Bioaccumulation and Transfer of Zinc in Soil Plant and Animal System: A Health Risk Assessment for the Grazing Animals.

Fu Chen
University
Fatima Ghulam Muhammad
University
Zafar Iqbal Khan (zikhan11@gmail.com)
University of Sargodha
Kafeel Ahmad
University
Ifra Saleem Malik
University
Asma Ashfaq
University
Majida Naeem
University
Muhammad Nadeem
University of Sargodha
Jing Ma
University
Muhammad Umer Farooq Awan
University
Shahzadi Mahpara
University
Shahid Mehmood
University

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Abstract

Heavy metal pollution has thoroughly worldwide apprehensions due to the instantaneous growth of industries. Farming regions are irrigated mainly with wastewater which contains both municipal and industrial emancipations. Therefore, three sites irrigated with ground, canal and municipal-wastewater in the District Jhang were selected to determine the zinc accumulation and its transfer in the soil, plant and animal food-chain. Soil, forage and animal samples of cow, buffalo and sheep were selected from each site. Various pollution indices were also measured to assess the zinc exposure to grazing animals. Zinc concentration was ranged as 18.85-35.59mg/kg in the soil, 26.42-42.67 mg/kg in the forage and 0.982-2.85mg/kg in the animal samples. Investigated zinc concentration in soil and forages was found to be within the recommended WHO/FAO limits but blood samples exceeding the standards of NRC (2007). Maximum level of pollution load index (0.427-0.805mg/kg) and enrichment factor (0.373-0.894 mg/kg) for zinc was noticed upon waste water irrigation. Daily intake (0.039 to 0.082 mg/kg/day) and health risk index (0.130 to 0.275 mg/kg/day) of zinc metal was higher in the buffaloes that feed on waste water irrigated forages. Bio-concentration factor (0.840 to 2.01mg/kg) for soil-forage was >1 represented that these plants accumulated the zinc concentration into their tissues and raised health issues in grazing animals on consumption of waste water contaminated forages. Overall findings of this study, suggested that animal herds should be monitored periodically to devise preventive measures regarding the toxic level of heavy metals availability to livestock.

1. Introduction

The term “Pollution” derived from “Polluere” which is a Latin word means “to defile or to make foul”. Any substance that deteriorates the eco-quality and disturbs the supremacy of life is called pollutant. Environmental pollution is the occurrence of these defilements in the environment which are detrimental for living creature (Duruibe et al., 2007). Heavy metal pollution in environment is a serious dilemma in almost all states of world but it is a forefront threat in the emerging countries due to inadequate reserves, standard, strategies and organizational supervision (Were et al., 2008). Heavy metals are defined as the elements that are dense beyond 4.5g/cm3 and produce free electrons in a reaction. They are known as good conductor of heat and electricity, having high boiling and melting points, ductile, non-transparent and shiny in appearance (Szyczewski et al., 2009). They are omnipresent elements, durable and have long biotic half life. The examples of heavy metals are cobalt, copper, iron, manganese, zinc, lead, cadmium and vanadium (Duman et al., 2019; Hu et al., 2018)

Biological monitoring is a methodical strategy which is practiced to locate the dispersion proliferation and accretion of heavy metals in the natural world. It is centered on the selection of representative tissue and fluids from the living biota and examines them for an extended period of time to determine its status. Mostly sampling of soil, vegetation, agrarian outputs are obtained from the metropolitan stretch to study heavy metal content in them. It provides better relation among ecological toxins and their consecutive effects on living organisms (Dogan et al., 2014)

In order to calculate risks associated with the livestock exposure to toxic metals, take into account diet selecting behaviors, fluctuation in climatic conditions and herbage growth that affect the metal bioavailability (Fritsch et al., 2011). Livestock is exposed to these toxic metals by drinking polluted water and by ingestion of polluted vegetation. Few elements like Fe, Cu, Zn, and Mn are required by animals as essential constituent but their high level in the feedstuff imposes health hazards on the yield and developmental process. Zinc enhances the development and growth of animals (Jiang et al., 2011). Zn repaired the damage DNA and RNA. Almost 200 enzymes use Zn as a coenzyme. It controls the gene expression of specific proteins by defending their conversion to unwanted forms and

enhances the metabolic process (Massanyi et al., 2001). It plays an important role in the normal functioning of cells especially in the cell division, synthesis of carbohydrates and proteins. In various enzymes, the cadmium swaps up the zinc atom. For that reason, an excessive amount of zinc plays important role to reduce the cadmium toxicity (Khan et al., 1990).

The health hazards of toxic metal in animal body depend on the metal intake, interaction of metal with the internal stability of organism as well as category of animate being used (Danish et al., 2014). With the advancement of industries and urbanization, emitted waste water incorporates the toxic metals into air, water, soil, flora and fauna which compel the researchers to analyze the various chemical dynamics of environment to detect the degree of deformation in natural environment. Therefore, present study was also conducted to analyze the effect of various irrigation sources on the accumulation of zinc in soil, forage samples and possible health risks assessment in animals via intake of these forages.

2. Material And Methods

1.2.1 Study area:

The position of district Jhang is 71°-37° to 73°-13° longitudes toward east as well as 30°-37° to 31^{o}-59^{o} latitude toward North of Punjab, having total area of 8,809 square kilometers. Its border touches to Hafizabad and Sargodha District in the North, Toba Tek Singh & Faisalabad District in the East, Muzaffargrah District in the southwest and Layyah & Bhakkar District toward the west (Badar et al., 2017). In this district, summer duration is prolonged nearly of seven months, which starts from April and persists until October. May, June and July are hottest months (Sindhu et al., 2012).

1.2.2 Sampling locations:

Sampling can be done during the period of 2019-2020. The collection of soil, fodder crops/forages, and animal samples was done from three different sites of District Jhang. Site-1 named was Jhang (Jh-I) use ground water irrigation source, Site-2 was Shorkot (Sh-II) use canal water irrigation source and Site-3 was Ahmad Pur Sial (Aps-III) use municipal wastewater for irrigation purpose.

1.2.3 Forage/fodder crops collection:

Three replicates of each forage sample (*Acacia nilotica, Capparis deciduas, Zea mays, Medicago sativa* and *Pennisetum glaucum)* was taken and kept in polythene bag and transported to lab. All the replicate samples of forages were cleansed with distilled water to remove dust particles and externally deposited contamination. These samples were firstly air-dried then put in an oven for 7 days at 70-75C.Then they were grounded and kept in label sealed bags for digestion.

1.2.4 Soil collection:

Three replicates of fodder soil sample collected from depth of 0-20cm. The samples were collected in air-tight bags. Samples were dried in air and then kept in an oven at 70-75C for approximately 7 days. When all the samples are fully dried, they were stored in labeled sealed bags for next step.

1.2.5 Animal samples collection:

This experimental study selected the following animals: Cow, Buffalo and Sheep. Selected animal samples include in this study are blood, hair and feces of each category. 10 animals of each category were used to collect samples from every site and these animals mainly feed on the collected forages of pastures.

From the jugular vein of animals, the blood samples were collected in sealed test tubes. The blood samples were centrifuged for 15minutes at 3500rpm, to separate blood plasma then stored at -20C, until digestion (Karademir, 2007).

The hair samples were also collected from the bodies of selected animals and are properly labeled them. These samples were rinsed with acetone and then follow distilled water to remove external contamination. Further, they were dried in oven for at least 3-4 days and then they are ready for digestion (Gabryszuk et al., 2018)

The collected fecal samples are taken directly from the selected animals upon excretion to avoid further contamination (Svane and Karring, 2019). Labeled samples are stored in plastic bags. Firstly, they were air dried (3 days) and then kept in oven nearly 3-4 days.

1.2.6 Soil digestion:

The powdered soil of 2 gram was added to the digestion tube. Add 20ml concentrated H_2SO_4 into the tube. Run the digestion chamber for 30 minutes. Further add 10ml H_2O_2 to assist digestion. And again run the chamber until colorless solution was obtained. Solution is filtered and makes a final volume 60ml through distilled water addition. The final volume of sample was stored in plastic bottles for further metal analysis.

1.2.7 Forage/fodder crops digestion:

Plants samples were digested through dry ashing process. Firstly, all the crucibles were washed with distilled water and dried in an oven to remove all moisture content. Add 2 gram of grinded powdered forage sample in the crucible and burn the organic content at 550C and then let it to cool down at room temperature. Ash content of each crucible was dissolved by adding 2.5ml of Hydrochloric acid in it. If some ash remained un-dissolved then heat it on the hot plate until complete digestion take place. The prepared solution was filtered by making a final volume of 60ml and kept in plastic bottles for digestion purpose (Siddique et al. 2014)

1.2.8 Animal samples digestion:

For blood digestion, add 5ml of H_2SO_4 and 2 ml of blood plasma in digestion tube. Heat the mixture to dissolve the organic content until yellow color appears. Further 2ml of H_2O_2 was added and heated until it turns into colorless solution. This mixture is cooled, filtrate through paper and store up to a final volume of 60ml in plastic bottles for metal analysis.

For hair and fecal samples, 2g of each sample was weighted for their digestion. The measured sample were mineralized with H_2SO_4 and H_2O_2 (4:1) reagents. After complete digestion, final volume of 60ml was made by adding distilled water. Finally stored for analysis after their filtration

1.2.9 Heavy metal analysis:

All the processed samples are then analyzed through Atomic Absorption Spectrophotometer to find out Zn concentration in all samples. To ensure the quality of results and to precise the analytical techniques, all the apparatus tools are washed with the distilled water and dried in air prior to usage. For the validation of results, the

replicates are analyzed for each sample and compare with the international standards. The concentrations of all the samples are taken in mg/kg (solid sample) or mg/l (liquid sample).

1.2.10 Statistical analysis:

The data of soil, forage and animal are analyzed statistically. The mean concentrations are set up in these samples and next presentation of data was established through SPSS-16 and Two-way ANOVA.

1.3 Indices for pollution exposure assessment:

1.3.1 Bio-concentration factor

The concentrated level of metal uptake in forage tissues was determined by Cui et al. (2004) formula:

BSF = Metal concentration analyzed in forage samples / Metal concentration analyzed in soil samples.

1.3.2 Pollution load index:

It is used to determine the contamination of heavy metals in soil. Its formula given by Liu et al. (2005) is:

PLI = Metal concentration analyzed in soil samples / Metal reference value in soil

The reference value for Zn is 44.19 mg/kg (Singh et al., 2010)

1.3.3 Enrichment factor (EF)

Enrichment factor is calculated by formula (Buat-Menard & Chesselet, 1979)

$$\textbf{Enrichment Factor} = \frac{(\text{Metal concentration in forage/ Metal concentration in soil})\text{sample}}{\text{Metal concentration in plant/ Metal concentration in soil})\text{standard}}$$

1.3.4 Daily intake of metals

The formula to find the daily intake of metals (DIM) is:

$$\textbf{DIM} = \frac{\text{Analyzed metal concentration in forage} * \text{ Conversion Factor} * \text{Daily food intake}}{\text{Average body weight of animals}}$$

For the conversion factor, value is 0.085 (Jan et al. 2010). For cows, daily intake of food is 12kg and average body weight is 600kg whereas the daily intake and average weight of sheep is 1.3kg and 75kg respectively (Johnsen and Aaneby, 2019). For buffaloes, average weight is 550kg and their daily intake is 12.5kg (Briggs and Briggs 1980)

1.3.5 Health risk index

The formula to find the HRI is (USEPA 2002)

HRi = Daily intake of metal / Oral reference dose

Oral reference dose for Zn is 0.3 mg/kg (USEPA, 2010)

3. Results And Discussion

1.4.1 Soil analysis:

The Zn analysis of variance showed that significant effect of treatments ($p<0.001$) was found on Sites while reverse ($p \geq 0.05$) was true for Soil and Site*Soil (Table 1) The Zn concentration in soil samples varied from 18.85-35.59 mg/kg (Table 2). The minimum concentration was observed in soil of *C. decidua* at site Jh-I whereas *P. glaucum* soil showed maximum concentration at the site Aps-III (Figure 1)

Present concentration of zinc in soil was lesser than acceptable limit (300mg/kg) of WHO/FAO (2007). Similarly, Narwal et al. (2013) stated that Zn deficiency was prevailed in almost 50% of the world-soil. The predominant climatic conditions, source of irrigation and the applied practices brought differences in the content of heavy metals. The reported zinc level of Eissa and Almaroai (2019) in soil was 600 mg/kg which was found to be higher than the current results. Pathak et al. (2010) was also recorded the higher level of zinc (211.96 mg/kg) than the present study. The findings of Murtaza et al. (2012) and Orisakwe et al. (2017) were lowered than the present values. In China, Lu et al. (2015) reported that wastewater irrigated sites showed higher concentration of zinc as compared to area that employ ground water source for irrigation which was in accordance with present findings. Normally the Zn concentration in the agricultural soil must be placed between 10-100mg/kg (Mertens and Smolder, 2013). Many factors such as the category of soil (saline, sandy, calcareous in nature), distribution of phosphorous and nitrogen as well as the content of organic matter are associated with insufficient amount of zinc in the soil (Sadeghzadeh, 2013).

1.4.2 Forage Analysis:

According to variance of analysis, non-significant difference ($p \geq 0.05$) for the zinc metal was noticed in the Site, Forage and Site*Forage treatments (Table 1). In collected forage samples, Zn concentration was present in the ranged of 26.42-42.67 mg/kg (Table 2). Waste water irrigated *Z. mays* presented the maximum zinc concentration but ground water irrigated M. sativa presents minimum concentration of this metal (Figure 1)

The present outcomes of zinc were lower than 60 mg/kg value given by WHO/FAO (2007) and recommended that deficient amount of Zn metal was present within the plants. Maximum concentration of Zn was examined in the forage *Z. mays* because it roots were scattered in the top soil which effectively absorb the content of zinc, chromium, nickel and lead from the contaminated soil and accrete them in various parts (Lu et al., 2015). Ogundiran et al. (2012) and Udiba et al. (2013) study evaluated the higher zinc content than present forages. They documented that either plants absorb this metal from the polluted soil directly or the deposition take place in those organs that are exposed to the polluted air. The recorded values were closer to the results of Raja et al. (2015) that utilize waste water to irrigate crops of Faisalabad. In turn, Orisakwe et al. (2017) had reported the lower concentration of Zn ($3.205–6.910$ mg kg^{-1}) in Zamfara state of Nigeria. Normally plants ranged the Zn content between 30-100mg/kg on the basis of dry matter but the toxicity occurs when it surpassed the limit of 300mg/kg (Noulas et al., 2018). Karyotis et al. (2011) acknowledged that the annual plants accrete 4 times higher amount of Zn, in contrast to perennial grazing land.

Table 1 ANOVA table for Zn concentration in soil, forage and animal samples of District Jhang

Zn metal	Soil			Forage		
Source of Variation	Site	Soil	Site*Soil	Site	Forage	Site*Forage
Degree of freedom	2	4	8	2	4	8
Mean Square	192.827***	16.394^{ns}	31.409 ^{ns}	58.369 ^{ns}	65.567 ^{ns}	71.527 ^{ns}
Animal						
Source of Variation	Site	Animal	Source	Site* Animal	Site* Source	Animal *Source	Site*Animal*Source
Degree of freedom	2	2	2	4	4	4	8
Mean Square	18.657***	2.173 ^{ns}	3.029*	0.181 ^{ns}	0.937 ^{ns}	0.938 ^{ns}	0.413 ^{ns}

***,* =Significant at 0.001and 0.05 level; ns= Non-significant

Table 2 Mean concentration of Zn (mg/kg) differed in collected soil and forages of various sites

Forages	Jh-I	Sh-II	Aps-III
Soil Samples			
A. nilotica	27.41±2.26	27.63±3.02	31.83±0.775
C. decidua	18.85±3.12	25.98±1.42	32.83±1.63
Z. mays	24.06±2.94	29.08±2.34	28.04±3.15
M. sativa	26.72±1.52	27.09±3.12	26.89±2.64
P. glaucum	22.32±0.852	28.85±2.29	35.59±0.650
Forage Samples			
A. nilotica	36.51±2.54	33.08±3.01	35.33±3.15
C. decidua	37.90±1.37	38.23±2.70	38.83±1.37
Z. mays	31.93±2.69	38.28±1.45	42.67±1.54
M. sativa	26.42±2.62	27.76±2.56	40.43±1.41
P. glaucum	34.67±2.29	39.41±1.35	29.89±3.02

1.4.3 Animal analysis:

Analysis of variance showed that Site ($p< 0.001$) and Source ($p<0.05$) were significantly varied with the presence of Zn but the opposite trend ($p>0.05$) was analyzed within Animal, Site*Animal, Site*Source, Animal* Source and Site*Animal* Source (Table 1). Zn concentration varied in the animal samples as 0.982-2.85mg/kg (Table 3). The

blood samples were varied in concentration of Zn as 1.37-2.53 mg/l. The minimum concentration was present in the sheep blood that grazed on site Jh-I, while buffalo blood of Aps-III exhibited maximum concentration of Zn metal. The samples of hair were ranged for Zn concentration between 1.25-2.29mg/kg. The maximal concentration of hair was examined in the sheep at Aps-III while minimal concentration was found in the buffalo of Jh-I. The faeces samples were differed from 0.982-2.85 mg/kg in the Zn concentration. The lowest concentration was found in the cow faeces at site Jh-I and the buffalo faeces of Aps-III showed the highest concentration of this metal (Figure 2)

NRC (2007) described that the normal blood level of Zn lies within the range of 0.8-1.20mg/l which was lesser than observed range. In Nigeria, Milam et al. (2017) stated the mean value of Zn in blood samples of sheep as 1.115 mg/l which was found to be lowered but a high level of zinc (20.58 mg/kg) was noticed in Egypt by Diab and Donia (2018). Olmedo- Juarez et al. (2012) study revealed the poorer level of Zn metal with reference to this study. Orisakwe et al. (2017) studied the mean level of Zn in the blood cattle as 2.0400mg/l that was within this range. The depleted level of zinc in blood demonstrate that its poorer absorption take place. The absorption of Zn was hindered in the presence of secondary metabolites (oxalic acid, tannins) because they act as chelating agents. Exposure of cows to the higher Pb concentration can also demote the absorption of Zn (Zhang et al., 2010). Hashem et al. (2017) presented the range of zinc as 85.7-141.1 mg/kg in the animal hairs collected from the cows, buffalo, goat and sheep. Szczegielniak et al. (2012) documented the high Zn-content fluctuated from 124-215 mg/kg of animal hairs assembled from different centres. The results of these two studies were placed beyond the analyzed range. The existing values were lower than the obtained results of various authors (Stoklasova et al., 2020; Pieper et al., 2017). As compared to the observed values, a prominent difference of elevated Zn concentration was studied by Ogundiran et al. (2012) in the cow feces of control (56.5 mg/kg) and contaminated sites (83.6 mg/kg). The enhanced level of Zn also showed correspondence with the findings of Svane and Karring (2019) whereas the Omonona et al. (2019) studied the 0.04-0.17 mg/kg value in the dry season that was identified as a lowered one as compared to examined range of this study. Animal body receives the Zn metal mainly from Soil-Plant system. However its concentration varies with the age factor (Szczegielniak et al., 2014). Normally Zn is added to the feed of animals to enhance its nutritional value (Lu et al., 2015) but too much profusion of its concentration can contaminate the dairy products derived from these animals (Zhang et al., 2012).

Table 3 Mean concentration of Zn differed in various sources of animals (Mean ± S.E)

Animals	Sources	Sampling Locations		
		Jh-I	Sh-II	Aps-III
Cow	Blood	1.68±0.264	1.90±0.163	2.49±0.306
	Hair	1.25±0.177	1.50±0.212	1.77±0.299
	Feces	0.982±0.145	1.71±0.267	1.93±0.303
Buffalo	Blood	2.01±0.257	2.28±0.306	2.53±0.317
	Hair	1.25±0.175	1.88±0.326	1.99±0.283
	Feces	1.04±0.183	2.11±0.321	2.85±0.339
Sheep	Blood	1.37±0.158	1.66±0.333	2.30±0.336
	Hair	1.35±0.296	1.75±0.258	2.29±0.270
	Feces	1.18±0.205	1.99±0.327	2.12±0.293

1.4.4 Bio-concentration factor

The observed BCF values for Zn were varied from 0.840 to 2.01 (Table 4). In the present study, the *C. decidua* exposed to the maximum concentration of zinc at Jh-I while the minimum value was present in the *P. glaucum* of Aps-III.

Alrawiq et al. (2014) stated that the observed BCF 1 showed that the forages accumulated the absorbed Zn content in their tissues. These recorded values were lower than the detected amount (0.10-0.84) of Balabanova et al. (2015). On the other hand, Mahmoud and Ghoneim (2016) depicted the higher BCF values of this metal as 1.32-2.82 in the Zefta drain of Egypt.

Table 4 BCF, PLI and EF of Zn metal differed in the various sites of District Jhang

Forages	Jh-I	Sh-II	Aps-III
BCF			
A. nilotica	1.33	1.2	1.11
C. decidua	2.01	1.47	1.18
Z. mays	1.327	1.32	1.52
M. sativa	0.989	1.02	1.5
P. glaucum	1.55	1.37	0.84
PLI			
A. nilotica	0.62	0.625	0.72
C. decidua	0.427	0.588	0.743
Z. mays	0.544	0.658	0.635
M. sativa	0.605	0.613	0.609
P. glaucum	0.505	0.653	0.805
EF			
A. nilotica	0.592	0.532	0.493
C. decidua	0.894	0.654	0.526
Z. mays	0.59	0.585	0.677
M. sativa	0.44	0.456	0.668
P. glaucum	0.691	0.607	0.373

1.4.5 Pollution load index

The PLI results for Zn metal ranged from 0.427-0.805 in the soil samples (Table 4). The minimum value was practiced in the *C. decidua* of ground watered soil while the maximum value was represented in the *P. glaucum* cultivated at waste watered site.

Overall, the values of pollution load index were observed to be less than 1 which indicated that all the sites are unpolluted (Harikumar et al., 2009). Singh et al. (2010) reported the reference content of Zn in soil as 44.19 which were seemed to be higher than the present range. Similarly, Bao et al. (2014) also studied the higher PLI values (1.03-1.14) in the soil that is irrigated with the sewage water for 40 years. The value reported by Ezemokwe et al. (2017) was 0.05 which observed to be lowered than the observed concentration.

1.4.6 Enrichment factor

The zinc outcomes for enrichment factor were present between 0.373-0.894 mg/kg (Table 4). The Jh-I site exhibited the maximum enrichment in the *C. decidua* fodder while the minimum EF was found in the *P. glaucum* of Aps-III.

Alghobar and Suresha (2015) declared the values of EF in the WW (0.67) and TWW (0.80) that were lower than the present range. According to Barbieri (2016), the lowered values (EF<2) means that insufficient enrichment of zinc take place. As compared to present results, a higher level of Zn in Giza Governorate (1.18-1.71) and Sudan (37.9&12.1) was described by Sherif et al. (2015) and Taha et al. (2013) respectively.

1.4.7 Daily intake of metal & Health risk index

The Zn daily intake varied from 0.039 to 0.082 mg/kg/day. Minimum intake was studied in the sheep that are feed on the *M. sativa* of Jh-I site while the buffaloes of Aps-III perceive the maximum concentration by grazing on the *Z. mays***.** The HRI value for Zn was amounting from 0.130 to 0.275 mg/kg/day. Maximum value of HRI was analyze in the buffaloes browse on the waste water irrigated forages while the minimum level was assessed in the sheep of Jh-I (Table 5).

Present values of daily intake were lowered than the range (0.041-0.115mg/kg/day) found by Ahmad et al. (2020). The obtained Zn concentration was greater in response to DIM values (0.039-0.769mg/kg/day) suggested by Nadeem et al. (2020) at different waste water irrigated sites. When the present HRI values for Zn were related with the work of these researchers, it can be concluded that the higher zinc range was reported by Ahmad et al. (2020) (0.19-0.72 mg/kg/day) and Nadeem et al. (2020) (0.13-2.67 mg/kg/day) in the contaminated sites of Sargodha region. Although, there was no health danger associated with the animals by browsing on these contaminated sites because HRI values was less than unity (USEPA, 2002).

Table 5 Daily intake and health risk assessment of Zn metal in animals in various sites of District Jhang

Daily intake of metal (DIM)									
Forages	Cow			Buffalo			Sheep		
	Jh-I	Sh-II	Aps-III	Jh-I	Sh-II	Aps-III	Jh-I	Sh-II	Aps-III
A. nilotica	0.062	0.056	0.06	0.071	0.064	0.068	0.054	0.049	0.052
C. decidua	0.064	0.065	0.066	0.073	0.074	0.075	0.056	0.056	0.057
Z. mays	0.054	0.065	0.073	0.062	0.074	0.082	0.047	0.056	0.063
M. sativa	0.045	0.047	0.069	0.051	0.054	0.078	0.039	0.041	0.06
P. glaucum	0.059	0.067	0.051	0.067	0.076	0.058	0.051	0.058	0.044
Health risk index (HRI)									
A. nilotica	0.207	0.187	0.200	0.235	0.213	0.228	0.179	0.162	0.174
C. decidua	0.215	0.217	0.220	0.244	0.246	0.250	0.186	0.1877	0.191
Z. mays	0.181	0.2169	0.242	0.206	0.247	0.275	0.157	0.1879	0.210
M. sativa	0.150	0.157	0.229	0.170	0.179	0.260	0.130	0.136	0.199
P. glaucum	0.196	0.223	0.169	0.223	0.254	0.192	0.170	0.194	0.147

4. Conclusion

This research concluded that zinc concentration in the soil was significantly varied within sites. Maximum concentration of this metal in forages and soil was examined mainly at wastewater irrigated site but lesser than WHO/FAO limits. Animal samples absorb the zinc concentration beyond their standards. Thus, zinc supplements in the animal feed should be avoided. Results from bio-concentration index determined that a large amount zinc metal was transferred from the soil to forage tissues due to waste water irrigation. Therefore, proper manageable use of waste water can be consumed by local farmers for irrigation purpose. Although wastewater is a power supply of organic matter that enhances soil fertility but the presence of heavy metals and soluble salts can contaminate the food chain. On the other hand, heavy metals are considered as constant pollutants of environment as they don't undergo degradation via any microbial and chemical activity. That's why recommended that waste water effluent must be treated before their appliance on farming lands as heavy metals are present in it and these metal contaminated forages effect the health of grazing animals.

Declarations

Ethics declarations

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The authors declare that the manuscript has not been published previously.

Consent to participate: All authors voluntarily to participate in this research study.

Consent to publish: All authors consent to the publication of the manuscript.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

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Author Contributions: ZIK and KA supervised the study. FC, FGM and JM were responsible for writing the manuscript. AA, MN, SM1 was responsible for conducting the experiments and the data analysis. ISM , MUFA, SM2 and MN were responsible for analyzing and interpreting the data. All authors read and approved the final manuscript**.**

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Figures

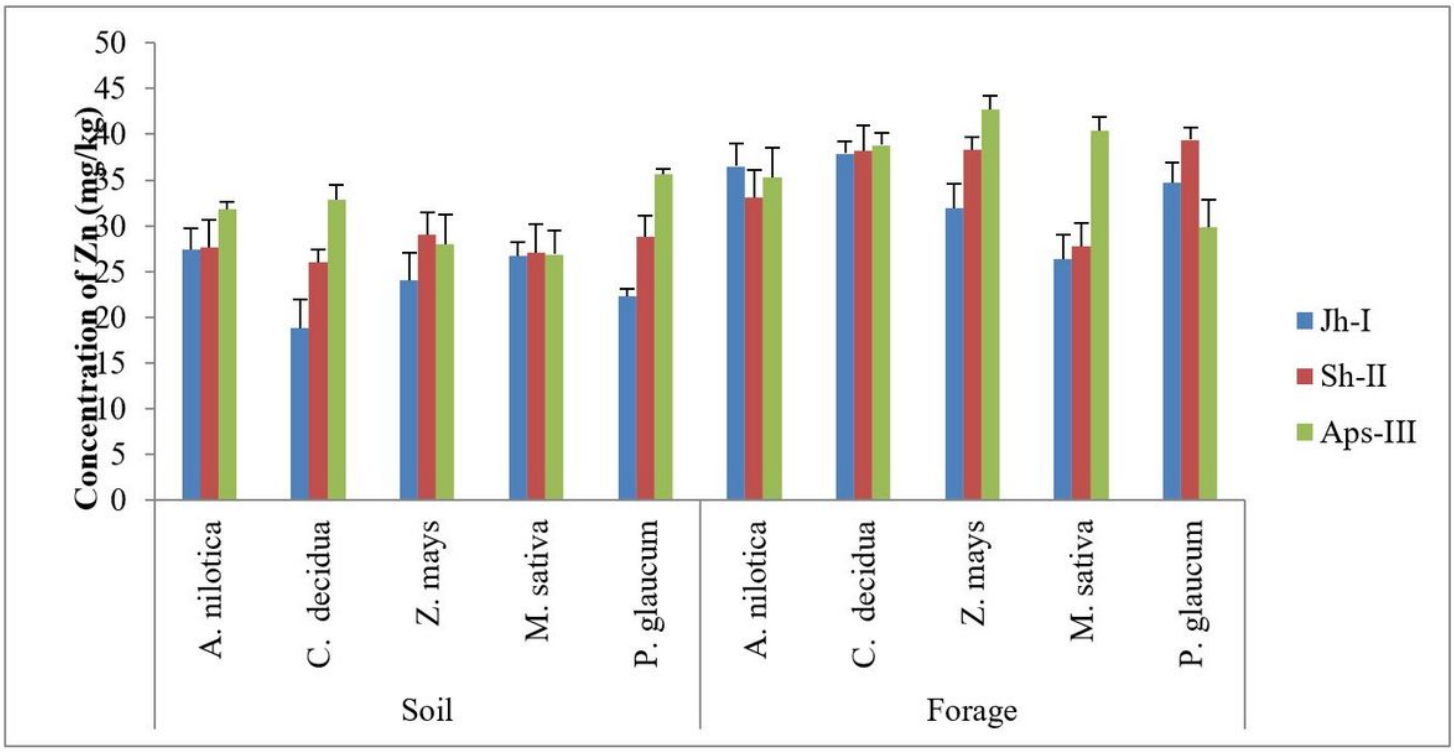

Figure 1

Variations of Zn concentration in soil and forage samples at various sites of District Jhang

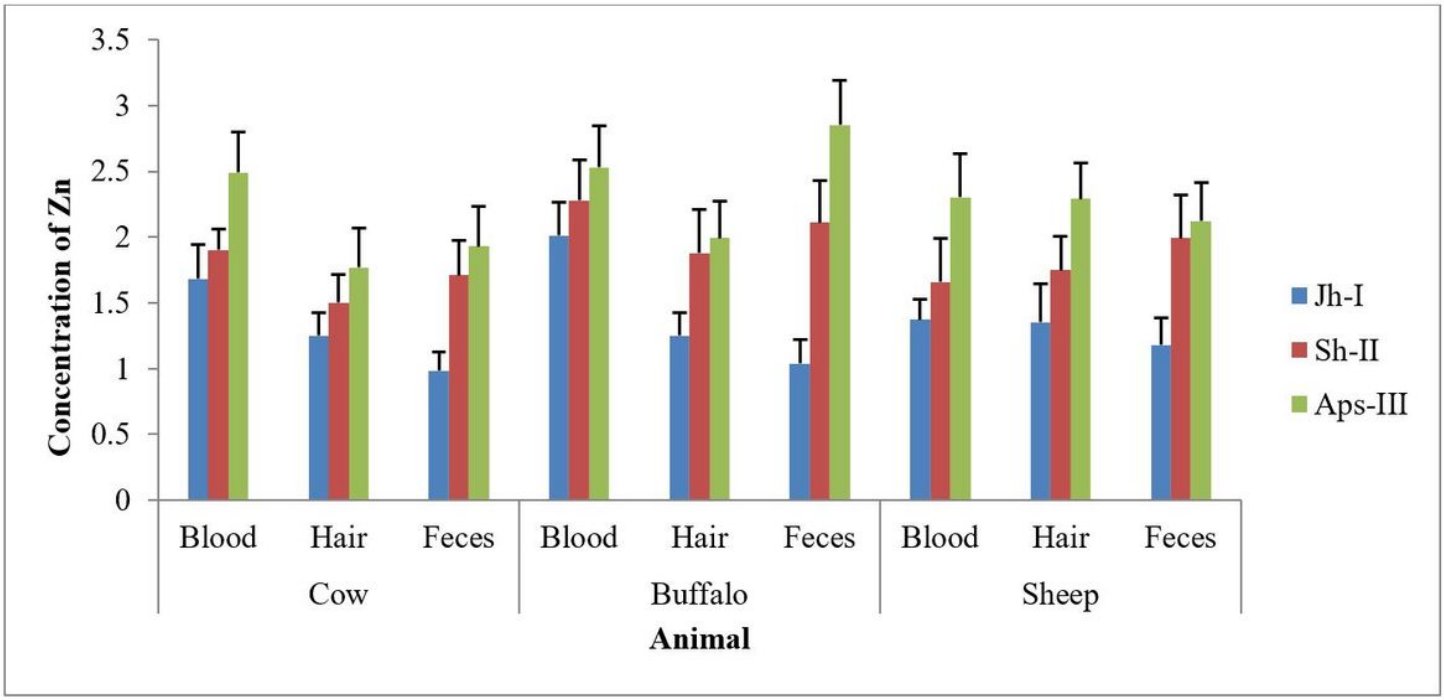

Figure 2

Variation of Zn concentration in different animal sources of District Jhang