Journal of Applied and Natural Science 4 (2): 291-296 (2012)

JANS

Enrichment of various metals in *Abelmoschus esculentus* grown in wastewater irrigated soil area of Dehradun city, India

Chakresh Pathak*, A. K. Chopra and Sachin Srivastava

Department of Zoology and Environmental Science Gurukula Kangri University, Haridwar-249404 (Uttarakhand), India
Corresponding authors E-mail:chakreshpathak@yahoo.co.in

Received:August 6, 2012; Revised received:September 29, 2012; Accepted:November 8, 2012

Abstract: The accumulation of metal contents in soil from wastewater (WW) irrigation is a cause of serious concern due to their potential accumulation in vegetables and food products growing in such areas. The results revealed the concentration of Pb (0.17±0.03 mg/l), Cu (0.10±0.04 mg/l), Zn (1.06±0.08 mg/l), Ni (0.08±0.02 mg/l), Cd (0.07±0.02 mg/l) and Cr (0.07±0.02 mg/l) in the WW of Bindal river used for irrigation of *Abelmoschus esculentus*. The maximum metal contents were observed for Pb (43.89±6.13 mg/kg), Cu (20.92±3.19 mg/kg), Zn (46.77±6.51 mg/kg), Ni (39.95±8.02 mg/kg), Cd (15.57±2.17 mg/kg) and Cr (125.70±23.01 mg/kg). The enrichment factors (EF) in WW irrigated soil were found to be in the order of Cd (2.46) > Cr (2.42) > Zn (1.67) > Cu (1.36) = Ni (1.36) > Pb (1.31). The concentration of Pb was maximum (57.99±1.54 mg/kg) in roots, Cu (33.91±2.13 mg/kg) in the leaves; Zn (81.70±2.99 mg/kg) in roots, Ni (86.10±3.19 mg/kg) in stem; Cd (20.39±1.99 mg/kg) and Cr (76.78±3.04 mg/kg) in leaves of *A. esculentus*. The EF values of 2-5 for Pb, Cu, Ni, Cd and Cr except Zn for fruits, leaves and roots of *A. esculentus* showed moderate enrichment of these metals. The values for Cd and Cr showed moderate enrichment of the stem of this plant. The study concluded that the WW irrigation increased the metallic contents of soil, which in turn were accumulated in different parts of *A. esculentus* that may cause potential health risk in human beings.

Keywords: *Abelmoschus esculentus*, Enrichment factor, Heavy metals, Soil, Wastewater

INTRODUCTION

The presence of heavy metals in the atmosphere, water, soil and food products represent a critical problem in most of the countries all over the world. Wastewater (WW) irrigation is practiced not only due to water scarcity but also due to nutritional value and environmental protection (Jagtap *et al*., 2010). The WW is recognized to have direct effect on the chemical properties of the soil. It affects supply of minerals- macro and micro nutrients for plant growth, soil pH, soil buffer capacity, and soil cation exchange capacity. It also increases soil nitrogen, phosphorus and potassium, while heavy metal levels tend to generally increase in soil with the increase in number of years of irrigation (Rusan *et al*., 2007). Long-term use of WW on agricultural lands contributes significantly to the buildup of the elevated levels of these metals in the soils and plants (Nyamangara and Mzezewa, 1999, Mapanda *et al*., 2005 and Sharma *et al*., 2007) which is of serious concern. The industrial WW carries appreciable amount of toxic metals including heavy metals (Cao and Hu 2000, Nan *et al*., 2002, Singh *et al*., 2004 and Mapanda *et al*., 2005).

Incidence of metallic poisoning arising from contamination of water, soil, food and feeding stuffs are well documented (Ezeonu, 2004). Trace metals may result to cardiovascular disease, depressed growth, impaired fertility, nervous and immune system disorders, increased spontaneous abortions, and elevated death rate among infants (Yuzbas *et al*., 2003). The presence of pollutants like heavy metals in urban and industrial WW results in contamination of water and soil. Household effluents, drainage water, business effluents atmospheric deposition and traffic related emissions transported with storm water into sewage system carry number of pollutants and enrich the urban WW with heavy metals (Oliveria, 2007).

The present study was undertaken to evaluate the concentration of various metals (Pb, Cu, Zn, Ni, Cd and Cr) in WW and their enrichment in soil and the plant Abelmoschus esculentus (Lady's finger) grown in WW irrigated soil of Dehradun city.

MATERIALS AND METHODS

Study area: Dehradun city, the capital of Uttarakhand state is located on 30° 19' North latitude and 78° 04' East longitude. The area being irrigated WW for a long term of about 15 to 20 years, is situated near Bindal river, on Haridwar-bypass road in Dehradun city. The river passes through the middle of the city being converted in to a WW channel with discharge of WW of city. The WW is used for irrigation in nearby agricultural fields where the plant Lady finger's (*Abelmoschus esculentus*) is grown near Bindal river area (Site I) during monsoon season. The plant *A. esculentus* grown in Tube well water (TWW) irrigated area of Guler ghati (Site II) about 10-12 Km away

ISSN : 0974-9411 (Print), 2231-5209 (Online)

from Bindal river area was taken as control.

Sampling: Water samples of the WW channel of Bindal river were collected in one litre polythene bottles, immediately acidified with HNO_3 and transported to the laboratory where these samples were filtered through Whatman 42 filter paper and stored in refrigerator at 40^0C. Soil samples were collected from the root area of *A. esculentus*. Composite samplings were carried out by taking soil samples from selected four experimental plots. The soil and Lady finger's plant samples of the same plot were composited separately for analysis. The different parts of the plants samples were washed with double distilled water and were separated manually and dried at room temperature. The different parts were chopped separately and dried in hot air oven at 60ºC for 48 hrs. The samples were then ground into powder form and stored into the polythene bags separately for further analytical use. Soil samples were dried in air at room temperature and sieved through 2 mm sieve.

Analysis: The water samples (10 ml), dried soil and grinded different parts of Lady's finger (0.5 gm) samples were digested with 10 ml of concentrated Nitric acid and 5 ml of concentrated Perchloric acid 80ºC until the solution became transparent (APHA, 2005). The solution was diluted with distilled water, filtered through Whatman No. 42 filter paper and volume was made up to 50mL. Heavy metals such as Lead (Pb), Nickel (Ni), Zinc (Zn), Cadmium (Cd), Copper (Cu) and Chromium (Cr) in the digested aliquots were determined by Atomic Absorption Spectrophotometer (AAS) (ECIL, Model No. 4129).

Enrichment factor (EF): The enrichment factor (EF) was used to evaluate the soil contamination in respect to heavy metals in soil and in plant grown on contaminated site with reference to soil and plant grown on uncontaminated soil (Kisku *et al*., 2000).

$$EF = \frac{\text{Concentration of metals in soil or plant parts at contaminated soil}}{\text{Concentration of metals in soil or plant parts at uncontaminated soil}}$$

RESULTS AND DISCUSSION

Heavy metal contents in wastewater used for irrigation

The mean ± SD values of heavy metals viz. Pb, Cu, Zn, Ni, Cd and Cr in WW channel of Bindal river used for irrigation of agricultural soil where Lady finger's (*A. escluentus*) being grown (Site I) and TWW of Guler ghati (Site II) of Dehradun being grown of Lady finger's are given in Table 1.

The concentration of heavy metals viz. Pb (0.17±0.03 mg/l), Cu (0.10±0.04 mg/l), Zn (1.06±0.08 mg/l), Ni (0.08±0.02 mg/l) and Cr (0.07±0.02 mg/l) in WW used for irrigation of soil were below the safe limit, while that of Cd (0.07±0.02 mg/l) was above the safe limit (Pescod, 1992). The present mean values of Pb, Cu, Zn concentration of WW were lower than the mean metal concentrations of Pb (4.26 mg/l), Cu (1.56 mg/l), Zn (1.91 mg/l) and Ni (0.68 mg/l) of the water used for irrigation at peri-urban region of Titagarh reported by Gupta *et al.* (2008) and also lower than Cd (1.015±0.005 mg/l), Cr (1.121±0.001mg/l), Cu (1.333±0.153 mg/l), Pb (0.212±0.081 mg/l) and Zn (2.267±0.153 mg/l) in water used for irrigation at Ramgarh Lake, Gorakhpur reported by Singh *et al.* (2011).

Table 1. Heavy metals content in water used for irrigation at Site I (ES) and Site II (CS) during monsoon season (2008-09).

Metals	Site I	Site II	Safe limit[a]
Pb	0.17±0.03*	0.03±0.01	0.5
Cu	0.10±0.04*	0.03±0.00	0.2
Zn	1.06±0.08*	0.03±0.01	2.0
Ni	0.08±0.02	BDL	0.2
Cd	0.07±0.02	BDL	0.01
Cr	0.07±0.02	BDL	0.1

Significance: $p<0.05$ (*), BDL-Below detection limit (**a)** Safe limit of heavy metals in irrigation water for agricultural purpose (Pescod, 1992).

The paired two sample 't' test for the metals such as Pb, Cu, Zn, Ni, Cd, and Cr revealed that the concentrations of Pb, Cu, Zn were significantly ($P<0.05$) higher in WW, while that of Ni, Cd and Cr were insignificantly ($P>0.05$) higher in WW in comparison to the control TWW (Table 1). Positive correlations were found for Zn-Pb (0.16864), Zn-Cu (0.370224), Ni-Pb (0.604075), Ni-Zn (0.887386), Cd-Cu (0.998073), Cd-Zn (0.311863), Cr-Cu (0.947107), Cr-Zn (0.052526), and Cr-Cd (0.965196) (Table 2).

Heavy metal contents in soil: During the present study, the concentrations of heavy metals like Pb (43.89±6.13 mg/kg), Cu (20.92±3.19 mg/kg), Zn (46.77±6.51 mg/kg), Ni (39.95±8.02 mg/kg), Cr (125.70±23.01 mg/kg) in WW irrigated soil were below the permissible limits of Indian standards (Awashthi, 2000) while that of Cd (15.57±2.17 mg/kg) was above the permissible limit of Indian standards (Table 3).

The level of heavy metals in the soil was lower than that reported by Mishra and Tirpathi (2008) for Pb (123.50±18.40 mg/kg), Cu (77.8±15.2 mg/kg), Zn (122.30±17.90 mg/kg) and lower for Cd (3.4±1.1 mg/kg) and Cr (56.3±8.9 mg/kg) in the soil irrigated with treated wastewater at Varanasi and also than the values reported by Gupta et al. (2008) for Pb (130.45±19.43 mg/kg), Cu (89.98±21.79 mg/kg), Zn (217.08±25.46 mg/kg), Ni (103.67±17.53 mg/kg), Cd (30.72±5.67 mg/kg), Cr (148.41±19.35 mg/kg) in WW irrigated soil at Titagarh, West Bengal, India. Pathak *et al.* (2010) also found higher concentration of Pb (86.41±36.37 mg/kg), Cu (57.24±13.88 mg/kg), Zn (211.96±19.77 mg/kg), Ni (59.09±42.54 mg/kg) and lower concentration of Cr (21.21±1.45 mg/kg) and Cd (6.50±0.66 mg/kg) in agricultural soil near Bindal river during summer season in the year of 2008 and also higher for Pb (83.22±2.50 mg/kg), Cu (90.13±9.41 mg/kg), Zn (254.65±32.95 mg/kg) and lower for Ni (35.27±2.51 mg/kg)

Table 2. Correlation coefficient between metal levels in WW used for irrigation.

Metals	Pb	Cu	Zn	Ni	Cd	Cr
Pb	1					
Cu	-0.8532	1				
Zn	0.168643	0.370224	1			
Ni	0.604075	-0.09974	0.887386	1		
Cd	-0.88392	0.998073	0.311863	-0.16129	1	
Cr	-0.97546	0.947107	0.052526	-0.41378	0.965196	1

Table 3. Heavy metals content in soil at Site I (ES) and Site II (CS) during monsoon season (2008-09).

Metals	Site I	Site II	EF	Permissible limits of Indian standard
Pb	43.89±6.13 (+30.66%)	33.59±4.07	1.31	250-500
Cu	20.92±3.19* (+36.38%)	15.34±1.88	1.36	135-270
Zn	46.77±6.51* (+66.32%)	28.08±6.52	1.67	300-600
Ni	39.95±8.02* (+36.54%)	29.26±7.84	1.36	75-150
Cd	15.57±2.17* (+150.32%)	6.22±1.09	2.46	3–6
Cr	125.70±23.01* (+141.94%)	51.96±4.04	2.42	n/a

% increase/decrease in comparison to control site, Significance: $p<0.05$(*), EF: Enrichment factor, n/a: not available

Table 4. Correlation coefficients between metal levels in WW irrigated soil.

Metals	Pb	Cu	Zn	Ni	Cd	Cr
Pb	1					
Cu	-0.12917	1				
Zn	-0.95985	-0.15417	1			
Ni	-0.4285	-0.84063	0.664743	1		
Cd	0.175573	-0.9989	0.107624	0.814275	1	
Cr	-0.37822	0.966814	0.103366	-0.67436	-0.97774	1

Cd (5.42±1.32mg/kg) Cr (25.22±2.38 mg/kg) in sewage water irrigated soil at Haridwar reported by Pathak et al. (2011). In present study, there was a remarkable increase of Zn (+66.32%), Cd (+150.32%), Cr (+141.94%) in WW irrigated soil in comparison toTWW irrigated soil. The paired two sample t test for the metals revealed that that the concentrations of Cu, Zn, Ni, Cd and Cr were significantly ($P<0.05$) higher and that of Pb was insignificantly ($P>0.05$) higher in WW irrigated soil than that observed in TWW soil (Table 3). The positive correlations were found for Ni-Zn (0.664743), Cd-Pb (0.175573), Cd-Zn (0.107624), Cd-Ni (0.814275), Cr-Cu (0.966814), Cr-Zn (0.103366) (Table 4).

EF for Soil: The values of EF of various metals in the WW irrigated soil are given in Table 3. The values were found in the order of Cd (2.46) > Cr (2.42) > Zn (1.67) > Cu (1.36) = Ni (1.36) > Pb (1.31). According to the Sutherland (2000), five contamination categories of soil are established on the basis of EF. In the present study, EF >2 for Cd (2.46) and Cr (2.42) indicated moderate enrichment, while Zn, Cu, Ni and Pb < 2 indicated minimal enrichment category of the soil near Bindal river. These EF values for Zn, Cu, and Pb were found lower than the values of EF for Zn (4.8), Cu (6.5) and Pb (15.5) observed by Mishra and Tripathi (2008) in WW irrigated soil of Varanasi and lower than the Cu (9.62) and Zn (7.96) as reported by Pathak *et al*., (2011) in SW irrigated soil and also lower than Cd (3.00) observed by Gupta *et al*., (2008) in WW irrigated soil of Tetagarh.

Heavy metals contents in different parts of *A. esculentus*

The results of the total metal concentrations (Pb, Cu, Zn, Ni, Cd, Cr and Hg) observed in different parts of the *A. esculentus* grown in WW and TWW irrigated soil (Table 5). During the present study, the concentrations of heavy metals determined were based on vegetables dry weight. Concentrations of the metals in edible part (fruits of *A. esculentus*) were more than the permissible limits of Indian standard.

Table 5. Heavy metals content (dry wt. basis - mg/kg) in *Abelmoschus esculentus* grown on site I (ES) and site II (CS) during monsoon (2008-09).

Sample	Site	Dry wt. %	Pb	Cu	Zn	Ni	Cd	Cr
Fruit	Site I	23.04	26.43±1.87*	24.65±2.48	58.29±6.55	58.96±2.49*	15.04±1.17*	64.62±2.52*
	Site II	18. 53	10.29±1.82	11.77±1.33	35.03±4.22	22.26±1.64	4.64±1.52	30.98±3.46
Leaves	Site I	16.93	43.30±3.63*	33.91±2.13*	73.29±2.03*	62.87±3.05*	20.39±1.99	76.78±3.04*
	Site II	27.40	13.59±1.65	16.29±2.00	44.63±2.54	24.37±3.60	7.02±1.36	36.79±2.07
Stem	Site I	28.47	43.47±2.92	26.77±2.06*	65.10±2.58	86.10±3.19*	18.04±1.53*	74.10±4.04*
	Site II	23. 57	31.72±3.11	14.04±2.71	40.69±5.71	57.58±9.35	6.00±0.57	33.45±3.06
Root	Site I	35.37	57.99±1.54*	28.98±2.77	81.70±2.99*	62.41±2.61	16.35±1.05	72.33±2.17*
	Site II	36.84	34.96±3.14	15.65±3.01	51.43±0.90	45.35±8.06	5.20±1.59	29.09±3.07

*Significance : $p<0.05$

The concentration of the metals varied differently in various parts of *A. esculentus* irrigated with WW. The maximum concentration of Pb (57.99±1.54 mg/kg) was in roots and minimum (26.43±1.87 mg/kg) in fruits. The Cu concentration was maximum (33.91±2.13 mg/kg) in the leaves and minimum (24.65±2.48 mg/kg) in fruit. The Zn concentration was maximum (81.70±2.99 mg/kg) in roots and minimum (58.29±6.55 mg/kg) in fruits. The Ni concentration was found maximum (86.10±3.19 mg/kg) in the stem and minimum (58.96±2.49 mg/kg) in fruits. The Cd concentration was found maximum (20.39±1.99 mg/kg) in the leaves and minimum (15.04±1.17 mg/kg) in fruits. The Cr concentration was maximum (76.78±3.04 mg/kg) in the leaves and minimum (64.62±2.52 mg/kg) in fruits of this plant (Table 5).

The concentration of Pb (26.43±1.87 mg/kg), Cu (24.65±2.48 mg/kg), Zn (58.29±6.55 mg/kg), Ni (58.96±2.49 mg/kg), Cd (15.04±1.17 mg/kg) and Cr (64.62±2.52 mg/kg) found in edible part (fruits) of *A. esculentus* were higher than that observed by Lone *et al.*, (2003) for Pb (9.35 mg/kg), Cu (22.91 mg/kg), Zn (20.05 mg/kg), Ni (20.80 mg/kg) Cd (2.60 mg/kg) and Cr (3.09 mg/kg) in Okra grown in sewage water irrigated soil at Hassanabdal area district Attock, Pakistan and also higher than that observed in edible part (fruit) of Lady's finger grown in sewage water irrigated soil for Pb (21.249±24.3 mg/kg), Cu (9.533±3.748 mg/kg), Zn (51.54±29.65 mg/kg), Ni (4.524±0.342 mg/kg) Cd (1.05±0.212 mg/kg) and Cr (3.1±2.404 mg/kg) reported by Sharif *et al.*, (2010).

Similarly, Singh and Kumar (2006) reported that Pb (1.1 to 6.7 mg/kg) and Cd (1.1 to 6.7 mg/kg) were lower in the edible part (fruit) of Lady's finger irrigated with WW at New Delhi than the present concentrations of the metals observed in *A. esculentus* grown in WW irrigated area. The Pb (15.03 mg/kg), Zn (34.70 mg/kg), Ni (10.86 mg/kg), Cd (2.81 mg/kg) and Cr (6.64 mg/kg) were also recorded to be higher in the edible part (fruit) of Lady's finger in comparison to the concentrations of these metals such as Pb (15.03 mg/kg), Zn (34.70 mg/kg), Ni (10.86 mg/kg), Cd (2.81 mg/kg), Cr (6.64 mg/kg) in the edible part (fruit) of Lady's finger grown in industrial effluents irrigated soil at Dhaka (Ahmed and Goni, 2010) .

Table 6. Enrichment factor (EF) for Lady's finger (*Abelmoschus esculentus)* grown at WW irrigated soil.

Sample	Pb	Cu	Zn	Ni	Cd	Cr
Fruit	2.57	2.09	1.66	2.65	3.24	2.09
Leaves	3.17	2.08	1.64	2.58	2.91	2.09
Stem	1.37	1.91	1.60	1.50	3.01	2.22
Root	1.66	1.85	1.59	1.38	3.14	2.49

In present study, the results of paired two sample t-test for various metals (Table 5) revealed that the concentration of Pb was significantly ($P<0.05$) higher in fruit, leaves and root; Cu in leaves and stem; Zn in leaves and root; Ni in fruit, leaves and stem; Cd in fruit and stem and Cr in fruit, leaves, stem and root grown in WW irrigated soil in comparison to TWW soil.

The results of EF of the heavy metals in *A. esculentus* are summarized in Table 6. EF is a good tool to know the variation of metal concentration by anthropogenic and natural source that depends on the bioavailability of metals concentration in soil, chemical forms, uptake capability of growth rate of different plant species (Adamo *et al.*, 2005; Vald'es *et al.*, 2005).

The EF values of 2-5 for Pb, Cu, Ni, Cd and Cr except Zn in fruit and leaves of A. esculentus showed moderate enrichment. The value for Cd and Cr showed moderate enrichment in stem and root. The values of EF were lower than the values reported by Singh *et al.*, (2010) for Cd (8.04), Pb (31.38), Zn (6.14), Ni (10.45) and Cr (14.35) in Lady's fingers grown in WW irrigated soil at Varanasi However, in the present study, the values of EF were higher than the values reported by Chary *et al.*, (2008) for Zn (0.81), Cr (0.59), Cu (0.06), Ni (0.02), Pb (0.61) in Lady's finger grown at sewage water irrigated soil at Musi river, Hyderabad.

Conclusion

The WW used for irrigation of *A. esculentus* contained Pb, Cu, Zn, Ni and Cr below the permissible limit while the Cd was above the permissible limit. However, there was

an increase in the concentration of Cr (141.94%), Cd (150.32%) and Ni (66.32%) in WW irrigated soil in comparison to TWW irrigated soil. The concentration of Pb, Cu, Zn, Ni and Cr was low, while that of Cd was more in the soil than the permissible limits of Indian standards. The EF for Cd and Cr indicated moderate enrichment of the WW irrigated soil. Among the different metallic concentrations, the concentration of Pb, Cu, Zn, Ni, Cd and Cr in different parts (fruit, leaves, stem and root) of *A. esculentus* was manifold than the prescribed limit of Indian standards. In view of the high level concentration of these metals in *A. esculentus* being grown in WW irrigated soil, the fruits (vegetables) are not fit for consumption by human beings and may cause severe health risks. There is need for the control of increase in metal contamination of the soil and vegetables growing in the area being irrigated with WW near Bindal river in Dehradun city.

REFERENCES

Adamo, P., Arienzo, M., Imperato, M., Naimo, D., Nardo, G., and Stanziones, D. (2005). Distribution and partition of heavy metals in surface and sub-surface sediments of Naples city port. *Chemosphere*, 61: 800–809.

Ahmad, J.U. and Goni, M.A. (2010) Heavy metal contamination in water, soil, and vegetables of the industrial areas in Dhaka, Bangladesh. *Environ Monit Assess*, 166:347–357.

APHA (2005). Standard methods for the examination of water and wastewater (21st Edn.). Washington, D.C.: American Public Health Association.

Awashthi, S. K. (2000). Prevention of Food Adulteration Act No. 37 of 1954. Central and State rules as amended for 1999 (3rd ed.). New Delhi: Ashoka Law House.

Cao, Z.H. and Hu, Z.Y. (2000). Copper contamination in paddy soils irrigated with wastewater. *Chemos*, 41 (1–2): 3–6.

Chary, N. S., Kamala, C.T. and Suman Raj, D. S. (2008). Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotoxicology and Environmental Safety*, 69: 513–524.

Ezeonu, E. (2004). Chemistry in Nigeria. A National Magazine of Chemical Society of Nigeria. 2 (2): 17–20.

Gupta, N., Khan, D. K. and Santra, S. C. (2008). An assessment of heavy metal contamination in vegetables grown in wastewater-irrigated areas of Titagarh, West Bengal, India. *Bull Environ Contam Toxicol*., 80:115–118.

Jagtap, M.N., Kulkarni, M.V. and Puranik, P.R. (2010). Flux of heavy metals in soils irrigated with urban wastewaters. *American-Eurasian J. Agric. & Environ. Sci*., 8 (5): 487-493.

Kisku, G.C., Barman, S.C. and Bhargava, S. K. (2000). Contamination of soil and plants with potentially toxic elements irrigated with mixed industrial effluent and its impact on the environment. *Water, Air and Soil Pollution*, 120:121-137.

Lone, M.I., Saleem, S., Mahmood, T., Saifullah, K. and Hussain, G. (2003) Heavy metal contents of vegetables irrigated by sewage/tubewell water. *Int. J. Agri. Biol*., 5 (4):533-535.

Mapanda, F., Mangwayana, E.N., Nyamangara, J. and Giller, K.E. (2005). The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. *Agric. Ecosyst. Environ*., 107: 151–165.

Mishra, A. and Tripathi, B.D. (2008). Heavy metal contamination of soil, and bioaccumulation in vegetables irrigated with treated waste water in the tropical city of Varanasi, India. *Toxicol Environ Chem*., 90 (5): 861–871.

Nan, Z., Li, J., Zhang and Cheng, G. (2002). Cadmium and zinc interaction and their transfer in soil–crop system under actual field conditions. *Sci. Total Environ*., 285: 187–195.

Nyamangara, J. and Mzezewa, J. (1999). The effects of long-term sewage sludge application on Zn, Cu, Ni and Pb levels in clay loam soil under pasture grass in Zimbabwe. *Agric. Ecosyst. Environ*., 73:199–204.

Oliveria, A.S., Bocio, A., Trevilato, T.M.B., Takayanagui, A.M.M., Domingo, J.L. and Segura-Munoz, S.I. (2007). Heavy metals in untreated/treated urban effluent and sludge from a biological wastewater treatment plant. *Env. Sci. Pollut. Res*., 14: 483-489.

Pathak, C., Chopra, A.K., Kumar, V. and Saxena, S. (2010) Heavy metals contamination in waste-water irrigated agricultural soil near Bindal river, Dehradun, India. *Poll Res*, 29(4):583–587.

Pathak, C., Chopra, A.K., Kumar, V. and Sharma, S. (2011) Effect of sewage-water irrigation on physico-chemical parameters with special reference to heavy metals in agricultural soil of Haridwar city. *J. Appl. & Nat. Sci*., 3(1):108–113.

Pescod, M.B. (1992). Wastewater treatment and use in agriculture. FAO irrigation and drainage.

paper 47, Food and Agriculture Organization of the United Nations, Rome.

Rusan, M. J. M., Hinnawi, S. and Rousan, L. (2007). Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. *Desalination*, 215: 143–152.

Sharma, R. K., Agarwal, M. and Marshall, F. (2007). Heavy metals contamination of soil and vegetables in suburban areas of Varanasi, India. *Ecotoxicol. Environ. Safety*, 66:258-266.

Sharif, M., Nergis, Y. and Farooq, M.A. (2010). Soil contamination from toxic elements irrigated with mixed industrial effluent and its environmental impact on the urban area of Karachi, Pakistan. *American-Eurasian J. Agric. & Environ. Sci*., 9(5):584-591.

Singh, K.P., Mohon, D., Sinha, S. and Dalwani, R. (2004). Impact assessment of treated/untreated wastewater toxicants discharge by sewage treatment plants on health, agricultural, and environmental quality in wastewater disposal area. *Chemos*., 55: 227–255.

Singh, S. and Kumar, M. (2006). Heavy metal load of soil, water and vegetables in peri-urban Delhi. *Environmental Monitoring and Assessment*, 120: 79–91.

Singh, A., Sharma, R. K., Agrawal, M., Marshall, F.M. (2010). Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food and Chemical Toxicology*, 48: 611–619.

Singh, J., Upadhyay, S.K., Pathak, R.K. and Gupta, V. (2011). Accumulation of heavy metals in soil and paddy crop (oryza sativa), irrigated with water of Ramgarh lake, Gorakhpur,

U.P., India. *Toxicological & Environmental Chemistry*, 93:462-473.

Sutherland, R.A. (2000). Bed sediment-associated trace metals in an urban stream, Oahu, Hawaii. *Environmental Geology*, 39: 611 – 37.

Vald'es, J., Vargas, G., Sifeddine, A., Ortlieb, L. and Guinez, M. (2005). Distribution and enrichment evaluation of heavy metals in Mejillones Bay, Northern Chile: Geochemical and statistical approach. *Marine Pollution Bulletin*, 50: 1558–1568.

Yuzbasi, N., Sezqin, E., Yildirim, M., Yildirim, Z. (2003). Survey of lead, cadmium, iron, copper and zinc in kasar cheese. *Food Add. Contam*., 20: 464–469.