Pak. J. Bot., 44(2): 781-785, 2012.

WASTEWATER IMPACT ON PHYSIOLOGY, BIOMASS AND YIELD OF CANOLA (*BRASSICA NAPUS* L.)

IMDAD ULLAH KHAN[1]*, MUHAMMAD JAMIL KHAN[1], NAQIB ULLAH KHAN[2], MOHAMMAD JAMAL KHAN[2], HABIB UR RAHMAN[1], ZARINA BIBI[1] AND KALIM ULLAH[1]

[1]*Faculty of Agriculture, Gomal University, Dera Ismail Khan, Pakistan*
[2]*Khyber Pakhtunkhwa Agricultural University, Peshawar, Pakistan*
**Corresponding Author: imdadullah62@yahoo.com*

Abstract

The impact of domestic/municipal wastewater (mww) of Dera Ismail Khan, Pakistan was assessed through its effects on biomass, physiology and yield of canola (*Brassica napus* L.). The pot experiments were conducted in a completely randomized design with three replications in net house during winter season 2006-07 and 2007-08 at Gomal University, Dera Ismail Khan, Pakistan. Treatments included were T_0 (tube well/tap water), T_1 (20% mww), T_2 (40% mww), T_3 (80% mww) and T_4 (100% mww/raw-form municipal wastewater). The quality and chemical composition of wastewater was deviating from international (Anon., 1985) as well as NEQS (2005) standard. Analysis of wastewater showed that biochemical oxygen demand (BOD), chemical oxygen demand (COD), sodium adsorption ratio (SAR) and total suspended solids (TSS) were above the permissible limit of irrigation. In pods per plant, the reduction was 61.55% by recording 110 pods per plant with T_4 (100% mww) as compared to control T_0 (286.1 pods per plant). Similarly pod length (reduced by 59.72%), seeds per pod (reduced by 42.53%), Seeds per plant (reduced by 82%), seed weight per plant (reduced by 88%), 100-seed weight (reduced by 19.54%) and straw yield (reduced by 54.23%) were significantly reduced by applying 100% wastewater. The most affected yield contributing traits were seeds per plant and seed weight per plant with 82% and 88% reduction, respectively due to T_4 (100% mww). On average, the decrease was 60% in the first stage and a further decrement of 4.83% was observed when the obtained seeds were re-sown in 2007-08. Results revealed that utilizing municipal wastewater of the area under investigation for irrigation purpose of food and feed crops might not be safe. The major reason seems to be the high salinity and sodium adsorption ratio that restricted crop growth and yield.

Introduction

Continued environmental contamination of waters with heavy load of toxic metals has become a threat for existence of flora and fauna in the existing ecosystem of world (Shamsi *et al*., 1983). A variety of sewage pollutants like pathogen, oxygen-demanding wastes, organic and inorganic substances, plant food nutrients, radioactive, oil and grease all get their way into the environment (Abedi & Najafi, 2001). Though domestic sewage contains disease causing microbes but the major constituents observed in polluted water were organic chemicals and plant nutrients while studying biochemical aspects of rice grown with wastewater (Kakar *et al*., 2006; Al-Makhdoom, 2006). Heavy metals like Cu (Copper), Zn (Zinc), Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni) and selenium (Se) which released into water from industrial activities, automobile exhausts, mining and also from soils were observed in *Solanum melongena* by irrigating with wastewater (Al-Nakshabandi *et al*., 1997; Faryal *et al*., 2007; Khan *et al*., 2008; Mushtaq & Khan, 2010).

Developing countries like Pakistan produces huge amount of untreated domestic and industrial water that ultimately find its way to surplus water supplies. The wastewater being rich source of organic matter and plant nutrients and promote crop growth, used by poor farming communities for irrigation that also reduce dependency on chemical fertilizers. However, on the other hand, the untreated wastewater contains heavy metals (Cu, Zn, Pb, Cd, Cr, Ni) which are not only toxic to human health but also pollutes the environment (Al-Enezi, 2004). Prolonged and continuous use of wastewater for growing vegetables, fodders and other major crops may result in soil build up of heavy metals and salinity that may be phyto-toxic (Adriano, 1986; Ghafoor *et al*., 2004; Qadir & Oster, 2004).

Adverse effects of toxic elements present in municipal wastewater were observed on *Phaseolus mungo* and *Lens culinaris* crops with bad impact on soil properties and environment (Azmat & Khanum, 2005). Plant's life tied processes like photosynthesis, plant-water relation and mineral nutrition stress, as well as respiration due to Pb accumulation resulted dry root, stem and leaves with decrease intake of mineral ions like Na, K, Ca, Mg, Fe, Mn and Zn. Municipal sewage water of cities which contain heavy metals have inhibitory effect on plant chloroplasts and enzymes when excised leaves were exposed to solution and adverse effect on seed setting which eventually affect the crop yield (Clijsters & Assche, 1985). Khedkar & Dixit (2003) also mentioned that *Spinacea oleracea* irrigated with sewage water contained heavy *met*als, potentially toxic, which become part of our food-chain through plant uptake, however, their ratio of accumulation depends on its solubility in soil solution (Chaney, 1990).

Pakistan has a population of over 160 million and is one of the few countries that are almost completely dependent on a single river system for all its agricultural water demands. The Indus River and its tributaries provide water to over 16 million hectares of land, situated mainly in arid and semi-arid zones of the country. A rapidly growing population, saline groundwater, a poorly performing irrigation distribution system, and recurrent droughts have led to increased water shortages. Under these conditions, the use of untreated urban wastewater for agriculture has become a common and widespread practice. These experiments aims at investigating the wastewater use looking at environmental and health risks together with nutritive value of wastewater. Therefore, the present research work was undertaken to characterize the quality of municipal wastewater of Dera Ismail Khan, Pakistan and its effects on the yield related traits of canola (*B. napus* L.).

Materials and Methods

Experimental procedure and layout: Pot experiments were conducted in net house during 2006-07 and 2007-08 at Central Research Laboratory (CRL), Gomal University, Dera Ismail Khan, Pakistan. Dera Ismail Khan lies between 31°, 49′ North latitude and 70°, 55′ East longitude. Five treatments i.e. T_0 (tube well/tap water), T_1 (20% municipal wastewater, mww), T_2 (40% mww), T_3 (80% mww) and T_4 (100% or raw-form mww) were used in completely randomized design (CRD) with three replications. Fresh and healthy seeds of *B. napus* L. cultivar "Bulbul" was procured from Agricultural Research Institute, Dera Ismail Khan, Pakistan. Polyvinyl chloride (PVC) dust bins having volume of 7166 cm^3 were used as pots instead of earthen pots with the idea to restrict any leakage or absorption of wastewaters as is the likelihood in earthen pots. Soil was collected from agricultural fields having clay loam texture and around seven kg soil was filled in each pot. Six seeds were sown at two equidistant places in each pot during each year and after germination two seedlings were kept per pot. Municipal wastewater collected in labeled plastic cans in sufficient quantity from main drain passing through city near *Shobra Hotel* located near Army Training Center, Dera Ismail Khan, Pakistan and analyzed for various physico-chemical properties (Table 1). Various concentrations were made by fresh dilutions of wastewater before irrigation and were supplied to the plants as a soil drench. The recommended basal dose of NPK was also applied. Regular watering through calculated quantity of wastewater was made to the experiments during both years.

Table 1. Physico-chemical characteristics of wastewater, tube well water and FAO as well as National Environ. Quality Standards (NEQS).

Parameters	Concentration	MWW	TWW	FAO	NEQS
pH		10.31	8.01	6.5-8.5	6 to 9
EC	dSm-1	3.87	2.07	3	Nil
SAR		36.23	9.33	15	NIL
COD	mgL-1	1589	89	Nil	400
BOD	mgL-1	850	57	Nil	250
Grease +oil		14.3	Nil	Nil	10
CO_3	mgL-1	2.53	0.51	Nil	NGVS
HCO_3	mgL-1	11.19	2.8	600	NGVS
Cl^{-1}	mgL-1	2631	213	1100	1000
Cu	mgL-1	1.7	Nil	0.1	1
Fe	mgL-1	15.35	1.73	5	8
Zn	mgL-1	5.83	0.09	Nil	5
Mn	mgL-1	2.21	0.06	0.2	1.5
Ni	mgL-1	2.55	Nil	5	1
Cd	mgL-1	0.41	Nil	0.01	0.1
Pb	mgL-1	2.19	Nil	2	0.5
Cr	mgL-1	1.69	Nil	0.1	1
Sulphate	mgL-1	1435	135	1000	1000
Phosphorus	mgL-1	26	1.12	Nil	15
NO_3-N	mgL-1	87.2	5.27	Nil	Nil

NGVS = no guideline value set. * no set value either of the 2.

The pH and electrical conductivity (EC) of the wastewater sample was determined using pH and EC meter (Richards, 1954). The concentration of Ca plus Mg was determined by titration with EDTA, whereas Na and K were determined by flame photometer. The SAR was determined by the formula as given by Richards (1954):

$SAR = Na/ (Ca\ Mg)^{½}$

The chemical oxygen demand (COD) and biological oxygen demand (BOD) was determined by following standard procedure of Clesceri *et al*., (1989). The wastewater samples was collected in bulk from wastewater drain and were subsequently used for irrigating the crop and the heavy metals (Cu, Fe, Mn, Zn, Ni, Cd, Pb and Cr) were determined using atomic absorption spectrophotometer. Nitrogen (NO3-N) in water sample was determined using kjeldahl method.

Traits measurement and statistical analysis: Data were recorded during both years on reproductive growth parameters viz., pods per plant, pod length (cm), seeds per pod, seeds per plant, seed weight per plant (g), 100-seed weight (g) and straw yield per plant (g). Data were subjected to analysis of variance (ANOVA) techniques appropriate for CRD to compare mean differences as outlined by Steel and Torrie (1980). Regression analysis was also carried out according to Gomez & Gomez (1984).

Results and Discussion

Physico-chemical make-up of the municipal wastewater: The physico-chemical composition of municipal wastewater showed that pH of the raw-form municipal wastewater was 10.31 of extreme basicity. Likewise, other chemical characteristics i.e. electrical conductivity (EC) and sodium adsorption ratio (SAR) were 3.87 and 36.23, respectively and were higher than FAO standards. Moreover, both chemical oxygen demand (COD = 1589 mg L^{-1}) and biochemical oxygen demand (BOD = 850 mg L^{-1}) were on the upper-most level of NEQS i.e., 400 and 250, respectively. The concentration of carbonates (CO_3) 2.53 and bi-carbonates (HCO_3) 11.9 were also higher than the recommendations (Anon., 1992). The concentration of heavy metals was higher than the permissible limits of set by FAO for irrigation purposes. The analysis of the water indicates that pH and higher SAR values may be irrigation limiting factors determining its suitability for irrigation.

Effect of wastewater on reproductive growth parameters: Physical appearance of the *B. napus* plants was heading toward weakness after 3rd weak of germination and as the irrigation of various concentrations of wastewater progressed. Both fresh as well as dry weights were reduced significantly and the reduction in yield was linear being maximum when 100% wastewater was used for irrigation. Plants in control (T_0) and 20% mww (T_1) concentrations pots resembled i.e. the differences were non-significant as compared to 40% mww (T_2), 80% mww (T_3) and 100% mww (T_4). Results revealed that from T_2 to T_4, there was a gradual and up-stair toxic effects, and adversely affect the plant health as wastewater concentration was enhanced from 40-100% (Fig. 1 & 2). It was also noticed that reproductive pods per plant reduced from 286.1 (T_0) to 110 (T_4) and 90.0 to 69.2 during 1st and 2nd years of study, respectively (Fig.1a). Similarly, the reduction in pod length was from 7.15 to 2.88 cm and 6.85 to 2.58 cm (Fig. 1b), seeds per pod from 17.28 to 9.93 and 17.08 to 9.63 (Fig. 1c), seeds per plant from 4497.74 to 805.13 and 4630.9 to 691.32 (Fig. 1d), seed weight per plant from 87.4 to 10.08 g and 81.95 to 7.13 g (Fig. 2e), and 100-seed weight reduced from 17.4 to 14 g and 17.5 to 11.5 g (Fig. 2f & Table 2). All the reproductive growth parameters were declined steeply and were directly proportional to the increase in wastewater concentration. However, overall the decline was 61.55% in pods per plant, 59.72% in pod length, 42.53% in seeds per pod, 82% in seeds per plant, 88% in seed weight per plant, 19.54% in 100-seed weight, and 54.23% reduction was noticed in straw yield (Fig. 1 & 2). The worst affected yield traits were seeds per plant and seed weight per plant with 82 and 88% decrease, respectively. However, over 1st and 2nd year, the decline was 60 and 64.83%, respectively. Similar results have been reported by Kafeel *et al*., (2011), Bazai & Achakzai (2006), Farid (2006) Kang *et al.,* (2007) and Khan *et al*., (2009) in canola. Adverse and toxic effects of municipal wastewater on the growth performance and yield of certain vegetables i.e. spinach, lettuce, carrot, radish and sugar beet was also observed by Tamoutsidis *et al*., (2002). The decreased and unsatisfactory growth because of irrigation with metal contaminated wastewater has also been reported in sunflower (*H. annuus* L.) (Saxena, 1987; Meagher, 2000; Andaleeb *et al*., 2008). Results indicated that the major adverse effect on plant growth emerged due to high pH and SAR whereby the heavy metals were present in higher concentration but the elevated pH is not conducive to the phytoavailability, thus the reduction might not be due to toxicity of metals.

Table 2. Regression coefficients for various reproductive growth traits in *B. napus* L.

Parameters	Year	A	B	r2
Pods per plant	1	-1.7855	264.1	0.9943
	2	-1.826	284.23	0.9903
Pod length	1	-0.046	7.319	0.972
	2	-0.046	7.06	0.982
Seeds per pod	1	-0.083	17.73	0.962
	2	-0.086	17.68	0.947
Seeds per plant	1	-37.74	4436.9	0.993
	2	-40.49	4484.63	0.983
Seed weight per plant	1	-0.82	88.33	0.986
	2	-0.82	82.45	0.959
100 seed weight	1	-0.03	17.77	0.935
	2	-0.06	18.09	0.972
Straw yield	1	-0.24	43.33	0.969
	2	-0.29	43.76	0.978

A, b and r^2 are the intercept, linear coefficients and coefficients of determinations with $p \leq 0.01$.

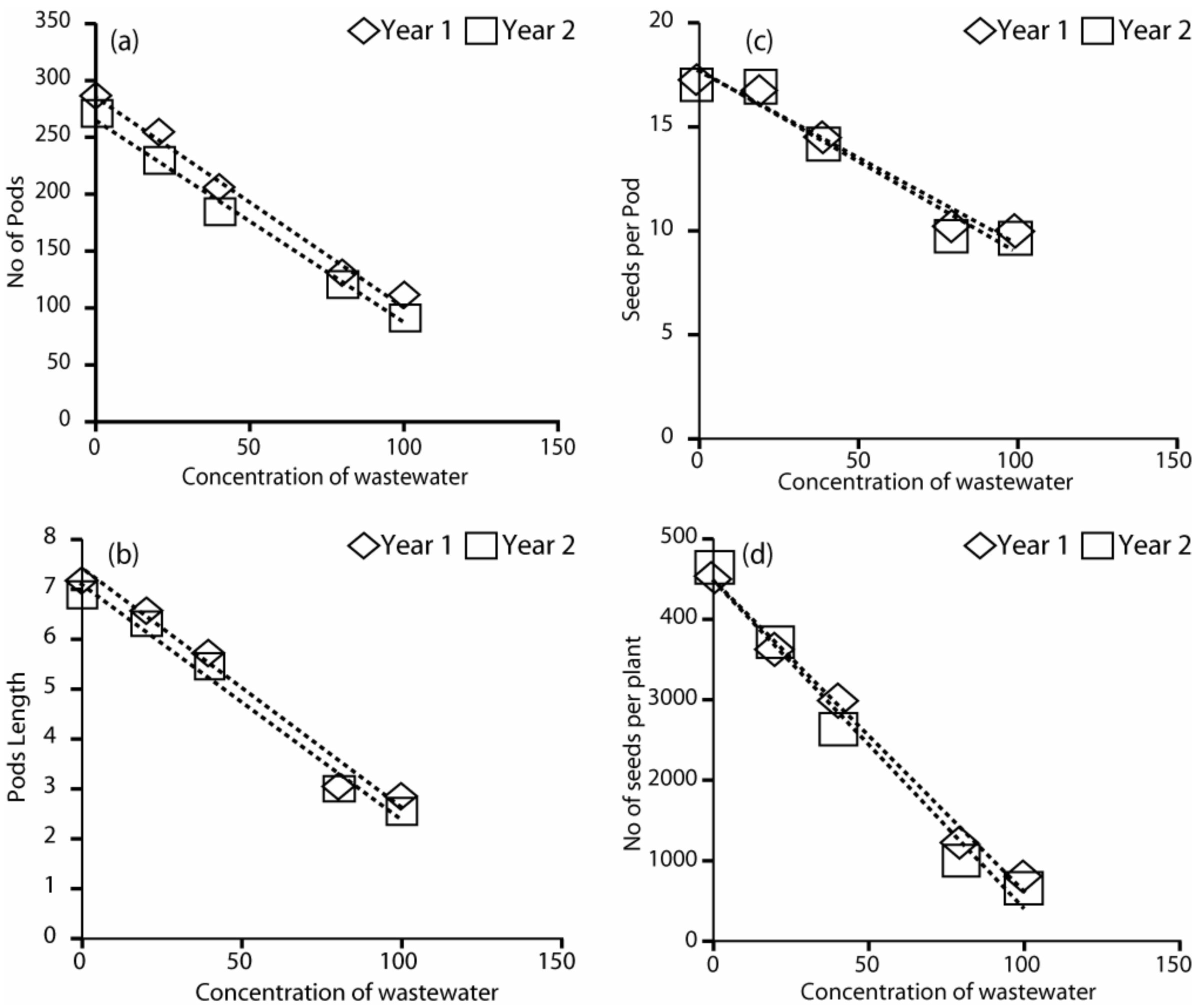

Fig. 1. Effect of various concentrations of wastewater on reproductive growth traits of *B. napus* L.

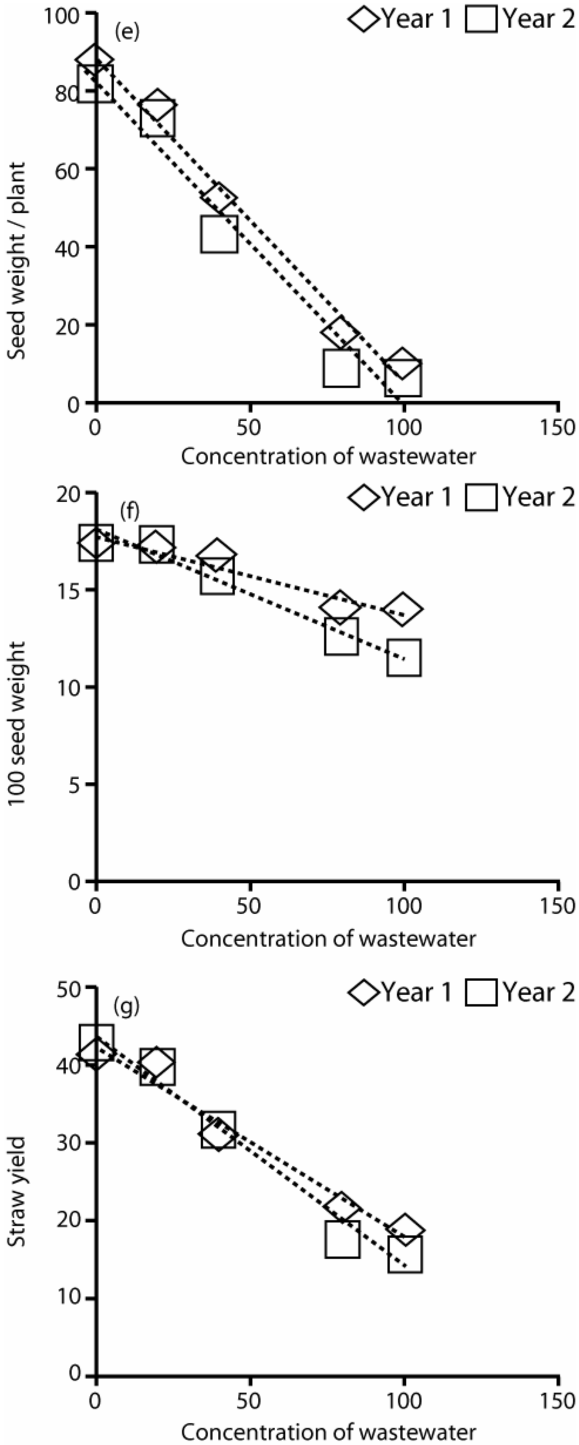

Fig. 2. Effect of various concentrations of wastewater on reproductive growth traits of *B. napus* L.

2nd Year experiment: After getting the seed from harvested plants of 1st year experiment (2006-07), the same experiment was repeated during 2007-08 and more severe effects were noticed during 2nd year. In 2nd year experiment, it was observed that there was further reduction in number of pods and the higher reduction was noted in T_4. Similarly pod length was reduced by 3.61% in T_4. There was also reduction in the number of seeds and the decrease was from 8.98 to 5.22% in T_2 and T_3 and seed weight per plant reduced from 7.36 and 9.7% in T_2 and T_3, respectively. As mentioned before, that high pH and SAR along with heavy metal might have adversely affected seed setting and plant's life tied processes. Results revealed that 100 seed weight was also reduced by 9.04 and 14.74% in T_3 and T_4, respectively whereas, vegetative growth reduced by 11.14 and 8% in T_3 and T_4, respectively. In physico-chemical analysis of wastewater, it was evident that different heavy metals like Cu, Zn, Fe, Mn, Cd and Pb were present in varying concentrations. These heavy metals became obstruction and restricted various plant physiological and growth processes. Ahmad *et al*., (2011) also proved that wastewater has higher concentration of heavy metals which are potent to retard plant growth and development and adversely affect the yield. Moreover, with application of wastewater the pH, EC as well as SAR values of the soil increases which change the environment of rhizosphere into saline one and also retard the plant growth. Similar findings have also been mentioned by Raziuddin *et al*., (2011) and Ahmad *et al*., (2011) in canola. Stobart *et al*., (1985) and Somashekaraiah *et al*., (1992) reported severe chlorosis on older leaves but less on younger leaves and decline in chlorophyll content in shoots of metal treated plants resulted mostly from its enhanced degradation or reduced synthesis in germinating seedlings of *Vigna radiata*. Panda & Choudhary (2005) observed disturbance in nutrient uptake and metabolism as a result of increased metal content in the growth environment, caused reduction in overall growth of many plants. Similarly, Sharma & Dubey (2005) reported that flower and pod senescence as a consequence of metal toxicity which leads to production of less number of viable pods and seeds, reduced yield under metal stress in rice crop. Likewise, Hussain *et al.*, (2006) conducted pot experiment on the effect of Lead and Chromium applied @ 20 and 40 mg L^{-1}, respectively on growth performance of mash bean and found that both metals retarded the growth as compared to control.

Results revealed that straw yield was gradually decreased as the concentration of wastewater increased from 20 to 80/100% mww (Fig. 2g & Table 2). The decreased response of straw yield was linear to the different wastewater concentrations during both years. The increased concentrations of wastewater also raise the soil pH, EC and SAR values and soil turned to saline which reduce the plant ability to absorb nutrients needed for vegetative growth. Kaker *et al*., (2010) and Raziuddin *et al*., (2011) also determined similar results in *B. napus* and *B. juncea*. Smits and Pilon (2002) observed that Fe^{2+} and Cu^{2+} cause oxidative stress. Others like Hg^{2+} and Cu^{2+} being very reactive to thiol (-SH) groups and interfere with protein structure and functions. Chhonkar (2004) and Kakar *et al*., (2010) also mentioned that there could be no likelihood of major differences in the physico-chemical properties and toxic metals of municipal wastewater of various locations.

Conclusion

Municipal wastewater application reduced plant vegetative as well as reproductive growth in addition to adverse effects on soil health and environment and it was probably due to High pH, SAR and salinity. Therefore, standard measures must be exercised where municipal wastewater is the only option to irrigate the crops.

References

Abedi, M.J. and P. Najafi. 2001. *Wastewater treatment and use in agriculture*. Report of Iranian National Committee on Irrigation and Drainage, pp. 240.

Adriano, D.C. 1986. Trace Elements in the Terrestrial Environment. Springer-Verlag New York Inc. pp. 390-420.

Al-Enezi, G.M.F. Hamoda and N. Fawzi. 2004. Ion exchange extraction of heavy metals from wastewater sludges. *J. Environ. Sci. Health*, 39(2): 455-464

Al-Makhdoom, M. 2006. Some biochemical aspects of rice plants grown in soils affected by wastewater. *Water Res*., 6(1): 225-229.

Al-Nakshabandi G.A., M.M. Saqqar, M.R. Shatanawi, M. Fayyad and H. Al-Horani. 1997. Some environmental problems associated with the use of treated wastewater for irrigation in Jordan. *Agric. Water Manag*. 34(1): 81-94.

Andaleeb, F., M.A. Zia, M. Asharaf and Z.M. Khalid. 2008. Effect of chromium on growth attributes in sunflower (*H. annuus* L.). *J. Environ. Sci.*, 20(12): 1475-1480.

Anonymous. 1992. FAO. Wastewater Treatment and Use in Agriculture. Corporate Document Repository, Natural Resources Management and Environment Department.

Azmat, R. and R. Khanum. 2005. Effect of chromium on uptakes of minerals in Bean plant. *Pak. J. Biol. Sci*., 8(2): 281-283.

Bazai, Z.A. and A.K.K. Achakzai. 2006. Effect of wastewater from Quetta city on the germination and seedling growth of lettuce (*Lactuca sativa* L.). *J. Appl. Sci*., 6(2): 380-382.

Chaney, R.L. 1990. Twenty years of land application research, Part-1. *Bio Cycle,* 31: 54-59.

Chhonkar, P.K. (2004) Phytoremediation: A 'Green Cure' for heavy metal contaminated soils. *J. Indian Soc. Soil Sci*., 52(4): 357-373

Clesceri, L.S., A.E. Greenberg and R. Trussell. 1989. Standard methods for examination of water and wastewaters. 17th ed. Amer. Publ. Health Assoc. and Amer. Water user Assoc. pp.754.

Clijsters, H. and F.V. Assche. 1985. Inhibition of photosynthesis by heavy metals. *Photosynth Res*., 7: 31-40.

Farid, S. 2006. Status of cadmium concentration in soil and vegetable irrigated with city effluents. *China J. App. Env. Biol*., 12(3): 414-419.

Faryal, R., F. Tahir and A. Hameed. 2007. Effect of wastewater irrigation on soil along with its micro and macro flora. *Pak. J. Bot*., 39(1): 193-204.

Ghafoor, A., M. Qadir, M. Sadiq, G. Murtaza and M.S. Brar. 2004. Lead, copper, zinc and iron contaminations in soils and vegetables irrigated with city effluent on urban agricultural lands. *J. Ind. Soc. Soil Sci*., 52: 114-117.

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research, 2nd ed. John Wiley and Sons Ltd., Singapore.

Hussain, M., M.S.A. Ahmad and A. Kausar. 2006. Effect of lead and chromium on growth, photosynthetic pigments and yield components in mash bean. *Pak. J. Bot*., 38(5): 1389-1396.

Kafeel, A., A. Ejaz, M. Azam, Z.I. Khan, M. Ashraf, F. Al-Qurainy, A. Fardous, S. Gondal, A.R. Bayat and E.E. Valeem. 2011. Lead, cadmium and chromium contents of canola irrigated with sewage water. *Pak. J. Bot*., 43(2): 1403-1410.

Kakar, R.G., M. Yasinzai, A.U. Salarzai, F.C. Oad and M.H. Siddiqui. 2006. Irrigation with sewage water: Assessment of water quality, nutrients and heavy metal distribution. *Asian J. Plant Sci*., 5(3): 438-440.

Kakar, S.U.R., A. Wahid, R.B. Tareen, S.A. Kakar, M. Tariq and S.A. Kayani. 2010. Impact of municipal wastewater of Quetta city on biomass, physiology and yield of canola (*B. napus* L.). *Pak. J. Bot*., 42(1): 317-328.

Kang, M.S., S.M. Kim, S.W. Park, J.J. Lee and K.H. Yoo. 2007. Assessment of reclaimed wastewater irrigation impact on water quality, soil and rice cultivation in paddy fields. *J. Environ. Sci. Health*, 42(4): 439-445.

Khan, M.A., S.S. Shaukat and M.A. Khan. 2008. Economic benefits of irrigation of maize with treated effluent of waste stabilization ponds. *Pak. J. Bot*., 40(3): 1091-1098.

Khan, M.A., S.S. Shaukat and M.A. Khan. 2009. Growth, yield and nutrient content of sunflower (*H. annuus* L.) using treated wastewater from waste stabilization ponds. *Pak. J. Bot*., 41(3): 1391-1399.

Khedkar, D.D. and A.J. Dixit. 2003. Effects of wastewater on growth pattern of *Spinacea oleracea. Res. J. Environ. Pollut.* 2(4): 45-49.

Meagher, R.B. 2000. Phytoremediation of toxic elemental and organic pollutants. *Current Opinion in Plant Biol.*, 3: 153-162.

Mushtaq, N. and K.S. Khan. 2010. Heavy metals contamination of soils in response to wastewater irrigation in Rawalpindi region. *Pak. J. Agri. Sci*., 47: 215-224.

NEQS, 2005. National Environmental Quality Standards for municipal and liquid industrial effluents.

Panda, S.K. and S. Choudhary. 2005. Chromium stress in plants. *Braz. J. Plant Physiol.,* 17(1): 95-102.

Qadir, M. and J.D. Oster. 2004. Crop and irrigation management strategies for saline-sodic soils and waters aimed at environmentally sustainable agriculture. *Sci. of the Total Environ*., 323: 1-19.

Raziuddin, Farhatullah, G. Hassan, M. Akmal, S.S. Shah, F. Mohammad, M. Shafi, J. Bakht and W. Zhou. 2011. Effects of cadmium and salinity on growth and photosynthesis parameters of *B.* species. *Pak. J. Bot*., 43(1): 333-340.

Richard, L.A. 1954. Diagnosis and improvement of saline and alkali soils USDA. Agri. Hand book 60.

Saxena, M.C. 1987. Agronomy of chickpea. 207-232 *In*: M.C. Saxena and K.B. Singh, (Eds.). The Chickpea C.A.B. International, Wallingford, Oxen, U.K.

Shamsi, S.R.A., I. Hussain, S. Naheed and D. Ariz. 1983. On the quality and biota of fresh and polluted waters of Deg Nullah (stream) in Pakistan. *Pak. J. Sci. Res*., 35(2): 163-169.

Sharma, P. and R.S. Dubey. 2005. Lead toxicity in plants. *Braz. J. Plant Physiol.,* 17(1): 35-52.

Smits, E.P. and M. Pilon. 2002. Phytoremediation of metals using transgenic plants. *Critical Rev. in Plant Sci.*, 21: 439-456

Somashekaraiah, B.V., K. Padmaja and A.R.K. Prasad. 1992. Phytotoxicity of cadmium ions on germinating seedlings of mungbean (*Phaseolus vulgaris*): Involvement of lipid peroxides in chlorophyll degradation. *Physiol. Plantarum*, 85(1): 85-89

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics, a biological approach, 2nd ed. Mc Graw Hill, Inc. New York, Toronto, London.

Stobart, A.K., W.T. Griffiths, I.A. Bukhari and R.P. Sherwood. 1985. The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. *Physiol. Plantarum,* 63(3): 293-298.

Tamoutsidis, E.I., S. Papadopoulos, J. Zotis and T. Mavropoulos. 2002. Wet sewage sludge effect on soil properties and element content of leaf and root vegetables. *J. Plant Nutr*., 25: 1941-1955.

(Received for publication 08 March 2010)