



# A study on the transfer of chromium from meadows to grazing livestock: an assessment of health risk

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## Abstract

The present investigation was performed in different district of Punjab to determine the chromium level in soil, forages, and blood plasma of the small ruminants (goat and sheep). The results showed that the mean concentrations of chromium in the soil of Sargodha, Mianwali, and Bhakkar districts were ranged from 1.8 to 2.3, 3.01 to 4.99, and 2.04 to 2.87 mg/kg, respectively. The chromium level was higher in Mianwali compared with Sargodha and Bhakkar. The mean concentrations of chromium in forages which were used for feeding purposes were found between 0.672 and 1.631, 1.493 and 2.612, and 0.7804 and 1.047 mg/kg for Sargodha, Mianwali, and Bhakkar, respectively. The mean concentrations of chromium in blood plasma of goat which consumed the contaminated forages were between 0.666 and 1.269 mg/L in Sargodha, 0.657 and 0.752 mg/L in Mianwali, and 1.39 and 2.37 mg/L in Bhakkar. In blood plasma of sheep, the residual levels of chromium in the districts Sargodha, Mianwali, and Bhakkar were ranged from 0.673 to 1.384, 0.83 to 1.086, and 1.496 to 3.611 mg/L, respectively. In the present research, there was a higher concentration of chromium in blood plasma of sheep from Bhakkar and the lowest was in Sargodha. The chromium level in blood samples was found to be higher than the tolerable level of 1.0 mg/L in all districts. In light of these results, it was concluded that chromium levels in the soil and forages of all sites were present within the safe limit while in blood samples of sheep and goats were found to be above the acceptable limit. Sheep and goats also consume water from river, streams, and ponds and other contaminated water sources. So it might be the reason of higher level of chromium in their blood plasma. Hence, the proper monitoring of study sites will be necessary to prevent the excessive accumulation of chromium in ruminants in the near future.

**Keywords** Heavy metal, Pollution, Blood plasma, Ruminant, Pakistan

## Introduction

In many countries, livestock is the source of milk, daily cash income, and food security for small farmers since immemorial times. The source of feeding for livestock is forages, which is mostly pleasant feed. This sentence may be modified as " The shortage of forages is increasing day by day as 2% land area under forages cultivation decreased after each decade (Sarwar et al. 2002; Khan et al. 2020). Also, the contaminated soil affects the plant growth, and affected forage plants may cause the problem in animals. Cultivation of crops in contaminated soils can cause bioaccumulation of heavy metals in the edible parts of crops which are used as feed for livestock. Thus, it may cause heavy metal pollution in animal manures (Li et al. 2020; Wajid et al. 2020). The usage of these plants and animals may affect the health of human (Shad et al. 2014; Ahmad et al. 2018a, b).

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The heavy metal contamination may cause the major threat to terrestrial and aquatic environment (Unver et al. 2015; Ugulu et al. 2016; Khan et al. 2019a, b). When contaminated water is used for irrigation, pollutants or unwanted material accumulates on the surface of soil (Khan et al. 2018a, b). These heavy metals are transferred from soil to plant and plant to animals or herbivores; specific metals show different rate of transfer, which depend on the different characteristics of soil such as clay contents, organic matter, and pH (Rajaganapathy et al. 2011; Dogan et al. 2014a, b; Unver et al. 2015; Zeng et al. 2020). Ruminants grazed freely on pasture land and are good environmental pollution indicators. The contaminated feed of ruminants may cause health problems such as fitness problems and rate of reproduction is low as well as cancerous disease in animals (Bires et al. 1995; Ahmad et al. 2011).

Chromium is the most critical metal in an environment which has destructive effects and it is potentially harmful for dairy production system as a bioaccumulated toxin (Ugulu 2015a, b; Khan et al. 2018c). The contamination in the environment is caused by chromium, and the source of this metal is tanning as well as polishing, paints, electroplating, and pigment formation. Chromium is present in trivalent form which is not soluble in water and in the presence of oxygen; trivalent is converted into hexavalent chromium. Bronchitis, asthma, cancer, and DNA damage are caused by hexavalent chromium (Peralta et al. 2009).

Sargodha, Mianwali, and Bhakkar are present in semi-arid areas of Pakistan. These areas contain a lot of herd of sheep and goats. These goats and sheep consume forages to meet their feeding requirements. Various environmental and anthropogenic sources cause accumulation of potential toxic metals in forages (Khan et al. 2019c, 2019d; Huma et al. 2019). When ruminant consume these forages, these toxic metals enter the body and become part of the food chain. Therefore, this study aims to assess the chromium level in soil, forages, and blood plasma of the small ruminants (goat and sheep) and to assess the bioaccumulation and the animal health risk from consumption of forages of this metal.

## Materials and methods

### Area of study

The present study was conducted in different districts of Punjab such as Sargodha (32.0740° N, 72.6861° E), Mianwali (32.5839° N, 71.5370° E), and Bhakkar (31.6082° N, 71.0854° E). Three sites from each district were chosen for the collection of different samples of soil, forages, and blood of animals (goat and sheep). These three districts were

selected because no work was reported in these areas on these parameters.

Sargodha, the California of Pakistan, is an important district of Punjab. It is situated 172 km northwest of Lahore, Pakistan. Sargodha comprises different industries and is the center of agricultural trade. Different interchanges connect it at various locations. Plain, fertile lands, small hills, and cold and hot weather are characteristics of Sargodha. Temperature in winter recorded was as low as the freezing point and maximum temperature reaches up to 50 °C (122 °F) in the summer.

In the sixteenth century, the name Mianwali was derived from a local saint, Mian Ali. It is located on the eastern bank of the Indus. Mianwali was positioned between 71–08° and 71–57° east longitudes, and 32–10° and 33–15° north latitudes. The annual maximum temperature recorded was 47 °C and minimum temperature recorded was 19 °C. Maximum rainfall occurs in the month of July. Some important crops such as mung, mash, Verin, barley, Eruca, fennel, wheat, oat, mustard, and peanut are cultivated in this area. There are three types of soil in this district: sandy, clay, and loamy. Due to ruthless cutting of forest for fuel and timber purposes, the forest-covered area is very low. Most of the area is semi-arid and a very small area is irrigated and the source of the irrigation is the canals of the river Indus.

In Pakistan, after slicing of Mianwali area, a new district named as Bhakkar in 1982 was established. It is located on the left side of the bank of the Indus River and at an altitude of 159 m. The Bhakkar district is situated between Chenab and the Indus River. It is located on deserted plains of Thal Desert and consists of a riverine tract along the Indus, called Kaccha. It consisted of sandy land and of semi-rectangular shape. Bhakkar district occupies a total area of 8153 km.

### Sample collection

Ten samples of soil, forages, and blood were collected from each site randomly. Ten samples of each were combined and made three composite samples of soil, forage, and blood plasma.

### Soil

For collection of soil samples, three different sites were selected in three districts (Sargodha, Mianwali, Bhakkar). With the help of stainless steel auger, 12 to 15-cm-deep layers for all selected sites were dug up (Sanchez and Lacombe 1976). Soil samples were collected from selected sites of the three districts. From each investigated site, ten samples were obtained from three districts and stored in a plastic bag.

**Table 1** Operation conditions for analysis of chromium by using atomic absorption spectrophotometer

Element	Chromium
Wavelength (nm)	422.7
Slit width (nm)	0.7
Lamp current (mA)	10
Air flow rate (L/min)	15
Acetylene flow rate (L/min)	2.8
Burner height (mm)	9

## Forage

Forages were also collected from the sites which were selected for soil sampling by mean of sterilized apparatus. Only those forage species were collected which were commonly used for feeding of the small ruminants (sheep and goat). Forage species which were selected as samples include berseem (*Trifolium alexandrinum*), bajra (*Pennisetum glaucum*), and oat (*Avena sativa*). Ten samples of these forages were taken from three sites of the three districts. Distilled water was used to eliminate impurities and with HCl. The samples were dried to remove moisture content and were placed in sunlight.

## Blood plasma

Blood samples of goats and sheep were collected from Bhakkar, Mianwali, and Sargodha in 2016. Goats and sheep of 1-year-old were selected for sampling. Each district was divided into three sites. Five goats of each site were selected, and data were pooled into one mean value. From the jugular vein, a sample of blood was collected through the needle of the syringe which was firstly sterilized. A vacuum was created into evacuated tubes and, to avoid from clotting, was retained in the heparinized Na citrate voiles rapidly. At 3000 rpm, blood and blood plasma were centrifuged for 15 min, stored in polyethylene tubes, and frozen at  $-20\text{ }^{\circ}\text{C}$ .

## Sample preparation

### Soil

Soil samples collected from the three districts were dried in open air and then placed in the oven for three days at  $72\text{ }^{\circ}\text{C}$ . Soil samples were dried until no moisture content remained in the soil then removed from the oven, and the samples were weight by using an electric balance. Digestion of these samples was done by “wet digestion method” (Vukadinovic and Bertic 1988). 1 gram of soil was taken and 10 mL nitric acid was added in a beaker and placed overnight. The next day, the samples were digested on a hot plate;  $\text{H}_2\text{O}_2$  was also added drop by drop until a colorless solution appeared. Then, the

**Table 2** Analysis of variance for chromium concentrations in soil, forage, and blood plasma in different districts of Punjab

Chromium	Sargodha	Mianwali	Bhakkar
Soil	0.198 <sup>ns</sup>	0.890 <sup>ns</sup>	2.361 <sup>ns</sup>
Forage	0.843 <sup>ns</sup>	1.058 <sup>ns</sup>	0.055 <sup>ns</sup>
B.G.	0.297 <sup>ns</sup>	0.009 <sup>ns</sup>	0.752 <sup>ns</sup>
B.S.	0.415 <sup>ns</sup>	0.0069 <sup>ns</sup>	3.704 <sup>ns</sup>
Degree of freedom	2	Error	8

<sup>ns</sup> non-significant, B.G. blood of goats, B.S. blood of sheep

samples were removed from the hot plate and placed for cooling and then distilled water was added up to 50 mL, filtered through Whatman filter paper of  $42\text{ }\mu\text{m}$  size. In labeled bottles, the samples were stored for further processing.

## Forage

Forage samples collected from the three districts were dried in open air and then placed in the oven for three days at  $72\text{ }^{\circ}\text{C}$ . The forage samples were dried until no moisture content was present then removed from the incubator and weight of the samples was done using an electric balance. Samples were digested by using the “wet digestion method” (Vukadinovic and Bertic 1988).

## Blood plasma

Blood samples of goats and sheep collected from the three districts were frozen at  $-20\text{ }^{\circ}\text{C}$ . Blood samples were removed from the freezer and digested by using the “wet digestion method” (Vukadinovic and Bertic 1988).

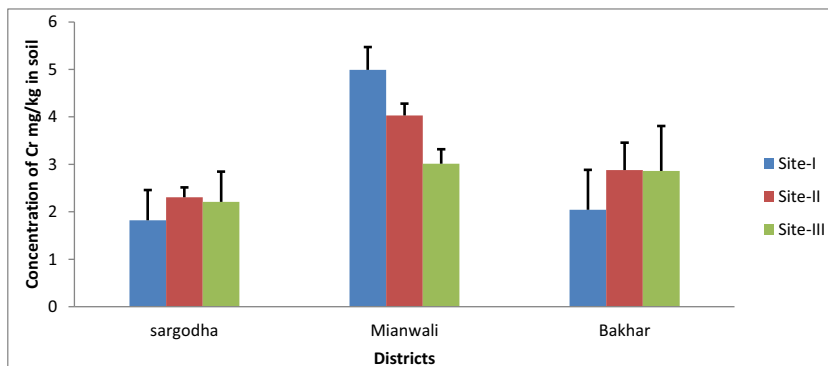
## Digestion

One milliliter of blood samples (goats and sheep) and 1 g of forage samples were taken for digestion and mixed in 10 mL  $\text{HNO}_3$  overnight. Samples were put on a hot plate and  $\text{H}_2\text{O}_2$  was added drop by drop and fumes get evaporated. The process remains continued until a colorless solution appeared. The samples were digested and removed from digestion chamber and the solution was left for cooling. Distilled water was added to make its volume up to 50 mL and then the solution was filtered through a  $42\text{-}\mu\text{m}$  size Whatman filter paper. These samples were stored in plastic bottles until analysis (Khan et al. 2017).

## Quality control

Precision and accuracy of analysis were assured through repeated analysis of samples with the National Institute of Standard and Technology, Standard Reference Material

**Fig. 1** Mean concentration of chromium in soil of different districts of Punjab



(SRM 1570), for all the heavy metals. The results were found within  $\pm 2\%$  of the certified value. Quality control measures were taken to assess the contamination and reliability of data. Bank and drift standards (Sisco Research Laboratories Pvt. Ltd., India) were determined after five determinations to calibrate the instrument. The coefficients of variation of replicate analysis determined after different determinations for precisions of analysis and variations below 10% were considered correct.

**Chromium analysis**

Atomic absorption spectrophotometer (AA-6300 Shimadzu, Japan) was used to evaluate the concentration of chromium in soil, forage, and blood plasma of goats and sheep. The operation conditions for determination of chromium are shown in Table 1.

**Statistical analysis**

For statistical analysis, SPSS 22 software was used. One-way ANOVA was used to find out the mean concentration of metals. To find out the relationship between metals of soil and forages, correlation coefficient was used. Significance between the mean was tested at 0.05, 0.01, and 0.001 level of probability as suggested by Steel and Torrie (1980).

**Pollution load index**

Pollution load index was used to measure the contamination of metals in investigated soil following the method of Liu et al. (2005).

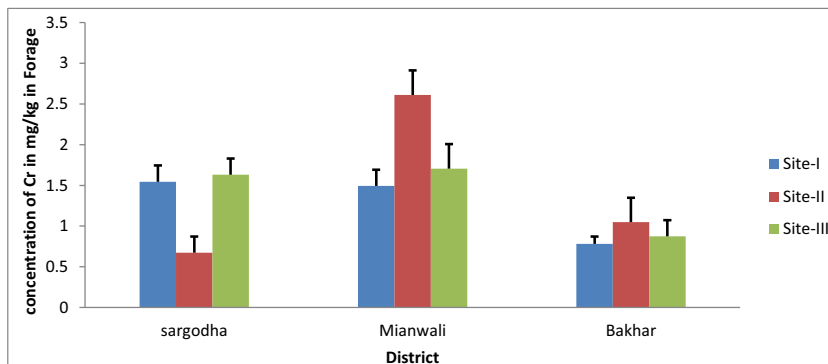
$$PLI = \text{Metal contamination in investigated soil} / \text{reference value of metal in soil}$$

**Bioconcentration factor**

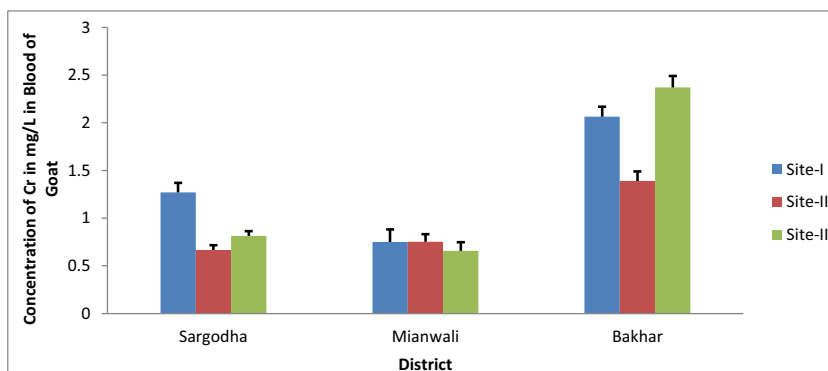
The most important component of animal contact to metal through the food chain is bioconcentration factor.

Bioconcentration factor was used to assess the content of metals in soil-forages and forage-ruminant blood in milligram per kilogram (Cui et al. 2004).

**Fig. 2** Mean concentration of chromium in forage of different districts of Punjab



**Fig. 3** Mean concentration of chromium in blood of goat of different districts of Punjab



BCF = Concentration of metals in forages/concentration of metals in soil  
 BCF = Concentration of metals with blood plasma/concentration of metals in forage.

On the basis of these consequences, individual metals in the soil samples to the forage plants defined as the ratio between the concentration of heavy metals in forage plants and their individual concentration in the soil samples and correspondingly total metal in the forage plants and their individual concentration in the blood plasma samples were evaluated.

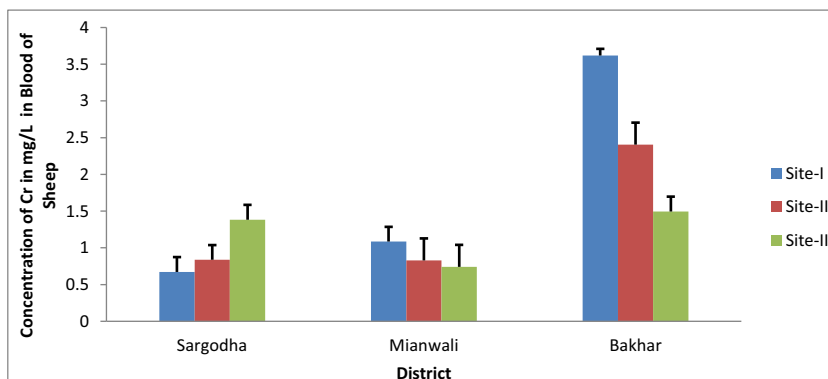
## Results and discussion

### Soil

The results of ANOVA showed a non-significant effect ( $p < 0.05$ ) of the sites on chromium in soil (Table 2). In the Sargodha district, the mean concentrations of chromium in soil, which was used in cultivation, were between 1.8 and 2.3 mg/kg. In Mianwali, the mean concentrations of chromium in soil were between 3.01 and 4.99 mg/kg. In Bhakkar, the mean concentrations of chromium were between 2.04 and 2.87 mg/kg (Fig. 1).

The chromium amount in soil was higher than the critical level of 0.02 mg/kg as examined by Anderson et al. (1973).

**Fig. 4** Mean concentration of chromium in blood of sheep of different districts of Punjab



The values reported from the present findings were much lower than the values created by Kabata-Pendias and Wiacek (1985) who suppose 75 mg/kg as the critical load for the chromium present in the soils. Ahmad et al. (2019) recorded lower level of chromium (1.05 to 1.36 mg/kg) in soil compared with the current findings. Commercial products and ashes from coal and industries are major reservoirs of chromium that enter into the soil (Nriagu and Pacyna 1988). The solid waste materials released on landfill are the major sources of chromium (Barceloux and Barceloux 1999; Kimbrough et al. 1999). However, the level of chromium is much higher in soil than in crop plants (Underwood and Suttle 1999).

### Forages

The results from ANOVA exhibited a non-significant effect ( $p < 0.05$ ) of the sites on chromium in forages (Table 2). In the Sargodha district, the mean concentrations of chromium in forages which was used for feeding purposes were between 0.672 and 1.631 mg/kg. In Mianwali, the mean concentrations of chromium in forages were between 1.493 and 2.612 mg/kg. In Bhakkar, the mean concentrations of chromium were between 0.7804 and 1.047 mg/kg (Fig. 2).

**Table 3** Correlation of chromium between soil-forage and soil-blood of plasma goats and sheep

Soil	Soil-forages	Soil-B.G	Soil-B.S	Forage-B.G.	Forage-B.S.
Sargodha	- 0.590	- 0.99-9*	0.529	0.626	0.373
Mianwali	0.941	0.643	- 0.109	0.345	- 0.439
Bhakkar	- 0.371	0.820	0.990	0.227	- 0.234

B.G blood of goats, B.S blood of sheep

Chromium is an essential element for plant. Chromium is required for plants in small amount, but in excess amount, it can be dangerous to plants (Nargus 2004). The permissible maximum limits of chromium in forages was 50 mg/kg recommended by WHO (1996). The present values of chromium at forages of all sites were found within the limit. The toxic impact of chromium to the animals using these plants cannot be noticed, because in the current findings, the level of chromium was below the toxic limit. Chromium proved to be very unsafe for livestock health if a diet consists of greater levels of chromium than the optimum level as described by Anonymous (1980) and Ahmad et al. (2019).

**Blood plasma of goat and sheep**

The results from the analysis of variance of the data showed a non-significant effect ( $p < 0.05$ ) of the sites on chromium in the blood of goats (Table 2). In the Sargodha district, the mean concentrations of chromium in blood plasma of goat which consumed these forages were between 0.666 and 1.269 mg/L. In Mianwali, the mean concentration of chromium in blood plasma of goats was between 0.657 and 0.752 mg/L. In Bhakkar, the mean concentrations of chromium were between 1.39 and 2.37 mg/L (Fig. 3).

The results from the analysis of variance of the data showed a non-significant effect ( $p < 0.05$ ) of chromium in the blood of

**Table 4** Bioconcentration of chromium in different districts of Punjab

BCF of chromium	Site	Sargodha	Mianwali	Bhakkar
Soil-forages	1	0.848	0.467	0.381
	2	0.291	0.648	0.364
	3	0.738	0.566	0.305
Forages-blood of goat	1	0.822	0.503	2.644
	2	0.991	0.287	1.326
	3	0.498	0.385	2.714
Forages-blood of sheep	1	0.436	0.727	4.637
	2	1.247	0.317	2.294
	3	0.848	0.435	1.714

**Table 5** Pollution load index of chromium in different district of Punjab

PLI of chromium	Sargodha	Mianwali	Bhakkar
1	0.201	0.352	0.225
2	0.254	0.444	0.317
3	0.244	0.332	0.315

sheep collected from different sites of the three districts (Table 2). In the Sargodha district, the mean concentrations of chromium in blood plasma of sheep which consumed these forages were between 0.673 and 1.384 mg/L. In Mianwali, the mean concentrations of chromium in blood plasma of sheep were between 0.83 and 1.086 mg/L. In Bhakkar, the mean concentrations of chromium were between 1.496 and 3.611 mg/L (Fig. 4).

In the current research, there was a higher concentration of chromium in the blood of sheep from Bhakkar and the lowest was in the group of sheep from the Sargodha district. The chromium is an essential element and helps the body to use fat, protein, and sugar, but in excess amount, it exerts toxic effect. Higher levels of chromium cause reduction in insulin in blood sugar and also cause flushing and itching (Shekhawati et al. 2015). The chromium level in blood samples was found to be higher than the tolerable level of 1.0 mg/L. Milam et al. (2017) recorded lower ranged of chromium in blood of ruminants compared with present findings.

**Correlation**

Person correlation coefficient method was used to find correlation between metal concentrations (Pentecost 1999). From soil-forages, chromium showed negative non-significant correlation. Chromium showed negative significant ( $p \leq 0.05$ ) correlation from soil-blood plasma of goats. Positive non-significant correlation for chromium was observed from soil-blood plasma of sheep in Sargodha samples. The perfect positive correlation was observed in soil to forage and soil to plasma of goats, and the negative correlation was present between soil and plasma of sheep in Mianwali samples. Perfect negative correlation was observed in soil to forage and forage to plasma of sheep, and the positive correlation was observed in soil to plasma of goat, soil to plasma of sheep, and forage to plasma of goat in Bhakkar samples (Table 3).

The non-significant correlation for chromium between soil and forage, forage to blood plasma of goat, and forages and blood plasma of sheep might be due to edaphic factors that might lead to the reduction in uptake of chromium. The non-significant correlation was studied between soils to forage and forage to blood, which leads to chromium imbalance among soil, plant, and animals.



## Bioconcentration factor

The bioconcentration factor of chromium in forage was greater in Sargodha samples related to Bhakkar samples. The minimum bioconcentration factor of chromium was seen in Bhakkar while the maximum bioconcentration factor of chromium was observed in Sargodha. The bioconcentration factor of chromium in plasma of goats in Bhakkar samples was higher compared with Sargodha and Mianwali. Similarly, bioconcentration factor of chromium in plasma of sheep of Bhakkar and Sargodha samples was higher compared with Mianwali (Table 4).

Bioconcentration factor is a key component to determine the level of exposure through the food chain. Bioconcentration factor for chromium at Bhakkar site was greater than 1 while at Sargodha and Mianwali sites was less than 1. The value of bioconcentration factor greater than 1 indicates that plant can accumulate heavy metals while BCF less than 1 shows that plant just absorb metal (Singh et al. 2011).

## Pollution load index

Pollution level in plants or soil was measured by a method, i.e., PLI. Chromium showed higher contamination of soil in Mianwali compared with Sargodha (Table 5).

Pollution severity in soil can be measured by the pollution load index. According to this method, soil is considered to be polluted if the pollution load index value was bigger than 1, while the soil is considered to be clean or less dirty if the pollution load index value is less than 1 (Harikumar et al. 2009).

## Conclusion

The level of potential toxic metals in animals depends on the level of metals in the forages they consume and environmental concentration of these metals. The concentration of metals in the blood of sheep and goat varied among the three sites. In blood samples of goat and sheep, the highest concentration of chromium was observed at Bhakkar site. In the current findings, the level of chromium in forages of all sites was found within the safe limit, while in blood of all samples was found to be above the tolerable limit. The bioconcentration factor of chromium from soil to forage, forage to blood of sheep, and forage to blood of goats at Bhakkar site was greater compared with the other two sites. The values of the pollution load index at all sites were less than 1 indicating good soil quality. So, the chromium toxicity was seen in sheep and goats of Sargodha, Mianwali, and Bhakkar sites. However, more wide-ranging sampling is needed to study such forages, and further investigation on contamination of other crops is required.

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