Journal of Environmental Biology
ISSN : 0254-8704

J. Environ. Biol.
24 (1), 107-112 (2003)

Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants.

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(Received : 09 July, 2001 ; Accepted : 18 January, 2002)

Abstract *: Excess of cadmium (Cd) induced changes in oxidative scenario and water status of plants viz., total water content, specific water content, water saturation deficit (WSD) and transpiration of Brassica juncea plants grown in soil pot culture. Although lower and marginal levels of excess cadmium (100 and 250 ppm) improved growth but higher levels (500 ppm) caused significant suppression. Significant accumulation of proline, an indicator of water stress, occurred at higher level of Cd. Gradual increases in activities of certain antioxidant enzymes such as catalase and peroxidase alongwith increased lipid peroxidation are suggestive of disturbed oxidative metabolism. Taking together, the deleterious effects of Cd and its effects on oxidative metabolism clearly indicate enhanced generation of reactive oxygen species (ROS) to be instrumental in producing toxic effects of Cd. The excess levels of Cd also decreased the concentrations of soluble protein and chlorophylls and increased the ratio of chlorophyll a/b.*

Key words *: Cadmium toxicity, Brassica juncea, Water relations, Catalase, Peroxidase, Lipid peroxidation, Proline, Oxidative stress.*

Introduction

Cadmium is one of the most toxic heavy metal pollutants for human beings, animals and plants. It enters in the environment mainly from industrial processes and phosphorus fertilizers and then is transferred to the food chain (Wagner, 1993). When accumulated in the plant tissues, it causes alterations in catalytic efficacy of enzymes (Van Assche and Clijsters, 1988; Somashekaraiah *et al.*, 1992; Romero-Puertas *et al.*, 1999. Piqueras *et al.*, 1999), damage to the cellular membranes (Fu and Brouillette, 1987) and inhibit the root growth (Wilkins, 1978). These changes result in inhibition of chlorophyll biosynthesis and photosynthesis (Singh and Singh, 1987), mineral nutrient uptake (Greger and Lindberg, 1987) and water stress (Barcelo and Poschenrieder, 1990 and Kastori *et al.*, 1992). Information on plant water relations and oxidative metabolism, which constitute a fundamental mechanism in plant's life and they are affected due to metal phyto-toxicity, is still scanty. The present investigation relates the effects of excess Cd on plant water relations and oxidative metabolism in mustard plants grown in soil pot culture under glasshouse conditions.

Materials and Methods

Plant material and culture condition : Indian mustard (*Brassica juncea* L. cv. Varuna) plants were grown in soil pot culture in a glasshouse. The soil was air dried and mixed thoroughly. Nearly 2.5 kg of soil was filled in bitumen painted clay pots. The physico- chemical characteristics of soil are given in Table 1. There were six replications for each treatment. Seeds were sown in polythene trays and watered with deionised water. The seedlings were transplanted in clay-pots filled with soil on 10th day after sowing. A basal treatment of 200 mg kg^{-1} soil as $Ca(NO_3)_2$ and 100 mg P kg^{-1} soil as KH_2PO_4 and 100 mg kg^{-1} soil as $MgSO_4$ was applied to all plants. The pots were randomized every 4 to 5 days and watered daily to field capacity. After 30 days of growth, pots were grouped in 4 lots each with 6 pots. The first lot was treated as control and no cadmium was supplied. Other 3 lots were treated with cadmium @ 100, 250 and 500 ppm as $CdCl_2$. The glasshouse conditions during experiment {light intensity (PAR), temperature (maximum and minimum) and humidity ranged from 960 to 1400 $\mu E\ m^{-2}\ s^{-1}$, 24 to 28 and 10 to 18^{0}C and 40 to

50% (9.00 AM) respectively}. The average day length during the period was 10.5 ± 0.2 hours.

Growth, diffusive resistance (DR), transpiration (E) and water status : Data were recorded as plant height and fresh and dry matter yields (oven dried at 70°C for 24 hours). At 4 and 8 days of Cd treatment, measurements of DR and E were made under glasshouse conditions between 9 to 10 AM on the abaxial surface of fourth expanded leaf using a Li-Cor (Lincoln, NE) steady state porometer (Model LI-1600). The same leaves were sampled for measuring water status of the leaf. Determination of water saturation deficit (WSD) was made by measuring fresh and hydrated (incubated in GDW for 3 hours at 10°C in the dark) and oven-dried weights of 30 discs of leaf (3 replicates of 10 discs each). The area of entire leaves was measured with Li-Cor portable area meter (Model LI- 3000 A).

Chlorophylls, proline and soluble proteins : Chlorophyll content was measured according to method of Arnon (1949). The concentration of free proline was determined in fresh leaf tissue with acid ninhydrin complex in toluene (Bates *et al.* 1973). Soluble protein was estimated with a colorimetric method using folin phenol reagent (Lowry *et al.* 1951). Bovine serum albumin (BSA) served as a standard.

Enzyme assays and lipid peroxidation : Ten percent homogenate of fresh finely chopped, and pre-chilled leaf lamina was prepared in phosphate buffer. The homogenate was then centrifuged and supernatant was used for enzyme assays. Catalase activity was measured as described by Bisht (1972) and peroxidase activity by modified method of Luck (1963). The activities of enzymes were expressed per milligram of soluble protein in the enzyme extracts. Lipid peroxidation in the leaf tissue was determined in terms of malondialdehyde (MDA, a product of lipid peroxidation) content by thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). The leaf tissue was homogenized in 0.1% (w/v) trichloroacetic acid (TCA) following centrifugation at 10,000 g for 5 minutes. Supernatants were treated with 0.5% (w/v) TBA [prepared in 20% (w/v) TCA] at 95°C and cooled quickly. MDA concentration was determined after subtracting optical density for nonspecific absorbance (600 nm) from the absorbance values at 532 nm, using an extinction coefficient of 155 mM^{-1} cm^{-1}.

The data have been analysed for its statistical significance at P = 0.05 by analysis of variance.

Results and Discussion

Plant growth and visible effects : Plants receiving lower and marginal level (100 and 250 ppm) Cd exhibited non-significant increase in fresh and dry matter yields but at higher level (500 ppm) of Cd, significant reduction in plant height (Fig. 1) and fresh and dry matter was observed (Table 2). Initiation of wilting was observed in plants receiving 500 ppm of Cd at 4th day of treatment. At this level white necrotic patches appeared that increased in area as the duration advanced. The chlorosis started from the margins of younger leaves and subsequently progressed toward midrib of leaves in both marginal and higher levels (250 and 500 ppm) of Cd. The marginal growth of leaf lamina in these treatments was restricted resulting in downward curling of leaves.

Water relations : Leaves of mustard plants receiving lower and marginal levels (100 and 250 ppm) of Cd exhibited non-significant increase in total water content, specific water content and lower WSD. These parameters decreased significantly in leaves of plants receiving 500 ppm of Cd (Table 2). The lower and marginal levels of Cd (100 and 250 ppm) decreased the DR and concomitantly increased E while at higher level (500 ppm) of Cd increased DR and decreased E (Table 3).

Chlorophylls, proline and soluble proteins : The chlorophyll concentration decreased with increasing Cd and the chlorophyll a/b ratio increased significantly with increasing Cd (Table 4). The proline content registered an increase with increasing level of Cd (Table 2). Concentration of soluble proteins decreased with increase in Cd supply (Table 4).

Activity of antioxidant enzymes and lipid peroxidation : The activity of antioxidant enzymes viz. catalase and peroxidase increased significantly with increase in Cd supply (Table 4).

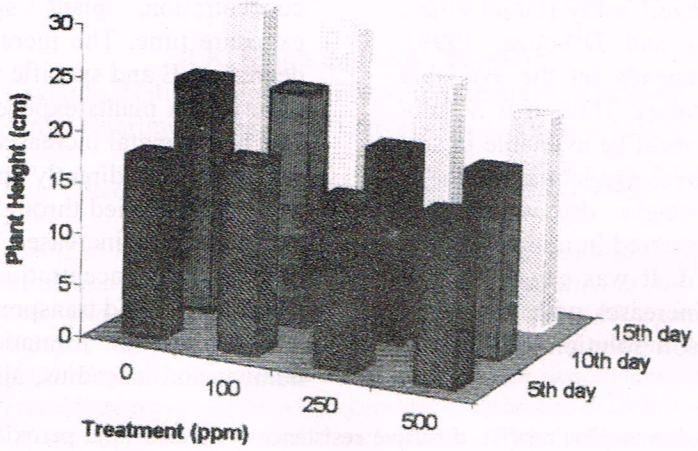

Fig. 1 : Effect of cadmium on plant height of mustard (*B. juncea*) plants growth in soil pot culture at 5, 10 and 15 days after cadmium treatment.

Table – 1 : Physico-chemical characteristics of soil used as culture medium for the pot culture experiment.

Physical character		Chemical character	
Bulk density (gm lit^{-1})	1.44	pH	8.36
Particle density (gm lit^{-1})	2.00	Electrical conductivity (d Sm^{-1})	0.16
Porosity (%)	28.00	Calcium carbonate (%)	0.5
Saturation percentage	37.50	Organic matter (gm kg^{-1})	6.86
Water holding capacity (%)	24.50	Nitrogen (%)	0.05
Texture sand (%)	32.00	Potassium (ppm)	120.0
Silt (%)	57.00	Copper (ppm)	2.55
Clay (%)	11.00	Zinc (ppm)	3.15
Textural class	Silt loam	Iron(ppm	10.46
		Manganese (ppm)	5.54

Table – 2 : Effect of Cd on fresh and dry weight, water contents [total water content (TWC), specific water content (SWC), water saturation deficit (WSD)] and proline concentration in leaves of mustard (*B. juncea*) plants grown in soil pot culture.

Treatment (ppm Cd)	Fresh weight (g $plant^{-1}$)	Dry weight (g $plant^{-1}$)	Water contents			Proline (µmol g^{-1} fresh wt.)
			TWC (g $leaf^{-1}$)	SWC (g dm^{-2})	WSD (%)	
Control	5.13	0.645	1.54	1.79	6.5	0.342
100	6.39	0.725	2.01	2.10	5.5	0.340
250	5.51	0.648	1.88	1.94	6.0	0.352
500	2.84	0.336	0.57	0.79	15.7	3.548
$LSD_{0.05}$	0.59	0.45	0.36	0.56	0.88	0.660

The MDA content (an index of lipid peroxidation) also increased with increasing supply of Cd (Table 3). These observations clearly suggest an enhanced generation of free radicals and thereby causing oxidative stress.

The insignificant increase in fresh and dry weight of plants receiving lower and marginal level of Cd (100 and 250 ppm) supply may possibly, be attributed to meager availability of Cd in the soil relatively at higher pH (8.36). The

soil pH is a primary factor influencing the availability of Cd and therefore increased sorption of Cd with increasing pH on soil particle surfaces would reduce Cd phyto-availability (Grant *et al.*, 1998 and Rarnchandran and D'Souza, 1999). Sorption of ions also depends on the available surface area on clay micelles. Thus, if it is fully saturated, the metal ions must be available in soil solution with resultant phyto-availability of metal ions. Decreased fresh weight, dry weight and visual toxic symptoms appeared in mustard plants receiving 500 ppm of Cd. It was suggested that uptake of Cd by plants increases with increasing concentration of Cd in soil solution (Mullins *et al.*, 1986).

The effects of heavy metals on plant water relations are quite complex and are dependent on the type of metal and its concentration, plant species, genotype and exposure time. The increased DR and WSD and decreased E and specific water content have been observed in plants exposed to higher level of Cd. The heavy metal increased the stomatal resistance not only when directly applied on guard cells but also when applied through the roots (Lanoreux *et al.*, 1978). The increased WSD, decreased suggest that high Cd concentration probably decreased the water uptake and transport as heavy metals inhibit the root growth, formation of root hairs, vessel number and its radius, all of which together lead

Table – 3 : Effect of Cd on transpiration (E), diffusive resistance (DR) and lipid peroxidation in the leaf tissue of mustard (*B. juncea*) plants grown in soil pot culture.

Treatment (ppm Cd)	Transpiration ($\mu g\ cm^{-2}\ sec^{-1}$)	Diffusive resistance ($s\ cm^{-1}$)	Transpiration ($\mu g\ cm^{-2}\ sec^{-1}$)	Diffusive resistance ($s\ cm^{-1}$)	Lipid peroxidation ($\mu mol\ MDA\ g^{-1}$ fresh wt.)
	4th day		8th day		
Control	3.50	3.43	2.91	2.99	4.68
100	4.84	2.25	5.39	2.15	5.05
250	3.60	3.55	4.75	1.93	6.12
500	0.38	72.50	0.40	71.43	8.68
$LSD_{0.05}$	0.48	0.62	0.57	0.63	1.36

Table – 4 : Effect of cadmium on concentrations of chlorophylls, soluble proteins and activities of catalase and peroxidase of mustard (*B. juncea*) plants grown in soil pot culture.

Treatment (ppm Cd)	Total chlorophyll ($mg\ g^{-1}$ fresh wt.)	Chlorophyll a/b ratio	Soluble protein ($mg\ 100\ mg^{-1}$ fresh wt.)	Catalase (Diffusive resistance ($\mu mol\ H_2O_2$ decomposed mg^{-1} protein)	Peroxidase (unit* μg^{-1} protein)
Control	2.742	2.838	4.26	479	9.25
100	2.369	3.091	3.92	571	24.38
250	1.958	3.211	3.88	567	27.73
500	1.251	3.328	3.60	608	32.19
$LSD_{0.05}$	0.42	0.38	0.83	8.71	2.39

*Change of optical density of 0.01 in the sample reaction mixture over the blanks per minute of reaction time.

to increased resistance to the water flow into and within roots (Barcelo and Poschenrieder, 1990). The slight or insignificant increase in the rate of E and specific water content, decrease in DR and WSD at low Cd supply levels are similar to the results of Hagemeyer *et al.* (1986). Low Cd concentration may decrease root growth without producing any toxic effect in the leaves. However, in leaf Cd can influence water relation by causing changes in photo-assimilate partitioning or increase the photosynthesis, which bring about increased turgor of leaves, stomatal opening and E (Barcelo and Poschenrieder, 1990).

The concentration of chlorophyll a and b decreased significantly in plants subjected to high level of Cd treatment. The decrease in chlorophyll concentration might be attributed to the involvement of Cd in inhibition of heme biosynthesis and chlorophyll formation by interacting with the functional -SH group of sulphydryl requiring enzymes involved in the pathway (Nandi and Shemin, 1968 and Stobart *et al.*, 1985). Somashekaraiah *et al.* (1992) suggested that increased activity of lipoxygenase in response to Cd might contribute to decreased level of chlorophylls. Significant increase in chlorophyll a/b ratio in plants supplied Cd could be explained on the basis of relative decreases noticed for chlorophyll a & b. It was observed (data not given) that magnitude of decrease in chlorophyll b was greater than chlorophyll a. This relative difference is reflected in the increased ratio. The greater decrease in chlorophyll b due to excess Cd may have resulted either from any or in combination of these mechanism (a) greater degradation of chlorophyll b, (b) its enhanced conversion to chlorophyll a or (c) reduced conversion of chlorophyll a to chlorophyll b. The occurrence of such interconversions has been reported by Ito *et al.* (1996).

The increased accumulation of proline in the leaf tissue might be caused by increase in WSD in plants exposed to higher doses of Cd. Proline is accumulated by several plants under various stress conditions such as low temperature (Stefl *et al.*, 1978) nutrient deficiencies (Sharma and Sharma, 1987 and Sharma *et al.*, 1995), toxicity of heavy metals (Kastori *et al.*, 1992 and Bassi and Sharma, 1993) and water stress (Carceller and Fraschina, 1980). The increased activity of catalase and peroxidase in plants exposed to higher level of Cd is in accordance to the results of Romero-Puertas *et al.* (1999). The increased activity of catalase and peroxidase, the prime antioxidant enzymes responsible for the detoxification of H_2O_2 was conducive to improved tolerance to Cd toxicity. Cd causes peroxidation of essential membrane lipids probably by generation of ROS. In fact, increased lipid peroxidation in plants treated with Cd was noticed and is in accordance of earlier observation (Somashekaraiah *et al.*, 1992 and Piqueras *et al.*, 1999).

Exposure of mustard plants to high levels of cadmium could be considered as abiotic elicitor of oxidative stress even in soils with high pH (8.36) that subsequently damage the essential membrane lipids and also affects uptake of water and thereby causing water stress in plants.

Acknowledgements

Authors are highly grateful to Prof. P.N. Sharma and Prof. S.S. Bisht, Botany Department, Lucknow University, Lucknow for their help, suggestion and facilities during experiment. Authors are also thankful to Dr. V. K. Garg, Scientist, N.B.R.I., Lucknow and Dr. G.N. Singh, Dy. Director, Agriculture Department, Lucknow, for reading the manuscript.

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