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Monitoring of Zinc Profile of Forages Irrigated with City Effluent

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Abstract

Wastewater contains a surplus amount of trace metals that contaminate the soil and crops. A pot trial was performed to determine the impact of wastewater on the zinc accumulation in forages and their associated health risk. Forages both of summer (Zea mays, Echinochloa colona, Pennisetum typhoideum, Sorghum vulgare, Sorghum bicolor, Sesbania rostrata, and Cyamopsis tetragonoloba) and winter (Trifolium alexandrinum, Medicago sativa, Brassica campestris, Trifolium resupinatum, Brassica juncea, and Brassica napus) were grown with sewage water and tap water treatment. The experiment was laid down in a completely randomized design with five replicates. The concentration of zinc in water, root and forage samples were analysed by atomic absorption spectrophotometer. In tap water, the zinc value was 0.498 mg/L and in wastewater 0.509 mg/L, respectively. The maximum level of zinc in the forages leaves was 3.582 mg/kg found in Brassica napus grown in the winter season. The maximum observed value for zinc bioconcentration factor in Brassica juncea was (2.88) grown in winter. The values of pollution load index for zinc were found less than 1. The values of daily intake of metal and health risk index for zinc in all forages were less than 1 indicated that consumption of these forages was free of risk.

Keywords: Bioaccumulation, Pollution load index, Forage, Health risk index, Zinc.

Introduction

The shortage of water is a major problem all over the world, and many parts of the world are facing this problem day by day [1]. This problem of water shortage is solved by alternate sources of irrigation [2]. The wastewater is a source of some nutrients essential for soil fertility, but it also contains toxic metals that contaminate the soil and crops [3]. The metals Ni, Pb, Zn, Cd, Cu, Cr, and Mn from wastewater contaminate the agricultural land and crops grown there and

become the part of the food chain and cause various health hazards in human [4, 5].

The wastewater irrigation is beneficial if it imparts no negative impact on crops as well as human health [6]. However, heavy metals due to their residing natures cause pollution in the environment and ultimately in humans [7]. The water sites such as sewage, canal water and tube-well water used for fields having different food crops. The root apices of

plants are impassable with heavy metals due to their immature cells and low-density cell walls. Metals are taken up by plant from contaminated soil and then transfer to the upper parts of the plants [8].

Zinc (Zn) is considered a vital element for metabolism in animals and plants, but if it exceeds the level severe losses to life occur [9]. Zn has great importance as a catalytic element for over 300 enzymes, such as carbonic anhydrase, alcohol dehydrogenase, alkaline phosphatase, Cu-Zn superoxide dismutase, and DNA-RNA polymerase [10]. Also, mitosis division of a cell is distressed due to Zn activity [11]. The activity and permeability of membranes are decreased by the Zn attack because it affects the movement of ions and enzymes there [12]. The necrosis of shoots caused by Zn and it also can destroy the plant cell finally [13]. Zn interrupts the root function [14]. Additionally, Cd, Pb, and Zn decrease plant uptake level of necessary elements like Mn, but a greater amount of Zn can cause a lack of development and reproduction [15].

The current research was conducted to determine the impact of Zn on pollution severity and transfer of Zn in forages and humans through soils.

Materials and Methods Study Area

The current research (pot trial) was performed at the Department of Botany, the University of Sargodha, Pakistan at coordinates 32.0740° N, 72.6861° E.

Plant Cultivation

Summer cultivation: 4 types of forages Bajra (*Pennisetum typhoideum* Rich.), Sanwak (*Echinochloa colona* L. Link), Jowar (hybrid) (*Sorghum bicolor* L. Moench), Jantar (*Sesbania rostrata* Bremek & Oberm.), Maize (Zea mays L.), Local jowar (Sorghum vulgare Pers.), Gawara (Cyamopsis tetragonoloba L. Taub.). were planted in 70 pots (35 control and 35 experimental) below 4-5 cm of soil. The physicochemical parameters of soil are given in Table 1. The experiment was laid down in a completely randomized design (CRD) with 5 replicates. The chemical composition of canal and sewage water is given in Table 2. Pots were irrigated twice a week.

Winter cultivation: Six winter forages were sown; Berseem (Trifolium alexandrinum L.), Sarsoon (Brassica campestris L.), Luscern (Medicago sativa L.). Indian mustard Czern.). (Brassica juncea L. Chatala (Trifolium resupinatum L.), and Canola (Brassica napus L.). Forages were planted in 60 including 30 control (Tap water irrigated) and 30 experimental pots (Sewage water). The plants were harvested on 6-10-2016.

Table 1. Physicochemical properties of water.

Properties of water	Tap water	Sewage water
Electrical Conductivity (µS/cm)	1890	7750
Calcium+ Magnesium (Ca ²⁺ +Mg ²⁺) (meq/L)	5.2	18.5
Sodium (Na ⁺) (meq/L)	13.7	59.0
Carbonate (CO ² ₃ -) (meq/L)	0.4	0.8
Bicarbonate (HCO ₃) (meq/L)	8.2	9.6
Chloride (Cl ⁻) (meq/L)	6.4	51.7
Sodium Adsorption Ratio (SAR)	8.5	19.4
Residual Sodium Carbonate (RSC)	3.4	Nil

Table 2. Physicochemical 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)	5.64	8.42	3.01	4.51
Organic matter (%)	0.90	0.83	0.96	0.76
Available phosphorus (mg/kg)	8.8	7.0	8.6	7.4
Available potassium (mg/kg)	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

Samples Collection

Plastic bottles were washed with distilled water and samples of sewage and tap water (100 mL each) were taken in plastic bottles. Conc. HNO₃ (1 mL) was added in water to prevent the activity of microorganisms. Samples (130) were stored in a refrigerator before the digestion. Soil samples were sun dried and then oven-dried for 3 days at 75°C to removes excess moisture. After drying and grinding, these samples were digested.

Zinc Analysis

Zn contents were analysed by running samples in atomic absorption spectrophotometer (AAS-6300 Shimadzu Japan).

Statistical Analysis

Zn values for water, soil and forage samples were analysed by Statistical Package of Social Sciences (SPSS 23). Independent samples t-test was used to determine whether tap water and sewage water irrigation made a statistically significant difference in the samples.

Bioconcentration Factor

Bioconcentration factor (BCF) was used to determine the transfer of metals from soil to the edible part of the plant [16].

$$BCF = \frac{Concentration{\left(\frac{mg}{kg}\right)}of\ heavy\ metal\ in\ plant}}{Concentration{\left(\frac{mg}{kg}\right)}of\ heavy\ metal\ in\ soil}}$$

Pollution Load Index

Pollution severity of soil can be well analysed by using the following formula [17].

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

The reference value of Zn was (44.19 mg/kg).

Daily Intake of Metals

Daily Intake of Metals (DIM) was computed according to the following formula [18].

$$DIM = \frac{Concentration \ of \ metal \times Daily \ int \ ake \ of \ for age}{Average \ body \ weight}$$

Average body weight was taken as 550 kg.

Health Risk Index

Health risk index (HRI) was calculated by the following formula [19].

 $$R_{\rm f}D$$ values for Zn was 0.3 mg/kg/day [20].

Results and Discussion Zinc Content in Water

According to independent samples t-test results, the difference between heavy metal values in tap and sewage water samples was statistically significant (p<0.01). The determined Zn value for tap water and sewage water was 0.498 and 0.509 mg/L, respectively (Table 3). The Zn content in the present findings was found within the permissible limit of 2.0 mg/L given by Pescod [21]. The Zn values in the present findings were higher than the findings of Tariq *et al.* [22] (0.1 mg/L) in tap water and by Murtaza *et al.* [23] (0.210 mg/L) for sewage water. Salawu *et al.*

[24] found a higher Zn value (4.236 mg/L) in sewage water. The present Zn values in water were lower than the findings of Kumar and Chopra [25] (2.17-8.80 mg/L) for borewell and industry effluent. Khaskhoussy et al. [26] reported a similar range (0.20-0.55 mg/L) for Zn in freshwater and treated wastewater. Kumar and Chopra [25] analyzed that the higher level of various metals in the wastewater might be due to the application of various chemicals used in the industry. Among the household products, the medicated (anti-dandruff) shampoos contain Zn pyrithione and the high Zn concentrations will thus raise the Zn inputs to the sewage waters. Also, the differences in the Zn values determined in the various studies can be potentially originated from the study areas of the studies.

Table 3. Zinc content in water (mg/L).

Tap water	Sewage water	p
0.498±0.1274	0.509±0.0506	0.001**
Permissible maximum l	imit ^a	2.0 mg/L

^{**:} Significant at 0.01 level, Source: aPescod [21]

Zinc in Soil

Independent sample t-test showed that the Zn content in the soil samples of C. tetragonoloba, S. vulgare, B. juncea, and T. alexandrinum were statistically different (p<0.01). The order as a result of tap water irrigation (TWI) was: P. typhoideum> Z. mays> B. napus> B. campestris> S. bicolor> E. colona> T. resupinatum> B. juncea> T. alexandrinum> vulgare> *C*. S. tetragonoloba>M. sativa> S. rostrata. The sequence was as: *M*. sativa> C. tetragonoloba> Z. mays> B. campestris> B. napus> P. typhoideum> T. alexandrinum> S. bicolor> E. colona> B. juncea> S. rostrata> T. resupinatum> S. vulgare for sewage water irrigation (SWI). The maximum values of Zn were found in the soil of M. sativa (2.871

mg/kg) and the minimum was found in the soil of *S. rostrate* (0.129 mg/kg) (Table 4). The values of Zn were found within the permissible maximum limits of 200 mg/kg established by USEPA [27]. These Zn values were contradicted as reported by some researchers (12.13 mg/kg) as in October and 8.47 mg/kg in June [28]. However, Kumar and Chopra [25] noticed a higher range of Zn in soil (3.75-4.15 mg/kg).

Table 4. Zinc content (mg/kg) in soil grown with different forages.

Forage	Tap water	Sewage water	р
Torage		Summer	
Z. mays	1.546±0.0026	1.824±0.0172	$0.193^{\rm ns}$
P. typhoideum	1.594±0.0498	1.798±0.0021	0.105^{ns}
C. tetragonoloba	0.153±0.0089	2.850±0.0379	18.198 ^{ns}
E. colona	0.462±0.0364	0.480±0.0536	0.001**
S. rostrata	0.129±0.0317	0.372±0.0187	0.148 ^{ns}
S. bicolor	0.522±0.0292	0.708±0.0232	0.086^{ns}
S. vulgare	0.294±0.0028	0.298±0.0137	0.001**
	Winter		
B. campestris	1.278±0.0018	1.822±0.0169	0.740^{ns}
B. napus	1.544±0.0192	1.805±0.0043	0.069^{ns}
B. juncea	0.468±0.0347	0.488±0.0520	0.001**
M. sativa	0.156±0.0084	2.871±0.0310	18.435 ^{ns}
T. resupinatum	0.154±0.0379	0.370±0.0188	0.117^{ns}
T. alexandrinum	0.298±0.0043	0.301±0.0160	0.001**
dF	24	t	-1.108
Permissible maxim	um limit ^a	200 mg/kg	

^{****:} Significant at 0.05 and 0.01 levels, ns: non-significant, Source: $^{\rm a}{\rm USEPA}\,[27]$

Khaskhoussy *et al.* [26] found a higher range for Zn (59.5-74.5 mg/kg) in soil irrigated with freshwater and treated wastewater. Zn accumulation in the soil might be due to various factors metals in water, biological processes, soil and water properties. The activities of soil microflora are affected adversely due to the binding of Zn ions with soil particles when irrigation is applied [29] as shown in Fig. 1.

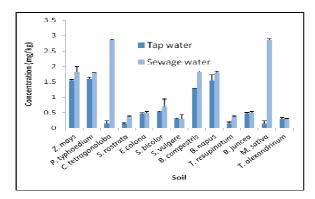


Figure 1. Zinc contents in soil

Zinc in Root

According to the results of the independent samples t-test, the difference between the heavy metal values of the plant root samples as a result of tap and sewage irrigation was statistically significant except for C. tetragonoloba S. bicolor and M. sativa plants (p<0.01 and p<0.05). The order of Zn values as a result of TWI alexandrinum>B. juncea> E. colona> napus> В. bicolor> В. S. vulgare> Р. campestris> *C*. tetragonoloba> typhoideum> Z. mays> S. bicolor> resupinatum> S. rostrata. While as a result of SWI was: T. alexandrinum> M. sativa> C. tetragonoloba> S. bicolor> B. jucea> E. colona> В. napus> S. vulgare> campestris> Z. mays> P. typhoideum> S. rostrata> T. resupinatum. The highest Zn content was in the root was 0.390 mg/kg in T. alexandrinum grown in winter and the lowest 0.075 mg/kg in S. rostrata grown in summer (Table 5). Asdeo [30] and Masona et al. [31] found a higher Zn range as 6.32-8.92 mg/kg and 24-120 mg/kg, respectively. Hassan et al. [32] reported a greater value of Zn in plants (35.3 mg/kg). Khaskhoussy et al. [26] found a higher trend of Zn root than present study and Keller et al. [33] observed that various plants with different root systems had diverse reactions and tolerances to heavy metals and minimum heavy metal concentrations in tissues could promote plant growth (Fig. 2).

Table 5. Zinc concentration (mg/kg) in roots of forage samples irrigated with tap and sewage water.

Forage	Tap water	Sewage water	p	
9	Summer			
Z. mays	0.120±0.0021	0.190±0.0016	0.012*	
P. typhoideum	0.123 ± 0.0018	0.183 ± 0.0018	0.009**	
C. tetragonoloba	0.133 ± 0.0017	0.310 ± 0.0019	0.088^{ns}	
E. colona	0.235 ± 0.0016	0.258 ± 0.0014	0.001**	
S. rostrate	0.075 ± 0.0015	0.138 ± 0.0017	0.010*	
S. bicolor	0.118 ± 0.0018	0.305 ± 0.0019	0.088^{ns}	
S. vulgare	0.147 ± 0.0146	0.238 ± 0.0017	0.021*	
	Winter			
B. campestris	0.138±0.0063	0.193±0.0028	0.008**	
B. napus	0.185 ± 0.0017	0.216 ± 0.0034	0.002**	
B. juncea	0.236 ± 0.0021	0.266 ± 0.0021	0.002**	
M. sativa	0.130 ± 0.0017	0.325 ± 0.0046	0.095^{ns}	
T. resupinatum	0.105 ± 0.0016	0.155 ± 0.0017	0.006**	
T. alexandrinum	0.367 ± 0.0023	0.390 ± 0.0017	0.001**	
dF	24	t	-1.138	
Permissible maxim	num limit ^a	50 mg/kg		

^{*, **:} Significant at 0.05 and 0.01 levels, Source: aWHO [6]

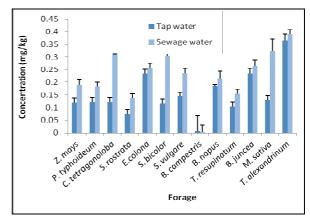


Figure 2. Zinc contents in root irrigated with tap and sewage water

Zinc in Leaves

According to the results of the independent samples t-test, the difference between the heavy metal values of the plant leaf samples as a result of tap and sewage irrigation was statistically significant except for *B. napus* and *B. juncea* plants (p<0.01 and p<0.05). The level of Zn in leaves of forages at TWI was found in following order: *B. napus* B. juncea> T. resupinatum> Z. mays> C. tetragonoloba> M. sativa> P. typhoideum> E. colona> S. rostrata> S.

bicolor> S. vulgare> T. alexandrinum> B. campestris. While as a result of SWI was: B. *T*. napus> В. iuncea> S. bicolor> resupinatum> P. typhoideum> Z. mays> S. rostrata> T. alexandrinum> S. vulgare> E. colona> Z. mays> M. sativa> B. campestris. The highest Zn content in the forages leaves was 3.582 mg/kg occurred in B. napus grown in the winter season and the lowest was 0.073 mg/kg in B. campestris also grown in winter (Table 6). The current Zn values were found within the permissible limit of 50 mg/kg established by WHO [6]. According to this finding, it seems like no risk for metal toxicity. Khan et al. [34] reported higher Zn concentrations varied from (25.88 to 42.24 mg/kg) with the lowest values during October and the highest during January. However, Kumar and Chopra [25] observed a lower range of Zn (8.28-11.60 mg/kg) in crops. Kansal et al. [35] found a higher range of Zn in different plant parts in maize (38-53 mg/kg) and berseem (25-46 mg/kg) irrigated with tube-well and sewage water. The lowest Zn prerequisite of livestock varies with the chemical form or combination of the diet [36] (Fig. 3).

Table 6. Zinc contents (mg/kg) in leaves of forages.

Forego	Tap water	Sewage water	р	
Forage	Summer			
Z. mays	0.084 ± 0.0023	0.126 ± 0.0024	0.004**	
P. typhoideum	0.085 ± 0.0017	0.259 ± 0.0023	0.075*	
C. tetragonoloba	0.125±0.0176	0.199 ± 0.0025	0.014*	
E. colona	0.196 ± 0.0023	0.257 ± 0.0025	0.009**	
S. rostrate	0.189 ± 0.0017	0.240 ± 0.0017	0.007**	
S. bicolor	0.143 ± 0.0627	0.286 ± 0.0018	0.051*	
S. vulgare	0.187 ± 0.0017	0.213±0.0019	0.002**	
	Winter	•		
B. campestris	0.073±0.0019	0.123±0.0018	0.006**	
B. napus	1.275±0.0017	3.582 ± 0.0026	13.300^{ns}	
B. juncea	0.265 ± 0.0019	1.350 ± 0.0177	2.943 ^{ns}	
M. sativa	0.086 ± 0.0018	0.125 ± 0.0021	0.004**	
T. resupinatum	0.214 ± 0.0222	0.285 ± 0.0017	0.013*	
T. alexandrinum	0.188 ± 0.0015	0.223±0.0016	0.003**	
dF	24	t	-1.257	
Permissible maxim	um limit ^a	50 mg/kg		

NS: non-significant, * **: Significant at 0.05 and 0.01 levels, Source: aWHO [6]

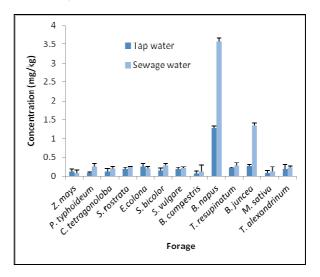


Figure 3. Zinc contents in leaves of forages

Bioconcentration Factor

The values of BCF in plants due to TWI was found in the following descending sequence: B. napus> C. tetragonoloba> T. resupinatum> S. rostrata> T. alexandrinum> B. juncea> E. colona> B. campestris> Z. mays> P. typhoideum> S. vulgare> M. sativa> S. bicolor. As a result of SWI was: B. juncea> S. bicolor> B. napus> S. rostrata> T. resupinatum> T. alexandrinum> E. colona> vulgare> *C*. tetragonoloba> campestris> P. typhoideum> Z. mays> M. sativa. BCF value was higher in B. juncea (2.88) and the minimum in M. sativa (0.0433)(Table 7). Lu et al. [37] found lower Zn BCF value (0.26 mg/kg) in maize shoots as compared to the present study. Alrawiq et al. [38] observed a lower range (0.296-0.196) for Zn BCF after irrigation with different treatments. Asdeo [30] also reported a lower value (0.4049) for BCF in millet. It was reported by Pawan et al. [29] that the ions of Zn associated with metal pollution caused by the property of Zn ions to bind with the soil particles and they also get dissolved in the water found in soil.

Table 7. Bioconcentration factor of zinc in forages.

Irrigation water Forage Tap Sewage Summer Z. mays 0.046 0.082 0.047 P. typhoideum 0.162 C. tetragonoloba 0.819 0.869 E. colona 0.516 0.598 0.742 S. rostrata 1.543 S. bicolor 0.211 2.446 0.457 0.475 S. vulgare Winter 0.052 B. campestris 0.672 1.984 0.826 B. napus 0.552 B. juncea 2.888

0.043

0.770

0.628

0.547

1.386

0.739

Pollution Load Index

M. sativa

T. resupinatum

T. alexandrinum

The order of PLI due to TWI was: P. typhoideum> Z. mays> В. napus> campestris> S. bicolor> E. colona> alexandrinum> S. vulgar> T. resupinatum> C. tetragonoloba> S. rostrata> B. campestris. The order of soil PLI value according to the plant due to SWI was: M. sativa> tetragonoloba> B. campestris> B. napus> S. bicolor> Z. mays> P. typhoideum> S. colona> juncea> rostrata> resupinatum> T. alexandrinum> S. vulgare. The highest PLI was noticed in M. sativa (0.0649) and the lowest value showed by S. vulgare (0.0066) (Table 8). Bao et al. [39] found higher PLI for Zn in soil (1.04, 1.14, 1.03) in three different zones irrigated with the long-term sewage water. Ahmad et al. [40] also noticed higher values of PLI for Zn (1.528) in soil treated with sewage and canal water. The higher PLI suggests that there was more contamination of heavy metals in the area.

Table 8. Pollution load index for zinc in soil.

	PLI Irrigation water		
Forage			
	Тар	Sewage	
	Sur	nmer	
Z. mays	0.033	0.0402	
P. typhoideum	0.036	0.0406	
C. tetragonoloba	0.0039	0.0645	
E. colona	0.0104	0.0108	
S. rostrate	0.0029	0.0084	
S. bicolor	0.0118	0.0160	
S. vulgare	0.0064	0.0065	
	Winter		
B. campestris	0.029	0.0412	
B. napus	0.035	0.0408	
B. juncea	0.0105	0.0109	
M. sativa	0.0037	0.0649	
T. resupinatum	0.0034	0.0083	
T. alexandrinum	0.0067	0.0068	

Daily Intake of Metal and Health Risk Index

The values of DIM for Zn due to TWI was found in the following sequence: B. juncea> colona> *T*. napus> E. resupinatum> S. rostrata> T. alexandrinum> S. vulgare> S. bicolor> P. typhoideum> C. tetragonoloba> Z. mays> M. sativa> B. campestris. While due to SWI was found in following descending sequence: B. napus> B. juncea> E. colona> T. resupinatum> S. vulgare> M. sativa> S. bicolor> S. rostrata> iuncea> Т. alexandrinum> tetragonoloba> B. campestris> Z. mays. The maximum DIM value calculated for Zn in B. napus (0.0813) and the minimum in B. campestris (0.00164) (Table 9). Roggeman et al. [41] noticed higher mean DIM value (7368-4216 mg/kg) in winter and summer value (3698-2110 mg/kg) in herds of cows as compared to the present study. Lawal et al. [42] earlier found similar DIM Zn values (0.0068-0.0062) in spinach leaves grown around Kubanni River in two farmlands. In the present results, the values of DIM were

lower than 1 and it suggests that health risk was linked with the use of such contaminated forages. The maximum HRI observed value showed by *B. napus* (0.965) and the minimum value by *B. campestris* (0.0054 mg/kg). Khan *et al.* [43] gave higher HRI Zn value (0.537-0.609) and Lawal *et al.* [42] observed lower HRI Zn value (0.040-0.021) in spinach leaves grown around Kubanni River in two farmlands. Khan *et al.* [44] gave similar mean HRI value (0.09-0.10) in wastewater irrigated sites. Health risk index depends on the physico-chemical characteristics of the soil, type of forage being consumed and the rate of the consumption of forages.

Table 9. Daily intake of metals and health risk index of zinc in forages.

	DIM Irrigation water		HRI	
Forego			Irrigat	ion water
Forage	Tap	Sewage	Tap	Sewage
	Summer			
Z. mays	0.0020	0.0029	0.0065	0.0093
P. typhoideum	0.0029	0.0058	0.0064	0.0195
C. tetragonoloba	0.0028	0.0045	0.0094	0.0150
E. colona	0.0058	0.0068	0.0144	0.0198
S. rostrata	0.0042	0.0054	0.0142	0.0181
S. bicolor	0.0032	0.0064	0.0107	0.0215
S. vulgare	0.0040	0.0048	0.0142	0.0160
	Winter			
B. campestris	0.0016	0.0027	0.0054	0.0092
B. napus	0.0289	0.0813	0.0271	0.965
B. juncea	0.0060	0.0306	0.0200	0.202
M. sativa	0.0019	0.0028	0.0064	0.0094
T. resupinatum	0.0048	0.0064	0.0161	0.022

Conclusion

T. alexandrinum

Wastewater irrigation readily contaminates the soil and agricultural land. In the present research work, the level of Zn was high in different parts of forages that were

0.0050

0.0041

0.0142

0.0168

irrigated with the sewage water. The concentration of Zn in all parts of forages treated with sewage water were higher than those treated with tap water. The values of Zn in both treatments were found within the permissible limit. The values of health risk index in all forages were less than 1. Thus, it was concluded that forages treated with tap and sewage water were safe for human consumption.

Conflict of Interest

The authors declare that there is no conflict of interest in this paper.

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