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Cadmium uptake by some *Chenopodium* spp. as model of phytoremediation technology

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Abstract

Cadmium (Cd) is extremely toxic heavy metal which adversely affects the growth of plants. Phytoextraction one from among of phytoremediation technology methods has proven to remediate media contaminated with heavy metals. The objective of this research is find out whether Chenopodium species (*Ch. ambrosoidae*, *Ch. album* and *Ch. quinoa*) can be used as a Cd hyperaccumulator from through study effects of Cd on growth of species seedlings. Therefore, three Cd concentrations (10, 20 and 30 ppm) with addition to control have conducted to archive aim of study. Different parameters have tested in present study include growth, physiological and biochemical parameters of growth seedlings stage. Results indicate that the inhibitory effect had observed on all growth, physiological and biochemical traits of early growth seedlings stage. *Chenopodium* species have many tolerance mechanisms against Cd stress such as membrane stability, stability pigments and activity of antioxidant enzymes under Cd treatments. Data indicated that *Ch. quinoa* get the better of another two species in Cd uptake and total tolerance index of Cd, so, we demonstrated that *Ch. quinoa* suitable for phytoextraction.

Keywords: Cadmium, *Chenopodium* spp., phytoremediation, antioxidants enzyme and chlorophyll content.

Introduction

Heavy metal contamination is a cause of major environmental hazards worldwide, leading to losses in agricultural yields and harmfully affecting human health when contaminants enter the food chain. Heavy metal is a pernicious problem affecting the productivity and quality of economically valuable crops (**Wagner, 1993**). Excess concentrations of some heavy metals in soils such as Cd, Cr, Cu, Ni, and Zn have caused the disruption of natural aquatic and terrestrial ecosystems (**Meagher, 2000**). Cd inhibits the photoactivation of photosystem 2 (PS2) by inhibiting electron transfer. Thus, Cd could lead to the generation of reactive oxygen species (ROS) such as the superoxide anion (O_2), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH), but generates oxidative stress by interfering with the antioxidant defiance system (**Benavides *et al*., 2005**) indirectly by production of a disturbance in the chloroplasts. In addition, other reports suggested that Cd may stimulate the production of ROS in the mitochondrial electron transfer chain (**Heyno *et al*., 2008**). Cd has been shown to cause delay in induce membrane damage, impair food reserve mobilization by increased cotyledon/embryo ratios of total soluble sugars, glucose, fructose and amino acids (**Rahoui *et al.*, 2010**), mineral leakage leading to nutrient loss (**Sfaxi-Bousbih *et al*., 2010**).

Oxidative stress defense mechanisms also play an important role against metal toxicity in plants (**Briat and Lebrun, 1999**). A variety of proteins function as scavengers of ROS such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidases (POD), and glutathione reductase (GR), and non-enzymatic scavengers, including, but not limited to, glutathione (GSH), ascorbic acid (ASA), carotenoids, and tocopherols. **Yilmaz and Parlak (2011)** reported that the observed high tolerance of *Groenlandia densa* to Cd stress was partially due to high activity of CAT. A decrease in POD activity caused by Cd was reported in mustard (*Brassica juncea*), **Markovska *et al*., (2009**). On the other hand, decline in the enzymatic activity of CAT have been associated with Cd toxicity in *Pharsalus vulgaris* (**Chaoui *et al*., 1997**), *Phaseolus aureus* (**Shaw, 1995**), *Helianthus annuus* (**Gallego *et al*., 1996**), and *Pisum sativum* (**Sandalio *et al*., 2001**).

The genus *Chenopodium* consists of 120 species, nine of which are found in Egypt (**Boulos, 2005**). Many species of *Chenopodium* have been reported to possess numerous medicinal properties in ancient texts (**Bakshi *et al*., 1999**). Chenopodium has a high level of resistance to adverse conditions like drought, frost, soil salinity and heavy metals (**Bhargava *et al*., 2003b and Garcia *et al*., 2003**). The aim of this research is study capacity of Chenopodium species used in uptake of Cd and study the different species response to Cd treatments on nutrients uptake, growth parameters and physiological and biochemical traits in three species of Chenopodium used as plants phytoremediation for Cd accumulation.

Materials and Methods

This study was carried out at the Plant Physiology Laboratory, Botany Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, one season during 2016. *Chenopodium* species seeds (*Ch. ambrosoidae*, *Ch. album and Ch. quinoa*) were brought from Agriculture Research Center, Field Crop Research Institute, Giza, Egypt.

Design experiment.

The hydroponically experiment carried out in growth chamber to evaluate effect of Cd on Chenopodium seedlings. We collected Chenopodium species seedlings from mix light clay soil (Soil: Sand: Patmos with value 1:1:1) in plastic pots diameter of 17 cm with weight 2kg, after 18 days were have collected and rinsed with tap water to remove any soil particles. The seedlings were placed in plastic pots with Hoagland's half strong Hoagland's solution used as macronutrient sources KH_2PO_4 (0.74M) – KNO_3(1M) – Ca $(NO_3)_2.4H_2O$ (0.42M) and $MgSO_4.7H_2O$(0.41M). For micronutrient sources H_3BO_3 (8.87mM) – $MnCl_2.5H_2O$ (1.77mM) – $ZnSO_4.7H_2O$ (0.31mM) – $CuSO_4.5H_2O$ (0.32mM) – (NH4) $6MO7O_{24}.4H2O$ (0.026mM) and $FeSO_4.7H_2O$ (2.59mM) (**Hoagland and Arnon, 1953**) as control (4 liter for pot) non treated for one week to let them adapt to the new environment, and develop roots, and grow three weeks in hydroponics before the Cd treatment was started. Then uniform and healthy seedlings were chosen randomly in triplicate to treat them with Cd (as $CdCl_2$. H_2O) doses (10, 20 and 30 ppm) with addition to control. After 7 days duration from exposed of Cd the plants were harvested and rinsed with distilled water to remove any trace of Cd on root seedlings, then different growth parameters (fresh and dry weight and leaf area) were measured. Then seedlings separated to root and shoot (leaves and stem), to determinates Cd metal accumulation, and physiological and biochemical parameters determination.

Growth parameters measurement:-

The growth parameters of plants seedling were recorded after 7d from Cd treatments as following: **Fresh weight (F.Wt)**: The whole seedling was surface dried with the blotting paper and their fresh weight was recorded. **Dry weight (D.Wt)**: The same seedlings were used and dried in oven for 24 h at 80°C and weighed again, this represented the dry matter. **Leaf area (L.A):** Leaf area is measured by counting grids covered by leaf as cm^2 using grid method according to (**Caldas *et al.*, 1992)**.

Physiological and biochemical traits.

Leaf relative water content (RWC) was estimated according to **Weatherley (1950)** and calculated as follows: LRWC (%) = (Fresh weight - Dry weight) / (Turgid weight - Dry weight) x 100. **Electrolyte leakage (EL)** was determined as electrical conductivity (EC %) according to **Hassanein *et al.* (2012)**. According to this formula: **EC (%)** = (C1/C2) × 100. Where C1 and C2 are the electrolyte conductivities measured before and after boiling, respectively.

Membrane stability index (MSI): determination of MSI was done by employing electrical conductivity (E.C.) of leaf. Measured by conductivity meter with equation following:

MSI = [1 – EC1/EC2] x100. **(Sairam and Tyagi, 2004)**.

Antioxidants enzymes assay:

Enzyme extraction: Fresh leaves samples (0.2 g) were ground in liquid N_2 and homogenized in an ice-bath in 4 ml of homogenizing solution containing 50 mM potassium phosphate buffer and 1% (w/v) polyvinylpyrrolidone (pH 7.8). The homogenate was centrifuged at 14000 rpm at 4°C for 10 min and the resulting supernatant was used for enzymes assays. CAT action was precise according to **(Aebi, 1984)**. POD activity was measured by the method of **(Chance and Maehly, 1955)**. The determination of PPO activity was done according to **(Duckworth and Coleman, 1970).**

Chlorophyll content assay*:* Chlorophyll levels were estimated in first true leaf of seedling by the spectrophotometric method described by **Lichtentaler and Wellburn (1985)**. Chlorophyll was expressed as methanol 90% as follows:

Chlorophyll a (Chl a) = 15.65 ×A666 - 7.340 × A653

Chlorophyll b (Chl b) = 27.05 × A653 - 11.21× A666

Where A666 and A653 are absorbance at A653, A470 and A666 nm.

Determination of Cd and macro-micronutrients in different tissues seedling.

After 7 days of under Cd treatment, the seedlings of Chenopodium were taken and washed in distilled water, and then representative portions were wet digested by using Modified method of (**Kaiser *et al*., 1972**) digestion method.

Potassium (K) determined by flame photometry (Jen way PFP7) as described by **Page *et al.*, (1982).** Calcium (Ca) assayed by atomic absorption spectrometry as described by **Chapman and Pratt (1982).** The concentrations of Fe and Zn were measured with an atomic absorption spectrometry as described by **Chapman and Pratt (1982).**

Statistical methods

All data were statistically analyzed using the CoStat package (Version# 6.3). Differences between treatments were determined by the least significant difference (LSD - P≤0.01) from the analysis of variance (ANOVA).

Results and Discussion:

Effect of Cd on growth parameters of Chenopodium species.

Effect of Cd on root and shoot biomass of Chenopodium species seedlings*:* The total biomass (fresh weight F.Wt and dry weight D.Wt of root and shoot) of Chenopodium species seedlings were measured in response to Cd stress after 7 days to

treatment are presented in Table-1. Gradual, the biomass of seedlings were decreased with increase Cd doses as compared with control. F.Wt and D.Wt of shoot and F.Wt of root were significantly ($P \leq 0.01$) in all species. But, variation of root D.Wt of *Chenopodium* species was non-significantly. Response of *Chenopodium* species against Cd was different. For example, *Ch .quinoa* is the loosest in decreasing of both F.Wt and D.Wt of root (0.063 and 0.006 g/plant) and shoot (0.47 and 0.069 g/plant) at 30 ppm Cd concentration as compared with another two species used at the same concentration. From the results of growth parameters of this experiment, it was concluded that *Ch. quinoa* had the ability to grow in medium contaminated with Cd, and termed as more tolerant plants when compared with other species. While, *Ch.ambrosoidae* is the highest decreasing of biomass at same concentration of Cd. Root symptom under Cd toxicity included browning (fig. not showed). Those results of reduction of shoot and root dry biomass caused by Cd application has been demonstrated in many plants, including eggplant (**Arao *et al.*, 2008**), maize (**Ekmekçi *et al.*, 2008**), safflower (**Shi *et al* 2010**), soybean (**Shamsi *et al.*, 2010**), tomato (**Haouari *et al.*, 2012**) and transgenic and wild type tobacco (**Dağhan *et al.*, 2013**). The reduction of shoot and root biomass as a result of the increasing Cd supply might be attributed to prominent decreases in shoot and root biomass and changes in the rate of net photosynthesis that reduces the supply of carbohydrates or proteins (**Yakup *et al.*, 2015**).

Effect of Cd stress on leaf area (L.A) of Chenopodium specie: L.A was examined as one of the important parameters in monitoring plant growth and development under the experimental conditions studied. In general, increasing Cd concentrations caused a significantly decreased ($p \leq 0.01$) linearly in L.A with all Chenopodium species seedlings (Table-1). The data indicated that *Ch. ambrosoidae* was the loosest in decreasing of L.A (calculated as tolerance index of Cd Table-2) was 84.17%, 67.91% and 66.32% at 10, 20 and 30 ppm Cd respectively. While *Ch. album* was 54.10%, 53.97% and 50.01% and *Ch. quinoa* was 60.77%, 43.57% and 23.90% respectively at the same Cd concentrations.

Table 1. Effect of Cd on growth parameters of Chenopodium species.

Species	Treatments	Root		Shoot		L.A (cm^2/leaf)	GR (%)
		F.Wt (g/plant)	D.Wt (g/plant)	F.Wt (g/plant)	D.Wt (g/plant)		
Ch.ambrosoidae	Ctrl.	0.050	0.008	0.294	0.051	0.77	19.70
	10 ppm	0.024	0.002	0.173	0.031	0.74	15.46
	20 ppm	0.009	0.001	0.087	0.016	0.28	11.74
	30 ppm	0.009	0.001	0.006	0.001	0.25	7.46
Ch.album	Ctrl.	0.307	0.020	0.763	0.113	4.02	50.13
	10 ppm	0.189	0.014	0.565	0.112	3.69	47.97
	20 ppm	0.113	0.007	0.294	0.046	3.30	33.23
	30 ppm	0.043	0.004	0.220	0.035	2.93	25.19
Ch.quinoa	Ctrl.	0.099	0.007	0.655	0.093	5.98	95.53
	10 ppm	0.058	0.004	0.409	0.070	4.15	75.89
	20 ppm	0.057	0.004	0.478	0.078	3.87	33.99
	30 ppm	0.063	0.006	0.473	0.069	3.32	31.70
LSD 1%		0.069	N.S	0.16	0.082	0.95	18.17

F.Wt fresh weight, D.Wt dry weight, L.A leaf area, G.R growth rate, LSD 1% least significant degree at probability 1%.

Cd induces various visible symptoms of phytotoxicity in leaf, such as leaf roll, chlorosis and necrosis, growth retardation, and finally death (**Tran and Popova, 2013**). The reduced L.A in *Chenopodium* species was associated with the reduced leaf expansion as a result of reduced cell size and small intercellular spaces (**Djebali *et al.*, 2005**). Cd may affect photosynthesis at different levels, including stomatal conductance, Calvin cycle enzyme activity, photosynthetic pigments, thylakoid ultrastructure, and electron transport activity (**Vassilev *et al.*, 1997**).

Effect of Cd on growth rate (GR) of *Chenopodium* species*:* GR of *Chenopodium* species under Cd stress was measured (Table-1). Difference notices were observed at this parameter when measured of *Chenopodium* species under the Cd treatments. GR significantly ($p \leq 0.01$) decreased with increase Cd concentrations in comparison all species controls. The decreasing of F.Wt and D.Wt of root and shoot and L.A due to reduction in GR with three species of Chenopodium. GR values for *Ch. ambrosoidae*, *Ch. album* and *Ch. quinoa* were (19.70%, 50.13% and 95.53%) respectively as compared with control. We observed that *Ch. album* is the lowest in decreasing of GR values (95.70%, 66.29% and 50.25%) at 10, 20 and 30 ppm of Cd. While, GR values of *Ch. ambrosoidae* and *Ch. quinoa* were (78.50%, 59.59% and 37.86 %) and (79.44%, 35.59% and 33.19%) respectively at the same Cd concentrations used.

Table 2. Cd tolerance index of growth parameters for *Chenopodium* species.

Species	Treatments	Root		Shoot		L.A	GR
		F.Wt	D.Wt	F.Wt	D.Wt		
Ch.ambrosoidae	10 ppm	27.78	58.65	60.04	96.87	84.17	78.50
	20 ppm	15.96	29.63	30.34	37.11	67.91	59.58
	30 ppm	15.63	2.10	19.53	32.51	66.32	37.85
Ch.album	10 ppm	68.68	74.07	99.39	91.65	54.10	95.70
	20 ppm	35.68	38.54	40.74	81.91	53.97	66.29
	30 ppm	21.58	28.78	30.88	72.86	50.01	50.24
Ch.quinoa	10 ppm	57.27	62.42	75.10	69.42	60.77	79.44
	20 ppm	62.22	73.03	83.44	64.77	43.57	35.58
	30 ppm	87.50	72.21	89.58	55.55	23.90	33.18

Our results according to **Ernst *et al.*, (1992)** when reported that growth reduction can be useful as an indicator to show the toxicity of metals. Heavy metals reduced the cell wall elasticity of roots and root elongation are reduced (**Hiedri *et al.*, 2005**). High reduction of growth rate can be due to the different effect of heavy metals on the reduced elongation and absorb minerals (**Vassilev, 2003)**. Cd interferes with photosynthesis, respiration and nitrogen metabolism in plants can lead to reduced growth, followed by biomass is reduced (**Gouia *et al.*, 2001**).

Effect of Cd stress on physiological and biochemical traits in *Chenopodium* species.

Effect of Cd on water relationships parameters of Chenopodium species*:* The water relationships include relative water content (RWC), membrane stability index (MSI) and electrolyte leakage (E.L) of *Chenopodium* species were measured under Cd concentrations are presented in Table 3. The results indicate that significantly decreasing ($p \leq 0.01$) on both RWC and MSI and increasing in E.L was detected under different Cd concentrations of *Chenopodium* species seedlings treated as compared with control Cd untreated. The results indicated that *Ch.quinoa* was higher than *Ch. album* and *Ch. ambrosoidae* in RWC and MSI values, which recorded (66.39%, 65.80% and 41.60%) respectively for RWC and (81.09%, 52.12% and 46.64%) for MSI respectively at high concentration of Cd (30 ppm). E.L significantly increased in *Chenopodium* species under Cd stress as compared with control untreated. *Ch. album* was maximum increasing of E.L (189.29%), while, *Ch. ambrosoidae* was (162.14%) and *Ch. quinoa* (126.01%) respectively at 30 ppm Cd. Cd stress is an intricate phenomenon which includes osmotic stress, specific ion effect, nutrient deficiency and these stresses can produce ROS (**Ghosh and Singh, 2005**). Cell membrane damage caused by Cd stress in plants correlated with ROS. Plants have enzymes and antioxidant compounds to inhibit the ROS and the cultivars which able to synthesis these compounds are tolerant (**Memon *et al.*, 2001**).

Effect of Cd on antioxidant enzymes activity of *Chenopodium* species*:* Activity of antioxidant enzymes CAT, POD and PPO in *Chenopodium* species were determined after 7 d under different of Cd doses (Table-3). The activity of antioxidant enzymes CAT, POD and PPO in the leaves of different species increased in response to Cd treatments as compared with control Cd untreated. We observed that Cd levels caused significantly increased in activity of CAT, POD and PPO in *Ch. ambrosoidae*, that was recorded 2.27, 2.20 and 1.67 times for CAT, and 3.07, 2.67 and 1.67 times for POD and 1.80, 2.20 and 1.00 times for PPO times at 10, 20 and 30 ppm as compared with control. In *Ch. album* POD and PPO increased with increase Cd treatment concentrations, the increasing values were 3.00, 4.50 and 6.00 times for POD and 1.00 and 1.17 times for PPO as compared with control. But, don't note any increase in CAT of *Ch. album* at all Cd concentrations used. CAT and POD of *Ch. quinoa* increase especially at high Cd doses (30 ppm), where increasing of activity antioxidant enzymes were 4.35 times for CAT and 3.00 times for POD at high Cd level compared with control Cd untreated. While, don't note any increase in PPO of *Ch. quinoa* at all Cd concentrations used. The higher activity of antioxidant enzymes CAT, POD, PPO was in proportion to the progressive increase in the concentration of Cd (0, 10, 20 and 30 ppm). Moreover, the percent increase in antioxidant enzymes was more in *Ch. ambrosoidae* then *Ch. album* and *Ch. quinoa*.

Table3. Effect of Cd on water relationships and antioxidant enzymes activity of *Chenopodium* species.

Species	Treatments	RWC (%)	MSI (%)	EL (%)	Enzyme Activity (%)		
					CAT	POD	PPO
Ch.ambrosoidae	Ctrl.	151.61	39.56	60.44	100.00	100.00	100.00
	10 ppm	95.70	29.69	70.31	226.67	306.67	180.00
	20 ppm	72.27	19.28	80.72	220.00	266.67	220.00
	30 ppm	63.08	18.45	98.16	166.67	166.67	100.00
Ch.album	Ctrl.	72.03	65.22	34.78	100.00	100.00	100.00
	10 ppm	65.69	55.40	44.60	29.17	300.00	58.33
	20 ppm	54.04	41.85	58.15	33.33	450.00	100.00
	30 ppm	47.40	33.99	66.01	12.50	600.00	116.67
Ch.quinoa	Ctrl.	98.95	57.91	42.10	100.00	100.00	100.00
	10 ppm	77.78	56.87	43.13	75.00	220.00	54.17
	20 ppm	76.00	55.26	44.74	210.00	260.00	62.50
	30 ppm	65.69	46.95	53.05	435.00	300.00	54.17
LSD 1%		22.14	9.11	14.36	92.7	135.84	57.58

Where: (RWC) relative water content, (MSI) membrane stability index, (EL) electrolyte leakage, (CAT) catalase , (POD) peroxidase, (PPO) poly phenol oxidase, LSD 1% least significant degree at probability 1%.

Aly and Mohamed (2012) working on *Brassica juncea* mentioned that the higher activity of antioxidative enzymes offers a greater detoxification efficiency which provides better resistance to a plant variety against heavy metal induced oxidative stress. These findings revealed the importance of the antioxidant enzymes in response to Cd toxicity in *Chenopodium* species seedlings. Under most conditions, H_2O_2 in plants can be efficiently scavenged by CAT, POD (**Foyer and Noctor, 2005).**

Induction of antioxidant enzymes has been observed in Arabidopsis (**Xie *et al*., 2012**), rice (**Xu *et al.,* 2013**) and alfalfa (**Jin *et al.,* 2013a**). We observed increased CAT activity at a low Cd concentration and reduced activity at a high Cd concentration. **El-Beltagi *et al.,* (2010)** found the same results in radish. These enzymes are regarded as bio indicators of heavy metal toxicity and play important roles in scavenging ROS like H_2O_2 to reduce oxidative damage. In tobacco, the activity of CAT increased during continuous exposure to Cu, Cd, and Pb (**Cvetanovska, 2010**).

POD activity in pea genotypes increases with Cd sensitivity and is a biomarker for metal toxicity in plants (**Metwally *et al.,* 2005**). Previous reports showed that rapid changes in PPO activity were proposed that may be involved in necrosis development around damaged leaf surfaces and in defense mechanisms against insects and plant pathogen attack (**Thipyapong *et al.,* 2007**). PPO activity in some plant species was observed under heavy metal stress, and showed increase compared to the control (**Saffar *et al*., 2009**). The induction of PPO activity might be due to its role in phenolic compound synthesis, which plays an important role in detoxification of heavy metals in plants (**Ruiz *et al.,* 1999**). On the basis of these results, our results indicated that changes of PPO activity might participate in the defense mechanism of licorice plants against Cd toxicity.

Effect of Cd on pigments concentration of Chenopodium species:

The concentration of photosynthetic pigments including Chlorophyll-a (Chl-a) and Chlorophyll-b (Chl-b) were determinate are shown in Table 4. The pigment concentrations in seedlings don't take one trend in response to Cd concentrations in contaminated medium. Where, in sometimes the pigments decreased in some species of *Chenopodium* and another sometimes increased in other species.

For example, data indicated that Cd tolerance index of Chl-a and Chl-b of *Ch. ambrosoidae* in general were decreased with increase of Cd concentrations by (83.92% and 64.43% and 55.22%) for Chl-a, but witch recorded (75.82%, 115.96% and 91.91%) for Chl-b with increase Cd concentration, while in *Ch.album* Chl-a and Chl-b increased by (171.07%, 144.87% and 166.89%) for Chl-a and (79.29%, 152.23% 134.35%) for Chl-b with increase (10, 20 and 30 ppm) Cd doses respectively.

Chl-a concentration in *Ch. quinoa* increased by (117.49%, 137.05% and 241.97) at 10, 20 and 30 ppm Cd. Contrarily Chl-b reduced by (10.07%, 7.27% and 3.07%) with the same of Cd concentrations. Supply of lower concentration of Cd slightly stimulated and enhanced chlorophyll formation in greening maize leaf segments (**Meeta *et al.,* 2007**). Some author's demonstrated that chlorophyll pigments concentration increased under high Cd concentration.

Rehman *et al.,* (2011) indicated that the total chlorophyll content increased in the Tomato plants treated with 10, 20, 30 and 40 ppm Cd compared with control.

Table 4. Effect of Cd concentrations on Chl-a and Chl-b of *Chenopodium* species.

Species	Treatments	Chl-a (mg/g f.wt)	Chl-b (mg/g f.wt)	Cd TI (%)	
				Chla	Chlb
Ch.ambrosoidae	Ctrl.	8.81	4.74		
	10 ppm	7.39	3.59	83.92	75.82
	20 ppm	5.67	5.49	64.34	115.96
	30 ppm	4.86	4.35	55.22	91.91
Ch.album	Ctrl.	3.86	1.73		
	10 ppm	6.60	1.37	171.07	79.29
	20 ppm	5.59	2.63	144.87	152.32
	30 ppm	6.44	2.32	166.89	134.35
Ch.qiunoa	Ctrl.	2.44	5.27		
	10 ppm	4.30	0.53	117.49	10.07
	20 ppm	5.05	0.38	137.05	7.27
	30 ppm	5.89	0.16	241.97	3.07
LSD 1%		1.93	1.78	———	———

Where: Chl-a chlorophyll a, Chl-b chlorophyll b, TI tolerance index.

Mohsen and Ali, (2013) showed that the application of Cd at lower level (100 µM Cd) as Cd chloride resulted increase in chlorophyll-a and chlorophyll-b compared with the control in Dill (*Anethum graveolens*) plant under current hydroponic system in the greenhouse.

The reasons of chlorophyll pigments increase is unknown, but, may be increases in leaf thickness tended to compensate slightly for the negative effects on leaf chlorophyll as response of salinity (**Longstreth *et al.*, 1984**), and heavy metal (**Manios et al., 2003**) stresses. Or recent reports have confirmed that anthocyanin can function as antioxidants and thus alleviate toxic effects of ROS in plant cells (**Gould *et al.*, 2002 and Neill *et al.*, 2002**). These results are results are agreement with reported of **Drazkiewicz *et al.*, (2003) and Jia *et al.*, (2012)** who found that chlorophyll content increased with Cd stress. In this context, it is believed that under stress situations, their main function is the quenching of the ROS generated by stress and may be reason in increasing of chlorophyll under Cd stress (**Neill and Gould, 2003**).

Determination of Cd and macro-micronutrients of Chenopodium species seedlings.

Effects of Cd on some macro and micro nutrients of *Chenopodium* spp. used were studded in Table-5. Results showed that Cd treatment had significantly effect on macronutrient and micronutrient contents of plants used. Accumulation of Cd increased with increase Cd in solution at all doses. Up take Cd treatments impact on uptake of (K, Ca, Zn and Fe). K content of *Chenopodium* spp. used was significantly decreased at all Cd treatments in all species of *Chenopodium*. Decreasing K uptakes by plants with Cd treatments were also reported by (**Veselov *et al.*, 2003)** for wheat and **(El-Kafafi and Rizk, 2013)** for Cowpea seedlings. Such decreases in K concentrations may be related to ATP-ase, responsible for active K uptake (**Lindberg and Wingstrand, 1985)**.

Table 5. Determination of Cd and nutrient elements (mg/g.d.wt) of *Chenopodium* species seedlings.

Species	Treatments	Cd	K	Ca	Fe	Zn
Ch. ambrosoidae	Ctrl.	0.70	63.70	86.00	7.53	2.12
	10 ppm	6.21	63.90	86.00	6.09	3.27
	20 ppm	6.78	50.30	90.00	3.77	3.34
	30 ppm	9.56	52.90	89.00	3.46	2.81
Ch. album	Ctrl.	0.60	81.50	111.00	7.27	4.14
	10 ppm	5.20	58.80	97.00	6.72	1.46
	20 ppm	5.61	51.90	86.00	3.51	2.60
	30 ppm	7.08	55.10	90.00	1.31	2.44
Ch.quinoa	Ctrl.	0.69	65.30	101.00	2.87	1.87
	10 ppm	5.56	63.20	95.00	2.86	1.29
	20 ppm	6.23	60.10	84.00	2.40	2.44
	30 ppm	6.56	54.30	85.00	3.81	1.65
LSD 1%		4.99	14.70	12.80	3.50	1.24

The increasing of Cd concentrations significantly decreased Ca concentration of seedlings tissues. The increasing concentration of Cd in the external medium replaces Ca at the binding site by other

heavy metal cations at the exterior surface of the plasma membrane, thereby increasing Ca requirement. Cd decreases Ca concentration because of the competition between Cd and Ca at both Ca channels, and intracellular Ca binding proteins **(Nelson, 1986)**.

Fe and Zn don't have one tend under Cd treatments in three species of Chenopodium. For example, Fe concentration was increase at 30 ppm Cd only in both *Ch. album* and *Ch. quinoa*, while, decrease in *Ch. ambrosoidae* at all Cd doses used compared with their controls. The results indicated that Zn concentration in *Ch. ambrosoidae* increased with increase Cd treatments in comparison with control, but the same concentration decreased in both *Ch. album* and *Ch. quinoa.*

Moreover, **Sandalio *et al.*, (2001)** reported that K, Ca, Fe and Zn concentrations decreased with increases of Cd in an aerated full nutrient media. However, **Zhang *et al.*, (2002)** found that, while K, Fe, and Zn concentrations increased in wheat genotypes at the seedling stage. As reported by **Jiang *et al*., (2004)** and **Yakup *et al*., (2015)** the nutrients mainly affected by Cd in Indian mustard were K, Ca, Fe and Zn. Recently, Cd supply increased macronutrients and decreased micronutrients concentrations in different plants (**Rezvani *et al*., 2012)**. Specific mechanisms seem to be involved to maintain homeostasis, i.e. a balance between having enough essential metals available for metabolic functions and at the same time avoiding toxicity and to keep nonessential metals below their toxicity thresholds (**Clemens, 2006**). Considering our results, the variation in nutrient concentrations as effect of Cd could be due to a series of defense mechanisms, e.g. phytochelatins (PCs) production, expressed by *Chenopodium* species to avoid toxicity.

Conclusion

Phytoremediation is one of the best methods used for removing the heavy metals from contaminated media. Removal of heavy metals by cheapest plants like *Chenopodiaceae* is one of the finest, ecofriendly and cost effective methods. In summary, we have determined the toxicity of selected heavy metals on early seedling growth in the model plant species. In the present study *Chenopodium* species plant absorbs more than 30 ppm of Cd. As this is the cheapest plant grown and removed, could be the best method in phytoremediation (phytoextraction). The present research study indicates exposure of Cd metal in hydroponic solution up to 30 ppm L^{-1} concentrations during the period of 7 days shows that *Chenopodium* species have up taken 80-85% of Cd from hydroponic solution which will have direct application to remediate toxic metals from the contaminated medium in their different tissues, and it has high bioconcentration factor and translocation factor. And Cd supply increased some macronutrients and decreased some micronutrients concentrations in different species. The results indicated that Chenopodium species studded were different in physiological and biochemical defense mechanisms as response against of Cd. The decreasing of F.Wt and D.Wt of both root and shoot and L.A. in *Ch. quinoa* was looser than *Ch. ambrosoidae* and *Ch. album.* Also, the increasing of RWC, MSI values, stability of photosynthetically pigments and activity of antioxidant enzymes in *Ch. quinoa* was the highest from *Ch. ambrosoidae* and *Ch. album*. In addition, *Ch. quinoa* had high ability for Cd uptake and high values of total tolerance index against Cd from another two species. That is the reasons why, *Ch. quinoa* considers efficient in phytoextraction as among phytoremediation methods.

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إمتصاص الكادميوم بواسطة أنواع من نبات الزربيح كنموذج لتقنية المعالجة النباتية

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يعتبر عنصر الكادميوم من أكثر العناصر الثقيلة سمية والذى يؤثر سلبا على نمو النباتات. تراكم العناصر الثقيلة في الأنسجة المختلفة للنباتات أحد أهم طرق المعالجة النباتية للتخلص أو لإمتصاص العناصر الثقيلة من البيئات الملوثة بها. الهدف من هذا البحث هو تحديد أي من أنواع نبات الزربيح الثلاث تحت الدراسة (زربيح أمبروسويدى وزربيح ألبيوم وزربيح كينوا) ذو كفاءة مرتفعة في إمتصاص وتراكم الكادميوم داخل أنسجته المختلفة وذلك من خلال دراسة تأثير الكادميوم على نمو نباتات تحت الدراسة خلال مرحلة النمو المبكر. تم عمل ثلاثة تركيزات من الكادميوم (10، 20 و30 جزء في المليون) بالإضافة إلى معاملة الكنترول. تم تقديربعض قياسات النمو وسمات فسيولوجية وبيوكيماوية للنباتات محل الدراسة خلال هذه المرحلة من النمو. تشير النتائج إلى وجود تأثير ضار لتركيزات الكادميوم المختلفة على كل القياسات التي تم قياسها وتقديرها خلال هذه التجربة. كما دلت النتائج على قدرة الأنواع النباتية الثلاث على إمتصاص الكادميوم من خلال مجموعها الجذرى ونقلها وتراكمها داخل المجموع الخضرى بها كأحد طرق المعالجة النباتية. كما لوحظ أيضا إختلاف رد فعل أنواع نبات الزربيح الثلاث المستخدمة في البحث لإجهاد عنصر الكادميوم من خلال تعدد آليات الدفاع الموجودة داخلها كثبات الأغشية والثبات النسبى لهدم صبغات البناء الضوئى المختلفة كذلك نشاط بعض مضادات الأكسدة. بمقارنة الأنواع النباتية الثلاث لتحديد أفضلها كنموذج في المعالجة النباتية تبين أن النوع "كينوا" قد تفوق على النوعين الآخرين في قدرته على إمتصاص وتراكم الكادميوم داخل أنسجته وكذلك في مجموع مؤشرات التحمل لإجهاد الكادميوم.