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RESEARCH ARTICLE

Transfer of metal element in soil plant chicken food chain: health risk assessment

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Abstract
This investigation was done for the assurance of potassium amassing in four assortments of maize (grains, shoot and root), soil, and water and in seven tissues of chickens (kidney, liver, heart, bone, gizzard, breast meat). The analysis of variance showed significant differences for potassium concentration in water in all sources of water; however, the season and variety significantly influenced the quantity of potassium in cereals. The corn varieties MMRI, Sadaf, and Pearl behaved differently when treated with water from various sources. Water taken from sewage had a higher concentration of potassium compared to canal and groundwater that is why the maize plants irrigated with this water had a higher grouping. Data regarding potassium concentration in different body parts of chicken showed that season and treatment have a significant effect on the potassium concentration in chicken organs. The variety was non-significant for the potassium concentration only in the bone. Season × Variety interaction was only significant in blood, meat heart, and gizzard. Season × Treatment and Variety × Treatment interactions were significant in the heart, kidney, and gizzard. The potassium contents were higher in the chicken body parts that were reared on grains irrigated with sewage water as compared to other groups. The potassium contents were higher in the chicken meat (96.23 ± 0.00) reared on grains of the Pearl variety raised with the sewage water. In a nutshell, the irrigation of grains with sewage water led to accumulation of nutrients greater than those irrigated with ground or canal water.

Keywords Potassium · Chicken · Body part · *Zea mays* · Contaminated water

Introduction

Water shortage is a matter of concern in the whole world. Taking into account the scarcity of conventional water sources, due to water demand increases linked to population growth and to agricultural water usage (< 80% of total water consumption), there is an urgent need to make available alternative water sources for agriculture replacing the high-quality water required for human consumption (Toze 2006). In reality, wastewater is considered an enormous nutrient source for the irrigated plant (Rattan et al. 2005) and falls in a good permissible range for most crops according to heavy metal contents. In addition, their high nitrogen and other nutrient element content significantly reduce or even exterminate the exigency for chemical fertilizers (Khan et al. 2019). Wastewater is a preferred marginal water source since its supply is reliable and uniform (Manjunatha et al. 2017). Costs of this water sources are low compared with those of other non-conventional irrigation water sources (e.g., desalinization) since agricultural reuse of urban wastewater serves also to dispose of treated urban sewage water (Haruvy and Sadan 1994; Hristov et al. 2021). Avian species, for example, winged creatures and ducks, are naive to bioaccumulation of

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toxins basically through the use of despoil food (Gochfeld 2000). For the higher trophic dimension of the food web, chickens can be useful as a utile biomarker animal group for ecological regulation (Kim et al. 2003). Natural contaminants are usually packed in the tissues of chickens which can be used to estimate the severe exposure, as chickens are fed an ample variety of feedstocks (Scheuhammer 1987). Through the utilization of contaminated diets, the metals accumulated in chickens have been studied in a couple to evaluate the potential human hazard from poultry utilization (Zhuang et al. 2009). It has been observed that when our kidneys are somehow malfunctioning, an accumulation of potassium will occur that disturbs heartbeat. Potassium also affects breathing; inhalation of dust; and irritation on eyes, nose, throat, and lungs etc. It also affects photosynthesis and stomatal opening and closure. The present endeavor was initiated keeping in view the importance and effects of potassium in plant and animal body and its translocation in soil-plant-animal continuum, comparing the soils with different irrigation sources.

Materials and methods

Study territory

A sample trial was planted in plastic sacks, carried out in the University of Sargodha. The territory of Sargodha has rich land equipped for generating all types of crops especially cereals including wheat, rice, and corn utilized not only by humans but also for broiling cobs and grub for domesticated animals, and nourishment supply source (Fig. 1).

Collection of seed

Seeds of two white varieties Pearl (white) and Sadaf (white), and two yellow varieties Sahiwal-2002 (yellow) and MMRI (yellow) were obtained from leading Corn and Millet Research Institute, Yousafwala, Sahiwal, Pakistan. The seeds were sown in 20-kg sandy loam soil packs with routine irrigations with canal water (CWT), groundwater (GWT), and sewage (SWT) at the College of Agriculture, University of Sargodha, Pakistan. During the experiment, soil bags were irrigated with 2 l of water to every treatment since sowing to the picking. Soil and water samples were analyzed before the initiation of the study. The four seeds were planted in each bag under standard agronomic practices in two seasons, i.e., spring and autumn. Remaining grains were collected and fed to the domestic chickens (Missouri golden) breed (age 2 days) kept in a small cage. The chickens were fed until maturity (45 days) on grind corn grains (variable factors) mixed with standard chicken feed (constant factor). The chickens were then slaughtered to check the potassium translocation in the bones, kidney, breast meat, blood, gizzard, heart, and liver.

Digestion of samples

Samples of water (Radojevic et al. 1999) and oven-dried soil were digested (Vukadinovic and Bertic 1988). Ten-gram samples (stem, roots, seeds, and leaf) were taken and digested using the methods delineated by Anar et al. (2000) and investigated to determine the concentration of potassium in each part of the corn (stem, roots, seeds, and leaf). According to the designed method by Mohammed et al. (2016)), chicken samples for the analysis were prepared individually except the blood which was digested by the technique as given by Memon et al. (2007). Atomic absorption spectrophotometer (model: AA 6300, Shimadzu, Japan) was used for the analysis of potassium in every sample after assimilation. SPSS version 13 was used for statistical analysis (Steel et al. 2006). The experiment was carried out in a completely randomized design. The differences between the mean concentration values were found at $p > 0.05$ (non-significant); $p < 0.05$ (significant); and $p < 0.01$ (highly significant) probability levels.

Results

Water

The analysis of variance of data for potassium concentration in water showed that there was a significant difference in potassium concentration in all sources of water (Table 1, Fig. 2).

The concentration of potassium in soil

The analysis of variance of data for potassium concentration in soil showed that there was a significant difference in potassium concentration in soil in both experiments conducted in varying seasons (Table 2, Fig. 3).

The concentration of potassium in plant

The analysis of variance of data for potassium in plant parts showed that season and variety affected significantly the concentration of potassium only in grains, but in all the parts of plants, treatment significantly affected the concentration of potassium. Season × Variety, Variety × Treatment, and Season × Variety × Treatment interactions were non-significant in all parts of the plants. Season × Treatment interaction was non-significant in the root (Table 3).

Grains

In experiment 1 (spring season), the highest (90.01 ± 0.71 mg/kg) portion of potassium for cereals was present in MMRI variety at the treatment of sewage water and the lowest (76.88 ± 3.86 mg/kg) potassium content was present in grains

Fig. 1 Photo courtesy: Google Maps

at the groundwater treatment of MMRI variety. In experiment 2 (autumn season), the supreme level of potassium (86.33 ± 1.28 mg/kg) found at sewage water treatment was that in grains of Sadaf and the lowest (62.54 ± 1.21 mg/kg) concentration of potassium at groundwater treatment was found in Sadaf variety (Fig. 4).

Shoot

In experiment 1 (spring season), the highest level (82.53 ± 2.86 mg/kg) of potassium for the shoot was found in MMRI variety at groundwater treatment and the lowest (76.64 ± 14.22 mg/kg) potassium content was observed in the shoot of the Sahiwal-2002 range at canal water treatment. In experiment 2 (fall season), the extreme level of potassium (94.39 ± 0.43 mg/kg) was found in the shoot of the Pearl variety at sewage water treatment and the lowest (71.69 ± 0.80 mg/kg) potassium content was found in the Sahiwal-2002 variety at groundwater treatment (Fig. 4).

Table 1 Analysis of variance for potassium in ground, sewage, and canal water

Mean squares		
Source of variations	Degree of freedom	Potassium
Source	2	67.2290*
Error	6	111.684

Significant at 0.05, 0.01, and 0.001 levels. ns: at $P > 0.05$

*significant at $p < 0.05$

Root

In experiment 1 (spring season), the highest (90.19 ± 3.24 mg/kg) level of potassium for root at sewage water treatment was found in the MMRI variety and the lowest (71.69 ± 3.65 mg/kg) potassium content was in the root of the Sahiwal-2002. In experiment 2 (autumn season), the potassium content (97.09 ± 1.66 mg/kg) was found the highest at sewage water treatment in the root of the Pearl variety and the lowest (75.61 ± 0.79 mg/kg) potassium content was found in the Sahiwal-2002 variety at groundwater treatment (Fig. 4).

Distribution of potassium in the body parts of the chicken

The analysis of variance of data for potassium concentration in different body parts of chicken showed that season and treatment affected significantly the potassium concentration in all body portions of chicken. The variety was non-significant for potassium concentration only in the bone. Season × Variety interaction was only significant in blood, meat heart, and gizzard. Season × Treatment and Variety × Treatment interactions were significant in the heart, kidney,

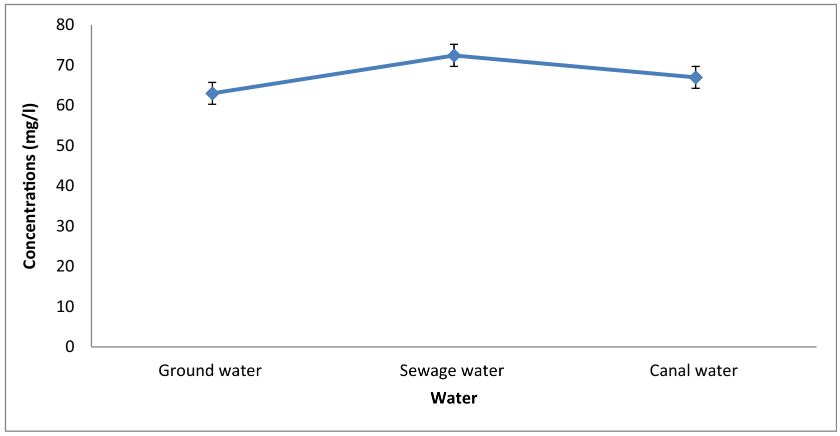

Fig. 2 Concentration of potassium in canal water treatment (CWT), underground (GWT), sewage (SWT), and in both experiments conducted in varying seasons

and gizzard. Season × Variety × Treatment interaction was significant only in the blood and heart (Table 4).

Blood

In experiment 1 (spring season), the maximum potassium content (42.27 ± 0.74 mg/kg) was noticed in the blood of the chickens that were fed the Sahiwal-2002 variety grains and these grains were irrigated with sewage water, and it was observed that chickens that were fed with the Pearl variety grains grown in groundwater indicated the minimum level (11.44 ± 1.35 mg/kg) of potassium in their blood. Experiment 2 (autumn season) indicated that chickens that were fed with the Sahiwal-2002 variety grains grown in sewage water have the maximum potassium content in their blood (81.60 ± 3.30 mg/kg) and the lowest potassium content (43.43 ± 0.80 mg/kg) was found in the blood of chickens that used the Pearl variety of grains grown in groundwater (Fig. 5).

Bone

Experiment 1 (spring season) showed that the highest potassium content was found in the bones (92.28 ± 1.18 mg/kg) of chickens fed grains of the Sahiwal-2002 variety irrigated with sewage water and the lowest (78.32 ± 0.68 mg/kg) was in the chickens fed the Sadaf variety grains grown in groundwater. In experiment 2 (autumn season), the maximum potassium content was found in the bones (97.54 ± 1.70 mg/kg) of chickens fed grains of the Sadaf variety grown in sewage water and the lowest (81.54 ± 1.04 mg/kg) was in those fed the Pearl variety grains grown in groundwater (Fig. 5).

Breast meat

Experiment 1 in spring season shows that the greater rate of potassium in meat should be (94.73 ± 2.28 mg/kg) found in chicken due to use of grain variety of Pearls also raised in sewage water added in their diet; minimum ratio was indicated in chicken meat (74.10 ± 2.91 mg/kg) which uses MMRI grain variety in canal water added to their diet. Experiment 2 in the autumn season, the Pearl grain variety raised in sewage water has the higher quantity of potassium (96.23 ± 0.01 mg/kg) and the lower quantity was identified using the Sadaf grain variety in groundwater (84.86 ± 3.01 mg/kg) (Fig. 5).

Liver

Experiment 1 in spring season shows the highest amount of potassium level was found in the liver of chicken (93.76 ± 3.51 mg/kg) fed the Pearl grain variety grown in sewage water and the lowest value was found in the liver (68.88±0.88 mg/kg) of those fed the MMRI variety grains grown in groundwater. In experiment 2 of autumn season, the greater quantity of potassium was found (98.83 ± 1.12 mg/kg) in the liver of chicken which consumed the Pearl grain variety raised in sewage water in the diet of chickens, whereas the lower

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

Mean squares			
Source of variations	Degree of freedom	Experiment 1 (Spring season)	Experiment 2 (Autumn season)
Source	2	77.4526	77.7254*
Error	6	74.290	72.261

Significant at 0.05, 0.01, and 0.001 levels. ns: at $P > 0.05$

*significant at $p < 0.05$

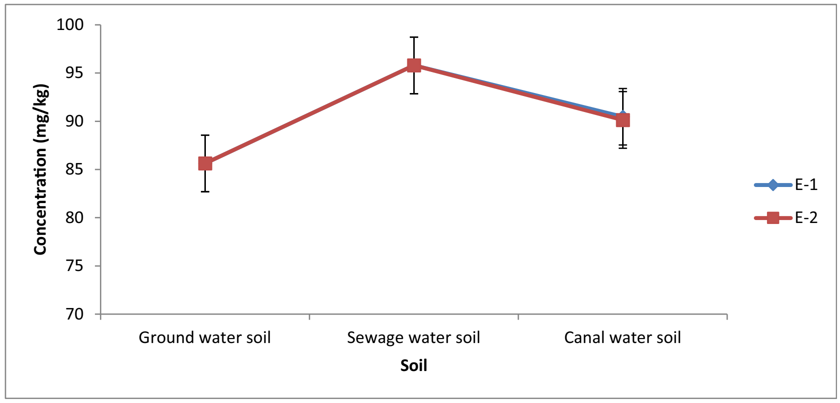

Fig. 3 Concentration of potassium in soil irrigated with the canal water treatment (CWT) underground (GWT), sewage (SWT), and in both experiments conducted in varying seasons

concentration was found in those fed the MMRI variety (84.19±3.99 mg/kg) in groundwater (Fig. 5).

Heart

The results of experiment 1 which was done in spring season gave the maximum ratio of potassium in the heart portion of chicken (65.80 ± 3.80 mg/kg) in which the Pearl variety of grains irrigated with water of sewage was given as their diet; on the other hand, groundwater-irrigated Sahiwal-2002 variety grains in chicken diet show the minimum level of potassium found in the heart (30.72 ± 0.53 mg/kg). Experiment 2 in autumn season shows the conclusion with the highest quantity of potassium in the chickens' heart (85.61 ± 0.01 mg/kg) that were fed the Pearl variety grains irrigated with sewage water, whereas the lowest value was found (30.52 ± 1.00 mg/kg) in chicken fed the MMRI grains grown in groundwater (Fig. 5).

Kidney

Experiment 1 in spring season shows that the variety of Pearl grains which were irrigated with sewage water and were given to chicken indicates the greater amount of potassium (30.56 ± 0.44) in chickens' kidneys and the Sahiwal-2002 grains which were grown in groundwater gave the lower value (47.40 ± 0.40 mg/kg) in the kidneys of chicken. Doing experiment 2, results indicated that the maximum quantity of potassium was found in the kidneys of chickens fed the Pearl grains irrigated with sewage water (28.53 ± 0.01 mg/kg) and utilizing the Sahiwal-2002 grains with groundwater gave the value 15.84 ± 0.01 mg/kg in the kidneys of chicken (Fig. 5).

Gizzard

The maximum concentration of potassium in chicken gizzard from those fed the Pearl grains irrigated with sewage water was 91.43 ± 3.43 mg/kg, and the minimum ratio (53.68 ± 1.32 mg/kg) was found in the gizzard of chicken which were fed Sadaf grains with groundwater; these were the results of experiment 1 in spring season. Whereas experiment 2 in autumn season gave high value of potassium in chicken gizzard (97.05 ± 0.85 mg/kg) from those fed the MMRI grains in sewage water to their diet, and the low concentration was 84.81 ±

Table 3 Analysis of variance (ANOVA) of the trait grain shoot and root of four corn varieties differing in concentrations of potassium under diverse irrigation regimes in varying seasons

Mean squares				
Source of variations	Degree of freedom	Grains	Shoot	Root
Season	1	1623.598**	66.538^{ns}	71.940^{ns}
Variety	3	142.680**	44.5815^{ns}	9.6537^{ns}
Treatment	2	1064.459**	305.934*	987.003**
S × V	3	26.5827^{ns}	59.301^{ns}	82.795^{ns}
S × T	2	127.140*	338.144**	28.9222^{ns}
V × T	6	35.8668^{ns}	43.0079^{ns}	6.2681^{ns}
S × V × T	6	13.1098^{ns}	35.5223^{ns}	46.7524^{ns}
Error	48	274.379	648.999	486.474

Significant at 0.05, 0.01, and 0.001 levels. ns: at $P > 0.05$

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

*significant at $p < 0.05$

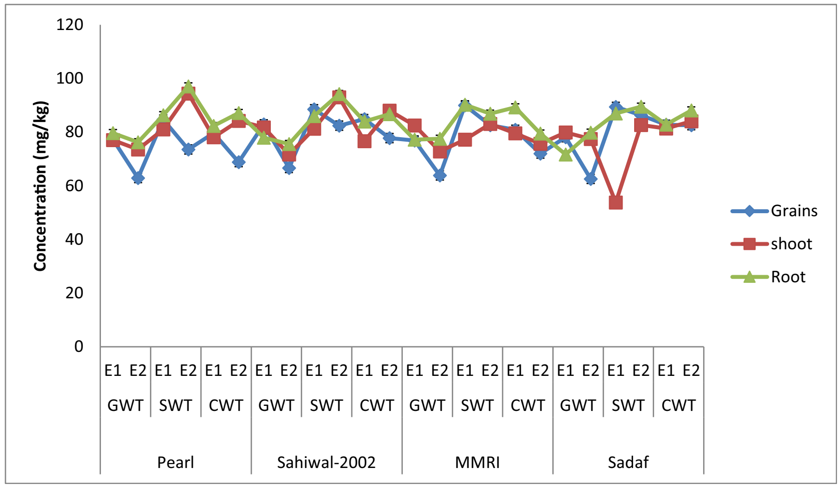

Fig. 4 Potassium concentration (mg/kg) in plant portions of various varieties of canal water treatment (CWT), underground (GWT), sewage (SWT), and in both experiments conducted in varying seasons

0.01 mg/kg found from the gizzard of those fed the Sahiwal-2002 grains irrigated with groundwater (Fig. 5).

Discussion

The presence of high-level potassium in groundwater can be accredited to processing facilities which are pinpoint impact from land application of wasted water because usually groundwater always has low potassium level. Wastewater taken from wineries and piggeries showed the elevated levels of the downgradient (Smiles and Smith 2004; Kumar and Kookana 2006). Adverse health effects caused by potassium in wastewater are still unknown (USEPA 1980). Usual water used for drinking purpose is not seen to set up a threshold level for potassium. Twelve-milligrams per liter concentration of potassium which was maximally admissible for human consumption in water was found by the European Community (EEC 1992). This identification of such value was totally criticized as it has no physiological/toxicological justification and was found as unnecessarily lower for health and nutritional aspects (Grossklaus 1992).

It was reported that ratio of heavy metals and nutrients was higher in the municipal waste effluent rather than well water (Khan et al. 2019). N, K, P, and Ca are in appreciable quantity found in sewage, reflected in the build-up of such nutrients in the soil irrigated with sewage; according to our study, the sewage water has higher concentration of potassium in it which was also high in sewage-irrigated soil. Phosphorous and potassium quantity was observed in soils of different places in which sewage was applied (Ryan et al. 2006). Findings similar to this recent one has also been concluded by Saha et al. (2010) and Masto et al. (2009) for sewage-irrigated soil. Potassium ranged from 524 to 334 kg/ha at New Delhi soil irrigated in sewage water (Masto et al. 2009). On the other hand, the observation done by Saha et al. (2010) potassium—283 μg/g was reported in Bhopal soil due to sewage irrigation.

Table 4 Analysis of variance (ANOVA) for distribution of potassium in the body parts of the chicken

Mean squares								
Source of variation	Degree of freedom	Blood	Bone	Meat	Liver	Heart	Kidney	Gizzard
Season	1	15,276.214**	408.864**	580.770**	1111.158**	2061.666**	3600.118**	2767.117**
Variety	3	758.505**	16.9179^{ns}	104.186**	129.908**	1288.894**	184.196**	46.3063**
Treatment	2	1531.158**	415.522**	541.410**	432.910**	968.296**	213.647**	1614.092**
S × V	3	115.451**	37.6177^{ns}	91.161**	4.9139^{ns}	590.701**	2.6932^{ns}	69.673**
S × T	2	47.2354^{ns}	4.5332^{ns}	30.7935^{ns}	36.1685^{ns}	177.378**	31.1876**	442.451**
V × T	6	32.1924^{ns}	15.3052^{ns}	30.0311^{ns}	5.0805^{ns}	60.956**	13.4029**	4.5195^{ns}
S × V × T	6	49.5703*	33.5380^{ns}	29.4289^{ns}	5.4982^{ns}	79.850**	2.7608^{ns}	7.5951^{ns}
Error	24	151.636	149.458	134.656	112.395	88.298	34.191	53.585

Significant at 0.05, 0.01, and 0.001 levels. ns: at $P > 0.05$

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

*significant at $p < 0.05$

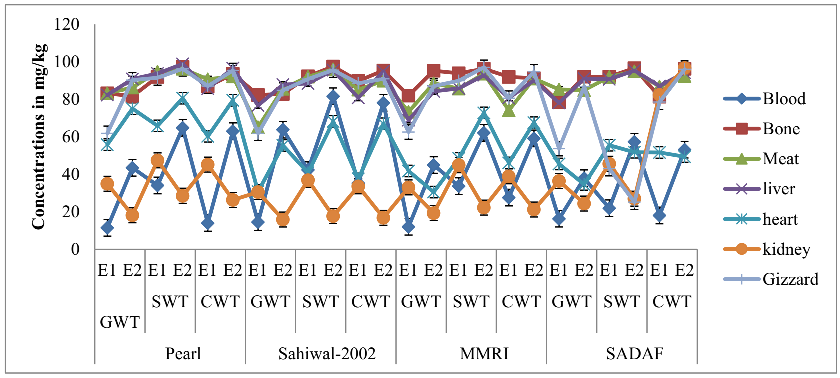

Fig. 5 Potassium concentration in seven body parts of the chicken in both experiments conducted in varying seasons reared on grains of different varieties irrigated with ground water (GW), sewage water (SW), and canal water (CW)

The long term effect of mixed wastewater irrigation on soil properties, citrus fruit quality in terms of heavy metal contamination was evaluated and found the higher concentration of potassium in mixed sewage water compared to fresh water (Hoda and Galal 2015). The soil samples and plants irrigated with mixed water showed a higher concentration of potassium compared to those irrigated with fresh water. Rai et al. (2015) conducted research to appraise the effect of sewage water and canal water on the properties of soil and they also found the higher concentration of potassium in sewage-irrigated soil compared to canal water–irrigated soil. Parkpain et al. (2000) also reported increased nutrients in the soil treated with sewage sludge.

Plant

In the present research, overall, the higher concentration of potassium in the grain, shoot, and root was found in the varieties irrigated with sewage water compared to those irrigated with canal and groundwater. These results are consistent with the study conducted by Abolfazl et al. (2010) where study indicated that irrigation with wastewater appreciably increased the macro element (N, P, and K) contents in corn forage by irrigation with wastewater. This increase could be associated with the quantity of enough nutrient elements present in wastewater. Present investigations about accumulation of potassium along with other metals in upper soil layers as well as in various parts of plants, irrigated with domestic wastewater, were in line with earlier work done by Gadallah (1994); Moazzam et al. (2009); Harati et al. (2012); Tavassoli et al. (2010); Rusan et al. (2007); Amin (2011); Alizade et al. (2001); and Khaskhoussy et al. (2013).

In the present research during both seasons, each variety accumulated potassium differently in all parts. The variations in heavy metal concentrations in various parts of the plants have been ascribed to compartmentalization and translocation through the vascular system (Kim et al. 2003).

Pinamonti (1998) revealed that using sewage effluent for the irrigation of vineyard and the potassium uptake gave results that the removal of potassium was interlinked with the level of adding effluent.

Research results gave potassium uptake of various plants like Japanese water iris, dwarf yellow daylily, Cleopatra, and garlic by the effects of high potassium irrigation waters. There was a significant difference shown between plant species of potassium uptake but was not observed in between different wastewater irrigation treatments (Wu et al. 1996). So, the conclusion drawn was that high potassium in wastewater on these ornamental plants does not seem to develop any mineral concentration stress or reduction in growth. In forages, potassium accumulates at very different concentrations. Pederson et al. (2002) reported that potassium contents were higher in stems and leaves of annual ryegrass than in similar portions of the legumes Ball clover (*Trifolium glomeratum*) and Austrian winter pea (*Pisum sativum*).

A study done by Allhands et al. (1995) shows that after irrigation with municipal wastewaters, warm-season Bermuda grasses (*Cynodon dactylon*) have more potassium uptake capacity. For all the warm-season Bermuda grasses, a linear rising ratio in potassium plant uptake with harvest interval was indicated relative to the control studied. The loaded seasonal rate of 97 potassium kg/ha, estimation of Bermuda grass, was 7.43-mg/ha dry matter yield, and corresponding values for winter rye (*Secale cereal*) were 4.25 mg/ha and 66 potassium kg/ha. Recent studies are concerning in showing the effects of using potassium wastewaters in higher rates which have reached grasses because they are widely identified to influence increasing rates of potassium removal potential (Grattan and Grieve 1992).

Animal

Group of chickens that utilized sewage water–irrigated grains as their feed showed a higher concentration of potassium in all their body parts compared to those that had canal water treatment and ground water treatment grains in their diet. Present research values for potassium in the body parts of chicken were lower than those found by Połtowicz and Doktor (2013) who conducted a study to evaluate the macro mineral (including potassium) ratio in leg and breast muscles along with their link with quality of meat in broiler chickens (slaughter age) and found a result that muscles of legs have more Na whereas breast muscles were rich source of P, Mg, and K. Poultry meat additionally contains vitamins and animal proteins a valuable mineral source according to human nutrition (Lombardi-Boccia et al. 2005). The ratio of these components are not only dependent on how much macro- and microelements are present but are also varied due to the breed, sex, health, slaughtering techniques, their muscle types, and how animals are housed (Doyle 1980; Pesut et al. 2005; Horbańczuk et al. 1998; Zapata et al. 1998; Sadoval et al. 1999; Skrivan et al. 2005), and Keeton and Eddy 2004) reported that components of mineral have an essential functioning in few elements (such as Na, Ca, Mg, P, and K) and skeletal muscle metabolism which are responsible for normal muscle function and play a vital role in various enzymatic processes.

Presently, it is been concluded that every part has different ways of absorbing potassium. Zarkadasc et al. 1987) noted considerable differences in potassium content according to the type of avian muscles. Zapata et al. (1998) reported values of 327.99-mg and 100-g tissue for potassium in broiler chicken light meat, and 280.25 mg/100-g tissue of potassium in dark meat.

Conclusion

The present research revealed overall the higher concentration of potassium in the grain, shoot, and root found in the varieties that were irrigated with sewage water compared to canal and groundwater. This increase is associated with the magnitude of adequate nutrient elements present in enormous quantity in wastewater. The present investigations addressed the accumulation of potassium in chicken reared on grains produced by the application of wastewater. Group of chickens that were fed wastewater-irrigated plant grains as their feed showed the higher concentration of potassium in all their body parts compared to those that had canal- and groundwater-irrigated grains in their diet. The results of the study provided a baseline data for future research so that a comprehensive awareness campaign may be initiated pertaining health issues related to using sewage water.

Availability of data and materials Data and material is available for research purpose and for reference.

Authors contribution Fu Chen, Zafar Iqbal Khan, and Kafeel Ahmad conceived and designed the study which was supervised by Ijaz Rasool Noorka. Jawaher Alkahtani and Mohamed Soliman Elshikh critically revised the manuscript and approved the final version. Zille Huma executed the experiment and compiled data. Fu Chen and Yongjun Yang statistically analyzed the data and helped in chemical analysis. Jing Ma interpreted the results. Fu Chen, Sonaina Nazar, and Humayun Bashir wrote the manuscript. Mudasra Munir and Asma Ashfaq, Ifra Saleem Malik, and Razia Sultana helped in sample collection and chemical analysis.

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Declarations

Ethics approval Ethical approval was taken from Department Ethical Review Committee to conduct study. 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, and all methods were performed in accordance with relevant guidelines and regulations

Consent to participate Informed consent was taken from formers to conduct the study and to collect the samples. They were briefed about the research plan in details.

Consent for publication Written consent was sought from each author to publish the manuscript.

Competing interests The authors declare no competing interests.

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