EVALUATION OF CADMIUM AVAILABILITY IN MILK THROUGH FODDERS GROWN IN LONG TERM WASTEWATER IRRIGATED SOIL: IMPLICATION FOR PUBLIC HEALTH

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Abstract

Municipal wastewater is the main source of irrigation in the vicinities of Jhang city therefore the current study was carried out to evaluate cadmium content in soil, forage, and milk at three sites in Jhang city, Punjab, Pakistan. Wastewater is used on large scale for irrigation purposes at selected sites. All samples were analyzed for Cadmium by Atomic Absorption Spectrophotometer. Different health indices were also studied to check Cd flow in the food chain. The level of all metals in the plant was found within safe limits. Cadmium level was higher in soil samples collected from Site-III than other sites. Cadmium showed a maximum value (0.0604 to 0.502 mg/kg) in the soil as compared to other samples. Fodders were found to accumulate cadmium from 0.016 to 0.1018 mg/kg. Cd content was found to be 0.082 to 0.285 mg/L, 0.0284–0.0723 mg/L in water and milk, respectively. BCF, PLI values were also found to be less than 1. EDI and THQ of Cd are found within permissible limits in the milk of cows feeding on fodders irrigated with wastewater and groundwater. So the use of wastewater for long-term irrigation purposes should be stopped as serious health concerns in animals as well as humans are showing due to increase of metal toxicity in plants and animals.

Keywords: *Wastewater, Fodders, Cow milk, Bio-concentration factor, Pollution load index*

Introduction

In cities, environmental pollution has been enhanced due to a large amount of effluents produced from human settlements and industries which are collectively called municipal waste (public waste). The amount of the effluents has been increased due to speedy growth population and increased number of industries in the country (Dogan *et al*., 2014).

Food safety and its quality is threatened by excessive accumulation of toxic metals in soil and crops after using wastewater. Irrigation of crops using wastewater for a long period causes the amassing of heavy metals in the soil and plants affecting its quality (Muchuweti *et al*., 2006).

Wastewater may have a variety of heavy metals such as lead, cadmium, chromium, manganese, nickel, copper, and zinc depending upon the source of waste In many cases, the heavy metal contamination due to the continuous use of effluents and industrial wastes frequently results in the buildup of elevated metal levels in soil (Gosh *et al*., 2006).

The environment is polluted due to release of massive amounts of heavy metals to soils and ground water. Many anthropogenic behaviors such as cultivation, use of automobiles for transportation, and production of industrial wastes from industries, are that consistent causes of these metals pollution. Crops become contaminated with heavy metals when they cross the borderline of soil and roots (Khan *et al*., 2018b). As heavy metals become an important part of the animal body utilizing a contaminated food chain. In contaminated soils, the collection of bioavailability of metals becomes a matter of great concern.

Livestock has played an important part in the production of healthy food, food security and fitness of the people. Fodders supplied to livestock, are a great source of microelements. For livestock, the main source of nutritional elements is also fodder plants. Health and productivity of livestock also depends on the mineral status of these in their bodies due to various feeding habits. The objective of this study is to assess the transfer of cadmium from different sources of wastewater to the soil, fodders and milk, and its possible health risks on human health.

Materials and Methods

Study area: Jhang is the 18th largest city in Pakistan. It is one of the oldest and central cities of Pakistan. It is recognized as agricultural city. The climate of Jhang city is extreme hot and cold in summer and winter, respectively. In months of May, June, July and August, temperature reaches upto 52 degree centigrade. However, monsoon also showers in these months. The maximum and minimum mean temperature is 50 ºC and 29 ºC in summer but in winter it is 21 ºC and 4.5 ºC respectively.

Wastewater irrigation in urban and peri-urban areas of Jhang city is common practice due to the easy availability of the wastewater including all effluents of the city such as domestic and industrial wastes. Sites were selected based on wastewater irrigation areas. Site-I is known as Jhang chiniot road, Site-II Jhang Faisalabad road, and Site-III was Jhang Sargodha road. Site-II and Site-III were wastewater irrigated but Site-I was irrigated with tube well water. Site-I is also considered as a control site.

Plant sampling: Each site was divided into 5 plots. All soil, fodders samples were collected from each plot of the site and made into one composite sample. Three replicates of soil and fodders were prepared. Samples were collected during 2018-2019.

A total of 72 plant samples (24 samples from each site) were collected at random from three sites. Plant samples were rinsed with distilled water and placed in paper bags. Each sample was assigned an identity. The saag, lucern, bajra, barseem and corn plants were common crops across three sites. Eight different types of fodders were collected from selected three sites which are given in table 1.

Table 1: List of fodders collected from three sites

Sr. No.	Common names	Scientific names
1	Maize	*Zea mays*
2	Millet	*Pennisetum glaucum*
3	Mustard	*Brassica campestris*
4	Barseem	*Trifolium alexandrinum*
5	Lucern	*Medicago sativa*
6	Bathu	*Chenopodium album*
7	Jai	*Avena sativa*
8	Methi	*Trigonella foenum-graecum*

Sampling of soils across three sites: Total 72 composite samples of soil from three sites were collected. Composite soil samples (one from each corner and one from the center of fields) were collected from each site. Soil samples (10 g each) were placed into sealed plastic bags and carried to the laboratory for heavy metals analysis.

All soil and plant samples were air dried and placed in an incubator for 5 days at 70 ^oC.

Water sampling: Water samples were also collected from all the sites used for irrigation purpose. About 10ml of water was collected from each site in plastic bottles. All water samples were collected from the irrigation sources. Water samples were also digested and subjected to Atomic Absorption spectrophotometer (AAS).

Milk sampling: A total of 75 raw cow milk samples were collected from randomly selected the cattle farms located in the study area and 25 cows were selected from each site for the samples. An alaquat of 20 mL milk sample was taken and stored at -20 °C for further process.

Digestion of samples: Fodder crop samples were washed with distilled water. Edible parts of each plant and soil samples were oven-dried at 70 ^oC. Dried plant samples and soils samples were ground into a fine powder using a grinder and stored in plastc bags for acid digestion. A wet digestion method was used for both plant and soil samples. Sample (1g) was digested in a flask with the addition of 4.0 mL of H_2SO_4 and 8.0 mL of H_2O_2 by placing it in the digestion chamber. Digested solution was filtrated by using whatman filter paper. In order to make final volume of the sample to 50 ml, distilled water was added. All prepared samples were kept in labeled bottles for additional analysis.

Milk digestion: In a digestion flask, 10 ml of 65 % HNO_3 and 3 mL of 30 % H_2O_2 were used to digest 10 mL of milk and heated until the solution became clear. Whattman filter paper 40 was also used for filtration and use distilled water to make a final volume of 20 mL.

Metal Analysis: Digested samples were analyzed for metal presences using Atomic Absorption Spectrophotometer Perkin-Elmer AAS-5000 (Perkin-Elmer Corp., 1980). Samples were analysed for Cadmium (Cd) concentration in all samples. The observed mean concentrations were compared with permissible values and were used for further calculations to evaluate possible risks. Mean content of heavy metal were also observed in soils in which different fodders were grown to compute pollution load index (PLI) and Bio-concentration factor

Target health quotient (THQ) of cadmium in groundwater and selected fodders were calculated to know whether said crop produce at each site poses a carcinogenic health risk to exposed population or not.

Statistical analysis: All results obtained from AAS were analyzed using Statistical version SPSS. During statistical analysis significance level was found out at probability levels of 0.05, 0.001and 0.01.

Bio-concentration factor: Bio-concentration factor (BCF) is determined using a formula worked out by Cui *et al*. (2004).

$$\text{Bio-concentration factor} = \frac{(M)^{Plant}}{(M)^{Soil}}$$

Where,

$(M)^{Plant}$ = Concentration of metal (mg/kg) in plant

$(M)^{Soil}$ = Concentration of metal (mg/kg) in soil

2.11. Pollution load index (PLI)

Pollution Load Index is determined by following formula given by Liu *et al*. (2005).

$$\text{Pollution Load Index} = \frac{(M)^{IS}}{(M)^{RS}}$$

Where,

$(M)^{IS}$ = Concentration of metal (mg/kg) in investigated soil

$(M)^{RS}$ = Reference value of metal in soil

Reference value of cadmium in soil is 1.49 (Singh, et al., 2010a).

2.12. Estimated Daily Intake (EDI)

Estimated daily intake of metals (EDI) was computed as given by Chaoua *et al.* (2008).

$EDI = C_{metal} \times I \times C_{factor} / B_{average\ weight}$

Where C_{metal} is mean concentration of metal in milk (mg/L), I is daily intake of milk (kg/day) and its value was taken as kg/person/day. C_{factor} is conversion factor (0.085). $B_{average\ weight}$ is average body weight (kg) taken as 60 kg.

2.13. **Target Health Quotient (THQ)**

THQ is described as the ratio of estimated daily intake of metals in milk to the oral reference dose (RfD) and was calculated with suggestion of Chaoua *et al.* (2008).

$THQ = EDI / R_fD$

EDI = Estimated daily intake of metal

RfD = Oral reference dose

If THQ > 1.0 for single metal indicates that health of consumers is at high risk or it is carcinogenic. Oral reference dose for cadmium is 0.001 (USEPA, 2010).

Results and Discussion

Soil: Cd^{+2} concentration in soil ranged significantly ($p<0.001$) at three different sites (Table 2). Among all soils, cadmium was found to be significantly higher (0.502 mg/kg) in S3 sample at Site-III than other samples. Less Cd level (0.0604 mg/kg) was also observed at Site-I in S2 sample. Table 3 showed varied quantities of Cd in all soil samples. Heavy metals are accumulated in upper layer of soil due to its retention and less mobility. The passage of metals in soil was blocked due to sand and clay particles (Hassan *et al*., 2013). Cd concentration in all soil samples was found less as compared to value (11.22 mg/kg) reported by Chaoua *et al*. (2018). Cd level (2.001, 2.16 mg/kg) in soil suggested by Khan et al. (2017) was found to be high than our results. Wang *et al.* (2015) reported similar Cd^{+2} concentration (0.05 mg/kg) in soil samples. Cd concentration in soil irrigated with ground and waste water was found within tolerable limits (3.0-6.0 mg/kg) given by WHO/FAO, (2007).

Fodders: Descriptive statistics analysis revealed considerable effects ($p<0.001$) on Cd^{+2} content in fodders irrigated with wastewater (Table 2). Cd content in fodders varies considerably from 0.016 mg/kg in F4 to 0.1018 mg/kg in F6. The maximum value of fodder was recorded at Site-III (Table 4). It has been found that Cd is transported from root to shoot by using symplast pathway. Possibilities for transfer of Cd in shoot automatically increases with increase in Cd content in the root. Cadmium values were following with guidelines for maximum limit (0.2 mg/kg) in fodder plants given by WHO/FAO, (2007). Wang et al. (2015) suggested Cd concentration (0.05-1.17 mg/kg) which was similar to values as estimated in the present study. Cd concentration (0.06 mg/kg) given by Aurangzeb et al. (2011) was similar to our findings. Higher cadmium content (2.175 mg/kg) in edible part of fodders was reported by Chaoua *et al.* (2018) than our findings.

Water: Effect of significance level ($p<0.05$) of water samples was recorded on Cd content (Table 2). Cadmium concentration in water samples varied from 0.082 to 0.285 mg/L. Maximum value was recorded at Site-III (Table 5). Wastewater drainage as a result of various industrial practices contains a number of toxic metals counting Cd. This water may also pollute ground water because of its higher concentration. In this research, Cd concentration was found within the range (0.1 mg/L) recommended by Hassan *et al*. (2013) in waste water samples. All water samples have highest Cd value as compared to value (0.036 mg/L) proposed by Aurangzeb *et al*. (2011). Ahmad *et al*. (2018) suggested Cd concentration (1.69-1.88 mg/L) was higher than examined values. Current investigated Cd concentration was found to be high than allowed limit (0.005 mg/L) confirmed by (USEPA, 2010).

Milk: Statistical analysis revealed significant effect of sites ($P<0.01$) on Cd level in cow milk (Table 2). A range of Cd content (0.0284–0.0723 mg/L) was detected in milk samples (Table 6). Toxicity due to cadmium accumulation in humans may cause kidney failure, skeletal and liver disorders Hassan *et al*. (2013). Higher cadmium level (0.92 mg/L) reported by Younus *et al.* (2016) in milk samples from farms and market situated near wastewater drains in Jhang city, Punjab, Pakistan in contrast to our values.

Cd level (0.083-0.145 mg/kg) suggested by Aslam *et al*. (2011) in cow milk samples was high than range examined in this study. Lower results for Cd (0.001 mg/kg) were evaluated by Ismail *et al*. (2015) than present work.

Bio-concentration factor: BCF values of cadmium in fodder samples were given in Table 7. Maximum BCF value of Cd (0.8929) in F6 sample was examined at Site-III. At Site-I, sample F3 exhibited least value (0.09239) for Cd transfer from soil to plant. The BCF range of cadmium (0.14 - 0.13) given by Khan *et al*. (2013) in samples was observed between range of current study. Value of BCF for Cd (0.5 mg/kg) was observed by Alrawiq *et al*. (2014) was similar to this study.

Pollution load index PLI for Cd concentration in soil samples collected from three sites was shown in Table. 8. PLI value of Cd (0.337) in S3 sample was highest at Site-III in comparison with other samples. Minimum value for Cadmium was found to be 0.033 in S2 at Site-I. Similar PLI for soil cadmium (0.11-0.12) was recorded by Ahmad *et al*. (2016) as compared to present work. The range (1.11-3.317) for Pollution load index of Cd given by Siddique *et al*. (2019a) was found higher in contrast to current values. Results confirmed that contamination of soil increased by application of wastewater for agricultural crops.

Estimated Daily Intake (EDI): Daily intake of Cd for all milk samples varied widely it is lower for milk collected from control areas but increases gradually in polluted areas. Daily intake of Cd in this study varied from 0.00004 to 0.0001 mg/kg (Table 9). Maximum Daily intake of Cd was noticed at Site-II and lower value was detected at Site-I. Chaoua *et al*. (2018) estimated daily intake (0.001 mg/kg) for Cd was higher than present findings. Ismail *et al*. (2017) suggested similar Daily intake value (0.00015 mg/kg) for Cd in samples as compared with current EDI values. Salah *et al*. (2013); Hassan *et al*. (2013) had estimated highest EDI value (0.158 mg/kg) for Cd.

Target Health Quotient (THQ): The value of THQ for Cd via intake of cow's milk was 0.0403- 0.0597 (Table 10). Highest THQ was observed for samples collected from Site-III. Target Health Quotient was used to evaluate risks coupled with consumption milk contaminated with trace metals. Khan *et al*. (2015) also reported more than 1.0 value for Cd THQ in vegetables. THQ for Cd (1.41) given by Chaoua *et al*. (2018) was found to be high than findings of this study. Current THQ range for Cd was greater than (0.0026) given by Younus *et al*. (2016).

Conclusion

Cows feeding on fodders grown in wastewater irrigated soil can lead to accumulate cadmium in milk. Target health quotient of milk was highest at long term wastewater irrigated agricultural farms. Statistical analysis indicated that water, contaminated fodder, wastewater contaminated soil are common sources contributing heavy metal contamination in milk. The wastewater containing heavy metals is not suitable to irrigate agricultural fields to grow fodder for animals or other plants. The government should make legislation for treatment of wastewater before its use in agricultural fields to avoid food chain contamination to save public health from health risks being caused by intake of contaminated raw milk of cows feeding at wastewater irrigated agricultural fields.

Table 2: Analysis of variance of Cd in soil, fodder, water and milk

Source	**Degree of freedom**	**Mean Square**	
		Soil	**Fodder**
Sites	2	20.837***	2.727***
Soil	7	2.792***	.188***
Sites * Soil	14	.941***	.140***
Error	72	.034	.009
Water			
Source of Variation	**Degree of freedom**	**Mean Square**	
Sites	2	.041*	
Error	9	.009	
Milk			
Source of Variation	**Degree of freedom**	**Mean Square**	
Sites	2	.247***	
Error	72	.031	

*, **, ***: Significant at 0.05, 0.01 and 0.001 levels; ns: non-significant

Table 3: Concentration of cadmium (mg/kg) in soils

Sites	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**
Site-I	0.0604	0.0492	0.3193	0.0797	0.0904	0.0558	0.081	0.0631
Site-II	0.0825	0.1168	0.0744	0.0865	0.0972	0.098	0.0802	0.0986
Site-III	0.0993	0.0967	0.5022	0.144	0.1655	0.114	0.1623	0.1447

Table 4: Concentration of cadmium (mg/kg) in fodders

Sites	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Site-I	0.0307	0.0252	0.0295	0.016	0.0223	0.0336	0.032	0.0175
Site-II	0.0535	0.0484	0.0396	0.049	0.0423	0.084	0.068	0.0757
Site-III	0.0819	0.0688	0.0673	0.0903	0.0727	0.1018	0.095	0.0973

Table 5: Concentration of Cd (mg/L) in water

Sites	**Cd**
Site-I	0.0825
Site-II	0.1755
Site-III	0.2855

Table 6: Concentration of Cd (mg/L) in milk

Sites	**Cd**
Site-I	0.02845
Site-II	0.07232
Site-III	0.04218

Table 7: Bio-concentration factor for cadmium

Sites	F1	F2	F3	F4	F5	F6	F7	F8
Site-I	0.508278	0.512195	0.09239	0.200753	0.246681	0.602151	0.395062	0.277338
Site-II	0.648485	0.414384	0.532258	0.566474	0.435185	0.857143	0.84788	0.767748
Site-III	0.824773	0.711479	0.13401	0.627083	0.439275	0.892982	0.585336	0.672426

Table 8: Pollution load index for cadmium

Sites	S1	S2	S3	S4	S5	S6	S7	S8
Site-I	0.040537	0.03302	0.214295	0.05349	0.060671	0.03745	0.054362	0.042349
Site-II	0.055369	0.078389	0.049933	0.058054	0.065235	0.065772	0.053826	0.066174
Site-III	0.066644	0.064899	0.337047	0.096644	0.111074	0.07651	0.108926	0.097114

Table 9: Estimated Daily Intake for Cd

Sites	**Cd**
Site-I	0.000040304
Site-II	0.000102453
Site-III	0.000059755

Table 10: Target Health Quotient for Cd

Sites	**Cd**
Site-I	0.040304
Site-II	0.102453
Site-III	0.059755

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