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A study the risk assessment of nickel element profile in diversely irrigated pastures in relation to livestock requirement is a sub-tropical environments and climate

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Abstract
Nickel (Ni) is an essential metal for plant metabolism, substantial amounts of Ni can have detrimental effects on the environment. Because of its widespread occurrence and persistence, which contribute to soil pollution and environmental risks, nickel (Ni) is the subject of this research as a major environmental contaminant. The main goal of the study is to evaluate the amounts of Ni in various crop cultivars such as (sugarcane, brassica, berseem, alfalfa, maize, and oat) and soils cultivated under different water regimes (groundwater, canal water, sewage) in Sargodha, Pakistan. Ni concentrations were analyzed in ecological settings such as water and soils, as well as in plant components including roots, shoots, and leaves. Furthermore, various pollution indices were utilized to evaluate the phytostabilization potential, aiming for a comprehensive understanding of Ni contamination's environmental impact. The results showed that the mean Ni concentration in different water regimes varied from. 0.073 to 0.78 mgL^{-1} at location 1 and location 3. 10.45 to 20.57 mgkg-1, At SITE I, *S. officinarum* and at SITE III, *Z. mays* in soil. 1.23 to 11.83 mgkg-1 in crops, B. campestris at SITE I, and Z. mays at SITE III. 0.43 to 2.89 mgL-1 in non-lactating buffaloes at SITE I and dry buffaloes consequently. Findings of the study also showed that the levels of nickel in crops, water, soil, and buffalo serum are all within acceptable bounds. All indices—contamination factor (CF), transfer factor (TF), enrichment factor (EF), estimated daily intake (EDI), and hazard quotient (HQ) for Ni—are less than 1, used to determine health risks posed by heavy metals. However, using wastewater for irrigation on a regular basis could pose risks to food chain organisms.

Keywords: Ni contamination, Environmental pollution, Crop varieties, Water regimes, Soil contamination, Phytostabilization potential

Introduction

Pakistan has a total irrigated land area of 18.84 million hectares. Among this, 7.79 million hectares are irrigated through canals, while the remaining agricultural land is irrigated through groundwater, with tube wells and canals accounting for the majority of the 7.7 million hectares (Yu *et al*., 2022) [55]**.** Pakistan, characterized by a moderately arid environment, faces

a serious shortage of freshwater. The availability of water is decreasing due to climate change and population growth globally, posing a threat to agricultural sustainability (Mekonnen & Hoekstra, 2020) [34]. However, this may indirectly impact the microbiological characteristics of the soil (Becerra-Castro *et al*., 2015) [6]. Subsequently wastewater is the only source of irrigation for fodder crops and plants, when it is applied for irrigation purposes, a lot of nutrients and hazardous chemical components collect in the plants, forages, and crops utilised by the organisms. Therefore, the unfavourable fertility, productivity, and soil improvement of this water impact the quality concerns of the produced fodder crops and soil as well as the yield of crops (Maqsood *et al*., 2022) [32].

Forages are planted primarily for livestock production but they are also a rich and outstanding source of nutrients, many of which are often unique to each forage species (Tava *et al*., 2022) [45] In this study, nickel (Ni) was selected as a significant environmental pollutant and toxic substance due to its widespread availability and high persistence, leading to soil pollution and serious environmental issues (Khan *et al*., 2019) [26]. Exposure to nickel can result in various adverse health effects in individuals, including allergies, kidney and heart problems, lung fibrosis, and lung and nasal cancer. Although the precise molecular mechanisms underlying nickel toxicity remain unclear, mitochondrial dysfunction and oxidative stress are believed to play a fundamental role in the toxicity of this metal (Wajid *et al*., 2020). The essentiality and recommended dietary intake levels of Ni for humans are not established. Nickel indirectly induces genotoxic effects by interfering with the DNA repair system, leading to Ni accumulation in breast tissues and potentially contributing to malignant tumors. Pastures that support livestock grazing are often highly vulnerable to risks because forage production is influenced by precipitation. Climate change exacerbates these risks, causing both intra- and interannual variability that complicates proactive planning. Prolonged drought and associated precipitation variability frequently result in a reduction in livestock numbers (Yilmaz, 2020).

Nickel is a highly mobile element in the natural environment, readily absorbed by plants in proportion to its soil concentration until it reaches toxic levels. Plants have evolved efficient physiological and biochemical mechanisms for the uptake, translocation, and accumulation of microelements, even at low concentrations. These mechanisms are also utilized for absorbing toxic substances with chemical properties similar to microelements. Plant species and varieties exhibit diverse abilities to accumulate heavy metals. Understanding these patterns is crucial for the potential utilization of these plants in phytoremediation of soils, waters, and sediments (Ogunkunle *et al*., 2014).The goal of this study is to evaluate the potential hazards associated with consuming contaminated forage crops cultivated in various irrigation systems at different locations an also determining the concentrations of HMs in the soil, feed, water and buffalo blood, analyze the flow of heavy metals from water, soil, feed, and animals, explore potential health risks using an assortment of parameters.

Materials and Methods

Design of experiment

The research was conducted in Sahiwal, a tehsil in the Sargodha division, 172 km west of Lahore. It is located approximately 30 kilometers from the M-2 highway. The city sits at an elevation of 190 meters above sea level. Sahiwal experiences extreme heat of up to 50°C in summer and intense cold in winter. Sahiwal is situated about 37 km from the Sargodha-Jhang road and around 5 km from river Jhelum. The town was established during the reign of Emperor Sher Shah Suri. It was once well-known for its business activities due to population growth and economic development. Farmers often use this untreated water for irrigation purposes, which poses risks to crops, vegetables, forage, and citrus farms.

Map of the study area

Climate

Sahiwal faces temperatures between 0 and 500 C. Though the summer months of July and August are hot and dry, rain is brought on by the monsoon season. September represents the end of of the monsoon season and the start of loud overflowing rains.

Vegetation

Tehsil Sahiwal is basically a city of agricultural importance. Alongside vegetables for human consumption, a large number of forage and forage crops are also produced for the animal to accomplish their requirement. There are two types of forage crops both kharif and rabi crops. Kharif crops include millet, sorghum, maize, etc. in Rabi crops Lucerne, mustard, berseem mustard.

Table 1: Names of selected Sites

Sampling site	Tehsil	Irrigation type
1	Jhamtawala	Ground water
2	Sahiwal	canal water
3	Vijh	Sewage water

Table 2: List of forages investigated and collected from selected sites

	Common name	Botanical name	Family	Parts use for sampling
I	Sugarcane	*S. officinarum*	*poaceae*	Stem and leaves
II	SAAG or Mustard	*B. campestris*	brassicaceae	Stem,leaves
III	Berseem	*T. alexandrinum*	leguminosae	Stem, leaves
IV	Alfafa or loosan	*M. sativa*	fabaceae	Stem, leaves
V	Corn or maize	*Z. mays*	Poaceae	Stem, leaves
VI	Oat or jodar	*A. sativa*	Poaceae	Leaves

The selection of specific forage types for sampling was based on several factors aimed at capturing a comprehensive understanding of the nutritional composition and potential contaminants present in the diet of animals in the Sahiwal Tehsil region. The selected forages, including sugarcane, brassica, berseem, alfalfa, maize, and oat, are commonly

consumed by animals such as buffaloes, which are prevalent in agricultural settings like Sahiwal Tehsil. By sampling these forages, we gain insights into the typical diet of animals in the region. Each forage type offers a unique nutritional profile, including variations in protein, carbohydrate, fiber, and mineral content. Sampling multiple forage types allows for a comprehensive assessment of the nutritional diversity available to animals in the area. The selected forages hold agricultural significance in the region, either as main crops or as supplementary feed for livestock. Understanding the quality and safety of these forages is crucial for optimizing animal health and productivity in agricultural systems. By sampling a variety of forage types from different sites with distinct irrigation practices (groundwater, canal water, sewage water), we can assess potential variations in forage quality and contamination levels associated with different water sources used for irrigation. These forage types have been the subject of previous research and are commonly studied in the context of animal nutrition and agricultural practices. Sampling these forages allows for comparisons with existing literature and contributes to ongoing research efforts in the field. the selection of these forages was driven by the desire to capture a representative sample of the typical diet of animals in Sahiwal Tehsil, assess nutritional diversity, investigate potential contamination risks associated with irrigation practices, and contribute to broader research objectives in animal nutrition and agricultural sustainability.

Collection of samples

Site Selection

The selection of three distinct sites within the Sahiwal Tehsil, namely Jhamtawala, Sahiwal, and Vijh, was strategic in assessing the diverse irrigation practices and their potential impacts on various environmental components. Each site represented a different irrigation source, thereby providing a comprehensive understanding of the effects of ground water (GW), canal water (CW), and sewage water (SW) irrigation on the sampled elements. Site 1, designated as GW 1, represented an area reliant on groundwater irrigation, which is often characterized by varying mineral compositions and potential contaminants. Site 2, identified as CW 2, represented an area where canal water was the primary irrigation source, typically sourced from rivers or reservoirs, which may introduce different pollutants or sedimentation compared to groundwater. Lastly, Site 3, known as SW 3, represented an area where sewage water was utilized for irrigation, presenting unique challenges related to nutrient levels, microbial content, and potential chemical contaminants. By selecting these specific sites, the study aimed to capture the spectrum of irrigation practices commonly employed in the region and evaluate their potential implications on forages, soil, water, and animal blood. The inclusion of three duplicates for each sample from every site ensured robustness and reliability in the data collected, allowing for comprehensive analysis and comparison over the sampling period from November 2022 to May 2023. This strategic selection of sites and sampling approach provides a holistic understanding of the environmental dynamics within the Sahiwal Tehsil and facilitates informed decision-making for sustainable agricultural practices and environmental management.

Sampling of water

Samples of water were collected from three sites in Tehsil Sahiwal. Each site yielded 100 ml of water samples sourced from three types of irrigation: canal water (CW), groundwater (GW), and sewage water (SW). The samples were collected in polypropylene bottles pre-treated with a 1% nitric acid solution. They were then transported to laboratories and stored at 4°C until analysis. (Yang *et al*., 2020).

Sampling of soil

Approximately 1kg of soil samples were collected from the same locations where fodder samples were collected at three selected sites. The soil was gathered from a depth of 0-15 cm. A total of 54 soil samples were taken. Replicates of soil were taken carefully. After evaluating soil samples to assess their moisture material, they were dried in the air for a period of 24 hours to remove any extra moisture. Next, the soil samples were further dehydrated in a microwave to remove any remaining moisture. Finally, the soil samples were beaten in a pestle and mortar, with about 2g of each specimen remaining for the mixing approach after sieving all samples. For subsequent investigation, these soil samples have been stored in either plastic or paper bags (Tovihoudji, 2018).

Sampling of forages

The sampling process for forage collection involved meticulous procedures to ensure representative and reliable samples for analysis. Three areas within Sahiwal Tehsil, namely Jhamtawala, Sahiwal, and Vijh, were designated for sampling. These sites were chosen to represent different irrigation practices: groundwater (GW 1), canal water (CW 2), and sewage water (SW 3) irrigation. Forages were sampled from locations where buffaloes frequently browsed, ensuring the collection of plants typically consumed by animals. Six different forage types were targeted for sampling. Sugarcane, Brassica, Berseem, Alfalfa, Maize, Oat. For each type of forage, three duplicate samples were collected from each site, resulting in a total of 18 samples (6 forages × 3 duplicates).The size of each forage sample depended on the availability and abundance of the forage at the sampling locations. However, a standardized approach was followed to maintain consistency across samples. After collection, the forage specimens were placed in see-through paper baggies to prevent contamination and ensure visibility. The collected forage samples undergo thorough cleaning in distilled water to remove any external debris or contaminants. Subsequently, the cleaned forage samples were allowed to dry for 72 hours, both in ambient air and in a 75°C oven, to ensure complete dehydration. Once dried, the forage samples were weighed and processed into a fine powder to facilitate further analysis. A standardized 2g sample was extracted from each forage specimen for subsequent processing and examination. this sampling approach ensured the collection of representative forage samples from different sites and irrigation practices within Sahiwal Tehsil, facilitating comprehensive analysis of their nutritional content and potential contaminants (FEDERATION, 2022)

Selection of Experimental animals and blood sampling

The experimental design of the study involved a systematic sampling approach to assess the physiological responses of buffaloes to different fodder samples obtained from three selected sites within Sahiwal, Jhamtawala, and Vijh.. The buffaloes were categorized into five groups based on lactation and age, namely: calves, pregnant buffaloes, lactating buffaloes, non-lactating buffaloes, and dry

buffaloes. Selection criteria included age (5-6 years old) and body weight (550-600 kg). Five buffaloes were chosen for each physiological stage from each site, resulting in a total of 15 buffaloes per site. The selected buffaloes were then fed the given fodder samples under standard feeding and management conditions to ensure consistency across the experiment. A 15 ml blood sample was collected via venipuncture of the jugular vein. Once daily for thirty-two consecutive days during the winter months of November and February. Samples were collected using disposable 10cc syringes and transferred into heparinized tubes containing ADTA-K3 as an anticoagulant. Plasma was harvested within 30 minutes of collection. The samples were stored frozen at -20°C until analysis. This sampling design aimed to evaluate the physiological responses of buffaloes to the provided fodder samples, providing insights into their nutritional quality and potential effects on animal health and performance across different physiological stages and geographical locations (Sejian *et al*., 2012).

Samples Preparation

Water

A beaker containing 5 milliliters of tap water and a few drops of an acidic solution was combined with 2 milliliters of a hydrogen peroxide solution, and the mixture was heated until smoke began to emerge. This process was repeated until the liquid became readily apparent at the point when it was filtered using filter paper and put in a bottle (Srinivas *et al*., 2014)

Dilution and filtration

After digesting the samples, they were diluted with freshly made distilled water to reach a total volume of 50ml. Dilution was crucial to ensure that the concentration of analyses fell within the detectable range of the analytical instrumentation. filters were employed to purify the diluted samples, removing any particulate matter or impurities that could interfere with subsequent analysis.all samples were transferred into glass containers to prevent contamination and maintain sample integrity. For metal analysis, a suitable spectroscopic technique, such as atomic absorption spectrometry (AAS), was employed. Spectroscopic techniques have inherent detection limits, which dictate the minimum concentration of analyses that can be reliably detected and quantified. These limits must be considered during sample preparation and analysis to ensure accurate results.

Atomic absorption spectroscopy

The concentration of the heavy metal nickel (Ni) was determined using atomic absorption spectroscopy, utilizing instruments such as the Shimadzu double beam AA-6300 and the Perkin Elmer Analyst 400. To ensure quality assurance, natural matrix certified reference material (CRM-1570) was measured, and duplicates were analyzed for each batch of samples to confirm the reliability of the results. Careful handling of the samples was observed to prevent contamination, with all glassware meticulously washed and double distilled water employed throughout the evaluation process. An analysis was conducted to validate different procedures by homogenizing the tested samples with varying amounts of standard solutions.

Digestion of samples

Soil

5 kilogram of soil and 2 ml of sulfuric acid were introduced to the digesting chamber. Before digestion, the overall concentration of potentially dangerous substances in the soil was determined. The use of nitric acid (2.5ml), 30% peroxide of hydrogen (0.5ml), and the acid hydrochloric (7.5ml) were all utilized (Gad, 2012).

Forages

The forage (5g) was placed in to each crucible, which was subsequently weighted again. It underwent heating until ash created and then after dispersing it out, it maintained its temperature for 24 hours. Ash was scaled once again and dispersed into 2.5 ml of HCL after the filtration solution had been adjusted to 50 ml without purified water (Khan *et al*., 2018) [27].

Evaluation of metal profile by statistical analysis

Statistical analysis

The statistical analysis, including one-way ANOVA and correlation, maintained significance levels at 0.001, 0.01, and 0.05, as outlined by Steel and Torrie (1980). SPSS software (version 20) was employed for this analysis. One-way ANOVA was specifically used to explore seasonal fluctuations in mean metal concentrations among soil, forages, and blood serum samples from the five buffalo categories. This statistical approach facilitated an examination of potential variations and relationships within the dataset.

Graphs were created using SPSS or other visualization tools such as graphpad prism 8, Common types of graphs used to represent ANOVA results. Overall, the use of graphs enhances the interpretation of ANOVA results by providing a visual representation of the observed patterns and trends in the data.

Contamination Factor (CF)

The degree of metal contamination can be determined by calculating the contamination factor (CF). A ratio, or CF, is defined as the average metal value divided by the metal concentration in the organic matter. It is incredibly useful for assessing the pollution over time and is computed as follows in equation no.1

$$\mathrm{CF} = \frac{c\ heavy\ metal}{c\ background} \quad (1)$$

According to (Sivakumar *et al*., 2016) CF 1 exhibits little contaminants. Significant saturation is 1 CF 3. There is a lot of contaminants with 3CF6.

Transfer Factor (TF)

The Transfer Factor (TF) measures the quantity of a heavy metal that enters the plant from the soil it grows on. (Yan *et al*., 2021) as given below the equation no.2

$$\mathrm{TF} = \frac{c\ plant}{c\ soil} \quad (2)$$

C represents the amount of the heavy metal present in the plant, which is equivalent to the concentration of that heavy

metal in the soil. "Metal hyper accumulators" are plants with very high metal absorption rates. (Gall *et al*., 2015) [16].

Enrichment factor (EF)

The description of the Enrichment Factor Formula described by (Buat-Menard & Chesselet, 1979) [8] as given in equation 3.

$$EF = \frac{metal\ concentration\ in\ crop/\ concetration\ in\ soil\ sample}{\text{metal concentration in crop/metal concentration in soil sample}} \quad (3)$$

Estimated Daily Intake (EDI)

Using the estimated daily intake (EDI), target hazard quotient (THQ), estimated cancer risk (ECR), and hazard index (HI), the potential health effects of the metals were computed and examined. The EDI is expressed in ($mgkg^{-1}$/day) using equation 4.

$$EDI = (C \times DI \times CF)/BW \quad (4)$$

Where (C) represents the metal concentration in mg/kg, (DI) denotes the daily intake, (BW) stands for the reference body weight, and (CF) signifies the conversion factor.

The suggested provisional tolerable daily intake levels (PTDI) established by JEFCA were compared to the EDI values. This was done to determine whether or not the suggested daily levels had been surpassed. (Amarh *et al*., n.d.)

Hazard Quotient (HQ)

Using hazard quotient, considered one could assess the detection level danger associated with ingesting contaminated food according to (Sharma *et al*., 2016) [40] by equation 5.

$$HQ = \frac{(D)\times(\text{Cmetal})}{(RfD)\times BO} \quad (5)$$

Where:

(D) Represents the daily intake of food (kg/day),

(C) Signifies the concentration of the metal (mg/kg), and

(RfD) denotes the reference oral dose of the metal (mg/kg of body weight/day).

Results and Discussion

Ni concentration in collected water, soil, crops and blood samples:

Analysis of variance for data indicated that non-significant effect (ns) was showed on Ni concentration by the Treatment and treatment*water and significant effect (p > 0.001) was shown on Ni concentration by the Water (Table 3).

An analysis of variance for the data revealed that the treatment, the soil, and the treatment all had a significant impact (p<0.001) on Ni concentration. *Soil (Table 3).

Analysis of variance for data indicated that non-significant effect (ns) was showed on Ni concentration by the Treatment, Crops and treatment*Crops (Table 3)

Analysis of variance for data indicated that significant effect (p<0.05) was showed on Ni concentration by the Treatment and significant effect (p > 0.01) of Ni concentration was shown on Blood and significant effect (p > 0.001) of Ni concentration was shown on treatment*Blood (Table 3).

Table 3: Analysis of variance of data for Ni in collected water, soil, crop, blood samples

Water			Soil		
Source of variance (S.O.V)	**Degree of Freedom (DF)**	**Adj mean square**	**Source of variance (S.O.V)**	**Degree of Freedom (DF)**	**Adj.mean square**
Treatment	2	0.07240^{ns}	Treatment	2	220.111***
Water	2	0.66067***	Soil	5	4.948***
treatment*water	4	0.03409^{ns}	treatment*Soil	10	2.963***
Error	18	0.02661	Error	36	0.000
Total	25		Total	53	
Crop			Blood		
Sum of Variance (S.O.V)	**Degree of Freedom (DF)**	**Adj.mean square**	**Sum of Variance (S.O.V)**	**Degree of Freedom (DF)**	**Adj.mean square**
Treatment	2	305.5^{ns}	Treatment	2	2167.1*
Crops	5	604.3^{ns}	Blood	5	2009.5**
treatment*crops	10	629.8^{ns}	treatment*Blood	10	2079.2***
Error	36	627.9	Error	36	515.1
Total	53		Total	53	

*, **, *** = Significant at 0.05, 0.01, and 0.001 levels. "ns" for non-significant.

Ni proportion in water, soil, crops and blood

The data analyzed for heavy metal analysis of water was described in (Table 4, Fig.2)

The mean concentration of Ni in water at all sites were ranged from 0.073 to 0.76 mgL^{-1}. At SITE I, minimum value (0.073 mgL^{-1}) of Ni was observed at location 1 and maximum value (0.76 mgL^{-1}) of Ni was found at location 3. At SITE II, minimum value (0.12 mgL^{-1}) of Ni was observed at location 1 and maximum value (0.56 mgL^{-1}) of Ni was found at location 3. At SITE III, minimum value (0.17 mgL^{-1}) of Ni was observed at location 1 and maximum value (0.68 mgL^{-1}) of Ni was found at location 2. (Table 4, Fig.2 (A).

The mean concentration of Ni in soil at all sites were ranged from 10.45 to 20.57 $mgkg^{-1}$. At SITE I, minimum concentration (10.45 $mgkg^{-1}$) of Ni was observed in *S. officinarum* and maximum value (12.68 $mgkg^{-1}$) of Ni was found in *Z. mays*. At SITE II, minimum concentration (13.45 $mgkg^{-1}$) of Ni was observed in *S. officinarum* and maximum value (15.72 $mgkg^{-1}$) of Ni was found in *T. alexandrinum*. At SITE III, minimum value (16.65 $mgkg^{-1}$) of Ni was observed in *B. campestris* and maximum concentration (20.57 $mgkg^{-1}$) of Ni was found in *Z. mays.* (Table 4, Fig. 2(B)

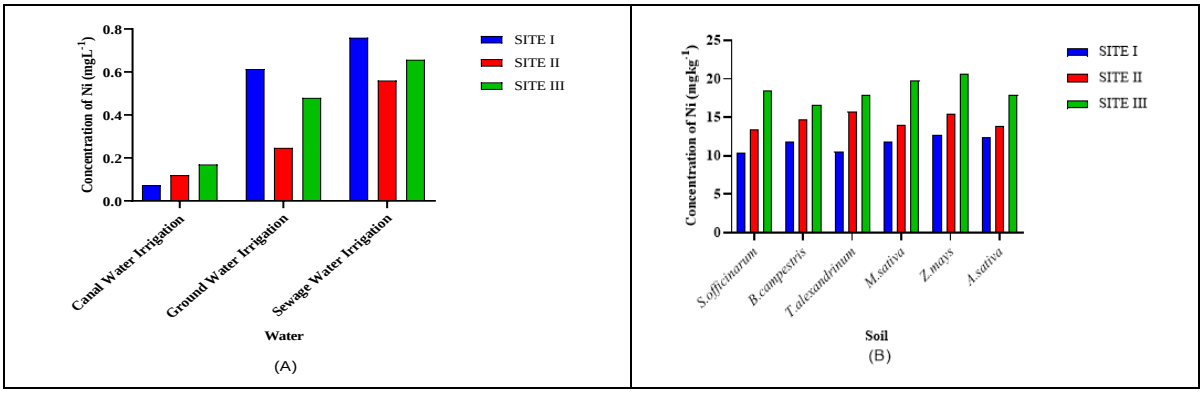
The mean concentration of crops at all sites were ranged from 1.23 to 11.83 $mgkg^{-1}$. At SITE I, minimum concentration (1.23 mgkg-1^{-1}) of Ni was observed in *B. campestris* and maximum value (3.27 $mgkg^{-1}$) of Ni was found in *Z. mays*. At SITE II, minimum concentration (4.93 $mgkg^{-1}$) of Ni was observed in *M. sativa* and maximum value (3.16 $mgkg^{-1}$) of Ni was found in *A. sativa*. At SITE III, minimum value (8.34 $mgkg^{-1}$) of Ni was observed in *B. campestris* and maximum

concentration (11.83 mgkg^{-1}) of Ni was found in *Z. mays.* (Table 4, Fig.2 (C).

The mean concentration of Ni in blood samples at three sites were ranged from 0.43 to 2.89 mgL^{-1}. At SITE I, minimum value of Ni (0.43 mgL^{-1}) was observed in Buffaloes non-lactating and maximum value (2.89 mgL^{-1}) of Ni was found in Buffaloes dry. At SITE II, minimum value of Ni (0.96 mgL^{-1}) was observed in two specimens Buffaloes calf and buffaloes lactating and maximum value (1.97 mgL^{-1}) of Ni was found in Buffaloes non-lactating. At SITE III, minimum value of Ni (0.56 mgL^{-1}) was observed in Buffaloes lactating and maximum value (2.78 mgL^{-1}) of Ni was found in Buffaloes non-lactating. (table 4, Fig.2 (D).

Table 4: Concentration of Ni in collected water, soil, crops and blood samples (Mean ± S.E)

Water			
Location	**SITE I**	**SITE II**	**SITE III**
Location 1	0.073±0.037	0.12±0.027	0.17±0.027
Location 2	0.61±0.027	0.24±0.028	0.68±0.017
Location 3	0.76±0.045	0.56±0.027	0.65±0.038
Soil			
Crops	**SITE I**	**SITE II**	**SITE III**
S. officinarum	10.45±0.618	13.45±0.670	18.44±0.515
B. campestris	11.77±0.677	14.66±0.277	16.65±0.581
T. alexandrinum	10.56±0.677	15.72±0.281	17.97±0.577
M. sativa	11.76±0.481	13.94±0.281	19.77±0.515
Z. mays	12.68±0.377	15.39±0.381	20.57±0.577
A. sativa	12.34±0.381	13.89±0.481	17.94±0.581
Crops			
Crops	**SITE I**	**SITE II**	**SITE III**
S. officinarum	1.86±0.277	3.84±0.252	9.57±0.37
B. campestris	1.23±0.377	4.93±0.477	8.34±0.37
T. alexandrium	1.63±0.277	3.68±0.477	8.72±0.37
M. sativa	3.23±0.377	3.16±0.477	9.46±0.37
Z. mays	3.27±0.277	4.13±0.481	11.83±0.3
A. sativa	2.57±0.277	6.80±0.481	10.71±0.3
Blood			
Buffaloes	**SITE I**	**SITE II**	**SITE III**
Buffaloes calf	0.77±0.099	0.96±0.057	1.69±0.088
Buffaloes lactating	1.67±0.097	0.96±0.088	0.56±0.088
Buffaloes non lactating	0.43±0.099	1.97±0.088	2.78±0.088
Buffaloes pregnant	1.39±0.088	1.45±0.088	2.33±0.098
Buffaloes dry	2.89±0.088	1.56±0.088	1.27±0.088



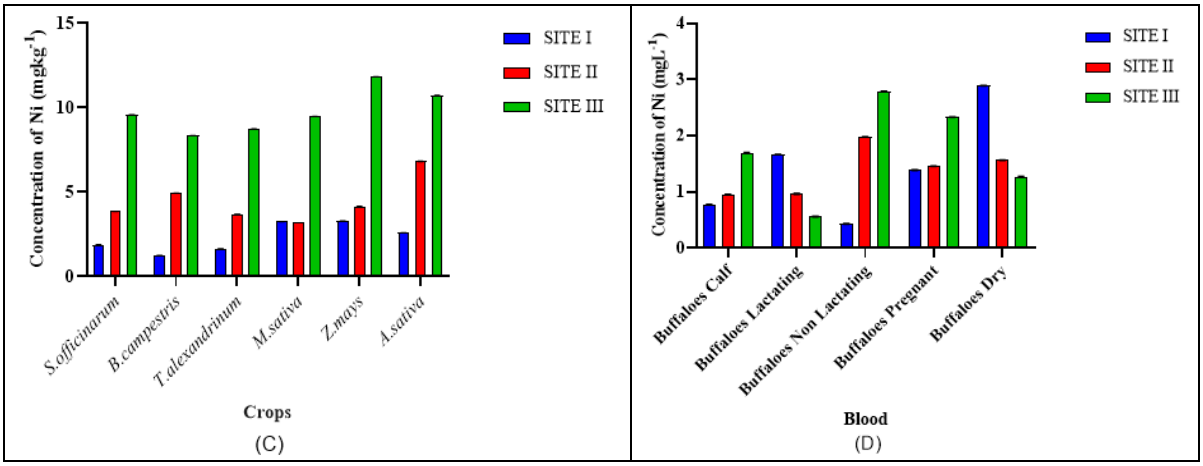


Fig 2

Fluctuation in level of Ni concentration (mgkg^{-1}) in water (A), Soil (B), Crops (C), Blood (D) samples collected from different Sites.

Contamination factor for Ni

Contamination factor for Ni summarized in below the (table 5.Fig. 3)

The contamination factor (CF) of the Ni at all sites were ranged from 1.1537 to 2.2704. The order of contamination factor of Ni in soil of forage crops at SITE I, was S. officinarum > *T. alexandrinum* > *M. sativa* > *B. campestris* > *A. sativa* > *Z. mays.* At SITE II, least concentration was found in S. officinarum and Maximum CF was observed in *T. alexandrinum.* at SITE III, minimum CF was observed in *B. campestris* and maximum CF was observed in *Z. may.* (table 5, Fig.3 (A).

Transfer factor for Ni

The transfer factor for Ni ranged from 0.1045 to 0.5967 in forages. The highest proportion of TF was observed at SITE III, with a value of 0.5967 in *A. sativa*, while the lowest content of Transfer Factor was observed at SITE I, with a value of 0.1045 in *B. campestris* (see Table 5, Fig.3(B).

Enrichment factor for Ni

The values of enrichment factor were fluctuated from 0.0014 to 1.7798 at different sites. The peak value of Ni was found in *S. officinarum* (1.7798) at SITE I and lowest was detected in *B. campestris* (0.0014) at SITE I. At SITE I, the order of enrichment factor in forages was observed as *B. campestris* > *T. alexandrinum* > *A. sativa* > *Z. mays* > *M. sativa* > *S. officinarum*. At SITE II, the order of enrichment factor was observed as *M. sativa* > *T. alexandrinum* > *Z. mays* > *B. campestris* > *A. sativa* > *S. officinarum*. At SITE III, the order of enrichment factor was seen as *M. sativa* > *T. alexandrinum* > *B. campestris* > *S. officinarum* > *Z. mays* > *A. sativa* (refer to Table 5, Fig. 3 (C).

Estimated daily intake of Ni

The highest EDI value was detected in Z. mays (0.0201) at SITE III, while the lowest was observed in *B. campestris* (0.0010) at SITE I. At SITE I, the order of EDI was *B. campestris* > *T. alexandrinum* > *S. officinarum* > *A. sativa* > *M. sativa* > *Z. mays.* At SITE II, the order of EDI was *M. sativa* > *T. alexandrinum* > *S. officinarum* > *Z. mays* > *B. campestris* > *A. sativa*. At SITE III, the order of EDI was *B. campestris* > *T. alexandrinum* > M. sativa > *S. officinarum* > *A. sativa* > *Z. mays* (refer to Table 5,Fig.3 (D).

Hazard quotient for Ni

The value of Ni in HQ was fluctuated between 0.1045 to 1.0055 at SITE I in *B. campestris* and at SITE III in *Z. mays.* At SITE I, maximum in *Z. mays (*0.2779) and minimum value of Ni in *B. campestris (*0.1045). At SITE II, the highest value of Ni was observed in *A. sativa* (0.5785), while the minimum value of Ni was observed in *M. sativa* (0.2686). At SITE III, highest value of Ni was detected in *Z. mays (*1.0055) while lowest value of Ni was seen in *B. campestris (*0.7089). (table 5,Fig.3 (E).

Table 5: Heavy metal pollution indices of nickel for soil and Crops

CF				EF			
Crops	**SITE I**	**SITE II**	**SITE III**	**Crops**	**SITE I**	**SITE II**	**SITE III**
S. officinarum	1.1537	1.4852	2.0353	*S. officinarum*	1.7798	1.1418	0.0074
B. campestris	1.2991	1.6181	1.8384	*B. campestris*	0.0014	0.0048	0.0071
T. alexandrinum	1.1655	1.7354	1.9834	*T. alexandrinum*	0.0022	0.0033	0.0069
M. sativa	1.2983	1.5393	2.1821	*M. sativa*	0.0039	0.0032	0.0068
Z. mays	1.3995	1.6990	2.2704	*Z. mays*	0.0036	0.0038	0.0082
A. sativa	1.3627	1.5334	1.9808	*A. sativa*	0.0029	0.0070	0.0085
TF				EDI			
Crops	**SITE I**	**SITE II**	**SITE III**	**Crops**	**SITE I**	**SITE II**	**SITE III**
S. officinarum	0.1242	0.7969	0.5189	*S. officinarum*	0.0031	0.0065	0.0162
B. campestris	0.1045	0.3362	0.5007	*B. campestris*	0.0020	0.0083	0.0141
T. alexandrinum	0.1543	0.2340	0.4852	*T. alexandrinum*	0.0027	0.0062	0.0148
M. sativa	0.2745	0.2265	0.4785	*M. sativa*	0.0054	0.0053	0.0160
Z. mays	0.2578	0.2687	0.5751	*Z. mays*	0.0055	0.0070	0.0201
A. sativa	0.2081	0.4899	0.5967	*A. sativa*	0.0043	0.0115	0.0182

HQ			
Crops	**SITE I**	**SITE II**	**SITE III**
S. officinarum	0.1581	0.3264	0.8134
B. campestris	0.1045	0.4190	0.7089
T. alexandrinum	0.1385	0.3128	0.7412
M. sativa	0.2745	0.2686	0.8041
Z. mays	0.2779	0.3516	1.0055
A. sativa	0.2184	0.5785	0.9103

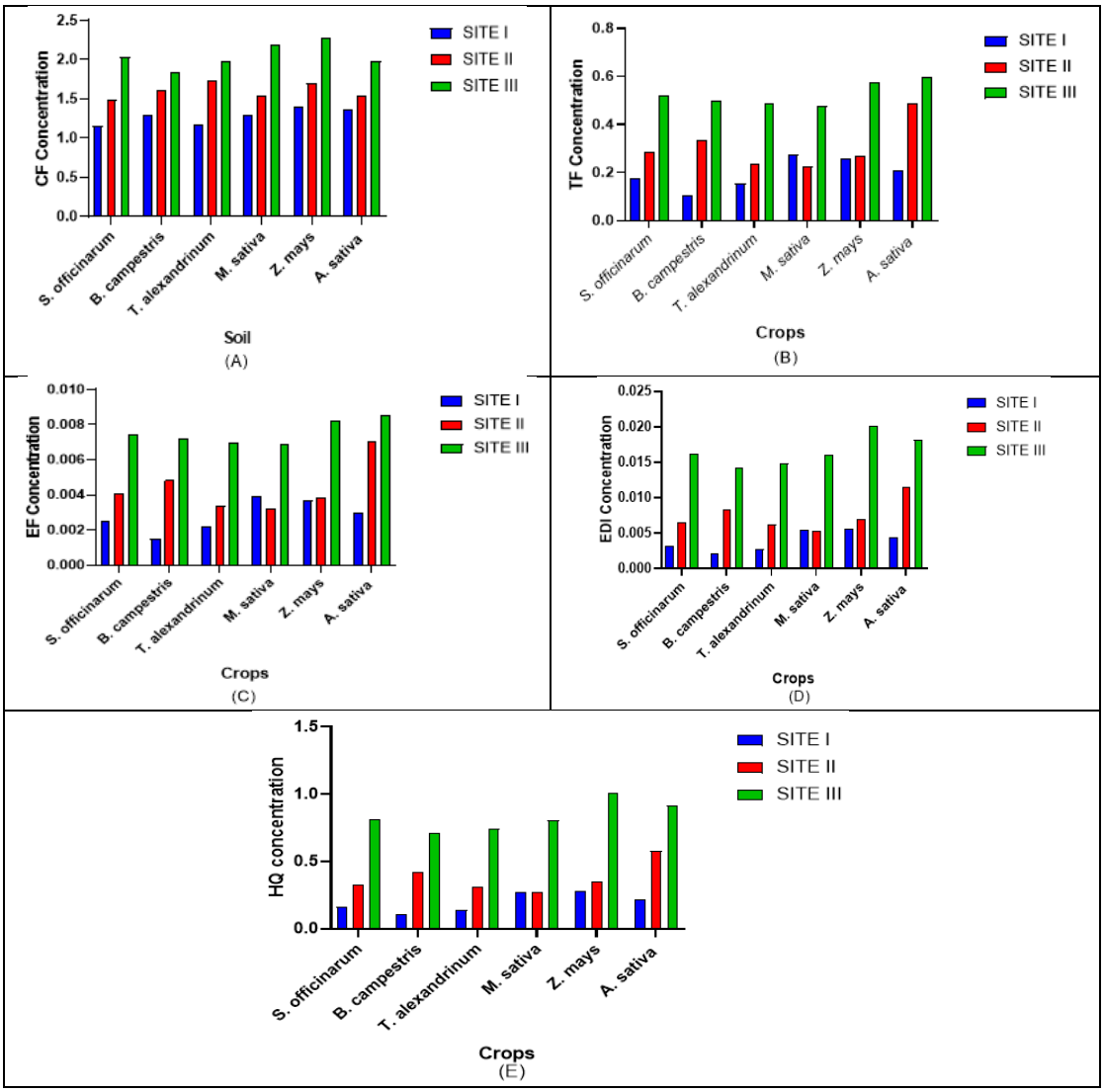


Fig.3

Alteration in the value of Contamination Factor (A), Transfer factor (B), Enrichment factor (C), Estimated daily intake (D) and Hazard quotient (E) for Ni at selected sites

Correlation of Nickel concentration in water soil forage and blood

The study utilized Pearson correlation matrices with correlation coefficients (r) to analyze the relationship between metal uptake in specimens. Negative correlations suggest that recent intake of certain metals may influence the absorption of others by the feed, while strong positive connections between metal properties may stem from common manufacturing or biological as well as chemical similarities. A non-significant negative correlation indicates a metal imbalance between variables, whereas a non-significant positive correlation is attributed to soil factors. At SITE I, a negative and non-significant correlation (ns) was observed in water-soil, soil-crop, and crop-blood relationships. At SITE II, a significant ($p > 0.01$) and positive correlation between water-soil was noted, while a negative and non-significant correlation was observed in other relationships as shown in the table below (Table 6, Fig. 4,5,6).

Table 6: Correlation of Ni between water-soil, Soil-forage, Forage-Blood

SITES	Water- Soil	Soil-Crop	Crop-Blood
SITE I	-0.211 ^{ns}	-0.316 ^{ns}	-0.158 ^{ns}
SITE II	0.745 **	-0.208 ^{ns}	0.120 ^{ns}
SITE III	-0.034 ^{ns}	-0.439 ^{ns}	-0.060 ^{ns}

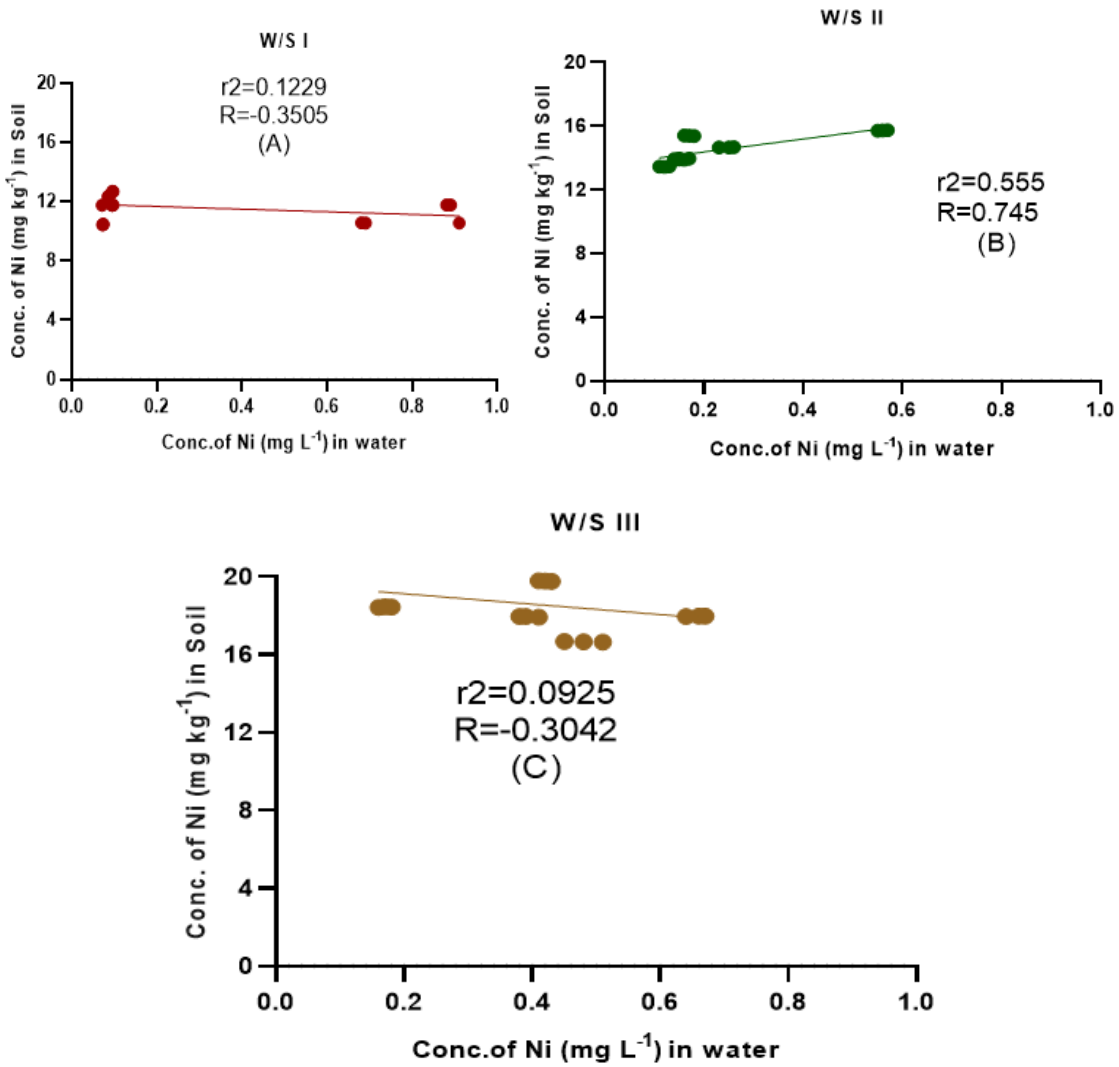
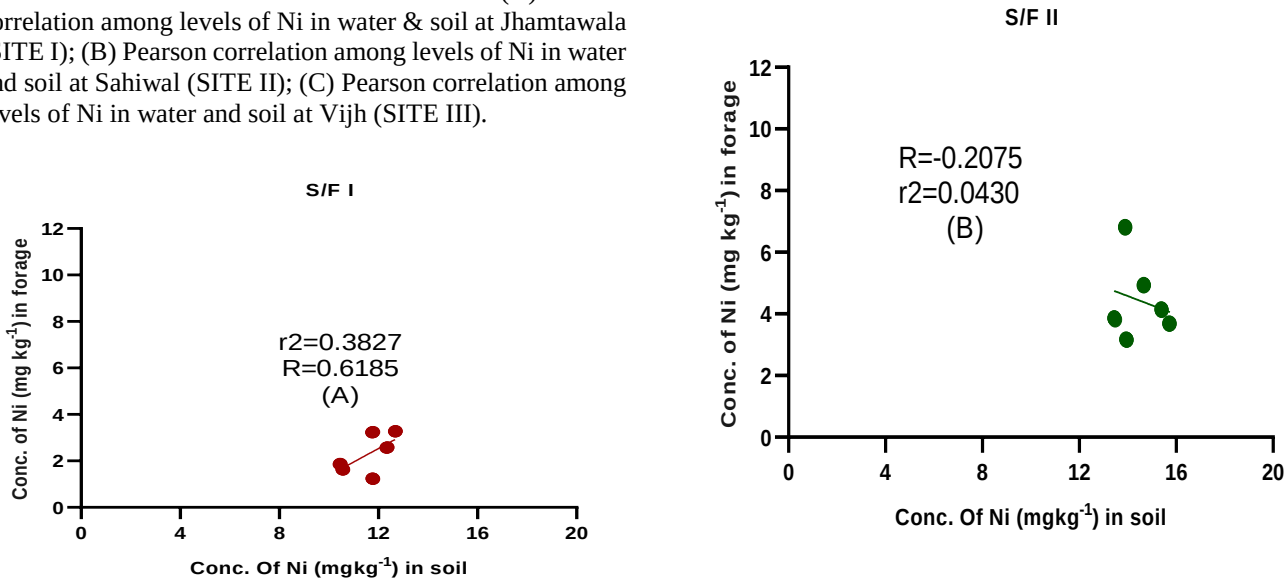


Fig. 4

Pearson correlation of Ni between water and soil: (A) Pearson correlation among levels of Ni in water & soil at Jhamtawala (SITE I); (B) Pearson correlation among levels of Ni in water and soil at Sahiwal (SITE II); (C) Pearson correlation among levels of Ni in water and soil at Vijh (SITE III).



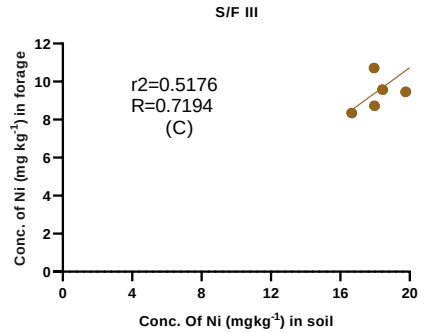


Fig. 5

Pearson correlation of Ni between soil and forage: (A) Pearson correlation among levels of Ni in soil & forage at Jhamtawala (SITE I); (B) Pearson correlation among levels of Ni in soil & forage at Sahiwal (SITE II); (C) Pearson correlation among levels of Ni in soil & forage at Vijh (SITE III).

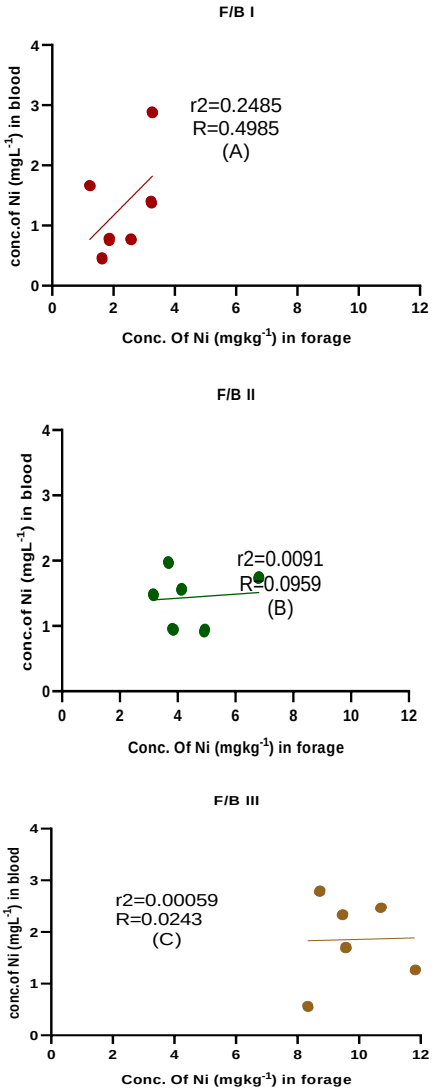


Fig. 6

Pearson correlation of Ni between forage and blood: (A) Pearson correlation among levels of Ni in forage & blood at Jhamtawala (SITE I); (B) Pearson correlation among levels of Ni in forage & blood at Sahiwal (SITE II); (C) Pearson correlation among levels of Ni in forage & blood at Vijh (SITE III).

Discussion

Nickel is essential for the healthy growth and development of plants as well as for a variety of morphological and physiological processes, including productivity and seed germination. On the other hand, plants' metabolic processes are influenced by high concentrations of nickel, which suppresses enzyme activity, photosynthetic electron transport, and chlorophyll production (Genchi *et al*., 2020) [17]. Our current observation of Ni in water was varied between 0.07 and 0.76 mgL^{-1}, which is greater than the values given by Christoforidis and Stamatis, (2009) [10] and those analyzed by Ayalew *et al*. (2020) [5] which are 0.003 mgL^{-1} and 0.0009. as reported by Andresen *et al*. (2008) [4] the concentration measured is less than 1.34 mgL^{-1} .According to Muhmood *et al*. (2015) [35] the present value of Ni, which is 0.27 mgL^{-1}, lies between the range of values of 0.073 to 0.76 mgL^{-1}.

At all sites, overall Ni content in the soil ranged from 10.45 to 20.57 $mgkg^{-1}$. Wasike *et al*. (2019) [51] noticed reduced Ni content in soil, which were 2.6 $mgkg^{-1}$, in irrigation-irrigated soil. Our previous concentration was greater than the 21.0 $mgkg^{-1}$. reported by Jiang *et al*. (2017) [24] but significantly higher than the 0.009 $mgkg^{-1}$reported by Christoforidis and Stamatis, (2009) [10]. Our findings vary more than the Critical Level, 2.87 $mgkg^{-1}$as published by He *et al*. (2019) reported previous concentration of nickel, 0.02 $mgkg^{-1}$ which was lower than the present measured amount of nickel in soil and was triggered by a high intake of objects containing heavy metals.

The forages' current concentrations at all sites ranged from 1.23 to 11.83 $mgkg^{-1}$.Ni concentration in forages was found to be 0.32 $mgkg^{-1}$which was greater than the amount reported in a study by Hasan *et al*. (2023) [20]. The Ni content in forages irrigated with wastewater reported by Usman *et al*. (2018) [49] ranged from 1.02 $mgkg^{-1}$ to 10.17 $mgkg^{-1}$of dry weight, which is lower than the Ni levels previously recorded. The research we conducted found that the nickel levels in various forages were higher than those reported by Ugulu *et al*. (2019) [26]. Our result was lower than that of Tahir *et al*. (2017) [44] who examined the levels of nickel (Ni) in forages growing at a site that was irrigated with hudiara drain water. They used a critical limit of Ni of 5.00 $mgkg^{-1}$(Lazzarini *et al*., 2009) [28] in order to divide the data into safe and risky forages. According to Li *et al*. (2019) Ni concentration in forages fluctuated between 3.24 to 39.25 $mgkg^{-1}$ at polluted and unpolluted sites, which is higher than the current study Meniman *et al*. (2009) [33] and demonstrates that the site was not significantly metal-polluted. The critical limit of nickel in forages, which has been estimated at 5 $mgkg^{-1}$ was exceeded. Ni concentrations ranged from 0.43 to 2.89 mgL^{-1} in blood samples taken from three separate locations. The concentration of Ni in blood specimens reported by Ali *et al*. (2020) ranged from 0.21 to 0.28 mgL^{-1} which was lower than in our investigation. Our reported figure exceeds the recommended limit for Ni in animal blood, which is 0.4 mgL^{-1} Council (1996) [11]. A metal presence indicator In comparison to Hussain *et al*. (2021) [22] study from two years ago, our investigation found a greater concentration of Ni in plasma (0.0007 mgL^{-1}).

The contamination factor (CF) for Ni at several sites ranged from 1.1537 to 2.2704. At SITE III, *Z. mays* had the highest

CF and S. officinarum had the lowest CF at SITE I. A contamination factor was use to assess the degree of soil contamination. In contrast to the current findings, Benhaddya & Hadjel, (2014) [7] observed higher values of the contamination factor (5.45). The values of CF found in the current research were less than those found by Ita & Anwana, (2017) [23] which recorded values of 3.95. Heavy metal poisoning of soil such as Nickel poisoning has been recognized as a serious environmental and human health problem due to their non-biodegradability and susceptibility to accumulate in plant and animal tissues (Shaheen *et al*., 2020) [39].

The Ni transfer factor (TF) in forages distinct between sites and varied from 0.1045 to 0.5967 in forages. On the location I and location III, the least and maximum TF, respectively, were visible. The "transfer factor" refers to how permeable an ingredient is to plants. Shi *et al*. (2020) [41] had a lower value of 0.7510 than the most recent finding. In comparison with the current research Salama (2018) [37] showed a lower concentration of transfer factor for Ni (0.0036) Than present research. Caunii *et al*. (2015) [9] suggested the Greater transfer factor values (3.67) were attained in comparison to the findings of this investigation.

Analyses for Ni revealed that the enrichment factor (EF) varied among different sites, ranging from 0.0014 to 1.7798. The highest concentration of Ni was detected in *S. officinarum* (1.7798) at SITE I, while the lowest proportion was observed in *B. campestris* (0.0014). Nickel E.F didn't show any noticeable differences from the season. According to the current study findings, the Ni efficiency had been greater than the 2.68 by Kamani *et al*. (2017) [25]. According to Dimitrijević *et al*. (2016) [12] standards, Ni E.F. is regarded as having a higher content of 4.39. Ghrefat *et al*. (2011) [18] which is less than the present value of our investigations, account for an EF in close vicinity to 0.003.

The EDI values for Nickel were found to range from 0.0010 to 0.0201 in various places. Türkmen *et al*. (2010) [47] published the EDI value of Nickel, which is 0.75–0.96 higher than existing values. This demonstrates that the environment's creatures are ingesting or absorbing a specific substance from that plant species in significantly higher amounts. If the EDI exceeds standard safety requirements, the organisms exposed to this substance may be at hazards for health issues Adeel *et al*. (2017) [1]. The value of EDI in forage crops 0.011 was shown by Makedonski *et al*. (2017) [31], which is within our current values. According to Salama (2018) [37] a less significant figure of 0.0023 than the current value which was reported in our results.

The HQ for Ni ranged from 0.1045 to 1.0055 at SITE I in *B. campestris* and SITE III in *Z. mays*. according to Dinake *et al*. (2023) [13] HQ is 1.95-4.52 This concentration exceeded the value that we currently possess. The HQ for Ni is within our acceptable limit of 0.53-0.55, according to a study Goel *et al*. (2011) [19] reported this value is within the lowest value when compared to our most recent exploration. Maigari *et al*. (2016) [30], state that HQ assesses any possible risks associated with being around heavy metals that are not cytotoxic.

Conclusion

Heavy metals are abundant in waste water, which is either directly or indirectly added to the soil where fodder crops are grown. This raises the concentration of metals in crops over the amount that is permitted. Animals feed on these polluted fodder crops, and a high quantity of metal builds up in their blood, which is carcinogenic to people. The outcomes of the analysis show that the amounts of nickel in crops and soil are within safe limitations, and that all nickel indices, such as CF, TF, EF, EDI, and HQ, are less than 1. But ongoing sewage and irrigation with canal water that contains a lot of Ni might further pollute the soil and crop cultivars in the tehsil Sahiwal region, endangering the health of both people and plants. Therefore, in regions where wastewater irrigation is extensively watered, measures like bioremediation to reduce heavy contaminants in soil and water regimes may be advantageous.

Ethical Approval for Research

The sampling protocols were approved by the Institutional Animal Ethics Committee, University of Sargodha (Approval No.25-A18 IEC UOS). All the experiments performed on animal complied with the rules of the National Research Council. In this study involving human participants, informed written consent to take part in the research have been obtained prior to the commencement of the study. The samples were taken from local farms by the consent of their owners by taking them into full confidence regarding the security of their animals as neglected nails and hooves can lead to discomfort or infection and it's a usual procedure as a part of animal care in farms. Keeping in view all Ethical aspects of Research whole work was done. In this Research no animal was sacrificed and only Blood was used. I'm addition, other animal parts such as hairs hooves etc are often used for analytical. purpose for experimentation.

Approval of whole work was taken from the Ethical Committee of the University. Director ORIC has constituted an independent Research Ethics and Support Committee (hereinafter referred to as RESC) of the University to ensure compliance with ethical standards, legal aspects and professional standards in research process undertaken at University of Sargodha. At present, RESC comprises up to sixteen (16) members and is headed by Director, ORIC. RESC was formed by the recommendations of Director ORIC which included the Chairman, 05 Deans and 03 Directors and the same was approved by the worthy Vice Chancellor.

Consent for publication

All subjects, gave their consent for the publication of details within the text ("Material") to be published in the above Journal and Article.

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Competing Interests

The authors declare that they have no competing interests

Availability of data and materials

All data generated or analyzed during this study are included in this research article

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