

## Bioaccumulation and translocation of cadmium in forages grown in soil irrigated with city effluent: An evaluation

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### 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 at Department of Botany, University of Sargodha to evaluate the possible health risks of heavy metals in forages. Forages both for summer and winter were grown with different water treatments (sewage and tap water). The concentration of cadmium 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 Cd value was 0.54 mg/L and in sewage water, it was 0.56 mg/L. In soil the calculated Cd value was lower than USEPA standards. The maximum Cd in root was calculated in winter forages (1.15 mg/kg). The calculated value for Cd in leaves was higher than the maximum permissible limit 0.5 mg/kg by World Health Organization. The maximum observed value for cadmium bioconcentration factor in *Medicago sativa* was (9.77) grown in winter. The maximum pollution load index observed for Cd was 1.148. The maximum value for daily intake of metal observed was 0.028 mg/kg/day and minimum observed was 0.00948 mg/kg/day.

**Keywords:** Bioaccumulation, Heavy metals, Forages, Health risk index.

### Original Research Article

## INTRODUCTION

More than 50% population of Pakistan lives in dry and semi-arid regions where it is difficult to irrigate agriculture with fresh water. Main irrigation system of Pakistan depends on Indus River having canals and its tributaries. Pakistan population was 136 million in 1988 and predictably will be double in 2025 (Ahmad *et al.*, 2006). Use of fresh water for agriculture, industrial and domestic needs is also increasing with increasing population. Therefore, there is a danger of fresh water scarcity in the coming years (Seckler *et al.*, 1998; Ahmad *et al.*, 2017).

Wastewater irrigation in agricultural areas is gaining popularity to solve this problem. The

advantageous use of agricultural lands is in danger due to presence of toxic substances such as heavy metals and trace minerals like; Ni, Zn, Cd, Pb, Cr, Cu and Mn etc. in wastewater (Luo *et al.*, 2012; Khan *et al.*, 2018a). Heavy metal pollution is very dangerous because it can be hardly detected. Sewage irrigation for a long time tends to accumulate heavy metals in soil (Lucho-constantino *et al.*, 2005). It has been reported that after 5 to 60 years of sewage irrigation the heavy metal concentrations in soil may exceed the standard limit (Mapanda *et al.*, 2005).

The irrigation with sewage water can be useful if it has no negative effects on food crop yield, soil pollution and human health (WHO, 1996; Durkan *et al.*, 2011; Khan *et al.*, 2018b). However,

heavy metals in every source of sewage water can produce pollution to the humans and the environment because of their non-renewable and steady nature (Zhuang *et al.*, 2009). The root apices of plants are impassable to heavy metals due to their immature cells and low-density cell walls. These pick-up metals from impure soil and then transfer to the above ground parts (Tung & Temple, 1996; Ahmad *et al.*, 2018a).

The cadmium (Cd) is highly detrimental and hazardous metal even when present in little amount. In plants, stunting and phenomenon of chlorosis are mostly observed symptoms arising from excessive Cd amount. Different diseases like bone, kidney and cardiovascular disorders can arise because of Cd in humans (Steenland & Boffeta, 2000; Ugulu *et al.*, 2016; Ahmad *et al.*, 2018b).

The present research was carried out with the objectives to investigate (1) the effect of sewage water irrigation on metal uptake by forages (2) the transfer of metals from soil to forages (3) pollution severity of soil due to metal (4) and health risk of grazing livestock consuming metal 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. River Jhelum is present between Northern and Western side and River Chenab is present in the East area of the city. Temperature range is 0-50°C in this region. Major crops grown in Sargodha are rice and sugarcane. Sargodha is known for the production of citrus fruits. Different green vegetables are also grown in Sargodha.

Present study was performed at Department of Botany, University of Sargodha (Figure 1). Forage crops were sown in both summer and winter seasons in this department.

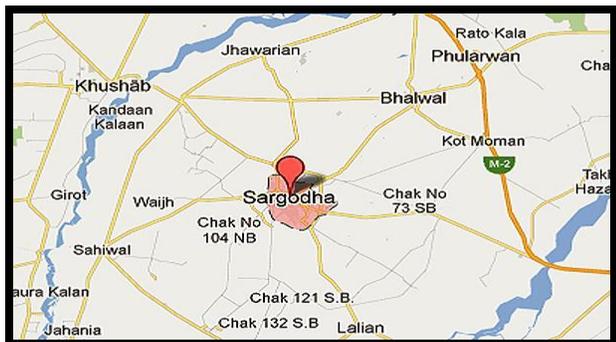


Fig. 1: Location map of experimental area

### Plant cultivation

Summer cultivation: Healthy seeds of seven forages were collected and sown in summer season. Seventy pots were taken and filled with fertile soil. Pots were placed in Department of Botany, University of Sargodha. Crops include 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 (*Sesbaniastrata* Bremek & Oberm.), and Gawara (*Cyamopsis tetragonoloba* (L.) Taub). Seeds were placed below 5 cm of soil. 35 control pots were irrigated with tap water and 35 experimental pots were irrigated with sewage water that was taken from city effluent of Sargodha. 5 replicates of control and 5 replicates of experimental were treated. Pots were irrigated regularly. Summer forages are shown in figure 2.



Fig. 2: Summer forages

Winter cultivation: Six winter forages were sown; Berseem (*Trifolium alexandrinum* L.), Lucerne (*Medicago sativa* L.), Sarsoon (*Brassica campestris* L.), Chatala (*Trifolium resupinatum* L.), Canola sarsoon (*Brassica juncea* (L.) Czern.), and Ghobisarsoon (*Brassic 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. Winter forages are shown in figure 3.



**Fig. 3:** Winter forages

### Samples collection

Plastic bottles were washed with polypropylene acid. For the sake of irrigation 100 mL samples of both sewage and tap water were taken in plastic bottles. To avoid from the actions of microorganisms almost 1 mL of concentrated  $\text{HNO}_3$  was mixed in water and stored in plastic bottles until analysis.

Total 130 samples of fertile soil were taken for summer and winter irrigated with both tap and mixed sewage water. To remove moisture samples were placed in sunlight firstly and then in oven for at least 3 days at  $75^\circ\text{C}$ .

The plants were uprooted on 6-10-2016. Sampling was done one time. Samples were washed with distilled water, dried 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  $75^\circ\text{C}$  for a week so that all the moisture was removed. After drying, the samples were removed from oven, grinded into fine powder with electrical grinder and then subjected to digestion.

### Cadmium analysis

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

### Statistical analysis

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

### Bioconcentration factor (BCF)

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

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

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

### Pollution load index (PLI)

The concentrations of metals have been determined at specific sites by using pollution load index (PLI) (Liu *et al.*, 2005).

$$\text{PLI} = \frac{\text{Metal concentration in investigated soil}}{\text{Reference value of the metal in soil}}$$
  
Reference value of Cd was (1.49 mg/kg).

### Daily intake of metal (DIM)

Daily intake of metal (DIM) was measured by the corresponding equation (Chary *et al.*, 2008);

$$\text{DIM} = \frac{C_{\text{metal}} \times D_{\text{food intake}}}{B_{\text{average weight}}}$$
  
Where,  $C_{\text{metal}}$  is concentration of metals in forages,  $D_{\text{food intake}}$  is the daily intake of forages and  $B_{\text{average weight}}$  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 (HRI)

It was calculated to determine the overall threat of exposure to all heavy metals through ingestion of specific forages. This shows the danger to cattle feed on contaminated forages. Daily ingestion of metals in food crops divided by the oral reference dose was said to as health risk index (HRI) (USEPA, 2002).

$$\text{HRI} = \frac{\text{DIM}}{\text{RfD}}$$
  
RfD values for Cd was 0.001 mg/kg (Singh *et al.*, 2010).

## RESULTS AND DISCUSSION

### Cadmium in water

Analysis of variance showed significant ( $p < 0.05$ ) effect of the Cd concentration in irrigation water both for tap and sewage water (Table 1). In the present study, the Cd values for tap water and sewage water observed were 0.54 and 0.56 mg/L, respectively. These Cd values were higher than the permissible maximum limits of 0.010 mg/L as established by Chiroma *et al.* (2014). The wastewater of city, region and most of the small-scale industries situated in the range, release their wastes and dust particles. Also, some domestic waste products are released into the water channel due to which high Cd values in the water samples were detected (Salawu *et al.*, 2015).

**Table 1: Cadmium concentration in water (mg/L)**

Tap water	Sewage water	Mean square
0.54±0.015	0.56±0.016	0.001**
Degree of freedom 1		Error 9
Maximum permissible limit <sup>a</sup>	0.010 mg/L	

\*\* : Significant at 0.01 level, Source: <sup>a</sup>Chiroma *et al.* (2014)

Different studies conducted in Europe showed that various household products such as cleaners, fabric conditioners, washing powders, hair conditioners and shampoos, etc. are important reasons of Cd pollution in the sewage waters. Among them, the high Cd concentrations found in the washing powders can be explained by the differences in the composition of phosphate ores used in their production. Reducing the amount of phosphate in washing powders could lead to a reduction in Cd in sewage waters. The concentrated washing powders have lower potentially toxic substances contents than the traditional powders and are designed to be used in smaller quantities.

The values obtained in the present work were lower (0.54-0.56 mg/L) than the values reported by Tariq *et al.* (2006) and Murtaza *et al.* (2010). Salawu *et al.* (2015) calculated the Cd value (0.037 mg/L) for sewage water and this value was higher compared to the present Cd value. The present Cd values in tap water were lower to the values found by Kumar & Chopra (2015) who found

0.36-2.75 mg/L of Cd in borewell and industrial water. Since precautionary measures for heavy metals in food crops, water and soil have not been determined in Pakistan now (Hassan *et al.*, 2013). So, no reliable information is available on risk levels. Hence, wastewater is being used extensively for irrigation purposes.

### Cadmium in soil

According to the plant sown in the soil samples all treatments showed significant ( $p < 0.05$ ) effects on Cd concentration according to analysis of variance for *Z. mays*, *P. typhoedium*, *E. colona*, *S. bicolor*, *S. vulgare*, *B. campestris*, *B. napus*, *B. juncea*, *T. alexandrim* while non-significant effect was observed for *C. tetragonoloba*, *S. rostrata*, *T. resupinatum*, *M. sativa*. As a result of tap water irrigation, order of concentration of Cd was: *B. campestris* > *B. napus* > *P. typhoedium* > *T. alexandrinum* > *B. juncea* > *S. vulgare* > *E. colona* > *S. bicolor* > *Z. mays* > *S. rostrata* > *T. resupinatum* > *M. sativa* > *C. tetragonoloba*. As a result of sewage water irrigation, order of concentration of Cd was: *B. campestris* > *B. napus* > *P. typhoedium* > *S. vulgare* > *S. bicolor* > *T. alexandrinum* > *Z. mays* > *B. juncea* > *E. colona* > *S. rostrata* > *C. tetragonoloba* > *M. sativa* > *T. resupinatum*. The maximum concentration of Cd in the soil sample was 1.71 mg/kg observed in *B. campestris* grown in winter and the lowest mean concentration was 0.092 mg/kg observed in *C. tetragonoloba* (Table 2, Figure 4). The present Cd value for soil was lower than the maximum permissible limit for Cd soil 3 mg/kg reported by USEPA (1997). In soil, cadmium mobility under different conditions like cationic exchange capacity, organic carbon and pH range observed. As compared to the other studies, the present soil Cd values were greater than the values found by Mido & Satake (2003) who found soil Cd to be 0.065 mg/kg in samples from the areas irrigated with recycled and non-recycled water. This was due to the less adsorption properties in the soil. Kumar & Chopra (2015) reported range of (0.66-0.84 mg/kg) Cd soil in agricultural crops of *T. aestivum*, *B. juncea* and *H. vulgare* irrigated with effluent water and the present range (0.092-1.715 mg/kg) was higher. Khaskhoussy *et al.* (2013) reported higher range (5.30-17.0 mg/kg) for Cd soil irrigated with fresh water and treated wastewater. The differences between the values of the studies might

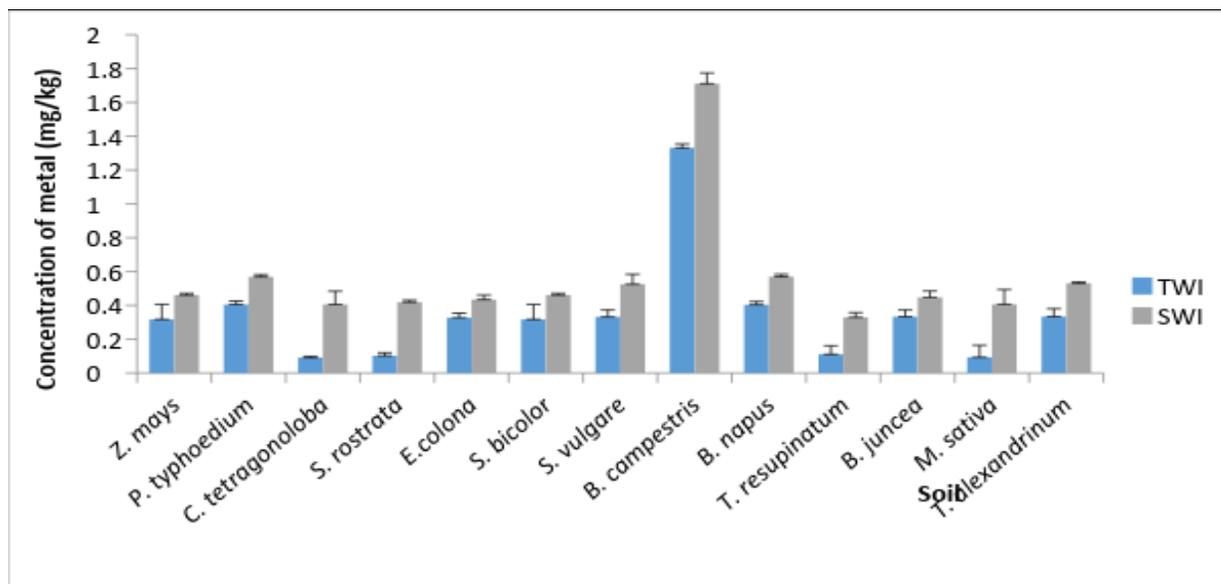
be due to various factors like soil pH, amount of organic matter, redox potential of soil and rate of addition of metals mainly affect their adsorption and retention in soil (Khan *et al.*, 2015).

**Table 2: Cadmium concentration in soil grown with different forages**

Forage	Irrigation water (mg/kg)		Mean square
	Tap	Sewage	
<b>Summer</b>			
<i>Z. mays</i>	0.320±0.0088	0.46±0.0095	0.052*
<i>P. typhoedium</i>	0.40±0.0141	0.56±0.0208	0.066*
<i>C. tetragonoloba</i>	0.092±0.0070	0.41±0.0078	0.246 <sup>ns</sup>
<i>S. rostrata</i>	0.102±0.0017	0.42±0.0123	0.250 <sup>ns</sup>
<i>E. colona</i>	0.33±0.0026	0.43±0.0026	0.029*

<i>S. bicolor</i>	0.321±0.0088	0.462±0.0095	0.052*
<i>S. vulgare</i>	0.332±0.0041	0.54±0.0058	0.093*
<b>Winter</b>			
<i>B. campestris</i>	1.33±0.0023	1.71±0.0632	0.360*
<i>B. napus</i>	0.41±0.0145	0.57±0.0192	0.069*
<i>T. resupinatum</i>	0.11±0.0050	0.330±0.0027	0.120 <sup>ns</sup>
<i>B. juncea</i>	0.34±0.0040	0.44±0.0037	0.032*
<i>M. sativa</i>	0.094±0.0070	0.40±0.0087	0.244 <sup>ns</sup>
<i>T. alexandrinum</i>	0.335±0.0046	0.53±0.0070	0.097*
<b>Degree of freedom</b>	1	<b>Error</b>	9
<b>Maximum permissible limit<sup>a</sup></b>	3mg/kg		

\*: Significant at 0.05 level, ns: non-significant, Source: <sup>a</sup>USEPA (1997)



**Fig. 4:** Fluctuation of cadmium in soil grown with different forages

### Cadmium in roots

All treatments showed significant ( $p < 0.05$ ) effect on the Cd concentration according to analysis of variance for *Z. mays*, *P. typhoedium*, *C. tetragonoloba*, *S. rostrata*, *E. colona*, *S. bicolor*, *S. vulgare*, *B. napus*, *T. resupinatum*, *B. juncea*, *M. sativa*, *T. alexandrinum* while non-significant effect was observed in *B. campestris* and as a result of tap water irrigation, order of Cd concentration was observed as: *T. alexandrinum* > *B. juncea* > *T. resupinatum* > *B. napus* > *B. campestris* > *S. bicolor* > *P. typhoedium* > *S. vulgare* > *E. colona* > *S. rostrata* > *Z.*

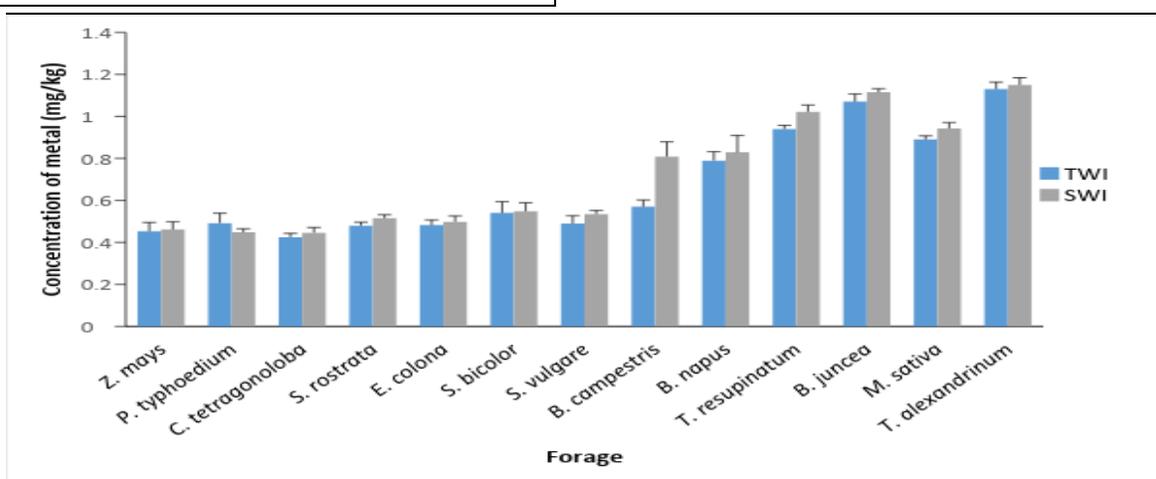
*mays* > *M. sativa* > *C. tetragonoloba*. As a result of sewage water irrigation, order of Cd concentration was observed as: *T. alexandrinum* > *B. juncea* > *T. resupinatum* > *M. sativa* > *B. napus* > *B. campestris* > *S. bicolor* > *S. vulgare* > *S. rostrata* > *E. colona* > *Z. mays* > *P. typhoedium* > *C. tetragonoloba*. The maximum mean concentration of Cd in the root was 1.15 mg/kg provided by *T. alexandrinum* grown in winter and the lowest mean concentration was 0.91 mg/kg provided by *C. tetragonoloba* grown in summer (Table 3, Figure 5).

**Table 3: Cadmium concentration in roots of forage samples irrigated with tap and sewage water**

Forage	Irrigation water (mg/kg)		Mean square
	Tap	Sewage	
<b>Summer</b>			
<i>Z. mays</i>	0.453±0.042	0.461±0.037	0.001**
<i>P. typhoedium</i>	0.492±0.047	0.448±0.017	0.005*
<i>C. tetragonoloba</i>	0.425±0.018	0.446±0.025	0.001**
<i>S. rostrata</i>	0.480±0.016	0.515±0.017	0.003*
<i>E. colona</i>	0.482±0.025	0.498±0.028	0.001**
<i>S. bicolor</i>	0.54±0.054	0.548±0.041	0.001**
<i>S. vulgare</i>	0.49±0.037	0.535±0.017	0.004**

<b>Winter</b>			
<i>B. campestris</i>	0.57±0.032	0.809±0.07	0.147 <sup>ns</sup>
<i>B. napus</i>	0.79±0.042	0.829±0.08	0.003**
<i>T. resupinatum</i>	0.94±0.017	1.022±0.032	0.019*
<i>B. juncea</i>	1.07±0.037	1.115±0.017	0.004**
<i>M. sativa</i>	0.89±0.017	0.943±0.028	0.006**
<i>T. alexandrinum</i>	1.13±0.033	1.15±0.034	0.001**
<b>Degree of freedom</b>	1	<b>Error</b>	9

\*, \*\*: Significant at 0.05 and 0.01 levels, ns: non-significant

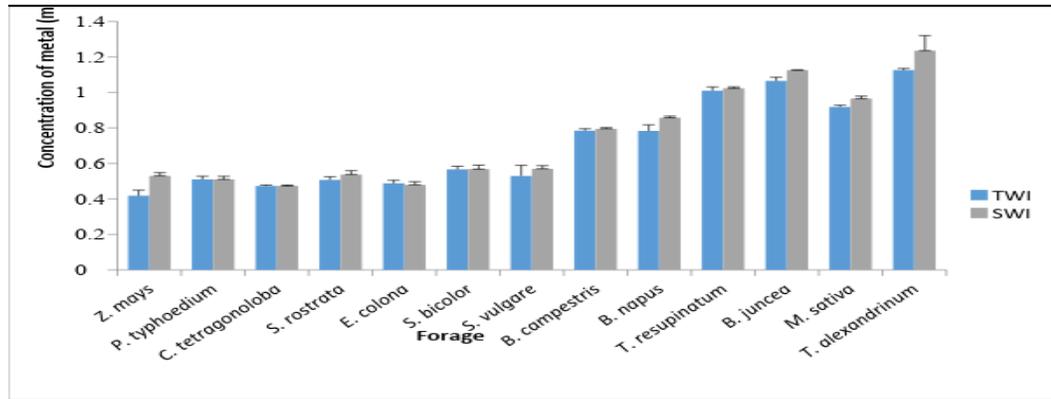
**Fig. 5: Fluctuation of cadmium in roots of forages**

Khaskhoussy *et al.* (2013) reported Cd in root irrigated with fresh water and treated waste water as 1.0 and 1.8 mg/kg, respectively. These values were higher as compared to the present calculated range (0.91-1.15 mg/kg). Asdeo (2014) also investigated higher Cd range (1.39-3.24 mg/kg) in roots of millet. Jarvis *et al.* (1976) observed that the absorbed Cd was excessively transferred from soil to roots to shoots in lettuce than other crops (ryegrass etc.). This greater movement of Cd from roots to shoots caused by active translocation or insufficient metal absorption to be fixed as well as soluble chelators in the plant roots or may be exchanged with those elements moving through the roots. There are various factors that affect the bioavailability of elements to plants like 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.

#### Cadmium in leaves

All treatments showed significant ( $p < 0.05$ ) effect on the Cd concentration in all forage samples. As a result of tap water irrigation, the order of concentration was: *T. alexandrinum* > *B. juncea* > *T. resupinatum* > *M. sativa* > *B. campestris* > *B. napus* > *S. bicolor* > *S. vulgare* > *P. typhoedium* > *S. rostrata* > *E. colona* > *C. tetragonoloba* > *Z. mays*. As a result of sewage water irrigation, the order of concentration was: *T. alexandrinum* > *B. juncea* > *T. resupinatum* > *M. sativa* > *B. napus* > *B. campestris* > *S. vulgare* > *S. bicolor* > *S. rostrata* > *Z. mays* > *P. typhoedium* > *E. colona* > *C. tetragonoloba* (Table 4, Figure 6).



**Fig. 6:** Fluctuation of cadmium in leaves of forages

The maximum Cd concentration in the leaves was 1.24 mg/kg observed in *T. alexandrinum* grown in winter season and the lowest mean concentration was 0.417 mg/kg observed in *Z. mays* grown in summer season. The calculated value for Cd in leaves was higher than maximum permissible limit 0.5 mg/kg by WHO (1996). So, it can be poisonous. As Cd has greater poisonous ability so, it is unnecessary element of water and food (Oluwole *et al.*, 2013). On the other hand, the shoot Cd concentrations in shoot samples of various forages in present study were lower as compared to the optimum level (5.0 mg/kg) for foraging livestock proposed by Anonymous (1980). Kumar & Chopra (2015) reported higher Cd range (3.54-5.09 mg/kg) in *B. juncea*, *T. aestivum* and *H. vulgar* when irrigated with effluent of water and Asdeo (2014) also reported higher Cd range (0.98-1.37 mg/kg) in millet leaves. As sewage water has greater concentration of Cd than tap water therefore, forages which are irrigated with sewage water would have greater Cd concentration. Due to higher Cd concentration of sewage water compared to that in tap water, it is expected that forages irrigated with sewage water would have greater Cd concentration. Ahmad *et al.* (2018b) reported that Cd translocation was greatly influenced by soil pH. However, Cd accumulation may be due to the characteristics and behavior of a plant species in determining the amounts of heavy metals that were taken up from soils. Such diversity may reflect different rates of absorption and transport from root to shoot.

**Table 4: Cadmium concentration in leaves of different forages**

Forage	Irrigation water (mg/kg)		Mean square
	Tap	Sewage	
<b>Summer</b>			
<i>Z. mays</i>	0.418±0.018	0.530±0.0032	0.032*
<i>P. typhoedum</i>	0.510±0.0017	0.512±0.0016	0.001**
<i>C. tetragonoloba</i>	0.470±0.0016	0.473±0.0018	0.001**
<i>S. rostrata</i>	0.507±0.0016	0.531±0.0017	0.001**
<i>E. colona</i>	0.488±0.0018	0.479±0.0049	0.001**
<i>S. bicolor</i>	0.570±0.00120	0.568±0.0017	0.001**
<i>S. vulgare</i>	0.530±0.0016	0.570±0.0017	0.004**
<b>Winter</b>			
<i>B. campestris</i>	0.784±0.0064	0.796±0.0093	0.001**
<i>B. napus</i>	0.783±0.0017	0.86±0.0028	0.014*
<i>T. resupinatum</i>	1.010±0.0016	1.022±0.0063	0.001**
<i>B. juncea</i>	1.064±0.0040	1.13±0.0108	0.009*
<i>M. sativa</i>	0.910±0.0070	0.96±0.0017	0.005**
<i>T. alexandrinum</i>	1.120±0.0077	1.25±0.0092	0.030*
<b>Degree of freedom</b>	1	<b>Error</b>	9
<b>Permissible maximum limit</b>		0.5 mg/kg	

\*, \*\*: Significant at 0.05 and 0.01 level, Source: WHO (1996)

### Bioconcentration factor

The order of BCF for Cd in plant samples irrigated with the tap water was: *T. resupinatum*>*B. juncea*>*M. sativa*>*T. alexandrinum*>*B. napus*>*E. colona*>*S. bicolor*>*S. rostrata*>*C. tetragonoloba*>*S. vulgare*>*Z. mays*>*P. typhoedium*>*B. campestris*. The order of BCF for Cd in plant samples irrigated with sewage water was: *M. sativa*>*T. resupinatum*>*S. rostrata*>*C. tetragonoloba*>*T. alexandrinum*>*B. juncea*>*B. napus*>*S. bicolor*>*Z. mays*>*S. vulgare*>*P. typhoedium*>*E. colona*>*B. campestris* (Table 5). The maximum observed value for Cd BCF in *M. sativa* was (9.77) grown in winter and the lowest observed value in *B. campestris* (0.465) also grown in winter. Victor (2014) found Cd BCF range of 0.20-0.60 in maize leaf and these values were higher than present range. Alrawiq *et al.* (2014) calculated higher BCF range for Cd (0.461-0.358) after irrigation with recycled and non-recycled water. When the BCF is >1, this shows that plant stores metals. Asdeo (2014) investigated lower BCF value (0.1890) as compared to the present study. The pollutants of heavy metals and physiological characteristics of plants are responsible for changes in BCF values.

**Table 5: Bioconcentration factor of cadmium in forages**

Forage	Bioconcentration factor	
	Tap	Sewage
<b>Summer</b>		
<i>Z. mays</i>	0.904	1.669
<i>P. typhoedium</i>	0.897	1.257
<i>C. tetragonoloba</i>	1.165	5.135
<i>S. rostrata</i>	1.211	5.170
<i>E. colona</i>	1.488	1.133
<i>S. bicolor</i>	1.229	1.782
<i>S. vulgare</i>	1.084	1.593
<b>Winter</b>		
<i>B. campestris</i>	0.465	0.589
<i>B. napus</i>	1.503	1.936
<i>T. resupinatum</i>	3.060	9.211
<i>B. juncea</i>	2.513	3.190
<i>M. sativa</i>	2.373	9.77
<i>T. alexandrinum</i>	2.325	3.363

### Pollution load index

The sequence for soil PLI according to plant samples irrigated with tap water was: *B. campestris*>*P. typhoedium*>*B. napus*>*S. vulgare*>*B. juncea*>*T. alexandrinum*>*E. colona*>*S. bicolor*>*Z. mays*>*T. resupinatum*>*S. rostrata*>*C. tetragonoloba*>*M. sativa*. The sequence for soil PLI in plants irrigated with sewage water was: *B. campestris*>*B. napus*>*P. typhoedium*>*T. alexandrinum*>*S. vulgare*>*S. bicolor*>*Z. mays*>*B. juncea*>*E. colona*>*S. rostrata*>*M. sativa*>*C. tetragonoloba*>*T. resupinatum* (Table 6). The results showed minimum PLI value in *M. sativa* (0.0630) and the maximum in *B. campestris* (1.1479) both grown in winter season. Khan *et al.* (2015) found lower range (0.131-0.467) for Cd PLI in soil. Bao *et al.* (2013) found similar PLI for Cd in soil (1.12, 1.29, 1.15) in three different zones irrigated with long term sewage water. Ahmad *et al.* (2014) found higher Cd PLI value (2.179) in soil irrigated with canal and sewage water. Pollution load index greater than 1 indicate that the area was contaminated while if the PLI is found less than 1 it shows that area of sampling was very much polluted. The lower PLI suggests that there was less rush of industries in Sargodha.

**Table 6: Pollution load index for cadmium in soil**

Forage	Pollution load index	
	Tap	Sewage
<b>Summer</b>		
<i>Z. mays</i>	0.203	0.308
<i>P. typhoedium</i>	0.272	0.381
<i>C. tetragonoloba</i>	0.061	0.272
<i>S. rostrata</i>	0.068	0.281
<i>E. colona</i>	0.219	0.291
<i>S. bicolor</i>	0.213	0.309
<i>S. vulgare</i>	0.233	0.352
<b>Winter</b>		
<i>B. campestris</i>	0.893	1.147
<i>B. napus</i>	0.271	0.382
<i>T. resupinatum</i>	0.074	0.221
<i>B. juncea</i>	0.223	0.300
<i>M. sativa</i>	0.063	0.273
<i>T. alexandrinum</i>	0.224	0.356

### Daily intake of metals and health risk index

Daily intake of metal was determined to assess the heavy metals in forages both for tap and sewage water. Daily intake of metal values in plant samples calculated for tap water irrigation was in the order of: *T. alexandrinum*>*B. juncea*>*T. resupinatum*>*M. sativa*>*B. napus*>*B. campestris*>*S. bicolor*>*S. vulgare*>*S. rostrata*>*E. colona*>*C. tetragonoloba*>*P. typhoedium*>*Z. mays*. Daily intake of metal for sewage water irrigation was in the order of: *T. alexandrinum*>*B. juncea*>*T. resupinatum*>*M. sativa*>*B. napus*>*B. campestris*>*S. vulgare*>*S. bicolor*>*S. rostrata*>*P. typhoedium*>*E. colona*>*C. tetragonoloba*>*Z. mays* (Table 7).

The maximum value for DIM observed in *T. alexandrinum* was 0.028 mg/kg/day and minimum observed DIM in *P. typhoedium* was 0.00948 mg/kg/day. Health risk index for Cd was calculated. The minimum value in tap water for HRI was observed in *B. juncea* (20.18) and in sewage water the maximum observed value in *T. alexandrinum* was 25.068 both grown in winter. Roggeman *et al.* (2013) found higher mean DIM value of 24-31 mg/kg/day in winter and summer value was 24-18 mg/kg/day in herds of cows. Lawalet *et al.* (2017) found lower DIM Cd value in spinach leaves grown around the Kubanni River in two farmlands. In the current results the values of DIM were lower than 1 and it suggests that no risk of health is associated with the consumption of such contaminated forages (Radwan & Salama, 2006). Lawal *et al.* (2017) found lower HRI Cd value (0.110-0.058) in spinach leaves grown around river Kubanni in two farmlands. The current value for Cd HRI was higher than the findings of Khan *et al.* (2015) (8.744-10.46) in wastewater irrigated sites. According to Sajjad *et al.* (2009) if the HRI is found greater than 1, it means a serious health risk is associated with the consumption of contaminated forages.

**Table 7: Daily intake of metal (mg/kg/day) and health risk index of cadmium in forages**

Forage	Daily intake of metal		Health risk index	
	Tap	Sewage	Tap	Sewage
<b>Summer</b>				
<i>Z. mays</i>	0.0095	0.0120	9.489	12.045
<i>P. typhoedium</i>	0.0094	0.0115	10.590	11.59

<i>C. tetragonoloba</i>	0.0107	0.0127	10.738	10.84
<i>S. rostrata</i>	0.011	0.0130	11.045	12.53
<i>E. colona</i>	0.010	0.0118	11.079	10.89
<i>S. bicolor</i>	0.013	0.0138	12.863	12.89
<i>S. vulgare</i>	0.0120	0.0129	10.045	12.95
<b>Winter</b>				
<i>B. campestris</i>	0.0170	0.0180	17.829	18.079
<i>B. napus</i>	0.0177	0.0194	17.784	19.477
<i>T. resupinatum</i>	0.0229	0.0232	19.238	23.954
<i>B. juncea</i>	0.0242	0.0255	20.181	23.568
<i>M. sativa</i>	0.0208	0.0219	19.875	21.931
<i>T. alexandrinum</i>	0.0255	0.0280	12.568	25.068

### CONCLUSION

Irrigation with polluted water may contaminate readily the soil and cultivated land. Cadmium is poisonous metal. In the experiment the soil, root and forage samples irrigated with sewage water showed a greater amount of metal. The accumulation of heavy metals from soil to plant varied according to treatment; however, it did not follow any particular pattern. Forage samples also showed higher amounts than the permissible maximum limit. So, consumption of such forages may be hazardous for animal and human health. Thus, wastewater must be treated properly to reduce the concentration of heavy metals in water, soil and forages to protect the animal health.

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### CONFLICTS OF INTEREST

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

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