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COPPER BIOACCUMULATION AND TRANSLOCATION IN FORAGES GROWN IN SOIL IRRIGATED WITH SEWAGE WATER

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Abstract

Wastewater is a source of some nutrients essential for soil fertility, but it includes various types of contaminants like heavy metals that pollute the soil and crops. In this regard, this study aimed to evaluate the possible health risks of copper (Cu) accumulation in forages irrigated with wastewater. Forages both of summer and winter were grown with different water treatments (sewage water and tap water) in the Department of Botany, University of Sargodha. The concentrations of copper in water, root and forage samples were determined. Moreover, the bioconcentration factor, pollution load index, daily intake of metals and health risk index were calculated. In tap water, the copper value was 0.072 mg L^{-1} and that in sewage water 0.077 mg L^{-1}. In soil, the calculated copper value was lower than the USEPA standards. The maximum copper in root was determined in winter forages (0.208 mg kg^{-1}). The maximum bioaccumulation factor for copper was observed in *Trifolium resupinatum* (8.2230) grown in winter. The maximum pollution load for copper was found in *Brassica campestris* (0.2853) grown in winter. The maximum value for the daily intake of metals observed was 0.045, and maximum observed health risk index was 1.136.

Key words: Bioaccumulation, Copper, Forage, Irrigation, Health risk index.

Introduction

Agriculture in peri-urban areas mainly depends on irrigation with domestic wastewater enriched with different types of metals (Ahmad *et al.,* 2019; Amman *et al*., 2002) because the use of freshwater is not accessible for irrigation practices all the time (Ahmad *et al.,* 2018). Wastewater irrigation in agricultural areas is gaining popularity to solve this problem (Khan *et al.,* 2018a, b). So, wastewater and industrial ejections are used for irrigation chiefly in countryside areas. The wastewater irrigation affects agricultural soils because the different organic and inorganic elements occur in wastewater. The irrigation with sewage water can be useful if it has no adverse effects on food crops' yields, soil pollution and health of humans (WHO, 1996; USEPA, 1992). However, heavy metals in every source of sewage water cause pollution to the humans and the environment because of their non-renewable and steady nature (Zhuang *et al.,* 2009; Khan *et al.,* 2019a, b). The use of wastewater irrigation in agricultural lands is in danger due to the presence of toxic substances and/or heavy metals and trace minerals in wastewater (Luo *et al*., 2012; Ugulu *et al.,* 2019). Heavy metals can be divided into two groups according to their beneficence and toxicity. As, Cd, Cr, Hg, and Pb are directly toxic to organisms, even in small doses (Dogan *et al*., 2010; Severoglu *et al*., 2015). However, Cu, Fe, Mn, Ni, and Zn are required for organisms as essential elements in small amounts and, their inadequate intake causes deficiency symptoms (Nadeem *et al*., 2019). Nevertheless, they could lead to poisoning at higher concentrations (Baslar *et al*., 2009; Ugulu *et al*., 2009; Durkan *et al.*, 2011).

Different anthropogenic activities like mining, metal processing, fertilisers industry, fungicides, industrial and domestic wastewater and traffic emissions cause the emission of copper (Dogan *et al.,* 2014a, b; Unver *et al.,* 2015; Ugulu *et al*., 2016). The root apices of plants are impassable with heavy metals due to their immature cells and low-density cell walls. These pick-up metals from contaminated soil and then transfer to above-ground parts (Tung & Temple, 1996; Khan *et al*., 2019a, b). Copper is an essential micronutrient for plants, but an excess amount of copper exerts a toxic effect on plants (Ugulu *et al.*, 2012; Sahin *et al.*, 2016). Excess amount of copper shows symptoms such as necrosis and chlorosis, inhibition of root growth, leaf discolouration and stunting (van Assche & Clijsters, 1990). The excess and deficiency of copper can cause disorder in plant growth and development by affecting the various physiological processes in the plant (Marschner, 1995; Ugulu, 2015; Khan *et al.,* 2019c).

The present research was undertaken to observe the effect of sewage water irrigation on copper uptake by forages, (2) determine the transfer of copper from the soil to forages, (3) pollution severity of soil due to copper, (4) and health risk of grazing livestock via consumption of copper contaminated forages.

Materials and Methods

Study area: The present study was conducted in Sargodha City, Pakistan. In Sargodha, summer is very hot while the winter season is cold. The Sargodha has an extreme climatic condition, too hot in summer with the highest temperature ranging from 45°C to 50°C and low in winter with temperature ranging from 4°C to 25°C. The average rainfall is about 410 mm annually. River Jhelum flows between Northern and Western side and River Chenab on the Eastern side of the city. Major crops grown in Sargodha are rice and sugarcane. The primary reason for the renown of Sargodha is the production of citrus fruits. Different green vegetables are also grown in Sargodha.

The experimental design of the present study was performed at the Botany Department of the University of Sargodha. Forage crops were sown both of summer and winter season in this department.

Plant cultivation: Summer cultivation: Healthy seeds of seven forages were collected from Ayub Agricultural Research Institute and sown in summer season in pots. Pots were placed in Department of Botany, University of Sargodha. Seventy pots were taken and filled with fertile soil. The physicochemical parameters of soil are given in Table 1. Crops were Maize (*Zea mays* L.), Sanwak (*Echinochlo acolona* (L.) Link), Pearl millet (*Pennisetum glaucum* (L.) R. Br.), jawar (*Sorghum vulgare* Pers.), Grain sorghum (*Sorghum bicolor* (L.) Moench), Jantar (*Sesbania rostrata* Bremek & Oberm.), and Gawara (*Cyamopsis tetragonoloba* (L.) Taub.). Seeds were placed below 4-5 cm of soil. Thirty-five (35) control pots were irrigated with tap water, and 35 experimental pots were irrigated with mixed sewage water that was taken from city effluent of Sargodha. Chemical composition of canal and sewage water is given in Table 2. Five replicates of control and five replicates of experimental were treated equally. Pots were irrigated twice a week.

Winter cultivation: Six winter forages were sown; Berseem (*Trifolium alexandrinum* L.), Luscern (*Medicago sativa* L.), Sarsoon (*Brassica campestris* L.), Persian clover (*Trifolium resupinatum* L.), Indian mustard (*Brassica juncea* (L.) Czern.), and Canola (*Brassica napus* L.). Totally 60 pots were taken and filled with fertile soil. Thirty control pots were irrigated with tap water, and 30 experimental pots were irrigated with mixed sewage water taken from city effluent of Sargodha. All other procedure was the same as summer cultivation.

Samples collection: With the help of polypropylene acid, washing of plastic bottles was done. For the sake of irrigation, 100 mL samples of both sewage and tap water were taken in plastic containers. To avoid polluted actions of microorganisms, almost 1 mL of concentrated HNO_3 was mixed in water. Before the digestion, the samples were stored in a refrigerator.

Total of 130 samples of fertile soil was taken for summer and winter irrigated with both tap and mixed sewage water. To remove moisture from these samples, they were placed in sunlight firstly and then in an oven for at least three days at 75ºC.

The forage plants were uprooted on 6-10-2016. Samples were collected and washed with distilled water, dried with a paper towel and cut into two pieces as; roots and shoots. Fresh weight of samples was measured. Then the plants were dried at room temperature for two weeks and placed in an oven at 75ºC for a week to remove that the whole moisture. After drying these samples were removed from the oven, ground into a fine powder with an electrical grinder and finally digestion was done.

Copper analysis: Determination of copper in digested samples was done by using atomic absorption spectrophotometer (AA-6300 Shimadzu Japan). The standard calibration curve was drawn for copper.

Statistical analysis: The average concentration of heavy metals in soil samples, forage crops, and water was determined. For forages, water and soil samples data One-way ANOVA was applied using the SPSS 20 (Statistical Package for Social Sciences).

Table 1. Physico-chemical properties of water.

Properties of water	Tap water	Sewage water
Electrical conductivity (µS cm^{-1})	1890	7750
Calcium + Magnesium (Ca^{2+}+Mg^{2+}) (meq L^{-1})	5.2	18.5
Sodium (Na^{+}) (meq L^{-1})	13.7	59.0
Carbonate ($CO^{2}{}_{3}$-) (meq L^{1})	0.4	0.8
Bicarbonate (HCO_3^{-}) (meq L^{-1})	8.2	9.6
Chloride (CL^{-}) (meq L^{-1})	6.4	51.7
Sodium Adsorption Ratio (SAR)	8.5	19.4
Residual Sodium Carbonate (RSC)	3.4	Nil

Table 2. Physico-chemical properties of soil.

Properties of soil	S-C*	S-E**	W-C***	W-E****
Depth	0-15	0-15	0-15	0-15
pH	7.7	8.1	7.9	8.1
Electrical conductivity (mS cm^{-1})	5.64	8.42	3.01	4.51
Organic matter %	0.90	0.83	0.96	0.76
Available phosphorus (mg kg^{-1})	8.8	7.0	8.6	7.4
Available potassium (mg kg^{-1})	240	160	200	170
Saturation %	36	38	40	38
Texture	Loamy	Loamy	Loamy	Loamy

*S-C: Summer control, ** S-E: Summer experimental, *** W-C: Winter control, **** W-E: Winter experimental

Bioconcentration factor: A parameter which is used to determine the shifting of minute elements from soil to forages is known as bioconcentration factor (BCF). It was determined as the ratio between the concentration of specific metals in the plant and the same in the consistent soil (Cui *et al.*, 2004).

$$\text{BCF} = \frac{\text{Concentration of heavy metal in plant}}{\text{Concentration of heavy metal in the soil}}$$

where Concentration of heavy metal in the soil as well as in forages was taken in mg kg^{-1}.

Pollution load index: The concentration of metals has been determined at specific sites by using pollution load index (PLI) (Liu *et al.*, 2005).

$$\text{PLI} = \frac{\text{Metal concentration in investigated soil}}{\text{Reference value of the metal in soil}}$$

The reference value of copper was (8.39 mg kg^{-1}).

Daily intake of metals: Daily intake of metal (DIM) was measured by the corresponding equation;

$DIM = C_{metal} \times D_{food\ intake} / B_{average\ weight}$ (Chary *et al.*, 2008)

where, C_{metal} is the concentration of metals in forages, $D_{food\ intake}$ is the daily intake of forages and $B_{average\ weight}$ is average body weight. Average body weight referred to as 550 kg per cattle, and average daily forage consumption per person is taken as 12.5 kg.

Health risk index: To measure the overall threat of exposure to all heavy metals through ingestion of specific forages health risk index was calculated. This showed that the danger to cattle which used contaminated forages. Daily ingestion of metals in food crops divided by the oral reference dose was said to act as a health risk index (HRI) (USEPA, 2002).

$$HRI = DIM/R_fD$$

R_fD values for copper was 0.04 mg kg^{-1} (USEPA, 2010).

Results and Discussion

Copper in water: Analysis of variance showed significant (p<0.05) effect on the copper concentration in irrigation water both for tap and sewage. The measured value of tap water was 0.072 mg L$^{-1,}$ and in sewage water was 0.077 mg L^{-1} (Table 3). The amount of heavy metals under study were higher than the maximum permissible limits of 0.020 mg L^{-1}, established by (Pescod, 1992). Copper accumulation in the sewage water comes mainly from corrosion and leaching of plumbing, fungicides (cuprous chloride), pigments, household products, wood preservatives, larvicides (copper acetoarsenite) and antifouling paints. Among the household products, cleaners are more effective than the others in terms of copper accumulation in sewage waters. However, copper concentrations in sewage water are directly proportional to water hardness. Hard water (high pH) is more aggressive to Cu and Zn plumbing thus increase the leaching.

When the values were determined in the present study compared to other studies, the copper values were lower than the found by Tariq *et al.*, (2006) (0.072 mg L^{-1}) and Murtaza *et al.*, (2010) (0.223 mg L^{-1}) in sewage water from different nearby cities of Pakistan. Salawu *et al.*, (2015) and Kumar & Chopra (2015) also found higher values for sewage water (1.842 mg L^{-1}) in borewell and industry effluent (1.26-6.88 mg L^{-1}), respectively. Khaskhoussy *et al.*, (2013) found a similar range for copper water 0.02-0.09 mg L^{-1} for tap and sewage water. The differences in the copper values obtained in the various studies could depend on the study areas.

Copper in soil: All treatments showed significant (p<0.05) effect on copper concentration according to the analysis of variance in *P. glaucum, C. tetragonoloba, S. rostrata, E. colona, S. vulgare, B. napus, B. juncea, M. sativa, T. alexandrinum* while non-significant effect in *Z. mays, S. bicolor, B. campestris, T. resupinatum*. The order of copper concentration in tap water irrigation was: *B. napus>P. glaucum>M. sativa>C. tetragonoloba>Z. mays>B. juncea>E. colona> T. alexandrinum> S. rostrata> S. vulgare>B. campestris>T. resupinatum>S. bicolor*. The order of copper concentration in sewage water irrigation was: *B. campestris>P. glaucum>B. napus>T. resupinatum>M. sativa>C. tetragonoloba>Z. mays>S. bicolor>B. juncea>E. colona>S. rostrata>S. vulgare>T. alexandrinum* (Table 4, Fig. 1).

Brassica napus samples in winter forage have the highest concentration (2.384 mg kg^{-1}) while the lowest mean concentration (0.066 mg kg^{-1}) observed in *S. bicolor* in the summer forage. The present value was lower than the observed maximum permissible limit for copper soil 50 mg kg^{-1} established by USEPA (1997). Also, the copper soil value was lower than found by Khan *et al.*, (2017) (3.838 mg kg^{-1}) in soil irrigated with the sewage water. However, the Khaskhoussy *et al.*, (2013) found the higher range for copper (9.0-13.7 mg kg^{-1}) in soil irrigated with freshwater and treated wastewater. The copper values in soil were similar to those values as found by Kumar & Chopra (2014-2015) (2.37 mg kg^{-1}) in soil irrigated with borewell water, and the value in *B. juncea* (4.37 mg kg^{-1}) irrigated with effluent water. The metals accessibility and bioaccumulation are directed by various environmental factors such as salinity, moisture contents of soil, pH, solubility and chemical speciation of the metal, occurrence of other metals, soil mineralogy, and texture. Díaz-Barrientos *et al.*, (2003) observed that soil organic matter showed higher affinity with copper metal. It was analyzed by Vandenbossche *et al.*, (2015) the concentration of $CaCO_3$ contents in the studied soil may be one of the reasons behind the Zn and Cu concentrations of the soil. All these variables could be the reasons for the differences in the results of the studies mentioned above.

Table 3. Copper concentration in water (mg L^{-1}).

Tap water	Sewage water	Mean square
0.072 ±0.0113	0.077 ± 0.0073	0.001**
Degree of freedom	1	Error 9
Permissible maximum limit[a]	0.20 mg L^{-1}	

**: Significant at 0.01 level, Source: [a]Pescod (1992)

Table 4. Copper concentration (mg kg^{-1}) in soil grown with different forages.

Soil	Irrigation water		Mean square
	Tap	Sewage	
		Summer	
Z. mays	0.328 ± 0.0131	1.749 ± 0.0649	5.045[ns]
P. glaucum	2.290 ± 0.0039	2.383 ± 0.0025	0.022*
C. tetragonoloba	2.056 ± 0.0030	2.070 ± 0.0293	0.001**
S. rostrata	0.086 ± 0.0053	0.146 ± 0.0121	0.009**
E. colona	0.179 ± 0.0292	0.233 ± 0.0022	0.007**
S. bicolor	0.066 ± 0.0023	1.053 ± 0.0075	2.435[ns]
S. vulgare	0.067 ± 0.0017	0.078 ± 0.0053	0.001**
		Winter	
B. campestris	0.287 ± 0.0021	2.394 ± 0.0034	11.104[ns]
B. napus	2.292 ± 0.0031	2.384 ± 0.0023	0.021*
T. resupinatum	0.265 ± 0.0162	2.145 ± 0.0017	10.816[ns]
B. juncea	0.183 ± 0.0316	0.483 ± 0.0021	0.224*
M. sativa	2.054 ± 0.0044	2.073 ± 0.0294	0.001**
T. alexandrinum	0.078 ± 0.0045	0.088 ± 0.0053	0.001**
Degree of freedom	1	Error	9
Permissible maximum limit[a]		50 mg kg^{-1}	

*,**: Significant at 0.05 and 0.01 level, ns: non-significant, Source: [a]USEPA (1997)

Copper in root: All treatments showed significant (p<0.05) effect on copper concentration according to the analysis of variance in all the forages. The sequence of the observed values in plants as a result of tap water irrigation was: *B. campestris>M. sativa>B. napus>C. tetragonoloba>S. bicolor>S. vulgare>T. alexandrinum>P. glaucum>B. juncea>T. resupinatum>Z. mays>E. colona>S. rostrata.* The sequence of the observed values in plants as a result of sewage water irrigation was: *M. sativa>B. campestris>C. tetragonoloba>B. napus>T. alexandrinum>S. bicolor>B. Juncea>S. vulgare>E. colona>P. glaucum>T. resupinatum>S. rostrate* (Table 5, Fig. 2). The highest mean concentration of copper in the root was 0.208 mg kg^{-1} occurred in *B. campestris* grown in winter, and the lowest mean concentration was 0.037 mg kg^{-1} observed in *S. vulgare* grown in summer. Daping *et al.*, (2015) found higher copper root value (20.2 mg kg^{-1}) in *B. campestris* during the June and August. Asdeo (2014) also found higher copper range (12.83-16.32 mg kg^{-1}) in millet. In the winter season, due to low winds and rainfall level, it is not possible for the forages to absorb sufficient heavy metals either by the roots or pores of stomata. The difference between the absorbed concentration of heavy metals in dry and wet seasons can also be endorsed to several factors like weather conditions and geological characteristics such as low mineral contents in the sample of soil within the study region (Lawal *et al.*, 2017).

Copper in leaves: All treatments showed significant (p<0.05) effect on copper concentration according to the analysis of variance in *Z. mays, P. glaucum, C. tetragonoloba, S. rostrata, E. colona, S. bicolor, S. vulgare, B. campestris, B. napus, T. resupinatum, M. sativa, T. alexandrinum,* while the non-significant effect was observed in *B. juncea*. The sequence of the observed values in plants as a result of tap water irrigation was: *B. juncea>M. sativa>B. napus>Z. mays>S. bicolor>S. vulgare>S. rostrata>T. resupinatum>C. tetragonoloba>E. colona> P. glaucum>T. alexandrinum>B. campestris.* The sequence of the observed values in plants as a result of sewage water irrigation was: *B. juncea> M. sativa>T. resupinatum>P. glaucum>T. alexandrinum>S. rostrata>S. bicolor>Z. mays>B. napus> E. colona>S. vulgare>C. tetragonoloba>B. campestris* (Table 6, Fig. 3). The winter forage *B. juncea* showed the highest mean concentration (2.00 mg kg^{-1}) and the minimum mean concentration (0.074 mg kg^{-1}) was also observed in *B. campestris* in the winter forage. Copper values presented in this study were lower than the maximum allowable limit of 20 mg kg^{-1} established by WHO (1996). According to this finding, it can be said that there was no risk for metal toxicity in the study area. Kumar & Chopra (2015) found higher copper range (9.73-10.99 mg kg^{-1}) than the present copper range in *B. juncea*, *T. aestivum* and *H. vulgare* flooded with effluent water (0.074-2.000 mg kg^{-1}). Ahmad *et al.*, (2018) observed that the addition of heavy metals like copper in plants considerably increased due to sewage water irrigation.

The current copper values were lower than those found by Khan *et al.*, (2009a) who found copper range (6.48-6.90 mg kg^{-1}) at different sampling periods. They also observed various disorders in animals like diarrhoea, anaemia, decreased growth, weakness and infertility, fragile bones due to the deficiency of copper.

Table 5. Copper concentration (mg kg^{-1}) in root irrigated with tap and sewage water.

Root	Irrigation water		Mean square
	Tap	Sewage	
		Summer	
Z. mays	0.0405 ±0.0026	0.052 ± 0.0031	0.001**
P. galucum	0.080 ± 0.0017	0.0825 ± 0.0017	0.001**
C. tetragonoloba	0.0900 ± 0.0627	0.1475 ± 0.0016	0.008*
S. rostrata	0.0375 ± 0.0039	0.0575 ± 0.0039	0.001**
E. colona	0.040 ± 0.0017	0.087 ± 0.0018	0.006**
S. bicolor	0.086 ± 0.0028	0.109 ± 0.0021	0.001**
S. vulgare	0.037 ± 0.0017	0.095 ± 0.0017	0.001**
		Winter	
B. campestris	0.112 ± 0.0045	0.208 ± 0.0021	0.023*
B. napus	0.100 ± 0.0017	0.138 ± 0.0026	0.004**
T. resupinatum	0.050 ± 0.0016	0.080 ± 0.0017	0.002**
B. juncea	0.055 ± 0.0018	0.107 ± 0.0016	0.007**
M. sativa	0.101 ± 0.0030	0.290 ± 0.0031	0.089*
T. alexandrnium	0.084 ± 0.0023	0.130 ± 0.0017	0.005**
Degree of freedom	1	Error	9
Permissible maximum limit[a]		20 mg kg^{-1}	

*,**: Significant at 0.05 and 0.01 levels, Source: [a]WHO (1996)

Bioconcentration factor: Bioconcentration factor was calculated for forages irrigated with both tap and sewage water and the following sequence was observed. The sequence of the observed value in plants as a result of tap water irrigation was; *B. juncea>S. vulgare>S. rostrata>T. alexandrinum>Z. mays>E. colona>B. campestris>M. sativa>B. napus>P. glaucum>T. resupinatum>C. tetragonolba>S. bicolor*. The sequence of the observed value in plants as a result of sewage water irrigation was: *T. resupinatum>B. juncea>S. bicolor>S. vulgare>T. alexandrinum>E. colona>S. rostrata>Z. mays>M. sativa>B. napus>B. campestris>P. glaucum>C. tetragonoloba* (Table 7).

The highest value in *T. resupinatum* (8.2230) was observed, and the lowest value was found in *S. bicolor* (0.007576). Bioconcentration factor mean value in the present study was higher than the range found by Alrawiq *et al*., (2014) (0.266-0.589) after irrigation with recycled and non-recycled water. If the BCF>1 showing that the plants stored metals. The present value higher than Asdeo (2014) (0.3440) for copper BCF. Higher BCF suggests low retention of metals in the soil while lower BCF suggests that metals are in tight bonding with the soil, and they do not get transferred to forage. Bioconcentration factor also depends upon soil pH (Zhang *et al*., 2007).

Pollution load index: The sequence of PLI value according to the plant samples irrigated with tap water was: *B. napus>P. glaucum>M. sativa>C. tetragonoloba>S. bicolor>Z. mays>B. campestris>B. juncea>E. colona>T. alexandrinum>S. rostrata>S. vulgare>T. resupinatum* (Table 7).Pollution load index value according to the plant samples irrigated with sewage water was: *S. bicolor>B. campestris>B. napus>P. glaucum>M. sativa>C. tetragonoloba>Z. mays> B. juncea>E. colona>S. rostrata>T. resupinatum>S. vulgare>T. alexandrinum.* The highest value for PLI was observed in *B. campestris* 0.2853, and the minimum value was found in *T. resupinatum* 0.0077. These copper PLI values were higher than those reported by Khan *et al*., (2017) in the soil. Bao *et al*., (2013) found higher copper PLI in soil (1.20, 1.27, 1.16) in three different zones irrigated withthe long-term sewage water. Ahmad *et al*., (2014) also found higher copper PLI value (1.151) in soil irrigated with canal and sewage water.

Daily intake of metals and health risk index: The sequence of DIM value according to the plant samples irrigated with tap water was*: B. juncea>M. sativa>B. napus>P. glaucum>Z. mays>S.bicolor>S. vulgare>S. rostrata>T. resupinatum> C. tetragonoloba>E. colona>B. campestris>T. alexandrium.* The sequence of DIM value according to the plant samples irrigated with sewage water was: *B. juncea>M. sativa>T. resupinatum>B. napus>P. glaucum>E. colona>T. alexandrinum>S. vulgare>S. rostrata>S. bicolor>C. tetragonoloba>Z. mays>B. campestris* (Table 8). The maximum DIM value observed in *B. juncea* was 0.0455, and the minimum value observed in *T. alexandrinum* was 0.00170. Roggeman *et al*., (2013) found higher mean DIM value (101-78 in winter and summer value (105-80) in herds of cows. Khan *et al*., (2017) found similar DIM value (0.065-0.098) after irrigation with ground and sewage water. In the current results, the values of DIM were lower than 1, and it suggests that no health risk is associated with the consumption of such contaminated forages.

Health risk index, both for tap water and sewage water irrigation, was calculated. The highest HRI value in *B. juncea* (1.136) and the minimum HRI value in *B. campestris* (0.326). Khan *et al*., (2015) found higher HRI value (1.337-1.717) in wastewater irrigated sites (Table 8). According to Khan *et al*., (2018c, d), the HRI depends on the chemical composition and the physical characteristics of the soil, the type, and rate of consumed forage.

Table 6. Copper concentration (mg kg^{-1}) in leaves of forages.

Leaves	Irrigation water		Mean square
	Tap	Sewage	
	Summer		
Z. mays	0.076 ± 0.0269	0.160 ± 0.0026	0.017*
P. glaucum	0.251 ± 0.0023	0.312 ± 0.0017	0.009**
C. tetragonoloba	0.095 ± 0.0016	0.100 ± 0.0017	0.001**
S. rostrata	0.122 ± 0.0017	0.128 ± 0.0018	0.001**
E. colona	0.080 ± 0.0018	0.201 ± 0.0116	0.037*
S. bicolor	0.100 ± 0.0015	0.133 ± 0.0176	0.003**
S. vulgare	0.125 ± 0.0014	0.130 ± 0.025	0.001**
	Winter		
B. campestris	0.074 ± 0.0017	0.075 ± 0.0017	0.001**
B. napus	0.258 ± 0.0018	0.326 ± 0.0037	0.012*
T. resupinatum	0.113 ± 0.0016	0.535 ± 0.0086	0.445*
B. juncea	0.780 ± 0.0215	2.000 ± 0.0176	3.721[ns]
M. sativa	0.296 ± 0.0021	0.810 ± 0.0231	0.662*
T. alexandrinum	0.075 ± 0.0176	0.134 ± 0.0057	0.009**
Degree of freedom	1	Error	9
Permissible maximum limit[a]		20 mg kg^{-1}	

*,**: Significant at 0.05 and 0.01 levels, ns: non-significant, Source: [a]WHO (1996)

Table 7. Bioconcentration factor and pollution load index of copper in forages.

Forage	BCF		PLI	
	Irrigation water		Irrigation water	
	Tap	Sewage	Tap	Sewage
	Summer			
Z. mays	0.087	0.437	0.039	0.208
P. glaucum	0.105	0.136	0.273	0.284
C. tetragonoloba	0.045	0.048	0.245	0.256
S. rostrata	1.473	0.839	0.010	0.017
E. colona	0.445	0.862	0.021	0.027
S. bicolor	0.007	2.094	0.059	1.125
S. vulgare	1.625	1.902	0.008	0.0093
	Winter			
B. campestris	0.021	0.262	0.034	0.285
B. napus	0.112	0.136	0.275	0.286
T. resupinatum	0.052	8.223	0.0077	0.255
B. juncea	4.140	4.250	0.021	0.057
M. sativa	0.142	0.392	0.246	0.257
T. alexandrinum	0.847	1.711	0.0092	0.0105

Table 8. Daily intake of metal and Health risk index of copper in forages.

Forage	DIM		HRI	
	Irrigation water		Irrigation water	
	Tap	Sewage	Tap	Sewage
	Summer			
Z. mays	0.0026	0.0037	0.0409	0.093
P. glaucum	0.0057	0.0071	0.143	0.178
C. tetragonoloba	0.0020	0.0022	0.054	0.057
S. rostrata	0.0027	0.0028	0.069	0.072
E. colona	0.0018	0.0045	0.045	0.114
S. bicolor	0.0020	0.0032	0.053	0.077
S. vulgare	0.0028	0.0029	0.073	0.0780
	Winter			
B. campestris	0.0012	0.0017	0.033	0.041
B. napus	0.0058	0.0074	0.146	0.185
T. resupinatum	0.0025	0.0121	0.064	0.303
B. juncea	0.0177	0.0454	0.443	1.136
M. sativa	0.0067	0.0184	0.168	0.460
T. alexandrinum	0.0017	0.0030	0.043	0.075

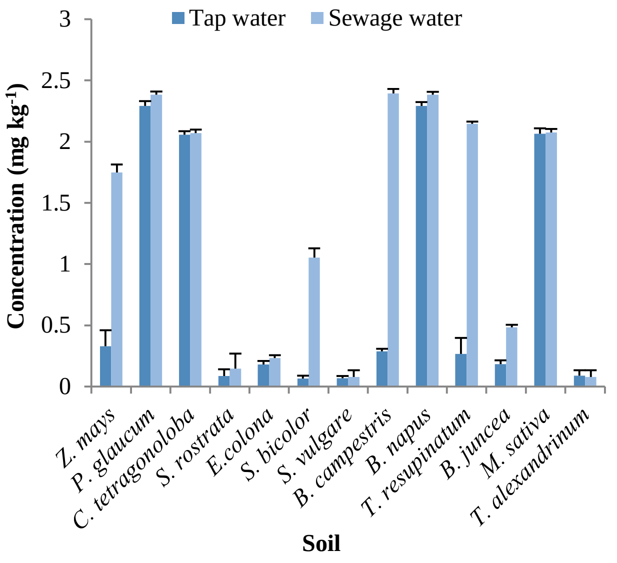

Fig. 1. The fluctuation of copper in soil grown with different forages.

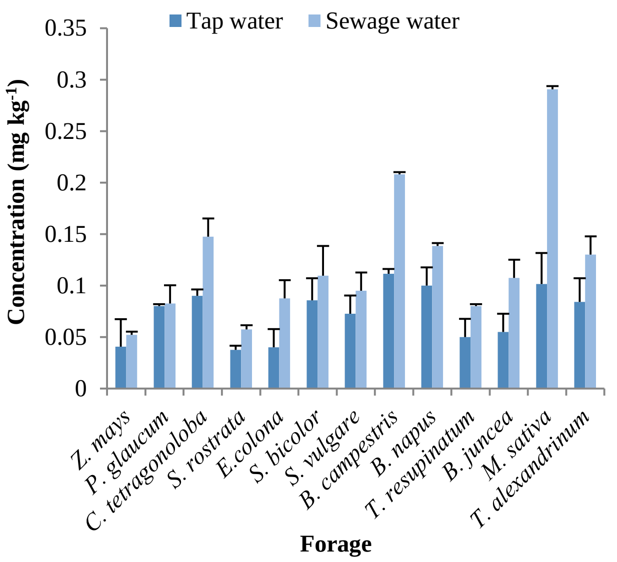

Fig. 2. The fluctuation of copper in root irrigated with tap and sewage water.

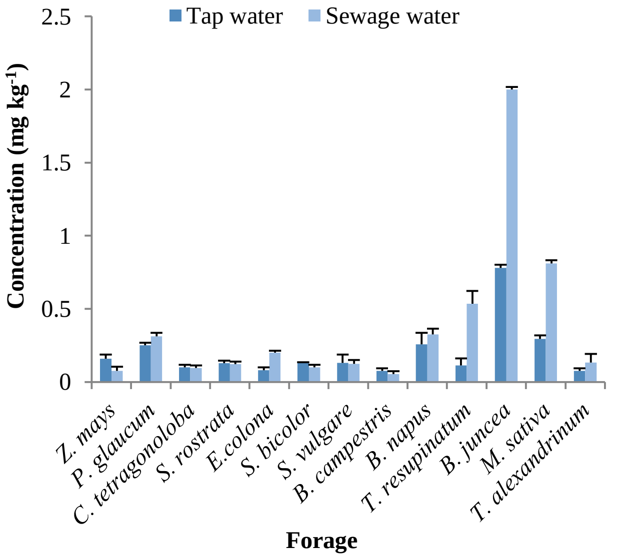

Fig. 3. The fluctuation of copper in leaves of forages.

Conclusion

Irrigation with polluted water may contaminate the soil and cultivated land readily. In the experiment, the soil, root and forage samples irrigated with sewage water showed a higher amount of metal. The accumulation of heavy metals from soil in plant varied according to treatment; however, they did not follow any particular pattern. Forages samples didn't show a higher amount than the maximum permissible limit. So, the consumption of such forages may be safe. However, more wide-ranging sampling is needed to study such forages, and further investigation on the contamination of other crops is required.

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