Journal of Biological Sciences 7 (1): 34-40, 2007
ISSN 1727-3048

Effect of Sewage Water on Growth, Metabolism and Yield of Bean

I.M. Zeid and H.M. Abou El Ghate
Department of Botany, Faculty of Science, Helwan University, Cairo 11792, Egypt

Abstract: Seed germination and early growth of bean (*Phaseolus vulgaris*) seedlings were stimulated by irrigation with sewage water. The stimulation of growth was accompanied by induction in synthesis of pigments, carbohydrates, nucleic acids, nitrogenous compounds, except amino acids-N decreased. The activity of amylase, invertase and protease enzymes increased may be due to the presence of some mineral ions which act as activators for enzymes. Treatment of sewage water by precipitation, rice residue or EDTA decreased its enhancing effect. Protein banding patterns of the produced seeds indicated that the appearance of new bands having high molecular weights was the marked feature in sewage water irrigated plants. The concentration of Zn, Cu, Cd and Pb increased greatly in the different plant organs in response to sewage application. Treating the sewage by precipitation, rice residue, or EDTA reduced their accumulation, particularly with precipitation treatment.

Key words: Enzyme activity, germination, growth, heavy metals, protein banding

INTRODUCTION

Sewage water and sewage sludge is used nowadays to improve physical, chemical properties and fertility index of soil in order to increase production per unit area. Pollution with ions of heavy metals result from excessive and/or unplanned uses of sewage water. In small quantities, certain heavy metals are nutritionally essential for a healthy life e.g., Fe, Cu, Mg and Zn. Increased loading of heavy metal ions in water and soil produce increases in mutations or cell death in plants and increases in human health hazards as the heavy metals enter and concentrated in the food chain through uptake by plants and ingestion by animals (Sancherz *et al*., 1999).

The residual effect of sewage or sewage sludge applications increased significantly plant height, fresh and dry weight, grain yield, number of pods/plant and seed yield (Lutrac *et al*., 1982), as well as leaf area and leaf biomass (Brown, 1981). Harangozo *et al*. (1984) added that pretreatment of *vicia faba* (broad bean) seeds with sewage water led to increase length of both root and shoot. Sewage sludge increased chlorophyll content and oxygen evolution (Drewa *et al*., 1993), as well as total soluble sugars and sucrose content (Mellberye *et al*., 1982). Nitrogen content also increased by sewage sludge application in wheat straw (Roszyk *et al*., 1989) and in pea and cotton plants (Lewis *et al*., 1992). Nitrate-nitrogen content decreased in plants irrigated with sewage (Belyuchenko and Dronov, 1988). Fresquez *et al*. (1990) reported that most nutrients including plant nitrogen were linearly increased as a result of sludge amendment. However, Ito *et al*. (1991) observed that sewage sludge application at the rate of 60 t ha^{-1}, led to inhibition of growth due to rapid mineralization of nitrogen, which enhances accumulation of toxic ammonia to growth. The activity of proteinase and peroxidase increased in potato stem and root, respectively in response to sewage water application (Chakrbarti and Chakrabarti, 1987).

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 mealand 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),

Corresponding Author: I.M. Zeid, Department of Botany, Faculty of Science, Helwan University, Cairo 11792, Egypt

the results of their study indicated that the pH of the treated soil samples rose after incubation, changes in 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 aims to study the effect of sewage water usage for irrigation of crops e.g., bean (*Phaseolus vulgaris*). The study included germination, seedling and fruiting stages with respect to germination percentage, growth, some enzyme activities, Hill reaction and some metabolites content, as well as the protein banding patterns of the produced seeds, as indication of gene expression, as affected by heavy metals in the sewage water. The concentrations of Zn, Cu, Cd and Pb were measured in the different plant organs.

MATERIALS AND METHODS

Sewage water was obtained from Arab Abou saed region, south of Helwan. The experiment was carried out to study the effect of sewage water usage on germination and growth of bean (*Phaseolus vulgaris*) as one of the plants cultivated in the sewage-polluted area. Its effect on photosynthetic pigments, carbohydrates, soluble protein, DNA, RNA, nitrogenous compounds and some enzyme activities were also studied. Some treatments e.g., precipitation, rice residue and EDTA were applied to reduce the accumulation of heavy metals in sewage water irrigated plants. 1N NaOH was added for precipitation of heavy metals, then filtered through cotton plug and neutralized before using for irrigation. Rice residue was used as 0.5 kg/kg 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 10 000 g for 20 min and the supernatant obtained was used directly for determining enzyme activity. For assaying the activity of α- and α-amylases, 3,5-dinitrosalicylic acid reagent was used according to Bergmeyer (1974). Acid-invertase enzyme was assayed by the method of Malik and Singh (1984). Protease activity was assayed according to the method described by Bergmeyer (1974). Fresh leaves were extracted in 70% ethanol and completed to a known volume with distilled water and used for estimation of total sugars using anthrone reagent (Umbriet *et al*., 1959), while the reducing sugars were determined by using Nelson reagent according to Naguib (1969). Photosynthetic pigments were estimated in 85% acetone extracted leaves according to Metzener *et al*. (1965). For isolation of chloroplasts, according to the method of Aronoff (1946) and Osman *et al*. (1982), fresh leaves were blended in cold buffer with 0.4 M sucrose, (pH 7.8), 3 mM $MgCl_2$, 4 mM sodium ascorbate and 0.1% bovine serum albumin. The suspension was centrifuged at 4°C (1 min at 800 g). The pellet was resuspended in the isolation buffer and centrifuged for 5 min at 300 g and the supernatant was then centrifuged for 10 min at 1000 g. Chloroplasts (residue) were resuspended in the buffer solution. Hill reaction of the isolated chloroplasts was measured by using potassium ferricyanide as electron acceptor. Borate buffer (pH 8.0) extract of dry leaves was used according to Naguib (1969) for determination of soluble nitrogenous compounds e.g., nitrate-N, amino acid-N, peptide-N and total soluble-N. Total nitrogen was measured by digesting the dry leaves in 50% sulphoric acid and 35% perchloric acidand 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. Protein banding patterns were examined in the Central Laboratory of Horticulture Research Center, Giza, Egypt. Separation of proteins was performed using Sodium Dodicyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

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

Sewage water application increased germination percentage of bean seeds to become 98% compared with control seeds (92.5%). The increased germination percentage because of sewage application was associated with a stimulating effect on the hydrolytic enzymes α- and β-amylases and proteases. Treatment of sewage water by precipitation, rice residue or EDTA led to a significant decrease in its stimulatory effect on germination percentage and enzymatic activities (Table 1). Application of sewage water significantly increased growth criteria e.g., shoot and root lengths, fresh and dry masses of bean seedlings, as well as the yield criteria e.g., number of pods/plant, length of pods, fresh and dry weights of pods. Treatment of sewage water by precipitation, rice residue or EDTA reduced its stimulatory effect. The beneficial effects of sewage application may be due to the greater capacity of sewage to supply nutrients to the plant and to improve soil properties (Heckman *et al*., 1986). These nutrients may activate the hydrolytic enzymes during germination, which in turn increase the amount of the hydrolyzates e.g., glucose and amino acids, which are required for growth of embryo axes (Zeid and Shedeed, 2006). The obtained results also support the suggestion of Staniforth and Smith (1991) who attributed the increased crop yield as a result of increasing rate of sewage application to the available nutrients supplied particularly nitrogen, which is reflected in the increased total nitrogen content of the crop.

Application of sewage water positively affected synthesis of photosynthetic pigments and photosynthetic activity (Hill reaction). Treatment of sewage water with precipitation, rice residue or EDTA reduced this increment (Table 2). The stimulatory effect of sewage water on pigments content could be attributed to the fact that sewage water enhances the rate of biosynthesis of chlorophyll a and b. The increased content of chlorophyll was in parallel with the enhancement of plant growth i.e., there is an intimate relationship between growth and chlorophyll content. El-Maghraby and Gomaa (1992) reported that sewage water application increased number of green leaves and leaf area per plants, or it may be increase both of macro and micronutrients elements in soil, which is essential for the plant growth and photosynthetic pigments.

Total soluble sugars, polysaccharide and total carbohydrates content were higher in sewage water irrigated plants compared with controls and decreased by treating the sewage by precipitation, rice residue, or EDTA (Table 2). The effect of precipitation treatment was more obvious. The increased content of carbohydrates in sewage water irrigated plants may be due to the presence of some mineral ions e.g., Mn and Cu that stimulate the two photosystems. Mn^{+2} is required for PSII (O_2 evolving system) and there is also a direct interaction between copper and ferredoxin on the reducing site of PSI. Cu^{++} stimulate the rate of overall electron transfer from water to NADP (Marschner, 1986).

DNA and RNA were significantly increased in sewage water irrigated plants (Table 3), may be due to the enrichment of sewage water with heavy metals such as Zn, which is a component of carbonic anhydrase, RNA polymerase and DNA polymerases. Treatment of sewage water with precipitation, rice residue or EDTA reduced its stimulatory effect on germination, growth, photosynthetic pigments, Hill reaction, carbohydrates, nucleic acids and hydrolytic enzymes activity due to the removal or chelation of heavy metals present in sewage water. Precipitation treatment was more effective. Kasan and Baecker (1989), Azenha *et al*. (1995) and Namasivayam and Ranganthan (1998) reported that EDTA treatment was effective as a chelator for sludge bound metal-ions.

Table 1: Effect of sewage water and treated sewage water on germination, enzyme activities during germination μg g^{-1} f.m. h^{-1}), growth of seedlings (15-day-old) and yield (90-day-old) of bean

Sewage water	Treat-ments	Germ.	α-amylase	β-amylase	Protease	Shoot length cm	Shoot f.m (g)	Shoot d.m. (g)	Root length (cm)	Root f.m. (g)	Root d.m. (g)	No. of pods/ plant	Pod length (cm)	Pod f.m.	Pod d.m
0	Control	92.50	44.60	43.60	2.55	11.50	1.04	0.035	3.60	0.32	0.013	20.00	5.60	2.64	0.75
Sewage water	0	98.00	62.50	54.20	5.22	17.40	3.97	0.099	6.40	0.79	0.089	36.00	8.68	4.40	1.60
	Pptn.	90.00	45.40	43.30	2.77	11.70	1.03	0.055	3.70	0.35	0.018	21.00	5.70	2.88	0.56
	Rice residue	93.00	51.70	46.20	3.81	13.20	1.95	0.065	4.10	0.62	0.063	30.00	7.10	3.70	0.64
	EDTA	97.00	60.00	52.20	4.41	15.30	2.78	0.078	5.50	0.71	0.074	36.00	8.07	4.30	0.72
LSD at 0.05		0.87	0.41	0.42	0.07	0.19	0.36	0.005	0.06	0.004	0.002	0.50	0.12	0.13	0.005
LSD at 0.01		1.23	0.49	0.51	0.09	0.32	0.49	0.007	0.08	0.007	0.005	0.76	0.28	0.17	0.007

Pptn: Precipitation

Table 2: Effect of sewage water and treated sewage water on the leaf content of photosynthetic pigments, Hill reaction (μM [ferricyanide] g^{-1} chlorophyll s^{-1}), carbohydrate content (mg g^{-1} dm) and enzyme activities (μg g^{-1} fm h^{-1}) in leaves of bean seedlings (15-day-old)

Sewage water	Treatments	Chl. *a*	Chl. *b*	Caroten -oids	Total pigments	Hill reaction	Total soluble sugars	Polysacc-harides	Total carbohyd-rates	α-amylase	β-amylase	Invertase
0	Control	3.25	1.60	0.83	5.68	30.5	140.80	70.72	211.52	64.50	71.50	39.40
Sewage Water	0	6.50	2.90	1.73	11.13	57.2	154.40	75.50	229.90	72.10	79.30	46.60
	Pptn.	3.09	1.61	0.89	5.59	28.1	141.00	71.01	212.01	65.40	72.20	40.90
	Rice residue	5.50	2.54	1.62	9.60	49.7	144.00	76.00	220.00	70.30	76.20	43.20
	EDTA	3.99	2.06	1.032	7.082	54.9	151.30	74.70	226.00	71.00	78.20	45.10
LSD at 0.05		0.015	0.023	0.024	-	1.3	0.77	0.45	1.20	0.22	0.34	0.82
LSD at 0.01		0.019	0.026	0.032		1.8	0.81	0.55	1.90	0.34	0.64	1.40

Pptn: Precipitation

Soluble nitrogenous compounds e.g., peptides, total soluble nitrogen, as well as total nitrogen increased in response to sewage water, while the nitrate-N and amino acids nitrogen content decreased (Table 3). However precipitation, rice residue or EDTA treatments reduced the accumulation of peptides, total soluble nitrogen, as well as total nitrogen, while the nitrate-N and amino acids nitrogen content increased, particularly with precipitation treatment. The reduced content of amino acids by sewage water application may be attributed to the stimulation of nitrate reduction process and consequently the ammination process to form protein (Ito *et al*., 1991). Marschner (1986) reported that Mn at certain levels plays a direct role in oxidation-reduction reaction and play an essential role in protein biosynthesis. Boardmen (1975) stated that the role of Mn in NO_3-N reduction step is expected as a kind of indirect relationship between Mn toxicity and N-assimilation by plants.

Activities of α- and β-amylases, invertase (Table 2) and protease (Table 3) also increased in leaves of sewage water irrigated plants. This activation may be due to the uptake of some mineral ions e.g., Mn, Zn and Fe, which stimulate these enzymes and consequently increase the cellular content of soluble sugars, reducing sugars and peptides, as indicated in Table 2 and 3. On the other hand, application of precipitation, rice residue or EDTA decreased this activation, but still higher than that of the controls.

Concerning the protein banding patterns, which indicated the effect of heavy metals on gene expression, the appearance of protein bands having high molecular weights was the marked feature in the sewage water irrigated plants (Table 4 and Fig. 1). In general, the seeds of control plants showed 24 bands with molecular weights ranged between 195.4 and 5.3 KDa. Seeds produced from plants irrigated with sewage water exhibited a new 9

Table 3: Effect of sewage water and treated sewage water on the leaf content of nitrogenous compounds (mg g^{-1}dm), DNA and RNA (mg g^{-1} fm) and protease activity (μg g^{-1} fm h^{-1}) in leaves of bean seedlings (15-day-old)

Sewage water	Treatments	NO_3-N	Amino acid-N	Peptide-N	Total soluble-N	Total-N	DNA	RNA	Protease
0	Control	2.43	9.45	5.58	19.41	53.01	151.2	187.4	2.84
Sewage water	0	0.67	5.89	12.89	29.21	97.40	162.4	197.6	4.79
	Pptn.	2.21	9.41	6.70	20.50	59.60	151.0	188.0	3.00
	Rice residue	1.34	7.40	9.40	24.30	82.30	156.0	193.0	3.81
	EDTA	0.79	6.40	11.30	28.10	91.20	160.0	195.0	4.01
LSD at 0.05		0.13	0.25	0.65	0.59	2.80	1.6	2.4	0.06
LSD at 0.01		0.25	0.33	0.96	0.87	3.30	2.7	3.1	0.08

Pptn: Precipitation

Table 4: Protein profile of the produced seeds by bean plants in response to sewage and treated sewage water irrigation

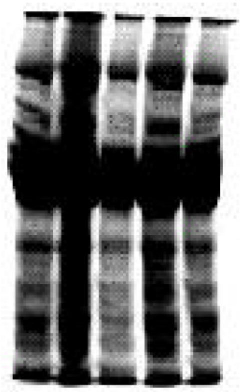
	Control		Sewage water		Sewage water+ Pptn.		Sewage water+ Rice residue		Sewage water+ EDTA	
Band No.	Relative Front	Mol. Wt.	Relative Front	Mol. Wt.	Relative Front	Mol Wt.	Relative Front	Mol Wt.	Relative Front	Mol Wt.
1	0.015	195.40	0.066	210.00	0.015	195.40	0.066	203.00	0.066	200.0
2	0.109	124.30	0.095	140.00	0.109	124.30	0.095	128.50	0.095	140.0
3	0.134	118.00	0.149	107.00	0.134	118.00	0.149	107.40	0.149	107.0
4	0.172	96.10	0.172	96.20	0.172	96.10	0.172	96.10	0.172	96.1
5	0.198	80.00	0.184	84.90	0.184	84.60	0.184	84.20	0.184	84.9
6	0.222	98.11	0.223	69.11	0.198	80.00	0.223	69.03	0.223	96.1
7	0.294	64.51	0.294	64.54	0.222	98.11	0.294	63.54	0.294	62.5
8	0.331	55.50	0.310	45.40	0.294	64.51	0.310	42.10	0.310	45.4
9	0392	50.40	0.321	53.50	0.310	45.10	0.321	48.40	0.321	53.5
10	0.414	47.74	0.414	47.73	0.331	55.50	0.414	47.23	0.414	46.1
11	0.454	46.10	0.451	46.11	0.392	50.40	0.451	46.00	0.451	46.1
12	0.497	45.94	0.493	45.84	0.414	47.74	0.493	38.11	0.493	45.8
13	0.542	36.86	0.541	36.84	0.454	46.10	0.541	36.72	0.541	36.8
14	0.609	32.50	0.610	32.70	0.497	45.94	0.610	32.40	0.610	32.7
15	0.679	30.11	0.681	30.43	0.542	36.86	0.681	30.43	0.681	28.4
16	0.712	29.50	0.710	29.45	0.609	32.50	0.710	29.45	0.710	29.5
17	0.736	29.20	0.760	29.20	0.679	30.11	0.760	29.20	0.760	29.2
18	0.813	28.50	0.813	28.40	0.712	29.50	0.813	28.20	0.813	28.4
19	0.826	25.40	0.874	25.80	0.736	29.20	0.874	25.50	0.874	25.8
20	0.875	20.10	0.895	22.40	0.813	28.50	0.895	22.40	0.895	22.4
21	0.935	17.50	0.932	17.40	0.826	25.40	0.932	17.40	0.932	17.4
22	0.949	13.30	0.949	13.90	0.895	22.10	0.949	13.70	0.949	13.9
23	0.978	9.11	0.978	9.11	0.935	17.50	0.978	9.11	0.978	9.1
24	0.984	5.3	0.991	7.30	0.949	13.3	0.991	-	0.991	-
25	-	-	-	-	0.978	9.11	-	-	-	-
26	-	-	-	-	0.984	5.3	-	-	-	-

Pptn: Precipitation

Table 5: Concentration of Cu, Zn, Pb and Cd ($\mu g\ g^{-1}$ dm) in the different plant organs of bean as affected by sewage and treated sewage water

Sewage water	Treatments	Heavy metals	Root	Stem	Leaves	Fruits
0	Control	Cu	10	8	8	4
		Zn	280	8	2	3
		Pb	0	0	0	0
		Cd	0	0	0	0
Sewage water	0	Cu	60	38	40	120
		Zn	810	330	600	760
		Pb	200	190	80	170
		Cd	160	58	58	82
	Pptn.	Cu	30	9	4	6
		Zn	520	320	160	80
		Pb	190	0	0	0
		Cd	110	0	0	0
	Rice residue	Cu	160	91	60	110
		Zn	770	70	66	60
		Pb	230	130	70	120
		Cd	160	48	40	61
	EDTA	Cu	280	92	86	120
		Zn	790	520	470	590
		Pb	790	180	160	780
		Cd	200	54	32	190

Pptn: Precipitation



1 2 3 4 5

Fig. 1: Electrophotograph of protein banding patterns of bean seeds produced from plants irrigated with: 1- control water, 2- sewage water, 3- sewage water treated with precipitation, 4- sewage water treated with rice residue and 5- sewage water treated with EDTA

polypeptides with molecular weight 210, 140, 107, 84.9, 69.11, 45.4, 53.5, 22.4 and 7.3 KDa. At the same time, 9 bands with M.Wt. of 195.4, 124, 118, 80, 98.11, 55.5, 50.4, 20.1 and 5.3 KDa disappeared, in comparison with the control. Seeds produced from plants irrigated with sewage water after precipitation treatment showed a more or less similar protein bands to that of the control plants, with only new three bands having molecular weights of 84.6, 45.1 and 22.1 KDa appeared. Seeds produced from plants irrigated with sewage water treated with rice residue, showed 23 bands and the mobilities ranged between 0.066 and 0.978. Major variation was indicated by the appearance of protein bands similar to that of sewage water irrigated plants, but have lower molecular weights e.g., 203, 128.5, 84.2, 69.03, 48.4 and 38.11 KDa. Only one protein band with mobility of 0.991 disappeared. EDTA treated sewage water resulted in seeds having only 23 bands. Three new bands with lower molecular weights (200, 62.54 and 28.43 KDa) than that of sewage irrigated plants were observed and also one band of mobility 0.991 was completely missed.

The appearance or disappearance of new bands was attributed either to alternation in the structural genes, or changes in the expression of regulatory genes involved in regulating these genes due to mutagenic effect of heavy metals present in sewage water. Mutational events occurring in the regulatory genes may lead to inhibition or constitutive expression of concerned genes and this will result in the disappearance of some bands or changes in some band intensities i.e., heavy metals present in sewage water result in an increase in the transcription of a number of stress-induced genes and lead to the accumulation of their polypeptides. Giordani *et al.* (2000) observed an accumulation of transcripts after exposure to trace metals such as copper and cadmium. The biological effects of heavy metals have been investigated for many years by many authors.

Irrigation of bean plants with sewage water led to accumulation of Zn, Cu, Pb and Cd in all plant organs (Table 5). Precipitation, rice residue, or EDTA treatments reduced this accumulation, but still more than plants irrigated with control water. Precipitation treatment was more effective. Antonovics *et al.* (1971) reported that one of the major concerns with the utilization of sewage water and sludge in agricultural crop production is the metal enrichment of plant tissue. Dowdy and Larson (1975) stated that the accumulation of metals in vegetable tissue was higher than in the edible fruit, root and tuber tissues following sewage application. Accumulation of Zn, Cu, Cd and Pb in plants irrigated with sewage water was also, observed by Maclean *et al.* (1987) and Berton *et al.* (1989).

CONCLUSIONS

In fact, the sewage water application increased germination percentage, growth, metabolic activities and the produced yield of bean, but the concentration of heavy metals, which have hazardous effects on human and animal health, was higher than permissible for health. Electrophoretic protein banding pattern, which is a mirror image for transcription events occurring during gene expression, indicated the appearance of new bands having higher molecular weights and disappearance of others. Finally, it could be suggested that sewage water must not be used without treatment to reduce the accumulation of heavy metals in the edible plants.

REFERENCES

Antonovics, J., A.D. Bradshow and R.G. Turner, 1971. Heavy metal tolerance in plants. Advan. Ecol. Res., 71-85.

Aronoff, S., 1946. Photochemical reduction of chloroplast grana. Plant Physiol., 21: 393- 409.

Ashwell, G., 1957. Methods in Enzymology. Inter-Science Publishers, Inc. New York.

Azenha, M., M.T. Vasconcelos and J.P.S. Cabral, 1995. Organic ligands reduce copper toxicity in *Pseudomonas syringae*. Environ. Toxicol. Che., 14: 369-373.

Belyuchenko, I.S. and A.V. Dronov, 1988. Contents of nitrate-nitrogen and heavy metals in soils and plants irrigated with sewage water. Akademi Sel' Skokozyaistvennykh Nauk, 11: 15-17.

Bergmeyer, H.U., 1974. Method of Enzymatic Analysis. Verlage Chemic, Winheim, Academic Press, New York. London.

Berton, R.S., O.A. Camargo and J.M.S. Valadares, 1989. Nutrient absorption by maize and response to addition of sewage sludge to five soils in Sao Paulo State. Revista Brasileira de Ciencia do Solo, 13: 187-192.

Boardmen, N.K., 1975. Trace Elements in Photosynthesis. In: Trace Elements in Soil-plant-animal Systems. Nicholas, P.J. and A.R. Yan, (Eds.), Academic Press, N.Y., pp: 199.

Brown, S., 1981. A comparison of the structure, primary productivityand transpiration of cypress ecosystems in Florida. Ecological Monographs, 51: 403-427.

Chaney, A.L. and E.P. Marbach, 1962. Modified reagents for determination of urea and ammonia. J. Chem., 8: 180-182.

Charkrabarti, C. and T. Charkrabarti, 1987. Effect of sewage water and sludge application on growth, enzyme activity and micronutrients in potato crop. IAWPC (Indian ASSOC Water Pollut. Control) Tech. Ann., 14: 67-78.

Dische, Z. and Z. Schwartz, 1973. Thin Layer Chromatography. Microchem. Acta, 2, 13. Cited by: Stahal, E. (Ed.), 2nd Edn., Springer Verlage, Berlin.

Dowdy, R.H. and W.E. Larson, 1975. The availability of sludge has borne metals to various vegetables crops. J. Environ. Qual., 4: 278-282.

Drewa, G., D. Andruszczak, M. Chesy, B.Kowalska, L. Kozica-Raszeja, K. Palgan and Z. Zbytniewski, 1993. Seasonal changes in the levels of detergents, chlorophyll (a) and oxygen in the Brada River. Polski Archiwan Hydrobiologii., 39: 153-160.

El-Maghraby, S.S. and M.A. Gomaa, 1992. Effect of sewage sludge as a fertilizer on syrup yieldand some agronomic characters of sweet sorghum. Egypt. J. Applied Sci., 7: 300-317.

Fresquez, P.R., R.E. Francis and G.L. Dennis, 1990. Sewage sludge effects on soil and plant quality in a degraded semi-arid grassland. J. Environ. Qual., 19: 324-329.

Giordani T, L. Natal, B.E. Maserti, S. Taddeiand and A. Cavallini, 2000. Characterization and expression of d DNA sequences encoding putative type-II metallothionins in the sea-grass *posidonia oceanica*. Plant Physiol., 123: 1571-1582.

Harangozo, M.G., A. Kovaces and M.M. Horvath, 1984. Examination of the effect of sewage disinfectant in broad bean seedlings. Botanikai-kolemenyek, 71: 221-223.

Heckman, J.R., J.S. Angle and R.L. Chancey, 1986. Soybean nodulation and nitrogen fixation in soil previously amended with sewage sludge. Biol. Fertil. Soils, 2: 181-185.

Ito, J., A. Tamura and T. Yamane, 1991. The effect of successive applications of sewage sludge to crops and soils. Bull. Shimane Agric. Exp. Station, 25: 84-100.

Kasan, H.C.and A.W. Baecker, 1989. An assessment of toxic metal biosorption by activated sludge from the treatment of coal-gasification effluent of a petrochemical plant. Water Res. Oxford., 23: 795-800.

Lewis, J.A., R.D. Lumsden, P.D. Minllner and A.P. Keinath, 1992. Suppression and damping of peas and cotton in the field with composts to sewage sludge. Crop Prod., 11: 260-266.

Lutrac, M.C., W.K. Robertson and J.A. Cornell, 1982. Heavy application of liquid-digested sludge on three ultisols: 11. Effect of mineral uptake and crop yield. J. Environ. Qual., 11: 288-287.

Maclean, K.S., A.R. Robinson and H.M. Macconnell, 1987. The effect of sewage sludge on the heavy metal content of soils and plant tissue. Commun. Soil. Sci. Anal., 18: 1303-1316.

Malik, C.P.and M.B. Singh, 1984. Plant Enzymology and Histo-Enzymology. Kalyani Publishers.

Marschner, H., 1986. Mineral Nutrition of Higher Plants. Academic Press.

Mejre, M.A. and J.K. Bulow, 2001. Chelation strategy in heavy metal resistance of plants. Special issue. Environmental contamination, toxicology and health. Chemosphere, 41: 197-207.

Mellberye, M.E., D.D. Hemphilland V.V. Volk, 1982. Sweet Corn growth on incinerated sewage sludge amended soil. J. Environ. Qual., 11: 160-163.

Metzener, H., H. Rau and H. Senger, 1965. Untersuchungen zur Synchronisier barteit einzelner Pigmentanangel-mutanten von Chlorella. Planta, 65: 186.

Naguib, M.I., 1969. Effect of various nitrogenous sources and/or colchicines on the colourimetry of nitrogen components of plant tissue. Bull. Fac. Sci., Cairo Univ., 43: 1-5.

Namasivayam, C. and K. Ranganthan, 1998. Effect of organic ligands on the removal of Pb, Niand Cd by waste Fe^{+3}/Cr^{+3} hydroxide. Water-Res., 32: 969-977.

Osman, M.E.H., H. Metzner and Karin Fisher, 1982. Effect of nitrate on thylakoid reaction. I. Influence of photosynthetic electron transport. Photosynthetica, 16: 7-12.

Roszyk, E., S. Spiak and S. Roszyk, 1989. The influence of sewage sludge on yield and chemical composition of plants. Polish. J. Soil. Sci., 22: 79-84.

Sancherz, P.G., L.P. Fernandez, L.T. Trejo, G.J. Elcantar and J.D. Cruz, 1999. Heavy metal accumulation in beans and its impact on growth and yield under soilless culture. International symmpositan on growing media and hydroponics. Acta Hortic., 481: 617-623.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 6th Edn., Iowa, State University Press, Ames.

Staniforth, A.R. and S.R. Smith, 1991. The use of sewage sludge-straw based compost as a fertilizer for agriculture. Agricultural Progress, 66: 23-35.

Umbriet, W.W., R.H. Burris, J. F. Stauffer, P.P. Cohen, W.J. Johanse, G.A. Lee Page, V.R. Potter and W.C. Schneider, 1959. Monometric technique a manual description method, applicable to study of describing metabolism. pp: 239. Burgess Publishing Company. (c.f., RazaK, A.A., 1976).

Xiong, L.M., 1994. Plant materials effect of cadmium adsorption by soil. Agrobiol. Res., 47: 160-167.

Zeid, I.M. and Z.A. Shedeed, 2006. Response of alfalfa to putrescine treatment under drought stress. Biol. Plant, 50: 635-640.