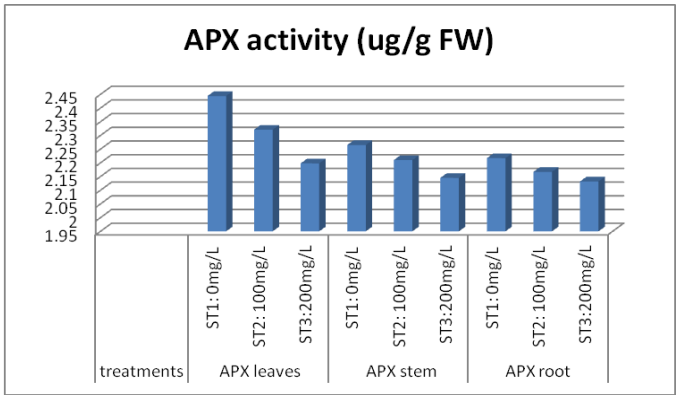

Figure 2. Figure shows reduced APX activity with increment in AsO stress level

3.3 MDA contents

According to Figure 3, all the three parts of water lily roots, stems and leaves have increased MDA content on exposure to S1 and S2 levels of arsenic oxide stress as compared to that group which is devoid of this contamination.

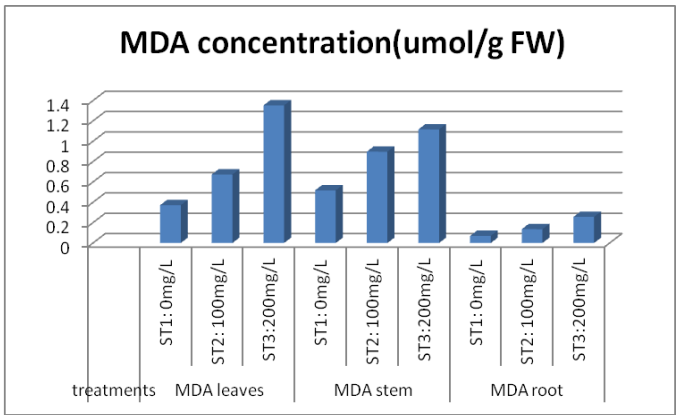

Figure 3. Figure represents an increase in MDA content at both levels of AsO

3.4 Hydrogen peroxide concentration

It is evident from results of figure 4 that roots, stems, and leaves of water lily have raised hydrogen peroxide concentrations in response to applied both levels of arsenic contamination. Comparing both levels of stress, it appeared that second level was more crucial to water lily plants.

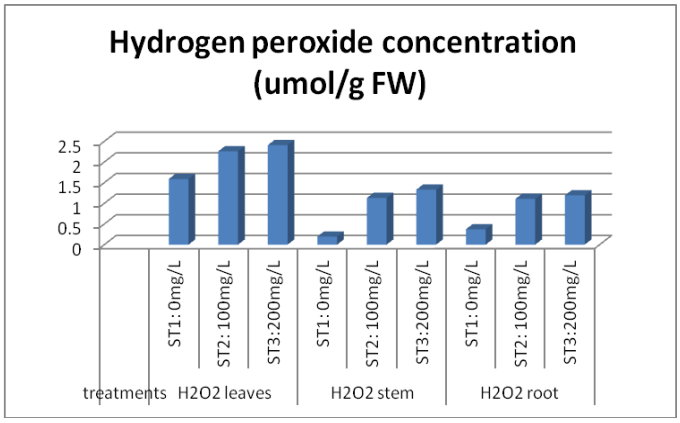

Figure 4. showing increased hydrogen peroxide concentrations at S2 and S3 stress levels.

3.5 Total phenolics

The results shown in figure 5 clarify that at both levels of AsO contamination S2 and S3 to water lily plant on the whole showed reduction in total phenolics content and among both these stress levels second one was more intense.

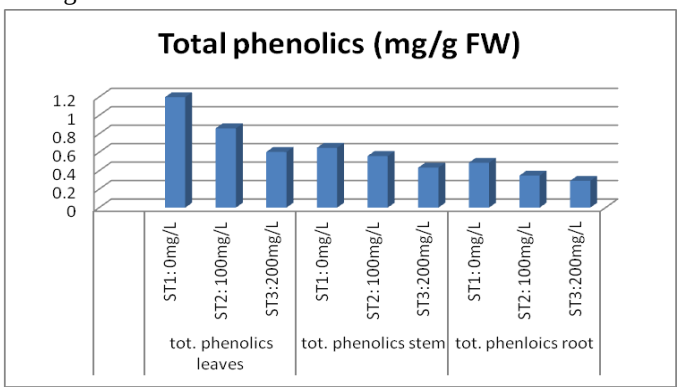

Figure 5. Decline in total phenolics with increase in AsO concentration

Our current research work has portrayed that water lily plant does absorb arsenic oxide from water to reduce arsenic contamination from water but this plant itself cannot resist from harms of this heavy metal stress after certain concentration level. Considering, all above results, it is very clear that stress of 100 and 200 mg/L causes increase in H_2O_2 and MDA content due to which reactive oxygen species are produced in larger amount as a result of which osmotic lysis of cells occurs and a condition of disturbance built up in cellular environment. On the other hand, reduction in phenolics and ascorbate peroxidase activity inhibits auxins from generating ROS scavenging proteins such as Glutathione S- transferase whose function is to denature ROS species. Thus, effective attempts are required to get rid of arsenic toxicity as it is causing alarming disruptions in ecosystem. Although, lily plant is very much popular for its phytoremediation potentials but it could not cope up with this heavy metal toxicity for longer duration.

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