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Effect of lead on malondialdehyde, superoxide dismutase, proline activity and chlorophyll content in *Capsicum annum.*

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Abstract

This study investigated toxic impacts of lead over the concentrations of malondialdehyde (MDA), super oxide dismutase (SOD), proline activity and chlorophyll content in *Capsicum annum*. Despite a reduction in the growth of the plant, its MDA, SOD and proline contents were increased, whereas its chlorophyll content was decreased under the heavy metal stress, corresponding to the concentration of the metal ion. Increased amount of MDA was indicative for the formation of free radicals in plants under heavy metal stress, while increased levels of SOD and proline were pointed to the occurrence of a scavenging mechanism. The decrease in chlorophyll content is an indication of reduction in the growth of the plants leading to a decrease in the yield.

Keywords: Lead, Free radicals, MDA, SOD, Proline, Chlorophyll and *Capsicum annum*.

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INTRODUCTION

The industrialization and urbanization during the past few years have given rise to serious problems of environmental pollution. General increases in the level of heavy metals pose a pervasive threat to the natural ecosystem (Meenakshi et al., 2006). Heavy metals make a significant contribution to the environmental pollution as a result of human activities such as mining, smelting, electroplating, energy, fuel production, power transmission, intensive agriculture, sludge dumping and military operations (Nedelkoska and Doran, 2000). Their presence is a risk for primary and secondary consumers and ultimately human population (Zeller and Feller, 1999). Lead (Pb) is one of the most abundant, ubiquitous toxic elements that pose a critical concern to human and environmental health. It causes multiple direct and indirect effects on plant growth and metabolism, along with visible symptoms including stunted growth and small leaves, as well as leading to membrane disorganization and reduced photosynthesis (Sharma & Dubey, 2005; Ahmad *et al.*, 2008). In addition, it is generally accepted that toxic levels of heavy metal can affect a variety of physiological processes in plants. One of the major consequences is the production of large quantities of Reactive Oxygen Species (ROS), which damages the proteins, lipids and DNA (Schützendübel & Polle, 2002). Therefore, a mechanism to interrupt such an autocatalytic process is required. Under normal circumstances, concentration of oxygen radical remains low because of the activity of protective enzymes, including superoxide dismutase (SOD), catalase, and ascorbate peroxidase (Asada, 1984). In resistant forms, stress condition may enhance protective processes such as accumulation of compatible solutes and increase in the activities of detoxifying enzymes. Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation that indicates the free radical production and the consequent tissue damage (Ohkawa et al., 1979). SOD is a metalloenzyme that catalyzes the dismutation of superoxide anion into oxygen and hydrogen peroxide (Beyer et al., 1991). Proline accumulates heavily in several plants under stress, thereby providing protection to the plants against damage by ROS. Proline accumulation, accepted as an indicator of environmental stress, is also considered as an osmoprotectant. Proline accumulation in plant tissues has been suggested to result from:

(a) A decrease in proline degradation,
(b) An increase in proline biosynthesis,
(c) A decrease in protein synthesis or proline utilization, and
(d) Hydrolysis of proteins (Charest and Phan, 1990).

Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity (Parekh, 1990).

Capsicum annum (a spice) is one of the major cash crops of southern Tamilnadu, which is seriously affected by the heavy metals accumulated in the soil that remains as the residues of chemical fertilizers and insecticides. Hence the present study is aimed to understand the effects of lead toxicity on the growth and changes of SOD, MDA, proline activity and chlorophyll content of pot cultured *C.annum* seedlings in order to better understand the defensive mechanisms of *C.annum* under heavy metal stresses.

MATERIALS

Capsicum annum seeds were brought from Tamil Nadu Agriculture College, Killikulam, Tuticorin.

METHODS

The seeds were soaked in distilled water for overnight and it was sown in the soil, the seeds were germinated after 48hrs. After the germination of the seedlings, it was subjected to stress (Lead acetate) with a concentration gradient of 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm and a set was maintained as control. The leaves were harvested on 10^{th}, 20^{th} and 30^{th} day for the biochemical analysis. Malondialdehyde (MDA) was measured by colorimetric method following the procedure of Stewart and Bewley, (1980). Superoxide dismutase (SOD) activity was assayed by the measurement of its capacity to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT) (Beauchamp & Fridovich, 1971). Proline was determined following the procedure of Bates *et al*. (1973). The amount of chlorophyll was estimated by the procedure of Arnon (1949).

RESULTS AND DISCUSSION

MDA content increases with the increasing heavy metal concentration, indicating a concentration-dependent free radical generation (**Table 1 & Fig. 1**). The MDA content on 10th day ranged from 1.01 to 1.93 mg/g fw, on 20th day 1.23 to 2.15 mg/g fw and on 30th day 1.45 to 2.46 mg/g fw, whereas in control it was 0.06 to 0.09 mg/g fw which indicates the low production of ROS. Similar results were obtained in a study done by Soleimanzadeh et al., (2010) in *Helianthus annus* under drought stress. This result suggests that stress directly or indirectly leads to the production of oxygen radicals, which results in the increased lipid peroxidation and oxidative stress in the plant, thus results in increased MDA.

Table 1: Effect of lead at various concentrations on MDA, SOD, Proline and Chlorophyll content.

	Concentration of lead	10th day	20th day	30th day
Amount of malondi aldehyde (mg/g fw)	10 ppm	1.01	1.23	1.45
	20 ppm	1.3	1.45	1.55
	30 ppm	1.58	1.73	1.94
	40 ppm	1.75	1.9	2.32
	50 ppm	1.93	2.15	2.46
	control	0.06	0.08	0.09
Amount of super oxide dismutase (mg/g fw)	10 ppm	79.13	83.32	90.56
	20 ppm	84.07	88.45	94.67
	30 ppm	90.43	94.89	98.33
	40 ppm	94.2	99.52	103.86
	50 ppm	97.75	102.21	106.29
	control	70.31	72.64	75.73
Amount of proline (mg/g fw)	10 ppm	150.43	155.52	159.77
	20 ppm	161.95	165.21	191.29
	30 ppm	168.47	192.38	233.64
	40 ppm	173.91	201.08	245.47
	50 ppm	213.04	268.68	305.42
	control	90.21	90.62	91.34
Amount of total chlorophyll (mg/g fw)	10 ppm	0.44	0.33	0.24
	20 ppm	0.33	0.27	0.22
	30 ppm	0.24	0.22	0.18
	40 ppm	0.22	0.11	0.09
	50 ppm	0.07	0.04	0.03
	control	0.56	0.6	0.65

SOD activity is increased with increasing heavy metal concentration (Table 1 & **Fig. 2**). The content increased gradually from 79.13 to 97.75 mg/g fw on the 10th day, 83.32 to 102.21 mg/g fw on the 20th day and 90.56 to 106.29 mg/g fw on the 30th day, whereas in control it was 70.31 to 75.13 mg/g fw. Up-regulation of SODs is essential for combating the oxidative stress and catalyzing the dismutation of superoxide into oxygen and hydrogen peroxide. In plants environmental adversities often lead to the increased generation of ROS and consequently, production of SOD has been proposed to be an important mechanism in plant stress tolerance (Alscher *et al.*, 2002). Similar result was observed by Nan Jiang et al., (2010), where the SOD activity increased significantly in *Luffa cylindrica* seedlings under different lead concentration. The increased SOD activity in response to lead stress appears to be the need for combating oxidative stress. SOD activity is of more relevance in heavy metal stress studies for the maintenance of the overall defense system of a plant subjected to oxidative damage (Alscher *et al.*, 2002). Based on the above results, SOD is believed

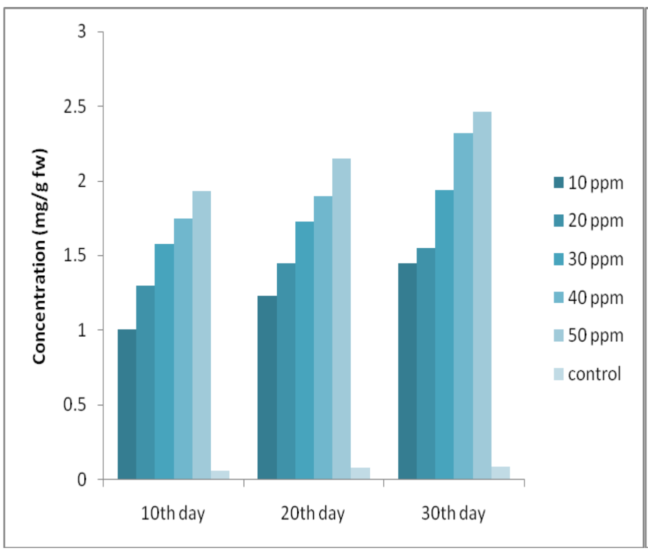

Fig 1: Effect of Lead on MDA content in *Capsicum annum*.

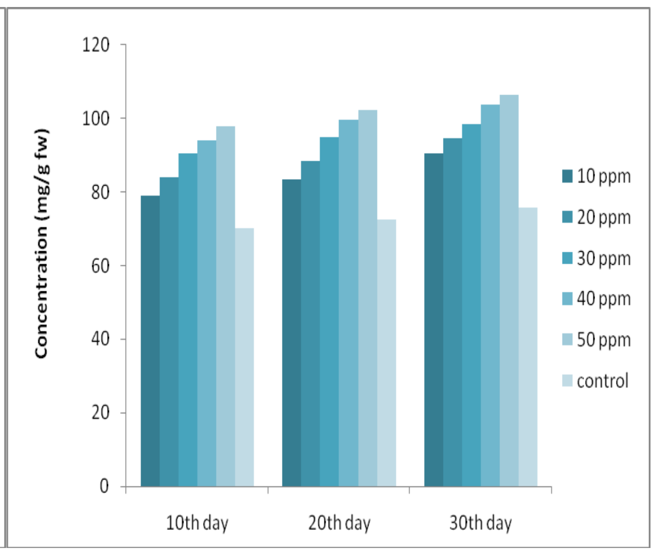

Fig 2: Effect of Lead on SOD content in *Capsicum annum*.

to be one of the most important factors of the plant biochemical defense against lead toxicity, which is actively involved in the self-regulation of plant metabolism.

Increase in the osmoprotectant proline content was directly proportional to the heavy metal concentration (Table 1 & **Fig. 3**). The proline content increased from 150.43 to 213.04 mg/g fw on the 10th day, 155.52 to 268.68 mg/g fw on the 20th day and 155.77 to 305.42 mg/g fw on the 30th day, whereas the control ranged from 90.21 to 91.34 mg/g fw with more or less no change in the proline content with increase in days. Similar results were observed by Fikriye and Omer (2005) in *Phaseolus vulgaris* when treated with heavy metals. The increase in proline content may be due to a decrease in the activity of the electron transport system (Alia et al., 1993) leading to the accumulation of NaDH and H^+ ions. Proline accumulation (presumably through synthesis from glutamic acid) might be an adaptive mechanism for reducing the level of accumulated NADH and the acidity; ($2NADH+2H^+$) is used for synthesizing each molecule of proline from glutamic acid (Venekemp et al., 1987). Proline as a cytosolic osmoticum and a scavenger of OH^- radical that can interact with cellular macromolecules such as DNA, protein and membranes and stabilize the structure and function of such macromolecules (Kavi Kishor et al. 2005). Binding with metal ions due to the chelating ability of proline (an imino acid) can also be a defense mechanism for survival.

A decreased trend was observed in the chlorophyll content with response to the increased metal ion concentration (Table 1 & **Fig. 4**). The decrease of the photo pigment was in the range 0.44 to 0.07 mg/g fw on the 10th day, 0.33 to 0.04 mg/g fw on the 20th day and 0.24 to 0.03 mg/g fw on the 30th day, where a gradual increase was observed in the controlled set (0.56 to 0.65 mg/g fw), indicating the normal functioning of the photosynthetic enzymes. Such an investigation was carried out by Shafi and Agnihotri (2010) in *Cicer arietinum* when treated with cadmium and mercury. Decreased chlorophyll content associated with heavy metal stress may be due to the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Heavy metals severely inhibit the plant growth and even cause plant death by disturbing the uptake of nutrients and thereby decreasing the photosynthesis via degradation of chlorophyll (Zhang *et al*., 2007).

CONCLUSION

From the observed results, it is concluded that the activity of malondialdehyde, superoxide dismutase and proline activity increased in response to the increased metal ion concentration from 10 ppm to 50 ppm. The increased malondialdehyde content indicates the production of free oxygen radical, whereas the increased superoxide dismutase and proline content indicates the scavenging mechanism of the plants against the ROS produced in response to the metal stress. The chlorophyll content of the metal stressed leaves decreased with increased concentration of metal ion from 10 ppm - 50 ppm. The decreased chlorophyll content is a visible symptom of reduced growth. The defense mechanism of the plants against metal stress was

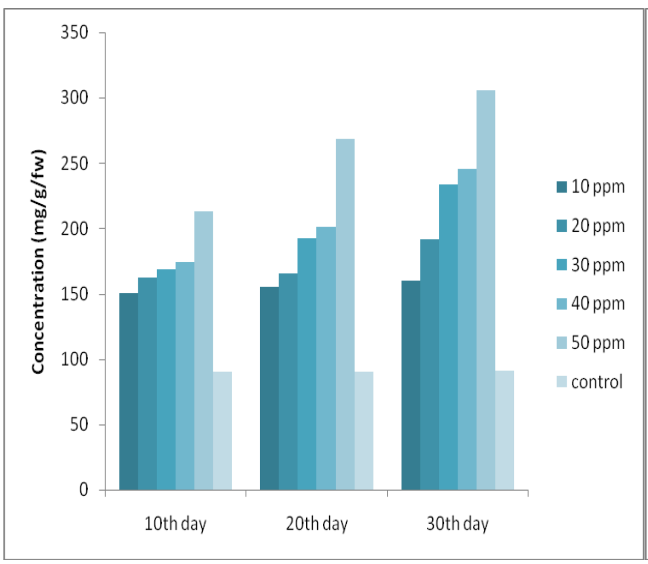

Fig 3: Effect of Lead on proline content in *Capsicum annum*

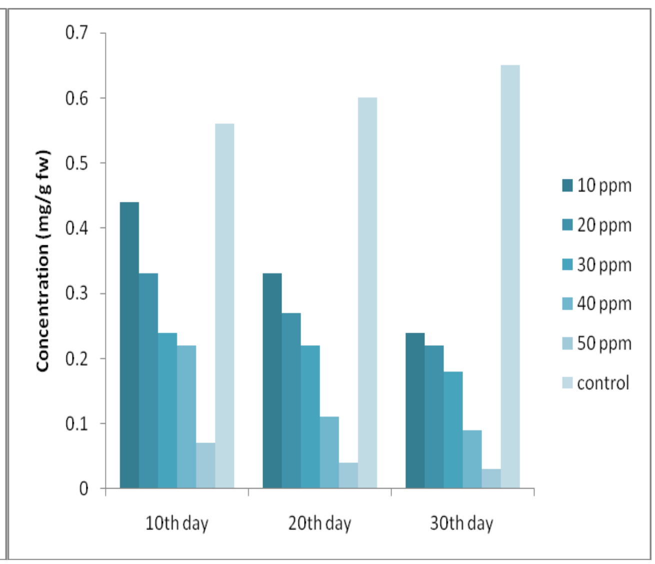

Fig 4: Effect of Lead on chlorophyll content in *Capsicum annum*

thus observed with the increased production of SOD and proline, and the reduced growth by the decrease in the chlorophyll content.

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