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Research Article

Assessment of Zn and Pb in buffaloes fed on forages irrigated with wastewater in Sahiwal, Sargodha, Pakistan

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Abstract

This study was designed to find the Pb and Zn concentration in forages, soil and blood samples that were taken from polluted sites. Sampling was performed in two seasons from five sites in tehsil Sahiwal. Atomic Absorption Spectrophotometer (AAS) was used for estimation of metal concentration. The samples at site-V had higher values as compared to other sites. Zn was found lower in blood while higher in soil and forages while lead had positive and non-significant correlation in soil, forage and blood due to its accumulation in lower concentration. The value for pollution load index was lower than standard value for both metals. Transfer had lower values indicated that antagonistic effect of metals present in soil or the crops are resistant to metals. Daily intake of both metals had lower values. Health risk index values were higher for both metals than the standard value which indicates that both these metals are not good for animals grazing on sampling sites. EF values for both metals were in order of Pb>Zn but lower than standard. The result from present study showed that there is increase in the concentration of metal at sampling sites due to waste water irrigation.

Keywords: Blood plasma; Enrichment factor; Forage; Lead; Pollution load index; Zinc

Introduction

Heavy metals can be distinct as with density higher than 5 mg/ml **[1]** and include molybdenum, lead, nickel, copper, arsenic, zinc, cadmium, vanadium, chromium, strontium, aluminum, cobalt and rare earth metals are also included in it **[2]**. They are nontoxic like Se and they are only toxic when their concentration exceed a limit that makes them effective and they are only termed heavy metals when their concentration cross a limit and poses toxic effects **[3, 4]**. Metals like Zn, Cu, Co, Mo and Mn are essential nutrients for growth of plants if taken in limit while Ar, Cd and Cr are carcinogenic compounds **[5]**. Streams going along urban focuses are utilized for water system of the vegetables developed on their banks. Streams water have frequently been accounted for to be infected by overwhelming metal and the greater part of these terrains are polluted with substantial chemicals through mechanical effluents, slime, sewage and vehicles pollution **[6-10]**. Wind direction, rainfall, characteristics of highways, seeded strip, the waste emitted from the factories and the wastewater irrigation are the major factors that increase the metal concentration **[11, 12]**. The uptake and accumulation of overwhelming chemicals in fodders is impacted by means of many factors, for example, atmosphere, humidity conditions, centralizations of irresistible nature of soil, chemicals in soils, intensity of plants and season of collection **[13, 14]**. Heavy metals like Zinc, Copper, Lead and Mercury have been found to cause undesirable impacts on the root system of forage plants **[15]**. Zinc is a fundamental supplement in people and creatures and is vital for the capacity of countless. Zinc takes part in different enzymes work, in carbon fixation and in synthesis of protein **[16]**.

The aim of research was to analyze Zinc and Lead absorption in soil and various varieties of forage crops irrigated with contaminated water, in relation to the requirements of buffaloes consuming those forages.

Study area

Experiment was conducted in the Tehsil Sahiwal of Sargodha division. Sahiwal is located at a distance of 37 km from Sargodha Jhang road and is almost 5 km away from river Jhelum. Samples were taken from five sites. All these sites receive wastewater irrigation.

Sample collection

Samples of soil and forages along with three replicates were collected from the experimental sites. These were then sealed in labeled paper bags .Forage sample contained three plant species *Trifolium alexanderium (Barseem)*, *Zea mays* (corn), *Sorghum bicolor* (Millet). These samples were collected from the same site from where soil sample were taken. Samples were dried in air and then in oven for 72ºC for 48 hours to attain constant dry weight. Fifteen samples of buffalo blood were taken along with three replicates. After collection samples were centrifuged at 2500 rpm for 2 minutes to obtain plasma. The plasma was transported to the laboratory in a cool box and kept frozen before experiment at -20°C.

Sample preparation for analysis

Wet digestion method was used for digestion. HNO_3 (2 ml) and H_2O_2 (4 ml) were used for digestion. 2 grams of soil and forage were taken and heated for 30min and then cooled. H_2O_2 were added in this sample until a colorless solution was obtained. Distilled water was used to make the volume 50ml. Determination of Zn and Pb concentration was performed with the help of an atomic absorption spectrophotometer (Model: AA-6300, Shimadzu, Japan) equipped with a graphite furnace (AA6300 & GFAEXi7i).

Statistical analysis

SPSS was used to find analysis of variance and correlation of data.

Bio concentration factor

It was calculated using Microsoft excel by the following formula:

BCF = mean concentration of metal in fodder/ mean concentration of metal in soil

Enrichment Factor (EF)

"It was determined by the procedure of Caselles *et al.* **[17]** (Table 1).

$EF = [M]_{fodder} / [M]_{Soil} / [M]_{fodder\ S} / [M]_{soil\ S}$

Pollution load index (PLI)

Pollution load index was calculated by the formula given by Liu *et al.* **[23]** (Table 2).

PLI= metal concentration in examined soil/metal concentration in reference soil".

Table 1. Standard concentrations of heavy metals in fodder and soil (mg/kg)

Metal	Co	Cr
Standard soil	44.19	8.15
Standard fodder	0.60	2.00

[18-22].

Table 2. Reference values of heavy metals in soil (mg/kg)

Reference values of heavy metals in soil		
Metals	**Zn**	**Pb**
Reference values	44.19	8.15

[18-20]

Daily intake of metal (DIM)

DIM was calculated by following Sajjad *et al.* [24] (Table 3).

DIM = Cmetal × Dfood intake × C conversion factor⁄ Baverage weight.

Health risk index (HRI)

Health risk index value can be calculated by daily intake of metal and oral reference dose (RfD) Cui *et al.* [30] (Table 4).

Health risk index (HRI) = DIM/ RfD"

Non-significant effect was observed at (p>0.05) at site-I in fodder samples of summer, blood samples of summer and winter and summer samples of soil while significant (>0.001) in all other samples at all sites (Table 5).

Table 3. Tolerable daily intake limit (mg kg-1day-1)

Tolerable daily intake limit		
Heavy Metals	**Zn**	**Pb**
TDI	60	0.214

[21, 22, 24]

Table 4. Oral reference dose for heavy metals (mg/kg/day)

Oral reference dose		
Metal	**Zn**	**Pb**
RfD	0.3	0.0035

[22, 24]

Table 5. Analysis of variance for Cr concentration in soil, fodder and blood samples at different sites

	Site-I	Site-II	Site-III	Site-IV	Site-V
Summer Soil	24.99ns	69.277***	2.016***	29.934**	0.059***
Winter soil	4.624***	3.219***	414.792***	0.162***	4.136***
Summer Fodder	0.000ns	0.013***	7.805***	16.103***	349.149***
Winter Fodder	19.384ns	0.010***	0.001***	264.291***	3.6837***
Summer blood	0.000ns	0.152*	0.035***	10.749***	401.503***
Winter blood	0.056ns	0.000*	15.096*	306.763***	51.494***

*,**,***= Significant at 0.05, 0.01 and 0.001

Concentration of Zn in soil sample

Higher concentration of Zn in soil samples was observed at site-V (18.25-18.84 mg/kg) and lower concentration at site-I (1.375-1.2933 mg/kg) during summer season. Moreki [31] reported lower Zn concentration as compared to the present investigation (Table 6 & Figure 1).

Concentration of Zn in fodder samples

The maximum value of Zn in fodder samples was found at site-V (9.8425-22.431 mg/kg) and the minimum at site-I (1.10-1.95 mg/kg).All values are higher than the PML recommended by FAO/ WHO [21] as 0.6 mg/kg (Table 7 & Figure 2).

Table 6. Zn content in soil samples

Sample	Site-1	Site-2	Site-3	Site-4	Site-5
Soil collected with Summer Fodder					
Zea mays	1.29±0.22	2.106±0.22	13.58±0.22	17.43±0.2	18.84±0.14
Sorghum bicolor	1.683±0.2	6.40±0.28	15.9625±0.2	16.065±0.14	18.995±0.1
Trifolium alaxandrium	1.37±0.14	3.65±0.144	10.262±0.14	18.10±0.14	18.25±0.28
Soil collected with Winter Fodder					
Zea mays	2.116±0.2	1.301±0.22	14.19±0.288	16.3±0.022	20.72±0.144
Sorghum bicolor	1.13±0.14	1.5250±0.28	2.0750±0.28	16.01±0.28	18.79±0.22
Trifolium alaxandrium	1.07±0.28	1.1141±0.22	1.4058±0.22	15.95±0.22	17.627±0.28

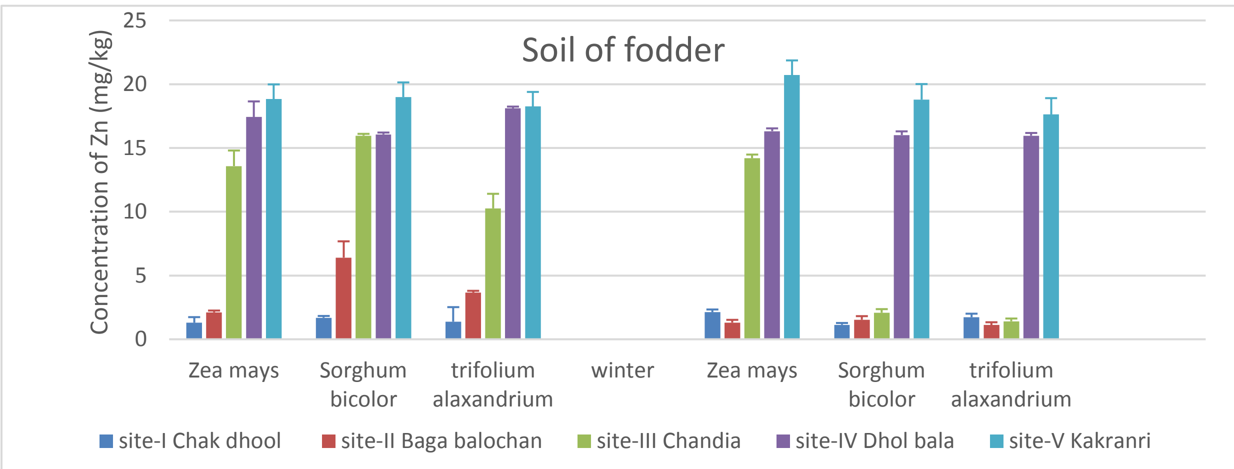

Figure 1. Fluctuations in mean concentration of Zn in soil samples at different sites during summer and winter season

Table 7. Zn content in fodder samples

Sample	Site-1	Site-2	Site-3	Site-4	Site-5
Summer Fodder					
Zea mays	1.95±0.144	1.608±0.22	2.955±0.144	2.0250±0.918	21.552±0.28
Sorghum bicolor	1.1175±0.144	1.90±0.144	2.1575±0.144	2.519±0.22	3.8416±0.22
Trifolium alaxandrium	1.100±0.1443	1.1875±0.144	2.165±0.144	3.0875±0.144	6.7858±0.22
Winter Fodder					
Zea mays	1.1066±0.83	2.132±0.144	6.36±2.721	3.982±0.144	9.842±0.144
Sorghum bicolor	1.082±0.144	2.232±0.144	2.76±2.501	2.941±0.699	22.431±0.22
Trifolium alaxandrium	1.625±0.144	2.137±0.144	1.45±0.144	5.335±0.144	10.20±0.144

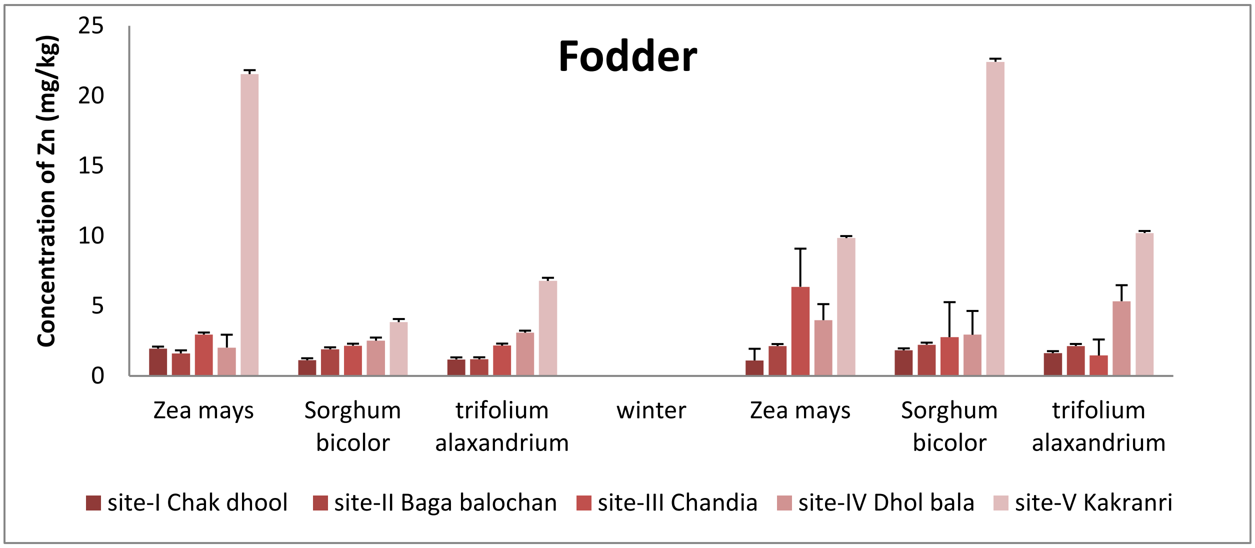

Figure 2. Fluctuations in mean concentration of Zn in fodder samples at different sites during summer and winter season

Concentration of Zn in blood samples

The maximum value of Zn in summer blood samples was found at site-V (1.97-5.3575mg/l) and the minimum at site-I (1.1575-0.1750mg/l). In winter the maximum concentration was found at site-V (4.495-19.683mg/l) and the minimum at site-I (1.75-1.3472mg/l) (Table 8 & Figure 3).

Table 8. Mean concentration of Zn in blood samples (mg/l)

S. No.	Site-I	Site-II	Site-III	Site-IV	Site-V
summer blood					
1	1.1575±0.144	1.70±0.1443	1.285±0.144	1.4875±0.144	5.3575±0.83
2	1.1750±0.1443	1.205±0.144	1.205±0.144	1.4875±0.1487	2.20±0.1443
3	1.160±0.144	1.2050±0.144	1.425±0.144	1.425±1.144	1.970±0.1443
Winter blood					
4	1.75±0.144	1.160±0.144	1.2875±0.144	1.585±0.144	4.4950±1.933
5	1.3472±0.149	1.1550±0.14	1.0425±0.14	1.870±0.144	19.683±0.02
6	1.200±0.144	1.1450±0.14	1.969±0.22	2.81750±0.14	9.095±0.144

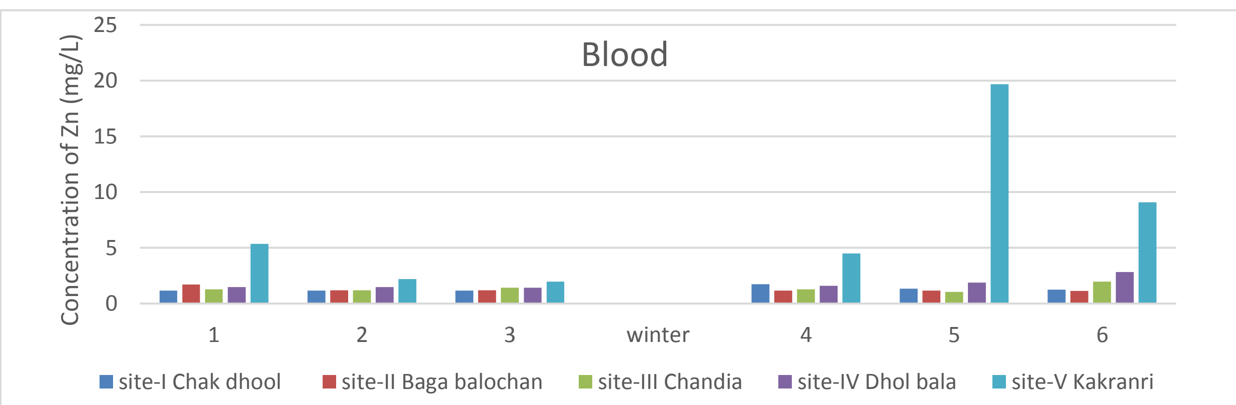

Figure 3. Fluctuations in mean concentration of Zn in blood samples at different sites during summer and winter season

Correlation

Significant correlation was observed for *Sorghum bicolor* and *Trifoloum alaxandrium* from fodder to blood samples of both seasons and for *Zea mays* in only summer. Non-significant and positive correlation was observed for *Z. mays* from soil to blood in summer (Table 9).

Pollution load index

Zn was found to be high for site-V. But at all the sites value of PLI was not more than 1 which indicates the lower contamination of the sites (Table 10).

Bio-concentration factor

Zn was observed higher at site-V for *T. alaxandrium* in summer season and lower for *Z. mays* at site-I for summer season (Table 11).

Daily intake of metal

Zn had value higher for *Z. mays* at site-V and lower for *Z. mays* at site-I. DIM were found higher at site-V (Table 12).

Table 9. Correlation between soil-fodder-blood

Correlation Coefficient						
	Zea mays		***Sorghum bicolor***		***Trifolium alaxandrium***	
	Soil-fooder	Fodder-Blood	Soil-Fodder	Fodder-blood	Soil-fooder	Fodder-blood
Summer	0.571ns	0.988**	0.864	0.930*	0.809	0.987**
Winter	0.882*	0.772	0.721	0.999**	0.913*	0.957*

** Correlation is significant at the 0.01 level (2-tailed)

Table 10. Pollution load index for Zn

Pollution load index						
Site	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Site-I	0.0292	0.047	0.038	0.025	0.031	0.039
Site-II	0.047	0.029	0.144	0.034	0.082	0.025
Site-III	0.307	0.321	0.361	0.046	0.232	0.031
Site-IV	0.394	0.3692	0.363	0.425	0.409	0.39
Site-V	0.426	0.468	0.429	0.425	0.412	0.39

Table 11. Bio concentration factor for Zn

Bio concentration factor						
Site	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**summer**	**Winter**
Site-I	0.15	0.52	0.66	0.20	0.85	0.94
Site-II	0.763	0.47	0.29	0.57	0.32	0.37
Site-III	0.21	0.44	0.13	0.18	0.21	0.33
Site-IV	0.11	0.24	1.50	1.33	0.17	1.03
Site-V	1.14	1.63	1.61	1.19	1.91	1.46

Table 12. Daily intake of metal for Zn

Daily intake of metal						
Site	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Site-I	0.06	0.039	0.0399	0.065	0.042	0.058
Site-II	0.057	0.076	0.067	0.079	0.042	0.076
Site-III	0.105	0.227	0.077	0.098	0.07	0.052
Site-IV	0.072	0.142	0.089	0.105	0.110	0.190
Site-V	0.769	0.351	0.13	0.801	0.24	0.364

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Health risk index

HRI for Zn was found higher for *S. bicolor* in summer and for *Z. mays* in winter at site-V. Lower health risk index value was found at site-I for *Z. mays* in winter season (Table 13).

Enrichment factor

Enrichment factor was observed higher for site-V for *T. alaxandrium* in winter and lower for *Z. mays* at site-I for summer season (Table 14).

Table 13. Health risk index for Zn

Health risk index						
Site	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Site-I	0.23	0.13	0.13	0.21	0.14	0.19
Site-II	0.19	0.25	0.22	0.26	0.14	0.25
Site-III	0.35	0.75	0.25	0.32	0.25	0.17
Site-IV	0.24	0.47	0.29	0.35	0.36	0.63
Site-V	2.56	1.17	0.45	2.67	0.807	1.21

Table 14. Enrichment factor for Zn

Enrichment factor						
Site	***Z. mays***		***S. bicolor***		***T.alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Site-I	0.0015	0.007	0.009	0.0027	0.00504	0.0045
Site-II	0.0033	0.0064	0.004	0.00212	0.004	0.007
Site-III	0.0029	0.0060	0.00183	0.0181	0.00286	0.0140
Site-IV	0.02	0.01	0.019	0.0024	0.00231	0.012
Site-V	0.015	0.02	0.021	0.0162	0.0116	0.026

Lead (Pb)

All the samples showed highly significant value at all the sites in both seasons (<0.001) (Table 15).

Concentration of Pb in soil samples

The values for summer soil at site-V were higher (0.0825-0.0950 mg/kg) and at site-I was lower (0.1858-0.3075 mg/kg) and the values for winter higher at site-V (0.875-0.95 mg/kg) and lower at site-I (0.040-0.235mg/kg) (Table 16 & Figure 4).

Table 15. Analysis of variance for Pb concentration in soil, fodder and blood samples at different sites

	Site-I	**Site-II**	**Site-III**	**Site-IV**	**Site-V**
Summer Soil	0.000***	0.000***	0.016***	0.055***	0.000***
Winter soil	0.001***	0.000***	0.002***	0.003***	0.000***
Summer Fodder	0.005**	0.001***	0.000***	0.000***	0.001***
Winter Fodder	0.000***	0.002***	0.000**	0.020***	0.000***
Summer blood	0.002***	0.001***	0.002***	0.011***	0.000*
Winter blood	0.021***	.000***	.002***	.000***	.000***

*,**,***= Significant at 0.05, 0.01 and 0.001

Table 16. Mean concentration of Pb in soil samples (mg/kg)

	Site-I	Site-II	Site-III	Site-IV	Site-V
Soil samples collected in summer					
Zea mays	0.18±0.244	0.55±0.244	0.450±0.244	1.80±0.244	1.950±0.244
Sorghum bicolor	0.320±0.244	0.0575±0.244	0.3150±0.244	1.650±0.244	1.900±0.244
Trifolium alaxandrium	0.307±0.244	0.04±0.244	0.310±0.244	1.62±0.244	1.82±0.244
Soil samples collected in winter					
Zea mays	0.19±0.244	0.350±0.244	0.550±0.244	1.82±0.244	1.95±0.244
Sorghum bicolor	0.23±0.244	0.325±0.244	0.550±0.244	1.62±0.244	1.90±0.244
Trifolium alaxandrium	0.40±0.244	0.242±0.244	0.450±0.244	1.62±0.244	1.87±0.244

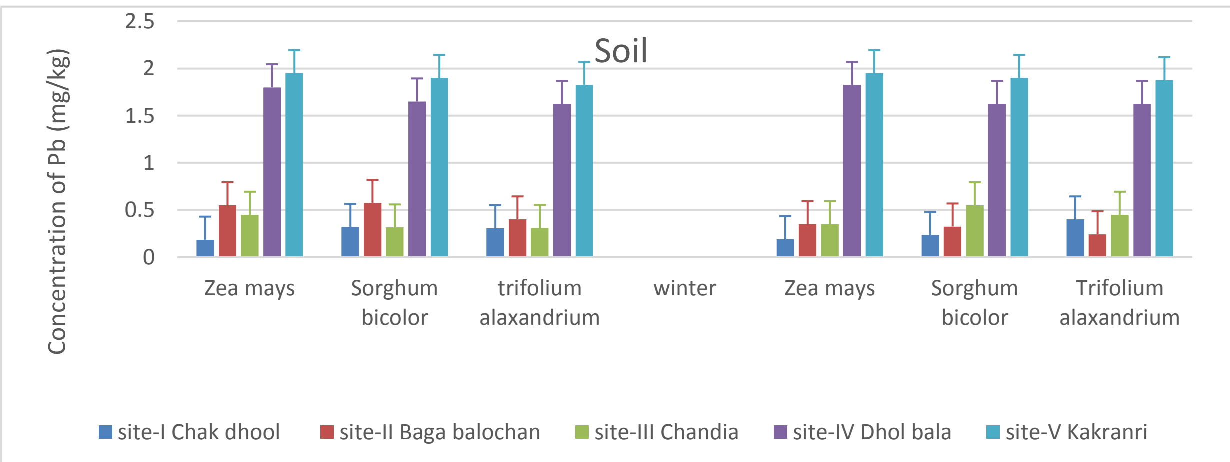

Figure 4. Fluctuations in mean concentration of Pb in soil at different sites during summer and winter season

Concentration of Pb in fodder samples

The maximum value of Pb was found in summer at site-v (1.290-1.320 mg/kg) and the minimum at site-I (1.075-1.13 mg/kg). The same concentration effect of sites were found during winter season values are (2.2375-2.280 mg/kg) and (1.115-1.1175 mg/kg) for site-V and site-I respectively (Table 17 & Figure 5).

Concentration of Pb in blood samples

Higher concentrations of Pb in blood samples were found at site-V (2.1975-2.23 mg/kg) and lower values were observed at site-I (1.1075-1.135 mg/kg) during summer season. In winter season, higher concentrations were found at site-V (2.2050-2.2075 mg/kg) and lower at site-I (1.0325-1.1475 mg/kg) (Table 18 & Figure 6).

Correlation coefficients of fodder samples

Positive and non-significant correlation was observed for all crops from soil to fodder in both seasons. Significant correlation was observed for fodder to blood samples of *Sorghum bicolor* in both seasons and only winter samples of I Zeay mays and *Trifolium alaxandrium* (Table 19).

Table 17. Mean concentration of Pb in fodder samples (mg/kg)

	Site-I	Site-II	Site-III	Site-IV	Site-V
Fodder samples collected in summer					
Zea mays	0.750±0.244	1.1500±0.244	1.1450±0.244	1.165±0.244	1.32±0.244
Sorghum bicolor	1.110±0.244	1.145±0.244	1.135±0.244	1.16±0.244	1.31±0.24
Trifolium alaxandrim	1.130±0.244	1.135±0.244	1.130±0.244	1.145±0.244	1.29±0.244
Fodder samples collected in winter					
Zea mays	1.11±0.244	1.140±0.244	1.167±0.244	1.23±0.244	2.28±2.244
Sorghum bicolor	1.11±0.244	1.132±0.244	1.157±0.244	1.14±0.244	2.24±0.244
Trifolium alaxandrium	1.11±0.244	1.137±0.244	1.140±0.244	1.13±0.244	2.23±0.244

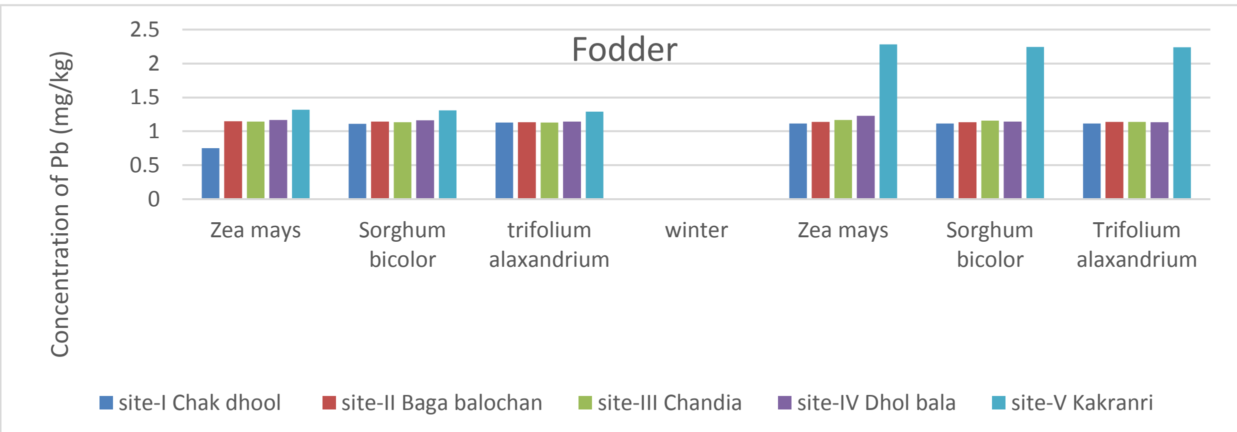

Figure 5. Fluctuations in mean concentration of Pb in Fodder at different sites during summer and winter season

Table 18. Mean concentration of Pb in blood samples (mg/l)

	Site-I	Site-II	Site-III	Site-IV	Site-V
Blood samples collected in summer					
1	1.135±0.144	1.1825±0.144	1.1875±0.144	1.1950±0.144	2.230±0.144
2	1.120±0.144	1.170±0.144	1.1875±0.144	1.1950±0.144	2.2175±0.144
3	1.1075±0.144	1.1575±0.144	1.180±0.144	1.1875±0.144	2.1975±0.144
Blood samples collected in winter					
4	1.325±0.144	1.180±0.144	1.1675±0.144	1.190±0.144	2.2125±0.144
5	1.375±0.144	1.170±0.144	1.170±0.144	1.1850±0.144	2.2050±0.144
6	1.1475±0.144	1.155±0.144	1.1575±0.144	1.1775±0.144	2.2075±0.144

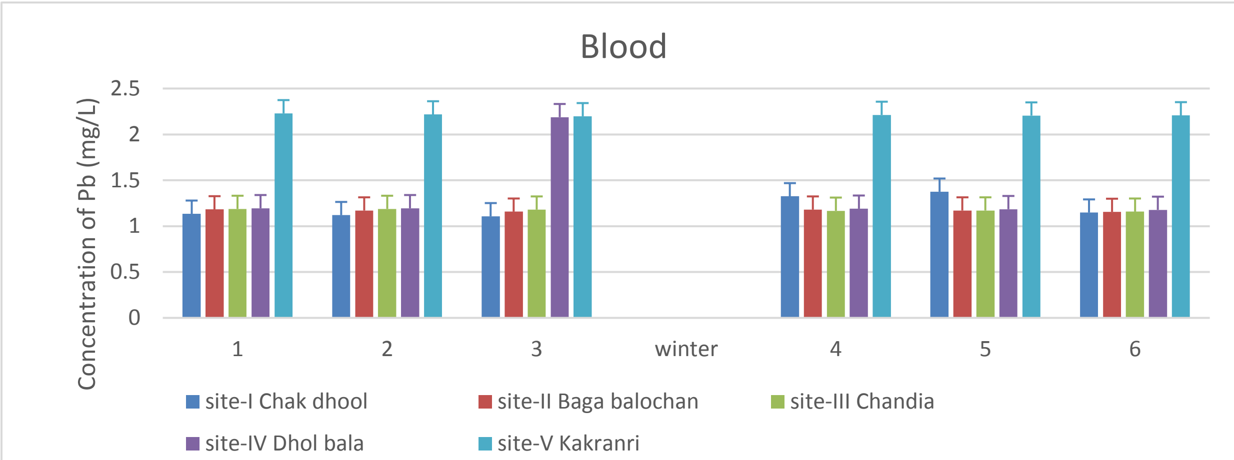

Figure 6. Fluctuations in mean concentration of Pb in fodder at different sites during summer and winter season

Table 19. Correlation between soil-fodder-blood

	Correlation Coefficient					
	Zea mays		***Sorghum bicolor***		***Trifolium alaxandrium***	
	Soil-fodder	Fodder-Blood	Soil-Fodder	Fodder-blood	Soil-fooder	Fodder-blood
Summer	.718	.603	.818	.982**	.742	.680
Winter	.708	.979**	.710	.975**	.698	1.000**

** Correlation is significant at the 0.01 level (2-taile

Pollution load index

Higher PLI value was observed at site-IV for *T. alaxandrium*in summer season and lower value was observed at site-I for *T. alaxandrium* in winter (Table 20).

Bio-concentration factor

Z. mays had higher values at site-V in summer and lower value was observed at site-I for *S. bicolor* in summer season (Table 21).

Daily intake of metal

Daily intakes of metal value for Pb were higher at site-V for all the samples and lower at site-I for the summer samples *of Z. mays* (Table 22).

Health risk index

HRI value was found the highest for winter samples of *Z. mays* and *S. bicolor*. Second highest HRI was observed for *T. alaxandrium*. The lowest HRI was observed in the summer samples of *Z. mays* (Table 23).

Enrichment factor

Highly enriched samples were observed for *T. alaxandrium* at site-V in winter season. The lower value of enrichment was observed for summer samples of *Z. mays* (Table 24).

Table 20. Pollution load index for Pb

	Pollution load index					
Site	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Site-I	0.022	0.023	0.039	0.028	0.037	0.0049
Site-II	0.067	0.042	0.070	0.039	0.049	0.029
Site-III	0.055	0.067	0.03	0.06	0.038	0.055
Site-IV	0.22	0.22	0.202	0.199	0.325	0.19
Site-V	0.23	0.23	0.23	0.233	0.22	0.23

Table 21. Bio concentration factor for Pb

Bio concentration factor						
Site	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Site-I	0.70	0.67	0.66	0.68	0.64	0.706
Site-II	0.69	1.19	1.9	0.704	0.704	1.181
Site-III	2.54	2.12	1.16	2.104	2.83	2.53
Site-IV	2.79	3.25	3.59	3.484	3.64	2.090
Site-V	5.81	4.03	4.74	4.69	3.67	3.46

Table 22. Daily intake of metal for Pb

Daily intake of metal						
Site	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Site-I	0.0022	0.0033	0.0034	0.0034	0.0034	0.0033
Site-II	0.0034	0.0034	0.0035	0.0034	0.0034	0.0036
Site-III	0.0035	0.0034	0.0034	0.0035	0.0034	0.0035
Site-IV	0.0035	0.0035	0.0034	0.0035	0.0035	0.0034
Site-V	0.0040	0.0039	0.0040	0.003	0.0070	0.0068

Table 23. Health risk index

Health risk index						
Site	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**summer**	**Winter**
Site-I	0.65	0.96	0.98	0.96	0.97	0.97
Site-II	0.99	0.99	0.98	0.98	0.98	0.99
Site-III	0.99	0.99	0.98	0.99	0.98	0.99
Site-IV	0.99	1.012	1.003	1.066	0.99	0.99
Site-V	1.144	1.137	1.12	1.97	1.95	1.94

Table 24. Enrichment factor for Pb

Enrichment factor						
Sites	***Z. mays***		***S. bicolor***		***T. alaxandrium***	
	Summer	**Winter**	**Summer**	**Winter**	**summer**	**Winter**
Site-I	0.15	0.16	0.16	0.17	0.17	0.17
Site-II	0.17	0.28	0.48	0.28	0.51	0.29
Site-III	0.62	0.52	0.69	0.51	0.79	0.62
Site-IV	0.99	0.85	0.90	0.88	0.85	0.89
Site-V	0.166	0.171	1.42	1.164	1.15	6.85

Discussion

High values of Zn in soil samples were observed at site-V (18.25-18.84 mg/kg) and lower at site-I (1.375-1.2933 mg/kg) during summer season. The Zn values of the collected soil samples were lower than the permissible limit recommended by WHO **[21]** as 44.19mg/kg. Also, Moreki **[31]** reported lower Zn concentration as compared to the present investigation.

The maximum amount of Zn in fodder samples was found at site-V (9.8425-22.431 mg/kg) and the minimum at site-I (1.10-1.95 mg/kg).Values are higher than the PML recommended by FAO/ WHO **[21]** as 0.6 mg/kg.

Zinc is an important element for living Ciba *et al.* [1]. It plays an important part in biochemical reactions, gene copying, multiplication of genetic chain, and formation of enzyme substrate complex. The maximum Zn values in blood samples were found between19.683 to 0.175 mg/kg. Zn concentrations in blood samples were lower than permissible limit (60 mg/kg) recommended by USEPA and WHO/FAO [21, 25]. Lead is ranked second most dangerous on the priority list of USEA [26]. Human actions are the main contributor of increase in concentration of lead and cadmium. Emission of these metals in the terrestrial and aquatic environment contributes in local pollution. Pb values in soil samples were higher at site-V (0.0825-0.0950mg/kg) and lower at site-I (0.040-0.235mg/kg). Permissible limit of Pb in soil is 0.3 mg/kg suggested by FAO/WHO [21]. Value of Pb was lower than the permissible limit. This means that this soil is good for the growth of plants.

Higher concentrations of Pb in fodder samples were found in summer at site-V (2.2375-2.280mg/kg) and the lower concentrations at site-I (1.115-1.1175mg/kg).All plant samples were within range of recommended by [18-20] as 2mg/kg except of the site-V. Higher concentration in fodder of winter than soil may be due to the presence of electronic chips and battery reserves in irrigated water [27].

Higher amount of Pb in blood samples were found at site-V (2.1975-2.23 mg/kg) and lower values were observed at site-I. All the blood samples were above the tolerable intake limit given by Food and Nutrition Board, USEPA and WHO/FAO [21, 25, 28] as 0.214mg/kg indicating susceptibility of animals from lead. Increase in age of animal lead to increase in fat and decrease in protein content of meat which cause increase in absorption of metals like Lead and Cadmium [29, 32].

Pollution load index (PLI)

PLI value greater than 1 show that these metals can cause natural hazard and researched destinations require appropriate checking for controlling metal develop in the soil [33-36]. PLI values for Zn and Pb was lower than 1 which showed that this soil is uncontaminated.

Bioconcentration factor

Higher values were observed for *T. alaxandrium* and lower for *Z. mays* for Zn and for Pb high for *Z. mays* and low for *S. bicolor*.

Daily intake of metal

The value of Zn was lower than tolerable daily intake 60 [21, 22, 25, 37, 38]. Higher value of intake was observed for *T. alaxandrium* and lower for *Z. mays*

For Pb was determined by using formula of Sajjad *et al.* [24]. Tolerable daily intake of metal for lead is 0.214 in present investigation all the values were lower than tolerable limit.

Health risk index

HRI value at Site-V was higher for both Zn and Pb. Highest values were observed for summer samples of *Z. mays* at winter samples of *S. bicolor* at site-V

Enrichment factor

It is the retention of heavy metal in soil to its reference value [1, 39-41]. Lower value of EF indicates more retention of metal in soil while high value of EF indicates less retention in soil. Zn had lower values for EF while Pb had higher value.

Conclusion

Farmers in Pakistan utilized wastewater as irrigation to save money. Wastewater is heavily contaminated with toxic metals and contains surplus amount of micronutrients required for the growth of plants. The results revealed that the mean levels of Zn and Pb were higher in waste water but were within acceptable range in forages and blood of buffaloes fed on such type of forages in Tehsil Sahiwal, Sargodha. However, bioaccumulation factor for Pb was quite high in forages and blood of buffaloes as compared to Zn. The values of HRI for Zn and Pb were higher at some sites. Prolonged use of wastewater for irrigation may cause issues as elevated level of metals accumulation which might reach toxic levels for humans.

Authors' contributions

Conceived and designed the experiments: Zafar Iqbal Khan and Kafeel Ahmad
Performed the experiments: Maria Ghazal
Analyzed the data: Humayu Bashir and Muhammad Nadeem
Contributed materials/ analysis/ tools: Mubeen Akhter, Pervaiz Akhter, Ijaz Rasool Noorka, Aima Iram Batool, Saqib Rehman, Ammara Ainee and Ifrah Khan
Wrote the paper: Maria Ghazal and Muhammad Nadeem

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