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ORIGINAL ARTICLE

Arsenic Induced Oxidative Stress and Role of Scavenging Enzymes in Phytoremediation by *Pteris vittata* and *Eichhornia crassipes*

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Arsenic pollution is a growing menace in major parts of West Bengal, India and Bangladesh. Arsenic phytoremediation abilities of two common plants *Pteris vittata* and *Eichhornia crassipes* growing abundantly under natural tropical conditions of India were biochemically analyzed. Reactive oxygen species, taking total peroxide and malondialdehyde contents as the parameters, indicate the extent of arsenic induced oxidative stress, while the activities of the scavenging enzymes catalase, peroxidase and superoxide dismutase indicate the comparative effectiveness of the two plants in arsenic detoxification. Total peroxide and MDA contents were significantly higher in all samples of *Eichhornia* as compared to *Pteris* throughout the experimental period while the three scavenging enzymes *viz.* catalase, peroxidase and superoxide dismutase exhibited higher activities in *Pteris* with increasing arsenic concentration while *Eichhornia* showed a reverse trend. The comparative study reveals that *Pteris vittata* is the more efficient plant in combating and tolerating arsenic stress, as revealed by the results obtained of biochemical constituents and enzymatic profile. Of the two selected plant species, *Pteris* is found to be more effective in arsenic removal can serve as a cheap and easily available green source for arsenic detoxification.

Key words: Arsenic toxicity, oxidative stress, reactive oxygen species, plant antioxidant defense, stress amelioration

Arsenic (As) pollution is on the rise globally and is a looming environmental threat. Arsenic is found chiefly as arsenate (AsV) and arsenite (AsIII) showing a broad range of solubility controlled by the ionic environment and pH. Arsenate behaves as a phosphate chemical analog, taking the help of phosphate transporters to enter the plant system, resulting in disparity of phosphate supply (Finnegan and Chen, 2012). Within the cell, arsenate disrupts the phosphate-controlled metabolism including ATP synthesis causing toxicity. Arsenite toxicity is mainly due to its affinity to react with thiol (−SH) groups of enzymes and proteins with cysteine residues, disturbing their structure and function (Hasanuzzaman *et al*., 2015).

Phytoremediation, an important eco-friendly technology, uses different techniques, such as uptake, transport, translocation, and detoxification, to remediate such harmful metals and metalloids. Hyperaccumulator plants take up metals and metalloids from soils, translocate and store them in their above ground biomass (Reeves, 2006). Similarly, arsenic hyperaccumulator plants have also developed different approaches to accumulate and withstand high concentrations of As. Arsenic toxicity induces oxidative stress due to abundant production of reactive oxygen species (ROS). The hyperaccumulator plants by their strong antioxidative defenses could constitute an important arsenic detoxification strategy.

The objective of this work was to compare the arsenic phytoremediation abilities of two common plants *Pteris vittata* and *Eichhornia crassipes* both growing abundantly under natural tropical conditions of India. Arsenic pollution is a growing menace in major parts of West Bengal, India and Bangladesh. The two selected plant species, if found effective in arsenic removal could serve as cheap and easily available green sources for detoxification. An analyses of their reactive oxygen species, taking total peroxide and malondialdehyde contents as the parameters, could indicate the extent of arsenic induced oxidative stress, while the activities of the scavenging enzymes catalase, peroxidase and superoxide dismutase could indicate the comparative effectiveness of the two plants in arsenic detoxification.

MATERIALS AND METHODS

Pteris vittata and Eichhornia crassipes used for the experimental work were ensured to be of identical age. *Pteris* plants were germinated from spores and 4 month old plants were acclimatized in a hydroponic system to promote root growth. After acclimatization in 0.2 strength Hoagland nutrient solution for 2 weeks, the plants were transferred into 0.2-strength Hoagland nutrient solution containing different concentrations of arsenic as As (V), as sodium arsenate ($Na_2HAsO_4.7H_2O$). The finally selected concentrations of arsenic are 0, 130-133 and 267-270 µM. The plants were harvested at three intervals - 1, 5 and 10 days post arsenic treatment. *Eichhornia* was collected from local ponds and thereby grown, acclimatized and allowed to reproduce under laboratory conditions. The daughter plants obtained were of the same age and were taken as test materials. *Eichhornia* was also subjected to the same treatment with arsenic in the form of sodium arsenate. The treated plants were collected at three intervals, i.e. 1, 5 and 10 days post arsenic treatment. Control sets were maintained throughout for comparison.

The test materials were subjected to biochemical and enzymatic analyses using standard methods and tests. Estimation of total peroxide was done by ferrithiocyanate method of Thurman *et al.* (1972) and Malondialdehyde (MDA) was estimated by the method of Heath and Packer (1968). Catalase enzyme activity was assayed by the method of Gasper and Lacoppe (1968), peroxidase enzyme activity was assayed spectrophotometrically according to Chance and Maehly (1955) and superoxide dismutase (SOD) was assayed by the method of Marshall and Worsfold (1978). All the experiments were carried out in triplicates and then subjected to statistical analyses using analysis of variance (ANOVA) table.

RESULTS

The results showed that total peroxide content (Fig.1) and MDA content (Fig.2) was significantly higher in all samples of *Eichhornia* as compared to *Pteris* throughout the experimental period. The results of the enzymatic analyses revealed an identical trend in all the three assayed enzymes viz. catalase (Fig.3), peroxidase (Fig.4) and SOD (Fig.5). The three scavenging enzymes exhibited higher activities in *Pteris* with increasing arsenic concentration while *Eichhornia* showed a reverse trend.

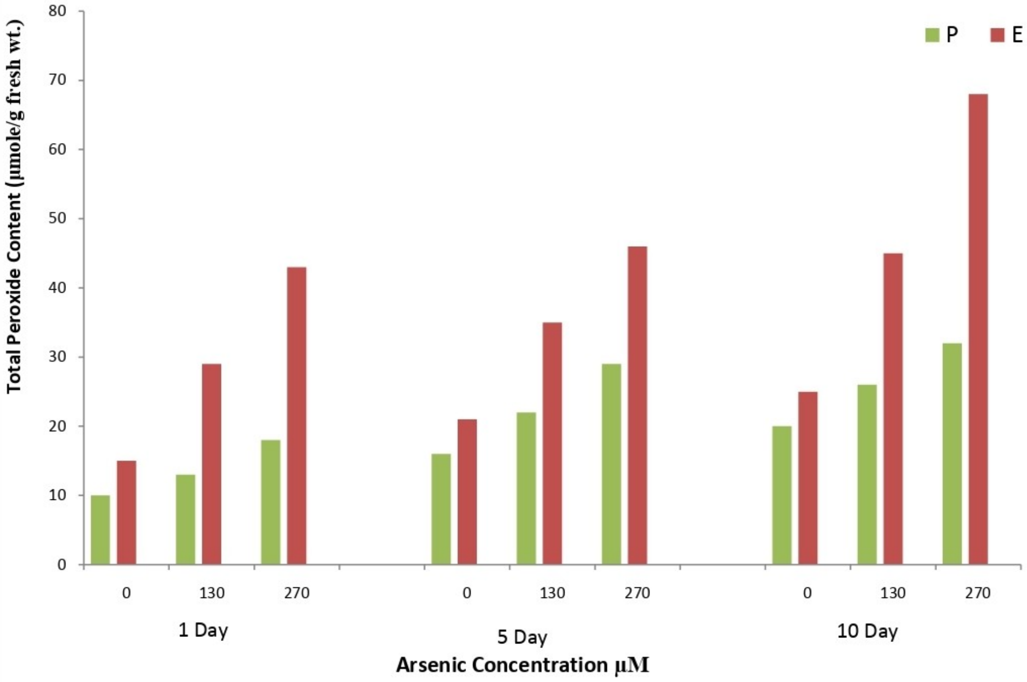

Figure. 1. Total Peroxide Content

P – *Pteris*, E - *Eichhornia*

S.E.= 0.74

C.D. =0.78 (5%)

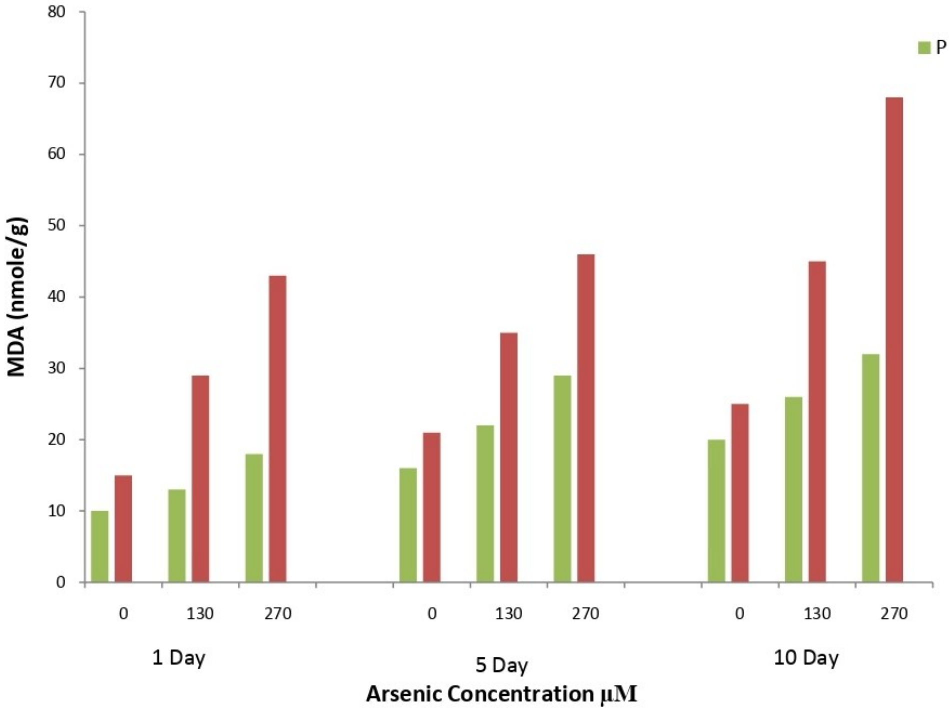

Figure 2. Malondialdehyde Content

P – *Pteris*, E - *Eichhornia*

S.E.= 0.73

C.D. =0.75 (5%)

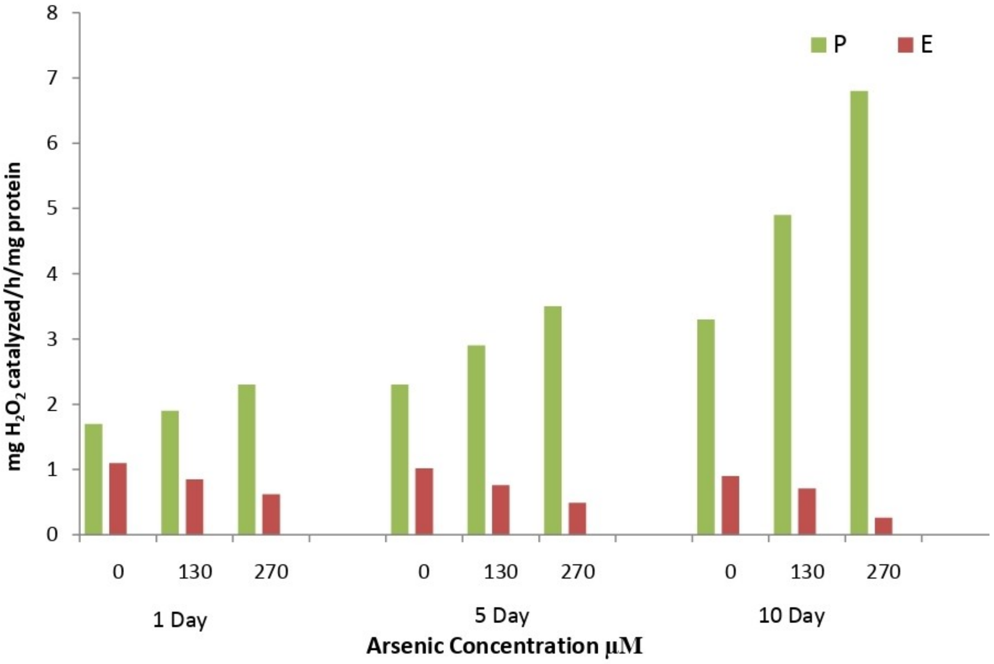

Figure 3. Catalase enzyme activity

P – *Pteris*, E - *Eichhornia*

S.E.= 0.26

C.D. =0.27 (5%)

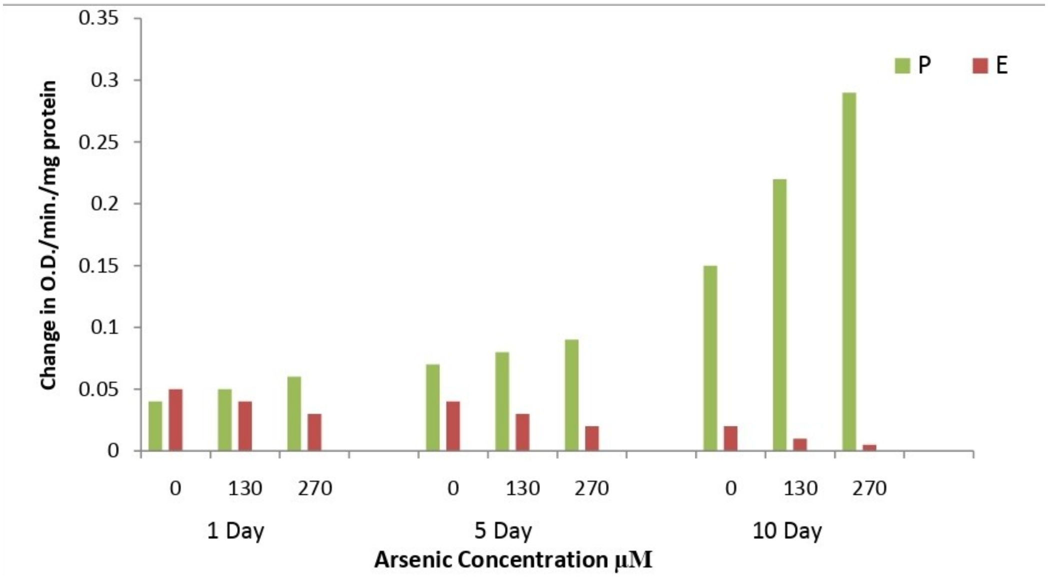

Figure 4. Peroxidase enzyme activity

P – *Pteris*, E - *Eichhornia*

S.E.= 0.07

C.D. =0.07 (5%)

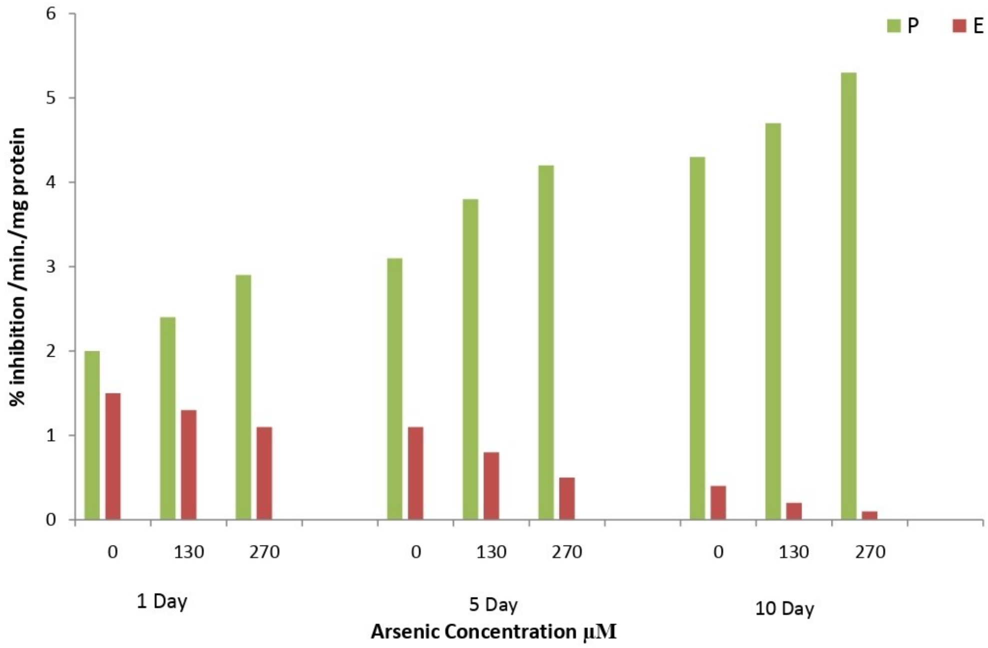

Figure 5. Superoxide dismutase (SOD) enzyme activity

P – *Pteris*, E - *Eichhornia*

S.E.= 0.07

C.D. =0.07 (5%)

DISCUSSION

In my earlier work in 2014 most of the arsenic concentration was reported in the fronds (*Pteris*) or leaves (*Eichhornia*) (Sen, 2014a,b). Further biochemical and enzymatic analyses was carried out with frond and leaf samples. The results obtained indicated *Pteris vittata* to be more efficient in arsenic uptake than *Eichhornia*. Arsenic concentration was high in 10 day old samples of *P. vittata* as compared to *Eichhornia* exhibiting lower uptake (Sen, 2014a,b). While *Eichhornia* showed significantly reduced concentrations of biochemical constituents *viz.* chlorophyll, carotenoids, protein, amino acid, nitrogen, total carbohydrates and total phenols even in low doses of arsenic, *Pteris* appeared to be relatively less affected. The significantly higher starch content in *Eichhornia* under arsenic toxicity is possibly an adaptation for survival under stress.

The Brake fern, *Pteris vittata*, has the remarkable ability to hyperaccumulate arsenic in its shoots, with shoot concentrations reaching levels ~100-fold higher than soil concentrations (Ma *et al.* 2001). *Pteris* takes up arsenate, arsenite and MMA, the maximum amount nearly 93% concentrated in the fronds (Ma *et al.* 2001). Detailed work has been carried out in *Pteris vittata* to study arsenic phytoextraction (Fayiga and Saha, 2016) as an *in situ* alternative to alleviate arsenic toxicity of soils through phytoremediation (Tack and Meers, 2010; Wan *et al*., 2016). *Pteris* is used in phyto-extraction of arsenic under a wide array of soil physico-chemical conditions (Ciurli *et al*., 2014; da Silva *et al*., 2018; Sen 2014c). Tu and Ma (2002) after a greenhouse study reported that *P. vittata* could phytoextract up to 38 mg As/plant after 20 weeks of plant growth which meant nearly a 25% reduction in soil arsenic content.

Arsenic is known to induce oxidative stress in plants by generating various ROS (Sen 2016a) resulting in a range of responses in plants, including readjustment of transport and metabolic processes and growth inhibition (Hartley-Whitaker *et al*. 2001). In this work total, peroxide (Fig. 1) and MDA content (Fig. 2) were found to be higher in all treated *Eichhornia* samples as compared to *Pteris*. Several reactive oxygen species (superoxide anion, hydroxyl radicals, hydrogen peroxide and lipid peroxide) are generated in the cell wall and within the cell, which affect membrane permeability, enzyme activity, photosynthetic activity, plant biomass (Karabel *et al*. 2003; Nguyen *et al*. 2003).

In my work, all the three scavenging enzymes *viz.* catalase (Fig.3), peroxidase (Fig.4) and SOD (Fig.5) showed higher activities in all samples of *Pteris* as compared to *Eichhornia*. This could be well correlated with low content of peroxide and MDA in all samples of *Pteris*. The scavenging enzymes of *Pteris* effectively remove the harmful peroxides and MDA thus making *Pteris* more effective than *Eichhornia* in combating arsenic stress. Like all other abiotic stresses, exposure of plants to arsenic stress increases ROS accumulation due to the disruption of electron transport chains (ETC) in mitochondria and chloroplasts, glycolate oxidase activation, antioxidant and scavenging enzymes inactivation, and GSH depletion according to the studies of Gupta *et al*., 2013; Fayiga and Saha, 2016. Karimi and Souri, 2016 reported a positive correlation between greater antioxidant content and As tolerance in hyperaccumulator plants. According to Singh *et al.* (2006) the large GSH pool helps to combat As-induced oxidative stress and improves As tolerance in *Pteris*. Moreover, ROS, particularly H_2O_2, play an important role as signaling molecules which participate in the complex network regulating cell responses to As (Sharma 2012, Thao et al., 2015).

In my earlier work while *Eichhornia* showed significantly diminished concentrations of chlorophyll, carotenoids, protein, nitrogen, amino acid, total phenols and carbohydrates even in low concentrations of arsenic, *Pteris* appeared to be comparatively unaffected (Sen 2016b, 2019). According to Tu and Ma (2003) arsenic stress creates conditions where the energy level exceeds the quantity that can be dispelled by chloroplast metabolism in the thylakoids. As a result, the electron transport processes in the thylakoid membranes are hampered and lethal symptoms appear.

Plants usually contain trace concentrations of several contaminants which at higher concentrations cause harm. At low levels, plants can usually metabolize or dispose of these compounds without any significant injury. Generally, at high contaminant concentrations in soil or water, plants often suffer (like *Eichhornia*, in the earlier study, Sen 2014a,b) and/or die because of their inability to metabolize these harmful elements. In the same study by Sen (2014a, b) *Pteris* was found to survive and thrive even after considerable arsenic accumulation. However, some plants can, (like *Pteris*, in the present study) survive and/or thrive even when they accumulate high concentrations of toxic elements (Sen 2016a, b; 2019).

From the results obtained, *Pteris vittata* appears to be more suitable than *Eichhornia crassipes* for its possible use in phytoextraction of arsenic-contaminated soils. The comparative study reveals that *Pteris vittata* is the more efficient plant in combating and tolerating arsenic stress, as revealed by the biochemical constituents (Fig.1 and 2) and enzymatic profile (Fig.3,4 and 5). The physiological status of *Eichhornia* reveals the inefficiency of the plant to deal with arsenic stress and Arsenic-induced oxidative stress. The activities of the scavenging enzymes *viz*. catalase, peroxidase and SOD are significantly diminished under oxidative stress and are thus largely responsible for the prevailing stressful conditions (Sen 2016a,c). Since *Pteris* is a better accumulator of arsenic and is physiologically more adapted to cope with arsenic stress (as evidenced by obtained results), it is more suitable for phytoremediation (phytoextraction) in cases of arsenic pollution or contamination as compared to *Eichhornia*.

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CONFLICT OF INTERESTS

The author declare that he has no potential conflicts of interest.

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