Heavy Metal Accumulation in Soil, Forage and Blood Plasma of Horses in Central Punjab, Pakistan

Ilker Ugulu[1]*, Zafar Iqbal Khan[2], Kafeel Ahmad[2], Humayun Bashir[2], Naunain Mehmood[3], Asma Zafar[2] and Muhammad Sohail[2]

[1]*Faculty of Education, Usak University, Usak, Turkey*
[2]*Department of Botany, University of Sargodha, Sargodha, Pakistan*
[3]*Department of Zoology, University of Sargodha, Sargodha, Pakistan*

ABSTRACT

The main purpose of the present study was to determine and evaluate the heavy metals in soil, forage and blood plasma of horses in Central Punjab, Pakistan. Concentrations of metals, cobalt (Co), selenium (Se), manganese (Mn) and iron (Fe) in these samples were determined. Atomic Absorption Spectrophotometer was used to determine the concentration of metals. The concentrations of all the analysed heavy metals differed significantly with different seasonal periods. The mean Fe, Co, Mn and Se values in blood plasma samples of 4 years (Cohort-8), 3 years (Cohort-9) and 2 years (Cohort-10) cohorts were 1.34, 0.18, 0.026 and 0.019 mg/L; 1.23, 0.17, 0.026 and 0.015 mg/L and 1.19, 0.15, 0.029 and 0.015 mg/L, respectively. No statistically significant changes were observed for heavy metal concentrations in plasma samples of horses according to two sampling seasons. The present results indicated that heavy metals in soil, forage and blood plasma samples were lower than the recommended critical levels except for the Mn accumulation in forage samples.

INTRODUCTION

Chronic environmental exposure to heavy metals represents a real threat to the biosphere (Ugulu, 2015a). Heavy metals, in the environment above the natural background concentrations, disturb the biological balance of ecosystems, can produce toxic effects on wildlife and pose an additional threat to human and animal health after finding their way into the food chain (Wieczorek-Dabrowska *et al*., 2013). Although these metals occur naturally in the environment, human activities such as the metal industry and battery manufacturing cause significant additional anthropogenic releases of metals (Tariq *et al*., 2021; Ugulu *et al*., 2022). The impact of heavy metals on the environment can be a serious threat to the stability of ecosystems and living organisms (Chen *et al*., 2021). All living beings must cope with element stress, either from

exposure to non-essential toxic elements or from depletion, or excess of essential elements (Ugulu, 2015b).

The nutritional needs of plants are fulfilled through the element composition of the soil (Dogan *et al*., 2011; Khan *et al*., 2021). Consequently, these elements become part of animal bodies while foraging (Wajid *et al*., 2020; Ugulu *et al*., 2021). Hence soil at a specific geographical location shows variations in element composition and it may result in element excesses or deficiencies in animals grazing on these soils (Reis *et al*., 2010). Accordingly, disease resistance in animals is affected by the heavy metal content in the soil composition of any area (Ahmad *et al*., 2018; Khan *et al*., 2018; Siddique *et al*., 2019).

The requirement to identify the degree of exposure and the effects of contaminants in living organisms has led to numerous biomonitoring studies (Sahin *et al*., 2016). Direct monitoring of air, soil, water, and sediments can be useful to define the degree of pollution in a particular area; however, it does not provide a measure of bioavailability and resultant uptake by biota or people (Carneiro *et al*., 2016). It is only through direct biomonitoring (the analysis of pollutants in fluids and tissues of organisms) that the actual exposure of living beings can be properly determined and related to the levels in the environment (Gómez-Ramírez *et al*., 2014). In general, the aims of monitoring the animals include data collection to estimate human health risks, to determine the contamination

---

*　Corresponding author: ilkerugulu@gmail.com

of the food chain, to define the levels of environmental contamination, to identify negative effects on animals and evaluate spatial or temporal trends in pollution levels (Carneiro *et al*., 2016; Khan *et al*., 2019). In this direction, the main purpose of the present study was to determine the heavy metal values, iron (Fe), manganese (Mn), cobalt (Co) and selenium (Se) in pasture soil, forage and animals by using a systematic approach.

MATERIALS AND METHODS

The present research was performed to analyse the concentration of the heavy metals in the samples collected from the blood plasma of horses, the forage they used and the soil where the forage was planted. The samples were collected twice from the experimental station with a time interval of six days. The difference in the metal concentrations was checked during their transfer from soil to forage and forage to blood plasma. The concentrations of four metals, Fe, Co, Mn, and Se were analysed in various soil, forage and blood samples.

Study area

The present investigation was performed in the district Sargodha of Punjab City which is situated in the northwest of Pakistan. It is a trade centre for various agricultural as well as industrial products. Sargodha mainly consisted of flat and fertile plains and some small hills are also located in the southeast part of the city. The climate of the city is extremely hot and cold. Maximum temperature reaches up to 50 °C in summer and at a freezing point in winter. Average monthly rainfall is recorded at about 82 mm in district Sargodha (Khan *et al*., 2019a, b).

The area covered for a barn in this Remount Depot is about 200 acres. The animals present on this farm include mauls and horses. The soil of this area is loamy to clay and very fertile for the fodder growth of animals, especially for horses and mauls. The pH of this soil is about 7.6. Different varieties of forages are grown in this field for animals. These species of forages include barsem (*Trifolium alexandrinum* L.), bajra (*Pennisetum glaucum* L.) and oat (*Avena sativa* L.).

Collection of samples

Soil samples were collected from three different places, selected randomly at distances of 10 acres in the field. Sample spots were dug out up to 12–15 cm deep by stainless steel auger. Samples were collected two times, once in winter and then in summer and 15 samples were accumulated during each sampling time. Samples were dried in the open air, stock up in sealed paper bags and put in an incubator at 70 °C for 3 days for complete dryness.

The forage samples were also collected in the same field from where soil samples were taken out. Sterilized apparatus was used to collect the samples. The collected forage samples include barsem (*Trifolium alexandrinum*), bajra (*Pennisetum glaucum*) and oat (*Avena sativa*). A sampling of forage was done two times, once in summer and then in winter. For accuracy, 15 samples were collected during each sampling period. To remove any dust particles or other contaminants, each sample was washed with distilled water. All the samples were dried in the open air and then in an incubator at 60 °C to remove all the moisture contents.

Blood samples were collected two times, once in summer and then during the winter season. Total of thirty horses was selected, ten from each age cohort i.e., CH-10 (2 yrs), CH-09 (3 yrs) and CH-08 (4 yrs). The blood samples were taken from the vein of the horses by using a sterilized needle. The blood was then transferred to the vials containing heparinized Na-Citrate solution, to avoid the formation of any clots. The centrifugation method was used to separate plasma from blood. The sample was centrifuged at the rate of 3000 rpm for a period of 15 to 30 min. The separated plasma was packed, labelled and put into the freezer at 20 °C.

Preparation of samples and analysis

The soil samples were digested according to the wet digestion method. About 1.0 g of sample was taken in a flask and mixed with H_2SO_4 (4 mL) and H_2O_2 (8 mL). The flask was placed in a chamber for heating at 75 °C. After about 30 min, when escaping of vapours ceased down, more H_2O_2 (2 mL) was added and heat it again in the digestion chamber. This process was continued till the sample become colourless. After the completion of digestion, the digested materials were removed and filtered through Whatman filter paper # 42. In filtrate, double distilled water was added to increase the volume up to 50 mL. The prepared sample was saved in a labelled polyethylene bottle.

Forage samples were digested by the wet digestion method. Weigh out 1.0 g of the forage sample and transfer it into the digestion flask. Add 2 mL H_2SO_4 and 4 mL H_2O_2 and placed the flask in the digestion chamber for heating at 75 °C. After about 30 min, when escaping of fumes was stopped, then more H_2O_2 (2 mL) was added. The sample was again heated by placing it in the digestion chamber. The process was repeated continuously till the colour of the sample disappeared. Finally, the digested mixture was taken out from the chamber and added double distilled water to make its volume up to 50 mL. Filtered the mixture through Whatman filter paper # 42 and stored it in labelled polyethylene bottles.

A plasma sample (1 mL) was poured into the digestion flask and added H_2SO_4 (1 mL) and H_2O_2 (2 mL). The mixture was then placed in the digestion chamber for about 30 min at 75 °C. When evaporation of the fumes is close down then the sample is removed from the chamber and added more H_2O_2 (2 mL). The process of removal of the sample and addition of H_2O_2 is continued till the sample becomes transparent. Finally, the digested material was removed and added double distilled water to make the volume up to 60 mL. The diluted material was filtered and stored in labelled polyethylene bottles.

Analysis of metals

Atomic Absorption Spectrophotometer (Perkin-Elmer AAS–5000) was used to determine the concentration of Fe, Co, Mn, and Se in the samples of soil, forage and blood plasma of horses.

Bioconcentration factor (BCF) and correlation

Transfer factor is used to find out the concentration of metals which is accumulated in animals through the food chain (Ugulu *et al*., 2021a, b; Ahmad *et al*., 2023). It is used to find out the efficiency of different species of plants and animals for the accumulation of different metals. It can be estimated in plants as concentration of metal in forage/ concentration of metal in soil, while in blood plasma as concentration of metal in plasma/ concentration of metal in forage.

The Pearson correlation coefficient method was used to create a relationship between metal concentrations in soil-forage, soil-plasma and forage-plasma models (Yorek *et al*., 2010).

RESULTS AND DISCUSSION

Iron (Fe)

The analyses on the selected heavy metals for this study showed that Fe content ranged from 86.67 to 88.80 mg/kg (Table I, Fig. 1). The maximum permissible level of Fe accumulation in soil was reported as 50000 mg/kg by World Health Organization (WHO), Food and Agricultural Organization (FAO), Standard Guidelines in Europe (Chiroma *et al*., 2014) and USEPA (2002). The lower concentration of Fe than the safe limits at the study area may be due to the removal of potentially toxic metals by the plants grown in this area and due to the leaching of heavy metals into the deeper layer of the soil (Singh *et al*., 2010). However, Khan *et al*. (2016a) determined the Fe values between 4.79-9.70 mg/kg in irrigated soil samples collected from Sargodha-Pakistan while Khan *et al*. (2016a) defined as ranged from 6.04 to 6.77 mg/kg in irrigated soil samples gathered from different agricultural

areas Sargodha-Pakistan. Also, Ahmad *et al*. (2016b) identified the Fe values varied from 34.09 to 42.05 mg/kg and Khan *et al*. (2016b) identified the Fe values varied from 20.22 to 25.11 mg/kg in irrigated soil samples from Khushab and Jhang City of Pakistan, respectively. The range values of the present study are below the mentioned studies. On the other hand, Khan *et al*. (2016c) studied the soil samples along the roadside and canal side of Sargodha and determined the average Fe value as 634 mg/kg.
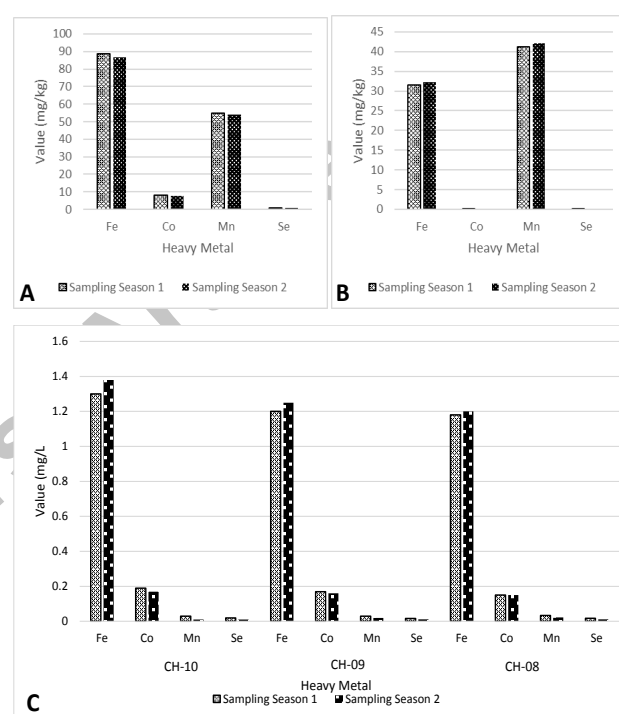

Fig. 1. Mean metal concentrations in soil (A), forage (B) and blood plasma of horses (C) during summer (sample season 1) and winter (sample season 2).

In forage samples, the Fe content varied from 31.48 to 32.23 mg/kg (Table I). The maximum permissible level of Fe accumulation in plants was reported as 425 mg/kg by WHO, FAO, Standard Guidelines in Europe (Chiroma *et al*., 2014) and USEPA (2002). The range values of Fe in forage samples were lower than this permissible level of Fe accumulation in plant samples (Fig. 1).

When the recent studies examining the accumulation of heavy metals in plants and forage samples in Punjab-Pakistan examined; Ahmad *et al*. (2016a) detected the Fe values between 32.61-42.63 mg/kg in *Coriandrum sativum* L. samples grown in contaminated water irrigated agricultural sites of Sargodha. Ahmad *et al*. (2016b) conducted a study in the peri-urban areas of Khushab and

Table I. Heavy metal concentrations in soil and forage samples (Mean±SEM, range from Min-Max, mg/kg) and horses blood plasma (Mean±SEM, range from Min-Max, mg/L) during summer and winter seasons.

Sample/ Sampling period	N	Samples			
		Fe	Co	Mn	Se
Soil					
Summer	15	88.80±0.053 (83.10-93.27)	8.23±0.016 (6.15-9.42)	55.02±0.059 (53.41-58.10)	1.043±0.058 (0.81-1.18)
Winter	15	86.67±0.041 (83.65-89.91)	7.87±0.099 (6.38-8.56)	53.90±0.049 (52.25-56.78)	0.99±0.032 (0.79-1.08)
Maximum permissible limits		50,000	50	80	3
Forage					
Summer	15	31.4865±0.025 (28.82-34.37)	0.094±0.012 (0.06-0.13)	41.1735±0.021 (38.26-44.65)	0.0705±0.001 (0.04-0.09)
Winter	15	32.2335±0.041 (30.84-34.45)	0.091±0.003 (0.055-0.12)	42.0775±0.024 (39.28-44.62)	0.0965±0.009 (0.07-0.10)
Maximum permissible limits		425	1	30	0.1
Blood plasma of horses					
2 years old (CH-10)					
Summer		1.30± 0.021 (1.22-1.5)	0.19± 0.004 (0.16-0.22)	0.033±0.002 (0.02-0.04)	0.022±0.001 (0.015-0.03)
Winter		1.38± 0.015 (1.24-1.49)	0.17± 0.002 (0.12-0.21)	0.018± 0.004 (0.005-0.03)	0.017±0.001 (0.01-0.025)
3 years old (CH-9)					
Summer		1.20±0.012 (1.11-1.32)	0.17±0.002 (0.13-0.23)	0.032±0.015 (0.025-0.04)	0.017±0.0021 (0.005-0.025)
Winter		1.25±0.017 (1.20-1.31)	0.16±0.002 (0.13-0.19)	0.020±0.001 (0.010-0.03)	0.012±0.003 (0.005-0.02)
4 years old (CH-8)					
Summer		1.18±0.004 (1.03-1.32)	0.15±0.007 (0.11-0.21)	0.03±0.002 (0.02-0.04)	0.018±0.001 (0.005-0.03)
Winter		1.20±0.003 (1.14-1.3)	0.15±0.003 (0.10-0.2)	0.02±0.001 (0.01-0.03)	0.012±0.099 (0.005-0.02)

Table II. Analysis of variance of data of heavy metals in blood plasma of horses.

Source of variation	D.F	Mean squares											
		Iron			Cobalt			Manganese			Selenium		
		CH_{10}	CH_{09}	CH_{08}	CH_{10}	CH_{09}	CH_{08}	CH_{10}	CH_{09}	CH_{08}	CH_{10}	CH_{09}	CH_{08}
Sampling periods	1	0.018[ns]	0.006[ns]	0.001[ns]	0.001[ns]	0.003[ns]	0.007[ns]	0.001[ns]	0.004*	0.012[ns]	0.001[ns]	0.002[ns]	0.001[ns]
Error	8	0.011	0.004	0.010	0.001	0.001	0.002	0.001	0.002	0.002	0.003	0.001	0.002

*, significant at 0.05 levels; ns, non-significant.

defined the Fe values ranged from 30.28 to 37.63 mg/kg in *Daucus carota* L. samples, Ahmad *et al*. (2016c) determined the Fe values varied from 50.1 to 78.4 mg/kg in *Solanum melongena* L. samples grown in a long-term wastewater-irrigated agricultural land of Sargodha. Khan *et al*. (2016b) identified the Fe values between 39.39 to 42.94 mg/kg in *Abelmoschus esculentus* (L.) Moench as a contaminated vegetable from sewage water collected from Jhang City.Khan *et al*. (2016c) studied vegetables such as *Brassica campestris* L*., Spinacia oleracea* L. samples grown along the roadside and canal side of Sargodha and determined the average Fe values ranging from 11.20 to 28.56 mg/kg and finally Khan *et al*. (2016d) defined the Fe values varied from 38.96 to 41.14 in *Cucurbita maxima* samples irrigated with domestic wastewater in Jhang. Although these values in different studies are close to each other, differences between the accumulation values may be originated from using the different plant species in these studies (Khan *et al*., 2019c).

According to the analysis results, the mean Fe values

in blood plasma samples ranged from 1.18 to 1.38 mg/L (Table I). The highest mean Fe value was obtained for CH-10 in the second sampling period (Fig. 1). The present values were within the permissible range of Fe (225 mg/L) as set by NRC (1996). Higher concentrations of Fe in plasma and plants indicated that there was no need for supplementation to enhance the Fe concentration for the animals. This contrasted with other areas of Pakistan where Fe concentration in plasma was found below the critical value. Statistically, a non-significant difference in Fe levels was obtained for all cohorts of horses i.e., CH-10 (2 yrs), CH-09 (3 yrs) and CH-08 (4 yrs) (Table II).

Table III. Correlation between soil–forage–blood plasma.

Metal	Soil: forage	Forage: blood plasma	Blood plasma: soil
Iron	0.191	−0.507	−0.267
Cobalt	−0.101	0.143	−0.101
Manganese	−0.281	0.356	−0.246
Selenium	−0.532	−0.519	0.479
Arsenic	−0.235	0.473	−0.248

The soil-forage model displayed a positive correlation while soil-plasma and forage-blood plasma showed a highly negative correlation (Table III). Edaphic factors could be a reason for this contrasting relationship of Fe between different mediums (Ugulu et al., 2019a, b). The Fe uptake could be reduced as a result of this non-significant relationship. The Fe imbalance could thus be produced between different media.

BCF for Fe was higher in the first sampling season (0.3719) than second sampling season (0.3546; Table IV). A conclusion to this trend could be the high sensitivity of forage grown in the second sampling period as high BCF was obtained for this season. This discrepancy in BCF for Fe in two sampling periods could be due to the low absorption ability of forages grown in the first sampling season. Trace metal contents in plants are reflective of plant type, age, soil pH, nature and climate. BCF values for Fe in different studies were determined as 0.86-0.89 and 1.16-1.63 by Ahmad et al. (2016b, c), respectively. The BCF value for the present study is quite low compared to these studies. On the other hand, horses of the age bracket of 4 years had high plasma BCF for Fe as compared to other age groups of horses bred on the same farm. However, all age groups of horses showed low BCF value than 1. BCF ≤ 1 demonstrate that animal can just retain but not collect trace metals; when a BCF>1, it demonstrates that animal can accumulate trace metals (Rasheed et al., 2020; Yang et al., 2020). Rajkowska and Protasowicki (2013) studied the distribution of trace metals in fish tissues and reported that the BCF values of Fe in different tissues such as muscles, skin, gills and digestive tract varied from 27 to 1599. These values are quite higher than the BCF values presented in this study.

Cobalt (Co)

According to the AAS analysis results, the accumulation levels of Co varied between 7.87 and 8.23 mg/kg (Table I). The Co concentrations were below the maximum permissible limit for soil (50 mg/kg) was reported by WHO, FAO, Standard Guidelines in Europe (Chiroma et al., 2014) and USEPA (2002). Khan et al. (2016b, d) in their studies conducted in Jhang City, Pakistan identified the Co values between 17.18-21.13 and 18.12-19.65 mg/kg in soil samples contaminated from waste-water, respectively. Also, Ahmad et al. (2016b) identified the Co values varied from 13.4 to 19.7 mg/kg in soil samples contaminated from sewage water in

Table IV. Transfer factor from soil to forages and forage to blood plasma during summer and winter seasons.

Transfer factor		Fe	Co	Mn	Se	As
Sampling period 1 (summer)						
Soil-forage	S-F	0.354568	0.01141	0.748215	0.067542	0.573539
Forage-blood plasma	F-CH10	0.041383	2.042553	0.000801	0.312057	0.568118
	F–CH09	0.038239	1.882979	0.000777	0.241135	0.570734
	F-CH08	0.037572	1.659574	0.000826	0.255319	0.54943
Sampling period 2 (winter)						
Soil-forage	S-F	0.371911	0.011554	0.780572	0.097455	0.690015
Forage-blood plasma	F-CH10	0.043092	1.901099	0.000428	0.176166	0.51011
	F–CH09	0.038811	1.824176	0.000475	0.124352	0.503993
	F-CH08	0.037259	1.725275	0.000547	0.124352	0.469669

S, Soil; F, Forage; CH10, 2 years old cohorts; CH9, 3 years old cohorts; CH8, 2 years old cohorts.

Jhang City and Ahmad *et al*. (2016c) determined the Co values between 14.3-26.2 mg/kg in soil samples collected from long-term wastewater-irrigated agricultural land of Sargodha, Pakistan.

The concentration of Co levels in forage samples varied between 0.091 and 0.094 mg/kg in both seasons (Table I). The maximum permissible level of Co accumulation in plants was reported as 1 mg/kg by WHO, FAO, Standard Guidelines in Europe (Chiroma *et al*., 2014) and USEPA (2002). The range values of Co in forage samples were lower than this permissible level of Co accumulation in plant samples. In Pakistan, Ahmad *et al*. (2016b) defined the Co values ranged from 0.72 to 0.89 mg/kg in *Daucus carota* samples in the peri-urban areas of Khushab City and Ahmad *et al*. (2016c) determined the Co values varied from 0.64 to 1.16 mg/kg in *Solanum melongena* samples grown in a long-term wastewater-irrigated agricultural land of Sargodha. In Jhang City, Khan *et al*. (2016b, d) identified the Co values between 0.48-0.49 and 0.57-0.73 mg/kg, respectively in *Abelmoschus esculentus* and *Cucurbita maxima* samples contaminated with wastewater.

In blood plasma samples, the mean Co values varied between 0.15 and 0.19 mg/L (Table I). The lowest value of Co was obtained in the plasma of the CH–08 group in both sampling periods while the highest value was obtained for CH-10 in the first sampling period but even the highest value did not exceed the permissible limit of 0.25 mg/L (McDowell *et al*., 1984). Rehman *et al*. (1998) in their studies performed in Eastern Sudan reported a similar value of Co in the blood serum of grazing cows. According to the ANOVA results, the Co mean levels in the plasma of horses for all three groups (CH-08, CH-09 and CH-10) were non-significant ($p > 0.05$) (Table II).

Forage-plasma medium showed a positive correlation while plasma-forage and soil-forage mediums displayed a negative correlation (Table III). Edaphic factors could be a reason for this contrasting relationship of Co between different mediums (Munir *et al*., 2019). This might potentially cause imbalances among different media for Co concentration emphasizing supplementation needs.

BCF for Co in the second sampling period (0.0116) was higher than in the first sampling period (0.0114; Table IV). According to BCF ≤ 1 demonstrate that plant can just retain but not collect metals, it cannot be said that the forage samples in the present study accumulate the metals from the soil. BCF values for Co in different studies were determined as 0.045-0.054 and 0.05 by Ahmad *et al*. (2016b, c), respectively. The BCF values identified in these Pakistani studies support the BCF values of the present study. The CH-10 group had the highest BCF in the first sampling season. Metal absorption might be affected by forage varieties, age, climatic conditions, soil nature

and pH (Carneiro *et al*., 2016; Khan *et al*., 2023). Metal concentrations in forages were used to calculate BCFs, which indicated that among examined metals Co was the most readily absorbed by horses. The presented research has shown also that forage was a good source of Co, which penetrated horses tissues.

Manganese (Mn)

In soil samples, the level of Mn varied between 53.90 and 55.02 mg/kg (Table I). The concentrations of Mn in soil samples in the study area are below WHO (1996) and USEPA (1997) maximum permissible levels in soils of 80 mg/kg. In addition, the Mn values in the present study are below the mean Mn accumulation values in soil samples in urban areas of Azerbaijan's oil industrial region were determined as 410-2170 mg/kg by Khalilova and Mammadov (2016). However, the present Mn values are above the following values determined in different cities of Pakistan by Ahmad *et al*. (2016b, c), Khan *et al*. (2016b, c, d), respectively: 15.93-23.83 mg/kg in Khushab City, 18.9-34.8 mg/kg in Sargodha city, 12.44-14.68 mg/kg in Jhang city, the average 230 mg/kg in Sargodha city and 17.68-18.14 mg/kg in Jhang city.

The Mn levels obtained from the forage samples ranged between 41.17-42.07 mg/kg (Table I). The maximum permissible level of Mn accumulation in vegetables was reported as 30 mg/kg by USEPA (1997). The range values of Mn in forage samples were higher than this permissible level of Mn accumulation in plant samples. When the studies performed in the different cities of Pakistan were considered, the following higher values than the present study were identified by Ahmad *et al*. (2016b) in *Daucus carota* samples grown in Khushab, Ahmad *et al*. (2016c) in *Solanum melongena* samples grown in Sargodha, Khan *et al*. (2016b) in *Abelmoschus esculentus* in Jhang and Khan *et al*. (2016d) in *Cucurbita maxima* samples in Jhang, respectively: 43.27-46.73, 74.4-79.5, 55.40-57.36 and 61.12-61.13 mg/kg. However, Khan *et al*. (2016c) studied the vegetables such as *Brassica campestris* and *Spinacia oleracea* samples grown along the roadside and canal side of Sargodha and determined the average Mn values ranged from 1.22 to 10.65 mg/kg.

In terms of blood plasma of horses, summer season samples had the highest value of Mn (0.034 mg/L=CH-08) while the lowest value was obtained for CH-10 (0.018 mg/L) in winter (Table I). The mean level of Mn was lower than the tolerable level of 0.05 mg/L (NRC, 1996). Higher manganese doses than this level can cause poisoning in animals. Mn poisoning can be diagnosed through the dosage of this metal in the liver, Mn concentration above 0.07 mg/kg is considered a poisoned animal (NRC, 1996). Statistically, the Mn level of all groups of horses was

significant according to both sampling seasons ($p<0.05$) (Table II).

Opposing trends for correlation were observed; a positive correlation between forage-blood plasma and a negative correlation between soil-forage and soil-blood plasma (Table III). Mn is also an essential plant nutrient, playing a key role in several physiological processes, particularly photosynthesis. Mn deficiency is a widespread problem, most often occurring in sandy soils, organic soils with a pH above 6 and heavily weathered, tropical soils (Alloway, 2008). In this direction, the reason for the negative correlation between soil-forage may be originated from edaphic factors of the study area (Khan *et al*., 2020a, b).

As the BCF values were investigated for Mn, the BCF value for winter samples (0.7806) was higher in the soil-forage medium than summer BCF values (0.7482; Table IV). BCF values of soil-forage medium for Mn in different studies determined the range as 1.81-2.93 and 2.33-3.92 by Ahmad *et al*. (2016b, c), respectively. The BCF values identified in these Pakistani studies are fairly higher than the BCF values of the present study. On the other hand, the BCF value of the forage-blood plasma medium of the summer sampling period is higher than the winter sampling period value. The highest BCF value between these samples, cohort CH-08 of horses had higher BCF (0.00082) in summer. Rajkowska and Protasowicki (2013) determined the range of BCF values between 8 and 1035 in fish tissues. As in Fe, these values are quite higher than the BCF values presented in this study.

Selenium (Se)

In soil samples, the mean levels of Se varied between 0.99 and 1.04 mg/kg (Table I). The maximum permissible level of Se accumulation in soil was reported as 3 mg/kg by WHO (1996) and USEPA (1997). The range values of Se in soil samples were lower than this permissible level of Se accumulation in soil. The soil Se values in different cities of Pakistan were determined by Ahmad *et al*. (2016a, b, c), Khan *et al*. (2016b, d) as follows: 1.81-2.51, 1.70-2.76, 2.44-3.33, 1.81-2.02 and 1.96-2.16 mg/kg, respectively. Current study findings were comparable with these studies carried out in Pakistan where Se values were low.

Trace metal analysis showed that the range of Se was 0.070-0.096 mg/kg (Table I). Mean Se levels were below the acceptable level of 0.1 mg/kg (NRC, 1996). Current study values were in contrast to other studies carried out in Pakistan. Ahmad *et al*. (2016a) detected the Se values between 0.25-0.45 mg/kg in *Coriandrum sativum* samples grown in contaminated water-irrigated agricultural sites of Sargodha. Ahmad *et al*. (2016b) performed a study in the peri-urban areas of Khushab and defined the Fe values

ranged from 0.50 to 0.51 mg/kg in *Daucus carota* samples grown in soil samples contaminated from sewage water. Ahmad *et al*. (2016c) determined the Mn values varied from 0.72 to 0.83 mg/kg in *Solanum melongena* samples grown in a long-term wastewater-irrigated agricultural land of Sargodha. Khan *et al*. (2016b) identified the Mn values between 0.54 to 0.55 mg/kg in *Abelmoschus esculentus* as a contaminated vegetable from sewage water collected from Jhang city. Khan *et al*. (2016d) defined the Mn values varied from 0.45 to 0.77 mg/kg in *Cucurbita maxima* samples irrigated with domestic wastewater in Jhang. Also, McDowell *et al*. (1984) and Khan *et al*. (2018a, b) obtained lower values in comparison to the current study.

Between the blood plasma samples, the CH-10 group of horses had the highest value of Se (0.030 mg/L; Table I). The Se level was similar to the permissible level of 0.03 mg/L reported by McDowell *et al*. (1984). The Se is vital for the growth and general health of animals. Animal health is affected by Se deficiency/excess in diet (Fordyce, 2005). Although in many published studies, Se poisoning has not been exactly explained, because there are controversies in search results published, fault of dosage of Se in animal tissue, fault of explicitness on diagnosis and fault of the description of clinical signs (Reis *et al*., 2010). The sampling period had a non-significant effect on mean Se levels in the blood plasma of horses according to the statistical analysis ($p>0.05$) (Table II).

Soil-blood plasma system had a positive correlation, but soil-forage and forage-blood plasma has a negative correlation (Table IV). As in the other metals presented in this study, it could be due to edaphic factors. The Se uptake might be reduced by displaying non-linear movement of Se from soil to plants and then to animals.

When the BCF values were evaluated for Se, in contrast to the BCF values of Fe and Mn, forage-blood plasma mediums were higher than soil-forage mediums in both winter and summer sampling periods. For the soil-forage mediums, the winter samples had a higher BCF value (0.0975) than the summer BCF value (0.0675; Table IV). Deficiency in Se uptake was noticed in forages grown in summer. Low bioavailability or low bioaccumulation capacity of forage varieties could be cited as possible reasons for this outcome. In Pakistan, for the BCF values of the soil-forage medium, Ahmad *et al*. (2016a, b, c) and Khan *et al*. (2016b) determined the ranges as 0.14-0.18, 0.18-0.29, 0.25-0.29 and 0.28-0.29, respectively. These BCF values are higher than the BCF values of the present study. BCF ≤ 1 demonstrate that animal can just retain but not collect metals; when a BCF>1, it demonstrates that animal can accumulate metals (Ugulu *et al*., 2009; Munir *et al*., 2019). In this direction, it can be said that there was

no Se accumulation in the blood plasma of horses.

CONCLUSION

This research provides data on heavy metal pollution in Sargodha. The present results indicated that heavy metals in soil, forage and blood plasma samples of the research materials of the present study were lower than the recommended critical levels except for the Mn accumulation in forage samples. A positive correlation was observed for forage and blood plasma to a certain degree for Co, As and Mn but for other media, metal correlations were negative and insignificant except for Fe. It is therefore suggested that regular monitoring of heavy metals in soils/ plants is essential to prevent excessive build-up of these metals in the environment. Also, new research on the other heavy metals unevaluated in the present study like Cd, Cr, Cu, Hg and Zn may be useful.

ACKNOWLEDGEMENT

IRB approval
There is no human subject in the present study. For animal samples, all the study protocols were approved by the Institutional Animal Ethics Committee, University of Sargodha (Approval No. 25-A18 IEC UOS).

Ethical statement
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 were compiled with the rules of the National Research Council and all methods were performed following relevant guidelines and regulations.

Statement of conflict of interest
The authors have declared no conflict of interest.

REFERENCES

Ahmad, K., Ashfaq, A., Khan, Z.I., Ashraf, M., Akram, N.A., Yasmin, S., Batool, A.I., Sher, M., Shad, H.A., Khan, A., Rehman, S., Ullah, M.A. and Noorka, I.R., 2016a. Health risk assessment of heavy metals and metalloids via dietary intake of a potential vegetable (*Coriandrum sativum* L.) grown in contaminated water irrigated agricultural sites of Sargodha, Pakistan. *Hum. Ecol. Risk Assess.*, **22**: 597-610. https://doi.org/10.1080/10807039.2015.1095630

Ahmad, K., Khan, Z.I., Yasmin, S., Ashfaq, A., Noorka, I.R., Akram, N.A., Shad, H.A., Hussain, A., Arshad, F., Sher, M., Tahir, H.M., Bashir, H. and Zafar, A., 2016b. Contamination of soil and carrots irrigated with different sources of water in Punjab, Pakistan. *Environ. Earth Sci.*, **75**: 426. https://doi.org/10.1007/s12665-016-5348-4

Ahmad, K., Khan, Z.I., Ashfaq, A., Akram, N.A., Ashraf, M., Yasmin, S. and Tufarelli, V., 2016c. Contamination and accumulation of heavy metals in brinjal (*Solanum melongena* L.) grown in a long-term wastewater-irrigated agricultural land of Sargodha, Pakistan. *Fresen. environ. Bull.*, **25**: 2404-2410.

Alloway, B.J., 2008. Micronutrients and crop production: An introduction. In: *Micronutrient deficiencies in global crop production* (ed. B.J. Alloway). Springer. https://doi.org/10.1007/978-1-4020-6860-7

Carneiro, M., Colaço, B., Colaço, J. Faustino-Rocha, A.I., Colaço, A., Lavin, S. and Oliveira, P.A., 2016. Biomonitoring of metals and metalloids with raptors from Portugal and Spain: A review. *Environ. Rev.*, **24**: 63-83. https://doi.org/10.1139/er-2015-0051

Chen, F., Khan, Z. I., Zafar, A., Ma, J., Nadeem, M., Ahmad, K., Shehzadi, M., Wajid, K., Bashir, H., Munir, M., Malik, I. S., Ashfaq, A., Ugulu, I., Dogan, Y., Yang, Y., 2021. Evaluation of toxicity potential of cobalt in wheat irrigated with wastewater: health risk implications for public, *Environ. Sci. Pollut. Res.,* **28**: 21119-21131. https://doi.org/10.1007/s11356-020-11815-8

Chiroma, T.M., Ebewele, R.O. and Hymore, F.K., 2014. Comparative assessment of heavy metal levels in soil, vegetables and urban grey waste water used for irrigation in Yola and Kano. *Int. Ref. J. Eng. Sci.*, **3**: 1-9.

Dogan, Y., Ugulu, I., Durkan, N., Unver, M.C. and Mert, H.H., 2011. Determination of some ecological characteristics and economical importance of *Vitex agnus-castus*. *Eurasia. J. Biosci.*, **5:** 10-18. https://doi.org/10.5053/ejobios.2011.5.0.2

Fordyce, F., 2005. Selenium deficiency and toxicity in the environment. In: *Essentials of medical geology, impacts of the natural environment on public health* (eds. O. Selinus, B. Alloway, J.A. Centeno, R.B. Finkelman, R. Fuge, U. Lindh and P. Smedley).

Elsevier Academic Press, London.

Gómez-Ramírez, P., Shore, R.F. and Van Den Brink, N.W., 2014. An overview of existing raptor contaminant monitoring activities in Europe. *Environ. Int.*, **67**: 12-21. https://doi.org/10.1016/j.envint.2014.02.004

Khalilova, H. and Mammadov, V., 2016. Assessing the anthropogenic ımpact on heavy metal pollution of soils and sediments in urban Areas of Azerbaijan's oil industrial Region. *Pol. J. environ. Stud.*, **25**: 159-166. https://doi.org/10.15244/pjoes/60723

Khan, Z.I., Ahmad, K., Rehman, S., Siddique, S., Bashir, H., Zafar, A., Sohail, M., Ali, S.A., Cazzato, E. and De Mastro, G., 2016a. Health risk assessment of heavy metals in wheat using different water qualities: Implication for human health. *Environ. Sci. Pollut. Res.,* **24:** 947-955. https://doi.org/10.1007/s11356-016-7865-9

Khan, Z.I., Ahmad, K., Ashraf, M., Parveen, R., Arshad, F., Hussain, A., Bibi, Z., Akram, N.A., Noorka, I.R. and Mustafa, I., 2016b. Risk assessment of heavy metal toxicity through contaminated vegetable from sewage water: Implications for populace health. *Hum. Ecol. Risk Assess.*, **22:** 302-311. https://doi.org/10.1080/10807039.2015.1052959

Khan, Z.I., Ahmad, K., Ashraf, M., Parveen, R., Bibi, Z., Mustafa, I., Noorka, I.R., Tahir, H.M., Akram, N.A., Ullah, M.F., Yaqoob, R., Tufarelli, V., Fracchiolla, M. and Cazzato, E., 2016c. Assessment of toxicological health risk of trace metals in vegetables mostly consumed in Punjab, Pakistan. *Environ. Earth Sci.*, **75**: 433. https://doi.org/10.1007/s12665-016-5392-0

Khan, Z.I., Ahmad, K., Ashraf, M., Parveen, R., Bibi, Z., Mustafa, I., Noorka, I.R., Tahir, H.M., Akram, N.A., Ullah, M.F., Yaqoob, R., Tufarelli, V., Fracchiolla, M. and Cazzato, E., 2016d. Risk assessment of heavy metal and metalloid toxicity through a contaminated vegetable (*Cucurbita maxima*) from wastewater irrigated area: A case study for a site-specific risk assessment in Jhang, Pakistan. *Hum. Ecol. Risk Assess.*, **22:** 86-98. https://doi.org/10.1080/10807039.2015.1055709

Khan, Z.I., Ahmad, K., Safdar, H., Ugulu, I., Wajid, K., Bashir, H. and Dogan, Y., 2018a. Manganese bioaccumulation and translocation of in forages grown in soil irrigated with city effluent: An evaluation on health risk. *Res. J. Pharma. Biol. Chem. Sci.,* **9**: 759-770.

Khan, Z.I., Ugulu, I., Umar, S., Ahmad, K., Mehmood, N., Ashfaq, A., Bashir, H. and Sohail, M., 2018a. Potential toxic metal accumulation in soil, forage and blood plasma of buffaloes sampled from Jhang, Pakistan. *Bull. environ. Contam. Toxicol.*, **101**: 235-242. https://doi.org/10.1007/s00128-018-2353-1

Khan, Z.I., Ugulu, I., Ahmad, K., Yasmeen, S., Noorka, I.R., Mehmood, N. and Sher, M. 2018b. Assessment of trace metal and metalloid accumulation and human health risk from vegetables consumption through spinach and coriander specimens irrigated with wastewater. *Bull. environ. Contam. Toxicol.*, **101**: 787–795. https://doi.org/10.1007/s00128-018-2448-8

Khan, Z.I., Nisar, A., Ugulu, I., Ahmad, K., Wajid, K., Bashir, H. and Dogan, Y., 2019a. Determination of cadmium concentrations of vegetables grown in soil irrigated with wastewater: Evaluation of health risk to the public. *Egypt. J. Bot.*, **59**: 753-762.

Khan, Z.I., Malik, I.S., Ahmad, K., Wajid, K., Munir, M., Ugulu, I. and Dogan, Y., 2019b. Efficacy of transfer of heavy metals in wheat grown in municipal solid waste amended soil. *Catrina Int. J. environ. Sci.*, **20**: 31-38. https://doi.org/10.21608/cat.2020.85765

Khan, Z.I., Ahmad, K., Rehman, S., Ashfaq, A., Mehmood, N., Ugulu, I. and Dogan, Y., 2019c. Effect of sewage water irrigation on accumulation of metals in soil and wheat in Punjab, Pakistan. *Pak. J. anal. environ. Chem.*, **20**: 60-66. https://doi.org/10.21743/pjaec/2019.06.08

Khan, Z.I., Arshad, N., Ahmad, K., Nadeem, M., Ashfaq, A., Wajid, K., Bashir, H., Munir, M., Huma, B., Memoona, H., Sana, M., Nawaz, K., Sher, M., Abbas, T. and Ugulu, I., 2019d. Toxicological potential of cobalt in forage for ruminants grown in polluted soil: A health risk assessment from trace metal pollution for livestock. *Environ. Sci. Pollut. Res.*, **26**: 15381–15389. https://doi.org/10.1007/s11356-019-04959-9

Khan, Z.I., Safdar, H., Ahmad, K., Wajid, K., Bashir, H., Ugulu, I. and Dogan, Y., 2020a. Copper bioaccumulation and translocation in forages grown in soil irrigated with sewage water. *Pak. J. Bot.*, **52**: 111-119. https://doi.org/10.30848/PJB2020-1(12)

Khan, Z.I., Ugulu, I., Sahira, S., Mehmood, N., Ahmad, K., Bashir, H. and Dogan, Y., 2020b. Human health risk assessment through the comparative analysis of diverse irrigation regimes for Luffa (*Luffa cylindrica* L.) Roem.). *J. Water, Sanitation, Hyg. Dev.,* **10**: 249-261. https://doi.org/10.2166/washdev.2020.132

Khan, Z.I., Ugulu, I., Zafar, A., Mehmood, N., Bashir, H., Ahmad, K. and Sana, M., 2021. Biomonitoring of heavy metals accumulation in wild plants growing at soon valley, Khushab, Pakistan. *Pak.*

J. Bot., **53**: 247-252. https://doi.org/10.30848/PJB2021-1(14)

Khan, Z.I., Liu, W., Mubeen, I., Alrefaei, A.F., Alharbi, S.N., Muhammad, F.G., Ejaz, A., Ahmad, K., Nadeem, M., Shoukat, J., Ashfaq, A., Mahpara, S., Siddique, S., Ashraf, M.A., Memona, H., Batool, A.I., Munir, M., Malik, I.S., Noorka, I.R. and Ugulu, I., 2023a. Cobalt availability in the soil plant and animal food chain: A study under a peri-urban environment. *Braz. J. Biol.*, **83**: e270256. https://doi.org/10.1590/1519-6984.270256

Khan, Z.I., Ahmad, K., Ahmad, T., Zafar, A., Alrefaei, A.F., Ashfaq, A., Akhter, S., Mahpara, S., Mehmood, N. and Ugulu, I., 2023b. Evaluation of nickel toxicity and potential health implications of agriculturally diversely irrigated wheat crop varieties. *Arab. J. Chem.,* **16**: 104934. https://doi.org/10.1016/j.arabjc.2023.104934

Liu, W.X., Liu, J.W., Wu, M.Z., Li, Y., Zhao, Y. and Li, S.R., 2009. Accumulation and translocation of toxic heavy metals in winter wheat (*Triticum aestivum* L.) growing in agricultural soil of Zhengzhou, China. *Bull. environ. Contam. Toxicol.*, **82**: 343–347. https://doi.org/10.1007/s00128-008-9575-6

McDowell, L.R., Ellis, G.L. and Conrad, J.H., 1984. Mineral supplementation for grazing cattle in tropical regions. *World Anim. Res.*, **52**: 1-10.

Munir, M., Khan, Z.I., Ahmad, K., Wajid, K., Bashir, H., Malik, I.S., Nadeem, M., Ashfaq, A. and Ugulu, I., 2019. Transfer of heavy metals from different sources of fertilizers in wheat variety (Galaxy-13). *Asian J. biol. Sci.*, **12**: 832-841. https://doi.org/10.3923/ajbs.2019.832.841

NRC (National Research Council), 1996. *Nutrient requirements of beef cattle, 7th ed*. National Academy Press, Washington.

Rajkowska, M. and Protasowicki, M., 2013. Distribution of metals (Fe, Mn, Zn, Cu) in fish tissues in two lakes of different trophy in Northwestern Poland. *Environ. Monit. Assess.*, **185**: 3493-3502. https://doi.org/10.1007/s10661-012-2805-8

Rasheed, M.J.Z., Ahmad, K., Khan, Z.I., Mahpara, S., Ahmad, T., Yang, Y., Wajid, K., Nadeem, M., Bashir, H., Ashfaq, A., Munir, M., Malik, I.S., Noorka, I.R., Kiran, M., Qamar, M.F. and Ugulu, I., 2020. Assessment of trace metal contents of indigenous and improved pastures and their implications for livestock in terms of seasonal variations. *Rev. Chim.*, **71**: 347-364. https://doi.org/10.37358/RC.20.7.8253

Rehman, A., Kincad, M.M. and Elzubejr, P.I., 1998. Mineral deficiencies in grazing cattle in Kardofan

and Darfur regions in the eastern Sudan. *Trop. Anim. Hlth. Prod.*, **30**: 123-135.

Reis, L.S., Pardo, P.E., Camargos, A.S. and Oba, E., 2010. Mineral element and heavy metal poisoning in animals. *J. Med. med. Sci.*, **1**: 560-579.

Sahin, I., Akcicek, E., Guner, O., Dogan, Y. and Ugulu, I., 2016. An investigation on determining heavy metal accumulation in plants growing at Kumalar Mountain in Turkey. *Eurasian J. Biosci.*, **10**: 22-29.

Siddique, S., Ahmad, K., Khan, Z.I., Wajid, K., Nadeem, M., Ugulu, I., Ghazzal, M., Akhter, P., Bashir, H., Noorka, I.R., Ashfaq, A., Munir, M., Malik, I.S., Akhtar, M., Shehzadi, M. and Muqaddas, H., 2019. Sodium status of soil, forages, and small ruminants of Punjab, Pakistan. *Pure appl. Biol.,* **8**: 1950-1961.

Singh, A., Sharma, R.K., Agrawal, M. and Marshall, F.N., 2010. Health risk assessment of heavy metals via dietary intake of food stuffs from the wastewater irrigated site of a dry tropical area of India. *Fd. Chem. Toxicol.,* **48**: 611–619. https://doi.org/10.1016/j.fct.2009.11.041

Tariq, F., Xiukang, W., Saleem, M.H., Khan, Z.I., Ahmad, K., Malik, I.S., Munir, M., Mahpara, S., Mehmood, N., Ahmad, T., Memona, H., Ugulu, I., Fiaz, S. and Ali, S., 2021. Risk assessment of heavy metals in basmati rice: Implications for public health. *Sustainability,* **13**: 8513. https://doi.org/10.3390/su13158513

Ugulu, I., 2015a. Determination of heavy metal accumulation in plant samples by spectrometric techniques in Turkey. *Appl. Spectros. Rev.*, **50**: 113-151. https://doi.org/10.1080/05704928.2014.935981

Ugulu, I., 2015b. Development and validation of an instrument for assessing attitudes of high school students about recycling. *Environ. Educ. Res.*, **21**: 916-942. https://doi.org/10.1080/13504622.2014.923381

Ugulu, I., Akhter, P., Khan, Z.I., Akhtar, M. and Ahmad, K., 2021b. Trace metal accumulation in pepper (*Capsicum annuum* L.) grown using organic fertilizers and health risk assessment from consumption. *Fd. Res. Int.*, **140**: 109992. https://doi.org/10.1016/j.foodres.2020.109992

Ugulu, I., Aydin, H., Yorek, N. and Dogan, Y., 2008. The impact of endemism concept on environmental attitudes of secondary school students. *Nat. Montenegr.,* **7**: 165-173.

Ugulu, I., Baslar, S., Dogan, Y. and Aydin, H., 2009. The determination of colour intensity of *Rubia tinctorum* and *Chrozophora tinctoria* distributed in Western Anatolia. *Biotech. Biotechnol. Equip.,* **23:**

410-413. https://doi.org/10.1080/13102818.2009.1 0818451

Ugulu, I., Bibi, S., Khan, Z.I., Ahmad, K., Munir, M. and Malik, I.S., 2022. Potentially toxic metal accumulation in spinach (*Spinacia oleracea* L.) irrigated with industrial wastewater and health risk assessment from consumption. *Bull. environ. Contam. Toxicol.,* **109**: 1117–1125. https://doi. org/10.1007/s00128-022-03606-3

Ugulu, I., Khan, Z.I., Aslam, Z., Ahmad, K., Bashir, H. and Munir, M., 2021a. Potentially toxic metal accumulation in grains of wheat variety Galaxy-2013 irrigated with sugar industry wastewater and human health risk assessment. *Euro-Mediterr. J. environ. Integr*., **6**: 38. https:// doi.org/10.1007/s41207-020-00203-w

Ugulu, I., Khan, Z.I., Rehman, S., Ahmad, K., Munir, M., Bashir, H. and Nawaz, K., 2019a. Trace metal accumulation in *Trigonella foenum-graecum* irrigated with wastewater and human health risk of metal access through the consumption. *Bull. environ. Contam. Toxicol.,* **103**: 468-475. https:// doi.org/10.1007/s00128-019-02673-3

Ugulu, I., Khan, Z.I., Rehman, S., Ahmad, K., Munir, M., Bashir, H. and Nawaz, K., 2019b. Appraisal of trace element accumulation and human health risk from consuming field mustard (*Brassica campestris* Linn.) grown on soil irrigated with wastewater. *Pak. J. anal. environ. Chem.,* **20**: 107-114. https:// doi.org/10.21743/pjaec/2019.12.14

Ugulu, I., Khan, Z.I., Safdar, H., Ahmad, K. and Bashir, H., 2021. Chromium bioaccumulation by plants and grazing livestock as affected by the application of sewage irrigation water: Implications to the food chain and health risk. *Int. J. environ. Res.,* **15**: 261–274. https://doi.org/10.1007/s41742-021-00311-7

Ugulu, I., Khan, Z.I., Rehman, S., Ahmad, K., Munir, M. and Bashir, H., 2022b. Heavy metal accumulation in goosefoot (*Chenopodium album* L.) irrigated with wastewater. *Pak. J. anal. environ. Chem.,* **23**: 225-236. https://doi.org/10.21743/pjaec/2022.12.05

Ugulu, I., Khan, Z.I., Sahira, S., Ahmad, K., Mehmood, N. and Dogan, Y., 2022a. Determination of heavy metal accumulation in wastewater irrigated pumpkin (*Cucurbita maxima* Duch.) by spectroscopic method. *Arab. J. Geosci.*, **15**: 1238. https://doi.org/10.1007/s12517-022-10519-2

USEPA (US Environmental Protection Agency), 1997. *Exposure factors handbook. Volume II-Food Ingestion Factors*. USEPA, Washington DC, EPA/600/P-95/002F.

USEPA (US Environmental Protection Agency), 2002. *Exposure factors handbook. Volume II-food ingestion factors*. EPA/600//P-95/002Fa. Office of Research and Development, Washington.

Wajid, K., Ahmad, K., Khan, Z.I., Nadeem, M., Bashir, H., Chen, F. and Ugulu, I., 2020. Effect of organic manure and mineral fertilizers on bioaccumulation and translocation of trace metals in maize. *Bull. environ. Contam. Toxicol.*, **104**: 649–657. https:// doi.org/10.1007/s00128-020-02841-w

WHO (World Health Organization), 1996. *Trace elements in human nutrition and health*. World Health Organization, Geneva.

Wieczorek-Dabrowska, M., Tomza-Marciniak, A., Pilarczyk, B. and Balicka-Ramisz, A., 2013. Roe and red deer as bioindicators of heavy metals contamination in north-western Poland. *Chem. Ecol*., **29**: 100-110. https://doi.org/10.1080/02757 540.2012.711322

Yang, Y., Khan, Z.I., Ahmad, K., Ullah, M.F., Nadeem, M., Bashir, H., Munir, M., Malik, I.S., Arshad, N., Ma, J., Chen, F. and Ugulu, I., 2020. Assessing health risk in livestock through quantification of iron in forages, soil and buffalo blood from Sargodha, Pakistan. *Rev. Chim*., **71**: 221-229. https://doi.org/10.37358/RC.20.9.8332

Yorek, N., Sahin, M. and Ugulu, I. 2010. Students representations of the cell concept from 6 to 11 grades: Persistence of the fried-egg model. *Int. J. phys. Sci.*, **5**: 15-24.