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Bioaccumulation of Cadmium in *Gallus* Fed in Punjab, Pakistan: A Human Health Concern

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Abstract. *A research project was conducted to investigate the cadmium bioaccumulation in chickens fed. In this direction, cadmium concentration was determined in maize plant parts and in seven tissues of chickens to study the potential human risk of consumption of these chickens by finding out the cadmium target hazard quotient - THQ (<1). In addition, the pollution load index for soil was also determined. The concentration of cadmium in maize and chicken body parts was determined by atomic absorption spectrophotometer. During the spring, maximum cadmium level (01.60 ± 0.47 mg / kg) was noticed in seeds of cultivar Sadaf irrigated with sewage and waste water. During autumn, the highest concentration of cadmium (0.99 ± 0.04 mg / kg) was noticed in seeds of cultivar MMRI irrigated with sewage water. Results indicated that the transfer of cadmium into chickens via grains owes to the irrigated of these plants with waste water. Pollution load index - PLI values in canal and sewage water treatment were greater than 1.00, indicated that soil is polluted. The results were also debated and related with acceptable values to appraise whether this species pose any health risk to human after its consumption or not. Though the target hazard quotient values for the poultry edibles were less than 1.00, indicated that these parts even from the group of chickens reared on the sewage water treated grains were harmless for human health. But higher Cd concentration in the Gallus group reared on the seeds grown on waste water indicated the need of an ample investigation to combat the problems related to flow of toxic heavy metals in a food chain.*
Keywords: *Cadmium, Sewage water, Maize, Gallus Chickens*

1.Introduction

In both developing and developed countries, environmental degradation is gaining much more importance. Life supporting component of environment are air, water and soil. Rapid industrialization, urbanization and increasing population can pollute these components. Water pollution is the most important problem throughout the world and it is the global cause of various diseases in humans [1]. The enormous amount of industrial, commercial and domestic waste water is discharged directly into fresh water resources such as rivers, streams, lakes, canals and tanks and contaminate these fresh water resources and makes them unfit for human [2]. Sewage water contains heavy metals such as nickel, cobalt, chromium and cadmium along with many nutrients. Accumulation of heavy metal in edible part of crops causes various clinical problems in human by becoming the part of the food chain [3-5]. Now a day's sewage water irrigation becomes more common in many parts of the world [6].

Waste water and industrial discharges comprise of the combination of useful as well as injurious components. Some of these components provide a good percentage of minerals to plants, for that reason rhizosphere let them accumulate for their clearance and disposal. But there may be a higher concentration of such Cd, Ni, Pb and Cr in untreated sewage and industrial effluents [7- 9].

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Predominantly in the world the major entanglement is the food safeness because of the contamination of heavy metal and human health peril via food chain. Soil can be an implied source of adulteration for plants as well as for animals, once it is contaminated with metals [10].

Members of Class *Aves*, including *Gallus* as well as fowl, are vulnerable to accumulate contaminants principally by utilization of desecrate food [11]. Chickens can be used as a functional standard to check environmental containment, for the pyramid type food web level [12].

Cd available in shallow layers of soil, is an invariably poisonous metal and can result in plants damage as well as to a number of living organisms. It can disturb a cultivar of enzymatic activities, hinder the photosynthesis mechanism, disturb water and nutrients uptake mechanism and also cause oxidative stress in plants, [13]. Similarly, Cd can cause kidney ailments, bone problems and heart diseases with some other malfunctions of vital organs in humans [14]. The present research was planned with objectives: (1) to quantify the metal contents in root, shoot & grains; (2) to analyze level of Cd in body parts of chicken (3) to assess the health risk index via intake of adulterated maize grains.

2.Materials and methods

2.1.Plant cultivation

Experiment was a soil-based culture and was conducted in plastic bags at Agriculture College, University of Sargodha, Pakistan. Maize and Millet Research Institute Yousafwala, Sahiwal, was approached to obtain seeds of four different maize cultivars including, Pearl (white), MMRI (yellow), Sahiwal 2002 (yellow) and Sadaf (white). Current investigation followed completely randomized design (CRD). After maturity of all maize cultivars, their grains were threshed from cobs. One portion of grains was sorted for Cd analysis and second portion was fed to chickens.

Chicken (Missouri golden breed) were used as an indicator to assess the metal translocation ratio. 2 days old chickens were bought from a hatchery in Sargodha. To keep the *Gallus*, a small enclosure was prepared according to the number and size of chickens' domestication needs. Two separately allocated chickens were fed the feed made of the maize grains obtained by repeating three treatments according to irrigation waters. Up to their maturity, these chickens were nourished with the maize grains (variable constant) obtained for conducting the study along with the standard chickens feed (constant factor).

2.2. Sample collection

Chickens after 45 days were slaughtered and their body parts were separated for analysis to assess the movement transfer of Cd from maize to hens. Wet digestion method was used for the preparation of samples. Analysis was done by using the method of Atomic Absorption Spectrophotometer; model AA-6300 Shimadzu Japan. The result data were then subjected to statistical analysis using SPSS software.

2.3. Pollution load index

Pollution load index (PLI) is the indication of metal concentration at a given site [15].

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

2.4.Target hazard quotient

Target hazard quotient (THQ) can be computed as following [16].

$$THQ = (EF \times ED \times FIR \times C/RfD \times BW \times AT) \times 10^{-3}$$

where,

EF = Exposure frequency (days/year)
ED = Exposure duration (year)
FIR = Food ingestion rate
(Ingestion rate by chicken = 32.7 g per individual per day),
C = Concentration of metal in tissues of chicken (mg per kg dry weight)
RfD = Oral reference dose of metal ($mgkg^{-1}day^{-1}$)

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BW = Average body weight (70 kg)
TA = Average exposure time for noncarcinogens (365 day/year × ED)

3.Results and discussions

3.1. Cadmium concentration in water sample

ANOVA of data for concentration of cadmium in water samples depicted a significant variation of cadmium concentration in all water sources (Table 1). Mean Cd contents in tap water, sewage water and canal water were 0.01, 1.98 and 1.91 mg/L respectively (Figure 1). In both seasons, all the waters [ground water (GW), sewage water (SW) and canal water (CW)] applied for watering were from the same sources and the values for Cd in water were same in both seasons. The maximum PML for Cd in water is 0.01mg/L. The Cd concentration in all water samples crossed the limit set by PML except that of ground water [17]. The order for the Cd concentration of waters was descending trailed sewage/waste H_2O > canal H_2O > ground H_2O (Figure 1). While comparing Cd values with all waters, it was higher in soil samples. This higher Cd value may be contributed to repeated irrigation of target soil with water impregnated with Cd. The soil trend on Cd values was the similar to that found for water samples i.e. sewage>canal>ground one (Figure 2).

Table 1. Analysis of variance (mean squares) for cadmium regarding water used for irrigation during the experiment

Mean squares		
Source of variation	Degree of freedom	cadmium in water
Source	2	3.7381**
Error	6	0.0016

**: Significant at 0.01 level

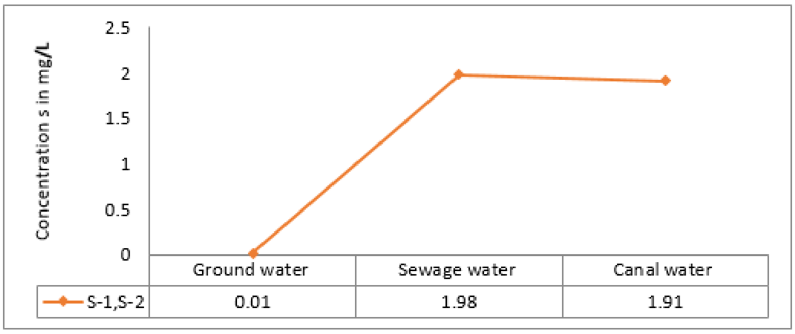

Figure 1. Concentration of cadmium in water

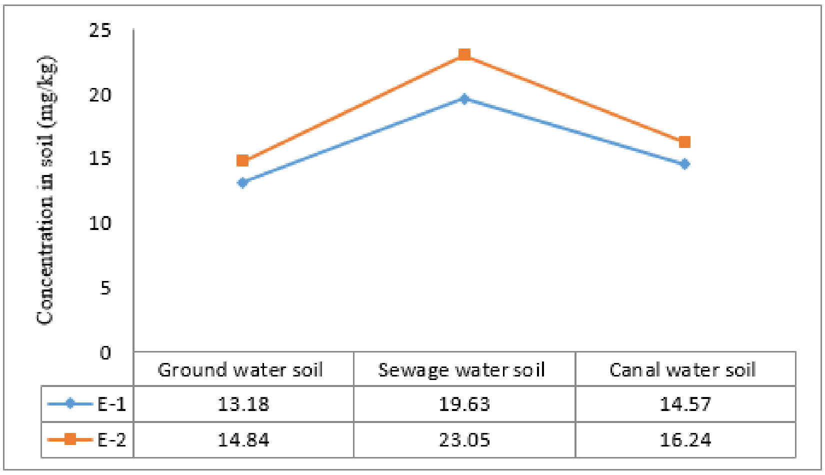

Figure 2. Concentration of cadmium in soil (mg/kg) irrigated with canal, ground or sewage water in two experiments

3.2.Cd concentration in soil

ANOVA of the data containing cadmium content in soil demonstrated a significant difference in metal concentration in soils treated with different sources of water (Table 2). For experiment 1 (spring period) the maximum level of cadmium in soil was found in sewage water treatment (SWT) (19.73 mg.kg) followed by canal water treatment (CWT) (14.57 mg/kg) and ground water treatment (GWT) (13.18 mg/kg). For experiment 2 (spring period) the highest level of cadmium in soil was found in SWT (23.05) followed by CWT (16.24 mg/kg) and GWT (14.84 mg/kg) (Figure 2). Sewage water had the elevated concentration of metal in it hence the site watered with SW presented the bigger level of Cd as the magnitude of metal accumulation in sewage water-irrigated soils depends upon the duration of exposure to contaminated water [18, 19]. In soil irrigated with sewage water, the metal values were above the permissible limits of FAO / WHO [20] (i.e. 3 mg/kg), in both seasons. Further noticed interestingly, that canal water's Cd content were on higher side while in soil, Cd concentration was comparatively lower. There would be likely hood this caused by different edaphic factors of soil like pH, redox potential, amount of organic matter in soil and rate of addition of the metal which influenced the rate of adsorption and preservation in the soil [21].

Table 2. Analysis of variance for cadmium in soil (mg/kg) irrigated with canal, ground or sewage water in two experiments

Mean square			
Source of variation	Degree of freedom	soil cadmium E-1	soil cadmium E-2
Source	2	3.6104**	4.1281**
Error	6	0.1091	0.0998

**: Significant at 0.01 level

3.3.Concentration of cadmium in different plant parts

ANOVA of figures collected from content of cadmium in maize grains, shoot and root of plants, is presented in Table 3. During both experimental seasons (autumn & spring), varieties and treatments affected significantly the level of cadmium concentration. Mutual interaction of season × variety, season × treatment, season × cultivar × treatment was also significant.

Table 3. Analysis of variance (mean squares) of data for maize grains, shoot and root of 04 cultivars differing in concentrations of cadmium when plants were grown in pots and irrigated with ground, sewage and canal water in both experiments conducted in different seasons

Mean squares				
Source of variation	Degree of freedom	cadmium grains	cadmium shoot	cadmium root
Season (S)	1	0.9656**	0.8190**	2.1081*
Cultivar(V)	3	0.1527*	0.6058**	0.2882ns
Treatment (T)	2	4.3037**	7.6753**	10.0529**
S×V	3	0.7928**	0.2094ns	0.5323ns
S×T	2	0.2405**	0.1711ns	0.3407ns
V×T	6	0.0482ns	0.2037ns	0.0638ns
S×V×T	6	0.2378**	0.1220ns	0.2106ns
Error	48	0.0399	0.0897	0.4454

**, **: Significant at 0.05 and 0.01 levels, ns: non-significant S: Seasons; V: variety; T: treatment

For experiment 1 (spring season) maximum concentration of cadmium (1.60 ±0.47 mg/kg) for grains existed in cultivar Sadaf treated with sewage water and minimum (0.01±0.00 mg/kg) Cd concentration in grains of cultivars Pearl and MMRI irrigated by ground water. In case experiment-2 (the autumn period) highest cadmium level of 0.99 ± 0.04 mg/kg was noticed in MMRI cultivar grains when watered with waste water, though the lowest level of cadmium was 0.01±0.00 mg/kg in grains of cultivar MMRI grown with ground water (Fig. 3). Similarly, in case of experiment-1 (spring period) highest Cd level

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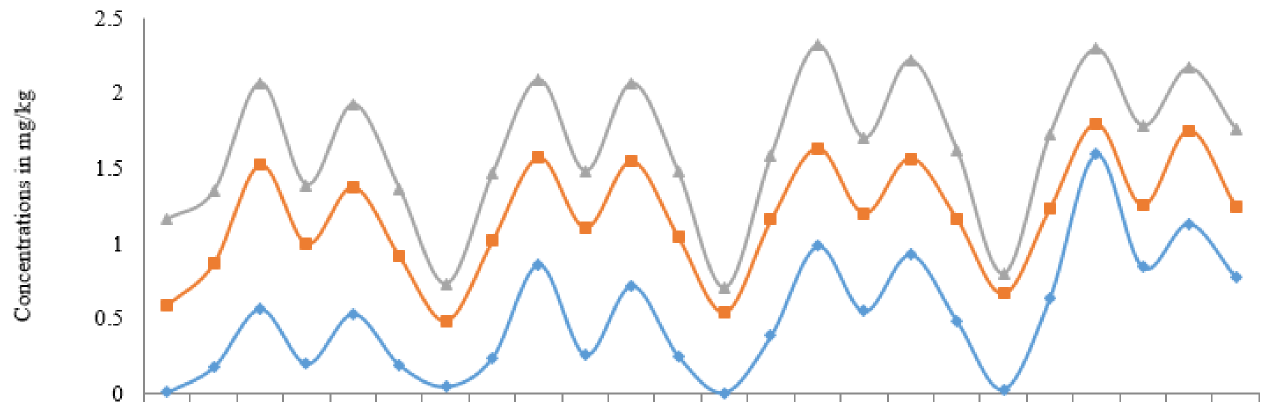
for plant shoot (1.80 ± 0.08 mg/kg) was noticed for cultivar Sadaf at treatment with sewage water while the lowest level (0.49±0.06 mg/kg) was noticed in shoot of cultivar Sahiwal, watered with ground water. However, in experiment-2 (autumn period) the highest Cd level of 0.99±0.04 mg/kg was recorded in cultivar MMRI that was treated with sewage water while the lowest Cd concentration (0.30±0.00 mg/kg) was recorded in cultivar Pearl grown with ground water irrigations (Figure 3). On other hand for experiment-1 (spring season) the maximum level of cadmium (2.30±0.18 mg/kg) in roots was noticed in cultivar Sadaf grown with sewage water and minimum Cd concentration (0.70±0.06 mg/kg) was noticed in MMRI cultivar's roots grown with ground water irrigations. In experiment 2 (autumn period) maximum Cd level (2.27±0.17 mg/kg) was found in Sahiwal cultivar's shoot grown with sewage water treatment while minimum one was recorded in cultivar MMRI grown with ground water (Figure 3).



	Pearl						Sahiwal-2002						MMRI						Sadaf					
	GWT		SWT		CWT		GWT		SWT		CWT		GWT		SWT		CWT		GWT		SWT		CWT	
	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
Grains	0	0.17	0.56	0.2	0.53	0.19	0.04	0.24	0.86	0.26	0.72	0.25	0	0.38	0.98	0.55	0.93	0.48	0.03	0.63	1.6	0.84	1.13	0.78
shoot	0.59	0.87	1.52	1	1.38	0.91	0.49	1.02	1.57	1.1	1.55	1.04	0.54	1.16	1.63	1.2	1.57	1.16	0.67	1.23	1.79	1.25	1.75	1.25
Root	1.16	1.35	2.07	1.39	1.93	1.36	0.73	1.46	2.09	1.48	2.07	1.47	0.7	1.59	2.32	1.7	2.22	1.62	0.79	1.72	2.3	1.78	2.17	1.76

Figure 3. Concentration of cadmium in grains, shoot and root of four varieties of maize irrigated with ground, sewage and canal water in both experiments conducted in varying seasons

All varieties exhibited different designs for accumulation of cadmium under changed treatments (ground, sewage and canal water) during two seasons (autumn & spring). Maize grains obtained from the test for feed of chickens under tail were impregnated with considerable amount of heavy metal in them and those led to accretion of the metal in chicken bodies. From the above outcomes and results comparison of cadmium concentration among four varieties of maize under three treatments in both seasons can be made easily.

In the present experimental studies performed during both of the seasons (autumn and spring), all four varieties of maize [Pearl (white), Sadaf (white), Sahiwal 2002 (yellow), MMRI (yellow)] and treatments (sewage, ground. canal water) were affected significantly by the Cd concentrations. Every cultivar exhibited different concentrations of cadmium in each of its part with each treatment. It may be reasoned by difference in cultivars, like Wahid and Ghani [22]; likewise reported by Abbas et al. [23] that species and varietal differences in crops varies in metal uptake, independent of treatments and seasons. Studies done by Kabata-Pendias and Pendias [24], showed that a number of factors regulate the availability of mineral nutrients to plants, such as condition of rhizosphere and climate, genotypic traits of plant, agricultural practices, and interaction of plant roots towards minerals in response to seasonal fluctuations. Grains were found to accumulate the least Cd concentration as compared to other plant parts and which was confirmed by other researchers too [25, 26]. The lower metal content in grains can be explained by the high affinity of metal ions to roots, thus poor transportation causes lesser accumulation in grains [27, 28].

In current study, the descending order for cadmium concentration was recorded as root > shoot > grain amongst all maize cultivars regardless of the treatments involved in E-1. Payus et al. [29] recorded a similar Cd concentration tendency in maize plant i.e. root > shoot > grain. However, in E-2, the cultivars Pearl and Sadaf exhibited a dissimilar findings i.e. Pearl and Sadaf exhibited a different sequence, i.e. root > grains > shoot at CWT. This deviation can be contributed to plants characteristics and physiology of different among species and cultivars for the quantification of Cd metal which was absorbed from soils. Further complicated mechanism of diversity may cause different rates of absorption and transportation of minerals from roots to shoots. Diversity of rhizosphere and architecture of plant root modify the movement and metal uptake by roots [30- 32].

The PML concentration of Cd define by FAO/WHO [33] in maize and cereals grains is 0.2 mg/kg [34]. In current study during E-1 (spring period) the maximum mean Cd contents in grains recorded was 1.60 ± 0.47 mg/kg recorded in cultivar Sadaf grown by SWT and was comparatively bigger than given by Chinese standards. On other hand the minimum Cd concentration to the tone of 0.01±0.00 mg/kg was recorded in cultivars Pearl and MMRI grains grown with GWT. Payus et al. [29] determined the similar values in maize grains.

In E-2 (autumn period) maximum Cd level 0.99±0.04 mg/kg was noticed in MMRI grains at STW which was lower than that recorded by Payus et al. [29], and minimum concentration of cadmium (0.01±0.00 mg/kg) was in MMRI grains at GWT. Hence, during both seasons concentration at sewage water irrigated pots the values for cadmium were high and it was confirmed by Payus et al. [29]. WHO recommends 0.02 mg/kg as PML for Cd in plants [35].

In current study, by E-1 (spring period) the maximum Cd level for shoot (1.80±0.08 mg/kg) was recorded in cultivar Sadaf grown by SWT while in E-2 (autumn period) maximum level of cadmium (0.99±0.04 mg/kg) was recorded in shoots of cultivar MMRI grown by SWT that was bigger than the values determined by Payus et al. [29].

In E-1, on other side (spring period) the lowest 0.49±0.06 mg/kg was recorded from the shoot of cultivar Sahiwal grown by GWT and in E-2 (autumn period) and minimal concentration of cadmium 0.30±0.00 mg/kg was recorded in cultivar Pearl grown by GWT that was comparatively higher than that recorded by Payus et al. [29]. During both seasons the values for cadmium in plants in current study were on higher side than the WHO standards and the same was irrespective of treatments and varieties involved.

In the study for E-I (spring period) the maximum Cd level in roots was recorded in Cultivar Sadaf grown by SWT (2.30±0.18 mg/kg) and the minimum concentration of Cd was recorded in roots of cultivar MMRI grown by GWT (0.70±0.06 mg/kg). While in E-2 (autumn period) maximal level of Cd 2.27±0.17 mg/kg was recorded in shoots of cultivar Sahiwal-2002 grown by SWT and minimal cd concentration 0.70±0.23 mg/kg was recorded in cultivar MMRI grown by GWT and during both seasons' values for Cd were comparatively higher than noticed by Payus et al. [29].

In present research, the high concentration of cadmium was shown by the SWT plants. SWT exhibited the higher Cd concentration in all maize varieties during both seasons that was trailed by CWT, and was the minimum in GWT pots as the sewage water shows the higher Cd level. This confirms with study by Schmidt [36] that toxic metals, including Cd, Cu, Ni, Zn and Pb were frequently existed in waste water and in significant concentrations.

3.4.Distribution of cadmium in chickens' anatomic parts

ANOVA of data for cadmium in chickens' bone and meat, blood, heart, liver, kidney and gizzard is presented in Table 4. Seasons, cultivars & treatments affected significantly the cadmium concentration in chickens' blood, heart, bone and kidney while treatments accounted non-significantly the cadmium concentration in chickens' meats, livers and gizzards. Interaction between season × variety was significant too, for all chickens' anatomic parts, except chickens' hearts. On same lines interaction between season × treatment was significant for chickens' bones and their livers only but interactions between cultivar × treatment and interactions among season × cultivar × treatment were non-significant in all chickens' body parts except for bones.

Figure 4 is presenting that during experiment-1 (spring period) the maximum Cd contents for blood of chickens (0.24±0.24 mg/kg) was recorded in the chickens that were fed with grains of cultivar Sadaf, grown by applying canal water and the minimum contents of Cd were recorded in the chickens' blood (0.01±0.00 mg/kg) who were fed grains of cultivar Sahiwal, grown by ground water irrigation. While during experiment-2 (autumn period) maximum Cd contents were recorded in the chickens' blood (0.73±0.00 mg/kg) who were fed grains of cultivar Sadaf, grown by sewage water irrigation and minimum Cd contents (0.33±0.00 mg/kg) were recorded in the blood of chickens fed on grains of cultivar Pearl grown with ground water.

Table 4. Analysis of variance (mean squares) for cadmium for blood, bone and meat

Mean squares								
Source of variation	Degree of freedom	Blood	Bone	Meat	Liver	Heart	Kidney	Gizzard
Season (S)	1	1.9445**	0.9761**	3.6603**	7.5724**	15.6865**	14.8074**	23.1505**
Cultivar(V)	3	0.0485**	0.0449**	0.0091**	0.4889**	0.0416**	0.0258**	0.1676**
Treatment (T)	2	0.0308*	0.0502**	0.0032ns	0.0355ns	0.0241**	0.0101*	0.0150ns
S×V	3	0.0818**	0.0353**	0.0119**	0.4080**	0.0092ns	0.0142**	0.1814**
S×T	2	0.0022ns	0.0341**	0.0046ns	0.2186**	0.0103ns	0.0013ns	0.0166ns
V×T	6	0.0035ns	0.0116**	0.0004ns	0.0719ns	0.0038ns	0.0033ns	0.0102ns
S×V×T	6	0.0067ns	0.0149**	0.0009ns	0.0524ns	0.0043ns	0.0010ns	0.0041ns
Error	24	0.0061	0.0022	0.0013	0.0384	0.0034	0.0020	0.0139

**, **: Significant at 0.05 and 0.01 levels, ns: non-significant S: Seasons; V: variety; T: treatment

During experiment-1 study (spring period) minimum Cd concentration for chickens' bone (0.51±0.01 mg/kg) was recorded among chickens who were fed grains of cultivar Sadaf, grown by ground water irrigation and maximum value of Cd was recorded amongst the chickens' bone (0.57±0.02 mg/kg) who were fed on grains of cultivar Sahiwal grown by applying sewage water. While in experiment-2 (autumn period) minimum Cd concentration was noticed in chickens' bones (0.43±0.08 mg/kg) who were fed on grains cultivar MMRI grown by ground water irrigation and the maximum Cd contents (1.01±0.02 mg/kg) were recorded in chickens fed on grains of cultivar Sadaf grown by applying sewage water (Figure 4).

The minimum Cd concentration in experiment-1 (spring period) for meat was recorded (0.52±0.01 mg/kg) in chickens who were fed on grains of Sahiwal cultivar grown by ground water irrigation while maximum Cd contents were recorded (0.63±0.01 mg/kg) in meat of the chickens who were fed grains of cultivar Sadaf grown by canal water irrigation.

In study experiment-2 (autumn period) the minimum Cd contents were recorded in chickens' meat (1.03±0.00 mg/kg) who were fed grains of Pearl cultivar grown on ground water irrigation and the maximum Cd contents (1.20±0.01 mg//kg) were recorded in meat of chickens fed with grains of Sadaf grown by applying sewage water. After consumption of variedly grown and hence treated grains of different maize cultivars, chickens' meat exhibited different designs for Cd accumulation during both of the seasons under study (Figure 4).

The highest Cd concentration in study experiment-1 (spring period) for chickens' liver was recorded 1.68±0.35 mg/kg for chickens who were fed grains of Pearl cultivar grown by irrigating with ground water while the lowest Cd contents were found in the liver (0.26±0.01 mg/kg) of chickens who were fed grains of Sahiwal cultivar grown by applying ground water. In study experiment-2 (autumn period) maximum Cd concentration was recorded in the chickens' liver (1.82±0.05 mg/kg) who were fed grains Sadaf cultivar, grown by sewage water application while minimum Cd contents (1.25±0.00 mg/kg) were recorded in livers of chickens fed on grains of cultivar Sahiwal grown by ground water. After utilization of variously treated grains of different maize cultivars, liver of chickens exhibited different order for cadmium accumulation during both the seasons (Figure 4).

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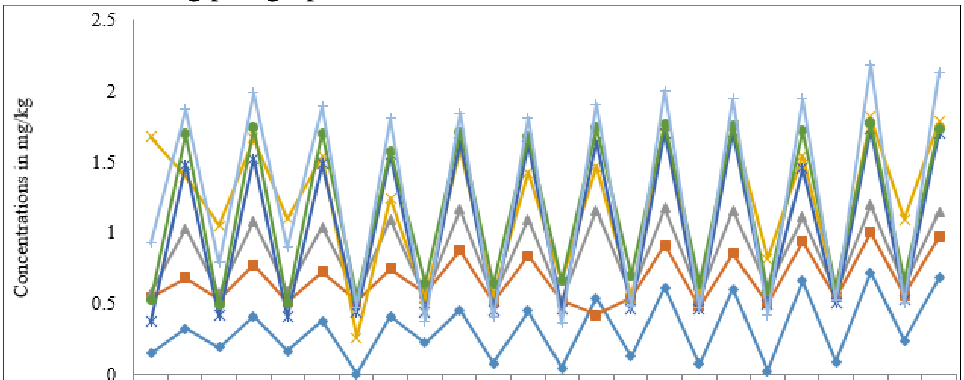
The maximum contents of cadmium in experiment-1 (spring period) in chickens' heart (0.54±0.00 mg/kg) were recorded in chickens who were fed grains of cultivar Sadaf grown with canal water irrigation while the minimum Cd contents were noticed in heart (0.38±0.03 mg/kg) of the chickens who were fed grains of Pearl cultivar grown by ground water irrigation. In study experiment-2 (autumn period) maximum Cd concentration was recorded in chicken-heart (1.73±0.02 mg/kg) of chickens who were fed grains of Sadaf cultivar grown by applying sewage water while the minimum Cd contents (1.46±0.05 mg/kg) were recorded by consumption of grains of Sadaf cultivar grown on ground water irrigation. Following consumption of variedly grown grains of different cultivars, chickens' heart exhibited different Cd accumulation pattern during both of the seasons (Figure 4).

The maximum Cd concentration in experiment 1 (spring period) for chickens' kidney (0.70±0.01 mg/kg) was noticed in chickens fed on grains of MMRI cultivar grown on application of sewage water and the minimum Cd contents were recorded in chickens' kidney (0.50±0.03 mg/kg) who were fed on grains of Pearl cultivar grown on sewage water. In experiment study 2 (autumn period) maximum Cd concentration was recorded in the kidney (1.77±0.07 mg/kg) of chickens who were fed grains of Sadaf cultivar grown on application of sewage water while the minimum Cd contents (1.58±0.02 mg/kg) were recorded on feeding of grains of Sahiwal cultivar grown on ground water irrigation. Hence after consumption of variously grown and treated grains from different maize varieties kidney of chickens exhibited different Cd accumulation patterns during both of the seasons (Figure 4).

The maximum Cd concentration in experiment-1 (spring period) for chickens' gizzard recorded 0.93±0.03 mg/kg was recorded for chickens who were fed on grains of Pearl cultivar grown with ground water and the minimum Cd contents were recorded in chickens' gizzard (0.37±0.06 mg/kg) who were fed on grains of MMRI cultivar grown by ground water. While in experiment study 2 (autumn period) the maximum Cd concentration was recorded in the gizzard (2.18±0.01 mg/kg) of chickens who were fed on grains of Sadaf cultivar grown by sewage water while the minimum Cd contents (1.81±0.01 mg/kg) were recorded on feeding of grains of Sahiwal cultivar grown by ground water and canal water. Hence after consumption of variedly treated grains of different maize varieties, gizzard of chickens exhibited different cadmium accumulation pattern during both of the seasons (Figure 4).

Similarly, after consumption of differently treated grains of different maize varieties, blood of chickens exhibited different cadmium accumulation patterns during both of the seasons (Figure 4). Details are given in following paragraphs.



	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
	GWT		SWT		CWT		GWT		SWT		CWT		GWT		SWT		CWT		GWT		SWT		CWT	
	Pearl						Sahiwal-2002						MMRI						SADAF					
Blood	0.2	0.3	0.2	0.4	0.2	0.4	0	0.4	0.2	0.5	0.1	0.5	0.1	0.5	0.1	0.6	0.1	0.6	0	0.7	0.1	0.7	0.2	0.7
Bone	0.5	0.7	0.5	0.8	0.5	0.7	0.5	0.8	0.6	0.9	0.5	0.8	0.5	0.4	0.5	0.9	0.5	0.9	0.5	0.9	0.5	1	0.6	1
Meat	0.6	1	0.6	1.1	0.6	1	0.6	1.1	0.5	1.2	0.5	1.1	0.5	1.2	0.6	1.2	0.6	1.2	0.6	1.1	0.6	1.2	0.6	1.1
liver	1.7	1.4	1.1	1.7	1.1	1.5	0.3	1.2	0.5	1.6	0.6	1.4	0.7	1.5	0.5	1.8	0.6	1.8	0.8	1.5	0.6	1.8	1.1	1.8
heart	0.4	1.5	0.4	1.5	0.4	1.5	0.4	1.5	0.4	1.6	0.5	1.6	0.5	1.6	0.5	1.7	0.5	1.7	0.5	1.5	0.5	1.7	0.5	1.7
kidney	0.5	1.7	0.5	1.7	0.5	1.7	0.6	1.6	0.6	1.7	0.6	1.7	0.7	1.7	0.7	1.8	0.6	1.8	0.6	1.7	0.6	1.8	0.7	1.7
Gizzard	0.9	1.9	0.8	2	0.9	1.9	0.5	1.8	0.4	1.8	0.4	1.8	0.4	1.9	0.5	2	0.5	1.9	0.4	1.9	0.5	2.2	0.5	2.1

Figure 4. Concentration of cadmium in blood, bone and meat in two experiments

In current research study, the values for cadmium level in chickens' blood during E-1 (spring period) was 0.24±0.24 mg/L and in E-2 (autumn season) was 0.73±0.00 mg/L, which were fed grains of Sadaf cultivar grown by applying CW and grains of Sadaf cultivar grown with SW respectively were recorded on higher side than the values reported by Zhuang et al. [34] (0.042±0.016 mg/L) in chickens who were fed on rice grains feed, grown with contaminated soil, in their study. On the other side, in their study [34] the values for cadmium in the blood of chickens' which consumed uncontaminated feed was 0.027±0.021 mg/L. While in current study the lowest Cd contents in experiment -1 (Spring period) were recorded in the chickens' blood (0.01±0.00 mg/L) of those birds who were fed grains of Sahiwal cultivar grown by applying GW and in expriment-2 (autumn period) the minimum (0.33±0.00 mg/L) Cd value in blood was resulted after consumption of grains of Pearl cultivar grown by applying GW, hence Cd values were slightly lower for experiment-1 and slightly higher for expriment-2 than that reported by Zhuang et al. [34] however the impact resulted by consumption of contaminated feed in both studies was fairly similar. The GWT grains exhibited the minimum Cd levels in chickens' blood than SWT and CWT maize grains. However, fortunately the Cd values recorded from all blood samples, regardless of the study seasons / periods, cultivars and treatments used for the birds, were within the permissible limits of FAO/WHO (0.5–1.0 mg/L).

In present experimental study, during both of the seasons, viz Spring and Autumn, the maximum (E-1, (0.57±0.02 mg/kg); E-2, 1.01±0.02 mg/kg) and the minimum (E-1, 0.51±0.01 mg/kg; E-2, 0.43±0.08 mg/kg) Cd concentrations of bones was much less than those reported by Eeden [37] while working on Red knobbed Coots, and Kim et al. [38] while conducting study on heavy metals in tissues including bones, of five shorebird species including bone). Our values vary with that of their findings, which may be due to numerous factors like growth, age, breeds, mode of feeding, etc., which may affect accumulation and transportation of metals within the body of birds [39, 40].

The maximum admissible cadmium levels in chicken tissue are 0.5 mg/kg fixed by Saudi Arabia standards and by Anonymous [41]. All the samples of breast meat contained metal content greater than the PML during both of the study seasons. Similarly, cadmium concentrations in meat recorded in present study was on higher side as compared with that noticed by Mariam et al. [42] who found cadmium content of 0.33 mg/kg, 0.37 mg/kg and 0.31 mg/kg for lean beef meat, mutton and broiler meat, respectively. Additionally, the higher Cd contents were recorded than those reported by Kalisińska and Salicki [43] who performed an experimental study to determine Pb as well as Cd contents in *Gallus* tissue samples.

In current study, the Cd values in liver exceeded those reported by Ramadan et al. [44] while reported cadmium contents in liver i.e. 0.085 mg/kg and 0.079 mg/kg prevailing at two poultry sheds, EL-Akoria and Gernada respectively. In first experiment E-1 (spring season) the maximum Cd contents (1.68±0.35 mg//kg) in chickens' liver were recorded who consumed grains from Pearl cultivar grown by applying GWT, though grains of Pearl cultivar with GWT exhibited the least Cd contents in them but chickens' liver showed the highest cadmium, as not surprising fact, because of kidney and liver of birds can hoard 65 % of total Cd that enters into chickens' body [45]. Possibly contaminated air may be a source for cadmium accumulation in the chickens' body augmented with Cd ingestion through contaminated feed [46]. Among the chickens which consumed grains of Sahiwal grown with GWT, the contents of Cd (0.26±0.01 mg/kg) in liver was recorded the least ones in E-1 (spring season) and the value was within the PML fixed by FAO/WHO (0.5-1.0 mg/kg) while the other values were over and above than standards of FAO/WHO. Chickens liver stored Cd varyingly according to the cultivars they consumed under different treatments. In E-2 (autumn period), cadmium concentrations in the chickens' liver, maximum (1.82±0.05 mg/kg) and minimum (1.25±0.00 mg/kg) were in chickens who were fed, SW grown grains of Sadaf cultivar and GW grown grains of Sahiwal cultivar. Values of Cd in chickens' liver were greater when compared to those of Siberian gull of reserve Hara biosphere, Southern Iran (1.1 mg/kg) [47] and black-crowned night heron birds (1.00 mg/kg) [12]. Consequently, it may be recommended that elevated levels of Cd in birds livers are exhibited when fed with grains containing a cadmium contents (0.08±0.01 mg/kg). Though the grains contained cadmium within PML set by Chinese coarse cereals including the maize seeds i.e. 0.2 mg/kg [33] but it can't be nullified that even an essential metal becomes toxic if ingested excessively [48, 49, 50, 51].

In our study the maximum concentrations of Cd in heart were (E-1, 0.54±0.00 mg/kg; E-2; 1.73 mg/kg) and minimum were (E-1, 0.38±0.03 mg/kg; E-2, 1.46±0.05 mg/kg). The maximum Cd values in chickens' heart were recorded in those birds who were fed on CW and SW grains and less was reported in those who were fed on GW grown grains. Though the CW and SW treated grown grains had Cd contents of harmless limits 0.2 mg/kg [33] but they showed the elevated concentration than GW treated grown grains. The present results concurred with the results of Millaku et al. [52] where the values for cadmium in sparrows' heart in contaminated area (1.214±0.617 µg/g) was on higher side as compared with the reference area (0.228±0.179 µg/g). Similar to our results, the higher values were found in those heart samples of chicken who were fed on grains with high cadmium levels. Hence, the accumulation of heavy metal in an animal's body not only depends on the feed they consumed but also on the habitat quality in which they nourish, because the tissue amassing of metals is also dependent on the pollution of air, soil and plants in a given environment especially in baby birds [53].

In present study maximum concentrations of Cd in kidney were (E-1-0.70±0.01 mg/kg; E-2; 1.77±0.07 mg/kg) while minimum contents were (E-1, 0.50±0.03 mg/kg; E-2, 1.58±0.02 mg/kg). The high Cd level in kidney was noticed in chickens that were fed on comparatively higher concentration of cadmium in the grains than the chickens who consumed less cadmium comprising grains. This established that more the concentration of the metal in the feed had resulted in more danger its accumulation in kidneys of chickens. It also was long-established by the Zhuang et al. [34] by dividing birds in two clusters (fed on contaminated rice feed) with control (fed on uncontaminated feed) and recorded that first cluster showed (4.64±1.56 mg/kg) as compared to one control cluster that showed (1.73±0.52 mg/kg) and hence exhibited the higher concentration of cadmium in their kidney. Similarly, Herzig et al. [54] divided chicken in to four groups (K, HA, Cd and Cd + HA.) on the basis of the type of the feed they consumed. The group which had the pure cadmium in their diet showed the maximum concentration of cadmium in the tissues of the hens included kidney (4.99±1.57 mg/kg). However, the above values were above than our research and may be contributed to the variations in crop and bird species selected in the both studies. In E-2 (autumn period) maximum Cd concentration in chickens' kidney were found higher than the permissible limits set by the European Commission Directive (EC) 466/2001 i.e., 1.0, of fresh weight [41].

The maximum Cd contents (E-1- 0.93±0.03 mg/kg and E-2- 2.18±0.01 mg/kg) and minimum ones (E-1- 0.37±0.06 mg/kg and E-2-1.81±0.01 mg/kg) in chickens' gizzard were found higher than those reported by Okoye et al. [63] i.e. (0.156 ± 0.019 mg/kg for layers, 0.131 ± 0.032 mg/kg for broilers, and 0.243 ± 0.039 mg/kg for local chickens got from various sheds) and Iwegbue et al. [55] by collecting chickens' gizzard samples from different seven vicinities in Nigeria. However, the higher Cd values were reported by Begum and Sehrin [56] while working on heavy metals in different parts, of pigeon included gizzards in Bangladesh. Additionally, the gizzard samples in our study research was determined Cd levels which are above the harmless limits fixed by standards recommended by FAO/WHO (0.5–1.0 mg/kg). The cause of high values, in current study, as compared with above mentioned researchers' work, was probably because feed grains had substantial cadmium in them.

Above results as well as discussion recorded that each body part of chickens involved, accumulated different cadmium levels during both of the study seasons that may be contributed to variation in feeds as confirmed by several researchers [34, 54]. In E-1, the maize feed grains obtained the higher Cd contents as related to that of E-2 ones. Contrarily, the chickens' body parts exhibited the higher cadmium contents in E-2, as related to E-1 ones, as Cd assimilation and accretion in birds' tissues is dependent upon a variety of other factors like nourishing materials and vitamin, age group and gender of birds [57]. Hence, Cd concentration in feed is not the sole indicator of the metal concentration in the tissue's absorption of Cd in gastrointestinal tract of chicken is affected by low-calcium diet which enhanced the absorption and accumulation of Cd in tissues [58].

3.5.Pollution load index for cadmium

During both of the seasons, values PLI were higher for soils of SWT and CWT. PLI > 1.0 indicated that presence of Cd can result in risk, and soils were not safe for cultivation and crop production (Fig. 5) [15] **(**Reference value for Cd in soil as suggested by Singh et al. [59] 1.49 µg/g). Outcomes for PLI (Cd) in current study, were in conformity with those reported by Khan et al. [60] who assessed the amount of accumulation of metals including Fe, Zn, Pb, Ni, Mo, Cu, As, Se, in mustard (*B. campestris* L.), where it was watered with ground, sewage and canal waters in Punjab, Pakistan. Resultantly, higher values for PLI of all study metals were recorded in all soils watered with sewage water, trailed by canal water and ground water. The current research study also recorded similar trend for PLI of Cd in order of, sewage water > canal water > ground water.

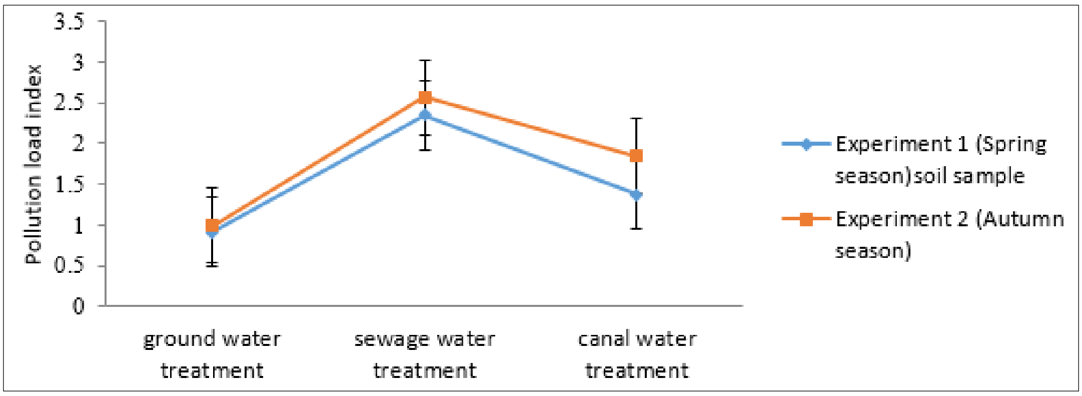


Figure 5. Pollution load index for cadmium in soil irrigated with ground water, sewage water and canal water in two experiments conducted in varying seasons

3.6.Target Hazard Quotient for cadmium

THQ values were recorded within safe limits for three chicken body parts (meat, liver and gizzard) among all chickens including from all the groups. This indicated that chickens were safe for human consumption but anatomic parts of chickens from the groups reared on SW grown grains exhibited the higher THQ values as compared with CW and GW grown grains. This again indicated the possibility of health risk (Fig. 6). During both of the seasons' studies, the chickens' anatomic parts exhibited the THQ less than 1.0, health risk estimates, irrespective of treatments and varieties, determined on the base of the Cd levels recorded in the chickens' liver, breast muscle and gizzard. Eatables and nutritious parts of all chickens involved in study, although they consumed different feed grains of maize, showed THQ less than 1.0, confirmed that, they were safe for human consumption during both of the seasons, except the gizzard (THQ 1.019) of the chickens who were fed on grains of Sadaf cultivar grown on sewage water in E-2. A similar assumption in Nigeria, was reported by Oforka et al. [61], they discovered that the excessive exposure of chickens to metals including Cd, Pb, Zn, Mn, and Ni had not posed any looming risk to human health, even by eating chicken meat, liver, and gizzard. Generally, present results of study were in conformity with the preceding studies conducted in Malaysia on eggs of domestic hens. Abduljaleel and Othman [62] vide the risk quotient, concluded that there was no apparent health risk to local population, by eating eggs of domestic hens who were tamed in the areas of heavy metals.

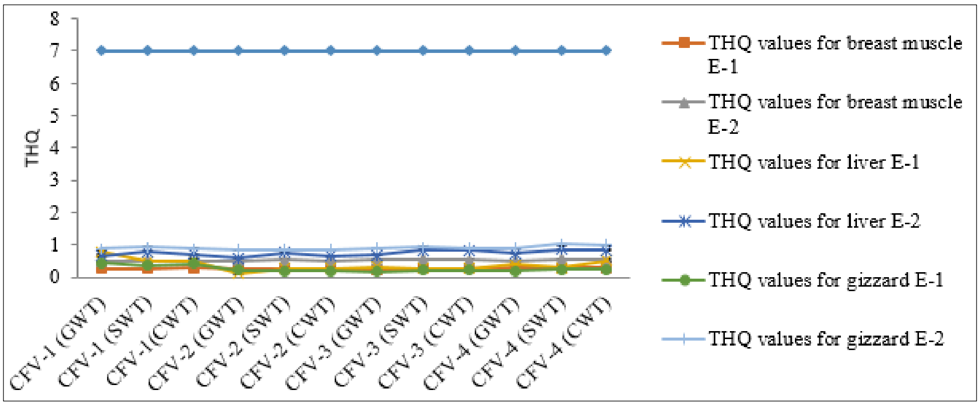


Figure 6. Health risk estimate for cadmium ingestion from chicken breast muscle and viscera (liver and gizzard) of experiment 1 (spring) and experiment 2 (autumn)

1. Conclusions

Irrigation of food and feed crops with polluted and waste water may contaminate food chain with heavy metals like Cd. The Cd concentration in grains of four varieties of maize under instant study was recorded above the permissible limit guided by FAO/WHO. A linear increase in cadmium concentration was detected in seven chickens' anatomic parts which were fed with grains grown on sewage water, as the Cd concentrations were higher in these maize varieties grains as compared to the grains which were grown on canal and ground water. The PLI values of canal and sewage irrigated soils were greater than 1.0, indicated that the soils were contaminated with Cd. THQ values for cadmium were recorded lower than 1.0 for the experimental chickens' meat, liver and gizzard, hereby demonstrating that the human population consuming the above edibles, is unlikely to face any obvious hazardous effect.

Ethics

The protocols followed during the course of study were in accordance with the Institutional Animal Ethics Committee, University of Sargodha (Approval No.25-A18 IEC UOS) and under the rules of National Research Council [64].

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