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Effects of different effluents on the yield quality and heavy metals accumulation in spinach (*Spinacia oleracea* L.)

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Abstract

Heavy metals are one of the important types of contaminants that can be found on the surface and in the tissue of fresh vegetables. In this regard, a study was conducted to see the impact of different effluents on the growth, yield and heavy metals content of spinach grown in pots. The plants were treated with 6 different effluents viz. 10% effluents of sugar mill, sewage water, soap factory, ghee mill, slaughterhouse and Control. Data on weight of leaves $plant^{-1}$ (g), number of leaves $plant^{-1}$, yield ha^{-1}, Vitamin C and heavy metals (Fe, Pb, Zn, Ni, Cd) were recorded. The results indicated that irrigation with effluents significantly affected all parameters studied. Sewage water, sugar mill effluent and slaughterhouse wastage significantly improved the yield and growth characteristics while ghee mill and soap factory effluents reduced these traits. Irrigation with sewage water increased 11.88% yield while the increment with slaughterhouse wastage and sugar mill effluent was 11.41% and 9.6%. Ghee mill and soap factory effluents reduced 8.9% and 7.38%, respectively. Heavy metal concentrations were increased with all types of effluents. The transfer factors showed that the accumulation of metals was in the order of Pb> Fe >Zn > Cd.

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Introduction

Spinach is a green edible plant and can be cultivated several times a year (Bashir and Bentel, 1994). With the development of industrialization, a huge amount of wastewater (WW) is discharged into water bodies which resulted in serious pollution problem in the water environment that negatively impact to the ecosystem and human life. Vegetables are mostly grown in the peri urban area of big cities which are commonly irrigated with municipal and industrial WW. Wastewaters contain valuable plant nutrients and serve as important source of nutrients and irrigation water for crops. Water and nutrients are the most critical input in agriculture, therefore, harvesting the nutrients and irrigation potential of WW are of prime importance for maximizing the production of vegetables, cereals and cash crops. Wastewaters contain appreciable amounts of micro-nutrients as well as heavy metals (Brar, 2002; Rusan, 2007). Edible parts of plant contain higher level of heavy metals and micro-nutrients than other parts while root crops accumulate less heavy metal than leafy plants (Hundal and Arora, 1993; Adhikari *et al.,* 1998).

Improved crop growth and higher yields of leafy vegetables have been obtained by sewage WW irrigation. During the year 2011, the total world production of spinach was 20.793 million tonnes. China being the largest spinach producing country with 18.783 million m. t., followed by USA (0.409 million m. t), Japan (0.264 million m. t) and Turkey (0.222 million m. t) while Pakistan ranked 9th with the production of 0.104 million m. t. (UN FAO, 2012). Chemical composition of spinach shows that it contains water 91.4%, carbohydrate 3.6%, sugar 0.4%, dietary fiber 2.2%, protein 2.9%, vitamin C 28.0mg 100g^{-1}, K 558mg 100g^{-1}, P 49mg 100g^{-1}, Fe 2.71mg 100g^{-1}, Zn 0.53mg 100g^{-1} and Na 79mg 100g^{-1} (USDA Nutrient database, 2012). Similarly, spinach contains water 90.8-92.5%, Pb 5.09-5.54ppm, Cd 4.16-4.46mg kg^{-1}, Zn 40.08-49.75mg kg^{-1}, Fe 42.49-42.82mg kg^{-1} and Cr 0.39-70.79mg kg^{-1} while heavy metal concentration in spinach leaves was determined and recorded as, Zn 21.11-50.00ppm, Pb 2.5-149.5ppm, Cd 0-1.50ppm, Cu 7.29-34.70ppm, Mn 71.57-134.57ppm and Cr 0.39-70.79ppm. The heavy metal were in order of Mn>Pb> Zn> Cu> Cr> Cd. Highest Pb and least Cd contents in spinach grown in the vicinity of an industrial area among Pb, Cu, Cr, Zn, and Cd metals were also analyzed (Farooq *et al.*, 2008; Ramesh and Murthy, 2012; Nankishore, 2014).

Considering the merits and demerits of use of industrial and municipal wastewaters for vegetable production, the present study was undertaken to figure out the effects of different effluents on the growth, yield, yield attributing traits and bioaccumulation of heavy metals like Fe, Pb, Zn and Cd in spinach.

Materials and methods

Planting of plant

Eight to ten healthy and uniform spinach seeds were planted in each pot containing 20kg soil with well-mixed FYM @ 20 t ha^{-1} and NPK @) 100-60- 60kg ha^{-1}.

Application of Fertilizers

All P, K and half N were applied at the time of sowing while the remaining half N was top-dressed after one month. Sowing was done during 3rd week of October in 2012 and 2013.

Thining

After the establishment of plants these were then thinned to four plants pot^{-1}. The effluents from each source were diluted to 10 times with irrigation water prior to application.

Experimental Design

Pots were arranged in a completely randomized design (CRD) with six treatments/ effluents repeated four times.

Treatments detail

The treatments included; T_1: Sugar mill effluent, T_2: Sewage water, T_3: Soap factory effluent, T_4: Ghee mill effluent, T_5: Slaughter house wastage and T_6: Control. All standard cultural practices were followed during the course of development of crop.

Parameters recorded

Data on weight of leaves plant^{-1} (g), number of leaves plant^{-1}, leaf area, yield pot^{-1} and yield ha^{-1}, pH, Vitamin C and heavy metals (Fe, Pb, Zn, Ni, Cd) were taken and analyzed statistically by computing Analysis of variance (ANOVA) and Least Significant Difference (LSD) according to Steel *et al.*, (1997) using Microsoft Excel and Statistix-8 computer software package.

Results and discussion

Leaves weight per plant (g)

The weight of leaves $plant^{-1}$ of spinach was significantly $\leq$ 0.05 affected by application of effluents from different sources (Table 1). During both years of study, the significantly highest weight of leaves $Plant^{-1}$ of 24.90 and 25.12g were noticed in plants irrigated with sewage water (T2). While, lowest weight of leaves $plant^{-1}$ (12.75 and 13.000g) was found in plants irrigated with ghee mill effluent (T3). All treatments differed significantly from each other during both years. The sewage water, slaughterhouse wastage and sugar mill effluent enhanced 36.44, 24.38 and 5.75% weight of leaves $plant^{-1}$ as compared to check during 1st year and 37.49, 26.44 and 6.73% in 2nd year, respectively. Application of ghee mill and soap factory effluents reduced weight of leaves $plant^{-1}$ by 30.14 and 21.53% in 1st year while 28.85 and 18.99% than control in 2nd year.

The greater weight of leaves $plant^{-1}$ with application of sewage water, slaughterhouse wastage and sugar mill effluents revealed that these wastewaters were richer in macro and micronutrients needed for better plant growth. The reduction in weight of leaves with the application of ghee mill and soap factory effluents suggests that these effluents were injurious for plant growth due to higher EC, TDS and pollution stress which retarded plant growth and destroyed soil structure. In accordance with these observations Nawaz *et al.*, (2006) recorded improvement in various crops with use of WW while Ale *et al.*, (2008) documented negative effect on growth of crops due application of wastewaters at higher

Number of leaves per plant

Significant variation existed in number of leaves $plant^{-1}$ of spinach by the application of different industrial and municipal effluents (Table 1). The maximum enhancement in leaves count (19.23% and 14.81%) was recorded in sewage water irrigated plants followed by slaughterhouse wastage with 11.54 and 11.11% during 1st and 2nd year, respectively.

The application of ghee mill and soap factory effluents resulted in 30.77 and 15.38% reduction in leaves count during 1st year while 27.78 and 16.67% decrease as compared to control in 2nd year, respectively. The results decipher that nutrients rich wastewaters like sewage, slaughterhouse and sugar mill wastes promoted growth of plant and increased leaf count while those deficient in plant nutrient reduced vegetative growth of crops and number of leaves $plant^{-1}$. These results coincide with the findings of Reddy and Borse (2001) and Ale *et al.*, (2008).

Table 1. Weight of leaves $plant^{-1}$, number of leaves $plant^{-1}$ and leaf area of Lettuce as affected by irrigation with different effluents.

Effluent/Wastewater	Weight of leaves $plant^{-1}$		Number of leaves $plant^{-1}$		Yield (ha^{-1})	
	1st year	2nd year	1st year	2nd year	1st year	2nd year
T_1: Sugar Mill Effluent	19.30 c	19.50 c	13.50 c	13.50 b	7.72 c	7.80 c
T_2: Sewage Water	24.90 a	25.12 a	15.50 a	15.50 a	9.96 a	10.05 a
T_3: Ghee Mill Effluent	12.75 f	13.0 f	9.00 e	9.75 d	5.10 f	5.20 f
T_4: Soap Factory Effluent	14.32 e	14.80 e	11.00 d	11.25 c	5.73 e	5.90 e
T_5: Slaughterhouse Wastage	22.70 b	23.10 b	14.50 b	15.00 a	9.08 b	9.24 b
T_6: Control	18.25 d	18.27 d	13.00 c	13.50 b	7.30 d	7.35 d
$LSD_{0.05}$	0.575	0.678	0.692	0.745	0.131	0.310

Mean followed by similar letter(s) do not differ significantly at 5% level of significance.

Yield (t ha^{-1})

Spinach yield (t ha^{-1}) was substantially influenced by application of effluents from different sources (Table 2). As compared to check, the highest increase in spinach yields of 36.44 and 36.73% were recorded with sewage water during 1st and 2nd year, respectively indicating that sewage water provided macro and some micronutrients, which are responsible for better plant development. Sewage water was followed by slaughterhouse wastage producing 24.38 and 25.71% more spinach yields during 1st and 2nd year, respectively. The highest reduction in yield (30.14 and 29.25%) was found with ghee mill effluent, succeeded by soap factory effluent with 21.51 and 19.73% decrease, showing that these effluents adversely affected the yield by remarkably reducing weight, number, weight and area of leaves. It can be inferred that nutrients rich wastewaters augmented yields while nutrient deficient effluents declined yields. These results are analogous to finding of Bhandhari (2014) who advocated that nutrients embodied in wastewaters increase yields as much or more than a combination of tap water and chemical fertilizer. However, Ale *et al.*, (2008) recorded yield suppressing trends with distillery effluent irrigation fields.

Chemical Composition

Water Content (%)

Water content in spinach leaves was also significantly influenced by irrigation with different wastewaters (Table 3). During both years, the highest water content of 92.00 and 91.95% was found in control plants (T6), followed by statistically similar sewage water (T2) which was also statistically akin to slaughterhouse wastage (T5) that in turn was significantly identical to soap factory effluent (T4). The statistically lowest water content (88.00%, 88.30%) was observed in plants irrigated with sugar mill effluent succeeded by ghee mill wastage (T3) and both treatments differed significantly from one another. These results correspond to values reported by Nankishore (2014), USDA Nutrient database (2012) and Barzegar *et al.*, (2007).

Table 2. Vitamin C (mg $100g^{-1}$), Water content (%) and Fe (mg kg^{-1}) of Lettuce as affected by irrigation with different effluents.

Effluent/Wastewater	Vitamin C (mg $100g^{-1}$)		Water content (%)		Fe (mg kg^{-1})	
	1st year	2nd year	1st year	2nd year	1st year	2nd year
T_1: Sugar Mill Effluent	88.00 e	88.30 e	275.50 c	276.00 b	60.62 c	61.00 c
T_2: Sewage Water	91.56 ab	91.62 ab	282.50 a	285.20 a	65.20 b	65.50 b
T_3: Ghee Mill Effluent	89.60 d	89.60 d	262.50 e	264.20 d	50.50 d	51.00 d
T_4: Soap Factory Effluent	90.85 c	90.87 c	265.00 e	266.00 d	42.36 e	43.30 e
T_5: Slaughterhouse Wastage	91.00 bc	91.35 bc	270.72 d	271.16 c	68.42 a	68.63 a
T_6: Control	92.00 a	91.95 a	278.70 b	276.00 d	24.62 f	24.62 f
$LSD_{0.05}$	0.581	0.527	2.930	4.229	0.829	1.403

Mean followed by similar letter(s) do not differ significantly at 5% level of significance.

Table 3. Pb (mg kg^{-1}), cd (mg kg^{-1}) and Zn (mg kg^{-1}) of Lettuce as affected by irrigation with different effluents.

Effluent/Wastewater	Pb (mg kg^{-1})		cd (mg kg^{-1})		Zn (mg kg^{-1})	
	1st year	1st year	1st year	2nd year	1st year	2nd year
T_1: Sugar Mill Effluent	1.440 b	1.440 b	0.035 b	0.037 c	25.50 b	26.00 b
T_2: Sewage Water	1.620 a	1.620 a	0.045 a	0.047 a	29.75 a	30.00 a
T_3: Ghee Mill Effluent	0.090 d	0.090 d	0.032 b	0.035 cd	20.55 c	21.00 d
T_4: Soap Factory Effluent	1.300 c	1.300 c	0.041 a	0.042 b	24.70 b	24.80 c
T_5: Slaughterhouse Wastage	0.070 d	0.070 d	0.030 bc	0.033 d	18.83 d	19.00 e
T_6: Control	0.055 e	0.055 e	0.025 c	0.025 e	15.22 e	15.23 f
$LSD_{0.05}$	0.072	0.072	0.0050	0.0026	0.990	0.739

Mean followed by similar letter (s) do not differ significantly at 5% level of significance.

Vitamin C (mg $100g^{-1}$)

Data presented in Table 3 illustrates the ascorbic acid content (mg $100g^{-1}$) of spinach leaves slurries as affected by application of different effluents. It varied from 262.50 to 282.50 and 264.20 to 285.20 mg $100g^{-1}$ in 1st and 2nd year's crops, respectively.

During 1st year, the maximum vitamin C content was noticed in plants irrigated with sewage water (T2) followed by control (T6), sugar mill effluent (T1) and slaughterhouse wastage (T5). However, all the four effluents differed significantly. The least value was found in ghee mill effluent (T3), preceded by soap factory effluent (T4), which were statistically at par but differed significantly from all other treatments. In 2nd year, the significantly highest ascorbic acid content was recorded in T2, superseded by statistically identical sugar mill effluent (T1) and control (T6) with 276.00mg $100g^{-1}$ each. The statistically lowest vitamin C content was determined in plants receiving ghee mill effluent that differed statistically from all other treatments, followed by soap factory effluent. US Nutrient database (2012) reports are in agreement with these results.

Heavy Metals

Fe (mg kg-1)

The Fe content of spinach irrigated with different effluents showed significant ($P \leq 0.05$) variation (Table 4). In two successive years of study, the slaughterhouse wastage treated plants possessed the maximum Fe contents of 68.62 and 68.63mg kg^{-1} during 1st and 2nd year, respectively. It was preceded by sewage water (T2), sugar mill (T1), ghee mill (T3) and soap factory effluent (T4). The minimum Fe content was found in slurries of leaves collected from check pots. However, all the treatments differed significantly from each other. During 1st year, 2.78 times increase in Fe content was noticed in T5 (slaughterhouse wastage), followed by T2 (2.65 fold), T1 (2.46 fold), T3 (2.05 fold) and T4 (1.72 fold). The accumulation of Fe in T5, T2, T1, T3 and T4 in 2nd year crop was 2.79, 2.66, 2.48, 2.07 and 1.76 times, respectively, suggesting that Fe concentration in spinach was directly related to concentration of Fe in the wastewaters. Slaughterhouse wastage was rich in Fe as compared to other effluents. Analogous to these results Antoniadis (1998) reported that heavy metal concentrations in plants depend on the concentrations of the metals in the soil and irrigation source. Mohammad and Latif (2010) found that total Fe in spinach was greater than that of lettuce.

Table 4. Transfer factor (TF) of metals in lettuce with respect to effluents.

Metal	Sugar M. Effluent	Sewage water	Ghee Mills Effluent	Soap Factory Effluent	Slaughterhouse Wastage	Metal Mean
Fe	2.47	2.65	2.06	1.74	2.78	2.34
Pb	26.73	29.63	1.68	23.82	1.34	16.64
Zn	1.69	1.96	1.36	1.62	1.24	1.57
Ni	1.75	1.62	2.03	1.82	1.12	1.66
Cd	1.44	1.84	1.32	1.64	1.24	1.49
Effluent	6.82	7.54	1.69	6.13	1.53	

Pb (mg kg-1)

The data pertaining to Pb content in spinach leaves as influenced by application of different effluents depicted that significant variation existed in Pb content among the treatments. During both years, the significantly highest Pb content (1.620 and 1.640mg kg^{-1}) was found in plants irrigated with sewage water (T2) that differed statistically from all other treatments. It was succeeded by sugar mill effluent (T1) and soap factory effluent (T4). However, all the three treatments varied significantly. The lowest Pb content (0.055mg kg^{-1}) was recorded in check plants (T6) succeeded by T5 (slaughterhouse) and T3 (ghee mill effluent), which did not differ statistically. The maximum permissible limit for Pb has been recommended as 0.30mg kg^{-1} by FAO/WHO (2001), suggesting that all samples were within the safe limit. The present results are in conformity with Singh *et al.*, (2010) and Begum *et al.*, (2010) who found the presence of high levels of lead in leafy vegetables.

Zn (mg kg-1)

Zinc is an essential mineral that is naturally present in some foods. It is involved in numerous aspects of cellular metabolism and is required for the catalytic activity of enzymes and in immune function, wound healing and protein synthesis. Zinc also supports normal growth and development. Zinc content in spinach varied significantly by application of effluents from different sources (Table 4). It ranged from 15.22 to 29.75mg kg^{-1} and 15.23 to 30.00mg kg^{-1} during 1st and 2nd year, respectively. In 1st year, the highest Zn content was recorded in sewage water (T2) treated plants followed by statistically similar T1 (25.50mg kg^{-1}) and T4 (24.70mg kg^{-1}). The least Zn content (15.22mg kg^{-1}) was found in T6 followed by T5 and T3 which differed significantly from each other. During 2nd year, maximum Zn content was recorded in T2 superseded by T1, T4, T3, T5 and control. All treatments varied significantly from each other. The composition of effluents showed highest Zn content in sewage water and sugar mill effluent suggesting that increased Fe concentration in irrigation water resulted in higher Fe content in spinach leaves. The Zn contents in all samples was within the safe limit (100.0mg kg^{-1}), recommended by FAO/WHO, (2001). In accordance with these results, Sridhara *et al.*, (2008) also found varied Zn concentration in leafy vegetables of wastewater irrigated areas. Likewise, Ramesh and Murthy (2012) recorded higher Zn content in sewage water treated spinach (24.32-48.31mg kg^{-1}) compared to control (21.11mg kg^{-1}).

Cd (mg kg^{-1})

Differential response of industrial and municipal effluents was observed regarding Cd content in spinach leaves during both years of experiment. It ranged from 0.025 to 0.045 and 0.025 to 0.047mg kg^{-1} during 1st and 2nd year, respectively. The statistically highest Cd content was observed in sewage water irrigated plants (T2) during both years. The lowest Cd content (0.025mg kg^{-1}) was reported in control plants (T6) that differed significantly from all other treatments except T5. During 2nd year, T2 was followed by statistically different T4 and T1 which was significantly similar to T3 that in turn was statistically identical to T5. The significantly lowest Cd content was registered in T6 that varied statistically from all other treatments. The study showed that Cd content in all samples fell below the safe limit of 0.10mg kg^{-1} FAO/WHO (2011), which is indicator that spinach grown under these effluents is safe for consumption without the risk of environmental toxicants. These findings are in agreement with reports released by Ramesh and Murthy (2012), Nankishore (2014) and indicating remarkable variation in Cd content and accumulation in spinach receiving wastewaters from different sources. The study also revealed direct relationship between the levels of Cd in effluents and its absorption by spinach plants. The composition of effluents shows that sewage water and soap factory effluents contained greater quantity of Cd as compared to other wastewaters. Likewise, Perveen *et al.*, (2012) reported high levels of Cd in vegetables irrigated with sewage water. The Cd levels reported in this study are far less than those reported by Chandorkar and Deota (2013) and Nankishore (2014).

Relative accumulation of heavy metals

Relative efficiency of transfer of heavy metals from soil to spinach plant (TF) was also studied and results are presented in Table 6. The perusal of data shows that highest mean TF (16.64) was recorded for Pb followed Fe, Zn and Cd with TF of 2.34, 1.66, 1.57 and 1.49, respectively. The magnitude of TF for heavy metals was in order of Pb> Fe > Zn > Cd. Concerning effluents, the mean highest TF (7.54) was observed for sewage water succeeded by sugar mill effluent (6.82), soap factory effluent (6.13), ghee mill effluent (1.69), and slaughterhouse wastage (1.53). The ranking of effluents with respect to different metals on the basis of TF was as follows:

Fe: Slaughterhouse wastage > Sewage > Sugar mill > Ghee mill > Soap factory

Pb: Sewage > Sugar mill > Soap factory > Ghee mill > Slaughterhouse wastage

Zn: Sewage > Sugar mill > Soap factory > Ghee mill > Slaughterhouse wastage

Ni: Ghee mill > Soap factory > Sugar mill > Sewage > Slaughterhouse wastage

Cd: Sewage > Soap factory > Sugar mill > Ghee mill > Slaughterhouse wastage

Analogous to these observations, considerable variations in TF of metals have been recorded by Puschenreiter *et al.*, (2005), reporting TF for Cd (5.00), Pb (0.13) and Zn (1.27). Mahdavian and Somashekar (2008) investigated heavy metals abundance in the vegetables and fruits in the order of >Pb>Zn > Cd, while Harati *et al.*, (2003) and Harati *et al.*, (2011) recorded concentration of metals in Spinach leaves is the sequence of Fe> Zn>Pb> Ni> Cd.

Conclusion

Our results indicated that irrigation with effluents significantly affected all parameters studied. Sewage water, sugar mill effluent and slaughterhouse wastage significantly improved the yield and growth characteristics while ghee mill and soap factory effluents reduced these traits. Irrigation with sewage water increased 11.88% yield while the increment with slaughterhouse wastage and sugar mill effluent was 11.41% and 9.6%. Ghee mill and soap factory effluents reduced 8.9% and 7.38%, respectively. More extended study is required to collect adequate data which would help in obtaining exact picture of the problem.

Conflict of interest

The authors have no conflict of interest to disclose.

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