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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (6): 874-879, 2007
ISSN 1028-8880

Response of Bean to Some Heavy Metals in Sewage Water

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Abstract: Seed germination and early growth of bean (*Phaseolus vulgaris*) seedlings were significantly reduced when irrigated with a mixture of Cu, Zn, Cd and Pb at concentrations of sewage water. Leaf content of photosynthetic pigments, carbohydrates, nucleic acids and total nitrogen also decreased. Cd and Pb significantly inhibited the activity of α and β-amylases and protease enzymes during germination. Treatment of the mixture solution by precipitation, rice residue or EDTA decreased its retarding effect. The effect of precipitation was more pronounced. Whilst the lower concentration of Cu and Zn stimulated germination, growth and metabolism of bean seedling, the higher concentrations showed inhibitory effects. Even at very low concentrations of Cd and Pb germination, growth and metabolism of bean significantly decreased. Precipitation treatment alleviated the adverse effects of higher concentrations of Zn, Cu, Cd and Pb. Whilst rice residue and EDTA treatments slightly enhanced germination of seeds treated with high concentrations of Zn and Cu, they did not affect germination of seeds treated with Cd or Pb.

Key words: Bean, Cd, Cu, EDTA, enzyme activity, germination, Pb, Zn

INTRODUCTION

Throughout the world, there is now a growing concern about pollution of the environment with different types of polluting agents, particularly ions of heavy metals owing to their possible accumulation in various plant organs in levels exceeding the maximum permissible limits accepted by FAO (Gupta and Gupta, 1998).

Heavy metals can be accumulated in cells via uptake systems responsible for essential cations. Once enter the cell, heavy metal cations, especially those with high atomic numbers such Hg^{+2}, Cd^{+2} and Ag^{+}, tend to bind to SH groups and may inhibit the activity of sensitive enzymes (Nies, 1999). Zayed and Amin (2002) reported that *Trifolium alexandrium* plants originated from lead polluted and non-polluted seeds differ in germination behavior, dry weights, resistance index and lead accumulation. They also found a considerable variation in total protein and minerals contents.

Heavy metals generally cause mutations or cell death in plants and increase in human health hazards as the heavy metals enter and concentrated in the food chain through uptake by plants and ingestion by animals (Sanchez *et al*., 1999). Heavy metals, such as Cd, Cu, Zn, Bi, Ni, Fe, Hg and Pb, may be toxic when present at concentrations higher than required for optimum growth conditions. Cadmium, the best-known toxic heavy metal, is a ubiquitous toxic metal that is capable of modulating immune responses. In small quantities, certain heavy metals are nutritionally essential for a healthy life. Some of these are referred to as the trace elements (e.g., iron, copper, manganese and zinc).

Chelation process is simply defined as process by which a molecule encircles and binds (attaches) to the metal and removes it from the medium. It occurs mainly through the induction of metal binding peptides and proteins such as metallothioneins and polyhistidindes (Mejre and Bulow, 2001). EDTA is one of the oldest chelating agents, Namasivayam and Ranganthan (1998) reported that Citrate and EDTA considerably decreased the adsorption of Ni^{2+}, while Cd^{2+} adsorption decreased significantly in the presence of acetate and citrate. Azenha *et al.* (1995) found that the exposure of *P. syringae* cells to 100 μM copper alone led to its death, suggesting that copper was responsible for cell death. EDTA significantly reduced both the amount of copper bound to the cells and cell death, indicating that, not only strong chelating agent but also weak and moderate copper ligands can effectively antagonize copper toxicity. Residues such as molasses, blood meal and silage effluents, containing various aliphatic carboxylic acids, sugar acids and amino acids or other precursor compounds have a potential to serve as extractants.

Xiong (1994) reported that Cd adsorption significantly increased when the soil had previously been incubated with the rice straw and a milvetch (Astralagus shoot), the results of their study indicated that the pH of the treated soil samples rose after incubation, changes in

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pH were hence considered responsible for increasing Cd adsorption by plant material incubated soil samples. Apple Residues (AR) were used for removal of the heavy metals Cu, Pb and Cd with higher selectivity for Pb than Cu and Cd. The presence of organic ligands decreased the capacity for Cu removal due to metal-ligand complex formation. AR may be used as effective and inexpensive adsorbent for metal removal from aqueous solution (Sung *et al*., 1998).

The present research investigated the effect of some heavy metals e.g., Zn, Cu, Cd and Pb, which present in sewage water in high concentrations, on germination, growth, enzyme activities during germination and some metabolites during growth of bean. The effect of each ion was also investigated individually at different concentrations. The various heavy metal solutions were also treated by precipitation, or chelation by rice residue or EDTA to reduce their accumulation in plant cell.

MATERIALS AND METHODS

In a pot experiment, seeds of bean (*P. vulgaris*) were irrigated with solution contains mixture of the heavy metals Zn, Cu, Pb and Cd at concentrations similar to that of sewage water (625, 112, 86 and 194 $\mu g\ mL^{-1}$, respectively). Lead was added as lead acetate, while copper, zinc and cadmium were added as sulphate salts. Each ion was supplied to the plant in the Hoagland's nutrient solution at three concentrations (lethal, high and low). Zn concentrations were 70, 40 and 10 mM, while Cu concentrations were 40, 10 and 1 mM. Cd concentrations were 1.9, 1 and 0.1 mM, while Pb concentrations were 90, 50 and 10 mM. Some treatments e.g., precipitation, rice residue and EDTA were applied to reduce the effect of heavy metals accumulation in plants. 1N NaOH was added for precipitation of heavy metals, then filtered within cotton plug and neutralized before using for irrigation. Rice residue was used as 0.5 kg kg^{-1} soil and EDTA was used at 50 mM concentration. Seeds of bean were obtained from the Agricultural Research Center, Ministry of Agricultural, Giza, Egypt.

For enzyme assay, plant material was prepared by macerating the tissues with a chilled pestle and mortar at 0-4°C. The tissue homogenate was centrifuged at 10000 g for 20 min and the supernatant obtained was used directly for determining enzyme activity. For assaying the activity of α-and β-amylases, 3,5-dinitrosalicylic acid reagent was used according to Bergmeyer (1974). Protease activity was assayed according to the method described by Bergmeyer (1974). Total carbohydrates were determined by using anthrone reagent after hydrolysis of the dry matter in 1N H_2SO_4, according to the method described by Umbriet *et al*. (1959). Photosynthetic pigments were estimated in 85% acetone extracted leaves according to Metzener *et al*. (1965). Total nitrogen was measured by digesting the dry leaves in 50% sulphoric acid and 35% perchloric acid and its ammonia content was estimated using Borthelot reaction which carried out according to Chaney and Marbach (1962). DNA was measured according to Dische and Schwartz (1973) by using diphenylamine reagent. RNA was determined using the method adopted by Ashwell (1957) using orcinol reagent.

Statistical analysis was carried out according to Snedecor and Cochran (1980) using analysis of variance and the significance was determined using LSD values at p = 0.05 and 0.01.

RESULTS AND DISCUSSION

Application of mixture of Zn, Cu, Cd and Pb ions, at the same concentrations present in sewage water, reduced germination percentage of bean seeds to become 67% (Table 1). This inhibitory effect could be attributed to the effect of Cd and Pb (non-metabolized), which are very toxic at low concentrations, as indicated in Table 4 and 5. Sewage water contains other nutrients cause, in general, induction in plant growth and has eutrophication properties, improve to some extent both physical and chemical properties of soil and plant growth (Zeid and Abou El Ghate, 2006). Voldares *et al.* (1983) reported that heavy metals introduced into the soil do not exhibit the same behaviour as the heavy metals released from sludge itself. Precipitation treatment alleviated this inhibitory effect and increased germination percentage to become 97%. Rice residue and EDTA treatments were non-effective on germination percentage. Reduction in germination percentage by this mixture could be attributed to its inhibitory effect on the activity of the hydrolytic enzymes α and β-amylases and proteases during germination (Table 1). However, precipitation treatment to the mixture solution led to increasing the activity of these enzymes, which in turn increase the amount of hydrolyzates e.g., glucose and amino acids which are required for growth of embryo axes (Zeid and Shedeed, 2006). Growth of shoot was negatively affected by the mixture of these heavy metals and was associated with a decrease in chlorophyll a (Chl. a), Chl. b and carotenoids. Reduction in the leaf content of the photosynthetic pigments resulted in decreasing the carbohydrate content. Total nitrogen, as well as DNA and RNA contents also negatively affected by the uptake of these heavy metals (Table 1). Precipitation treatment effectively reduced the harmful effect on growth and metabolism, followed by rice residue and EDTA treatments.

Table 1: Effect of mixture of heavy metals (Cu, Zn, Cd and Pb) at concentrations of sewage water on germination percentage, enzyme activities ($\mu g\ g^{-1}$ f.m. h^{-1}) during germination, shoot length (cm), photosynthetic pigments, total carbohydrates and total-N (mg g^{-1} d.m.) and nucleic acids (mg g^{-1} f.m.) of bean seedlings (15-day-old)

Heavy metals mixture	Treatments	Germ	α-amylase	β-amylase	Protease	Shoot length	Chl. a	Chl. b	Caroten-oids	Total carbohydrates	Total-N	DNA	RNA
0	Control	99.0	46.10	45.50	3.11	16.6	3.23	2.02	0.92	215.5	56.7	152.2	183.4
Heavy metals	0	67.0	32.60	36.10	0.92	8.2	1.30	0.90	0.71	201.6	32.4	144.0	157.9
mixture	Pptn.	97.0	43.70	44.50	3.00	16.1	3.20	2.00	0.91	215.1	46.6	151.3	183.0
	Rice residue	68.0	38.40	40.60	1.98	10.7	2.70	1.90	0.86	208.0	40.2	149.0	167.0
	EDTA	65.0	34.30	38.30	1.00	9.4	1.80	1.10	0.79	203.0	35.6	146.0	159.0
LSD at 0.05		1.1	0.53	0.62	0.05	0.1	0.07	0.06	0.02	0.7	1.5	1.2	0.8
LSD at 0.01		1.9	0.61	0.91	0.08	0.2	0.16	0.09	0.04	0.9	2.8	1.9	1.0

Pptn: Precipitation

Table 2: Effect of Zn on germination percentage, enzyme activities ($\mu g\ g^{-1}$ f.m. h^{-1}) during germination, shoot length (cm), photosynthetic pigments, total carbohydrates and total-N (mg g^{-1} dm) and nucleic acids (mg g^{-1} fm) of bean seedlings (15-day-old)

Zn (MM)	Treatments	Germ	α-amylase	β-amylase	Protease	Shoot length	Chl. a	Chl. b	Caroten-oids	Total carbohy-drates	Total-N	DNA	RNA
0	Control	99	51.2	49.2	3.00	14.9	3.23	1.70	0.87	213.5	43.00	156.2	189.4
10	0	100	66.4	58.6	4.90	17.2	4.90	1.92	1.30	220.0	56.40	191.5	210.4
40		52	36.1	30.5	1.65	6.1	1.60	0.78	0.40	130.1	32.50	72.4	96.1
70		0	0.0	0.0	0.00	0.0	0.00	0.00	0.00	0.0	0.00	0.0	0.0
10	Pptn.	99	52.9	50.2	2.10	13.9	3.30	1.60	0.84	202.6	42.30	144.2	181.0
40		91	50.2	49.0	3.60	9.1	3.41	1.63	0.82	198.2	40.00	145.2	177.0
70		91	50.1	49.1	2.87	10.2	3.25	1.50	0.89	210.1	39.50	139.0	173.3
10	Rice residue	97	62.5	54.6	3.40	15.8	3.95	1.80	0.97	206.7	51.20	187.4	198.2
40		61	42.4	36.1	2.01	6.5	2.50	0.16	0.60	120.0	33.80	77.6	97.9
70		9	14.1	14.5	1.64	2.4	0.17	0.04	0.03	16.4	8.90	18.1	20.9
10	EDTA	97	65.2	57.2	4.00	16.5	4.21	1.90	1.09	218.4	54.40	190.5	202.6
40		61	37.5	33.3	1.89	6.2	1.90	0.94	0.50	131.5	30.60	73.1	98.0
70		9	8.2	5.5	0.095	1.3	0.20	0.07	0.02	11.7	4.40	16.2	2.2
LSD at 0.05		0.9	0.5	0.64	0.09	0.3	0.06	0.03	0.03	1.3	0.52	1.2	0.8
LSD at 0.01		1.1	0.7	0.82	0.10	0.5	0.08	0.08	0.05	3.6	0.73	1.9	1.0

Pptn: Precipitation

Zinc uptake at 10 mM increased germination percentage to 100% (Table 2) may be due to its stimulatory effect on the activity of the hydrolytic enzymes during germination. Shoot length of bean seedlings also increased by the application of Zn at low concentration. Growth induction was associated with enhancement in biosynthesis of photosynthetic pigments, as well as the total carbohydrate content. Total nitrogen and nucleic acids contents also increased (Table 2). On the other hand, the higher concentration (40 mM) significantly decreased the enzyme activities during germination and consequently, reduced the germination percentage to become 52%. Shoot length also decreased at this concentration and was associated with marked reduction in leaf content of photosynthetic pigments, carbohydrates, total nitrogen and nucleic acids. Uptake of Zn at 70 mM concentration was lethal for seed germination. Precipitation treatment alleviated its inhibitory effect on the activity of the hydrolytic enzymes and germination, which became 91% at both high concentration and the lethal concentration. Growth and leaf content of photosynthetic pigments, carbohydrates, total nitrogen and nucleic acids markedly increased after precipitation of higher concentrations of Zn. Rice residue and EDTA treatments were less effective than precipitation, since the germination percentage became 61% at 40 mM and 9% at 70 mM of Zn. Hopkin (1999) reported that the moderate levels, generally, play an important role in plant growth and productivity. They act as activators or co-factors in all vital processes of plant life. In contrast, the relatively elevated levels induced harmful effects on most physiological responses (Bonnet *et el.*, 2000; Saleh and Abdel-Kader, 2000). Moreover, lower concentrations increase photosynthetic criteria (chlorophyll biosynthesis and PSII activity). Zn is a component of enzymes like carbonic anhydrase, alcohol dehydrogenase and RNA polymerase. It also activates various enzymes e.g., dehydrogenase, aldolases, isomerases, transphosphorylases and RNA and DNA polymerases. It has been found essential for CO_2 evolution and utilization, carbohydrate and phosphous metabolisms and synthesis of RNA and auxins. Zinc plays an important role in protein synthesis, as amino acids and amides accumulate in zinc-deficient plants. A close relationship between zinc and chlorophyll formation is found. Stunted growth and little leaf are related to disturbances in the metabolism of auxins (IAA in particular), since zinc is required for synthesis of tryptophan (a precursor for IAA synthesis).

Copper uptake at 1 mM exhibited a stimulatory effect, similar to that of Zn at low concentration (10 mM). Germination percentage became 100% and the hydrolytic

Table 3: Effect of Cu on germination percentage, enzyme activities (μg g^{-1} f.m. h^{-1}) during germination, shoot length (cm), photosynthetic pigments, total carbohydrates and total-N (mg g^{-1} dm) and nucleic acids (mg g^{-1} fm) of bean seedlings (15-day-old)

Cu (mM)	Treatments	Germ	α-amylase	β-amylase	Protease	Shoot length	Chl. a	Chl. b	Carotenoids	Total carbohydrates	Total-N	DNA	RNA
0	Control	99.0	54.4	47.50	2.67	14.90	3.23	1.70	0.83	213.5	55.60	156.2	189.4
1	0	100.0	68.7	59.30	3.88	17.60	5.30	2.30	1.03	247.8	58.20	185.5	213.7
10		44.0	34.3	32.30	0.87	5.70	2.10	0.93	0.61	166.7	30.80	89.6	120.5
40		0.0	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.0	0.0
1	Pptn.	99.0	54.9	48.60	2.12	10.50	3.51	1.22	0.78	211.4	47.50	142.4	160.8
10		95.0	54.9	48.50	2.33	11.10	3.08	1.46	0.80	212.5	46.50	103.2	127.4
40		94.0	55.6	48.10	2.33	10.70	3.73	1.62	0.89	210.5	45.90	100.5	110.5
1	Rice residue	100.0	64.5	54.40	2.70	15.60	4.50	1.97	0.77	241.6	46.20	160.8	172.4
10		53.0	45.4	37.40	2.01	8.30	2.40	1.03	0.62	168.0	30.50	93.0	124.0
40		21.0	11.1	12.00	1.51	3.10	0.61	0.14	0.05	51.4	0.00	26.3	30.7
1	EDTA	100.0	66.2	57.30	3.11	16.90	4.90	2.40	1.01	243.0	41.80	183.7	186.0
10		47.0	39.5	34.50	1.89	6.30	2.20	1.10	0.63	152.0	25.90	90.8	122.0
40		10.0	7.0	5.06	0.078	2.20	0.23	0.06	0.03	13.0	0.00	20.0	24.5
L.S.D at 0.05		1.0	0.9	0.55	0.05	0.30	0.08	0.07	0.03	1.9	0.77	2.7	1.5
L.S.D at 0.01		1.7	1.5	0.84	0.09	0.40	0.10	0.18	0.07	3.2	0.92	4.1	1.9

Pptn: Precipitation

Table 4: Effect of Cd on germination percentage, enzyme activities (μg g^{-1} f.m. h^{-1}) during germination, shoot length (cm), photosynthetic pigments, total carbohydrates and total-N (mg g^{-1} d.m.) and nucleic acids (mg g^{-1} f.m.) of bean seedlings (15-day-old)

Cd (mM)	Treatments	Germ	α-amylase	β-amylase	Protease	Shoot length	Chl. a	Chl. b	Carotenoids	Total carbohydrates	Total-N	DNA	RNA
0	Control	99.0	47.1	44.6	2.38	14.9	3.23	1.70	0.87	213.5	53.00	156.2	189.4
0.1	0	77.0	28.1	30.3	0.67	10.2	1.85	0.68	0.25	89.1	38.70	73.4	93.5
1		40.0	16.7	10.2	0.26	3.7	0.52	0.17	0.07	51.0	24.80	32.7	55.3
1.9		0.0	0.0	0.0	0.00	0.0	0.00	0.00	0.00	0.0	0.00	0.0	0.0
0.1	Pptn.	98.0	47.0	42.3	2.27	129.0	3.02	1.35	0.83	206.5	51.70	102.5	163.5
1		93.0	46.4	42.2	2.25	12.7	3.66	1.52	0.84	203.1	51.50	100.8	155.7
1.9		92.0	44.5	41.4	2.15	12.2	3.42	1.29	0.80	201.7	51.60	101.3	152.2
0.1	Rice residue	78.0	36.9	38.5	0.72	10.5	2.09	0.92	0.29	93.4	41.10	82.9	98.4
1		43.0	20.3	16.4	0.58	5.9	0.68	0.26	0.08	54.1	28.60	40.3	73.7
1.9		0.0	0.0	0.0	0.00	0.0	0.00	0.00	0.00	0.0	0.00	0.0	0.0
0.1	EDTA	77.0	30.0	32.4	0.41	10.3	1.89	0.73	0.27	91.8	34.20	76.6	83.8
1		41.0	18.0	11.4	0.39	4.6	0.57	0.19	0.07	52.2	27.20	35.2	65.0
1.9		0.0	0.0	0.0	0.00	0.0	0.00	0.00	0.00	0.0	0.00	0.0	0.0
LSD at 0.05		1.0	0.9	0.7	0.07	0.7	0.05	0.04	0.02	1.5	0.57	2.7	1.7
LSD at 0.01		1.7	1.2	0.9	0.08	0.9	0.07	0.06	0.04	2.4	0.71	4.1	2.7

Pptn: Precipitation

enzymes were activated during germination. Shoot length, photosynthetic pigments, carbohydrates, total-N and nucleic acids contents were also increased by the influence of 1 mM Cu (Table 3). Higher concentration (10 mM) significantly decreased germination percentage and growth, as well as the different metabolites content. Copper at 40 mM concentration was lethal for seed germination.

Copper acts as an electron carrier and as a part of certain enzymes. Cytochrome oxidase is a mixed copper-iron protein catalyzing the terminal oxidation in mitochondria. It is a constituent of plastocyanin (a protein component forming part of photosynthetic electron transport chain), polyphenol oxidase and ascorbic acid oxidase and nitrate reductase. It may also play a catalytic role in nitrogen fixation. Precipitation treatment alleviated the effect of Cu ions at higher concentrations and reduced its stimulation at the low concentration. Rice residue and EDTA treatments increased the germination percentage at the lethal concentration (40 mM) to become 21 and 10%, respectively, but the plant growth and the leaf content of photosynthetic pigments and the different metabolites was very low, particularly the total nitrogen content, which was not detected. These results support the findings of Sunda and Lewis (1978) and Toro ***et al.*** (2003) who reported that copper toxicity decreased with increasing binding of copper by natural ligands. Azenha ***et al.*** (1995) also found that EDTA significantly reduced both the amount of copper bound to the cells and cell death and reduced the free copper concentration from 100 to 5 μM in the medium.

On the contrary, Cd at very low concentration (0.1 mM) was very toxic. Activity of amylases and protease during germination and consequently, the germination percentage markedly decreased, as well as the seedling growth and the leaf content of pigments, carbohydrates, total-N and nucleic acids (Table 4). Increasing the concentration of Cd markedly increased its inhibitory effect on germination, growth and metabolism. Cd at 1.9 mM completely inhibited germination. However, treating the Cd solutions with precipitation alleviated the

Table 5: Effect of Pb on germination percentage, enzyme activities (μg g^{-1} f.m. h^{-1}) during germination, shoot length (cm), photosynthetic pigments, total carbohydrates and total-N (mg g^{-1} dm) and nucleic acids (mg g^{-1} f.m.) of bean seedlings (15-day-old)

Pb (mM)	Treatments	Germ.	α-amylase	β-amylase	Protease	Shoot length	Chl. a	Chl. b	Carotenoids	Total carbohydrates	Total-N	DNA	RNA
0	Control	99.0	49.2	42.5	2.94	14.9	3.23	1.70	0.80	213.50	43.00	156.20	189.4
10	0	71.0	31.2	30.3	0.96	10.3	2.01	0.72	0.23	111.00	45.70	110.80	140.7
50		60.0	20.6	18.4	0.37	7.4	0.92	0.19	0.07	66.50	18.20	84.70	100.2
90		0.0	0.0	0.0	0.00	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0
10	Pptn.	97.0	48.9	42.3	2.48	13.7	3.22	1.64	0.83	210.50	48.30	137.80	177.4
50		96.0	48.1	42.2	2.44	13.9	3.21	1.66	0.81	211.16	42.10	126.50	171.6
90		97.0	46.6	41.4	2.15	13.7	3.41	1.68	0.86	209.40	42.00	126.40	168.4
10	Rice residue	76.0	37.2	36.7	1.72	7.2	2.70	0.79	0.34	116.00	37.60	114.50	122.5
50		67.0	25.3	24.3	0.64	8.4	1.02	0.32	0.09	71.70	24.30	91.40	111.8
90		0.0	0.0	0.0	0.00	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0
10	EDTA	72.0	30.0	32.4	0.41	3.3	2.02	0.73	0.23	112.70	36.20	111.30	117.9
50		62.0	22.0	20.2	0.49	8.0	0.95	0.21	0.08	67.20	21.30	86.00	99.2
90		0.0	0.0	0.0	0.00	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.0
LSD at 0.05		1.1	0.6	0.9	0.06	1.1	0.06	0.05	0.03	2.30	0.47	2.60	1.5
LSD at 0.01		1.9	0.7	1.2	0.08	2.3	0.07	0.07	0.04	3.50	0.56	0.38	1.9

Pptn: Precipitation

adverse effects of Cd on germination and metabolism of bean. Rice residue and EDTA treatments were less effective and did not affect germination at the highest concentration.

Pb showed a similar effect as Cd at low, high and lethal concentrations. Increasing the concentration from 10 to 50 mM increased its inhibitory effect on germination, growth and metabolism of bean (Table 5). Precipitation treatment considerably alleviated these adverse effects of Pb at all concentrations used. Rice residue and EDTA were less effective at low and high concentrations, but non-effective at the lethal concentration. Similar effect of Pb and EDTA on germination of onions, peas, radishes, carrots, lettuces and Brussels sprouts have been observed by Ilic and Milenkovic (1997). Nies (1999) suggested that heavy metals cations, once enter the cell, especially those with high atomic numbers such Hg^{+2}, Cd^{+2} and Ag^{+}, tend to bind to SH groups and may inhibit the activity of sensitive enzymes. Some heavy metals cations such as Cd^{+2} and Cu^{+2} may interact with physiological ions, including Mg^{+2} and Ca^{+2} there by inhibiting the function of the, respective physiological cation.

In conclusion, Zn and Cu are metabolized by the plant and play essential roles at low concentrations, but the higher concentrations are toxic. On the other hand, Cd and Pb are non-metabolized by the plant and exhibit high toxicity even at low concentrations. Continuous irrigation with sewage water leads to more accumulation of many heavy metals in plant organs, in concentrations exceed the permissible limits accepted by FAO and affect human health. Finally, in spite of the enhancement effect of sewage water on vegetative growth and productivity of plant crops (Zeid and Abou El Ghate, 2006), it must not be used without treatment for reducing the heavy metals accumulation in plant cells.

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