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BRIEF COMMUNICATION

Membrane lipid peroxidation, free radical scavangers and ethylene evolution in *Amaranthus* as affected by lead and cadmium

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Abstract

Membrane lipid peroxidation, activity of free radical scavangers and ethylene evolution of *Amaranthus lividus* seedlings were used to determine the lead and cadmium (1, 10, 100 and 1000 μM) induced phytotoxicity. Malondialdehyde (MDA) accumulation and higher lipoxygenase activity (LOX) was found in the 7-d-old treated seedlings. The activities of free radical scavangers like peroxidase, catalase and superoxide dismutase declined considerably with the concomitant rise in hydrogen peroxide level. Heavy metal treatment also caused decline in ethylene evolution in germinating seedlings.

Additional key words: free radicals, heavy metals, lipoxygenase, malondialdehyde.

Stress induced membrane damage is related to membrane lipid peroxidation which is mainly caused by free radicals (Eltsner 1982, Gregor and Lindsberg 1986, Cakmak and Watar 1991). These free radicals and H_2O_2 are major source of active oxygen species, such as superoxide ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), singlet oxygen ($^1O_2$) *etc.*, which in turn interact with protein and lipid components of membrane, causing damage (*e.g.* Gregor and Lindsberg 1986). Lipoxygenase (LOX) mediates polyunsaturated fatty acid oxidation, which again cause membrane deterioration. Hydrogen peroxide also accumulates in plant tissues in the absence of natural scavangers such as peroxidase (POD), catalase (CAT) and superoxide dismutase

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Abbreviations: CAT - catalase, EFE - ethylene forming enzyme, LOX - lipoxygenase, MDA - malondialdehyde, POD - peroxidase, PUFA - polyunsaturated fatty acid, RLR - relative leakage ratio, SOD - superoxide dismutase, SE - standard error.

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(SOD). Hydrogen peroxide along with different free radicals also causes cytotoxity and even causes peroxidation of membrane lipids (Scandalios 1993). Different abiotic stresses may also causes disruption of membrane structure, resulting in loss of ethylene forming enzyme (EFE) activity (Yang 1984, Bhattacharjee and Mukherjee 1995).

The aim of the present study was to determine the effect of Pb and Cd stress in terms of MDA accumulation, ethylene evolution and activities of LOX, POD, CAT and SOD, so that they can be used as diagnostic criteria of biological test for evaluation of heavy metal induced phytotoxicity.

Seeds of stress tolerant plant *Amaranthus lividus* were first washed with distilled water, then surface sterilised with 0.1 % $HgCl_2$ for 5 min and washed twice with sterile distilled water for 15 min and then allowed to imbibe water for 4 h. Water imbibed seeds were germinated in Petri dishes on filter paper soaked with solution of $PbCl_2$ and $CdCl_2$ of different (0, 1, 10, 100 and 1000 μM) concentrations in darkness (temperature 24 ± 2 °C and relative humidity 78 - 80 %) for 7 d.

For the estimation of relative leakage ratio (RLR) the method of Shcherbakova and Kacperska-Palacz (1980) was used with some necessary modifications (Bhattacharjee and Mukherjee 1996). To determine the membrane injury index, the method of Sullivan (1972) was followed. For the measurement of ethylene evolution the procedure of Bhattacharjee and Mukherjee (1995) was followed. Ethylene concentration was determined by *Nucon* gas layer chromatography using *Porapack-T* column and flame ionising detector. MDA was estimated as thiobarbituric acid reactive material from seedling extract following the method of Heath and Packer (1968). For the extraction and estimation of LOX, method of Peterman and Siedow (1985) was followed. H_2O_2 was extracted and estimated following the method of MacNevin and Arnon (1953). For determination of POD, CAT and SOD activities procedures of Kar and Mishra (1976), Snell and Snell (1971) and Giannopolitis and Ries 1977), respectively, were used. In all the cases enzyme activities were measured according to Fick and Qualset (1975).

With the increase in the $PbCl_2$ and $CdCl_2$ concentrations there was a corresponding increase in the extent of membrane injury, which is supported by RLR data (Table 1). There was more leakage of cellular UV-absorbing substances with the increasing concentrations of heavy metals. This is in agreement with the findings of Rauser and Hanson (1965) and Bhattacharjee and Mukherjee (1996). The extent of injury is however found to be more in case of $CdCl_2$ than $PbCl_2$ under the same concentration.

Proportional to the increased heavy metal stress the level of MDA (reflects the membrane lipid peroxidation of *Amaranthus* seedlings) also increased (Table 1). LOX which catalyzes membrane lipid peroxidation by oxidising polyunsaturated fatty acids (PUFA), also increased significantly over water control. So, Pb and Cd induced or facilitated lipid peroxidation and thereby disorganized membrane structure, corroborating well with the findings of Grossman and Leshem (1988) and Bhattacharjee and Mukherjee (1996) in a similar stress study.

The amount of ethylene also declined gradually with the increasing concentration of $PbCl_2$ and $CdCl_2$ in the media (Table 1) and this was also found to be minimum at

the concentration of 1000 μM $CdCl_2$. This drop in ethylene formation might be due to heavy metal induced changes in membrane integrity that results in loss of EFE activity in plant tissues (Yang and Hoffman 1984, Bhattacharjee and Mukherjee 1996).

Table 1. Effect of different concentrations of heavy metals ($PbCl_2$ and $CdCl_2$) on relative leakage ratio (RLR), membrane lipid peroxidation (MLP), lipoxygenase activity (LOX) and ethylene evolution in germinating *Amaranthus* seedlings (7-d-old). Values are mean of four replicate ± SE.

Heavy metal conc. [μM]	RLR [A_{280}/A'_{280}]	MLP [nmol MDA g^{-1} (d.m.)]	LOX [U g^{-1}(d.m.) s^{-1}]	C_2H_4 evolution [nmol g^{-1}(d.m.) d^{-1}]
H_2O (control)	0.160±0.0004	110±0.58	0.0455±0.0002	0.467±0.002
$PbCl_2$: 1	0.170±0.0003	116±0.49	0.0491±0.0001	0.406±0.001
10	0.200±0.0004	128±0.37	0.0500±0.0002	0.373±0.002
100	0.210±0.0007	205±0.67	0.0539±0.0003	0.264±0.003
1000	0.350±0.0002	227±0.43	0.0695±0.0001	0.162±0.002
$CdCl_2$: 1	0.190±0.0004	122±0.19	0.0508±0.0002	0.382±0.001
10	0.215±0.0004	135±0.21	0.0516±0.0002	0.378±0.002
100	0.310±0.0007	217±0.63	0.0700±0.0003	0.199±0.003
1000	0.400±0.0003	245±0.44	0.0858±0.0001	0.152±0.001

The data of injury index hinted the electrolyte leakage values under $PbCl_2$ and $CdCl_2$ stresses. This showed a concomitant increase in membrane injury in *Amaranthus* seedlings along with the concentration of heavy metals (Table 2). The

Table 2. Effect of different concentrations of heavy metals ($PbCl_2$ and $CdCl_2$) on injury index, free radical scavanging enzymes and H_2O_2 content in *Amaranthus* seedlings (7-d-old). Value are mean of four replicate ± SE.

Heavy metal conc. [μM]	Injury index [%]	Enzyme activities [U g^{-1}(d.m.) s^{-1}] CAT	POD	SOD	H_2O_2 content [μmol g^{-1}(d.m.)]
H_2O (control)		0.070±0.002	0.025±0.002	0.083±0.002	88.5±0.58
$PbCl_2$: 1	2.85±0.03	0.071±0.002	0.025±0.003	0.081±0.001	89.0±0.60
10	3.95±0.02	0.052±0.001	0.023±0.001	0.072±0.002	110.0±1.10
100	9.50±0.07	0.051±0.001	0.015±0.002	0.068±0.003	154.0±0.33
1000	18.20±0.04	0.041±0.003	0.015±0.001	0.061±0.001	170.0±0.39
$CdCl_2$: 1	2.90±0.03	0.069±0.005	0.026±0.002	0.085±0.004	93.0±0.54
10	3.90±0.04	0.050±0.003	0.018±0.001	0.066±0.002	108.0±0.67
100	12.50±0.23	0.056±0.001	0.014±0.001	0.062±0.002	174.0±0.30
1000	27.30±0.30	0.035±0.002	0.012±0.002	0.050±0.001	190.0±0.28

activities of POD, CAT and SOD showed gradual decline over untreated control in *Amaranthus* seedlings under increasing magnitude of $PbCl_2$ and $CdCl_2$ stress. The concentration of H_2O_2 also increased concomitantly with the increasing concentration

of $PbCl_2$ and $CdCl_2$ in the medium (Table 2). The extent of quantitative increment of H_2O_2 was maximum at 1000 μM $CdCl_2$ with maximum decline in activities of free radical scavangers. H_2O_2 is generally produced by dismutation of superoxide ($O_2^{\cdot-}$) (Eltsner 1982). Superoxide may again react with H_2O_2 generating more toxic reactive free radicals like $OH^{\cdot}$, $^1O_2$ *etc.*, which may in turn cause membrane damage (Scandalios 1993).

The decreased SOD activity under Pb and Cd stress favours accumulation of superoxide, which contribute to membrane damage. The decreased activity of POD and CAT may be one of the causes of accumulation of H_2O_2 under heavy metal stress. Although the activity of SOD under toxic heavy metal concentration minimize the generation of H_2O_2 from superoxide, the simultaneous decrease in activity of POD and CAT could also be a plausible explanation for accumulation of H_2O_2. This H_2O_2 may in turn be responsible for macromolecular degradation in seedlings including membrane deterioration via lipid peroxidation (Thompson *et al.* 1987, Scandalios 1993).

It may be concluded that *Amaranthus* seedlings under Pb and Cd stress may result in loss of semipermeable nature of cell membranes. The extent of injury is dependent on magnitude of heavy metal stress. The quantitative relation observed in *Amaranthus lividus* seedlings between membrane lipid peroxidation, ethylene evolution, specific changes in the activities of free radical scavangers, RLR and injury index delivers reliable biological test system for evaluation of heavy metal induced phytotoxicity.

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