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Effects of Copper and Lead on Germination, Accumulation and Phenolic Contents of *Spinancea oleracea* and *Lycopersicum esculentum*

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Abstract: Effects of copper 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 (*S. oleracea and L. esculentum*). Maximum inhibition was recorded in plants when treated with 150ppm lead as compared to copper treated sample and control.

Key words: Copper, lead, germination, accumulation and phenols

Introduction

The main sources of elevated heavy metals concentration in soils are agricultural, manufacturing, mining, waste disposal practices and the use of sewage sludge as fertilizer in agricultural fields (Salgare, 1991). Heavy metals concentration is introduced due to the application of metals containing agrochemicals such as pesticides and fertilizers (Shaukat *et al.*, 1999).

Lead, copper, chromium, mercury are used extensively in industry (Raihan *et al.*, 1995). Lead is released in the environment by mining, smelting, refining lead based products. pesticides, vehicular exhaust and burning of coal and industrial rabbish (Dix, 1981).

Copper is an essential micro nutrient for normal plant growth, when provided in trace amount. Excessive use however becomes poisonous in many species (Foy *et al.*, 1974).

Several investigations have reported that heavy metal contaminations have drastic effects on plan such as inhibition of germination (Shuakat *et al.*, 1999), reduction in plant growth and yield (Iqbal *et al.*, 1991) and altering normal metabolic pathway, including respiration and photosynthesis by disruption of cellular enzymes (Krupa *et al.*, 1993).

Plant species, commercial varieties, cultivar of crop plants and ecotype have wide range of tolerance to heavy metals (Al-Helal, 1995). Although mechanism of heavy metals tolerance has been recorded in several species yet, no specific mechanism has so far been elucidated (Baker and Walker 1990). Verkleg and Schat (1990) in a review concluded that available studies only permit comprehensive 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 on 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 copper 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 (Ried *et al.*, 1992).

Materials and Methods

Collection and Sterilization of Seeds: Seeds of *Lycopersicum esculentum* and *Spinacia oleracea* were obtained from National Institute of Agriculture and Biology Faisalabad and were surface sterilized with 1% mercuric chloride solution for 10min. washed with running tap water followed by washing with deionized 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 period of a week. Finally the water was decanted and sand was saturated with Hoagland nutrient solution to be used in the experiment.

Sowing, Treatment and Sampling: Ten seeds of both vegetables were sown in each pot respectively. Different concentrations of copper and lead chloride (10, 50, 100 and 150ppm) were prepared in Hoagland solution. The plants were irrigated with these solutions, served as treated and Hoagland (alone) treated served as control. Germination test was performed in sterilized petri plates (9cm) having Whatman No. 1 filter paper. Ten seeds of both vegetables were placed on filter paper containing 5ml of treated Hoagland solution. Each set of petri plates were kept on growth chamber at 30 C2 light intensity at the top of the petri plates was 1950flux. 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 copper 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).

Results

Germination:

Effect of copper chloride and lead chloride on seed germination *S. oleracea:* Lead chloride caused greater reduction in germination percentage of *S. oleracea* than did copper chloride (Fig. 1b). Germination was inhabited significantly ($P < 0.001$) by lead chloride at 50ppm onwards. While in case of copper chloride, considerable increase in germination particularly at high concentration was observed.

Effect of copper chloride and lead chloride on seed germination of *L. esculentum*: Lead showed marked toxicity and significantly($P < 0.001$) inhibited seed germination of *L. esculentum* at all concentration as compared to control and copper (Fig. 1a). Germination was almost completely inhibited at 150ppm of lead as compared to copper, where no significant inhibition was recorded.

Accumulation:

Accumulation of copper and lead in the leaves of *S. oleracea*: The result obtained and illustrated in Tables 1 & 2 showed significant accumulation of lead in the leaf of *S. oleracea* as compared to copper ($P < 0.001$). However, maximum

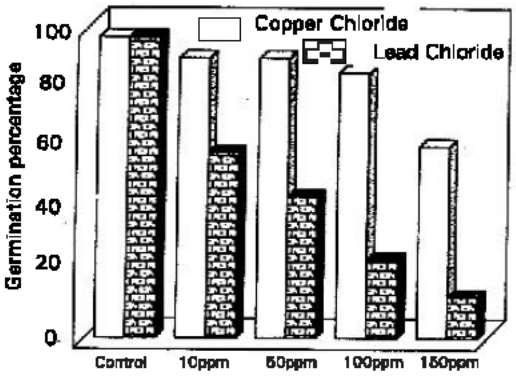

Fig. 1a: Effect of copper and lead chloride on germination of *Lycopersicum esculentum L.*

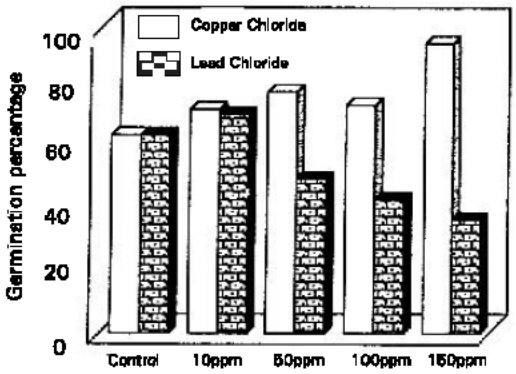

Fig. 1b: Effect of copper and lead chloride on germination of *Spinacia oleracea L.*

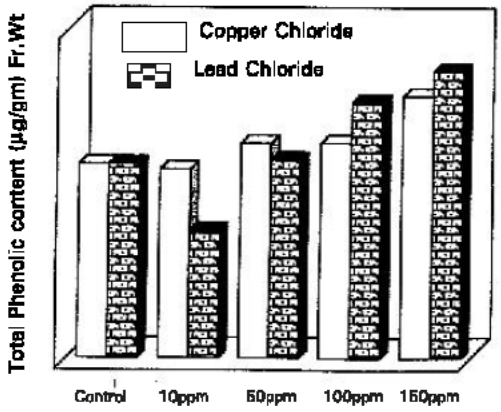

Fig. 2a: Effect of copper and lead chloride on total phenolic content of *Lycopersicum esculentum L.*

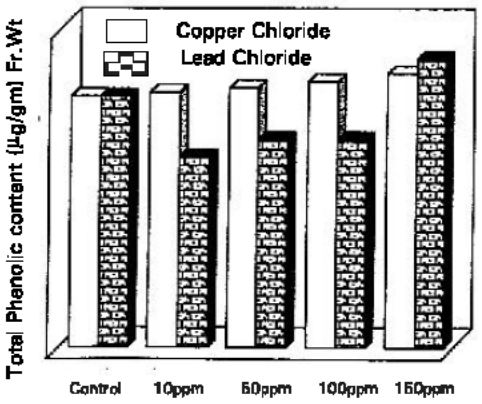

Fig. 2b: Effect of copper and lead chloride on total phenolic Content of *Spinacia oleracea L.*

Table1: Weekly Changes in the amount of Cu accumulated (ppm/gm Fr. Wt.) in the leaves of *Spinacia oleracea* L.

No. of 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	1.0773 ± 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

Correlation (r = 0.984) (F = 1298.56, P <0.001)

Table 2: Weekly Changes in the amount of Pb accumulated (ppm/gm Fr. Wt.) in the leaves of *Spinacia oleracea* L.

No. of 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	0.170 ± 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 ±0.010	0.536 ± 0.08	3.020 ±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

Correlation (r = 0.784, F = 1798.46, P <0.001)

Table 3: Weekly Changes in the amount of Cu accumulated (ppm/gm Fr. Wt.) in the leaves of *Lycopersicum esculentum* L.

No. of 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

Correlation (r = 0.892, F = 1258.26, P <0.001)

Table 4: Weekly Changes in the amount of Pb accumulated (ppm/gm Fr. Wt.) in the leaves of *Lycopersicum esculentum* L.

No. of 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 ± 0.08	3.120 ±0.018	4.020 ±0.04
6	0 ±0	0.530 ±0.020	0.750 ± 0.020	3.738 ±0.020	4.655 ± 0.022

Correlation (r = 0.884, F=1898.26, P<0.001)

accumulation was found when plants were treated with lead chloride at 150ppm. Accumulation was in directional proportion with applied concentration of lead and copper.

Accumulation of copper and lead in the leaves of *L. esculentum:* Application of lead showed significant (P<0.001) accumulation in the leaves of *L. esculentum* as compared to copper (Table 3 & 4). However, maximum accumulation was recorded in plants when treated with lead chloride at150ppm.

Phenolic content: In general heavy metals elevated the phenolic content over control in the seedling of both *S. oleracea* and *L. esculentum* and greater increase occurred at 150ppm (Fig. 2a, b). Both plant species showed more or less same trend with respect to phenolic content. Lead chloride caused greater increase in phenolic content than copper chloride.

Discussion: The salt of heavy metals like lead and copper had an inhibitory effect on germination of the two tested species. Inhibition of seed germination by the heavy metals has often been reported (Al-Helal, 1995). The inhibitory effect of salts on seed germination could be the result of ionic toxicity (Redman, 1974) or due to an osmotic effect (Michael *et al.*, 1972) or it could be due to decreased level of auxin resulting from enhanced destruction of auxin by metal ion (Mukherji and DasGupta, 1972) *Lycopersicum* and *Spinecea* are usually grown in field that are less polluted particularly 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 according to Mehrag (1993) are (1) detoxification of metals (2) metal compartmentalization within cell (3) restricted transportation (4) formation of metal binding polypeptides (5) limited heavy metals are taken up by tolerant plant than non tolerant (6) chealating by organic acids (7) membrane may have some role in metal tolerance and accumulation. The results also suggests that accumulation of lead is much faster and greater than copper. The indifferent plant also showed different level of accumulation. The reasons for differential response of leaves to heavy metals are not known but it might be due to impart, to more rapid accumulation in shoot than root (Al-Helal, 1995). Dayton *et al.* (1972) suggested that lead accumulation rate was high as compared to other heavy metals. It is well known that stress condition elevate the phenolic content of plants (Reid *et al.*, 1992; Siddiqui and Ahmed, 1996) The enhanced phenolic content observed in the test species could be such response. The high level of phenolic compounds particularly at high concentration of lead and copper could be at least in part responsible for germination inhibition.

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