Journal of Applied and Natural Science 6 (2): 797 - 803 (2014)

JANS

Bioaccumulation of heavy metals in *Spinacea oleracea* grown in distillery effluent irrigated soil

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Received: October 18, 2014 Revised received: December 01, 2014 Accepted: December 10, 2014

Abstract: The aim of the present study was to estimate the accumulation of heavy metals in *Spinacea oleracea* plant grown in Distillery Effluent (DE) irrigated soil. The results revealed that there was an increase in the metal contents Fe (+2.39%), Zn (+14.27%), Ni (+70.45%), Cd (+34.15%)and Cr (+20.46%) of soil irrigated with DE. In case of *S. oleracea* grown in the DE irrigated soil, it was observed that there was maximum concentration of Fe (353.24±7.94 mg/kg) and Zn (78.95±7.59 mg/kg) in leaves and that of Cr (54.19±8.39 mg/kg), Cd (7.73±1.41 mg/kg) and Ni (66.47±3.65 mg/kg) in root. The value of Bio-concentration factor (BCF) was found maximum for Cr (2.00) in comparison to other metals in the *S. oleracea* irrigated with DE. The value of Transfer factor (TF) was found maximum for Zn (TF- 1.51) for the soil irrigated with DE in comparison to soil irrigated with Bore well water (BWW). The DE can be a source of contamination to the soil as some toxic metals may also be transferred to roots and then to leaves in *S. oleracea*. The practice of continuous irrigation of agricultural land by DE may increase the risk of metal contamination in growing food crops to cause human health risks.

Keywords: Bio-concentration factor, Distillery effluent, Heavy metals, *Spinacea oleracea,* Transfer factor

INTRODUCTION

The occurrence of heavy metals in the environment and foodstuff represents a critical problem in all over world. Heavy metals in waste water come from industries and municipal sewage and are one of the main causes of water and soil pollution. The discharge of heavy metals as a byproduct of various human activities has been accompanied by large scale soil pollution. So much so that contamination levels in these soils were either more than normal levels or expected to soon reach those levels (Yargholi *et al.*, 2008). Long-term use of irrigation on agricultural lands contributes significantly to the buildup of the elevated levels of these metals in soils and plants (Nyamangara and Mzezewa, 1999, Mapanda *et al.*, 2005 and Sharma *et al.*, 2007) which is of serious concern.

The mobility of heavy metals, their bioavailability and related eco-toxicology to plants depend strongly on their specific chemical forms or ways of bindings. Metal chemistry, speciation and thus bioavailability are known to be dependent on soil physicochemical parameters. Strong binding of heavy metals to the soil particles reduces the bioavailability resulting in less plant uptake. Soil erosion and plant uptake are the ways for loss of metals from soil. Still, heavy metals are considered to be less mobile and thus less bioavilable in soil rich in organic matter and clay contents (Jagtap *et al.*, 2010). Most heavy metals infiltrate and accumulate in the top soil. Accumulation of heavy metals in soil is incremental and in the long term results in increased levels of contamination so much so that contamination levels may reach limits that can constitute a real threat to food safety for human civilization. The risk associated with the exposure to trace metals in food products has aroused widespread concern in human health. Acute and chronic symptoms, dizziness, nausea, vomiting, diarrhea, sleeping disorders, loss of appetite and reduced conception rate are the symptoms of heavy metal toxicity. These metals are also connected 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 and Bakircioglu *et al.*, 2011).

In many developing countries including India, farmers are irrigating their crop plants with industrial effluents having high level of several toxic metals (Cu, Cd, Cr, Zn, Fe, Ni, Mn and Pb) due to the non-availability of alternative sources of irrigation water (Chandra *et al.*, 2009).The distilleries and breweries are considered as a major polluter of the environment. Distilleries are one of the 17 most polluting industries listed by the

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

Central Pollution Control Board (Singh and Yadav, 2012). At present, there are 319 distilleries in India with an installed capacity of 3.29 billion litre of alcohol (Malaviyaand Sharma, 2011). In addition, the distillery effluent is characterized by high biochemical oxygen demand (BOD), chemical oxygen demand (COD), phenolic compounds, sulphate and heavy metals (Pant and Adholeya, 2007).

Spinacea oleracea (Spinach) is an edible flowering plant in the family of Amaranthaceae. It is a cool season crop that is easily grown here through winter and in early spring. It is a leafy vegetable and it had greater potential of accumulating heavy metals in their edible parts than grain or fruit crops and has hypoglycemic properties. The leaves are cooling, emollient, antipyretic, diuretic, maturant, laxative, digestible, anthelmintic, useful in urinary concretion, inflammation of the lungs and the bowels, sore throat, pain in joints, thirst, lumbago, cold and sneezing, sore eye, ring worm, scabies, leucoderma, soalding urine, arrest vomiting, biliousness, flatulence and have been used in the treatment of febrile conditions (Subhash *et al.,* 2010).

Keeping in view of the medical importance of *S. oleracea,* the present study was undertaken to observe the bioaccumulation of heavy metals in *S. oleracea* grown in Distillery effluent (DE) irrigated soil.

MATERIALS AND METHODS

Experimental design: The present field study was conducted in the experimental garden of the Department of Zoology and Environmental Sciences, Gurukula Kangri University, Haridwar (29^{o}55'10.81" N and 78^{o}07'08.12" E) during the period November, 2008-February, 2009 to study the evaluation of selected heavy metals level in *S. oleracea* grown in DE irrigated soil with Bore well water (BWW) taken as control. Twelve microplots (each of diameter. 30 cm) were used for growing the *S. oleracea*. The experiments were conducted under completely randomized design and were replicated six times.

Soil analysis: The soil was analyzed after harvesting the crop for the physico-chemical parameters such as soil pH and Electrical conductivity (EC) were determined at soil/water ratio of 1:1 using glass electrode pH meter and EC meter, Organic carbon (OC), Total Kjeldahl Nitrogen (TKN), Available Phosphorous (P), Available Potassium (K) and heavy metals (Zn, Fe, Ni, Cr and Cd) were determined as per standard methods (APHA, 2005; Chaturvedi and Sankar, 2006).

The soil samples as well as the leaves and roots of *S. oleracea* were dried separately in air at room temperature and sieved through a 2 mm sieve. Samples were digested in Nitric acid (HNO_3) and Perchloric acid ($HClO_4$) acid as per the method described in AOAC (Association of Official Analytical Chemists, 1990). After digestion, all samples were filtered through Whatmann No. 42 filter paper and in each case volume was made with 50 ml. Heavy metals such as Zinc (Zn), Iron (Fe), Nickel (Ni), Chromium (Cr) and Cadmium (Cd) were determined in the digested aliquot by Atomic Absorption Spectrophotometer (AAS) (Make–ECIL, Model No. 4129) using a specific lamp of particular metal using appropriate drift blank.

Data analysis: The heavy metal contents in both the *S. oleracea* and adjoining soil were expressed as means and standard deviation of six replicates calculated in Microsoft Office Excel (2007) and Axum 50.

Bio-concentration factor (BCF): The BCF for *S. oleracea* for all metal is expressed by the following formula:

$BCF = C_M / C_S$

where BCF represents the bioaccumulation factor of *S. oleracea*; C_M is the metal concentration in *S. oleracea* root and C_S is the metal content of growth media (soil) (Yoon *et al.*, 2006).

Transfer factor (TF): TF was calculated for the amount of threat and associated exposure due to waste water irrigation and resulting heavy metal accumulation in edible portion of test vegetables. It was calculated following the formula given by (Cui *et al*., 2004, Singh *et al*., 2010).

RESULTS AND DISCUSSION

Physico-chemical characteristics and heavy metal contents of soil after irrigation: The mean ± SD of variousphysico-chemical parameters viz. pH, EC, OC, TKN, K, P and heavy metals contents in soil after irrigation with 100% concentration of DE irrigated soil and with BWW irrigated soil for 60 days harvesting are given in Table 1.

The industrial effluents play an important role in altering the physico-chemical characteristics and heavy metal contents of soil.The long-term application of DE has been proved useful in significantly increasing OC, TKN, K, P and enzymatic activities in the soil but tended to build up harmful concentration of sodium that could be chelated by bioamendments. Short-term application of 50% DE along with bioamendments proved to be the most useful in improving the properties of sodic soil (Kaushik *et al.,* 2005). Effluent irrigation generally adds significant quantities of salts to the soil environment such as sulfates, phosphates, bicarbonates, chlorides of the cations sodium, calcium, potassium and magnesium that stimulate the growth at lower concentration but inhibit at higher concentration (Patterson *et al.,* 2008).It has been reported reported that DE (spent wash) discharged as wastewater contains various nutrients and toxic chemicals that can contaminate water and soil which may affect the common crops if used for agricultural irrigation (Kannan and Upreti, 2008).

In the present study, the soil characteristics have been found to change on irrigation with 100% concentration of DE after 60 days of *S. oleracea* crop harvesting. The

Table 1. Physico-chemical properties and heavy metal contents in soil after irrigation with BWW (control) and DE irrigated

S.No.	Parameters	BWW irrigated	DE irrigated	Permissible limit(a)	Permissible limit(b)
1	pH	8.05±0.06	7.75±0.06 (-3.87%)	-	-
2	EC (dsm^{-1})	0.14±0.06	0.72±0.37* (+80.55%)	-	-
3	OC %	0.80±0.14	1.20±0.47 (+33.33%)	-	-
4	K (mg/kg)	130.75±10.44	583±13.88* (+77.57%)	-	-
5	N (mg/kg)	1.82±0.17	2.41±0.15* (+24.48%)	-	-
6	P (mg/kg)	57.66±5.60	93.53±6.16* (+38.35%)	-	-
7	Zn (mg/kg)	43.44±4.26	52.17±4.73* (+16.73%)	300–600	300
8	Fe (mg/kg)	177.49±6.71	287.82±7.84* (+38.33%)	-	1,000
9	Ni (mg/kg)	63.02±6.07	206.51±9.63* (+69.48%)	75–150	60
10	Cr (mg/kg)	18.53±2.89	27.03±5.90 (+31.45%)	NA	50
11	Cd (mg/kg)	6.49±1.88	8.45±2.57 (+23.20%)	3–6	1

(a) Source: Awashthi (2000), (b) Source: Kabata-Pendias and Pendias (1992), *indicates significant increase (P <0.05).

irrigation with 100% concentration of DE significantly increased the EC (+431.99%), K (+345.89%), TKN (+32.28%) and P (+62.22%) and there was a slight decrease of pH (-3.87%) in comparison to BWW irrigated soil. Kumar and Chopra (2012) also observed significant increase in the EC (84.13%), K^+(31.59%) and TKN (1723.32%) after irrigation of soil with 100% distillery effluent concentration upto 90 days for *Trigonellafoenum-graecum*, while Srivastava*et al*. (2012) observed significant increase in the EC (+63.46%), OC (+3746.63%), K (+48.39%), TKN (+1449.18%), P (+338.83%) in soil irrigated with distillery effluent after 60 days of irrigation for *Abelmoschus esculentus*. pH is an important parameter as many nutrients are available only at a particular range of pH for plant uptake (Kumar and Chopra, 2011). Under acidic conditions, elements such as Fe, Al, Mn, and heavy metals (Zn, Cu and Cr) become highly soluble and may create problems for vegetation (Charman and Murphy, 1991).

Among various metals, the concentrations of Zn (52.17±4.73 mg/kg), Fe (287.82±7.84 mg/kg) and Cr (27.03±5.90 mg/kg) in DE irrigated soil were below the official Indian Standard (Awashthi, 2000; Kabata-Pendias and Pendias, 1992), whereas Ni (206.51±9.63 mg/kg) and Cd (8.45±2.57 mg/kg) concentrations in DE irrigated soil were several folds higher than the permissible limits official Indian Standard as cited in Awashthi (2000) and Kabata-Pendias and Pendias (1992).

The study revealed that the level of heavy metals (Zn-52.17±4.73 mg/kg, Fe-287.82±7.84 mg/kg, Ni-206.51±9.63 mg/kg, Cr-27.03±5.90 mg/kg and Cd-8.45±2.57 mg/kg) in soil irrigated with DE were observed to be higher than the values reported by Kumar and Chopra (2011) for Zn-8.86±0.26 mg/kg, Fe-10.67±2.21 mg/kg, Cr-1.98±0.07 mg/kg and Cd-0.94±0.02 mg/kgin DE irrigated soil atHaridwar, while the present values were lower than the values reported by Gupta *et al.* (2007) for Fe-20,401.3 mg/kg, Zn-230.8 mg/kg, Cr-293.7 mg/kg and Ni-26.9 mg/kg in tannery waste water irrigated soil. Similarly, Chandraju *et al.* (2010) observed lower concentration of DTPA Fe -240.0 mg/kg and higher concentration of DTPA Zn - 65.0 mg/kg in soil irrigated Distillery spent wash for 30 days as compared to the concentration of Fe (287.82±7.84 mg/kg) and Zn (52.17±4.73 mg/kg) in soil irrigated for 60 days with DE during the present study.

In present study, the results of paired two sample t-test for metals (Zn, Fe, Ni, Crand Cd) revealed that the concentrations of Zn, Fe and Ni were significantly (P<0.05) higher in DE irrigated soil than that observed in BWW irrigated soil, while the concentrations of Cr and Cd were insignificantly (P>0.05) higher in DE irrigated soil. It was also observed that the DE irrigation increased the amount of heavy metals such as Zn, Ni and Fe in the soil (Table 1). The Pearson's correlation at 5 % level between physicochemical parameters and heavy metals in DE irrigated soil indicated that OC-pH, K-pH, Zn-pH, Cr-pH, P-EC, Fe-EC, TKN-OC, Zn -K, Cd-TKN, Ni-Fe, Cd-Ni had a strong positive correlation (Table 2).

Heavy metal contents in roots and leaves of *S. oleracea*: The metal contents in leaves and roots of *S. oleracea* are indicated in Table 3.Among various heavy metals, the concentrations of Ni (36.99± 2.39/ 66.47±3.65 mg/kg), Cr (21.62±0.83/54.19±8.39 mg/kg) and Cd (6.47±0.74/7.73±1.41 mg/kg) in roots/leaves were found to be above the permissible limits except in

Table 2. Correlation (r values) between physico-chemical parameters and heavy metals in*S. oleracea* irrigated with DE.

	pH	**EC**	**OC**	**K**	**TKN**	**P**	**Zn**	**Fe**	**Ni**	**Cr**	**Cd**
pH	1.0000										
EC	-0.6268	1.0000									
OC	0.5239	-0.6239	1.0000								
K	0.5407	-0.6457	-0.1343	1.0000							
TKN	0.0626	-0.3987	0.8827	-0.4424	1.0000						
P	-0.4809	0.5433	-0.9951	0.2273	-0.8990	1.0000					
Zn	0.8417	-0.8348	0.3091	0.8846	-0.0920	-0.2246	1.0000				
Fe	-0.9559	0.5469	-0.6936	-0.2778	-0.2821	0.6735	-0.6725	1.0000			
Ni	-0.8800	0.2532	-0.1016	-0.5339	0.3702	0.0740	-0.6934	0.7865	1.0000		
Cr	0.5347	0.1434	-0.3955	0.4788	-0.7636	0.4095	0.4221	-0.3861	-0.8688	1.0000	
Cd	-0.5759	-0.1231	0.3391	-0.4617	0.7208	-0.3516	-0.4358	0.4402	0.8940	-0.9979	1.0000

case of Zn which was higher only in leaf part of *S. oleracea* than the permissible limits of FAO/WHO standard, Codex Alimentarious Commission (1984) and Indian standards (Awashthi, 2000).

The present study revealed that heavy metal content of Zn (78.95±7.59 mg/kg) and Fe (353.24±7.94 mg/kg) in leaves of *S. oleracea* irrigated with DE were found higher than the Zn (33.1±1.9 mg/kg) and Fe (309±27.0 mg/kg) in leaves of *S. oleracea* irrigated with waste water recorded by (Arora et al. 2008). Similarly, the concentrations of Zn (78.95±7.59 mg/kg), Cr (21.62±0.83 mg/kg) and Cd (6.47±0.74 mg/kg) were recorded to be higher in leaves of *S. oleracea* than Zn (62.24 mg/kg), Cr (4.08 mg/kg), Cd (0.39 mg/kg) present in leaves of spinach grown in waste water irrigated soil of Varanasi reported by Mishra and Tripathi (2008).

Earlier studies by Chandra *et al.* (2009) recorded the lower concentrations of heavy metals such as Zn (21.04±3.76 mg/kg), Fe (340.08±12.36 mg/kg), Ni (16.80±2.08 mg/kg), and Cd (2.18±0.56 mg/kg) except Cr (50.62±4.20 mg/kg) in leaves of wheat plant, while Zn- 48.24±2.86 mg/kg, , Ni- 3.16±0.53 mg/kg, Cr-15.76±2.08 mg/kg, and Cd-2.04±0.43 mg/kg except Fe-560.46±13.48 mg/kg in leaves of mustard plant grown in DE and tannery mixed effluent irrigated soil at Unnao (U.P.), India as compared with the heavy metals in leaves of *S. oleracea*. However the present value of Zn (78.95±7.59) mg/kg and Fe (340.08±12.36 mg/kg) in leaves were lower than that observed for Zn (297.40 mg/kg) and Fe (509.40 mg/kg) in Spinach irrigated with waste water in the area Shahre Rey, Iran reported by Bigdelhi and Seilsepour (2008).

The content of Zn (34.55±5.58 mg/kg), Cr (54.19±8.39 mg/kg) and Cd (7.73±1.41 mg/kg) in root of *S. oleracea* irrigated with DE were found higher than the Zn (0.781±0.02 mg/kg), Cr (0.511±0.04 mg/kg) and (Cd-0.052±0.003 mg/kg) in root of Spinach grown in the vicinity of an industrial area Faisalabad, Pakistan. Whereas, the concentration of Zn, Fe, Ni, Cr, and Cd in the root part of *S. oleracea* were recorded to be higher than the concentration of Zn-18.26±2.24 mg/kg, Fe-128.36±10.86 mg/kg, Ni-5.14±1.02 mg/kg, Cr - 10.16±2.05 mg/kg, and Cd-2.12±0.34 mg/kg in root of wheat plant and Zn-61.26±4.06 mg/kg, Ni- 7.28±1.16 mg/kg, Cd-2.26±0.16 mg/kg except Fe-432.57±7.06 mg/kg, Cr-60.26±4.16 mg/kg in root of mustard plant grown in DE and tannery mixed effluent irrigated soil at Unnao (U.P.), India.

The t-test showed that the contents of metals were increased significantly ($P<0.05$) for the Zn, Fe and Ni in both leaves/roots, while Cr was significantly ($P<0.05$) increased only in roots and Cd in leaves parts of *S. oleracea* (Table 3).

Accumulation and transfer of metals from soil to *S. oleracea:* Soil-to-plant transfer is one of the key components ofhuman exposure to metals through foodchain. The ratio of metals between soil and plants

Table 3. Heavy metal contents (mg/kg) for *S. oleracea* grown in BWW and DE irrigated soil.

Metals	Parts	BWW irrigated	DE irrigated	Permissible limits (a)	Permissible limits (b)
Zn	Leaves	40.63±4.53	78.95±7.59*	60	50
	Root	24.18±4.04	34.55±5.58*		
Fe	Leaves	169.52±3.49	353.24±7.94*	450	–
	Root	139.98±7.47	334.12±7.14*		
Ni	Leaves	30.96±2.86	36.99±2.39*	20	1.5
	Root	35.24±6.08	66.47±3.65*		
Cr	Leaves	20.57±1.79	21.62±0.83	5	20
	Root	27.66±7.71	54.19±8.39*		
Cd	Leaves	3.52±0.56	6.47±0.74*	0.3	1.5
	Root	5.46±1.08	7.73±1.41		

Source (a) FAO/WHO standard (Codex Alimentarious Commission, 1984), Source (b) Indian standard (Awashthi, 2000), *indicates significant increase (P< 05).

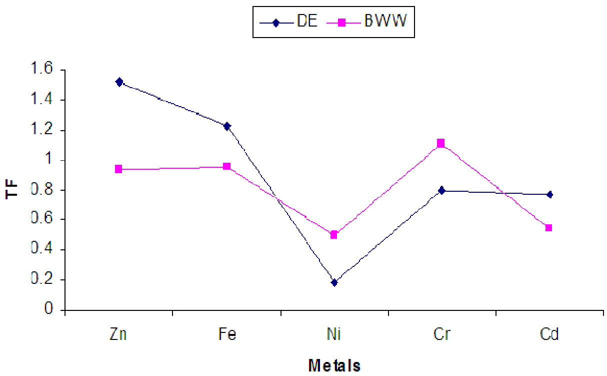

Fig. 1. *TF for S. oleracea grown in DE irrigated soil.*

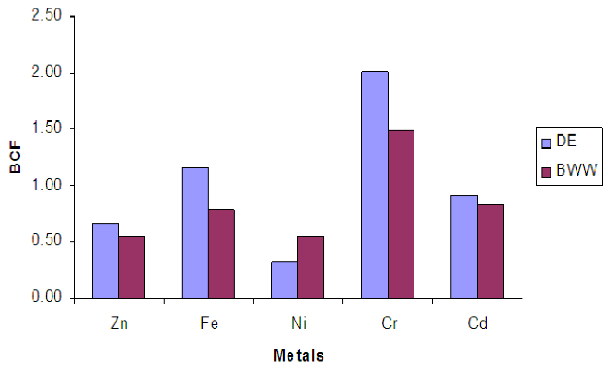

Fig. 2. *BCF for S. oleracea grown in DE and BWW irrigated soil.*

is an important criterion for the selection of crop plants for the cultivation in soils contaminated with high level of toxic metals (Bose *et al.*, 2008).

In the present study TF values for Zn (1.51) and Fe (1.23) were usually higher than those for Ni (0.18), Cr (0.80) and Cd (0.77) in *S. oleracea* grown in DE irrigated soil (Fig. 1). The present TF values of Zn and Cr were higher than that for Zn (0.660), Cr (0.194) while the values of Ni and Cd were lower than that observed for Ni (0.827), Cd (0.996) in Spinach grown at wastewater irrigated soil (Singh *et al.*, 2010). Among all the metals, TF of Zn and Fe were higher than other metals indicating their more mobility than other metals. The BCF values were found for Zn (0.66), Fe (1.16), Ni (0.32), Cr (2.00) and Cd (0.91) in *S. oleracea* grown in DE irrigated soil (Fig.2).

Both BCF and TF have been used to estimate a plant's potential for phytoremediation purpose (Yoon *et al.*, 2006). The ratio of metals between soil and plants is an important criterion for the selection of crop plants for the cultivation on soils contaminated with high level of toxic metals (Bose *et al.*, 2008). Variations in TF among different vegetables may be attributed to differences in the concentration of metals in the soil and the differences in element uptake by different vegetables (Cui *et al.*, 2004; Zheng *et al.*, 2007, Singh *et al.*, 2010).

Conclusion

The study concluded that the DE irrigation increased the EC, OC, K, TKN, and P and decreased the pH of the soil. The EC and K values were recorded manifold higher in comparison to other TKN, OC, and P. The DE irrigation improved the soil nutrient status. However, the higher concentration of heavy metals such as Ni, Cd and Cr in the soil irrigated with DE may lead to toxicity of soil. In *S. oleracea* grown in DE irrigated soil, there was an increase of Zn, Fe, Cr concentration in leaves and that of Ni and Cd in root. BCF and TF values of Zn, Cr, Fe more than one showed high mobility affinity of these metals being transferred from roots to leaves resulting in their greater accumulation and thus capable tolerate higher levels of Zn, Cr and Fe.

It is suggested that these effluents should be properly diluted prior to discharge from industries to bring down the metals concentration well within the prescribed limits to maintain soil health for irrigation purposes for the maximum yield of this crop and also to avoid risk for the accumulation of toxic metals in edible parts of the crop.

ACKNOWLEDGEMENT

The University Grant Commission, New Delhi, India is acknowledged for providing the financial support in the form of UGC research fellowship (F.7–70/2007 BSR) to Mr. Chakresh Pathak.

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