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Zinc asseveration in fodder, forages, soil, grazing animals, hairs, blood, urine and feces collected from different districts of Punjab. Pakistan

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Abstract

Livestock plays a significant role to fulfill the nutritional requirements of humans. Research studies indicated the potential of forages and fodder species to withstand the shortage of livestock/animal fodder and forage can at least partly be met by making use of suitable and potential fodder and forage species. Although the wastewater or sewage water provides immediate relief to forage and fodder species but at the same. The article is focused on the reckoning of Zinc in fodder, forages, soil, grazing animals' hairs, blood, urine and feces collected from different districts of Punjab (Sargodha, Jhang, Layyah and Bhakkar, their five sites (one control/groundwater and four wastewater sources), three animals (Goat, Sheep and Cow) and four sources (Blood, Hair, Urine and Feces).The samples were prepared by prescribed sample preparation, standard and wet digestion methods, to determine heavy metals in the forage and soil samples and then passed to Atomic Absorption Spectrophotometer to examine Zinc (Zn).The results revealed that Zn values in the soil, forage and animals' blood, heir, feces, urine samples were in diverse nature. The Zn were found in safe limit as recommended by the world health organization. The current study gives significant information about the mineral relationship between soil, forage and animals.

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Introduction

The man's life is closely associated with agriculture and livestock, particularly in rural areas of the country. It is the biggest industry containing livestock, which bears hot and humid conditions, round the clock and converts average and even poor-quality fodder and forages into valuable milk and meat Noorka and Khaliq, 2007. Under the changing climatic conditions, the survival of livestock is an integral part of our society. It is not only the motivation for rearing livestock. Noorka, 2019. Survival ship is standing on the top priority for the animal's self. Indeed, many environmental factors like climate, geographical conditions, agronomic conditions, feed and food availability and genetic variations play a significant role to enhance the nutritive value of fodder for livestock. Noorka *et al.,* 2017; 2020; Ali *et al.*, 2020.

The quality of fodder for livestock varies from place to place. Feed resources mainly consist of two categories in our country as conventional feed resources and non-conventional feed resources. (Habib and Siddiqui, 1994). Green roughages include green crops, shrubs and forbs which are part of a range of grasses, silages, sugar beet tops, sugarcane tops, and leaves of higher trees.

In the modern world those countries in which grain is used as feed for ruminants, take 75 % nutrition from forages for ruminants. On the other hand, those countries which did not depend upon grains as fodder source would contribute 95 % of their nutrition for ruminants from roughage (Bulla *et al.,* 1977). Thomas and Howarth, 2000. Berseem or Egyptian clover (Trifolium alexandrinum) is the major winter fodder. Oats (Avena sativa) has become a very important crop in the past fifteen years. Persian Clover or Shaftal (Trifolium resupinatum) is a crop of ancient cultivation.

The commonest fodder is "Sarson" *Brassica campestris* var. *sarson* but "toria" *Brassica campestris* subsp. *oleifera* var. *toria* and mustard or "rai", *Brassica juncea* are also used as fodder. Barley (*Hordeum sativum*) and *H. vulgare* are grown as a minor winter crop. Vetches (*Vicia* spp.) perform well in trials but have not gained popularity as fundamental. Dry fodder like wheat straw, dried maize, sorghum and millet herbage are also used for the animal. The demand for irrigation water is continuously increasing in arid and semi-arid countries (Al-Salem, 1996). Heavy metals are accumulated from soil crops, pesticides, fertilizers (Onianwa *et al*., 2001; Audu and Lawal, 2005).

The main uses of wastewater include among other agricultural irrigation (Rattan *et al.,* 2001). The extent of the build-up of metals in wastewater-irrigated soils depends on the period of its applications (Bansal *et al.,* 1992).

Forage and fodders (Buffalo herb (*Medicago sativa),* Hay (Conserved forage plants), Straw, Silage, Compressed & Pelleted feeds, Oils & Mixed rations, Sprouted grains, Legumes/Grains. Conventional Fodders, Barley, Birdsfoot trefoil, Brassicas (Kale, Rapeseed (Canola), Rutabaga (swede), Turnip, Chau moellier). In Pakistan, it is a common practice to use polluted water as supplemental irrigation to combat irrigation water shortage for the growing of forage and fodder in the immediate surroundings of our big cities. Noorka and Heslop-Harrison, 2019. This water is a rich source of metals like Fe, Mn, Cu, Zn, Pb which may accumulate in soil and may be toxic to the plants and also because deterioration of soil (Khan *et al.* 2009) reported that the use of wastewater, chemicals and fertilizers in agricultural land cause heavy metal pollution and cause various health hazards in human. Although Pakistan world's largest canal irrigation system but shrinking freshwater resources have compelled farmers to use wastewater as supplemental or sometimes sole water resources. Raw sewage is widely used on agricultural soils in urban areas of developing countries to meet water shortages where treatment and safe effluent disposal facilities are limited or non-existent. Keeping in view the objectives like the determination of Zn in fodder and forages under both normal and wastewater application.

Material and methods

Samples and sampling

Collection of forage, fodders, animal (blood, hair, urine and feces samples) and soil samples. Sampling and collections of forage, soil and animal samples were carried out from all sites. Fresh samples of forages and fodder were randomly collected from different forage and fodder sites of Sargodha and Jhang, Layyah and Bhakkar randomly, Figure1, which supply most of the forages and fodder consumed in both winter and summer seasons. Animal blood samples were taken from Sargodha, Layyah, Jhang, and Bhakkar Districts. The animal includes the goat, sheep and cow. Samples of Forage and fodder samples were collected in new, clear polyethylene bags. Similarly, forage and fodder samples cultivated on a piece of land irrigated with fresh groundwater resources with different sample areas, at the Sargodh and Jhang, Layyah and Bhakkar were collected to serve as controls.

Courtesy = Google maps

Fig. 1. The study area containing four districts Sargodha, Jhang, Layyah and Bhakkar of Punjab Province, Pakistan.

Site 1. Control (groundwater)

Site 2 waste water

Site 3 waste water

Site 4 waste water

Site 5 waste water

Site 2-5 were selected in respective areas where polluted urban sewage water was used as an irrigation source and without the applications of chemical fertilizers, manures, herbicides and pesticides. The representative samples were obtained from soil collected from different sites and examined under the Hi-tec lab, College of Agriculture, University of Sargodha.

District 1= Sargodha

District 2= Jhang

District 3= Layyah

District 4= Bhakkar

Animal 1= Goat

Animal 2= Sheep

Animal 3= Cow

Source 1= Blood

Source 2= Hair

Source 3= Feces

Source 4= Urine

Digestion of samples

All samples containing soil, forage and fodder were dried at 105°C for one day. Then 1 g dried sample was digested in 15 mL of HNO_3, H_2SO_4 and $HClO_4$ mixture (5:1:1) at 80°C until it converts to colorless. After filtering the digests, the final volume of each sample was raised to 50 ml and then stored in plastic bottles until analysis.

Determination of heavy metals

Levels of all macro, micro, essential, toxic and heavy metals in the forage and soil samples were determined using an SP 1900 Pye Unicam Atomic Absorption Spectrophotometer (AAS) equipped with an air–acetylene burner.

The mean values of six determinations per sample were recorded.

Sample preparation

Samples of soil and forage were kept at 72°C till it's dryness. Then it was converted into powder and 1g was used to search heavy metals. Apparatus used for the digestion was Digestion flasks of 100 ml, beakers (50ml and 100ml). Measuring cylinder (50 ml), pipette (10 ml), Stirrer, Hotplate, filter paper, Gloves, Sulphuric acid (H_2SO_4) 70% and Hydrogen peroxide (H_2O_2) 50% and newly organized condensed water for sample preparation.

Wet digestion method

The dried samples were digested with conc. HNO_3 and H_2O_2, 1:2 on a hot plate. Digestion continued until a colorless solution appeared. After cooling, dilute all the samples then filtered through Whatmann filter paper No. 42.

Metals concentration

All the processed samples were then passed to Atomic Absorption Spectrophotometer (Perkin-Elmer Corp., 1980) to find heavy metals in samples. The metals to be examined were Zinc (Zn). The Standard solution of different metals was made from the Stock solution, to get the standardizable curve.

Statistical analysis

Data collected for each parameter were analysed for analysis of variance, by using SAS software version 9.2 (SAS, 2008). The results were compared with the International standards revealed by USEPA (US Environmental Protection Agency). 2002. WHO/FAO, 2007

Result and discussion

Soil

The analysis of variance (ANOVA) depicted that Zn values were significantly (P<0.05) affected by the district while a non-significant (P > 0.05) effect was shown by sites and district x sites (Table 1).

Table 1. Analysis of variance of Zn for soil.

Source	df	Sum of Squares	Mean Square	F value
District	3	425.470	141.823	19.132*
Sites	4	17.379	4.345	.586 NS
District x Sites	12	103.485	8.624	1.163*
Error	40	296.517	7.413	
Total	60	52366.317		

(P>0.05) Non-significant =NS; (P<0.05) Significant =*

Table 2. Analysis of variance of Zn for forages.

Source	df	Sum of Squares	Mean Square	F value
District	3	223.002	74.334	10.102*
Sites	4	38.237	9.559	1.299*
District * Sites	12	69.555	5.796	.788*
Error	40	294.333	7.358	
Total	60	94270.940		

(P>0.05) Non-significant =NS; (P<0.05) Significant =*

The values of Zn in soil ranged from 23.05 mg/kg to 34.99 mg/kg. The lowest value was observed at site 5 of district Sargodha while the highest value was present at site 5 of district Layyah. Fig. 2. Zn values in soil samples of the present study were much lower than the critical level (60 mg/kg) mentioned by the World Health Organization.

Table 3. Analysis of variance of Zn for animals.

Source	df	Sum of Squares	Mean Square	F value
District	3	67.112	22.371	26.468*
Animal	2	.397	.199	.235 NS
Source	3	20.021	6.674	7.896*
District * Animal	6	1.175	.196	.232 NS
District * Source	9	8.518	.946	1.120*
Animal * Source	6	3.841	.640	.757*
District * Animal * Source	18	7.825	.435	.514 NS
Error	432	365.121	.845	
Total	480	2183.936		

(P>0.05) Non-significant =NS; (P<0.05) Significant =*

Forage

Variance analysis of Zn data in forages showed a significant (P < 0.05) effect of district, sites and District x sites (Table 2). The value of Zn in forages ranged from 34.06 to 43.28 mg/kg. Fig. 3. The lowest value was found in site 5 of district Sargodha while the highest was found at site 3 of district Bhakkar (Table 2). Gill, 2014. Zn level in the forages investigated in the present study was below the permissible value (50 mg/kg) suggested by the World Health Organization.

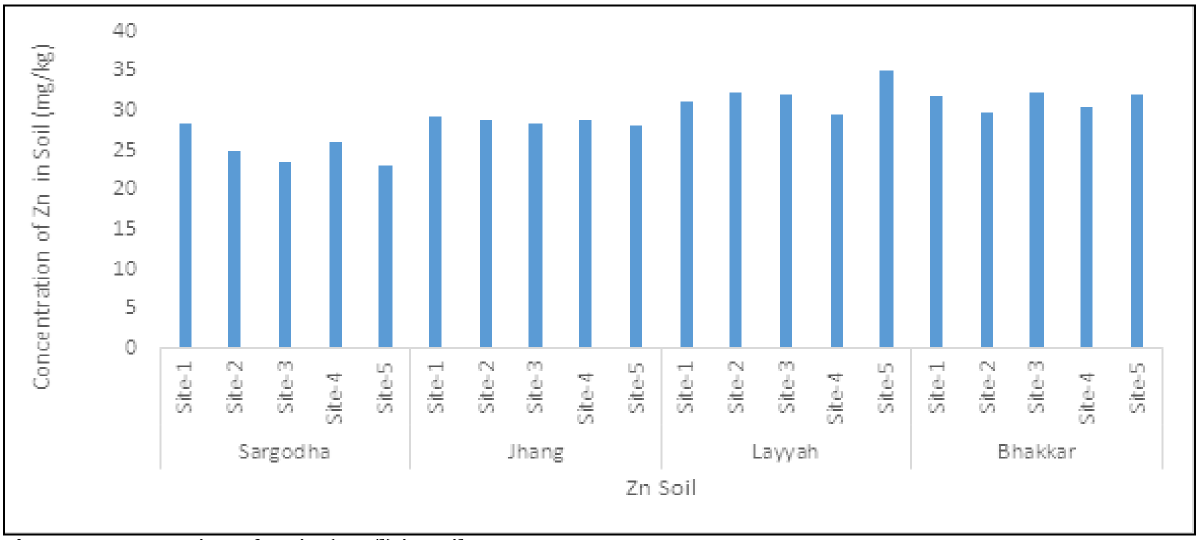

Fig. 2. Concentration of Zn in (mg/l) in soil.

Animals

Analysis of Variance performed on data of Zn in animals revealed the significant impact of District and source while the no-significant impact of animal, district x animal, district x source, animal source and district x animal x source on values of Zn in animals (Table 3). The value of Zn ranged from 0.82 to 2.66 mg/kg. Fig. 4. The lowest value was exhibited in the urine of sheep present in district Sargodha while the highest existed in blood collected from the goat of district Bhakkar (Table 3). Zn concentration of blood samples in the present study was greater than the permissible limits (1.45 mg/L). NRC (2007).The mean values Zn concentration was revealed in figure 4 while the mean values of Zn concentration among the blood, heirs, feces and urine samples of cow, sheep and goat in study areas.

The results obtained are in agreement with Ahmad *et al.*, 2011 and Bao *et al.*, 2014.

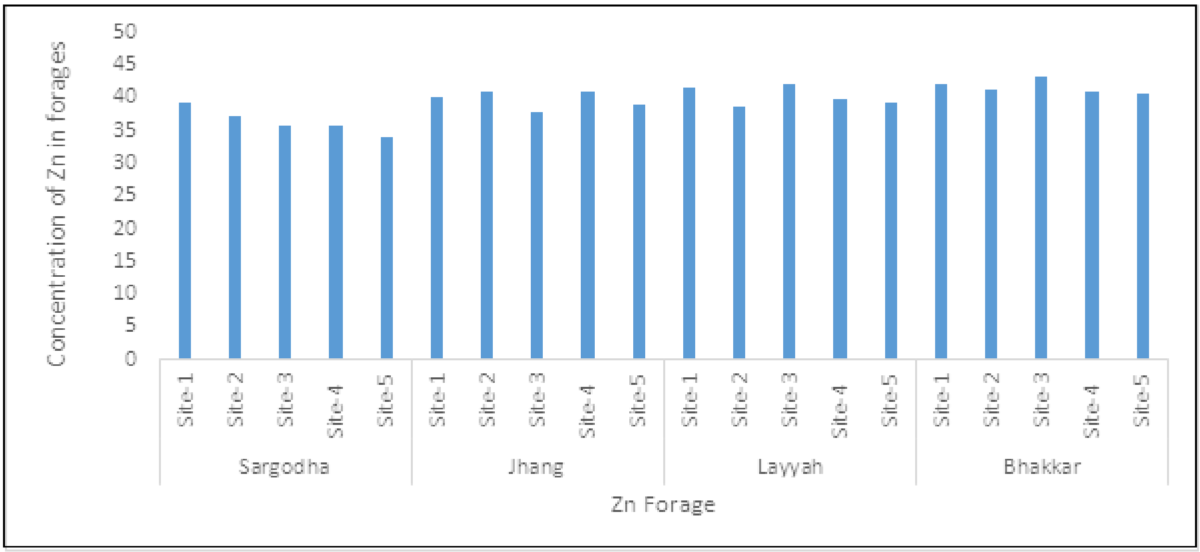

Fig. 3. Concentration of Zn in different parts of Forages (mg/kg).

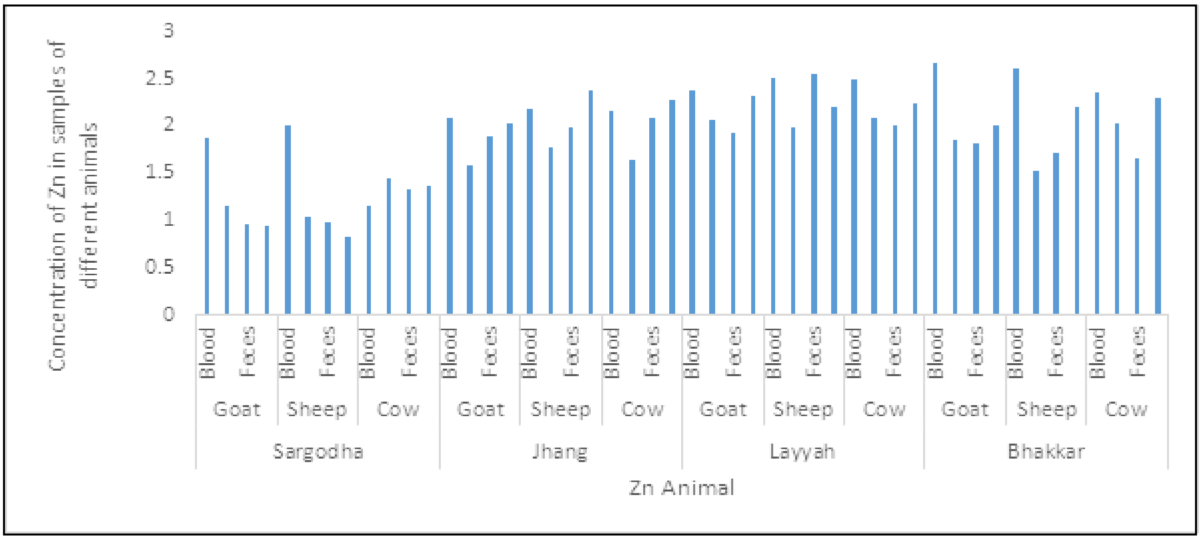

Fig. 4. Concentration of Zn, (mg/l) in different samples of different animals (mg/kg).

Conclusion

It was concluded that the values of Zn were within the safe limit recommended by the world health organization. The values of heavy metals such as Zn values in the soil forage and animals' blood, heir, feces and urine samples are on and off nature.

The results of the present study depicted the monitoring of heavy metal contamination of the study area and other adjacent areas in Pakistan as the results are of alarming nature approaching the toxic levels for future research.

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