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TOXICOKINETIC STUDIES OF ANTIOXIDANTS OF AMARANTHUS TRICOLOUR AND MARIGOLD (*CALENDULA OFICINALIS L.*) PLANTS EXPOSED TO HEAVY METAL LEAD

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ABSTRACT: Environmental contamination of soil by toxic metals is a serious problem worldwide due to their accumulation in food chain and continued persistence in ecosystem. Conventional technologies involve physical displacement or chemical replacement, generating yet another problem of toxic sludge. Some species of plants act as phytoaccumulators of heavy metals. During accumulation, plants exhibit some defense mechanism to overcome the stress due to heavy metal exposure through their antioxidants. In the present work an attempt has been made to study the toxicokinetic behaviour of the antioxidant enzymes such as superoxide dismutase, catalase and glutathione and total antioxidant activity of the plants *Amaranthus tricolor* and Marigold (*Calendula oficinalis* L.) exposed to heavy metal Lead. The metal has significant effect on the enzymes activities and the results are discussed.

Key words: Heavy metals Lead, toxicokinetics, antioxidants, *Amaranthus Tricolor* and Marigold (*Calendula oficinalis* L.).

INTRODUCTION

Metals are naturally found in the earth's crust at various concentrations. The development of various industries, mining activity, irrigation with waste water and the application of sewage sludge to agricultural lands have increased the release of metals into our ecosystem, causing serious environmental problem and posing threats to human health. In short, contamination of soils by heavy metals is of wide spread occurrence as a result of human, agricultural and industrial activities.

Toxic heavy metals such as cadmium (Cd), lead (Pb) and mercury (Hg) have become one of the major pollutants due to their significant release into environments via natural and anthropogenic activities (Chen et al., 2009). Lead is a naturally occurring, bluish – grey lustrous metal found in small amounts within the earth's crust. Based on the frequency of occurrence, toxicity and the potential for human exposure, lead is second on the list of the 'Top twenty Hazardous substances according to the agency for Toxic substances and Disease Registry (ATSDR, 2000). Lead is considered one of the most frequently encountered heavy metals of environmental concern and is the subject of much remediation research. Although Lead is not an essential element for plants, it gets readily absorbed and accumulated in different plant parts, and shows greater possibility of it entering the food chain. Many studies have shown that food crops accumulate trace metals in their tissues when grown on contaminated soil (Liu et al., 2005; Muchuweti et al., 2006; Sharma et al., 2007; Nabulo et al.,2010).

It has been discovered that plant-metal interaction can be utilized for industrial and environmental benefits.

As a cost effective and environmentally-friendly alternative, the use of plants to remedy soils contaminated with inorganic and organic xenobiotics has gained increasing attention in recent years, giving rise to the phytoremediation concept (Doty, 2008;Macek et al., 2008; Eapen et al., 2007; Eapen and D'Souza, 2005; Cherian and Oliveira, 2005; Macek et al., 2004).

Phytoremediation defines the use of plants to extract, sequester, and/or detoxify various kinds of environmental pollutant (Salt et al., 1998). It is a newly evolving field of biotechnology that uses plants to clean-up polluted soil, water, and air (Salt et al., 1998). The thriving plants show a particular potential for remediation, they act as natural vacuum cleaners sucking pollutants out of the soil and depositing them in the leaves and shoots. Thus the key plant characteristic required is the 'Heavy metal tolerance.

However, the contaminants accumulated in the plants impose stress on the plants. In general factors that impose stress on plants tend to trigger a plant's innate defense mechanism, to cope up with the stress. A wide range of protective mechanisms exist in plants which involve non – enzymic antioxidants and enzymic antioxidants.

Plant species differ markedly in uptake and tolerance to Lead and other heavy metals. Metal-hyperaccumulating plants typically store large amounts of metals in their aerial parts (Baker and Walker, 1990), which renders hyperaccumulators highly suitable for phytoremediation. To exploit full potential of phytoremediation, it is obligatory to investigate the mechanisms responsible for tolerance and hyperaccumulation, using natural hyperaccumulators as model plant species (Lombi et al., 2001).

Though some heavy metals are essential as micronutrients, uptake of higher concentrations of heavy metal is found to be toxic for plants. Certain metals are known to produce/act as catalysts for the production of free radicals in biological systems (Aust, 1989; Dietz et al., 1989). Many heavy metals like Fe, Cu, Cd, Cr, Zn, etc. have been shown to cause oxidative damage in various higher plants (Luna et al., 1994; Prasad and Freitas,1999; Panda and Patra, 2000).

Life in an oxygen-rich atmosphere has led to the evolution of biochemical adaptations that exploit the reactivity of active oxygen species (AOS). AOS consist of free radicals, such as superoxide, hydroxyl radicals, hydrogen peroxide, and singlet oxygen (Noctor and Foyer, 1998). The role of amaranthus as an under exploited plant with promising economic value has recently been recognized by the National Academy of Sciences (NAS,1984). The Amaranthaceae family consists of hardy, weedy, herbaceous, fast-growing, cereal-like plants (Opute, 1979), with a seed yield of upto 3 tons / hectare when grown in monoculture for 3-4 months, and a vegetable yield of 4.5 tons dry matter/hectare after 4 weeks (Grubben and Van Sloten, 1981). An increase or decline in the activity of antioxidants is a direct indication of the adaptive response of plants to avoid the metal toxicity.

Amranthus tricolor belongs to the family *Amaranthaceae* and species *A. tricolor.* It is an ornamental plant also known as Joseph's coat, (after the Biblical figure Joseph who is said to have worn a coat of many colours) cultivars have striking yellow, red and green foliage. It is rich in minerals Ca, Fe, Mg, Zn, Cu, Mn, K, P and vitamins A, B6 and Vitamin C, riboflavin. Marigold belongs to the family *Asteraceae* and the Botanical name is *Calendula oficinalis* (Linn). It is a garden flower with pale green leaves and golden orange flowers. Hence in the present study it is aimed to investigate the toxicity of Pb on the antioxidative enzymes of plants *Amaranthus tricolor* and *Calendula oficinalis.*

MATERIALS AND METHODS

Top soil upto 15cm depth, was collected from the fertile agricultural lands of Kaniyambadi Village, near Vellore town. The samples were air dried, crushed to powder and sieved in 0.5mm mesh. The sieved soil samples were stored in polythene bags. This soil is taken as control. The toxicity of Lead as Lead nitrate was evaluated for both the plants and the sublethal concentration (300 mg/kg) was mixed with 1500 grams of soil..

Amaranthus tricolor and Marigold seeds were sown and in the fertile soil and the saplings were planted in the in six pots each. The plants were exposed to the metal contaminated soils for a period of 30 days and vitamins and minerals factors, and antioxidant factors were tested by the following standard methods.

Preparation of the homogenate

Cold aqueous extracts were prepared by homogenizing 10 g of each fresh plant materials in 100 ml of water for 5 minutes. The cooled and macerated mixtures were filtered through Whatman No.1 paper and were dried in an air oven at 50ºC. The collected dried extracts were stored in the refrigerator at 4ºC. Various parameters of the control and lead contaminated soil exposed plant extracts were estimated by standard methods. Ascorbic acid was estimated by USP method, (1980), α-Tocopherol by the method of Desai (1984), estimation of reduced Glutathione by the method of Ellman et al., (1959), estimation of catalase by Beers and Seizer (1952) method, DPPH free radical scavenging activity by Blosi (1958) method, estimation of SOD by the method of Misra and Fridovich (1972).

RESULTS AND DISCUSSION

Some heavy metals at low doses are essential micronutrients for plants, but in higher doses they may cause metabolic disorders and growth inhibition for most of the plants species (Fernandes and Henriques, 1991; Claire et al., 1991). Researchers have observed that some plants species are endemic to metalliferous soils and can tolerate greater than usual amounts of heavy metals or other toxic compounds. In the present study the selected plants Amaranthus tricolour and Marigold show tolerance to lead contaminated soils.

Table: 1 – Biochemical and mineral factors of Amaranthus tricolour and Marigold leaves

Factors	*Amaranthus tricolor*	Marigold (Calendula oficinalis L.)
Carbohydrates g/100g	6.7	25.6
Protein g/100g	3.83	16.6
Fat g/100g	0.8	0.78
Chlorophyll µg/100g	8.8	180.8
Total amino acid g/100g	3.6	5.8
Proline g/100g	3.1	778.7
Total Phenolics mg/100g	485	85.75
β- Carotene µ/100g	11.9	37.5
Ascorbic acid µ/100g	126	122
Thiamine µ/100g	0.14	0.19
Total Ash g/100g	9.2	3.2
K mg/100g	510	7.2
Na mg/100g	32.4	5.4
Fe mg/100g	3.8	4.7
Ca mg/100g	446	216

Table – 1 shows the biochemical factors and mineral status of the two selected plants, *Amaranthus tricolor* and *Marigold*. Among the two plants Marigold shows higher levels of carbohydrates and proteins. Total phenolics, Ascorbic acid, and mineral status of *Amaranthus tricolor* showed higher levels than marigold plant leaves. Proline accumulation is a common metabolic response of higher plants to water deficits, and salinity stress, and has been the subject of numerous reviews over the last 20 years (Stewart and Larher, 1980; Delauney and Verma, 1993; Samaras et al., 1995). Marigold shows higher levels of proline when compared to Amaranthus leaves.

Table – 2 : Levels of antioxidant factors of the leaf parts of Amaranthus tricolor plant exposed to the control and lead contaminated soils

Factors	Control			Experimental		
	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days
AOA %	84	85	90	66	73	75
SOD µgm / minute / mg protein	512	516	518	560	586	637
GSH mg/g	10.02	11.4	12.1	12.4	13.5	13.6
Catalase µmol/min/g	5.84	6.54	6.45	6.36	7.02	7.42

Table – 3 : Levels of antioxidant factors of the leaf parts of plant *Calendula oficinalis* (Marigold) exposed to the control and lead contaminated soils

Factors	Control			Experimental		
	10 Days	**20 Days**	**30 Days**	**10 Days**	**20 Days**	**30 Days**
AOA %	93	102	115	69	63	61
SOD µgm / minute / mg protein	846	854	859	879	914	937
GSH mg/g	17.8	18.5	19.2	18.9	19.8	20.3
Catalase µmol/min/g	5.62	6.17	6.87	7.66	8.3	9.76

Tables- 2 and 3 show the antioxidant enzymes such as superoxide dismutase (SOD), Glutathione reductase(GSH) and Catalase and percentage antioxidant activity of the *Amaranthus tricolor* and Marigold plants respectively in the control soil and sublethal lead contaminated experimental soil samples exposed for 10, 20 and 30 days. The results clearly show that the percent antioxidant activity increased in the control soil whereas it decreased in the experimental soil exposed plant leaves.

The antioxidant enzymes SOD, GSH and catalase increase with increase in the time of exposure. There is induction of oxidative stress in growing plant parts due to enhanced production of reactive oxygen species (ROS). Enzymes SOD, GSH of catalase appear to play a pivotal role in combating Pb – induced oxidative injury in the said plants. Increase in the values of these enzymes in response to Pb can be regarded as an important adaptive response of the plants to avoid Lead toxicity for their ultimate survival during periods of stress. The level of the antioxidant enzymes SOD and CAT may determine the sensitivity of plants to lipid peroxidation (Kanazawa et al., 2000). The response of antioxidant defense system in hyperaccumulator to Cd and other metals have indicated several distinct patterns, which varied among various plant species and tissue analyzed. The increase in SOD activity in response to stresses has been attributed to synthesis of the enzyme (Slooten et al., 1995).

Conclusion

Thus in the present study it is clearly indicated that the two plants Calendula oficinalis (Marigold) and *Amaranthus tricolor* have good mineral , antioxidant, vitamin status and high antioxidant activity too, which helped the plant to survive the high lead toxicity. The increase in antioxidant enzymes prove the plants efficacy to overcome the metal stress and hence the plants can be used for the phytoremediation of lead contaminated soils since the plants show high lead accumulation efficiency also. Among the two plants Calendula oficinalis (Marigold) showed higher antioxidant potential.

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