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Research Article

Variation in contamination status of cadmium in forages and blood plasma of livestock in different districts of Punjab, Pakistan

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Abstract

The pollution in the environment causes many health problems such as industrial, agriculture, mining and natural process adds many harmful substances in the surrounding and damage the environment. These toxic substances make the part of the food chain due to which livestock is at high exposure to risk. The present investigation was carried out in different district of Punjab to find out the cadmium levels in soil, forages as well as in the blood of the ruminants like goat and sheep. The cadmium contents in soil of district Sargodha, Mianwali and Bhakhar were ranged from 4.57 to 4.94, 2.98 to 3.95, 1.65 to 2.98 mg/kg respectively. The level of cadmium was higher in Sargodha as compare to Mianwali and Bhakkar. The mean concentrations of cadmium in forages which were used for feeding purpose were found between 2.745-3.432, 2.245-3.196 and 1.9-2.036 mg/kg for Sargodha, Mianwali and Bhakhar, respectively. The mean concentrations of cadmium in blood of goat which consumed the contaminated forages were between 2.152-2.436, 1.727-2.39 and 2.3-2.62 mg/L for Sargodha, Mianwali and Bhakhar, respectively. In blood of sheep, residual levels of cadmium in district Sargodha, Mianwali and Bhakhar were ranged from 2.152 to 2.436, 1.794 to 2.286 and 1.97 to 2.066 mg/L, respectively. All these values of cadmium for blood samples were within the permissible limit set by WHO.

Keywords: Bioconcentration factor; Cadmium; Forage; Goat; Pollution load index; Sheep

Introduction

The pollution in the environment causes many health problems such as industrial, agriculture, mining and natural process adds many harmful substances in the surrounding and damage the environment [1]. These toxic substances make the part of the food chain [2]. The safe feed product is essential for animal health and welfare as well as human health and healthy feed product reduce the risk of toxicity in animals and human health [3, 4]. Biological functions may be affected by heavy metals especially due to bioaccumulation in the food chain because these pollutants present in the environment [5, 6].

Metals present in the environment can be transported and accumulated in plants and animals and through food chain affect the humans [7, 8]. Metals are transported and biomagnified from one trophic level to the next may accumulate more toxin in the food of higher animals as compare to their food contains [9].

The cadmium in the environment can easily be entered in the food chain and may cause various deleterious effects in plants, human and animals and result in undesirable physiological and biochemical changes [10]. In industrial areas, the level of cadmium and lead is high which cause the hormonal changes of plasma and abnormal liver function [11, 12].

Cadmium and lead are accumulated in human body and extreme accumulation may cause the severe health problems [13]. Different diseases as kidney, bone and blood diseases, nerves system and cardiovascular may cause due to lead and cadmium because these metals are carcinogenic in nature. The life span or life length reduces 9-10 years due to contamination of these metals in soil and plants. Lead is dangerous for young children because it affects the growth of children and growth of these children which live around past smelter is disturbed due to the presences of pollution of lead. Copper and zinc play important role in plant but the high level or toxicity of these metals in food causes health trouble in animal as well as in human. Gastrointestinal cancer may cause due the contamination of heavy metals in soil and plant [14, 15].

This study was planned to investigate the cadmium level in soil, forages as well as blood plasma of the small ruminants like goat and sheep and to evaluate its deleterious effects in soil and of using Cd contaminated forages on lives.

Materials and methods

This study was carried out in different Punjab districts of Pakistan to evaluate the toxic effects of Cd in soil, forages and ruminants by using specific systematic technique and to investigate the deficiencies or excesses which affect the forage yield and animals' health.

Study area

The present study was conducted in different districts of Punjab such as Sargodha, Mianwali and Bhakkar. Three sites from each district were chosen for collection of different samples of soil, forages and blood of animals (goat and sheep).

Sargodha is important district of Punjab. It is situated 172 km northwest from Lahore, Pakistan. Temperature in winter recorded as low as freezing point and maximum temperature as high as 50 °C in the summer. It is located on eastern bank of Indus.

Mianwali positioned between 71-08º to 71-57º East longitudes, and 32-10º to 33-15º North latitudes. Annual maximum temperature recorded as 47 ºC and minimum temperature recorded as 19 °C. Mianwali falls in semi-arid zone and only some area is irrigated with the canals of river Indus.

In Pakistan after slicing of Mianwali area a new district named as Bhakkar in 1982 was established. It is located in 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.

Samples collection

Ten samples of each soil, forages and blood were collected from each site randomly. These samples were combined and make 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, Bhakhar). With the help of stainless-steel auger, 12 to 15 cm deep soil layers for selected sites were dug up [16]. From each investigated site ten samples were obtained from three districts, stored in plastic bag.

Forage

Forages samples (10) include Barsem (*Trifolium alexandrinum* L.), Bajra (*Pennisetum glaucum* (L.) R. Br.) and Oat (*Avena sativa* L.) were also collected from three sites of three districts which were selected for soil sampling by mean of sterilized apparatus. Only those forages species were collected which were commonly used for fed of the small ruminants (sheep and goat). Distilled water was used to eliminate impurities and with HCl. Samples were dried to remove moisture content were placed in sunlight.

Blood plasma

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

Samples preparation

Soil and forage

Soil and forage samples collected from three districts were air dried and then placed in oven at 72 ^{o}C until dried and weighed by analytical balance. Wet digestion was adopted to digest the samples [17]. 1g sample, 10 mL nitric acid was taken in a beaker and placed it overnight. Next day samples were digested on hot plate, H_2O_2 was also added drop by drop until colorless solution was appeared. Then removed from hot plate and placed it for cooling and then distill water was added up to 50 mL, filtered through Whatman filter paper of 42 µm size. In labeled bottles samples were stored for further process.

Blood plasma

Blood samples of goats and sheep collected from three districts were frozen at -20 ^{o}C. Blood samples removed from freezer and samples of blood were digested by same procedure [17].

Cadmium analysis

Cadmium content in soil, forage and blood of goat and sheep was assessed by atomic absorption spectrophotometer (AA-6300 Shimadzu Japan).

Statistical analysis

SPSS 22 software was used for ANOVA and to find out the mean values of metals and relationship between metals of soil and forages correlation coefficient was used at significance level 0.05 [18].

Pollution load index

Pollution load index (PLI) was used to measure the contamination of metals in investigated soil following the method of Liu *et al*. [19].

PLI= Metal concentration in soil / Reference metal value of soil

Bioconcentration factor

Bioconcentration factor (BCF) was used to assess the content of metals in soil-forages and forage-ruminants blood in (mg/kg) following Cui *et al*. [20].

BCF= Metals contents in forages/ Metals contents in soil

BCF= Metals contents in blood / Metals contents in forage.

Results

Soil

The results from ANOVA showed that sites have non-significant effect ($p<0.05$) on

cadmium content in soil (Table 1). In district Sargodha, the mean concentrations of cadmium in soil which used by cultivation were between 4.57 to 4.94 mg/kg. In Mianwali, the cadmium contents in soil were between 2.98-3.95 mg/kg. In Bhakhar, cadmium contents were between 1.65-2.98 mg/kg (Figure 1). The level of cadmium was higher in Sargodha as compare to Mianwali and Bhakkar.

Forages

The sites showed non-significant effect ($p<0.05$) on cadmium content in forages collected from different three districts (Table 1). In district Sargodha, the mean concentrations of cadmium in forages used for feeding purpose were between 2.745-3.432 mg/kg. In Mianwali, the mean values of cadmium content in forages were between 2.245-3.196 mg/kg. In Bhakkar, the mean values of cadmium content were between 1.90-2.036 mg/kg (Figure 2).

Blood of goat

The sites showed non-significant effect ($p<0.05$) on cadmium content in blood samples of goats (Table 1). In district Sargodha, the mean concentrations of cadmium in blood of goat which consumed the contaminated forages were between 2.152-2.436 mg/L. In Mianwali, the mean concentrations of cadmium in blood of goats were between 1.727-2.39 mg/L. In Bhakkar, the cadmium contents were between 2.3-2.62 mg/L (Figure 3).

Blood of sheep

The sites showed non-significant effect ($p<0.05$) on cadmium content in blood samples of sheep (Table 1). In district Sargodha, the mean concentrations of cadmium in blood of sheep which consumed the contaminated forages were between 2.152-2.436 mg/L. In Mianwali, the mean concentrations of cadmium in blood of sheep were between 1.794-2.286 mg/L. In Bhakkar, the mean concentrations of cadmium were between 1.97-2.066 mg/L (Figure 4).

Correlation

In the present study Cd content from soil-forages, forage-blood samples and soil-blood samples showed negative and non-significant correlation (Table 2). Positive significant correlation was observed from forage-blood samples in Sargodha. Negative correlation was observed in soil and forage, soil to blood and positive between forage to blood in Mianwali. Negative correlation was observed in soil and forage, soil to blood of goat, forages to blood of sheep and positive between soil to blood of sheep and forage to blood of goat in Bhakkar Samples. Cadmium content also revealed non-significant positive correlation between soil and blood of goat, between forage and blood of goat and non-significant negative between soil and forage which lead to cadmium imbalance among soil, plant and animals. It can be said that non-significant correlation between soil-forage-blood which might be due to edaphic factors.

Bioconcentration factor

Bioconcentration factor of cadmium content in forage samples from Mianwali was higher as compared to forage samples from Sargodha. Higher BCF value of cadmium in Mianwali and Bhakkar samples was observed while the lowest BCF value of cadmium was observed in Sargodha. The BCF value of cadmium in blood of goats in Bhakkar samples was higher as compared to Sargodha and Mianwali samples. Likewise, the BCF of cadmium in blood of sheep in Bhakkar samples was higher and lower BCF values of cadmium were in Sargodha and Mianwali (Table 3).

Pollution load index

Pollution level in plants or soil was measured by method i.e. pollution load index. The soil of Sargodha District showed higher PLI value for cadmium as compared to Mianwali and Bhakkar (Table 4).

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Table 1. Analysis of variance for cadmium content in soil, forage and blood samples in different districts of Punjab

Cadmium	Sargodha	Mianwali	Bhakhar
Soil	.134^{ns}	.067^{ns}	.114 ^{ns}
Forage	.402^{ns}	.698^{ns}	.002*
BG	.073^{ns}	.351^{ns}	.101^{ns}
BS	1.199^{ns}	.183^{ns}	.004^{ns}
Degree of freedom	2	Error	8

ns: non-significant, *: Significant at 0.05 level, BG: Blood of goat, BS: Blood of sheep

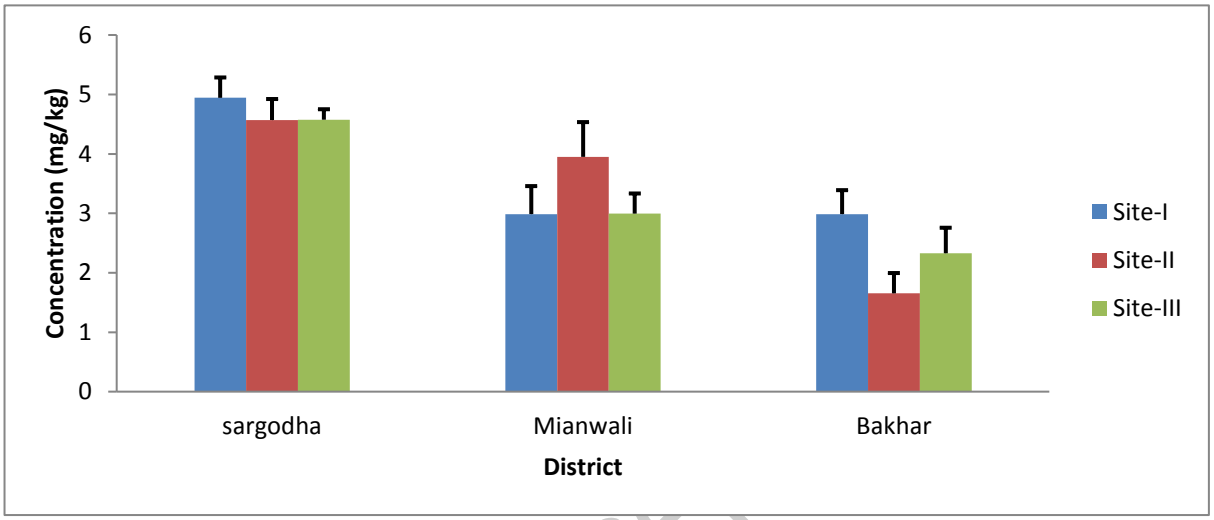

Figure 1. Mean concentration of cadmium in soil of different districts of Punjab

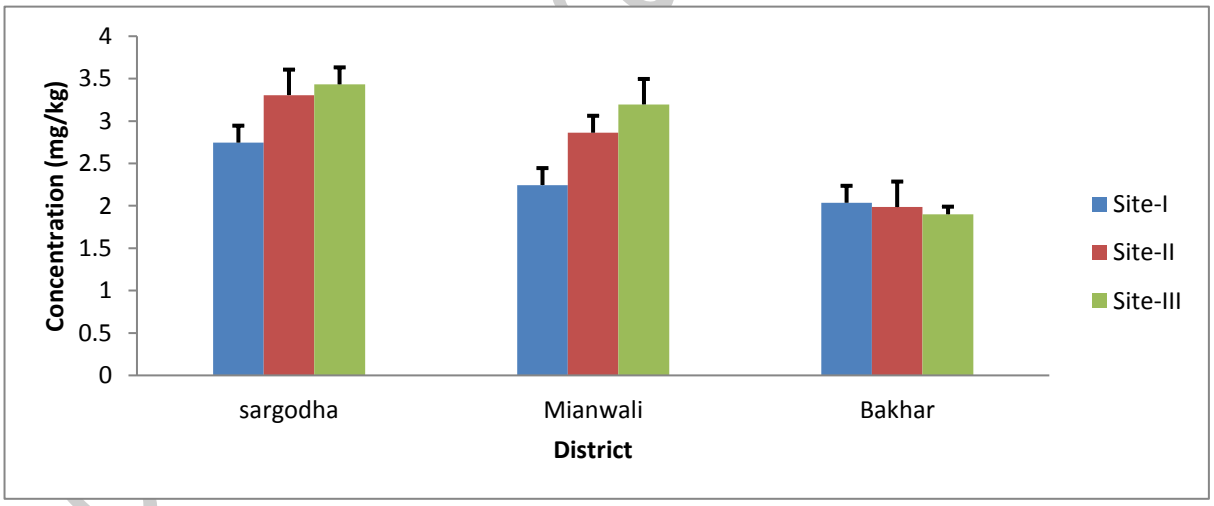

Figure 2. Mean concentration of cadmium in forages of different districts of Punjab

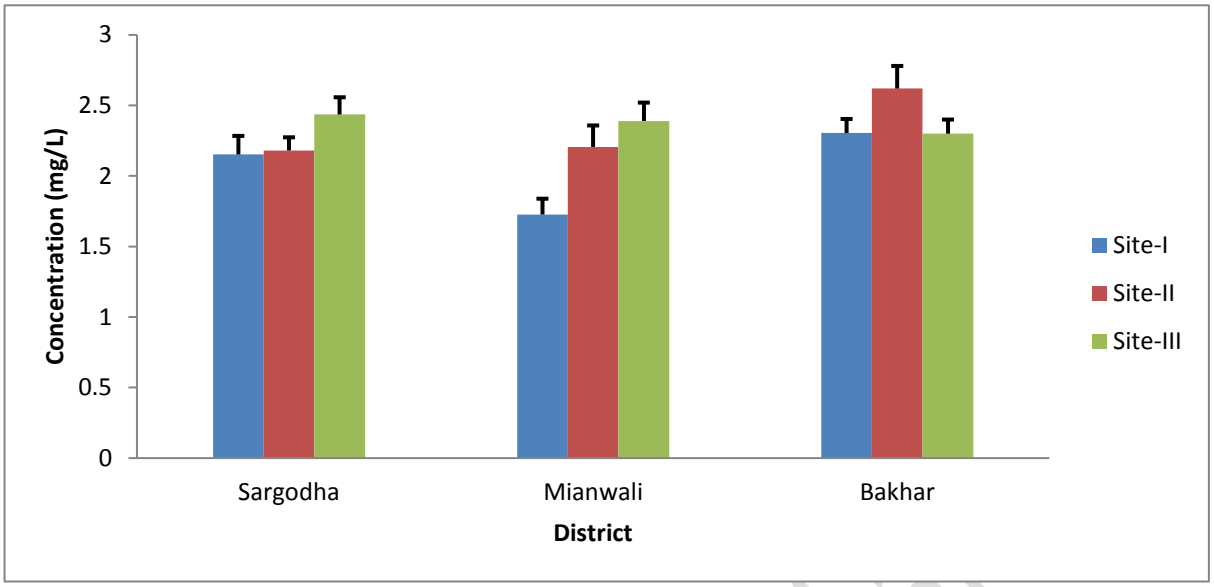

Figure 3. Mean concentration of cadmium in blood of goat of different districts of Punjab

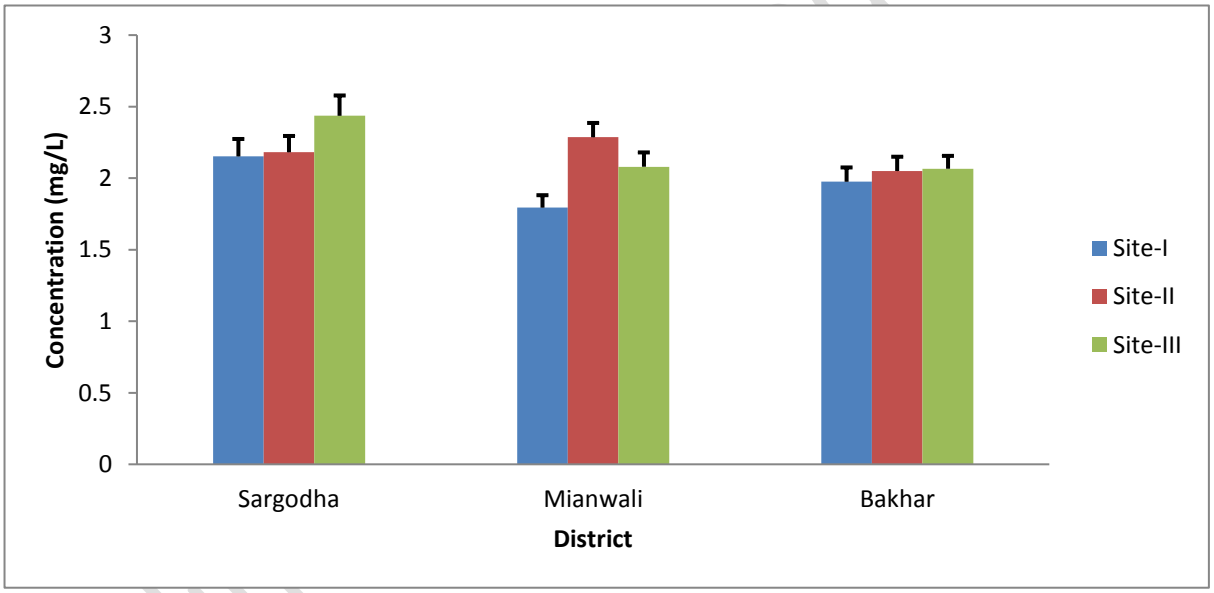

Figure 4. Mean concentration of cadmium in blood of sheep of different districts of Punjab

Table 2. Correlation of cadmium between soil-forage and soil-blood of goat and sheep

District	Soil-Forages	Soil-BG	Soil-BS	Forages-BG	Forages-BS
Sargodha	-.983	-.569	-.569	.711	.721
Mianwali	-.003	-.084	-.708	.997	.708
Bhakkar	-.884	-.991	.230	.938	-.657

BG= Blood of goats, BS= Blood of sheep

Table 3. Bioconcentration factor of cadmium in different districts of Punjab

Parameter	Site	BCF		
		Sargodha	Mianwali	Bhakkar
Soil-Forages	1	0.555	0.751	0.681
	2	0.723	0.970	1.200
	3	0.750	1.067	0.816
Forages-Blood of goat	1	0.784	0.769	1.131
	2	0.659	0.77	1.319
	3	0.709	0.747	1.211
Forages-Blood of sheep	1	0.784	0.799	0.970
	2	0.659	0.798	1.032
	3	0.709	0.651	1.087

Table 4. Pollution load index of cadmium in different districts of Punjab

Site	PLI		
	Sargodha	Mianwali	Bhakkar
1	3.317	2.005	2.005
2	3.067	1.979	1.11
3	3.070	2.011	1.56

Discussion

Cadmium is present naturally in Earth's crust. It joins with other elements such as oxygen (cadmium oxide), or sulfur (cadmium sulfate, cadmium sulfide) chlorine (cadmium chloride). All soil and rocks, including coal and mineral fertilizers, consists of some amount of cadmium [21]. The higher Cd content was found in the soil samples than the critical value of 3.00 mg/kg as reported by Mc Dowell *et al*. [22] except in Bhakkar district. On the other hand, these findings were higher than the values given by Logan and Miller [23] for Sargodha district compared to other two districts (Mianwali and Bhakkar). However, cadmium concentration in the soil samples of the present research was lower than as given by Pierce *et al*. [24]. The cadmium content in agricultural soils depends upon the parent rocks and fertilizers used and the low amounts of cadmium observed during present studies might be due to the leaching of cadmium. The level of cadmium in the present studies was below than the toxic level but higher than determined by Oluokun *et al*. [25] in Nigeria and Lopez *et al*. [26].

The outcomes showed that high cadmium content in forage samples of Sargodha prone to toxic level as compared to forage varieties of Mianwali and Bhakkar that were resistant to cadmium as low cadmium bioconcentration factor was seen. The low concentration of cadmium in forages of Mianwali and Bhakkar is affected by the soil period. During recent study, BCF values were observed lower as compared to the suggested by Mapanda *et al*. [27].

Critical value was 0.030 mg/kg reported by Kloke [28] much lower than the cadmium levels. In plants, concentration of cadmium was 3.0 mg/kg as recommended by Ciecko *et al*. [29] which was similar with present study. The higher cadmium concentrations more than normal level have greater threat for livestock by Aksoy *et al*. [30]. Concentration of cadmium in blood was higher than the Gowda *et al*. [31]. The mean concentration of cadmium was lowest in Mianwali and highest in Bhakkar. Current research values were more than those reported by Ubwa *et al*. [32].

In current research, there was a higher cadmium concentration in blood plasma samples of sheep from Sargodha district. On the other hand, the lowest concentration was observed in the blood plasma of sheep from Bhakkar district. The current research values were higher than the values reported by Ahmad *et al*. [33] who reported that

values for blood cadmium was 0.160±0.020 ppm in the street garbage group and 0.008±0.004 ppm in the indoor group. So, this study indicated that the type of feed taken by an animal was responsible about the concentration of metals in their body.

According to the WHO [34] blood concentrations <0.1 mg/L are considered satisfactory and all samples in current research from three districts were within the safe limit. There was a variation in values of cadmium blood from all districts. Such variation is because sheep grazed freely on contaminated environment and drink water from ponds, stream and other possible contaminated water source. Sheep in the process are exposed to the high levels of heavy metals in the environment, these agree with that reported by Nwude *et al*. [21].

Bioconcentration factor of cadmium in forage was higher in Mianwali samples as compared to Sargodha samples. Higher BCF value of cadmium in Mianwali and Bhakkar samples was observed while the lowest BCF value of cadmium was observed in Sargodha. Low cadmium in plants in Mianwali and Bhakkar may be due to the low cadmium uptake by forage species and periods have also affected the metal transmission and low cadmium level in animals present in Sargodha. The rate of metal uptake by plant has been affected by nature of soil, plant age, plant species, soil pH, and climate.

To determine the contamination status and inconsistency in soil, pollution load index was examined. The values of PLI>1 indicated that soil is contaminated, while PLI<1 considered as uncontaminated [35]. The PLI for cadmium was greater than 1 in all sites of three districts and soil considered to be contaminated. The values of PLI in present findings were similar to those recorded by Ahmad *et al*. [8].

Ethics

All the study protocols were approved by the Institutional Animal Ethics Committee, University of Sargodha (Approval No.25-A18 IEC UOS). All the experiments performed complied with the rules of the National Research Council [36] and all methods were performed in accordance with relevant guidelines and regulations.

Conclusion

In present findings, concentration of cadmium in forages of all sites was surpassed the acceptable limit given by FAO/WHO, while in blood samples it was found within the critical limit given by WHO. Bioconcentration factor of cadmium from soil to forage, forage to blood of sheep and forage to blood of goat at Bhakkar site was greater as compared to other two sites. The values of PLI at all sites was greater than 1 indicated that soil is contaminated. So proper monitoring of soil is necessary to prevent the excessive buildup of cadmium in soil-plant-animal continuum.

Authors' contributions

Conceived and designed the experiments: ZI Khan, K Ahmad & IR Noorka, Performed the experiments: S Siddique, M Ghazzal & K Wajid, Analyzed the data: M Akhtar & H Bashir, Contributed reagents/ materials/ analysis tools: M Nadeem & I Ugulu, Wrote the paper: P Akhter, IS Malik, A Ashfaq & M Munir.

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