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Speciation of two heavy metals in pastures and animals: An assessment of health risk

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Abstract

The aim of study was access the cobalt and lead contamination in soil, forages and animals. Heavy metal pollution is a matter of prime significance in natural environment. Through food chain toxicity of heavy metals and their bioaccumulation potential are transferred into humans .Higher concentrations of metallic compounds are toxic to living organisms but these are essential to maintain body metabolism. Intake of food crops polluted with heavy metals is chief food chain channel for human exposure. Animals are exposed to heavy metal stress by the intake of richly contaminated food crops those are chief part of food chain. We collected samples of soil, plant, animal blood, hair and faeces to find contamination through wet digestion process in lab and metal analysis. Different forages were collected to study Zn amount in forages whilst soil and animals in Mianwali Pakistan. The health risk index (HRI) calculation was our major concern in this study. Our present findings also emphasized on the assessment of bio-concentration factor (BCF). We also calculated other significant indices i.e. Pollution load index (PLI), daily intake of metal (DIM), Health Risk Index (HRI) and Enrichment factor (EF). While the experimentation result showed different concentrations of metal in different seasons. When the Zn concentration in forages was (32.59-42.17mg/kg) and in soil (21.82-35.09 mg/kg). Soil samples showed higher level of (PLI) Pollution load index. Bio-concentration of zinc was (1.03-1.57mg/kg). It can be concluded as regular monitoring of the level metal is essential evaluate the contamination status.

Key words: Seasonal variation, Health risk index, Bio concentration, Pollution Load Index, Pakistan

Introduction

In different ecological systems and atmosphere heavy metals are harsh contaminants of environment. By anthropogenic actions alterations of land use patterns, population explosion, industrial activities and intensive farming the soil quality is worsening in whole world (Pathak et al.2015). In soil the heavy metal pollution is cause of hazardous problems in agricultural ecosystem and cause potential detrimental effects (Bhatti et al. 2016; Tian et al. 2017).

Manipulation and the accessibility of some crucial metals in the body of living being is produced by level of Bio-concentrations of some heavy metals. In food chain heavy metal load is measured by degree of contamination at specific area. Toxicity of heavy metals is measured by assessment of biomagnifications in trophic levels from soil-forages-animal continuum and its bioaccumulation beside this by evaluation of contamination in fodder crops and water (Has-Schön et al. 2006; Saleemi et al. 2019).

Zinc (Zn) is necessary for six enzyme classes e.g, ligases, hydrolases, lyases, erases, oxidoreductases, transferases and it is fundamental constituent in numerous metabolical reactions in forages. Hence, suitable concentrations of Zn (Zinc) should be present in soil for survival of plants. In unpolluted environment iron zinc (Zn) constitutes 8–100 mg/kg in forages (Nagajyoti et al. 2010). Metals cause great hazards to plant survival and growth when present above the critical limits (Suresh 2005).

With soil pH Zinc is readily obtainable by forages and trace amount of metals are lixiviate into subsurface water table since in acidic soil pH, the action mechanism of Zn^{2+} ions intensify counter effect for zinc (Zn) exchange sites, ingestion of heavy metals by principal contamination routes of the trophic chain is by virtue of utilization of forages contaminated by metal traces is carcinogenic, are underlying of activating cell mutation in forages and nervous system disturbances in organisms. Some forage species accumulate or tolerate more heavy metals than others without clear toxicity symptoms (Singh et al. 2010).

Forages and animals accumulate Zn in them that are risky for human these trace metals are transported through food chain (Hongyu et al. 2005; Ahmad et al. 2018b). From one trophic level to higher trophic level biomagnifications and transport of Zinc take place by this more bioaccumulation of toxins occur in food of animals (Monteiro et al. 1996). Due to soil and forage heavy metal bio-accumulation produce the gastrointestinal cancer in animals (Zhuang et al. 2009; Dogan and Ugulu 2013).

Microelement (Zn) is element that required in lesser amount for animals and plants that is present in environment and is essential for organisms for sustainability of life i.e. minerals and vitamins.

Macronutrients and micronutrients of all types fulfill the particular nutritious needs of animals and forages. In soil-forage-animal continuum heavy metals and other mineral nutrients perform a significant role in metabolic, catabolic, biochemical, biological, chemical and enzymatic activities of living cell in organisms (Pais and Jones 1997).

MATERIALS AND METHODS

Study Area

District Mianwali is located in south-western region of province Punjab. This district is consists of the plains of western area of salt range. It is situated near the Sakesar hill. Mianwali district has boundaries with Khushab, D.I Khan, Bhakkar and Bannu districts. This district is part of Sargodha Division. Temperature of this area ranges from 47°C maximum and 19°C minimum per annum. In Mianwali the maximum rain fall occur in July about 6.6mm annual mean rain fall is about 3.3 mm. Soil condition of this area characterize as loamy, sandy and clay soil. Pea nut, mung, mash, mustard, Eruca, fennel, wheat, barley and oat are important crops. Forest cover area is very low because trees are used as fuel and timber. Canal irrigation system is very less developed, only a little area is irrigated with Indus river irrigation system (Ghani et al. 2016; Qureshi et al. 2007).

Sample collection from sites

In district Mianwali four sites were selected for sampling. The 3 samples of agricultural soil, forages and animal blood, hair and faeces were taken to examine the metal profile of soil-forage-animal continuum. The samples were taken from Wan bhachran site, Mianwali, Esakhel and Piplan. S1 (Summer), S2 (Autumn) and S3 (Winter) was selected for sampling. The samples were taken randomly from sites.

Soil sample collection

In the district Mianwali four sites were selected to collect the samples. 3 samples of soil were collected with equal distances in the field. Stainless steel auger was used to dig up the upper layer of soil about 12-15 cm (Siddique et al. 2019). These samples were packed into plastic bags to avoid the mixing of other chemical compounds into it. Samples were stored in laboratory and labeled then metal analysis was performed. For each sample three composite samples were made. The collected samples were firstly air then oven drying at 72^{0}C for 2 days. The samples were placed in incubators at 70 ^{0}C temperature for 5 days.

Forage sample collection

Sterilized apparatus were used to collect the forage samples. Forage and soil were collected from same field and place. Only those forages were selected for taking samples that are used as common feed of livestock. 3 samples of each forage plant were taken from the sampling area. The samples were washed with distilled water to clear impurities and dirt. These samples were dried to eliminate moisture in the freshly collected samples. The collected samples were dried for further process. These are following species that were selected for sampling.

Calotropis procera	Apocynaceae	Apple of Sodom
Dactyloctenium aegyptium	Poaceae	Egyptian crowfoot grass
Parthenium hysterophorus	Asteraceae	carrot grass
Rumex dentatus	Polygonaceae	Jangali palak
Ziziphus jujube	Rhamnaceae	red date, Chinese date

Animal blood plasma, hair and faeces sample collection

Blood samples of cow, buffalo and sheep of Mianwali was taken in 2020. Young animals within age of two years were selected for sampling. Blood was collected from four sites of district Mianwali. Animal blood was calculated from 10 animals (Cow, buffalo and sheep) each from each sampling site and heavy metal evaluation was done. Sterilized syringe was used to obtain the blood samples. The grazing ruminant’s blood was taken from the vein. The vacuum was created in evacuated tubes while collecting blood to minimize the extent of clotting. The blood was collected in heparinized Na-citrate voiles quickly. For 15 minutes blood was centrifuged at 3000 rpm and blood plasma was separated. Polyethylene tubes were used to store the blood plasma and frozen at -20 ^{0}C. Hair and faeces samples were also collected and stored for the further digestion process.

Sample measurement and preparation

Arrangement and preparation of samples involves the digestion process. This method of digestion is called wet digestion. It has following steps.

1.Digestion 2.Dilution 3. Filtration 4. Analysis of samples

Acid and hydrogen per oxide is used for complete digestion process. Distilled water is added after digestion into prepared samples for dilution purpose. After that filtration of samples occur. In next step Atomic Absorption Spectrophotometer (AAS) is an apparatus through which metal analysis is done.

Apparatus and chemicals for digestion

Chemicals that are used for digestion process includes the 10 mL nitric acid ,70% Sulphuric acid (H_2SO_4), 50% Hydrogen peroxide (H_2O_2) and newly synthesized condensed water or distilled (H_2O). The apparatus for digestion includes digestion flasks of 100 ml, measuring cylinder (50 ml), beakers (50ml) and (100ml), pipette (10 ml), filter paper, stirrer, hotplate and gloves.

Digestion of soil, forages and Animal samples

Digestion of soil and forages and animal samples (blood, hair and faeces) include various steps. First of all the samples are air dried and followed by oven dried process at 72^0C for 5 days until the moisture content is removed. When plants are completely dried they weighed with electrical balance. Standard procedure of digestion was applied to digest the samples (Siddique et al. 2019). 1gm sample was weighed by electrical balance and placed in a beaker of 50ml. 10 ml nitric acid was added to beaker and was kept overnight. Hot plate was used for digestion of particular sample by pouring H_2O_2 drop wise until solution becomes transparent. Cooling at room temperature was done. For dilution purpose 50 ml distilled water was added to solution. To filter the solution Whatman filter paper of 42 µm was used. Then this prepared solution was kept in plastic bottles for metal profile evaluation.

Blood samples collected from the Mianwali district were stored and freezed at -20 ^{0}C. For digestion process the samples were from freezer and digested with same standard procedure as applied to soil and forages (Siddique et al. 2019).Hair sample was sun dried and was cut into pieces of 1.0-2.9 cm. De-ionized water was used to wash the samples and ethanol was also applied to wash. Oven drying process was carried out and for 4 hours and then desiccator cooling was performed (Hashem et al. 2017). Faeces samples were collected from cow, buffalo and sheep after air drying and oven dry the samples were submitted for digestion (Nicholson et al. 1999).

Metal profile evaluation analysis

The prepared samples were then analyzed for metal contents by Atomic Absorption Spectrophotometer (Perkin-Elmer Corp., 1980). Nutritional minerals that were evaluated in the sample was Zn. Standard solution was prepared to get the standardized curve. The metal analysis was done by running the samples through Atomic Absorption Spectrophotometer. This apparatus is equipped with a graphite furnace. The each metal is measured according to value of standard solution. The amount of each metal occurring in the sample is obtained in absolute farm. While sample is run through the Atomic Absorption Spectrophotometer the little quantity of sample is sprayed at the flame. Atomic resonance absorption line by element is calculated and measured. The apparatus is convenient for analysis. Any radiation that is emitted by flame had no effect on the working of apparatus. The absorption method is independent of the excitation potential of the spectral line used.

Evaluation Indices:

Bio concentration Factor (BCF):

For assessment of metal (mg/kg) transport from agricultural soil and forages that are growing on this soil, a BCF is applied (Cui et al. 2004).

BCF for soil to forage

(BCF) = Level of metal in forage /Level of metal in soil

Pollution load index (PLI)

Liu et al. (2005) described a formula which was used to find this indices.

$$\text{Pollution Load Index} = \frac{(M)IS}{(M)RS}$$

Where,

(M)IS = (mg/kg) Concentration of metal that occurs in soil to investigate

(M)RS= Soil reference value of metal

Reference values for soil in Zn was taken as 44.9 suggested by Singh et al. (2010)

Enrichment factor (EF)

Formula for Enrichment factor is described by Buat-Menard and Chesselet (1979).

$$\text{Enrichment factor (EF)} = \frac{\text{(Conc. of metal in plant/Conc. of metal in soil) sample}}{\text{(Conc. of metal in plant/Conc. of metal in soil) standard}}$$

According to FAO/WHO (2001) standard reference value for Zn was used 99.4 mg/kg.

Daily intake of metals (DIM)

Daily intake of metal (DIM) can be calculated by following equation

$DIM = Cfactor \times C_{metal} \times D_{food\ intake} / B_{average\ weight}$ Sajjad et al. (2009).

where,

C_{metal} is the concentration of metals in forages,

$D_{food\ intake}$ is the daily intake of forages,

$B_{average\ weight}$ is the average body weight.

For calculating this daily intake of metal the conversion factor was taken 0.085 (Jan et al. 2010). Daily intake metal for cow was calculated by using animal body weight 600 kg and daily forage intake 12 kg while for sheep body weight was taken 75 kg and daily forage intake 1.3 kg (Johnsen and Aaneby 2019). To calculate the DIM for buffalo body weight was taken 550 kg and daily forage intake (TDI) was taken 12.5 kg (Yang et al. 2020).

Health Risk Index (HRI)

Health risk index is the ratio of daily intake of metals in the forages to oral reference dose (RfD) and was calculated by the help formula (USEPA 2002).

$$\text{Health risk index (HRI)} = DIM / R_fD$$

DIM = Daily intake of heavy metal

R_fD = Oral reference dose

An HRI > 1.0 for any single metal indicates that the health of consumer population is at risk or it is carcinogenic (USEPA 2013). According to FAO/WHO (2013) oral reference dose for Zn was taken as 0.3 (mg/kg/day).

Result and Discussion for Zinc metal

Table 1 Analysis of variance table for Zn concentration in soil, forages and animals

Source	Degree of freedom	Mean Square

Zn in Soil		
Season	2	171.594***
Soil	4	5.59^{ns}
Season x soil	8	22.24*
Zn in forages		
Season	2	70.785***
Forage	4	19.87^{ns}
Season x Forage	8	11.283^{ns}
Zn in Animals		
Season	2	19.025***
Animal	2	1.302ns
Source	2	3.971**
Season x Animal	4	0.163ns
Season x Source	4	0.704ns
Animal x Source	4	0.668ns
Season x Animal x Source	8	0.587ns

Table 2: The mean concentration of Zn (mg/kg) in soil and forages

	Seasons		
Samples	**S 1**	**S 2**	**S 3**
Zinc in Soil of forages			
Soil of forage C. procera	26.91±2.36	29.47±1.06	31.33±0.64
Soil of forage D. aegyptium	25.02±1.25	25.48±1.42	32.33±1.63
Soil of forage P. hysterophorus	23.56±2.94	28.58±2.44	32.21±1.10
Soil of forage R. dentatus	26.22±1.52	28.92±1.77	26.39±2.64
Soil of forage Z. jujube	21.82±0.852	28.35±2.29	35.09±0.31

Zinc in forages

C. procera	36.01±2.94	39.25±1.51	41.49±0.677
D. aegyptium	37.40±1.47	37.73±2.70	38.33±0.355
P.hysterophorus	36.10±1.54	37.78±0.25	42.17±1.64
R. dentatus	32.59±0.932	33.93±1.14	39.93±1.41
Z. jujube	34.17±2.391	38.91±1.35	36.05±0.399

Table 3: The Mean concentration of Zn in animals blood, hair and faeces (mg/kg).

Source	**Animal**	**S1**	**S2**	**S3**
Blood	Cow	1.87±0.230	1.89±0.163	2.48±0.395
	Buffalo	1.24±0.177	1.69±0.229	1.96±0.233
	Sheep	0.927±0.135	1.70±0.267	1.93±0.353
Hair	Cow	1.10±0.257	2.27±0.304	2.52±0.387
	Buffalo	1.24±0.175	1.87±0.326	1.98±0.283
	Sheep	1.03±0.183	2.10±0.321	2.84±0.449
Faeces	Cow	1.37±0.158	1.95±0.317	1.33±0.420
	Buffalo	1.44±0.263	1.7±0.258	1.17±0.369
	Sheep	1.34±0.214	1.98±0.357	0.923±0.293

Table 4: Pollution indexes for Zinc

Forages	**Seasons**

	S 1	S 2	S 3
BCF (Zn)			
C. procera	1.34	1.33	1.32
D. aegyptium	1.49	1.48	1.19
P. hysterophorus	1.53	1.32	1.31
R. dentatus	1.24	1.17	1.51
Z. jujube	1.57	1.37	1.03
PLI (Zn)			
C. procera	0.599	0.656	0.698
D. aegyptium	0.557	0.567	0.720
P. hysterophorus	0.525	0.637	0.717
R. dentatus	0.584	0.644	0.588
Z. jujube	0.486	0.631	0.782
EF (Co)			
C. procera	0.595	0.592	0.589
D. aegyptium	0.665	0.658	0.527
P. hysterophorus	0.681	0.588	0.582
R. dentatus	0.553	0.522	0.673
Z. jujube	0.696	0.610	0.457

Table 5: Daily intake metal and Health risk index for Zinc

Forages	Cow			Buffalo			Sheep		
	S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
DIM (Zn)									
C. procera	0.061	0.067	0.071	0.069	0.076	0.080	0.053	0.058	0.061
D. aegyptium	0.064	0.064	0.06	0.072	0.073	0.074	0.055	0.055	0.056
P.hysterophorus	0.061	0.064	0.072	0.070	0.073	0.081	0.053	0.056	0.062

R. dentatus	0.055	0.058	0.068	0.063	0.066	0.077	0.048	0.050	0.059
Z. jujube	0.058	0.066	0.061	0.066	0.075	0.070	0.050	0.057	0.053

HRI (Zn)

C. procera	0.204	0.222	0.235	0.232	0.253	0.267	0.177	0.193	0.204
D. aegyptium	0.212	0.214	0.217	0.241	0.243	0.247	0.184	0.185	0.188
P.hysterophorus	0.205	0.214	0.239	0.232	0.243	0.272	0.177	0.186	0.207
R. dentatus	0.185	0.192	0.226	0.210	0.218	0.257	0.160	0.167	0.196
Z. jujube	0.194	0.220	0.204	0.220	0.251	0.232	0.168	0.191	0.177

Result and Discussion

The results from ANOVA of Zn for soil samples depicted significant effect ($p<0.05$) of season and season × soil while the reverse was true for Soil. The analysis of variance exhibited the significant effect ($p< 0.05$) of Zn season while non-significant ($p> 0.05$) in forages and season x forage. The ANOVA of Zn in animal demonstrated significant ($p<0.05$) impact on season and source while exhibited non-significant effect ($p> 0.05$) on Season × Animal, Season × Source, Animal × Source and Season × Animal × Source (Table 1).

The level of Zn in soil samples varied within range 21.82 mg/kg minimum and 35.09 mg/kg maximum (Table 2). The minimum Zn value was noticed in soil of *Z. jujube* in S1 and its maximum value was observed in soil of *Z. jujube* during S3 (Fig 1). The present study had the Zn value below the permissible limits of 300 mg/kg suggested by WHO /FAO (2001).

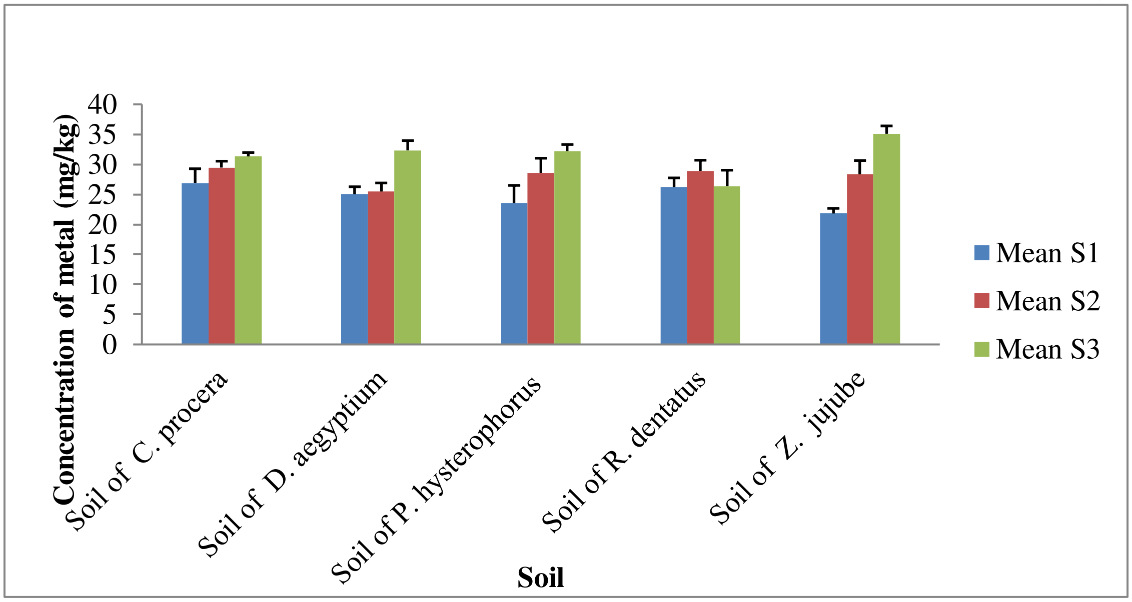

Fig 1: Mean concentration of Zn in soil of forages mg/kg

The Zn concentration occurred in different amounts in forage samples. The minimum concentration of Zn was recorded in *R. dentatus* during S1while the minimum amount of Zn was noticed 32.59 mg/kg (Table 2). The maximum amount of Zn was recorded in *P.hysterophorus* during S3 while the maximum value of Zn was 42.17 mg/kg (Fig 2). The present value of Zn in forages had the values within the permissible limits of 50 mg/kg suggested by WHO (1998).

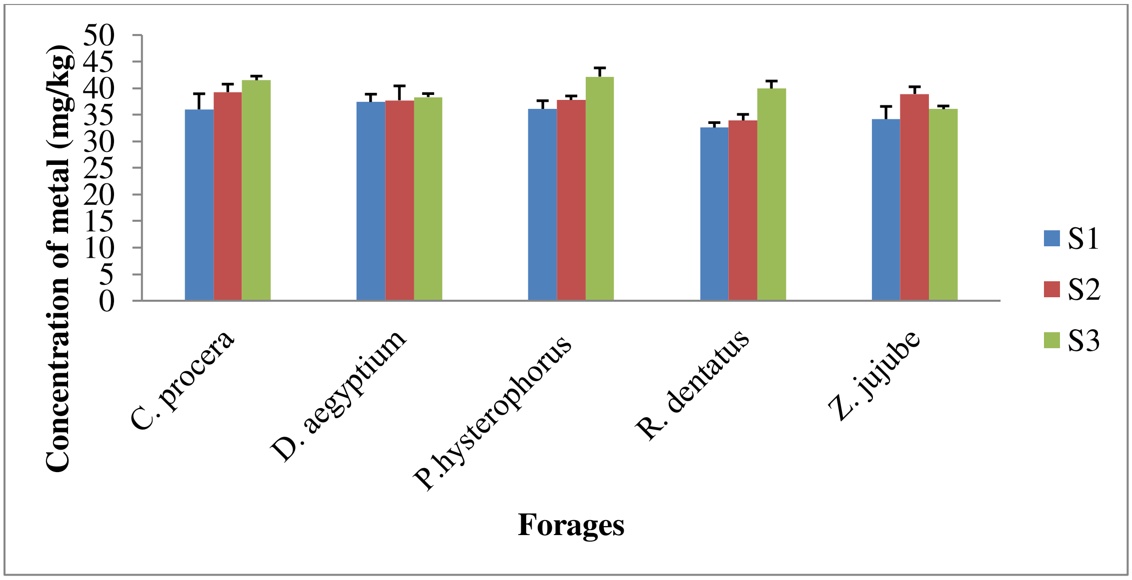

Fig 2: Mean concentration of Zinc in forages mg/kg

The Zn metal concentration varied in all samples of blood. The mean value ranged from 0.927-2.48 mg/kg correspondingly. The minimum level of blood was present in sheep during S1 and maximum level was observed in cow blood of S3 (Table 3).The present investigation of Zn was found in the range of permissible limit 1.45 mg/l as suggested by NRC (2007). The Zn level in hair sample was ranged minimum to maximum 1.03- 2.84 mg/kg (Fig 3). In S1 season the sheep hair had minimum values. In S3 the sheep had maximum level of Zn. In the faeces samples the Zn concentration had minimum level 0.923 mg/kg in sheep during S3. The maximum concentration of Zn was noticed 1.98 mg/kg during sheep of S2.

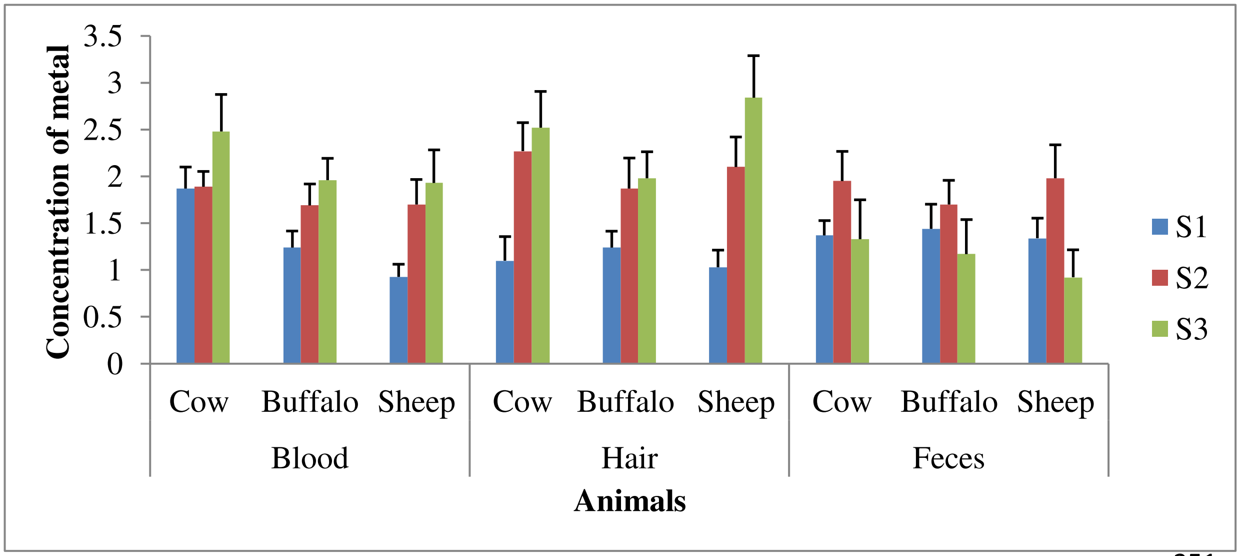

Figure 3: The Zn concentration in Animals blood, hair and faeces in different seasons

Bio-concentration factor for Zn from soil to forage varied in the range from 1.03-1.57 mg/kg. The higher content of heavy metal was present in *Z. jujube* of S1 while lower amount of heavy metals occurred in *Z. jujube* of S3.The value of pollution load index for Zn varied within the range from (0.486-0.782 mg/kg). The lower value was noticed in *Z. jujube* of S1 while the higher amount was observed in *Z. jujube* of S3.The values of enrichment factor for Zn ranged from minimum concentration to maximum concentration (0.457-0.696 mg/kg).The minimum concentration was noticed in *Z. jujube* during S3 (Table 4).The daily intake of metal for Zn ranged from 0.048-0.081 from minimum to maximum. The lowest value of daily intake of metal was depicted by sheep during S1 while the highest DIM was noticed in buffalo during S3.The health risk index for Zn ranged from 0.160-0.272 from lower to higher values. The health risk index (HRI) was depicted lowest in sheep during S1 while the highest value found in the buffalo during S3 (Table 5).

The current concentration of Zn in soil was found lower as compared to concentration of Zn (0.83-37.33 mg/kg) as reported by Fosu-Mensah et al. (2017). Level of Zn in this study was lower than permissible limits of Zn 250 mg kg−1 (UNEP 2013). The amount of Zn (24-179 mg/kg) reported by Muhammad et al. (2011) was greater than the present values of Zn in soil samples. Zn was bio-accumulated in forages that were grown in wastewater irrigated soil. The uptake of Zn trace and its toxicity increased in forages due to sewage water irrigation as compare to irrigation by ground water (Rusan et al. 2007).

The Zn value found in forages of current investigation was found to be lower than Zn (17.4-202 mg/kg) as recorded by Ogundiran et al. (2012). In our present work the value of Zn was found greater than reported Zn concentration in forages (18.67 mg/kg to 25.83 mg/kg) by Raeside et al. (2012). Our present investigation was found lower than those Zn concentration (211.7 mg·kg-1 to 901.7 mg·kg-1) recorded by Zhang et al. (2014). Raeside et al.(2012) stated that zinc metal played a significant role in absorption of Cu so its amount should be increased upto 100 mg Zn/kg; hence a very low level of Zn was present in that forages. The calculated value of zinc in present work was also low as compare to suggested value of Raeside et al. (2012).

In present findings the Zn concentration in cow blood plasma was lower than the findings of Mohajeri et al. (2014). The current study Zn was recorded greater than the Zn values reported in cow blood by Noaman et al. (2012). Zn bioaccumulation in blood plasma is directly affected by seasonal variations. Zn accumulation is decreased due to hyper-thermal stress so that's why Zn value varies in the samples of different season (Radostits et al. 2007). Environmental pollutant Zn was higher at the sampling sites due to metal containing forage consumption by the ruminants (Mohajeri et al. 2014). The observed value of Zn in buffalo blood was higher than the critical values as suggested by the Underwood and Suttle (1999). In our results were below the limits reference values as suggested by the results of Noaman et al. (2012). Our present investigations were lower for Zn concentration in sheep than those recorded by Shen et al. (2020). Our findings were higher in Zn concentration present in sheep blood plasma as compare to results of Šoch et al. (2011). This present study exhibited the lower Zn values in sheep blood than the values given by Popovic et al. (2010). These findings provided the greater value of Zn in cow hair and lower values in buffalo hairs as compare to results of the Kumar and Kewalramani (2011). Reason for the greater accumulation of Zn is due to mineral supplementation to ruminants at the farms of sampling sites. Different forage species have different chemical composition and metallic constituents; hence, minerals have interaction with Zn in animal body (Kumar and Kewalramani 2011). Our investigation about Zn in sheep wool and buffalo hair was respectively lower and higher as compare to the values found by Hashem et al. (2017). His recorded values for faeces were also greater for Zn in cow and buffalo than present study. Zn metal is excreted out in faeces so comparatively less metal is accumulated in the animal body. Fluctuating amount of Zn in animal samples is present due to numerous factors such as supplementation of mineral, antagonistic affects of elements and changes in climate (Šoch et al. 2013).

Our present calculated values of BCF for Zn was lower than fidings of Al-Rashdi and Sulaiman (2013). Current findings Zn bio-concentration factor was higher than the findings of Albornoz et al. (2016). The observed values of bio-concentration factor (BCF) Zn in soil to forage was similar with the recorded values of Meng et al. (2013). The present Zn bio-concentration was lower than the given value of Zhang et al. (2014).

Our result for PLI was similar to the results given by Ogbeibu et al. (2014). Our present investigation for pollution load index was higher than those recorded by Barakat et al. (2012). These findings were lower than concentration of pollution load index for Zn as compare to the results of Ngole and Ekosse (2012). The pollution load index in present work was (PLI< 1) so the sampling site was not polluted.

The maximum concentration was noticed in *Z. jujube in S1.*The results demonstrated in present finding for enrichment factor was lower than the range of (EF<2) as described by the Barakat et al. (2012). The results of enrichment factors showed the similarities with the results of Alghobar and Suresha (2015). Current findings for EF were lower than those values given by the Uduma and Awagu (2014). In this present experiment the enrichment factor was (EF<2) hence, there was deficient enrichment of zinc.

The investigated value of DIM for Zn was higher than the observed values of Chaoua et al. (2019). The found value of daily metal intake was lower than the daily intake metal of Zn by Orisakwe et al. (2017). In our work the (DIM<1) for Zn that can be characterized as non-toxic concentration.

Health risk index for Zn was found higher as compared to the HRI given by Chaoua et al. (2019). HRI for Zn was observed lower than the recorded by Orisakwe et al. (2017). Health risk index was observed (HRI<1) for Zn that non toxic.

Conclusion

It was concluded that seasonal changes gave different fluctuating concentrations of metals and sites also gave fluctuating metal readings in soil-forage-animal continuum. In soil and forage samples collected from semi-arid environment was found safe according to FAO/WHO. In animal samples Zn was found safe according to NRC standards. Bio-concentration factor and pollution load index for Zn was noticed greater than 1. Enrichment factor was also in safe limits for all metals that was less than 1. Daily intake metal and health risk index was found less than 1.

Ethical Statement

All the study protocols were approved by Institutional Animal Ethics Committee, University of Sargodha (Approval No. 25-A18 IEC UOS). All the experiments performed compiled with the rules of National Research Council and all methods were performed in accordance with relevant guidelines and regulations.

Ethical Approval: Ethical approval was taken from Department Ethical Review Committee to conduct study as animals were involved in this study So ethical approval was very essential

Consent to Participate: Informed consent was taken from formers to conduct the study and to collect the samples. They were briefed about the research plan in details.

Consent to Publish: Written consent was sought from each author to publish the manuscript.

Authors Contributions: Fu chen, Laraib Saqlain, jing Ma and Zafar Iqbal Khan conceived and designed the study and critically revised the manuscript and approved the final version study was supervised by Kafeel Ahmad and Asma Ashfaq. Razia Sultana, Fatima Ghulam Muhammad, Majida Naeem and Ayesha maqsood executed the experiment and compiled data. Muhammad Nadeem statistically analyzed the data and help in chemical analysis. Yongjun Yang interpreted the results and critically edited and revised the manuscript. Ifra Saleem Malik and Mudasra Munir helped in sample collection and chemical analysis

Competing Interests: There is no competing interest in the publication of this manuscript.

Availability of data and materials: Data and material is available for research purpose and for reference.

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Figures

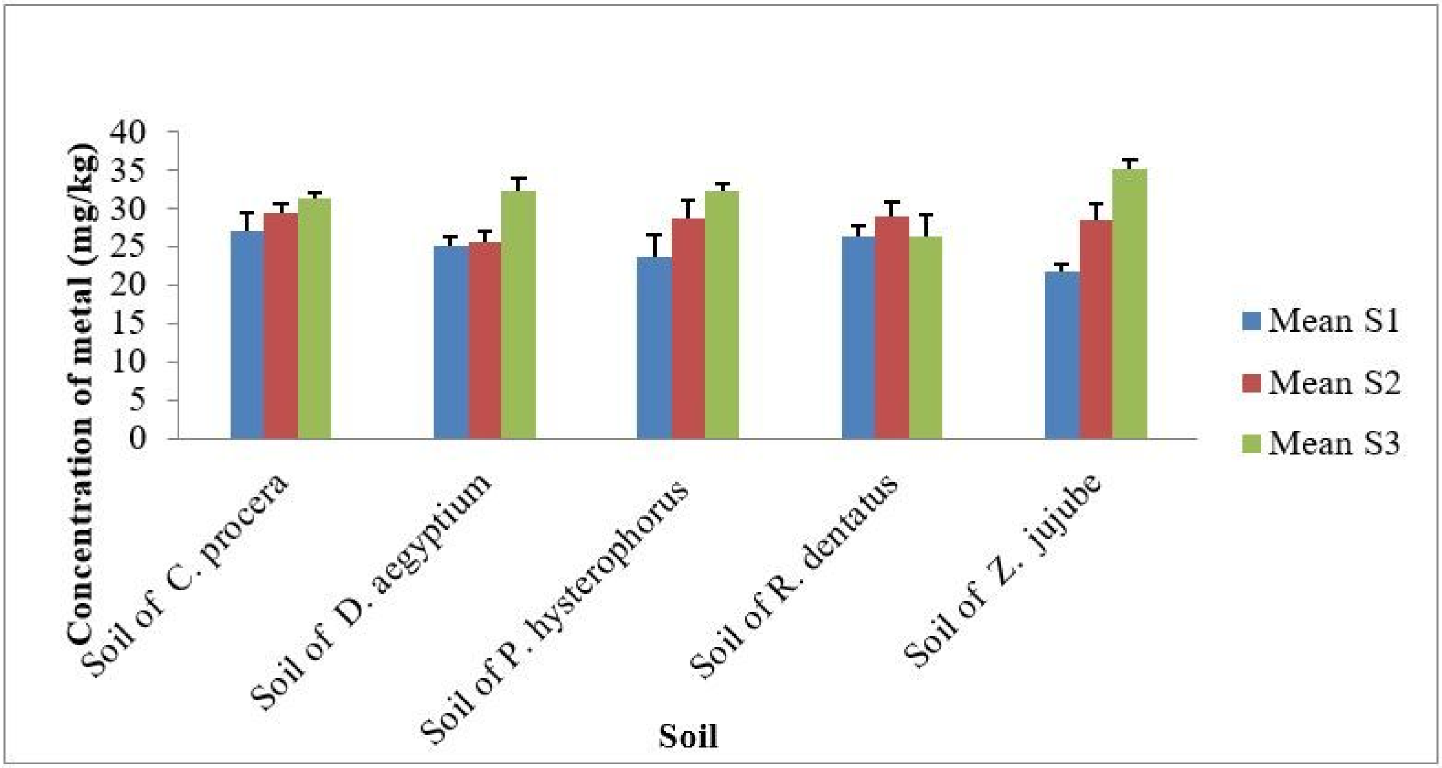

Figure 1

Mean concentration of Zn in soil of forages mg/kg

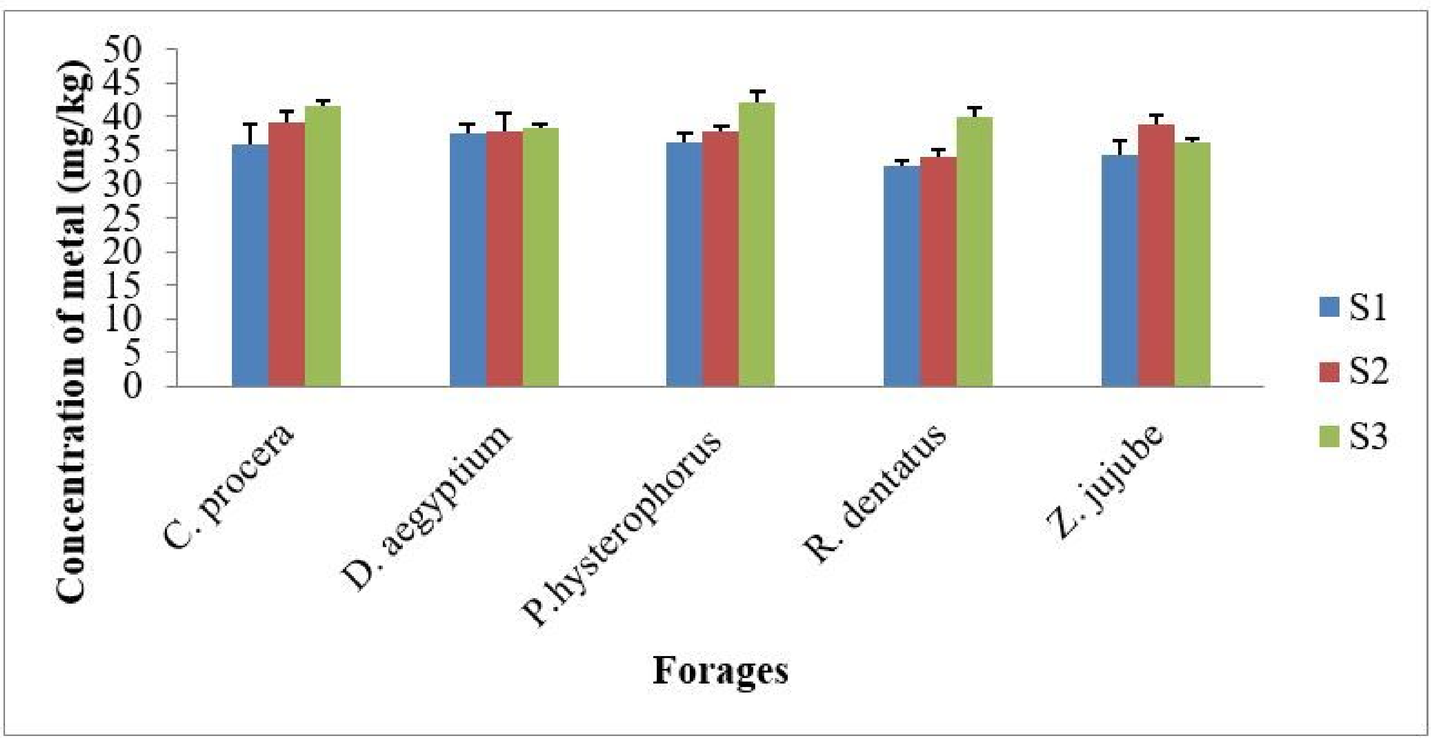

Figure 2

Mean concentration of Zinc in forages mg/kg

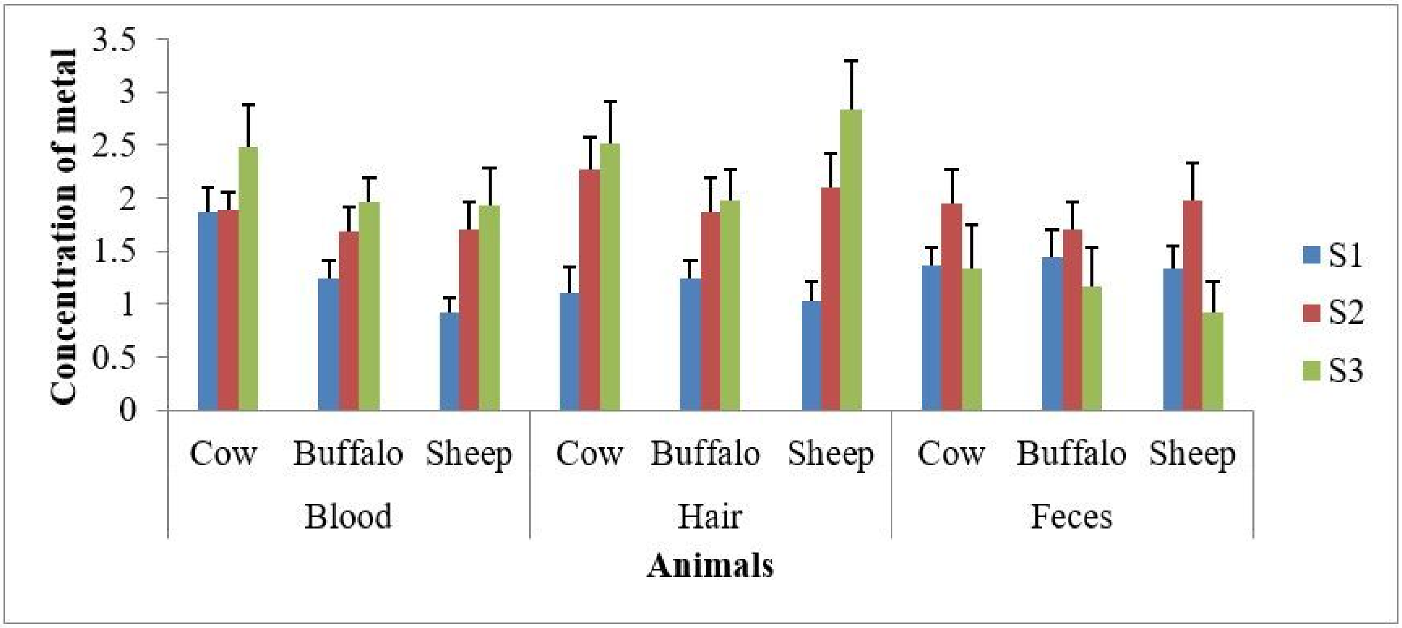

Figure 3

The Zn concentration in Animals blood, hair and faeces in different seasons