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PROLONGED WASTEWATER IRRIGATION AND HEAVY METAL ACCUMULATION IN SOILS, VEGETABLES AND FODDER CROPS

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Abstract: Worldwide wastewater loaded with toxic heavy metals, used as a reliable source for irrigating a variety of crops, have some risks and restrictions. The extent of wastewater heavy metal accumulation was investigated during 2009-10 in soils, vegetables and fodder crops grown in the periphery of District Dera Ismail Khan, Pakistan. The wastewater exhibited extreme basicity (pH 10.31) and the ratios of electrical conductivity, sodium adsorption, carbonates and bi-carbonates, and chloride concentration were beyond the prescribed standards suggesting that the use of wastewater could be a limiting factor. In addition, wastewater had abnormally higher levels of Fe, Cu, Zn, Ni, Mn, Pb, Cr, and Cd. Generally, in wastewater irrigated soil, the heavy metal concentration was higher than tube-well irrigated soil and was found beyond the permissible limits. Majority of the vegetables and fodder crops tested here accumulated excessive amounts of Cd, Cu, Pb and Zn, which were beyond the permissible limits. The concentrations of Cr, Ni, Mn and Fe on the contrary were below the permissible limits in vegetables and fodder crops. Utilization of wastewater for irrigating the food and feed crops might not be safe, which could possibly be due to high salinity and sodium adsorption ratio leading eventually to adverse impact on growth and yield of crops. Soils and crops irrigated with wastewater should closely be monitored for heavy metals deposition so that the human health problems could be avoided.

Keywords: Wastewater, heavy metals, vegetables, fodder crops, human and livestock health

1. INTRODUCTION

Pedosphere, hydrosphere, atmosphere, lithosphere or biosphere, are adversely affected by environmental pollution (Lone et al., 2008). Due to anthropogenic activities and desire, land and water resources are over exploited and degraded. Water pollution is generated due to ill-planned and untreated sewage and factories effluent disposal into the canals and watercourses. Water pollution has become global issue and every man's business, ultimately making ground water resources unfit (Mashiatullah et al., 2005). Globally, it is a common practice that municipal and industrial effluents are used in agriculture. Due to greater use of fresh water and constantly declining trend of irrigation water resources, the wastewater discharged into channels reaches to the cultivated area located at the periphery of the municipality. A wide variety of fodder and vegetable crops are irrigated with that wastewater (Ensink et al., 2002; Sharma et al., 2007).

Use of wastewater as an irrigation alternative is practiced in most of the countries due to shortage and scarcity of fresh irrigation water. It is also well known fact, that wastewater is a reliable source of water used by farmers for irrigation. In using wastewater, the risks and benefits are tied together and go parallel with each other. Even though, wastewaters provides valuable plant nutrients and organic matter yet it contains toxic heavy metals which accumulate in soil and through uptake, into the crops (Horswell et al., 2003; Liu et al., 2005; Kahlown et al., 2006). However, the metal concentrations in plants vary with plant species (Secu et al., 2008). Opportunities and risks, both are there for the farming sector due to use of wastewater (Singh et al., 2004; Chen et al., 2005). Some of the essential plant nutrients are Cu, Fe, and Zn while others like, Cd, Cr, Ni and Pb are toxic to plants (Baker et al., 1991). However, the accumulation of heavy metals in soils beyond certain limits may result in serious environmental problems due to their negative impact on crop production and human health and livestock (Arar, 1988). Due to natural tendency, the plants accumulate the toxic heavy metals due to their solubility in water. Heavy metals are ubiquitous due to excessive use in industrial sector which discharge a substantial amount into environment that after uptake may damage the mental and neurological functions, influence neurotransmitter production and utilization, and alter numerous metabolic processes (Singh et al., 2004; Chen et al., 2005). Systems where toxic elements can have negative effects include the blood and cardiovascular, detoxification pathways (colon, liver, kidneys, skin), endocrine (hormonal), energy production pathways, enzymatic, gastrointestinal, immune, nervous (central and peripheral), reproductive, and urinary (Greenwood & Earnshaw, 1986). The clinical course of the copper sulphate intoxicated patient is often complex involving intravascular hemolysis, jaundice and renal failure (Saravu et al., 2007).

Heavy metals when present in the food chain retard the process of hydrolysis e.g. α amylase, phosphatase, RNAse and proteins. By replacing metal ions from metaloenzymes, the heavy metals interfere with enzyme action. Heavy metals also reduces photosystem-II activity and causes structural change in chloroplast and thus reduces photosynthesis, availability of CO_2, lowers stomatal conductance, reduces total lipids, glycolipids and neutral lipids, interfere with membrane permeability and reduces respiration in leaves (Agarwal, 2002). As investigated by Raza et al., (2013), the seedlings length and dry weights were severely affected by cadmium. Toxic level of Pb inhibits germination, reduces rate of photosynthesis, transpiration, gaseous exchange in leaves and total chlorophyll production by altering relative proportion of chlorophyll A and B. Similarly, toxic levels of nickel and chromium showed drastic effects on dry matter production and yield (Agarwal, 2002).

Vegetables and fodder crops are the potential source of important nutrients both for humans and livestock. Vegetables provide protein, vitamins, iron and calcium to the human health (Arai, 2002). Higher amounts of heavy metals are accumulated by vegetables especially the leafy ones being capable of more absorption in leaf tissues, cultivated on contaminated sites (Al-Jassir et al., 2005; Chary et al., 2008). In various parts of Turkey, Demirezen & Ahmet (2006) investigated the concentrations of heavy metals such as Cd, Pb, Zn, Cu and Ni in different vegetables. Radwan & Salama (2006) examined the levels of heavy metals (lead, cadmium, copper and zinc) in fruits and vegetables of Egypt and found the heavy metals above the permissible limits. Similar studies were undertaken by Fytianos et al., (2001) who reported heavy metals beyond the international standards in vegetables grown in industrial area of North Greece.

Dera Ismail Khan, Pakistan is an old and extreme southern district of the Khyber Pakhtunkhwa province, Pakistan with an area of 7,326 km^2 (2,829 sq. miles) sharing its borders with Punjab on south, South Waziristan and Baluchistan in the west, accommodating a population of about one million. Because of no way-out, in-time and continued availability and cheap source of irrigation, the wastewater use as irrigation water is in vogue for more than fifty years. The farmers without considering its health implications or pre-treatment, use raw wastewater for irrigating fodder and vegetable crops grown in the periphery. These crops are then sold into local market.

Considering the negative impacts of heavy metals on soil fertility and crop production, the present research work was designed to determine the concentration of heavy metals in tube-well water, wastewater, soil, vegetables and fodder crops. Furthermore, to determine the extent of pollution because of prolonged and permissible use of raw wastewater for growing vegetables and fodder crops with least health implications.

2. MATERIALS AND METHODS

The farmer's cropped fields used for the studies were situated in the periphery of North-East, East and South-East of the municipality of Dera Ismail Khan, Pakistan. The wastewater heavy metals accumulation was investigated in soil, vegetables and fodder crops (grown for the last five decades). The laboratory work was carried out during 2009-10 at the Central Research Laboratory (CRL), Gomal University, Dera Ismail Khan (31°, 49′ North latitude and 70°, 55′ East longitude), Pakistan.

2.1. Physico-chemical analysis of wastewater and tube-well water samples

Wastewater and tube-well water samples were collected for physico-chemical characteristics in pre- acid-washed polypropylene 500 ml bottles and 1 ml of concentrated HNO_3 was added to the samples to avoid microbial activity and soon refrigerated after collection (Table 1). The pH and EC of wastewater and tube-well water was determined using pH meter and electrical conductivity meter, respectively (Richards, 1954). The Ca and Mg concentrations of both types of water were determined by titration with EDTA, whereas the flame photometer was used to determine Na and K. The SAR values were determined according to Richards (1954).

$$SAR = Na^{+}/\sqrt{(Ca^{++}\ Mg^{++})/2}$$

Similarly, the chemical oxygen demand (COD) and biological oxygen demand (BOD) were determined following the standard procedure of Clesceri et al., (1989). The heavy metals (Fe, Cu, Pb, Zn, Cd, Cr, and Mo) in wastewater and tube-well water were determined by atomic absorption spectrophotometer (Buck Scientific Accusys-211, Germany) using their respective standards for quality control. Nitrogen (NO_3-N) in water samples was determined by micro-kjeldahl method (Bremner & Keeney, 1965).

2.2. Collection of crop samples

Composite crop samples of vegetables and fodder crops grown in farmer's field situated in the periphery of North East, East and South East of the municipality of Dera Ismail Khan, Pakistan were collected. The vegetable and fodder farms received wastewater irrigation continuously for five decades. The harvested crops are retail sold in the local market of Dera Ismail Khan. A total of six sound and healthy samples of each crop, and in total 6480 and 3888 vegetable and fodder crop samples, respectively were collected from three different locations (having 36 plots at each location). The test vegetable crops included tomato, eggplant, okra, bitter gourd, ridged gourd, lettuce, spinach, turnip, cauliflower and purslane leaves, while fodder crops included berseem, maize, sorghum, millet and wild oat. Moreover, for heavy metals analysis, the crop samples were collected before the blossoming stage. Similarly, the soil samples from tube-well and wastewater irrigated soil were collected in labeled plastic bags.

2.3. Preparation and preservation of crop samples

After collection, the vegetables and fodder crop samples were shifted to the laboratory with in 30 min., washed with distilled water and oven dried (70 °C for 72 h). After drying, the crop samples were ground manually in pastel and mortar and homogenized. The ground crop sample (0.2 g) was dissolved in 10 ml concentrated HCl in a 100-ml beaker. The beaker was covered with a watch glass, and the contents were boiled on a hot plate for approximately 30 min. The contents were then evaporated to nearly dryness. After cooling, 20 ml of 0.1 M HCl was added, and the contents were gently boiled. The contents were quantitatively transferred into 100 ml volumetric flask by filtering through Whatman No. 2 filter paper. The residues were thoroughly washed with 0.1 M HCl and the volume was adjusted with the same solution to 100 ml. The digest obtained was analyzed for various heavy metals using flame Atomic Absorption Spectrophotometer.

2.4. Statistical analysis

Using descriptive statistics, the various variables were calculated with MS-Excel program.

3. RESULTS AND DISCUSSION

The wastewater with alkaline pH used for vegetables and fodder crops exhibited extreme electrical conductivity (EC) and sodium adsorption ratio (SAR) as 3.87 and 36.23, respectively which were beyond the prescribed standards of Food and Agricultural Organization (FAO, 1992) (Table 1). In contrast to the FAO standards, carbonates and bi-carbonates concentration (2.53 and 11.19 mg L^{-1}), respectively were also higher in the wastewater. Wastewater quality in the context of crop irrigation can be seen from pH and SAR figures, which revealed that use of wastewater could be a limiting factor. Similarly, chloride concentration was higher by 40% than that of tube-well irrigated soil which may be a problem in agronomic practices. The chemical composition of the used wastewater biochemical oxygen demand (BOD), chemical oxygen demand (COD) and sodium adsorption ratio (SAR) were above the permissible limits (FAO, 1992; NEQS, 2005; Kakar et al., 2010; Khan et al., 2012). Preliminary study of physico-chemical properties of the soil irrigated with tube-well and wastewater is given in table 2.

3.1. Heavy metals in tube-well and wastewater

The heavy metals concentration in tube-well water and wastewater varied greatly (Table 3). In wastewater, the extent of Fe was 15.35 mg L^{-1} which was much higher than tube-well water (1.73 mg L^{-1}) and was the highest among all the heavy metals which exceeded the permissible limits of FAO (5 mg L^{-1}) and NEQS (8 mg L^{-1}). Other heavy metals like Cu, Pb, Cd, Cr, and Ni were not detected in the tube-well water. On the contrary, the Cu concentration in wastewater was 1.7 mg L^{-1} against the permissible limits of 0.1 mg L^{-1} of the FAO.

Table 1. Physico-chemical characteristics of tube-well water and wastewater in comparison to FAO, NEQS and WHO standards

Parameters	Concentration	Tube-well water	Wastewater	FAO	NEQS	WHO
pH	-	8.01	10.31	6.50-8.50	6-9	-
EC	dsm^{-1}	2.07	3.87	3.00	Nil	-
SAR	-	9.33	36.23	15.00	Nil	-
COD	mg L^{-1}	89.00	1589.00	Nil	400.00	-
BOD	mg L^{-1}	57.00	850.00	Nil	250.00	-
Grease & Oil	-	Nil	14.30	Nil	10.00	-
CO_3	mg L^{-1}	0.51	2.53	Nil	NGVS	-
HCO_3	mg L^{-1}	2.80	11.19	600	NGVS	400
Cl-	mg L^{-1}	213	2631.00	1100	1000	400
Fe	mg L^{-1}	1.73	15.35	5.00	8.00	5.00
Cu	mg L^{-1}	Nil	1.70	0.10	1.00	0.20
Zn	mg L^{-1}	0.09	5.83	Nil	5.00	5.00
Mn	mg L^{-1}	0.06	2.21	0.20	1.50	0.20
Ni	mg L^{-1}	Nil	2.55	5.00	1.00	0.20
Cd	mg L^{-1}	Nil	0.41	0.01	0.10	0.01
Pb	mg L^{-1}	Nil	2.19	2.00	0.50	5.00
Cr	mg L^{-1}	Nil	1.69	0.10	1.00	0.10
SO_4	mg L^{-1}	135.00	1435.00	1000	1000	0.50
Phosphorus	mg L^{-1}	1.12	26.00	Nil	15.00	-
NO_3-N	mg L^{-1}	5.27	87.20	Nil	Nil	-

FAO - Food and Agriculture Organization; NEQS - National environmental quality standards; WHO - World Health Organization; NVGS - No guidelines value standards; EC - Electrical conductivity; SAR - Sodium adsorption ratio; COD - Chemical oxygen demand; BOD - Biochemical oxygen demand

Table 2. Physico-chemical properties of the soil irrigated with tube-well and wastewater

Parameters	Units	Soil irrigated with Tube-well	Soil irrigated with wastewater	WHO
Soil texture	Silt clay loam	Nil	Nil	-
pH	-	8.10	7.84	4-8.5
EC	Dsm^{-1}	0.96	1.63	4.00
CO_3	-	Nil	Nil	-
HCO_3	mg L^{-1}	196.36	243.90	-
Cl-	mg L^{-1}	273.52	385.60	-
Ca Mg	mg L^{-1}	213.82	249.39	-
Org. Carbon	%	7.00	0.81	-
Potassium	ppm	8.08	19.78	>80
Sodium	ppm	11.20	13.63	-
Phosphorus	kg ha^{-1}	14.06	20.82	>7

WHO - World Health Organization

Table 3. Heavy metals in tube-well water and wastewater

Heavy metals	Tube-well water (mgL-1)			Wastewater (mgL-1)		
	Mean	Range	SD	Mean	Range	SD
Cd	ND	ND	ND	0.41	0.38 - 0.44	0.03
Cr	ND	ND	ND	1.69	1.64 - 1.77	0.05
Cu	ND	ND	ND	1.70	1.65 - 1.81	0.60
Fe	1.73	1.67 - 1.77	0.04	15.35	14.36 - 16.05	0.71
Ni	ND	ND	ND	2.25	2.51 - 2.59	0.04
Mn	0.06	0.04 - 0.09	0.02	2.21	0.19 - 0.25	0.03
Pb	ND	ND	ND	2.19	2 - 2.31	0.12
Zn	0.09	0.07 - 0.11	0.2	5.83	5.34 - 6.18	0.31

ND - Not detected; SD - Standard deviation

In wastewater, the Zn (5.83 mg L^{-1}) was slightly above the recommended standards of NEQS. In wastewater, the concentrations of Ni, Mn, Pb, Cr, and Cd were found as 2.25, 2.21, 2.19, 1.69 and 0.41 mg L^{-1}, respectively which also deviated from the permissible limits of FAO and NEQS. In the past studies, the higher concentrations of heavy metals were noted in wastewater than tube-well water (Rattan et al., 2005). Prolong use of sewage/ industrial water as irrigation water may cause buildup of heavy metals up to toxic levels for plant and animal health (Kirkhan, 1983). In previous studies, higher levels of Cu, Mn, Pb and Cd have also been reported in sewage water collected from Peshawar, Pakistan (Ehsan et al., 2011). In a similar study, Jagtap et al., (2010) also reported higher contents of Zn, Cu, Pb, Ni, Cd and Cr in wastewater collected from Rawalpindi, Pakistan.

3.2. Heavy metals concentration in soil irrigated with tube-well water and wastewater

The prolonged raw wastewater irrigation resulted in many-fold increase in concentration of heavy metals in the soil. For example, in wastewater irrigated soil, the Cd concentration was 7.11 mg kg^{-1} (88.6% higher than tube-well irrigated soil) which was more than those of the permissible limits of WHO (Table 4). Even though, the highest concentration of Zn (59.83 mg kg^{-1}) was observed in wastewater irrigated soil, yet it was lower than the WHO permissible limits. However, these heavy metals were not detected in the tube-well irrigated soils. In agreement to these finding, Singh et al., (2004) and Sharma et al., (2007) also reported the higher concentration of Zn in wastewater irrigated soil. Differences in concentration of heavy metals in soils between urban and rural zone have been reported (Secu et al., 2008). The concentration of other metals like, Fe (81.74%), Cu (97.17%), Pb (94.65%) Cr (97.96%) and Mn (96.39%) were higher in wastewater irrigated soil than tube-well irrigated soil (WHO, 1989). In wastewater irrigated soil, except Cd, the concentration of other heavy metals were within the permissible limits which could possibly be due to continuous accumulation of metals by the crops grown in this area (Singh et al., 2010). Soils were heavily tainted, and the heavy metals ranged from 9.1-2593 (Cu), 1.1-27 (Cd), 83-8040 (Pb) and 60-11445 mg kg-1 (Zn) (Big et al., 2012). The Cd had the highest absorption capacity in vegetables, and soil pH had no clear effect on metal accumulation in leaf vegetables (Chang et al., 2014). Harmanescu et al., (2011) findings revealed that in contaminated areas, the analyzed metals (Mn, Zn, Cu, Cd and Pb) were higher over normal content in plant tissues. Bashir et al., (2014) findings revealed that total metal contents in soil were in the order of Mn > Co > Zn > Cr > Ni > Cu > Cd.

3.3. Heavy metals concentration in vegetables irrigated with wastewater

Accumulation of heavy metals in the shoot and root tissues of vegetables of the farmer's field irrigated with municipal wastewater was variable (Table 5). The trend of variability in absorption of heavy metals by these vegetables and fodder crops could be attributed to their differential efficiency and capabilities (Zurera et al., 1989). In tomato shoots, the concentration of Cd, Cu and Pb were 6.17, 237.13 and 174.23 mg kg^{-1} while in root tissues the concentration was 3.50, 107.21 and 121.27 mg kg^{-1}, respectively (Table 5). The concentration of such metals in root and shoot tissues of tomato plants were significantly higher than those recommended by WHO, FAO and NEQS. In a similar study, Liu et al., (2006) also observed greater accumulation of Cu in other vegetables. In the present study, Cd, Cu and Pb in eggplants were 7.51, 247.26 and 178.29 mg kg^{-1} in shoot and 3.73, 119.12 and 133.12 mg kg^{-1} in root tissues, respectively (Table 5).

Table 4. Heavy metals in soil irrigated with tube-well water and wastewater

Heavy metals	Soil irrigated with tube-well water (mg kg^{-1})			Soil irrigated with wastewater (mg kg^{-1})			WHO (mpl) (mg kg^{-1})
	Mean	Range	SD	Mean	Range	SD	
Cd	0.81	0.76 - 0.86	0.04	7.11	6.00 - 1.57	0.64	1.0
Cr	0.37	0.30 - 0.43	0.05	18.15	17.89 - 18.34	0.21	100
Cu	0.71	0.66 - 0.79	0.05	25.13	24.21 - 25.87	0.61	100
Fe	3.51	3.42 - 3.60	0.07	19.23	18.67 - 20.0	0.55	NGVS
Ni	ND	ND	ND	13.71	13.65 - 13.77	0.52	20
Mn	0.12	0.09 – 016	0.03	3.33	0.26 - 0.38	0.06	500
Pb	1.39	1.25 - 1.65	0.16	26.01	25.57 - 26.48	0.33	500
Zn	ND	ND	ND	59.83	57.91 - 61.24	1.23	250

ND - Not detected; SD - Standard deviation; mpl - Maximum permissible limit; WHO - World Health Organization; NVGS - No guidelines value standards

Table 5. Heavy metals concentration in vegetables irrigated with wastewater

Vegetables	Shoot /Root	Cd	Cr	Cu	Fe	Ni	Mn	Pb	Zn
	Shoot and tissues (dm. mg kg^{-1})								
Tomato	Shoot	6.17	0.67	237.13	387.13	5.31	4.25	174.23	77.11
	Root	3.50	0.35	107.21	157.25	2.12	2.29	121.27	31.07
Egg plant	Shoot	7.51	0.43	247.26	395.12	4.21	5.04	178.29	83.21
	Root	3.73	0.31	119.12	167.16	1.23	3.31	133.12	37.53
Okra	Shoot	5.88	0.64	218.61	174.32	2.13	5.14	172.21	116.3
	Root	4.54	0.40	102.37	64.22	2.15	3.64	134.76	81.36
Bitter gourd	Shoot	6.31	0.51	187.12	167.15	3.55	3.89	133.53	91.37
	Root	4.83	0.34	84.71	122.32	1.87	2.17	121.17	57.62
Ridged gourd	Shoot	13.03	1.97	173.21	115.25	5.17	4.12	123.14	130.19
	Root	13.03	1.97	173.21	115.25	2.21	2.56	123.14	130.19
Lettuce	Shoot	0.29	0.18	19.13	21.11	4.11	2.21	23.32	23.45
	Root	0.29	0.18	19.13	21.11	1.08	0.94	23.32	23.45
Spinach	Shoot	0.06	0.59	91.23	79.25	2.14	3.35	113.00	85.96
	Root	0.03	0.59	91.23	79.25	0.87	0.51	94.33	69.03
Turnip	Shoot	1.56	0.59	55.21	46.15	5.41	4.85	57.42	53.36
	Root	2.14	0.84	79.61	69.63	0.95	2.16	73.31	76.57
Cauliflower	Shoot	2.71	0.72	93.15	79.81	7.12	5.61	113.15	83.31
	Root	1.07	0.38	68.61	51.17	2.29	3.67	98.27	83.31
Purslane leaves	Shoot	0.34	0.22	59.59	51.47	6.33	5.72	53.57	64.09
	Root	0.23	0.17	49.37	43.27	2.57	1.98	46.57	50.13
WHO (mpl)		0.20	2.30	73.30	425.00	67.90	500.00	0.30	99.40
Dm - dry matter; WHO - World Health Organization; mpl - Maximum permissible limit									

However, the Fe contents in tomato (387.13 and 157.25 mg kg^{-1}) and eggplant (395.12 and 167.16 mg kg^{-1}) in shoot and root tissues, respectively, even-though did not reach beyond the permissible limits, yet it was highest among other heavy metals. Moreover, the Cr, Ni, Mn and Zn in tomato and eggplant were found within the permissible limits. In a similar study, Bashir et al., (2014) noted that concentrations of all the toxic metals in edible parts of the vegetables were above the critical level. Demirezen & Ahmet (2006) analyzed different samples of vegetables and reported high concentrations of Pb (3.0-10.7 mg kg^{-1}) which poses health risks to human life.

In okra, there was maximum concentration of Cd (5.88 and 4.54 mg kg^{-1}), Cu (218.61 and 102.37 mg kg^{-1}) and Pb (172.21 and 134.76 mg kg^{-1}) in shoot and root tissues, respectively and Zn (116.3 mg kg^{-1}) in okra shoot, were noted above the permissible limits. Other heavy metals in okra were within the permissible range, but the amounts of Fe was substantially greater than other metals in shoot and root tissues (Lăcătușu & Lăcătușu, 2008).

In shoot and root tissues of bitter gourd, the concentration of Cd was: 6.31, 4.83 mg kg^{-1}, Cu: 187.12, 84.31 mg kg^{-1} and Pb: 133.53, 121.17 mg kg^{-1} In ridged gourd, the extent of Cd, Cu, Pb, Zn were 13.03, 173.21, 123.14 and 130.19 mg kg^{-1} in shoot and 13.03, 173.21, 123.14 and 130.19 mg kg^{-1} in root tissues, respectively. It was also noticed that Cd, Cu, Pb and Zn concentrations in shoots were identical to those found in root tissues of ridged gourd. However, the concentrations of all other heavy metals were below the permissible standards. Muchuweti et al., (2006) reported the level of Pb (6.77 mg kg^{-1}) in vegetables irrigated with wastewater to be higher than WHO safe limit. Higher amounts of heavy metals are reported to disturb the physiological functions of plants leading eventually to the death of plants (Garbisu & Alkorta, 2001; Schmidt, 2003; Khan et al., 2012, 2014).

The green leafy lettuce absorbed all the heavy metals but below the recommended standards with the exception of Cd and Pb with equal concentrations of 0.29 and 23.32 mg kg^{-1} in shoot and root tissues, respectively. High levels of heavy metals accumulation was reported in vegetables like lettuce, cabbage and carrot (Harmanescu et al., 2011). In wastewater irrigated vegetables, the loose-leaf lettuce, lettuce, Chinese cabbage and Chinese leaf mustard showed higher values of Hg, Pb, and Cr, and estimated that the residents of the Pearl River Delta (PRD) had 70% target hazard quotient through consumption of these vegetables (Chang et al., 2014). In the present studies, spinach accumulated a bit higher concentration than permissible limits of Cu and Pb (91.23 and 113.00 mg kg^{-1}) in shoot and 91.23 and 94.33 mg kg^{-1} in root tissues, respectively. While comparing the uptake of heavy metals by spinach and bitter gourd, Bashir et al., (2014) reported that Ni, Cr, Zn, and Mn accumulated more in spinach than bitter gourd. However, in the present studies the metals absorption was lower than permissible limits which might be attributed to low level of pollution in water, soil and soil structure. Other heavy metals were observed below the allowable maximum permissible limits of the spinach. Farooq et al., (2008) also reported a similar higher level of Cu in spinach. In contrast, Chang et al., (2014) found that that pak-choi (Chinese cabbage) had the lowest capacity of heavy metal enrichment in all the six leafy vegetables, and Cd had the highest capacity for transferring from soil into vegetables. Due to higher transpiration rate, the leafy vegetables accumulate more heavy metals than non-leafy. Chary et al., (2008) also reported highest enrichment factor for heavy metals through leafy vegetables. Khedkar and Dixit (2003) also mentioned that *Spinacea oleracea* irrigated with sewage water contained heavy metals, potentially toxic, which become part of our food-chain through plant uptake, however, their ratio of accumulation depends on its solubility in soil solution (Chaney, 1990). Adverse toxic effects of the municipal wastewater on the growth and yield of certain vegetables like spinach, lettuce, carrot, radish and sugar beet have been reported (Tamoutsidis et al., 2002).

In case of turnip, the Cd and Pb exhibited the concentrations of 1.56 and 57.42 mg kg^{-1} in shoot and 2.14 and 73.31 mg kg^{-1} in root tissues, respectively which were beyond the permissible limits. However, the concentration of other heavy metals in turnip was significantly lower. Likewise, the cauliflower had beyond the permissible limit concentration of Cd, Cu and Pb i.e. 2.71, 93.15 and 113.15 mg kg^{-1} in shoot and 1.07, 68.61 (slightly below) and 98.27 mg kg^{-1} in root tissues, respectively. However, the Cr, Fe, Ni, Mn and Zn accumulation was below the permissible limits in the cauliflower. Moreover, the purslane used as a substitute of spinach, contained 0.34 and 53.57 mg kg^{-1} of Cd and Pb in shoot and 0.23 and 46.57 mg kg^{-1} in root, respectively. Higher concentrations of Pb, Cd, and Zn were observed in purslane, however, Cd was more toxic to *P. oleracea* seedling, compared to Pb and Zn, and roots of *P. oleracea* seedlings were more sensitive than shoots (Naz et al., 2013). Harmanescu et al., (2011) also concluded that in metals contaminated areas, higher accumulations than allowable limits were observed for Zn, Cu, Cd and Pb in parsley roots and leaves, carrot roots, cabbage, lettuce and cucumber. Farooq et al. (2008) reported varied concentrations of Pb, Cu, Cr, Zn and Cd in the leaves, stems and roots of spinach, coriander, lettuce, radish, cabbage and cauliflower. However, contents of Cu, Zn, Cr, Pb and Cd were below the acceptable levels (FAO, 1992; WHO). They further concluded that only leaves of spinach, cabbage, cauliflower, radish and coriander contained higher concentrations of Cu, Cd, Cr, Zn and Pb as compared to other parts of each vegetable. In vegetables, the heavy metals ranges were 5.8-196 (Cu), 0.3-5.2 (Cd), 0.5-15 (Pb) and 21-126 mg kg^{-1} (Zn), and the plants showed retention of heavy metals in soil (Big et al., 2012).

3.4. Heavy metals concentration in fodder crops irrigated with wastewater

Berseem (*Trifolium alexandrinum*), which is an annual fodder crop and cultivated all over the world, accumulated Cd, Cu and Pb to the extent of 13.4, 127.6 and 165.7 mg kg^{-1} in shoot and 5.96, 76.3 and 118.6 mg kg^{-1} in root tissues, respectively and Cr (2.48 mg kg^{-1}) in shoot tissues only were beyond the permissible limits of FAO, WHO and NEQS (Table 6). However, accumulation of other heavy metals in berseem were: Fe 70; Ni 6.72; Mn 7.22 and Zn 55.1 mg kg^{-1} in shoot and 45.99, 3.18, 3.15 and 21.9 mg kg^{-1} in root tissues, respectively. Likewise, high bio-mass crop maize, used as fodder and cereal, accumulated Cd, Cu, Pb and Zn (7.31, 254.45, 184.3 and 207.3 mg kg^{-1}) in shoot and root (4.93, 208.51, 57.20 and 178.75 mg kg^{-1}) tissues, respectively which were beyond the permissible limits (Table 6). Millet which is used also as fodder and grain crop, had Cd and Pb (4.74 and 65.42 mg kg^{-1}) in shoot and root (3.03 and 51.36 mg kg^{-1}) tissues, respectively and these concentrations were above the FAO and WHO permissible limits. Concentration of other heavy metals in maize and millet were below the permissible limits. Similarly, Khan et al., (2012, 2014) have reported maximum accumulation of Zn (271.30 mg kg^{-1}) in maize shoot. The concentration of Cu, Zn and Pb in shoots and roots of *Brassica napus*, grown as oil and fodder crop followed the order: of Cu > Zn > Pb > Cd.

Table 6. Heavy metals concentration in fodder crops irrigated with wastewater

Fodder crops	Shoot/ Root	Cd	Cr	Cu	Fe	Ni	Mn	Pb	Zn
		Shoot & root tissues (dm. mg kg^{-1})							
Berseem	Shoot	13.40	2.48	127.6	70.00	6.72	7.22	165.70	55.10
	Root	5.96	1.17	76.30	45.99	3.18	3.15	118.60	21.90
Maize	Shoot	7.31	0.93	254.5	197.00	9.12	12.13	184.30	207.30
	Root	4.93	0.39	208.50	170.75	3.85	2.25	157.20	178.75
Millet	Shoot	4.745	0.36	72.17	120.2	8.16	9.33	65.42	75.80
	Root	3.03	0.22	58.08	54.94	2.96	3.54	51.35	56.10
Brassica napus	Shoot	5.21	0.71	210.81	151.73	6.37	8.75	179.61	197.33
	Root	2.11	0.13	187.11	157.12	1.23	0.91	149.19	167.81
Sorghum	Shoot	6.90	0.75	119.35	59.99	7.89	8.13	94.37	104.18
	Root	4.89	0.44	91.29	49.23	3.31	3.11	77.33	83.14
Wild oat	Shoot	2.33	0.17	47.14	43.21	5.89	6.34	39.18	42.27
	Root	1.21	0.09	38.12	31.25	1.78	2.37	41.57	44.32
WHO (mpl)		0.20	2.30	73.30	425.00	67.90	500.00	0.30	99.40

Dm - dry matter; WHO - World Health Organization; mpl - Maximum permissible limit

Other heavy metals absorbed by *Brassica* remained below the permissible limits. In a study, about 75% reduction in root and shoot dry matter yield of Canola and Indian mustard was observed when these plants were grown in metal contaminated soils (Turan & Esringu, 2007). Similarly, Kakar et al., (2010) reported deleterious impact of various heavy metals on growth and yield of *B. napus* and *B. juncea*. Mechanistically, some of the heavy metals such as Pb and Cd adversely affects photosynthesis, respiration and other important metabolism of plants (Paolacci et al., 1997). Apart from plants, microbial communities inhabiting soil are also reported to be adversely affected due to high metal concentration in soils (Giller et al., 1998; Kozdroj & Elsas, 2001; Kurek & Bollag, 2004).

Similarly, the highest accumulation of Cd (6.9 mg kg^{-1}), Cu (119.35 mg kg^{-1}), Pb (94.37 mg kg^{-1}) and Zn (104.18 mg kg^{-1}) was noted in sorghum shoot tissues, while in root tissues the values were 4.89, 91.29, 77.33 and 83.14 mg kg^{-1}, respectively and were beyond the permissible limits. In wild oat, the concentration of Cd (2.33, 1.21 mg kg^{-1}) and Pb (39.18, 41.57 mg kg^{-1}) in shoot and root tissues, respectively were beyond the permissible limits, however, other heavy metals were under permissible standards. Previous investigations revealed that Pb causes retardation of plant growth and inhibition of seed germination (Iqbal & Shazia, 2004; Sharma et al., 2007), with significant negative impacts on seedling biomass, root and shoot lengths (Uveges et al., 2002). Zinc is one of the essential elements for many physiological processes in plants, however, in higher concentration it become toxic (Baccio et al., 2005). The Zn significantly changes mitotic activity (Rout & Das, 2003), disturbs membrane integrity and permeability (Stoyanova & Doncheva, 2002) and finally kills the plant cell (Change et al., 2005). Furthermore, the Pb, Zn and Cd also reduces the plant uptake rate of essential elements like Mn, Fe, K, Mg and Ca (Wu et al., 2007), which further aggravate the toxicity to plants and results in reduction in plant height and weight.

Prolonged and continuous use of wastewater for growing vegetables, fodders and other major crops may result in soil build up of heavy metals and salinity that may be phyto-toxic (Ghafoor et al., 2004; Qadir & Oster, 2004). Adverse effects of toxic elements found in wastewater were observed on *Phaseolus mungo* and *Lens culinaris* crops with badly effect the soil properties and environment (Azmat & Khanum, 2005). Wastewater irrigation contaminated the farm-land and the various crops, resulted with heavy metals beyond the recommended standards. Moreover, with application of wastewater the pH, EC as well as SAR values of the soil increases which change the environment of rhizosphere into saline and also retard the plant growth.

4. CONCLUSIONS

Wastewater use in irrigating the vegetables and fodder crops, exceeded the WHO, FAO and NEQS permissible limits for heavy metals, and soil resulted with increased contents of heavy metals. Utilization of wastewater for irrigating the food and feed crops might not be safe, and causes also high salinity and sodium adsorption ratio which restrict the crops growth and yield. Soil and crops irrigated with wastewater should be closely monitored for heavy metals accumulation so that it may not result into health concern.

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