



# Appraisal of Trace Element Accumulation and Human Health Risk from Consuming Field Mustard (*Brassica campestris* Linn.) Grown on Soil Irrigated with Wastewater

Ilker Ugulu<sup>\*1</sup>, Zafar Iqbal Khan<sup>2</sup>, Sidrah Rehman<sup>2</sup>, Kafeel Ahmad<sup>2</sup>, Mudasra Munir<sup>2</sup>, Humayun Bashir<sup>2</sup> and Khalid Nawaz<sup>2</sup>

<sup>1</sup>Faculty of Education, Usak University, Usak, Turkey.

<sup>2</sup>Department of Botany, University of Sargodha, Sargodha, Pakistan.

\*Corresponding Author Email: [ilkerugulu@gmail.com](mailto:ilkerugulu@gmail.com)

Received 25 March 2019, Revised 02 September 2019, Accepted 10 November 2019

---

## Abstract

The aim of the present research was to determine the trace metal accumulations in *Brassica campestris* irrigated with three different water regimes (groundwater, canal water, and sugar mill water). The analysis was conducted by Atomic Absorption Spectrophotometer to evaluate the concentration of minerals in the soil and in vegetables. The heavy metals investigated in this study were Cd, Cu, Cr, Fe, Zn, Ni, and Mn. Trace metal concentrations in soil samples ranged from 0.30 to 0.39, 0.14 to 0.30, 0.25 to 0.39, 1.47 to 3.98, 0.37 to 0.41, 0.8 to 8.78 and 0.36 to 0.36 mg/kg for Cd, Cr, Cu, Fe, Ni, Zn and Mn, respectively. Among the three treatments, the mean concentrations of Fe and Zn were higher than other metal accumulations for all treatments. The contents of Cd, Cr, Cu, Fe, Ni, Zn and Mn in *Brassica campestris* ranged from 0.35 to 0.44, 0.32 to 0.89, 0.09 to 0.73, 1.93 to 3.02, 1.11 to 1.82, 0.36 to 0.43 and 0.37 to 0.40 mg/kg, respectively. Statistical analyses showed that the treatments have a non-significant effect ( $p > 0.05$ ) on concentrations of metals in *Brassica campestris* collected from three sites for Cd, Cr, Cu and Mn and significant effect on Fe, Zn, and Ni.

**Keywords:** Heavy metals, Vegetable, Wastewater, Health risk, Biomonitoring.

---

## Introduction

In many arid and semi-arid zones, freshwater is used for different purposes other than irrigations that's why competition exists, causing a reduced distribution of freshwater to cultivation [1]. Due to this reason, higher demand from other consumers and decreasing sources of water quality for irrigation are compelling growers to use wastewater [2]. The scarcity of freshwater is being replenished by the conjunctive use of municipal wastewater (industrial effluents and sewage) for developing vegetables particularly in regions around cities and groundwater for growth of cereals and food crops [3].

Consumption of food crops grown in soils irrigated with contaminated water having a high

amount of heavy metals can exert a direct impact on human health [4-6]. Unnecessary deposition of heavy metals in soil due to wastewater irrigation not only cause soil pollution but also disturb the safety and nutritional value of food [7]. Uptake of heavy metals occurs in vegetables and accumulate in their different eatable and inedible parts; the quantity of these toxic compounds is so high that it causes health problems in both human beings and animals that eat them [8-9].

Vegetables play an important role in our food chain. Vegetables are not only a valuable source of nutrients but also provides vital food components such as vitamins, proteins, carbohydrates and calcium which have a prominent

impact on human health [10-12]. Leafy vegetables, for example spinach, cultivated in polluted land store a considerable amount of heavy metals than that grown in unpolluted soil reason is that they captivate heavy metals with the help of their roots [13]. Both eatable and nonedible parts of vegetables store heavy metals [14-17]. Some vegetables have greater ability to accumulate a higher quantity of heavy metals than others [18-20]. The capability of vegetables to accumulate these toxic compounds mainly depends upon the type and nature of vegetable [21].

The present study was performed to (1) assess the health hazard effects caused by heavy metals in Brassica sp. samples from different irrigations, (2) determine the health risk index by leafy vegetables irrigated with sugar mill water and (3) evaluate accumulation and translocation of heavy metals in vegetables, soil, and water.

## Materials and Methods

### Study Site

The proposed study was conducted under field conditions in Khushab. Khushab is the district of province Punjab, Pakistan. The climate of Khushab is extremely hot in summer and moderate cold in winter. The maximum temperature in summer is about 50°C and the minimum temperature recorded in winter is 12°C.

### Plant Cultivation

Seeds of *Brassica campestris* were grown at the end of October 2016 in 60 small plastic pots. Four replicates, 15 pots were set in each replication about 2.5 kg of soil was put in each pot. Ten seeds of vegetable were sown in each plastic pot. Different amounts of groundwater, canal water, and sugar mill water were applied in experimental pots for irrigation purpose. Mill water was obtained from Kooch e Noor Sugar mill, Khushab. After maturation of vegetables four plants were left in each pot other were eradicated. Different concentrations of urea fertilizer were applied for better development of vegetables. Soil samples were taken out from each pot at the depth of 5 cm. Vegetable leaves were harvested at the end of April 2017. The vegetable samples were dried in an open environment and ground in a domestic grinder. Fine powder of vegetable samples was

kept in the oven for three days at 75°C temperature. After that samples were completely dried removed from the oven and digested by “wet digestion method”.

### Metal Analysis

The analysis was conducted by Atomic Absorption Spectrophotometer (Shimadzu model AA-6300) to evaluate the concentration of minerals in the soil and in vegetables. The heavy metals investigated in this study were Cd, Cu, Cr, Fe, Zn, Ni and Mn.

### Statistical Analysis

Correlations and variance of data were calculated by SPSS (Statistical Package for Social Sciences). Variance of vegetables and soil was obtained by applying one-way ANOVA. Correlations for vegetables and soil were calculated. Mean significance was at 0.05, 0.001 and 0.01 probability levels given by Steel and Torrie [22].

### Quality Control Analysis

To avoid any infectivity that may affect laboratory performance, appropriately clean equipment was used. Acid and other chemicals required for digestion and spectrophotometry were bought from a superior company to give surety for correctness and precision. Standards were prepared carefully. Measurement of soil and vegetable samples was done on the basis of dry weight. Analyses were performed three times for each sample.

### Bioconcentration Factor (BCF)

Bioconcentration factor was calculated as a ratio of the concentration of metal in plants (on a dry weight basis). It was used to evaluate the uptake of heavy metals from soil and their bioaccumulation in vegetable using the following formula:

$$\text{Bioconcentration factor} = C_{\text{veg}} / C_{\text{soil}}$$

Where  $C_{\text{veg}}$  is the metal concentrations in plant tissue, mg/kg fresh weight and  $C_{\text{soil}}$  is the metal concentrations in soil, mg/kg dry weight [23].

### Daily Intake of Metals (DIM)

For finding out consumer-based health risks certain methods are taken into consideration. One of them is the daily intake of metals.

$$\text{Daily intake of metals} = C_{\text{metal}} \times C_{\text{food intake}} / B_{\text{average weight}}$$

Where  $C_{\text{metal}}$  represents the concentration of metals in grains,  $C_{\text{food intake}}$  represents the daily food intake and  $B_{\text{average weight}}$  represents average body weight. The daily food intake was taken as 0.345 mg/kg and the average body weight is taken as 60 kg.

### Health Risk Index (HRI)

It was measured to find the overall risk of exposure to all heavy metals via ingestion of particular food crops. This shows the maltreatment to people who consume contaminated foodstuff. It is defined as a ratio of daily intake of metals in food crops to the oral reference dose [24].

$$\text{HRI} = \text{Daily intake of metals} / \text{Oral reference dose}$$

An HRI <1 for any metal in vegetable means that consumer population faces a serious risk of health. However, HRI >1 does indicate a considerable health risk to the organisms consuming these vegetables.

### Pollution Load Index (PLI)

Based on the concentration factor of each metal in the soil, PLI gave us an estimation to the metal contamination status and the necessary action that should be taken. PLI was estimated as given by Liu *et al.* [25] for each treatment following equation:

$$\text{PLI} = \text{Determined level of metal in examined soil} / \text{Reference value of soil metal.}$$

## Results and Discussion

### Trace Metal Accumulation in Soil Samples

In the present study, trace metal concentrations in soil samples ranged from 0.31 to

0.42, 0.14 to 0.30, 0.25 to 0.39, 1.47 to 3.98, 0.37 to 0.41, 0.8 to 8.78 and 0.36 to 0.36 mg/kg for Cd, Cr, Cu, Fe, Ni, Zn and Mn, respectively. Among the three treatments, the mean concentrations of Fe and Zn were higher than other metal accumulations for all treatments (Fig. 1). At treatment -I (GWI), the mean concentrations of metals in soil were in the order: Fe > Zn > Ni > Mn > Cd > Cr > Cu. At treatment II (CWI), the mean concentrations of metals in soil were in the order: Zn > Fe > Cd > Cu > Ni > Mn > Cr. At treatment III (SWI), the mean concentrations of metals in soil was in the order: Zn > Fe > Ni > Cu > Mn > Cd > Cr (Fig. 1).

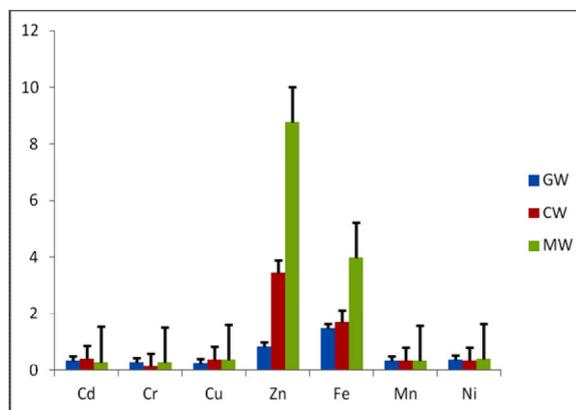


Figure 1. Fluctuation in heavy metals in soil *Brassica campestris* (mg/kg)

The results from the variance of the data showed that the treatments have a non-significant effect ( $p > 0.05$ ) on concentrations of metals in soil which was used to grow vegetables for Mn, Cu, Ni and Zn while significant effect on Cd, Fe and Cr in the soil of *Brassica campestris* (Table 1).

Maximum permissible limits of the Cd, Cr, Cu, Fe, Ni, Zn and Mn accumulation in soil were reported as 3, 100, 50, 21000, 50, 200 and 2000 mg/kg, respectively by USEPA [26]. All metal concentrations studied in the present research remained below these limits under all treatment conditions. Constant uptake of trace metals by crops and infiltration into deeper soil layers could be possible explanations for the relatively low levels of these metals in the soils even at wastewater-irrigated sites as the geological composition of soils affect metal concentrations in agricultural lands [27].

Table 1. Analysis of variance for heavy metals in the soil of *Brassica campestris*.

Source of Variation SOV	Degree of freedom df	Mean Squares						
		Cd	Cr	Cu	Fe	Ni	Zn	Mn
Treatments	4	.012*	.031*	.025 <sup>ns</sup>	7.693**	.002 <sup>ns</sup>	65.673 <sup>ns</sup>	.001 <sup>ns</sup>
Error	10	.002	.005	.022	.544	.001	39.256	.000

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

Haq *et al.* [28] reported that the mean values of Zn, Cu, Fe and Mn in soil irrigated with effluent were 2.78, 13.95, 14.32 and 9.58 mg/kg, respectively and these values are greater than the values presented in this study except for Zn. Also, Manzoor *et al.* [29] indicated that the level of Cr, Fe, Cd, Ni and Zn in soils which was irrigated with industry effluents were 0.370, 1.082, 0.017, 0.180 and 0.055 mg/kg, respectively in which values of Fe and Zn were less than the present study.

#### Trace Metal Accumulation in Vegetable Samples

Heavy metal accumulations in *Brassica campestris* samples gathered from where soil samples were taken are as follows: The contents of Cd, Cr, Cu, Fe, Ni, Zn, and Mn ranged from 0.35 to 0.44, 0.32 to 0.89, 0.09 to 0.73, 1.93 to 3.02, 1.11 to 1.82, 0.36 to 0.43 and 0.37 to 0.40 mg/kg, respectively. In treatment I (GWI), the mean concentrations of metals in *Brassica* were in the order: Zn>Fe>Ni>Mn>Cd>Cr>Cu. In treatment II (CWI), the mean concentrations of metals were in the order: Fe>Zn>Cd>Ni>Mn>Cu>Cr.

In treatment III (MWI), the mean concentrations of metals were in the order: Zn>Fe>Cr>Cu>Ni>Mn>Cd (Fig. 2). Among the three treatments, the mean concentrations of Fe and Zn were higher than the other metals and mean concentrations of Cr and Cu were less (Fig. 2). The results from the variance of the data showed that the treatments have a non-significant effect ( $p>0.05$ ) on

concentrations of metals in *Brassica campestris* samples collected from three sites for Cd, Cr, Cu and Mn and significant effect on Fe, Zn and Ni (Table 2).

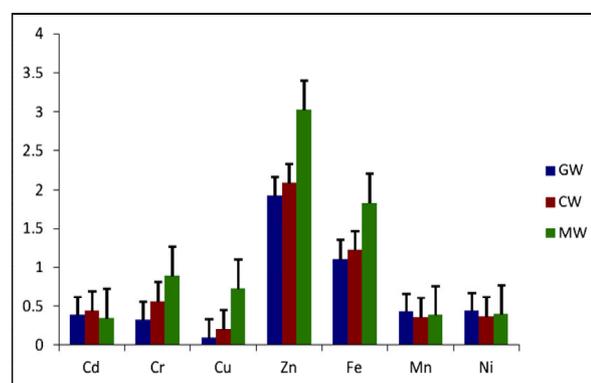


Figure 2. Fluctuation in heavy metals *Brassica campestris* (mg/kg)

Maximum permissible limits of the Cd, Cr, Cu, Fe, Ni, Zn and Mn accumulation in plants were reported as 0.1, 5, 73, 425, 67, 100 and 500 mg/kg, respectively by WHO, FAO/WHO, Standard Guidelines in Europe [30]. The range values of metal accumulation in *Brassica campestris* samples in the present study were lower than the maximum permissible limits in plant samples except for Cd. The Cr, Ni and Cd values in the present study were lesser than the values reported by Parveen *et al.* [31] as 0.8, 0.4 and 0.4 mg/kg, respectively. This result may be due to their vegetables were cultivated in sewage water. According to the results presented by Latif *et al.* [32], all vegetables which were grown in sewage water irrigated areas showed high concentrations of Cr, Cd and Ni.

Table 2. Analysis of variance for heavy metals in *Brassica campestris*.

Source of Variation SOV	Degree of freedom df	Mean Squares						
		Cd	Cr	Cu	Fe	Ni	Zn	Mn
Treatments	4	.008ns	.595ns	.468ns	259.367*	.006*	10.604***	.005ns
Error	10	.007	.530	.301	46.324	.001	1.064	.004

\*,\*\*\* significant at 0.05 and 0.001 levels; ns, non-significant

### Bioconcentration Factor of Trace Metals

In treatment I (GWI), transfer factor for Mn and Zn was higher than Fe and Cu. In treatment II (CWI), transfer factor for Cd and Fe was greater as compared to Zn and Cu. In treatment III (MWI), transfer factor for Cr and Cd was higher than Fe and Cu. Analysis results of a range of metals in three treatments Cu revealed minimum value (Table 3). The order of BCF values for treatment I: Zn>Mn>Ni>Cr>Cd>Fe>Cu, treatment II: Fe>Cd>Cr>Ni>Mn>Zn>Cu and treatment III: Cr>Cd>Mn>Ni>Zn>Fe>Cu.

Several agents of climatic and edaphic origin, as well as plant age, development stage, and life-cycle phase, might influence rates of metal uptake [1]. In the present study, the BCF values for Cr and Cd were the highest for Mill water irrigated leafy vegetables. Mahakalkar *et al.* [33] determined the transfer factor values were in the order as Zn>Fe>Cu>Ni>Mn. This result does not coincide with the findings of this study.

### Daily Intake of Metal (DIM)

Daily intake of metals for Fe and Zn was higher and Cr was the lowest value in all treatments. The order of DIM values for treatment I was: Zn>Fe>Ni>Mn>Cd>Cr>Cu. The order for treatment II was: Fe>Zn>Cd>Ni>Mn>Cu>Cr. The order of DIM for treatment III was: Zn>Fe>Cu>Ni>Mn>Cd>Cr (Table 4). DIM values for all metals were below the permissible limit given by WHO [30]. DIM value for Fe was (0.006-0.012) lesser than 0.329 mg/kg per day, given by Santos *et al.* [34].

### Pollution Load Index (PLI)

The pollution load index (PLI) in mint grown with three diverse irrigations was in the following arrangement. Order of PLI in treatment I (GWI) was Cd>Ni>Cr>Cu>Fe>Zn>Mn, in treatment-II (CWI) was Cd>Zn>Cu>Ni>Fe>Cr>Mn and in treatment-III (MWI) was Cd>Zn>Fe>Cu>Ni>Cr>Mn (Table 5). The maximum PLI was observed for Cd and the minimum PLI was observed for Mn at all treatments. Harikumar *et al.* [35] suggested that if the value of PLI is greater than 1 then food is contaminated if less than 1 then it is not contaminated. In the present study, PLI values for all metals were lesser than 1, it means that these vegetables can be consumed.

### Health Risk Index (HRI)

According to the analysis results, the health risk index of Cd was more than 1 in each treatment which was above the permissible limit. At treatment-I (GWI), the control order for HRI of the metals at treatment-I (GWI) and treatment-II were: Cd>Ni>Mn>Zn>Cu>Fe>Cr. The order for health risk index of the metals at treatment -III was: Cd>Ni>Cu>Zn>Mn>Fe>Cr (Table 6). Cd value was more than 1, consumers of such vegetables in which HRI of metal was greater than 1 will be at risk [16]. HRI values for Cd (2.04-2.54) and Cr (0.001- 0.0006) in the study of Khan *et al.* [20] were higher than the values presented in this study.

Table 3. Bioconcentration factor for *Brassica campestris*.

Irrigation	Heavy Metal						
	Cd	Cr	Cu	Fe	Ni	Zn	Mn
I	1.083	1.086	0.376	0.752	1.162	2.294	1.187
II	1.047	1.068	0.512	1.311	1.003	0.60	0.99
III	1.133	3.036	0.385	0.459	0.975	0.549	1.057

Table 3. Bioconcentration factor for *Brassica campestris*.

Irrigation	Heavy Metal						
	<i>Cd</i>	<i>Cr</i>	<i>Cu</i>	<i>Fe</i>	<i>Ni</i>	<i>Zn</i>	<i>Mn</i>
I	1.083	1.086	0.376	0.752	1.162	2.294	1.187
II	1.047	1.068	0.512	1.311	1.003	0.60	0.99
III	1.133	3.036	0.385	0.459	0.975	0.549	1.057

Table 4. Daily intake of metal for *Brassica campestris*.

Irrigation	Heavy Metal						
	<i>Cd</i>	<i>Cr</i>	<i>Cu</i>	<i>Fe</i>	<i>Ni</i>	<i>Zn</i>	<i>Mn</i>
I	0.00223	0.00189	0.00056	0.00639	0.00256	0.01109	0.00248
II	0.00254	0.00090	0.00116	0.01276	0.00213	0.01199	0.00208
III	0.00204	0.00113	0.00422	0.01051	0.00232	0.02774	0.00223

Table 5. Pollution load index for *Brassica campestris*.

Irrigation	Heavy Metal						
	<i>Cd</i>	<i>Cr</i>	<i>Cu</i>	<i>Fe</i>	<i>Ni</i>	<i>Zn</i>	<i>Mn</i>
I	0.2407	0.0333	0.030	0.0259	0.0424	0.0190	0.0077
II	0.2835	0.0161	0.0470	0.0297	0.0409	0.0777	0.0078
III	0.21015	0.03245	0.04715	0.0699	0.0456	0.1988	0.0078

Table 6. Health risk index for *Brassica campestris*.

Irrigation	Heavy Metal						
	<i>Cd</i>	<i>Cr</i>	<i>Cu</i>	<i>Fe</i>	<i>Ni</i>	<i>Zn</i>	<i>Mn</i>
I	2.2353	0.00126	0.01401	0.0091	0.128	0.036	0.060
II	2.54437	0.00060	0.0291	0.01824	0.106	0.0399	0.050
III	2.04125	0.00342	0.1057	0.015	0.116	0.092	0.054

### Correlation

The results revealed a positive non-significant correlation of Cr, Cu, Fe, Ni and Zn and negative non-significant correlation of Mn (Table 7). Cd shows significant result while antagonistically in findings of Parveen *et al.* [31]. The positive relationship shows shared presence and advancement of the heavy metals in soil, although negative relationship demonstrates their competition to involve the same destinations in soil trade base or cross-section.

Table 7. Metal correlation between soil-vegetable of *Brassica campestris*.

Correlation	
Metal	Soil-vegetable
Cd	.999*
Cr	.646
Cu	.630
Fe	.244
Ni	.229
Zn	.962
Mn	-.675

\* Correlation is significant at the 0.05 level (2-tailed).

## Conclusion

Environmental contamination by heavy metals released from industrial effluent is one of the major challenging issues in many countries. Concentrations of metals in vegetables depend upon distance from pollution sources, and the quality of irrigation water. The range values of metal accumulation in plant samples in the present study were lower than the maximum permissible limits in plant samples except for Cd. Bioconcentration factor for Cr and Cd were the highest for Mill water irrigated leafy vegetables. The accumulation of metals on plants depends on different factors, including genetic specialties in addition to characteristics of metals and surfaces of vegetables, climatic factors, and intake from the soil. Sugar industry effluent contains minerals and toxic metals. So, it is necessary to give proper treatment to sugar industry wastewater before its application on agricultural land.

## References

- Z. I. Khan, I. Ugulu, S. Umar, K. Ahmad, N. Mehmood, A. Ashfaq, H. Bashir and M. Sohail, *Bull. Environ. Contam. Toxicol.*, 101 (2018) 235.  
<https://doi.org/10.1007/s00128-018-2353-1>
- K. Ahmad, K. Nawaz, Z. I. Khan, M. Nadeem, K. Wajid, et al., *Fresen. Environ. Bull.*, 27 (2018) 846.  
<file:///C:/Users/AYESHA%20ALI/Downloads/EFFECTOFDIVERSEREGIMESOFIRRIGATIONON.pdf>
- Z. I. Khan, I. Ugulu, S. Sahira, K. Ahmad, A. Ashfaq, N. Mehmood and Y. Dogan, *Int. J. Environ. Res.*, 12 (2018) 503.  
<https://doi.org/10.1007/s41742-018-0110-2>
- I. Ugulu, *Appl. Spectrosc. Rev.*, 50(2) (2015) 113.  
<https://doi.org/10.1080/05704928.2014.935981>
- I. Ugulu, M. C. Unver and Y. Dogan, *Oxid. Commun.*, 39 (2016) 765.  
<http://scibulcom.net/ocr.php?gd=2016&bk=1>
- M. C. Unver, I. Ugulu, N. Durkan, S. Baslar and Y. Dogan, *Ekoloji*, 24 (2015) 13.  
<https://doi.org/10.5053/ekoloji.2015.01>
- Y. Dogan, I. Ugulu and N. Durkan, *Pak. J. Bot.*, 45 (2013) 177.
- I. Ugulu, S. Baslar, *J. Alternative Compl. Med.*, 16 (2010) 313.  
<http://doi.org/10.1089=acm.2009.0040>
- M. Nadeem, T. M. Qureshi, I. Ugulu, M. N. Riaz, Q. U. An, Z. I. Khan, K. Ahmad, A. Ashfaq, H. Bashir and Y. Dogan, *Pak. J. Bot.*, 51 (2019) 171.  
[http://doi.org/10.30848/PJB2019-1\(14\)](http://doi.org/10.30848/PJB2019-1(14))
- Y. Dogan, I. Ugulu and S. Baslar, *Ekoloji*, 19 (2010) 88.  
<http://doi.org/10.5053/ekoloji.2010.7512>
- N. Durkan, I. Ugulu, M. C. Unver, Y. Dogan and S. Baslar, *Trace Elem. Electroly.*, 28 (2011) 242.  
<http://doi.org/10.5414/TEX01198>
- I. Ugulu, Y. Dogan, S. Baslar and O. Varol, *Int. J. Environ. Sci. Technol.*, 9 (2012) 527.  
<https://doi.org/10.1007/s13762-012-0056-4>
- Y. Dogan, S. Baslar and I. Ugulu, *Appl. Ecol. Environ. Res.*, 12 (2014) 627.  
[http://doi.org/10.15666/aeer/1203\\_627636](http://doi.org/10.15666/aeer/1203_627636)
- Y. Dogan, M.C. Unver, I. Ugulu, M. Calis and N. Durkan, *Biotech. Biotechnol. Equip.* 28 (2014) 643.  
<http://doi.org/10.1080/13102818.2014.947076>
- Z. I. Khan, K. Ahmad, H. Safdar, I. Ugulu, K. Wajid, H. Bashir and Y. Dogan, *Res. J. Pharmaceut. Biol. Chem. Sci.*, 9 (2018) 759.  
<WOS:000447016800094>
- Z. I. Khan, I. Ugulu, S. Umar, K. Ahmad, N. Mehmood, A. Ashfaq, H. Bashir and M. Sohail, *Bull. Environ. Contam. Toxicol.*, 101 (2018) 235.  
<https://doi.org/10.1007/s00128-018-2353-1>
- I. Ugulu, *Biotech. Biotechnol. Equip.*, 29 (2015) 20.  
<http://doi.org/10.1080/13102818.2015.1047168>
- N. Yorek, I. Ugulu and H. Aydin, *Comput. Intel. Neurosc.* 2476256, (2016) 1.  
<http://dx.doi.org/10.1155/2016/2476256>
- I. Ugulu, *Biotech. Biotechnol. Equip.*, 23 (2009) 14.
- Z. I. Khan, I. Ugulu, K. Ahmad, S. Yasmeen, I. R. Noorka, N. Mehmood and M. Sher, *Bull. Environ. Contam. Toxicol.*, 101 (2018) 787.  
<https://doi.org/10.1007/s00128-018-2448-8>

21. Z. I. Khan, K. Ahmad, S. Rehman, A. Ashfaq, N. Mehmood, I. Ugulu and Y. Dogan, *Pak. J. Anal. Environ. Chem.*, 20 (2019) 60.  
<http://doi.org/10.21743/pjaec/2019.06.08>
22. R.G.D. Steel and J.H. Torrie, Principles and procedures of statistics. A Biometrical Approach, 2. McGraw-Hill, New York, (1980).  
<https://onlinelibrary.wiley.com/doi/abs/10.1002/bimj.19620040313>
23. Y. J. Cui, Y. G. Zhu, R. H. Zhai, D. Y. Chen, Y. Z. Huang, Y. Qui and J. Z. Liang, *Environ. Int.*, 30 (2004) 785.  
<https://doi.org/10.1016/j.envint.2004.01.003>
24. USEPA. Preliminary remediation goals, Region 9. United States Environmental Protection Agency, Washington, DC, (2002).  
<https://semspub.epa.gov/work/02/103453.pdf>
25. W. H. Liu, J. Z. Zhao, Z. Y. Ouyang, L. Soderlund and G. H. Liu, *Environ. Int.*, 31 (2005) 805.  
<https://doi.org/10.1016/j.envint.2005.05.042>
26. D. Mendil, O. D. Uluozlu, M. Tuzen and M. Soylak, *J. Hazard. Mater.* 165 (2009) 724.  
<https://doi.org/10.1016/j.jhazmat.2008.10.046>
27. A. Singh, R. K. Sharma, M. Agrawal and F.M. Marshall, *Food Chem. Toxicol.*, 48, (2010) 611.  
<https://doi.org/10.1016/j.fct.2009.11.041>
28. M. U. Haq, H. K. Puno, R. A. Khattak and M. S. Saif, *J. Chem. Soc. Pak.*, 26 (2004) 443. <https://jcsp.org.pk/ArticleUpload/1379-6171-1-RV.pdf>
29. S. Manzoor, M.H. Shah, N. Shaheen, A. Tariq and M. Khaliq, *J. Chem. Soc. Pak.*, 26 (2004) 429.  
<https://jcsp.org.pk/ArticleUpload/1377-6169-1-RV.pdf>
30. T. M. Chiroma, R. O. Ebebele and F. K. Hymore, *Int. Refereed J. Eng. Sci.*, 3 (2014) 1.  
[www.irjes.com > Papers > vol3-issue2](http://www.irjes.com/Papers/vol3-issue2)
31. S. Parveen, A. Samad, W. Nazif and S. Shah, *Pak. J. Bot.*, 44 (2012) 1923.  
[https://www.pakbs.org/pjbot/PDFs/44\(6\)/15.pdf](https://www.pakbs.org/pjbot/PDFs/44(6)/15.pdf)
32. M. I. Latif, M. I. Lone and K. S. Khan, *Soil Environ.*, 27 (2008) 29.  
<http://www.se.org.pk/File-Download.aspx?archivedpaperid=148>
33. A. S. Mahakalkar, R. R. Gupta and S. N. Nandeshwar, *Curr. World Environ.*, 8 (2013) 463.  
<https://doi.org/10.12944/CWE.8.3.16>
34. E. E. Santos, D. C. Lauria and C. L. Porto da Silveira, *Sci. Total Environ.*, 327 (2004) 69.  
<https://doi.org/10.1016/j.scitotenv.2004.01.016>
35. P. S. Harikumar, U. P. Nasir, M. P. Mujeebu, A. Rahma, *Int. J. Environ. Sci. Tech.*, 6 (2009) 225.  
<https://doi.org/10.1007/BF03327626>