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Evaluation of Toxic Effect of Copper and Cadmium on Growth, Physiological Traits and Protein Profile of Wheat (*Triticum aestivium* L.), Maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.)

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Abstract: A pot experiment was carried out in order to investigate the effect of deleterious concentration of copper and cadmium either individual or in combination on three cereal crops i.e. wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and sorghum (*Sorghum bicolor L.*) after 2 and 4 weeks. The results showed that cadmium applied alone caused significant reduction in growth traits and photosynthesis ($P< 0.05$ or $P <0.01$). The effect was less pronounced when Cu applied alone or in combination with Cd^{+2}. The same trends were also observed with leaf osmotic potential and relative water content under the same treatments. The stress associated protein, profiles in leaves at 14 kDa in wheat, 35 and 54 kDa in maize were disappeared, compared to others treatments, in response to 75 Cu, 150 Cu, 75 Cd^{+2}, 150 Cd^{+2} and 150 Cd^{+2}, 75 Cu +75 Cd^{+2} µmol/L after four weeks, respectively. A 60 and 16 kDa a soluble protein was over expressed only in sorghum under 150 Cd^{+2} µmol/L and in wheat under 150 Cd^{+2} µmol/L and 75 Cu^{+} + Cd^{+2} µmol/L. These proteins designated as cadmium stress inducible protein (CSIP) and may be appositive marker to cadmium stress tolerance in sorghum and wheat. It could be concluded that, in all three crops, the effect of different treatments were more toxic in order to Cd^{+2} < Cu^{+} + Cd^{+2} < Cu < control either after 2 or 4 weeks. Also, sorghum was more tolerance to heavy metal stress followed by wheat and maize; and it could help alleviate crop losses in area affected by heavy metals stress.

Key words: Heavy metals residues % Tolerance % Growth and physiological traits % Genetic variances % Stress protein

INTRODUCTION

Heavy metal toxicity is one of the major current environmental health problems and potentially dangerous due to bioaccumulation through the food chain and in plant products for human consumption [1]. Therefore, heavy metal contaminations of soils and plants have become an increasing problem. Particularly, amongst the heavy metals, Copper (Cu) and cadmium (Cd) are caused increasing international concern due to its toxicity is generally considered to be much higher than those of other heavy metals and it is readily taken up by plants [2]. Copper in small concentration is an essential micronutrient for all form of life such as growth, physiological process [3], protein trafficking machinery, oxidative phosphorylation and structure element in regulatory protein [4], different metabolic pathway including ATP synthesis and acting in the prothetic group of many oxidizing enzymes [5]. However, as a result of the formation of organo copper complexes, excess copper can be consider as a toxic elements leading to reduces shoot and root growth by inhibits cell elongation and cell cycle [6]decrease of chlorophyll content, leaf expansion, disturbance of DNA conformation and damage chromatin and damages the plasma membrane causing ion efflux [7]. While Cadmium (Cd) is a major environmental pollutant and one of the most toxic metals in plants, it has inhibitory effects on seed germination, leaf area ratio and relative water content and plant growth, chlorophyll synthesis indicated that a growth analysis is a useful index of cadmium toxicity [8].

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Metal pollution is a multi element propel, in many cases, it is more appropriate to study combined heavy metals on plants may be quite different form those of individual pollutants due to interaction between heavy metals. The association of copper and cadmium in environment and their chemical similarity can lead to interaction between these two ions, resulting in the lowering of cadmium toxicity [9]. Plants are often damaged due to increasing the pollution in the atmosphere by toxic chemical. As it's often not feasible to modify the environment to suit the plants, efforts are being made to modify the plants to suite the harvest environmental conditions. Metals tolerant plants can help in reclaiming degraded land. Wheat, maize and sorghum have been chosen in this study, first, because it is important food source as mention previously, second, because they are monocotyledon and most studies on heavy metals toxicity have been carried out on dicotyledons and third, because wheat and maize and sorghum particularly useful C3 and C4 plant, respectively.

The exposure of plant to heavy metals ion, as one of a biotic stress, is known to induce several stress associated with protein and peptides such metallothionins [10, 11, 12]. These protein and peptide function in cellular regulation and homeostasis during metal stress. Hansch and Mendel [13] indicated that the excess of Cu and Cd concentrations may induce significant toxic effect by altering the protein function and enzymes activity. Toxicity may result from the binding of metal sulfhydryl group in the protein, leading to inhibition of activity or disruption of the structure [14]. Several studies on toxic of heavy metals indicated that Cu and Cd changes in the protein profiles on several plants [15] observed that plant when exposed to high concentrations of heavy metals, including Cu and Cd, produce low molecular mass peptide. A 51 KDa soluble protein was over-expressed in wheat seedling by the treatment of seeds before germination with 50 µM $CdCl_2$, this protein designated as cadmium stress associated protein [12].

The aim of present work was to study the changes induced by cooper and cadmium either single or combined on different growth and physiological traits; expression of some protein and assertion the correlations among these traits in three different crops.

MATERIALS AND METHODS

The present research work was conducted at the Biotechnology Lab, Biological Department, Faculty of Science, North Jeddah, KAU. Three cultivars, named Sakha 93, Giza 649 and Giza 2, belong to three different crops, wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.), respectively were obtained from Field Crops Research Institute, Agriculture Research Center, Giza, Egypt. The grains were kept at room temperature and each plastic pot (14 x 17 cm) was filled up with 0.5 kg of sand and peat-moss at 1:1 ratio. Different concentration of copper (75 or 150 µmol/L) as $CuSO_4.5H_2O$, cadmium (75 or 150 µmol/L) as $3CdSO_4.8H_2O$ and different combination of copper and cadmium concentration 37.5 or 75 µmol/L were applied after germination. The pots trials were conducted in growth chamber using a completely randomized design with three replications and 10 grains per pots were used in this study. The pots were maintained in growth chamber under 16/8 hrs light/dark, photoperiod with 100 µmol/m^2/s illumination at 25±1°C. After germination the seedling were irrigated with tap water every 2-3 days as control treatment and different concentration of copper or cadmium. After 2 and 4 weeks ten plants from each treatment were taken at random for measuring the following parameters:

Growth Traits: Shoot length (cm), shoot fresh weight (g), root fresh weight (g) and number of leaves (n). Dry weight of shoot and root (g) were recorded after dried in forced oven at 70°C for 72 hrs [16]. Flag Leaf Area (FLA) was measuring according to House [17]: FLA = L x W x A where L is leaf length, w is leaf maximum width and A is constant equal 0.75.

Physiological Traits: Osmotic Potential (OP): The fresh samples of leaves were collected from each cultivar to determine electric conductivity (EC). The EC multiplied by factor 0.36 to present OP (-bar) Relative Water Content (RWC): the RWC in leaves was determined according to Morgan [18] as a percentage.

Chlorophyll Extraction and Measurement: For the chlorophyll analysis fully expended leaves (0.1 g) were ground and extracted with 5 ml of 80% (v/v) acetone overnight at 4°C, centrifuged at 3000 x g for 3 min. and the mixture was filtered and absorbencies were determined at 645, 663 and 450 nm using spectrophotometer (Spectro 23 RS). Concentration of chlorophyll a, chlorophyll b and carotenoids were estimated by the equation of Witham *et al.* [19].

Protein Profiles: In order to study the protein profiles under heavy metals stress, the protein extraction of different treatments was carried out at 4°C. After 2 and 4 weeks leaves from the sowing were collected and frozen

at -80°C until used. Protein extraction was conducted by homogenizing 1 g of frozen leaves with 1.5 M Tris-HCl buffer, pH 8.8 in clean Eppendorf tube. Then centrifuged at 5000 rpm for 10 min, The supernatant of each sample (contains protein extract) was kept in deep-freeze until use for electrophoresis analysis SDS-polyacrylamide gel electrophoresis was performed in 12% acrylamide slab gels following the system of Laemmli [20]. The protein content was determined by the method of Lowery *et al.* [21] using bavin serum albumin (sigma) as standard. Volume of 50 µl protein extract was added to 20 µl volume of treatment buffer and 50 µl of each sample was loaded in the gel with addition to BLUeye prestained protein leader (Genedirex) having molecular mass ranging 17-135 kDa. After the electrophoresis run was completed, gels of SDS-protein was stained in Coomassie Brilliant Blue R250 (CBB) solution for 12-18h and then rinsed in destaining solution until the dark background became colorless except blue protein bands and photographed.

Analysis of Heavy Metals: After 2 and 4 weeks of treatment, the leaves form all treatments and non treatments were harvested, washed thoroughly with deionized water the leafs tissue were cut into small pieces and oven dried at 80°C for two days. Oven-dried material was weighed and 1 gram of the leaves was placed in Te?on vessels. The plant material was digested by the diacid method [22]. The volume of the extract was made up to 50 mL with distilled water and the metal content in the extract were analyzed for Cu and Cd by ICP-MS (ELAN DRC II, Perkin Elmer).

Statistical Analysis: Results were expressed as mean ± SD (standard deviation). All data were subjected 3 way ANOVA analysis to calculate the least significant difference (LSD) at $p<0.05$ and $p<0.01$ with CoStat computer program.

RESULTS AND DISCUSSION

Effect of Different Concentration of Cu^{+} and Cd^{+2} on Growth Traits: Data presented in Table 1 indicated that the mean average of shoot fresh weight (SFW), Root fresh weight (RFW) and root dry weight (RDW) of wheat, maize and sorghum were the highest comparing with others treatments after 2 and 4 weeks the greatest values of the studied traits were obtained under 150 µmol/L of Cu^{+}. These results are in agreement with those obtained by Inmaculada [23] which found that, for healthy plant growth and development copper must be acquired from soil, transport through the plant, distributed and compartmentalized within different tissues and its content carefully regulated within different cells and tissue. On the other hand the reduction in growth traits was observed after 4 weeks at (75 µmol/L of Cu^{+}), this reduction was positive relation with concentration and the duration where more dosage of copper are accumulated in plants to toxic effect which inhibit growth and to interfere with important cellular process such as photosynthesis. Amin and Amal [24] observed that, significantly decreased of shoot length, root length and number of root after exposure plants to different concentrations of Cu (0.0, 25, 50 and 100 µmol/L) after 15 days. The inhibitory action of excess copper in shoots length, root length and dry matter may be due to reduction in cell division and toxic effects of Cu on photosynthesis [25, 26].

The data of wheat, maize and sorghum the concentrations of 75 and 150 µmol/L of Cd^{+2}caused a large decrease in all traits after 2 weeks and complete inhibition was occurred after 4 weeks compared to control (Table 1). Ouzounidou [27] indicated that cadmium in high concentration is more phytotoxicity than other heavy metals. This may be due to the fact that Cd^{+2} are more mobile than other heavy metals. Ali *et al.* [28] indicated that the reduction in growth characters during stress may due to water potential hampered nutrient uptake, reduction in meristem cells and oxidative stress.

It is obvious from the results that the growth, morphological and physiological characters under different treatment were affected by the presence of Cu^{+} and Cd^{+2} but combined effect of both Cu^{+} and Cd^{+2} was less synergistic on the traits under study and less reduction in the average of these traits was showed compared to Cd^{+2} as single (Tables 1-3). The results are in line with same earlier finding by Ali *et al.* [29], they suggested that the additional supply of Cu^{+} in the Cd^{+2} causes certain degree of recovery in plant growth and the toxicity of Cd^{+2} can be circumvented by the addition of copper. This influence of combined effect of copper with cadmium was more effective in sorghum than wheat and maize plants (Table 1). This result are supported by Jeff *et al.* [30] who found that under the same stressed environment such as heavy metals the adaption and yield stability of sorghum is more enhanced than that of poaceae plants. This may be sorghum is more able to prevented oxidative stress by a cell antioxidative system whose more efficiency in sorghum than wheat or maize.

Effect of Heavy Metals on Physiological Characters: Leaf Osmotic Potential (LOP): LOP of wheat, maize and sorghum plants under different concentration of Cu^{+} and

Table 1: Effect of different concentration of Cu, Cd^{+2} and Cu + Cd^{+2} on some growth and physiological traits in wheat (*Triticum aestivum* L.).

Traits			Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
2 weeks	Cont.		1.01 ± 0.17	0.83 ± 0.06	0.40 ± 0.49	0.17 ± 0.02
	Cu	75 µmol/L	0.95 ± 0.14	0.74 ± 0.06*	0.09 ± 0.01**	0.13 ± 0.02**
		150 µmol/L	0.73 ± 0.06**	0.82 ± 0.12	0.03 ± 0.01**	0.12 ± 0.02**
	Cd^{+2}	75 µmol/L	0.62 ± 0.03**	0.50 ± 0.15**	0.07 ± 0.01**	0.03 ± 0.01**
		150 µmol/L	0.58 ± 0.10**	0.44 ± 0.03**	0.19 ± 0.14**	0.07 ± 0.02**
	Cu + Cd^{+2}	75 µmol/L	0.79 ± 0.02**	0.65 ± 0.09**	0.07 ± 0.01**	0.08 ± 0.01**
		150 µmol/L	0.75 ± 0.15**	0.86 ± 0.05**	0.19 ± 0.14**	0.16 ± 0.02
4 weeks	Cont.		2.50 ± 0.20	3.20 ± 0.10	0.32 ± 0.02	0.69 ± 0.09
	Cu	75 µmol/L	2.03 ± 0.15*	1.33 ± 0.15**	0.23 ± 0.02**	0.26± 0.01**
		150 µmol/L	2.30 ± 0.20	1.91 ± 0.18**	0.27 ± 0.06**	0.36 ± 0.01**
	Cd^{+2}	75 µmol/L	1.63 ± 0.21**	1.97 ± 0.12**	0.20 ± 0.00**	0.27 ± 0.03**
		150 µmol/L	1.01 ± 0.09**	1.44 ± 0.20**	0.13 ± 0.01**	0.26 ± 0.01**
	Cu + Cd^{+2}	75µmol/L	1.40 ± 0.26**	0.97 ± 0.01**	0.14 ± 0.02**	0.20 ± 0.03**
		150 µmol/L	0.65 ± 0.04**	0.73 ± 0.06**	0.09 ± 0.01**	0.15 ± 0.02**

The values are means ± SD

*or ** indicated significant difference from the control at p # 0.05 and p # 0.01, respectively

Table 1: Continue

Traits			Shoot length (cm)	Root length (cm)	Leaf area index (cm^2)	Leaf osmotic potential (-bar)	Relative water content (%)
2 weeks	Cont.		17.67 ± 1.15	12.67 ± 1.18	2.57 ± 0.25	1.29 ± 0.27	84.33 ± 2.08
	Cu	75 µmol/L	19.67 ± 1.53**	10.00 ± 1.00**	2.13 ± 0.15*	3.83 ± 0.29**	75.67 ± 3.06**
		150 µmol/L	20.00 ± 2.00**	11.33 ± 1.53	2.97 ± 0.49	5.17 ± 0.29**	70.67 ± 1.15**
	Cd^{+2}	75 µmol/L	19.33 ± 1.15**	7.00 ± 1.00**	2.33 ± 0.15*	3.17 ± 0.29**	71.00 ± 1.73**
		150 µmol/L	14.00 ± 1.61**	7.00 ± 1.73**	2.30 ± 0.10*	4.17 ± 0.29**	67.67 ± 2.52**
	Cu + Cd^{+2}	75 µmol/L	18.00 ± 0.91	9.33 ± 1.31*	1.34 ± 0.23**	5.50 ± 0.50**	70.00 ± 1.73**
		150 µmol/L	14.33 ± 1.06**	11.67 ± 0.76	1.20 ± 0.10**	5.83 ± 0.29**	66.67 ± 2.89**
4 weeks	Cont.		19.00 ± 1.10	12.33 ± 1.53	5.43 ± 0.36	1.69 ± 0.19	86.33 ± 2.08
	Cu	75 µmol/L	23.00 ± 1.00*	19.00 ± 1.00**	6.26 ± 1.22*	4.10 ± 0.17**	72.67 ± 3.06**
		150 µmol/L	25.00 ± 1.00**	15.33 ± 0.58**	5.00 ± 0.46*	6.00 ± 0.30**	68.00 ± 2.65**
	Cd^{+2}	75 µmol/L	20.33 ± 0.58	14.33 ± 0.58*	5.02 ± 0.45*	4.00 ± 0.20**	68.00 ± 1.73**
		150 µmol/L	14.33 ± 2.08**	13.00 ± 1.00	1.04 ± 0.58**	4.47 ± 0.45**	65.00 ± 5.00**
	Cu + Cd^{+2}	75µmol/L	20.33 ± 2.52	15.33 ± 1.15**	3.70 ± 0.17**	5.07 ± 0.12**	69.00 ± 1.00**
		150 µmol/L	15.67 ± 2.08**	10.00 ± 1.00*	2.69 ± 1.04**	5.10 ± 0.17*8	64.00 ± 1.73**

The values are means ± SD

*or ** indicated significant difference from the control at p # 0.05 and p # 0.01, respectively

Table 2: Effect of different concentration of Cu, Cd^{+2} and Cu + Cd^{+2} on some growth and physiological traits in maize (*Zea mays* L.)

T raits			Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
2 weeks	Cont.		2.89 ± 0.10	3.59 ± 0.09	0.24 ± 0.03	0.69 ± 0.09
	Cu	75 µmol/ L	2.75 ± 0.05*	3.71 ± 0.23*	0.23 ± 0.01*	0.39 ± 0.03**
		150 µmol/ L	3.93 ± 0.15**	3.07 ± 0.15**	0.26 ± 0.02*	0.70 ± 0.07*
	Cd^{+2}	75 µmol/ L	1.70 ± 0.20**	1.22 ± 0.12**	0.11 ± 0.02**	0.15 ± 0.03**
		150 µmol/ L	2.40 ± 0.26*	2.97 ± 0.22**	0.18 ± 0.02**	0.45 ± 0.01**
	Cu + Cd^{+2}	75 µmol/ L	3.71 ± 0.17**	3.27 ± 0.06*	0.30 ± 0.00**	0.39 ± 0.02**
		150 µmol/ L	3.33 ± 0.06**	2.87 ± 0.06**	0.24 ± 0.02**	0.25 ± 0.02**
4 weeks	Cont.		3.00 ± 0.30	4.10 ± 0.20	0.27 ± 0.06	0.78 ± 0.08
	Cu	75 µmol/ L	3.93 ± 0.06**	4.35 ± 0.05*	0.35 ± 0.02**	0.45 ± 0.03**
		150 µmol/ L	5.37 ± 0.25**	3.63 ± 0.15*	0.52 ± 0.05**	0.71 ± 0.06
	Cd^{+2}	75 µmol/ L	1.28 ± 0.03**	2.27 ± 0.06**	0.11 ± 0.01**	0.36 ± 0.03**
		150 µmol/ L	1.90 ± 0.09**	3.53 ± 0.15*	0.25 ± 0.04*	0.47 ± 0.03**
	Cu + Cd^{+2}	75µmol/ L	4.57 ± 0.11**	5.60 ± 0.10**	0.43 ± 0.04**	0.93 ± 0.04**
		150 µmol/ L	3.73 ± 0.21**	4.93 ± 0.15*	0.34 ± 0.01**	0.34 ± 0.10**

The values are means ± SD

*or ** indicated significant difference from the control at p # 0.05 and p # 0.01, respectively

Table 2: Continue

Traits			Shoot length (cm)	Root length (cm)	Leaf area index (cm^2)	Leaf osmotic potential (-bar)	Relative water content (%)
2 weeks	Cont.		25.00 ± 2.00	14.00 ± 2.00	3.30 ± 0.06	1.50 ± 0.36	89.33 ± 4.04
	Cu	75 µmol/L	20.33 ± 1.04*	16.00 ± 1.61**	3.17 ± 0.25*	3.07 ± 0.06**	74.67 ± 4.51**
		150 µmol/L	26.33 ± 1.53	18.67 ± 1.15*	2.50 ± 0.26**	3.23 ± 0.25**	69.33 ± 2.31**
	Cd^{+2}	75 µmol/L	25.00 ± 1.73	21.33 ± 1.53	2.20 ± 0.10**	3.90 ± 0.26**	70.00 ± 0.00**
		150 µmol/L	24.67 ± 0.58*	17.00 ± 1.00*	2.60 ± 0.69**	3.73 ± 0.25**	68.00 ± 1.73**
	Cu + Cd^{+2}	75 µmol/L	21.67 ± 7.64*	15.67 ± 2.04*	3.70 ± 0.61	3.23 ± 0.25**	70.00 ± 0.00**
		150 µmol/L	21.00 ± 1.73*	16.67 ± 2.89*	2.83 ± 0.40**	4.67 ± 0.29**	65.00 ± 4.36**
4 weeks	Cont.		34.33 ± 1.15	20.00 ± 1.60	21.83 ± 3.06	1.83 ± 0.21	89.33 ± 4.04**
	Cu	75 µmol/L	30.67 ± 1.15**	16.00 ± 2.65*	17.18 ± 0.59**	3.20 ± 0.30**	66.67 ± 1.53**
		150 µmol/L	32.00 ± 2.58*	23.00 ± 4.58**	22.53 ± 5.95	3.43 ± 0.49**	65.00 ± 5.00**
	Cd^{+2}	75 µmol/L	31.33 ± 0.58**	22.67 ± 2.08**	15.80 ± 0.75**	3.93 ± 0.21**	57.33 ± 2.52**
		150 µmol/L	21.67 ± 3.51**	20.33 ± 4.16**	11.00 ± 0.35**	3.43 ± 0.59**	54.00 ± 1.73**
	Cu + Cd^{+2}	75µmol/L	25.00 ± 1.73**	13.00 ± 0.11	12.15 ± 2.78**	3.40 ± 0.17**	58.67 ± 6.35*8
		150 µmol/L	26.67 ± 3.06**	20.00 ± 5.00**	14.23 ± 2.00**	4.63 ± 0.60**	51.67 ± 2.89**

The values are means ± SD

*or ** indicated significant difference from the control at p # 0.05 and p # 0.01, respectively

Table 3: Effect of different concentration of Cu, Cd^{+2} and Cu + Cd^{+2} on some growth and physiological traits in sorghum (*Sorghum bicolor* L.).

Traits			Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
2 weeks	Cont.		0.57 ± 0.05	0.24 ± 0.03	0.07 ± 0.01	0.02 ± 0.01
	Cu	75 µmol/L	0.37 ± 0.02**	0.12 ± 0.01**	0.02 ± 0.01**	0.02 ± 0.01
		150 µmol/L	2.40 ± 0.26**	0.21 ± 0.03*	0.02 ± 0.01**	0.22 ± 0.02**
	Cd^{+2}	75 µmol/L	0.84 ± 0.08**	0.21 ± 0.01*	0.07 ± 0.01	0.01 ± 0.00
		150 µmol/L	1.28 ± 0.05**	0.25 ± 0.02*	0.07 ± 0.01	0.02 ± 0.00
	Cu + Cd^{+2}	75 µmol/L	0.64 ± 0.04*	0.30 ± 0.06**	0.08 ± 0.01	0.22 ± 0.02**
		150 µmol/L	0.71 ± 0.05*	0.42 ± 0.02**	0.13 ± 0.00**	0.14 ± 0.18**
4 weeks	Cont.		3.25 ± 0.26	1.67 ± 0.19	0.43 ± 0.02	0.31 ± 0.03
	Cu	75 µmol/L	2.30 ± 0.18*	0.52 ± 0.12**	0.33 ± 0.04**	0.13 ± 0.01**
		150 µmol/L	2.07 ± 0.15*	0.42 ± 0.07**	0.23 ± 0.01**	0.12 ± 0.02**
	Cd^{+2}	75 µmol/L	1.39 ± 0.06**	0.36 ± 0.01**	0.18 ± 0.00**	0.07 ± 0.00**
		150 µmol/L	0.81 ± 0.08**	0.34 ± 0.04**	0.15 ± 0.03**	0.03 ± 0.00**
	Cu + Cd^{+2}	75µmol/L	0.77 ± 0.05**	0.27 ± 0.05**	0.13 ± 0.02**	0.04 ± 0.00**
		150 µmol/L	1.23 ± 0.11**	0.45 ± 0.01**	0.11 ± 0.02**	0.04 ± 0.00**

The values are means ± SD

*or ** indicated significant difference from the control at p # 0.05 and p # 0.01, respectively

Table 3: Continue

Traits			Shoot length (cm)	Root length (cm)	Leaf area index (cm^2)	Leaf osmotic potential (-bar)	Relative water content (%)
2 weeks	Cont.		16.33 ± 0.58	6.67 ± 1.09	4.40 ± 0.10	1.77 ± 0.25	86.67 ± 1.53
	Cu	75 µmol/L	17.33 ± 1.15	8.00 ± 1.00*	3.50 ± 0.36*	4.03 ± 0.45**	75.67 ± 2.31**
		150 µmol/L	16.33 ± 1.73	8.00 ± 1.30*	2.40 ± 0.10**	4.67 ± 0.58**	68.33 ± 4.04**
	Cd^{+2}	75 µmol/L	19.67 ± 2.51**	9.67 ± 3.79*	2.23 ± 0.15**	3.00 ± 0.00**	69.00 ± 2.65**
		150 µmol/L	25.67 ± 1.15**	8.33 ± 1.53*	1.17 ± 0.25**	4.23 ± 0.25*8	66.33 ± 2.31**
	Cu + Cd^{+2}	75 µmol/L	18.67 ± 3.06**	8.00 ± 0.99*	2.10 ± 0.20**	5.90 ± 0.17**	68.67 ± 2.31**
		150 µmol/L	19.33 ± 2.08**	8.00 ± 0.00*	1.80 ± 0.78**	5.67 ± 0.29**	65.67 ± 5.13**
4 weeks	Cont.		32.33±2.08	11.00 ± 1.00	9.36 ± 2.61	2.00 ± 0.10	90.67 ± 1.15
	Cu	75 µmol/L	24.00 ± 2.65**	9.33 ± 1.53	8.42 ± 2.10	3.67 ± 0.29*	70.00 ± 2.00**
		150 µmol/L	26.67 ± 1.53**	7.67 ± 1.15**	7.73 ± 1.28	5.90 ± 0.36**	66.00 ± 5.29**
	Cd^{+2}	75 µmol/L	20.00 ± 1.73**	9.00 ± 2.65	4.12 ± 0.51**	3.03 ± 0.15*	62.33 ± 2.52**
		150 µmol/L	16.00 ± 1.00**	7.00 ± 1.00**	3.01 ± 1.28**	5.23 ± 0.25**	60.67 ± 1.15**
	Cu + Cd^{+2}	75µmol/L	12.33 ± 1.53**	9.33 ± 2.08	2.56 ± 0.28**	5.60 ± 0.26**	60.00 ± 0.00**
		150 µmol/L	17.67 ± 4.04**	6.50 ± 0.50**	2.36 ± 0.76**	5.10 ± 0.10**	61.67 ± 1.53**

The values are means ± SD

*or ** indicated significant difference from the control at p # 0.05 and p # 0.01, respectively

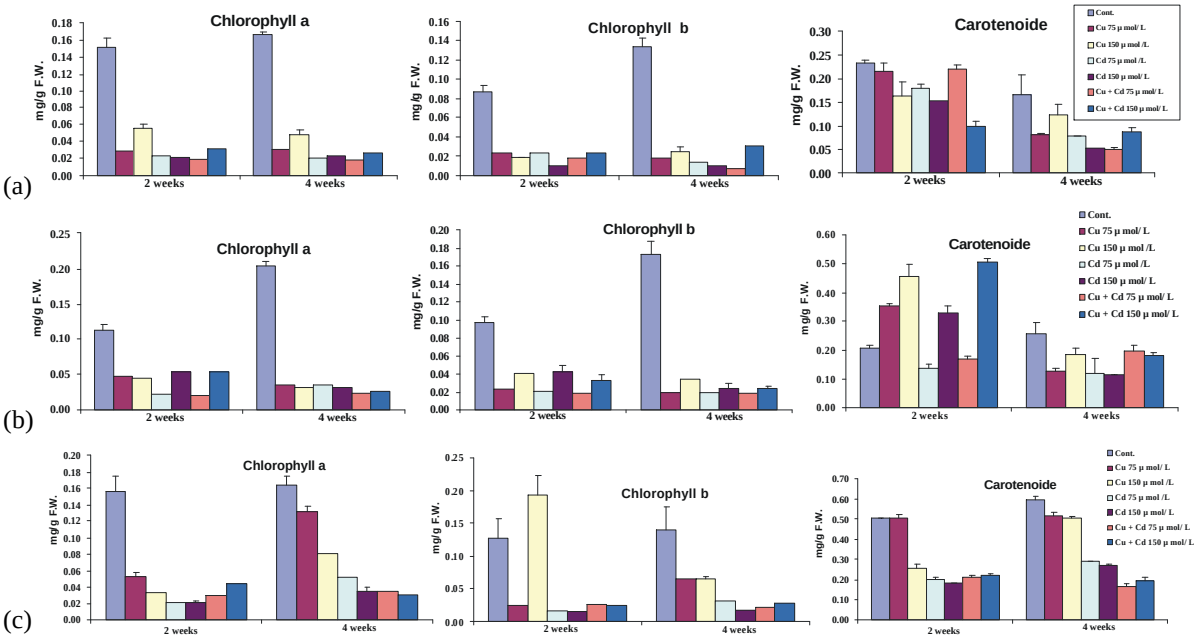

Fig. 1: Effect of different concentration of Cu, Cd and Cu+Cd on chlorophyll a, chlorophyll b and carotenoids after 2 and 4 weeks from sowing of a: wheat(*Triticum aestivium* L.), b: maize (*Zea mays* L.) and c: sorghum (*Sorghum bicolor* L.).

Cd^{+2} differed significantly and decreased by influence of heavy metals. Under normal condition, LOP varied from (1.29–bar) in wheat after two weeks to (2.00–bar) in sorghum after 4 weeks. Comparing to effect of different concentration of heavy metals on LOP for different plants, that Cu^{+} and Cd^{+2} application was more effective at (75 µmol/L Cu+75 µmol/L Cd^{+2}) than the others (Table 1-3). Also, the results indicated that the wheat and maize was more affected by Cu^{+} and Cd^{+2} stress than sorghum. Chinnusamy and Zhu [31] have suggested that plant survival depends on maintaining a positive turgor, which is indispensable for expansion growth of cells and stomata opening. Most of Cd in these plants accumulated in the cytoplasm of root, whereas Cu is readily translocated to the shoots and accumulates in cell wall as well as cytoplasm.

Relative Water Content (RWC): The results in Tables 1,2 and 3 showed that relative water content was also affected by different concentration of Cu^{+} and Cd^{+2} and the plants revealed different response to Cu^{+} and Cd^{+2} stress. Higher concentration of combination Cu^{+} and Cd^{+2} decreased significantly RWC compared to others treatments. Variation between the different plants was observed and reduction of RWC was greatest in maize (54%) after four weeks under 150 µmol/L Cd^{+2}. This variation in the average of RWC between different types of plants was supported by Pasternak [32] which suggested that the mechanism for protection against LW loss as follows: increase the cuticle thickness or high root/shoot ratio and osmotic adaption by absorption of elements which were compartmented in the vacuoles. Costa *et al.* [33] showed that cadmium disrupts the plant water relations and its negative effect can be observed in the uptake, transport and transpiration of water in plants. Vassilev *et al.* [34] showed that Cd stress decreased the RWC in barely leaves by 2-3%.

Chlorophyll a, Chlorophyll b and Carotenoids: Data in Fig. 1 showed that Cu^{+} and Cd^{+2} stress caused significant reduction ($P<0.01$) in the content of chlorophyll a, chlorophyll b and carotenoids in wheat, maize and sorghum after 2 and 4 weeks. In case of control plants, the content of chlorophyll a, chlorophyll b and carotenoids were higher after 4 weeks than 2 weeks (Fig. 1). However, 150 µmol/L Cd^{+2} caused a conspicuous decrease in chlorophyll a, chlorophyll b and carotenoids in wheat and maize plants but sorghum exhibited lesser damage (0.04, 0.02 and 0.21) than wheat (0.02, 0.01 and 0.05) and maize (0.03, 0.02 and 0.11) compared with control. The reduced chlorophyll content in Cd^{+2} treated plants are due to both to inhibition of its biosynthesis [35] and the

Table 4: Analysis of variance for the effect of different treatments of Cu, $Cd.^{+2}$ and Cu + $Cd.^{+2}$ on growth and physiological traits in wheat, maize and Sorghum after two and four weeks

		MS					
SOV	d.f	Shoot length	Root length	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight
Crop species	2	658.538**	1038.6**	50.80**	109.25**	0.2001**	1.79**
Treatment	6	8.924**	14.678**	5.115**	2.215**	5.89**	0.185**
Duration	1	30.64**	98.67**	14.48**	0.464*	43.29**	0.056**
Crop species x Treatment	12	2.662**	23.509**	2.414**	1.913**	0.0341**	0.065**
Crop species x Duration	2	3.36**	10.604^{ns}	0.469**	9.617**	0.0263*	0.232**
Treatment x Duration	6	8.582**	7.569	1.641**	1.77**	1.767**	12.41**
Error	96	10.780	7.569	0.1329	0.08322	0.083	0.0064
Total	125	--	--	--	--	--	--
CV (%)		15.176	21.418	19.139	17.00	46.92	29.09

SOV = source of variance; MS= Mean square; df= degree of freedom; * and ** significant at 5 % and 1 %, respectively

Table 4: Continue

		MS					
SOV	d.f	Leaf area index	Osmotic pressure	Leaf water content	Chlorophyll a	Chlorophyll b	Carotenoids
Crop species	2	527.98**	0.0126^{ns}	53.37**	0.0026**	0.0080**	0.508**
Treatment	6	50.40**	36.42**	101.3**	0.044**	0.0254**	0.1203**
Duration	1	118.33**	1.252**	216.1**	0.0032**	4.804^{ns}	0.0388**
Crop species x Treatment	12	4.796ns	0.318**	14.37*	0.001**	0.00297**	0.0319**
Crop species x Duration	2	42.89**	0.0026^{ns}	18.28^{ns}	0.0029**	3.751^{ns}	0.0469**
Treatment x Duration	6	27.32**	1.5036**	25.76**	3.304**	0.0022**	0.0156**
Error	96	2.706	0.0902	25.756	7.519	5.774	0.0013
Total	125	--	--	--	--	--	--
CV (%)		29.51	7.064	3.633	16.509	60.432	18.54

Table 5: Correlation between different growth and physiological traits under different concentration of Cu, Cd and Cu + Cd for wheat, maize and sorghum

Variables	Shoot length	Root length	Shoot fresh weight	Root fresh weight	Shoot dry weight
Carotenoids	0.070^{ns}	-0.399**	-0.944^{ns}	-0.307**	0.003^{ns}
Chlorophyll a	0.107^{ns}	-0.134^{ns}	0.122^{ns}	-0.019^{ns}	0.155^{ns}
Chlorophyll b	0.225*	-0.068^{ns}	0.133^{ns}	0.082^{ns}	0.319**
Leaf water content	0.268**	0.062^{ns}	0.154^{ns}	0.213*	0.248**
Leaf osmotic potential	-0.144^{ns}	-0.008^{ns}	0.056^{ns}	-0.052^{ns}	-0.108^{ns}
Leaf area index	0.712**	0.451**	0.598**	0.382**	0.523**
Root dry weight	0.464**	0.598**	0.754**	0.848**	0.524**
Shoot dry weight	0.452**	0.403**	0.695**	0.515**	--
Root fresh weight	0.254**	0.648**	0.785**	--	--
Shoot fresh weight	0.627**	0.544**	--	--	--
Root length	0.544**	--	--	--	--

- (* and **) significant different at 5% and 1%, respectively
- (ns) non significant different at 5 and 1%

Table 5: Continue

	Root dry weight	Leaf area index	Leaf osmotic potential	Leaf water content	Chl. a	Chl. b
Carotenoids	-0.196*	0.066*	-0.330**	0.421**	0.557**	0.483**
Chlorophyll a	0.188*	0.137*	-0.553**	0.597**	0.714**	--
Chlorophyll b	0.257**	0.222*	0.715**	0.772**	--	--
Leaf water content	0.310**	0.155^{ns}	0.738**	--	--	--
Leaf osmotic potential	-0.163^{ns}	-0.078^{ns}	--	--	--	--
Leaf area index	0.413**	--	--	--	--	--

- (* and **) significant different at 5% and 1%, respectively
- (ns) non significant different at 5 and 1%
- Chl. = Chlorophyll

activation of its enzymatic degradation [36]. Although, Cu^{+} was an essential micronutrient for growth at low level, it could be stronger inhibitor of photosynthesis when Cu in excess [37]. The results was in line with our data, which indicated that under 75 µmol/L of Cu the value of chlorophyll a, chlorophyll b and carotenoids were more higher in both plants than 150 µmol/L Cu either after three and 2 weeks. The loss in chlorophyll content could be due to peroxidation of chloroplast membranes or replacing of magnesium in chlorophyll molecule by copper [38].

Analysis of Variance and Correlation: Analysis of variance of growth and physiological characters are shown in Table 4. According to Table 4, the analysis of variance for all the characters showed high significant differences, in additional an interaction among different type of plants, treatment and duration of measuring sample was significant ($P<0.01$), this indicates the presence of variability for all characters under study. Also only, insignificant differences in root length, leaf area, relative water content and chlorophyll b were showed in the interaction between types of plants and duration. The results of present study are in agreement with those reported by Lin *et al.* [39]. The correlation coefficient among quantitative traits were computed and presented in Table 5. The results showed that significant positive correlation between shoot length (SL) and LWC, LA, chlorophyll a, RDW, SDW,RFW, SFW, RL, Root length (RL) and LA, RDW, SDW, RFW, SFW; Shoot Fresh Weight (SFW) and LA, RSW, SDW, SFW; Shoot dry weight (SDW) and chlorophyll b, LWC, LA, RDW; Leaf Water Content (LWC) and chlorophyll a, b and carotenoids. Also, this positive and significant association of leaf area with chlorophyll a, b and carotenoids indicated that increased leaf area index would simultaneously increase photosynthesis pigments and hence directly improve grain yield. These finding suggested that the characters showing positive and significant correlation could be effectively be utilized in crop improvement programme under undesirable environment such as heavy metals polluted soils. However, significant and negative correlation was also found between root fresh weight, root dry weight and osmotic pressure.

Effect of Heavy Metals (Cu^{+} and Cd^{+2}) on the Protein Patterns Using SDS-PAGE: Detection of proteins due to heavy metal stress in wheat, maize and sorghum (Fig. 2 and Table 6) which levels are altered by copper and cadmium stress was done by comprising patterns from control with patterns from copper and cadmium treated plants. SDS-PAGE analysis showed presence of protein bands ranged from 1 to 245 kDa (Fig. 2). One of these bands at (55 kDa) was common among wheat, maize and sorghum and one band at (23 kDa) between maize and sorghum. Incidentally at different concentration of copper and cadmium protein bands with molecular weight (MWs) 15 kDa was disappear after four weeks from treatment with different concentration of Cu,Cd^{+2} and Cu+Cd^{+2} in wheat. Also, two protein bands with MWs (35 and 54 kDa) were inhibited after four weeks from treatment with 75 µmol/L Cd^{+2}; 150 µmol/L Cd^{+2} and 75 µmol/L Cu^{+} + 75 µmol/L Cd^{+2}) in maize. One possible explanation for completely disappearance of these protein patterns only after four weeks from treated with heavy metals is that genes responsible for certain proteins had been completely suppress after four weeks as a results of excess stress, especially under Cd^{+2} stress, on tissues. Therefore, the developed tissue after four weeks had lost their ability to synthesis this protein under this stress. On the other hand, the newly synthesis protein bands was observed at MWs (60 kDa) only under 150 µmol/L Cd^{+2} in sorghum and another band at 16 kDa in wheat under 150 µmol/L Cd^{+2} and 75 µmol/L Cu^{+} + 75 µmol/L Cd^{+2}.These bands might indicated the Cd^{+2} stress induced a stress related gene to produce this protein and consequently, this bands can be considered as an adaptive bands to Cd^{+2} stress inducible protein (CSIP). Our results are in agreement with the findings of Lee *et al.* [40] which indicted that, plants when exposed to high concentrations of heavy metals, including Cd^{+2}, produce low molecular mass peptide at 51 KDa. Mitter [12] designated this protein as cadmium stress associated protein (CSAP). Appeared protein banding at 63 kDa only after four weeks from treatment with Cd^{+2} and not appeared under other treatments may be as a results from the additional supply of copper with the cadmium dosage causes certain degree of recovery plant growth, this hypothesis is in agreement with those reported Ali *et al.* [29].

It is already known that higher plants not only respond to heavy metals treatments by the synthesis of phytoclelatins or related peptide but also the synthesis of stress related proteins [41] and this protein might have helped for encountering their inhibitory effects. In addition, excess heavy metals are said to generate oxidative stress due to an increase in the levels of reaction oxygen species (ROS) which affect mainly amino acids, protein and nucleic acids [42]. Expression of CSIP in sorghum after four weeks from treatments with high concentration of cadmium (150 µmol/L) and not expressed

Wheat (*Triticum aestivum* L.)

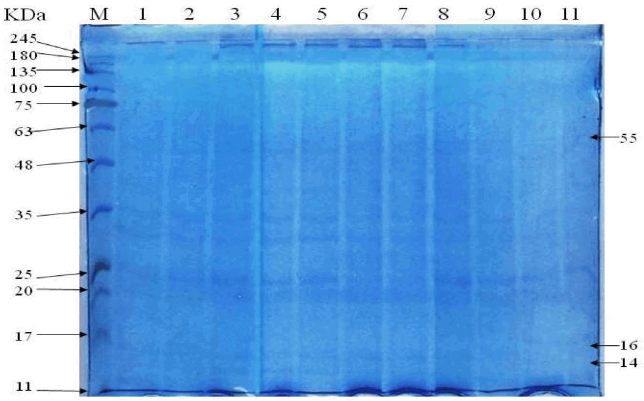

Fig. 2a

Maize (*Zea mays* L.)

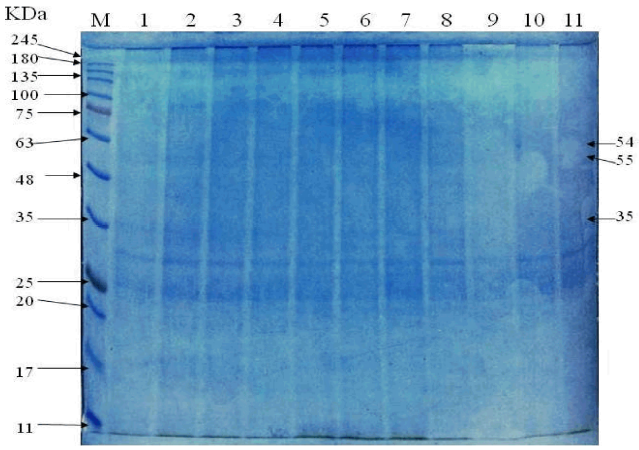

Fig. 2b

Sorghum (*Sorghum bicolor* L.)

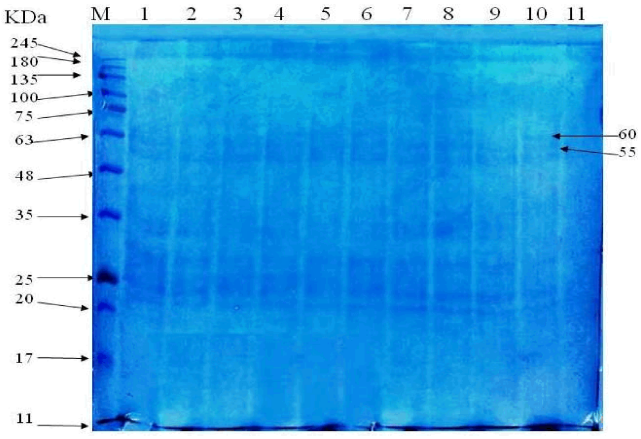

Fig. 2c)

Fig. 2: Protein Profile on SDS-PAGE of a) wheat, b) maize and c) sorghum under heavy metal stress. Lane 1 to 11:1=control; [2=75 µmol.L^{-1} Cu; 3=150 µmol.L^{-1} Cu; 4=75 µmol.L^{-1} Cd; 5=150 µmol.L^{-1} Cd; 6=75 µmol.L^{-1} Cu + 75 µmol.L^{-1} Cd] after two weeks and [7=75 µmol.L^{-1} Cu; 8=150 µmol.L^{-1} Cu; 9=75 µmol.L^{-1} Cd; 10=150 µmol.L^{-1} Cd; 11=75 µmol.L^{-1} Cu + 75 µmol.L^{-1} Cd] after four weeks. M= marker

Table 6a: Protein Profile of three different crops of a) wheat, b)maize and c) sorghum under different concentration of Cu; Cd and Cu + Cd after two and three weeks using different pattern of soluble protein

MW(kDa)	1	2	3	4	5	6	7	8	9	10	11
133	+	+	+	+	+	+	+	+	+	+	+
128	-	+	+	+	+	+	+	+	-	-	-
55	+	+	+	+	+	+	+	+	+	+	+
54	+	+	+	+	+	+	+	+	+	+	+
32	+	+	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+	+	+	+
22	+	+	+	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+	+	+	+
16	-	-	-	-	-	-	-	-	-	+	+
14	+	+	+	+	+	+	-	-	-	-	-

Table 6 b: Zea Gel's

MW(kDa)	1	2	3	4	5	6	7	8	9	10	11
131	+	+	+	+	+	+	+	+	+	+	+
97	+	+	+	+	+	+	+	+	+	+	+
55	+	+	+	+	+	+	+	+	+	+	+
54	+	+	+	+	+	+	+	+	-	-	-
35	+	+	+	+	+	+	+	+	-	-	-
26	+	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+	+

Table 6 c: Sorghum Gel's

MW(kDa)	1	2	3	4	5	6	7	8	9	10	11
68	-	-	-	-	-	-	-	-	-	+	-
63	+	+	+	+	+	+	+	+	+	+	+
55	+	+	+	+	+	+	+	+	+	+	+
58	+	+	+	+	+	+	+	+	+	+	+
37	+	+	+	+	+	+	+	+	+	+	+
33	+	+	+	+	+	+	+	+	+	+	+
28	+	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+	+	+	+	+

Lane 1 to 11:1=control; [2=75 µmol.LG^{1} Cu; 3=150 µmol.LG^{1} Cu; 4=75 µmol.LG^{1} Cd; 5=150 µmol.LG^{1} Cd; 6=75 µmol.LG^{1} Cu + 75 µmol.LG^{1} Cd] after two weeks and [7=75 µmol.LG^{1} Cu; 8=150 µmol.LG^{1} Cu; 9=75 µmol.LG^{1} Cd; 10=150 µmol.LG^{1} Cd; 11=75 µmol.LG^{1} Cu + 75 µmol.LG^{1} Cd] after four weeks. (+) = presence bands and (–) = absent bands

in wheat or maize under same treatment, this might be the different in signal transduction pathway among, wheat, maize and sorghum, that lead to gene induction under this stress only in sorghum. This results are confirm with those obtained by Jeff *et al.* [30], which observed that sorghum was greater than other poaceae plants in tolerant to heavy metals stress.

Heavy Metals Concentration: Concentrations of copper and cadmium were detected in leaves of, sorghum, wheat and maize after 2 and 4 weeks of exposure are presented in Table 6. Copper and cadmium concentrations in untreated control plants remained stable during the experiment. In this study, we observed that the levels of heavy metals in the tested plants increased with the increased concentration of Cu or Cd. Our study regarding the interaction between heavy metals level and Cu and Cd content with the previous researches of Deng *et al.* [43]. From Table 7, it can be noticed that the content of Cu in different plants used in this study significantly increased with Cu concentration increase and reached maximum when Cu concentration was 150 µmol/L after 4 weeks of treatments. Cu content in different plant leaf was in order of sorghum **9** wheat **9** maize respectively. Meanwhile, the content of Cu was a positive correlation with Cu concentration (Table 7). The highest rate for copper residues about 2.99 and 2.31 and 1.81µg/g for sorghum, wheat and maize respectively, of treatment with 150 µmol/L after 4 weeks.

It was reported that the extent of cadmium accumulation in plants changed depending on the plant species [44]. Briefly, important differences in cadmium

Table 7: Copper and cadmium concentrations in wheat, maize and sorghum (µg/g) fresh weight after 2 and 4 weeks of treatments

		Concentration of heavy metals µg.gG^{1} fresh weight					
		Wheat (*Triticum aestivum* L.)		Maize (*Zea mays* L.)		Sorghum (*Sorghum bicolor* L.)	
Heavy metals used	Initial concentrations of heavy metals µmol/L	2 Weeks	4 Weeks	2 Weeks	4 Weeks	2 Weeks	4 Weeks
Cu	Control	0.031 ± 0.001	0.044 ± 0.001	0.058± 0.002	0.061 ± 0.001	0.056 ± 0.002	0.069 ± 0.001
	75	0.95 ± 0.12**	1.32 ± 0.13**	0.72 ± 0.11**	0.84 ± 0.13**	1.12 ± 0.16**	1.11 ± 0.14**
	150	1. 27 ± 0.20**	2.28 ± 0.23**	0.92 ± 0.12**	1.81 ± 0.23**	0.69 ± 0.001**	2.99 ± 0.32**
Cd	Control	0.027 ± 0.01	0.037 ± 0.001	0.014 ± 0.001	0.021 ± 0.001	0.048 ± 0.001**	0.037 ± 0.001
	75	0. 89 ± 0.01**	1.53 ± 0.21**	0.74 ± 0.001**	1.03 ± 0.21**	1.16 ± 0.24**	2.83 ± 0.34**
	150	1.18 ± 0.01**	2.33 ± 0.22**	1. 16 ± 0.33**	3. 17 ± 0.12**	2. 47 ± 0.13**	3.34 ± 0.22**
Cu + Cd	37.5 Cu	0.15 ± 0.01**	0. 59 ± 0.02*	1.35 ± 0.22**	2. 86 ± 0.33**	0. 54 ± 0.03**	0. 94 ± 0.03**
	37.5 Cd	0. 35 ± 0.02**	1.12 ± 0.21**	0. 28 ± 0.01**	0. 23 ± 0.01**	0. 39 ± 0.002**	2 30 ± 0.2**
Cu + Cd	75 Cu	0.96 ± 0.24**	1.64 ± 0.03**	0. 67 ± 0.04**	1. 54 ± 0.003**	0.90± 0.002**	1. 78 ± 0.04**
	75 Cd	0.77 ± 0.003**	1.20 ± 0.04**	0. 55 ± 0.06**	0.76 ± 0.002**	0.92 ± 0.007**	1.08 ± 0.008**

Data are means ± SD of three independent experiments

*or ** indicated significant difference from the control at p # 0.05 and p # 0.01, respectively

uptake and transfer among plant genotypes have been reported by the researchers in these studies. Although Cd^{+2} concentrations in leaves increased for the applied Cd^{+2} of 75 or 150 µmol/L, the observed Cd^{+2} levels for these groups were found higher 8-12 times than for the control groups depending on growth period. Furthermore, Cd concentrations in sorghum was found to be higher significantly up to 10-fold than those in their control of. While cadmium concentrations of wheat slightly increased as dependent on the growing period for control group. Also, It is obvious from the results in Table 7, that when plants was treated with a mixture of copper and cadmium with the concentrations of 37.5 or 75 µmol LG^{1} led to increasing in concentration of copper in all the treatment plants. While, this led to lack of concentration of cadmium after two and four weeks of treatment. Heavy metal contamination has become a worldwide problem by disturbing the normal functions of rivers and lakes [45]. Phytoextraction has been proposed as an inexpensive, sustainable, in plant-based technology that makes use of natural hyper accumulators as well as high-biomass-producing crops to help. Rehabilitate soils contaminated with heavy metals without destructive effects on soil Properties [46, 47].

The uptake of Cu and Cd by various plants is discussed in this section. Metal accumulation by the plants was affected by many factors. In general, variations in plant species, the growth stage of the plants and element characteristics control absorption, accumulation and translocation of metals. Furthermore, physiological adaptations also control toxic metal accumulations by sequestering metals in the leaves [48]. Copper is an essential element and enzyme cofactor for oxidase, however, plants can accumulate toxic levels of copper [46]. The accumulation was highest in the sorghum, when it was treated with 75 or 150 of cop µmol/L after 4 weeks. In this study, the least effective plant species in concentrating copper was maize. Here also the maximum absorption was by sorghum .and the least absorption was maize. Cadmium is a toxic metal and a probable carcinogen associated with zinc mining and Industrial operations. Successful cadmium phytoextraction must increase cadmium mobilization in to soil solution in order to maximize the transfer of cadmium to plant shoots [49]. In the previous studies it was found that the extent of cadmium accumulation in plants differs between parts of plant and species, Wu *et al.* [50] reported that cadmium concentration in different organs of cotton increased with increasing Cd levels in the nutrient solution in the following order: root > petiole > xylem > fruiting branch, leaf > phloem in vegetative organs and seed coat, seed nut > boll shell > fiber in reproductive organs. A dramatic increase in Cd^{+2} concentrations of roots with increasing Cd concentrations in the nutrient solution was observed by Wu *et al.* [50] and Zhao *et al.* [51]. Also the tolerant mechanisms of Cd tolerant plants have been reported previously by Zhou and Song [52]. They included two strategies: exclusion and accumulation, with the accumulation strategy, plants accumulated high amounts of Cd in the tissue, with only a small amount of Cd being stored in the roots and the rest being all translocated to the shoots.

CONCLUSION

Considering data obtained on growth and physiological parameters, it was clear that heavy metal have been shown to cause changes in plants and harmful

effect of the different treatments on the wheat, maize and sorghum was consistently in order Cu < Cu+Cd < Cd, also the plants were in descending order based on their tolerance to heavy metals as follow: sorghum, wheat and maize. On the other hand, analysis of soluble protein by SDS-PAGE analysis are useful molecular tools to distinguished between sorghum, wheat and maize under heavy metals stress conditions, where 60 and 16 kDa soluble protein designated as a cadmium stress inducible proteins (CSIP). In conclusion, it can be suggested that considering more growth, physiological and protein profiles related to salt tolerance can be useful in our better understanding on physiological and genetically aspects of salinity tolerance mechanisms in different genus.

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