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Investigation of Nickel in soil, forages and blood plasma of buffaloes with respect to seasonal variations

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ABSTRACT

Heavy metal accumulation in forage poses a threat of toxic effects on the cattle. This study includes collection of forage, soil and buffalo blood samples to analyze heavy metals in them. Forages, soil and buffalo blood were collected from six sites of sampling. Out of which 5 sites were on the roadside and one site was away from the road. Grinding of the collected samples of soil and forage was followed by their acid digestion. Buffalo blood samples were centrifuged and the blood serum was separated which was then digested using acids. The atomic absorption spectrophotometer was then used to analyze the heavy metals in the digested samples. The results were analyzed statistically. Two-way ANOVA was used to find out variance and correlation in all samples. The nickel concentration found in the samples of soil, forage and blood was highest in the samples collected from site II (Chak 50 SB). The bio concentration factor found for nickel was highest in the samples of site II. On the other hand, the bio concentration factor for blood and forage was highest in the samples collected form site IV (Shaheenabad Road). The correlation result showed that Ni was positively significant when the soil and forage was correlated unlike the result of correlation for blood and forage which was negatively significant. The daily intake of metals and health risk index was found highest in the samples collected from site IV. The pollution load index was found highest in the samples collected from site V (Bhalwal Road).

Keywords: Nickel, Sites, Atomic absorption Spectrophotometer, Variance

INTRODUCTION

Various kinds of pesticides, polycyclic aromatic hydrocarbons, heavy metals, inorganic fertilizers and other such pollutants contaminate the environment (Gong *et al*., 2004)These pollutants are emitted from various sources like Agriculture, commercial and vehicular emission, industries etc. (Srivastava, 2001) . Among the worst sources of the pollution are the anthropogenic activities like fuel combustion, construction, traffic and waste disposal etc. Although that industrial areas are moved away from the residential areas but the road side traffic and railway terminals still remains the sources of pollution. Heavy metals like Zn, Fe and Ni are also

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released from the components of pipe, alloy, tyre in the motor vehicles. These heavy metals enter into the soil and get accumulated. Not only the soil fauna and flora are affected by the elevated concentrations of the heavy metals but they also affect the forages, vegetables and fruits etc. These heavy metals leach down to the underground water through run off. The heavy metals released into environment by vehicles are dispersed by rainfall or by wind and thus they enter into the soil ecosystem. Thus in order to check the pollution caused by vehicles, the chemical and biological analysis of the soil samples may be performed in order to estimate the extent of pollution.

Among the heavy metals, nickel as component of important enzymes urease was discovered in 1975 (Taksihima *et al*., 1998). The nickel is an important part of the urease enzyme required for nitrogen metabolism and it is also an essential nutrient for the growth of plants. But the nickel is phytotoxic in large amount. The nickel toxicity appears as growth inhibition, disturb sugar transport, necrosis, chlorosis, wilting and affect seed germination. The pollution due to Nickel per year is almost 150,000 and 180,000 metrics tons. The sources of nickel entrance in the environment are mostly roadside traffic emissions, industrial production and fossil fuel consumption etc. The disturbed nitrogen metabolism affects the growth and reproduction in plants etc. The concentration of nickel in polluted soil (26000 ppm) is about 20-30 times greater than the nickel permissible limit. Nickel is also found to disturb the uptake of phosphorus by plants.

With the increase in the population day by day, urbanization and industrialization had become a serious issue. Organisms have to eat polluted feeds and they are helpless to live in a highly contaminated environment. The animal feeding areas close to the roads are mainly subjected to the heavy metal contamination. Heavy metals after getting entry into food chain are found responsible for various disorders (Makridis & Daras, 2012). The need of hour is that the heavy metals concentration of the road side forages, soil and the blood of animals like buffalo rearing on these forages should be analyzed. This study aims at determining of Ni concentrations in the automobile contaminated soil, forage and blood of buffalo so that some precautionary measure could be adopted.

MATERIALS AND METHODS

Study area

The area selected for study was Sargodha, which is the 11th most populated metropolitan city in Pakistan, is located between 32°3" E and 32°7"E 72°38" and between N and 72°43".This city of Pakistan has hot and cold climate. Flat surface of Sargodha has an altitude of 190m (Topo Contour, 2015).The Sargodha city has an area of about 52km^2 and is having population of 0.7 million. Major parts of a country are connected with Sargodha by road and rail**.**

This study was done on roadside contaminated forages, soil and buffalo blood of various areas of Sargodha city. The six different roadsides of Sargodha were selected to collect the samples of forages, soil and buffalo blood serum. The sampling for this study was done in two seasons' winter and summer in December 2016, January 2016 and May 2016,June 2016, respectively.

COLLECTION OF SAMPLES

Forage and soil samples

The roadsides selected for sampling were 50 Chak, Shaheenabad, Bhalwal, Faisalabad, and Mateela. The site away from the road was Dera Saudi. 120 samples of forage and soil samples were collected in winter and summer. The two g samples of each forage and soil were kept in plastic bags after their air and oven drying(at 70-75^{0}C for 7 days).

Blood samples

The samples of the Buffalo blood were collected by sterilized needles from the vein (jugular) of Buffalo. The blood was protected from clotting by placing them in heparinized Na-citrate vials. The blood serum samples were centrifuged for 15-30min at 3000rpm.After centrifugation the samples of serum were placed in labeled vials in a freezer at 20^{0}C.

Forage

125 ml conical flask was taken in to which about 0.5g of the sample of forage was added. Along with this 25ml of concentrated (55%), 5 ml of concentrated (72%) hydrochloric acid (HCl), 5 ml of concentration (98%) sulphuric acid (H_2SO_4) were also added to this flask in order to digest the sample. This whole solution was heated till the appearance of white fumes.

Soil

The soil samples were digested by utilizing 1g of the soil samples adding 1ml of per chloric acid and 5ml of concentrated trioxonitrate acid. This mixture was added to the digestion flask, This mixture was heated upto 80-90 ^{0}C until the white fumes started to appear(Allen *et al*., 1976).The above solution was cooled and diluted upto 50ml.

Blood

Blood samples were digested using concentrated nitric acid and per chloric acid. The 0.5 g sample of blood serum was digested by using 10ml of nitric acid and 5ml of per chloric acid in a digestion flask. Heating of this solution resulted in a clear solution which is our desired digested sample (Richards, 1968).

Dilution and filtration

All the digested samples were diluted by freshly prepared distilled water making their volume upto 50ml.The dilution of the samples was followed by their filtration and labeling. All the samples were then reserved in plastic bottles.

Formation of standard solution for heavy metal analysis

To standardize the atomic absorption spectrophotometer to find the precise value, it was needed that standard solution of the heavy metals (Ni, Cd, Cu, Fe, Cd, Cr, Co) to be analyzed be prepared.

Instrumentation

The instrumentation used in this study was the atomic absorption spectrophotometer Model AA-6300 which was used to analyze the heavy metals in the digested samples. This technique involves the measurement of the absorbed radiations by the heavy metal in the sample. The extent up to which the radiations are absorbed is estimated by reading the spectra produced on sample excitation. This instrument follows the Beer-Lambert Law.

Statistical analysis

SPSS software (version 20) and Two-way ANOVA were the used to find the variance for the concentration of heavy metals in soil, forage and buffalo blood serum. Statistical significance between the mean was tested at 0.05, 0.01 and 0.001 level of probability as suggested by Steel &Torrie (1980).

Bio-concentration factor

Bio-concentration factor formula was calculated by the following equation according to Smith *et al*. (2013)

BCF = Content of heavy metal in forage / Content of heavy metal in corresponding soil (mg kg^{-1})

Daily intake of metal

The estimated daily intake dose (EDI) was calculated as follows:

$C_i \times IR/BW$

In this equation BW (kg) stands for the body weight of buffalo (550 kg per cattle (Briggs & Briggs 1980), IR (g per day) the average daily consumption of forages by buffalos which is ,12.5 kg where Ci (mg kg−1) is the concentration of heavy Ni in forages.

Health risk index

The formula given below (USEPA, 2002) was used to find health risk index:

$HRI=DIM/R_fD$

From the integrated risk information system, RfD values for Ni was 0.02 mg/kg/day (USEPA, 2010).

Pollution load index (PLI)

In order to find out the heavy metal concentration in soil samples the Pollution load index was calculated. The formula of the Pollution load index was calculated by formula given by Liu *et al*. (2005).

RESULTS

Analysis of soil

Nickel affected non-significantly to the sites, seasons and sites x seasons, respectively (Table 1). The concentration of Nickel in the six sites of sampling was of the order: Site II>Site V>Site III>Site VI>Site IV>Site I respectively (Table 2, Figure 1).

Table 1: Analysis of variance for Nickel in soil at six sites of sampling

Degree of freedom	Source of Variation	Mean Squares
		Ni
Sites	5	1.644^{ns}
Seasons	1	7.84^{ns}
Sites × seasons	5	3.41^{ns}
Error	36	1.393

Ns =non-significant, significant at 0.05=*, significant at 0.01=** Significant at 0.001=***.

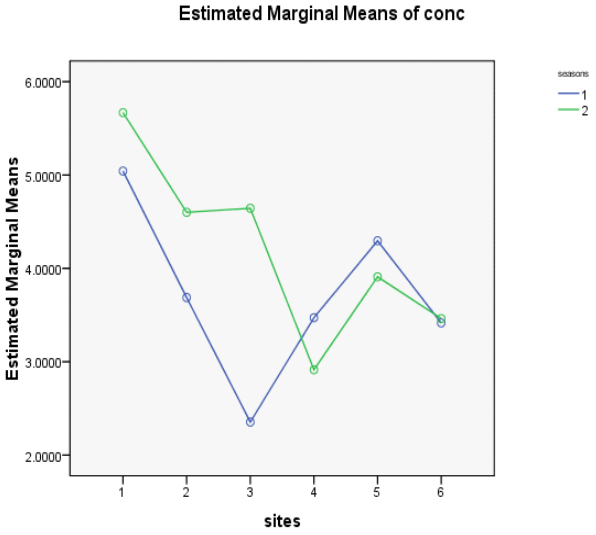

Fig. 1: The fluctuation of Ni in soil at six sites of sampling

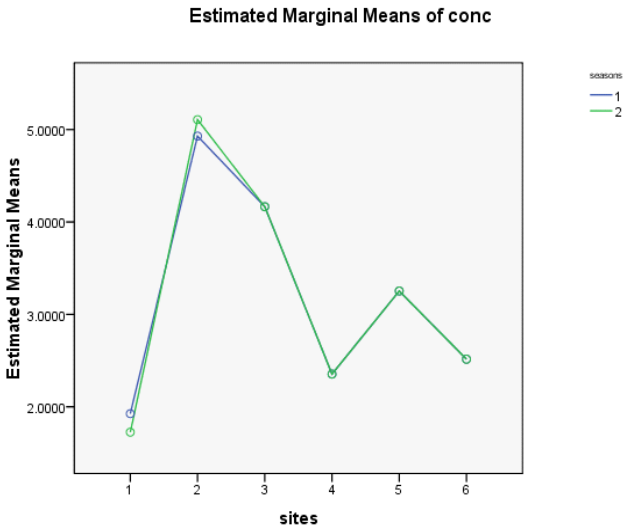

Fig. 2: The fluctuation in Ni in forage at six sites

Analysis of forage

Sites effected non-significantly while Seasons and Sites × Seasons affected significantly on the concentration of Ni (Table 2). The concentration of Nickel in the six sites of sampling was of the order: Site II>Site III>Site V>Site VI>Site IV>Site I (Figure 2).

Reported that the difference of heavy metal concentrations in forages depend on the type of soil, the fertility of soil, the pH and the type of forage being studied (Huston *et al*., 2006). The lower concentrations of the heavy meals in forage sample may be due to lesser automobile exhaust as compared to the larger cities. Heavy metal levels studied by John *et al*. (2013) showed higher concentration of Cd, Cr, Cu and Zn as compared to that found in this study. On the other hand the concentration of Ni was found lowest as found by Bahadur *et al*. (2011) According to Word *et al*. (1977) it was reported that the accumulation of Ni along with other heavy metals in soils is caused by the emission of dangerous and poisonous gases from the vehicles.

Table 2: Analysis of variance for Nickel in forage at six sites of sampling

Degree of freedom	Source of Variation	Mean Squares Ni
Sites	5	11.70 ^{ns}
Seasons	1	0.001 ***
Sites × seasons	5	0.029 **
Error	36	0.801

Significant at 0.05=*, significant at 0.01=**, Significant at 0.001=***, and ns=non-significant

Analysis of blood

Site and Season affected non-significantly on the concentration of Ni while Sites × Seasons affected non-significantly on the concentration of Ni (Table 3).Concentration of Ni was found higher at Site II and lowest at Site I (Figure 3). The nickel concentration in the study done by Nwede *et al*., (2010) was Ni-0.41mg which was higher unlike the concentrations of Ni in current study.

Table: 3 Analysis of variance for Nickle in buffalo Blood at six sites of sampling

Degree of freedom	Source of Variation	Mean Squares Ni
Sites	5	4.15 ^{ns}
Seasons	1	39.78 ^{ns}
Sites × seasons	5	3.454 **
Error	36	2.088

Significant at 0.001=***, significant at 0.01=**, Significant at 0.05=* and ns=non-significant

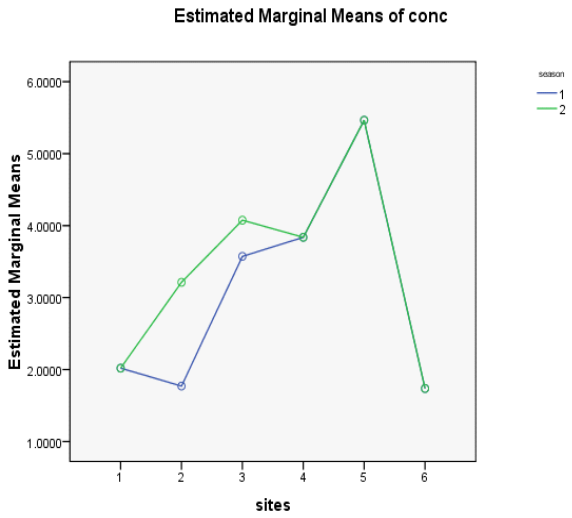

Fig. 3: The fluctuation of Ni in blood at six sites of sampling

BCF for forage and soil

The bio concentration factor for forage and soil at the six sites of sampling was of the order: Site II>Site III>Site V>Site >Site IV>Site VI> site I (Table 4).

The study done by Kamal *et al*. (2015) showed higher concentration of nickel (Ni-0.08 mg/kg) as compared to the current study. Higher BCF of Ni was due to the low retention of heavy metals in soil and their continuous transport to the forages unlike the lower BCF which means a tight bonding exists between forage and soil. Zhang *et al*., 2007 and Cui *et al*., 2004) have reported that the soil pH also affects the BCF

Table 4: Bio concentration factor from soil to forage and from forage to blood

Sites	Soil-Forage	Forage-Blood
Site I	0.601	0.409
Site II	1.89	0.86
Site III	1.58	0.73
Site IV	0.90	1.32
Site V	1.09	1.105
Site Vi	0.803	1.72

Bio-concentration factor for forage to blood

The Bio concentration factor for blood and forage at the six sites of sampling was of the order: Site VI>Site IV>Site V>Site II>Site III>Site I (Table 4).

The soil properties like its pH influence the mobility of heavy metals in soil. It may be possible that the pH of examined soil affect the bioconcentration factor of heavy metal. According to Liu *et al*. (2006) the bioconcentration factor if found greater than 1 suggest that the plants can accumulate heavy metals in them. Alloway & Ayres, 1997 reported that the extent of the heavy metal uptake by forages depends upon their age, edaphic factors and the climatic factors.

Correlation

Concentration of Ni (0.324) between soil and forage was found positive non-significant while Concentration of Ni (-0.382) between forage and blood was found negative non-significant. The trend of correlation found by Chakresh *et al*., (2012) was different as compared to that found in this study. It was found highest for Ni. Relationship of heavy metals suggesting imbalance of the heavy metals between soils was one of the reason behind negative non-significant correlation. Effective translocation of heavy metals from soil was one of the reasons behind the positive correlation found for Ni (Amlan *et al*., 2012).

Daily intake of metals

The order of the DIM was: Site IV>Site I>Site II>Site III>Site VI>Site V (Table 5).

The daily intake of metal for Nickel was found higher than reported by Lente *et al*. (2011). In the current results the values of DIM were lower than 1 it suggests that no risk of health is associated with the consumption of such contaminated forages (Radwan & Salama (2006).

Table 5: Daily intake of metal and health risk index via consumption of forage from six different sites of Sargodha District

Sites	DIM	HRI
Site I	0.05	1.03
Site II	0.048	2.09
Site III	0.043	2.15
Site IV	0.059	2.95
Site V	0.042	2.145
Site Vi	0.049	0.138

Health risk index

The order of HRI for Ni was of the order: Site IV>Site III>Site V>Site II>Site I>Site VI (Table 5). The values of nickel were higher in the samples collected and examined in this study contrary to the study done by Zahra *et al*. (2014). The value of the health risk index if found greater than 1 means a serious health risk is associated with the consumption of this contaminated forage and vice versa. USEPA. (2002) According to Sajjad *et al*. (2009) if the HRI is found greater than 1 It mean a serious health risk is associated with the consumption of roadside contaminated forages. Health risk index depends on the chemical composition and the physical characteristics of soil, type of forage being consumed and rate of the consumption of forages.

Pollution load index

The order of PLI for Ni was: Site V>Site VI>Site III>IV>Site I> Site II (Table 6).
The pollution load index observed by Ahmad *et al*. (2014) in samples of forage and soil was lower as compared to that found in this study. Pollution load index if found greater than I indicate the more contamination in the examined area unlike the pollution load index less than 1 which means that area is less polluted and there is less automobile concentration on the sampling site.

Table 6: The pollution load index in soil of forage obtained from six sites of sampling

Study sites	Ni
Site I	0.29
Site II	0.04
Site III	0.38
Site IV	0.35
Site V	0.45
Site VI	0.371

DISCUSSION

The mean values of Ni found in present study were higher. The elevated concentrations of heavy metals in the roadsides were due to the pollution by factories and traffic smoke found in the vicinity of sampling sites. On the other hand the lower values of heavy metal suggest lesser traffic in the area of sampling as compared to larger cities. In addition to that the concentration of heavy metals in soil depends on the type of soil. Arid and semi-arid soils have a higher average of trace elements concentration than those of temperate and boreal regions as well as the humid tropic zones (Fengxiang, 2007).The lower concentrations of the Ni in forage were due to the lesser automobile exhaust in the Sargodha as compared to larger city. Differences may be related to variations in botanical composition of selected diets, soil pH, soil fertility etc. (Huston *et al*., 2002).In the blood samples Ni was found higher in our study unlike that found by Nwede *et al*., (2011). The concentrations of heavy metals such as Ni retained in the sensitive organs of body like liver and kidneys which further leads to poisoning by them (Minervino *et al*., 1999).Metallurgical waste and road side smoke should be properly managed in order to be safe from their toxic effects.

Bio concentration factor

The higher bio concentration factor of heavy metals suggest low retention of metals in soil and heavy metal get readily transported into the forages while on the other hand lower bio concentration factor suggest that heavy metals are in tight bonding with the soil and they do not get transferred readily to forage. The bio concentration factor also depends upon soil pH (Zhang *et al*., 2007; Cui *et al*., 2004).The soil properties like its pH influence the mobility of heavy metals in soil. At low pH, high mobility of heavy metals was occurred (Celechovska *et al*., 2008). In addition to this the translocation of heavy metals was controlled by pressure of leaf transpiration and the pressure of root (Violante *et al*., 2010).

Pollution load index

The pollution load index greater than 1 indicated more contamination in the study site. The lower pollution load index suggests that there was less rush of industries in Sargodha. The pollution load index is the measure of assessing soil quality where a value of PLI > 1 would indicate deterioration of site quality, where values of PLI < 1 denote perfection, PLI = 1 presents that only baseline levels of pollutant present (Thomilson *et al*., 1980).

Correlation

The value of correlation for Ni was similar to those found by Bushra *et al*. 2014(Ni-0.09).The weak relationship between soil and forage leads to positive non-significant correlation while strong relationship between soil and forage leads to non-significant correlation.

Health Risk Index

The value of health risk index for Ni was found higher as compared to that found by Zahra *et al*. (2014). The trend of the values of health risk

index was the same in present study as compared to that studied by Asma *et al*.(2015). According to USEPA(2002), if the health risk due to consumption of contaminated forage was found greater than 1 then its means there is an obvious health risk on the buffalos but if the health risk was found more than 1 than there is no health risk to Buffalo.

CONCLUSION

Nickel accumulation in the roadside forages, soil and grazing buffalos was found. Higher concentration of Ni in samples of blood forages and soil suggested nickel is readily transferred from one trophic level to another. The alarming rate of nickel is highly toxic to the living systems as it cannot be easily removed from the buffalo bodies. So, need of hour is that strategies should be applied to get rid of the nickel toxicity.

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