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Assessment of Trace Metal Contents of Indigenous and Improved Pastures and Their Implications for Livestock in Terms of Seasonal Variations

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Abstract: *The research was aimed to determine seasonal effects on trace metals levels in soil, forages and blood plasma of animals. The mean cadmium, chromium and copper values in soil samples in different sampling seasons were ranged from 6.97 to 4.10, 0.060 to 0.72 and 3.54to 4.08 mg/kg, respectively, while, in forage samples were between 0.671-0.697, 1.57-2.22 and 6.75-7.06 mg/kg, respectively. Higher Cd, Cr and Cu concentrations were observed in blood plasma of young buffaloes during summer season, in dry buffaloes during spring season and in young buffaloes during autumn season, while lower Cd, Cr and Cu contents were noticed in blood plasma of lactating buffaloes in summer season. The highest bio-concentration factor value from soil to forage was determined for Cr while from forage to blood plasma of buffaloes was detected for Cd. The Cd, Cr and Cu correlation of soil with blood plasma were positive for all samples.*

Keywords: *buffalo, forage, soil, trace metal*

1. Introduction

In the balanced diet of organisms minerals play vital role throughout their life. If mineral concentration is improper, the activities of animals in livestock minimized. For popper growth of livestock animals various macro and micro minerals are essential [1]. The macro minerals such as phosphorus, calcium and magnesium play an important role for proper lactation, growth and reproduction of ruminants. On the other hand, the micro-minerals like trace metals such as cadmium, chromium and copper are very important to improve human and animal health [2].

In animal's feed the minerals requirement cannot be denied because they have crucial role in the normal physiological functions of animal body, their growth and reproduction. Therefore, in the mineral requirements of animals there is high level of uncertainty depending upon level of production, age, breed, dietary antagonist, animal adaptation and interrelationship with other nutrients [3]. Soil properties and mineral profile affect the mineral deficiencies in grazing livestock [4].

Mineral imbalances in soil and forages have long been held responsible for low production and reproductive problems among grazing ruminants in the world. In spite of very much importance of minerals in ruminant nutrition, very little information is available on the mineral status of livestock in Pakistan.

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There are number of methods to evaluate the mineral status of livestock, but animal fluid and tissue mineral concentration are better indicators of the availability of most minerals. Grazing animals frequently obtain their mineral nutrition by consuming water, soil, leaves, tree branches etc, rather than entirely from forages. So the livestock fluid and tissue mineral concentrations are more accurately related to animal environmental conditions, in meeting the mineral requirements for their production [5].

Mineral deficiencies, imbalances and toxicities have been reported to inhibit ruminant production systems [6]. So, there is need to explore the forage mineral status which fulfil the nutritional requirements of buffaloes. It establishes baseline data that is essential for formulating ways of supplementing minerals to improve buffalo production. This work will provide baseline information to farmers and animal nutritionists on the seasonality of trace metals in soil, plant material and blood plasma of buffaloes, which is essential for formulating supplements to improve productivity. It will provide a description of trace metal levels in buffaloes for diagnosing the deficiencies and toxicities to institute corrective or preventive measures. In this direction, the main objective of the present study was to determine trace metal contents and particularly to find out the effect of season on the levels of trace metals in forages and blood plasma of animals.

2. Materials and methods

2.1. Study site

Khushab is that district of Punjab which was elicited for demonstrative study (Figure 1). Khushab is populated with 102,793 inhabitants and is roughly160 km from Lahore.

Figure 1. Geographical location of study area

2.2. Samples collection and preparation

2.2.1. Soil

Ten feeding sites were selected randomly from each agro-ecological zone i.e. from where the fodder samples under study were collected. Soil samples were taken from half to 1ft depth four times in a year of summer, winter, autumn and spring seasons. Soil samples after air dried, were placed in hot air oven till constant weight and ground, stored in labelled bags for further processing. After that, samples were digested [7]. Soil sample (1g) was digested with H_2SO_4 (4mL) and H_2O_2 (8mL) in digestion chamber. After digestion, sample diluted, filtered and saved in labelled plastic bottles.

2.2.2. Forage

The sterilized apparatus can be used for sampling from similar site while these forages that were grazed by buffaloes. Composite fodder samples were collected that were being served to the buffaloes at the time of blood sample collection (Table 1). Traditionally mixed fodders (at least two fodder

species) are served to the buffaloes. The collected forage samples were digested with 2 mL of sulphuric acid solution plus 4 mL of hydrogen per oxide solution and put in digestion chamber. After digestion, sample diluted, filtered and saved in labelled plastic bottles.

Table 1. The collected forage samples during different seasons

Sampling	Season	Common Name	Scientific name
1st	Summer	Maize Jowar	*Zea mays* *Sorghum bicolour*
2nd	Autumn	Bajra Guar	*Pennisetum glaucum* *Cyamopsis tetragonoloba*
3rd	Winter	Barseeem Lucerne	*Trifolium alexandrinum* *Medicago sativa*
4th	Spring	Sarsoon Barseem	*Brassica campestris* *Trifolium alexandrinum*

2.2.3. Blood plasma

The Nili Ravi buffaloes maintained under the existing field conditions comprised of the study animals. In current research thirty buffaloes (Nili-Ravi) were selected and divided into three categories lactating, non-lactating and young. Four samplings were done in different seasons (summer, autumn, winter and spring) and 10 blood plasma samples were collected from each category of Nili-Ravi buffaloes during each sampling period. The sterilized needle was used for blood sampling from the jugular vein of standing position buffalo and these samples were placed immediately in the heparin of sodium citrate voiles to blocked the clot forming. Centrifugation was done for serum separation from blood plasma. Then in last serum was poured in labelled small voiles and stored at -20^0C. For digestion, same procedure was followed as in forages digestion.

2.3. Quality control

Being repeated study of samples assured the accuracy and precision of analysis versus nutritional institute of standard and technology, (SRM 1570) is the standard reference material for heavy metals. Within ±2% resulted readings were observed of the documented worth. For the assessment of contamination and reliability of data, quality control measures had been taken. To calibrate the instrument, bank and be adrift were observed after five findings. For opposite determinations and for the precision of analysis and variations that could be considered right if it will be less than 10%, after the determination of coefficient of replicate analysis.

2.4. Preparing of standard

For receiving accurate results, instruments must be calibrated with standard before the using for analysis. Standard preparation is also necessary for the washing of glassware that will be used in analysis. Take sample in a beaker and weighing on an analytical balance, it has dissolved in little amount of water or in another solvent and placed on a hot plate to make speedy dissolution. Before making further processes, we must be sure that the sample had fully dissolved. A measured amount of solution was transferred in a volumetric flask. Thoroughly wash the glassware like a beaker, funnel etc. and stirring the flask with deionized water so that to ensure, that dissolved samples have been entirely shifted to 2nd flask. Thoroughly rinse the beaker with distilled water and up to mark little below the 100 mL line. Then deionized water was added carefully with dropper until the meniscus touched up to 100 mL mark. For good mixing, hold the stopper tightly and agitate the flask again and again. Make sure that uniform solution filled in the flask. Must be the same concentration of the solution and it can ensure that its bottom is exactly at 100 mL if needed.

2.5. Instrumentation

As a result of wet digestion, atomic absorption spectrophotometer was used to analyzed the forage and blood plasma samples (AA 5000) for the assessment of mineral concentration in all collected samples. Trace metals like zinc, iron and lead were evaluated in exhibition analytic process. The detective limits of AAS for the above metals are given in (Table 2).

Table 2. Detection limits of atomic absorption spectrophotometer

Elements	Detection limits
Cadmium	0.8 (Flame AA)
Chromium	3 (Flame AA)
Coppe	15 (Flame AA)

2.6. Physico-chemical analysis of water and soil samples

The *p*H of all soil samples was calculated via usage of 1:2 (w/v) soil and water ratio. The *p*H of soil was measured via pH meter. Electrical conductivity was determined via adding 5grams soil in 10 mL of distilled water to make a suspension (1:2w/vas in) [8](De Vos et al. 2007).

2.7. Statistical analysis

Data obtained from each parameter was analysed statistically using the SPSS software, descriptive analysis, ANOVA, transfer factor and correlation was worked out [9]

2.8. Correlation

Pearson's correlation coefficient was used to find out the relationship of diverse heavy metals in soil and plants.

2.9. Bio concentration factor

It is applied to examine the proportion of metal contents in plants to that in corresponding soil. It was used to evaluate the uptake of metals from soil and their bioaccumulation in vegetable using the following formula:

BCF soil-forage =Metal contents in forage/Metal content in soil

And similarly, bioconcentration factor (BCF) from forage to blood plasma:

BCF forage-plasma = Metal contents in plasma/Metalcontents in forage

3. Results and discussions

3.1. Physiochemical characterization of water

The *p*H value of water samples was the highest at winter sampling season and the lowest pH value was found summer sampling season (Table 3). Electrical conductivity is another important indicator to check the ionic content of water. In present study, the range of EC in all treatments was ranged from 280 to 321 dS/m Present results showed higher EC values than the values determined by Pandey and Singh [10]. They observed EC value 7.8 dS/m in their research. According to USDA, the EC value of irrigation water quality and the EC value of standard limit for irrigation should be lower than 0.7 and 3 dS/m, respectively. It was revealed that present value for EC was not in permissible limits.

The value of Ca^{2+} and Mg^{2+} varied 92-112 and 32-38 mg/L respectively and Chloride ranged from 47 to 55 mg/L with mean concentration of 47, 49, 50, and 55 mg/L for summer, spring, autumn and winter seasons, respectively. The range of total dissolved solid was 130 to 135 mg/L with the mean

concentration of 6.2, 38.2, 540, and 894 mg/L respectively. Total hardness and alkalinity varied 95-100 and 100-121 mg/L respectively (Table 3).

Table 3. Physiochemical characterization of water at different sampling seasons

Parameters	WHO desirable limit	WHO Max. permissible limit	Sampling season			
			Summer	Autumn	Winter	Spring
pH	7-8.5	6.5-9.2	7.8	8	8.2	7.9
Calcium (mg/L)	75	200	92	100	112	98
Magnesium (mg/L)	50	150	32	36	34	38
Chloride (mg/L)	200	600	47	50	55	49
Electrical conductivity (us/cm)	-	-	280	300	321	289
Total dissolved solid (mg/L)	500	1000	130	130	135	135
Total hardness ($CaCo_3$) (mg/L)	100	500	100	98	95	97
Total alkalinity as $CaCo_3$ (mg/L)	_	500	112	100	112	121

Conductivity is directly related with various water properties. Alkalinity, pH and hardness affect the toxicity of many substances in the water [11].

3.2. Physiochemical characterization of soil

The *p*H value of soil samples was highest at summer sampling season and the lowest pH value was found at spring sampling season (Table 4). Range of electrical conductivity among all sites was observed between 0.22-0.29 ms/cm. No risk of salinity and sodicity was found in the soil at three sites as EC values were lower than the threshold values of EC (4 dS/m). Textural class of the soil samples for four seasons was loamy with a *p*H range of 8.1-8.9 (Table 4).

Table 4. Physiochemical characterization of soil at different sampling seasons

Parameter	Unit	Sampling season			
		Summer	Autumn	Winter	Spring
Ph		8.9	8.5	8.7	8.1
Electrical conductivity	dS/m	0.24	0.25	0.22	0.29
Organic matter	(%)	0.68	0.67	0.7	0.71
Phosphorus	Ppm	8.5	9	9.5	9.2
Potassium	Ppm	132	135	137	130
Saturation	%	44	44	44	44
Texture		loamy	loamy	loamy	loamy

According to laboratory test, the soil samples are deficient with organic matter percentage contents and need fertilizers to enhance its fertility. Soil *p*H is the only feature of soil that clarifies a whole

image of the medium for plant growth comprising soil mineralogy, method for nutrient supply, salinity/sodicity position and soil exposure to air, destiny of nutrients added and final weather situations of the area. The numerical values are each time within limit of 8.0-8.4 in normal soil where as in Pakistan *p*H of sodic soils might reach 10.00. Therefore, a reduction in soil *p*H because of some land management strategy is every time considerable and effects in eventual alteration of soil medium into positive one and remaining translation into improved yields. Outcomes on *p*H were alike by manure alone or its mixture by fertilizer at similar level of application. Numerical values were minute less next to wheat in the similar treatments representing constant progressive effect of manure on such soil limitation. Smiciklas-Wright et al. [11], Pattanayak et al. [12], Yadav and Khirwar [13] also observed a decrease in soil pH after the use of organic materials.

3.3. Cadmium contents of samples

3.3.1. Soil

The mean Cd levels in soil samples during various sampling seasons are listed in Table 5 and Table 6. According to ANOVA, the mean Cd concentrations in soil samples affected non-significantly (p>0.001) by sampling seasons. The mean Cd contents in different sampling seasons were ranged from 6.97 to 4.10 mg/kg. Higher mean Cd contents were found in soil samples collected during autumn sampling season and lower Cd concentrations were observed during summer sampling season (Figure 2). The mean values of Cd contents in the different sampling seasons were 4.10 mg/kg (summer), 6.97 mg/kg (autumn), 4.73 mg/kg (winter) and 5.17 mg/kg (spring). The order of the obtained Cd mean values in all soil samples were determined as autumn>spring>winter>summer. The mean Cd contents in soil samples exhibited inconsistent pattern of variation along the sampling seasons.

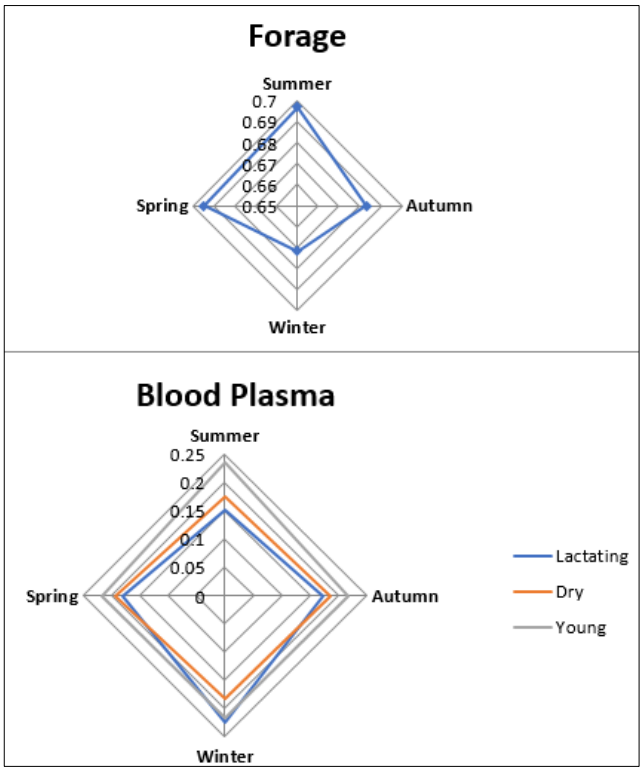

Figure 2. Fluctuations in cadmium values in soil, forage and blood plasma during different seasons

Ruminant health is influenced by minerals uptake from feed, but minerals in feed depends on availability from soil [14]. The determined Cd values in this study are minimum than the Cd content (3 mg/kg) as noted by McDowell [15]. In contrast to this, recent results were in accordance with Cd present in soil concentration as specified before [16]. Thus, the recent results were lower than the concentrations of Cd in soil as described before [17-19]. The soil fertility has to be maintained by

adding organic matter into soil. Nutrients are made available to the plants when certain factors are fulfilled [20]. The presence of major minerals and trace minerals in the fodders mainly depends on their level in the soil on which the fodder is grown [21].

3.3.2. Forage

The average Cd contents in forage during various sampling seasons are summarized in Table 5 and Table 6. According to the analysis of variance, the mean Cd concentrations in forage samples affected non-significantly (p>0.05) by sampling seasons. The mean Cd contents in different sampling seasons were ranged from 0.671 to 0.697 mg/kg. Higher mean Cd contents were found in forage samples collected during summer sampling season and lower Cd concentrations were observed during winter sampling season (Figure 2). The mean values of Cd contents in the different sampling seasons were 0.697 mg/kg (summer), 0.682 mg/kg (autumn), 0.671 mg/kg (winter) and 0.694 mg/kg (spring). The order of the obtained Cd mean values in all forage samples were determined as summer>spring> autumn>winter. The mean Cd contents in forage samples exhibited inconsistent pattern of variation along sampling seasons.

Cadmium concentration found in the present investigation was higher than values (0.03 mg/kg) established by Kloke [22]. The maximum Cd content in plants was recommended about 3.0 mg/kg by Cicek et al. [23]. In this study Cd contents were minimum than the recommended toxic level by Aksoy [24] and has no potential threat for livestock. The Cd level investigated at present was also lower than the earlier as accounted by Gowda et al. [25]. Various factors determine the bioavailability of minerals in plant systems [26].

3.3.3. Blood plasma

The average Cd contents in blood plasma samples of buffaloes during various sampling seasons are summarized in Table 5 and Table 6. While the Cd concentration in blood plasma significantly (p<0.001) affected by sampling seasons in lactating buffaloes, it was non-significantly (p>0.05) affected by the sampling seasons in dry and young buffaloes. The mean Cd values in blood plasma of lactating buffaloes were 0.151 mg/L (summer), 0.174 mg/L (autumn), 0.224 mg/L (winter), and 0.181 mg/L (spring). The mean Cd concentration in blood plasma of dry buffaloes were 0.175 mg/L (summer), 0.187 mg/L (autumn), 0.182 mg/L (winter) and 0.195 mg/L (spring). The average Cd contents in blood plasma of young buffaloes were 0.236 mg/L (summer), 0.217 mg/L (autumn), 0.217 mg/L (winter), and 0.218 mg/L (spring). Higher mean Cd concentrations were observed in the blood plasma of young buffaloes during summer season and lower mean Cd contents were noticed in the blood plasma of lactating buffaloes in summer sampling season (Figure 2). The detected orders of the Cd contents were in lactating buffaloes as winter>spring>autumn>summer, in dry buffaloes as spring>autumn>winter>summer and in young buffaloes as summer>spring>autumn> winter.

Table 5. Analysis of variance for cadmium concentrations in soil, forage and blood plasma influenced by different seasons

Source of variation	Degrees of freedom	Mean Squares				
		Soil	Forage	Blood plasma		
				Lactating buffaloes	Dry buffaloes	Young buffaloes
Sampling Season	3	45.391 ns	0.004ns	0.029***	0.002ns	0.002 ns
Error	36	0.483	0.008	0.001	0.001	0.001

*** = Significant at 0.001 level, ns = non-significant

Table 6. Descriptive analysis for cadmium concentrations in soil, forage and blood plasma influenced by different seasons

Parameter	Soil	Forage	Blood plasma		
			Lactating buffaloes	Dry buffaloes	Young buffaloes
Mean	5.242	0.687	0.184	0.185	0.223
Std. Error±	0.132	0.015	0.005	0.004	0.004
Median	4.823	0.670	0.179	0.178	0.221
Mode	3.975[a]	0.765	0.107[a]	0.137	0.241
Std. Deviation	1.440	0.161	0.051	0.047	0.0412
Minimum	2.983	0.356	0.103	0.101	0.101
Maximum	8.455	0.994	0.326	0.326	0.320

a. Multiple modes exist. The smallest value is shown

Ruminant health directly relates with feed quality [27,28,29] The values found in the present investigation are higher than the Cd contents (0.1 mg/L) reported by WHO [30]. One of the environmental sources of trace minerals is weathering of soil which becomes the serious pollution agents [31,32].

In animal diets, the maximum tolerated concentration of Cd is 0.5 mg/kg [33,34]. If diet contain 5 to 30 mg Cd dose it is thought to be toxic for buffaloes. Many observations illustrated that Cd poisoning mechanism is based on ingested dose, mineral flow in organism, during of exposure to mineral element metal chemical form, age and species of animal [35,36]. Cd damages adhesion between DNA, energy metabolism and cells [37-40]. It also rigorously interferes on cell metabolism as a result death of mice cell occurs [41,42]. Based on type of cell this mineral element induces two types of cell death: necrosis and apoptosis [43].

3.4. Chromium contents of samples

3.4.1. Soil

The mean Cr contents in soil samples during various sampling seasons are showed in Table 7 and Table 8. According to ANOVA, the mean Cr contents in soil samples affected significantly (p<0.01) by sampling seasons. The mean Cr concentrations in different sampling seasons varied from 0.060 to 0.72 mg/kg. Higher mean Cr values were observed in soil samples collected during summer sampling season and lower Cr concentrations were found during winter sampling season (Figure 3). The mean values of Cr contents in various sampling seasons were 0.060 mg/kg (summer), 0.062 mg/kg (autumn), 0.072 mg/kg (winter) and 0.061 mg/kg (spring). The order of the observed mean Cr contents in all soil samples were determined as summer>spring>autumn>winter. The mean Cr contents in soil samples showed inconsistent pattern of variation during various sampling seasons.

Table 7. Analysis of variance for chromium concentrations in soil, forage and blood plasma influenced by different seasons

Source of variation	Degrees of freedom	Mean squares				
		Soil	Forage	Blood plasma		
				Lactating buffaloes	Dry buffaloes	Young buffaloes
Sampling Season	3	0.001**	2.923 ns	0.297***	0.078 ns	0.193 ns
Error	36	0.000	1.013	0.014	0.012	0.025

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

Table 8. Descriptive analysis for chromium concentrations in soil, forage and blood plasma influenced by different seasons

Parameter	Soil	Forage	Blood plasma		
			Lactating buffaloes	Dry buffaloes	Young buffaloes
Mean	0.064	1.74734	0.597	0.624	0.567
Std. Error±	0.0012	0.154208	0.024	0.017	0.018
Median	0.063	1.58150	0.628	0.634	0.539
Mode	0.057	1.435[a]	0.423[a]	0.682	0.544[a]
Std. Deviation	0.0132	1.689261	0.222	0.176	0.192
Minimum	0.042	1.095	0.109	0.245	0.208
Maximum	0.097	19.880	0.998	0.998	0.976

a. Multiple modes exist. The smallest value is shown

Chromium values in soil were more than Cr content (0.02 mg/kg) determined by Anderson et al. [44]. Variuos factors determine the fate of minerals in soil [45]. In the present study, the Cr content in soil is higher than the values reported by Baron et al. [46] and lesser than the values recognized by [26]. who measured that in soils critical value of Cr is 75 mg/kg. Industrial effluents and discharge of electric utilities influence Cr contents in soils [47]. The potential sources of Cr exposure are slag and solid waste produced during processes of chromate processing [48,49]. Moreover, Cr is found abundantly in soil rather in crops [50]. The mean Cr contebt was lower in recent study when compared to Cr content (44.72mg/kg) as explained by Wu et al. [51] and reported by Ghanem et al. [52], but higher as reported by Rui et al. [53]. These results appeared to be in line with the findings from Kelly [54].

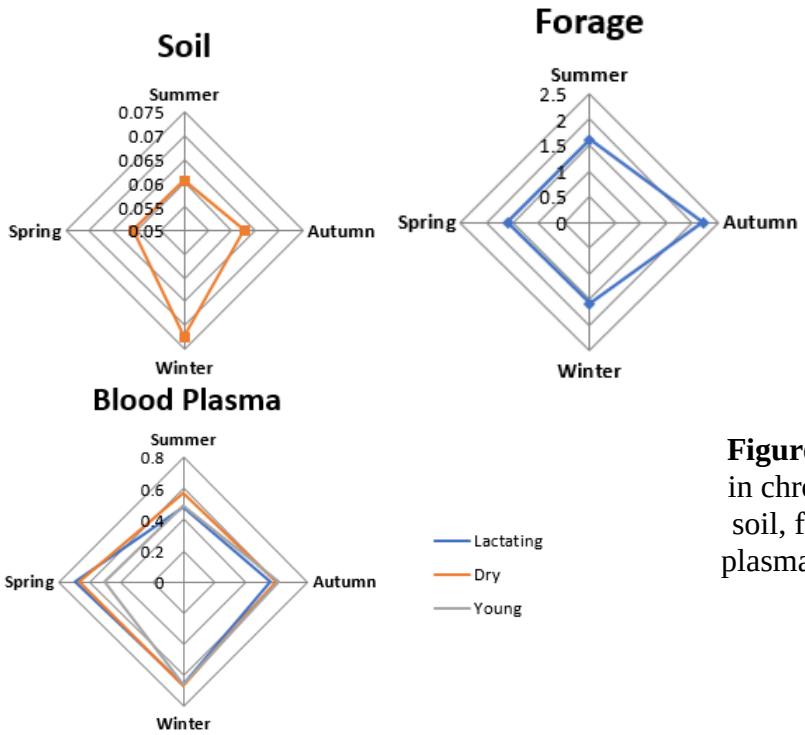

Figure 3. Fluctuations in chromium values in soil, forage and blood plasma during different seasons

3.4.2. Forage

The mean Cr contents in forage during various sampling seasons are presented in Table 7 and Table 8. According to the analysis of variance, the Cr mean contents in forage samples affected non-significantly (p>0.05) by sampling seasons. The mean Cr concentrations in different sampling seasons varied from 1.57 to 2.22 mg/kg. Higher mean Cr values were observed in forage samples collected

during summer sampling season and lower Cr concentrations were found during winter sampling season (Figure 3). The mean values of Cr contents in the various sampling seasons were 1.60 mg/kg (summer), 2.22 mg/kg (autumn), 1.57 mg/kg (winter) and 1.59 mg/kg (spring). The order of the observed mean Cr contents in all forage samples were determined as autumn>summer>winter>spring. The mean Cr contents in forage samples showed inconsistent pattern of variation during various sampling seasons.

In the recent study all mean values of Cr observed were minimum than the values (3 mg/kg) observed by McDowell [55]. All mean values of Cr contents in forage were lesser than values reported by Ahmad et al. [56]. If higher levels of Cr are present in diet than tolerated level, Cr was accounted to be very toxic for livestock [57].

The forage plants may absorb metal deposits on the plant parts surfaces exposed to a polluted environment as well as toxic metals from soil under specific conditions. Moreover, for forage plants heavy metals present in fertilizers is the other source of metal pollution. Despite of all this, metals like Cu, Co, Zn, Mn and Cr are essential for plant and animal growth in trace amounts [58]. In the metabolism of living organisms, Cr also plays crucial role like other metals [58,59].

3.4.3. Blood plasma

The mean Cr contents in blood plasma during various sampling seasons are listed in Table 7 and Table 8. According to the analysis of variance, blood plasma Cr content non-significantly affected by sampling seasons in young and dry buffaloes but it was significantly (p<0.01) affected by the sampling seasons in lactating buffaloes. The mean values of Cr in blood plasma of lactating buffaloes were 0.475 mg/L (summer), 0.557 mg/L (autumn), 0.658 mg/L (winter) and 0.694 mg/L (spring). Cr content in blood plasma of dry buffaloes were 0.568 mg/L (summer), 0.594 mg/L (autumn), 0.668 mg/L (winter) and 0.666 mg/L (spring). The average contents of Cr in blood plasma of young buffaloes were 0.492 mg/L (summer), 0.612 mg/L (autumn), 0.656 mg/L (winter) and 0.506 mg/L (spring). Higher Cr contents were observed in dry buffaloes in the spring Cr and lower mean Cr contents were noticed in lactating buffaloes in summer sampling season (Figure 3). The noticed order of Cr contents was summer<autumn<winter<spring in lactating buffaloes, spring<summer<autumn<winter in dry buffaloes and summer<spring<autumn<winter in young buffaloes [59]. In blood Cr concentration was higher than the average value (0.34 mg/L) studied by Christensen et al. [60], while its value was lower in calves than the critical value as mentioned earlier. Cr absorption in fasting animals is low with approximate range of 0.522 to 3% because of Cr supplementation [61]. In recent study lower values found are than the Cr contents as observed by Burr [62]. In these specific area, levels of Cr in blood samples indicated Cr deficiency. Cr is very important to improve human and animal health [2]. Mineral supplementation is necessary like supplementation of Cr to the ruminants at this investigated area.

3.5. Copper contents of samples

3.5.1. Soil

The mean Cu concentrations in soil during various sampling seasons are summarized in Table 9 and Table 10. No significant seasonal or sampling intervals effect was observed on the mean Cu contents in soil samples. The mean Cu values in different sampling seasons were ranged from 3.54 to 4.08 mg/kg. Higher mean Cu contents were found in soil samples collected during winter sampling season and lower Cu concentrations were observed during spring sampling season (Figure 4). The mean values of Cu contents at various sampling seasons were 3.65mg/kg (summer), 3.84mg/kg (autumn), 4.08 mg/kg (winter) and 3.54 mg/kg (spring). The order of the observed mean Cu values in all the soil samples were determined as winter>autumn>summer>spring. The mean Cu contents in soil samples exhibited inconsistent pattern of variation at different sampling seasons.

Table 9. Analysis of variance for copper concentrations in soil, forage and blood plasma influenced by different seasons

Source of variation	Degrees of freedom	Mean squares				
		Soil	Forage	Blood plasma		
				Lactating buffaloes	Dry buffaloes	Young buffaloes
Sampling season	3	1.675 ns	0.713 ns	0.011 ns	0.002 ns	0.002 ns
Error	36	0.370	1.233	0.001	0.001	0.001

Ns = non-significant

Table 10. Descriptive analysis for copper concentrations in soil, forage and blood plasma influenced by different seasons

Parameter	Soil	Forage	Blood plasma		
			Lactating buffaloes	Dry buffaloes	Young buffaloes
Mean	3.783	6.916	0.218	0.206	0.235
Std. Error±	0.093	0.131	0.006	0.006	0.005
Median	3.663	6.732	0.222	0.194	0.236
Mode	3.292[a]	8.725	0.287	0.287	0.172[a]
Std. Deviation	1.017	1.435	0.068	0.060	0.054
Minimum	1.427	4.265	0.107	0.107	0.101
Maximum	6.387	9.825	0.387	0.346	0.379

a. Multiple modes exist. The smallest value is shown

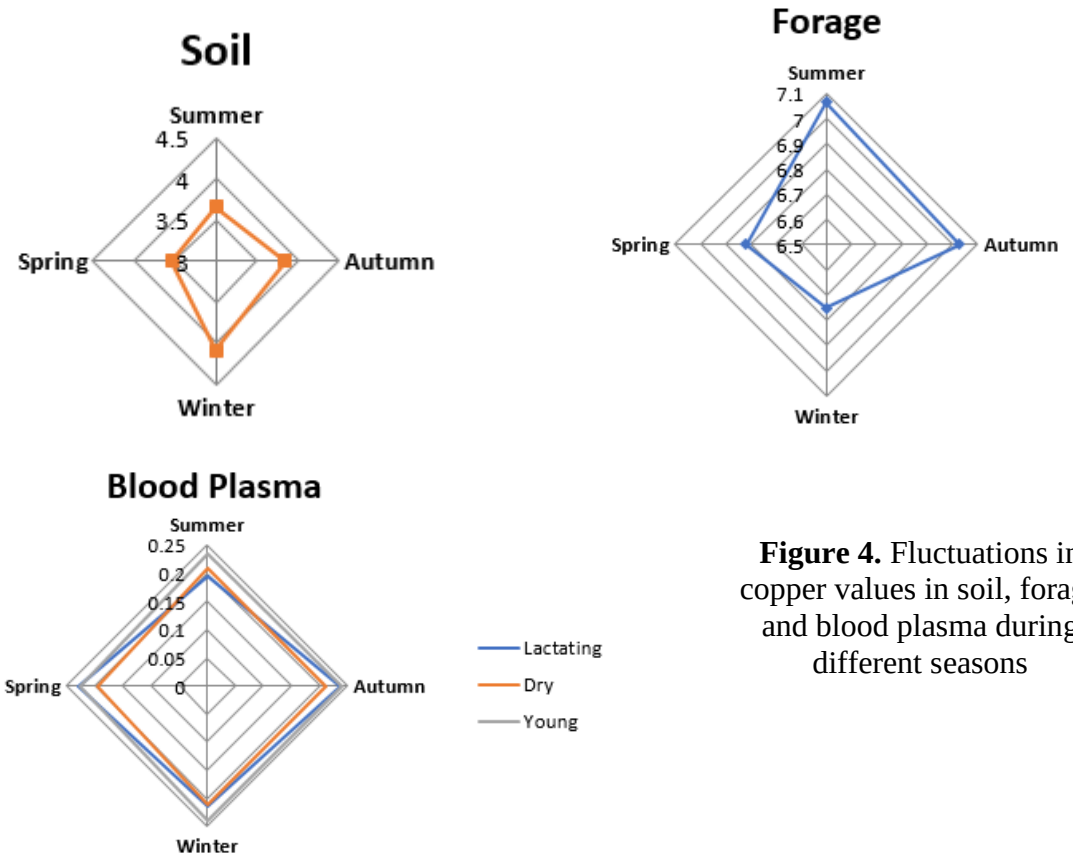

Figure 4. Fluctuations in copper values in soil, forage and blood plasma during different seasons

All Cu concentrations in the present study were more than the findings (1 mg/kg) reported by McDowell et al. [63]. The present results were lower than observed by various researchers [64-68] and higher than reported by Khan et al. [69] and Yadav and Khirwar [70] reported comparable results.

3.5.2. Forage

The average values of Cu in forage during various sampling seasons are showed in Table 9 and Table 10. No significant seasonal or sampling intervals effect was noticed on the mean Cu contents in forage samples. The mean Cu values in different sampling seasons were ranged from 6.75 to 7.06 mg/kg. Higher mean Cu contents were found in forage samples collected during summer sampling season and lower Cu concentrations were observed during winter sampling season (Figure 4). The mean values of Cu contents at various sampling seasons were 7.06 mg/kg (summer), 7.02 mg/kg (autumn), 6.75 mg/kg (winter) and 6.81 mg/kg (spring). The order of the observed Cu mean values in all forage samples were determined as summer>autumn>spring>winter. The mean Cu contents in forage samples exhibited variable pattern of findings at different sampling seasons.

In this research all mean values of Cu investigated were lesser (10 mg/kg) as described by McDowell et al. [63]. These Cr values were different from the values calculated in Indonesia by Prabowo et al. [70] and the minimum than the values determined by [71,72] in Guatemala. The values determined by [27, 28, 73] in Pakistan were higher than the Cu values investigated in recent observation. In forages the concentration of Mo and S has opposing effect on Cu concentration [74]. In forage the Cu^{2+}low concentration is due to its interaction with other metals. Cu contents were reduced as the Fe level increases which also so the antagonistic affect upon each other [75,76].

3.5.3. Blood plasma

The average Cu values in blood plasma during various sampling seasons are showed in Table 9 and Table 10. The mean Cu contents in blood plasma non-significantly (p>0.05) affected by sampling seasons in all the three categories of buffaloes (lactating, dry and young). The mean values of Cu in blood plasma of lactating buffaloes were 0.193 mg/L (summer), 0.236 mg/L (autumn), 0.213 mg/L (winter) and 0.230 mg/L (spring). The Cu contents in blood plasma of dry buffaloes were 0.210 mg/L (summer), 0.209 mg/L (autumn), 0.211 mg/L (winter) and 0.193 mg/L (spring). The average contents of Cu in blood plasma of young buffaloes were 0.233 mg/L (summer), 0.240 mg/L (autumn), 0.239 mg/L (winter) and 0.224mg/L (spring). Higher mean Cu contents were observed in young buffaloes during autumn season and lower mean Cu contents were noticed in lactating buffaloes in summer sampling season (Figure 4). The detected order of Cu mean contents was autumn>spring> winter>summer in lactating buffaloes, winter>summer>autumn>spring in dry buffaloes and autumn> winter>summer>spring> in young buffaloes.

Copper contents in the present study were less (0.65 mg/L) as reported by McDowell [77]. Cu content found in the present investigation was lower as observed by Bhardwaj et al. [78] and in comparison with values noticed by Khan et al. [17]. In general, the deficiency of Cu affects the reproduction and physiological functions. In haemoglobin formation and Fe transportation, Cu plays crucial role. The activity of estrus in ruminants is disturbed by the deficiency of Cu [79]. Therefore, Cu supplementation of the animals is necessary.

Copper content in growing tissues in which liver is included as part of the carcass, is about 1.15 mg/kg based primarily on studies of buffaloes and sheep. Excess of absorbed Cu is stored in liver, where concentrations of Cu can be much more depending upon diet. The dietary Cu required supplying the Cu needed for lactation growth and maintenance will change with animal age and various factors. Reproductive inefficiency osteoporosis, cardiac failure, poor growth, fragile bones and anemia (hypochromic macrocytic) considered by depressed estrus also noticed in Cu deficiency [80].

3.6. Bioconcentration factor

Bioconcentration factor was evaluated as said by Sajjad and Khan [81] who stated it as metal relative tendency to be accumulated by a plant species. In general, BCF indicates bioavailability of metal at a position on a plant species. Plants uptake metals and it is affected by different factors such as metal in soils, *p*H of soil, organic matter content, cation exchange capacity, types, varieties and age of plants. Generally, it is accepted that the metal in soil is dominant factor. It is due to the reason that different BCF have been reported for the same species of vegetables, their different parts such as roots and leafy parts by different authors. In this study BCF calculated depends upon plant total metal contents irrespective of its different parts [82].

BCF of Cd from soil-forage-blood plasma is summarized in Table 11. The determined BCF for Cd content from soil to forage during different sampling seasons were 0.721 (summer), 0.451 (autumn), 0.654 (winter) and 0.616 (spring). The observed transfer factor for Cd content from forage to blood plasma in lactating buffaloes were 1.272 (summer), 2.602 (autumn), 2.357 (winter) and 1.944 (spring); in dry buffaloes were 1.474 (summer), 2.796 (autumn), 1.914 (winter) and 2.090 (spring); in young buffaloes were 1.991 (summer), 3.248 (autumn), 2.278 (winter) and 2.337 (spring). The highest transfer factor for Cd content was found in forage-young buffalo at autumn sampling season and the lowest was in soil-forage during autumn sampling season. Cadmium uptake by vegetables is a function of forms of Cd in soils. The presence of soluble compounds or ions temperature, soil organic matter, total Cd content, redox potential and pH, are the properties of soil influencing Cd uptake in vegetables [83]. Cd is the most dangerous metals because of its high mobility and its small concentration at where it effect on vegetables begin to appear [84]. The determined BCF for Cr from soil to forage during different sampling season was 53.971 (summer), 57.804 (autumn), 33.134 (winter) and 51.674 (spring). The noticed BCF for Cr content from forage to blood plasma in lactating buffaloes were 0.011 (summer), 0.007 (autumn), 0.019 (winter) and 0.017 (spring); in dry buffaloes were 0.013 (summer), 0.008 (autumn), 0.020 (winter) and 0.016 (spring);in young buffaloes were 0.012 (summer), 0.008 (autumn), 0.019 (winter) and 0.012 (spring). The minimum transfer factor for Cr content was found in forage-lactating buffalo at autumn sampling season and the maximum was in soil-forage during autumn sampling season. The Cr had the highest BCF value and this could be attributed to the low retention rate of the metal in soil and therefore it is more mobile in the soil.

Table 11. Bioconcentration factor of cadmium, chromium and copper from soil- forage-blood plasma

Metal	Samplings season	Bioconcentration factor			
		Soil to forage	Forage to blood plasma		
			Lactating buffaloes	Dry buffaloes	Young buffaloes
Cd	Summer	0.721	1.272	1.474	1.991
	Autumn	0.451	2.602	2.796	3.248
	Winter	0.654	2.357	1.914	2.278
	Spring	0.616	1.944	2.090	2.337
Cr	Summer	53.971	0.011	0.013	0.012
	Autumn	57.804	0.007	0.008	0.008
	Winter	33.134	0.019	0.020	0.019
	Spring	51.674	0.017	0.016	0.012
Cu	Summer	8.256	0.014	0.015	0.017
	Autumn	7.615	0.018	0.016	0.019
	Winter	6.887	0.019	0.019	0.022
	Spring	8.552	0.018	0.015	0.017

The detected BCF for Cu concentrations from soil to forage during different sampling seasons were 8.256 (summer), 7.615 (autumn), 6.887 (winter) and 8.552 (spring). The determined transfer factor for Cu concentration from forage to blood plasma in lactating buffaloes were 0.014 (summer), 0.018 (autumn), 0.019 (winter) and 0.018 (spring); in dry buffaloes were 0.015 (summer), 0.016 (autumn),

0.019 (winter) and 0.015 (spring); were 0.017 (summer), 0.019 (autumn), 0.022 (winter) and 0.017 (spring). The lowest transfer factor for Cu concentration was found in forage-lactating buffalo at summer sampling season and the highest was in soil-forage during spring sampling season. Hofmann et al. [85] pointed out that the vegetables grown around industrial spots cause health hazard to poor communities, especially to the children and also observed higher Cu uptake in vegetables. The concentrations of Cu in vegetables usually do not rise to the levels where toxicity occurs. In the roots excess of Cu accumulates even under conditions of Cu toxicity and very minute quantity is carried to the aerial parts of vegetables [21].

3.7. Correlation

By using Pearson correlation coefficient method relationship between metal concentrations was established and presented in Table12. The investigated Cd correlation of soil with forage was negative and no significant (r=-0.262) while it was positive and no significant between forage-blood plasma (r=0.006) and blood plasma-soil (r=0.994).The observed Cr correlation of soil with forage was negative and no significant (r=-0.211) while it was positive and no significant between forage-blood plasma (r=0.405) and blood plasma-soil (r=0.595).The noticed Cu correlation of soil with forage was negative and no significant (r=-0.349) and forage-blood plasma (r=-0.013), while it was positive and no significant between blood plasma-soil (r=0.987).

Table 12. Correlation of various minerals between Soil-Forage, Forage-Blood plasma and Blood Plasma-Soil

Metal	Soil-Forage	Forage-Blood plasma	Blood plasma-Soil
Cd	-0.262	0.006	0.994
Cr	-0.211	0.405	0.595
Cu	-0.349	-0.013	0.987

These findings corroborate with some earlier studies which also exhibited weak or no correlations among different parameters [27, 71].

4. Conclusions

It was concluded that the values of physico-chemical properties of soil and water samples were found within the safe limit recommended by world health organization. The values of heavy metals such as Cd values in the soil samples, and Cr, values in blood plasma samples were higher than their permissible limits. The values of correlation between soil-forage for Cd, Cu and Cr were negative and non-significant and from from forage to bloodplasm were positive and significant. According to the results of the present research, it can be said that there is a need for a continuous monitoring of contamination level of heavy metals in the study area and other areas in Pakistan since these metals can increase to toxic levels.

Ethics

All the study protocols were approved by Institutional Animal Ethics Committee, University of Sargodha (ApprovalNo.25-A18 IEC UOS). All the experiments performed complied with the rules of National Research Council (1996) and all methods were performed in accordance with relevant guidelines and regulations.

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References

1.AKHTAR, M.S., ASIMFAROOQ, A., AKBARLODHI, L., MUHAMMAD, S., MAZHARAYAZ, M., LASHARI, M.H., HUSSAIN, M., Studies on serum macro and micro minerals status in repeat breeder and normal cyclic Nili-Ravi buffaloes and their treatment strategies, *African. J. Biotechn.,* **13**, 2014.
2.SALEH, A.A., EBEID, T.A., EID, Y.Z., The effect of dietary linseed oil and organic selenium on growth performance and muscle fatty acids in growing rabbits, *Pak. Vet. J.,* **33**, 2013, 450-454
3.ENGLE, T.E., Trace minerals, vitamins and preventative nutrition, *Feed stuffs.,* **73**, 2001, 11-18.
4.MCDOWELL, L.R., VALLE, G., Macro minerals in forages. In: D.I. GIVENS, E. OWEN, R.F.E. OXFORD AND H.M. OMED (Eds.) Forage Evaluation in Ruminant Nutrition. CAB International, Wallingford, UK, 2000, p. 373.
5.ARTHINGTON, J.D. Essential trace minerals for grazing cattle in Florida: University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS, 2000.
6.PASTRANA, R., MCDOWELL, L.R., CONRAD, J.H., WILKINSON, N.S. Macromineral status of sheep in the Paramo region of Colombia. *Small. Rumin. Res.,* **5**, 1991, 9-21
7.VUKADINOVIC, V., BERTIC, B., *Agrochemistry and Plant Nutrition*. University J.J.Strossmayer in Osijek, Faculty of Agriculture (in Croatian) Osijek, Croatia, 1988.
8.DE-VOS, B., LETTENS, S., MUYS, B., DECKERS, J.A., Walkley–Black analysis of forest soil organic carbon: recovery, limitations and uncertainty. *Soil Use Manag.,* **23**, 2007, 221-229
9.STEEL, P., KÖNIG, C.J., Integrating theories of motivation. *Acad. Manag. Rev.,* **31**, 2006, 889-913.
10.KUMAR, N., SINHA, D.K., Drinking water quality management through correlation studies among various physicochemical parameters: A case study. *Int. J. Envıron. Sci.,* **1**, 2010, 0976 – 4402
11.SMICIKLAS-WRIGHT, H., MITCHELL, D., MICKLE, S., COOK, A., GOLDMAN, J., Foods commonly eaten in the United States: quantities consumed per eating occasion and in a day. US Department of Agriculture NFS report 2002, p. 252
12.PATTANAYAK, S., NAYAK, S.S., PANDA, D.P., DINDA, S.C., SLENDE, U., JADAV, A., Hepatoprotective activity of crude flavonoids extract of Cajanus scarabaeoides (L.) in paracetamol intoxicated albino rats. *Asian J. Pharm. Boi. Res*., **1**, 2011, 22-27.
13.YADAV, S., KHIRWAR, S.S., Soil plant animal relationship of zinc in milch buffaloe of Zind district in Haryana, *Indian J. Anim. Sci.*, **70,** 2000, 965-967
14.ASHRAF, M.Y., KHAN, A., ASHRAF, M., Sodium transfer from soil, forage, feed and water to lactating and non-lactating buffalos under arid environment, *Buffalo Bull.*, **26**, 2007, 29-35
15.MCDOWELL, L.R., Nutrition of Grazing Ruminants in Warm Climates. Academic Press New York, 1985, p. 443.
16.LOGAN, T.J., MILLER, R.H., Background Levels of Heavy Metals in Ohio Farm Soils. Research Circular 275. The Ohio State University Agricultural Research and Development, Wooster, Ohio, 1983, p. 1.
17.KHAN, Z.I., AHMAD, K., AKRAM, N.A., MUSTAFA, I., IBRAHIM, M., FARDOUS, A., GONDAL, S., HUSSAIN, A., ET AL., Heavy Metals Concentration in Soil-Plant-Animal Continuum under Semi-Arid Conditions of Punjab, Pakistan. *Pak. J. Zool.,* **47**, 2015, 377-382.
18.PIERCE, M.L., MOORE, C.B., Absorption of arsenite and arsenate on amorphous iron hydroxide, Water Res., **16,** 1982, 1247.
19.KHAN, Z.I., ASHRAF, M., AHMAD, K., VALEEM, E.E., Periodic evaluation of potassium transfer from soil and forage to small ruminants on an experimental station in southern Punjab, Pakistan, *Pak. J. Bot.,* **42**, 2010, 1353-1360
20.RASHID, A., MEMON, A.R., BASHIR, E., MIAN, H.R. Soil and Plant Analysis Laboratory Analysis. ICARDA, Allepo, Syria and NARC, Islamabad, Pakistan, 2001.
21.SINGH, A.Y., Equilibria availably and pollution hazard of sludge born nickel and cobalt in soil and plant. Ph.D. thesis. Rajendera Prasad Agricultural University PUSGI, Bihar, India, 2005.

22.KLOKE, A., Richtwerete ’80 orientierungsdaten fur tolerierbare Gesamtgehalte einiger Elemente in Kulturboden. Mih Vdlufa **1**, 1980, 9-11
23.CICEK, N., ZHANG, Q., ZUBRISKI, S., EVANS, C., Fate of selected heavy metals during gasification of municipal biosolids. *J. Resid. Sci. Technol*., **1**, 2004, 223-230
25.AKSOY, A., HALE WH, DIXON JM (1999) Capsella bursa-pastoris (L.) Medic. as a biomonitor of heavy metals. Sci Total environ 226:177-186
25.GOWDA, N.K.S., MALATHI, V.S., JASH, S., ROY, K.S., Status of pollutants and trace elements in water, soil, vegetation and dairy animals in industrial area of Bangalore. *Indian J. Dairy Sci*., **56**, 2003, 86-90
26.KABATA-PENDIAS, A., PENDIAS, H., Trace elements in Soil and Plants. CRC Press Inc., Boca Raton, 1992.
27.MTIMUNI, J.P., Identification of mineral deficiencies in soil, plant and animal tissues as constraints to cattle production in Malawi. Ph.D. Dissertation. Univ. Florida, Gainesville, 1982.
28.KHAN, Z.I., HUSSAIN, A., ASHRAF, M., MCDOWEL, L.R., Mineral Status of Soils and forages in southwestern Punjab-Pakistan: Micro-minerals. *Asian-Aust. J. Anim. Sci.*, **19**, 2006,1139-1147
29.KHAN, Z.I., ASHRAF, M., AHMAD, K., MUSTAFA, I., DANISH, M., Evaluation of micro mineral composition of different grasses in relation to live stock requirements. *Pak. J. Bot*., **39**, 2007, 719-728
30.*** WHO, Study Group. Recommended health-based limits in occupational exposure to heavy metals.Friberg L Vahter M. Assessment of exposure to lead and cadmium through biological monitoring: results of a UNEP/WHO global study 1983. *Environ. Res.*, **30**, 1983, 95-128
31.MERIAN, E., Metals and their Compounds in the Environment. VCH, Weinheim-New York-Basel-Cambridge, 1991.
32.O’NEILL, P., Heavy Metals in Soils. Blackie Academic and Professional, London, 1995.
33.MCDOWELL, L.R., Minerals in Animal and Human Nutrition. Academic Press, New York, 1992.
34.***NRC, Minerals In: National Research Council, editors. Nutrient requirements of dairy cattle. Washington: National Academy Press, 2001, p. 105-61.
35.KUESTER, R.K., WAALKES, M.P., GOERING, P.L., FISHER, B.L., MCCUSKEY, R.S., SIPES, I.G., Differencial hepatotoxicity induced by cadmium in Fisher 344 and Sprague-Dawley rats. *Toxicol. Sci.*, **65**, 2002, 151-159.
36.STANEVIČIENĖ, I., SADAUSKIENĖ, I., LESAUUSKAITĖ, V., IVANOVIENĖ, L., KAŠAUSKASAND, A., IVANOV, L., Subacute effects of cadmium and zinc ions on protein synthesis and cell death in mouse liver. Medicina (Kaunas) **44**, 2008, 131-136.
37.SHIMODA, R., NAGAMINE, T., TAKAGI, H., MORI, M., WAALKES, M.P., Introduction apoptosis in cells by cadmium: quantitative negative correlation between basal or induced metallothionein concentration and apoptotic rate. *Toxicol. Sci*., **64**, 2001, 208-215.
38.WAISBERG, M., JOSEPH, P., HALE, B., BEYERSMANN, D., Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicol.*, **192**, 2003, 95- 117.
39.SMALINSKIENĖ, A., GAILEVIČIŪTĖ, R., LESAUSKAITE, V., SAUDAUSKIENĖ, I., ABDRAKHMANOV, O., IVANOV, L., Effects of cadmium and zinc íons on mitotic activity and protein synthesis in mouse liver, *Medicina (Kaunas)*, 41, 2005, 506-511.
409.NEWAIRY, A.A.., EL-SHARAKY, A.S, BALDRELDEEN,. M.M. ET AL., The hepatoprotective effects of selenium against cadmium toxicity in rats. *Toxicol.*, **242**, 2007, 23-30.
41.KIM, S.C., CHO, M.K., KIM, S.G., Cadmium induced non-apoptotic cell death mediated by oxidative stress under condition of sulfhydryl deficiency. *Toxicol. Lett.*, **144**, 2003, 325-336.
42.IVANOV, L., LESAUSKAITE, V., IVANOVIENE, L., SADAUSKIENE, I., GAILEVICIŪTE, R., ET AL., Effects of cadmium ions on the initial stage of translocation and the cell death in mouse liver. *Medicina (Kaunas,)* **41,** 2005, 47-53.
43.HABEEBU, S.S., LIU, .J, KLAASSEN, C.D., Cadmium-induced apopitosis in mouse liver. *Toxicol. Appl. Pharmacol.*, **149**, **1998,** 203-209.

44.ANDERSON, A.J., MEYER, D.R., MAYER, F.K., Heavy metal toxicities levels of nickel, cobalt and chromium in the soil and plants associated with visual symptoms and variation in growth of an oat crop. *Austral. J. Agri. Res.*, **24**, 1972, 557-571.

45.MCBRIDE, M.B., Environmental chemistry of soils. Oxford University Press, New York, 1994.
46.BARON, D., STANLEY, J.T., PALMER, C.D., Identification of two Fe-chromate minerals in chromium (IV) - contaminated Soil. *Environ. Sci. Technol.*, **30**, 1996, 964-968
47.NRIAGU, J.O., PACYNA, J.M., Quantitative assessment of worldwide contamination of air, water and soil by trace metals. *Nature*, **333,** 1988, 134-139.
48.BARCELOUX, D.G., Chromium. *J. Toxicol. Clin. Toxicol* .,37, 1999, 173-194/
49.KIMBROUGH, D.E., COHEN, Y., WINER, A.M., CREELAM, L., MABUNI, C.A., critical assessment of chromium in the environment. *Crit. Rev. Environ. Sci. Technol*., **29**, 1999, 1-46.
50.UNDERWOOD, E.J., SUTTLE, N.F., The Mineral Nutrition of Livestock. Midlothian, UK, 1999.
51.WU, Y.G., YOUNING, X.U., JIANGHUA, Z., SIHAI, H.U., Evaluation of ecological risk and primary empirical research on heavy metals in polluted soil over Xiaoqinling gold mining region, Shaanxi, China, Trans. *Nonferrous Me.t Soc. China*, **20,** 2010, 688-694.
52.GHANEM. M., SHALABY, A.H., SHARAWY, S., SALEH, N., Factors Leading to Endometritis in Dairy Cows in Egypt with Special Reference to Reproductive Performance. *J. Reprod. Devel.,* **48**, 2002, 371-375.
53.RUI, L.F., FEN, K.L., QING, G.X, WEI, H., WANG, Y.F., LONG, H.W., Traffic-Related heavy metal accumulation in soils and plants in Norwest China. *Soil Sedim. Contam*., **16,** 2007, 473-484
54.KELLY, J., THORNTON, I., SIMPSON, P.R., Urban geochemistry: a study of influence of anthropogenic activity on heavy metal content of soils in traditionally industrial and non-industrial areas of Britain. *Appl. Geochem.*, **11**, 1996, 363-370.
55.MCDOWELL, L.R., Nutrition of Grazing Ruminants in Warm Climates. Academic Press, New York, 1985.
56.AHMAD, K., KHAN, Z.I., ASHRAF, M., VALEEM, E.E., SHAH, Z.A., MCDOWELL, L.R., Determination of forage concentration of lead, nickel and chromium in relation to the requirement of grazing ruminants in the salt range. *Pak. J. Bot*., **41**, 2009, 61-65.
57.ANONYMOUS, Analytical Methods for Atomic-Absorption Spectrophotometry. Perkin-Elmer, Norwalk, Connecticut, 1980.
58.TOKALIOGLU, S., KARTA, L.S., GUNIS, A.A., Determination of heavy metalin soil extracts and plant tissues at around of zinc smelter. *Int. J. Environ. Analyt. Chem.*, **80**, 2000, 210-217.
59.MCDOWELL, L.R., Minerals in animals and human nutrition. Elsevier Science BV. Amsterdam, The Netherlands, 2003.
60.CHRISTENSEN, J.M., HOLST, E., BONDE, J.P., KNUDSEN, L., Determination of chromium in blood and serum – evaluation of quality control procedures and estimation of reference values in danish subjects. *Sci. Total Environ.*, **132**, 1993, 11–25
61.LI, Y.C., STOECKER, B.J., Chromium and yogurt effects on hepatic lipid and plasma glucose and insulin of obese mice. *Biol. Trace Element Res.,* **9**, 1986, 233-242.
62.BURR, R.G., Plasma-Zinc Levels. Lancet **I**, 1974, 879.
63.MCDOWELL, L.R., CONRAD, J.H., ELLIS, G.L., LOOSLI, L.K., Minerals for grazing ruminants intropical regions. Gainesville, University of Florida, 1983.
64.GUPTA, V.K., GUPTA, S.P., RAMA, K., Micro nutrient status of Haryana soils. *Haryana Farmings*, **25**, 1995, 12-14.
65.KHAN, Z.I., AHMAD, K., DANISH, M., MIRZA, M.A., MIRZAEI, F., Selenium profile in blood plasma of grazing sheep: A case study in specific ranch in Punjab, Pakistan. Agri. Sci., **3**, 2012, 470.
66.KHAN, Z.I., AHMAD, K., MUKHTAR, M.K., MIRZAEI, F., HUSSAIN, G., Assessment of pasture and plasma minerals of cows: A case study in Pakistan. *Agri. Sci.*, **4**, 2013, 57

67.KHAN, Z.I., AHMAD, K., AKRAM, N.A., MUSTAFA, I., IBRAHIM, M., FARDOUS, A., GONDAL, S., ET AL., Heavy Metals Concentration in Soil-Plant-Animal Continuum under Semi-Arid Conditions of Punjab, Pakistan, *Pak. J. Zool.*, **47**, 2015, 377-382
68.KAPADIY, F., SIDDIQUEE, G., Blood plasma trace elements in repeat breeding Mehsana buffaloes, *The Indian J. Anim. Reprod*, 33, 2015.
69.KHAN, Z.I., AHMED, K.., ASHRAF, M., VALEEM, E.E., JAVED, I., A Comparative Study on Mineral Status of Blood Plasma of Small Ruminants and Pastures in Punjab, Pakistan, *Pak. J. Bot*., **41**, 2008, 67-72.
70.PRABOWO, A., MCDOWELL, L.R., WILKINSON, N.S., WILCOX, C.J., CORNAD, J.H., Mineral Status of Grazing cattle in South Sulawesi, Indonesia; 1 Macrominerals. *Am. J. Anim. Sci.,* **4**, 1990, 111-120.
71.TEJADA, R., MCDOWELL, L.R., MARTIN, F.G., CONRAD, J.H., Mineral element analyses of various tropical forages in Guatemala and their relationship to soil concentrations, *Nutr. Rep. Int.,* **32**, 1985, 313-323.
72.TEJADA, R., MCDOWELL, L.R., MARTIN, F.G., CONRAD, J.H., Evaluations of cattle trace mineral status in specific region of Guetemala, *Trop Agric (Trinidad),* **6**, 1987, 55-60
73.MCDOWELL, L.R., KIATOKO, M., BERTRAND, J.E., CHAPMAN, H.L ET AL., Evaluating the nutrition status of beef cattle from four soil order regions of Florida: II. Trace min. J. Anim. Sci., **55**, 82, 38-47.
74.DAVENDRA, C., Trees and shrubs and sustainable sources. Proceedings of the world conference on animal production. *Endmonton,* **10**, 1993, 119-136.
75.PHILLIPO M, HUMPHRIES WR, GARTHWAITE PH., The effect of dietary molubdenum and iron on copper status and growth in cattle, *J. Agri. Sci.,* **109**, 1987, 315.
76.DAVIS, G.K., MERTZ, W., Copper. In: Trace Elements in Human and Animal Nutrition (Mertz, W., ed.), 5th Ã©d., Academic Press, San Diego, CA, 1987, p. 301-364.
77.MCDOWELL, L.R., Minerals for Grazing Ruminants in Tropical Regions. Extension Bulletin, Anim. Sci. Dept. Centre for Trop. Agric., Univ. Florida, p. 81
78.BHARDWAJ, R.L., ROY, K.S., SRIVASTAVA, A.K., Normal levels of some macro-and micro-minerals and hormones in the ovarian tissues of pregnant and cyclic buffaloes (bubalus bubalis), *Indian J. Anim. Sci*., **68**, 1998, 448-449.
79.SUTTLE, N.F., MCLAUCHLIN, M., Predicting the effects of dietary molybdenum and sulphur on the availability of copper to ruminants, *The Proc. Nutr. Soci.,* **35**, 1976, 22A-23A.
80.UNDERWOOD, E.J., The Mineral Nutrition of Livestock. Common wealth Agricultural Bureaux, Slough, England, 1981.
81. SAJJAD, M., KHAN, F.A., Genetic Diversity among Sugarcane Cultivars in Pakistan, *American-Eurasian J. Agri. Environ. Sci.,* **6**, 2009, 730-736.
82.ADRIANO, D.C. Trace Elements in the Terrestrial Environment. Springer, New York, 1986.
83.RUPA, T.R., RAO, C.S., RAO, A.S., SINGH, M., Effects of farmyard manure and phosphorus on zinc transformations and phyto availability in two alfisols of India. *Biores. Technol*., **87**, 2003, 279-288.
84.BENAVIDES, M.P., GALLEGO, S.M., TOMARO, M.L., Cadmium toxicity in plants. *Braz. J. Plant. Physiol.,* **17**, 2005, 21-34.
85.HOFMANN, M., GUSCHEL, M., BERND, A., BEREITER-HAHN, J., KAUFMANN, R., TANDI, C., KIPPENBERGER, S., Lowering of tumor interstitial fluid pressure reduces tumor cell proliferation in a xenograft tumor model, *Neoplasia***, 8**, 2006, 89-9.

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