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Evaluation of Cobalt Concentration in the Forages and Small Ruminant Blood Collected from Various Agroecological Regions

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***Abstract**: Like animals plants required nutrients for their growth and existence. Cobalt is also important element for plant and animal growth, however higher levels of Co might have toxic potential. The study was aimed to investigate Co level in samples of forages, soil, and blood plasma of sheep and goat in Bhakkar, Mianwali and Sargodha district. The results showed that district Mianwali excelled in level of Co as compared to Bhakkar and Sargodha. The factors such as bio-concentration factor and pollution load index explained the movement of cobalt along the soil-plant-animal continuum.*

***Keywords**: Co, forages, pollution soil, ruminant*

1.Introduction

The pollution in the environment such as industrial, agriculture, mining and natural process add many harmful substances in the surrounding and cause many health problems. These toxic substances make the part of food chain and when we study about the contamination impact of these toxic substances then we should focus on these livestock which have high exposure to these toxic substances. The safe feed product is essential for animal health and welfare as well as human health and healthy feed product reduce the risk of toxicity in animals and human health. Biological functions may effect by heavy metals especially due to bio-accumulation in food chain because these pollutants present in environment [1]. There is more than one of the more ways of contamination food chain to enter the metals into the animal system, hence important and dominant concern of monitoring metals in soil, food stuff and water. Eventually, grasses absorb and accumulate the more elements from soil and ruminant mostly feed on these grasses. Different research found the result that the trace metals are transferred from contamination soil to plants then transfer livestock. Transfer of metals from the environment to agricultural foods is one of the preferential subjects for the researchers who study environmental pollution [2]. Commonly, metals are not transfer from soil to plant in large quantities, but it is possible that some metals may take up in large quantities and it depend on many factor such as metal bioavailability of metals for uptake in the soil-plant system, plant species, soil properties. The chemical nature of plants affects the mobility and phyto-availabilty of metals.

The metals which are absorbed by plants are sometime retained in the root of plants. Different studies proof that the foliage of plants may take up lead and plants of different species may have the ability to accumulate the high concentration of lead in certain parts.

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The PH of soil is above 6.5 then the availability of lead to plants is extreme low [3]. The problem of metals is strictly connected with disaster such as biological weathering of soil, volcanic activity, fires, erosion, as well as with man-made activities such as (metal processing and agriculture). The anthropogenic and natural activities show a negative effect on the safety and quality of food chain. Contaminations are increase in environment and affect the heath of animals and human. Metals which are considerably put in environmental contamination such as Cu, Hg, Pb, Zn, Cd and three non-essential metals (Cd, Hg, Pb) have been studied more of the time. Continuous intake of metals may lead chronic effect of animal and human healthiness, particularly when their dietary content surpasses the tolerable limit [4]. The excess of heavy metal may cause the problems such as brain disorders, kidney and liver diseases, these complications may rise the death rate over the long period of time [5-6]. Deficiency of minerals in livestock is linked with soil characteristics and it may affect production as well as pasture in the most area of world which includes both the major elements and trace elements. Many problems of health are associated by the toxicity or excessive use of minerals such as Copper, Molybdenum, Iron, Sodium and Potassium. Developing countries rely on livestock as income resource and limited research was done on soil status, availability of forage for grazing animals instead of animal nourishment [7].

Soil is the main source of trace elements in plants and enters in human and animals through food chain. Chemical properties of metals and their uptake by plants are affected due to soil physical condition, drainage and aeration. Use of fertilizer and wastewater application add trace elements in soil. Some of these metals may be toxic while on the other hand some are essential for human, animals and plants [8]. To find out pollution level of food chain used common method as valuation of metals in blood of livestock or evaluation of mineral profile status of blood of livestock. In the blood of livestock, the concentration of heavy metal may increase and have significant effect on the physiological and biochemical variations in the organism, these fluctuations may lead to organ damage such as kidney problems, male and female reproductive organs. Animals which graze on pasture land have a high risk as compare to the other animals because these animals use the forage directly without washing. The degree of accumulation in animal's tissues is associated with metals in soils and forage and positively correlated with the minerals concentration in the soil [9].

Cobalt, as a heavy metal, is harmful in the case of an excessive amount. Cobalt functions as vitamin B12 responsible for metabolism. Vitamin B12 is also associated with functions of iron and copper in hematopoietic and deficiency of Co results in anemia in ruminants. Cobalt deficiency results loss of appetite and harsh emaciation [10]. There is need to explore the forage mineral status so that nutritional requirements of goats and sheep can be fulfilled in Pakistan. This work will provide baseline information to farmers and animal nutritionists on the seasonality of minerals in soil, plant material and plasma of goat and sheep, which is essential for formulating supplements to improve productivity. It will provide a description of mineral levels in goats and sheep for diagnosing deficiencies and toxicities in order to institute corrective or preventive measures. This work was carried out in different districts in Pakistan to evaluate the cobalt profile in soil, forages and ruminants by using specific systematic techniques, and to investigate the mineral deficiencies or excesses which affect the forage yield and animals' health.

2. Materials and methods

2.1 Selection of Site

Sargodha, Mianwali and Bhakkar Districts were selected for present investigation. Sargodha an important district of Punjab situated between two rivers Chenab and Jehlum is 172 km from Lahore. Temperature is extreme in Sargodha District, in winter minimum (0°C) and upto 50°C in the summer. Mianwali is situated near Indus river with average annual high temperature recorded as 47°C and minimum temperature as 19°C. District Bhakkar was established in 1982, located along Indus river with sandy land and extreme temperature conditions in summer and winter.

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2.2 Collection of Samples

Ten Samples were taken from 10 sites randomly in each district. These samples were thoroughly mixed to get composite samples in triplicate. Soil samples were taken with stainless-steel auger, 1.0 to 1.5 ft deep [11] and stored in plastic bag. Forages such as Bajra (Pennisetum glaucum), Barsem (Trifolium Alexanderium) and Oat (Avena sativa) were selected and sun dried. From jugular vein, blood samples of five sheep and goats were taken in the voiles, plasma separated and was frozen at -20^{o}C.

2.3 Sample Preparation and Analysis

Soil and forages samples were sun dried and then in oven dry for three day at 72^{o}C. After drying, these samples (1g each) and blood plasma (1mL each) were digested by following standard wet digestion procedure [12] and then diluted to 50 mL, filtered and stored for further processing. To evaluate cobalt content in samples, standard Co solutions were also prepared and run in Atomic Absorption Spectro-photometer (AA-6300 Shimadzu Made in Japan).

2.4 Statistical Analysis

Data was statistically processed using Minitab 16 software. One-way ANOVA and LSD was used [13].

2.5 Pollution Load Index (PLI)

PLI was measured by the method of Liu et al. [14].

PLI =Metal content in investigated soil ⁄ reference value of metal in soil

2.6 Bio-concentration factor (BCF)

Bio-concentration factor was determined following Cui et al. [15].

BCF = Metals content in forages/metals content in soil

BCF= Metals content in blood plasma/ Metals content in forage

3. Results and discussions

3.1 Soil

Cobalt is a non-essential metal and involve in various metabolic processes of plants. ANOVA revealed non-significant effect ($p<0.05$) Co content on site in relation to soil (Table 1). In district Sargodha, Co content in soil which was used by cultivation was 2.709 to 3.506 mg/kg. In Mianwali, Co content in soil was between 5.592-5.943 mg/kg. In Bhakkar, Co content was between 2.115-4.246 mg/kg (Figure 1). The critical values (5mg/kg) reported by Kabata-Pendias and Pendias [16] are lower than the Co content values obtained. The critical value was lower than Co content in Mianwali district. These values were more closely related to the findings by Khan [17] which examined the Co content in Punjab, Pakistan. These results suggest that there must be add the Co containing fertilizers to enhance the plant growth and development.

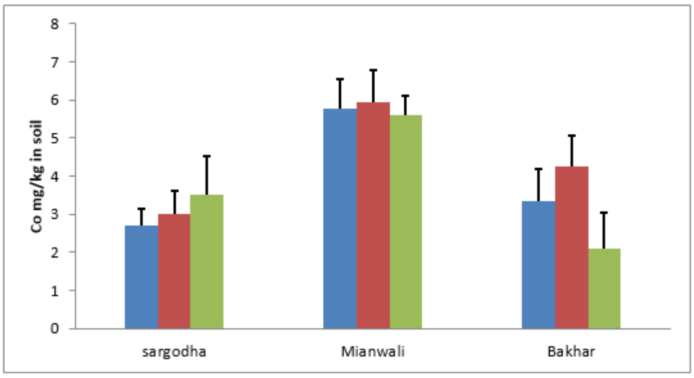

Figure 1. Cobalt content in soil of districts in Punjab

3.2 Forages

Co content varied non-significant ($p<0.05$) in forages collected from various sites (Table 1). In district Sargodha, Co content in forages which used for feeding purpose was ranged from 2.008 to 2.84 mg/kg. In Mianwali, Co content in forages was between 1.34-1.972 mg/kg. In Bhakkar, Co content was between 0.766-1.608 mg/kg (Figure 2). A trace amount of cobalt facilitates proper plant growth [18]. Higher level of cobalt in soil does not mean that it must be available for plants [19]. Cobalt interact with phosphorus and copper is harmful for plants [20]. Higher Mn level enhance the cobalt uptake by plants. Co content was the lowest in Mianwali and higher in Bhakkar. The critical values are higher the normal forage values 0.1 mg/kg reported by McDowell [21]. The same results were built up Pakistan and Florida by Khan [17] and Espinoza et al. [20] respectively. Forage Co^{2+} levels were not much higher that of tolerant level as it does not reached to toxic level. In Nicaragua similar findings were present [22].

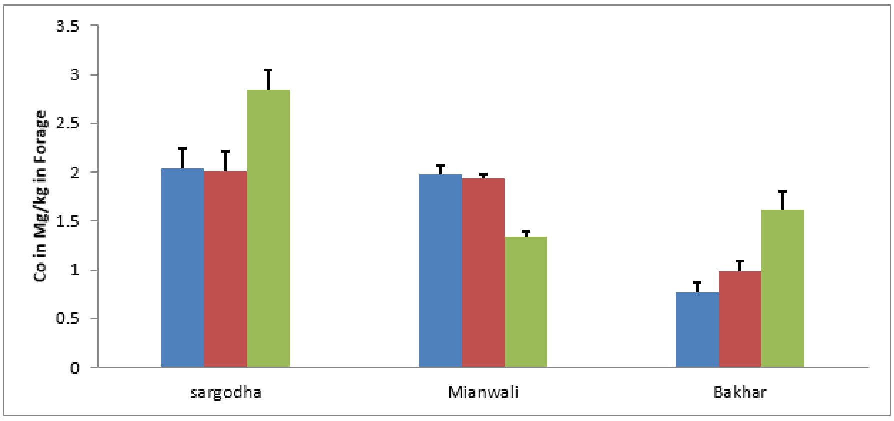

Figure 2. Cobalt content in forage samples

3.3 Blood of goat

Co content depicted non-significant effect ($p<0.05$) of sites on blood of goat (Table 1). In district Sargodha, Co content was between 1.400-1.497mg/L. In Mianwali, Co content in blood of goat was between 0.696-0.785 mg/L. In Bhakkar, Co content was between 2.376-2.465 mg/L (Figure 3). Unlike sheep, goats have been shown to be less sensitive to low levels of feed cobalt concentration [23].

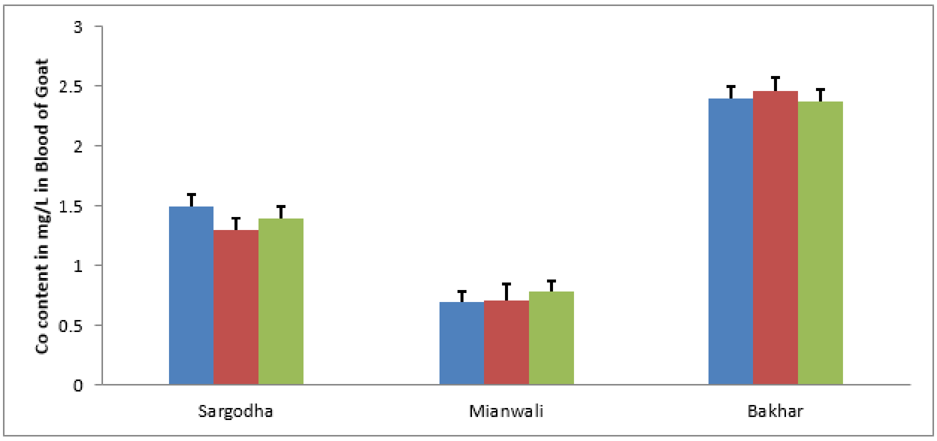

Figure 3. Cobalt content in blood samples of goat

3.4 Blood of sheep

Co content varied non-significantly ($p<0.05$) in blood of sheep (Table 1). In district Sargodha, Co content was between 0.820-1.219 mg/L in blood of sheep. In Mianwali, Co content in blood of sheep

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was between 0.148-0.250 mg/L. In Bhakkar, Co content was between 3.990-5.118 mg/L (Figure 4). Higher Co content was observed in blood of sheep from Bhakkar and the lowest from Mianwali district. The lack of Co leads to disorder called WLD. WLD, characterized by increased accumulation of fat in the liver due to lack of cobalt and vitamin B12, is one of most important diseases of sheep [24-25].

Table 1. ANOVA for cobalt content in various samples

Co	Sargodha	Mianwali	Bhakhar
Soil	.485^{ns}	.088 ^{ns}	2.635 ^{ns}
Forage	.671 ^{ns}	.052 ^{ns}	0.573 ^{ns}
B.G.	.032 ^{ns}	.007 ^{ns}	0.007 ^{ns}
B.S.	.142 ^{ns}	.098 ^{ns}	1.118 ^{ns}
df	2	**Error**	8
ns= non-significant, B.G= Blood of Goats, B.S= Blood of Sheep			

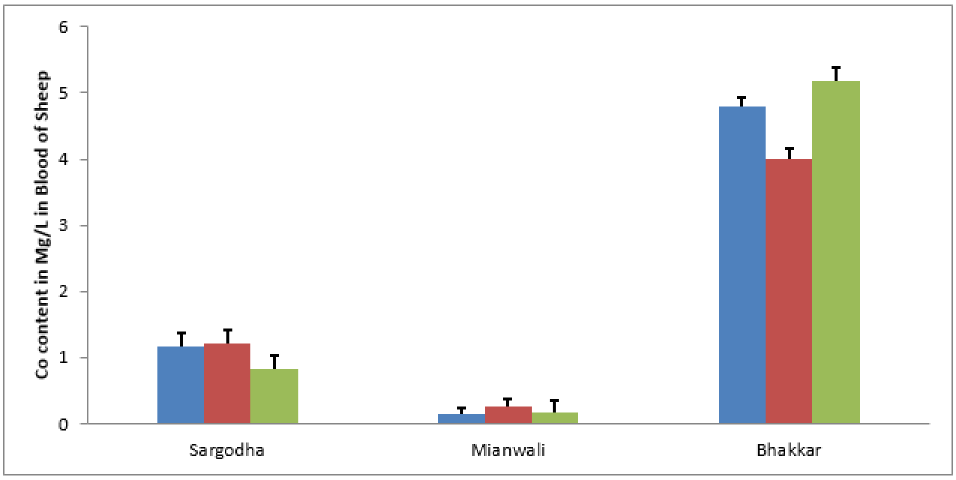

Figure 4. Cobalt content in blood of sheep

3.5 Correlation

Pearson correlation coefficient method was adopted to search relationship between metal content [26]. Positive non-significant correlation exists in Co content and soil-forage and negative non-significant relationship for Co was observed from soil-blood of goat and sheep. Cobalt showed positive non-significant correlation between forage to plasma of goat and negative and significant ($p \leq 0.05$) correlation between forages to plasma of sheep in Sargodha samples. Perfect positive correlation was observed in soil to forage and soil to plasma of sheep and negative was present between soil to plasma of goat in Mianwali samples. Perfect positive correlation was observed in forage to plasma of sheep and goat and negative was present between soil to forages, soil to plasma of goat and sheep in Bhakkar samples (Table 2). The non-significant correlation for Co between soil and forage, forage and blood plasma of sheep and between soil and blood plasma which might be due edaphic factors that might have led to the reduction in uptake of Co. Co also revealed non-significant positive correlation between forage and blood plasma of sheep and goat and non- significant negative correlation between soil and plasma of goat which lead to Co imbalance among soil, plant and animals.

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

Soil	Soil-Forage	Soil-B.G	Soil- B.S	Forage-B.G	Forage-B.S
Sargodha	.908	-.354	-.875	.071	-.997*
Mianwali	.870	-.846	.655	-.999*	.198
Bhakkar	-.860	-.708	-.989	.248	.926
B.G= Blood of Goats, B.S= Blood of Sheep					

3.6 Bio-concentration factor (BCF)

BCF is a key component to determine the level of exposure through food chain. BCF of Co in forages was greater in Sargodha related to Bhakkar and Mianwali. The least BCF of Co was observed in Mianwali while high BCF of Co was seen in Bhakkar. BCF of Co in blood plasma of goat in Bhakkar and Mianwali sampling was higher as compared to Sargodha sampling. Similarly, BCF of Co in blood plasma of sheep of Bhakkar sampling was higher as compared to Sargodha and Mianwali (Table 3). BCF for Co at Bhakkar site was >1 while at Sargodha and Mianwali sites was <1. The values of BCF > 1 indicate that plant can accumulate heavy metals while BCF <1 shows that plant just absorb metal [27]. Low Co in plants of Sargodha may be due to the low Co uptake of forage species on same soil have also affected the metal transmission and in animal little Co level present in Mianwali and Sargodha sampling. The rate of metal uptake by plant could have been affected by nature of soil, plant species, plant age, soil *p*H, and climate. The BCF values in the present study were found lower than suggested by Xue et al. [28].

Table 3. Bioconcentration factor of cobalt

BCF of Co	Sites	Sargodha	Mianwali	Bhakkar
Soil-Forages	1	0.754	0.341	0.228
	2	0.665	0.326	0.232
	3	0.809	0.239	0.760
Forages-Blood of Goat	1	0.733	0.352	3.127
	2	0.643	0.365	2.502
	3	0.492	0.586	1.477
Forages-Blood of Sheep	1	0.573	0.075	2.712
	2	0.607	0.129	4.051
	3	0.289	0.131	3.221

3.7 Pollution load index

Pollution severity in soil can be measured by pollution load index. According to this method soil is considered to be polluted if pollution load index value was > 1, while soil is considered to be clean or less dirty if pollution load index value is <1 [29]. The contamination factor for Co in soil of Mianwali was higher than Sargodha and Bhakkar (Table 4). The value of PLI at all sites was <1 indicated soil is non-polluted.

Table 4. Pollution load index

PLI Co	Sargodha	Mianwali	Bhakkar
1	0.0416	0.089	0.052
2	0.0464	0.091	0.065
3	0.0539	0.086	0.032

4. Conclusions

In present findings, cobalt content in blood of sheep and goat was the highest in Bhakkar district. BCF of Co from soil to forage, forage to blood of sheep and forage to blood of goat at Bhakkar site was greater as compared to other two sites. The value of PLI at all sites was <1 indicated that soil is un-contaminated.

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