

Comparative studies on the Effect of Antioxidant properties of the Plants *Helianthus annus* and *Solanum nigrum* Exposed to the Heavy Metal Chromium

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Abstract

Large areas of soils have been contaminated by heavy metals, which are deleterious to the existence, reproduction and development of living organisms including plants, animals and microorganisms. The contamination of nature compartments by heavy metals has become a serious environmental problem. Due to the high cost of conventional clean-up technologies, there is an increasing interest in the remediation of contaminated sites using biological environmental friendly technique. One such method is Phytoremediation - A technique in which soil contamination due to industrial activities is minimized. It is a method, which isolates and detoxifies the heavy metal pollutants. Phytoremediation of soil contaminated by heavy metals has been widely accepted as a cost-effective and environmental-friendly clean-up technology. During the exposure of plants to contaminated soils the antioxidant defence system helps the plant to protect itself from the damage. Antioxidants are substances used by the body to protect itself from damage caused by oxidation due to toxic environment. *Helianthus annus* (Sunflower) and *Solanum nigrum* (Black night shade) are common plants grown in all parts of the world. In the present work the plants *Helianthus annus* and *Solanum nigrum* were exposed to the heavy metal Chromium contaminated soils and the biochemical factors, vitamins and minerals factor and antioxidant factors were investigated. From the results it is evident that the heavy metal chromium affects significantly the biochemical factors, vitamins and minerals factor and antioxidant factors in both the plants. While comparing the antioxidant property between the two plants, *Solanum nigrum* is found to be having higher antioxidant property than *Helianthus annus*.

Key Words: *Phytoremediation, Helianthus annus, Solanum nigrum, antioxidant factors.*

INTRODUCTION

The sunflower (*Helianthus annus*) is an annual plant native to the Americas in the family Asteraceae, with a large flowering head (inflorescence).

The black nightshades (*Solanum nigrum* L. and related species) are worldwide weeds of arable land, gardens, rubbish tips, soils rich in nitrogen, in moderately light and warm situations which occur from sea to montane levels. They are, however, also widely used as leafy herbs and vegetables, as a source of fruit and for various medicinal purposes.

Helianthus annus and *Solanum nigrum* was exposed to the metal Chromium contaminated soils and the biochemical factors, vitamins and minerals factors and antioxidant factors were tested and the results were discussed.

MATERIALS AND METHODS

Preparation of polluted soil

Top soil upto 15 cms 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 0.5 mm mesh. The sieved soil samples were stored in polythene bags.

Different concentrations of Sodium dichromate were mixed with 500 gms of the soil samples and the saplings of the *Helianthus annus* and *Solanum nigrum* plants was planted in different pots. The toxicity of the cadmium metal on the both the plants was investigated after a time period of two weeks.

The sublethal concentration and half of sublethal concentration of the Sodium dichromate solution were selected for the toxicity studies of the chromium on the *Helianthus annus* and *Solanum nigrum* plants.

The plants were exposed to the above metal contaminated soils for four weeks and the biochemical factors, vitamins and minerals factors and antioxidant factors were tested as per standard methods.

- Determination of total carbohydrates by Anthrone method
- Protein estimation by Lowry's method (Lowry et al, 1951)
- Estimation of protein, total free amino acids, proline, reduced glutathione (Moron et al.), superoxide dismutase (Misra and Fridovich, 1972) and catalase (Beers and Siezer, 1952) are done by colorimetric method
- Estimation of free fatty acids (Titration)
- DPPH free radical scavenging activity (Blosi, 1958)

RESULTS AND DISCUSSION

In the present research work an attempt has been made to study the effect of Chromium metal polluted soils on the various antioxidant factors, vitamins and minerals factors and biochemical properties of the plants *Helianthus annuus* and *Solanum nigrum*.

Table – 1 represents the Biochemical factors of the plants *Helianthus annuus* and *Solanum nigrum* grown in fertile garden soil, sublethal and half of sublethal Chromium polluted soil. The total carbohydrates, total proteins, fats, chlorophyll, Total amino acid and the amino

acid except proline were considerably reduced in the plants sample of both *Helianthus annuus* and *Solanum nigrum* exposed to heavy metal Chromium contaminated soil.

This may be because proline a non essential amino acid is synthesized in the living organism whenever it is subjected to stress such as high or low temperatures, high salinity, sodicity, high heavy metal exposures etc.

Proline accumulation is a common metabolic responses 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; Thompson, 1980; Stewart, 1981; Hanson and Hitz, 1982; Rhodes, 1987; Delauney and Verma, 1993; Samaras et al, 1995; Taylor, 1996; Rhodes et al, 1999).

Proline protects membranes and proteins against the adverse effects of high concentrations of inorganic ions and temperature extremes (Pollard and Wyn Jones, 1979; Paleg et al, 1981) Proline may also function as a protein-compatible hydrotrope (Srinivas and Balasubramanian, 1995), and as a hydroxyl radical scavenger (Smirnoff and Cumbes, 1989).

Table – 1 Biochemical Factors of the plants *Helianthus annuus* and *Solanum nigrum*

Factors	<i>Helianthus annuus</i>			<i>Solanum nigrum</i>		
	Control	Sublethal Cr	Half of sublethal Cr	Control	Sublethal Cr	Half of sublethal Cr
CHO g/100g	6.7	4.1	4.3	7.7	5.3	6.8
Protein g/100g	3.83	1.77	2.01	7.63	5.42	6.42
Fat g/100g	0.8	0.41	0.56	0.8	0.69	0.75
Chlorophyll µg/100g	16.8	11.8	12.2	20.8	15.4	17.1
Total amino acid g/100g	3.6	2.1	2.9	4.1	3.3	3.51
Proline g/100g	3.1	5.4	5.1	4.8	6.1	4.1

Table – 2 Vitamins and Minerals Factors of the plants *Helianthus annus* and *Solanum nigrum*

Factors	<i>Helianthus annus</i>			<i>Solanum nigrum</i>		
	Control	Sublethal Cr	Half of sublethal Cr	Control	Sublethal Cr	Half of sublethal Cr
Total Phenolics mg/100g	374	312	331	910	598.6	716.6
β- Carotene µg/100g	28.9	18.7	21.6	44.3	38.4	40.4
Ascorbic acid µ/100g	126	98	103	480	375	458
Thiamine µ/100g	0.14	0.09	0.107	0.15	0.13	0.14
Total Ash g/100g	9.2	7.53	8.35	3.0	1.9	2.9
K mg/100g	510	435	475	410	324.5	363.5
Na mg/100g	32.4	25.5	28.5	33	27.8	30.2
Fe mg/100g	3.8	2.15	2.95	52	32.9	43.14
Ca mg/100g	446	325	385	430	312	410

Table - 2 shows the vitamin and mineral content of the plants *Helianthus annus* and *Solanum nigrum* grown in fertile garden soil, sublethal and half of sublethal Chromium polluted soil.

The factors were reduced significantly in the heavy metal contaminated soil. The vitamins A, B and C and total phenolics and other minerals were significantly high in the normal plant showing that the plant is rich in antioxidant phytochemicals.

Table - 3 shows the antioxidant enzyme levels and the percentage antioxidant activity of the

plants *Helianthus annus* and *Solanum nigrum* plant. The antioxidant activity was found to be the maximum when the plants exposed to control soil. The same has been reduced much in the plants exposed to sublethal Chromium and half of sub lethal Chromium exposed soil

This shows that the free radical scavenging activity is reduced much in the plant exposed to heavy metal contaminated soils. The enzymes Superoxide dismutase, catalase and glutathione were increased significantly in the plant sample exposed to heavy metal contaminated soils.

Table –3 Antioxidant properties of the plants *Helianthus annus* and *Solanum nigrum*

Factors	<i>Helianthus annus</i>			<i>Solanum nigrum</i>		
	Control	Sublethal Cr	Half of sublethal Cr	Control	Sublethal Cr	Half of sublethal Cr
AOA %	86	68	71	110	66	75
SOD µgm / minute / mg protein	617	484	535	518	630	737
GSH mg/g	10.02	7.1	8.4	16.1	17.4	19.3
Catalase µ/mol/min/g	5.85	4.1	4.72	7.85	7.86	8.92

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) The availability of heavy metals to plants and, thus, their toxicity depends on complex rhizospheric reactions involving not only exchange processes between soil and plants but also microbial activities.

Heavy metals and antioxidative defences

There is ample evidence that exposure of plants to excess concentrations of redox active heavy metals such as Fe and Cu results in oxidative injury (De Vos et al., 1992; Gallego et al., 1996; Wecks and clijsters, 1996; Mazhoudi et al., 1997; Yamamoto et al., 1997). The ability of plants to increase antioxidative protection to combat negative consequences of heavy metal stress appears to be limited since many studies showed that exposure to elevated concentrations of red ox reactive metals resulted in decreased and not in increased activities of anti-oxidative enzymes.

Exposure to heavy metals also provoked pronounced responses of antioxidative systems, but the direction of the response was dependent on the plant species and tissue analyzed, the metal used for the treatment and the intensity of the stress. However, some common reaction patterns can be found.

In most cases, exposure to heavy metals initially resulted in a severe depletion of GSH (Cd: *Rauvolfia serpentina*: Grill et al., 1987; pine *Schutzendubel* et al., 2001; carrot di Toppi et al., 1999; tobacco: Vogeli-Lange and Wagner, 1996; Cu: *Silence cucubalus*: de Vos et al., 1992; Cu or Cd: *Arabidopsis*: Xiang and Oliver, 1998; Ni and Zn pigeonpea: Rao and Sresty, 2000; Fe, Cu or Cd: sunflower leaves: Gallego et al., 1996). This is a common response to Cd caused by an increased

consumption of glutathione for phytochelatin production (Zenk, 1996; Mehra and Tripathi, 1999).

The significance of phytochelatin for protection from heavy metals has frequency been reviewed (Rauser, 1995; Zenk, 1996; Mehra and Tripathi, 1999) and therefore, will be summarized here only briefly. Phytochelatins sequester heavy metals. For Cd, the formation of Cd-thiolate (Cd-S) complexes in phytochelatins has been shown (Strasdeit et al., 1991).

The chelated metals are transported to the tonoplast, taken up to active transport systems, and deposited in the vacuole (Tommasini et al., 1998; Rea, 1999). This mechanism contributes to the protection from heavy metal toxicity in several plant species and in some fungi as well (Ishikawa et al., 1997).

From the above results it is evident that the heavy metals Chromium affects significantly the biochemical factors, vitamins and mineral factors and antioxidant enzymes in both the plants. Among the two plants the antioxidant property of *Solanum nigrum* is found to be comparatively higher than *Helianthus annuus*.

CONCLUSION

In the present work the plants *Helianthus annuus* and *Solanum nigrum* was exposed to the Chromium metal contaminated soils and the biochemical factors, vitamins and mineral factors and antioxidant factors were tested. The toxicity of the Chromium metal on *Helianthus annuus* and *Solanum* plant was investigated and the results were tabulated. From the above results it is evident that the heavy metal Chromium affects significantly the biochemical factors, vitamins and minerals factors and antioxidant enzymes in both the plants *Helianthus annuus* and *Solanum nigrum*. Among the two plants the overall antioxidant property of *Solanum nigrum* is found to be comparatively higher than *Helianthus annuus*.

REFERENCES

- [1]. Angelone M, Bini C. 1992. Trace elements concentrations in soils and plants

- of western Europe. In: Adriano DC, ed. *Biogeochemistry of traces metals*. Boca Raton, FL: Lewis Publishers, 19-60.
- [2]. Anilkumar, (1989). In: Environmental Chemistry. Edition –2, Willy eastern Limited – pp 67.
- [3]. Anon. 1994. Phytoremediation for Wastewater Cleanup. *Enhanced Energy Recovery & Refining News* 18(1):4 (August 1994).
- [4]. Baccouch S, Chaoui A, El Ferjani E. 1998. Nickel-induced oxidative damage and antioxidant responses in *Zea mays* shoots. *Plant Physiology and Biochemistry* 36, 689-694.
- [5]. Barnett NM, Naylor AW 1966 Amino acid and protein metabolism in Bermuda grass during water stress. *Plant Physiology* 41: 1222-1230.
- [6]. Baubles, A., E.A. Decker and F.M. Clydes Dale, 2000. An antioxidant effect of aqueous extracts from wheatbased ready-to-eat breakfast cereals. *Food Chem.*,
- [7]. Bergmann L, Rennenberg H. 1993. *Glutathione metabolism in plants*. In: DeKok LJ, Stulen I, Rennenberg H, Brunold C, Rauser W, eds. *Sulphur nutrition and assimilation in higher plants*. The Hague: SPB Academic Publishing, 109-124.
- [8]. Berti, W.R. and Cunningham, S.D., (2000): In *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment*. (ed. Raskin, I.) – Wiley-Interscience, John Wiley and Sons, Inc. New York, NY.; pp 71- 88.
- [9]. Bielawski W, Joy KW. 1986. Reduced and oxidized glutathione and glutathione reductase activity in tissue of *Pisum sativum*. *Planta* 169, 267.
- [10]. Blaylock, M.J., Huang, J.W., (2000): Phytoextraction of metals. In: Raskin, I., Ensley, B.D. (Eds.), *Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment*. – John Wiley and Sons, New York; pp. 53–70.
- [11]. Blois, M.S., 1958. Antioxidant determination by the use of a stable free radical. *Nature* 181, 1199–1200.
- [12]. Boggess SF, Aspinall D, Paleg LG 1976 Stress metabolism. IX. The significance of end-product inhibition of proline biosynthesis and of compartmentation in relation to stress-induced proline accumulation. *Aust. J. Plant Physiology* 3: 513-525.
- [13]. Chaoui A, Mazhoudi S, Ghorbal MH, El Ferjani E. 1997. Cadmium and Zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris L.*). *Plant Science* 127, 139-147.
- [14]. Clemens, S.; Palmgren, M.G. and Krämer, U. A long way ahead: understanding and engineering plant metal accumulation. *Trends in Plant Science*, 2002, vol. 7, p. 309-315.
- [15]. Cobbett, C.S. (2000): Phytochelatins and their role in Heavy Metal Detoxification. – *Plant Physiology*, 123; 825-832.
- [16]. Delauney AJ, Verma DPS 1993 Proline biosynthesis and osmoregulation in plants. *Plant J.* 4: 215-223.
- [17]. De Vos RCH, Vonk MJ, Vooijs R, Schar H. 1992. Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus*. *Plant Physiology* 98, 853-958.
- [18]. Di Toppi LS, Lambardi M, Pazzagli L, Cappugi G, Gabbriellini R. 1999. Response to cadmium in carrot in vitro plants and cell suspension cultures. *Plant Science* 137, 119-129.
- [19]. Dushenkov, D. (2003): Trends in phytoremediation of radionuclides. – *Plant and Soil*. 249; 167-175.
- [20]. Gerard, E., Echevarria, G., Sterckeman, T., and Morel, J.L. P. (2000): Availability of Cd to three plant species varying in accumulation pattern. – *J. Environ. Qual.* 29; 1117-1123.
- [21]. Godbold DL, Hittermann A. 1985. Effect of zinc, cadmium and mercury on root elongation of *Picea abies* (Karst.) seedlings, *Environmental Pollution* 38, 375-381.
- [22]. Godbold DL. 1991. Cadmium uptake in Norway spruce. *Tree Physiology* 9, 349-357.
- [23]. Ghosh, M., Singh, S. P. (2005): A comparative study of cadmium phytoextraction by accumulator and weed species. – *Environment Pollution*. 133: 365-371.
- [24]. Ghosh, M., Singh, S. P., Purohit, S.B. (2003): Comparative uptake

- and phytoextraction study of soil induced chromium by accumulator and high biomass weed species. (Communicated).
- [25]. Halliwell, "Superoxide Dismutase", The toxic effects of oxygen on plant tissues 1, Ed. L.W. Orley – London: CRC Press. Boca Ration, 89-123, (1982).
- [26]. Halliwell, B., 1996. Antioxidant in human health and disease. *Ann. Rev. Nutr.*, 16: 33-50.
- [27]. Ishikawa T, Li ZS, Lu YP, Rea PA. 1997. The GS-X pump in plant, yeast, and animal cells: structure function, and gene expression. *Bio Science Reports* 17, 189-207.
- [28]. Krämer, U. and Chardonnens, A.N. The use of transgenic plants in the bioremediation of soils contaminated with trace elements. *Applied Microbiology and Biotechnology*, 2001, vol. 55, no. 6, p. 661-672.
- [29]. Koppolua L., Agblover F.A., Clements L.D. (2003): Pyrolysis as a technique for separating heavy metals from hyperaccumulators. Part II: Lab-scale pyrolysis of synthetic hyperaccumulator biomass. – *Biomass and Bioenergy*. 25; 651 – 663
- [30]. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (November 1951). "Protein measurement with the Folin phenol reagent". *J. Biol. Chem.* **193** (1): 265–75.
- [31]. Mazhoudi S, Chaoui A, Ghorbal MH, El Ferjani E. 1997. Response of antioxidant enzymes to excess copper in tomato (*Lycopersicon esculentum*, Mill). *Plant Science* 127, 129-137.
- [32]. Mehra RK, Tripathi RD. 1999. Phytochelatins and metal tolerance. In: Agarwal SB, Agarwal M, eds. *Environmental pollution and plant responses*. Boca Raton, FL: CRC Press, Lewis Publisher, 367-382.
- [33]. PRASAD, M.N.V. Phyto remediation of metals and radionuclides in the environment: the case for natural hyperaccumulators, metal transporters, soil-amending chelators and transgenic plants. In: *Heavy metal stress in plants: from biomolecules to ecosystems*. Heidelberg, Springer-Verlag, 2nd ed., 2004, pp. 345-392.
- [34]. PRASAD, M.N.V. and STRZALKA, K. *Physiology and biochemistry of metal toxicity and tolerance in plants*. Dordrecht, Kluwer Academic Publishers, 2002, 432 p. ISBN 1-40-200468-0.
- [35]. Rao KVM, Sresty TVS. 2000. Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan*(L). Millspaugh) in response to Zn and Ni stresses. *Plant Science* 157, 113-129.
- [36]. Raskin, I. and Ensley, B. D. (2000): *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment*. – John Wiley & Sons, Inc., New York.; pp 53-70.
- [37]. Rauser WE. 1995. Phytochelatins and related peptides. Structure, biosynthesis, and function. *Plant physiology* 109, 1141-1149.
- [38]. Rea P. 1999. MRP subfamily ABC transporters from plants and yeast. *Journal of Experimental Botany* 50, 895-913.
- [39]. Reeves, R.D. (2003): Tropical hyperaccumulators of metals and their potential for phytoextraction. – *Plant and Soil*. 249; 57-65.
- [40]. Rudolph AS, Crowe JH, Crowe LM 1986 Effects of three stabilizing agents - proline, betaine and trehalose - on membrane phospholipids. *Arch. Biochem. Biophys.* 245: 134-143.
- [41]. Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Ensley BD, Chet I, Raskin I. 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology* 13, 468-474.
- [42]. Samaras Y, Bressan RA, Csonka LN, Garcia-Rios MG, Paino D'Urzo M, Rhodes D 1995 Proline accumulation during drought and salinity. In (N Smirnoff ed) "Environment and Plant Metabolism: Flexibility and Acclimation," Bios Scientific Publishers, Oxford, pp 161-187.
- [43]. Schutzendubel A, Schwanz P, Teichmann T, Gross K, Langenfeld-Heyser R, Godbold DL, Polle A. 2001. Cadmium-induced changes in antioxidative systems, H₂O₂ content and differentiation in pine (*Pinus sylvestris*) roots. *Plant Physiology* 127, 887-892.
- [44]. Singh, S. P., Ghosh, M. (2003): A Comparative study on effect of cadmium, chromium and lead on seed germination of weed and accumulator plant

- species. –Indian Journal of Environment Protection. 23(5); 513-518.
- [45]. Strasdeit H, Duhme AK, Kneer R, Zenk MH, Hermes C, Nolting HF. 1991. Evidence for discrete Cd(Scys)₄ units in cadmium phytochelatin complexes from EXAFS spectroscopy. *Journal of the Chemical Society Series of Chemical communications*, 1129-1130.
- [46]. Tommasini R, Vogt E, Fromenteau M, Hoertensteiner S, Matile P, Amrhein N, Martinoia E. 1998. An ABC-transporter of *Arabidopsis thaliana* has both glutathione-conjugate and chlorophyll catabolite transport activity. *The Plant Journal* 13, 773-780.
- [47]. TRAMPCZYNSKA, A.; GAWRONSKI, S.W. and KUTRYS, S. *Canna x generalis* as a plant for phytoextraction of heavy metals in urbanized area. *Zeszyty Naukowe Politechniki Slaskiej*, 2001, vol. 45, p. 71-74.
- [48]. TSAO, D. *Phytoremediation*. Heidelberg, Springer-Verlag, 2003. 206 p. ISBN 3540433856.
- [49]. VALDES, J.J. *Bioremediation*. Kluwer Academic Publishers, 2002. p. 169. ISBN 0792364597.
- [50]. Vogeli-Lange R, Wagner GW. 1996. Relationship between cadmium, glutathione and cadmium-binding peptides (phytochelatins) in leaves of intact tobacco seedlings. *Plant Science* 114, 11-18.
- [51]. Weckx JEJ, Clijster HMM. 1996. Oxidative damage and defense mechanisms in primary leaves of *Phaseolus vulgaris* as a result of root assimilation of toxic amounts of copper. *Physiologia Plantarum* 96, 506-512.
- [52]. Wise, D.L.; Trantolo, D.J.; Cichon, E.J.; Inyang, H.I. and Stottmeister, U. *Bioremediation of contaminated soils*. New York, Marcel Dekker Inc., 2002. p. 903. ISBN 0824703332.
- [53]. Xiang C, Oliver DJ. 1998. Glutathione metabolic genes co-ordinately respond to heavy metals and jasmonic acid in *Arabidopsis*. *The Plant Cell* 10, 1539-1550.
- [54]. Yamamoto Y, Hachia A, Matsumoto H. 1997. Oxidative damage to membranes by a combination of aluminium and iron in suspension-cultured tobacco cells. *Plant Cell Physiology* 38, 1333-1339.
- [55]. Zenk MH. 1996. Heavy metal detoxification in higher plants a review. *Gene* 179, 21-30.