Health Risk Assessment and Seasonal Trend of Metals in Arid-Soil-Forage Crops and Animals

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Abstract

Background: The aim of study was accessing the cobalt (Co) and lead (Pb) contamination in soil, forages and animals. The heavy metal ions bioaccumulation and escalating transfer to food chain has become main concern for mankind. Nevertheless, they play crucial role in organismal metabolism, however exceeding threshold level causes toxicity or even system failure. Since the plants are the main source of nutrition supply and carbohydrates for animal metabolism, therefore mankind exposure risk to food chain heavy metal toxicity increases significantly.

Method: To highlight toxicity levels and exposure risk we designed this field study in the nearby areas of district Mianwali, Punjab, Pakistan during different seasons, and carried out samplings of soil, native flora. While following research ethics we collected samples of blood, hair and feces from domestic herbivore animals. Traditional wet digestion was carried out in available laboratory facilities, Pb and Co heavy metal concentrations were analyzed with atomic absorption spectrum. With emphasis on health risk index (HRI) and bio-concentration factor (BCF), we also draw attention to pollution load index (PLI), daily intake of metal (DIM), Health Risk Index (HRI) and Enrichment factor (EF).

Results: Our findings revealed high Pb concentration (5.29-9.84 mg/kg) in forage during season 1. While our findings indicate concentrations of metal in different seasons. When the Co concentration in forages was 0.238 to 0.492 mg/kg and Pb was found in the range of 5.29-9.84 mg/kg). Soil samples showed higher level of (PLI) Pollution load index. Bio-concentration of cobalt and lead was (0.447- 1.106 and 0.717- 1.152 mg/kg) respectively.

Conclusions: Bio-concentration factor and pollution load index for Co and Pb was noticed significantly higher than 1. Whereas enrichment factor (EF) was under permissible limit for both metals, daily intake metal (DIM) and no harmful health risk concentration was found. Therefore, wide-scale regular monitoring for heavy metal risk assessment on seasonal basis are highly recommended to avoid or minimize further contamination.

1. Introduction

Extreme toxicity for the metal is due to reason that, dissimilar to organic matter, trace metals are not biodegradable although they merely alter their oxidation state as well as they are very exceedingly persistent in environment having a half-life greater than twenty years (Hadia-e-Fatima et al., 2018). recognized elements are termed as heavy metals and they are characterizing as universal pollutants having densities were greater than 5 gram per centimeter cube (Ashraf et al., 2019; Prieto et al., 2018). In the soil ecosystem heavy metal elements are components of soil. Accumulation of metal in soil and getting mixed with the groundwater is perhaps damaging to livestock, forages and other plants.

In soil heavy metals are present in combined state and are soluble with water. Some heavy metals occur in soil having soluble state of water. These metals are mobile because they are chelated with other metal and metalloids hence, they are easily available to forage plants. Heavy metals are incorporated into food chain in a direct pathway when these trace metals are accumulated in forages because forages absorb trace metals from water, air and soil (Chiroma et al., 2014).

Contaminants consisted of toxic consequences of organism's corpse by producing digestion related issues, malfunctioning of excretive system, immune system disorders, respiratory diseases, and glandular failure. Chronic disorders, lethal cancerous tumours, psychological illnesses, reducing growth and development and psychological illnesses are furthermore linked with toxicosis (Unver et al., 2015).

Forages species that are grown on contaminated soil become more contaminated by the heavy metals (Nadeem et al., 2019). Pasturage grown along boundaries of highways are promptly influenced by heavy metals stress by liberation of unhealthy smoke, these hazardous conditions have more dangerous impacts on forages as compared to others (Dogan et al., 2014a,b). Heavy metals enter food chain firstly they are accumulated into forage plant body and soil; through forage consumption heavy metals enter the body of livestock (Khan et al., 2018).

Cobalt is a trace metal in forages shown by its normal concentration level, its normal concentration found in their dry weight is almost $0.1-10 \mu\text{g g}^{-1}$. Many enzymes and coenzymes contain cobalt as their significant constituents. Physiological conditions of forages and their growth is altered by the amount of Co (cobalt) occurring in rhizosphere and soil. It commonly from complexes while interacting with other metals. Seasonal changes have influenced the bioavailability of heavy metals and mature growth and developments of forages (Kalaivanan et al., 2016).

Metabolic pathways of forage plants are directly affected by lead (Pb) accumulation in forages; although, it produces deficiencies of other metals, lead (Pb) is also responsible of damage to plant cells when its level is above permissible limits and cause toxicity (Bibi et al., 2014). When Pb level is less than permissible limits in the soil of an area this is not harmful for grazing ruminants and forage species. If canal water is used for irrigation purpose instead of industrial effluent water and sewage water, the Pb concentration is less in these plants, so the forages less hazardous for the grazing animals. Anthropogenic activities increase the lead (Pb) contamination above the permissible limits and change the extrinsic and intrinsic factors of pasture soil (Shad et al., 2014). This study aims to investigate the concentrations of lead (Pb) and Cobalt (Co) in soil, forage crops and animals. Current study was designed with following objectives; seasonal variations among bioconcentration, pollution load indices and daily intake metals for heavy metals concentrations.

2. Materials And Methods

2.1 Study Area:

District Mianwali is in south-western region of province Punjab. This district has plains of western area of salt range. It is situated near the Sakesar hill. Mianwali district has boundaries with Khushab, D.I Khan, Bhakkar and Bannu districts. Canal irrigation system is very less developed, only a little area is

irrigated with Indus river irrigation system. The native of this district is affiliated with bordering plant resources. District Mianwali is divided into three Tehsils such as Piplan, Mianwali and Esakhel. Mianwali city is the larger city. Its human population is about 140000. One of the popular areas is Kalabagh, brown salt hills, Kalabagh Dam, Indus river and Nawab of Kalabagh. At the south side of district tehsil Piplan is situated and towards north-west tehsil Esakhel is located. These areas are economic and commercial zones of the district (Ghani et al., 2016; Qureshi et al., 2007).

2.2 Sample collection from sites

In district Mianwali four sites were selected for sampling. Three samples of agricultural soil, forages and animal blood, hair and feces were taken to examine the metal profile of soil-forage-animal continuum. The samples were taken from Wan bhachran site, Mianwali, Esakhel and Piplan. The summer, autumn and winter seasons were selected for sampling. The samples were taken randomly from sites.

2.3 Soil sample collection

In the district Mianwali four sites were selected to collect the samples. 3 samples of soil were collected with equal distances in the field. Stainless steel auger was used to dig up the upper layer of soil about 12-15 cm (Siddique et al., 2019). These samples were packed into plastic bags to avoid the mixing of other chemical compounds into it. Samples were stored in laboratory and labeled then metal analysis was performed. For each sample three composite samples were made. The collected samples were firstly air then oven drying at 72°C for 2 days. The samples were placed in incubators at 70°C temperature for 5 days.

2.4 Forage sample collection

Sterilized apparatus was used to collect the forage samples. Forage and soil were collected from same field and place. Only those forages were selected for taking samples that are used as common feed of livestock. 3 samples of each forage plant were taken from the sampling area. The samples were washed with distilled water to clear impurities and dirt. These samples were dried to eliminate moisture in the freshly collected samples. The collected samples were dried for further process.

These are following species that were selected for sampling. *Calotropis procera* (Apocynaceae), *Dactyloctenium aegyptium* (Poaceae), *Parthenium hysterophorus* (Asteraceae), *Rumex dentatus* (Polygonaceae) and *Ziziphus jujube* (Rhamnaceae).

2.5 Animal blood plasma, hair and feces sample collection

Blood samples of cow, buffalo and sheep of Mianwali was taken in 2020. Young animals within age of two years were selected for sampling. Blood was collected from four sites of district Mianwali. Animal blood was calculated from 10 animals (Cow, buffalo and sheep) each from each sampling site and heavy metal evaluation was done. Sterilized syringe was used to obtain the blood samples. The grazing ruminant's blood was taken from the vein. The vacuum was created in evacuated tubes while collecting blood to minimize the extent of clotting. The blood was collected in heparinized Na-citrate voiles quickly. For 15 minutes blood was centrifuged at 3000 rpm and blood plasma was separated. Polyethylene tubes were used to store the blood plasma and frozen at -20°C. Hair and feces samples were also collected and stored for the further digestion process.

2.6 Sample measurement and preparation

Arrangement and preparation of samples involves the digestion process. This method of digestion is called wet digestion. It has following steps. Acid and hydrogen per oxide is used for complete digestion process. Distilled water is added after digestion into prepared samples for dilution purpose. After that filtration of samples occur. In next step Atomic Absorption Spectrophotometer (AAS) is an apparatus through which metal analysis is done.

2.7 Apparatus and chemicals for digestion

Chemicals that are used for digestion process includes the 10 ml Nitric acid ,70% Sulphuric acid (H_2SO_4), 50% Hydrogen peroxide (H_2O_2) and newly synthesized condensed water or distilled (H_2O). The apparatus for digestion includes digestion flasks of 100 ml, measuring cylinder (50 ml), beakers (50ml) and (100ml), pipette (10 ml), filter paper, stirrer, hotplate and gloves.

2.8 Digestion of soil, forages and Animal samples

Digestion of soil and forages and animal samples (blood, hair and faeces) include various steps. First, the samples are air dried and followed by oven dried process at 72°C for 5 days until the moisture content is removed. When plants are completely dried, they weighed with electrical balance. Standard procedure of digestion was applied to digest the samples (Siddique et al. 2019). 1gm sample was weighed by electrical balance and placed in a beaker of 50ml. A 10 ml nitric acid was added to beaker and was kept overnight. Hot plate was used for digestion of sample by pouring H_2O_2 drop wise until solution becomes transparent. Cooling at room temperature was done. For dilution 50 ml distilled water added to solution. To filter the solution Whatman filter paper of 42 μm was used. Then this prepared solution was kept in plastic bottles for metal profile evaluation.

Blood samples collected from the Mianwali district were stored and freeze at -20°C. For digestion process the samples were from freezer and digested with same standard procedure as applied to soil and forages (Siddique et al., 2019). Hair sample was sun dried and was cut into pieces of 1.0-2.9 cm. De-ionized water was used to wash the samples and ethanol was also applied to wash. Oven drying process was carried out and for 4 hours and then desiccator cooling was performed (Hashem et al., 2017). Feces samples were collected from cow, buffalo and sheep after air drying and oven dry the samples were submitted for digestion (Nicholson et al.1999).

2.9 Metal profile evaluation analysis

The prepared samples were then analyzed for metal contents by Atomic Absorption Spectrophotometer (Perkin-Elmer Corp, 1980). Nutritional minerals that were evaluated in the sample were Co and Pb. Standard solution was prepared to get the standardized curve. The metal analysis was done by running the samples through Atomic Absorption Spectrophotometer. This apparatus is equipped with a graphite furnace. Each metal is measured according to value of standard solution. The amount of each metal occurring in the sample is obtained in absolute form. While sample is run through the Atomic Absorption Spectrophotometer the little quantity of sample is sprayed at the flame. Atomic resonance absorption line by element is calculated and measured. The apparatus is convenient for analysis. Any radiation that is emitted by flame had no effect on the working of apparatus. The absorption method is independent of the excitation potential of the spectral line used.

2.10 Evaluation Indices:

2.10.1 Bio concentration Factor (BCF):

For assessment of metal (mg/kg) transport from agricultural soil and forages that are growing on this soil, a BCF is applied (Cui et al., 2004).

BCF for soil to forage

(BCF) = Level of metal in forage /Level of metal in soil

2.10.2 Pollution load index (PLI)

Liu et al. (2005) described a formula which was used to find these indices.

$$Pollution\ load\ index = \frac{(M)IS}{(M)RS} \quad (1)$$

Whereas,

(M)IS = (mg/kg) Concentration of metal that occurs in soil to investigate

(M)RS= Soil reference value of metal (Table 1).

2.10.3 Enrichment factor (EF)

Formula for Enrichment factor is described by Buat-Menard and Chesselet (1979). See the Table 2.

$$Enrichment\ factor\ (EF) = \frac{\frac{Conc.of\ metal\ in\ plant}{Conc.\ of\ metal\ in\ soil\ (Sample)}}{\frac{Conc.of\ metal\ in\ plant}{Conc.of\ metal\ in\ soil\ (Standard)}} \quad (2)$$

2.10.4 Daily intake of metals (DIM)

Daily intake of metal (DIM) can be calculated by following equation from Sajjad et al. (2009).

$$DIM = \frac{C_{factor} \times C_{metal} \times D_{food\ intake}}{B_{average\ weight}} \quad (3)$$

Whereas,

C_{metal} is the concentration of metals in forages,

$D_{food\ intake}$ is the daily intake of forages,

$B_{average\ weight}$ is the average body weight.

For calculating this daily intake of metal, the conversion factor was taken 0.085 (Jan et al., 2010). Daily intake metal for cow was calculated by using animal body weight 600 kg and daily forage intake 12 kg while for sheep body weight was taken 75 kg and daily forage intake 1.3 kg (Johnsen and Aaneby, 2019). To calculate the DIM for buffalo body weight was taken 550 kg and daily forage intake (TDI) was taken 12.5 kg (Yang et al., 2020).

Health Risk Index (HRI)

Health risk index is the ratio of daily intake of metals in the forages to oral reference dose (RfD) and was calculated by the help formula (USEPA, 2002).

$$\text{Health risk index (HRI)} = \frac{DIM}{R_f D} \quad (4)$$

DIM = Daily intake of heavy metal

R_fD = Oral reference dose (Table 3)

An HRI > 1.0 for any single metal indicates that the health of consumer population is at risk, or it is carcinogenic (USEPA, 2013).

3. Results

Concentration of Cobalt in soil

The variation of Co metal concentration ranged from minimum 0.420 mg/kg and maximum 0.532 mg/kg. The minimum value of Co in soil was observed in *Z. jujube* during S₃. The maximum value was noticed in the samples collected in S₃ (Fig 2). The permissible range of UNEP (2013) for Co metal is 100 mg/kg. The calculated amount of Co is less than permissible limit in our samples (Table 4 and Table 12).

Concentration of Cobalt in forage

The analysis of Co metal in forage sample had different concentration in different forages. The mean concentration for Co ranged from 0.238 mg/kg minimum to 0.492 mg/kg maximum. The minimum value was noticed in *C. procera* during S₃ (Fig 3). The maximum amount was exhibited by 0.492 mg/kg during period of S₁ in *Z. jujube* forage (Table 5 and Table 13). Our present calculated values for cobalt in forages were lower than permissible limits of 52 mg/kg by WHO (2001).

Concentration of Cobalt in animals

The blood had the values Co ranging from lower amount to higher amount (0.389-0.536 mg/l). The higher concentrations were observed in sheep during season S₂ and lower concentration during S₃ in buffalo. The presence of Co blood was noticed similar with the 0.5 mg/l as compared to the values given by Schweinzer et al. (2017). The hair had Co concentration ranging from (0.377- 0.537 mg/kg) the maximum concentration of Co was present cow of S₁ while the minimum concentration of Co was noticed in sheep of S₃ (Table 6 and Table 14). The Co value in feces ranged the minimum to maximum (0.383-0.518 mg/kg) the higher amount of Co was found in cow during S₂ and lower value was noticed in sheep during S₃ (Fig 1).

Bio-concentration Factor (BCF) of Cobalt

The Bio-concentration factor of Co was ranged from 0.447 to 1.106 mg/kg. The lowest concentration was noticed in *C. procera* in S₃. The maximum amount for BCF was noticed in *Z. jujube* in S₁ (Table 7).

Pollution Load Index (PLI) of Cobalt

The pollution load index (PLI) was found within the range between 0.072 mg/kg -0.102 mg/kg. The low concentration was observed in *C. procera* during S₁ (Table 8).

Enrichment Factor (EF) of Cobalt

The enrichment factor for Co metal was noticed in range between 0.081 mg/kg -0.172 mg/kg. The minimum concentration was noticed in *C. procera* during S₃. The maximum amount was found *C. procera* during S₁ (Table 9).

Daily intake of metal (DIM) of Cobalt

The daily intake of metal (DIM) for Co ranged from 0.00035-0.00095 mg/kg. The lower level was found in sheep during S₃ while the higher concentration was noticed in buffalo in S₁ (Table 10).

Health Risk Index (HRI) of Cobalt

The health risk index for Co ranged from 0.008 mg/kg-0.022 mg/kg. The minimum values were noticed in *P. hysterophorus* during S₃ while the higher amount was found in *Z. jujube* in S₁ (Table 11).

Concentration of Lead in Soil

The Pb concentration in soil samples had different amounts in each sample of soil. The amount of Pb varied in the range from minimum 6.60 mg/kg to maximum 10.89 mg/kg (Table 12 and 4). The maximum concentration of soil was exhibited by soil of forage *R. dentatus* during S₃ and minimum concentrations was observed in soil of forage *R. dentatus* during S₁(Fig 2). The permissible limit of Pb in UNEP (2013) is 200 mg/kg the evaluated value of Pb in soil samples is lower than the permissible values.

Concentration of Lead in forage

The analysis of variance for Pb showed the significant effect in season. It showed the non-significant effect in soil and Season $\times$ soil (Table 5). The amount of Pb in different samples was different in each forage samples. The minimum amount of Pb was noticed 5.29 mg/kg in *P. hysterophorus* during S1 (Table 13). The maximum value of Pb was present in 9.84 mg/kg in *R. dentatus* during S3 (Fig 3). The Pb concentration was found within the permissible limits of 10 mg/kg as given by (WHO, 1995).

Concentration of Lead in animals

The analysis of variance for Pb showed the significant effect in Season. The non-significant effect was shown by Animal, Source, Season $\times$ Animal, Season $\times$ Source, Animal $\times$ Source and Season $\times$ Animal $\times$ Source (Table 6). The Pb concentration in blood ranged from minimum to maximum (1.01-2.44 mg/l). The minimum value was noticed in sheep during S1 and maximum value was noticed in buffalo during S3 (Table 14). This current investigation for Pb in blood was greater as compared to 0.2 mg/l by Puls (1988). The hair had Pb value ranged from lower to higher (1.00-2.33 mg/kg). The maximum value of Pb in hair was noticed in cow during S3 and minimum value was found in buffalo in S1 (Fig 1). The Pb in animal feces varied in the range from minimum to maximum (0.63-2.22 mg/kg). The minimum value was observed in cow during S1 and maximum value was noticed in sheep of S2.

Bio-concentration Factor (BCF) of Lead

The bio-concentration for Pb was ranged from 0.717-1.152 mg/kg. The minimum amount was found in *P. hysterophorus* during S1 while the maximum amount was depicted by *D. aegyptium* in S3 (Table 7).

Pollution Load Index (PLI) of Lead

The Pb pollution load index was found similar within the range from 0.809-1.048 mg/kg. The minimum amount was exhibited by the *R. dentatus* in the S1 while the maximum value was depicted by *C. procera* during S3 (Table 8).

Enrichment Factor (EF) of Lead

The enrichment factor Pb was noticed within the range from 1.95-3.129 mg/kg. The lowest value was noticed in S1 while the highest value was found in S3 (Table 9).

Daily intake metal (DIM) of Lead

The daily intake metal (DIM) was ranged in Pb from 0.0190-0.0078 mg/kg. The minimum value was observed in S1 and was noticed in sheep while the maximum concentration was found in buffalo during S3 (Table 10).

Health Risk Index (HRI) of Lead

The health risk index for Pb varied within range from 1.95-4.75 mg/kg. The minimum concentration was noticed from sheep during S1 while the highest level was found in the buffalo in S3 (Table 11).

Discussion

Present Co concentration in soil was recorded lower than permissible limits 10 mg/kg given by WHO (2000). This current study for Co in soil was in accordance with the investigations given by Tahir et al., (2017). Our result for soil Co concentration was below as compared to the findings of Uren (2013). Nature of soil properties are responsible for occurrence of Co in soil moreover there are other factors that are too responsible for cobalt in soil such as interface of soil-forage root, mechanism of property and pedo-geochemical effects (Lange et al., 2014). The Pb in soil samples was observed lower than that of the values given by Angelova et al. (2010). Pb amount in present findings was greater as compared to the values given by Nica et al. (2012). The current investigation for Pb in soil was lower as compared to narrated concentrations by Boussen et al. (2013). Our present work Pb level in soil was calculated higher than those given by Salazar et al. (2012). Pb in soil is accumulated due to presence of oxides and hydroxides of elements, minerals occurring in clay and organic matter dissolved in soil. Change in pH promotes lead precipitation in soil in form of carbonates, phosphates and hydroxides etc. When Pb^{2+} ion form compounds with humic compounds these are less available to forage (Wuana and Okieimen, 2011). Chelating agents are also involved in increasing or decreasing Pb availability in soil and transfer into forage plants (Butcher, 2009).

These results for Co in forages were observed below than the investigation given by Tahir et al., (2017). Our investigation for Co in forages was noticed greater than values given by Comino et al. (2011). The Co concentration forages was similar with the observations given by Liu et al. (2020). There are many interspecific and interspecific factors in cadmium uptake by forages. Sometime surface of plant is also contaminated with metal rich soil so there is chance to transfer metal in samples so precautions measures should be taken. Some phenotypic variations are also responsible for different concentration of forage plants (Faucon et al., 2007; Van der Ent et al., 2013). The amount of Pb in forages was found similar with the concentration of Pb given by Angelova et al. (2010). In this present work the Pb level in forage was in high range than the values given by Popovic et al. (2010). This value for Pb in forages was found lower as compared to the investigations by Salazar et al. (2012). In this study the present work was found lower than the Pb level estimated in forages by Cui et al. (2018). Pb is accumulated in forages near endodermis, it enters in forages through root via apoplast pathway and crosses cortex in radial manner. Pb is translocated from root to shoot in forages and endodermis acts as partial barrier. So, Pb is accumulated in roots in higher concentrations and less shoot of forages Sharma and Dubey (2005).

This concentration of Co in blood was found in accordance with the results given by Ismael et al. (2015). The cobalt (Co) level in hair of ruminants was greater as compared to observations given by Hejna et al. (2018). This present study for Co-occurrence in sheep feces was lower than recordings of Abrahams (2013). Our work for Co amount in sheep blood was lower than the investigations by Tarhan et al. (2016). The value of Co in blood of cow was found similar with the findings by Abdel-Salam et al. (2013). The soil hydrogen ion concentration is responsible for Co transfer from soil-forage-animals. The lesser concentration of hydrogen ion is less effective and greater concentration is more effective in transfer of cobalt from soil to forages and forage to grazing ruminants (Čelechovská et al., 2008). The current study for Pb in livestock blood was found lower than depicted results by Ogundiran et al. (2012). Our Pb amount in cow blood was found greater as compared to the results given by Luna et al. (2019). This result for Pb in animal faeces was found lower as compared to the work of Ogundiran et al. (2012). Our findings for Pb in animal hair were observed higher as compared to the results given by Patra et al. (2017). Our findings for animal blood were found like the narrated value by Swarup et al. (2007). Human activities produce stress to environment and increase lead to the environment. The lead containing fodder is consumed by animal and lead accumulation takes place in the animal body parts (Abdel-Salam et al., 2013).

Safari et al. (2018) reported the similar results with the BCF of Co in current study. In these current findings our values were greater than Lotfy and Mostafa (2014). In our present recordings was observed lower as compared to the Kosiorek and Wyszowski (2019). The value for Pb bio-concentration was observed similar with the values given by Nawab et al. (2015). Our work was found higher than the values given by the Sakizadeh et al. (2016). The present findings were found greater than the values narrated by Yang et al. (2014).

Our work for PLI cobalt (Co) was found similar as compared to investigations of Chandrasekaran et al. (2015). The current study was greater than recorded values given by Sulaiman et al. (2019). Present findings were investigated lower for PLI as compared to values given by Pathak et al. (2015). The PLI of Pb in this study was noticed lower than the values (PLI>2) investigated by Nawab et al. (2015). Our recorded values of PLI lead were found in accordance with observations by Zhuang et al. (2020). These current study Pb pollution load index was found greater than the narrated values of Barakat et al. (2012).

The current recordings for Co metal for (EF) were lower than the values given by Abbaslou et al. (2014). In present finding of Co was observed greater as compared to the values given by Giridhar and Samireddypalle (2015). This finding for Co enrichment factor was similar with the values given by Jing et al. (2019). The enrichment factor (EF) of Pb was ranged in this trend from higher to lower 1.95-3.129 mg/kg. Barakat et al. (2012) gave the greater values as compared to our work for Pb enrichment factor. Our present recordings were found greater than the findings by Marrugo-Negrete et al. (2017). Enuneku et al. (2017) likely found our calculated Pb enrichment factor.

Our daily metal intake metal for Co was lower than daily intake of metal observed by Ismail et al. (2015). These current investigations for Co (DIM) were similar with results given by Iwegbue (2010). In current study HRI for Co was in accordance with the concentrations recorded by Simonsen et al. (2012). Present findings for health risk index (Co) were similar with recorded values by Khan et al. (2019). Our work for (DIM) Pb was lower as compared to the values recorded by Smith et al. (2010). The similar value of Pb daily metal intake was found in accordance with the observations by Nadeem et al. (2020). This present work for Pb health risk index was recorded greater than HRI calculated by Nadeem et al. (2020). In this finding the health risk index (HRI) Pb was observed higher than the values by Ugulu et al. (2020).

Conclusion

It was concluded that seasonal changes gave different fluctuating concentrations of metals and sites also gave fluctuating metal readings in soil-forage-animal continuum. In soil and forage samples collected from semi-arid environment Co and Pb was found toxic according to FAO/WHO. In animal samples Pb showed toxicity while the Co was found safe according to NRC standards. Bio-concentration factor and pollution load index for Co and Pb was noticed greater than 1. Enrichment factor was also in safe limits for all metals. Daily intake metal and health risk index was found less than 1.

Declarations

Author contribution

Fu Chen, Zafar Iqbal Khan and Kafeel Ahmad supervised the study. Laraib Saqlain, collected samples, Muhammad Arslan Ashraf and Laraib Saqlain wrote first draft. Laraib Saqlain conducted experiment. Muhammad Arslan Ashraf participated in statistical data analysis. All authors equally contributed to discussion.

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Ethics declarations

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

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

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

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

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

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 270 that manuscript has not been published previously.

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Tables

Table 1. Metal reference value in soil

Metals	Reference Values (mg/kg)	References
Co	5.23	Shad <i>et al.</i> (2014)
Pb	8.15	Singh <i>et al.</i> (2010)

Table 2. Reference value of metals and metalloids in forage

Metals and metalloids	Reference values Standard forages (mg/kg)	Reference values Standard Soil
Co	50 c	5.23 a
Pb	3 c	8.15 b

Source: a Shad *et al.* (2014), b Singh *et al.* (2010), c FAO/WHO (2001)

Table 3. Oral reference dose (mg/kg) for metals

Metals	RfD (mg/kg/day)
Co	0.043 b
Pb	0.004 b

Source b= USEPA (2010)

Table 4. Analysis of variance for Cobalt and Lead in soil forages with respect to seasons.

		Cobalt (Co)	Lead (Pb)
Source	Df	Mean Square	Mean Square
Season	2	0.003 ^{ns}	15.432 ^{**}
Soil	4	0.002 ^{ns}	1.99 ^{ns}
Season × soil	8	0.006 ^{ns}	1.916 ^{ns}

^{**} = Significant and ^{ns} = non significant

Table 5. Analysis of variance for Cobalt and Lead in forages (mg/kg)

		Cobalt (Co)	Lead (Pb)
Source	df	Mean Square	Mean Square
Season	2	0.045 ^{**}	34.702 ^{***}
Soil	4	0.006 ^{ns}	2.747 ^{ns}
Season x soil	8	0.007 ^{ns}	2.357 ^{ns}

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

Table 6. Analysis of variance for Cobalt and Lead in animal

		Cobalt (Co)	Lead (Pb)
Source	Df	Mean Square	Mean Square
Season	2	0.159***	26.844***
Animal	2	0.009 ^{ns}	0.522 ^{ns}
Source	2	0.02*	0.197 ^{ns}
Season × Animal	4	0.021**	1.331 ^{ns}
Season × Source	4	0.012 ^{ns}	1.324 ^{ns}
Animal × Source	4	0.014*	1.282 ^{ns}
Season × Animal × Source	8	0.01 ^{ns}	0.44 ^{ns}

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

Table 7. Results for BCF of Cobalt and Lead

BCF	Heavy metals					
	Cobalt (Co)			Lead (Pb)		
Season	S_1	S_2	S_3	S_1	S_2	S_3
<i>C. procera</i>	0.946	0.583	0.447	0.748	0.773	0.929
<i>D. aegyptium</i>	0.703	0.617	0.567	0.854	0.928	1.152
<i>P. hysterophorus</i>	0.716	0.727	0.557	0.717	1.067	0.780
<i>R. dentatus</i>	0.705	0.746	0.588	0.850	0.989	0.904
<i>Z. jujube</i>	1.106	0.691	0.600	0.833	0.862	1.025

Table 8. Results for pollution load index (PLI) of Cobalt and Lead

Heavy metal	Cobalt (Co)			Lead (Pb)		
Season	S_1	S_2	S_3	S_1	S_2	S_3
<i>C. procera</i>	0.071	0.085	0.102	0.946	1.044	1.048
<i>D. aegyptium</i>	0.087	0.099	0.085	0.825	1.038	0.930
<i>P. hysterophorus</i>	0.084	0.084	0.083	0.904	1.032	1.044
<i>R. dentate</i>	0.085	0.083	0.085	0.809	1.131	1.336
<i>Z. jujube</i>	0.085	0.082	0.080	0.833	1.139	1.045

Table 9. Results for (EF) of Cobalt and Lead

Heavy metal	Cobalt (Co)			Lead (Pb)		
Season	S_1	S_2	S_3	S_1	S_2	S_3
<i>C. procera</i>	0.172	0.106	0.081	2.033	2.101	2.523
<i>D. aegyptium</i>	0.128	0.112	0.103	2.320	2.521	3.129
<i>P. hysterophorus</i>	0.130	0.132	0.101	1.95	2.89	2.12
<i>R. dentatus</i>	0.128	0.136	0.107	2.31	2.69	2.45
<i>Z. jujube</i>	0.201	0.126	0.109	2.26	2.34	2.78

Table 10. Daily intake metal (DIM) of Cobalt and Lead

DIM		Heavy metals									
		Cobalt (Co)					Lead (Pb)				
animals	Forage	<i>C. procera</i>	<i>D. aegyptium</i>	<i>P. hysterothorus</i>	<i>R. dentatus</i>	<i>Z. jujube</i>	<i>C. procera</i>	<i>D. aegyptium</i>	<i>P. hysterothorus</i>	<i>R. dentatus</i>	<i>Z. jujube</i>
Cow	S_1	0.00059	0.00054	0.00053	0.00054	0.00084	0.0098	0.0097	0.0089	0.0095	0.0096
	S_2	0.00044	0.00054	0.00054	0.00055	0.0005	0.0112	0.0133	0.0152	0.0155	0.0136
	S_3	0.00041	0.00043	0.00041	0.00044	0.00043	0.0135	0.0148	0.0113	0.0167	0.0148
Buffalo	S_1	0.00067	0.00062	0.00061	0.00061	0.00095	0.0111	0.0111	0.0102	0.0108	0.0109
	S_2	0.00049	0.00062	0.00061	0.00063	0.00057	0.0127	0.0152	0.0173	0.0176	0.0155
	S_3	0.00046	0.00048	0.00046	0.0005	0.00048	0.0153	0.0169	0.0128	0.019	0.0169
Goat	S_1	0.00052	0.00047	0.00046	0.00046	0.00073	0.0085	0.0085	0.0078	0.0083	0.0083
	S_2	0.00038	0.00047	0.00047	0.00048	0.00044	0.0097	0.0116	0.0132	0.0134	0.0118
	S_3	0.00035	0.00037	0.00036	0.00038	0.00037	0.0117	0.0129	0.0098	0.0145	0.0129

Table 11. Results for (HRI) of Cobalt and Lead

HRI		Heavy metals									
		Cobalt (Co)					Lead (Pb)				
animals	Forage	<i>C. procera</i>	<i>D. aegyptium</i>	<i>P. hysterothorus</i>	<i>R. dentatus</i>	<i>Z. jujube</i>	<i>C. procera</i>	<i>D. aegyptium</i>	<i>P. hysterothorus</i>	<i>R. dentatus</i>	<i>Z. jujube</i>
Cow	S_1	0.013	0.013	0.012	0.012	0.019	2.45	2.44	2.25	2.38	2.41
	S_2	0.01	0.013	0.013	0.013	0.012	2.8	3.34	3.81	3.88	3.4
	S_3	0.009	0.009	0.009	0.01	0.01	3.37	3.71	2.82	4.18	3.71
Buffalo	S_1	0.016	0.014	0.014	0.014	0.022	2.79	2.77	2.55	2.71	2.73
	S_2	0.012	0.014	0.014	0.015	0.013	3.18	3.79	4.33	4.4	3.87
	S_3	0.011	0.011	0.011	0.012	0.011	3.83	4.22	3.21	4.75	4.22
Goat	S_1	0.012	0.011	0.011	0.01	0.017	2.13	2.11	1.95	2.07	2.08
	S_2	0.009	0.011	0.01	0.011	0.01	2.42	2.89	3.3	3.36	2.95
	S_3	0.008	0.009	0.008	0.008	0.008	2.92	3.22	2.45	3.62	3.22

Table 12. The mean concentration of Cobalt and Lead (mg/kg) in soil

Heavy metal	Cobalt (Co)			Lead (Pb)		
Season	S_1	S_2	S_3	S_1	S_2	S_3
Soil of forages						
Soil of forage <i>C. procera</i>	0.370±0.075	0.442±0.019	0.532±0.110	7.71±0.479	8.51±0.587	8.54±0.649
Soil of forage <i>D. aegyptium</i>	0.455±0.003	0.520±0.061	0.443±0.015	6.72±0.782	8.46±0.440	7.58±0.321
Soil of forage <i>P. hysterothorus</i>	0.437±0.009	0.440±0.056	0.433±0.009	7.37±1.29	8.41±1.22	8.51±0.673
Soil of forage <i>R. dentatus</i>	0.447±0.014	0.433±0.007	0.442±0.007	6.60±1.22	9.22±1.006	10.89±0.227
Soil of forage <i>Z. jujube</i>	0.445±0.009	0.427±0.014	0.420±0.013	6.79±1.08	9.29±0.642	8.52±0.371

Table 13. The mean concentration of cobalt and Lead in forages (mg/kg).

Heavy metal	Cobalt (Co)			Lead (Pb)		
Season	S_1	S_2	S_3	S_1	S_2	S_3
Forage type						
<i>C. procera</i>	0.350±0.03	0.258±0.03	0.238±0.04	5.77±0.988	6.58±0.323	7.93±0.16
<i>D. aegyptium</i>	0.320±0.08	0.322±0.06	0.252±0.07	5.74±1.38	7.85±1.27	8.73±0.42
<i>P. hysterophorus</i>	0.313±0.02	0.320±0.04	0.242±0.06	5.29±1.03	8.97±0.91	6.64±0.37
<i>R. dentatus</i>	0.315±0.05	0.323±0.05	0.260±0.02	5.61±2.02	9.12±0.51	9.84±0.37
<i>Z. jujube</i>	0.492±0.06	0.295±0.06	0.252±0.04	5.66±1.28	8.01±0.44	8.73±0.26

Table 14. The mean concentration of Cobalt and Lead in animals (mg/kg).

Heavy metal		Cobalt (Co)			Lead (Pb)		
Season		S_1	S_2	S_3	S_1	S_2	S_3
Source	Animal						
Blood	Cow	0.409±0.029	0.469±0.028	0.404±0.013	1.19±0.191	1.48±0.228	2.38±0.447
	Buffalo	0.432±0.041	0.473±0.014	0.389±0.015	1.25±0.226	1.25±0.312	2.44±0.462
	Sheep	0.471±0.028	0.536±0.026	0.404±0.012	1.01±0.158	1.67±0.347	1.60±0.335
Hair	Cow	0.537±0.047	0.510±0.010	0.411±0.015	1.09±0.169	2.20±0.432	2.33±0.438
	Buffalo	0.459±0.026	0.457±0.026	0.416±0.021	1.00±0.199	1.41±0.276	2.05±0.471
	Sheep	0.472±0.030	0.514±0.013	0.377±0.012	1.02±0.158	2.32±0.453	2.15±0.370
Feces	Cow	0.471±0.019	0.518±0.011	0.424±0.013	0.63±0.033	1.85±0.307	2.08±0.319
	Buffalo	0.482±0.023	0.386±0.014	0.406±0.014	1.25±0.281	2.02±0.331	1.87±0.335
	Sheep	0.512±0.025	0.427±0.012	0.383±0.015	1.13±0.164	2.22±0.373	2.19±0.340

Figures

Figure 1

Mean heavy metal ions concentration in animals during different seasons

Figure 2

Mean heavy metal ions concentration in different soils of forage during different seasons

Figure 3

Mean heavy metal ions concentration in different forages during different seasons