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Full Length Research Paper

Effect of heavy metal pollutants on sunflower

Rajeev Gopal* and Neena Khurana

Department of Botany, University of Lucknow, Lucknow-226007, India.

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The aim of this present study was to assess the tolerance of pollutant elements (Co, Ni, Cd, Cr and Pb) on visible foliar symptoms, tissue concentration and some biochemical parameters in sunflower plants grown at 0.25 mM in soil pot culture. At equimolar concentrations (0.25 mM), Cd induced the most severe visual toxicity effects and exhibited maximum oxidative damage as observed by accumulation of ThioBarbituric Acid Reactive Substances (TBARS) and lower antioxidant capacity than the plants is exposed to Co, Cd, Cr and Pb. The study suggests that the degree of oxidative damage assessed by the manifestation of external visual toxicity effects, tissue concentration and alteration in biochemical parameters were found to be in the order Cd>Cr>Ni>Co>Pb.

Key words: Sunflower, visible symptoms, TBARS, antioxidant, tissue concentration.

INTRODUCTION

Heavy metal contamination of soils is the major global environmental problem. It has increased considerably in last several years and a part is responsible for limiting the crop production. Elevated concentrations of both essential (Co and Ni) and non-essential (Pb, Cd and Cr) heavy metals pose a risk to the health of vegetarian wildlife and human beings (Salt et al., 1998). Among these metals Cd and Pb are considered as the most toxic metals in the environment (Oliveira et al., 2005; Fritioff and Greger, 2006) and are released into the environment from various sources (Landis and Yu, 1999; Sanita and Gabrielli, 1999). Even though these metals are not essential for plants but are readily absorbed by the most root systems because of their water solubility. The growth and metabolism of plants are adversely affected by the increasing levels of these metals in the soil environment (Balestrasse et al., 2003; John et al., 2009), besides this, Cd and Pb are known to accumulate in different plant parts and enter into the food chain. Therefore, pollution due to heavy metals is significant from nutritional and environmental point of view.

*Corresponding author.E-mail: rgbot@sify.com.

Abbreviations: TBARS, Thiobarbituric acid reactive substances; **GSH,** glutathione; **ROS,** reactive oxygen species; **TBAR,** thiobarbuteric acid reaction; **TCA,** trichloroacetic acid; **NBT,** nitro blue tetrazolium; **BDL,** below detect level; **EDTA,** ethylenediaminetetraacetic acid; **LSD,** least significant difference; **MDA,** malondialdehyde.

High concentrations of these metals cause toxicity in plants and have been shown to generate oxidative stress usually accompanied by an increase of reactive oxygen species including the level of hydrogen peroxide (H_2O_2), superoxide ($O^{\cdot}_2$) and hydroxyl radicals (OH^{-}) (Stohs and Baghi, 1995; Pietrini et al., 2003; Milone et al., 2003). Cd and Pb are also known to interrupt the photosynthetic electron transport chain which leads to generation of superoxide radical and singlet oxygen (Asada and Takahashi, 1987) and thus enhance the peroxidant status of the cell by reducing the antioxidant glutathione (GSH) pool, activating calcium dependent systems affecting the iron-mediated processes (Pinto et al., 2003). Cobalt and nickel being essential micronutrients are component of several enzymes and co-enzymes (Marschner, 2002) but excess levels of these have detrimental impact on growth of plants where they disturb several physiological processes (Parida et al., 2003; Gazewska and Sklodowska, 2007). However evidences indicate that Co and Ni phytotoxicity may be attributed to oxidative metabolism of plants (Pandey et al., 2009). Chromium is also considered as a serious environmental pollutant (Khan, 2007). Chromium exposure has also been described in plants to cause alterations in metabolism either by causing a direct effect on enzymes or other metabolites. Cr is a strong oxidant with a high redox potential accounting for a rapid induction in generation of Reactive oxygen species (ROS) and its resultant toxicity (Shanker et al., 2005). The studies on the antioxidative efficiency under metal stresses are well documented but reports on the changes in the antioxidative efficiency due

to excess Co, Ni, Cr, Cd and Pb induced oxidative stress under identical experimental conditions are very scarce. The present study focuses on the relative response of sunflower to stresses of heavy metals that is, Co, Ni, Cd, Cr and Pb in plants grown in soil-pot culture at an excess supply of these elements causing growth retardation and inducing visible foliar symptoms. Attempt has also been made to identify the metabolism of tolerance in sunflower to stresses of these metals.

MATERIALS AND METHODS

Sunflower (*Helianthus annuus* L. cv ASFH 378), plants were grown in soil contained in pots under glass house conditions at an excess supply of Co, Ni, Cd, Cr and Pb. The soil used in the experiment was taken from the sub-surface horizon of normal alluvial soil from district Barabanki, U.P. having a pH 7.1 (1:2 soil-water suspension), EC 0.3 dSm^{-1}, $CaCO_3$ 0.57% and organic carbon 0.3%. A basal dose of NPK (2:1:2) was missed in the processed soil and 5 Kg of air dried soil was filled in each polyethylene pot. The soil was watered to field capacity and incubated in the glass house for one week before seedling. Ten seeds were sown in each pot and after germination 4 seedlings of equal size were retained. After 20 days of normal growth, soil in each pot was treated with 500 ml of aqueous solution of Co, Ni, Cd (as SO_4), Cr (as dichromate) and Pb (as nitrate) at the rate of 0.25 mM. For each treatment three replicates were taken. One set of pot was allowed to grow plants without treatment to serve as control simultaneously. The pots were arranged in randomized block design. As visible symptoms of metal toxicity were initiated, fresh leaf samples from each treatment were collected and estimated for non-protein thiol and lipid peroxidation content and specific activity of antioxidant enzymes – peroxidase, superoxide dismutase and ascorbate peroxidase. Lipid peroxidation was estimated as the amount of malondialdehyde produced by thiobarbuteric acid reaction (TBAR) (Heath and Packer, 1968) with 2 ml of leaf extract in 0.1% trichloroacetic acid (TCA).

The leaves were homogenized (1:10 w/v) in 50 mM potassium phosphate buffer (pH 7.0) containing 1.0% polyvinyl pyrrolidone. For ascorbate peroxidase, 1mM sodium ascorbate was also added to the extraction medium. After centrifugation at 20,000 g for 20 min the supernatant was used for the assay of POD, SOD and APOD. POD activity was measured by a modified method of Luck (1963), in a reaction mixture containing 500 µ moles phosphate buffer (pH 6.0), 1ml 0.5% p-phenylenediamine, 1 ml 0.01% H_2O_2 and 1ml suitably diluted enzyme extract. After 5 min, reaction was stopped by adding 2 ml 5 N H_2SO_4 to the reaction mixture and change in absorbance of the supernatant was measured at 485 nm. Total SOD activity was assayed according to the method of Beauchamp and Fridovich (1971) based on the ability of the enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1mM ethylenediaminetetraacetic acid (EDTA) and soluble enzyme extract. Riboflavin was added in the last and the mixture was illuminated with cool fluorescent light for 30 min. The reaction was stopped by switching the light off. Corresponding unilluminated blanks were also taken.

The absorbance of solution was measured at 560 nm. One unit of SOD represented the amount of enzyme that causes inhibition of NBT by 50%. The APOD activity was assayed following the oxidation of ascorbate to dehydroascorbate at 290 nm by the method of Nakano and Asada (1981).

The reaction mixture consisted of 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM H_2O_2, 1 mM EDTA and enzyme extract (10 to 20 µg protein). Addition of H_2O_2 started the reaction and decrease in the absorbance was recorded after every 30 s interval up to 3 min. The amount of ascorbate oxidized was calculated using extinction coefficient of 2.8 mM cm^{-1}. For the determination of dry matter, plants were collected and sampled from each treatment at day-45, washed thoroughly to avoid loss and injury to roots with distilled water, separated into different parts and weighed for the determination of dry matter after oven drying at 70°C. All the dried samples were digested with a mixture of HNO_3:$HClO_4$ acids (10:1) and analysed for the concentration of Co, Ni, Cd, Cr and Pb in digested plant material by atomic absorption spectrophotometer. All determinations were made in duplicate and the data have been tested statistically for significance at 5% level of probability (Fisher Lysergic acid diethylamide method (LSD) using SigmaStat (ver. 2.03) software.

RESULTS

Visible symptoms

COBALT

The effect of Co supply appeared after 5 days of metal supply as interveinal chlorosis of young leaves starting from the base in addition to depression in growth and leaf area. The chlorosis later extended to apical part of lamina and intensified with increasing duration. The leaf size and height of plants was reduced to 50% of untreated plants.

NICKEL

Visible symptoms of Ni toxicity were initiated after 6 days as marked depression in growth followed by interveinal chlorosis of young leaves initiating from the base extending to the middle part of lamina on continued exposure. Subsequently the interveinal chlorotic areas became necrotic forming necrotic patches near the veins. These necrotic areas enlarged, dried and the leaves collapsed. Leaf area and height of these plants were reduced and depression was 61 and 54%, respectively of the control. Plants collapsed after 20 days of metal addition.

CADMIUM

Cadmium in excess caused reduction in size of young leaves with a marked decrease in growth after 5 days of metal addition. The lower leaves of Cd exposed plants were limped and basal part of the stem developed purple pigmentation which extended upwards in due course. Compared to control plants, flowering was delayed and head size was smaller in Cd treated plants. The depression in height and leaf area was 77% of untreated plants.

CHROMIUM

The symptoms of chromium toxicity appeared earlier that

is within 2 to 3 days of chromium addition as wilting of older leaves. Further growth of these plants was almost stopped and old leaves showed induction of senescence as yellowing at leaf apex. Later the entire lamina turned yellow along with leaf petiole and stem. Plants treated with Cr failed to survive beyond 10 days and collapsed due to severe Cr toxicity. A marked reduction was observed in height (79%) and leaf area (90%) at excess Cr.

LEAD

Sunflower plants appeared considerably tolerant to Pb excess. A supply of excess Pb was unable to induce any characteristic symptoms except for reduction in overall growth. Height of plants was reduced to 20% of control whereas reduction in leaf area was 28%. Compared to control, at excess Pb lower leaves appeared wilted and flowering was delayed and the size of head was decreased from that of control.

BIOMASS

Total biomass of sunflower was reduced after 15 days exposure to excess supply of each Co, Ni,Cr, Cd and Pb. Compared to control reduction in biomass was 94, 90, 81, 69 and 20%, respectively in excess supply of Cr, Ni, Co, Cd and Pb (Table 1).

Tissue concentration

COBALT

Compared to 11.2, 10.2 and 15.9 µg Co g^{-1} in leaves, stem and roots of untreated plants, the concentration of Co in respective plant parts was 241, 191 and 964 µg Co g^{-1} in Co excess, respectively. At 0.25 mM Co, the concentration of Co was maximum in roots followed by leaves and stem.

NICKEL

In leaves, stem and roots of Ni treated plants, the concentration of Ni was as high as 440, 660 and 1276 µg Ni g^{-1} as compared to 28, 8 and 15 µg Ni g^{-1} in respective parts of the control plants. The accumulation of Ni was about three times more in roots than leaves at 0.25 mM Ni.

CADMIUM

At 0.25 mM Cd, the distribution of Cd was 321 µg Cd g^{-1} in roots, 149 µg Cd g^{-1} in stem and 137 µg Cd g^{-1} in leaves as 0.72, 0.72 and 1.02 µg Cd g^{-1} in leaves, stem and root of control plants, respectively.

CHROMIUM

The chromium concentration was also highest in roots 610 µg Cr g^{-1} followed by stem 312 µg Cr g^{-1} and leaves 298 µg Cr g^{-1} while in control plants Cr was undetectable.

LEAD

In sunflower treated with excess Pb the concentration of Pb was 2224 µg Pb g^{-1} in roots whereas 552 µg Pb g^{-1} in leaves and 135 µg Pb g^{-1} in stem (Table 2).

Enzyme activity and lipid peroxidation

The specific activities of antioxidant enzymes POD, SOD and APOD increased at excess each of Co, Ni, Cd and Pb. The induction of POD activity was highest at excess Ni and lowest in Cd treated plants. However, the increase in SOD activity was maximum at excess Pb and that of APOD at excess Ni (Table 1). The 3,4-Methylenedioxyamphetamine (MDA) concentration also increased at excess supply each of Ni, Co, Cd and Pb and the increase was highest at excess Pb (Table 1).

DISCUSSION

Plants response to various types of stresses depends on its genetic make up therefore it behaves differently according to the tolerance mechanism it inhabits. Sunflower appears to be quite resistant to various pathogens because of its capacity to synthesize and accumulate phenolic compounds. Growth and metabolism of sunflower were affected variably by the stress of heavy metals such as Co, Ni, Cd, Cr and Pb. The excess of different metals under study resulted in stunted growth, reduced biomass accumulation and produced characteristic visible effects similar to those described by other workers in different plant species (Tewari et al., 2002; Zhou and Qiu, 2005; Gajewaska and Sklodowska, 2007). These observations are substantiated by a significant enhancement in the level of the metal in the plant tissue. The reduction in biomass was maximum in Cr excess followed by Ni, Co, Cd and Pb as compared to control plants. This may represent the cumulative effect of damaged or inhibited physiological function under stress condition. The observed decrease in dry matter production as a result of metal stress is in agreement with that reported earlier in plants other than sunflower (Ryser and Sauder, 2006; Pandey and Pathak,

Table 1. Effect of pollutant elements on various physical and biochemical parameters of sunflower in soil-pot culture.

Control	Cr	0.25 mM Co	Ni	Cd	Pb
		Height (cm)			
37.5	7.75*	19.5*	17.5*	8.75*	30
		Leaf area (cm^2)			
62.6	6.50	31.5*	24.2*	14.2*	44.8
		Biomass (g dry matter $plant^{-1}$)			
3.27	0.192	1.208*	1.322*	1.002*	2.385*
		Peroxidase (Change in O.D. mg^{-1} protein)			
0.013	0.142*	0.188*	0.787	0.112*	0.152*
		Superoxide dismutase (EU)			
1.71	2.44*	2.82	3.10	2.39*	2.50*
		Ascorbate peroxidase (µ mol ascorbate mg^{-1} protein oxidized)			
0.243	0.338*	0.561*	0.867	0.284*	0.614*
		Lipid peroxidation (n moles MDA 100 mg^{-1} fresh weight)			
11.2	12.1*	13.3*	13.8	14.2*	11.4*

The values carrying superscript star are significantly different from those of respective controls.

Table 2. Effect of pollutant elements on tissue concentration in different plant parts of sunflower in soil-pot culture (±SE).

Treatment	Plant part		
	Leaves	Stem	Root
		µg Co g^{-1}	
Control	11.2±0.23	10.2±0.46	15.9±1.22
0.25 mM Co	241±8.15	191±6.24	964±45.3
		µg Ni g^{-1}	
Control	28.0±1.22	8.30±0.35	15.8±0.65
0.25 mM Ni	440±33.2	660±42.3	1276±55.3
		µg Cd g^{-1}	
Control	0.72±0.09	0.72±0.08	1.02±0.02
0.25 mM Cd	137±4.56	149±4.33	321±7.34
		µg Cr g^{-1}	
Control	BDL	BDL	BDL
0.25 mM Cr	298±7.78	312±8.43	610±21.2
		µg Pb g^{-1}	
Control	BDL	BDL	9.72±0.56
0.25 mM Pb	552±21.4	135±3.58	2224±52.6

BDL = below detect level.

2006). Heavy metal similarly to other stress factors have been reported to induce over production of ROS in plant tissues to protect the plant from damages caused by ROS, plants possess low molecular weight antioxidants (ascorbic acid, reduced glutathione, carotenoids) and antioxidant enzymes such as superoxide dismutase, peroxidase and ascorbate peroxidase, that scavenge reactive oxygen species (Gratao et al., 2005) and avoid oxidative damage to plants.

In the present study Co, Ni, Cd and Pb toxicity in sunflower led to significant alterations in the activity of antioxidant defense mechanism. The activity of superoxide

dismutase responsible for mitigating the oxidative stress (Gratao et al., 2005) was significantly enhanced in the leaves of sunflower in the toxicity of each pollutant metal. Enhancement in SOD activity as a consequence of oxidative stress caused by excess of these metals is well documented (Dey et al., 2007; Zhang et al., 2007). Extent of the increase in the enzyme activity was highest in the presence of Pb. The observed increase in SOD activity in excess Ni is contrary to earlier observation for wheat (Gajewaska and Sklodowska, 2007). Exposure to sunflower plants to Co, Cd, Ni, Cr and Pb caused several fold increase in peroxidase activity in leaves. Induction of peroxidase with heavy metals reported earlier (Tewari et al., 2002; Pandey et al., 2009; Gajewska et al., 2007) has been suggested by its role in building up physical barrier against toxic metal entering the cell as well as in scavenging H_2O_2. Heavy metal stress also led to marked increase in ascorbate peroxidase another H_2O_2 scavenging enzyme is in agreement with the findings of other workers (Gajewaska and Sklodowska, 2007) suggesting implication of Mahler-peroxidase reaction. Membrane destabilization is generally attributed to lipid peroxidation, due to an increased production of free radicals under heavy metal stresses. In aerobic cells, the hydroxyl radical is known to be the most potentially toxic species. The unsaturated fatty acid components of membrane lipids are highly susceptible to hydroxyl attack and are peroxidized in its presence. The concentration of malondialdehyde MDA increased in leaves of sunflower under heavy metal stress condition (Yadav, 2010) with little increment in excess Pb may probably due to limiting peroxidizable fatty acid content in Pb stress condition.

In conclusion, the present findings affirm that, in common with other abiotic stresses excess intake of the heavy metals produce oxidative stress, and trigger antioxidative responses, but differ in their effectiveness to do so. At equimolar concentrations (0.25 mM), Cd induced the most severe visual toxicity effects and exhibited maximum oxidative damage as observed by accumulation of TBARS and lower antioxidant capacity than the plants exposed to Co, Cd, Cr and Pb. This was especially so in SOD and H_2O_2-eliminating enzymes POD and APOD which showed lower activity in Cd excess plants. Further, the study suggests that the degree of oxidative damage may also be assessed by the manifestation of external visual toxicity effects both of which were found to be in the order Cd>Cr>Ni>Co>Pb.

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