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ACCUMULATION OF HEAVY METALS BY LETTUCE (*LACTUCA SATIVA* L.) IRRIGATED WITH DIFFERENT LEVELS OF WASTEWATER OF QUETTA CITY

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Abstract

Heavy metal contamination of soils resulting from irrigation by wastewater is causing major concern due to the potential health risk involved. This study was therefore designed to investigate the heavy metal ions viz., Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+} and Cd^{2+} concentrations by lettuce (*Lactuca sativa* L.) grown in pots. This leafy vegetable was irrigated with 5 different concentrations of wastewater collected from 3 different localities viz., Chiltan town, Chiltan Ghee Mill (oil industry) and Zarghoon town of Quetta city. Tap water was used as background water. Plant samples were analyzed for their heavy metal contents using Atomic Absorption Spectroscopy (AAS). Results showed that localities, treatments and their interactions (except Cu^{2+}& Pb^{2+}) generally exhibited highly significant ($P\leq0.01$) influence on the accumulation of heavy metals. The maximum values of 9.71; 509.06; 32.47; 94.38; 8.58; 5.05 and 7.45 mg kg^{-1} for Cu^{2+}, total Fe^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+},Pb^{2+}, and Cd^{2+} respectively, were generally obtained in highest concentration of effluents. While maximum values of 9.02; 4.47 and 7.90 mg kg^{-1} of Cu^{2+}, Ni^{2+} and Cd^{2+} were obtained for Chiltan ghee mill effluents. However, maximum values i.e., 435.96 and 80.47 mg kg^{-1} of total Fe and Zn^{2+} were recorded for Chiltan town. The highest values of 30.09 and 6.32 mg kg^{-1} for Mn^{2+} and Pb^{2+} were noted in Zarghoon town, respectively. On the basis of grand mean values,the magnitude of heavy metals detected for various metals is in order: Fe > Zn >Mn> Cu >Pb> Cd > Ni. Results further demonstrated that the total concentrations of Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} and Ni^{2+} are within the recommended international standards set by WHO/FAO, but could be reached to toxic level either by the consecutive use of effluents or by increased dietary pattern of the consumers. Whereas, the amount of Pb^{2+} and Cd^{2+} are at par than the international maximum permissible limits. This study highlights the potential health risks associated with the cultivation and consumption of leafy vegetables on wastewater-contaminated soils. Wastewater used for irrigation around the peri-urban area of Quetta is highly polluted especially in term of Pb^{2+}& Cd^{2+} metals. Therefore, domestic and industrial waste of the city should be properly disposed and or recycled so as to avoid the present/ future health risks.

Introduction

Environmental pollution affects the quality of pedosphere, hydrosphere, atmosphere, lithosphere and biosphere (Lone *et al.,* 2008). Land and water are precious natural resources on which rely the sustainability of agriculture and the civilization of mankind. Unfortunately, they have been subjected to maximum exploitation and severely degraded or polluted due to anthropogenic activities. Linked with environmental pollution, water pollution is also a problem of worldwide concern and ground water is extremely polluted due to unplanned disposal of untreated domestic sewage and industrial effluents into watercourses (Mashiatullah *et al.,* 2005). Disposal of sewage water and industrial wastes is a great problem. Often it is drained to the agricultural lands where it is used for growing crops including vegetables. Wastewater irrigation poses several threats to the environment through contamination by nutrients, heavy metals, salts, and nitrates (Stagnitti *et al*., 1998). It also poses a number of potential risks to human health via the consumption of or exposure to pathogenic microorganisms, heavy metals or harmful organic chemicals (Stagnitti, 1999). Effective wastewater treatment can reduce pathogen levels, but in most developing countries it is not an option for the municipal authorities due to the high costs involved (Keraita *et al.,* 2002).

As demand for fresh water intensifies, the use of municipal or industrial wastewater in agriculture sector is frequently being seen as a common practice in many parts of the world (Ensink *et al.,* 2002; Sharma *et al.,* 2007). A rough estimate indicates that at least 20 million hectares in 50 countries are irrigated with raw or partially treated wastewater and likely to increase markedly during the next few decades as water stress intensifies (Hussain *et al.,* 2001; Scott *et al.,* 2004; Hamilton *et al.,* 2007). The major objectives of wastewater irrigation are that it provides a reliable source of water supply to farmers and has the beneficial aspects of adding valuable plant nutrients and organic matter to soil (Horswell *et al.,* 2003; Liu *et al.,* 2005), which are creating both opportunities and problems for the farming sector (Singh *et al.,* 2004; Chen *et al.,* 2005). Plants have a natural propensity to take up metals. Some of them like Cu^{2+}, Co^{2+}, Fe^{2+}, Mo^{2+}, Mn^{2+}, and Zn^{2+} are essential plant micronutrients (Baker *et al*., 1991), while few others like Hg^{2+}, Cd^{2+}, Ni^{2+} and Pb^{2+} are toxic to plants. However, such toxic effects are even varying from genotype to genotype of the same crop (Liu *et al.,* 2001). The toxic dose depends on the type of ion, ion concentration, plant species, and stage of plant growth (Memon & Schröder, 2009). Metal uptake differences by the leafy vegetables are attributed to plant differences in tolerance to heavy metals (Itanna, 2002). Leafy vegetables are high accumulator of metal ions as compared to root vegetables and legumes (Alexander *et al.,* 2006).

Quetta the capital of Balochistan province is of the largest trade centers in the country. It has a hardy, ugly and dull face, despite being the provincial metropolis nestled amidst the craggy mountains that form a ring around it, looks dusty and bone dry (Hein & Shabeer, 1992). The population explosion, urbanization, persistent drought, the influx of Afghan refugees and industrialization are the major factors showed the way to city a situation that the fresh water channels were replaced by the domestic sewage and industrial wastes. Vegetables found in Quetta are lettuce, spinach, carrots, radish, cabbage, potato, tomato, turnip etc. are commonly grown in peri-urban areas of Quetta and irrigated by the city effluents. The direct use of untreated wastewater is common in most cities, which are due to the lack of alternative water sources, and about 26% of the domestic vegetables are cultivated with wastewater in Pakistan (Ensink-Jeroen *et al.,* 2004). Quetta is one of the badly affected cities by different pollutions (Anonymous, 2002; Kakar *et al.,* 2004). Like many other cities of the country, such wastewater in Quetta is also directly used for irrigation to raise the net output of vegetables, which are considered as a major source of organic manure and plant nutrients as well (Ahmad *et al.,* 1992; Otobbang *et al.,* 1997). The use of city effluents for irrigation purpose may also result in accumulation of macro (Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}) and micro-essential elements (Fe^{2+}, Mn^{2+}, Zn^{2+}) as well as trace and toxic elements (Pb^{2+}, Ni^{2+}, Cd^{2+}, Cr^{2+}, and Co^{2+}) in phytotoxic concentrations (Qadir&Ghafoor, 1997; Shah &Riazullah, 2003), which may adversely affect the human metabolism. Therefore, wastewater application on agricultural lands and their heavy metal accumulation by vegetables is a cause of serious concern due to the potential public health impacts. It is often argued that heavy metals such as Cd^{2+}, Ni^{2+} or Pb^{2+} in sludge, when applied to soils, may enter in the food chain through plants or animals, contaminate surface and ground water, and thus cause health hazards (Hue, 1994; Cui *et al.,* 2005; Bi *et al.,* 2006). This study was therefore; mainly aimed to determine the concentration of accumulated trace elements by lettuce irrigated with different sources (localities) and concentrations of wastewater.

Materials and Methods

In order to determine the concentration of heavy metals in lettuce (*Lactuca sativa* L.) it was irrigated with 5 different concentrations (treatments) of wastewater collected from three different localities of Quetta city viz., Chiltan town, Chiltan Ghee Mill (oil industry) and Zarghoon town. The treatments (T) were made by dissolving calculated amount of wastewater in tap water i.e., control, 25, 50, 75, and 100%. These treatments were then designated by letters T_1, T_2, T_3, T_4 and T_5, respectively. Each treatment was replicated thrice for each locality. Lettuce (*Lactuca sativa* L.) was grown in 45 pots contained well-mixed noncontaminated garden soil. They were then arranged in North-South direction at proper distance to avoid any possible external contamination alongwith the provision of possible uniform light condition. Ten healthy and uniform seeds of lettuce were sown at one-centimeter depth at an equal distance with the help of clean policeman. Pots were arranged in a completely randomized design (CRD) in an open field, and were then equally irrigated with respective treatments of wastewater. All standard agricultural practices were made during the course of germination, growth and development. Before initiation of flowers, plant samples were made by taking three leaves each from all treatments and replicates. Each treated plant sample was washed separately with 1% of HCl. They were then followed by 3-4 washings with de-ionized water to remove the foreign materials. These samples were then spread on blotting paper and air-dried, then oven dried at 70°C. After drying, leaf samples were crushed to powdered form in a grinder, and then converted into liquid following wet digestion procedure i.e., one gram of dried leaf powder samples placed in a beaker, 10 ml of concentrated HNO_3 was added and allowed to stand overnight. It was then heated with care on a hot plate until the production of red fumes ceased. The beakers were cooled and 2 ml of $HClO_4$ (70%) added to it, then again heated and allowed to evaporate to a small volume. Samples were filtered through the filter papers (Wattman 42) and transferred the filtrate to a 100 ml volumetric flask and the volume was made up to 100 ml with the help of de-ionized water (Adrain, 1973). The digest was thereafter analyzed for heavy metals including Cu^{2+}, Mn^{2+}, Fe^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, and Cd^{2+} by using an Atomic Absorption Spectrometer (Model ca-2380 Perkin-Elmer). Results obtained were statistically analyzed, following the procedure described by Steel &Torrie (1980). MSTAT-C computer software package was used for the said purpose.

Results and Discussion

Data presented in Table 1 deciphered that water sampling localities (A) and their different concentrations (B) in general highly significantly ($p \leq 0.01$) influenced the accumulation of all mentioned species of heavy metals. Whereas, interactions between A x B were found significant only for Fe^{2+}, Mn^{2+}, Zn^{2+}, $Ni^{2+}$and Cd^{2+}.

Results pertaining to mean values showed that both Cu^{2+} and total Fe^{2+} contents of lettuce were significantly and linearly increased as the concentrations of wastewater increased (Tables 2 & 3). Statistically maximum Cu^{2+} (9.72 mg kg^{-1}) and total Fe^{2+} contents (509.06 mg kg^{-1}) were obtained in highest dose of applied wastewater (T_5). However, by comparing the sampling localities, significantly high amount of Cu^{2+} (9.02 mg kg^{-1}) and total Fe (435.96 mg kg^{-1}) were found in Chiltan ghee mill and Chiltan town, respectively. Results also showed a highly significant interaction between treatments and localities for total Fe, but non-significant for Cu^{2+} contents. A maximum amount of Cu^{2+} and total Fe contents (10.73 and 580.91 mg kg^{-1}) were obtained in T_5 and T_4 of Chiltan ghee mill and Chiltan town effluents, followed by a minimum i.e., 5.33 and 210.65 mg kg^{-1} in T_1 of Chiltan town, respectively. Similar trend of results were also obtained by Achakzai & Bazai (2006) and Mohamad & Latif (2010) for spinach and water hyacinth respectively, but present values of Cu^{2+} and total Fe for lettuce are far lesser than those of spinach irrigated by the same wastewater. Following the criteria proposed by FAO/WHO (Table 9) the grand mean values of Cu^{2+} and total Fe are within the safe limit and is not in toxic range (Anonymous, 2001). Accumulation of these metals could be attributed to the use of effluents having low magnitude of mentioned metals for uptake and translocation by vegetable crops. Ramraj *et al.,* (2000), Demirezem & Aksoy (2006), Farooq *et al.,* (2008) and Zhuang *et al.,* (2009) have also obtained similar results with the same reasoning for lettuce, but contrasting results (12.13-32.80 mg kg^{-1}) were obtained by Itanna (2002) and Kumar *et al.,* (2009).

Table 1.Analysis of variance (ANOVA) for various heavy metals of lettuce subjected to various levels of wastewater collected from different localities of Quetta.

Variables	Sum of square			Mean square			F-Value of variables at an error of 28			CV (%)
	Localities (A)	Sewage water levels (B)	A x B	Localities (A)	Sewage water levels (B)	A x B	(A)	(B)	A x B	
Copper	23.808	75.909	2.038	11.904	18.977	0.255	16.0944 **	25.6581 **	0.3444 ns	10.74
Iron	7051.220	440158.036	57369.6596	35115.788	110039.509	7171.2072	15.9408 **	49.9523 **	3.2554 **	12.21
Manganese	579.979	701.788	1346.130		175.447		79.3292 **	47.9951 **	46.0307 **	7.26
Zinc	382.499	10655.998	1169.462	289.989	2664.000	168.266	5.1191 *	71.3044 **	3.9127 **	7.99
Nickel	44.384	91.733	8.556	191.255	22.933	146.183	38.3357 **	39.6164 **	1.8475 *	24.68
Lead	4.597	280.667	15.874	22.192	70.167	1.069	1.8718 *	57.1314 **	1.6157 ns	18.65
Cadmium	117.506	142.254	20.743	2.299	35.563	1.984	59.7256 **	36.1522 **	2.6358 *	17.61
				58.753		2.593				

* Slightly and * *highly significant at 5% and 1% level of probabilities, ns = non-significant and CV = coefficient of variation.

Table 2. Effect of five different levels of wastewater of Quetta city on the copper content (mg kg^{-1}) of lettuce.

Wastewater localities	Waste-water treatments (T)					Mean
	T_1	T_2	T_3	T_4	T_5	
Chiltan Ghee Mill	6.874	8.825	8.986	9.698	10.733	9.023 A
Chiltan Town	5.326	7.463	7.286	8.436	9.677	7.638 AB
Zarghoon Town	5.427	6.763	7.230	8.656	8.726	7.360 B
Mean	5.876 C	7.684 B	7.834 B	8.930 AB	9.712 A	8.007

LSD <0.05 and LSD <0.01 both for localities and treatments were 1.439 and 1.941, respectively

Table 3. Effect of five different levels of wastewater of Quetta city on the total iron (mg kg^{-1}) content of lettuce.

Wastewater localities	Waste-water treatments (T)					Mean
	T_1	T_2	T_3	T_4	T_5	
Chiltan Ghee Mill	244.450 gh	335.830 ef	292.430 ef	396.840 de	430.350 cd	339.980 B
Chiltan Town	210.650 h	391.010 de	491.293 bc	514.940 ab	580.910 a	435.961 A
Zarghoon Town	212.660 h	312.017 fg	399.960 cd	445.940 bcd	515.910 ab	377.296 AB
Mean	219.587 D	346.283 C	394.561 BC	452.573 AB	509.057 A	384.412

LSD <0.05 and LSD <0.01 both for localities and treatments were 78.50 and 105.9, respectively

Data concerned about Mn^{2+} exhibited that initial doses of applied wastewater samples significantly increased the Mn^{2+} level of lettuce, beyond that reduction is observed (Table 4). Whereas, in case of Zn^{2+}, significantly linear and progressive increase was recorded (Table 5). Statistically maximum Mn^{2+} (32.47 mg kg^{-1}) and Zn^{2+} contents (94.38 mg kg^{-1}) were obtained in T_2 and T_5 levels of wastewater, respectively. However, by comparing the site of samplings, significantly highest amount for Mn^{2+} (30.09 mg kg^{-1}) and Zn^{2+} (80.47 mg kg^{-1}) were obtained in Zarghoon and Chiltan towns followed by lowest viz., 21.50 and 73.63 mg kg^{-1} in Chiltan ghee mill effluents. Data also showed a highly significant interaction between localities and treatments (A x B). A maximum level of Mn^{2+} and Zn^{2+} (35.22 & 104.09 mg kg^{-1}) were recorded in T_2 and T_5 of Chiltan ghee mill and Chiltan town effluents. Whereas a minimumi.e., 12.57 and 46.11 mg kg^{-1} of the same metals noted in T_5 and T_1 of the same sampling sites, respectively. Few other researchers also obtained similar trend of results for spinach irrigated with the same kind and concentration of wastewater (Achakzai & Bazai, 2006), but present magnitude of Mn^{2+} and Zn^{2+} are much lesser than those recorded for spinach. Following the criteria proposed by FAO/WHO (Table 9) the grand mean values of Mn^{2+} (26.11 mg kg^{-1}) and Zn^{2+} (76.46 mg kg^{-1}) are within the safe limit and are not laid in toxic range (Anonymous, 2001). Hence the situation at this time does not seem to pose a great threat. However, with increase in lettuce consumption this situation could easily be changed. Therefore, periodical monitoring of contamination and consumption rates is necessary to assess the overall exposure level in the consumer's community. These results are in line with the findings of previous studies (Demirezen & Aksoy, 2006; Farooq *et al.,* 2008: Prabu, 2009) where they stated that Mn^{2+} and Zn^{2+} in lettuce were found in the range of 145.6 and 108.94 µg g^{-1}, respectively. While contrasting results were recorded by few others (Itanna, 2002; Akan *et al.,* 2008; Zhuang *et al.,* 2009; León *et al.,* 2010).

Table 4. Effect of five different levels of wastewater of Quetta city on the total manganese (mg kg^{-1}) content of Lettuce.

Wastewater localities	Waste-water Treatments (T)					Mean
	T_1	T_2	T_3	T_4	T_5	
Chiltan Ghee Mill	29.197 de	35.220 a	15.590 g	14.910 g	12.570 g	21.497 b
Chiltan Town	22.100 f	29.530 cde	34.077 ab	26.440 e	21.620 f	27.753 a
Zarghoon Town	21.150 f	32.650 bc	34.420 ab	32.250 bcd	29.970 cd	30.088 a
Mean	24.149 C	32.467 A	29.140 B	24.533 C	21.387 C	26.113

LSD <0.05 and LSD <0.01 both for localities and treatments were 3.198 and 4.314, respectively

Table 5. Effect of five different levels of wastewater of Quetta city on zinc content (mg kg^{-1}) of lettuce.

Wastewater Localities	Waste-water treatments (T)					Mean
	T_1	T_2	T_3	T_4	T_5	
Chiltan Ghee Mill	48.280 g	80.700 def	70.980 f	82.380 de	85.820 cd	73.632 AB
Chiltan Town	46.110 g	73.023 ef	81.760 de	97.370 ab	104.090 a	80.471 A
Zarghoon Town	51.110 g	75.030 ef	80.620 def	76.367 def	93.220 bc	75.269 AB
Mean	48.500 C	76.250 B	77.787 B	85.372 AB	94.377 A	76.457

LSD <0.05 and LSD <0.01 both for localities and treatments were 10.22 and 13.79, respectively

Data regarding mean values reflected that Ni^{2+} concentrations in lettuce are also significantly and linearly increased as the level of effluents increases (Table 6). Statistically maximum amount of Ni^{2+} (5.05 mg kg^{-1}) is obtained in highest dose of applied effluents (T_5) followed by lowest in background/tap water (T_1). However, by comparing the sampling localities, significantly high amount of Ni^{2+} (4.47 mg kg^{-1}) is recorded in Chiltan ghee mill. This could be due to the use of metal containers in food preservation and processing industry. The effluents from metal industry have also been reported to contribute enormously towards water contamination. Data also reflected a slightly significant ($p<0.05$) interaction between treatments and localities (A x B). A maximum level of Ni^{2+}(7.08 mg kg^{-1}) is obtained in T_5 of Chiltan ghee mill, followed by 0.40 and 0.60 mg kg^{-1} in T_1 of Zarghoon and Chiltan towns, respectively. However, the total amount of nickel accumulating in the plants is very low and even not the highest concentration of Ni^{2+}(3.083 mg kg^{-1}) approached the maximally admissible value given in the FAO/WHO (Table 9) standard (67.90 mg kg^{-1}). The results proved that lettuce belongs to crops with a low capacity of nickel uptake and accumulation. This might be due to limited availability of Ni^{2+} in the waste water or to a large extent this is because of its relatively short vegetation period (32 days). Poulik (1999), for instance, as well as other researchers also reached on the same conclusions (Demirezen & Aksoy, 2006; Prabu, 2009). Whereas, negligible amount of Ni^{2+} was detected by Kakar *et al.,* (2005) in tomato fruits irrigated by the effluents of Quetta city. However, few others obtained contrasting results for Ni^{2+} accumulation. The concentration of Ni^{2+} in various vegetable samples was higher than the limits set by WHO/FAO (Akan *et al.,* 2008, 2009; Khan *et al.,* 2008).

Table 6. Effect of five different levels of wastewater of Quetta city on nickel content (mg kg^{-1}) of lettuce.

Wastewater localities	Waste-water treatments (T)					Mean
	T_1	T_2	T_3	T_4	T_5	
Chiltan Ghee Mill	1.300 fgh	3.650 cd	5.270 b	5.020 b	7.080 a	4.465 A
Chiltan Town	0.600 gh	1.800 efg	2.740 de	2.210 ef	3.510 cd	2.172 B
Zarghoon Town	0.400 h	1.800 efg	2.760 de	3.540 cd	4.560 bc	2.612 B
Mean	0.767 C	2.417 B	3.590 B	3.590 B	5.050 A	3.083

LSD <0.05 and LSD <0.01 both for localities and treatments were 1.273 and 1.717, respectively

Mean values recorded for Pb^{2+} enumerated that there was a significant linear increase in the amount of Pb^{2+} as the concentration of wastewater increases. Statistically maximum amount (8.58 mg Pb kg^{-1}) was achieved in T_5 level of applied effluents (Table 7). Mean values also enumerated that water sampling localities were found significant by producing a maximum of 6.32 mg Pb kg^{-1} in Zarghoon town. While, reverse is true for interaction between sampling localities and its different levels. Present findings are also in agreement with those explained by other researchers (Vousta *et al.,* 1996; Kahlown *et al.,* 2006; Khan *et al.,* 2008; Manas *et al.,* 2009; León *et al.,* 2010). Following the criteria proposed by FAO/WHO (Table 9) the grand mean values of Pb^{2+} (5.94 mg kg^{-1}) is not within the safe limit, but lay in toxic range (Anonymous, 2001). Most other researchers also obtained similar findings (Demirezen & Aksoy, 2006; Kachenko & Singh, 2006; Akan *et al.,* 2008, 09; Bigdeli & Seilsepour, 2008; Gaw *et al.,* 2008; Khan *et al.,* 2008; Kumar *et al.,* 2009; Zhuang *et al.,* 2009). They noted that Pb^{2+}in lettuce generally ranges from 2.3-5.30 mg kg^{-1}, which super-passed the maximum permissible level of Pb^{2+} set by FAO/WHO. While few others noted that Pb^{2+} lying below the recommended maximum acceptable levels set by the joint FAO/WHO expert committee on food additives (Farooq *et al.,* 2008). In the present study the high level of Pb^{2+} in lettuce suggests that the wastewater used for irrigation was unfit for irrigation of crops in general and leafy vegetables in particular. Earlier studies revealed that metal uptake by plants not only occur by soil-root transfer, but also by direct transfer of contaminants from the polluted atmosphere to the aerial parts of plants (Uzu *et al.,* 2010). Therefore, atmospheric deposition could be another reason for elevated concentration of Pb^{2+} in lettuce. This study in term of Pb^{2+} level highlights the potential health risks associated with the cultivation and consumption of lettuce on wastewater-contaminated soils. The situation could be more worst if we do increase our consumption rate of lettuce in particular and other leafy vegetables in general as well as long-term exposure of soil to wastewater irrigation.

Table 7. Effect of five different levels of wastewater of Quetta city on lead content (mg kg^{-1}) of lettuce.

Wastewater localities	Waste-water treatments (T)					Mean
	T_1	T_2	T_3	T_4	T_5	
Chiltan Ghee Mill	2.100	4.850	5.770	6.980	7.970	5.534 AB
Chiltan Town	1.240	3.920	7.600	8.800	8.320	5.976 AB
Zarghoon Town	2.220	4.920	7.670	8.980	9.450	6.315 A
Mean	1.853 D	4.563 C	7.013 B	8.253 AB	8.580 A	5.942

LSD <0.05 and LSD <0.01 both or localities and treatments were 1.853 and 2.500, respectively

Data regarding mean values reflected that Cd^{2+} concentrations in lettuce is significantly increased by the initial doses of applied effluents, beyond that insignificant change is observed (Table 8). Numerically a maximum amount of Cd^{2+} (7.45 mg kg^{-1}) is obtained in highest dose of applied effluents (T_5) followed by lowest in background/tap water (T_1). However, by comparing the sampling localities, significantly high amount of Cd^{2+} (7.89 mg kg^{-1}) is recorded in Chiltan ghee mill effluents. Chiltan ghee mill is basically an oil industry, and such toxic level of Cd^{2+} in resultant lettuce could be due to the use of metal containers in food preservation and processing. The effluents from metal industry have also been reported to contribute enormously towards water contamination. Data also reflected a slightly significant ($P<0.05$) interaction between treatments and localities (A x B). A maximum level of Cd^{2+} (10.2 mg kg^{-1}) is obtained in T_5 of Chiltan ghee mill, followed by minimum values of 0.40 and 1.43 mg kg^{-1} in T_1 of Zarghoon and Chiltan towns, respectively. The results obtained suggest that lettuce is found to have more contaminant capability. Similar findings are also obtained by other researchers (Kahlown*et al.,* 2006; Gaw *et al.,* 2008; Yang *et al.,* 2009; Fu *et al.,* 2009; Mohamad & Latif, 2010). On the basis of grand mean value, the concentration of Cd^{2+} (5.63 mg kg^{-1}) in present study comes under the toxic range as proposed by FAO/WHO (Table 9). Analytical results of Cd^{2+} recorded by different researchers for various vegetable foodstuffs (including lettuce) also clearly indicated that vegetable samples values were well above the WHO critical toxic level (Vousta *et al.,* 1996; Bahemuke & Mubofu, 1999; Itana, 2002; Kachenko & Singh, 2006; Mapanda*et al.,* 2007; Akan *et al.,* 2008, 09; Khan *et al.,* 2008; Yorgholi *et al.,* 2008; Prabu, 2009; Kumar *et al.,* 2009; Zhuang *et al.,* 2009). While very few others recorded that Cd^{2+} is lying below the recommended maximum acceptable limit set by the joint FAO/WHO expert committee in food additives (Demirezen & Aksoy, 2006; Farooq*et al.,* 2008). However, negligible amount of Cd^{2+} is also detected by Kakar *et al.,* (2005) in tomato fruits irrigated by the same effluents of Quetta city. In the present study the accumulation of elevated concentration of Cd^{2+} in lettuce could be attributed to the use of effluents for their cultivation. The reason for accumulation is that Cd^{2+} is relatively taken by food crops and especially by leafy vegetables. Also it may be due to foliar absorption of atmospheric deposits on plant leaves.

Table 8. Effect of five different levels of wastewater of Quetta city on cadmium content (mg kg^{-1}) of lettuce.

Wastewater localities	Waste-water treatments (T)					Mean
	T_1	T_2	T_3	T_4	T_5	
Chiltan Ghee Mill	3.500 efg	6.860 b	8.940 a	8.980 a	10.210 a	7.898 A
Chiltan Town	1.433 h	4.990 cde	5.400 bcd	5.400 bcd	6.590 bc	4.763 B
Zarghoon Town	2.560 gh	3.100 fg	4.320 def	5.650 bcd	5.650 bcd	4.238 B
Mean	2.498 C	4.983 B	6.220 AB	7.010 A	7.453 A	5.633

LSD <0.05 and LSD <0.01 both for localities and treatments were 1.659 and 2.238, respectively

Mean values tabulated in Tables #. **2-8** followed by the same letter(s) within right side column (localities) and bottom rows (treatments) are not significantly different (p<0.05) using LSD test. Similarly values followed by the same letter(s) within column and rows (localities x treatments) in the center of each Table are not significantly different from each other.

Table 9. A comparison of heavy metal levels in cultivated lettuce (*Lactuca sativa* L.) and international maximum limits for vegetables.

Heavy metals	Mean concentration of metals (mg kg^{-1})	Permissible levels set by FAO/WHO ^{a}(mg kg^{-1})	Maximum levels prescribed by other authorities (mg kg^{-1})
Cu^{2+}	8.00	73.30a	73.30^{c}
Fe^{2+}	384.412	425.50a	425.50^{c}
Mn^{2+}	26.113	500.00a	500.00^{d}
Zn^{2+}	76.457	99.40a	50.0^{b}
Pb^{2+}	5.942*	0.30a	0.30^{b}
Ni^{2+}	3.083	67.90a	67.90^{c}
Cd^{2+}	5.633*	0.20a	0.20^{b}

^{a}Anonymous (2001), ^{b}Anonymous (1984 & 1989), ^{c}Weigert (1991) and ^{d}Pendias & Pendias (1992).*Metals found above permissible limit

Conclusion

It is concluded that as the concentration of wastewater increases, heavy metals are generally increases. This was also noted that heavy metals like Cu^{2+}, Ni^{2+} and Cd^{2+} are found significantly greater in Chiltan ghee mill effluents (oil industry). Analytical result of grand mean values for some trace metals viz., Cu^{2+}, total Fe, Mn^{2+}, Zn^{2+} and Ni^{2+} indicates that they are within the safe limit following the recommended international standards. Hence the situation at this moment does not seem to pose a great health threat. However, with increase in vegetable consumption as well as consecutive use of wastewater effluents in agriculture sector could easily change the scenario. Therefore, periodical monitoring of contamination and consumption rates is necessary to assess the overall exposure level in the community. On the basis of grand mean values,the magnitude of heavy metals detected for various metals is in order: Fe > Zn >Mn> Cu >Pb> Cd > Ni.

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