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

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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...
of the food chain, to define 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$_2$SO$_4$ (4 mL) and H$_2$O$_2$ (8 mL). The flask was placed in a chamber for heating at 75 °C. After about 30 min, when escaping of fumes ceased down, more H$_2$O$_2$ (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$_2$SO$_4$ and 4 mL H$_2$O$_2$ 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$_2$O$_2$ (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\textsubscript{2}SO\textsubscript{4} (1 mL) and H\textsubscript{2}O\textsubscript{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\textsubscript{2}O\textsubscript{2} (2 mL). The process of removal of the sample and addition of H\textsubscript{2}O\textsubscript{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 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).](image-url)
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.

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

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

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>D.F</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH10</td>
<td>CH09</td>
</tr>
<tr>
<td>Iron</td>
<td>0.018* 0.006*</td>
<td>0.001* 0.003*</td>
</tr>
<tr>
<td>Cobalt</td>
<td>0.010</td>
<td>0.001</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.001</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*, 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.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Soil: forage</th>
<th>Forage: blood plasma</th>
<th>Blood plasma: soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>0.191</td>
<td>–0.507</td>
<td>–0.267</td>
</tr>
<tr>
<td>Cobalt</td>
<td>–0.101</td>
<td>0.143</td>
<td>–0.101</td>
</tr>
<tr>
<td>Manganese</td>
<td>–0.281</td>
<td>0.356</td>
<td>–0.246</td>
</tr>
<tr>
<td>Selenium</td>
<td>–0.532</td>
<td>–0.519</td>
<td>0.479</td>
</tr>
<tr>
<td>Arsenic</td>
<td>–0.235</td>
<td>0.473</td>
<td>–0.248</td>
</tr>
</tbody>
</table>

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.

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

<table>
<thead>
<tr>
<th>Transfer factor</th>
<th>Fe</th>
<th>Co</th>
<th>Mn</th>
<th>Se</th>
<th>As</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sampling period 1 (summer)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil-forage</td>
<td>S-F</td>
<td>0.354568</td>
<td>0.01141</td>
<td>0.748215</td>
<td>0.067542</td>
</tr>
<tr>
<td>Forage-blood plasma</td>
<td>F-CH10</td>
<td>0.041383</td>
<td>2.042553</td>
<td>0.000801</td>
<td>0.312057</td>
</tr>
<tr>
<td></td>
<td>F–CH09</td>
<td>0.038239</td>
<td>1.882979</td>
<td>0.000777</td>
<td>0.241135</td>
</tr>
<tr>
<td></td>
<td>F-CH08</td>
<td>0.037572</td>
<td>1.659574</td>
<td>0.000826</td>
<td>0.255319</td>
</tr>
<tr>
<td><strong>Sampling period 2 (winter)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil-forage</td>
<td>S-F</td>
<td>0.371911</td>
<td>0.011554</td>
<td>0.780572</td>
<td>0.097455</td>
</tr>
<tr>
<td>Forage-blood plasma</td>
<td>F-CH10</td>
<td>0.043092</td>
<td>1.901099</td>
<td>0.000428</td>
<td>0.176166</td>
</tr>
<tr>
<td></td>
<td>F–CH09</td>
<td>0.038811</td>
<td>1.824176</td>
<td>0.000475</td>
<td>0.124352</td>
</tr>
<tr>
<td></td>
<td>F-CH08</td>
<td>0.037259</td>
<td>1.725275</td>
<td>0.000547</td>
<td>0.124352</td>
</tr>
</tbody>
</table>

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 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.
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 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.

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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.

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I. Ugulu et al.


