

Risk Assessment of Nickel Toxicity in Rams in a Semi-Arid Region Using Soil-Plant and Blood Plasma Samples as Indicators

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Abstract.- The present study was conducted at a livestock farm to assess whether the rams being reared therein are experiencing nickel (Ni) toxicity. To fulfill this objective, blood plasma, forage and soil samples were collected four times each after 30 days interval and analyzed through wet digestion for determining Ni concentration. The results showed a significant influence of sampling intervals on soil and forage Ni level, but a non-significant effect on blood plasma Ni. The values of soil and forage Ni in this study were higher than the critical levels for these attributes. The blood plasma Ni concentration was found to be much higher than the normal value for ruminants. Thus, toxic effects of Ni may be expected in the ruminants being reared at this farm. Soil and forage Ni levels were highly correlated, but the correlation between forage and blood plasma Ni levels was poor. Overall, blood plasma, soil and forage Ni concentrations were higher than the normal reference values. So application of fertilizer containing Ni to the soil of the farm as well as supplementation with Ni for grazing ruminants should be avoided.

Keywords: Sheep, ruminants, rams, soil, forage, nickel.

INTRODUCTION

Minerals have a significant influence on ruminant nutrition and metabolism, but mineral availability from soil for forages and livestock vary greatly (Ashraf *et al.*, 2007). Ruminant production is in fact related to the availability of appropriate levels of various mineral elements in the dietary sources (Underwood, 1981; Khan *et al.*, 2006, 2007, 2010), but the large amounts of mineral elements do not guarantee availability of desired amount of these elements to animals (Littledike and Goff, 1987). However, grazing ruminants fulfill their mineral requirements mostly from the pasture forages (Provenza, 1995; Underwood and Suttle, 1999; Khan *et al.*, 2005; Napolitano *et al.*, 2011).

Mineral imbalances change a large amount of physiological processes in livestock animals. For example, deficiency in dietary minerals led to reproductive failure in most ruminants (Hidiroglou, 1979). Similar to some other elements, the issue of

the importance of nickel(Ni) has gained much attention in recent years as researchers are curious to reveal its importance in a variety of trace elemental compounds involved in the proper metabolism of living systems. The importance of Ni depends on various factors, but the assessment of these factors is very intricate (Taylor, 1996). Nickel and other transition metals are essential for the synthesis of metallo-enzymes and, at the same time, these elements are toxic even at moderate concentrations. Interference of Ni with such processes has been reported long ago to pose a variety of severe hazardous effects (Hidiroglou, 1979). Many of problems in animals are often considered to be due to the various essential roles of nickel which it plays in protein synthesis in living systems (Schnegg and Kirchgessner, 1978; Sidhu *et al.*, 2005). Nickel has an interactive role with other materials importance for the proper biological functioning of various metabolic systems (Anke *et al.*, 1980; Dalton *et al.*, 1988; Jinwal *et al.*, 2009). Due to frequent use of nickel containing chemicals in industry, the release of Ni in the environment cannot be avoided. This is certainly a serious threat to the health of living organisms, because high amount of Ni is known to

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cause a large number of metabolic disorders in the organisms (Denkhaus and Salnikow, 2002). Nickel plays a vital role in different animals such as cows, goats and sheep (Phipps *et al.*, 2002; McDowell, 2003). The adverse effects of Ni deficiency in animals are numerous which include delayed skin eruptions, gestation period, low number of offspring, reduced hemoglobin, anaemia and hematocrit values, and impaired activity of several enzymes (WHO, 1991). Although a diseases caused due to Ni deficiency has not been reported in animals and humans, but some evidences regarding its importance in livestock and humans have been suggested by some researchers (Anke *et al.*, 1995; Nielsen, 1996; Fraga *et al.*, 2005). However, information on optimal and deficient nickel levels for animals, humans as well as plants is limited.

Considering the significance of Ni for animals and plants, the present study was designed to study the risk assessment of Ni of ruminants by appraising Ni levels in blood plasma, soil and forage. Animals employed in the present investigation were adult uncastrated male sheep (rams). It has been reported that sex of sheep affects mineral requirements through differences in growth rates and physiological functions (Anonymous, 1980). Rams grow faster than ewes and so require greater daily supply of minerals. This information would be useful for formulating mineral mixtures for animals as well as for soil amendments with appropriate fertilizers to maintain appropriate levels of Ni in soil-plant-animal continuum so as to protect the animals from the hazards of excess amount of Ni, if present in these sources.

MATERIALS AND METHODS

The present study was conducted during October 2008 through January 2009 at the Livestock Station, Khizerabad, Central Punjab, Sargodha (latitude 32°8'O'N; longitude 73°7'O'E; altitude 187 m). This livestock station was established in 1972. The soil of the station is clay to clay loam in texture with pH varies from 7.6-8.2. This farm covers an area of 1682 ha keeping 1400 cattle of Sahiwal breed and nearly 2000 sheep of Kajli breed. This area falls under semi-arid environment. The temperature varied from 5-23°C in winter during investigation period and annual precipitation was 180-200 mm.

Pastures description

The pastures had sown varieties of forages of different species. At the time of present survey mostly dominant forage species were *Avena sativa*, *Trifolium alexandrinum*, *Cichorium intybus*, *Medicago sativa*, *Chenopodium morale* and some grass species mainly *Cynodon dactylon*. These forages were the representatives of the area which were mostly grazed by the ruminants on this farm. The forages were fertilized with 25 kg urea/ha. However, the irrigation was carried-out using tube well as well as canal waters.

Animal description

In the present investigation, 20 healthy male uncastrated sheep (rams) of Kajli breed of 3-4 year age were used. Average body weight of these rams was in the range of 38-44 kg. Experimental animals belong to the same class of rams throughout the investigation. The class of rams adopted in this study was basically allowed to graze on the pastures in all seasons. However, these rams were fed with small amount of concentrates occasionally.

Sample collection

Forage and soil samples along with blood plasma from grazing rams were collected on monthly basis up to four months from October 2008 to January 2009. Soil and pasture samples were collected from five sites within each pasture. Five soil sub-samples collected from 20 cm deep and mixed to form amalgamated soil (Sanchez, 1981). At the same spot, five forage samples were collected consisting of the predominant forage species repeatedly grazed by the animals at the farm. To stimulate the grazing behavior of animals, the forage was maintained at a uniform height of 3-6 cm from the ground. They were rapidly washed first with tap water to remove surface contaminants followed by three washings with distilled water. Cloth bags were used to air dry the forage as well as soil samples.

The blood from rams was collected at the same time as soil and forage. Blood (15 mL) was drawn from the jugular vein using a sterilized syringe having heparin as an anticoagulant. Plasma was isolated by centrifugation and stored at -20°C for subsequent mineral analysis (Fick *et al.*, 1979).

Sample preparation and analysis

Oven-dried soil (1 g each) was transferred in digestion flasks each containing 5 mL H₂SO₄ in it and kept overnight at room temperature. Then hydrogen peroxide (H₂O₂; 25 mL) was added in each digestion flask and placed all flasks on a hot plate until complete digestion of the material. The final volume of the extract was made up to 50 mL using distilled water. After filtering the extract it was used for the analysis of Ni concentration as described following Wolf (1982).

Air-dried samples of forage were further oven-dried at 65°C and ground using Willy mill. The dried samples (1 g each) were digested in HNO₃ and HClO₄ (3:1) at 250°C for 3-4 h until the solution turned colorless (Koh and Babidge, 1986; AOAC 1990). The contents of each flask were diluted to a constant volume. The supernatants obtained from centrifugation were used for mineral analysis. To avoid spattering or swelling the plasma samples (1mL each) were pretreated with 50% HNO₃ on a heating plate until smoking ended to char the organic matter. Then these samples were subjected to wet digestion with HNO₃ and HClO₄ (3:1 v/v) at 250°C for 2 h until fumes appeared in the flasks. The residue was dissolved in 1% HCl and final volume made with distilled water. Atomic absorption spectrophotometer (AA-6300 and GFAEXi7i, Shimadzu, Japan) was used for the analysis of nickel from soil and forage (flame method) and plasma (graphite furnace method) samples.

Statistical treatment of data

The data obtained from all analyses were tested for significance at $P < 0.05$, 0.01 and 0.001 using the software SPSS. Correlations among soil, forage and plasma Ni concentrations were also drawn using this software.

RESULTS AND DISCUSSION

Soil

Analysis of variance of data showed that there were considerable effects of sampling intervals on soil Ni levels (Table I). Mean soil Ni concentrations ranged from 16.55-24.55 mg/kg. The lowest level of soil Ni was observed at fourth, while

Table I.- Analysis of variance of data for Ni concentrations in soil, forage and blood plasma at different sampling periods

Source of variance (S.O.V)	Degrees of freedom (df)	Mean Squares		
		Soil	Forage	Blood
Sampling periods	3	84.173*	91.241***	0.000138 ^{ns}
Error	16	17.426	8.028	0.00007

*, Significant at 0.05 level; ***, Significant at 0.001 level;

the highest at the third sampling interval. Soil Ni values fluctuated with an inconsistent trend of increase or decrease during the study (Fig. 1a). The values of soil Ni in this study were higher than the critical level i.e. 2 mg/kg as observed by Robinson *et al.* (1999). Ni concentrations ranging from 1.16-2.16 mg/kg for rural soils and 7.07-102 mg/kg for urban soils have been reported in the UK (Environmental Agency, 2007). The mobility of Ni in soil and its solubility increases with decreasing pH (Tye *et al.*, 2004; Kabata-Pendias and Mukherjee, 2007). Ni compounds are mainly soluble at a pH < 6.5 (Mcgrath, 1995) and Ni adsorption by soil decreases with increasing level of soil organic matter (Atsdr, 2005). Soil nickel, higher than the values found in the present study, has been reported previously (Sutton *et al.*, 2002). However, in contrast, considerably lower soil Ni than that of the present study has been observed by Mayland *et al.* (2006) and Suzuki *et al.* (2009). Excess of nickel in soils is one of the important factors causing reduced growth, but Ni phytotoxicity varies with Ni concentration in soil solution as well as with the plant species (Khalid and Tinsley, 2005; Rahman *et al.*, 2005). It is also known that the increase in Ni level in the nutrient solutions increases the concentrations of copper and Fe in roots and decreases in shoots (Rahman *et al.*, 2006). Furthermore, concentrations of Mn and Zn in the roots and shoots of plants decrease with increase in Ni supply in the nutrient solution (Tokalioglu *et al.*, 2000). Such an imbalanced uptake of a variety of essential mineral nutrients due to excessive amount of Ni present in the growth medium may lead to poor plant growth.

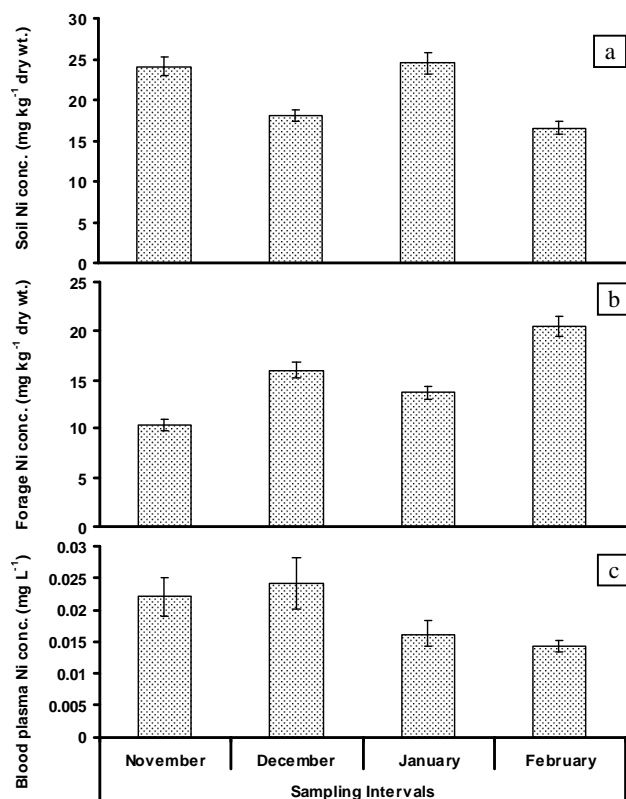


Fig. 1. Soil, forage, and blood plasma Ni concentration in relation to sampling intervals.

Forage

A considerable influence of sampling intervals on forage Ni levels were observed (Table I). Mean forage Ni concentrations ranged from 10.34-20.50 mg/kg. The pattern of decrease or increase in forage Ni levels was found to be inconsistent from first to the last sampling interval (Fig. 1b). Mean forage Ni levels were more than the normal value of 0.05-0.5 mg/kg necessary for grazing livestock (Anke *et al.*, 1983). These results support an earlier argument that Ni availability in nature exceeds the tentative requirement. Thus, Ni deficiency is not likely to occur under practical conditions (Anke *et al.*, 1983). High levels of Ni in plants can decrease Fe and Mn concentrations, and insoluble compounds usually are transformed into soluble in soil with low pH, which in turn raises the Ni levels in plants thereby leading to reduced plant growth (McDowell, 2003). Concentration of Ni in grasses is lower than that in soils, but legumes contain relatively more Ni (Underwood and Suttle

1999). High levels of Ni in forages were also reported previously by Ahmad *et al.* (2009) in the Salt Range, Pakistan. Higher value of forage Ni may have been due to high level of soil Ni found in the present study. The forage Ni values recorded in the present study were below the tolerable value of 50 mg/kg as suggested by NRC (1980). Normal Ni concentrations in plants range from 0.05 to 5.0 mg/kg, and the Ni concentration exceeding the upper limit is considered toxic for plants (Kabata-Pendias and Pendias, 1992). At vegetative stages, elevated Ni levels lower the shoot and root enlargement, affect branch development, deform various plant organs, induce abnormal flower appearance, decrease biomass production, disturb process of mitosis in root and cause Fe deficiency which results in chlorosis. In addition, higher level of Ni also influences mineral uptake by roots, affects plant metabolism, retards the process of photosynthesis and transpiration. Eventually, yields of forage crops for livestock are highly reduced due to excess amounts of Ni in soil and plants, which cause toxicosis in ruminants. The higher concentration of Ni in forage could not be attributed solely to root absorption, but it can be attributed to aerial sources as well (NRC, 1980).

Blood plasma of rams

Sampling interval effects on blood plasma Ni concentrations were non-significant (Table I). Mean blood Ni concentrations fluctuated from 0.014-0.024 mg/L. The highest concentration of Ni in blood plasma of rams was observed at the second, while the lowest value at the fourth sampling interval (Fig. 1c). Mean plasma Ni concentration increased till the second interval and then abruptly decreased at the third and fourth sampling intervals. Blood Ni concentration in this study was much higher than the normal value for blood plasma as established by Puls (1994). This higher plasma nickel values could be attributed to high Ni levels in soil and forages found in the present study. Similar plasma Ni values have previously been reported in cattle by Mayland *et al.* (2006), but in contrast, Yazar *et al.* (2006) have reported considerably high amount of Ni (0.2505 mg/L) in the blood plasma of goats in Turkey. These contrasting results may have been due to differences in geographical areas, diet

composition, sampling seasons, etc. (Erdogan *et al.*, 2002; Youde, 2002). It has been widely reported that gastrointestinal absorption of nickel is variable and it depends on the composition of diet supplied to an animal (Hausinger, 1993). Following parenteral administration of nickel salts, the highest nickel accumulation may occur in various tissues and fluids within the animal body (Anke *et al.*, 1983; Erdogan *et al.*, 2002; Youde, 2002).

Correlation among soil and forage and blood plasma Ni concentrations

Correlations coefficients were calculated between different variables for Ni concentration from samples collected across all sampling intervals. A strong negative correlation (-0.606) was found between forage and soil Ni. Correlation coefficient between soil and blood Ni was though positive, its value was very low (0.342). Forage and blood Ni correlated negatively (-0.25).

CONCLUSIONS

The present study showed that variable nickel concentrations are present in the soil, forage and blood plasma samples and were higher than the normal reference values. So application of fertilizers containing Ni to soil of the farm concerned as well as supplementation with Ni for grazing ruminants should be avoided as there could be potential hazards due to higher intake of Ni by the livestock at this specific ranch. Keeping in view the expected toxicity of Ni in ruminants, the mineral supplements containing essential metals like Fe, Mn, Ca, Zn, Cu, and Mg which can suppress or modify the toxic effects of nickel should be provided to animals to prevent the various toxicosis caused by this element at the studied animal farm.

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