ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Manganese Bioaccumulation and Translocation of in Forages Grown in Soil Irrigated with City Effluent: An Evaluation on Health Risk.

Zafar Iqbal Khan[1], Kafeel Ahmad[1], Hareem Safdar[1], Ilker Ugulu[2], Kinza Wajid[1], Humayun Bashir[1], and Yunus Dogan[2].

[1]Department of Botany, University of Sargodha, Sargodha, Pakistan.
[2]Buca Faculty of Education, Dokuz Eylul University, Izmir, Turkey.

ABSTRACT

Wastewater is a source of some nutrients essential for soil fertility but include various types of contaminants like heavy metals that pollute the soil and crops. In this regard, an experiment was carried out to evaluate the possible health risks of heavy metals in forages. Forages both of summer and winter were grown with different water treatments (sewage water and tap water) in Department of Botany, University of Sargodha. The concentration of manganese in water, root and forage samples was determined. Moreover, bioconcentration factor, pollution load index, daily intake of metals and health risk index were calculated. In tap water the manganese value was 0.063 mg/L and in sewage water 0.067 mg/L, respectively. In soil the calculated manganese value was lower than USEPA standards. The maximum average concentration of manganese in the leaves was 0.553 mg/kg occurred in *Sesbania rostrata*. The maximum observed value for manganese bioconcentration factor in *Trifolium resupinatum* was (4.495) grown in winter. The maximum pollution load index was observed for manganese (0.0302). The maximum value for daily intake of metals observed was 0.0125 and maximum observed health risk index was 9.84.

Keywords: Bioaccumulation, Forage, Health risk index, Manganese

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Fresh water provision for the life of flora and fauna is much essential to combat burgeoning population food requirements. About 90% of water was used for irrigation, only 6% of total accessible water was used for native purpose and 3% was used for industrial purposes. Deficiency of surface water store was a perplexing issue in Pakistan. City wastewater was used for irrigation of fields near urban areas and issue was resolved [1-3]. Numerous plant procedures like metabolism and growth require micronutrients at low level but if these nutrients touch high level then poisonousness could verify many disarrays in plants [4,5]. When the heavy metals move in the biome and in food chain, several disorders can be triggered like bone disorder, neural disorders, circulatory disorder, and renal disorder etc. in animals and humans [6-8]. Heavy metals make reactive oxygen species like hydrogen peroxide, hydroxyl radicals and superoxide radicals that are harmful for plants and animals [9].

Various studies have been conducted on metal contamination of topsoil by sewage water irrigation [10,11]. The risk of using sewage water for irrigation could lead to heavy metal build up in soil and crops and cause various health risk to human may limit the extensive use of sewage water in agriculture [12,13].

The concentration of heavy metals reduces in sewage water after sewage treatment process; however greater amount of heavy metals has been observed in the upper layer of soil where treated wastewater is used for irrigation purpose for 20 years [14]. By increasing the duration of irrigation these metals are transferred in the depth of soil [15]. It has been estimated that the concentration of heavy metals in soil would reach the maximum level after 50 to 100 years use of sewage water for irrigation purpose [16].

Manganese (Mn), which is an essential micronutrient for plants, has crucial functions in photosynthesis, carbohydrate and lipid biosynthesis, and oxidative stress protection. Manganese deficiency inhibits the plant growth and development. Also, it causes various plant deficiency symptoms such as interveinal chlorosis and tissue necrosis [17-19]. The manganese bioavailability for plants is indirectly related with pH of soil, thus greater Mn amount was perceived in forages cultivated on acidic soils. Poorly drained soils have more Mn content in plants [20]. It is also observed that cobalt absorption in forages may be decreased due to the greater amount of Mn in the soil [21].

The present research was performed to (1) observe the effect of sewage water irrigation on manganese uptake by forages, (2) determine the transfer of manganese from soil to forages, (3) define pollution severity of soil due to manganese and (4) examine health risk of grazing livestock via consumption manganese contaminated forages.

MATERIALS AND METHODS

Study Area

The present study was conducted in Sargodha City, Pakistan. In Sargodha, summer is very hot while winter season is cold. River Jhelum is present between Northern and Western side and River Chenab is present on East area of the city. Temperature ranges 0-50ºC in this region. Major crops grown in Sargodha are rice and sugarcane. The basic reason for the renown of Sargodha is production of citrus fruits. Different green vegetables are also grown in Sargodha.

The experimental design of the present study was performed at Botany Department of 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 and sown in summer season. Seventy pots were taken and filled them with fertile soil. Pots were placed in Department of Botany, University of Sargodha. Crops were Maize (*Zea mays* L.), Sanwak (*Echinochloa colona* (L.) Link), Bajra (*Pennisetum typhoideum* Rich.), Local jowar (*Sorghum vulgare* Pers.), Jowar (hybrid) (*Sorghum bicolor* (L.) Moench), Jantar (*Sesbania rostrata* Bremek & Oberm.), and Gawara (*Cyamopsis tetragonoloba* (L.) Taub.). Seeds were placed below 4-5 cm of soil. 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. 5 replicates of control and 5 replicates of experimental were treated equally. Pots were irrigated regularly.

Winter cultivation: Six winter forages were sown; Berseem (*Trifolium alexandrinum* L.), Luscern (*Medicago sativa* L.), Sarsoon (*Brassica campestris* L.), Chatala (*Trifolium resupinatum* L.), Canola sarsoon (*Brassica juncea* (L.) Czern.), and Ghobisarsoon (*Brassica napus* L.). Totally 60 pots were taken and filled with fertile soil. 30 control pots were irrigated with tap water and 30 experimental pots were irrigated with mixed sewage water that was taken from city effluent of Sargodha. All other procedure was 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 bottles. To avoid from polluted actions of microorganisms almost 1 mL of concentrated HNO_3 was mixed in water. Before the digestion the samples were stored in refrigerator.

Total 130 samples of fertile soil were 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 them in oven for at least 3 days at 75ºC.

The forage plants were uprooted on 6-10-2016. Sampling was done one time. Samples were washed with distilled water, dry with paper towel and cut into two pieces as; roots and shoots. Fresh weight of samples was measured. Then plants dried at room temperature for 2 weeks and put in oven at 75ºC for a week so that all the moisture removed. After drying these samples were removed from oven, grinded into fine powder with electrical grinder then digestion was done.

Manganese Analysis

Determination of manganese in digested samples was done by using Atomic Absorption Spectrophotometer (AA-6300 Shimadzu Japan). Standard calibration curve was drawn for manganese.

Statistical Analysis

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

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 elements in the plant and the same in the consistent soil [22].

BCF= Concentration of heavy metal in plant/Concentration of heavy metal in soil.

Where concentration of heavy metal in soil as well as in forages was taken in mg/kg.

Pollution Load Index

The concentration of metals has been determined at specific sites by using pollution load index (PLI) [23].

PLI= Metal concentration in investigated soil/Reference value of the metal in soil

Reference value of Mn was (46.5 mg/kg).

Daily Intake of Metals

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

$$DIM= C_{metal} \times D_{food\ intake} / B_{average\ weight}$$ [24]

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

Health Risk Index

It was calculated to measure overall threat of exposure to all heavy metals through ingestion of specific forages. This shows the danger to cattle which use contaminated forages. Daily ingestion of metals in food crops divided by the oral reference dose was said to as health risk index (HRI) [25].

$$HRI=DIM/R_fD$$

RfD values for Mn was 46.75 mg/kg/day [26].

RESULTS AND DISCUSSION

Manganese in Water

Analysis of variance showed significant (p<0.05) effect on the concentration of Mn in water both for tap and sewage water. Mn concentration in tap water was (0.063 mg/L) and in sewage water was (0.067 mg/L) (Table 1). The heavy metals under study were lower than the permissible maximum limits of 0.2 mg/L established by Pescod [27].

Table 1: Manganese concentration in water (mg/L)

Tap water	Sewage water	Mean square
0.063±0.0017	0.067±0.0130	0.003**
Degree of freedom	**1**	**Error 9**
Permissible maximum limit[a]	0.2 mg/L	

**: Significant at 0.01 level; Source: [a]Pescod [27]

However, the water Mn range was higher than the values found by Kumar and Chopra [28] (0.36-1.54 mg/L) in borewell and industrial water. Also, Barreto *et al*. [29] found value (0.030 mg/L) for Mn in wastewater and this was similar to present investigation. Manganese is presented in large amounts in various steel materials as a hardening agent. It also finds application in pharmaceutical preparations [30].

Manganese in Soil

According to the plant samples sown in the soil samples, all treatments showed significant (p<0.05) effect on the Mn concentration in soil according to analysis of variance in *C. tetragonoloba, E. colona, S. vulgare, B. juncea, M. sativa, T. alexandrinum* while non-significant effect was observed in *Z. mays, P. typhoideum, S. rostrata, S. bicolor, B. napus, B. campestris* and *T. alexandrinum*. The sequence of the observed values in plants as a result of tap water irrigation was: *T. alexandrinum>S. vulgare> B. napus>B. juncea>E. colona>Z. mays>B. campestris>C. tetragonoloba>S. rostrata>M. sativa>P. typhoideum>T. resupinatum>S. bicolor.* The sequence for sewage water irrigation was: *Z. mays>S. bicolor>B. napus>B. campestris>B. juncea>E. colona>S. vulgare>T. resupinatum>T. alexandrinum>M. sativa>C. tetragonoloba>S. rostrata>P. typhoideum*. The highest mean concentration of Mn in the soil was 1.415 mg/kg occurred in *Z. mays* grown in summer and the lowest mean concentration was 0.119 mg/kg occurred in *T. resupinatum* grown in winter (Table 2). The current value was lower than permissible maximum limits used 80 mg/kg established by USEPA [31]. Kumar and Chopra [28] found range of Mn soil (1.38-1.56 mg/kg) in agricultural crops viz; *T. aestivum*, *B. juncea* and

H. vulgare flooded with effluent water and the Mn value for tap water was lower but for sewage water was similar. Khan *et al*. [32] found higher Mn values in soil samples. It has been observed that pH of soil and organic matter can enhance solubility of Mn for plants [33] (Figure 1).

Table 2: Manganese concentration (mg/kg) in soil grown with different forages

Soil	Irrigation water		Mean square
	Tap	Sewage	
Summer			
Z. mays	0.297±0.0051	1.415±0.4275	3.122^{ns}
P. typhoideum	0.127±0.0104	0.346±0.0595	0.119^{ns}
C. tetragonoloba	0.268±0.0017	0.367±0.0373	0.025*
S. rostrata	0.128±0.0104	0.356±0.0595	0.119^{ns}
E. colona	0.328±0.0190	0.497±0.0517	0.071*
S. bicolor	0.1165±0.0301	0.675±0.0412	0.780^{ns}
S. vulgare	0.393±0.0626	0.468±0.0017	0.014*
Winter			
B. campestris	0.296±0.0034	0.600±0.0176	0.230^{ns}
B. napus	0.365±0.0415	0.616±0.0418	0.158^{ns}
T. resupinatum	0.119±0.0128	0.409±0.0023	0.210^{ns}
B. juncea	0.337±0.0203	0.499±0.0532	0.066*
M. sativa	0.313±0.0474	0.374±0.0371	0.009**
T. alexandrinum	0.398±0.0633	0.465±0.0027	0.011*
Degree of freedom	1	**Error**	9
Permissible maximum limit[a]		80 mg/kg	

*, **: Significant at 0.05 and 0.01, ns: non-significant, Source: [a]USEPA [31]

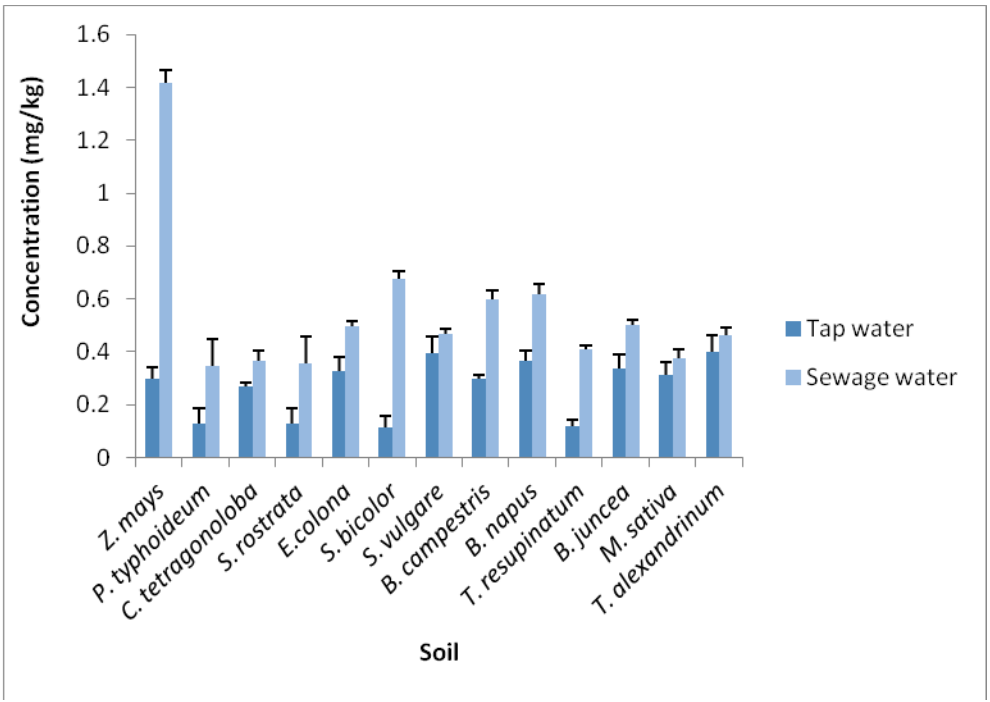

Figure 1: Fluctuation of manganese in soil grown with different forage

Manganese in Root

All treatments showed significant (p<0.05) effect on the Mn concentration in root samples according to analysis of variance in all forages. The sequence of the observed values in plants as a result of tap water irrigation was: *S. bicolor*>*S. vulgare*>*T. alexandrinum*>*B. campestris*>*Z. mays*>*M. sativa*>*P. typhoideum*>*E.*

colona>T. resupinatum>B. napus>S. rostrata>B. juncea>C. tetragonoloba. The sequence of the observed values in plants as a result of sewage water irrigation was: *S. bicolor>C. tetragonoloba>M. sativa>T. alexandrinum>S. vulgare>B. campestris>Z. mays>B. napus>P. typhoideum>T. resupinatum>S. rostrata>B. juncea>E. colona.* The highest mean concentration of Mn in the root was 0.455 mg/kg occurred in *S. bicolor* and the lowest mean concentration was 0.017 mg/kg occurred in *C. tetragonoloba* grown in summer (Table 3). In other study, Molahoseini *et al*. [34] found higher value (50.84 mg/kg) for Mn in root of forage corn. Kansal *et al*. [35] also found higher Mn range (26-37 mg/kg) in maize and range (25-38 mg/kg) in berseem irrigated with tube-well and sewage water. It has been reported that there were different factors such as plant genotype, the structure of plant root system, soil and climatic conditions and the response of plant species to elements related to the seasonal cycles can be affect the bioavailability of elements to plants (Figure 2).

Table 3: Manganese concentration (mg/kg) in roots of forage samples irrigated with tap and sewage water

Root	Irrigation water		Mean square
	Tap	Sewage	
Summer			
Z. mays	0.305±0.0017	0.338±0.0018	0.003**
P. typhoideum	0.139±0.0016	0.280±0.0015	0.051*
C. tetragonoloba	0.017±0.0016	0.40±0.0015	0.036*
S. rostrata	0.133±0.0017	0.225±0.0018	0.021*
E. colona	0.138±0.0017	0.163±0.0017	0.002**
S. bicolor	0.370±0.0021	0.455±0.0014	0.018*
S. vulgare	0.380±0.0018	0.385±0.0015	0.001**
Winter			
B. campestris	0.333±0.0017	0.358±0.0017	0.002**
B. napus	0.135±0.0025	0.312±0.0098	0.078*
T. resupinatum	0.143±0.00176	0.241±0.00231	0.024*
B. juncea	0.130±0.00177	0.175±0.0017	0.005**
M. sativa	0.293±0.00176	0.398±0.0034	0.028*
T. alexandrinum	0.367±0.0023	0.390±0.0017	0.001**
Degree of freedom	1	**Error**	9
Permissible maximum limit[a]		30 mg/kg	

*, **: Significant at 0.05 and 0.01 levels, Source: [a]WHO [36]

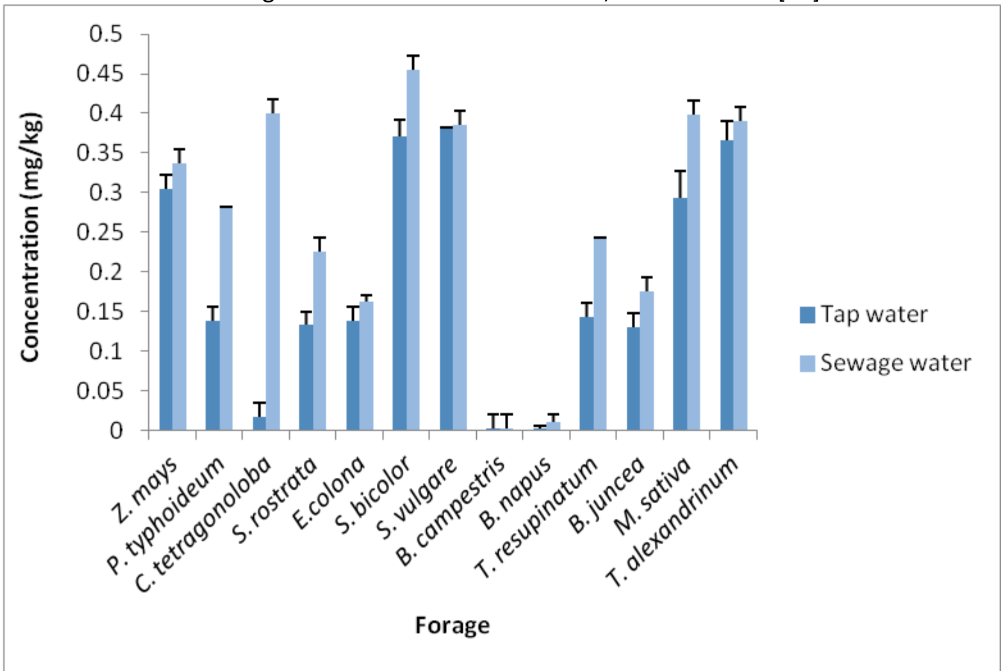

Figure 2: Fluctuation of manganese in root irrigated with tap and sewage water

Manganese in Leaves

All treatments showed significant ($p<0.05$) effect according to analysis of variance in all forages except for *T. resupinatum* was observed. The sequence of the observed values in plants as a result of tap water irrigation was: *S. rostrata>T. resupinatum>P. typhoideum> B. napus>M. sativa>S. vulgare>T. alexandrinum>Z. mays>C. tetragonoloba>E. colona>B. campestris>B. juncea>S. bicolor*. The order of sequence for sewage water irrigation was: *S. rostrata>T. resupinatum>S. bicolor>S vulgare>T. alexandrinum>C. tetragonoloba>M. sativa>B. napus>P. typhoideum>B. juncea>E. colona>Z. mays>B. campestris.* The maximum average concentration of Mn in the leaves was 0.553 mg/kg occurred in *S. rostrata* and the lowest mean concentration was 0.072 mg/kg occurred in *S. bicolor*. (Table 4). The permissible maximum limits used was 30 mg/kg established by WHO [38]. The present Mn value was lower so, there was no risk for metal toxicity.

Manganese range in leaves was lower than the found by Kumar and Chopra [28] (1.55-3.22 mg/kg) in *B. juncea*, *T. aestivum* and *H. vulgare* irrigated with discharge of glass industry. Khan *et al.* [39] (2009*)* found higher Mn values in forage in November (115.50 mg/kg) and January (76.65 mg/kg) and higher range of Mn (153.43-215.20 mg/kg), respectively. According to Moreki *et al*. [40] (2013) it was observed that continuous winds accelerate the drift of small minerals from both environment and exhaust fumes into the forages in the summer season (Figure 3).

Table 4: Manganese concentration (mg/kg) in leaves of different forages

Forage	Irrigation water		Mean square
	Tap	Sewage	
Summer			
Z. mays	0.190±0.0017	0.203±0.0017	0.001**
P. typhoideum	0.265±0.0016	0.283±0.0018	0.001**
C. tetragonoloba	0.153±0.0018	0.298±0.0039	0.054*
S. rostrata	0.543±0.0019	0.553±0.0017	0.001**
E. colona	0.150±0.0170	0.263±0.0017	0.032*
S. bicolor	0.072±0.0039	0.073±0.0093	0.001**
S. vulgare	0.261±0.0016	0.398±0.0597	0.047*
Winter			
B. campestris	0.140±0.0017	0.158±0.0017	0.001**
B. napus	0.262±0.0034	0.286±0.0026	0.001**
T. resupinatum	0.284±0.0040	0.535±0.0017	0.158[ns]
B. juncea	0.130±0.0310	0.268±0.0018	0.047*
M. sativa	0.145±0.0017	0.293±0.0015	0.054*
T. alexandrinum	0.260±0.0018	0.370±0.0025	0.030*
Degree of freedom	1	**Error**	9
Permissible maximum limit[a]		30 mg/kg	

*, **: Significant at 0.05 and 0.01 levels, ns: non-significant, Source: [a]WHO [36]

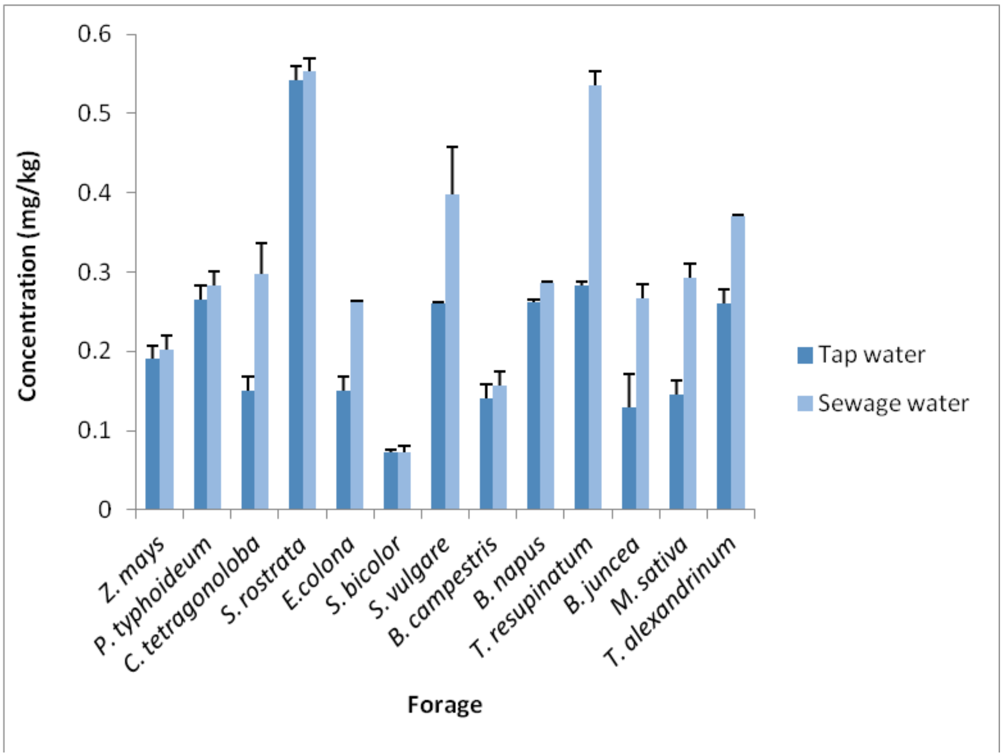

Figure 3: Fluctuation of manganese in leaves of different forages

Bioconcentration Factor

Bioconcentration factor was calculated for forages irrigated with both tap and sewage water and the following sequences were observed. The sequence of the observed values in plants as a result of tap water irrigation was: *S. rostrata>P. typhoideum>B. napus>T. resupinatum>S. vulgare>T. alexandrinum>C. tetragonoloba>M. sativa>E. colona>B. juncea>B. campestris>Z. mays>S. bicolor.* As a result of sewage water irrigation was: *T. resupinatum>S. rostrata>P. typhoideum>S. vulgare>C. tetragonoloba>E. colona>T. alexandrinum>B. juncea>M. sativa>Z. mays>S. bicolor>B. campestris>B. napus* (Table 5). The maximum BCF value in *T. resupinatum* (4.4957) and the minimum value in *S. bicolor* (0.10740). These Mn BCF values were higher than the values found by Alrawiq *et al.* [41] (0.221-0.490) for Mn after irrigation with recycled and non-recycled water. According to Liu *et al*. [42], the BCF if found greater than 1, it suggests that the plants can accumulate heavy metals in them.

Table 5: Bioconcentration factor of manganese in forages

Forage	BCF	
	Irrigation water	
	Tap	Sewage
Summer		
Z. mays	0.134	0.680
P. typhoideum	0.767	2.224
C. tetragonoloba	0.560	0.810
S. rostrata	1.570	4.350
E. colona	0.302	0.800
S. bicolor	0.107	0.618
S. vulgare	0.662	0.850
Winter		
B. campestris	0.233	0.531
B. napus	0.718	0.463
T. resupinatum	0.692	4.496

B. juncea	0.260	0.794
M. sativa	0.463	0.782
T. alexandrinum	0.653	0.796

Pollution Load Index

The sequence of soil PLI value according to the plant samples irrigated with tap water was: *T. alexandrinum> S. vulgare>B. napus>P. typhoideum>B. juncea>E. colona>Z. mays>M. sativa>B. campestris>C. tetragonoloba>T. resupinatum>S. rostrata>S. bicolor.* The sequence ofsoil PLI value according to the plant samples irrigated with sewage water was: *Z. mays>S. bicolor>S. vulgare>B. napus>B. campestris>B. juncea>E. colona>T. alexandrinum>C. tetragonoloba>M. sativa>T. resupinatum>P. typhoideum>S. rostrate* (Table 6). The maximum PLI for Mn showed in *Z. mays* (0.0302) and the minimum value showed in *C. tetragonoloba* (0.0057). Ahmad *et al*. [43] found higher PLI in soil at two sites (0.305-0.379) and irrigated with canal and sewage water (1.495), respectively. Pollution load index greater than 1 indicate that the area was contaminated while if the PLI was found less than 1 it shows that sampling area was un-polluted.

Table 6: Pollution load index for manganese in soil

Forage	PLI	
	Irrigation water	
	Tap	Sewage
Summer		
Z. mays	0.0063	0.0302
P. typhoideum	0.0074	0.0077
C. tetragonoloba	0.0057	0.0078
S. rostrata	0.0027	0.0073
E. colona	0.0070	0.0106
S. bicolor	0.0024	0.0144
S. vulgare	0.0084	0.0142
Winter		
B. campestris	0.0063	0.0128
B. napus	0.0078	0.0131
T. resupinatum	0.0028	0.0085
B. juncea	0.0072	0.0108
M. sativa	0.0066	0.0086
T. alexandrinum	0.0085	0.0099

Table 7: Daily intake of metals and health risk index of manganese in forages

Forage	DIM		HRI	
	Irrigation water		Irrigation water	
	Tap	Sewage	Tap	Sewage
Summer				
Z. mays	0.004	0.005	9.236	9.844
P. typhoideum	0.006	0.008	0.00012	0.00013
C. tetragonoloba	0.008	0.009	0.00015	7.292
S. rostrata	0.012	0.014	0.00026	0.00027
E. colona	0.009	0.010	0.00012	7.292
S. bicolor	0.001	0.002	3.50455	3.520
S. vulgare	0.007	0.009	0.00010	0.00019
Winter				
B. campestris	0.010	0.003	6.80603	7.656
B. napus	0.005	0.007	0.00011	0.00013
T. resupinatum	0.006	0.013	0.00013	0.00026
B. juncea	0.002	0.004	0.00014	6.319

M. sativa	0.003	0.006	0.00015	7.049
T. alexandrinum	0.015	0.017	0.00013	0.00017

Daily Intake of Metal and Health Risk Index

The sequence of DIM value according to the plant samples irrigated with tap water was: *S. rostrata>B. campestris>E. colona>C. tetragonoloba>S. vulgare>T. resupinatum>P. typhoideum>T. alexandrinum>B. napus>Z. mays>M. sativa>B. juncea>S. bicolor.* The sequence of DIM value according to the plant samples irrigated with sewage water was: *T. alexandrinum>T. resupinatum>S. rostrata>E. colona>S. vulgare>C. tetragonoloba>P. typhoideum>M. sativa>B. napus>B. juncea>Z. mays>B. campestris>S. bicolor* (Table 7). The maximum DIM value was observed in *S. rostrata* (0.0125) and the minimum value was observed in *S. bicolor* (0.00163). 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 for Mn was calculated. And highest range observed in *Z. mays* (9.844 mg/kg) grown in summer and the lowest in *S. vulgare* (0.0001 mg/kg) also grown in summer. Khan *et al.* [44] found lower HRI value (0.30-0.38) in wastewater irrigated sites.

CONCLUSION

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

CONFLICTS OF INTEREST: The authors declare that there are no conflicts of interest regarding the publication of this paper.

REFERENCES

[1] Ahmad K, Ashfaq A, Khan ZI, Bashir H, Sohail M, Mehmood N, Dogan Y. Metal accumulation in *Raphanus sativus* and *Brassica rapa*: An assessment of potential health risk for inhabitants in Punjab, Pakistan. Environ Sci Pollut Res 2018; 25(8):16676-16,685.

[2] Khan ZI, Ugulu I, Sahira S, Ahmad K, Ashfaq A, Mehmood N, Dogan Y. Determination of toxic metals in fruits of *Abelmoschus esculentus* grown in contaminated soils with different irrigation sources by spectroscopic method. Int J Environ Res 2018; 12(4): 503-511.

[3] Khan ZI, Ahmad K, Iqbal S, Ashfaq A, Bashir H, Mehmood N, Dogan Y. Evaluation of heavy metals uptake by wheat growing in sewage water irrigated soil. Hum Ecol Risk Assess 24(5): 1409-1420.

[4] Osma E, Ozyigit II, Leblebici Z, Demir G, Serin M. Determination of heavy metal concentrations in tomato (*Lycopersicon esculentum* Miller) grown in different station types. Rom Biotech Lett 2012; 17(1): 6962-6974.

[5] Ugulu I. Determination of heavy metal accumulation in plant samples by spectrometric techniques in Turkey. Appl Spectrosc Rev 2015; 50(2): 113-151.

[6] Dogan Y, Baslar S, Ugulu I. A study on detecting heavy metal accumulation through biomonitoring: Content of trace elements in plants at Mount Kazdagi in Turkey. Appl Ecol Environ Res 2014; 12(3): 627-636.

[7] Unver MC, Ugulu I, Durkan N, Baslar S, Dogan Y. Heavy metal contents of *Malva sylvestris* sold as edible greens in the local markets of Izmir. Ekoloji 2015; 24(96): 13-25.

[8] Ugulu I, Unver MC, Dogan Y. Determination and comparison of heavy metal accumulation level of *Ficus carica* bark and leaf samples in Artvin, Turkey. Oxid Commun 2016; 39(1): 765-775.

[9] Chailapakul O, Korsrisakul S, Siangproh W, Grudpan K. Fast and simultaneous detection of heavy metals using a simple and reliable microchip-electrochemistry route: An alternative approach to food analysis. Talanta 2007; 74: 683-689.

[10] Ahmad K, Nawaz K, Khan ZI, Nadeem M, Wajid K, et al. Effect of diverse regimes of irrigation on metals accumulation in wheat crop: An assessment-dire need of the day. Fresen Environ Bull 2018; 27(2): 846-855.

[11] Khan ZI, Ugulu I, Umar S, Ahmad K, Mehmood N, Ashfaq A, Bashir H, Sohail M. Potential toxic metal accumulation in soil, forage and blood plasma of buffaloes sampled from Jhang, Pakistan. Bull Environ Contam Toxicol 2018 (online first) https://doi.org/10.1007/s00128-018-2353-1

[12] Yadav RK, Goyal B, Sharma RK, Dubey SK, Minhas PS. Post-irrigation impact of domestic sewage effluent on composition of soils, crops and ground water - A case study. Environ Int 2002; 28: 481-486.

[13] Leblebici Z, Kar M. Heavy metals accumulation in vegetables irrigated with different water sources and their human daily intake in Nevsehir. J Agr Sci Technol 2018; 20(2): 401-415.

[14] Valentina L, Bouwer H, Akica B. Water quality considerations. In: Valentina L, Akica B (Eds.), Water Reuse for Irrigation: Agriculture, Landscapes, and Turf Grass, CRC Press, Boca Raton, FL, 2005; pp. 31-60.

[15] Xu J, Wu LS, Chang AC, Zhang Y. Impact of long-term reclaimed wastewater irrigation on agricultural soils: A preliminary assessment. J Hazard Mater 2010; 183: 780.

[16] Smith SR. Agricultural Recycling of Sewage Sludge and The Environment. CAB International, Wallingford, 1996.

[17] Baslar S, Kula I, Dogan Y, Yildiz D, Ay G. A study of trace element contents in plants growing at Honaz Dagi-Denizli, Turkey. Ekoloji 2009; 18(72): 1-7.

[18] Sahin I, Akcicek E, Guner O, Dogan Y, Ugulu I. An investigation on determining heavy metal accumulation in plants growing at Kumalar Mountain in Turkey. Eurasia J Biosci 2016; 10: 22-29.

[19] Vatansever R, Filiz E, Ozyigit II. In silico analysis of Mn transporters (NRAMP1) in various plant species. Mol Biol Rep 2016; 43(3): 151-163.

[20] Givens DI, Owen E, Axford RFE, Omed HM. Forage Evaluation in Ruminant Nutrition. CABI Publishing, Oxon, 2000.

[21] Norrish K. Geochemistry and minerology of trace elements. In: Nicholas D (Ed.), Trace Elements in Soil-Plant-Animal Systems, Academic Press, New York, 1975; pp. 55-81.

[22] Cui YJ, Zhu YG, Zhai RH, Chen DY, Huang YZ, Qui Y, Liang JZ. Transfer of metals from near a smelter in Nanning, China. Environ Int 2004; 30: 785-791.

[23] Liu WH, Zhao JZ, Ouyang ZY, Soderlund L, Liu GH. Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. Environ Int 2005; 31: 805-812.

[24] Chary NS, Kamala CT, Raj DS. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. Ecotoxicol Environ Saf 2008; 69(3): 513-24.

[25] USEPA. Preliminary Remediation Goals, Region 9. Office of Research and Development, US Environmental Protection Agency, Washington, DC., 2002.

[26] Singh A, Sharma RK, Agarwal M, Marshall F. Risk assessment of heavy metal toxicity through contaminated vegetables from waste water irrigated area of Varanasi, India. Trop Ecol 2010; 51(2): 375-387.

[27] Pescod MD. Wastewater Treatment and Use in Agriculture. Irrigation and drainage paper. 47. Food and Agricultural Organization, Rome, 1992.

[28] Kumar V, Chopra AK. Heavy metals accumulation in soil and agricultural crops grown in the Province of Asahi India Glass Ltd., Haridwar (Uttarakhand), India. Adv Crop Sci Tech 2015; 4: 203.

[29] Barreto AN, Do Nascimento JJ, Medeiros EPD, Nóbrega JAD, Bezerra JR. Changes in chemical attributes of a fluvent cultivated with castor bean and irrigated with wastewater. Rev Bras Eng Agríc Ambient 2013; 17(5): 480-486.

[30] Teo KC, Chen J. Determination of manganese in water samples by flame atomic absorption spectrometry after cloud point extraction. Analyst 2001; 126: 534-537.

[31] USEPA. Exposure Factors Handbook. Volume II-Food Ingestion Factors. EPA/600//P-95/002Fa. Office of Research and Development, US Environmental Protection Agency, Washington, DC., 1997.

[32] Khan K, Lu Y, Khan H, Ishtiaq M, Khan S, Waqas M, Wei L, Wang T. Heavy metals in agricultural soils and crops and their health risks in Swat District, northern Pakistan. Food Chem Toxicol 2013; 58: 449-458.

[33] Espinoza JE, McDowell LR, Wilkinson NS, Conrad JH, Martin FG. Monthly variation of forage and soil minerals in Central Florida. II. Trace minerals. Commun Soil Sci Plant Anal 1991; 22: 1137-1149.

[34] Molahoseini H, Feizi M, Seilsepour M. The concentration of some essential elements and cadmium in sunflower, turnip and forage corn under wastewater irrigation. In: Proc. The 1th International and the 4th National Congress on Recycling of Organic Waste in Agriculture. Isfahan, Iran, 26-27 April 2012. pp. 1-6.

[35] Kansal BD, Kumar R, Sokka R. The influence of municipal wastes and soil properties on the accumulation of heavy metals in plants. Heavy Metals in the Environment. CEC Consultants Ltd., Edinburgh, 1996; pp. 413-416.

[36] WHO Trace Elements in Human Nutrition and Health. World Health Organization, Geneva, 1996.

[37] Khan ZI, Ashraf M, Ahmad N, Ahmad K, Valeem EE. Availability of nutritional minerals (cobalt, copper, iron, manganese and zinc) in pastures of central Punjab for farm livestock. Pak J Bot 2009; 41(4): 1603-1609.

[38] Moreki JC, Woods TO, Nthoiwa PG. Estimation of the concentration of heavy metals in forages harvested around Dibete area, Botswana. Int J Innov Res Sci Eng Technol 2013; 2(8): 4060-4071.

[39] Alrawiq N, Khairiah J, Talib J, Ismail ML, Anizan BS. Accumulation and translocation of heavy metals in soil and paddy plant samples collected from rice fields irrigated with recycled and non-recycled water in MADA Kedah, Malaysia. Int J ChemTech Res 2014; 6(4): 2347-2356.

[40] Liu WX, Li HH, Li SR, Wang YW. Heavy metals accumulation of edible vegetables cultivated in agricultural soil in the suburb of Zhengzhou City, People's Republic of China. Bull Environ Contam Toxicol 2006; 76: 163-170.

[41] Ahmad K, Khan ZI, Yasmin S, Ashraf M, Ishfaq A. Accumulation of metals and metalloids in turnip (*Brassica rapa* L.) irrigated with domestic wastewater in the peri-urban areas of Khushab City, Pakistan. Pak J Bot 2014; 46(2): 511-514.

[42] Khan ZI, Ahmad K, Ashraf M, Parveen R, Mustafa I, Khan A, Bibi Z, Akram AN. Bioaccumulation of heavy metals and metalloids in luffa (*Luffa cylindrica* L.) irrigated with domestic wastewater in Jhang, Pakistan: A prospect for human nutrition. Pak J Bot 2015; 47(1): 217-224.