Research Article

Incidence and health risk assessment of grazing animals due to toxic metal residues (Cd and Mn) in Sahiwal, Sargodha

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Abstract

Ecological contamination due to toxic chemicals is the major issue. Present research was conducted to find out the concentration of Cd and Mn in fodder soil and blood. Five sites of Tehsil Sahiwal of District Sargodha were selected for research where mostly contaminated water was used for irrigation. Pollution load index as well as health risk index was also studied. The results show that cadmium and manganese exceeded the permissible limit of Cd (0.2) and Mn (500) mg/kg suggested by FAO/WHO. Enrichment factor showed lower values as compared to standard. Higher values were observed for Mn at site-V 3.09 mg/kg for summer samples of *Zea mays* and lower *Trifolium alexandrium* 1.17 mg/kg, while for Cd higher values were observed for *Zea mays* 2.80 mg/kg and lower for *Trifolium alexandrium* 0.20 mg/kg for during winter season.

Keywords: Cadmium; Health risk index; Manganese; Pollution load index

Introduction

The distribution of different characters of soil affects the grouping of metals in soils [1]. The uptake of overwhelming metals in fodders is impacted by numerous factors, atmosphere, humidity conditions, centralizations of the irresistible nature of soil, chemicals in soils, and intensity of

development of plants at season of collection [2, 3]. Plants absorbing metals in high level may result in great risk. They can deplete essential nutrient from human body and decreased immunity [4].

Study of dumpsites had shown that depending on the age location and contents soil contains different concentration of toxic

chemicals [5-7]. Since of their solidness, metals may enter natural compartments, in a few cases, numerous years underlying testimony contamination of soil and water frameworks may likewise emerge from decomposition of arranged item [8]. Toxic chemicals which have been found to cause adverse effect on the root system of forage plants include Zn, Cu, Pb and Hg [19].

Roots are the fundamental organs of plants through which they absorb overwhelming metals from the soil. In metal free soil, vegetables developed on overwhelming metal polluted soils gather higher measures of metals than those developed in unpolluted soils [4, 10-13]. Overwhelming chemicals are constant in the earth and are liable to bioaccumulate in natural pecking orders. They are effortlessly collected in eatable in fodder, as compared to other crops [14]. They

stay in eatable segment of leaves as opposed to grains of nourishment. Metals were added in fodder through roots and stored in aerial parts [15].

The purpose of present investigation was to analyze Cd, Mn concentration in soil and various varieties of forage crops irrigated with wastewater, in relation to the requirements of buffaloes consuming those forages”.

Materials and methods

Study site

Research was conducted in the Tehsil Sahiwal of Sargodha division. Sahiwal is situated 37 kilometers from Sargodha Jhang road and is almost 5 km away from river Jhelum. Samples were taken from five sites. All these sites receive wastewater irrigation (Figure 1).

Figure 1. Location map of study area

Sample collection

Samples along with (3 replicates) were collected. Soil and plant samples were taken, sealed in label paper bags. Forage sample contained three plant species *Trifolium alexandrinum* (Barseem) *Zea mays* L. (corn) *Sorghum bicolor* (Millet). All the forage and soil samples were dried in air and in an oven at 72°C for 48 hours to attain constant dry weight. Fifteen samples of buffalo blood were taken along with three replicates. After

collection samples were centrifuged at 2500 rpm for 2 minutes to obtain plasma. The serum was transported to the laboratory in a cool box and kept frozen at -20°C before investigation.

Sample preparation for analysis

Wet digestion method was used for digestion. HNO₃ (2 ml) and H₂O₂ (4 ml) were used for digestion. 2 grams of soil and forage were taken and heated for 30min and then cooled. H₂O₂ were added in this sample until a

colorless solution was obtained. Distilled water was used to make the volume 50ml. Determination of Zn and Pb concentration was performed with the help of an atomic absorption spectrophotometer (Model # AA-6300, Shimadzu, Japan) equipped with a graphite furnace (AA6300&GFAEXi7i).

Statistical analysis

SPSS was used to find analysis of variance and correlation of data.

Bio concentration factor (BCF)

Bio concentration factor from soil to forage was calculated using Microsoft excel by the following formula:

$BCF = \text{mean concentration of metal in fodder} / \text{mean concentration of metal in soil}$

Enrichment Factor (EF)

It was determined by Buat-Menard and Chesselet [16].

$EF = [M]_{\text{fodder}} / [M]_{\text{Soil}} / [M]_{\text{fodder S}} / [M]_{\text{soil S}}$

Pollution load index (PLI)

Pollution load index was calculated by the formula Liu et al. [17].

$PLI = \text{metal concentration in examined}$

soil/metal concentration in reference soil”.

Daily intake of metal (DIM)

DIM was calculated by following Sajjad et al. [18].

$DIM = C_{\text{metal}} \times D_{\text{food intake}} \times C_{\text{conversion factor}} / B_{\text{average weight}}$

Health risk index (HRI)

Health risk index value can be calculated by daily intake of metal and oral reference dose (RfD) Cui et al., [19].

Health risk index (HRI) = DIM/ RfD”

Results

Analysis of variance

Blood samples of all the sites had non-significant (>0.001) values (Table 1).

Concentration of Cd in soil samples

Higher concentration of Cd was found at site-V 0.60 to 0.783 mg/kg and lower at site-I 0.10 to 0.233mg/kg in summer season. In winter, highest concentration was found at site-V (0.90-0.925) and the lowest at site-I (0.142-0.58). Higher concentration was found during winter for *Z. mays* (Figure 2).

Table 1. Analysis of variance for Cd concentration in soil, fodder and blood

Sample	Site-I	Site-II	Site-III	Site-IV	Site-V
Summer Soil	0.000ns	0.002***	0.000 ns	0.002**	0.003**
Winter soil	0.006***	0.001***	0.001**	0.003**	0.002*
Summer Fodder	0.006***	0.001***	0.001*	0.003**	0.002**
Winter Fodder	0.013**	0.002***	0.000**	0.009***	0.001***
Summer blood	0.003***	0.001***	0.001***	0.002***	0.001***
Winter blood	0.000ns	0.000ns	0.001***	0.000ns	0.000ns

*, **, *** = Significant at 0.05, 0.01 and 0.001, ns = non-significant

Figure 2. Fluctuations in mean concentration of Cd in soil at different sites during summer and winter season

Concentration of Cd in fodder samples

A relatively high concentration of Cd was noticed at site-V in summer that ranged from 0.85 to 0.925. In winter, high concentration ranged from 0.094 to 0.10 and low 0.06 to 0.07 (Figure 3).

Concentration of Cd in blood samples

It was noted that blood samples collected at site-V in summer had more Cd values

(0.2125-0.70) than the samples collected in winter season (0.2017-0.2350). Less contaminated in both seasons was site-I and range of values were 0.1050-0.1325 and 0.120-0.1433. Between seasons, the most accumulation was found at site-V in winter (Figure 4).

Figure 3. Fluctuations in mean concentration of Cd in fodder samples at different sites during summer and winter season

Figure 4. Fluctuations in mean concentration of Cd in blood samples at different sites during summer and winter season

Correlation coefficients of fodder samples

Positive and non-significant correlation was observed for all of three forage crops from soil to fodder samples of summer and negative and non-significant correlation was observed for fodder-blood samples and soil to fodder samples in winter. *Trifolium alexandrium* had significant correlation for fodder-blood (Table 2).

Pollution load index

The PLI of Cd showed higher values for site-V. All the values are lower than 1 which indicated that soil was less contaminated in present investigation (Table 3).

Bio concentration factor

Bioconcentration factor values for Cd in fodder samples were higher at site-IV and site-V. The lowest BCF value was observed at site-I (Table 4).

Daily intake of metal

Site-V had higher DIM values as compared to other sites. Site-I and Site-2 had lower value of intake at daily basis (Table 5).

Health risk index

Health risk index for Cd was found to be higher than the limit set by WHO [20]. Site-V had higher values than all others (Table 6).

Enrichment factor

High EF value was found at site-V. Lower EF was observed at site-I. These observations revealed that site-V had higher risk as compared to other sites (Table 7).

Manganese (Mn)

Some of the samples at site-V of soil and blood had non-significant values ($p>0.001$). Other samples had significant value ($p>0.05$) (Table 8).

Concentration of Mn in soil sample

Higher Mn values ranged from 2.41 to 2.40 at site-V and lower values ranged from 1.16 to 1.205 at site-I in summer season. In winter season, higher Mn values ranged from 1.405 to 1.45) at site-V and lower values ranged from 1.17 to 1.1925 at site-I (Figure 5).

Concentration of Mn in fodder samples

The result showed that higher Mn values ranged from 3.5425 to 3.555 at site-V and lower values from 1.407 to 1.440 at site-I in summer season. In winter season, higher Mn values ranged from 2.52 to 2.536 and lower from 1.35 to 1.4416 (Figure 6).

Concentration of Mn in blood samples

High Mn concentrations in blood samples were found at site-V (1.485-1.9625) and lower values at site-I (1.3116-1.1050) in summer season. In winter season, higher Mn concentrations were found at site-V (1.6350-6.130) and lower values at site-I (0.2650-0.3575) (Figure 7).

Correlation coefficients of fodder samples

Significant correlation was observed for soil to fodder and fodder to blood samples for *Z.mays* and also for *Sorghum bicolor* and *Trifolium alexandrium* from soil to fodder. Positive and non-significant correlation was observed for soil to fodder and also for fodder to blood in winter (Table 9).

Pollution load index

Winter samples of *S. bicolor* showed high PLI at site-V. Summer samples of *T. alexandrium* showed lowest value of PLI at site-II. All the values were lower than 1 indicated that soil was not much contaminated (Table 3).

Bio-concentration factor

All the values were higher than 1 (Table 4).

Daily intake of metal

According to WHO 1994 its tolerable range is from 0.5-5 mg/kg/day all the values in present investigation was within range (Table 5).

Health risk index

All the values were higher than HRI was higher oral reference dose for Mn (0.041mg/kg) (Table 6).

Enrichment factor

Enrichment factor was high for all samples in present investigation. Highest enrichment was observed at site-V in both seasons and

lowest for site-I in both seasons for all the samples (Table 7).

Table 2. Correlation between soil-fodder-blood for Cd

Correlation Coefficient						
	<i>Z. mays</i>		<i>S. bicolor</i>		<i>T. alexandrinum</i>	
	Soil-fodder	Fodder-Blood	Soil-Fodder	Fodder-blood	Soil-fodder	Fodder-blood
Summer	0.847	-0.420	.592	-0.393	0.483	0.965**
Winter	0.027	-0.307	-0.313	-0.723	-0.384	-0.292

** Correlation is significant at the 0.01 level (2-tailed)

Table 3. Pollution load index for Cd and Mn

Pollution load index						
Site	<i>Z. mays</i>		<i>S. bicolor</i>		<i>T. alexandrinum</i>	
	Summer	Winter	Summer	Winter	Summer	Winter
Cd						
Site-I	0.067	0.38	0.156	0.083	0.0671	0.095
Site-II	0.1342	0.2234	0.1342	0.263	0.134	0.134
Site-III	0.449	0.067	0.33	0.201	0.449	0.184
Site-IV	0.33	0.44	0.201	0.480	0.20	0.33
Site-V	0.52	0.620	0.447	0.57	0.402	0.64
Mn						
Site-I	0.025	0.025	0.0259	0.025	0.0259	0.025
Site-II	0.0257	0.0271	0.0259	0.0265	0.0268	0.026
Site-III	0.026	0.027	0.028	0.027	0.028	0.028
Site-IV	0.027	0.028	0.028	0.029	0.028	0.030
Site-V	0.051	0.030	0.051	0.030	0.051	0.031

Table 4. Bio concentration factor for Cd and Mn

Bioconcentration factor						
Site	<i>Z. mays</i>		<i>S. bicolor</i>		<i>T. alexandrinum</i>	
	Summer	Winter	Summer	Winter	Summer	Winter
Cd						
Site-I	5.8	0.185	0.536	1.04	1.41	0.8
Site-II	1.665	0.48	1.955	0.14	1	0.9
Site-III	0.149	0.7	0.6	0.23	0.41	0.24
Site-IV	1	0.11	2.39	0.112	2.22	0.166
Site-V	1.18	0.120	1.349	0.101	1.43	0.10
Mn						
Site-I	1.17	1.232	1.15	1.203	1.18	1.14
Site-II	1.18	1.140	1.18	1.16	1.13	1.170
Site-III	1.20	1.15	1.11	1.13	1.104	1.10
Site-IV	1.93	1.13	1.89	1.089	1.47	1.04
Site-V	1.87	1.80	1.47	1.79	1.47	1.73

Table 5. Daily intake of metal for Cd and Mn

Daily intake of metal						
Site	<i>Z. mays</i>		<i>S. bicolor</i>		<i>T. alexandrinum</i>	
	Summer	Winter	Summer	Winter	Summer	Winter
Cd						
Site-I	0.001760	0.00038	0.00043	0.00384	0.000397	0.000349
Site-II	0.001011	0.001187	0.000607	0.00049	0.000167	0.000582
Site-III	0.000304	0.000911	0.000835	0.000213	0.00021	0.000205
Site-IV	0.001518	0.002177	0.002025	0.000228	0.000245	0.000253
Site-V	0.002808	0.002732	0.00260	0.000339	0.000266	0.000286
Mn						
Site-I	0.0507	0.051	0.0502	0.05	0.051	0.048
Site-II	0.051	0.051	0.051	0.051	0.050	0.051
Site-III	0.052	0.052	0.052	0.052	0.052	0.052
Site-IV	0.090	0.053	0.090	0.05	0.089	0.052
Site-V	0.12	0.090	0.126	0.090	0.126	0.09

Table 6. Health risk index for Cd and Mn

Health risk index						
Site	<i>Z. mays</i>		<i>S. bicolor</i>		<i>T. alexandrinum</i>	
	Summer	Winter	Summer	Winter	Summer	Winter
Cd						
Site-I	0.33	0.37	0.42	0.39	0.34	0.34
Site-II	0.22	0.24	0.60	0.16	0.58	0.56
Site-III	0.30	0.90	0.83	0.21	0.21	0.20
Site-IV	1.51	0.26	0.28	1.01	1.18	0.25
Site-V	2.80	2.73	2.60	2.17	2.01	1.76
Mn						
Site-I	1.23	1.25	1.22	1.25	1.25	1.17
Site-II	1.24	1.25	1.24	1.25	1.24	1.25
Site-III	1.29	1.28	1.27	1.27	1.27	1.26
Site-IV	2.20	1.31	2.21	1.29	2.17	1.29
Site-V	3.09	2.20	3.096	2.20	3.08	2.19

Table 7. Enrichment factor for Cd and Mn

Enrichment factor						
Site	<i>Z. mays</i>		<i>S. bicolor</i>		<i>T. alexandrinum</i>	
	Summer	Winter	Summer	Winter	Summer	Winter
Cd						
Site-I	0.0016	0.0024	0.0072	0.014	0.0013	0.0014
Site-II	0.0015	0.0065	0.00151	0.0018	0.013	0.0128
Site-III	0.002	0.0093	0.008	0.0030	0.005	0.0032
Site-IV	0.013	0.022	0.032	0.0262	0.029	0.0022
Site-V	0.015	0.077	0.018	0.018	0.019	0.0108
Mn						
Site-I	5.04	5.27	4.95	5.14	5.07	4.86
Site-II	5.07	4.87	5.05	4.98	4.84	5.006
Site-III	5.14	4.95	4.78	4.86	4.72	4.73

Site-IV	6.314	4.84	6.31	4.66	6.31	4.468
Site-V	8.09	7.72	8.007	7.6	8.28	7.434

Table 8. Analysis of variance for Mn concentration in soil, fodder and blood samples at different sites

	Site-I	Site-II	Site-III	Site-IV	Site-V
Summer Soil	0.003***	0.002***	0.007***	0.001***	0.000ns
Winter soil	0.000***	0.001***	0.002***	0.006***	0.002*
Summer Fodder	0.000***	0.001***	0.009***	0.000*	0.004**
Winter Fodder	0.000*	0.000***	0.001*	0.002*	0.031***
Summer blood	0.001***	0.051***	2.712***	0.030***	0.003***
Winter blood	0.697 ns	0.985***	0.012***	0.012***	20.521ns

*, **, *** = Significant at 0.05, 0.01 and 0.001, ns = non-significant

Figure 5. Fluctuations in mean concentration of Mn in soil at different sites during summer and winter season

Figure 6. Fluctuations in mean concentration of Mn in fodder at different sites during summer and winter season

Figure 7. Fluctuations in mean concentration of Mn in blood samples at different sites during summer and winter season

Table 9. Correlation between soil-fodder-blood for Mn

	Correlation coefficient					
	<i>Z. mays</i>		<i>S. bicolor</i>		<i>T. alexandrinum</i>	
	Soil-fodder	Fodder-Blood	Soil-Fodder	Fodder-blood	Soil-fodder	Fodder-blood
Summer	0.999**	0.925*	0.902*	0.651	0.900*	0.848
Winter	0.885*	0.978**	0.740	0.833	0.692	0.861

**. Correlation is significant at the 0.01 level (2-tailed)

Discussion

Industrial development and human activities cause the Cd most prevalent pollutant in agriculture soil and soil plant system. Cadmium accumulation in the environment is presently turning into an essential reason for environmental pollution. Cadmium is mainly toxic chemical found in soil for both plant and animals [12]. In the present study, Cd values in soil samples ranged from (0.60 to 0.783 mg/kg) in summer season and (0.10 to 0.233 mg/kg) in winter season. Values were lower than permissible limit of Cd is 3 mg/kg according to FAO/WHO [21]. It can be easily taken up by roots and effect on biochemical, morphological, physiological activities of plants and impact negatively. It may cause death of plants. Relatively high Cd in fodder samples was noticed at site-V in summer 0.85 to 0.925 mg/kg higher than permissible limit 0.1 mg/kg according to WHO [20]. Cd is easily available to plants as its exchangeable

capacity is higher and easily soluble in soil [22]. High Cd accumulation in the fodder samples studied in the present research may be due to this reason.

Cadmium has been considered as one of the main toxic metals that strive and act together with other essential metals such as Iron, Zinc and Copper, and bring shortages of these metals in organisms. In the present study, it was noted that blood samples collected at site-V in summer had more Cd values (0.2125-0.70) than the samples collected in winter season (0.2017-0.2350).

Higher Mn values were found at site-V (2.40-2.41). All values were lower than recommended by FAO/WHO [21] as 46.75mg/kg. Sparks [23] and Ness & Woolhouse [24] reported higher values as compared to present investigation. Mn takes part in photosynthesis in water splitting system in photosystem-II by providing electron Goussias et al. [25]. However, its

higher values are hazardous for plants Migocka and Klobus [26]. Higher Mn values in fodder samples ranged from 3.5425 to 3.555 at site-V and lower values from 1.35 to 1.4416 at site I. Mn permissible limit given by FAO/WHO [21] is 200mg/kg. Present Mn results were below than suggested value indicating that plants are less contaminated. Higher Mn concentrations in blood samples of buffaloes were found at site-V (1.6350-6.130mg/kg) and lower values at site-I (0.2650-0.3575 mg/kg). Tolerable intake of Mn in buffaloes is between 0.5-5mg/kg [27, 28]. All the samples contained lower value than suggested value except for winter blood at site-V.

The value of PLI higher than 1 showed that soil is contaminated and lower than 1 considered as unpolluted Harikumar et al. [29]. In present research all the values are lower than 1.

Differences in concentrations of Cd is due to binding of metal to plant roots, available metal soil, type of plants species, physio-chemical [30, 31].

Bioconcentration factor indicates the level of toxicity in animals Cui *et al.* [19] higher value was observed for *Z. mays* and lower for *S. bicolor*.

Tolerable daily intake limit for Cd is 0.07µg/kg and 0.5-5 µg/kg for Mn set by WHO [32]. Present studies showed the intake lower than TDI for both metals.

HRI values indicated the high values for both Cd and Mn that cause toxic effects on buffalo it is due to reason that in both seasons' livestock owners are more likely to graze their animals than harvesting on contaminated soils. Mn had highest values for health risk index.

Abundance of metals in soils than standard Hernandez et al. [33]. Lower values of EF for Cd and Mn indicate its high retention in soil. Present values were lower than Sherif et al. [34] for Cd.

Conclusion

This is the first study to find the heavy metal content in waste water irrigated soil in Tehsil Sahiwal of district Sargodha. Irrigation with waste water is common practice now a days. In this study Soil, Fodder and Blood sample showed a cooperative amount of metals, However it varied from site to site. Higher concentration were found at site-V and lower at site-I. All the values were within the range. Fodder samples of Cd showed higher values than permissible limit. Further research on contamination of fodder crops is required.

Authors' contributions

Conceived and designed the experiments: ZI Khan & K Ahmad, Performed the experiments: M Ghazzal, M Sohail, P Akhter & H Bashir, Analyzed the data: N Mehmood & K Wajid, Contributed reagents/ materials/ analysis tools: M Nadeem & M Akhtar, Wrote the paper: IS Malik, S Mahpara, A Ashfaq & M Munir.

References

1. Qishlaqi A & Moore F (2007). Statistical analysis of accumulation and sources of heavy metals occurrence in agricultural soils of Khoshk River Banks, Shiraz, Iran. *Am Eurasian J Agric Environ Sci* 2(5): 565-573.
2. Scott SH & Loeb GE (1996). Mechanics of feline soleus: II Design and validation of a mathematical model. *J Muscle Res Cell Motil* 17(2): 221-233.
3. Voutsas D, Grimanis A & Samara C (1996). Trace elements in vegetables grown in an industrial area in relation to soil and air particulate matter. *Environ Pollut* 94(3): 325-335.
4. Al-Jassir MS, Shaker A & Khaliq MA (2005). Deposition of heavy metals on green leafy vegetables sold on roadsides of Riyadh City, Saudi Arabia. *Bullet Environ Contamin Toxicol* 75(5): 1020-1027.
5. Udosen ED, Udossien EI & Ibok UJ (1990). Evaluation of some metals in

- the industrial wastes from a paint industry and their environment pollution implications. *Niger J Technol Res* 2: 71-77.
6. Odukoya OO, Bamgbose O & Arowolo TA (2000). Heavy metals in topsoil of Abeokuta dumpsites. *Global J Pure Appl Sci* 7: 467-472
 7. Haluschak P, Eilers RG, Mills GF & Grift S (1998). Status of selected trace elements in agricultural soils of southern Manitoba. Canada: Agriculture and Agri-Food Canada.
 8. Nordberg GF, Fowler BA, Nordberg M & Friberg L (2005). Handbook on the toxicology of metals.
 9. Khan ZI, Safdar H, Ahmad K, Wajid K, Bashir H, Ugulu I & Dogan Y (2019). Health risk assessment through determining bioaccumulation of iron in forages grown in soil irrigated with city effluent. *Environ Sci Pollut Res* 26(14): 14277-14286.
<https://doi.org/10.1007/s11356-019-04721-1>.
 10. Khan ZI, Safdar H, Ahmad K, Ugulu I, Wajid K, Bashir H & Dogan Y (2018). Manganese bioaccumulation and translocation of in forages grown in soil irrigated with city effluent: an evaluation on health risk. *Res J Pharm Biol Chem Sci* 9(5):759-770.
 11. Ahmad K, Wajid K, Khan ZI, Ugulu I, Memoona H, Sana M, Nawaz K, Malik IS, Bashir H & Sher M (2019). Evaluation of potential toxic metals accumulation in wheat irrigated with wastewater. *Bullet Environ Contam Toxicol* 102(6): 822-828.
<https://doi.org/10.1007/s00128-019-02605-1>.
 12. Ahmad K, Nawaz K, Khan ZI, Nadeem M, Wajid K et al (2018a). Effect of diverse regimes of irrigation on metals accumulation in wheat crop: an assessment-dire need of the day. *Fresen Environ Bullet* 27(2): 846-855.
 13. Ahmad K, Kokab R, Khan ZI, Ashfaq A, Bashir H, Munir M et al (2018b). Assessment of heavy metals in wheat variety “Chagi-2” under short-term wastewater irrigation. *Biologia (Pak)* 64(1): 15-25.
 14. Mapanda F, Mangwayana EN, Nyamangara J & Giller KE (2005). The effects of long-term irrigation using water on heavy metal contents of soils under vegetables. *Agric Ecosys Environ* 107: 151-156.
 15. Haiyan W & Stuanes A (2003). Heavy metal pollution in air-water-soilplant system of Zhuzhou City, Hunan Province, China. *Water Air Soil Pollut* 147: 79-107.
 16. Buat-Menard P & Chesselet R (1979). Variable influence of the atmospheric flux on the trace metal chemistry of oceanic suspended matter. *Earth Planet Sci Lett* 42(3): 399-411.
 17. Liu WH, Zhao JZ, Ouyang ZY, Soderlund L & Liu GH (2005). Impacts of sewage irrigation on heavy metals distribution and contamination in Beijing, China. *Environ Inter* 31: 805-812.
 18. Sajjad K, Farooq R, Shahbaz S, Khan MA & Sadique M (2009). Health risk assessment of heavy metals for population via consumption of vegetables. *World Appl Sci J* 6:1602-1606.
 19. Cui YJ, Zhu YG, Zhai RH, Chen DY, Huang YZ, Qui Y & Liang JZ (2004). Transfer of metals from near a smelter in Nanning, China. *Environ Int* 30(6): 785-791.
 20. WHO (1996). Toxicological evaluation of certain food additives and contaminants. 33rd Report of the Joint FAO/WHO Expert Committee on food additives,

21. FAO/WHO (2001). *Codex Alimentarius Commission*. Food additive and contaminants. Joint FAO/ WHO Food Standards Programme, ALINORM 01/12A, pp 1-289.
22. Liu JG, Qian M, Cai GL, Yang JC & Zhu QS (2007). Uptake and translocation of Cd in different rice cultivars and the relation with Cd accumulation in rice grain. *J Hazard Mater* 143: 443-447.
23. Sparks D (1995). *Environmental soil chemistry*. San Diego, Academic Press, pp 352.
24. Ness PJ & Woolhouse HW (1980). RNA synthesis in *Phaseolus* chloroplasts. 1. Ribonucleic acid synthesis and senescing leaves. *J Exp Bot* 31: 223-233.
25. Goussias C, Boussac A & Rutherford W (2002). Photosystem II and photosynthetic oxidation of water: an overview. *Phil Trans R Soc Lond B* 357: 1369-1381.
26. Migocka M & Klobus G (2007). The properties of the Mn, Ni and Pb transport operating at plasma membranes of cucumber roots. *Physiol Plant* 129: 578-587.
27. USEPA (2002). Solid waste and emergency response. In: AGENCY, USEP.
28. WHO/FAO (2007). Joint FAO/WHO food standard programme codex Alimentarius commission 13th session. Report of the thirty-eight session of the codex committee on food hygiene. Houston, ALINORM 07/30/13.
29. Harikumar PS, Nasir UP, Mujeebu MP & Rahma (2009). Distribution of heavy metals in the core sediments of a tropical wetland system. *Int J Environ Sci Technol* 6(2): 225-232.
30. Toth T, Tomas J, Lazor P, Bajcan D & Jomova D (2009). The transfer of metals from contaminated soils into agricultural plants in high Tatars region. *Czech J Food Sci* 27: 390-393.
31. Bose S & Bhattacharyya AK (2008). Heavy metal accumulation in wheat plant grown in soil amended with industrial sludge. *Chemosphere* 70: 1264-1272.
32. WHO (World Health Organization) (1994). *Quality Directive of Potable Water*, Geneva, second ed, pp. 197.
33. Hernandez LE, Carpena-Ruiz R & Garate A (1996). Alterations in the mineral nutrition of pea seedlings exposed to cadmium. *J Plant Nutr* 19: 1581-1598.
34. Sherif SEA, El-Kholy MM & Salem TM (2015). Risk assessment of trace elements toxicity through edible plants from irrigation canal at Giza Governorate, Egypt. *Iranica J Energy Environ* 6(1): 47-55.