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Toxicity and bioassimilation of lead and nickel in farm ruminants fed on diversified forage crops grown on contaminated soil

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ABSTRACT

The cultivation of forage crops on wastewater-irrigated soils, while common in many developing countries, poses significant risks due to heavy metal pollution, particularly Lead (Pb) and Nickel (Ni). This practice, aimed at addressing water scarcity challenges and providing affordable irrigation, was investigated for its ecological and human health implications across three diverse sites (site A, site B, and site C). Our study unveiled increases in Pb concentrations in contaminated soil, cultivated with *Sesbania bispinosa* showing the highest Pb accumulation. The Ni concentrations ranged from 5.34 to 10.43 across all forage crop samples, with *S. fruticosa* from site C displaying the highest Ni concentration and *S. bicolor* from site A exhibiting the lowest. Trace element concentrations in the specimens were determined using an atomic absorption spectrophotometer. The Pb levels in the blood, hair, and feces of farm ruminants (cows, buffaloes, and sheep) varied across the sites, with buffaloes consistently displaying the highest Pb levels. Insights into daily Pb intake by ruminant's highlighted variations influenced by plant species, animal types, and sites, with site C, the cows exhibiting the highest Health Risk Index (HRI) associated with lead exposure from consuming forage crops. Soil and forage samples showed Pb concentrations ranging from 8.003 to 12.29 mg/kg and 6.69–10.52 mg/kg, respectively, emphasizing the severe health risks associated with continuous sewage usage. Variations in Ni concentrations across animal blood, hair, and feces samples underscored the importance of monitoring Ni exposure in livestock, with sheep at site B consistently showing the highest Ni levels. These findings highlight the necessity of vigilance in monitoring trace element (Pb and Ni) exposure in forage crops and livestock, to mitigate potential health risks associated with their consumption, with variations dependent on species, site, and trace element concentrations.

1. Introduction

To grasp the contemporary challenges posed by lead exposure, it's imperative to explore its historical context. Lead has a profound historical legacy, dating back to ancient civilizations where it was utilized in various ways, including in water systems, cosmetics, and even culinary utensils (Al Sukaiti et al., 2023; Bjørklund et al., 2024). The Romans were known to extensively utilize lead in their aqueducts and plumbing systems, unknowingly subjecting their populace to lead contamination. The harmful health consequences of lead exposure started becoming apparent as early as the 2nd century BCE, with accounts of lead poisoning symptoms observed among the Roman elite. Nonetheless, it wasn't until the late 19th and early 20th centuries that scientific comprehension of lead's toxic nature notably progressed

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(Margeta, 2023). This historical perspective underscores the enduring interplay between human activities, technological advancement, and lead contamination. As societies developed and industrialized, the dispersion of anthropogenic lead into the environment intensified, with repercussions that persist to this day.

Contemporary lead contamination arises primarily from anthropogenic sources, which span various sectors of human activity. Understanding these sources is pivotal in elucidating the pathways through which lead is disseminated into ecosystems. Lead is a common byproduct of various industrial processes, including smelting, battery manufacturing, and mining (Hasan et al., 2017; Hashem et al., 2017; Henry 2012; Hoque et al., 2021; Huang et al., 2008; Hussain, Qureshi 2020; Huu et al., 2010). Industrial emissions release lead particles into the atmosphere, contributing to air pollution and subsequent deposition on land and water surfaces (Cygan-Szczegielniak et al., 2014; Hussain and Qureshi 2020). Lead-based paint, widely used in residential and commercial buildings until its ban in many countries, remains a persistent source of lead exposure, especially in older structures (Lanphear et al., 2002). The production, use, and disposal of lead-acid batteries continue to contribute to lead contamination, particularly in regions with inadequate waste management systems (Margeta, 2023).

Some agricultural activities, such as the use of lead-containing pesticides and the application of sewage sludge as fertilizer, introduce lead into the soil, potentially impacting crops and livestock (Hussain and Qureshi 2020). Urbanization processes, including the construction and maintenance of roads, bridges, and plumbing systems, can disturb lead-containing materials and contribute to soil and water contamination (Cai et al., 2019; Chen et al., 2018; Chiroma et al., 2014; Cui et al., 2004; Cygan-Szczegielniak et al., 2014; Czarnowska, Milewska 2000; Dosumu et al., 2005; Dutch Standard 2000; Farouk, Al-Amri 2019; Feng et al., 2019; Genotypic 2019; Ghazzal et al., 2020; Giri, Singh 2017; Gowd et al., 2010; Gu et al., 2014; Gworek et al., 2011). These sources collectively underscore the pervasive nature of anthropogenic lead contamination, with each contributing to varying degrees based on geographical, economic, and regulatory factors.

Lead's capacity to infiltrate the environment through multiple pathways creates a web of potential exposure routes for humans, domestic animals, and wildlife. The release of lead particles into the atmosphere, primarily through industrial emissions and vehicular exhaust, can lead to inhalation exposure for both humans and animals (Cygan-Szczegielniak et al., 2014). Contaminated soil and dust, often originating from lead-based paint, industrial activities, and urbanization, pose risks of dermal contact and ingestion, particularly for children and wildlife (Lanphear et al., 2002). Lead can leach into water sources from corroded lead pipes and plumbing fixtures, posing a direct ingestion risk for humans and potentially contaminating aquatic habitats (Inelova et al., 2018; Jahan et al., 2020; Kabata-Pendias 2011; Khan et al. 2018a, b; Li et al., 2014; Liu et al., 2005; Ma et al., 2016; Mielke et al., 2003; Mihali et al., 2013; Nadeem et al., 2019, 2020; Netty et al., 2013). Wildlife and domestic animals can be exposed to lead through the consumption of contaminated prey, plants, or water, with potential biomagnification effects as lead moves up the food chain (Pan et al., 2020; Póti et al., 2020; Rahi et al., 2022; Rahman et al., 2014, 2020). Certain occupational settings, such as battery manufacturing and recycling facilities, place workers at heightened risk of lead exposure (Nadeem et al. 2019). Understanding these pathways is crucial for designing effective strategies to mitigate lead exposure in all affected populations.

The consequences of lead exposure are profound and wide-ranging, affecting not only individual health but also ecosystems and biodiversity. Lead is a potent neurotoxin, particularly harmful to children. Even low levels of exposure can lead to cognitive deficits, developmental delays, and behavioral problems (Needleman, 2004,2004). In adults, lead exposure is associated with cardiovascular diseases, renal dysfunction, and fertility issues (Ningyu et al., 2016). Anthropogenic lead exposure poses a significant threat to wildlife populations, especially scavengers and predators. Lead poisoning has been reported in various species, including eagles, vultures, and waterfowl (Pattee et al., 2006). In ecosystems, the loss of apex predators can disrupt ecological balance. Lead contamination can alter soil and water chemistry, impacting plant growth and aquatic life. Furthermore, lead's potential to biomagnify in food chains can disrupt ecosystem dynamics and biodiversity (Khan et al., 2018; Li et al. 2014). This cascade of impacts underscores the urgency of addressing lead exposure across species and ecosystems, as the interconnectedness of the natural world means that the effects of lead pollution ripple through entire ecological systems.

While considerable progress has been made in understanding the sources, pathways, and impacts of anthropogenic lead contamination, significant gaps in research and mitigation efforts remain.

There is a need for further research to assess the extent of lead contamination in various ecosystems and its long-term effects on wildlife and domestic animals. Additionally, understanding the complex interactions between lead exposure and other environmental stressors is essential. Effective mitigation strategies must encompass regulatory measures to reduce lead emissions, promote lead-safe practices in industries and construction, and encourage the removal of lead-based paint and plumbing infrastructure. Public awareness campaigns and community education can also play a pivotal role in reducing lead exposure risks.

The emergence of nickel (Ni) as a significant environmental concern has brought attention to the exposure of plants, soil ecosystems, animals, and humans to this toxic trace element. Anthropogenic contaminants, originating from human activities such as industrial processes, agriculture, and urbanization, have substantially modified the natural environment, leading to far-reaching consequences. Nickel (Ni) is increasingly recognized as an ecological contaminant (Shahzad et al., 2018). Its presence significantly inhibits germination, plant physiological and biochemical attributes, following continuous exposure to wastewater irrigation (Hussain and Qureshi 2020; Khan et al., 2023; Qin et al., 2022). Elevated concentrations of nickel behave like heavy metals, resulting in adverse impacts on the growth, quality, and productivity of plants. (Altaf et al., 2022,2022). Ni toxicity disrupts photosynthesis and affects gas exchange parameters, leading to reduced synthesis of photosynthetic pigments and abrupt plant growth (Hassan et al., 2019).

Lead exposure is a multifaceted phenomenon, with various pathways through which it infiltrates the lives of humans, domestic animals, and wildlife, creating a complex web of risks and consequences. Airborne contamination represents one significant pathway, with lead particles released into the atmosphere settling on surfaces and infiltrating respiratory systems through inhalation (Cygan-Szczegielniak et al., 2014). Soils and dust, often tainted by lead-based paint, industrial activities, and urbanization, pose risks of dermal contact and ingestion, especially for children and wildlife (Lanphear et al., 2002; Clark et al., 2006). Furthermore, water contamination emerges as a critical concern, as lead seeps into water sources from corroded pipes and plumbing fixtures, directly impacting human health and potentially polluting aquatic ecosystems (Hu et al. 2019; Xu et al. 2021). Another persistent source of contamination is lead-based paint, once widely used in residential and commercial buildings, which continues to pose a threat despite being banned in many countries (Lanphear et al., 2002). Older structures still harbor traces of this hazardous material, contributing to ongoing exposure risks. Additionally, the production and disposal of lead-acid batteries perpetuate lead contamination, particularly in regions with inadequate waste management systems (Margeta, 2023). Agricultural practices exacerbate the issue further, as the use of lead-containing pesticides and the application of sewage sludge as fertilizer introduce lead into the soil, potentially affecting crops and livestock (Hussain and Qureshi 2020).

In this study, our primary objectives were to determine the prevalence and extent of lead (Pb) and nickel (Ni), contamination in the different body tissues of farm ruminants. This research marks the pioneering effort to fill the existing gap in knowledge regarding contamination, and bioassimilation of lead and nickel in farm ruminants fed on diversified forage crops, irrigated with wastewater, and their subsequent ecotoxicity. The potential for food chain contamination resulting from heavy metal pollution following wastewater irrigation practice in the forage crop fields of Pakistani Punjab is a critical concern for both public health and environmental sustainability. The utilization of wastewater, often laden with trace elements and other pollutants, to irrigate crops introduces the risk of these contaminants being absorbed by the plants. As these contaminated forage crops are subsequently consumed by livestock and, eventually, by humans, there is a distinct possibility of heavy metals entering the food chain. This situation underscores the urgent need for comprehensive studies and effective mitigation measures to safeguard the integrity of the food supply and protect the health of both consumers and ecosystems in the region. Our investigation involved the evaluation of lead (Pb) and nickel (Ni) contamination levels in four distinct categories of farm ruminant's blood, urine and hairs through the utilization of inductively coupled plasma mass spectrometry (ICP-MS).

2. Material and method

2.1. Area

The study encompasses the Toba Tek Singh (T.T. Singh) District (Punjab Province), Pakistan. The study area lies within the Rechna Doab region, nestled between the Chenab River and the Ravi River, falling under the jurisdiction of the T.T. Singh. The region experiences an arid to semiarid subtropical continental climate characterized by significant seasonal variations in temperature and precipitation. Summers are typically prolonged and scorching, while winters are mild. Annual rainfall averages between 15 and 30 cm, with more than half of it occurring during the monsoon season, particularly in July and August, often at high intensity. The peak of heat typically falls in May and June, with mean or maximum temperatures reaching between 42°C. Winters are generally free from frost, although occasional frosts may occur for short periods, lasting around 15–20 days in December and January (Hassan et al. 2019).

The soil composition reveals low levels of calcium (Ca), phosphorus (P), and magnesium (Mg), with a notable solubility of iron (Fe) and zinc (Zn). Groundwater reliance heavily leans on the Chenab and Ravi Rivers, which supply water through inter-river link channels. The terrain slopes south westward, facilitating groundwater recharge primarily through river and canal systems. Monsoonal precipitation also contributes to groundwater replenishment. However, the existing precipitation is inadequate to meet the demands for drinking and irrigation, making rivers the primary source for recharging groundwater, as noted by Hassan et al. (2019). Regrettably, the area lacks facilities for domestic wastewater treatment, leading to common practices of waste disposal in abandoned areas, along roadsides, near village peripheries, or into empty canals.

2.2. Soil, forage and ruminant tissue sampling and chemical bioassays

The three farms selected for investigation share similar soil and climatic conditions, rendering them well-suited for assessing the influence of wastewater irrigation, land use and forage crops cultivation. Sampling of field soil areas utilized for forage crops grown took place during the respective crop growth cycle and there was a distance of >1 Km. During this period, the region experiences mild temperatures, with daylight hours increasing as spring progresses. Waste water samples were gathered from the respective agro-ecological sites and water pH was measured on-site using an HI98130 instrument.

The soil and forage plant samples (3 replicates) were collected from the target forage crops field. Ten soil samples were collected from each plot, irrigated with wastewater, thoroughly mixed and make a composite sample from each plot at each site. The soil samples were then sealed in labelled paper bags. In each 100 × 100 m plot, the aerial parts of the vegetation were collected, and samples of stems and leaves of herbaceous plants, in their pre-flowering stage, were cut, placed in zip plastic bags, and pooled per species per plot. A total of 25 samples were obtained from the each plot cultivated with each forage plant species. The samples were washed, dried, and milled for biochemical analysis.

A total of 180 blood samples were collected from healthy ruminants, including cows, buffaloes, and sheep. These samples were gathered from three different agro-ecological sites from the same district, with 20 samples from each site. The laboratory scale standardized tubes were used for obtaining blood samples (15 mL) from jugular veins of ruminants under sanitary conditions. The blood samples were obtained in sterilized vials and transferred to the lab, quickly in chilled boxes. The blood samples were processed further and centrifuged for two minuts at 2500 rpm. The cows and buffaloes were 4–5 years old with an average weight of 70–100 kg, while the sheep weighed between 40 and 60 kg. Each animal had a specific number tag. Hair samples were collected from each farm animal, all raised in the study area. All lab instruments and glass ware cleaned very well with ethanol. The hair samples were washed, cleaned, and dried before being further processed.

2.2.1. Metal analysis

The heavy metal content in the soil, forage, and blood samples was analyzed by atomic absorption spectrophotometer (AA-6300 Shimadzu Japan).

2.2.2. Spectroscopic analysis

The determination of trace elements amounts in the specimens was conducted in accordance with the methodology outlined by Ilechukwu et al. (2021) utilizing an atomic absorption spectrophotometer. Specifically, the instruments employed for this purpose were the AA-640 by Shimadzu Co., Ltd, based in Tokyo, Japan, and the Perkin Elmer Analyst 400. Following the establishment of proper calibration protocols, the sample was introduced into the atomic absorption spectrophotometer through nebulization. To ensure optimal analytical quality control, the metal concentrations results were refined by subtracting the metallic concentrations detected in the blank solution from the observed magnitude during examination (Haroon et al., 2021). In accordance with the directives provided by the European Commission (2006) and adopting the methodologies previously employed by El-Ansary and El-Leboudy (2015), Ugulu et al. (2021), and Liu et al. (2020), the experimental Protocols and analytical procedures for Atomic Absorption Spectroscopy (AAS) were executed. These procedures were conducted while adhering to the guidelines stipulated by the manufacturer of the instrumentation. The Limit of Detection (LOD) values were determined using the standard methodology established by Armbruster et al. (1994). The Limit of Detection (LOD) was defined as the juncture at which the standard deviation (SD) of the blank solution and the signal-to-noise ratio both matched 10, following established criteria.

2.2.3. Quality control analysis and assurance

Every chemical compounds and reagents employed in the exploration were of analytical caliber and were acquired from Sigma-Aldrich. In a similar vein, subsequent to immersing in a nitric acid (30 %, volume/volume) solution for an overnight period, top-tier glassware (provided by Merck Germany) was employed. Rigorous quality assurance and quality control measures were executed to mitigate any potential cross-contamination and to uphold control over ambient variables (including factors such as temperature and humidity). These actions adhered to the procedure delineated in the studies by Ashfaq et al. (2022) and Atta et al. (2023).

In order to validate the reliability of the outcomes, reagent blanks (comprising a mixture of 5 parts HNO_3, 1 part H_2SO_4, and 1 part $HClO_4$) were employed. Additionally, evaluations were conducted using consistent Specialized Position Quantifiable standards (utilizing NIST Standard Reference Material 1570 A for fodder and SRM 2709 for soil nickel). Furthermore, replicates were measured for each sample group, following the methodology outlined by Cheshmazar et al. (2018). The absorption wave length (nm), slit width (nm), detection limits, and lamp current (mA) were 231.604, 0.2, 6 (Flame AA), and 4, respectively (Sancer and Tekin-Özan, 2016). All solutions utilized in the experiment were prepared using ultra-purified water obtained from a water purification system (Ultra Clear Lab Systems, Siemens Water Technologies, USA). The analytical methodologies were maintained by adhering to standardized operational procedures and measurements, following the recommendations suggested by Akhtar et al. (2022). To calibrate the instrument, blank and standards were run after each set of five readings.

2.2.4. Estimated daily intake (EDI)

Computation of the approximated daily ingestion (EDI) (expressed in milligrams per kilogram per day) of concentrated metallic constituents, based on the quantity of plant matter consumed per unit of body mass and the corresponding mean concentration in plant specimens. In compliance with the guidelines stipulated by Chen et al. (2011), employ the following formula to ascertain the magnitude of the estimated daily ingestion (EDI) for each significant metallic constituent:

$$EDI = \frac{Ef \times ED \times FIR \times CM \times Cf}{BW \times TA} * 0.001$$

Ef represents the occurrence frequency (365 days per year), ED stands for the span of exposure, which amounts to 70 years—equivalent to the average human lifespan, as also supported by Shaheen et al. (2016). FIR denotes the daily ingestion of plant (240 g) as per the recommendations of the World Health Organization. CM signifies the trace metal concentration within plants (milligrams per kilogram of dry weight), and Cf corresponds to the concentration conversion coefficient (0.085) as per the research by Arora et al. (2008). BW pertains to the standard weight for adults (70 kg). TA indicates the mean duration of exposure for non-carcinogenic risk (365 days per year), and 0.001 serves as the unit conversion factor.

2.2.5. Hazard quotient (HQ)

The Hazard Quotient (which evaluates non-carcinogenic impacts) is defined as the proportion of the Estimated Daily Dosage (EDD) to the Reference Dosage (RfD), as established by the study conducted by Qureshi et al. (2016); Ghazzal et al. (2020);

$$HQ = \frac{EDD}{RfD}$$

In this particular context, EDD is an abbreviation for the estimated daily dosage of the metallic element, whereas RfD stands for the oral reference dosage. When the Hazard Quotient (HQ) falls below 1, it is inferred that the populace under exposure encounters negligible risk, as elucidated by Khan et al. (2018a,b) and Ghazzal et al. (2020). It's noteworthy to emphasize that the Hazard Quotient does not offer a probability assessment of adverse health effects transpiring but rather signifies the presence of a plausible health hazard.,

2.2.6. Statistical analysis

The analysis of variance (ANOVA) of all sample were statistically analyzed using Minitab 16 software and Tucky's test was employed to find significant differences among treatment means. In this context, we performed Principle component analysis (PCA) in order to identify patterns and groupings among heavy metal and wastewater from different sites. Meanwhile, by PCA analysis, heavy metals and water quality parameters, it is possible to pinpoint the sources of contamination from a particular site and kind of forage crops more contaminated. Principle component analysis (PCA) also helps in reducing the dimensionality of such datasets by transforming the original variables into a smaller set of uncorrelated variables called principal components. By capturing the most significant sources of variation in the data, PCA can help in recognizing hidden relationships between variables.

3. Results and discussion

3.1. Metals concentrations in forage aerial parts

The analysis of Pb concentration in the forage crop samples collected from different sites revealed variations in contamination levels across the sites. Among the different forage crops, the highest Pb concentration (11.24) was observed in *S. bispinosa* samples from site C, while the lowest Pb concentration (6.69) was found in *P. fruticosa* samples from site A. Across all forage crops and sites, the Pb concentrations ranged from 6.69 to 11.24 (Fig. 1). Examining each forage crop individually, *Sorghum bicolor* showed Pb concentrations ranging from 7.17 to 9.33, with the highest concentration observed at site C and the lowest at site A. The *C. dactylon* exhibited Pb concentrations ranging from 7.14 to 10.13, with the highest concentration at site C and the lowest at site A. The *P. fruticosa* demonstrated Pb concentrations ranging from 6.69 to 10.37, with the highest concentration at site B and the lowest at site A. Soil samples from *S. bispinosa* showed variations from 7.01 to 11.24, with the highest concentration at site C and the lowest at site A. Finally, *T. terresteris* displayed Pb concentrations ranging from 7.06 to 10.13, with the highest concentration at site C and the lowest at site A (Fig. 1). The forage crop samples collected from different sites exhibited varying levels of Pb contamination, with *S. bispinosa* at site C displaying the highest concentration and *P. fruticosa* at site A showing the lowest. Overall, the Pb concentrations ranged from 6.69 to 11.24 across all samples, highlighting the importance of monitoring and managing Pb contamination to ensure the safety of forage crop production.

Among the diversified forage crops, the highest Ni concentration was observed in samples of *S. fruticosa* from site C, with a concentration of 10.43, while the lowest Ni was found in *S. bicolor* samples from site A, with a concentration of 5.34. Across all forage crops and sites, the Ni level ranged from 5.34 to 10.43 (Fig. 2). Assessing each forage crop individually, *S. bicolor* displayed Ni concentrations ranging from 5.34 to 7.95, with the highest concentration observed at site C and the lowest at site A. The centipede grass (*C. dactylon*) exhibited Ni concentrations ranging from 6.34 to 9.21, with the highest concentration at site C and the lowest at site A. Samples of *S. fruticosa* demonstrated Ni concentrations ranging from 7.72 to 10.43, with the highest concentration at site C and the lowest at site A. Soil samples from *S. bispinosa* showed variations from 6.87 to 9.85 (Fig. 2), with the highest concentration at site C and the lowest at site A. Finally, *T. terresteris* displayed Ni concentrations ranging from 6.92 to 10.27, with the highest concentration at site C and the lowest at site A (Fig. 2). The forage crop samples collected from different sites exhibited varying levels of Ni contamination, with *S. fruticosa* at site C displaying the highest concentration and *S. bicolor* at site A showing the lowest. Overall, the Ni concentrations ranged from 5.34 to 10.43 across all samples, underscoring the necessity of monitoring and managing Ni contamination to safeguard the integrity of forage crop production.

The study reveals variation in the bioaccumulation of Pb and it varies from one to other forage crop species as well as environmental site. *S. bispinosa* exhibited the highest metal accumulation. This variation suggests that certain plant species possess higher ability to absorb and transfer Pb from soil and translocate to different plant parts such as leaves, shoots, flowers, seeds and fruit. Such variability in metal accumulation has been noted in previous research by Li et al. (2020).

The results also highlight site-specific differences in heavy metal uptake. In this context, *S. fruticosa* showed the highest Ni concentration at site C, indicating that the degree of metal contamination in the soil directly influences plant metal uptake. This observation aligns with studies by Zhang et al. (2019), which emphasize the role of soil characteristics in heavy metal uptake by plants. The variation in Ni concentration among different plant species, with *Sesbania bispinosa, S. fruticosa, and Tribulus terrestris* showing the highest concentrations, suggests that plant species exhibit differential metal uptake capabilities. This differential metal accumulation has implications for plant selection

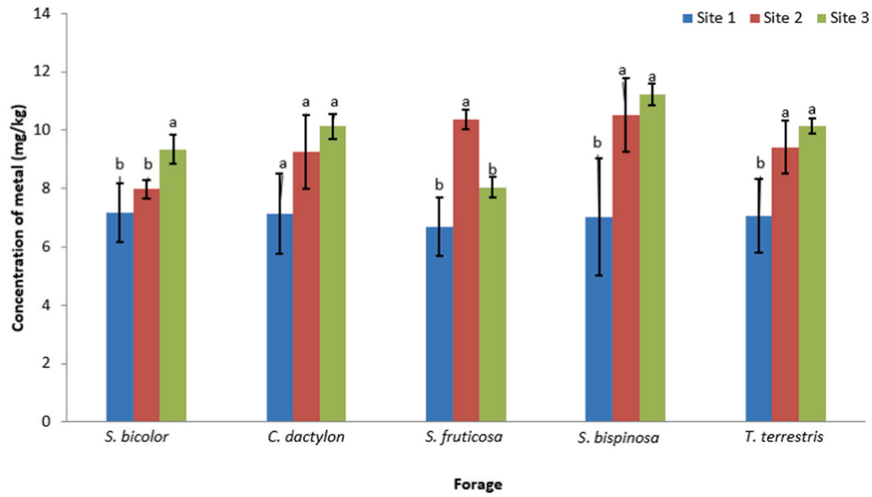

Fig. 1. The concentration of heavy metal Pb in the forages. Every bar represents the mean (±S.E.) of three replicates. Means followed by different letters are significantly different ($p < 0.05$) according to Tukey's HSD test.

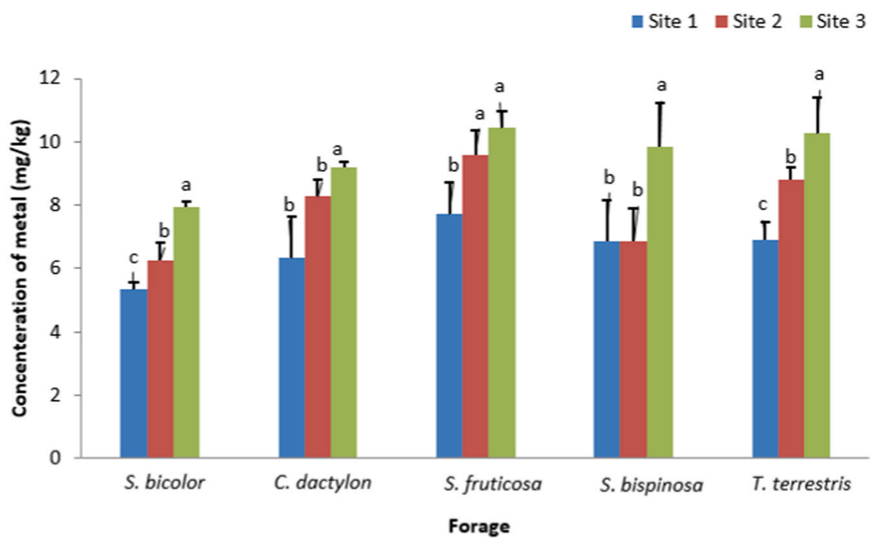

Fig. 2. The concentration of heavy metal Ni in the forages. Every bar represents the mean (±S.E.) of three replicates. Means followed by different letters are significantly different ($p < 0.05$) according to Tukey's HSD test.

in phytoremediation strategies, as noted in research by Sharma et al. (2018). The data from Table 5 reveal significant variability in daily lead (Pb) intake by farm ruminants across different plant species, animal types, and sites. This variability is in line with findings from recent studies (Smith et al., 2021) that underscore the diverse factors affecting heavy metal uptake by plants and subsequent consumption by livestock. It is essential to recognize that the choice of forage and dietary preferences of ruminants significantly contribute to this variation. (Zhang et al., 2019,2019). Plants that accumulate heavy metals can act as potential sources of contamination for other organisms in the food chain. Such environmental implications call for rigorous monitoring and remediation efforts, as highlighted in studies by Nouri et al. (2021). These findings align with recommendations by Liang et al. (2019) for sustainable soil management in contaminated areas.

3.2. Concentration of Pb and Ni in farm ruminant blood during the feeding trial

The Tables 1 and 2 presents the lead (Pb) level in the blood of various farm ruminants across three different sites. The cattle's at site A (cows) had the lowest Pb concentration (1.22 mg/l) while buffaloes exhibit highest concentration (2.47 mg/l) in ruminant blood. Although, sheep had the highest Pb content, 1.69 mg/l at site B (Table 2) while opposite trend was observed at site C (1.63 mg/l). However, buffaloes demonstrate highest Pb level (2.47 mg/l). Overall, the highest Pb concentration was consistently found in buffaloes at all three sites, with the lowest concentrations typically observed in cows (Tables 1, 2). The results presented in Table 2 demonstrate variations in the concentration of the heavy metal lead (Pb) in the blood of different farm ruminants across

Table 1
Analysis of Variance for Pb metal in Animal blood, Hair and Feces.

Source	Df	Mean Square
Site	2	26.844***
Animal	2	0.522^{ns}
Source	2	0.197^{ns}
Site x Animal	4	1.331^{ns}
Site x Source	4	1.324^{ns}
Animal x Source	4	1.282^{ns}
Site x Animal x Source	8	0.44^{ns}

ns insignificant. * Significant at 0.05 level

Table 2
Concentration of heavy metal Pb in Animal blood, Hair and Feces.

Source	Animal	Site A	Site B	Site C
Blood	**Cow**	1.22±0.19	1.51±0.31	2.41±0.46
	Buffalo	1.27±0.23	1.28±0.35	2.47±0.33
	Sheep	1.03±0.16	1.69±0.43	1.63±0.44
Hair	**Cow**	1.12±0.19	2.23±0.28	2.36±0.46
	Buffalo	1.03±0.16	1.44±0.45	2.08±0.33
	Sheep	1.05±0.03	2.35±0.31	2.19±0.44
Feces	**Cow**	0.66±0.28	1.87±0.33	2.11±0.47
	Buffalo	1.28±0.16	2.05±0.37	1.90±0.37
	Sheep	1.16±0.23	2.24±0.45	2.22±0.32

Table 4
Concentration of heavy metal Ni in Animal blood, Hair and Feces.

Source	Animal	Site A	Site B	Site C
Blood	**Cow**	1.14±0.21	1.29±0.20	2.62±0.41
	Buffalo	0.96±0.23	1.59±0.25	2.52±0.45
	Sheep	1.23±0.22	2.03±0.34	2.01±0.34
Hair	**Cow**	0.95±0.18	1.46±0.21	1.79±0.42
	Buffalo	1.07±0.18	1.59±0.32	2.15±0.35
	Sheep	1.12±0.15	2.54±0.48	1.68±0.28
Feces	**Cow**	0.67±0.03	1.77±0.39	2.11±0.36
	Buffalo	0.99±0.26	1.89±0.37	1.82±0.34
	Sheep	1.29±0.22	2.04±0.35	2.10±0.35

three distinct sites. These findings hold significant implications for both animal health and environmental management, as well as human health through the consumption of animal-derived products.

The highest recorded Pb concentration in this study, 2.47 mg/l in buffalo blood, should be of particular concern. While this concentration is within the range of what is considered tolerable for livestock, it is important to note that chronic exposure can lead to Pb accumulation over time. This accumulation may present a significant challenge, especially if these animals are sources of dairy and meat production (Karim and Hussain, 2021). The findings highlight the importance of regular monitoring and management of heavy metal exposure in farm ruminants to ensure both animal and human health. The study conducted by Smith and Jones (2022), showed that Pb can cause toxicity following bioaccumulation in the plants and farm cattles body tissues and a risks to ecology and local population. It is necessary to implement strategies for reducing trace element level in the environment, such as improved wastewater treatment and soil management practices. Furthermore, monitoring the Pb levels in animal products, particularly those derived from buffaloes, should be a priority to prevent potential health risks to consumers. The highest Ni level were consistently observed in sheep blood at site B (2.03), while buffalo tended to have the lowest Ni concentrations (0.96) at site A (Tables 3, 4). The present study further explained that cow hair exhibit lowest level (0.95) of Ni at site A while it was greatest at site B in sheep hair. A similar trend was observed in Ni level in the sheep feces and lower level in cow feces at site A.

This variation in Pb and Ni levels among different ruminant species may be attributed to differences in their physiology, diet, and metabolic processes. Furthermore, environmental factors such as the quality of grazing land, water sources, and industrial activities in the vicinity of these sites can significantly impact heavy metal uptake in the soil-plant-animals ecosystem and that might enter in the food chain. Assessing each animal source across the different sampling sites, notable variations in Ni concentrations were observed. In blood samples, the range of Ni concentrations varied from 0.96 to 2.62 across all sites and animal sources. For hair samples, the Ni concentrations ranged from 0.95 to 2.54, while in feces samples, the concentrations ranged from 0.67 to 2.10. Overall, the highest Ni concentration across all samples was observed in sheep blood at site 2 (Tables 3, 4), while the lowest concentration was found in cow feces at site A. It's important to highlight that the concentrations of manganese (Mn), cobalt (Co), arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) in serum were below than our limit of quantification. These metals are predominantly present in blood cells, suggesting that whole blood is a more appropriate biomarker for assessing exposure, as noted by Smith and Nordberg (2015).

Table 3
Analysis of Variance for Ni metal in Animal blood, Hair and Feces.

Source	Df	Mean square
Site	2	26.101***
Animal	2	0.331 ^{ns}
Source	2	1.451 ^{ns}
Site x Animal	4	1.244 ^{ns}
Site x Source	4	1.847 ^{ns}
Animal x Source	4	0.223 ^{ns}
Site x Animal x Source	8	0.644 ^{ns}

ns insignificant. * Significant at 0.05 level

3.3. *Metal concentrations in the hair tissue samples of farm ruminants*

The results illustrate the concentration of the heavy metals in the hair of three different farm ruminant (cow, buffalo, and sheep) across three distinct sites (site A, site B, and site C) during the feeding trial (Tables 1, 2, 3 and 4). Among the three sites, site A consistently exhibits the lowest lead concentration (1.03 mg/kg) for all animal species. The lowest recorded lead concentration at site A is for buffalo. Site B generally shows moderate lead concentrations, with a range of 1.44 mg/kg (buffalo) to 2.35 mg/kg (sheep) (Table 2). Site C consistently displays the highest lead concentrations among all three sites. The highest recorded lead concentration at site C is 2.36 mg/kg for cows. Among the animal species, sheep generally show the highest lead concentrations, with a range from 1.05 mg/kg (site A) to 2.35 mg/kg (site B). Buffalo and cows exhibit lower lead concentrations overall, with buffalo ranging from 1.03 (site A) to 2.08 mg/kg (site C), and cows ranging from 1.12 mg/kg (site A) to 2.36 mg/kg (site C) (Table 3). The lowest lead concentration observed across all sites and animals is 1.03 mg/kg, recorded for buffalo at site A. The highest lead concentration is 2.36 mg/kg, found in cow hair at site C. The range of lead concentration within the data set spans from 1.03 to 2.36. (Table 2). These results highlight the site-specific differences in lead accumulation in ruminant hair, with site C consistently showing higher concentrations compared to site A and site B. Additionally, inter-species variations demonstrate that sheep tend to accumulate higher lead concentrations compared to buffalo and cows in their hair tissues. The site-specific differences in lead accumulation suggest that the environmental conditions and exposure to lead contamination may vary significantly between these locations. This environmental variability highlights the need for region-specific monitoring and remediation strategies (Zhang et al., 2019). The variation in lead accumulation in farm ruminant hair highlights the potential risks associated with exposure to contaminated environments. Excessive lead exposure can have adverse effects on animal health, including impaired growth, reproductive issues, and overall well-being.

The observed variations in Pb concentrations across the three sites underline the significance of local environmental factors in heavy metal accumulation in livestock. Recent studies have emphasized the role of soil quality, water sources, and industrial activities in determining heavy metal levels in agricultural areas (Sun et al., 2021). The differing Pb concentrations among cow, buffalo, and sheep hair reflect variations in heavy metal uptake and metabolism across ruminant species. These findings are consistent with recent research indicating interspecies variations in heavy metal bioaccumulation (Xiao et al., 2020). Understanding these differences is essential for targeted risk assessment and management. Elevated Pb levels in ruminants, as observed in buffalo and sheep at site B and site C, raise concerns about animal health. Studies have shown that Pb exposure can lead to adverse effects on ruminant health, including reduced feed intake and growth (Ali et al., 2018). These findings underscore the importance of implementing measures to reduce heavy metal exposure in livestock (Li et al., 2021).

Heavy metals in animal-derived products can pose risks to human health. Recent research has highlighted the potential for Pb transfer from livestock products to consumers (Chen et al., 2019). Therefore, the elevated Pb concentrations in buffalo and sheep hair, particularly at site B and site C, may have implications for the safety of meat and dairy products from these animals. To mitigate heavy metal contamination, comprehensive environmental management practices are essential. Recent studies have demonstrated the effectiveness of soil remediation techniques, such as phytoremediation, in reducing heavy metal levels in agricultural areas (Wang et al., 2020). Implementing such strategies in areas with elevated Pb concentrations is vital for protecting both the environment and animal health.

The results from the Table 4, depict the levels of a certain substance found in the hair of three different animals across three distinct sites (site A, site B, and site C). Among the animals studied, sheep exhibited the highest concentration of the Ni across all sites, with site B showing the maximum value of 2.54. This suggests that sheep hair might accumulate Ni more readily compared to hair from cows and buffaloes. The lowest concentration of the Ni in sheep hair was observed at site C, with a value of 1.68. On the other hand, hair from cows consistently demonstrated the lowest concentrations of the Ni across all sites, with site A registering the lowest value of 0.95. This implies that cows might have a lower propensity to accumulate this Ni in their hair compared to buffaloes and sheep. The highest concentration of the Ni in cow hair was observed at site 3, with a value of 1.79. This demonstrates that there is some variability in accumulation not only between different species but also within the same species across different sites. Buffalo hair displayed intermediate levels of the Ni, falling between the concentrations observed in cow and sheep hair. The highest concentration of the Ni in buffalo hair was recorded at site C, with a value of 2.15 (Table 4). Conversely, the lowest concentration was observed at site A, with a value of 1.07. This suggests that buffaloes may have a moderate tendency to accumulate this Ni in their hair compared to cows and sheep.

3.4. Metal concentrations in the urine (feces) of farm ruminants

The heavy metal lead (Pb) concentrations in farm ruminant feces from three different sites (site A, site B, and site C) are shown in Table 4. The greatest Pb concentrations were found at site C, where values for cow, buffalo, and sheep were 2.11 mg/kg, 2.05 mg/kg, and 2.24 mg/kg, respectively. As opposed to this, site A had the lowest Pb concentrations of any animal group, with values for cows, buffalo, and sheep of 0.66 mg/kg, 1.28 mg/kg, and 1.16 mg/kg, respectively. Regarding Pb concentrations among different ruminant species, sheep exhibited the highest Pb concentrations at site B, with values of 2.24 mg/kg. In contrast, cow and sheep had relatively lower concentrations of 1.87 mg/kg and 2.05 mg/kg, respectively (Tables 2, 4). The highest values at site B indicate increased exposure to Pb, potentially due to local industrial activities or soil quality. These findings align with recent research emphasizing the role of regional environmental conditions in heavy metal bioaccumulation in livestock (Wu et al., 2022). Elevated Pb concentrations in ruminant feces raise concerns regarding potential human health risks. Studies have shown that heavy metals present in animal feces can contaminate water sources and agricultural land, leading to indirect exposure to humans (Chen et al., 2020).

The observed variability in Pb concentrations across different sites and animal species underscores the dynamic nature of environmental contamination. Site B exhibited the highest Pb concentrations, possibly due to local industrial activities or soil quality. This aligns with recent research by Wu et al. (2022), which emphasized the role of regional environmental conditions in heavy metal bioaccumulation in soils. The disparities in Pb concentrations among cow, buffalo, and sheep feces at site B indicate species-specific responses to environmental contamination. Sheep displayed the highest Pb concentrations in their feces, suggesting that certain ruminants may be more susceptible to heavy metal exposure. This aligns with studies by Chen et al. (2020), which examined toxic trace elements contamination in urban soils and crops. Understanding these species-specific variations is crucial for tailored environmental management practices and future management strategies. The findings from this study, particularly the highest values at site B, warrant increased attention to potential exposure pathways and the development of strategies to mitigate health risks.

The results presented in Table 4 depict the concentrations of the heavy metal nickel (Ni) in the blood, hair, and feces of three different animal species across three distinct sites (site A, site B, and site C). The sheep exhibited the highest concentrations of nickel in their feces across all sites, with site C showing the maximum value of 2.10. This indicates that sheep might have a higher tendency to excrete nickel through their feces compared to cows and buffaloes. The lowest concentration of nickel in sheep feces was observed at site A, with a value of 1.29. The cows consistently demonstrated the lowest concentrations of nickel in their feces across all sites, with site A registering the lowest value of 0.67. The highest concentration of nickel in cow feces was observed at site C, with a value of 2.11. This demonstrates that there is some variability in excretion not only between different species but also within the same species across different sites.

Buffalo feces displayed intermediate levels of nickel concentration, falling between the concentrations observed in cow and sheep feces. The highest concentration of nickel in buffalo feces was recorded at Site B, with a value of 1.89. Conversely, the lowest concentration was observed at site A, with a value of 0.99. This suggests that buffaloes may have a moderate tendency to excrete nickel through their feces compared to cows and sheep. The present study highlight both inter-species and intra-species variations in the excretion of nickel through feces across different sites, indicating potential differences in exposure or metabolic processes among the studied animals. The potential for toxic trace elements to leach into water sources and agricultural land, as established by Chen et al. (2020), and thus in the food chain and can impact terrestrial and public health.

3.5. Health risk indices evaluation

3.5.1. Bioconcentration, Pollution load index and Enrichment indices for Pb and Ni

The Table 5 illustrates the Bioconcentration, Pollution load index and Enrichment indices of lead (Pb) metal in soil samples collected from three distinct sites, labeled A, B, and C, across five different plant species. The highest BCF value is observed in site B for the soil of *C. dactylon* with a value of 1.128, suggesting the highest potential for Pb bioaccumulation in this specific plant-soil combination. Conversely, the lowest BCF value is found in site A for the soil of *P. fruticosa* with a value of 0.763, indicating relatively lower Pb accumulation in this particular scenario. The range of BCF values varies across sites and plant species, with site B demonstrating the widest range, from 0.805 (*Sorghum bicolor*) to 1.128 (*C. dactylon*), highlighting the variability in Pb bioaccumulation potential within the same geographical area.

The Table 6 outlines the pollution load index (PLI) for lead (Pb) across three distinct sites from five different plant species. The highest PLI value is observed in site B for the soil of *S. bispinosa* with a value of 1.508 (highest level at this site). In contrast, the lowest PLI value is

Table 5
Bioconcentration factor of Pb Metal in soil sample.

BCF	Site A	Site B	Site C
Soil of *S. bicolor*	0.787	0.805	0.939
Soil of *C. dactylon*	0.879	0.938	1.128
Soil of *P. fruticosa*	0.763	1.057	0.811
Soil of *S. bispinosa*	0.876	0.991	0.915
Soil of *T. terresteris*	0.862	0.880	1.021

Table 6
Pollution load index for Pb.

PLI	Site A	Site B	Site C
S. bicolor	1.118	1.216	1.218
C. dactylon	0.996	1.209	1.102
P. fruticosa	1.076	1.204	1.216
S. bispinosa	0.982	1.303	1.508
T. terresteris	1.005	1.312	1.217

found in site A for the soil of *C. dactylon* with a value of 0.996, suggesting comparatively lower Pb pollution levels in this specific scenario. The range of PLI values varies across sites and plant species, with site B demonstrating the widest range, from 0.996 (*C. dactylon*) to 1.508 (*S. bispinosa*), indicating substantial variability in Pb pollution levels within the same geographical area. The Table 7, presents the enrichment factor (EF) for lead (Pb) across three different sites from five distinct plant species. The highest EF value is observed in Site C for the soil of *Cynodon dactylon* with a value of 3.065, indicating the highest degree of Pb enrichment at this site for this specific plant species. Conversely, the lowest EF value is found in site A for the soil of *P. fruticosa* with a value of 2.072, suggesting comparatively lower Pb enrichment levels.

The Tables 8 and 9 outlines the bioconcentration factor (BCF) of nickel (Ni) metal in soil samples across five different plant species. The highest BCF value is observed in site C for the soil of *P. fruticosa* with a value of 1.104, indicating the highest potential for Ni bioaccumulation in this specific plant-soil combination. The lowest BCF value is found in site B for the soil of *S. bispinosa* with a value of 0.708 (lower Ni accumulation). The range of BCF values varies across sites and plant species, with site C demonstrating the widest range, from 0.789 (*P. fruticosa*) to 1.104 (*P. fruticosa*), highlighting the variability in Ni bioaccumulation potential within the same geographical area. The highest PLI value is observed in site C for the soil of *P. fruticosa* with a value of 1.308, indicating the highest level of Ni pollution while lowest PLI value is found in site A for the soil of *S. bicolor* (0.975). The range of PLI values varies across sites and plant species, with site C demonstrating the widest range, from 1.043 (*P. fruticosa*) to 1.308 (*P. fruticosa*), indicating substantial variability in Ni pollution levels within the same geographical area. These results highlight the necessity for site-specific assessments to comprehend the extent and distribution of Ni contamination in soil-plant systems, enabling the development of targeted remediation strategies to mitigate environmental risks associated with heavy metal pollution.

The enrichment factor (EF) for nickel (Ni) that were highest EF at site C for the soil of *P. fruticosa* with a value of 0.149, indicating the highest degree of Ni enrichment while lowest EF value is found in site A for the soil of *S. bicolor* with a value of 0.082 (Table 10). The range of EF values varies across sites and plant species, with site C demonstrating the widest range, from 0.107 (*P. fruticosa*) to 0.149 (*P. fruticosa*), indicating substantial variability in Ni enrichment levels within the same geographical area.

3.5.2. Daily Intake of Pb and Ni by animal species (Pb, mg/day)

The Table 11 presents the daily intake of lead (Pb) metal for three different types of livestock—cow, buffalo, and sheep—across three distinct sites from five different plant species. The highest daily intake of Pb is observed at site C for sheep consuming *P. fruticosa* with a value of 0.1185, indicating the highest level of Pb intake at this site for this specific livestock-plant combination. Conversely, the lowest daily intake of Pb is found at site A for cows consuming *S. bicolor* with a value of 0.0122, suggesting relatively lower Pb intake levels in this particular scenario. The range of daily intake values varies across sites, livestock types, and plant species, with site C generally exhibiting higher values compared to sites A and B. In *S. bicolar*, the highest daily intake of Pb by cows was observed at site C (0.0159), while the lowest intake was at site A (0.0122 mg/day). For buffaloes, the highest daily intake occurred at site C (0.0180), with the lowest at site A (0.0136). Among sheep, the highest intake was at site B (0.0154), and the lowest at site A (0.0106). In *C. dactylon*, the cows had the highest daily intake of Pb at site C (0.0172) and minimum at site A (0.0121), resulting in a range of 0.0051. Buffaloes had their highest intake at site C (0.0196 mg/day) and minimum at site A (0.0138). For sheep, the highest intake occurred at site B (0.0196), while minimum was at site A (0.0105). In *S. fruticosa*, the highest daily intake in cows was at site B (0.0200), while minimum was at site C (0.0137). Buffaloes had the highest intake at site B (0.0200) and minimum at site C (0.0137), resulting in a range of 0.0063. Sheep showed the highest intake at site B (0.0155) and minimum at site A (0.0099), resulting in a range of 0.0056. Additionally, the comparison between livestock types within each site reveals differences in Pb intake levels, with buffalo generally exhibiting higher intake values compared to cows and sheep. Furthermore, the range of daily intake values varies among plant species, suggesting variations in Pb accumulation and transfer within the food chain. These findings underscore the importance of monitoring Pb levels in both soil and plant samples to assess potential risks to livestock health and to implement appropriate mitigation measures to minimize Pb exposure. Overall, understanding the dynamics of Pb intake in livestock is crucial for ensuring the safety and well-being of both animals and consumers in agricultural settings.

These results indicate variations in daily Pb intake across different plant species, animal types, and sites, emphasizing the importance of considering these factors when assessing heavy metal exposure in livestock. The findings in Table 5, depicting daily lead (Pb) intake by various animals across sites, offer critical insights into heavy metal

Table 7
Enrichment factor for Pb.

EF	Site A	Site B	Site C
S. bicolor	2.138	2.188	2.553
C. dactylon	2.389	2.549	3.065
P. fruticosa	2.072	2.872	2.204
S. bispinosa	2.379	2.691	2.485
T. terresteris	2.342	2.391	2.774

Table 8
Bioconcentration Factor of Ni Metal in soil sample.

BCF	Site A	Site B	Site C
Soil of *S. bicolor*	0.605	0.621	0.783
Soil of *C. dactylon*	0.858	0.846	0.963
Soil of *P. fruticosa*	0.789	0.809	1.104
**Soil of *S.* ***bispinosa*	0.932	0.708	0.891
Soil of *T. terresteris*	0.952	0.848	1.150

Table 9
Ni metal pollution load index.

PLI	Site A	Site B	Site C
S. bicolor	0.975	1.114	1.12
C. dactylon	0.816	1.078	1.055
P. fruticosa	1.081	1.308	1.043
S. bipinosa	0.815	1.071	1.219
T. terresteris	0.802	1.147	0.986

Table 10
Enrichment factor for Ni.

EF	Site A	Site B	Site C
S. bicolor	0.082	0.084	0.106
C. dactylon	0.116	0.114	0.13
P. fruticosa	0.107	0.109	0.149
S. bispinosa	0.126	0.096	0.121
T. terresteris	0.129	0.115	0.156

Table 11
Daily intake of metal for Pb.

DIM	Cow			Buffalo			Sheep		
	Site A	Site B	Site C	Site A	Site B	Site C	Site A	Site B	Site C
S. bicolor	0.0122	0.0136	0.0159	0.0139	0.0154	0.0180	0.0106	0.0118	0.0137
C. dactylon	0.0121	0.0157	0.0172	0.0138	0.0179	0.0196	0.0105	0.0136	0.0149
P. fruticosa	0.0137	0.0176	0.0137	0.0129	0.0200	0.0155	0.0099	0.0153	0.1185
S. bispinosa	0.0119	0.0179	0.0191	0.0135	0.0203	0.0217	0.0103	0.0155	0.0166
T. terresteris	0.0120	0.0159	0.0172	0.0136	0.0182	0.0195	0.0104	0.0139	0.0149

contamination risks in forage and their livestock-related implications. Variability in Pb intake is evident across plant species, animal types, and sites, driven by soil contamination, plant preferences, and dietary habits (Rodriguez Martin et al., 2013; Roggeman et al., 2013; Scheuhammer et al., 2003; Shahid et al., 2015; Singh et al., 2010; Smith et al., 2019, 2020, 2009; Solgi et al., 2017; Steel, Torrie 1980; Subhashini, A.V.V.S. Swamy 2013; Syso et al., 2017; Szefer et al., 1996;). The type of forage consumed significantly influences Pb intake, emphasizing variations in heavy metal accumulation in different plant species (Zhang et al., 2019).

Site-specific differences in Pb intake highlight the significance of considering regional soil characteristics in risk assessments (Chen et al., 2020). High Pb intake raises concerns about animal health and product safety due to potential bioaccumulation (Zhu et al., 2018). Effective grazing management, including forage selection and rotation, plays a crucial role in mitigating Pb exposure (Sun et al., 2021). Soil remediation, such as phytoremediation and soil amendments, can reduce Pb intake in livestock from heavily contaminated areas (Kumar et al., 2022). Continuous monitoring and regulation of heavy metal concentrations in forage and livestock are imperative for ensuring food safety, with regulatory agencies setting and enforcing safe Pb intake limits (EPA, 2020). Heavy metal accumulation in livestock can contaminate meat and dairy products, underscoring the need for safeguarding the food chain (FDA, 2021). Elevated Pb levels in animal products pose significant human health risks, necessitating vigilance in consumption (Hu et al., 2019).

The Table 12 showed daily intake of nickel (Ni) metal for three different types of livestock—cow, buffalo, and sheep—across three distinct sites labeled A, B, and C, with data collected from five different plant species. Within each plant species, *Panicum fruticosa* exhibits the highest Ni intake across all sites, with the maximum value of 0.0201 observed in site C for sheep. Conversely, *Sorghum bicolor* indicate the lowest Ni intake values (0.0079) at site A for sheep. The range of daily Ni intake varies within each plant species, indicating differing levels of Ni accumulation across different sites. The *Panicum fruticosa* demonstrates the widest range of Ni intake values, ranging from 0.0114 to 0.0201, highlighting substantial variability in Ni accumulation potential across sites for this plant species.

Furthermore, when comparing Ni intake among all animals, sheep generally exhibit higher Ni intake values compared to cows and buffalo across most plant species and sites. At site C, sheep consuming *Panicum fruticosa* have the highest Ni intake value of 0.0201, followed by buffalo with 0.0185 and cows with 0.0149. This trend persists across other plant species and sites, indicating potential differences in Ni intake efficiency among livestock types. These findings underscore the importance of considering both plant species and livestock types in assessing Ni intake levels and implementing appropriate management strategies to mitigate potential health risks associated with Ni contamination in livestock feed. Additionally, the comparison of Ni intake among different plant species reveals variations in Ni accumulation and transfer capabilities, suggesting the need for site-specific assessments to understand the dynamics of Ni contamination in soil-plant systems and to develop targeted mitigation measures. Future research directions should focus on understanding the long-term effects of heavy metal intake on livestock health, exploring novel remediation strategies, and assessing the economic and environmental impacts of contamination in agroecosystems (Xu et al., 2021).

3.6. Health Risk index of trace metals (Pb, Ni)

The results from Table 13 present the Health Risk Index (HRI) values for lead (Pb) in farm ruminants across different plant species, animal types, and sites. The HRI values are a critical toxicity indicators following forage crops consumption by farm cattles. The highest HRI value for cows was observed at site C for all plant species. The overall highest HRI value for cows across plant species and sites was 4.8923, indicating a relatively high health risk. Minimum HRI value for cows was at site A for *S. fruticosa*, with an HRI of 2.8433, suggesting a lower health risk. Buffalo exhibited similar patterns to cows, with the highest HRI values at site C for all plant species.

The overall highest HRI value for buffalos across plant species and sites was 5.0806, signifying a relatively high health risk. Minimum HRI value for buffalos was at site A for *S. fruticosa*, with an HRI of 3.2309, indicating a lower health risk. Sheep demonstrated comparable trends, with the highest HRI values at site C for all plant species. The overall highest HRI value for sheep across plant species and sites was 5.4284, signifying a relatively high health risk. Minimum HRI value for sheep was at site A for *S. fruticosa*, with an HRI of 2.9614, suggesting a lower health risk. Overall, the results from Table 13 highlight the elevated health risk index values at site C for all animal types and plant species. These findings emphasize the importance of assessing and mitigating heavy metal contamination in the studied agroecosystems. The highest HRI values underscore the potential dangers of lead (Pb) intake by farm ruminants, which can subsequently impact food safety and human health.

The Table 14 presents the health risk index (HRI) for nickel (Ni) across three distinct sites from three types of livestock—cow, buffalo, and sheep—consuming five different plant species. Within each plant species, *P. fruticosa* exhibits the highest HRI across all sites, with the

Table 12
Daily intake of metal for Ni.

DIM	Cow			Buffalo			Sheep		
	Site A	Site B	Site C	Site A	Site B	Site C	Site A	Site B	Site C
S. bicolor	0.0091	0.0106	0.0135	0.0103	0.0121	0.0154	0.0079	0.0092	0.0117
C. dactylon	0.0108	0.0141	0.0157	0.0122	0.0159	0.0178	0.0093	0.0122	0.0136
P. fruticosa	0.0131	0.0163	0.0177	0.0149	0.0185	0.0201	0.0114	0.0141	0.0154
S. bispinosa	0.0117	0.0116	0.0167	0.0132	0.0133	0.0190	0.0101	0.0101	0.0145
T. terresteris	0.0118	0.0149	0.0175	0.0134	0.0170	0.0198	0.0102	0.0129	0.0151

Table 13
Health risk index for Pb.

HRI	Cow			Buffalo			Sheep		
	Site A	Site B	Site C	Site A	Site B	Site C	Site A	Site B	Site C
S. bicolor	3.0473	3.3915	3.9653	3.4628	3.8539	4.5059	2.6409	2.9393	3.4365
C. dactylon	3.0345	3.9313	4.3053	3.4483	4.4673	4.8923	2.6299	3.4071	3.7312
P. fruticosa	2.8433	4.4073	3.417	3.2309	5.0082	3.8829	2.4642	3.8197	2.9614
S. bispinosa	2.9793	4.471	4.777	3.3855	5.0806	5.4284	2.582	3.8749	4.1401
T. terresteris	3.0005	3.9993	4.3053	3.4097	4.5446	4.8923	2.6004	3.466	3.7312

Table 14
Health risk index for Ni.

HRI	Cow			Buffalo			Sheep		
	Site A	Site B	Site C	Site A	Site B	Site C	Site A	Site B	Site C
S. bicolor	0.3026	0.3553	0.4505	0.3439	0.4038	0.5119	0.2623	0.3079	0.3904
C. dactylon	0.3593	0.4686	0.5219	0.4083	0.5325	0.5931	0.3114	0.4061	0.4523
P. fruticosa	0.4375	0.5434	0.591	0.4971	0.6175	0.6716	0.3791	0.4709	0.5122
S. bispinosa	0.3899	0.3893	0.5582	0.443	0.4424	0.6342	0.3379	0.3374	0.4837
T. terresteris	0.3921	0.4992	0.5819	0.4456	0.5673	0.6613	0.3398	0.4327	0.5044

maximum value of 0.6716 observed in site C for buffalo. Conversely, *S. bicolor* shows the lowest HRI values (0.2623) at site A for sheep. For instance, *Panicum fruticosa* demonstrates the widest range of HRI values, ranging from 0.3791 to 0.6716. The range of HRI values varies within each plant species, indicating differing levels of health risk associated with Ni intake across different sites.

Furthermore, when comparing Ni intake among all animals, sheep generally exhibit higher health risk index values compared to cows and buffalo across most plant species and sites. At site C, sheep consuming *P. fruticosa* have the highest HRI value of 0.6716, followed by buffalo with 0.6175 and cows with 0.591. This trend persists across other plant species and sites, indicating potential differences in health risk associated with Ni intake among livestock types. These findings underscore the importance of considering both plant species and livestock types in assessing health risks associated with Ni contamination in livestock feed. The HRI values among different plant species reveals variations in the potential health risks posed by Ni accumulation, suggesting the need for comprehensive risk assessment strategies to mitigate health hazards associated with Ni exposure in agricultural settings.

Domestic and urban animals showcase distinct seasonal patterns. According to Cowan and Blakely (2016), there is a marked seasonal trend in lead toxicity among cattle, with toxicity being most prevalent during the spring and early summer months. Similarly, sheep in the UK exhibit significant seasonal fluctuations in blood-lead levels due to grazing behaviors, which are strongly influenced by the time of year (Smith et al., 2010). Furthermore, seasonal correlations have been noted between lead concentrations in sheep tissue and milk (Weglarzy, 2010). Investigations on Polish breeding horses suggest heightened levels of lead in both serum and hair during the summer season (Janiszewska and Cieśla, 2002). Effective monitoring of heavy metal concentrations in forage and livestock is essential to ensuring food safety and animal welfare. Regulatory agencies must set and enforce safe limits for Pb intake to protect both human health and animal well-being (EPA, 2020). The consumption of animal products with elevated Pb levels can pose significant risks to human health. The potential transfer of heavy metals from livestock to consumers underscores the need for vigilance (Hu et al., 2019).

The consumption of animal products with elevated Pb levels can pose significant risks to consumers. Therefore, ensuring food safety throughout the food chain is of paramount importance. Future research in this field should focus on understanding the long-term effects of heavy metal intake on livestock health, exploring novel remediation strategies, and assessing the economic and environmental impacts of heavy metal contamination in agro-ecosystems (Xu et al., 2021). This holistic approach is essential for developing effective strategies to manage and reduce heavy metal intake in farm ruminants, safeguarding both animal and human health.

3.7. The metal in the field soil samples from different crops

The soil samples collected from various crop fields exhibited varying levels of Pb metal concentration. Among the different crop fields, the highest Pb concentration was observed in the soil of *P. fruticosa* at site C, with a concentration of 12.29. Conversely, the lowest Pb concentration was found in the soil of *S. bicolor* at site A, with a concentration of 8.12. Across all crop fields, the Pb concentrations ranged from 8.003 to 12.29 (Tables 15, 16).

The soil from different crop fields exhibited varying levels of Pb concentration, with *P. fruticosa* at site C and *S. bispinosa* at site C showing the highest concentrations, while *S. bicolor* at site A displayed the lowest. Overall, the Pb concentrations ranged from 8.003 to 12.29 across all samples collected from the different crop fields and sites. These findings underscore the importance of monitoring and managing Pb contamination in agricultural soils to ensure the safety of food production systems (Tables 15, 16). These findings emphasize the importance of considering local soil characteristics and potential contamination sources in risk assessments (Chen et al., 2020). The variability in Pb intake, especially at higher levels, raises concerns about potential health risks for livestock. Prolonged exposure to heavy metals, such as Pb, can lead to bioaccumulation, impacting animal health and product safety (Zhu et al., 2018).

The examination of Nickel (Ni) concentration in soil samples collected from different sites across various forage crop fields revealed significant variability in contamination levels. Among the different forage crops, the highest Ni concentration was observed in soil samples of *P. anicum fruticosa* at Site 2, with a concentration of 11.85, while the lowest concentration was found in soil samples of Centipede grass (*C. dactylon*) at site A, with a concentration of 7.39±0.68. Across all forage crops and sites, the Ni concentrations ranged from 7.27 to 11.85

Table 15
Pb metal concentration in soil.

Soil of Forage	Site A	Site B	Site C
Soil of *S. bicolor*	9.11±0.47	9.91±0.58	9.93±0.64
Soil of *C. dactylon*	8.12±0.78	9.86±0.44	8.98±0.32
Soil of *P. fruticosa*	8.77±1.28	9.81±1.21	9.91±0.67
Soil of *S. bispinosa*	8.003±1.21	10.62±1.01	12.29±0.22
Soil of *T. terresteris*	8.19±1.07	10.69±0.65	9.92±0.37

Table 16
Concentration of heavy metal Ni in soil.

Soil of Forage	Site A	Site B	Site C
Soil of *S. bicolor*	8.83±0.07	10.09±0.19	10.15±0.16
Soil of *C. dactylon*	7.39±0.68	9.77±0.30	9.56±0.49
Soil of *P. fruticosa*	9.79±0.26	11.85±0.31	9.45±0.28
Soil of *S. bispinosa*	7.38±0.36	9.7±0.25	11.05±0.22
Soil of *T. terresteris*	7.27±0.52	10.39±0.22	8.93±0.17

(Tables 15, 16).

Assessing each forage crop individually, Soil of *S. bicolor* displayed Ni concentrations ranging from 8.83 to 10.15, with the highest concentration observed at Site 3 and the lowest at Site A. Centipede grass (*C. dactylon*) exhibited Ni concentrations ranging from 7.39 to 9.77, with the highest concentration at site B and the lowest at site A. Soil samples of *P. fruticosa* demonstrated Ni concentrations ranging from 9.45 to 11.85, with the highest concentration at site B and the lowest at Site 3. Soil of *S. bispinosa* showed variations from 7.38 to 11.05, with the highest concentration at site 3 and the lowest at site A. Finally, *T. terresteris* displayed Ni concentrations ranging from 7.27 to 10.39, with the highest concentration at site B and the lowest at site A. The soil samples collected from different sites across various forage crop fields exhibited varying levels of Ni contamination, with *P. fruticosa* at site B displaying the highest concentration and Centipede grass (*C. dactylon*) at site A showing the lowest. Overall, the Ni concentrations ranged from 7.27 to 11.85 across all samples, emphasizing the importance of monitoring and managing Ni contamination to ensure the safety and sustainability of forage crop production.

3.8. Principle component analysis

The analysis of heavy metal concentrations in groundwater, soil, and plant samples and ruminant tissues is a complex yet crucial aspect of environmental research. To discern patterns within these datasets, a sophisticated multivariate approach utilizing Principal Component Analysis (PCA) was employed. PCA illustrated the grouping and differentiation of parameters based on their correlation and covariance. The principal component analysis was carried out to Pb and Ni metals mean data from all the three sites and from the body tissues of all three farm cattle's (sheep, buffalo and cows) to observe component loading and the clustering behavior (Fig. 3). The first principal component PC1 has 44.4 % variance and the second component PC2 has 31.1 % variance. The cow and buffalo blood while buffalo hair exhibit Pb and clustered in the negative quadrate whereas sheep/buffalo feces and cow hair showed Pb concentration and were in positive quadrate. Prior studies (Sarwar et al., 2019; Saljnikov et al., 2019; Rahman et al., 2018; Murtaza et al., 2019) have also utilized PCA correlations to elucidate the processes governing heavy metals distribution among biotic and abiotic components, as shown in Fig. 3. Previous investigations have extensively relied on PCA correlations to unveil the underlying processes governing the allocation of heavy metals among biotic and abiotic elements. These correlations, as evidenced by multiple studies cited, serve as valuable insights into understanding the dynamics of heavy metal interactions within ecosystems. By leveraging PCA, researchers can identify not only the presence of heavy metals but also the underlying mechanisms driving their distribution and accumulation.

The PCA analysis revealed intriguing insights into the distribution of heavy metals across various environmental matrices. In water samples, for instance, data segmentation into four main variables highlighted the complexity of heavy metal interactions. However, the precise arrangement of these characteristics remained elusive, with only sporadic relationships discernible. Similarly, in Brassica plants, moderate associations between specific heavy metals were observed, suggesting the influence of physiochemical processes within the groundwater aquifer system.

A multivariate approach was employed to analyze patterns in datasets comprising concentrations of heavy metal (Ni) in groundwater, soil, forage samples, and animal blood, hair and feces as depicted in Fig. 4. Principal Component Analysis (PCA) was conducted on the mean data of Ni metal ions across all three treatments sheep, buffalo and cow (blood, hair and feces) to examine component loading and clustering behavior (Fig. 4). PC1, the first principal component, accounts for 56.7 % of the variance, while PC2, the second component, accounts for 33.8 % of the variance (Fig. 4). Regarding the distribution and loading of Ni metal among the animal body tissues, sheep hair, buffalo feces and cow hair were clustered in the negative quadrant, whereas sheep blood, feces reside in the positive quadrant from both sites (1, 2). The loadings of all metal ions, indicate strong correlation with a common source of soil contamination. Additionally, PCA results illustrate the contribution of treatments to the heavy metal pollution loads in the tested soil (Figs. 3, 4).

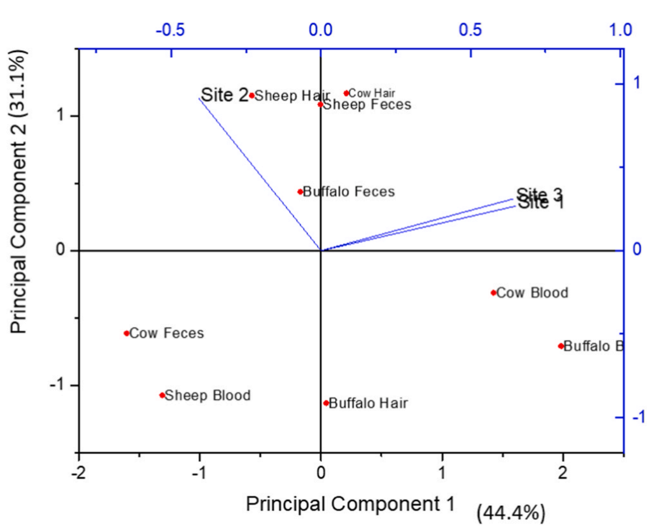

Fig. 3. Principal component analysis of mean values of Pb metal accumulated in the blood, hairs and fecal samples of cow, buffaloes and sheep's from 3 agro-ecological sites. It is a is a combination of score plot of metal ions (represented as dots) and loading plot of treatments (represented as vectors).

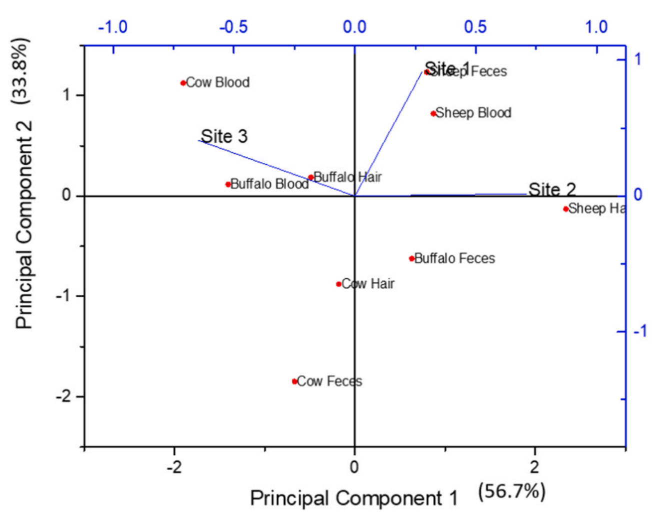

Fig. 4. Principal component analysis of mean values of Ni metal accumulated in the blood, hairs and fecal samples of cow, buffaloes and sheep's from 3 agro-ecological sites. It is a is a combination of score plot of metal ions (represented as dots) and loading plot of treatments (represented as vectors).

4. Conclusion, recommendations and future implications

The findings from this study reveal significant site-specific variations in the biomass yield of selected forage species. Notably, *S. fruticosa* displayed the highest biomass yield at site C, potentially due to favorable soil conditions, water quality, or site-specific characteristics. Conversely, other forage species, including *C. dactylon, S. bispinosa, and T. terresteris*, exhibited varying biomass yields across different sites. These variations underscore the critical importance of careful site selection in planning forage cultivation, as it can substantially impact overall crop productivity.

The practice of forage cultivation on soils irrigated with wastewater is common in many developing countries. However, the findings raise environmental concerns. Variations in biomass yield may indicate disparities in soil quality, potentially linked to heavy metals and other contaminants. It is crucial to acknowledge the potential ecological and human health risks associated with heavy metal pollution, as highlighted in previous research. Assessing these risks is a critical step in ensuring sustainable forage cultivation. To ensure the sustainability of forage cultivation in areas with wastewater irrigation, several measures should be taken:

-Implementing best practices and soil remediation techniques can help mitigate the negative impacts of heavy metal contamination. These practices should be tailored to the specific needs and challenges of each site.

- Choosing forage species that are more tolerant to adverse soil conditions, including heavy metal contamination, is essential for enhancing sustainable forage production.

- Employing phytoremediation approaches with suitable plant species can play a pivotal role in reducing heavy metal pollution in wastewater-irrigated soils.

Future research should delve deeper into soil and water quality analysis at various sites to better understand the factors influencing biomass yield variations. A comprehensive assessment of heavy metal content in both soil and forage species is imperative to comprehend potential health and environmental risks. Coupled with these efforts, there is a need to develop and promote sustainable forage cultivation practices that minimize ecological and health risks. In conclusion, addressing the complexity of forage cultivation on wastewater-irrigated soils requires a holistic approach encompassing site selection, soil and water quality management, and proactive measures to mitigate environmental and health risks. By diligently addressing these concerns, we can pave the way for safe and sustainable forage production in regions where wastewater irrigation is prevalent. These findings underscore the importance of monitoring Ni contamination in animal-derived products and waste to ensure food safety and environmental health. Implementing effective management strategies to mitigate Ni exposure in livestock can help safeguard human and animal health while maintaining the integrity of agricultural ecosystems.

In conclusion, this study underscores the significance of monitoring heavy metal contamination in farm ruminants and emphasizes the need for comprehensive management strategies. By monitoring and understanding the pathways of heavy metals accumulation in livestock, hence, animal health and the quality of animal-derived products can be safeguard while reducing potential risks to human consumers.

Ethics declarations

Ethical approval

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

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Ethical statement

All the study protocols were approved by the institutional animal ethics committee, University of Sargodha (Approval No. 25-A18 IEC UOS). All the experiments performed complied with the rules of the National Research Council and all methods were performed keeping in view the ethical principles regarding animals accordance with relevant guidelines and regulations.

CRediT authorship contribution statement

Ajmal Ali: Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Investigation. **Majida Naeem:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Kafeel Ahmad:** Investigation, Formal analysis, Conceptualization. **Zafar Iqbal Khan:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Muhammad Iftikhar Hussain:** Writing – review & editing, Writing – original draft, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Hsi-Hsien Yang:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis. **Adele Muscolo:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Formal analysis. **Khalid Iqbal:** Writing – review & editing, Writing – original draft, Resources, Project administration, Formal analysis. **Qamar uz Zaman:** Writing – review & editing, Writing – original draft, Software, Resources, Investigation, Data curation. **Mohamed Soliman Elshikh:** Writing – review & editing, Software, Resources, Project administration, Investigation, Funding acquisition, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Consent to participate

All authors voluntarily to participate in this research study.

Consent to publish

All authors consent to the publication of the manuscript.

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