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ORIGINAL ARTICLE

Effect of Cadmium and UV-B Solution on the Non-Enzymatic Antioxidants-Flavonoids, Proline and Lipid Peroxidation Content of Water Fern *Azolla microphylla* and *Azolla pinnata*

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ABSTRACT

The study was conducted to investigate the changes in flavonoid, proline and lipid peroxidation of Azolla microphylla and Azolla pinnata under treatment of cadmium and UV-B radiation. Individual treatment of 9ppm cadmium and 90 min UV-B significantly enhances the flavonoids, proline and lipid peroxidation of A. microphylla and A. pinnata. Interactive treatment of 9 ppm Cd+UV-B also causes significant increase in proline, flavonoid and lipid peroxidation of A. pinnatabut in case of A. microphylla, it was significant only for proline content. However, for flavonoid and lipid peroxidation of A. microphylla, the increment was not significant. Increment was found to be maximum under treatment 9 ppm Cd+UV-B since maximum stress was found under this condition. Results indicated that A. microphyllafound to be have better non-enzymatic antioxidants machinery than A. pinnata.

Key words: *Azolla microphylla, Azolla pinnata, cadmium, UV-B radiation, flavonoid, prolineand lipid peroxidation*

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INTRODUCTION

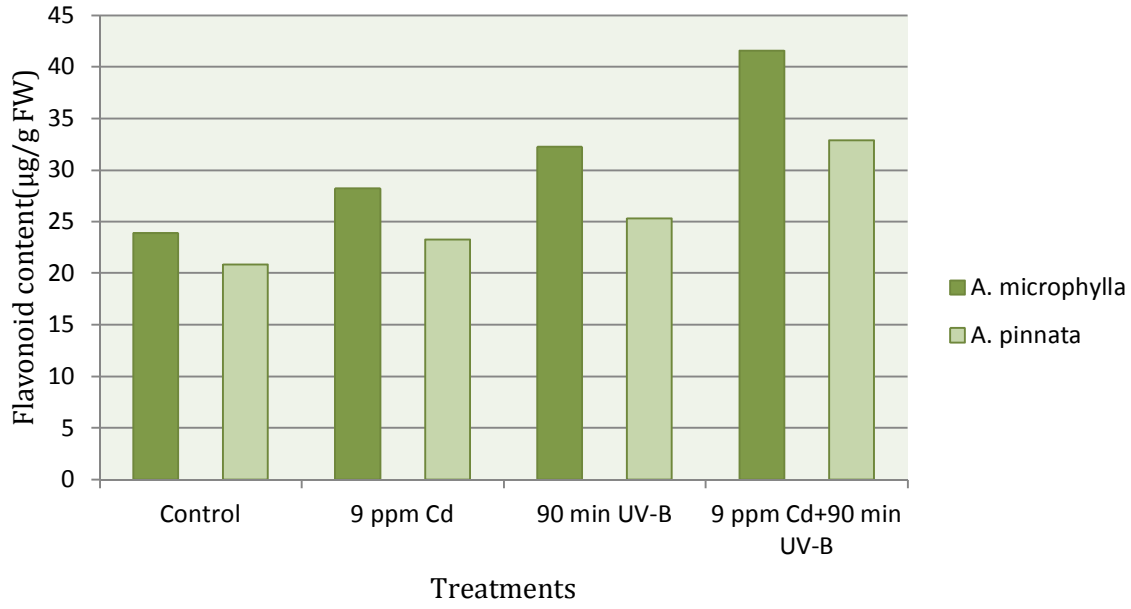
Heavy metal pollution is a major problem of present scenario which causes biological stress to living organisms. Cadmium is considered as most potent toxic pollutant released into environment by industrial discharge thus a threaten for aquatic organisms (Freiberg *et al.,* 1994). Beside heavy metal pollution, there is increase in environmental level of UV-B due to release of ozone depleting substances (Farman *et al.,* 1985). Water fern, *Azolla*, has been utilized in agriculture and also employed in bioremediation of waste water (Pabby *et al.,* 2004). Plants stimulates formation of reactive oxygen species (ROS) under stress condition (Hideg and Vass, 1996) and metabolizes ROS by increasing activity of several non-enzymatic antioxidants such as proline, flavonoids etc. (Arora *et al.,* 2002; Matysik *et al.,*2002). It was found earlier that individual and interactive effect of Cadmium and UV-B induced changes in *Riccia species* (Prasad *et al.,* 2004). *Azolla* as an aquatic plant and a potent biofertilizer of paddy fields exposed to simultaneous cd and UV-B stresses.

MATERIALS AND METHODS

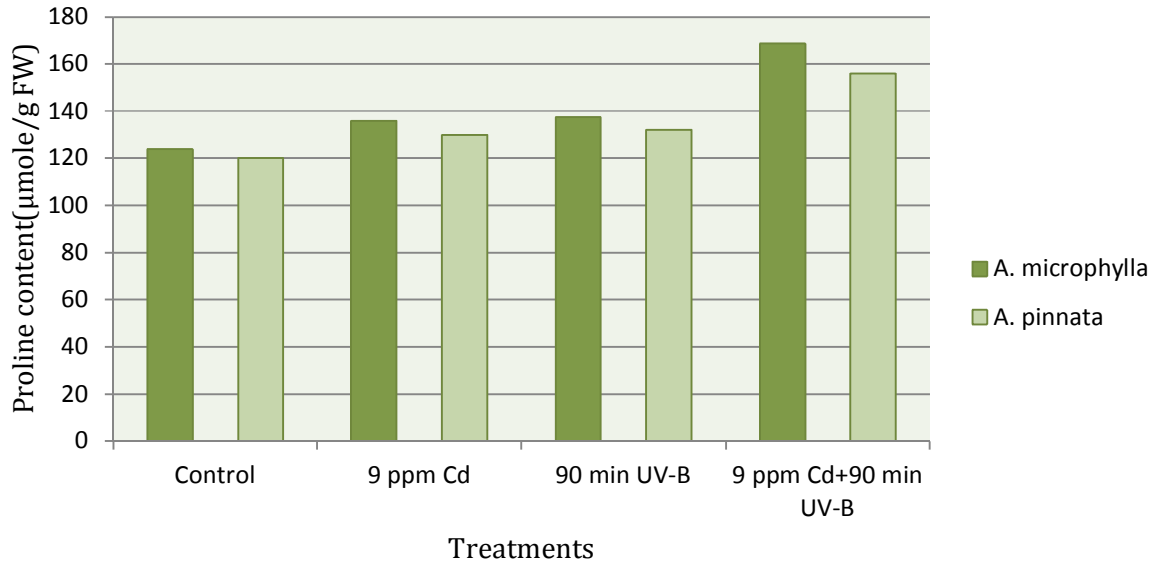
Fronds of *A. microphylla* and *A. pinnata* were grown in 2/5 strength of Hogland nutrient solution (Peers & Mayne, 1974). 9ppm cadmium chloride was added in the standard nutrient solution as a heavy metal source. UV-B treatment was provided to healthy fronds

by UV-B tube inside a UV-B chamber for 90 min. for interactive treatment *Azolla* was treated with 9 ppm $cdcl_2$ and kept in UV-B chamber. The flavonoids were determined according to the method of Mirecki and Teramura (1984). Proline content of the samples was determined spectrophotometrically by the method of Bates *et al.,* (1973). Lipid peroxidation was estimated by measuring the content of 2- thiobarbituric acid reactive substances in leaf homogenates, prepared in 20% trichloroacitic acid containing 0.5% 2-thiobarbituric acid and heated at 95^0C for 25 min. Malondialdehyde (MDA) content was determined spectrophotometrically at 532 nm and corrected for non-specific turbidity at A600.

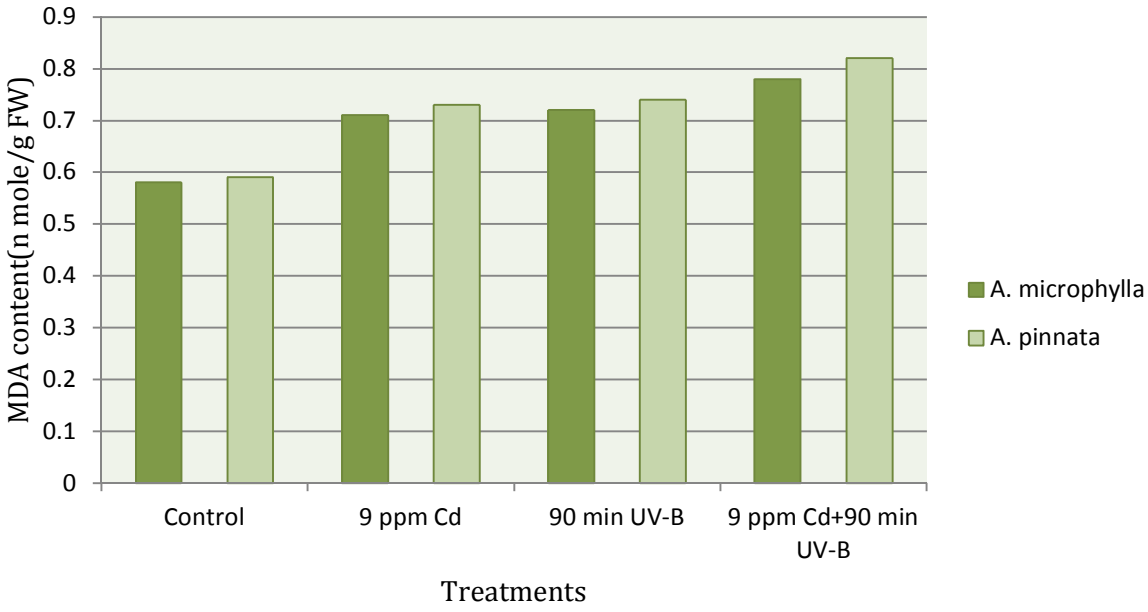
RESULTS AND DISCUSSION



Graph 1: Effetc of Cadmium and UV-B on Flavonoid Content (g/g Fresh Weight) of *A. microphylla* & *A. pinnata*



Graph 2: Effect of Cadmium and UV-B on Proline Content (µmoles/g Fresh Weight) of *A. microphylla* & *A. pinnata*



Graph 3: Effect of Cadmium and UV-B on Lipid Peroxidation (n mol/g Fresh Weight) of *A. microphylla* & *A. pinnata*

Flavonoids are ubiquitous plant secondary products with a vast array of biological functions including apparent role in stress protection. Absorption profile of flavonoids showed a significant increase under the treatment of heavy metal cadmium, in both *Azolla microphylla* and *Azolla pinnata.* Flavonoids in *A. microphylla* showed the increment of 18.7% in comparison to control while the increment was quite low in *A. pinnata* plants i.e. 12.1% under exposure of 9 ppm concentration of Cd. Results obtained through this study showed the similarity in increment of flavonoid content due to heavy metals cobalt and nickel in *Hibiscus sabdanffa*L by Aziz & Gad (2007). Application of UV-B radiation on *A. microphylla* and *A. pinnata* showed a significant increase in UV-B absorbing compound flavonoids. Exposure of UV-B radiation increased flavonoid content 36.2% and 22.4% by control in *A. microphylla* and *A. pinnata* respectively. Similar results due to UV-B irradiation were obtained by Ibrahim and Mostafa (2007) in *Azolla caroliniana* and by Masood *et al.,* (2008) in *Azolla pinnata* and *Azolla filiculoides.*There was a significant increment in flavonoid content due to interaction of Cd+UV-B in *A. pinnata*, however, this increment was not significant in *A. microphylla*. Flavonoid content increase up to 73.8% and 60.1% by control due to 9 ppm Cd+UV-B in *A. microphylla* and *A. pinnata* respectively. Flavonoids showed a higher value in UV-B and UV-B + Cd treated plants as compared to individual treatment with heavy metal or control plant was in the support of the results obtained by Mishra and Agrawal (2006) in pea plants.

Proline, an imino acid, is well known to get accumulated in wide variety of organisms ranging from bacteria to higher plants on exposure to abiotic stress. Present study showed the increment in proline content after treatment of cadmium and UV-B, singly and in combination in Azolla plants. Variation in proline content was significant due to all factors in both *Azolla microphylla* and *Azolla pinnata*. Presence of 9 ppm of Cd in nutrient solution increased proline content by 9.8% in *A. microphylla* where as 8.1% in *A. pinnata*. Increase in proline content due to lead nitrate in *Azolla microphylla* Kaulf and *Azolla filiculoides* Lam also reported by Muthukumaras *et al.,* (2000). UV-B radiation caused an increase in proline content by 11.1% and 10% by control in *A. microphylla* and *A. pinnata* respectively which was parallel to the result obtained by Masood *et al.,* (2008). Interaction of heavy metal (Cd) and UV-B increase proline content which was higher than

their individual treatments in *Azolla* plants. The maximum increment of proline was 36.3% and 30% in *A. microphylla* and *A. pinnata* respectively under the interactive treatment of 9 ppm Cd+UV-B. Exposure of heavy metal (Ni) + UV-B increased proline content in pea plants as reported by Singh *et al.,* (2009). Plants have been shown proline accumulation under environmental stresses (Ahmad & John, 2005).

Malondialdehyde (MDA) level was used as indicator for lipid peroxidation and represents a balance of oxidative stress that induced production of MDA in relation to various stresses. MDA can be regarded as a sink for oxidative radical. In present study, showed a significant marked increase in MDA level as a result of Cadmium treatment and the increase in Cd concentration enhanced the degree of lipid peroxidation in both *Azolla* plants. Increase of Lipid peroxidation was 23.66% in *Azolla microphylla* and 24.6% in *Azolla pinnata* due to Cd (9 ppm). Increase in Lipid peroxidation due to cadmium stress in *Lemnapolyrhizza* was also reported by John *et al.,* (2007). Lipid peroxidation was also found to be increased significantly due to UV-B radiation on both *A. microphylla* and *A. pinnata* which was 24.98% and 26.02% respectively by control. Interactive response due to Cd+UV-B on Lipid peroxidation increased significantly in *A. pinnata* which was not significant in *A. microphylla*. Results showed increment of 36.19% in *A. microphylla* and *in A. pinnata* it was 40.5% due to exposure of 9 ppm Cd+UV-B. Prasad *et al.,* (2004) observed a similar result due to interactive exposure of Cd+UV-B on *Riccia species.*

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