

## Research Article

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# Biotransfer of cobalt along a soil-plant-chicken food chain: Implication for public health

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### Citation

Zill-E-Huma, Zafar Iqbal Khan, Ijaz Rasool Noorka, Kafeel Ahmad, Muhammad Nadeem and Ilker Ugulu. Biotransfer of cobalt along a soil-plant-chicken food chain: Implication for public health. Pure and Applied Biology. Vol. 8, Issue 3, pp2015-2027. <http://dx.doi.org/10.19045/bspab.2019.80146>

Received: 15/05/2019

Revised: 10/07/2019

Accepted: 16/07/2019

Online First: 24/07/2019

### Abstract

This study was carried out for the determination of cobalt (Co) accumulation in four varieties of maize (grains, shoot and root) and in seven tissues of chickens (blood, bone, breast meat, kidney, liver, heart and gizzard) using wet digestion method by atomic absorption spectrophotometer. Representative samples of water, soil, grains and different chicken parts were assayed for Co contents. Compared to canal and ground water, sewage water had the highest concentration of Co, which is the reason why the maize plants irrigated with sewage water had the highest Co concentration. The group of chickens that were reared with grains treated by sewage water treated grains also showed the highest Co concentration in their body parts compared to the other groups. Enrichment factor and pollution load index for the soil, as well as bioconcentration, bioaccumulation and transfer factors were assessed to evaluate the transfer of Co from water to soil and to the plant. Target Hazard Quotient (THQ) was also calculated to estimate the potential human health risk of Co contamination from consumption of selected chicken parts (liver, breast meat and gizzard) by the local inhabitants and found the THQ values <1. THQ values for Co were less than 1.0 for meat, liver and gizzard which indicated that the exposed population was unlikely to experience obvious adverse effects on utilization of these poultry edibles.

**Keywords:** Biomonitoring, heavy metal, health risk

### Introduction

Heavy metals as a general collective term are a group of elements with atomic density greater than 4 g/cm<sup>3</sup> or five times or greater than water, which tend to release electrons in chemical reactions and form simple cations [1, 2]. In the living systems, heavy metals play a vital role in the biological processes till their concentration does not exceed the required limits because in the

elevated concentrations heavy metals are injurious to health [3-5]. With long non-biodegradable nature, the heavy metals could remain persistent in the environment for a long time [6, 7]. Superfluous effects are caused to various body parts by heavy metals as they tend to accumulate in body due to their long biological half-lives [8]. Heavy metals are found in soil naturally [9]. Toxic percentages of heavy metals are

released into environment by anthropogenic and geologic activities. These activities include utilization of pesticides and fertilizers in agriculture, mining, fossil fuel burning, smelting process of metals, batteries production and production of different metals in sewage sludge, industries, and public waste disposal [10-12]. Incessant irrigation with wastewater, results in accumulation of heavy metals in the soil, which ultimately affects the development of plant [13]. As the soils are easily exchanged and reached, humans are subjected to direct effect on health via pollution of soils [14]. Soils act as poisonous chemical channel may accumulate heavy metals from wastewater. Because of continuous absorption of toxins and changes in pH, the limit of soils to hold toxic metals being decreased accordingly soil may discharge heavy metals into groundwater or accessible for plant uptake [15]. The ability of heavy metals to assimilate in living system and then entering the food chain is the main reason behind their toxic nature [16, 17]. Food chain contamination is the consequence of wastewater irrigation through which heavy metals enter into human body. Heavy metal accumulation causes various disorders in the human body and moreover these metals (even in fewer amounts) could cause mental disorders in children [18]. Nowadays, the escalating insists of food safety has encouraged the research concerning the danger allied with the utilization of food stuffs contaminated with heavy metals and/or toxins [19]. Improvements in the food production and processing technology had increased the chances of contamination of food with various environmental pollutants, especially heavy metals. Ingestion of these contaminants by animals causes deposition of residues in meat [20]. Environmental contaminants are usually concentrated in the tissues of chickens which can be used to guesstimate the enduring or acute exposure, as chickens are fed an ample variety of feed stocks [21]. Through the utilization of

contaminated diets, the metals are accumulated in chickens have been studied in a few to assess the potential human risk from poultry consumption [22].

In the present study, we investigated the effect of short-term use of sewage, canal and groundwater on the cobalt accumulation in maize and in experimental chickens which were fed on grains obtained from the experimental maize. The main objective of this study was to evaluate the level of heavy metals along soil-plant and animal. Another objective of the current research was to estimate the target hazard quotient to evaluate whether experimental chicken parts are safe for human consumption or not.

## **Materials and methods**

### **Study site**

A soil culture experiment was planted in plastic bags carried out in the wirehouse, University College of Agriculture, University of Sargodha.

### **Sample preparation**

Seeds were collected from country leading Maize and Millet Research Institute (CMRI) Yousaf wala, Sahiwal, Pakistan including Pearl (white), Sahiwal-2002 (yellow), MMRI (yellow), Sadaf (white).

The experimental project was carried out using CRD (Completely Randomized Design) comprising three replication and three water regimes (ground water, sewage water and canal water). The sources of water remained a viable variable.

Soil bags containing 20 kg of sandy loam soil was used in the experiment. The field capacity was measured by volumetric means and 2 liters water of each treatment was used to irrigate the soil bags since sowing to the harvesting. The soil analysis was made before bag filling and each source of water analysis was made before the initiation of the study and found buffer for any metal in it. Two experiments were conducted in varying seasons that is Experiment 1 in Spring season and Experiment 2 in Autumn season.

The four seeds were sown in each bag and at three-leaf stage two weak seedlings were

uprooted for ample space of remaining seedlings. All standard agronomic practices were in use for good crop stand and stay green for potential yield corn. At maturity, the crop was harvested, the grains were separated from each cob. 10 g sample of grains, plant root, plant stem and plant leaves were subjected for metal analysis and the remaining's were used as feed for the experimental chickens.

The next phase of the experiment is to the transfer of metal from grains to animal. To check the metal transfer translocation ratio the 2 days old domestic chickens (Missouri golden) breed were purchased from the hatchery in Sargodha. A small cage was made according to the requirement of the chicken. Two chickens were separately allocated, and feed made by the corn grains obtained by three treatments with respect to water was given. The chickens were fed on grind corn grains (variable factors) mixed with standard chicken feed (constant factor) until 45 days of age/maturity. The chickens were then slaughtered to check the metal translocation and their body parts (blood, bone, breast meat, liver, heart, kidney and gizzard) were taken for the evaluation of transfer of cobalt from corn to chickens using wet digestion method.

### Digestion

Water samples, used for irrigation, were digested by using the method of Radojevic and Bashkin [23]. Soil samples were taken out from oven after five days and digested by wet digestion method delineated by Vukadinovic and Bertic [24]. To determine the concentration of Co in each part of corn plant (root, stem, leaf and seed), representative 10 g samples were taken and analyzed by using the method delineated by Anar *et al.* [25]. Chicken samples were prepared separately for analysis by adopting the method designed by Mohammed *et al.* [26] except the blood which was digested by the method as given by the Memon *et al.* [27].

### Analysis

After digestion, all the samples were analyzed for cobalt by atomic absorption spectrophotometer (AA 6300 Shimadzu Japan) with graphite furnace and Zeeman background corrector was used to determine soils and forage Co concentrations. SPSS version 13 was used for statistical analysis. The experiment will be carried out in completely randomized design. The differences between the mean concentration values were found at  $p > 0.05$  (non-significant);  $p < 0.05$  (Significant); highly significant ( $p < 0.01$ ) probability levels.

Target hazard quotient (THQ) was also calculated using the following equation [28].

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

where EF is the exposure frequency, ED is the exposure duration, FIR is the food ingestion rate and in the present study, chicken tissues ingestion rate was assumed to be 32.7 g/person/day, C is the metal concentration in tissues of chicken (mg/kg dry weight); RfD is the oral reference dose for metal, BW is the average consumer body weight (70 kg) and TA is the average exposure time for non-carcinogens (365 day/year  $\times$  ED).

### Quality control analysis

To avoid any infectivity 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. Quality control procedures were strictly followed for the entire sample to ensure the quality of results.

### Results

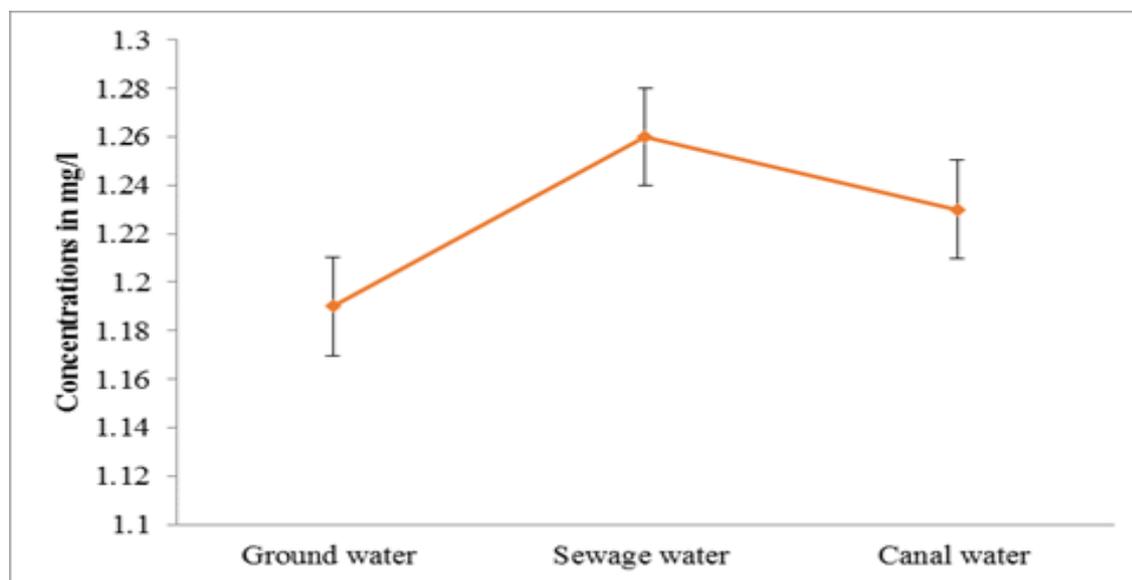
#### The concentration of cobalt in water

Analysis of variance of data for Co concentration is shown in (Table 1) which indicated that in all sources of water, there was a non-significant difference in Co concentration. The concentration of Co was high in sewage water compared to other water samples used for irrigation (Table 1; Figure 1).

**Table 1. Analysis of variance for cobalt in ground, sewage and canal water**

Mean squares		
Source of variation (SOV)	Degree of freedom (df)	Cobalt
Source	2	0.0037 <sup>ns</sup>
Error	6	0.0107

ns = Non-significant ( $P>0.05$ ); \* = Significant ( $P<0.05$ ); \*\* = Highly significant ( $P<0.01$ )

**Figure 1. The concentration of cobalt in water used in experiments**

#### The concentration of cobalt in soil

Analysis of variance of data for Co concentration in soil showed that there was a significant difference in Co concentration in both seasons. The concentration of Co was high in sewage water irrigated soil compared to ground water and canal water irrigated soil samples (Table 2; Figure 2).

#### The concentration of cobalt in plant samples

Analysis of variance of data for Co in grains, shoot and root is shown in Table 3, indicated that season variety and treatment significantly affected the concentration of Co in all parts of the plant. Season  $\times$  Treatment, Variety  $\times$  Treatment, Season  $\times$  Variety  $\times$  Treatment interactions were non-significant in all parts of the plant except Season  $\times$  Variety interaction was only non-significant in the shoot (Table 3).

**Table 2. Analysis of variance for cobalt in soil (mg/kg) irrigated with ground, sewage and canal water**

Mean Squares			
Source of variation (SOV)	Degree of freedom (df)	Experiment 1 (Spring season)	Experiment 2 (Autumn season)
Source	2	0.7214*	0.0477**
Error	6	0.0879	0.0006

ns = Non-significant ( $P>0.05$ ); \* = Significant ( $P<0.05$ ); \*\* = Highly significant ( $P<0.01$ )

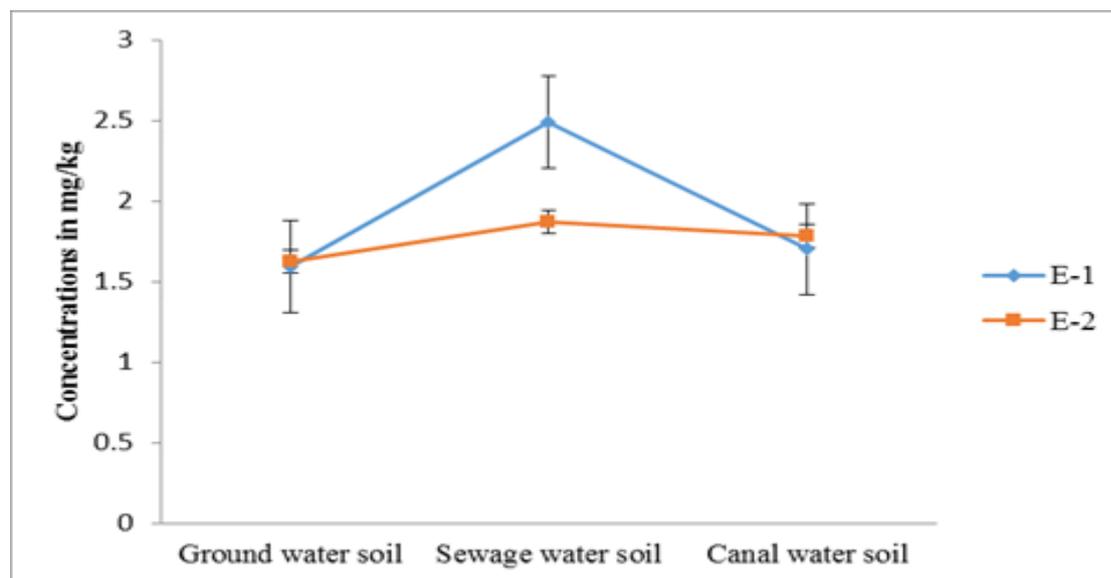


Figure 2. The concentration of cobalt in soil in two experiments

Table 3. Analysis of variance (Mean squares) of data for grains, shoot and root of four varieties of corn differing in concentrations of cobalt grown in pots under diverse irrigations (ground water, sewage water and canal water) in both experiments conducted in varying seasons

Source of Variation (SOV)	Degree of freedom (df)	Mean squares		
		Grains	Shoot	Root
Season	1	1.5167**	0.3850**	2.7907**
Variety	3	0.4222**	0.2369**	0.2287**
Treatment	2	0.0401**	0.0407**	0.0459**
SxV	3	0.1934**	0.0139ns	0.0661**
SxT	2	0.0030ns	0.0026ns	0.0058ns
VxT	6	0.0064ns	0.0009ns	0.0042ns
SxVxT	6	0.0022ns	0.0020ns	0.0028ns
Error	48	0.0034	0.0058	0.0056

ns = Non-significant ( $P > 0.05$ ); \* = Significant ( $P < 0.05$ ); \*\* = Highly significant ( $P < 0.01$ ); S, Seasons; V, variety; T, treatment, SxV, season into variety interaction; SxT, season into treatment interaction; VxT, Variety into treatment interaction; SxVxT, season into variety into treatment interaction

In both seasons, the highest concentration of Co (E-1,  $0.82 \pm 0.05$ ; Experiment 2 (Autumn season),  $0.84 \pm 0.06$ ) was found in

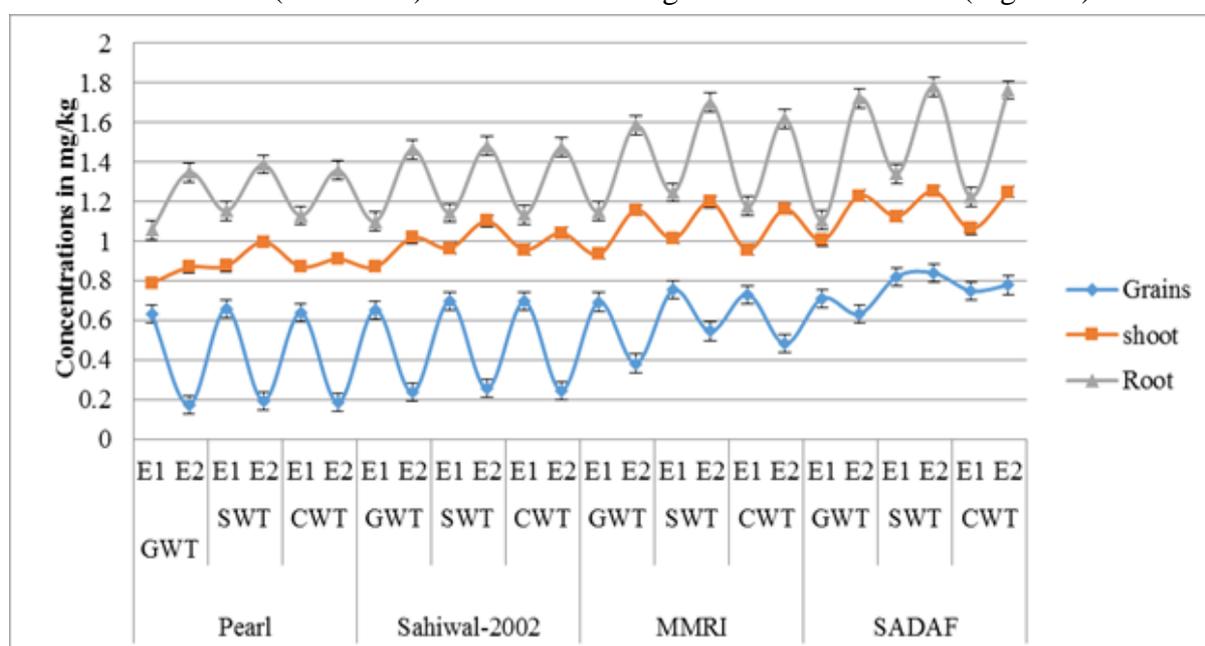
grains of Sadaf variety at sewage water treatment and the lowest (Experiment 1 (Spring season),  $0.63 \pm 0.03$ ; Experiment 2

(Autumn season),  $0.17 \pm 0.04$ ) was found in grains of MMRI variety at groundwater treatment (Figure 3).

In experiment 1 (Spring season), the highest level of Co for the shoot ( $1.13 \pm 0.02$ ) was found in Sadaf variety at sewage water treatment and the lowest concentration of Co ( $0.79 \pm 0.05$ ) was found in the shoot of Pearl variety at ground water treatment. In experiment 2 (Autumn season), the maximum level of Co ( $1.25 \pm 0.08$ ) was found in the shoot of Sadaf variety at sewage water treatment and canal water treatment and the minimum concentration of Co ( $0.06 \pm 0.00$ ) was found

in Pearl variety at groundwater treatment (Figure 3).

In Experiment 1 (Spring season), the highest level of Co for root ( $1.34 \pm 0.08$ ) was found in Sadaf variety at sewage water treatment and the lowest concentration of Co ( $1.06 \pm 0.02$ ) was found in the root of Pearl variety at groundwater treatment. In Experiment 2 (Autumn season), the maximum level of Co ( $1.78 \pm 0.01$ ) was found in the root of Sadaf variety at sewage water treatment and the minimum ( $1.35 \pm 0.04$ ) concentration of Co ( $0.06 \pm 0.00$ ) was found in Pearl variety at groundwater treatment (Figure 3).



**Figure 3. The concentration of cobalt in plant parts (Shoot, Root and Grains) in two experiments**

**Concentration of cobalt in chickens**

Analysis of variance of data for cobalt in various body parts of chickens was shown in (Table 4), indicating that seasons significantly affected the concentration of Co in all body parts. Varieties non-significantly affected the Co concentration only in blood and heart whereas treatment significantly affected the Co concentration in all parts of chicken except kidney and gizzard. Season × Variety interaction was significant only in meat and heart. On the other hand, Season × Treatment, Variety × Treatment and Season × Variety ×

Treatment interaction were significant only in bone (Table 4).

The maximum concentration of Co in blood during Experiment 1 (Spring season) ( $2.22 \pm 0.07$ ) was in chickens that used grains of Sadaf Variety raised with sewage water in their diet and the minimum level was found in blood ( $1.79 \pm 0.02$ ) of the chickens that used grains of Pearl Variety raised with ground and canal water in their diet. In Experiment 2 (Autumn season) the highest concentration of Co was found in the blood ( $3.09 \pm 0.21$ ) of chickens that utilized grains of Pearl Variety raised with

sewage water in their diet and the lowest ( $2.13 \pm 0.16$ ) was found on the utilization of the grains of MMRI Variety raised with groundwater (Figure 4).

The maximum concentration of Co in bone during Experiment 1 (Spring Season) ( $2.78 \pm 0.03$ ) was in chickens that used grains of Sadaf variety raised with sewage water in their diet and the minimum level was found in bone ( $2.22 \pm 0.02$ ) of the chickens that used grains of Sahiwal-2002 variety raised with groundwater in their diet. In Experiment 2 (Autumn Season), the highest concentration of Co was found in the bone ( $2.03 \pm 0.30$ ) of chickens that utilized grains of Pearl variety raised with sewage water in their diet and the lowest ( $0.07 \pm 0.03$ ) was found on the utilization of the grains of Sahiwal-2002 variety raised with groundwater (Figure 4).

The maximum concentration of Co in meat during Experiment 1 (Spring Season) ( $3.60 \pm 0.28$ ) was in chickens that used grains of Sadaf variety raised with sewage water in their diet and the minimum level was found in meat ( $2.90 \pm 0.04$ ) of the chickens that used grains of Pearl variety raised with groundwater in their diet. In Experiment 2 (Autumn Season), the highest concentration of Co was found in the meat ( $0.77 \pm 0.04$ ) of chickens that utilized grains of Sadaf variety raised with sewage water in their diet and the lowest ( $0.26 \pm 0.07$ ) was found on the utilization of the grains of Pearl variety raised with groundwater (Figure 4).

The maximum concentration of Co in the liver during Experiment 1 (Spring Season) ( $4.15 \pm 0.03$ ) was in chickens that used grains of MMRI variety raised with sewage water in their diet and the minimum level was found in liver ( $3.46 \pm 0.04$ ) of the chickens that used grains of Pearl variety raised with groundwater in their diet. In Experiment 2 (Autumn Season), the highest concentration of Co was found in the liver ( $1.11 \pm 0.02$ ) of chickens that utilized Sadaf variety grains raised with sewage water in their diet and the lowest ( $0.66 \pm 0.04$ ) was found on the utilization of the grains of

Pearl variety raised with groundwater (Figure 4).

The maximum concentration of Co in heart during Experiment 1 (Spring Season) ( $4.33 \pm 0.02$ ) was in chickens that used grains of Pearl variety raised with sewage water in their diet and the minimum level was found in heart ( $3.82 \pm 0.07$ ) of the chickens that used grains of Pearl variety raised with groundwater in their diet. In Experiment 2 (Autumn Season), the highest concentration of Co was found in the heart ( $1.26 \pm 0.05$ ) of chickens that utilized Sadaf variety grains rose with sewage water in their diet and the lowest ( $1.02 \pm 0.04$ ) was found on the utilization of the grains of Pearl variety raised with groundwater (Figure 4).

The maximum concentration of Co in kidney during Experiment 1 (Spring Season) ( $4.09 \pm 0.13$ ) was in chickens that used grains of Pearl variety raised with sewage water in their diet and the minimum level was found in kidney ( $3.52 \pm 0.14$ ) of the chickens that used grains of Sadaf variety raised with groundwater in their diet. In Experiment 2 (Autumn Season), the highest concentration of Co was found in the kidney ( $1.41 \pm 0.02$ ) of chickens that utilized grains of Sadaf variety raised with sewage water in their diet and the lowest ( $1.14 \pm 0.02$ ) was found on the utilization of the grains of Pearl variety raised with groundwater (Figure 4).

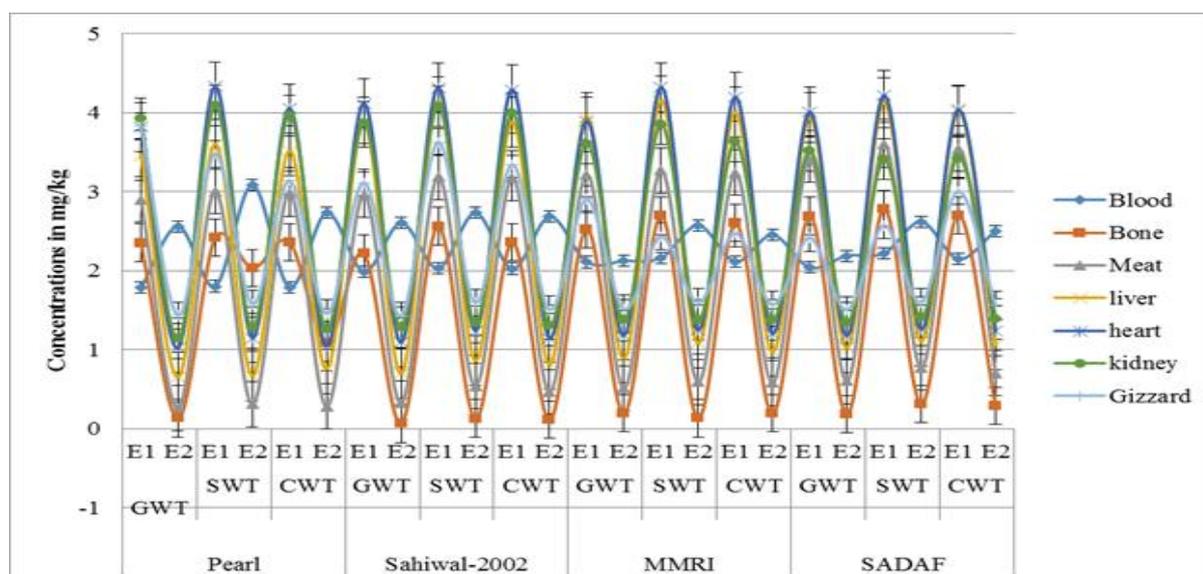
The maximum concentration of Co in gizzard during Experiment 1 (Spring Season) ( $3.63 \pm 0.09$ ) was in chickens that used grains of Sahiwal-2002 variety raised with sewage water in their diet and the minimum level was found in gizzard ( $2.42 \pm 0.29$ ) of the chickens that used grains of Sadaf variety raised with groundwater in their diet. In Experiment 2 (Autumn Season), the highest concentration of Co was found in the gizzard ( $1.60 \pm 0.07$ ) of chickens that utilized grains of Sahiwal-2002 variety raised with sewage water in their diet and the lowest ( $1.51 \pm 0.02$ ) was found on the utilization of the grains of

Sahiwal-2002 variety raised with groundwater (Figure 4).

**Table 4. Analysis of variance (Mean squares) of data for grains, shoot and root of four varieties of corn differing in concentrations of cobalt grown in pots under diverse irrigation (ground water, sewage water and canal water) in both experiments conducted in varying seasons**

Mean squares								
Sources of Variation (SOV)	Degree of freedom (df)	Blood	Bone	Meat	Liver	Heart	Kidney	Gizzard
Season	1	3.6658**	53.833**	88.319**	108.420**	105.658**	72.595**	26.9251**
Variety	3	0.0159ns	0.5064**	0.4992**	0.4672**	0.0380ns	0.1255*	0.5395**
Treatment	2	0.2104*	0.4544**	0.0767**	0.0994**	0.1980**	0.0379ns	0.0123ns
S×V	3	0.3645**	0.9188**	0.0176ns	0.0620**	0.0257ns	0.3067**	0.7267**
S×T	2	0.0961ns	0.1192*	0.0008ns	0.0153ns	0.0546ns	0.0070ns	0.0289ns
V×T	6	0.0124ns	0.1899**	0.0068ns	0.0059ns	0.0080ns	0.0070ns	0.1259ns
S×V×T	6	0.0111ns	0.2523**	0.0009ns	0.0023ns	0.0096ns	0.0092ns	0.1035ns
Error	24	0.0391	0.0221	0.0133	0.0051	0.0332	0.0390	0.0902

ns = Non-significant (P>0.05); \* = Significant (P<0.05); \*\* = Highly significant (P<0.01); S, Seasons; V, variety; T, treatment, S×V, season into variety interaction; S×T, season into treatment interaction; V×T, Variety into treatment interaction; S×V×T, season into variety into treatment interaction



**Figure 4. The concentration of cobalt in body parts of chickens in two experiments**

**Discussion**

In the present study, we aimed to evaluate accumulation and translocation of Co along soil-plant and animal. The maximum

permissible limit of Co in water according to WHO is 0.005 mg/L [9]. In the current research, all water samples contained Co above its safe limit. However, sewage

water showed the highest concentration of Co followed by canal water and ground water respectively. This result was in line with Al-Omran [29] who found that sewage effluent contained much higher amounts of Co, Cu, Fe, Mn, Cr, Pb and Ni compared to ground well water. Sewage water irrigation is also known to contribute significantly to the heavy metal content of soils [15]. The long-term application of treated and untreated wastewater has resulted in a significant build-up of heavy metals in the soil [13]. Sewage effluents are usually considered a prosperous source of other nutrients and organic matter, but they increased the levels of heavy metals, such as Mn, Fe, Co, Cu, Pb, Ni, Cr, Co and Cd in the soils irrigated with sewage water [9]. The present research also showed the highest concentration of Co in soil irrigated with sewage water compared to canal and ground water irrigated soils (Figure 5). Results were in line with Shad *et al.* [30] also found a higher concentration of Co in soil irrigated with sewage water than those irrigated with canal water. The concentration of Co in all soil samples were in safe limits that are 65 mg/kg [31].

In the current research, the roots showed the highest concentration of Co than shoot and the least value was shown by the grains in both seasons by all varieties same was reported by Mojiri and Hamidi [32]. They found higher concentrations of heavy metals in wheat plants treated with municipal wastewater and also observed more accumulation of metals in roots than in shoots. In the present research, sewage irrigated plant parts showed a higher concentration of Co compared to other treatments which were may be due to the presence of a higher concentration of Co in wastewater. Jayakumar and Jaleel [33] conducted a study based on exogenous Co in soybean. In his experiment, they treated soybean with different levels of Co (50, 100, 150, 200 and 250 mg kg) and found the highest concentration of Co in leaves, stem, seeds and root treated with 250 mg/kg Co and the least were observed in control.

Many studies have indicated that crops grown in metal-contaminated soil have higher concentrations of metals than those in uncontaminated soil [13]. Hussain *et al.* [34] conducted an experiment and found the higher concentration of heavy metals including Co in wastewater treated *Brassica napus* and *Lactuca sativa* compared to control. Shad *et al.* [30] found the higher concentration of Co in the shoot of wheat plants irrigated with sewage water compared to canal water irrigated plants. However, in their research grains from the wheat plants irrigated with sewage water accumulated the less amount of Co which was contrary to the results of the present research. Tremendous accumulation of heavy metals in agricultural soils in the course of wastewater irrigation may not only result in soil pollution but also lead to more heavy metal up-take by crops, affecting food quality and as well as food safety [13]. Brar *et al.* [35] also reported higher accumulation of metals in leaves and tubers of potato grown on sewage irrigated soils as compared with ground water irrigated soils. According to FAO/WHO the maximum permissible limit of Co in grains was 50 mg/kg and all the grains were within safe limit [36].

Various studies were conducted to evaluate the heavy metal concentration in plants. Amin *et al.* [37] studied the accumulation of various heavy metals (Cu, Ni, Co, Cr, Fe, Mn, Co and Pb) in green vegetables like *Allium sativum*, *Allium cepa*, *Solanum lycopersicum* and *Solanum melongena*, irrigated with wastewater in Mardan, Pakistan. Their study showed that heavy metals in vegetable grown on soil irrigated with wastewater were considerably higher compared to those of tube well water irrigated soil.

Similarly, Odai *et al.* [38] studied the concentration of heavy metals in cabbage, lettuce and cauliflower grown on urban waste dump sites. The levels of the two most toxic heavy metals (Cd and Pb) were far higher in the vegetables than the WHO/FAO recommended values and the

transfer factors of these two metals were also the highest suggesting that consumption of vegetables grown on such sites could be dangerous to human health. Contact to high levels of contaminants, like heavy metals, can result in unpleasant health effects, and organisms that are higher on the food chain are particularly at risk to bioaccumulative effects [13]. Metals like Hg, Cd, Cr, Pb, Ni, Co, and Zn are highly toxic to both flora and fauna components of the ecosystem [18]. The risk of heavy metal contamination in meat is of great concern for both food safety and human health because of the toxic nature of these metals at relatively minute concentrations [39]. In the present research the values for Co in all body parts were higher in chickens that consumed grains raised with sewage water and the lowest were observed in group of chickens that utilized grains raised with ground water in two experiments conducted in both seasons. Present research values of cobalt in blood and gizzard were higher compared to the values found by Abduljaleel *et al.* [40] in the blood of chicken and quail. Current research values of Co in bone,

heart, kidney and liver tissues were also higher than the values found by Metcheva *et al.* [41]. These researchers found concentration of different metals (Pb, Cd, Cu, Co, Mn and Co) in various body parts of brown skua (*Catharacta lonnbergi*), gentoo penguin (*Pygoscelis papua*) and in crabeater seal (*Lobodon carcinophagus*). The values may be higher due to the presence of a considerable amount of cobalt in the grains utilized by the chickens. Generally, birds get heavy metals via intake of food, drinking, and geophagy. The extent of heavy metal absorption varies and depending on many factors like species physiology, bioavailability and metal properties, in the environment. After absorption, metals circulate in the body, are excreted or get deposited in various body tissues [42].

The target hazard quotient (THQ) used for assessing health risk and in present research. THQ values of Co for meat, liver and gizzard were less than 1 and these values indicated that exposed population is unlikely to experience obvious adverse effects on utilization of these poultry edibles.

**Table 5. Health Risk Estimate for Co ingestion from chicken breast muscle and viscera (liver and gizzard) of Experiment 1 (Spring season) and Experiment 2 (Autumn season)**

Groups of chicken	Level of Exposure day/week	THQ values for breast muscle		THQ values for liver		THQ values for gizzard	
		E-1	E-2	E-1	E-2	E-1	E-2
CFV-1 (GWT)	7	0.067	0.006	0.080	0.015	0.089	0.033
CFV-1 (SWT)	7	0.070	0.007	0.084	0.015	0.081	0.036
CFV-1 (CWT)	7	0.069	0.006	0.082	0.017	0.073	0.034
CFV-2 (GWT)	7	0.069	0.007	0.090	0.016	0.072	0.033
CFV-2 (SWT)	7	0.074	0.012	0.096	0.020	0.084	0.037
CFV-2 (CWT)	7	0.074	0.010	0.090	0.019	0.078	0.035
CFV-3 (GWT)	7	0.075	0.011	0.092	0.020	0.067	0.035
CFV-3 (SWT)	7	0.076	0.013	0.096	0.025	0.056	0.037
CFV-3 (CWT)	7	0.075	0.013	0.093	0.022	0.057	0.036
CFV-4 (GWT)	7	0.079	0.014	0.092	0.023	0.056	0.035
CFV-4 (SWT)	7	0.084	0.017	0.096	0.025	0.060	0.037
CFV-4 (CWT)	7	0.082	0.016	0.093	0.024	0.070	0.036

Key; CFV1, Chicken feed on 'Pearl variety'; CFV2, Chicken feed on 'Sahiwal 2002' variety; CFV3, Chicken feed on 'MMRI' variety; CFV4, Chicken feed on 'Sadaf' variety. GWT (ground water treatment), SWT (sewage water treatment), CWT (canal water treatment)

## Ethics

All the study protocols were approved by the Institutional Animal Ethics Committee, University of Sargodha (Approval No.25-A18 IEC UOS). All the experiments performed complied with the rules of the National Research Council [43] and all methods were performed in accordance with relevant guidelines and regulations.

## Conclusion

Irrigation with polluted water may contaminate the food chain with heavy metals. The concentration of cobalt in grains of four varieties of maize was found within the permissible limit given by FAO/WHO. A linear increase in cobalt concentration was observed in seven body parts of the chickens that consumed sewage water raised grains as the concentration of cobalt were higher in these grains compared to the groups that utilized canal water and ground water treated grains in their diet. Target hazard quotient values for cobalt were less than 1.0 for meat, liver and gizzard indicating that utilization of these poultry edibles is safe.

## Authors' contributions

Conceived and designed the experiments: ZI Khan, Performed the experiments: ZE Huma, Analyzed the data: IR Noorka, Contributed reagents/ materials/ analysis tools: K Ahmad & M Nadeem, Wrote the paper: I Ugulu

## Acknowledgement

The Higher Education Commission, Pakistan is hereby acknowledged for providing the financial support through a research project # 20-3546/NRPU/R&D/HEC/14/536 titled "Toxicological Risk Assessment of Potential Pollutants on Fauna and Flora in Selected Areas of Punjab".

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