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SINGLE AND COMBINED TOXICITY EFFFECTS OF Cu AND Cd to GARDEN CRESS SEEDLINGS GROWTH AND OXIDIZING ENZYMES

Abstract

Environmental Pollution due to heavy metal (HM) is a global issue. The single and binary effects of Copper and Cadmium in Lepidium sativum grown in control and heavy metals are studied. Single and combined metal treatment led to major effects in the growth of root shoots elongation, fresh and dry weight. It was observed that effects of heavy metal mixture to plants are concentration depending. 600 ppm of Cu+Cd induced additive effects on root and shoot elongation. Growths of roots shoot and dry weight by Single and combined metal treatment causes severe effects. The interactive effects of heavy metals were also evaluated with Percent Phytotoxicity and Root Shoot (R/S) Ratio. Lowering in growth was due to the suppression in higher IAA oxidase activity. Peroxidase activity was most sensitive enzyme to heavy metals.

Key words: *growth, heavy metal, Garden cress, mixture toxicity, polyphenol oxidase IAA oxidase, peroxidase*

Introduction

Environmental Pollution due to heavy metal is a global sensitive issue (Bhat et al 2014). Heavy metals are reported to affect growth, morphology and metabolism of plants in several ways **(**Rastgoo, 2011). Gill and Tuteja, (2010) and Bhat et al (2014) studied on responses to heavy metal stress on plants. Xiong et al., 2006; Martínez-Peñalver et al.,2012 reported that Cu is an essential trace element for plant growth in less amount, higher concentration of Cu lowered plant growth, adverse effect on metabolism and enzymes activities, damages cell and inhibits photosynthetic activity. Severe biochemical, physiological and morphological effects of Cd is very well known. Growth of plants, enzyme activities , uptake of nutrient and translocation affected by Cd in plant (Sandalio et al., 2001, Larbi et al., 2002; López-Millán et al., 2009). In nature more than one chemical can have a greater negative impact than does the individual constituents of the mixture, it is very important to investigate the effects of single and mixture of heavy metal. To reveal the relationship between metal toxicity, oxidative enzymes, the effects single and combined heavy metals i.e. Cadmium (Cd), Copper (Cu) on Garden cress were studied. The purpose of the experiment was to study the single and mixture toxicities of Cu and Cd to Garden cress.

Materials and Methods

Garden cress (Lepidium sativum L. var local) was germinated in sterilized petriplates lined with Whatmann filter paper no. 1. Details are given below

DW (control),

200 ppm - 200 mg $CuCl_2$/$CdCl_2$/ l
600 ppm - 600 mg $CuCl_2$/$CdCl_2$/l
200 ppm (each) - 200 mg (each) Cu+Cd/l
600 ppm (each) - 600 mg (each) Cu+Cd/l

At 28 ± 2º C and up to 120h experiment was conducted under laboratory conditions. Heavy metal effects on Garden cress seedlings were observed.
For elongation, fresh weight and dry weight, ten seedlings from each treatment were used. The elongation of root and shoot was measured; mean was calculated and expressed as cm/seedling. Root and shoot were separated from 10 seedlings, their fresh weights were taken, and mean was calculated, dried at 80º C for 48h and dry weights were recorded and expressed as mg/seedling. The interactive effects of heavy metals were also evaluated with Percent Phytotoxicity and Root Shoot (R/S) Ratio.

For the following metabolism, control and 600ppm treated seedlings were analyzed.

1) **Polyphenol Oxidase Activity:**

The method of Kar and Mishra (1976) was used to determine Polyphenol oxidase activity. Polyphenol oxidase activity was calculated and expressed as OD/10 min/µg enzyme protein.

2) **Peroxidase Activity:**

To essay peroxidase activity George (1953) and Maehly (1954) method was employed and represented as difference in OD/30sec/µg protein.

3) **IAA Oxidase Activity:**

Method of Hare (1964) was used and OD was read at 530nm on Systronics 106 spectrophotometer. The following regression formula was prepared using IAA as standard.

$$X = 101.98\,Y + 7.79$$

The activity was calculated and expressed as µg IAA oxidized/h/µg protein.

RESULTS:

The data on root and shoot elongation, fresh weight and dry weight, % phytotoxicity, R/S ratio and oxidizing enzymes of Garden cress seedlings grown without and with Cu, Cd and Cu+Cd are presented as follows.

FIG.1: ELONGATION OF ROOT WITH CONTROL, SINGLE AND MIXTURE OF HEAVY METALS IN GARDEN CRESS SEEDLINGS

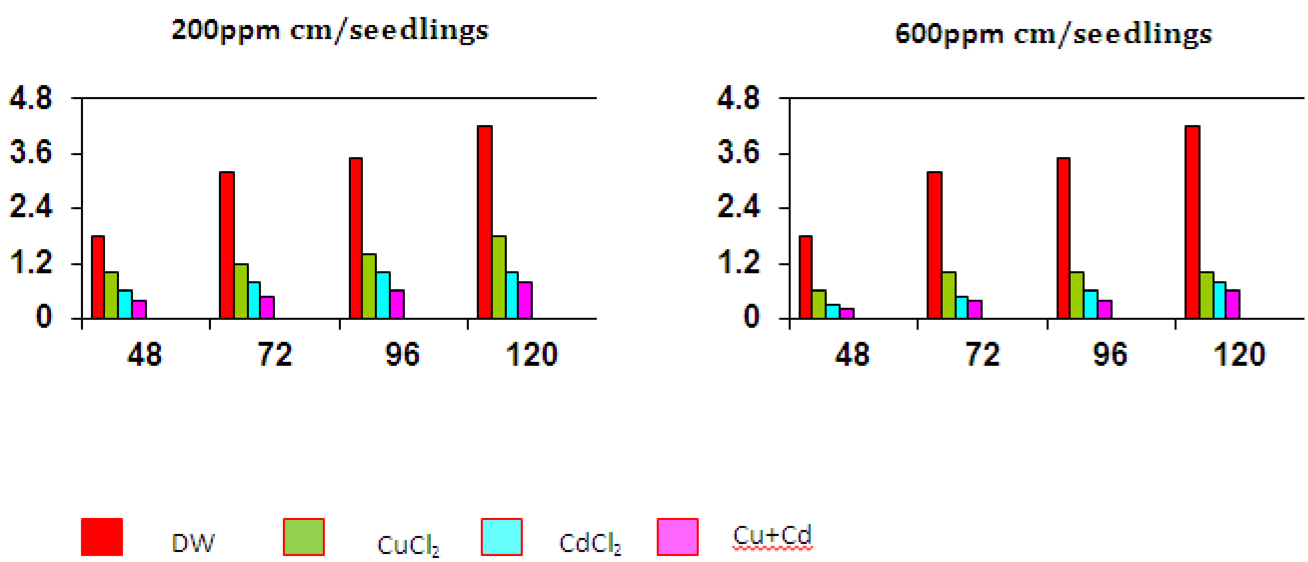


FIG. 2: FRESH WEIGHT OF ROOT WITH CONTROL, SINGLE AND MIXTURE OF HEAVY METALS IN GARDEN CRESS SEEDLINGS

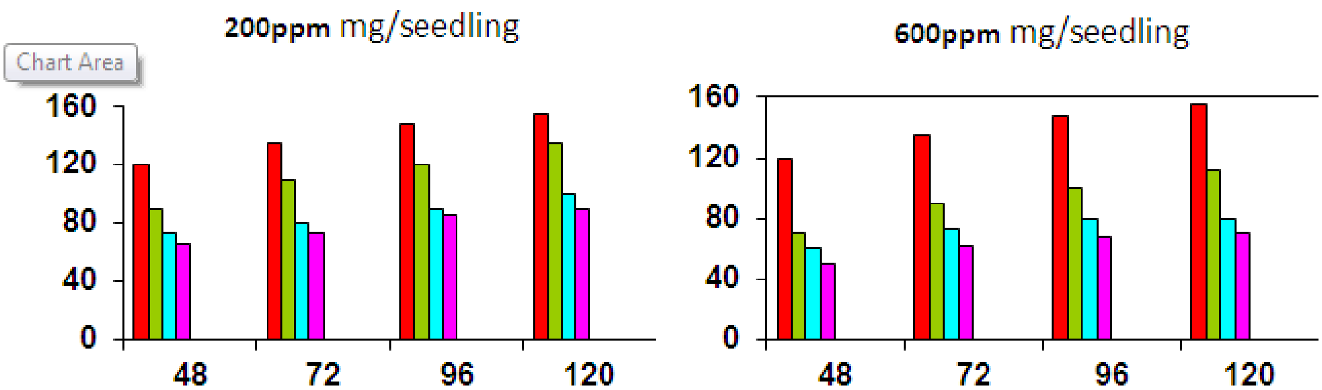


FIG.3: DRY WEIGHT OF ROOT WITH CONTROL, SINGLE AND MIXTURE OF HEAVY METALS IN GARDEN CRESS SEEDLINGS

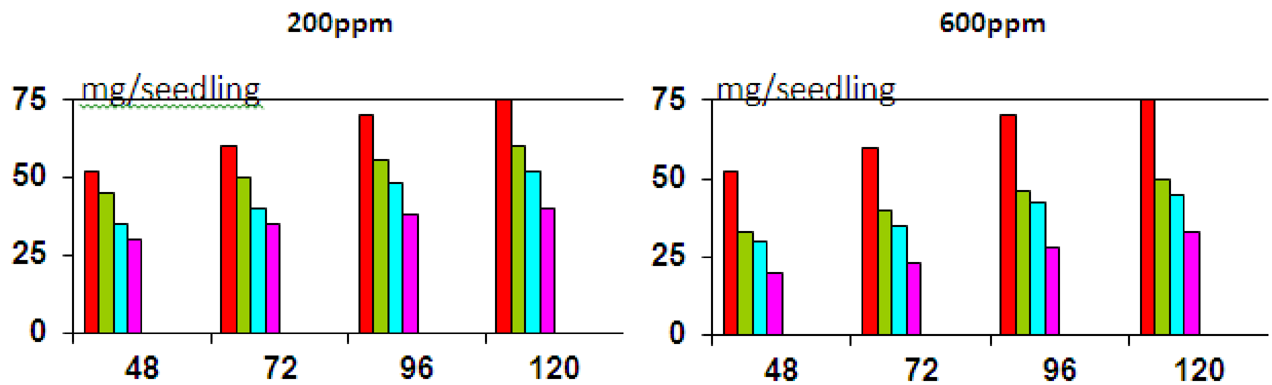


FIG. 4: ELONGATION OF SHOOT WITH CONTROL, SINGLE AND MIXTURE OF HEAVY METALS IN GARDEN CRESS SEEDLINGS

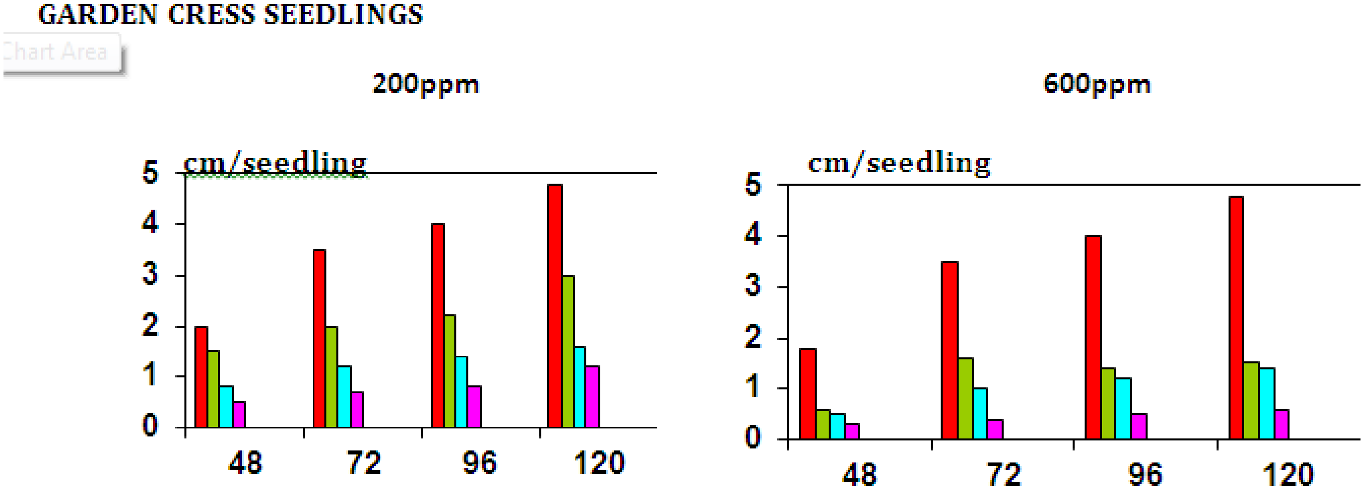


FIG. 5: SHOOT FRESH WEIGHT OF GARDEN CRESS SEEDLINGS GROWN WITHOUT AND WITH SINGLE HEAVY METAL AND MIXTURE OF HEAVY METALS

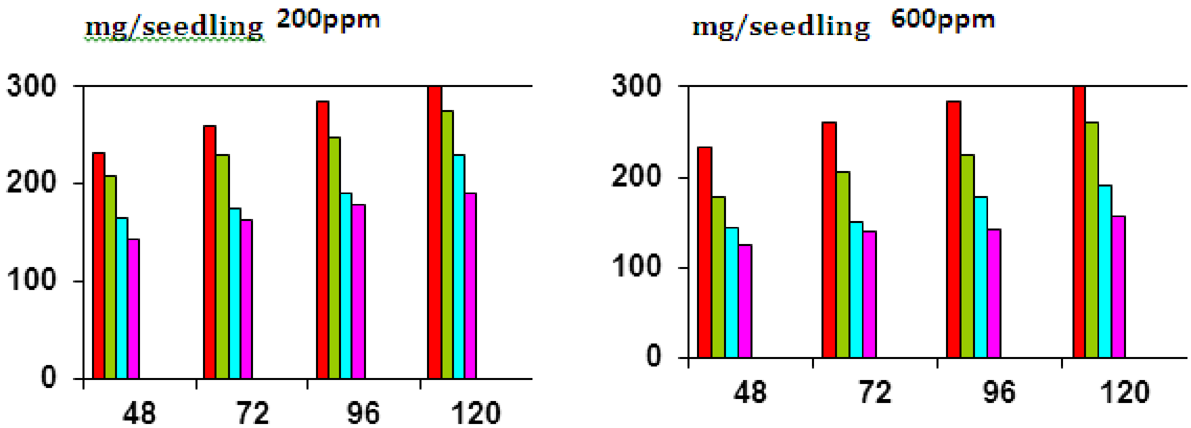


FIG.6: SHOOT DRY WEIGHT OF GARDEN CRESS SEEDLINGS GROWN WITHOUT AND WITH SINGLE HEAVY METAL AND MIXTURE OF HEAVY METALS

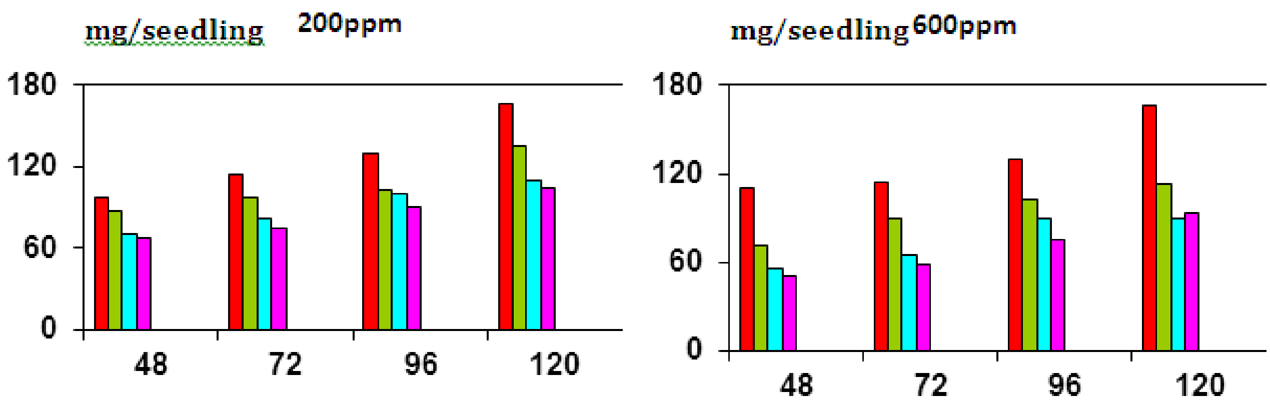
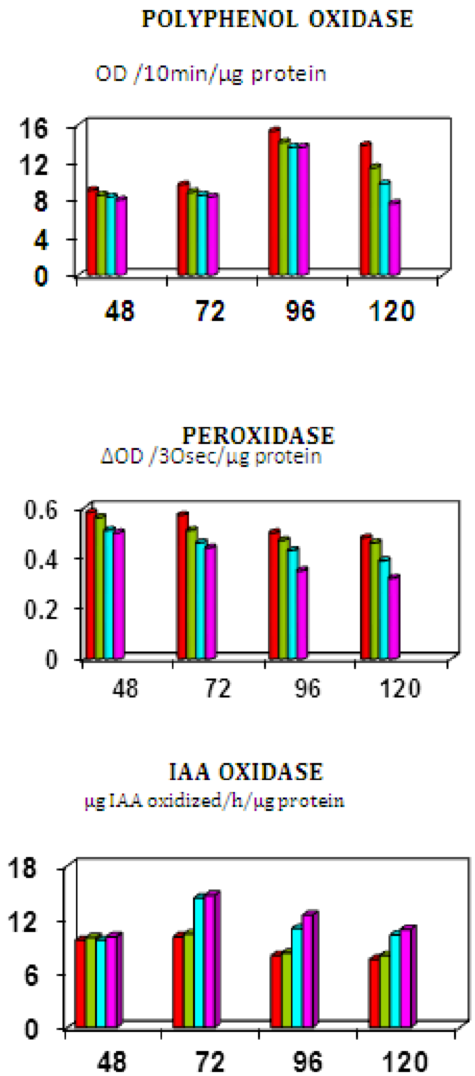


TABLE 1: % PHYTOTOXICITY AND ROOT SHOOT RATIO GARDEN CRESS SEEDLINGS GROWN WITHOUT AND WITH SINGLE HEAVY METAL AND MIXTURES OF HEAVY METALS

HEAVY METAL TREATMENT	% PHYTOTOXICITY GERMINATION PERIOD - h		R/S RATIO GERMINATION PERIOD-h	
	48	120	48	120
Control			0.9	0.8
200 ppm				
$CuCl_2$	44	57	0.6	0.6
$CdCl_2$	66	76	0.7	0.6
Cu+Cd	77	81	0.8	0.6
600 ppm				
$CuCl_2$	66	76	1	0.6
$CdCl_2$	83	81	0.6	0.5
Cu+Cd	88	85	0.6	1

FIG. 7: OXIDIZING ENZYMES OF GARDEN CRESS SEEDLINGS GROWN WITHOUT AND WITH SINGLE HEAVY METAL AND MIXTURE OF HEAVY METALS



CONCLUSIONS

The inhibitory effects were highly correlated with concentration of metals i.e. 600ppm concentration was more toxic than 200ppm of each metal. The inhibitory effect of single heavy metal was in the order of Cd>Cu. The phytotoxicity of heavy metals on seedling growth was noted even after 48h of heavy metal application. Response of seedlings in terms of growth to heavy metals may be evaluated by studying the growth response seedlings. All the heavy metal treatments stimulated IAA oxidase activity. The biochemical changes were noted even in 48h old seedlings. Binary interactions of Cd gave additive effects on growth and metabolism, the intensity of the effects depend upon nature of the metal, Cd were more phytotoxic than Cu, their interaction caused severe suppression in growth and metabolism. Before using the seeds for drug purpose, seeds must be analyses for presence of Cu and Cd.

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