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Hydrophytes lack potential to exhibit cadmium stress induced enhancement in lipid peroxidation and accumulation of proline

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Abstract

Investigations were carried out to evaluate if hydrophytes (viz. *Ceratophyllum*, *Wolffia*, and *Hydrilla*) can be used as markers to assess the level of heavy metal pollution in aquatic bodies. The potential of these hydrophytes for lipid peroxidation and accumulation of proline in response to cadmium (Cd^{2+}) pollution was studied. Hydrophytes were raised in artificial pond water (APW) supplemented with various levels of Cd^{2+}. Interestingly, unlike mesophytes none of the hydrophytes showed ability to accumulate proline. Infact, in response to Cd^{2+} pollution hydrophytes exhibited a decline in proline levels in comparison to controls but mesophytes (viz. *Brassica juncea*, *Vigna radiata* and *Triticum aestivum*) showed progressive increase in the level of proline with increase in the extent of Cd^{2+} pollution. Mesophytes showed six to nine-fold increase in the level of proline in response to 1 mM Cd^{2+}. The potential of the above hydrophytes for lipid peroxidation was also low under Cd^{2+} stress. In contrast, as expected a significant enhancement in the lipid peroxidation was observed in all three mesophytes in response to their exposure to Cd^{2+}. About two-fold increase in production of malondialdehyde (a cytotoxic product of lipid peroxidation) was recorded in mesophytes exposed to 1 mM Cd^{2+}. However, a decline in chlorophyll (Chl a and Chl b) levels was recorded in response to Cd^{2+}pollution both in hydrophytes as well as mesophytes. In summary, hydrophytes neither have potential to accumulate proline nor have ability to accelerate lipid peroxidation under heavy metal stress. This suggests that the adaptive mechanism(s) existing in hydrophytes to tackle heavy metal stress is distinct from that in mesophytes.

Keywords: Proline; Hydrophytes; Lipid peroxidation; Malondialdehyde

1. Introduction

Heavy metal pollution is one of the major threats to the environment. Effluents rich in heavy metals are often released by industries into nearby water bodies. Toxic levels of heavy metals influence major metabolic events, such as photosynthesis, respiration, nitrogen metabolism and protein synthesis in various living systems (Clijsters and Van Assche, 1985). They can directly interact and suppress functioning of various essential biological components, in particular the electron transport system in chloroplast as well as mitochondria and enzymes like Rubisco, nitrogenase and nitrate reductase (Alia et al., 1995; Devriese et al., 2001) either by directly interacting or replacing essential nutrients that are required for their

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functioning (Atal et al., 1991, 1993). Heavy metals are known to promote incomplete reduction of molecular oxygen leading to the generation of free radicals due to inhibition in photosynthetic electron transport. Oxidative damage involving lipid peroxidation has been observed in higher plants in the presence of toxic levels of Cd^{2+}, Cu^{2+}, Zn^{2+} and Fe^{2+} (Atal et al., 1991, 1993; Alia et al., 1995).

Cadmium is one of the major heavy metal pollutants released into the environment and easily absorbed by the plants. Cd^{2+} induced biological effects could result from its interaction with –SH groups of functional enzymes and/or due to ionic imbalance as a result of altered translocation of ions, such as Fe^{2+}, Zn^{2+}, Cu^{2+} or Mn^{2+} and/or metal ion substitution. Infact, Cd^{2+} induced deficiency or substitution of Fe^{2+}, Zn^{2+}, Mn^{2+} and/or Ca^{2+} seems to be the prime cause behind chlorosis and impaired photosynthetic electron transport (Clijsters and Van Assche, 1985; Atal et al., 1993). As Cd^{2+} is one of the major heavy metal pollutants that is often seen in water bodies, investigations were carried out to check if hydrophytes could be used as a means to assess cadmium pollution in water bodies. Proline accumulation and lipid peroxidation were tested to ascertain if they can be used for rapid evaluation of the extent of cadmium pollution. In this communication we are demonstrating for the first time that unlike in mesophytes, the levels of proline and lipid peroxidation cannot be used as markers for determining the extent of cadmium pollution in hydrophytes.

2. Materials and methods

As industrial pollutants are often leached into nearby fresh water ecosystems in and around Delhi (India), we have chosen the most common free floating and submerged hydrophytes namely *Ceratophyllum* (Ceratophyllaceae), *Hydrilla* (Hydrocharitaceae) and *Wolffia* (Lemnaceae) found in this region. Mesophytes namely *Brassica juncea* (Brassicaceae), *Vigna radiata* (Fabaceae) and *Triticum aestivum* (Poaceae) were used for comparison with hydrophytes. Physiological and biochemical studies were carried out to assess the response of both aquatic and terrestrial plants to heavy metal pollution.

The hydrophytes were collected from non-polluted fresh water bodies in Delhi and maintained in 100 l of artificial pond water (APW) for 16 weeks in cemented tubs (1.52 m × 0.61 m × 0.61 m). APW consisted of NaCl (0.0058 g/l), KCl (0.0076 g/l) and $CaCl_2$ (0.0115 g/l). Investigations on evaluating performance of the hydrophytes to cadmium were initiated by exposing uniformly looking plants of *Ceratophyllum*, *Hydrilla* and *Wolffia* to different levels of $Cd(NO_3)_2$ (0, 0.01, 0.1, 0.5 and 1 mM) in 51 APW in tubs (30 cm × 15 cm) under a light (80 μmol m^{-2} s^{-1})/dark cycle of 14/10 h at room temperature (28–30 °C). The number of plants of each species taken in each tub varied from 5 in case of *Hydrilla* and *Ceratophyllum* to 200 in case of *Wolffia*.

Ten day old plants of *B. juncea*, *V. radiata* and *T. aestivum* raised (from the seeds obtained from Indian Agricultural Research Institute, New Delhi), on cotton flooded with APW in cylindrical glass bottles (25 cm × 12 cm) were supplemented different concentrations (viz. 0, 0.01, 0.1, 0.5, and 1.0 mM) of $Cd(NO_3)_2$. Various biochemical estimations were carried out on day 6. Chlorophyll (Chl) from leaves was extracted using 80% acetone (v/v). The extract was centrifuged at 3000 × *g* for 6 min. The supernatant was used for pigment estimation. The Chl a and Chl b levels were calculated as described earlier (Atal et al., 1991) and expressed as μg g^{-1} fresh weight. Proline levels were estimated as described in Alia and Pardha Saradhi (1991). The proline content was expressed in μg g^{-1} fresh weight. TBA reactive MDA production in various plant systems was determined as described by Heath and Packer (1968). The amount of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

All experiments were carried out at least 5 times with two replicates each time. Statistical analysis was carried out using Duncan's Multiple range test to check the level of significance within the samples (Duncan, 1955).

3. Results and discussion

The chlorophyll content was comparatively higher in controlled conditions (APW) as compared to those grown in the presence of Cd^{2+} in both hydrophytes as well as mesophytes. Both Chl a and Chl b contents declined progressively with an increase in concentration of Cd^{2+} (Table 1). Cd^{2+} stress induced decline in Chl

Table 1
Chlorophyll content ($\mu g\, g^{-1}$ fresh weight) in shoots of hydrophytes and mesophytes exposed to different levels of Cd^{2+} stress

Cd^{2+} (mM)	Hydrophytes					
	Ceratophyllum		*Hydrilla*		*Wolffia*	
	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
0	390 ab ± 35.0	150 a ± 10.5	820 a ± 53.8	300 a ± 24.0	114 a ± 7.98	58 a ± 2.9
0.1	400 a ± 32.0	152 a ± 9.12	748 b ± 74.8	293 b ± 20.5	115 a ± 6.9	58 a ± 2.9
0.5	380 b ± 19.0	140 b ± 11.2	490 c ± 34.3	200 c ± 10.0	109 b ± 8.72	56 ab ± 2.8
1.0	360 c ± 21.6	105 c ± 5.3	300 d ± 18.0	110 d ± 5.5	104 b ± 5.2	55 bc ± 2.75
	Mesophytes					
	Brassica		*Vigna*		*Triticum*	
	Chl a	Chl b	Chl a	Chl b	Chl a	Chl b
0	689 a ± 58.7	253 a ± 24.2	712 a ± 56.9	278 ab ± 19.5	699 a ± 84.3	265 a ± 27.4
0.1	682 a ± 63.3	268 a ± 18.9	715 a ± 42.9	291 a ± 23.3	625 a ± 37.0	199 b ± 17.5
0.5	586 ab ± 46.9	228 ab ± 13.8	597 b ± 35.8	224 b ± 13.4	503 b ± 56.7	135 c ± 11.2
1.0	490 c ± 27.2	167 c ± 18.7	495 c ± 24.7	198 c ± 18.9	327 c ± 28.5	91.4 d ± 14.2

Values designated by different letters within a column differ significantly at $P \leq 0.05$ (Duncan's multiple range test). Values represent mean ± S.E. ($n = 5$).

a and Chl b content was more prominent in *Hydrilla* in comparison to that in *Ceratophyllum* and *Wolffia* grown in APW supplemented with 1 mM $Cd(NO_3)_2$. In general, heavy metal stress induced a significant decline in chlorophyll content, in *Hydrilla* and mesophytes. The decline in chlorophyll content in plants exposed to Cd^{2+} stress is believed to be due to (a) inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase (ALA-dehydratase) (Padmaja et al., 1990) and protochlorophyllide reductase (Van Assche and Clijsters, 1990) associated with chlorophyll biosynthesis; (b) impairment in the supply of Mg^{2+} and Fe^{2+} required for the synthesis of chlorophylls; (c) Zn^{2+} deficiency resulting in inhibition of enzymes, such as carbonic anhydrase (Van Assche and Clijsters, 1990); (d) the replacement of Mg^{2+} ions associated with the tetrapyrrole ring of chlorophyll molecule (Küpper et al., 1996). Similar decrease in chlorophyll content under heavy metal stress was reported earlier in cyanobacteria, unicellular chlorophytes (*Chlorella*), gymnosperms, such as *Picea abies* and angiosperms, such as *Zea mays*, *Quercus palustrus* and *Acer rubrum* (Siedlecka and Krupa, 1996). The loss in chlorophyll content can consequently lead to disruption of photosynthetic machinery.

In contrast to a almost similar pattern of decline in chlorophyll content in both hydrophytes and mesophytes in response to heavy metal stress, there has been a distinct variation among hydrophytes and mesophytes/terrestrial plants with regard to proline accumulation. 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 stresses (Pardha Saradhi et al., 1993) including heavy metal stress (Alia and Pardha Saradhi, 1991). Proline, a compatible solute is demonstrated to play role in maintenance of (a) cellular osmoticum; (b) NADPH/NAD(P^+) ratio; and (c) cytosolic pH, besides helping in detoxification of free radicals/toxic oxygen species (in particular singlet oxygen and hydroxyl radicals) (Alia and Pardha Saradhi, 1991; Alia et al., 1993, 1995, 1997). None of the hydrophytes tested showed any ability to accumulate proline upon their exposure to toxic levels of Cd^{2+}. In fact, all the hydrophytes tested showed relatively higher levels of proline in their respective controls in comparison to those exposed to Cd^{2+} stress. Surprisingly, the level of proline in these hydrophytes declined progressively with increase in the level of Cd^{2+} (Fig. 1). The decline in proline levels in *Ceratophyllum*, *Hydrilla* and *Wolffia* was as high as 50, 14 and 33%, respectively, over that of controls (Fig. 1). Similar results were recorded on exposure of these hydrophytes to other heavy metals as well as salt stress (results not shown).

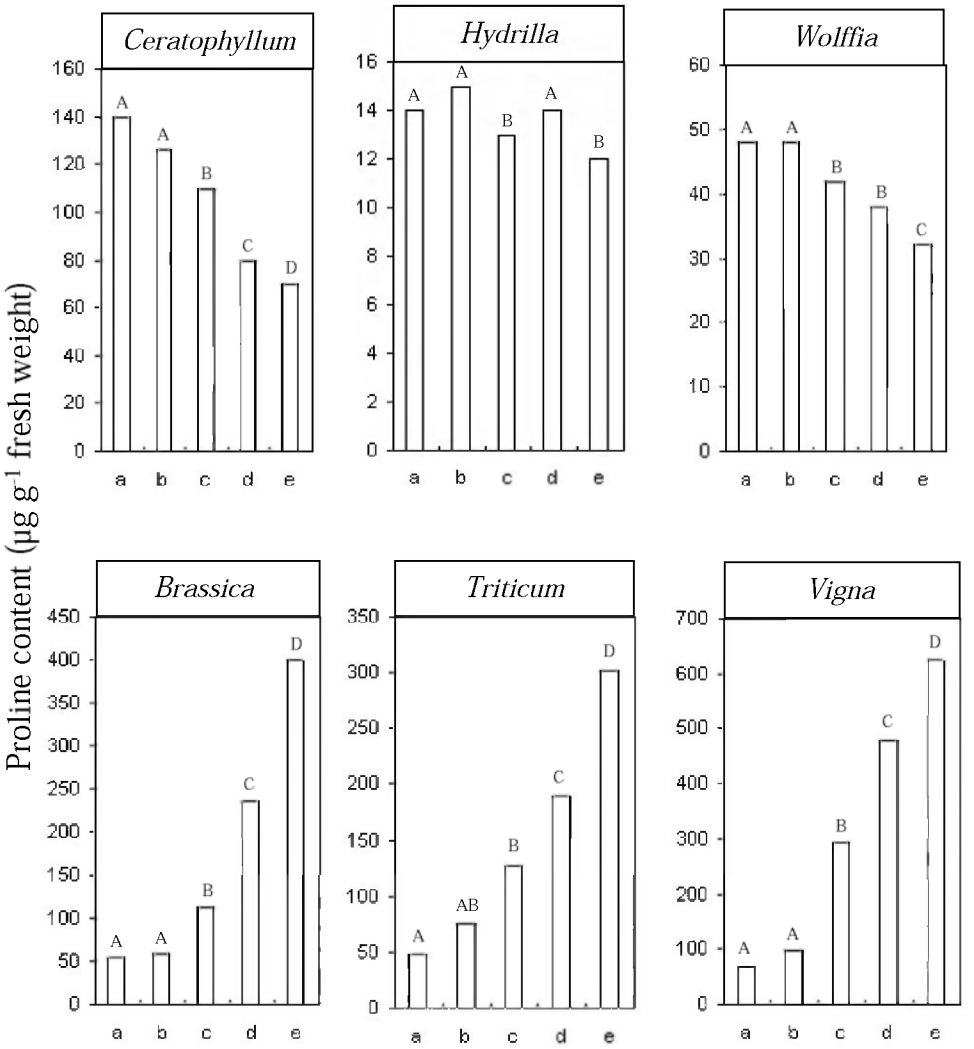

Fig. 1. Proline level in shoots of hydrophytes and mesophytes in response to varying levels of Cd^{2+} (a, b, c, d, e represent 0, 0.01, 0.1, 0.5, 1 mM) stress. Values represent Mean of five independent experiments. Values designated over the bars in different capital letters are significantly different at $P \leq 0.05$ level (Duncan's multiple range test).

These results convincingly demonstrated for the first time that hydrophytes lack potential to accumulate proline under heavy metal stress. Stress induced enhancement in the synthesis/accumulation of proline was also not observed in cyanobacteria. Cyanobacteria have been reported to accumulate a wide variety of organic compounds like carbohydrates, glucosylglycerol, tertiary sulphonium compounds and quaternary ammonium compounds in response to osmotic stress (Borowitzka, 1986). Like cyanobacteria, hydrophytic angiosperms (at least those species tested by us) seem to possess some other adaptive means to tackle/adapt to heavy metal and other abiotic stress conditions. As expected and reported earlier by our group (Alia and Pardha Saradhi, 1991; Alia et al., 1995; Arora and Pardha Saradhi, 1995), Cd^{2+} toxicity led to proline accumulation in shoots of terrestrial plants/mesophytes viz. *B. juncea*, *T. aestivum* and *V. radiata*. In contrast

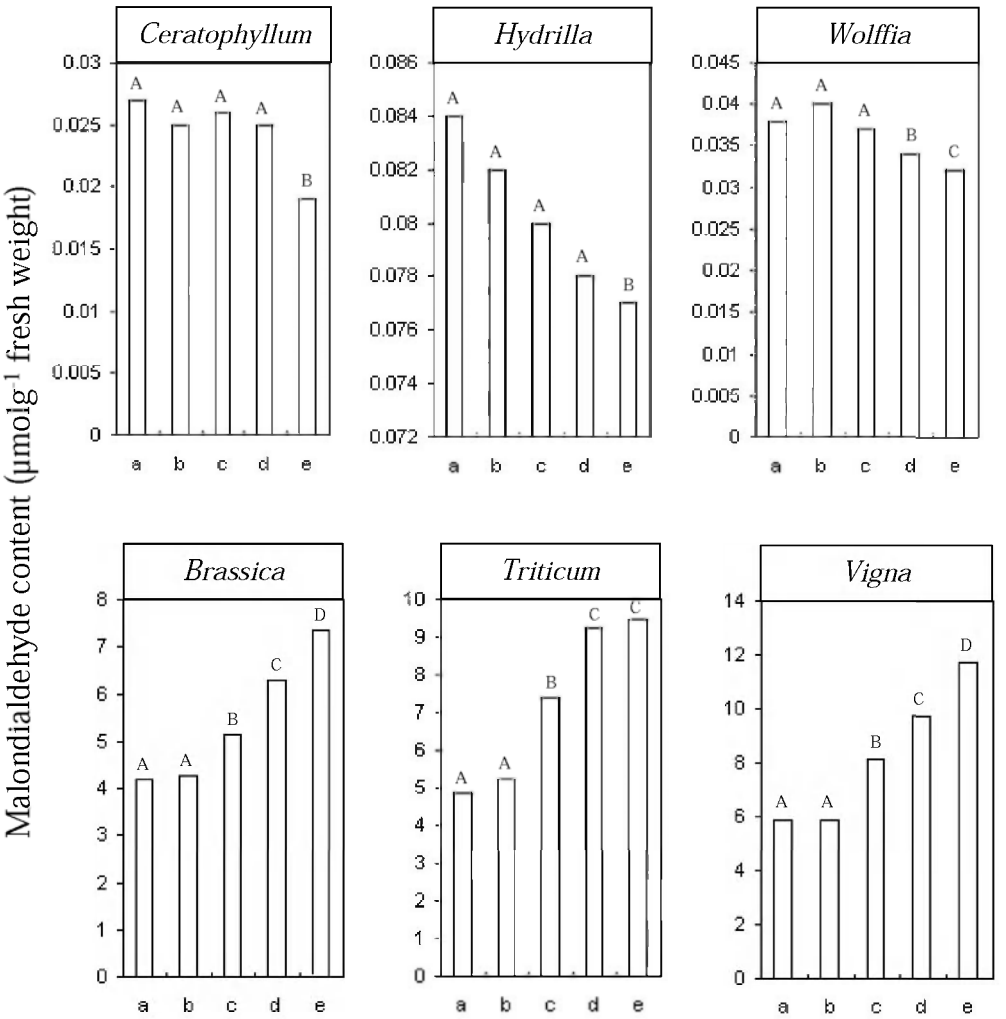

Fig. 2. MDA content in shoots of hydrophytes and mesophytes in response to varying levels of Cd^{2+} (a, b, c, d, e represent 0, 0.01, 0.1, 0.5, 1 mM) stress. Values represent Mean of five independent experiments. Values designated over the bars in different capital letters are significantly different at $P \leq 0.05$ level (Duncan's multiple range test).

to aquatic plants, proline levels in these mesophytes increased progressively with increase in Cd^{2+} concentration. The proline levels increased by seven, six and nine-fold over that of control in *B. juncea*, *T. aestivum* and *V. radiata* in response to 1 mM Cd^{2+} (Fig. 1). Enhancement in the levels of proline was also recorded in mesophytic plants upon their exposure to cadmium stress even at other developmental stages (data not shown).

Earlier several groups including ours have clearly demonstrated that plants exposed to heavy metals including Cd^{2+} show enhanced lipid peroxidation (Alia et al., 1991, 1995, 1997; Prasad et al., 1999). Increased production of alkoxy and peroxy radicals, leads to peroxidative damage to membranes through degradation of polyunsaturated fatty acids. This further leads to Cd^{2+} induced enhancement in lipoxygenase activity (Somashekaraiah et al., 1992). Moreover, under heavy metal stress including Cd^{2+} toxicity, normal translocation of electrons gets affected resulting in free radical production which in turn leads to lipid peroxidation (Atal et al., 1991; Somashekaraiah et al.,

1992; Alia and Pardha Saradhi, 1993; Pardha Saradhi et al., 1993). Interestingly, similar to that of proline, the level of MDA (a major cytotoxic product of lipid peroxidation and widely accepted to be an indicator of free radical/TOS production) in hydrophytes grown under control conditions was relatively higher than those exposed to Cd^{2+} pollution. Amongst hydrophytes, MDA content was maximum in *Hydrilla* (0.084 $\mu mol\, g^{-1}$ fresh weight) followed by *Wolffia* (0.038 $\mu mol\, g^{-1}$fresh weight) and *Ceratophyllum* (0.026 $\mu mol\, g^{-1}$fresh weight) under unstressed conditions. Surprisingly, MDA level in all the three hydrophytes declined on exposure to Cd^{2+}. In response to 1 mM Cd^{2+} the plants of *Ceratophyllum*, *Hydrilla* and *Wolffia* showed decline in MDA content by 29, 8 and 15%, respectively (Fig. 2). In contrast, as expected, MDA levels increased in all the three mesophytes viz. *B. juncea*, *T. aestivum* and *V. radiata* in response to Cd^{2+} stress. MDA content in shoots of *Brassica*, *Triticum* and *Vigna* seedlings raised in 1 mM Cd^{2+} was about two-fold higher than their respective controls (Fig. 2). Mesophytic plant species (*B. juncea*, *T. aestivum* and *V. radiata*) exposed to different levels of cadmium stress exhibited an enhanced lipid peroxidation even at other developmental stages. In general, irrespective of the development stage in mesophytic plant species, the extent of lipid peroxidation as well as proline accumulation increased with increase in Cd^{2+} toxicity (data not shown). In fact there seems to be correlation in accumulation of proline and lipid peroxidation. This is in accordance with our earlier reports wherein we had unequivocally demonstrated that there exists a direct correlation between lipid peroxidation and proline accumulation (Alia et al., 1993, 1995; Prasad et al., 1999).

4. Conclusion

The results presented in this communication unequivocally demonstrate for the first time that hydrophytes unlike mesophytes neither have potential to accumulate proline nor have ability to exhibit lipid peroxidation under heavy metal stress. This suggests that the adaptive mechanism(s) existing in hydrophytes to tackle heavy metal stress is distinct from those in mesophytes. At this stage it is difficult for us to predict any valid reason behind this seemingly unusual phenomenon. Further, investigations are required in order to elucidate the actual mechanism associated with it.

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