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Original Article

Evaluation of potential ecological risk assessment of toxic metal (lead) in contaminated meadows in the vicinity of suburban city: soil vs forages vs livestock

Avaliação de risco ecológico em potencial avaliação de metal tóxico (chumbo) em prados contaminados nas proximidades da cidade suburbana: solo x forragens x gado

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Abstract

Heavy metal toxicity is becoming an increasing concern for environmental, human and animal health. The current research analyzed the lead (Pb) contamination in the food chain under three different irrigation sources (ground, canal, and wastewater). Soil, plant and animal samples were collected from the Jhang district of Pakistan and processed with an atomic absorption spectrophotometer. Lead concentration varied in the samples as: 5.22-10.73 mg/kg in soil, 2.46-10.34 mg/kg in forages and 0.736-2.45 mg/kg in animal samples. The observed lead concentration in forage and animal blood samples was higher than the standard limits. The pollution load index (0.640-1.32) in soil showed that lead contamination mainly took place at the wastewater irrigating sites. Bio-concentration factor values (0.313-1.15) were lower than one in all samples except *Zea mays*, showing that lead metal was actively taken up by *Zea mays* tissues from the soil. Enrichment factor values ranged from 0.849-3.12, showing a moderate level of lead enrichment. Daily intake and health risk index varied between 0.004-0.020 mg/kg/day and 0.906-4.99, respectively. All the samples showed maximum lead concentration at the wastewater irrigating site compared to the ground or canal water application sites. These results recommended that consistent application of wastewater for forage irrigation must be avoided to prevent health hazards associated with lead in the animal and human food chain. Government must implement adequate strategies to protect the animal and human health from the harms of toxic heavy metals.

Keywords: lead, wastewater, accumulation, animal, Jhang, food chain.

Resumo

A toxicidade de metais pesados está se tornando uma preocupação crescente para a saúde ambiental, humana e animal. A pesquisa atual analisou a contaminação por chumbo (Pb) na cadeia alimentar sob três diferentes fontes de irrigação (solo, canal e águas residuais). Amostras de solo, plantas e animais foram coletadas no distrito de Jhang, no Paquistão, e processadas com um espectrofotômetro de absorção atômica. A concentração de chumbo nas amostras variou em: 5,22-10,73 mg/kg no solo, 2,46-10,34 mg/kg nas forragens e 0,736-2,45 mg/kg nas amostras de animais. A concentração de chumbo observada nas amostras de forragem e sangue animal foi superior aos limites padrão. O índice de carga de poluição (0,640-1,32) no solo mostrou que a contaminação por chumbo ocorreu principalmente em locais de irrigação de águas residuais. Os valores do fator de bioconcentração (0,313-1,15) foram menores que um em todas as amostras, exceto *Zea mays*, mostrando que o chumbo metálico foi ativamente absorvido pelos tecidos de *Zea mays* do solo. Os valores do fator de enriquecimento variaram de 0,849-3,12, mostrando um nível moderado de enriquecimento de chumbo. A ingestão diária e o índice de risco à saúde variaram entre 0,004-0,020 mg/kg/dia e 0,906-4,99, respectivamente. Todas as amostras mostraram concentração máxima de chumbo no local de irrigação de águas residuais em comparação com os locais de aplicação de água no solo ou no canal. Esses resultados recomendam que a aplicação consistente de águas residuais para irrigação de forragem deve

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ser evitada para evitar riscos à saúde associados ao chumbo na cadeia alimentar animal e humana. O governo deve implementar estratégias adequadas para proteger a saúde animal e humana dos danos dos metais pesados tóxicos.

Palavras-chave: chumbo, esgoto, acúmulo, animal, Jhang, cadeia alimentar.

1. Introduction

In Pakistan, livestock is the backbone of agriculture and fulfills nutritional needs of both rural and urban population (Rehman et al., 2016). Besides its role in nutrition, the livestock industry is also a significant source of employment in society. In rural areas, about 53 million population gains subsistence from livestock industry (Sindhu et al., 2012; Khan et al., 2019). The livestock sector is expanding very rapidly because all the basic needs of this sector, required to maintain sustainable ruminant growth, are present in Pakistan. According to Rehman et al. (2017), the livestock sector subsidizes nearly 56.3% of agriculture sector and constitutes 11% of GDP. The sustainable productivity of livestock depends on the variety of forages, climatic conditions and animals that are locally present (Uddin and Kebreab, 2020).

In Pakistan, although 16-19% of cropping area deals with major Rabi and Kharif crops farming, animals still face forage shortage due to lack of fresh water availability for irrigation purpose. On the other hand, forage crop also demands a higher supply of water in contrast to vegetable farming. Therefore, farmers use wastewater as a reliable source for agriculture over other conventional water sources (Khan et al., 2022; Hussain et al., 2021). According to an estimate, nearly 32,500 ha of land are irrigated with wastewater. About, 0.876 x 109 m^3 /yr wastewater is directly used in agriculture while 0.146 x 109 m^3 /yr is directly drained in the various irrigating canals which contaminate the canal water (Murtaza and Zia, 2012). Wastewater irrigation enhances soil nitrogen and potassium concentration, and in the case of phosphorus its availability in wastewater is much higher than any fertilizer application (Khan et al., 2020). But wastewater also contains toxic metals which affect the environmental and human health (Kalsom et al., 2020).

Forages are mineral suppliers for grazing ruminants which are required for their proper growth and reproduction. The minerals also stimulate the microbial activity in rumen and help to combat with diseases while their deficient supply affects the productivity and health of ruminants (Silva and Abdalla Filho, 2021; Khan et al., 2021). The uptake of metal by forages, used to feed herbivores, depends upon pH, clay, organic portion and the presence of oxides (Fe and Mn oxides) in the soil (Fayiga and Nwoke, 2017). The nutritional characteristics and their amount in forage determine the quality of animal products as well as animal health (Siddique and Ahmad, 2019). Waste water irrigation strategies enhance the heavy metal concentration in soil-forages and deform the natural ecosystem. Therefore, domestic animals also show poisoning by heavy metals, especially lead (Liu et al., 2020). Lead absorption mainly takes place in reticulum, and it disrupts enzyme function by displacing calcium or other bivalent cations. This process of absorption is enhanced by a shortage of essential elements (Liu et al., 2015). Lead is a bluish- grey naturally occurring heavy metal on the earth's crust which is used to make alloys, batteries, solder and ceramics. Industrial sources include gasoline leakage, fuel stabilizers, beverages, mining activities, paint, gun powder, fungicides, soldering rods and automobile exhausts. The recycling effluents of lead containing batteries can form the basis of lead poisoning in nations such as Pakistan (Rees and Fuller, 2020).

Lead is considered non-essential for soil and is normally in a range of 15-40 ppm (Pourrut et al., 2013). Lead has affinity for proteins due to S (sulphur) and N(nitrogen)-ligands. In plants, it limits the formation of carotenoids and plastoquinone which destroy the electron transport chain, deactivate enzymes and disrupt membrane permeability. Thus, lead toxically inhibits photosynthetic pigments and plant development (Yang et al., 2020a). Lead also reduces the lifespan and flexibility of red blood cells (RBCs) when it accumulates in the blood and organs of animals. It may cause osteoporosis, anaemia and disrupt manganese and iron metabolism. Animals absorb lead into the blood serum, lungs, liver, kidney, bones, cerebrum, hair and ribs (Stoklasova et al., 2020). The organic form of lead can cause chronic damage to the central nervous system (CNS). The inorganic form affects the gastrointestinal tract (GIT), urinary tract, central and peripheral nervous system, and leads to numerous neurological disorders. It also disrupts the haemoglobin synthesis, renal, hepatic and reproductive functioning of body. Lead inhalation causes hearing loss, hyperactivity and emotional destabilization (Jyothi, 2020; Engwa et al., 2019). Overall, lead deposition causes anaemia, muscle pain, feeble bone development, shrinkage of red blood cells and improper functioning of CNS (Briffa et al., 2020). About 10% of total pollution caused by heavy metals is attributed to Pb (Collin et al., 2022). Therefore, it is important to document lead levels to understand animal and human lead-hazards.

The rapid urbanization, industrialization and water scarcity conditions in District Jhang instigate the farmers to use municipal or industrial wastewater to irrigate their forage crops. Wastewater irrigation could potentially enhance the heavy metal exposure to human population by taking in contaminated forage crops and ruminant products. Heavy metal toxicity (Zn, Cu, As, Cd, Mn, Fe, Cr) has been investigated in soil-plant-animal and human food chain of various districts of Pakistan indicating metal toxicity in environment and components of animal/human food chain (Ahmad et al., 2022; Ejaz et al., 2022; Ge et al., 2021; Khan et al., 2021, Ghazzal et al., 2020; Yang et al., 2020a, b). Therefore, this study was carried out to assess the lead contamination in soil, forages and livestock of District Jhang, Pakistan. The objective of the present study was i) to quantify lead transfer in the soil-plant and animal continuum under different irrigating water sources, ii) to appraise lead associated risks for grazing animals and iii) to quantify the lead contamination status in study area via different indices.

2. Materials and Methods

2.1. Study area

Jhang district is situated between 30°-37° to 31°-59° latitude toward north and 71°-37° to 73°-13° longitude toward east (Figure 1). It is connected to Sargodha District in north, Faisalabad in east, Muzaffargrah District in south and Bhakkar and Layyah Districts in the west side. Three different sites of District Jhang were selected in this study where local forage crops are grown: Jhang (Jh-I) site used ground water irrigation, Shorkot (Sh-II) site used canal water irrigation and Ahmad Pur Sial (Aps-III) site used municipal wastewater irrigation.

2.2. Sample collection

Sampling was carried out during 2019-2020. Five forages were collected from each site: *Acacia nilotica* (Mimosaceae), *Capparis deciduas* (Capparaceae), *Zea mays* (Poaceae), *Medicago sativa* (Fabaceae) and *Pennisetum glaucum* (Poaceae). Five replicates of each forage sample with their respective soil samples (100 g) were taken from each site and saved in plastic bags. These samples were firstly dried in open air and then placed in an oven at 75 °C for almost 8 days. After this drying process, samples were stored in the sealed bags with proper labels for further processing (Khan et al., 2022).

Cows, buffaloes and sheep were the selected animal categories in this study. A total of thirty animals (at each site), ten belonging to each livestock category, were used to collect blood, hair, and fecal samples. All the animals were about 3-5 years in age and mainly fed on these collected forages. Blood samples (5ml) were taken from the animal jugular vein and centrifuged at 3500 rpm (15 minutes) to attain the blood plasma. Then, the plasma samples were stored at -20 °C (Ahmad et al., 2021). Animal hair and fecal samples were taken according to Chen et al. (2022a, b). Animal hair were rinsed with acetone and distilled water to clean the external contamination. All the hair and fecal samples were firstly air dried and then placed in oven for 4 days. After this drying process, samples were stored up in the labeled bags for further processing.

2.3. Sample digestion

About, 2 g of weighed sample was digested with 20ml of conc. H_2SO_4 in a digestion chamber for almost 30 minutes. Furthermore, 10ml H_2O_2 was added in the mixture and heated until the solution became transparent in color. The digested solution was filtered and a final volume of 60 mL was obtained by adding distilled water in it (Akhter et al., 2020).

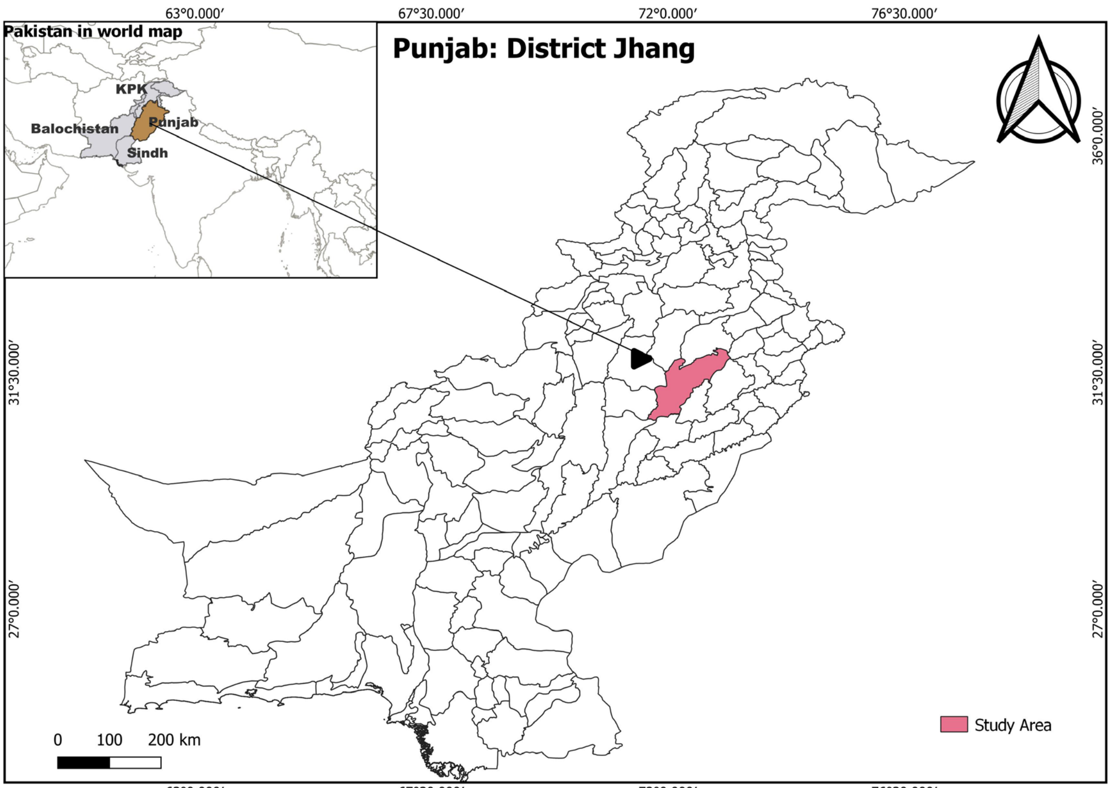

Figure 1. Map of the study area.

2.4. Lead analysis

All the digested solutions were processed through an Atomic Absorption spectrophotometer (Perkin-Elmer Corp. 1980) to analyze the lead concentration. Soil, fodder and animal sample data were presented statistically with the help of SPSS (version 20) and ANOVA analysis. All the samples were processed against certified reference material (CRM-1570) to gain quality results (Khan et al., 2022).

2.5. Bio-concentration factor (BCF)

BCF measures the metal concentration taken up in forage tissues (Cui et al., 2004). The Formula 1 is as under:

$$BCF\ soil - forage = (M)\ Forage \,/\, (M)\ Soil \quad (1)$$

2.6. Pollution load index (PLI):

PLI measures the metal contamination in the soil samples (Liu et al., 2005). It is calculated as Formula 2:

$$PLI = (M)\ Soil \,/\, (M)\ Reference\ soil \quad (2)$$

Soil reference value was 8.15 mg/kg in case of lead (Dutch Standard, 2000).

2.7. Enrichment factor (EF)

EF measures the metal value enriched in the soil samples (Buat-Menard and Chesselet, 1979). It is calculated by the following Formula 3:

$$Enrichment\ factor\ (EF) = \frac{\left(\frac{\text{metal concentration in forage}}{\text{metal concentration in soil}}\right)\text{sample}}{\left(\frac{\text{metal concentration in forage}}{\text{metal concentration in soil}}\right)\text{standard}} \quad (3)$$

2.8. Daily intake of metals (DIM)

DIM index was measured by Formula 4:

$$DIM = C_{metal} * CF * D_{food\ intake} \,/\, BW \quad (4)$$

C_{metal} stands for metal concentration in forage, CF stands for conversion factor which was 0.085, $D_{food\ intake}$ stands for daily food intake which is 12.5 kg for buffalo, 12 kg for cow and 1.3 kg for sheep (Chen et al., 2022b).

2.9. Health risk index (HRI)

HRI is measured by the following Formula 5 given by Cui et al. (2004):

$$HRI = Daily\ intake\ of\ metal \,/\, Oral\ reference\ dose \quad (5)$$

Lead oral reference dose is 0.0035 mg/kg/day (FAO, 2013).

3. Results

3.1. Lead analysis in soil samples

The concentration of Pb varied significantly at the three sampling sites (p<0.001) whereas soil and site by soil relationship showed non-significant effect for lead metal (p>0.05) (Table 1). The concentration of Pb in the soil fluctuated between 5.22-10.73 mg/kg. The ground water irrigated *C. decidua* soil showed minimum concentration at Jh-I. The *M. sativa* presented the maximum concentration of this metal, cultivated at wastewater irrigated soil of Aps-III (Table 2).

3.2. Lead analysis in forage samples

ANOVA results showed that the Pb concentration significantly varied in the forages (p<0.05) with respect to the three sites (p<0.001) and site*forage analysis showed significant results (p<0.01) (Table 1). The concentration of Pb in the collected forage samples lied within the range of 2.46-10.34 mg/kg. The *M. sativa* at Aps-III site showed maximum value of Pb and the minimum value was observed at the Jh-I that used ground water to grow *Z. mays* (Table 2).

3.3. Lead analysis in animal samples

ANOVA results showed that significant (p<0.001) variation of lead was found between the sites while non-significant (p>0.05) impact was observed in the animals, sources, site*animal, site*source, animal*source and site*animal*source (Table 1). Pb concentration

Table 1. ANOVA analysis for lead contamination in soil, forage and animal samples.

Pb	Soil				Forage		
Source of variation	**Site**	**Soil**	**Site*Soil**		**Site**	**Forage**	**Site*Forage**
Degree of freedom	2	4	8		2	4	8
Mean square	26.223***	3.755^{ns}	1.581^{ns}		67.711***	6.869*	6.251**
				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	27.211***	0.630 ^{ns}	0.801 ^{ns}	1.436 ^{ns}	1.564 ^{ns}	1.290 ^{ns}	0.591 ^{ns}

***,**,*, ^{ns}Significant at 0.001, 0.01, 0.05 and non-significant.

was determined to be 0.736-2.39 mg/l in the blood. The maximum concentration was observed in cow blood of site Aps-III and minimum was found in the sheep blood at the Jh-I site. The Pb concentration varied in the observed hair samples between 1.01-2.45 mg/kg. The lowest concentration was observed in the buffalo hair marked on the Jh-I and the highest concentration was noticed in the cow hair of Aps-III site. The Pb concentration in fecal samples varied between 0.994-2.33 mg/kg. The sheep feces showed minimum concentrations of Pb at Jh-I, while maximum was shown by buffalo feces sampled from Sh-II (Table 3).

Table 2. Mean lead concentration in soil and forage samples.

Forages	Jh-I	Sh-II	Aps-III
Soil samples (mg/kg)			
A. nilotica	6.87 ± 0.740	9.01 ± 0.587	9.44 ± 0.649
C. decidua	5.22 ± 0.938	8.96 ± 0.440	8.08 ± 0.321
Z. mays	7.87 ± 0.901	8.25 ± 0.715	9.10 ± 0.673
M. sativa	7.10 ± 0.899	9.72 ± 0.726	10.73 ± 0.592
P. glaucum	7.29 ± 0.809	9.09 ± 0.642	9.25 ± 0.370
Forage samples (mg/kg)			
A. nilotica	6.27 ± 0.958	7.08 ± 0.324	8.43 ± 0.172
C. decidua	4.57 ± 0.440	6.02 ± 1.06	7.89 ± 0.970
Z. mays	2.46 ± 1.01	9.47 ± 0.905	7.14 ± 0.366
M. sativa	4.11 ± 0.947	9.62 ± 0.503	10.34 ± 0.374
P. glaucum	6.16 ± 1.02	8.51 ± 0.440	9.23 ± 0.358

3.4. Pollution load index

The Pb level in the soil fluctuated from 0.640-1.32 as observed with the help of the pollution load index. The minimal concentration of PLI was 0.640 that was noticed in the fodder *C. decidua* sampled from the Jh-I location. Maximal concentration (1.32) of lead for PLI existed in the *M. sativa* located on the Aps-III sampling region (Table 4).

3.5. Bio-concentration factor

BCF results fluctuated between 0.313-1.15 in all the samples. A maximal value was found for *Z. mays* on Sh-II site, while the lowest value was found for the same plant but with ground water irrigation at the Jh-I site (Table 4).

3.6. Enrichment factor

The EF results suggested that Pb differed from 0.849-3.12. The maximum enrichment of lead was displayed by *Z. mays* at a canal water site. Ground watered, *Z. mays* grown on the Jh-I showed the least Pb enrichment (Table 4).

Table 3. Mean lead concentration (mg/kg) in blood, hair and fecal samples of animals.

Animals	Sources	Sampling locations		
		Jh-I	Sh-II	Aps-III
Cow	**Blood**	1.20 ± 0.191	1.49 ± 0.228	2.39 ± 0.257
	Hair	1.06 ± 0.219	1.06 ± 0.273	2.45 ± 0.239
	Feces	1.01 ± 0.158	1.68 ± 0.227	1.61 ± 0.235
Buffalo	**Blood**	1.10 ± 0.170	2.21 ± 0.222	2.34 ± 0.222
	Hair	1.01 ± 0.199	1.22 ± 0.294	1.88 ± 0.207
	Feces	1.03 ± 0.158	2.33 ± 0.233	2.16 ± 0.270
Sheep	**Blood**	0.736 ± 0.112	1.86 ± 0.207	2.09 ± 0.220
	Hair	1.26 ± 0.281	2.03 ± 0.231	1.88 ± 0.236
	Feces	0.944 ± 0.119	2.22 ± 0.204	2.20 ± 0.240

Table 4. Pollution indices for lead concentration at different sites.

Indices	BCF			EF			PLI		
	Jh-I	Sh-II	Aps-III	Jh-I	Sh-II	Aps-III	Jh-I	Sh-II	Aps-III
A. nilotica	0.913	0.786	0.893	2.48	2.13	2.43	0.843	1.11	1.16
C. decidua	0.875	0.672	0.976	2.38	1.83	2.65	0.640	1.10	0.99
Z. mays	0.313	1.15	0.785	0.849	3.12	2.13	0.966	1.01	1.12
M. sativa	0.579	0.990	0.964	1.57	2.69	2.62	0.871	1.19	1.32
P. glaucum	0.845	0.936	0.998	2.30	2.54	2.71	0.894	1.12	1.13

3.7. Daily metal intake and health risk index

This study observed a DIM range of Pb from 0.004 to 0.020 mg/kg/day. Minimal DIM was given by *Z. mays* of Jh-I that raised the cows and sheep. A maximal value of DIM was calculated in buffalo which feed on the *M. sativa* of Aps-III. The Pb range for HRI varied between 0.906-4.99. The highest HRI was noted in the *M. sativa* of Aps-III used to feed buffalo. The lowest HRI value was found in sheep that feed on the *Z. mays* of Jh-I (Table 5).

4. Discussion

WHO (2007) has recommended the lead concentration in agricultural soil to be in the range of 250-500 mg/kg. The lead concentration observed in the current study was lower than WHO (2007) limits. Sajid et al. (2017) observed a much higher Pb level in the sewerage irrigated soil (6.91-15.80 mg/kg) of Jhang. Ogundele et al. (2015) also studied higher Pb contamination in soil collected from the roadside (24-157.667 mg/kg) and a control site (33.667 mg/kg). However, Farrag et al. (2016) recorded lower Pb levels in wastewater (0.46 mg/kg) and reference soil (0.15 mg/kg). Similarly, low Pb absorption in soil was also found in the in the Lahore city (1.95-6.9 mg/kg) (Hamid et al., 2017). Vehicular emanations and the use of wastewater from industries or household activities can enhance Pb contamination in soil (Shen et al., 2019). However, all the studied soil samples were found to have lesser Pb levels than the suggested value of the EU (2002), which is 300 mg/kg and is considered safe for grazing animals. Pb mobility mechanism is associated with the pH, mineral elements and organic content in soil environment. The lower Pb mobility in soil of study area may be due to its alkaline nature which causes the available Pb to be less soluble in soil (Leogrande et al., 2019).

CERSPC (2009) prescribed the permissible concentration of Pb as 5.00 mg/kg in the plants. The present findings of Pb were much higher compared to CERSPC (2009). The present level was found to be much lower than the concentration given by Abah et al. (2017) in the control and waste dumpsite (0.15-0.20 and 0.44-0.60 mg/kg respectively). A higher Pb level was estimated by Shen et al. (2019) in the polluted pastures of China. Miclean et al. (2019) verified the lesser amount of Pb accumulation in the forages (0.15-2.24 mg/kg) to those reported by this research work. Reis et al. (2020) also showed similar values of Pb uptake in sewage irrigated forages of two different farms while lower amount of Pb was identified by the study of Khawla et al. (2019). Forages grown on wastewater sites of Marrakech (Chaoua et al., 2019) accumulated higher Pb (13.555-83.09 mg/kg) compared with current values. Overall, the observed range also surpassed the acceptable EU (2006) limits for Pb concentration that was 5 mg/kg but lower than the toxic concentration of 30 mg/kg reported in the forages to feed the dairy animals (Farrag et al., 2016). According to Shukla et al. (2018) the ability of plants to deposit metals is associated with both soil factors (Pb solubility and Pb-soil interaction) and forage type. Soil pH stimulates the Pb-solubility in soil and Pb is easily absorbed by forage tissues. Higher Pb level was analyzed in roots than aerial parts of forages. Pb contamination in forages highlights the toxicity of human actions in the environment. Wastewater irrigation, lead batteries, traffic discharge and corrosion of tires are the main points of Pb entry into the plant environment (Reis et al., 2020; Leogrande et al., 2019).

This study concluded that Pb accumulated beyond the tolerable limit of 0.1 mg/l recommended in animal blood (Milam et al., 2017), thus possibly passing on health dangers through the food chain, as already reported by many researchers. As the present concentrations were beyond the safe limits, too much profusion of Pb in the reticulum of grazing animals takes place which dislocates the calcium and other bivalent ions and ultimately interrupts the normal bodily activities (Liu et al., 2015).

Table 5. Analyzed DIM and HRI index of lead in various animals.

Forages	Cow			Buffalo			Sheep		
	Jh-I	**Sh-II**	**Aps-III**	**Jh-I**	**Sh-II**	**Aps-III**	**Jh-I**	**Sh-II**	**Aps-III**
DIM (mg/kg/day)									
A. nilotica	0.011	0.012	0.014	0.012	0.014	0.016	0.009	0.010	0.012
C. decidua	0.008	0.010	0.013	0.009	0.012	0.015	0.007	0.009	0.012
Z. mays	0.004	0.016	0.012	0.005	0.018	0.014	0.004	0.014	0.011
M. sativa	0.007	0.016	0.018	0.008	0.019	0.020	0.006	0.014	0.015
P. glaucum	0.010	0.014	0.016	0.012	0.016	0.018	0.009	0.013	0.014
HRI									
A. nilotica	2.66	3.01	3.58	3.03	3.42	4.07	2.31	2.61	3.11
C. decidua	1.94	2.56	3.35	2.21	2.91	3.81	1.68	2.22	2.91
Z. mays	1.05	4.02	3.03	1.19	4.57	3.45	0.906	3.49	2.63
M. sativa	1.75	4.09	4.39	1.98	4.65	4.99	1.51	3.54	3.81
P. glaucum	2.61	3.62	3.92	2.98	4.11	4.46	2.27	3.13	3.40

An absorbed lead content of about 99% fixes with body RBCs, is carried to various soft tissues (kidney & liver), and finally deposits in the bones. Sajid et al. (2017) estimated a high level of Pb, above the reference limits, in the serum of Lohi sheep grazing on the area with sewage drainage as well as in the control site where ground water was utilized to irrigate crops. The recent Pb range in the study area was higher than the Pb concentration in Lohi sheep raised on both the sewage polluted (0.98-1.84 mg/l) and control site (0.65 mg/l) (Sajid et al., 2017), suggesting Pb toxicity to grazing animals in the investigated locale. A lower concentration of Pb in cattle (0.99 mg/kg) and camel (0.86 mg/kg) hair was estimated by Darwish et al. (2018) in Sharkia Governorate, Egypt. Furthermore, Ogundiran et al. (2012) and Adesoye et al. (2014) demonstrated lower Pb content in the excreta of various domestic animals. When the present results were compared against the findings of Johnsen et al. (2019), all fecal samples of sheep showed higher Pb level in both R and J range (4.0 mg/kg and 5.0 mg/kg). The animals that feed only on the Pb-contaminated forages have the highest Pb level in excreted manure compared to animals feeding on grains mixed with fodder plants. Pb excretion is mainly associated with bile production. Pb enters from liver to bile and bile to small intestine and finally excreted through feces. Lead poisoning damages the immune system, causes blindness, and affects liver functioning in animal body (Akhter et al., 2020).

Chaoua et al. (2019) suggested a lower range of Pb transfer (0.319-0.922) in Morocco compared to the present work. Similarly, less transfer of Pb from soil-plant was also suggested by Reis et al. (2020) (0-0.03) in forages irrigated from the Vieira River, which received waste effluent from various sources. The BCF recorded in the current survey was also higher than the values observed by earlier researchers (Orisakwe et al., 2017; Miclean et al., 2019). Accretion of lead metal into the plants with respect to soil is analyzed by bio-concentration factor. In the current study, all the plants had BCF<1 except *Z. mays. Z. mays* is categorized as lead accumulator plant because it shows BCF>1 and may be the major reason for Pb toxicity in ruminants (Khan et al., 2022).

The pollution load index of >1 certified the lead contamination in the present area due to wastewater irrigation practice. The lead pollution index calculated in agronomic soil by Chukwu and Oji (2018) in Nigeria (0.8-3.8) and Yu et al. (2019) in Lin'an city (0.25- 41.35) was higher than the Pb values in our present research. The mean Pb contamination appraised by Reis et al., (2020) at the two farms of Brazil was found to be lesser than the recorded results of our survey. Our PLI level was lesser than Shu and Zhai (2014) observed in the South China. Conversely, a slightly higher PLI concentration for Pb was found in Iranian soil (0.8-3.8) (Jorfi et al., 2017). Metal concentration in agricultural soil is increased by sewage irrigation. Therefore, it could be speculated that various anthropogenic and agricultural practices caused Pb toxicity in soil environment (Ahmad et al., 2022).

Moderate level of Pb enrichment was recorded by Barbieri (2016). Inengite et al. (2015) specified greater Pb enrichment in the topsoil (2.01) and bottom soil (4.44), but a decreased level of enrichment (0.56) was ascertained in the findings of Hussain et al. (2015). Mohamed et al. (2014) found an enrichment range of 0.03-0.40 with a mean value of 0.56, which was lesser than the EF calculated in the current study. Likuku et al. (2013) anticipated EF values within the range of 1.01-3.58 which was higher than the present data. The current results of this study suggested moderate enrichment of Pb in the surveyed region.

The daily intake of Pb was higher than the reported results of Akhter et al. (2020) in the sheep of district Chakwal. Nadeem (2020) also highlighted lower daily intake in the buffaloes fed on the contaminated pastures of Sahiwal. Our DIM results were lower than the mean daily intake of herds in Galloway cows in both summer (68 mg/kg/day &157 mg/kg/day) and winter (145 mg/kg/day &75 mg/kg/day) seasons (Roggeman et al., 2013). Akhter et al. (2020) reported a HRI range as 0.35-1.35 mg/kg/day that was lower than the recorded results of our study. Similarly, these values were also lower than Nadeem (2020). Present results observed the HRI>1 which demonstrated that all the animals were susceptible to health hazards caused by Pb toxicity. These results showed that constant application of wastewater to irrigate forages must be avoided to prevent lead associated health hazards in the animal food chain.

5. Conclusion

Domestic animals mainly showed the lead poisoning because all the environmental components: soil and fodder plants constantly accreted lead metal which enhanced its exposure to animal and humans. Present results revealed that lead concentration in fodder crops were beyond the standards due to wastewater application. This study concluded that the level of Pb in blood was above the recommended values but lacked the appearance of poor health symptoms in grazing animals. Present results suggested that actions should be taken to prevent lead transfer from soil and fodder crops to animal tissues and finally to human beings. Government must provide practical strategies to secure the animal and human health. A well-coordinated campaign must be commenced to create awareness among the different sectors (especially farmers) regarding the contamination of food chain and health issues raised due to wastewater application.

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Ethical Approval

Institutional Human Ethics Committee of University of Sargodha (Approval No.25-A18 IEC UOS) has allowed all the protocols used in this experiment to the commencement of the study. The authors declare that manuscript has not been published previously.

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