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Research Article

Assessing the seasonal transfer of Pb and Cu from pasture to animals in the vicinity of coal mines in district Chakwal, Pakistan

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Abstract

The present investigation was aimed to search out Pb and Cu level in soil, fodder and blood samples of sheep at five different sites in district Chakwal of Punjab, Pakistan. The value of Pb observed in soil is much greater than acceptable limit 0.3 mg/kg recommended by FAO/WHO (2001), while in fodder and blood samples Pb values are lesser than tolerable limit. Value of Cu observed in soil and blood samples were lower than tolerated limit, while in fodder Cu values are higher. PLI values were found to be higher than 1 for both metals and higher values are recorded for Pb. BCF and DIM values are lower than 1 for Pb and Cu and higher values are recorded in Cu. HRI values are higher for Cu as compared to Pb. EF are found to be lower than 1 for both metals and varied from 0.82 to 0.24mg/kg. Pb showed a positive and significant correlation in both season however Cu showed a positive and non-significant correlation in winter season.

Keywords: Blood; Chakwal; Copper; Fodder; Lead; Pakistan; Plasma; Soil

Introduction

As grazing animals might not obtain mineral supplementations excluding common brine they rely upon fodder for their normal function and other necessities, only seldom can fodder entirely fulfill all requirements of

mineral [1, 2]. The proper working and extent of the yield of animals rely on well-adjusted and acceptable amounts of all the essential nutrients to fulfill their needs for a given physical stage [3]. For grazing animals for example goats, sheep and cows which get all or some of the nutrients needed from fodders, information of the composition of the nutrients of such fodder is therefore vital.

Biological properties of soil are influenced by the concentration of metals [4]. Metal presence in the soil can take route to food chain and might pose serious problems to higher organisms in food chain especially humans. Some heavy metals are called as xenobiotic since these metals perform no valuable function in the body of an animal and are even minor concentration can be very harmful. Cd, Hg, Bi, Ar, Be, Sb, Al, U, Pb and Ba are thought to be toxic metals. Increased concentrations of these metals are very toxic to animals and plants. Solubility of above-mentioned metals in water is considered as major issue of environment [5]. Bio-accumulation of metals in food chain results various health problems in humans and livestock [6]. Harm caused by metals depends on concentration of heavy metals, absorption by digestive systems and species of animals [7]. Animals that feed on fodder contaminated by lead had higher amount of lead in liver and kidney [8]. Pseudo-total concentration was found 182-498% higher and concentration of available metals was 196-1877% higher in soil of reclaimed mine compared to control soil [9].

Concentration of heavy metals in below ground tissue remains higher than above ground tissue [10]. Sheep and cattle that are reared freely indicate the levels of toxicity caused by pollution of heavy metals [11]. In domestic animal's various physiological problems like low fitness, reproduction problem, immunity decline and risks of cancer are caused by higher concentrations of these heavy metals [12]. In some studies, it was found that Zn, Cu, Pb, Hg etc., were present in considerably higher amount in soil and also in vegetables [13]. Cancer, blood disorders [14], neural problems [15] and gastrointestinal diseases arise from the toxicity of Pb in humans [16].

Some major factors that affect the degree of harm caused by heavy metals are age, sex, the time period of exposure and intake frequency [17]. Various metals are present in small amount in the milk and are necessary for some enzymes to function properly but higher absorption of metals in fodder results in increased concentration of heavy metals which affect normal body functioning [18]. Mineral insufficiencies, inequalities and excess of heavy metals have been a main factor to decline and stop the production system of ruminants [19].

It is essential to investigate the mineral and especially heavy metal status of fodders which meets the nourishing necessities of grazing animals in Pakistan. It creates a reference point data that is vital for expressing methods of metal intake to increase the production of goats and sheep. This study focused mainly on to know the abundance of the heavy metals for grazing cattle and to study health risk in ruminants from ingesting of fodder contaminated by heavy metals of coal mines. A serious consideration is required for regular monitoring of such toxic metals and metalloids.

Materials and methods

Fifteen samples of soil, blood and fodder i.e. aerial parts "(*Acacia nilotica*, *Ziziphus nummularia*, *Acacia modesta*)" were collected from five different sites from chakwal "(Pidh, Tobar, Ratoccha, Kalar Kahar road, and Choa Saiden Shah, Chakwal Road)." Samples were stored in polythene bags for analysis. Samples were sweep away with filter water. Soil and fodder samples were dried at 91°C for 48 hours [20]. Dried samples were pulverized into powder. 1 g of

each sample along with three replicates are taken into the flask, soaked in 40ml of nitric acid and left for night, complete dissolution of mixture.

Sample of blood were collected by using sterilized needle in ADTA-K3 tubes. After sampling blood was centrifuge to obtain plasma. Then, samples were digested on hot plate and add H_2O_2 drop wise till the solution becomes transparent. Cooled the digested samples, put it into the volumetric flask of 100ml and add the water to raise the volume at 50ml. All apparatus which are involved in the process of sampling and digestion were soaked in 35% HNO3 for 9 hours and then wash away with cleanse water **[21]**. Pb and Cu estimation was carried out by Atomic Absorption Spectrophotometer and flame type for Pb and Cu is Air- acetylene

Lamp current for Pb and Cu is 6.0 and 5.8 (mA).

Statistical analysis

SPSS 16 software was used for One-way (ANOVA) to find mean and standard deviation of metal in support of soil, fodder and sheep blood **[22]**.

Pollution load index (PLI)

PLI was measured by formula given by Liu *et al*. **[23]**.

PLI= 'metal content in examined soil sample / metal concentration in reference soil'

Reference value of Pb and Co in soil is 8.15 and 9.1 mg/kg Dutch standard **[24]**.

Bioconcentration factor (BCF)

It was measured by following formula **[25]**.

BCF = [M] Fodder samples / [M] Soil sample

Daily intake of metal (DIM)

Metals enter in body of organisms through diverse pathways, through skin contact, during breathing or by consuming contaminated fodder etc. **[26]**.

DIM = C metal × F conversion factor × D food intake/B average weight of sheep

DIM by ingesting of forages is 1.51 (kg per sheep-1) as stated by Sun *et al*. (2016) and normal weight of sheep is 45 kg was used **[27]**. Conversion factor of 0.085 was apply to transform fresh green plant mass to dry weight, as termed by Arao **[28]**. Tolerable daily intake limit of Pb and Cu is 0.21 and 3.01 (mg kg-1day-1).

Health risk index (HRI)

Oral reference dose value for Pb and Cu is 0.0035 and 0.04 (mg/kg/day) reported by World Health Organization **[29]**.

"Health risk index = Daily intake of Metal / RfD **[30]**"

Enrichment factor (EF)

It was calculated by formula of Buat-Menard and Chesselet **[31]**.

$$EF = \frac{[M]Fodder\ E/[M]SoilE}{[M]FodderS/\ [M]SoilS}$$

Average absorption of Pb and Cu in forages is 2 and 10 mg/kg and in soil is 8.15 and 8.39 mg/kg, respectively **[32]**.

Results and discussion

Lead (Pb)

Analysis of variance displayed significant ($P<0.05$) effect of site 4 on the concentration of Pb while non-significant effect on concentration at site 1, 3, 5 and 2 (Table 1).

The current study was conducted to assess metal accumulation in soil, forages and blood samples of sheep in different coal mining sites of district Chakwal, Pakistan.

The results from soil, fodder and blood sample investigation were assessed by matching with suggested acute levels of soil, fodder and blood metal concentrations. The results specified that in all soil samples metal absorptions inclined to rise in sizzling dry season, due to low soil moisture.

In the present study, the Pb amount in soil samples during summer season differed from 28.17 to 9.00 mg/kg while 28.28 to 10.03 mg/kg in winter (Table 2). The permissible limit of Pb in soil is 0.3 mg/kg recommended by FAO/WHO **[33]**. Value of Pb observed in current research is much higher than the permissible limit. This means that this soil is not good for the growth of plants. The current level of Pb in the soil in this study showed a

possible healthiness danger to the animals which are consuming these fodders which are grown on contaminated soil. Pastrana et al. [20] reported a range of Pb in soil which is quite similar to the present results. Aluko et al. [34] described Pb value in soil fluctuating from 1246 - 1596 mg/kg having a range greater as related to current findings.

Table 1. Analysis of variance Pb concentration in soil, fodder and blood samples at different sites

Sample	S-I	S-II	S-III	S-IV	S-V
Summer Soil	0.001***	0.000ns	0.005***	0.001***	0.007 ns
Winter soil	0.001 ns	0.000**	0.043***	0.003***	0.016***
Summer Fodder	0.826***	0.277***	3.650***	0.016***	0.001***
Winter Fodder	0.001***	0.003**	0.312 ns	0.004***	0.000**
Summer blood	0.009***	0.019***	0.004***	18.813***	0.008***
Winter blood	0.013***	0.006***	50.134***	0.002***	0.001***

, *: Significant at 0.01 and 0.001 levels, ns: non-significant

Table 2. Concentration of Pb in soil, forages and blood samples at different sites

Sample	S-1	S-2	S-3	S-4	S-5
Soil collected with summer fodder					
Acacia modesta	27.24±4.02	28.17±5.02	15.11±2.02	11.13±1.02	9.11±1.02
Ziziphus nummularia	26.20±4.03	27.16±5.00	17.11±3.02	11.15±1.08	9.01±1.02
Acacia nilotica	26.23±4.02	26.10±5.03	16.10±3.14	11.14±1.01	9.00±1.02
Soil collected with winter fodder					
Acacia modesta	28.28±4.02	25.10±4.03	16.09±3.03	10.24±1.02	13.05±2.02
Ziziphus nummularia	28.24±4.02	28.11±4.01	16.06±3.02	10.03±1.01	13.05±2.03
Acacia nilotica	27.23±4.02	25.18±4.01	13.09±3.01	10.04±1.01	13.06±2.01
Summer fodder					
Acacia modesta	3.37±1.44	2.67±1.01	1.28±0.65	1.14±0.45	1.06±0.28
Ziziphus nummularia	3.15±1.44	2.37±1.02	1.33±0.62	1.17±0.42	1.05±0.26
Acacia nilotica	3.30±1.14	3.31±1.00	1.44±0.63	1.15±0.43	1.08±0.22
Winter fodder					
Acacia modesta	4.04±1.14	2.94±1.00	2.05±0.72	1.12±0.39	1.15±0.19
Ziziphus nummularia	4.04±1.22	2.91±1.00	2.20±0.74	2.13±0.37	1.10±0.21
Acacia nilotica	3.05±1.22	2.98±1.00	2.63±0.78	2.03±0.32	1.19±0.26
Summer sheep blood					
1	9.64±2.01	7.33±1.01	5.39±1.01	3.06±1.01	2.11±1.02
2	8.63±1.01	7.24±1.01	4.44±1.01	2.08±1.01	2.06±1.01
3	9.61±1.01	8.27±1.01	5.38±1.01	2.16±1.01	1.01±1.02
Winter sheep blood					
1	8.23±2.01	6.24±1.01	4.11±1.01	1.03±1.01	1.09±1.02
2	7.52±1.01	7.28±1.01	4.49±1.01	2.06±1.03	1.08±1.03
3	8.33±1.02	6.36±1.01	3.41±1.01	2.07±1.02	1.08±1.02

In present findings, the value of Pb in the fodder samples during summer ranged from 3.37 to 1.05 mg/kg and in winter 4.04 to 1.10 mg/kg. Maximum permissible limit for Pb in fodder is 5.0 mg/kg according to CERSPC [35]. The values of Pb observed in present study are lower than this permissible limit. Dey et al. and Palacios et al. [36, 4] reported Pb concentration in fodder samples in the range which is higher as

compared to the current research. Plants which are growing inside or near the surrounding of coal mining areas have higher ability of accumulation of Pb and Cd. Plants can tolerate up to some extent of these heavy metal concentration but higher level of these metals can cause closure of stomata and oxidative stress in plants **[37]**.

Lead has shown very harmful effects on human health even at inferior intensities. Extent of Pb level above 0.01 mg/kg is damaging to health of humans, it might affect in probable nervous impairment to embryo, miscarriages, muscular-skeletal, and further obstacles in offspring below the age of three years **[38]**. According to USEPA, the regular consumption of Pb in food from 2 to 8 mg/kg of body weight/day can reason for death of most grazing animals **[39]**.

The observed Pb mean value in the blood of sheep during summer season ranged from 9.64 to 1.01 mg/L while 8.33 to 1.03 mg/L in winter. In winter season, the lead concentrations in the blood of animals were much higher in the vicinity of coal mining areas. Such a high mean Pb value might cause serious health issues for animal's i-e (goats, sheep, and cows). Sidhu et al. **[40]** reported Pb concentration in the blood of ruminants higher as related to present investigation. Dwivedi *et al*. **[41]** recorded Pb poisoning in the blood of cows and buffaloes having a value lower as compared to the current investigation.

Copper (Cu)

Analysis of variance results showed the significant ($P<0.05$) outcome on concentration of Cu at site-1, 2, 3, 4 and 5 during both seasons (Table 3).

In the present findings, Cu values in the soil samples during summer season fluctuated from 21.01 to 7.66 mg/kg and during winter season ranged from 19.24 to 7.50 mg/kg (Table 4). The tolerable limit of Cu in soil is 36 mg/kg recommended by FAO/WHO **[33]**. Cu concentration in soil samples in present study is lower than this allowable limit. Nikhil **[42]** recorded the Cu concentration in soil of coal-mining area. China having average concentrations of Cu is 26.4 mg/kg which is little bit higher as compared to current finding. Ladwani et al. **[43]** observed Cu absorption in the soil in range of .41-0.77 mg/kg which is much lower to the present study results.

In current research, the value of Cu in fodder samples during summer season ranged from 11.18 to 4.02 mg/kg and in winter 11.16 to 3.04 mg/kg. The maximum permissible limit for Cu in fodder is 10 mg/kg according to WHO **[29]**. The values of Cu observed in existing study are greater than allowed limit. Also, Cu absorption in feeds and fodder was much higher than reported by Yadav et al. **[44]**. On the other hand, Somasundaram et al. **[45]** perceived the value of Cu in fodder as 379.60 mg/kg and is much higher as per our results. Waseem et al. **[46]** described that absorptions of heavy metals in fodders were significantly pretentious by seasons and species both.

In animals which have deficiency of Cu have few biochemical and physiological changes have been accounted for example decreased craving, lessened development, and in addition hindrance of a few metabolic chemicals. Copper move into the blood might be managed by cystol protein that improves or obstructs the move of Cu into the blood. Copper retention may likewise be directed or more probable confined by metallothionin **[47]**. In current investigation, value of Cu in blood samples of sheep during summer season was 14.661 to 5.244 mg/L in summer, while in winter was 15.87 to 6.31 mg/L. Intake of extreme Cu (through diet) can affect the liver and kidney mutilation as well as anemia **[48]**.

Pollution load index value was higher in Pb as compared to Cu, in both metals the highest concentration was found at site 1 and 2 (Table 5). Concentration of both metals is higher in

winter seasons as compared to summer. PLI values range from 3.46 to 1.10mg/kg for Pb and 2.50 to 0.89 mg/kg for Cu. Among the different sites, contamination showed the impact of anthropogenic activities on soil overwhelming metals [49, 50].

BCF having values greater than 1 are regarded as unsafe for health of plants as well as animals [51]. Bioconcentration factor of Pb in fodder was greater than Cu (Table 6). BCF values are higher at site 1 and 2 as compared to the other sites, in winter concentrations of these metals are higher as compared to summer season. The Pb uptake from topsoil into forages may be influenced by soil type, species of plants and type of metal [51, 52].

DIM values for both metals were found to be lower than 1 which showed that the sheep which are grazing at that site are not at greater health risk (Table 7). DIM values are higher for Cu as compared to Pb. Increased intake of heavy metal in the diet marks several developmental disorders in the animals and human body, so regular checking of unsafe elements is necessary in faunae and human nutrition [53]. If the level of Pb exceed 30 mg/kg in food on a d.w basis it is extremely poisonous to the animals and higher uptake of causes various problems about synthesis of red blood [54].

Intake of metals in animals and human may happen through various pathways for example soil, plants, water, air and food [54-56], beside this bio-accumulation is the main source through which metals go into the animals and human body [55, 57-59]. HRI value was higher for Cu and ranged from 3.85 to 1.07 mg/kg which showed that sheep which are browsing on that site are at a higher health risk, for Pb value ranged from 1.35 to 0.35 mg/kg (Table 8). On site 1 and 2 contamination is higher as compared to other. Higher concentration of Cu decreases the functioning of immune system and levels of high-density lipoprotein [33, 60, 61].

Enrichment factor is the soil holding capacity of heavy metals [62]. The EF in metals is pointers used to diagnose the occurrence and extent of human made pollutant accumulation on the surface of the soil [63, 64]. Enrichment Factors values range within 0.5 and 1.5 shows that metal is exclusively from top soil resources or usual phenomena [56, 65-67]. EF values for both metals were found to be lower than 1 (Table 9).

Pb exhibited a positive and significant correlation from soil to fodder, soil to blood and fodder to blood in both seasons while Cu showed a positive and significant co relation in summer season, positive and non-significant correlation from soil to blood and fodder to blood in winter season (Table 10). Higher value of transfer coefficients displays comparatively reduced holding in soils or larger ability of forages to captivate metals [68, 69]. Low values of transfer amounts show strong sorption of metals to soil and from soil to plants animals [70].

Table 3. Analysis of Cu concentration in soil, fodder and blood samples at different sites

Sample	S-I	S-II	S-III	S-IV	S-V
Summer Soil	0.002***	0.008***	0.026***	0.018***	0.002***
Winter soil	0.135***	0.006***	0.093***	0.002***	0.002***
Summer Fodder	0.005***	0.021***	0.007***	0.015**	0.010***
Winter Fodder	0.010***	0.342***	0.042***	0.020***	0.091***
Summer blood	0.005***	0.018**	0.017***	0.042***	0.094***
Winter blood	1.303***	0.000***	0.002***	0.003***	0.003***

,*: Significant at 0.01 and 0.001 levels

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Table 4. Concentration of Cu in soil, forages and blood samples at different sites

Sample	S-1	S-2	S-3	S-4	S-5
Soil collected with summer fodder					
Acacia modesta	21.01±3.00	16.96±3.00	13.90±1.00	8.71±1.00	9.78±1.00
Ziziphus nummularia	20.05±3.00	16.98±3.00	13.81±1.00	7.66±1.00	8.78±1.00
Acacia nilotica	20.00±3.00	16.92±2.00	13.91±1.00	8.68±1.00	9.87±1.00
Soil collected with winter fodder					
Acacia modesta	19.24±2.00	15.02±2.00	12.98±1.00	10.89±1.00	7.50±1.00
Ziziphus nummularia	19.03±2.00	15.92±2.00	12.96±2.00	8.89±1.00	6.76±1.00
Acacia nilotica	18.08±2.00	15.98±2.00	12.93±1.00	10.82±1.00	7.80±1.00
Summer fodder					
Acacia modesta	11.18±3.00	8.23±2.00	7.02±1.04	4.97±0.94	5.11±1.08
Ziziphus nummularia	11.13±2.00	7.17±2.00	8.13±1.05	4.04±0.92	4.22±2.06
Acacia nilotica	11.15±3.02	8.29±3.00	9.05±1.01	4.02±0.91	5.13±1.07
Winter fodder					
Acacia modesta	10.05±2.00	9.13±2.00	8.21±1.06	3.50±0.81	4.10±1.05
Ziziphus nummularia	9.13±3.00	8.13±2.00	9.13±1.06	3.04±0.79	3.12±1.02
Acacia nilotica	11.16±2.00	10.31±3.00	8.41±1.08	3.20±0.74	4.12±1.05
Summer sheep blood					
1	9.18±3.00	14.66±3.58	7.62±2.050	6.03±1.048	6.037±1.03
2	9.68±1.00	14.66±3.05	6.45±2.022	6.23±1.096	5.244±1.04
3	8.68±1.00	14.62±3.05	7.41±2.030	5.26±1.068	5.427±1.03
Winter sheep blood					
1	8.64±1.00	15.87±3.02	7.45±2.036	8.33±1.048	7.193±1.04
2	8.59±1.00	15.69±3.05	7.46±2.033	8.47±1.096	6.53±1.04
3	8.57±1.00	14.63±3.03	6.31±2.048	7.34±1.046	7.126±1.02

Table 5. The values of pollution load index for Pb and Cu in soil

Pollution load index						
Site	***Acacia modesta***		***Ziziphus nummularia***		***Acacia nilotica***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
	Pb					
Site-I	3.34	3.46	3.21	3.46	3.21	3.34
Site-II	3.45	3.07	3.33	3.44	3.20	3.08
Site-III	1.85	1.97	2.09	1.97	1.97	1.60
Site-IV	1.36	1.25	1.36	1.23	1.36	1.23
Site-V	1.11	1.60	1.10	1.60	1.10	1.60
	Cu					
Site-I	2.50	2.29	2.38	2.26	2.38	2.15

Site-II	2.02	1.79	2.02	1.89	2.01	1.90
Site-III	1.65	1.54	1.64	1.54	1.65	1.54
Site-IV	1.03	1.29	0.91	1.05	1.03	1.28
Site-V	1.16	0.89	1.04	0.80	1.17	0.92

Table 6. Bioconcentration factor for Pb and Cu

Bioconcentration factor						
Site	***Acacia modesta***		***Ziziphus nummularia***		***Acacia nilotica***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
	Pb					
Site-I	0.12	0.14	0.12	0.14	0.12	0.11
Site-II	0.09	0.11	0.08	0.10	0.12	0.11
Site-III	0.08	0.06	0.07	0.12	0.08	0.20
Site-IV	0.10	0.10	0.10	0.21	0.10	0.20
Site-V	0.11	0.08	0.11	0.08	0.12	0.091
	Cu					
Site-I	0.53	0.52	0.55	0.47	0.55	0.61
Site-II	0.48	0.60	0.42	0.51	0.48	0.64
Site-III	0.50	0.63	0.58	0.70	0.65	0.65
Site-IV	0.57	0.32	0.52	0.34	0.46	0.29
Site-V	0.52	0.54	0.48	0.46	0.51	0.52

Table 7. Daily intake of metal for Pb and Cu

Daily intake of metal						
Site	***Acacia modesta***		***Ziziphus nummularia***		***Acacia nilotica***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
	Pb					
Site-I	0.03	0.04	0.03	0.04	0.03	0.04
Site-II	0.03	0.03	0.02	0.02	0.03	0.03
Site-III	0.01	0.02	0.01	0.02	0.01	0.03
Site-IV	0.03	0.01	0.01	0.02	0.01	0.02
Site-V	0.01	0.01	0.02	0.01	0.01	0.01
	Cu					
Site-I	0.15	0.13	0.15	0.12	0.15	0.15
Site-II	0.11	0.12	0.09	0.11	0.11	0.14
Site-III	0.09	0.11	0.11	0.12	0.12	0.11
Site-IV	0.06	0.04	0.05	0.04	0.05	0.04
Site-V	0.07	0.05	0.05	0.04	0.07	0.05

Table 8. Health risk due to Pb and Cu via consumption of contaminated forages

Health risk index						
Site	***Acacia modesta***		***Ziziphus nummularia***		***Acacia nilotica***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
	Pb					
Site-I	1.12	1.35	1.05	1.35	1.10	1.02
Site-II	0.89	0.98	0.79	0.97	1.10	0.99

Site-III	0.42	0.68	0.44	0.67	0.48	0.88
Site-IV	0.38	0.37	0.39	0.71	0.38	0.67
Site-V	0.35	0.38	0.35	0.36	0.36	0.39
	Cu					
Site-I	3.85	3.46	3.83	3.14	3.84	3.84
Site-II	2.83	3.14	2.46	2.80	2.85	3.55
Site-III	2.41	3.82	2.80	2.14	3.11	2.89
Site-IV	1.71	1.20	1.39	1.04	1.38	1.10
Site-V	1.76	1.41	1.45	1.07	1.76	1.41

Table 9. Enrichment factor for Pb and Cu

Enrichment factor						
Site	***Acacia modesta***		***Ziziphus nummularia***		***Acacia nilotica***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
	Pb					
Site-I	0.50	0.58	0.48	0.58	.51	0.45
Site-II	0.38	0.47	.35	.42	0.51	0.49
Site-III	0.34	0.28	0.31	0.51	0.36	0.81
Site-IV	0.41	0.44	0.42	0.86	0.42	0.82
Site-V	0.47	0.35	.47	0.34	0.48	0.37
	Cu					
Site-I	0.44	0.43	0.46	0.40	0.46	0.51
Site-II	0.40	0.50	0.35	0.42	0.41	0.54
Site-III	0.42	0.53	0.49	0.59	0.54	0.54
Site-IV	0.47	0.26	0.44	0.28	0.38	0.24
Site-V	0.43	0.45	0.40	0.38	0.43	0.44

Table 10. Correlation between soil-fodder, soil-blood and fodder-blood

Metal	**Soil to fodder**		**Soil to blood**		**Fodder to blood**	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Pb	0.953**	0.842**	0.954**	0.952**	0.942**	0.916**
Cu	0.951**	0.882**	0.671**	0.446	0.481	0.421

Conclusion

This is the first study to find seasonal transfer of Pb and Cu from pasture to animals in the vicinity of coal mines in district Chakwal, Pakistan**.** The concentration of Cu and Pb in soil, fodder and blood sample varied from site to site. The values of BCF for Pb and Cu were less than 1 at all sites. The values of HRI for Cu and Pb were greater than 1 at all sites indicated that the consumption of these forages are not free of risk.

Authors' contributions

Conceived and designed the experiments: ZI Khan IR Noorka & K Ahmad, Performed the experiments: M Akhtar, P Akhter & N Mehmood, Analyzed the data: K Wajid, M Ghazzal & ZF Rizvi, Contributed materials/ analysis/ tools: F Shaheen, IS Malik, A Ashfaq & M Munir, Wrote the paper: ZE Huma, H Bashir, S Mahpara & M Sohail.

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