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 3.54 to 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 proper 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](image)

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$_2$SO$_4$ (4mL) and H$_2$O$_2$ (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.

| Sampling | Season | Common Name | Scientific name |
|----------|--------|-------------|-----------------|
| 1st      | Summer | Maize       | Zea mays         |
| 2nd      | Autumn | Bajra       | Sorghum bicolor |
| 3rd      | Winter | Sarsoom     | Setaria italica  |
| 4th      | Spring | Barseem     | Brassica campestris |

**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°C. 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).

| Elements | Detection limits |
|----------|------------------|
| Cadmium  | 0.8 (Flame AA)   |
| Chromium | 3 (Flame AA)     |
| Copper   | 15 (Flame AA)    |

Table 2. Detection limits of atomic absorption spectrophotometer

2.6. Physico-chemical analysis of water and soil samples

The pH of all soil samples was calculated via usage of 1:2 (w/v) soil and water ratio. The pH of soil was measured via pH meter. Electrical conductivity was determined via adding 5 grams 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:

$$ BCF \text{ soil-forage} = \frac{\text{Metal contents in forage}}{\text{Metal content in soil}} $$

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

$$ BCF \text{ forage-plasma} = \frac{\text{Metal contents in plasma}}{\text{Metal contents in forage}} $$

3. Results and discussions

3.1. Physiochemical characterization of water

The pH 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 (CaCo₃) (mg/L)| 100                | 500                       | 100    | 98     | 95     | 97     |
| Total alkalinity as CaCo₃ (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 pH 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 pH 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   | 0.23   | 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 pH 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 pH of sodic soils might reach 10.00. Therefore, a reduction in soil pH 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 pH 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.

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.

| Source of variation | Degrees of freedom | Soil | Forage | Lactating buffaloes | Dry buffaloes | Young buffaloes |
|---------------------|-------------------|------|--------|---------------------|--------------|-----------------|
| Sampling Season      | 3                 | 45.391 *** | 0.004 ** | 0.029 ***           | 0.002 **     | 0.002 **        |
| Error               | 36                | 0.483 | 0.008  | 0.0               | 0.001        | 0.001           |

*** = Significant at 0.001 level, ** = non-significant
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 6. Descriptive analysis for cadmium concentrations in soil, forage and blood plasma influenced by different seasons

| Parameter       | Soil  | Forage | Blood plasma |
|-----------------|-------|--------|--------------|
|                 | Mean  | Std. Error | Median | Mode | Std. Deviation | Minimum | Maximum |
|                 | 5.242 | 0.132  | 4.823 | 3.975<sup>a</sup> | 1.440  | 2.983 | 3.455 |
|                 | 0.687 | 0.015  | 0.670 | 0.763 | 0.161  | 0.356 | 0.994 |
|                 | 0.184 | 0.005  | 0.179 | 0.107<sup>a</sup> | 0.051  | 0.103 | 0.326 |
|                 | 0.185 | 0.004  | 0.178 | 0.137 | 0.047  | 0.101 | 0.326 |
|                 | 0.223 | 0.004  | 0.221 | 0.241 | 0.0412 | 0.101 | 0.320 |

<sup>a</sup> Multiple modes exist. The smallest value is shown.

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<sup>**</sup> | 2.923<sup>**</sup> | 0.297<sup>***</sup> | 0.073<sup>m</sup> | 0.193<sup>m</sup> |
| Error               | 36                 | 0.600       | 1.013       | 0.014       | 0.012       | 0.025       |

<sup>**</sup>, <sup>***</sup> = Significant at 0.01 and 0.001 levels, <sup>m</sup> = non-significant
Chromium values in soil were more than Cr content (0.02 mg/kg) determined by Anderson et al. [44]. Various 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 content was lower in recent study when compared to Cr content (44.72 mg/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].

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 and autumn.
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 | Soil   | Forage | Mean squares | Blood plasma         |
|---------------------|--------------------|--------|--------|--------------|----------------------|
|                     |                    |        |        |              | Lactating buffaloes | Dry buffaloes | Young buffaloes |
| Sampling season     | 3                  | 1.675<sup>ns</sup> | 0.713<sup>ns</sup> | 0.011<sup>ns</sup> | 0.002<sup>ns</sup> | 0.002<sup>ns</sup> |
| Error               | 36                 | 0.370  | 1.213  | 0.001        | 0.001                | 0.001        |

<sup>ns</sup> = 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<sup>a</sup> | 0.063   | 0.131    | 0.006                | 0.006        | 0.005          |
| Median             | 3.663    | 6.732    | 0.222                | 0.194        | 0.236          |
| Mode               | 3.292<sup>s</sup> | 8.725    | 0.287                | 0.287        | 0.172<sup>s</sup> |
| Std. Deviation     | 1.017    | 1.435    | 0.068                | 0.060        | 0.034          |
| Minimum            | 1.427    | 4.265    | 0.107                | 0.107        | 0.101          |
| Maximum            | 6.387    | 9.825    | 0.387                | 0.346        | 0.379          |

<sup>a</sup> 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 Cu 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 Cu²⁺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, pH 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), 0.019 (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.

| Metal | Samplings season | Soil to forage | Bioconcentration factor to forage | Forage to blood plasma |
|-------|------------------|----------------|----------------------------------|-----------------------|
| 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              |
| 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 Table 12. 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 ($r=-0.987$).

| 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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