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# Tobacco, corn and wheat for phytoremediation of cadmium polluted soil

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## Abstract

Tobacco (*Nicotiana tabacum* L.) corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) seedlings were grown in three cadmium (Cd) concentration levels (10, 30 and 50 mg $kg^{-1}$) in a soil pot culture to analyze cadmium concentration, proline contents (leaves) and growth responses in the shoots and roots of corn, wheat and tobacco plants.The experiment was conducted at Botanical Garden, Department of Botany, Annamalai University, Tamil Nadu, during the period of January to March 2011. In the pot culture experiment. These plants were analysed on 15th sampling days, in soil amended with various levels of cadmium (viz, 10, 30 and 50 mg $kg^{-1}$). The inner surfaces of pots were lined with a polythene sheet. Each pot containing 3kg of air dried soil. Six seeds were sown in each pot. All pots were watered to field capacity daily. Plants were thinned to a maximum of three per pots, after a week of germination. Root and shoot cadmium concentrations of corn, wheat and tobacco increased with their exposure to the cadmium levels and the highest cadmium concentration occurred in roots (except tobacco), followed by the shoot. The highest cadmium concentration was regarded in shoot then root of tobacco plants. An increase in proline in the leaves of corn, wheat and tobacco seedlings exposed to cadmium occurred as well as a decreased shoots and roots biomass. Thus, cadmium levels negatively affected the corn, wheat and tobacco seedlings growth. When compared to corn and wheat, tobacco plants are identified hyperaccumulators of cadmium in polluted soil.

Keywords: Phytoremediation, Cadmium, Growth, uptake and accumulation

## INTRODUCTION

A rapid industrialization and its use in agriculture had led to regional and global redistribution of metals with consequent environmental pollution. The role of environmental pollution to produce various types of deleterious effects on diverse living system has been well established. Heavy metals are the most hazardous pollutants as they are non-degradable & get accumulated and become toxic both to plants & animals. Land and water are precious natural resources on which rely the sustainability of agriculture and the civilization of mankind. Unfortunately, they have been subjected to maximum exploitation and severely degraded or polluted due to anthropogenic activities. The pollution includes point sources such as emission, effluents and solid discharge from industries, vehicle exhaustion and metals from smelting and mining, and nonpoint sources such as soluble salts (natural and artificial), use of insecticides/pesticides, disposal of industrial and municipal wastes in agriculture, and excessive use of fertilizers (McGrath et al., 2001; Nriagu and Pacyna, 1988; Schalscha and Ahumada, 1998). Each source of contamination has its own damaging effects to plants, animals and ultimately to human health, but those that add heavy metals to soils and waters are of serious concern due to their persistence in the environment and carcinogenicity to human beings. They cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another (Garbisu and Alkorta, 2001; Gisbert et al., 2003). Therefore, heavy metal pollution poses a great potential threat to the environment and human health. Physicochemical approaches have been widely used for remedying polluted soil and water, especially at a small scale. However, they experience more difficulties for a large scale of remediation because of high costs and side effects. The use of plant species for cleaning polluted soils and waters named as phytoremediation has gained increasing attention since last decade, as an emerging cheaper technology. Numerous plant species have been identified and tested for their traits in the uptake and accumulation of cadmium. Mechanisms of metal uptake at whole plant and cellular levels have been investigated.

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## MATERIALS AND METHODS

### Seed materials

The certified seeds of tobacco (*Nicotiana tabacum* L.), Corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) were purchased from Tamil Nadu Agricultural University, Agricultural Research Station, Paramakudi, Ramanathapuram district. Seeds with uniform size, colour and weight were chosen for the experimental purpose.

### Experimental soil

The soil used in the experiment was sandy loam in nature and the pH of the soil was 7.2. It contains 126kg available N, 76kg available P and 98kg available K/ha, and micro nutrients of 18.32mg available Cu, 190.28mg Fe, 172mg Mn and 20.44mg Zn/kg, cadmium was not available in this experimental soil. The cadmium chloride (Cd $Cl_2$ ½ $H_2O$) was used as cadmium source.

### Pot culture experiment

The pot culture experiment was conducted at Botanical Garden, Department of Botany, Annamalai University, Tamil Nadu, during the

period of January to March 2011. Surface sterilized corn , wheat and tobacco seeds were sown in pots (15 cm in diameter) containing mixture of sandy loam soil in nature and plants were grown in pots containing untreated soil (Control) and soil mixed with various levels of cadmium (viz., 10, 30 and 50 mg $kg^{-1}$). The inner surfaces of pots were lined with a polythene sheet. Each pot contained 3kg of air dried soil. Six seeds were sown in each pot. All pots were watered to field capacity daily. Plants were thinned to a maximum of three per pots, after a week of germination. Each treatment including the control was replicated five times. Data points in the tables and figures represent the means.

## Growth Measurements

The plant samples were collected on 15th days after sowing. Three plants from each replicates of pot were analyzed for the various growth parameters. The following growth parameters: shoot and root dry weight was determined after drying of plant material in an oven $70^0$C. The plant samples were oven dried at 70°C for 48 h to a constant weight, after which dry weight of shoots and roots were determined by electronic balance.

## Assay for proline estimation

Proline was assayed as described by Bates et al. (1973).

Five hundred mg of plant tissue was homogenized in 10.0 ml of 3% aqueous sulphosalicylic acid. The homogenate was filtered through whatmann No. 42 filter paper. Two ml of acid ninhydrin (1.25 g ninhydrin in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid) and 2 ml of glacial acetic acid in a test tube was heated for an hour at 100°C. The reaction mixture was extracted with 4 ml toluene and mixed vigorously by using a vortex mixture for 15-20 sec. The chromophore containing toluene was aspirated from the aqueous phase. The absorbance of the toluene layer was measured in a spectrophotometer at 520 nm using toluene as blank.

## Cadmium

assayed as described by Khan et al., (1972; Slavin et al., (1983).

The plant material was repeatedly and carefully washed with distilled water to remove surface contamination, air dried, and wet ashed. One g of powdered sample was digested over night in 10 ml 50%, $HNO_3$, filtered, brought to a volume with distilled $H_2O$ and then 1 ml 10% $NH_4H_2PO_4$, as matrix modifier, was added. Cadmium was measured by an atomic absorption spectrophotometer AA-475 equipped with a graphite furnace (Varian GTA 95).

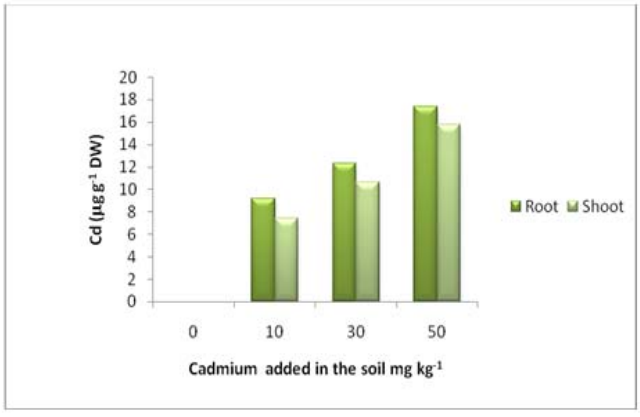


Fig:1 Uptake and accumulation of cadmium in corn plants.

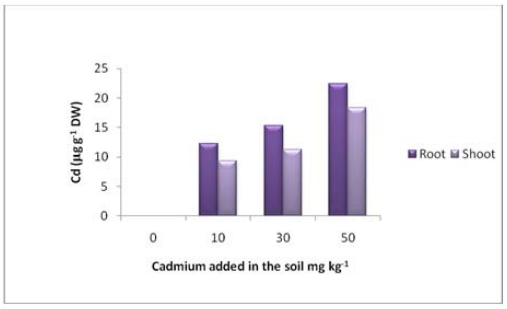


Fig: 2 Uptake and accumulation of cadmium in wheat plants.

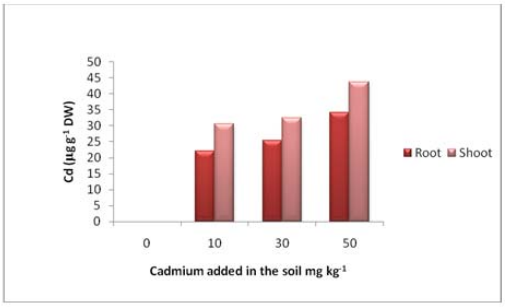


Fig. 3. Uptake and accumulation of cadmium in tobacco plants.

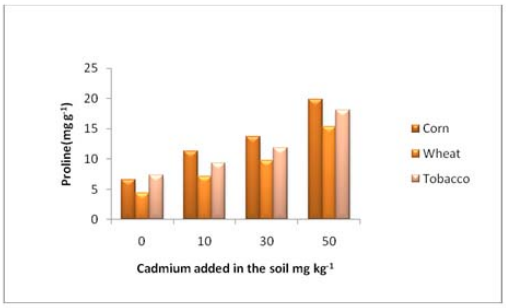


Fig: 4 Proline content of cadmium treated corn, wheat and tobacco plants.

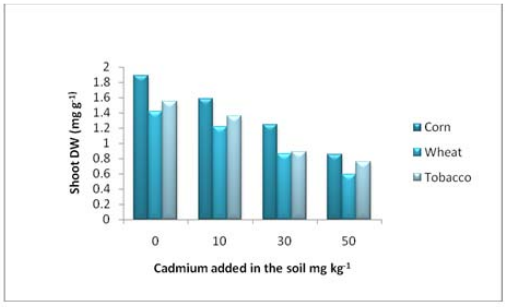


Fig: 5 Dry biomass of cadmium treated shoot of corn, wheat and tobacco plants.

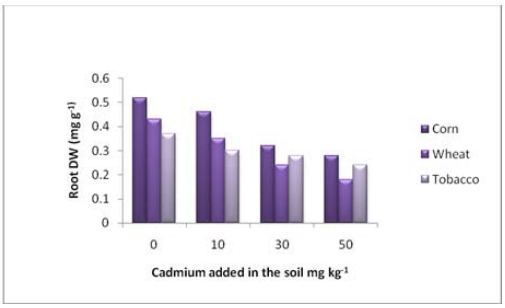


Fig: 6 Dry biomass of cadmium treated root of corn, wheat and tobacco plants.

## RESULTS AND DISCUSSION

### Root and Shoot Biomass

Root and shoot dry weight of corn, wheat and tobacco plants at various levels of cadmium are furnished in Fig. 5 and 6. The Maximum root (viz., 0.52, 0.43 and 0.37) and shoot (viz., 1.89, 1.42 and 1.25) dry weight of corn, wheat and tobacco was observed in control plants, in the sampling days. There was a progressive decline in root dry weight with an increase in cadmium (10, 30 and 50 mg $kg^{-1}$) level. The minimum root (viz., 0.28, 0.18 and 0.24) and shoot (viz., 0.85, 0.59 and 0.56) dry weight was recorded at 50 mg $kg^{-1}$ cadmium level in all the plants. When compared to these plants, the highest biomass observed in corn plants and lowest in tobacco plants in 15$^{th}$ sampling days. There was a progressive fall in the dry matter yield of root and shoot with the corresponding increase in cadmium (10-50 mg $kg^{-1}$) level in the soil. Similar results were obtained by several authors in a number of plants such as Poschenrieder *et al.* (1989) in bean, Vitoria *et al.*(2001) in radish, Kim *et al.*(2002) in cabbage and lettuce, Zhang *et al.*(2002) in wheat, Shukla *et al.* (2003) in wheat, Rai *et al.* (2005) in *Phyllanthus amarus*. The decrease in biomass due to cadmium stress might be due to low protein formation, resulting in the inhibition of photosynthesis, as well as hampering carbohydrate translocation (Samarakoon and Rauser, 1979). Taken up in excess by plants, this non-essential element directly or indirectly inhibits physiological processes such as respiration, photosynthesis, plant–water relationships, loss of cellular turgor, inhibiting the activity of the cell and its enlargement, nitrogen metabolism and mineral nutrition, resulting in poor growth and low biomass (Barceló and Poschenrieder, 1990; Gabbrielli *et al.,* 1990; Sanita di Toppi and Gabbrielli, 1999).

### Proline

The results showed in Fig. 4 indicated that the minimum proline content of corn, wheat and tobacco was occurred in control (viz., 6.056, 4.420 and 7.036) plants. With further increase of cadmium level (10, 30 and 50 mg kg-1), the proline content of corn, wheat and tobacco was strongly increased in 15Th sampling days. Maximum proline content was observed at 50 mg kg-1 cadmium level (viz., 19.820, 15.039 and 18.018) in all the plants. This would be evident from the study of Bavaji (1999) and Dinakar et al. (2008) in *Arachis hypogaea*, Mishra and Agrawal (2006) in spinach. Sun et al. (2007) in *Solanum nigrum* and Hasan *et al.* (2008) in chickpea. This investigation lends support to the findings of Schat *et al.* (1997) suggested that proline accumulation in plants under cadmium stress is due to the decrease of the plant water potential and the functional significance of this accumulation could be related to the water balance. It may be argued that proline accumulation helps to conserve nitrogenous compounds and protect the plant against heavy metal stress. These results also support the view that proline acts as a membrane stabilizing agent under stress conditions (Poschenrieder and Barcelo, 2004).

### Cadmium uptake and accumulation

Cadmium content of corn, wheat and tobacco plants raised in various level of cadmium is presented in Fig. 1,2 and 3. The cadmium content of root and shoots of corn, wheat and tobacco increased with an increase in cadmium level in the soil. The minimum accumulation in root (viz., 9.20, 12.26 and 22.12) and shoot (viz., 7.45, 9.33 and 30.60) was observed at 10 mg $kg^{-1}$ cadmium level in the soil. Maximum cadmium content in root (viz., 17.39, 22.45 and 34.24) and shoot (viz., 15.80, 18.40 and 43.62) was recorded at 50 mg $kg^{-1}$ cadmium level in the soil in all the plants. Showed this experiment, increasing cadmium level in the soil, increased the uptake and accumulation of cadmium contents in root and shoot of corn, wheat and tobacco plants. Similar observations as stated by Wangstrand *et al.* (2007) in winter wheat, Pehlivan *et al.* (2008) in sugar beet, Rascio *et al.* (2008) in rice and Sun *et al.* (2008) in *Solanum nigrum*. Our results are in agreement with the findings of (Vazquez et al., 1992) who reported that the cadmium accumulation in the roots was due to compartmentation of cadmium in the vacuoles. Cadmium accumulation in the roots was due to compartmentation of cadmium in the vacuoles (Vaszquez *et al.,* 1992). Roots are the first organs with contact to the toxic metal ions, and roots usually accumulate significantly higher amounts of metal than do shoots (Breckle, 1991). Wu *et al.* (2006) showed that the cadmium uptake in plants is correlated with the increasing amount of metal in the growing medium or soil.

## CONCLUSION

Showed this experiments, decrease in dry biomass of cadmium treated corn, wheat and tobacco plants, when compared to untreated plants. The loss of these may be due to inhibition in the uptake of mineral nutrients from the soil, inhibition of cell division, impairment of PSII activity, directly or indirectly inhibits physiological processes such as respiration, photosynthesis, plant–water relationships resulting in poor growth and low biomass. In addition, application of cadmium significantly increased the level of the proline in corn, wheat and tobacco leaves. The proline accumulation in corn, wheat and tobacco leaves played an important role in cadmium tolerance. Uptake and accumulation of cadmium increased in the root and shoots of treated plants with an increase in the cadmium level in the soil. The uptake and accumulation of cadmium was higher in roots than the shoot (except tobacco plants). The highest cadmium concentration was regarded in shoot then root of tobacco plants. When compared to corn and wheat plants, tobacco was identified hyper accumulator of cadmium polluted soil.

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