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Zafar Iqbal
*Department of Botany, University of Sargodha, Punjab, Pakistan*, zafar.khan@uos.edu.pk

Kausar Parveen
*Department of Botany, University of Sargodha, Pakistan*, kausar.parveen@gmail.com

Kafeel Ahmad
*Department of Botany, University of Sargodha, Pakistan*, kafeel.ahmad@uos.edu.pk

Tasneem Ahmad
*Pakki Thatti Research and Development Farm, Toba Tek Singh, Pakistan*, tasneem91@yahoo.com

Naila Riaz
*Department of Zoology, University of Sargodha, Pakistan*, naila.riaz@uos.edu.pk

*See next page for additional authors*



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# Monitoring of Cadmium and Lead Accumulation in Soil-Forage-Animal Continuum in Pasture Land Irrigated with Ground Water in Bhalwal, Punjab, Pakistan

## Cover Page Footnote

This article is only a small portion of Kausar Parveen thesis, M.Phil Scholar Department of Botany, University of Sargodha. The authors acknowledge all reviewers as well as those who provided assistance with sample preparation, data analysis, and article completion.

## Authors

Zafar Iqbal, Kausar Parveen, Kafeel Ahmad, Tasneem Ahmad, Naila Riaz, Abrar Hussain, Asma Ashfaq, Faiza Zubair, Ijaz Rasool Noorka, Abid Ejaz, Hafsa Memona, Shahzadi Mahpara, Allah Bakhsh Gulshan, Tayyaba Aziz, Tayyba Liaqat, Mumtaz Akhtar, Muhammad Umer Farooq Awan, Farzana Siddique, Abdllah Saleh Alsubail, and Rand Suleiman Alhuthayli

# MONITORING OF CADMIUM AND LEAD ACCUMULATION IN SOIL-FORAGE-ANIMAL CONTINUUM IN PASTURE LAND IRRIGATED WITH GROUND WATER IN BHALWAL, PUNJAB, PAKISTAN

**ZAFAR IQBAL KHAN[1], KAUSAR PARVEEN[1], KAFEEL AHMAD[1,2], TASNEEM AHMAD[3], NAILA RIAZ[4], ABRAR HUSSAIN[5], ASMA ASHFAQ[1], FAIZA ZUBAIR[4], IJAZ RASOOL NOORKA[6], ABID EJAZ[1], HAFSA MEMONA[7], SHAHZADI MAHPARA[8], ALLAH BAKHSH GULSHAN[9], TAYYABA AZIZ[1], TAYYBA LIAQAT[1], MUMTAZ AKHTAR[10], MUHAMMAD UMER FAROOQ AWAN[11], FARZANA SIDDIQUE[12], ABDLLAH SALEH ALSUBAIL[13], AND RAND SULEIMAN ALHUTHAYLI[14]**

[1]*Department of Botany, University of Sargodha, Punjab, Pakistan*
[2]*Department of Botany, Baba Guru Nanak University, Nankana Sahib, Punjab, Pakistan*
[3]*Pakki Thatti Research and Development Farm, Toba Tek Singh, Pakistan*
[4]*Department of Zoology, University of Sargodha, Punjab, Pakistan*
[5]*Department of Botany, University of Education, Township Campus, College road Lahore, Pakistan*
[6]*Department of Plant breeding and Genetics, University college of Agriculture, University of Sargodha, Punjab, Pakistan*
[7]*Queen Mary College, Lahore, Punjab, Pakistan*
[8]*Department of Plant breeding and Genetics, Ghazi University, Dera Ghazi Khan, Pakistan*
[9]*Department of Botany, Ghazi University, Dera Ghazi Khan, Pakistan*
[10]*Department of Zoology, Superior University Campus, Sargodha Pakistan*
[11]*Department of Botany, Government College University, Lahore Pakistan*
[12]*Institute of Food Science & Nutrition, University of Sargodha, Punjab Pakistan*
[13]*Ar-Rawdha, District Jeddah, Darweesh, Kayyal; St. 23433,2348, Abdullah Sultan, Ar-Rawdah, Jeddah,23435, Kingdom Saudi Arabia*
[14]*Hamraa District, Hail St, ‘JCHA 7324, 7324, 3190, Jeddah 23323 School 8521 Al Safaa, An Nahdah, 8481-, Jeddah 23523 Saudi Arabia.*

*Corresponding Author’s email: naila.riaz@uos.edu.pk

**ABSTRACT**

Recent study was directed to check the accumulation of Cd and Pb in pasture land treated with ground water. In particular the transfer of cadmium and lead from soil to forages and in turn to animal (buffaloes) was conducted in Bhalwal, Punjab, Pakistan which comes under sub-tropical environmental conditions. The Cd and Pb concentration in selected samples was explored by atomic absorption spectrophotometer (AA-6300 Shimadzu Japan). The results depicted the concentration of cadmium in water, soil, forages, milk and hair of buffaloes was in the range of 0. 00320 – 0.00866 $mgL^{-1}$, 1.9500 to 5.3000 mg/kg, 0.300 to 0.7100 $mgkg^{-1}$, 0.1033 to 0.4133 $mgL^{-1}$ and 0.037 to 0.0656 mg/kg, respectively. The lead concentration was ranged from 0.004 mg/L to 1.963 $mgL^{-1}$ for water, 5.960 -13.600 mg/kg for soil, 0.293 to 2.570 mg/kg for forages, 0.2166 to 6.100 mg/L for milk and 0.0206 to 0.074 mg/kg for hair samples. Various indices (BCF, PLI, EF, DIM and HRI) were examined and results presented that PLI and EF of Cd, EF and HRI for Pb was above 1 indicating that metal was causing pollution while value of BCF and DIM was below one. If exposed for an extended period of time through feed, forages with a higher Cd and Pb content may harm animal's cells, create respiratory issues and have an adverse effect on the animal's kidney, liver and lungs.

**Keywords**: Accumulation, cadmium, milk, lead, pollution.

## INTRODUCTION

In Pakistan one of the most important agricultural subsectors is livestock. It produces milk, meat, leather, manure and accounts for 12 % of the nation's GDP. This industry profoundly influences rural communities. Fodder, forage crops and soil minerals provide the nutritional demands of animals. However, livestock ingests animal tissue, including liver and kidney as well as blood samples, which at relatively low amounts may be harmful to both the health of animals and people. Toxic quantities of heavy metals in soil are taken up by plants which are then consumed by livestock and may be harmful to both animals' and people health (Ahmad et al., 2010).

The transfer and uptake of nutrients among the soil and plants as well as the quality of grazed soils are primarily influenced by grazing animals. A total of 67 species are grazed to varying degrees by animals according to the grazing status attributed to taxa. 25 of them are grasses, 14 are legumes, while 28 others are from a variety of groups. Because plants take up HMs which then pass through the food chain their accumulation in forages poses a risk to grazing animals. Trace element tolerance levels differ amongst animals and even within the same animal from day to day. The level at which a mineral element may have a negative effect is influenced by a variety of parameters including age, physiological status of the animal, nutritional status, level of different food components and biological availability of compounds. The occurrence of matelic elements its content in vegetation and on the surface of soil, duration of contact with polluted soil and pasture are the key factors influencing the buildup of potentially hazardous metals (PTM) by grazing animals (Chandio et al., 2020).

All living things depend heavily on microelements to function properly. Pathological situations frequently occur from excess or deficiency of certain chemical components, notably heavy metals in an organism. The most poisonous elements are lead and cadmium while the critical micronutrients nickel, zinc, manganese, cobalt, copper and iron are needed by both humans and animals. Whereas Mn, Fe, Ni, Co, Cu and Zn are essential micronutrients for both animals and humans. Chemical contamination bio-monitoring is becoming more crucial. The amount of toxic metals in a person's or living organisms bio-media (hair, blood, bones, urine, teeth, mother's milk, etc.) are revealed as one of the investigational surfaces that are available (Ahmadpour et al., 2012).

Compared to human hair, animal hair is a stronger index of contamination due to its exposure to contaminated soil through feed. The buildup level of toxic elements over the past years and months are reflected in the hair of animals, which is a larger duration of exposure. A biomarker for metal contamination is camel hair. Bactrian camel hair was measured for the presence of iron, manganese, zinc, copper, molybdenum, selenium and cobalt (Yang et al., 2021).

Although milk and its derivatives are of interest because of the variety of bioactive substances, fats, proteins and carbohydrates, they carry one of these fundamental elements of the human nutrition. Milk and its derivatives are one of the essential elements of the human diet especially considering its mineral content and protein (Maity et al., 2020). Because of the various diseases that these can induce in consumers particularly in youngsters, heavy metal contamination of it, among other compounds can pose a severe public health issue (Sharma et al., 2021).

Cadmium is ranked as the seventh most dangerous heavy metal by the ATSDR. At work or in the environment, both people and animals may be introduced to it because it is a by-product of the manufacture of zinc. When this metal is absorbed by people, it builds up over the course of a person's life. Metals that accumulate in plants are gradually taken up by them, where they accumulate along the food system until they ultimately spread in people community. According to the Agency for Harmful Substances and Disease Registry, over 500,000 US employees are subjected to hazardous Cd every year (Mutlu et al., 2012).Cd is mostly present in vegetables and fruits because of its extraordinary speed of translocation from soil/ to plant (Satarug et al., 2017).

The very poisonous Cd is recognized for its injurious possessions on cellular, oxidative stress, enzymatic and the induction of nutritional deficit in plants (Irfan et al., 2013). Because of its ability to absorb Cd, rice holds a distinctive place among crop plants. Rice/contain the "capability to absorb soil/Cd in lgrains, avoiding Ca, Fe and Zn (even if the soil holds 100 times more Zn than Cd)," according to Chaney et al., (2004) Farmers who consume refined rice that is lacking in, Zn, Ca and Fe face a serious threat as a result of this. Cd is a vastly poisonous noxious metal/that/is only found in the top layer of soil. Cd can harm numerous creatures, including plants. Cd can disrupt the mechanism of water and nutrient intake, photosynthesis, and oxidative stress in plants. It can also influence the activity of many different enzymes (Metwally et al., 2005).

The pervasive usage of lead has led to serious environmental degradation and health/ difficulties in numerous regions of the world. Pb is a highly toxic, shiny, silvery/metal that appears somewhat bluish in a dry environment. It initiates to blemish as soon as it is subjected to air, depending on the atmospheric factors, producing a complex chemical mixture (Sharma & Dubey, 2005). Food and smoking, residential sources, drinking water, the primary industrial operations are the sources of Pb exposure. In addition to lead paint and gasoline, other sources of lead include plumbing fixtures, energy storage systems, toys/and pipes. (Thurmer et al., 2002)

Pb is not a necessary element; hence plants lack Pb absorption pathways. However, it is yet unknown how this substance enters the tissue of the root. As an alternative ,it is attached to carboxylic/groups/ of mucilage[ uronic acids on root faces (Sharma & Dubey, 2005). Among other impacts the excess Pb in some plant species prevents chlorophyll synthesis, plant development and seed germination (Begonia et al., 2004). High levels of lead in a plant speed up the creation of reactive oxygen species (ROS), which in turn damages lipid membranes, destroys chlorophyll, disrupts photosynthetic activities and slows down the progression of the plant/as a whole (Najeeb et al., 2017).

This study's primary objectives were to examine the levels of heavy metals (Cd and Pb) and their bioaccumulation in the water, soil, forages and buffaloes tissues that being raised on pasture land in Bhalwal that receive frequent irrigation from ground water. The aim of the study was to look at the health risks in Buffaloes posed by specific components in forages because animals consuming such forage plants as their main source of mineral needs.

## MATERIALS AND METHODS

### *i. Study Area*

For this study, Bhalwal City in Punjab, Pakistan, was nominated. The current area was situated at latitude 32° 16′ and 15.9" N and longitude $72^0$ and 53′ 47.6” E. Four sites (Jhada, Kot Hakim Khan, Bhalwal and Chak No 10 SB) where ground water was used to irrigate the forages were selected for sampling of water, soil and forages. In January 2022, samplings from four sites of these chosen specimens were finished. For every site, replicates of every sample were collected.

The following forages are considered for heavy metals exploration: *Trifolium repens*, *Cynodon dactylon*, *Lolium perenne* and *Festuca arundinacea.*


**Figure 1: Map of Study Area**

### *ii. Sample collection*

#### *Water*

Sanitized plastic bottles were used for the collection of 100 ml of groundwater. To prevent it from microbial growth, concentrated nitric acid (1ml) was added.

#### *Forages*

About 500g of forage samples were kept in fresh water to remove all the dirt and then it was cleaned with deionized water. The samples were then put into an oven set to 70–75°C. the sample (10g) was proceeded to mortar and grinded to obtain a consistent mass. Lastly, the sample was stored in small airtight polythene bags for digestion and further analysis.

#### *Soil*

16 fused soil samples from all selected sites were collected. About 500g of soil was stored in polythene bag, washed with deionized water thrice and taken to the laboratory as soon as possible.

In the laboratory, the collected soil samples were dried in electrical oven at a temperature around 72°C to remove the moisture and then homogenized in mortar-pestle and reduced size to a fine powder.

#### *Animal Samples*

Sixteen buffaloes were selected of which hair and milk samples were collected. These buffaloes grazed on the same area from where the forage and soil samples were collected. Milk samples that were stored shortly after milking. For the following processing, a freezer was set at 20 °C to store 20 ml of milk. Sterilized scissors were used for cutting of hairs from neck and tail. To get rid of contaminants, distilled water was used to wash the hair samples. Following their collection, the hair samples were air-dried and placed into paper bags with labels. After that, all samples were baked for 48 hours at 90 0 C to get a constant dry weight.

### *iii. Digestion of Water, Soil, Forages and Animal Samples*

Each of the gathered water samples had 10 cc of concentrated HNO3 treated at 80 degrees Celsius. Each sample's final volume was increased to 50 ml by adding distilled water once the digestion process was finished, and they were then placed in plastic bottles for storage.

In the flask, there was around 2 g of soil and 20 ml of HNO3. The mixture that was left over was heated until 2-3 ml of the residual solution was kept. Following that, H2O2 was added, boiled once more, and allowed to cool until a clear solution was achieved (Naam et al., 2012).

$$\text{PLI}, = \frac{\text{Metal content in selected soil.}}{\text{Metal refrence value for soil}}$$

With the use of hydrogen peroxide (4 ml) and sulfuric acid (2 ml), each forage sample (1.0 g) was digested. After 30 minutes of heating in a digestion chamber, the samples were cooled. Two milliliters of hydrogen peroxide were applied to each of the cooled samples. After more heating, a colorless solution emerged from the combination. Each sample was finished up to a final volume of 50 milliliters using distilled water (Siddique et al.,2014)

Using two milliliters of sulfuric acid and four milliliters of hydrogen peroxide, each sample (1.0 g) of milk and hair was digested. After 30 minutes of heating in a digestion chamber, the samples were cooled. Two milliliters of hydrogen peroxide were applied to each of the cooled samples. After more heating, a colorless solution emerged from the combination. Each sample was brought up to a final volume of 50 milliliters using distilled water.

### *iv. Statistical Analysis*

With the aid of the SPSS software, data for a variety of attributes were statistically analyzed using correlation and one-way analysis of variance. The mean values were assessed for statistical significance at the 0.05, 0.01, and 0.001 probability levels as suggested by (Steel and Torrie, 1986).

### *Pollution Load Index*

Pollution Load Index was employed to evaluate the amount of metal contamination in the soil under investigation using the technique of Liu et al. (2005).

### *Bio-concentration factor*

The quantity of metal transferred from soil to the edible parts of forages was calculated by the bio-concentration factor (BCF) index. The equation of Cui et al. (2004) determines it.

$$\text{BCF} = \frac{\text{MV (Forage)}}{\text{MV (Soil)}}$$

Where,

MV (Fruit) = Metal value (mgKg-1) in forages

MV (Soil) = Metal value (mgKg-1) in soil

### *Enrichment Factor*

Enrichment factor was calculated by following Ahmad et al. (2016).

$$\text{EF} = \frac{\text{MV in sampled forage/MV in sampled soil}}{\text{MV}\left(\frac{\text{Forage}}{\text{Soil}}\right)\text{Standard}}$$

### *Daily Intake of Metals*

According to Shahid et al. (2015), there are a variety of methods for heavy metals to accumulate in Individual body, including through skin contact, inhaling and eating contaminated forages. The daily Consumption of heavy metals is calculated using the formula below.

$$\mathrm{DIM} = \frac{\mathrm{C} \times \mathrm{F} \times \mathrm{D\ food\ intake,}}{\mathrm{W}}$$

C represents the Concentration of metal in plants, F represents Conversion factor that was 0.085 (Jan et al., 2010), D food intake represents Forages intake per day, W is Average body weight of a buffalo was estimated to be 550 kg, and its daily forage need was estimated to be 12.5 kg (Yang et al., 2020).

### *Health Risk Index (HRI)*

It is estimated as the daily intake of metal to oral reference dosage ratio (DIM/RFD). The following formula was used to calculate the HRI:

$$\mathrm{HRI,} = \frac{\mathrm{DIM}}{\mathrm{RfD}}$$

DIM stands for daily intake of metals, while Rf D stands for the oral reference dose. If the HRI value is more than 1, users are subjected to extreme toxicity.

**Table 1: The Cadmium and Lead, reference values, standard reference values and oral reference dose in soil and forages were as follows.**

| Metal | Reference values in Soil | Standard reference values | | (RfD) | References |
|---|---|---|---|---|---|
| | | Soil | Forages | | |
| **Cadmium** | 1.49b | 0.2b | 1.49e | 0.0005 f | b(Hassan et al., 2013); e(Dosumu et al., 2005); f(USEPA, 2010) |
| **Lead** | 8.15 a | 3 c | 8.15a | 0.004d | a (Singh et al., 2010); d(USEPA, 2010); c(FAO/WHO,2001) |

## RESULTS

### *Cd and Pb Concentration in Irrigated Water*

The concentration of Cd and Pb ranged from 0.0032 to 0.00866 mg/L and 0.004 to 1.963 mg/L respectively. In comparison to other locations Jh-I presented least contamination for cadmium, but at C10-IV, due to the buildup of urban effluents, water is more contaminated. The means of Pb concentration was found high in water at C10-IV and was lowest at site Khk-II (Table 1).

### *Cd and Pb concentration in Soil samples*

The concentration of Cd and Pb were highly significat p<0.001 and ranged from 1.950 to 5.300 mg/kg and 5.960 - 13.600 mg/kg respectively. Estimated Cd mean value was highest in *L. perenne* soil and least in *T. repens* at Jh-I. The means of Pb concentration was found high in soil of *T. repens* at Bh-III and lowest mean value was detected in soil of *F. arundinacea* at Jh-I (Table 2 &3).

### *Cd and Pb concentration in forages/*

The concentration of Cd and Pb were highly significat p<0.001 but forages were non-significantly effected (P<0.05) on Pb concentration, their concentration were ranged from 0.300 to 0.7100 $mgKg^{-1}$ and 0.293 to 2.570 $mgKg^{-1}$. Comparatively *L. perenne* had the highest mean value of Cd at C10-IV and *F. arundinacea* had the lowest at Khk-II. The highest mean concentration of Pb was seen in *T. repens* at C10-IV and lowest in *F. arundinacea* at Jh-I (Table 4 and 5).

**Table 1: Cadmium and Lead concentration in ground water samples**

| Sites | Cd concentration in Water | | | |
|---|---|---|---|---|
| | *T. repens* | *C. dactylon* | *L. perenne* | *F.arundinacea* |
| **Jh-I** | 0.0050±0.0015 | 0.00450±0.00115 | 0.00800±0.00153 | 0.00320±0.0000058 |
| **Khk-II** | 0.0060±0.0015 | 0.00563±0.00177 | 0.004600±0.00116 | 0.008300±0.000577 |
| **Bh-III** | 0.00500±0.015 | 0.00660±0.00155 | 0.00580±0.000058 | 0.006400±0.0058 |
| **C10-IV** | 0.0060±0.0015 | 0.00700±0.0115 | 0.00866±0.006677 | 0.0071967±0.00118 |
| **Mean squares** | $0.000^{***}$ | $0.000^{***}$ | $0.000^{***}$ | $0.000^{***}$ |
| **Pb concentration in Water** | | | | |
| **Jh-I** | 0.070±0.0115 | 0.011±0.00115 | 0.016±0.00115 | 0.0250±0.00115 |
| **Khk-II** | 0.004±0.00115 | 0.008±0.00176 | 0.0230±0.00115 | 0.0450±0.00115 |
| **Bh-III** | 0.300±0.1154 | 0.970±0.01154 | 0.330±0.01154 | 0.250±0.01154 |
| **C10-IV** | 0.230±0.01154 | 1.300±0.1154 | 1.963±0.01763 | 0.760±0.01154 |
| **Mean squares** | $5.894^{ns}$ | $1.321^{ns}$ | $2.604^{ns}$ | $0.351^{ns}$ |

****Correlation-is significant)at 0.001 level ; ns= non-significant*

**Table 2: Analysis of variance data for Cd and Pb in Soil samples ($mgKg^1$)**

| Source Of Variation (S.O.V) | DF | Cd Mean Squares | Pb mean Squares |
|---|---|---|---|
| Sites | 3 | $21.2633^{***}$ | $60.6741^{***}$ |
| plants | 3 | $1.5903^{***}$ | $7.7629^{***}$ |
| Sites*plants | 9 | $1.3222^{***}$ | $13.9088^{***}$ |
| Error | 32 | 0.0195 | 0.0177 |

**** Significant at 0.001 level*

**Table: 3 Cadmium and Lead concentration in Soil**

| Sites | Cd concentration in Soil | | | |
|---|---|---|---|---|
| | *T. repens* | *C. dactylon* | *L. perenne* | *F.arundinacea* |
| **Jh-I** | 1.950±0.0115 | 2.420±0.01154 | 2.270±0.01154 | 3.460±0.01154 |
| **Khk-II** | 2.600±0.1154 | 3.370±0.01154 | 3.300±0.1154 | 4.300±0.1154 |
| **Bh-III** | 4.500±0.1154 | 4.100±0.01154 | 5.300±0.1154 | 4.134±0.00577 |
| **C10-IV** | 3.670±0.1154 | 5.130±0.09074 | 5.023±0.0384 | 4.753±0.0306 |
| **Pb concentration in Soil** | | | | |
| **Jh-I** | 7.430±0.01154 | 9.500±0.1154 | 6.960±0.01154 | 5.9600±0.0115 |
| **Khk-II** | 8.360±0.01154 | 6.060±0.01154 | 9.450±0.01154 | 7.600±0.1154 |
| **Bh-III** | 13.700±0.1154 | 9.700±0.1154 | 7.330±0.01154 | 11.600±0.1154 |
| **C10-IV** | 11.960±0.01154 | 9.30±0.1154 | 12.760±0.01154 | 14.700±0.1154 |

**Table 4: Analysis of variance data for Cd and Pb in Forage samples (mgKg$^{1}$)**

| Source Of Variation (S.O.V) | DF | Cd Mean Squares | Pb Mean Squares |
|---|---|---|---|
| Sites | 3 | 0.327013$^{***}$ | 7.13540$^{***}$ |
| Forages | 3 | 0.004547$^{***}$ | 0.03272$^{ns}$ |
| Sites*Forages | 9 | 0.013826$^{***}$ | 0.52259$^{***}$ |
| Error | 32 | 0.000404 | 0.03163 |

**** Significant at 0.001 level ; ns=non-significant*

**Table 5: Cadmium and Lead concentration in forages**

| Sites | Cd concentration in Forages | | | |
|---|---|---|---|---|
| | ***T. repens*** | ***C. dactylon*** | ***L. perenne*** | ***F.arundinacea*** |
| **Jh-I** | 0.320±0.0115 | 0.300±0.01154 | 0.41000±0.01154 | 0.4500±0.01154 |
| **Khk-II** | 0.300±0.0115 | 0.5333±0.01763 | 0.4700±0.01154 | 0.3700±0.01154 |
| **Bh-III** | 0.410±0.0115 | 0.7000±0.01154 | 0.3700±0.01154 | 0.510±0.005773 |
| **C10-IV** | 0.453±0.0088 | 0.4700±0.01154 | 0.2400±0.01154 | 0.4500±0.01154 |
| **Pb concentration in Forages** | | | | |
| **Jh-I** | 0.480±0.01154 | 0.4300±0.1154 | 0.340±0.17009 | 0.293±0.1467 |
| **Khk-II** | 0.730±0.01154 | 0.760±0.01154 | 0.660±0.01154 | 0.820±0.0115 |
| **Bh-III** | 1.020±0.1154 | 1.070±0.1154 | 2.300±0.1154 | 1.500±0.1154 |
| **C10-IV** | 2.570±0.2371 | 2.500±0.1154 | 1.700±0.1154 | 1.967±0.2404 |

**Table 6: Analysis of variance data for Cd and Pb in Milk samples (mgL$^{1}$)**

| Source Of Variation (S.O.V) | DF | Cd Mean Squares | Pb Mean Squares |
|---|---|---|---|
| Sites | 3 | 0.377006$^{***}$ | 0.002242$^{***}$ |
| Milk | 3 | 0.037700$^{**}$ | 0.001045$^{***}$ |
| Sites*Milk | 9 | 0.009435$^{ns}$ | 0.000702$^{***}$ |
| Error | 32 | 0.007396 | 0.000097 |

**** Significant at 0.001 level; ** Significant at 0.01 level; ns=non-significant*

**Table 7: Cadmium and Lead in milk samples**

| Sites | **Jh-I** | **Khk-II** | **Bh-III** | **C1-IV** |
|---|---|---|---|---|
| **Mean concentration of Cd** | 0.1033±0.103 | 0.3166±0.1675 | 0.2466±0.246 | 0.4133±0.212 |
| **Mean concentration of Pb** | 0.2166±0.088 | 0.366±0.5206 | 0.466±0.433 | 0.540±0.0692 |

**Table 8: Analysis of variance data for Cd and Pb in Hair samples (mgKg$^{1}$)**

| Source Of Variation (S.O.V) | DF | Cd Mean Squares | Pb Mean Squares |
|---|---|---|---|
| Sites | 3 | 1.22567$^{***}$ | 96.4263$^{***}$ |
| Hairs | 3 | 0.00085$^{***}$ | 0.6677$^{***}$ |
| Sites*Hairs | 9 | 0.04909$^{***}$ | 2.2439$^{***}$ |
| Error | 32 | 0.00131 | 0.0616 |

**** Significant at 0.001 level; ns=non-significant*

**Table 9: Cadmium and Lead concentration in hair samples**

| Sites | Jh-I | Khk-II | Bh-III | C1-IV |
|---|---|---|---|---|
| **Mean concentration of Cd** | 0.037±0.005 | 0.0463±0.0060 | 0.0603±0.0108 | 0.0656±0.048 |
| **Mean concentration of Pb** | 0.0206±0.0108 | 0.045±0.0235 | 0.074±0.0180 | 0.066±0.0056 |

**Table 10: PLI for Cadmium and Lead**

| Sites | Cd PLI | | | |
|---|---|---|---|---|
| | ***T. repens*** | ***C. dactylon*** | ***L. perenne*** | ***F.arundinacea*** |
| **Jh-I** | 0.696 | 0.864 | 0.810 | 1.235 |
| **Khk-II** | 0.928 | 1.203 | 1.178 | 1.535 |
| **Bh-III** | 1.607 | 1.464 | 1.892 | 1.476 |
| **C10-IV** | 1.310 | 1.832 | 1.794 | 1.697 |
| **Pb PLI** | | | | |
| **Jh-I** | 0.911 | 1.165 | 0.853 | 0.741 |
| **Khk-II** | 1.025 | 0.733 | 1.159 | 0.932 |
| **Bh-III** | 1.435 | 1.190 | 0.899 | 1.668 |
| **C10-IV** | 1.467 | 1.141 | 1.565 | 1.803 |

**Table 11: Bio concentration factor for Cadmium and Lead**

| Sites | Bio concentration factor for Cd | | | |
|---|---|---|---|---|
| | ***T. repens*** | ***C. dactylon*** | ***L. perenne*** | ***F.arundinacea*** |
| **Jh-I** | 0.164 | 0.115 | 0.132 | 0.109 |
| **Khk-II** | 0.088 | 0.095 | 0.921 | 0.093 |
| **Bh-III** | 0.091 | 0.114 | 0.069 | 0.058 |
| **C10-IV** | 0.123 | 0.072 | 0.101 | 0.094 |
| **Bio concentration factor for Pb** | | | | |
| **Jh-I** | 0.064 | 0.045 | 0.048 | 0.049 |
| **Khk-II** | 0.087 | 0.125 | 0.069 | 0.107 |
| **Bh-III** | 0.087 | 0.110 | 0.313 | 0.110 |
| **C10-IV** | 0.214 | 0.268 | 0.133 | 0.133 |

### *Cd and Pb Concentration in Buffalo Milk*

The findings from analysis of the variance of data revealed that the concentration of Cd and Pb were highly significat $p<0.001$ but Sites×milk for Cd was non-significantly effected $(0.05>P)$ and their concentration were ranged from 0.1033 to 0.4133 mg/L and 0.2166 to 0.540 mg/L. At C10-IV buffalo milk showed highest mean conc. of cadmium and lead, while least concentration was observed at Jh-I (Table 6 7).

### *Cd and Pb Concentration in Buffalo Hair*

The concentration of Cd and Pb were highly significant $p<0.001$ and their concentrations were ranged from 0.037 to 0.065 mg/kg and 0.0206 to 0.074 mg/kg. The buffaloes of Jh-I showed least mean concentration in milk. While in C10-IV, a higher mean content of Cd and Pb was detected (Table 8 and 9).

**Table: 12 Enrichment Factor for Cadmium and Lead**

| Sites | Enrichment Factor for Cd | | | |
|---|---|---|---|---|
| | ***T. repens*** | ***C. dactylon*** | ***L. perenne*** | ***F.arundinacea*** |
| **Jh-I** | 1.418 | 0.415 | 0.984 | 0.818 |
| **Khk-II** | 1.430 | 0.651 | 0.558 | 0.693 |
| **Bh-III** | 0.794 | 0.644 | 0.5200 | 0.432 |
| **C10-IV** | 1.712 | 0.621 | 0.7563 | 0.705 |
| **Enrichment Factor for Pb** | | | | |
| **Jh-I** | 0.048 | 0.0492 | 2.4766 | 3.166 |
| **Khk-II** | 0.069 | 0.1071 | 2.7866 | 2.020 |
| **Bh-III** | 0.313 | 0.110 | 3.900 | 3.233 |
| **C10-IV** | 0.133 | 0.133 | 3.986 | 3.10 |

**Table 13: Daily intake of Cadmium and Lead**

| Sites | Daily intake of Cd | | | |
|---|---|---|---|---|
| | ***T. repens*** | ***C. dactylon*** | ***L. perenne*** | ***F.arundinacea*** |
| **Jh-I** | 0.00062 | 0.00054 | 0.0006 | 0.000734 |
| **Khk-II** | 0.00059 | 0.00040 | 0.00064 | 0.000781 |
| **Bh-III** | 0.00079 | 0.00091 | 0.00072 | 0.000464 |
| **C10-IV** | 0.00088 | 0.00072 | 0.00099 | 0.000869 |
| **Daily intake of Pb** | | | | |
| **Jh-I** | 0.0009 | 0.00083 | 0.0006 | 0.00056 |
| **Khk-II** | 0.00147 | 0.00146 | 0.0012 | 0.00158 |
| **Bh-III** | 0.0019 | 0.00206 | 0.0044 | 0.00289 |
| **C10-IV** | 0.00496 | 0.00482 | 0.003 | 0.00379 |

**Table 14: Health risk index for Cadmium and Lead**

| Sites | Health risk index for Cd | | | |
|---|---|---|---|---|
| | ***T. repens*** | ***C. dactylon*** | ***L. perenne*** | ***F.arundinacea*** |
| **Jh-I** | 0.618 | 0.541 | 0.58 | 0.734 |
| **Khk-II** | 0.630 | 0.6730 | 0.665 | 0.780 |
| **Bh-III** | 0.792 | 0.908 | 0.715 | 0.4636 |
| **C10-IV** | 0.876 | 0.715 | 0.985 | 0.8693 |
| **Health risk index for Pb** | | | | |
| **Jh-I** | 0.2649 | 0.237 | 0.1876 | 0.1619 |
| **Khk-II** | 0.4029 | 0.419 | 0.3642 | 0.4525 |
| **Bh-III** | 0.5629 | 0.590 | 1.2694 | 0.8279 |
| **C10-IV** | 1.4185 | 1.379 | 0.938 | 1.085 |

## *Pollution Load Index (PLI) for Cd and Pb*

For all types of soils, evaluated PLI values for Cd and Pb were found to fall within the range of 0.696-1.892 and 0.733 -1.803. PLI of cadmium was minor in *T. repens* at Jh-I. While *L. perenne* had maximum value at Bh-III, and for Pb maximum PLI identified at C10-IV in *F arundinacea* which showed contamination of soil by anthropogenic activities. Least observed value was at Khk-II in soil of *C. dactylon* (Table 10).

***Bio concentration factor for Cd and Pb*:**

BCF values of Cd and Pb were ranged from 0.0580 – 0.164 mg/kg and 0.045 -0.268 mg/kg. For Cd, *T. repens* exhibited maximum value at Jh-I. Minimum concentration was detected by *F. arundinacea* at Bh-III. Maximum value of BCF for lead was inspected at C10-IV in *C. dactylon,* while least value was observed at Jh-I in *C. dactylon* (Table 11).

***Enrichment Factor for Cd and Pb***

The Cd and Pb concentration was ranged between 0.0415 to 1.7126 mg/kg and 0.048 to 3.986 mg/kg among all locations. The peak value for Cd and Pb in *T. repens* was observed at C10-IV, and the lowest value for Cd and Pb was detected in *C. dactylon* and *T. repens* at Jh-I (Table 12).

***Daily Intake of Cd and Pb by Buffaloes***

The range of the daily intake for cadmium and lead was 0.00040-0.00099 mg/kg/day and 0.0012-0.00496 mg/kg/day. Maximum value of cadmium and lead was observed at C10-IV while lowest value was demonstrated at site Khk-II (Table 13).

***Health Risk Index of Cd and Pb for Buffaloes***

HRI for cadmium and lead ranged from 0.4636 – 0.985 mg/kg/day and 0.161 to 1.418 mg/kg/day. Data showed that HRI for Cd was greater at C10-IV in contrast to every other site, while minimum value was detected at Bh-III. While in case of Pb *T. repens* had the largest concentration found in forage at C10-IV, while lowest value was detected in *F. arundinacea* (Table 14).

**DISCUSSION**

Water samples had level of Cd ranging from 0.00320 to 0.00866 mg/L. A number of hazardous metals, including Cd are present in wastewater drainage as a result of various industrial operations. Due to its higher concentration, this water might also contaminate groundwater. In this study, Cd concentration in water samples was found to be within the range (0.1 mg/l) reported by Hassan et al.( 2013). The Cd levels in all of the water samples are the lowest compared to the readings (0.036 mg/L) offered by Aurangzeb et al. (2011) and Ahmad et al. (2024). According to Ahmad et al.(2018) the Cd concentration (1.69-1.88 mg/l) was greater than the levels that were looked at.

Pb levels can vary from 0.004 mg/L to 1.963 $mgL^{-1}$. Pb concentrations in the current study were greater than those (0.08–0.34 mg/l) suggested by Farid and Baloch (2012). In waste water, Hassan et al.( 2013) showed a lower concentration of Pb (0.25 mg/L). Mousavi and Shahsavari (2014) examined wastewater at substantially less content (0.001–0.012 $mgL^{-1}$). In comparison to our findings for water, Aurangzeb et al. (2011) found the highest amount of Pb (6.12 mg/L). The main causes of Pb pollution in water sources are several industrial practices (Ugulu et al., 2022, Ugulu et al., 2023)

The range of Cd content in soil was 1.9500 to 5.3000 $mgKg^{-1}$. Due to soil's retention and limited mobility, contaminants tend to collect in top layer of soil. Sand and clay particles in the soil prevented the transport of heavy metals. Due to its severe toxicity, cadmium harms bones and the kidneys in particular in humans. All soil samples had lower Cd concentrations to that (11.22 $mgKg^{-1}$) provided by Chaoua et al. (2018). Cd content (2.001, 2.16 mg/kg) in soil recommended by Khan et al.(2017) was discovered to be lower than our findings. In soil samples, Wang et al. (2015) found a lower concentration of Cd (0.05 mg/kg) than these figures.

The range of the average Pb concentration in soil was determined to be 0 5.960 – 13.600 mg/kg. According to Frank Hsu and Taksa (2005) the ideal range of Pb concentrations in soil for growing forages is between 5 and 25 mg/kg. Mlay and Mgumia (2008) reported a greater content of Pb in soil when the consequences of the current analysis were compared to those of other comparable readings. The primary causes of soil Pb contamination are a number of industrial applications. In opposed to level (57.36 mg/kg) of lead in soil provided by Chaoua et al.(2018), the present Pb content in soil samples was found to be lower. In comparison to the current study, Farid and Baloch (2012) showed greater results for Pb levels (6.45-25.83 mg/kg) in soil.

The range of Cd content in forages is wide, ranging from 0.300-0.700 mg/kg. It has been discovered that the symplast pathway is how Cd is carried from the root to the shoot. Increasing the Cd level in the root immediately enhances the likelihood of Cd transfer to the shoot. Cadmium levels (0.2 mg/kg) in forage plants above the maximum allowable limit set by FAO, (2007). Wang et al.(2015) proposed a Cd concentration range (0.05-1.17 $mgKg^{-1}$), which was lower than concentration found in the present work. It was discovered that the Cd content (0.06 mg/kg) provided by Aurangzeb et al. (2011) was lower. In comparison to our findings, Chaoua et al.(2019) observed lower levels (2.175 mg/kg) in the edible section of forage.

Lead content in forages fluctuated from 0.293 to 2.570 mg/kg, according to the research's findings. In this study, the lead concentration in forage was lower than the risky level (30 $mgKg^{-1}$) set by (Oluokun et al., 2007). In comparison to concentrations of this metal discovered in the current investigation, the study conducted by Darwish and Pöllmann (2015) revealed a greater concentration of Pb (24.67 $mgKg^{-1}$). In related to the present work, Ghaderpour et al.(2018) reported lower Pb readings. Pb levels in forages were lower than those (6–16 mg/kg) recorded by Mbarki et al. (2018).

In milk samples, Cd levels between 0.1033 and 0.4133 mg/L were found. Human toxicity brought on by cadmium buildup may result in hepatic, skeletal, and kidney diseases (Zaidan et al., 2013) In contrast to our data, Younis et al. (2016) reported higher levels of cadmium (0.92 mg/L) in milk samples from farms and markets located near wastewater drains in Jhang city, Punjab, Pakistan. Aslam et al.(2011) showed a smaller range for the Cd content (0.083-0.145 mg/kg) in milk samples than what this study assessed. Ismail et al.(2015) examined Cd results that were lower (0.001mg/kg) than those of the current study.

Pb concentrations in milk samples at the four sites under examination fluctuated from 0.2166 to 0.54100 $mgL^{-1}$. The toxicity of milk and its products is increasing daily due to growing industrialization (Swarup et al., 2005) . Younis et al. (2016) reported the content (1.25 mg/L) in milk samples from dairy farms, which was higher than what was seen in this investigation. Aslam et al.(2011) indicated a value for Pb (41.762-43.414 mg/L) that was significantly higher than the amount of the same metal considered in this investigation. Ismail et al.(2015) reported a lower Pb concentration in milk samples (0.014 mg/kg) than what has since been discovered.

The content of Cd in the hair of buffaloes diversed from 0.037 - 0.0656 mg/L and these results were below as predicted by Cygan-Szczegielniak et al. (2014) and Krupa et al. (2006). The PLI for the Cd levels in soil samples taken from four different locations fluctuated from 0.696 to 1.892 mg/kg. Ahmad et al. (2016) found decreased PLI for soil cadmium (0.11-0.12) than the current study. The Samra Siddique et al.(2019) range (1.11-3.317) for the Pollution load index of Cd was found to be greater than the present values. Similar research was conducted by Ahmad et al.(2014) in which PLI value for Cd was 1.57. Results showed that applying wastewater to agricultural crops enhanced soil pollution.

At all sites, PLI values varied from 0.733 to 1.803 $mgKg^{-1}$. By using pollutant load index, the level of soil contamination was assessed at four separate locations. The PLI value for Pb in the current investigation was lower than the soil's reference value, which was stated by Singh et al. (2010) as 8.15. Pb had a higher pollution load level (2.96-3.77) in soil, according to Ahmad et al. (2016). The findings of Ashfaq et al. (2015) demonstrated a higher PLI level (2.84 mg/kg) for Pb in comparison to the results of the current investigation.

BCF fluctuated from 0.0580 to 0.164 mg/kg for Cd content in soil taken from four different locations. Compared to the current investigation, Alrawiq et al.(2014) evaluated the BCF concentration for Cd (0.5 mg/kg). The BCF range of cadmium (0.14-0.13) reported by Khan et al.(2017) was seen between range of the current study.

At all sites, BCF content ranked from 0.045 to.268 $mgKg^{-1}$. Pb's BCF content was less than 1, demonstrating that it has no hazardous effects on the forages and animals in the current study region. The results of this investigation are supported by the BCF value for Pb (0.22-0.39) found by Ahmad et al. (2014). Ahmad et al. (2016) reported BCF values (0.349-0.427) in ground and waste water samples that were found to be marginally higher than those in the present investigation. Lead content of BCF found in a recent research was more than Opaluwa et al. (2012) found during their analysis.

Present investigation revealed that content of EF for lead was greater than 1, which designates toxicity in the studied area. In contrast to what was discovered in the current research, Proshad et al. (2017) predicted a greater Pb concentration. Krishna et al. (2013) reported a lower concentration of EF for lead in comparison to the current study.

In this investigation, the daily Cd intake fluctuated from 0.000403 to 0.000985 $mgKg^{-1}$. According to current data, Chaoua et al. (2019) predicted daily consumption for Cd was higher at (0.001 mg/kg). In comparison to existing values, Ismail et al. (2017) proposed a slightly lower value of DIM (0.00015mg/kg) for Cd in samples. The maximum DIM value 0.158 mg/kg for Cd was estimated by Salah et al. (2013). According to the most recent Cd HRI results, Cd can have hazardous effects on animals that use forages growing in sewage water.

The present findings pointed to a daily consumption of Pb in human milk samples of (0.048-3.98 mg/kg). Chaoua et al. (2018) found that the DIM for Pb was lower (0.03 mg/kg) than the current range. Ismail et al. (2017) estimate of Pb daily intake (0.00006-0.00094 mg/kg) was discovered to be less than the existing DIM limits. The result of DIM was higher than what Ismail et al. (2015) had predicted with 0.0035 mg/kg.

The HRI concentration for Pb in the current investigation was greater than 1, which suggests that the forages the animals eat may be harmful to their health (Khan et al., 2023). A low HRI value was reported by Ahmad et al. (2018) in comparison to a recent experiment.

## CONCLUSION

The aim of this work was to determine the content of Cadmium (Cd) and lead (Pb) in milk and hair from buffaloes fed forage irrigated with ground water and to determine the rate of transfer of these elements from forages to milk and hair samples. From this research it was concluded that occurrence of both microelements and macro elements in forages is necessary to provide the dietary demands of grazing animals, as all biological and metabolic processes depend on the metal elements found in forages. Soil is enriched with heavy metals due to some anthropogenic and natural sources that cause contamination of forages and these polluted forages are grazed by animals, pollutants buildup in their body tissues that are ingested by humans and cause health risks. This research work was planned to gauge the level of toxic metals in soil, forages and animal tissues and to learn about the different metal indices to estimate the level of pollution.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHORS CONTRIBUTION

ZIK and KA supervised the research work. KP did the experimentation and wrote the manuscript. TA,NR,AH,AA and FZ review and analyze the manuscript. IRN, AE, HM,SM and ABG helps in statistical analysis and formal analyis. TA,TL,MA,MUFA and FA heps in formla analyis and methodology.

## CONSENT TO PARTICIPATE

All authors voluntarily to participate in this research study.

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