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EFFECTS OF CHROMIUM AND LEAD ON GERMINATION, ACCUMULATION AND PHENOLIC CONTENTS OF *GOSSYPIUM HIRSUTUM* (L.) AND *SOLANUM MELONGENA* (L.)

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ABSTRACT

Effects of chromium and lead chloride on germination, accumulation and stress phenolics were studied. Lead chloride significantly reduced the germination, increased accumulation and total phenols in two tested species (*Gossypium hirsutum* and *S. melongena*). Maximum inhibition was recorded in plants when treated with 150ppm lead as compared to chromium treated sample and control.

Key word: Chromium, lead, germination, accumulation and phenols, *G. hirsutum*, *S. melongena*

INTRODUCTION

Agricultural, manufacturing, mining, waste disposal practices and the use of sewage sludge as fertilizer in agricultural fields are the main causes of elevated heavy metals concentration in soils (Salgare, 1991). Lead, copper, chromium and mercury are used extensively in industry (Raihan *et al.*, 1995). Lead and chromium are released in the environment by mining, smelting, refining, tanning lead and chromium based products such as pesticides, vehicular exhaust and burning of coal and industrial rubbish (Dix, 1981).

Several reports have suggested that heavy metal contaminations have drastic effects on plants to cause inhibition of germination (Shuakat *et al.*, 1999), reduction in plant growth and yield (Iqbal *et al.*, 1991) and alteration of normal metabolic pathway thus negatively affecting respiration and photosynthesis by disruption of cellular enzymes (Krupa *et al.*, 1993).

Plant species, commercial varieties, cultivar of crop plants and ecotypes have wide range of tolerance to heavy metals (Al-Helal, 1995). Although mechanisms of heavy metals tolerance have been recorded in several species yet, no specific mechanism has been elucidated (Baker and Walker, 1990). Verkleg and Schat (1990) in a review concluded that available studies only permit inclusive and tentative mechanism of heavy metals tolerance in plant. Apparently, there is little information available pertaining to the effect of heavy metals on germination, growth and accumulation of crop plants in Pakistan (Iqbal *et al.*, 1991; Iqbal and Siddiqui, 1992).

The main objective of the present study was to investigate the effect of chromium and lead on germination, accumulation and total phenol contents, which is selected as an indicator and is elevated due to the presence of heavy metals (Reid *et al.*, 1992)

MATERIALS AND METHODS

Collection and sterilization of seeds: Seeds of *Gossypium hirsutum* (L.) and *Solanum melongena* (L.) were obtained from National Institute of Agriculture and Biology (NIAB), Faisalabad and were surface sterilized with 8% sodium hypochlorite solution for 10min. washed with running tap water followed by washing with de-ionized water.

Preparation of Pots: Experiment was conducted in plastic pots having a diameter of 16″, filled with acid washed sand, saturated with 1.1% HCl and left for a week. Afterwards, the sand was leached with double distilled water daily and was saturated in water during night for a one week period. Finally the water was decanted and sand was saturated with full strength Hoagland nutrient solution to be used in the experiment.

Sowing, Treatment and Sampling: For the test of total phenols and accumulation of lead and chromium, fifteen seeds of both plant species were sown in each pot respectively. Ten healthy seedlings were treated with three different concentrations of chromium and lead chloride (10, 50, 100 and 150ppm) as prepared in full strength Hoagland solution. The plants irrigated with these solutions, served as treated and the Hoagland (alone) served as control.

Germination test was performed in sterilized Petri plates (9cm) having Whatman No. 1 filter paper. Fifteen seeds of both vegetables were placed on filter paper containing 5ml of test and control Hoagland solution. Each set of Petri plates were kept in Hotpack refrigerated growth chamber under controlled conditions (30±2°C during 13h light periods from 5 a. m. to 6 p m. of about 8000flux white florescence light and 25±2°C during 11h darkness). A seed was considered germinated when the radicle had attained a length at least 1.5mm (Taylor, 1942).

Analysis: Leaf samples were collected randomly at fruiting. Accumulation of lead and chromium was analyzed by atomic absorption spectrophotometer using the method proposed in USDA Handbook for Diagnosis and Improvement of Saline and Alkali soil (1956). Total phenols were observed by the method of Swain and Hillis (1959). The data was statistically analysed.

RESULTS

Germination:

Application of heavy metals, lead in particular, significantly ($P<0.001$) inhibited seed germination of *G. hirsutum* as compared to control (**Fig. 1**). Germination of *G. hirsutum* was almost completely inhibited at 150ppm of lead compared to chromium. Use of chromium salt had little effects on germination of both plant species. However, significant increase in final percent germination was recorded in *S. melongena* when treated with chromium chloride at 150 ppm.

Phenolic content:

In general, heavy metals caused an elevation in the phenolic contents over control in the seedlings of both *G. hirsutum* and *S. melongena* and greater increase occurred at 150ppm (Fig. 1). Both plant species showed more or less same trend with respect to phenolic content. Chromium chloride caused greater increase in phenolic content than lead chloride.

Table 1.Weekly accmulation of lead in *S. melongena*.

Weeks	Cont	10ppm	50ppm	100ppm	150ppm
1	0± 0	0.540±0.015	0.766 ±0.008	1.026 ±0.012	1.850 ±0.011
2	0± 0	0.850± 0.011	1.077±0.031	1.533± 0.024	2.020 ±0.015
3	0± 0	1.133± 0.071	1.553 ±0.008	1.893± 0.014	2.753± 0.014
4	0 ±0	1.553± 0.008	2.000 ±0.011	2.020± 0.014	3.000± 0.003
5	0 ±0	1.850± 0.017	2.793± 0.012	2.743 ±0.017	3.543 ±0.017
6	0 ±0	2.045 ±0.006	3.063± 0.082	3.220± 0.015	3.966± 0.033

Table 2. Weekly accmulation of chromium in *S. melongena*.

Weeks	Cont	10ppm	50ppm	100ppm	150ppm
1	0± 0	0.126 ±0.012	0.153± 0.002	0.573± 0.014	1.626± 0.014
2	0± 0	0.17± 0.011	0.220 ±0.015	1.293± 0.020	2.013± 0.008
3	0± 0	0.230± 0.015	0.663± 0.008	1.993± 0.012	2.766 ±0.014
4	0 ±0	0.353± 0.014	0.423± 0.011	2.526 ±0.012	3.533 ±0.020
5	0 ±0	0.460± 0.100	0.536± 0.080	3.202± 0.015	4.030± 0.002
6	0 ±0	0.540± 0.020	0.740± 0.020	3.636 ±0.020	4.556 ±0.023

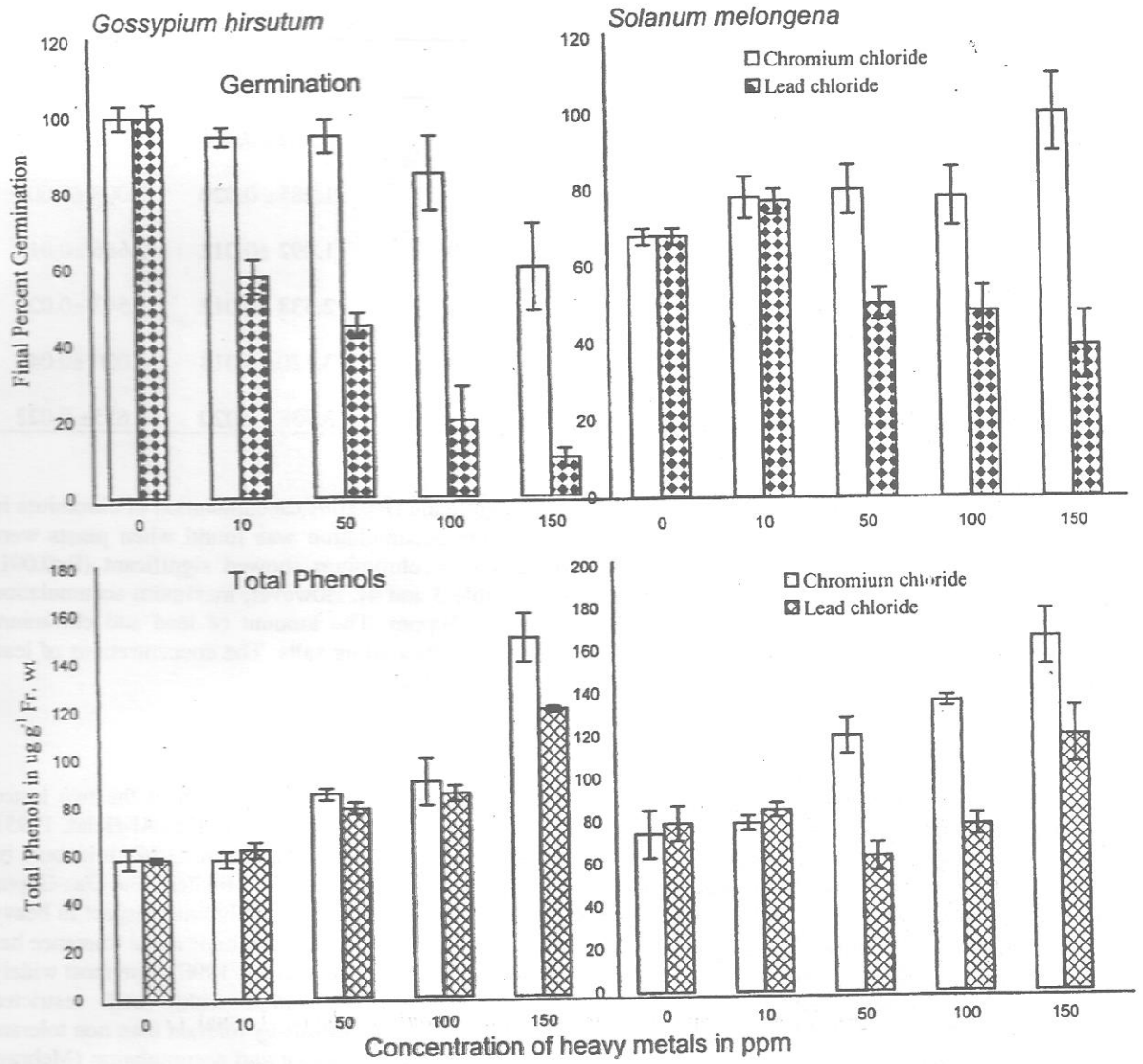

Fig.1. Effect of chromium and lead on germination and production f total phenols of *Gossypium hirsutum* and *Solanum melongena.*

Table 3. Weekly accmulation of lead in *G. hirsutum.*

Weeks	Cont	10ppm	50ppm	100ppm	150ppm
1	0± 0	0.340± 0.015	0.566 ±0.008	1.026± 0.012	1.650 ±0.011
2	0± 0	0.550± 0.011	1.083 ±0.031	1.433± 0.024	2.220± 0.015
3	0± 0	1.023 ±0.071	1.553 ±0.008	1.993 ±0.014	2.553± 0.014
4	0 ±0	1.553± 0.008	1.750± 0.011	2.220± 0.014	3.422 ±0.003
5	0 ±0	1.750± 0.017	2.693 ±0.012	2.763± 0.017	3.643± 0.017
6	0 ±0	2.055± 0.006	3.073± 0.082	3.520± 0.015	3.866 ±0.033

Table 4. Weekly accmulation of chromium in *G. hirsutum.*

Weeks	Cont	10ppm	50ppm	100ppm	150ppm
1	0± 0	0.129± 0.012	0.153± 0.004	0.564± 0.014	1.426± 0.012
2	0± 0	0.182± 0.013	0.220± 0.014	1.285± 0.020	2.003 ±0.005
3	0± 0	0.235 ±0.015	0.363± 0.006	1.892 ±0.012	2.666 ±0.012
4	0 ±0	0.365± 0.014	0.433 ±0.011	2.538 ±0.012	3.543 ±0.022
5	0 ±0	0.430 ±0.010	0.546 ±0.080	3.120± 0.018	4.020 ±0.040
6	0 ±0	0.530 ±0.020	0.750 ±0.020	3.738± 0.020	4.655± 0.022

Accumulation:

The result obtained are illustrated in Tables 1 and 2 showed significant ($P<0.001$) accumulation of chromium in the leaf of *S. melongena* as compared to lead. However, maximum accumulation was found when plants were treated with chromium chloride at 150ppm. Likewise, application of chromium showed significant ($P<0.001$) concentration in the leaves of *G. hirsutum* as compared to lead (Table 3 and 4). However, maximum accumulation was recorded in plants when treated with chromium chloride at 150ppm. The amount of lead and chromium accumulation is consistent with respect to applied concentration of corresponding salts. The concentrations of lead and chromium chloride were found more in *G. hirsutum* than in *S. melongena.*

DISCUSSION

The salt of heavy metals like lead and chromium had an inhibitory effect on germination of the two tested species. Inhibition in seed germination of rice and alfalfa by the heavy metals has been reported (Al-Helal, 1995). The inhibitory effect of salts on seed germination could be the result of ionic toxicity (Redmann, 1974) or it could be due to decreased level of auxin resulting from enhanced destruction of auxin by metal ion (Mukherji and Das-Gupta, 1972). *Gossypium* and *Solanum* are usually grown in fields that are less polluted specifically with respect to heavy metals. Therefore, they have shown high degree of susceptibility to heavy metals. Mechanism of metal tolerance has been studied in several species, yet no elucidation has been made so far (Balker and Walker, 1990). The most widely accepted mechanism includes detoxification of metals, metal compartmentalization within cell, restricted transportation, formation of metal binding polypeptides, uptake of limited heavy metals by tolerant than non tolerant plants, chelating by organic acids and some role of cell membrane in metal tolerance and accumulation (Mehrag, 1993). The results also suggest that accumulation of chromium is much faster and greater than lead. The indifferent plant also showed various levels of accumulation. The reasons for differential response of leaves to heavy metals are not known but it might be due to more rapid accumulation in shoot than root (Al-Helal, 1995). Contradictory to present study, Dayton *et al.* (1972) suggested that lead accumulation rate was high as compared to other heavy metals. However, present study revealed that chromium accumulation rate was high compared to lead. It might be due to the possibility of chromium which is an important cofactor of many iso-enzymes responsible for enhancing germination as is obvious in present study.

It is well known that stress conditions elevate the phenolic content of plants (Reid *et al.*,1992). The enhanced phenolic content observed in the test species could be such a response. It has been suggested that environmental factors including UV stress, pathogen invasion (hypersensitive reaction), herbicide action, heavy metal application are responsible to generate reactive oxygen species (ROS). Generation of reactive oxygen species (ROS) is characteristic for hypoxia and especially for reoxygenation. Of the ROS, hydrogen peroxide (H_2O_2) and superoxide (O_2^-) are both produced in a number of cellular reactions, including the iron-catalysed Fenton reaction, and by various enzymes such as lipoxygenases, peroxidases, NADPH oxidase and xanthine oxidase. To control the level of ROS and to protect cells under stress conditions, plant tissues contain several enzymes scavenging ROS (SOD, CAT, peroxidases and glutathione peroxidase), detoxifying LP products (glutathione S-transferases, phospholipid-hydroperoxide glutathione peroxidase and ascorbate peroxidase), and a network of low molecular mass antioxidants (ascorbate, glutathione, phenolic compounds and tocopherols) are formed (Olga *et al.*, 2002). The enhancement in

phenolic compounds particularly at high concentration of lead and chromium could be at least in part responsible for germination inhibition and at the same it also give protection to plants under metal stress.

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