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Impact of different effluents on the performance and heavy metal accumulation of Lettuce (*Lactuca sativa* L.).

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Abstract

A study was conducted to see the impact of different effluents on the growth, yield and heavy metals content of lettuce grown in pots during the year 2012-13 and 2013-14. The pots were arranged in a completely randomized design (CRD) with six treatments (effluents) replicated four times. The treatments included 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}, leaf area, yield ha^{-1}, pH, Vitamin C and heavy metals (Fe, Pb, Zn, Ni, Cd) were recorded and statistically analyzed by computing Analysis of variance (ANOVA) and using Least Significant Difference (LSD) test.

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. The highest yield and related characteristics were recorded with sewage water followed by slaughterhouse wastage and sugar mill effluent. Irrigation with sewage water increased 37.6% yield while the increment with slaughterhouse wastage and sugar mill effluent was 32.1 and 11.2%. Ghee mill and soap factory effluents reduced 30.8 and 14.5%, respectively. Heavy metal concentrations were increased with all types of effluents. The highest values for Pb, Zn and Cd were reported in plants irrigated with sewage water while Fe and Ni were highest in slaughterhouse wastage and ghee mill effluent applied plants, respectively. The transfer factors showed that the accumulation of metals was in the order of Pb > Fe = Zn > Ni > Cd.

Keywords: Lettuce (*Lactuca sativa* L.), effluents, growth, yield, composition, heavy metal accumulation

Introduction

Lettuce (*Lactuca sativa* L) belonging to family Compositae is most often grown as a leaf vegetable. It was originated around Asia Minor, Iran, Turkey and has been used as a salad crop for more than 2500 years. There are several types of lettuce, but three viz leaf, head and romaine are the most common. It is often used for salads, although it is also seen in other kinds of food such as soups, sandwiches and wraps; it can also be grilled (Zohay *et al*., 2012; Richard *et al*., 2012).

The world production of lettuce during calendar year 2011 was 23,622,366 metric tons. China ranked 1st with the production of 13,434,500 m. t (53%) followed by USA (17%) and India (4%)(FAO Statistics Database, 2012)

Lettuce is a good source of vitamin A, vitamin K, potassium as well as a minor source for several other vitamins and nutrients. A 100g lettuce provides 13 Kcal energy and contains water 95.63%, carbohydrate 2.23%, protein 1.35%, dietary fibre 1.1%, vitamin A 166 µg, thiamin 0.06 mg, riboflavin 0.062 mg, pantothenic acid 0.15 mg, vitamin K 102.3 µg, vitamin C 3.7 mg, P 33 mg, K 238 mg, Ca 35 mg, Fe 1.24 mg, Mg 13 mg and Zn 0.2 mg (USDA Nutrient Database, 2012).

Due to scarcity of quality fresh water for irrigation, the use wastewaters (WW) including municipal sewage water as well as industrial effluents has become a reasonable alternative. Wastewater is commonly used for irrigation of vegetables in many developed and developing countries like Pakistan particularly in the suburbs of big cities. Consequently, vegetables in addition to providing nutrients also accumulate toxic metals and cause health risks (Gosh *et al.*, 2012). The results of many studies on the use of wastewater (WW) have illustrated significant increase in crop yields than groundwater (GW) irrigated fields. Murtaza *et al*. (2003) have documented better crop growth particularly of leafy vegetables like spinach, lettuce, cauliflower, cabbage etc. grown on fields receiving sewage WW. Singh *et al*. (1991) recorded significant augmentation (3.38 g pot^{-1} to 8.85 g pot^{-1}) in dry matter yield of barseem (*Trifoliam sp.)* in pots irrigated with sewage WW than GW irrigated pots. Udayasoorian *et al*. (1999) conducted field experiments on maize, sunflower, groundnut and soybean using WW for irrigation and registered 19.3, 29.9, 5.9 and 4.8% higher grain yield, respectively over fields irrigated with GW. Contrarily, Sutapa and Bhattacharyya (2008) reported significant negative relationship between yield and concentration of Cd, Cu, Pb, Zn and Cr in root and shoot of wheat plant.

Neumann *et al*. (2014) cultivated lettuce on three different types of soil viz alluvial loam, loess loam and dilluvial loam and found that lettuce shoot fresh weight ranged from 5.32-9.07g plant^{-1} and the leaf of lettuce contained Fe 74.3-308, Mn 35.0-97.1 and Zn 34.5-47.8 mg kg^{-1}, respectively on dry weight basis. Nankishore (2014) analyzed four leafy vegetables including *Lactuca sativa*, *Brassica oleracea*, *Brassica chinensis* and

Basella alba, and showed remarkable variation in heavy metal contents like Cd, Pb, Cu, Zn, Co, Ni, Mn, and Fe. Mohamed *et al*. (2003) collected different kinds of leafy and non- leafy vegetables and reported high levels of Fe in lettuce, spinach, cucumber, tomatoes, green pepper and cabbage. The study also revealed that different elemental concentrations in each vegetable depended upon the selective uptake of the elements by the plant.

Achakzai *et al*. (2011) studied the effect of five different concentrations of wastewaters collected from Chiltan ghee mill, Chiltan town and Zarghoon town on heavy metals concentration in lettuce and found considerable variations in heavy metals. The maximum values for Fe, Mn, Zn, Ni, Pb, and Cd were registered with the highest concentration of effluents. The highest values of Cu, Ni and Cd were recorded in Chiltan ghee mill effluent while maximum concentration for Fe and Zn were reported in Chiltan town whereas Mn and Pb in Zarghoon town wastewaters, respectively. Perveen *et al*. (2012) collected forty samples each of soil, water and vegetables from forty different localities of Peshawar. The Ni, Pb and Cd contents in lettuce leaves were 47.6, 2.20 and 15.24 mg kg^{-1}, respectively. Positive correlations were observed between Ni, Pb, Cd and Cr of vegetables and irrigation water concentration. Kudirat and Funmilayo (2011) revealed that Cd and Zn in leafy vegetables ranged from 0.028 to 0.091 and 0.348 to 0.77 mg kg^{-1} on dry weight basis, respectively. Harati *et al*. (2013) reported relative accumulation of metals in lettuce leaves, spinach leaf, radish root and turnip tubers in order of Fe> Zn>Pb> Ni> Cd.

Keeping in view the beneficial and adverse effects of wastewaters, the present study was designed to quantify the effects of different effluents on the yield and quality of lettuce and to figure out the bioaccumulation of heavy metals like Fe, Pb, Zn, Ni and Cd.

Material and Methods

Twenty kg soil mixed with FYM @ 20 t ha^{-1} was transferred to earthen pot and NPK @) 100-60- 60 kg ha^{-1} were incorporated. All P, K and half N were applied at the time of sowing while the remaining half N was top-dressed after one month. Eight to ten healthy and uniform seeds of lettuce were sown during 3rd week of October in 2012 and 2013. The effluents from each source were diluted to 10 times with GW prior to application. The experiment as laid out in complete randomized design (CRD) with six treatments (effluents) replicated four times. 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. The pots were then equally irrigated with respective treatments of wastewater. All standard agricultural practices were followed during the course of germination, growth and development. Data on weight of leaves $plant^{-1}$ (g), number of leaves $plant^{-1}$, leaf area, yield (t ha^{-1}), pH, Vitamin C and heavy metals were recorded and statistically analyzed by computing Analysis of variance (ANOVA) and using Least Significant Difference (LSD) according to Steel *et al*. (1997) using Microsoft Excel and Statistix-8 computer software package.

Results and Discussion

Growth Characteristics

Weight of Leaves $Plant^{-1}$ (g)

Vegetative growth of a crop is associated with development of new shoots, leaves and leaf area. Irrigation with various effluents significantly $\leq$ 0.05 affected the weight of leaves $plant^{-1}$ of lettuce (Table 1). During 1st year, the significantly highest weight of leaves (28.25 g) was recorded in plants irrigated with sewage water (T2) followed by statistically similar (27.00 g) slaughterhouse wastage (T5). These were succeeded by sugar mill effluent (T1) and canal irrigated (check) plants (T6) that differed statistically. The significantly lower weight of leaves $plant^{-1}$ were found in plants irrigated with ghee mill effluent (T3) and soap factory effluent (T4) with 14.50 and 17.80 g respectively. In 2nd year of experiment, the significantly highest weight of leaves $plant^{-1}$ was observed in T2 (31.15 g), succeeded by T5, T1, T6, T4 and T3 with 30.00, 25.00, 21.50, 19.10 and 15.25 g leaves $plant^{-1}$, respectively. However, all treatments differed statistically from each other.

The results revealed that during both years, the maximum weight of leaves $plant^{-1}$ was reported in plants of T2 (sewage water) followed by T5 (slaughterhouse wastage) and T1 (sugar mill effluent). The higher weights of leaves $plant^{-1}$ can be attributed to provision of plant nutrients particularly N, P, K and some micro nutrients which promoted growth of lettuce while ghee mill and soap factory effluents were deficient in these nutrients and inhibited growth of plants. Sewage water, slaughterhouse and sugar mill effluent enhanced 31.09, 25.29% and 6.73% weight of leaves $plant^{-1}$ as compared to check (canal irrigation) during 1st year while 44.88%, 39.53% and 16.28% during 2nd year, respectively. However, ghee mill and soap factory effluents reduced 32.71 and 17.40% weight of leaves plant-1 than control in 1st year while the reduction was 29.07% and 11.16%, respectively during 2nd year. These results correspond to findings of Antil (2012) reporting positive effect of sewage irrigation on growth and yield of crops. Orhue *et al*. (2005) and Nawaz *et al*. (2006) recorded improvement in various crops with use of WW. Huma *et al*. (2012) illustrated that probably there are some essential organic compounds in wastewaters which may alleviate some part of negative impacts.

Number of Leaves plant^{-1}

The number of leaves plant^{-1} was increased significantly by application of sewage water, sugar mill effluent and slaughterhouse wastages but significant reduction was noticed by ghee mill and soap factory effluents (Table 1). During both year, the highest number of leaves plant^{-1} was registered in T2 (13.50), proceeded by T5 (12.75 and both were statistically identical. Likewise, T1 (11.00) and T6 (10.25) were also significantly similar, producing similar number of leaves in each treatment. The T4 and T3 were also statistically akin in reducing the number of leaves plant^{-1}. Amongst effluents, the sewage water, slaughterhouse wastage and sugar mill resulted in 31.71, 24.39 and 7.80% increase in number of leaves plant^{-1} during 1st year while the increment in 2nd year was 31.71, 26.83 and 7.80%, respectively. On the other hand, the application of ghee mill and soap factory effluents reduced the number of leaves plant^{-1} by 31.70 and 12.19% in 1st year while 26.83 and 9.56% in 2nd year.

The results show that number of leaves plant^{-1} of lettuce was considerably affected by application of industrial and municipal effluents during both years of study. During both years, the maximum numbers of leaves were counted in plants irrigated with sewage water followed by slaughterhouse wastage and sugar mill effluent indicating positive effect of effluents which contained appropriate amount of macro and micronutrients which resulted in higher number of leaves plant^{-1}. However negative impact of ghee mill and soap factory effluents was noticed on leaves count due to deficiency of essential plant nutrients, higher TDS and pollution stress. These results are in agreement with the findings of Porra (2002) and Kumar and Chopra (2012) who reported that municipal and industrial wastes contain optimum quantities of nutrients required for better vegetative growth of crops. Huma *et al*. (2012) noticed reduced vegetative growth of crops with WW due to greater salts contents and pollution stress of effluents.

Leaf Area (cm^2)

Leaf area is an important variable for most physiological and agronomic studies involving plant growth, light interception, photosynthetic efficiency, evapotranspiration and response to fertilizers and irrigation. Application of municipal and industrial effluents considerably influenced the leaf area of lettuce (Table 1). In both years, irrigation with sewage water (T2) substantially increased the leaf area (cm^2) preceded by slaughterhouse wastage (T5) and sugar mill effluent (T1) while ghee mill (T3) and soap factory (T4) effluents noticeably decreased the leaf area as compared to control. All treatments varied significantly from each other. The data showed 18.73 and 22.47% increase in leaf area in T2 during 1st and 2nd year, respectively. It was followed by T5 and T1 with 14.45 and 8.44% enlargement in leaf area during 1st year while 15.26 and 11 84% in 2nd year. The enhancement in leaf area might be attributed to availability of growth promoting nutrients from these wastages. The application of ghee mill and soap factory effluents decreased leaf area by 54.84 and 54.15%, respectively during 1st year whereas 53.68 and 51.91% during 2nd year, indicating that ghee mill and soap factory effluents proved injurious for plants growth, restricted the plant development and resulted in lower leaf area. These results are supported by Kumar and Chopra (2013) who recorded maximum LAI plant^{-1} of *T. foenum-graecum* with application of 10% from distillery effluent but decreased at higher concentrations. The reason might be due to higher salt content. Hassanain *et al*., (2013) reported that industrial effluents exhibited significant decrease in leaf area of lettuce and the magnitude of decrease was positively correlated with the concentrations of effluent.

Yield (t ha^{-1})

Considerable differences in yield (t ha^{-1}) of lettuce were reported due to application of different industrial and municipal wastages (Table 2). During 1st year, the maximum yield (11.30 t ha^{-1}) was recorded with application of sewage water (T2) followed by slaughterhouse wastage (T5), sugar mill effluent (T1), control (T6), soap factory (T4) and ghee mill effluents (T3) producing 10.82, 9.20, 8.62, 7.12 and 5.85 t ha^{-1} yields, respectively. Similarly, in 2nd year the highest yield (12.46 t ha^{-1}) was found in T2, succeeded by T5 (12.00 t ha^{-1}), T1 (10.00 t ha^{-1}), T6 (8.64 t ha^{-1}), T4 (7.64 t ha^{-1}) and T3 (6.10 t ha^{-1}).

The results reflected that sewage water, slaughterhouse wastage and sugar mill effluent enhanced lettuce yield (t ha^{-1}) by 31.09, 25.52 and 6.73% in 1st year while 44.5, 38.73 and 15.61% in 2nd year, respectively. However, irrigation with ghee mill and soap factory effluents curtailed the yield by 32.13 and 17.40% in 1st year and 29.48 and 11.67% in 2nd year, respectively. The explanation for enhancement may be due to provision of nutrients by effluents while reduction due to deficiency of nutrients. In line with these results, the yield enhancement has also been reported by Udayasoorian *et al*. (1999) who recorded 6.9 to 13.9% increase in yield in different sugarcane varieties grown with wastewater. Sharma and Kansal (1984) showed 23, 46 and 50% higher grain yield of wheat, rice and cotton with sewage wastewater, respectively. On the contrary, Ale *et al*. (2008) reported about 40% yield suppression with distillery effluent irrigation.

Chemical Composition

Water Content (%)

The data presented in Table 3 deciphered that use of various wastewaters significantly affected the water content of lettuce leaves. In 1st year, the maximum water content (95.13%) was reported in check plants (T6) followed by statistically identical sewage water (95.05%) which was also significantly similar to slaughterhouse wastage (94.90%). Likewise, slaughterhouse wastage (T5) was akin to T4 (soap factory effluent) with 94.82% water. The minimum water content (94.02%) was recorded in plants treated with sugar mill effluent (T1) proceeded by ghee mill wastage (T3) with 94.50% water. During 2nd year, the highest water content (95.15%) was estimated in T6 plants succeeded by T2, T5 and T4; all the four treatments were statistically alike. However, T2, T5 and T4 were also significantly similar to T3. The significantly lowest water was found in T1 (93.50%).

These results are in accordance with the finding of Hussain (1987) recording 94-96% water in lettuce plants. Dan *et al*. (2013) reported 49.11 to 86.40% moisture content in edible leaves of nine selected vegetables.

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

The vitamin C content in lettuce leaves was considerably influenced by application of different effluents (Table 3). It ranged from 20.85 to 42.20 and 21.00 to 45.65 mg $100g^{-1}$ during 1st and 2nd year crops, respectively. During both years, the highest values were recorded in plants receiving sewage water (T2) superseded by slaughterhouse wastage (T5), control (T6), sugar mill effluent (T1), soap factory effluent (T4) and ghee mill effluent (T3). All the treatments varied significantly from each other. These values are in line with the findings of Hussain (1987) recording 37 mg $100g^{-1}$ vitamin C.

pH

Application of different effluents significantly affected the pH of leaves slurries (Table 3). During 1st year the highest pH value (6.753) was noticed in sugar mill effluent applied plants (T1) that differed statistically from all other treatments. It was succeeded by statistically similar control (T6) and ghee mill effluent (T3). The significantly lowest pH (6.712) was observed in slurries of leaves receiving slaughterhouse wastage (T5) preceded by soap factory (T4) and municipal wastages (T2). However, all the three treatments varied statistically. In 2nd year, the highest pH was registered in T1 plants followed by T3 and T6 and all the three treatments were statistically akin. Likewise, T3 and T6 were also significantly similar to T2 and T4. The lowest pH value was observed in T5 plants which in turn was statistically identical to T4.

Heavy Metals

Fe (mg kg^{-1})

Iron is an essential element for the growth and development of plants and other organisms, including humans because it is a cofactor for fundamental biochemical activities such as energy metabolism, oxygen transport, and DNA synthesis (Sharma *et al*., 2009). Application of effluents from different industrial and municipal sources significantly increased the Fe content of lettuce (Table 4). During both years, the plants receiving slaughterhouse wastage (T5) contained the significantly highest Fe content (34.35 and 35.02 mg kg^{-1}) superseded by sewage water (T2), sugar mill (T1), soap factory (T4) and ghee mill (T3) effluents. The leaves collected from check pots possessed statistically the lowest Fe content (12.50 mg kg^{-1}). All treatments varied significantly ($P \leq 0.05$). During both years, the highest uptake of Fe (2.75 and 2.80 fold) was noticed in T5, preceded by T2, T1, T4 and T3 with 2.16, 1.98, 1.50 and 1.14 folds against control in 1st year and 2.19, 2.00, 1.53 and 1.16 folds in 2nd year, respectively. The high levels of Fe in lettuce irrigated with slaughterhouse and sewage water might be because of the fact that these wastewaters were enriched with Fe. The major ingredient of slaughterhouse wastage is blood which contains the highest level of Fe. Donnelly et al. (1978) reported 3.0 mg Fe l^{-1} in animal blood. In accordance with these results Perveen *et al*. (2012) enunciated high levels of heavy metals in vegetables irrigated with sewage water. Achakzai *et al*. (2011) reported that Fe content of lettuce was significantly and linearly increased as the concentration of wastewater increased. Compared to our results, higher levels of Fe in lettuce leaves have been documented by Nankishore, 2014 (108.15-115.85 mg kg^{-1}) and Mohamed *et al*., 2003 (324 mg kg^{-1}) that may be due to difference in cultivar, soil and effluents and their concentrations. Arora *et al*., 2008) found that trace elements differed in plants in order of Fe> Mn> Zn> Ni.

Zn (mg kg^{-1})

Zinc is essential for the growth and development of plants and other animals. Among heavy metals, it is the least toxic and is an essential component of many enzymes and nucleic acid metabolism (Antoniadis, 1998). Lettuce leaves collected from plants irrigated with different effluents possessed significantly variable amount of Zn (Table 4). It ranged from 10.00 to 26.12 mg kg^{-1} and 10.05 to 27.00 mg kg^{-1} during 1st and 2nd year, respectively. During both years, the maximum Zn content was recorded in lettuce irrigated with sewage water (T2) that differed statistically from all other effluents. It was succeeded by soap factory effluent (T4), sugar mill effluent

(T1), ghee mill effluent (T3) and slaughterhouse wastage (T5). All effluents varied significantly from each other. Sewage water irrigated plants contained 2.61 and 2.69 times more Zn as compared to control in 1st and 2nd year crop, respectively. Crops treated with soap factory, sugar mill and ghee mill effluents possessed 2.42, 1.89, 1.53 and 1.20 times Zn in 1st year while 2.40, 1.88, and 1.57 fold in 2nd year. The minimum enhancement of 1.20 and 1.23 fold was observed in slaughterhouse wastage during 1st and 2nd year, respectively. These results are analogous to findings of Sridhara *et al*., (2008) reporting varied Zn concentration in leafy vegetables of wastewater irrigated areas of Hyderabad, Andhra Pradesh. Likewise, Afolami *et al*. (2010) found that vegetables grown on contaminated soils contained greater Zn content than those grown on un-contaminated soils.

Ni (mg kg^{-1})

Nickel is also an essential element for plant growth that plays role in body functions including enzyme functions (Sobukola *et al*., 2010). Sources of Ni in water bodies are ghee, oil, chemical, kitchen and steel industries (Amin *et al*., 2014). The Ni content in lettuce was significantly elevated by the application of different industrial and municipal effluents (Table 4). During both years, the highest Ni content (2.87 and 2.91 mg kg^{-1}) was determined in plants treated with ghee mill effluent (T3) that differed significantly from all other wastewaters. It was followed by equally effective soap factory effluent (T4) and sewage water (T2). These were succeeded by statistically different sugar mill effluent (T1) and slaughterhouse wastage (T5). The significantly lowest Ni content (1.57 and 1.58 mg kg^{-1}) was observed in canal water irrigated plants (T6) that differed significantly from all other wastewaters. The highest accumulation of Ni was found in plants receiving ghee mill effluents (T3) with 1.83 times more Ni as compared to check plants during both years while 1.54, 1.51, 1.34 and 1.19 times Ni was noticed in crops from T4, T2, T1 and T5, respectively in 1st year. However, during 2nd year, 1.58, 1.52, 1.39 and 1.20 fold Ni was registered in T4, T2, T1 and T5, respectively. The highest values in plants irrigated with ghee mill effluent (T3) might be due to the fact that ghee mill effluent is rich in Ni as it is used as catalyst in ghee production. These results indicate a range of 1.57 to 2.89 mgNi kg^{-1} which is consistent with findings of Dan *et al*. (2013) with Ni 0.11-2.51 mg kg^{-1} in vegetable samples and Ebong *et al*. (2007) with 0.80-1.82 mg kg^{-1} in vegetables and fruits and but inconsistent with Yusuf *et al*., (2003) and Harati *et al*. (2011) reporting 25.08–51.03 and 15.38–30.31 mg kg^{-1} Ni in lettuce leaves collected from industrial and sewage water of village areas of Lagos, respectively.

Pb (mg kg^{-1})

Lead is non-essential elements with no biological function within the plant and is the most commonly occurring heavy metal contaminant because of its toxicity to human health and other organisms (Zheng *et al*., 2007). It is strongly adsorbed to organic matter in soils, more than any other heavy metal and therefore organic matter is a very important sink for Pb in polluted soils (Pendias and Pendias, 1992). Significantly variable amount of Pb was found in lettuce leaves as affected by irrigation with different effluents (Table 5). During both years of study, the highest concentration of Pb (1.222 and 1.250 mg kg^{-1}) was noticed in leaves of plants irrigated with sewage water (T2), followed by sugar mill effluent (0.780 and 0.812 mg kg^{-1}), ghee mill effluent (0.540 and 0.580 mg kg^{-1}), soap factory effluent (0.222 and 0.242 mg kg^{-1}), which varied significantly from each other. Statistically, the lowest Pb content was recorded in check plants (0.066 and 0.068 mg kg^{-1}) succeeded by statistically similar slaughterhouse wastage treated plants (0.084 and 0.090 mg kg^{-1}). The higher Pb in sewage water applied plants might be due to higher level of Pb in sewage water, consequently plants accumulated greater amount. The slaughterhouse wastage contained the least Pb content and plants irrigated with this wastage took up the minimum amount of Pb. The maximum assemblage of Pb (18.51 and 18.38 fold) was observed in sewage water treated plants during 1st and 2nd year, respectively. It was succeeded by sugar mill, ghee mill, soap factory effluents and slaughterhouse wastage with 11.82, 8.18, 3.36 and 1.27 fold augmentation in 1st year crop and 11.94, 8.53, 3.56 and 1.32 times build up in 2nd year produce, respectively. However, all treatments were with in the safe limit during both years of study. Analogous to these results, variable concentrations of Pb have been reported in lettuce viz. Dotse (2010) 1.08-3.76 mg kg^{-1}, Perveen *et al*. (2012) 2.20 mg kg^{-1}, Harati *et al*., (2011) 9.9 mg kg^{-1} and Dan *et al*., (2013) 0.13-3.42 mg kg^{-1} and Nankishore (2014) 2.76-3.17 mg kg^{-1}. He also stated that *L.sativa* and *B.alba* have more of these metal-binding compounds (Pb, Cd) than *B.oleracea* and have the tendency to compete with the microelements for uptake by plants.

Cd (mg kg^{-1})

Cadmium is a non-essential element for both plants and animals which is bio-available and toxic to human health and other organisms (Sharma *et al*., 2009). It interferes with metabolic processes in plants and can bio-accumulate in aquatic organisms and enters the food chain (Adriano, 2001). The Cd content in lettuce leaves harvested from different treatments receiving various types of effluents varied from 0.018 to 0.038 and 0.022 to 0.040 mg kg^{-1} during 1st and 2nd year, respectively (Table 5). The significantly highest Cd content was determined in sewage water irrigated (T2) plants during both years. It was preceded by T4 (0.028 mg kg^{-1}) and

T1 (0.022 mg kg^{-1}) during 1st year. The T1 was also statistically akin to T5. Likewise T3 and T6 were also significantly at par in raising Cd level of lettuce leaves. In 2nd year, the statistically remarkable Cd content was detected in T2 plants (0.040 mg kg^{-1}) and T4 (0.035 mg kg^{-1}) which was also statistically identical to T1 (0.030 mg kg^{-1}) that in turn was significantly alike to T5 (0.028 mg kg^{-1}). The lowest Cd content was observed in control (T6) that was statistically similar to T3 (0.025 mg kg^{-1}). The data revealed that sewage water irrigation intensified 2.11 and 1.82 fold Cd in plants as compared to control during 1st and 2nd year, respectively, followed by soap factory effluent, sugar mill effluent, slaughterhouse wastage and ghee mill effluent showing 1.56, 1.22, 1.11 and 1.0 times enhancement in Cd concentration during 1st year whereas 1.59, 1.36, 1.27 1.14 time increase was recorded during 2nd year, respectively. These results suggest that Cd content in plants increased with the increasing levels of Cd in effluents. The study also showed that Cd content in all treatments fell below the safe limit (0.1-5.0 mg/kg Cd) proposed by the FAO/WHO (2001). In accordance with our results, Latif *et al*. (2008) reported elevated levels of heavy metals in vegetables irrigated with sewage and untreated industrial effluents. Similar concentration for Cd was observed by Kudirat and Funmilayo (2011) reporting 0.028 to 0.09 mg kg^{-1}. However, higher levels of 2.8 and 15.24 mg kg^{-1} were found by Harati *et al*. (2011) and Perveen *et al*. (2012), respectively.

Relative Accumulation of Heavy Metals

Transfer Factor (TF) is an indicator of the plant accumulation behaviour and is expressed as the ratio of plant concentration divided by the concentration in soil/ wastewater. Table 5 shows the transfer factors of different heavy metals in lettuce with respect of effluents from various sources. The mean highest TF (8.68) was observed for Pb, followed equal value for Fe and Zn (1.92) succeeded by Ni (1.48) and Cd (1.43). The magnitude of TF for heavy metals was in order of; Pb > Fe = Zn > Ni > Cd. Regarding different effluents, the mean TF of metals was highest for sewage water (5.32) superseded by sugar mill effluent (3.36), ghee mill effluent (2.11), soap factory effluent (1.53) and slaughterhouse wastage (1.53). Mahdavian and Somashekar (2006) investigated heavy metals abundance in the vegetables and fruits and reported in the order as follows: Fe > Cr > Mn > Pb > Ni > Co > Zn > Cu > Cd. Similarly Puschenreiter *et al*. (2005) also recorded large differences in the transfer of Cd, Zn, Ni, Cu, Pb and Cr from soil to different parts of plants. Kursad *et al*. (2013) reported 3.5 to 340-fold higher amounts of the heavy-metals (Co, Cd, Pb, Mn, Ni and Cu) from soils irrigated with WW than irrigated with GW in fruit and vegetable samples.

Conclusion

It is concluded from the study that differential responses of wastewaters from different source were observed on the growth, yield, chemical composition and heavy metals accumulation of lettuce. Amongst effluents, sewage water, slaughterhouse wastage and sugar mill effluent significantly increased the yield and yield contributing traits of lettuce while ghee mill and soap factory effluents significantly decreased these parameters. Heavy metals including Fe, Pb, Zn, Ni and Cd contents of leaves were considerably increased with all types of effluents as compared to control. The transfer factors showed that the accumulation of metals was in the order of Pb > Fe = Zn > Ni > Cd.

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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}		Leaf Area (cm)	
	1st Year	2nd Year	1st Year	2nd Year	1st Year	2nd Yer
T_1: Sugar Mill Effluent	23.00 b	25.00 c	11.00 b	11.00 b	205.50 c	212.50 c
T_2: Sewage Water	28.25 a	31.15 a	13.50 a	13.50 a	225.00 a	232.70 a
T_3: Ghee Mill Effluent	14.50 d	15.25 f	7.00 d	7.50 c	85.58 e	88.00 f
T_4: Soap Factory Effluent	17.80 d	19.10 e	9.00 c	9.25 bc	86.88 e	91.37 e
T_5: Slaughterhouse Wastage	27.00 a	30.00 b	12.75 a	13.00 a	216.88 b	219.10 b
T_6: Control	21.55 c	21.50 d	10.25 b	10.25 b	189.50 d	190.00 d
$LSD_{0.05}$	**3.688**	**1.107**	**1.210**	**1.825**	**2.817**	**2.511**

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

Table 2. Yield (ha^{-1}) of Lettuce as affected by irrigation with different effluents.

Effluent/ Wastewater	Yield (t ha^{-1})	
	1st Year	2nd Year
T_1: Sugar Mill Effluent	9.20 c	10.00 c
T_2: Sewage Water	11.30 a	12.46 a
T_3: Ghee Mill Effluent	5.85 f	6.10 f
T_4: Soap Factory Effluent	7.12 e	7.64 e
T_5: Slaughterhouse Wastage	10.82 b	12.00 b
T_6: Control	8.62 d	8.64 d
$LSD_{0.05}$	**0.226**	**0.448**

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

Table-3. Water (%), Vitamin C (mg 100g $^{-1}$) and pH of Lettuce as affected by irrigation with different effluents.

Effluent/ Wastewater	Water (%)		Vitamin C (mg 100g-1)		pH	
	1st Year	2nd Year	1st Year	2nd Year	1st Year	2nd Yer
T_1: Sugar Mill Effluent	94.02 e	93.50 c	30.20 d	30.68 d	6.753 a	6.755 a
T_2: Sewage Water	95.05 ab	95.00 ab	45.20 a	45.65 a	6.732 c	6.735 b
T_3: Ghee Mill Effluent	94.50 d	94.65 b	20.85 f	21.00 f	6.740 b	6.745 ab
T_4: Soap Factory Effluent	94.82 c	94.84 ab	28.20 e	29.00 e	6.728 d	6.730 bc
T_5: Slaughterhouse Wastage	94.90 bc	94.85 ab	42.22 b	42.32 b	6.712 e	6.714 c
T_6: Control	95.13 a	95.15 a	40.00 c	40.20 c	6.742 b	6.743 ab
$LSD_{0.05}$	**0.198**	**0.397**	**1.473**	**1.318**	**0.0039**	**0.0172**

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

Table 4. Fe (mg kg^{-1}), Zn (mg kg^{-1}) and Ni (mg kg^{-1}) of Lettuce as affected by irrigation with different effluents.

Effluent/ Wastewater	Fe (mg kg^{-1})		Zn (mg kg^{-1})		Ni (mg kg^{-1})	
	1st Year	2nd Year	1st Year	2nd Year	1st Year	2nd Year
T_1: Sugar Mill Effluent	24.700 c	25.000 c	18.85 c	18.92 c	2.10 c	2.20 c
T_2: Sewage Water	27.000 b	27.400 b	26.12 a	27.00 a	2.37 b	2.40 b
T_3: Ghee Mill Effluent	14.220 e	14.520 e	15.28 d	15.82 d	2.87 a	2.91 a
T_4: Soap Factory Effluent	18.800 d	19.120 d	24.24 b	24.10 b	2.42 b	2.50 b
T_5: Slaughterhouse Wastage	34.350 a	35.020 a	12.00 e	12.32 e	1.87 d	1.90 d
T_6: Control	12.500 f	12.500 f	10.00 f	10.05 f	1.57 e	1.58 e
$LSD_{0.05}$	**0.491**	**0.548**	**0.431**	**0.462**	**0.083**	**0.171**

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

Table 5. Pb (mg kg^{-1}) and Cd (mg kg^{-1}) of Lettuce as affected by irrigation with different effluents

Effluent/ Wastewater	Pb (mg kg^{-1})		Cd (mg kg^{-1})	
	1st Year	2nd Year	1st Year	2nd Year
T_1: Sugar Mill Effluent	0.780 b	0.812 b	0.022 c	0.030 be
T_2: Sewage Water	1.222 a	1.250 a	0.038 a	0.040 a
T_3: Ghee Mill Effluent	0.540 c	0.580 c	0.018 d	0.025 cd
T_4: Soap Factory Effluent	0.222 d	0.242 d	0.028 b	0.035 ab
T_5: Slaughterhouse Wastage	0.084 e	0.090 e	0.020 c	0.028 c
T_6: Control	0.066 e	0.068 e	0.018 d	0.022 d
$LSD_{0.05}$	**0.030**	**0.028**	**0.0042**	**0.0058**

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

Table 6. Transfer Factor (TF) of Metals in lettuce with respect to Effluents

Metal	**Sugar M. Effluent**	**Sewage water**	**Ghee M. Effluent**	**Soap F. Effluent**	**Slaughterhouse Wastage**	**Metal Mean**
1. Fe	1.99	2.17	1.15	1.52	2.77	1.92
2. Pb	11.88	18.45	8.36	3.46	1.29	8.68
3. Zn	1.88	2.55	1.55	2.41	1.21	1.92
4. Ni	1.36	1.50	1.83	1.56	1.19	1.48
5. Cd	1.30	1.95	1.10	1.60	1.20	1.43
Effluent Mean	3.36	5.32	2.80	2.11	1.53	