

HEALTH RISK ASSESSMENT OF CHROMIUM AND LEAD IN A SOIL-PLANT-RUMINANT FOOD CHAIN AGAINST TERRESTRIAL SOIL POLLUTION GRADIENT

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Abstract

The present study was conducted to determine the concentrations of chromium (Cr) and lead (Pb) in soil, fodder, and animal (cow and buffalo) blood in Sargodha, Punjab, Pakistan. During the summer and winter seasons, samples were taken from three distinct locations. Using an atomic absorption spectrophotometer, the content of chosen heavy metals was determined after digestion using the wet technique (AASP). The Cr and Pb ranged in forage (0.0465-0.4884 & 0.1807-0.9440 mg/kg) and soil (0.0918-0.5100 & 0.3374-2.5153 mg/kg) was far lower to permitted limits. The blood means concentration of both Cr (0.1029-2.8197 mg/L) and Pb (0.1643-0.8037mg/L) was found to be higher than the permitted limit. The burning of fuels and the mixing of residential waste in drinking water sources might be the cause. The health risk index was found more than 1 in Pb, while HRI value was lower than 1 in case of Cr metal. Sewage water irrigated site of winter season showed highest value of HRI. Elevated Cr and Pb levels in cattle blood appear to be dangerous. As a result, the government must enact regulations and raise public awareness for the benefit of animal and, ultimately, human health.

Keywords: Chromium, Contamination, Forages, Health danger, Lead, Livestock.

Introduction

Pakistan's economy is based on agriculture, as it is a developing country. Pakistan covers 34.4 square kilometers of land as agricultural land. Arable land is the portion of agricultural land that is utilized to sustain

crop and pasture production (Ashraf *et al.*, 2017; Hussain *et al.*, 2022).

Biological communities such as bacteria, plants, and animals are employed as

indicators of pollution in the environment (Zaghloul *et al.*, 2020; Yu *et al.*, 2022). Traditionally, the global dairy farming sector has relied on pasture; however, in recent decades, there has been a widespread trend to supplement dairy production with additional feed (Foote *et al.*, 2015). However, because growth and grass characteristics are seasonal, frequent feed supplementation is required to address pasture deficiency and fodder plays an important role in cattle nutrition since it is an excellent source of minerals and a plant-based diet is readily digested in the rumen of grazing animals (Hussain *et al.*, 2022). Plants provide almost 90% of the mineral demands of grazing ruminants (Masters *et al.*, 2019). Domestic and modified grasses are among the most common forages for animals in Pakistan (Iqbal *et al.*, 2015). Mineral deficiency in the soil and fodder composition causes reproductive and growth issues in animals (Shukla *et al.*, 2018).

Metals having atomic number > 20 and density $> 5 \text{ g cm}^{-3}$ are regarded as Heavy metals which occur naturally in the environment (Ali and Khan, 2018). Even lower concentrations of specific heavy metals can pose acute health hazards in humans when they are exposed to these metals (Gall *et al.*, 2015; Khan *et al.*, 2019; Ahmad *et al.*, 2022, Khan *et al.*, 2022). From the last few years a lot of attention has been focused on the heavy metals monitoring and screening in environment (Elzwayie *et al.*, 2017; Ejaz *et al.*, 2022; Maqsood *et al.*, 2022). According to the report provided by various studies, developing countries such as Pakistan, Nigeria, Egypt and Iran represented above

heavy metal concentrations in milk as compared to whole globe (Boudebbouz *et al.*, 2021). Discharge of certain heavy metals into ash in the atmosphere and either by wet or dry deposition their transfer occur towards soil (Wang *et al.*, 2021). Soil acts as a reservoir for rapid accumulation of heavy metals and these metals are gradually diminished from soil through leaking towards the plants (Srivastava *et al.*, 2017; Ge *et al.*, 2022).

Heavy metals play fundamental role in contamination via food chain (Waqas *et al.*, 2015; Chen *et al.*, 2022). Heavy metals may get entry into the livestock body via feed, water drinking or even by dermal contact (Danish and Chan, 2016; Yu *et al.*, 2022; Akhtar *et al.*, 2022a). Food constituents comprising of animal products that fulfill human diet needs, get polluted when they are exposed to air, water and soil environmental media (Toussaint *et al.*, 2019). Assemblage of heavy metals occurs in crucial body organs including kidney and liver (Briffa *et al.*, 2020; Akhtar *et al.*, 2022b). Cell damages, skeletal damages, osteoporosis, renal failure, cancer of lungs, gastrointestinal problems, anemia and blood, hormonal disturbances is the hazardous influences of heavy metals on mankind health (Ismail *et al.*, 2015).

Pb has the potential to harm the cardiovascular and neurological systems. Lead is found in the form of sulphates and phosphates, which are inaccessible to plants (Oliver and Gregory, 2015; Akhtar *et al.*, 2022a). Pb mobility in the soil is halted due to the creation of complexes in conjunction with

organic materials (Rodriguez-Vila *et al.*, 2015). When used in excess, Pb is a dangerous heavy metal that causes health problems and pollution all over the world (Rehman *et al.*, 2015). Cr is a life-sustaining element for animals, but it may be lethal to forage even in little amounts (Saha *et al.*, 2017). The amount of Cr in different regions of the plant varies. Plant systems such as mineral feeding, photosynthesis, respiration, and water relations are all harmed by Cr polluted soil and water, according to Azmat and Khanum, (2005). Above all, a lack of knowledge about proper garbage disposal and failure to adhere to strict regulatory criteria has exacerbated the cause of environmental degradation (Khalid *et al.*, 2017). Industrialization, urbanization and gradual increase in population had turned into a critical issue (He *et al.*, 2016). Organisms are helpless to reside in extremely polluted environment and to intake contaminated feeds (Vardhan *et al.*, 2019). The poisonous heavy metals elimination from living beings is a concerning point (Ali *et al.*, 2019). In the following scenario heavy metals are piercing in every single environment of the earth crust. The main goal of this study was to investigate forages produced on various locations in tehsil Sahiwal of District Sargodha and the health hazards to cows and buffaloes grazing on Cr and Pb-contaminated forages.

Materials and methods

Study area

The area used for study is Sahiwal, situated in Sargodha, Punjab, Pakistan. Soil utilized for agriculture purpose is fertile because of the occurrence of Sahiwal near the

east bank of river Jhelum. Sahiwal (with diacritics) is its real name and is well known for its Wheat, Sugarcane, and Cotton and Rice crops. It is a subdivision (Tehsil) of Sargodha District and was approximately 37 km from Sargodha at main Sargodha-Multan highway. Geographical coordinates of the area are 31° 58'23"N and 72° 19'32"E. Soil utilized for agriculture purpose is fertile because of the occurrence of Sahiwal near the east bank of river Jhelum. During 2018-2019, research was carried out and samples were collected in both summer and winter season. Three sites, located at 5km distance from each other, were selected for present research. Site-I was treated with tube well water, Site-II was treated with canal water and Site-III was treated with sewage water.

Soil and Forage sampling

Total 240 samples (120 soils & 120 forage sample) were collected in both seasons. About 60 samples (20 from each site) were collected in each season. Each soil sample along with 4 replicates was taken from almost 15-30cm depth with the help of shovel. For all forage species, 4 replicates were also taken in polythene bag from each site. All the forage impurities were removed by washing with pure water. After air drying, the samples were oven dried for 3 days at nearly 70-75°C. Samples were crushed into fine powder with the help of grinder. About 1g of each powder sample was taken for wet digestion method given by Khan *et al.* (2021).

Blood sampling

Total 108 blood samples of cows and buffaloes were collected after taking consent from Institutional Review Committee. Total 54 samples (27 of cows and 27 of buffaloes) during each season (9 samples from each site) were taken in test tubes of 16 ×150 mm which were sealed. Jugular vein of cattle was preferred for the extraction of blood. When animals were in standing position, the blood was drawn by using sterilized needle. At 3000rpm the centrifugation was performed for about 15-30 minutes to separate the serum from blood. In labeled bottles, the serum was put and deposited at about -20°C in freezer. Samples (1mL each) were taken and processed for wet digestion method according to the procedure given by Khan *et al.* (2021).

Metal Analysis

Sampling digestion used wet acid. A digestion flask contained 10 mL of H₂SO₄ and 1 g of ground soil samples. The acid-soil combination was heated for 30 minutes. After adding 4 mL H₂O₂, heat was applied again until sample was cleared. Distilled water dilutes solutions up to 50 mL and was preserved for metal analysis.

Washing forage samples with tap followed by distilled water and oven dried at 65°C. A flask with 4 ml H₂SO₄ and 8 ml H₂O₂ digested 1g of forage samples. The digesting chamber was sampled when odours stopped dissipating. Each sample was boiled again in the digesting chamber with 2 ml of H₂O₂ repeatedly until sample became clear. Filtered

and added distilled water to make the sample 50 ml. Labeled bottles held all samples for analysis.

Health Risk Index

The oral reference dose (RfD) and daily metal intake determines the values of health risk index. The formula given by Cui *et al.* (2004) was used to calculate the HRI.

$$\text{HRI} = \text{DIM} / \text{RfD} \text{ (USEPA, 2002)}$$

The values of oral reference dose are described as Pb: 0.0036, Cr: 0.003mg/kg/day (USEPA, 2007).

Quality Control

To guarantee the accuracy and precision of analysis against, the samples were analyzed again and again. To estimate the accuracy and contamination of data quality control measures were adapted. In order to keep away from any contamination which disturbs the lab work, well washed apparatus with deionized water was utilized. To confirm the precision and accuracy, salts and chemicals which were utilized for atomic absorption spectrophotometry as well as in digestion were purchased from Sigma Aldrich Germany, available in local market. Standards were carefully prepared.

Statistical Analysis

All the results were statistical analyzed by Two-way ANOVA using SPSS. 20 software. Significant difference among samples means was computed at level of significance at 0.05, 0.01% as given by Khan *et al.* (2021).

Results

Metal analysis in Soil

Site, Soil, Season*Site, Season*Soil, Site*Soil and Season*Site*Soil showed significant effect on the Pb and Cr concentration in soil (**table 1**).

According to ANOVA, the Season,

Table 1. Variance analysis (ANOVA) of Cr and Pb in Soil Samples

Sources	Cr-Mean Square	Pb- Mean square
Season	0.028***	37.660***
Site	0.005**	5.328***
Soil	0.091***	0.534***
Season*Site	0.076***	4.469***
Season*soil	0.062***	0.222***
Site*Soil	0.015***	0.012***
Season*Site*Soil	0.015***	0.010***

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

During summer, Cr values varied from highest 0.5100mg/kg to lowest 0.0918mg/kg. The soil along with forage plant *S. vulgare* (**sweet Funnel**) at canal site indicated more Cr contamination while the soil along with forage *Z. mays* (**corn**) at sewage site exhibited minimum Cr concentration. During winter

season, higher and lower mean values of Cr are 0.2306mg/kg and 0.1075mg/kg respectively. In winter season soil samples taken along the *M. sativa* (**Lucerne**) treated with sewage water showed maximum concentration and lower value was observed in soil found around the *A. sativa* plant (**figure 1**).

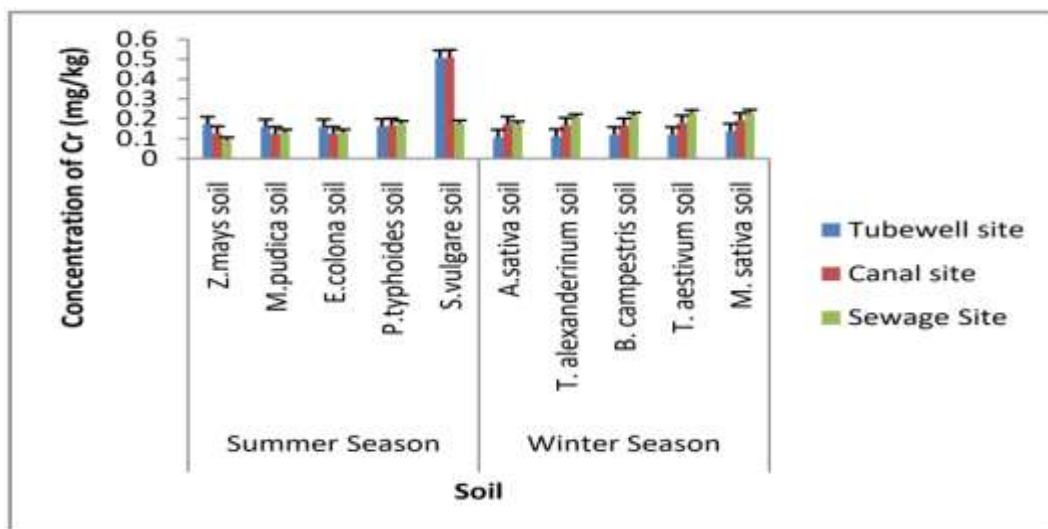


Figure 1. Cr concentration in soil samples during summer and winter

During summer, Pb values varied from highest 0.6310 to lowest 0.3374mg/kg. During summer season the soil taken along with canal water treated forage plant i.e. *S. vulgare* exhibited maximum (0.6310mg/kg) concentration of Pb whereas minimum (0.3374mg/kg) concentration was found in soil collected along *Z. mays* irrigated with tube well

water. In winter, mean values of Pb varied from highest i.e. 2.5153mg/kg to lowest i.e. 0.5955mg/kg. *M. sativa* soil irrigated with sewage water showed higher concentration of Pb while soil of *T. alexandrinum* (**Berseem**) plant treated with tube well water exhibited lower Pb dispersion (**figure 2**).

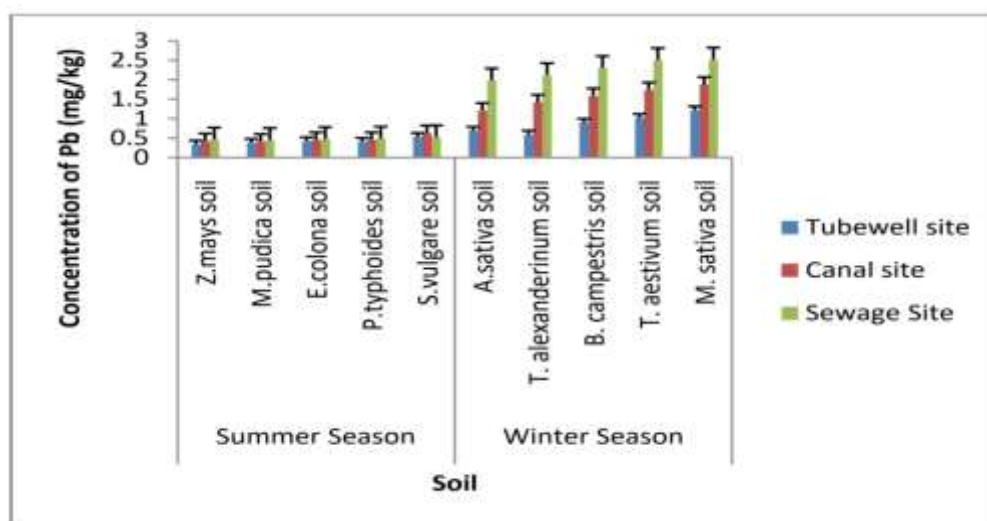


Figure 2. Pb concentration in soil sample during summer and winter

Metal analysis in Forages

The significant effect of Season, Site, Forages, Season*Site, Season*Forages, Site*Forages, and Season*Site*Forages was

shown by ANOVA on the concentration of Cr and Pb in forages except non-significant effect of Cr was observed by Season and Site (**table 2**).

Table 2. Variance analysis (ANOVA) of Cr and Pb in forages

Sources	Cr-Mean square	Pb-Mean square
Season	0.005 ^{ns}	3.247***
Site	0.002 ^{ns}	0.441***
Forages	0.108***	0.157***
Season*Site	0.079***	0.512***
Season*Forages	0.082***	0.028***
Site*Forages	0.022***	0.023***
Season*Site*Forages	0.031***	0.016***

***, ^{ns}= Significant at 0.001 and non-significant

The Cr value in varied from 0.4884 to 0.0465mg/kg in summer season. Tube well treated forage. *S. vulgare* showed the higher value i.e. 0.4884mg/kg and Sewage treated forage *P. typhoides* showed the lowered (0.0465mg/kg) value. The Cr value in forages

varied from 0.2424 to 0.0823mg/kg in winter season. Tube well water treated forage. *A. sativa* showed the higher value i.e. 0.2424mg/kg while *P. typhoides* from same treatment showed less (0.0465mg/kg) concentration (**figure 3**).

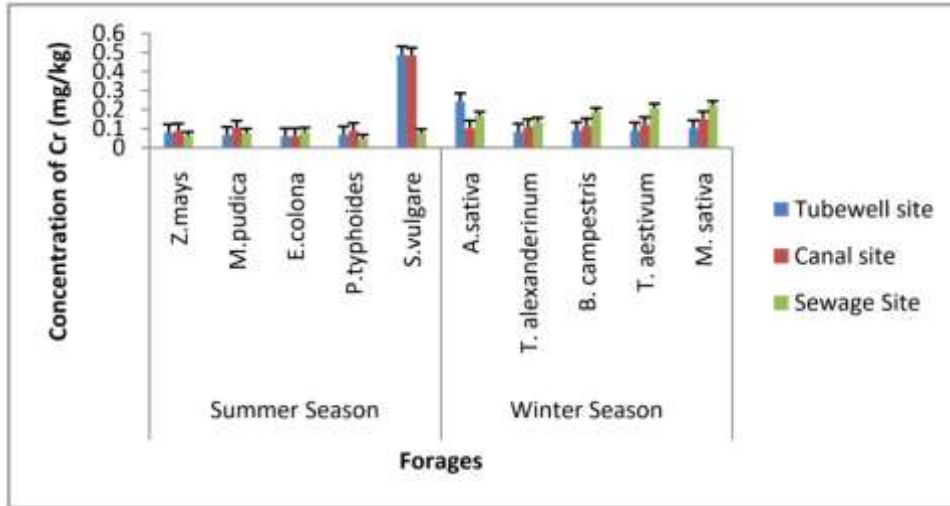


Figure 3. Cr content in forage samples during summer and winter

It was indicated by the result that in summer season more Pb concentration was observed in *S. vulgare* collected from tube well water treated site and less mean concentration was shown by *E. colona* irrigated with canal water. During summer Pb concentration differed from 0.5686 to 0.1807mg/kg. During

winter season, Pb concentration differed from 0.9440 to 0.3247mg/kg. Pb concentration level was found higher (0.9440 mg/kg) in *M. sativa* of sewage water irrigated site while lower Pb (0.3247mg/kg) level was observed in *A. sativa* collected from tube well water treated site in winter (**figure 4**).

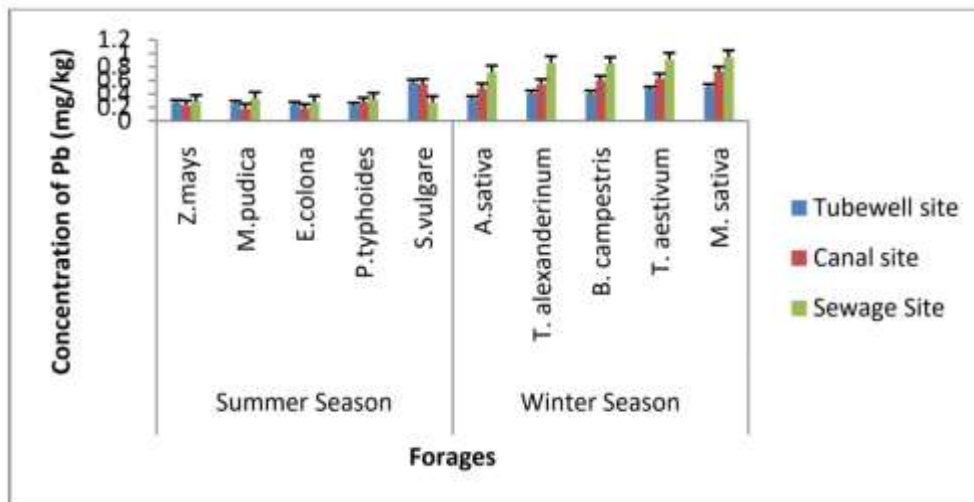


Figure 4. Pb in forage samples during summer and winter

Metal analysis in Blood

According to the ANOVA results Season, Site, Animals, Season*Site, Season*Animals, Site*Animals and

Season*Site*Animals had significant (P< 0.05) effect on the concentration of Cr and Pb in animal serum sample (**table 3**).

Table 3: Variance analysis (ANOVA) of Cr and Pb in blood

Sources	Cr-Mean square	Pb-Mean Square
Season	64.653***	2.477***
Site	5.374***	0.410***
Animals	3.539***	0.795***
Season*Site	3.906***	0.210***
Season*Animals	0.490**	0.021***
Site*Animals	1.923***	0.239***
Season*Site*Animals	1.945***	0.218***

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

According to summer season results it was found that Cr mean content was maximum i.e.0.4517mg/L in animal treated with forages of sewage site and minimum content i.e. 0.1029mg/L was found in animal serum of tube well site. The observed level of Cr was highest

in animal blood taken from sewage site while minimum Cr concentration was exhibited by canal site animal in winter season. During winter the mean concentrations of Cr were varied from higher (2.8197mg/L) to lower (0.6453 mg/L) (**figure 5**).

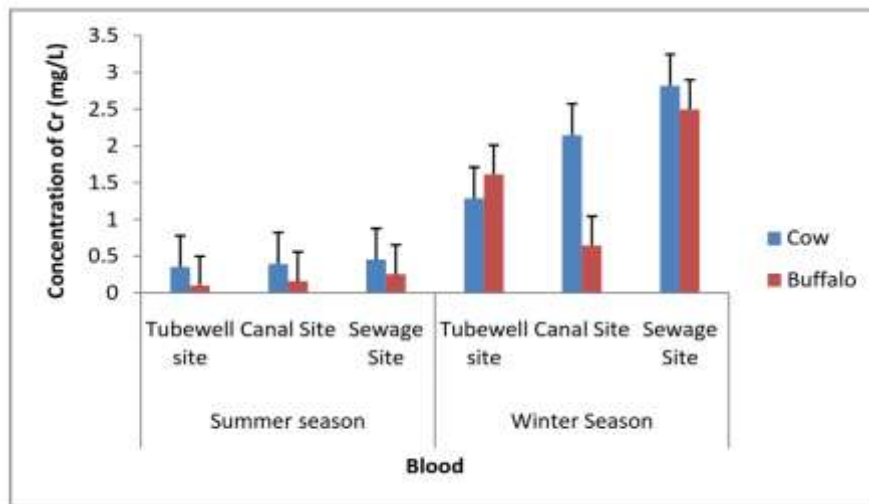


Figure 5. Concentration of Cr in Animal Blood samples during summer and winter

Sewage site samples of animal serum showed highest mean level of Pb during summer i.e. 0.3754mg/L and winter i.e.

0.8037mg/L. Whereas in summer least Pb level (0.1643mg/L) was found in blood serum samples of tubewell site animals and during

winter canal site animals showed less concentration of Pb (0.1682 mg/L). The recorded mean concentration range was 0.3754

to 0.1643mg/L in summer and 0.8037 to 0.1682mg/L in winter season respectively (figure 6).

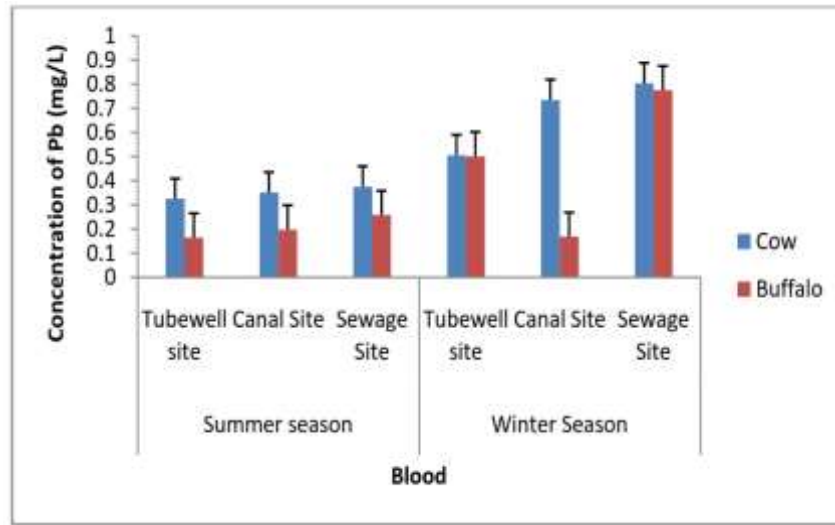


Figure. 6 Concentration of Pb in Animal Blood samples during summer and winter

Health Risk Index

Following results (Table 4) show that the HRI value of Cr below 1 has no possibility of any danger to the animals ingesting contaminated forages of the investigated region. The values of HRI for Pb were ranged from 3.4716 to 0.0970mg/kg in summer. The highest Pb value of HRI was shown by S.

vulgare irrigated with canal water while lower HRI level was exhibited by E. colona which was treated by the same canal water. During winter season, the health risk index showed variation from 5.9596 to 2.0500 mg/kg in M. sativa of sewage site and A. sativa of tube well water irrigated site (table 4).

Table 4: HRI values for Cr (chromium) and Pb (lead)

Summer Season		Z. mays	M. pudica	E. colona	P. typhoides	S. vulgare
TubewellSite	Cr	0.0509	0.0427	0.0376	0.044	0.3145
	Pb	0.1424	0.1358	0.1283	0.1212	0.3051
CanalSite	Cr	0.0563	0.0658	0.0399	0.0578	0.3123
	Pb	0.1249	0.0986	0.097	0.1479	3.4716
SewageSite	Cr	0.0396	0.0505	0.0539	0.03	0.0488
	Pb	0.1504	0.1752	0.1453	0.1684	1.6903
Winter Season		A. sativa	T. alexanderinum	B. campestris	T. aestivum	M. sativa
TubewellSite	Cr	0.1561	0.053	0.0583	0.0572	0.0645

	Pb	2.05	2.5852	2.5614	2.9136	3.1731
CanalSite	Cr	0.0664	0.0711	0.0732	0.0777	0.0968
	Pb	3.0565	3.4574	3.7743	3.983	4.6256
SewageSite	Cr	0.1076	0.0878	0.1205	0.1349	0.1435
	Pb	4.5404	5.4111	5.3271	5.7265	5.9596

Discussion

The range of soil Cr established by Ahmad *et al.* (2011) was exceeded the Cr range found in current study. Cary and Kubota, (1990) reported the range 2-50mg/kg as toxic Cr level in soil which was also higher as compared to Cr values observed in current study. So Cr concentration under the deadly limit has no potential danger for the health of livestock if forages grown on sewage water irrigated soil are fed to them in this particular study region. The range of Cr concentration in forages (2.16-4.12mg/kg) given by Ahmad *et al.* (2011) was far higher than Cr value observed in our study. Cr level in forages was found lower than the tolerable value of Cr (10.0mg/kg) suggested by Chief Editor Room of Standard Press of China (CERSPC, 2009). In present study, no injurious health effect of Cr can be expected in animals consuming forages which contained Cr concentration below the unsafe limit. The Cr concentration observed in present study (2.8197mg/L) was higher than Cr value (0.06mg/L) found in the study done by Puls, (1989). The recent mean value was also higher than that observed in the research done by Okareh and Oladipo, (2015). Cr accumulation animal body might depend upon the type of forage consumed by them. The Cr concentration more than the tolerable limit is poisonous for livestock and ruminant

reproductive capacity is poorly influenced by it (McDowell, 2003). The value of HRI for Cr observed in the current research was high as compared to that reported by Ahmad *et al.* (2018). The research performed by Khan *et al.* (2017) found 0.0024mg/kg for Cr which was lower than the recent study value.

The concentration of lead (Pb) in our study was found greater than soil Pb value reported by Ahmad *et al.* (2011). The value of Pb observed by Fardous *et al.* (2010) was 5 to 25 mg/kg, which was extremely high as compared to that found in investigated area. The concentration of lead (Pb) in recent study was found lower than soil Pb value reported by Sathyamoorthy *et al.* (2016). The trace quantity of lead in soil indicates pollution nonexistence might be due to the utilization of water for irrigation purpose which has no Pb toxicity. So forages grown on the soil of study area have no alarming influence on livestock because Pb concentration was found below the toxic level. Pb concentration reported by CERSPC (2009) was higher as compared to that observed in our investigation. The mean range of forage Pb suggested by Ahmad *et al.* (2011) was higher than the Pb value of our study. Pb concentration observed in recent study was lowered than suggested range of Sathyamoorthy *et al.* (2016). In sewage water irrigated forage more Pb concentration was found. Through the

restriction of cell elongation and cell division, Pb hinders the extension of root (Eun *et al.*, 2000). Some and Lagerkvist (2002) suggested that the cause of various physiological, hematological and neurological disorders is the lead. The Pb range reported by Swarup *et al.* (2006) was 0.21- 10.6 mg/kg that was greater to our values observed in this study. Okareh and Oladipo (2015) suggested lower mean value for Pb as compared to the following result. Fuel burning, application of sewage sludge and constant supply of fertilizers can lead to Pb accumulation. Khan *et al.* (2008) observed that Pb toxicity indicates various symptoms including brain damage, headache and anemia. In recent study, the value of HRI for Pb was found higher as compared to that observed in the investigation done by Ahmad *et al.* (2018). The forage quantity, forage type which is ingested and quality of soil determines the health risk index. Pb is a poisonous metal in humans and according to previous study it was specified that the transfer of Pb from environment to human beings occur mostly through the food chain (El fadeli *et al.*, 2014). In the current results HRI exceeded the safe limit 1 which specifies that the forages consumed by animals have potential health danger for them.

Conclusion

Sewage water irrigation in agricultural lands interprets Cr and Pb intoxication in ruminants as well as human beings. The Cr and Pb in forage and soil were far lower to permitted limits which indicates that the consumption of these forages has no danger for livestock health.

Based on these findings, it seems that this region has been subjected to the least amount of anthropogenic and industrial alteration. Animal blood from sewage sites had the highest Cr and Pb levels, whereas canal and tube well water feeding animals had low metal concentrations in summer season that may be due to heavy rain in summer that wash out and percolate metals from soil and its availability decrease for the plants while increase in winter due to less rain. In the blood the concentration of both Cr and Pb were found to be higher than the permitted limit which indicates gradual built up of metal in animal blood. Fuel burning, application of sewage sludge and constant supply of fertilizers can lead to contamination of Pb and Cr in forages that is main source of feed for animals. As a result metal level elevated in livestock blood and finally this metal toxicity is passed to human beings through food chain. Hence, government needs to make legislations and bring public attention for the sake of animal and finally human health.

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

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

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

Data availability: All data generated or analyzed during this study are included in this published article.

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