BIOCHEMICAL EFFECTS OF ZINC AND CADMIUM ON THE GROWTH PERFORMANCE OF WHEAT (*TRETICUM AESTIVUM* L.) GROWN ON SAND CULTURE

Eman Fadel Aly Awad Allah, Ibrahim H. Elsokkary, and Abdel Salam A. Abdel Salam

Received on: 9/9/2008 ***Accepted: 11/11/2008***

ABSTRACT

Sand culture experiments were carried out at the greenhouse of the Department of Soil and Water Sciences, Faculty of Agriculture, Alexandria University to study the effect of Zn and Cd on the growth of wheat (Triticum aestivum L.) variety Giza 168, element content, photosynthetic pigments content, carbonic anhydrase activity (CAA) and phytochelatins (PCs) contents.

The results showed significant increases in the fresh and dry weights of shoots and roots with increasing Zn concentrations in the nutrient solution and significant decreases in both with increasing Cd concentrations. However, Zn and Cd contents in the leaves and roots were increased with increasing Zn or Cd concentrations in the nutrient solution. The leaves contents of Chl a, Chl b and Carotenes were increased with increasing Zn treatments and were decreased with increasing Cd treatments. The results showed that Zn stimulated Chl b synthesis more than Chl a while Cd inhibited Chl b synthesis more than Chl a while carotenes synthesis was less inhibited by increasing Cd concentrations.

Significant increases in CAA in leaves of wheat plants were found with increasing Zn concentrations while significant decreases in CAA were found with increasing Cd concentrations. The activity of carbonic anhydrase enzyme is positively associated with Zn increases in leaves and is negatively associated with Cd increases in plant leaves. On the other hand, increasing Cd concentrations in the nutrient solution stimulated the synthesis of phytochelatins in leaves and roots of wheat plants than that of Zn. The results also showed that the concentrations of PCs were almost higher in roots than in leaves with either Zn or Cd treatment.

Transmission electron microscope (TEM) examinations of root tips of wheat seedlings exposed to 0.04 mg Cd L^{-1} for 28 days showed thickness of cell walls relative to the control. Also, granular depositions were observed, in cell walls of the epidermis at the outer cell layer cortex of Cd- treated roots than the control. The results clearly showed that Cd altered the nucleolus features where granular deposits occurred on nucleolus as compared with the control.

Key words: Wheat plant, Zinc, Cadmium, Photosynthetic Pigments, Carbonic Anhydrase, Phytochelatins.

INTRODUCTION

Zinc is an essential nutrient and is involved in several aspects of biochemical reactions in plant. On the other hand, cadmium is a nonessential element for plant. In spite of their physicochemical similarity, their absorption by plant roots, translocation and accumulation, in different plant organs, are not similar (Clemens et al., 2002).

The studies on the phytotoxic effects of Cd in higher plants have been focused principally on the inhibition of photosynthesis where long term exposure of plant to Cd may affect chlorophyll synthesis and chloroplast development in young leaves (Baszynski et al., 1980; Barcelo et al., 1988; Sheoran et al. 1990; Abo- Kassem et al., 1997; Öncel et al., 2000 and Shukla et al., 2003).

In response to excessive Cd uptake by plant, metal- binding nonprotein- SHs (phytochelatins:PCs) are produced. It has been reported that the synthesis of phytochelatins is induced by Cd^{2+} and other trace elements such as Cu^{2+} , Hg^{2+} , Pb^{2+} , and Zn^{2+}, but larger amounts of PCs are synthesized when exposed to Cd^{2+} (Gill, et al., 1985 ; Klapheck et al., 1995;and Keltjens and van Beusichem (1998).This suggests that PCs may have a role in heavy metal detoxification in plants (Vatamaniuk et al., 2001 and Cobbett and Goldsbrough.,2002)). The formation of Cd- PC complex is up to 1000 times less toxic to many plant enzymes than the free Cd^{2+} ion (Kneer and Zenk, 1992).

While Cd induces the synthesis of PCs greater than Zn, it reduces the activity of carbonic anhydrase enzyme (Aravind and Prasad, 2004) and Zn is an essential element for stimulating carbonic anhydrase catalytic activity (Silverman, 1991 and Marschner, 1990). It was found that in wheat genotypes suffering Zn deficiency, a sharp decline in carbonic anhydrase activity (CAA) was measured (Römheld and Marschner, 1991; Rengel,1995 and Pandey et al., 2002).

Despite the increasing knowledge about the diverse effects of Cd on the biochemical processes in plants, there is scarce informations about Cd effects on the structural and ultrastructural changes of different organs of Cd- treated plant. Most studies revealed that Cd caused more marked ultrastructural changes in the aerial parts of plants than in roots (Barcelo et al., 1988). This may be explained by both the capacity of roots to store Cd in a more inactive form and by the

Department of Soil and Water Sciences, Faculty of Agriculture, El-Shatby, Alexandria University, Egypt.

relatively low mobility of Cd within plants. Rauser and Ackerley (1987) and Barçelo et al. (1988) reported that despite of high Cd concentration in plant roots, plastid ultrastructure was hardly affected. However, Vázquez et al. (1992) found high Cd accumulation in the vacuoles and nuclei in roots of bean and this produced ultrastructural disorders. These conflicting results may be due to differences between Cd concentrations, plant species, differences between monocots and dicots and detection methods used by the authors.

The objectives of this study, therefore, were to investigate the comparative effects of Zn and Cd on plant growth, metals and phytochelatins contents, activity of carbonic anhydrase enzyme, and the ultrastructure of roots of wheat grown on sand culture.

MATERIALS AND METHODS

Sand culture experiment was carried out to investigate the effect of Zn or Cd on the growth performance of wheat grown in the glasshouse of Soil and Water Sciences department, Faculty of Agriculture, Alexandria University. The sand- water system had pH of 6.5 and total soluble salts of 9.6 mg/L. Seeds of wheat (*Triticum aestivum* L.) variety Giza 168 was used as the test plant.

Modified Zn-free nutrient solution of Hoagland and Arnon (Hewitt, 1966) was used as the base solution. Zinc- free stock nutrient solution was obtained by dissolving 10.2, 4.9, 2.3, 4.9 g/L KNO_3, $Ca(NO_3)_2$, $NH_4H_2PO_4$ and $MgSO_4.7H_2O$ respectively and 28.6, 18.1, 0.8, 1.0 mg/L H_3BO_3, $MnCl_2.4H_2O$, $CuSO_4.5H_2O$, $(NH_4)_6Mo_7O_{24}.4H_2O$, respectively. Iron-EDDHA was freshly prepared in a concentration of 100 mg/L. This solution is 10 times strength the modified Hoagland and Arnon nutrient solution. The working solution (base solution) was obtained by diluting 50 ml of the stock solution to 1 liter by distilled water to obtain half strength nutrient solution.

Stock Zn solution No.1 was obtained by dissolving 0.5191 gm $ZnSO_4.7H_2O$ in one liter distilled water. This solution contains 100 mg Zn L^{-1}. Stock Zn solution No. 2 was obtained by diluting 100 ml stock Zn solution No. 1 to one liter with distilled water. This solution contains 10 mg Zn/L. The Zn working solution was obtained by mixing 0, 2.5, 5, 10 ml stock Zn solution No.2 with 50 ml stock nutrient solution No.1 and made one liter by distilled water. The concentrations of Zn, therefore, in the working solution were 0,0.025,0.050,0.100 mg Zn L^{-1} in 0.5-strength modified nutrient solution . The pH of this working Zn solution was adjusted to 7.5 by 0.1M Tris (hydroxyl methyl)- aminothane buffer according to Hajiboland et al. (2003).

Stock Cd solution No.1 was obtained by dissolving 0.1632 gm $CdCl_2$ in one liter distilled water. This solution contains 100 mg Cd L^{-1}. Stock Cd solution No.2 was obtained by diluting 100 ml stock solution No.1 to one liter by distilled water. This solution contains 10 mg Cd L^{-1}. For preparing the Cd working solution; 0,1,2,4 ml stock Cd solution No. 2 was mixed with 50 ml stock nutrient solution No.1 and made to one liter with distilled water. This working solution contains 0, 0.01, 0.02, 0.04 mg Cd L^{-1} .The pH of this working Cd solution was adjusted to 7.5 by 0.1 M Tris (hydroxyl methyl)- aminothane buffer according to Hajiboland et al.(2003).

Experimental layout:

One Kg oven-dried pre-washed sand (Hewitt, 1966) was placed in a plastic pot of 15 cm inside diameter and 12 cm depth. The seeds of wheat were washed with tap water, then distilled water and surface sterilized by 10% H_2O_2 and then washed with distilled water (Hewitt, 1966). These seeds were germinated directly in the sand culture and watered with distilled water for 7 days. After 7 days from planting the wheat seedlings were thinned to 10 plants/pot and irrigated daily with 100 ml half strength nutrient solution containing Zn or Cd. Each treatment was repeated in six replicates and distributed randomly in a complete block design in the glasshouse.

The whole plants were collected after 15 and 30 days from planting, where three replicates were collected each time. The plants of each pot were washed with tap water, then distilled water, plotted between tissue paper to remove adhered water. Each plant was separated into leaves and roots, and their fresh weights were measured. Proportions of the fresh plant materials were oven-dried at 70^0 C for 48 hrs, and their weights were measured, ground in a stainless steel mill and stored for analysis (Chapman and Pratt, 1961). Half gram oven-dried plant material was subjected to wet digestion with concentrated analytical grade HNO_3, and H_2O_2 (Jones, 1989). The concentrations of Zn and Cd in the digested solution were measured by atomic absorption spectrophotometer (Varian, spectra AA220). The other proportions, of fresh leaves and roots, were preserved for the determination of the concentrations of photosynthetic pigments; chlorophyll a and b and carotenes (Horowitz, 1975), carbonic anhydrase activity (Barman,1974) and total phytochelatines (Ellman,1959). In addition, the ultrasructure of roots were examined by transmission electron microscope (TEM) according to Stempak and Ward (1964) and Reynolds (1963).The obtained results were statistically analyzed of the least significant difference, using PC-SAS software (SAS Institute, 1988).

RESULTS AND DISCUSSION

Plant Growth:

Table 1 showed significant increases in the fresh and dry weights of shoots and roots with increasing both Zn concentrations in the nutrient solution and plant age. However, the relative increases of roots weights were higher than those of shoots. This can be observed by the decreased shoots/roots ratios due to Zn concentrations. These data reveal that Zn had stimulated the growth of roots more than shoots of both plants collected after 15 and 30 days from planting. On the other hand, Table 2 showed significant decreases in the fresh and dry weights of shoots and roots with increasing Cd concentrations in the nutrient solution. However, the weights of shoots and roots increased with plant age. The results showed that the relative decreases of roots weights were greater than those of shoots. This can be noticed by the increased shoots/roots ratios with increasing Cd concentrations. This indicated that plant roots had adversely more affected by increasing Cd than shoots, and this adverse effect had increased with increasing plant age.

Trace Elements Contents:

Table 3 showed significant increases of Zn contents in leaves of wheat plant with increasing Zn concentrations in the nutrient solution. The relative increases of Zn contents in leaves, were 191.7, 219.3 and 239.3% for the 15 days old plants and were 113.5, 164.5 and 211.9 % for the 30 days old plants. These results showed high Zn translocation to leaves, and that the rate of Zn translocation to leaves had decreased with plant age.

Table 4 showed significant increases of Cd contents in leaves of wheat plant with increasing both Cd concentrations in the nutrient solution and plant age. The relative increases of Cd contents in leaves were 800, 2000 and 3400% for the 15 days old plant and were 650, 1900 and 2650% for the 30 days old plant. This indicates high Cd translocation to leaves and also the rate of Cd translocation to leaves had decreased with plant age. These results reveal similarity of the mode of both Zn and Cd translocation to plant leaves. However, the magnitude of Cd translocation to leaves was greater than that of Zn.

Photosynthetic Pigments Contents:

Table 3 showed significant increases in Chl a, Chl b, and carotenes contents in leaves of wheat plant with increasing Zn concentrations in the nutrient solution. The results showed that the ratio of Chl a/ Chl b decreased with Zn increasing which indicate that Zn stimulated Chl b synthesis more than Chl a. This can be observed from the data of the relative increases of Chl a which varied from 2.84 to 14.89% and from 5.23 to 15.69 % for 15 and 30 days old plants, respectively as compared with those of Chl b which varied from 13.33 to 46.67% and from 12.5 to 37.5% for 15 and 30 days old plants, respectively. The results also showed that Zn stimulated carotenes synthesis in leaves of 30 days old plants more than in leaves of 15 days old plants since the relative increases varied from 1.04 to 5.31% for the 15 days old plants and from 6.02 to 14.5% for the 30 days old plants. The results in Table 3 showed higher stimulating effect of Zn for the synthesis of Chl b than for Chl a and carotenes.

Table 4 showed significant decreases of Chl a, Chl b and carotenes with increasing Cd in the nutrient solution. Values of Chl a/ Chl b ratios increased with Cd increase for both leaves of 15 and 30 days old plants. The relative decreases of Chl a varied from 12.14 to 28.57% and from 9.21 to 22.37% for leaves of 15 and 30 days old plants, respectively. These values varied from 13.33 to 33.33% and from 12.50 to 37.50% for Chl b, respectively. These results showed that Cd inhibited the synthesis of Chl b more than Chl a. However, the relative decreases for carotenes varied from 2.20 to 11.02% and from 2.83 to 10.44% for 15 and 30 days old plants, respectively which indicate that carotenes synthesis was less inhibited by Cd as compared with Chl a and Chl b. The inhibition of Chl a, Chl b and carotenes synthesis due to increasing Cd in plant leaves was reported by Baszynski et al., (1980), Barcelo et al., (1988), Sheoran et al. (1990), Pasad, (1995), Abo- Kassem et al., (1997), Öncel et al., (2000)and Shukla et al., (2003) who suggested that the inhibition was attributed to damage of cell membrane which led to photosynthesis and oxidative stress in plant leaves.

Carbonic Anhydrasé Activity (CAA):

Table 3 showed significant increases of CAA in leaves of wheat plants with increasing Zn concentrations for both the two ages of plants. The relative increases in CAA were 13.64, 27.27, and 45.45% and were 10.00, 24.00 and 32.00% for 15 and 30 days old plants, with Zn concentrations of 0.025, 0.050, and 0.100 mg L^{-1}, respectively. This indicates higher stimulating effect of Zn for increasing CAA in leaves of younger plants than of older plants. It is clear from Table 3 that the activity of CA enzyme is associated with Zn concentrations in plant leaves. Römheld and Marschner (1991) and Rengel (1995) found that the activity of CA enzyme had sharply declined with Zn deficiency in plant. Also, Sasaki et al. (1998) found that Zn deficiency in plant induced a significant decrease in the activity of CA and with increasing Zn content in plant leaves the CAA was increased. Pandey et al. (2002) found that CAA is highly correlated with Zn supply to plant. Also,

Table 1- The mean values of the fresh and dry weights of shoots and roots of wheat plants collected after 15 and 30 days from planting as influence by Zn concentrations.

Zn Conc. mg L^{-1}	Fresh weight						Dry weight					
	Shoots		Roots		Shoots/Roots		Shoots		Roots		Shoots/Roots	
	15 d.	30 d.	15 d.	30 d.	15 d.	30 d.	15 d.	30 d.	15 d.	30 d.	15 d.	30 d.
0.000	0.285	1.245	0.213	0.850	1.34	1.47	0.055	0.212	0.017	0.078	1.34	1.48
0.025	0.289	1.270	0.218	0.899	1.33	1.41	0.061	0.267	0.020	0.093	1.37	1.56
0.050	0.293	1.317	0.223	0.967	1.31	1.36	0.067	0.299	0.028	0.127	1.40	1.59
0.100	0.298	1.372	0.227	1.168	1.31	1.18	0.078	0.323	0.033	0.143	1.46	1.60
LSD 0.05	0.001	0.025	0.001	0.040	----	----	0.001	0.023	0.001	0.013	-------	------
0.01	0.002	0.038	0.002	0.060	---	--	0.002	0.035	0.002	0.020		

Table 2- The mean values of the fresh and dry weights of shoots and roots of wheat plants collected after 15 and 30 days from planting as influence by Cd concentrations.

Cd Conc. mg L^{-1}	Fresh weight						Dry weight					
	Shoots		Roots		Shoots/Roots		Shoots		Roots		Shoots/Roots	
	15 d.	30 d.	15 d.	30 d.	15 d.	30 d.	15 d.	30 d.	15 d.	30 d.	15 d.	30 d.
0.00	0.287	1.244	0.214	0.841	1.34	1.48	0.055	0.213	0.017	0.085	3.24	2.51
0.01	0.280	1.223	0.205	0.782	1.37	1.56	0.050	0.192	0.014	0.076	3.57	2.53
0.02	0.278	1.182	0.198	0.744	1.40	1.59	0.047	0.161	0.012	0.067	3.92	2.40
0.04	0.271	1.121	0.186	0.701	1.46	1.60	0.041	0.130	0.010	0.048	4.10	2.71
LSD 0.05	0.002	0.016	0.003	0.020	----	----	0.001	0.020	0.001	0.009	------	------
0.01	0.003	0.024	0.004	0.030			0.001	0.023	0.002	0.014		

Table 3- The mean values of Zn (µg/g D.W), chlorophyll and carotenes content (mg/g F.W) and carbonic anhydrase activity (CAA,µg/g F.W) in leaves of wheat plants collected after 15 and 30 days from planting as affected by Zn concentrations.

Zn, mg/l	Zn		Chl a		Chl b		Chl a/Chlb		Carotenes		CAA	
	15 d	30 d	15 d	30 d	15 d	30 d	15 d	30 d	15 d	30 d	15 d	30 d
0.000	30.0	45.3	1.41	1.53	0.15	0.16	9.40	9.56	77.2	162.8	11.0	25.0
0.025	87.5	96.7	1.45	1.61	0.17	0.18	8.52	8.94	78.0	172.6	12.5	27.5
0.050	95.8	119.8	1.55	1.68	0.19	0.20	8.15	8.40	79.3	179.0	14.0	31.0
0.100	101.8	141.3	1.62	1.77	0.22	0.22	7.36	8.05	81.3	186.4	16.0	33.0
LSD 0.05	3.0	6.8	0.01	0.01	0.12	0.01	----	----	0.2	3.1	1.3	1.9
0.01	5.0	10.3	0.02	0.02	0.02	0.02			0.4	4.6	2.0	2.9

Table 4- The mean values of Cd (µg/g D.W), chlorophyll and carotenes content (mg/g F.W) and carbonic anhydrase activity (CAA ,µg/g F.W) in leaves of wheat plants collected after 15 and 30 days from planting as affected by Cd concentrations.

Cd, mg/l	Cd		Chl a		Chl b		Chl a/Chlb		Carotenes		CAA	
	15 d	30 d	15 d	30 d	15 d	30 d	15 d	30 d	15 d	30 d	15 d	30 d
0.00	0.1	0.2	1.40	1.52	0.15	0.16	9.33	9.50	77.1	162.8	11.5	24.7
0.01	0.9	1.5	1.23	1.38	0.13	0.14	9.46	9.86	75.4	158.2	8.7	22.3
0.02	2.1	4.0	1.05	1.30	0.11	0.13	9.55	10.00	71.4	153.9	7.5	19.5
0.04	3.5	5.5	1.00	1.18	0.10	0.11	10.00	11.80	68.6	145.8	5.7	17.0
LSD 0.05	0.1	0.1	0.01	0.02	0.01	0.01	------	------	0.2	4.1	0.7	1.5
0.01	0.2	0.2	0.02	0.03	0.02	0.02			0.3	5.2	1.0	2.3

Hacisalihoglu et al. (2003) found that high Zn supply to wheat plant had stimulated the CAA in plant leaves.

Table 4 showed significant decreases in the activity of CA enzyme in plant leaves with increasing Cd concentrations in the nutrient solution. The relative decrease at the lowest Cd treatment (0.010 mg Cd L^{-1}) was 24.35% for leaves of 15 days old plants and was 9.72% for leaves of 30 days old plants while at the highest Cd treatment (0.04 mg L^{-1}) these values were 50.43% and 31.17%, respectively. These results indicate high inhibition effect of Cd on the activity of CA enzyme with high Cd concentration treatment and with increasing plant age. It is also clear that the decrease of CAA is associated with increasing of Cd concentrations in plant leaves whether for 15 or 30 days old plants. Thus, it has been found that Cd-treated plant showed toxicity to CA enzyme (Aravind and Prasad, 2004). Fuhrer (1982) reported that the harmful effect of Cd might be due to its ability to inactivate CA enzyme through reaction with the SH-groups of proteins.

Phytochelatins(PCs)Content:

Table 5 showed marked increases of Zn concentrations in leaves and roots with Zn treatment and with plant age. Also, Zn transport from roots to leaves had increased with Zn treatment. The relative increases of Zn in leaves were 239.3 and 211.9% and in roots were 151.7 and 132.2% for 15 and 30 days old plants, respectively. This indicates that the rates of increasing Zn concentration in both shoots and roots were higher in younger plants (15 days old). However, the concentration of Zn in roots was almost higher than in leaves as indicated by Zn leaves/ roots ratio which is almost less than unity. This ratio increased from 0.73 to 0.75, for 15 and 30 days old plants, respectively treated with Zn which points out that the rate of Zn transport from roots to shoots had increased with plant age.

The concentrations of total phytochelatins (PCs) in plant leaves were increased with both Zn treatment and with plant age (Table 5). There is positive association between Zn and PCs concentrations in leaves and roots. The results showed that the concentrations of PCs in roots were higher than in leaves and there is marked translocation of PCs from roots to leaves. This is indicated by the ratios of PCs leaves/ PCs roots which had increased with both Zn treatment and plant age which indicate PCs transport from roots to shoots. However, PCs transport is almost low since the PCs leaves/ root ratio is less than unity. The results showed that translocation of PCs from roots to leaves are associated with Zn translocation from roots to leaves. This indicates high positive relation between Zn contents in plant leaves or roots and PCs synthesis.

Table 6 showed marked increases of Cd concentrations in leaves and roots with both Cd treatment and plant age as compared with the control plant. The relative increases in Cd concentrations in leaves and roots were 3400 and 3450% and were 2650 and 4167% for 15 and 30 days old plants, respectively. This indicates an accumulation of Cd in roots with increasing plant age rather than its translocation to leaves. This is revealed by values of Cd leaves/roots ratio which decreased in Cd-treated plants from 0.49 (15 days old plants) to 0.43 (30 days old plants).

Table 6 showed higher concentration of total phytochelatins (PCs) in roots than in leaves and in older plants than in younger plants. The relative increases of the concentrations of total PCs in leaves and roots were 285.5 and 169.4%, and 156.6 and 127.4 % for 15 and 30 days old plants, respectively. This indicates that the increasing rate of PCs in leaves was greater than in roots and this rate had decreased with plant age. This is evident by comparing values of PCs shoots/ roots ratios which increased from 0.57 to 0.72 for the control plants and deceased from 0.86 to 0.85 for Cd-treated plants. These results showed positive association between Cd and PCs concentrations in plant organs. Keltjens and van Beusichem (1998) observed a close positive relationship between Cd and PCs concentrations in the shoots of wheat plant grown in hydroponic culture containing Cd. They also found that PCs contents in roots were greater than in shoots. Stolt et al. (2003) found higher concentrations of PCs in roots than shoots of wheat seedlings grown in nutrient solution containing Cd and it was associated with high PCs accumulation.

Inspite of the highest levels of Zn in plant shoots and roots, PCs contents in shoots and roots were lower than those associated with Cd in plant organs. This point out that Cd enhanced PCs synthesis more than Zn. The PCs/Zn ratios in leaves were 2.8 and 2.0 and in roots were 2.6 and 1.9 for the control and Zn- treated 15 days old plants, respectively. For 30 days old plants, these ratios were 3.0 and 1.8 in leaves and 2.3 and 1.7 in roots, respectively. These results showed that PCs/Zn ratios are within 1.7-3.0. These ratios with respect PCs/Cd were 830.0 and 91.4 in leaves and 725.0 and 52.4 in roots of 15 days old plants, respectively, and were 670.0 and 65.6 in leaves, and 620 and 33.0 in roots of the control and Cd-treated 30 old days plants, respectively. These results reveal that PCs/Cd ratios are within 33.0- 830.0. These indicate that Cd had higher stimulating action for PCs synthesis than Zn in spite of the low level of Cd as compared with Zn in plant. Cobbett and Goldsbrough (2002) concluded that PCs biosynthesis occurs within minutes of exposure to low concentrations of Cd. They reported that PCs synthesis was activated more with

low concentrations of Cd than high concentrations of Zn in plant tissues.

Ultrastructure of Roots:

As shown in Plates 1-6, transmission electron microscope (TEM) examinations of root tips of wheat seedlings exposed to 0.04 mg Cd/L for 28 days showed that the root had thick cell walls (Plates 1 and 2). Also, several granular deposits are located in the cell wall of the epidermis at the outer cell layer of the cortex (Plates 1-4). In addition, Cd altered the nucleolus features where deposits occurred on nucleolus as compared with the control (Plates 5 and 6). This is due to the occurrence of high Cd concentration in roots which had been deposited in cell wall and nucleolus. Khan et al.,(1984) found that Cd accumulates in root cell walls and cause alteration in cell wall structure of roots of corn plant. The reduced internal membrane system may have more direct effect of Cd on plastid development with roots of been plant (Barcelo et al., 1988). Vázquez et al. (1992) found that Cd had accumulated in vacuoles and nuclei of roots of bean plant, and Cd grains had occurred in both the walls of the cortex parenchyma cells and localized in the nuclei and cytoplasm.

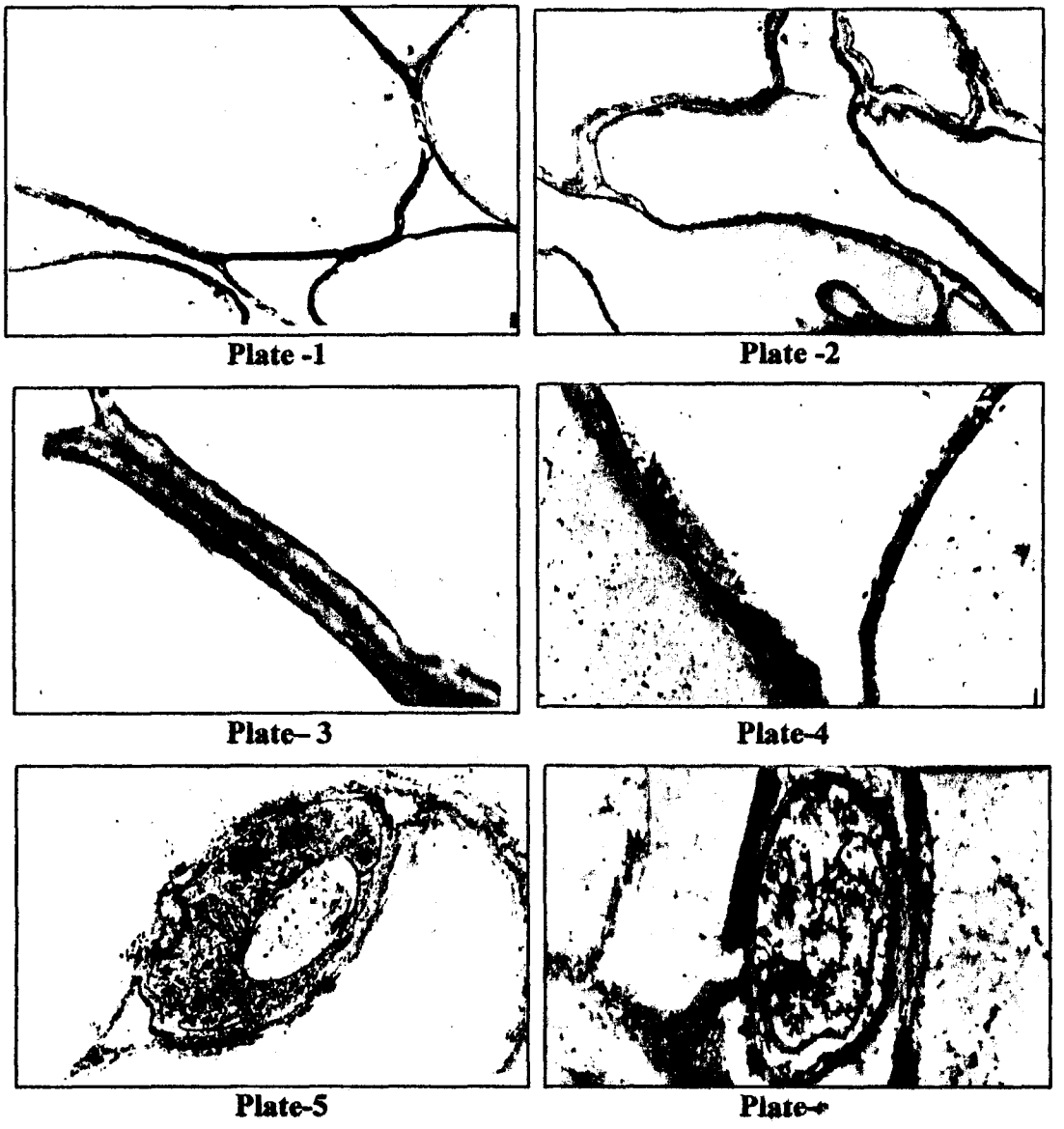
The accumulation of Cd in root cell walls may be responsible for the restricted transport of Cd from roots to shoots. This is evident when discussing Cd shoots/ Cd roots ratio which were almost less than unity and decreased in Cd- treated plant than that of the control plant (Table 4).On the other hand, Zn behaved in opposite trend where Zn shoots/ Zn roots ratio was higher in Zn-treated plant than in the control plant (Table). The restricted transport of Cd from roots to shoots was also reported by Khan et al., (1984), and Barcelo et al. (1988).

Table 5- The mean values of Zn (µg/g D.W) and total phytochelatins (PCs, mM SH/g F.W.) in leaves of wheat plants collected after 15 and 30 days from planting as influenced by Zn treatment.

Treat.	After 15 days						After 30 days					
	Leaves		Roots		Zn L/R	PCs L/R	Leaves		Roots		Zn L/R	PCs L/R
	Zn	PCs	Zn	PCs			Zn	PCs	Zn	PCs		
Control	30.0	83	55.7	145	0.64	0.54	45.3	134	81.1	186	0.56	0.72
0.1 mg Zn/l	101.8	206	140.2	268	0.73	0.77	141.3	259	188.3	310	0.75	0.84

Table6- The mean values of Cd (µg/g D.W) and total phytochelatins (PCs, mM SH/g F.W.) in leaves of wheat plants collected after 15 and 30 days from planting as influenced by Cd treatment.

Treat.	After 15 days						After 30 days					
	Leaves		Roots		Cd L/R	PCs L/R	Leaves		Roots		Cd L/R	PCs L/R
	Cd	PCs	Cd	PCs			Cd	PCs	Cd	PCs		
Control	0.1	83	0.2	145	0.50	0.57	0.2	134	0.3	186	0.67	0.72
0.04 mg Cd/l	3.5	320	7.1	372	0.49	0.86	5.5	361	12.8	423	0.43	0.85



Plates1-6: TEM micrographs of roots of wheat seedling treated with 0.4 mg Cd/l for 28 days. Plate 1: roots of the control showing outer cell wall(X=5000), plate 2: Cd-treated roots showing outer cell wall cortex (X=5000), plate 3: roots of the control showing cell wall (X=15000), plate 4:Cd- treated roots showing granules deposits on cell wall (X=15000), plate 5 : roots of the control showing nucleolus (X=25000), plate 6: Cd-treated roots show granules deposits on nucleolus (X= 25000).

REFERENCES

Abo – Kassem, E. M.; Sharaf El- Din, A.; Mohamed, Y.A.; and Foda, E. A., (1997). Effect of different cadmium concentration on growth, photosynthesis and ion relation of wheat. Egypt. J. Physiol. Sci. 21 (1), 41– 51.

Aravind, P.,and Prasad, M. N. V., (2004). Carbonic anhydrase impairment in cadmium– treated ceratophyllum demersum L. (free floating fresh water macrophyte): toxicity reversal by zinc. J. Anal. At. Spectram. 19: 52 – 57.

Barcelo, J ; Vazgeuz. M. D ; and Poschenrider, C. H. (1988). Structural and ultrastructure disorders in cadmium – treated bush bean plants (*Phaseolus Vulgaris* L.). New Phytol. 108 : 37 – 44.

Barman, T.E. (1974): Enzyme Hand Book. Vol. I. Springer Velag. Berlin and New York.

Baszynski, T., Wajda, L., Krol, M., Wolinska, D., Krupa, Z. and Tukendrof, A. (1980). Photosynthetic activities of cadmium – treated tomato plants. Physiol Plant. 48: 365 – 370.

Chapman, H. D. and P. F. Pratt (1961) : "Methods of Analysis for Soils. Plants, and Waters". University of California, Division of Agricultural Sciences.

Cobbett, C., and Goldbrough, P. (2002).Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis. Ann. Rev. Plant Biol. 53: 159-182.

Clemens, S., Palmgren, M. G., and Kramer, U. (2002). A long way ahead: Understanding and engineering plant metal accumulation. Trends in Plant Sci. 7: 309-315.

Ellman, a. l. (1959):Tissue Sulfhydryl Groups. Archives of Biochemistry and Biophysics, 82:70-77.

Fuhrer, J., (1982). Ethylene biosynthesis and cadmium toxicity in leaf tissue of beans (*Phaseolus Vulgaris* L.) Plant Physiol. 70 : 162 – 167.

Gill, E ; Winnacker, E. L., and Zenk, M.H.(1985). Phytochelatin : The principal heavy – metal complexing peptides of higher plants. Sci. 230 : 674 – 676.

Hacisalihoglu, G., Hart, J. J., Wang, Y. H., Cakmak, I., and Kochian, L. V. (2003). Zinc efficiency is correlated with enhanced expression and activity of zinc – requiring enzymes in wheat . Plant Physiol. 131 : 595 – 602.

Hajiboland, R., Yang, X.E., and Romheld, V. (2003): Effects of bicarbonate and high pH on growth of Zn- efficient and Zn- inefficient genotypes of rice, wheat and rye. Plant and Soil, 250:349-357.

Hewitt, E. J.(1966). Sand and Water Culture Methods Used in The Study of Plant Nutrition. Technical Communication No.22 Commonwealth Bureau, London.

Horowitz, W. (1975):" Official Methods of Analysis of the Association of Official Analytical Chemists". Twelfth Edition. Published by Association of Official Analytical Chemists. PO. Box 540, Benjamin Franklin station. Washington, D.20044.

Jones, J. B. Jr. (1989). "Soil Testing and Plant Analysis". Laboratory Inc. Athens Georgia USA Pp. 59.

Keltjens, W. G., and van Beusichem, M. L., (1998). phytochelatins as biomarkers for heavy metal stress in maize (*Zea mays* L.): and wheat (*Triticum aestivum* L.): combined effects of copper and cadmium. Plant and Soil. 203: 119 – 126.

Khan, D. H., Duckett, J. G., Frankland, B., and Kirkham, J. B. (1984). An x – ray microanalytical study of the distribution of cadmium in roots of Zea mays L. J. Plant Physiol. 115 : 19 – 28.

Klapheck, S. ; Schlunz, S. and Bergmann, L. (1995). Synthesis of phytochelatins and homophtochelatins in Pisum Sativum L. Plant Physiol. 107 : 515 – 521.

Kneer, R. and Zenk, M. H. (1992). Phytochelatins protect plant enzymes from heavy metal poisoning. Phytochemistry. 31: 2663 – 2667.

Marschner, H. (1990)."Mineral Nutrition of Higher plants". Academic Press. Harcout Brace Jovanovich, Publishers, New York.

Öncel, I., Keles, Y.,and Ustun, A. S., (2000). Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. Environ. Pollut. 107: 315 – 320.

Pandey, N., Pathak, G. C., Singh, A. K., and Sharma, C. P., (2002). Enzymic changes in response to zinc nutrition . J. Plant Physiol. 159 :1151 – 1153.

Pasad, M. (1995). Cadmium toxicity and tolerance in vascular plants. Env. Exp. Bot. 35 : 525 – 545.

Rauser, E. E., and Ackerley, C. A. (1987). Localization of cadmium in granules within differentiating and mature root cells. Canad. J. Botony. 65 : 643 – 646.

Rengel, Z. (1995). Carbonic anhydrase activity in leaves of wheat genotypes differing in Zn efficiency. J. Plant. Physiol. 147 : 251 – 256.

Reynolds, E.S. (1963). The Use of Lead Citrate at High pH as an Electronopague Stain in Electron Microscopy. J. cell Biol. 171: 208-212.

Römheld, V., and Marschner, H., (1991). Function of micronutrient in plants. In :" Micronutrients in Agriculture " 2 nd Ed. Mortvedt, J. J. number 4 in the Soil Sci. Soc. Am. Book Series Soil Sci. Soc. Am. In., Madison. Wisconsin. USA. Pp : 297 – 328.

Sasaki, H., Hirose, T., Watanabe, Y., and Ohsugi, R. (1998). Carbonic anhydrase activity and CO2 – transfer resistance in Zn – deficient rice leaves. Plant Physiol. 118 : 929 – 934.

SAS Institute. (1988). PC-SAS user's guide. Statisitics SAS Inst., Cary, NC.

Sheoran, I.S., Singal, H.R.and Singh, R. (1990). Effect of cadmium and nickel on photosynthesis and the enzyme of photosynthetic carbon reduction cycle in pigonpea (L). Photosynthesis Res. 23 : 345 – 347.

Shukla, U. C., Singh, J., Joshi, P. C.,and Kakkar, P., (2003). Effect of bioaccumulation of cadmium on biomass productivity, essential trace elements, chlorophyll biosynthesis, and macromolecules of wheat seedling. Biological Trace Element Research. 92, 257 – 273.

Silverman, D. N. (1991). The catalytic mechanism of carbonic anhydrase. Canad. J. Bot. 69 : 1070 – 1078.

Stempak, C.D. and R.T. Ward (1964).An Improved Staining Method for Electron Microscopy. J. cell Biol.12:414-420.

Stolt, J. P., Sneller, F. E. C., Bryngelsson, T., Lundborg, T., and Schat, H., (2003). Phytochelatin and cadmium accumulation in wheat. Environ. Experim. Botany. 49 :21 – 28.

Vatamaniuk, O. K., Bucher, E. A., Ward, J. T., and Rea, P. A.(2001) A new pathway for heavy metal detoxification in animals- phytochelatin synthesis is required for cadmium tolerance in Caemorhabditis elegans. J. Biol. Chem. 276: 20817-20820.

Vázquez, M. D., Poschenrieder, C. H., BarcelÓ, J., (1992). Ultrastructural effects and localization of low cadmium concentration in bean roots. New phytol. 120: 215 – 226.

الملخص العربي

التأثيرات البيوكيميائية للزنك والكادميوم على نمو القمح النامي في مزرعة رملية

إيمان فاضل على عوض الله- إبراهيم حسين العسكري- عبد السلام عباس عبد السلام

قسم علوم الأراضي والمياه-كلية الزراعة-جامعة الإسكندرية

قد تم اجراء تجارب المزرعة الرملية في صوبة قسم الأراضي والمياه كلية الزراعة جامعة الإسكندرية لدراسة تأثير الزنك والكادميوم على نمو نبات القمح صنف جيزة ١٦٨ و محتواه من صبغات البناء الضوئي و انزيم الكربونيك انهيدريز ومركبات الفيتوكلاتين.

أوضحت النتائج زيادة معنوية في الوزن الجاف والرطب للمجموع الخضري والجذري مع زيادة الزنك في المحلول المغذى. وأيضا انخفاض معنوي لنفس الخصائص مع زيادة الكادميوم في المحلول المغذى. وقد زاد محتوى الاوراق من الكلورفيل أ وب والكاروتين مع زيادة الزنك وانخفض محتواها من هذه الصبغات مع زيادة الكادميوم. أوضحت النتائج أن الزنك يساعد على تخليق كلورفيل ب عن كلورفيل أ بينما الكادميوم يمنع من تخليق كلورفيل ب بمقدار أكبر من كلورفيل أ و يخفض من تخليق الكاروتين ولكن بمقدار قليل.

أظهرت النتائج زيادة معنوية لنشاط أنزيم الكربونيك أنهيدريز في أوراق نبات القمح مع زيادة تركيز الزنك وأنخفاض معنوى لنشاط الانزيم مع زيادة تركيز الكادميوم. وقد وجد أن زيادة تركيز الكادميوم في المحلول المغذى ساعد فى الحث على تخليق مركبات الفيتوكلاتين فى اوراق وجذور نبات القمح. ومحتوى الجذور من مركبات الفيتوكلاتين تزيد عن محتوى الاوراق مع زيادة المعاملة بالزنك أو الكادميوم.

وقد تم اختبار جذور بادرات القمح المعرضة لتركيز ٠,٠٤ ملليجرام كادميوم/اللتر لمدة ٢٨ يوم بالميكروسكوب الإلكتروني فوجد زيادة سمك الجدر الخلوية للنباتات المعاملة مع وجود ترسيبات في صورة حبوب على هذه الجدر مقارنة بجذور النباتات الغير معاملة. و ظهر ايضا ترسيبات في النواة بخلايا جذور هذه النباتات مقارنة بجذور النباتات الغير معاملة.