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Trace mineral concentration of forages in connection with sheep dietary requirements

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Abstract

This paper describes the trace and ultra-trace mineral concentration in different forages utilized by sheep (Kajli and Fat tail) in the rangelands of Chakwal district, Punjab, Pakistan. The concentrations of Se, Mo, Zn, Mn, Cu, and Co were assessed using spectrophotometric methods after the digestion of forage samples. The dominant forage species consumed by sheep were *Buxus pappillosa, Prosopis juliflora, Ficus palmata, Acacia modesta, Trichodesma indica, Olea permiginea, Lantana camara, Justicia adhatoda, Morus nigra,* and *Ipomoea carnea*. The overall mean concentration of Co (1.27±0.24 mg/Kg of DM), Zn (35.14±1.28 mg/Kg of DM), and Cu (28.06±1.29 mg/Kg of DM) in forages were higher than the requirements for lactating ewes, while the concentration of Se was within the normal range. Molybdenum (0.030±0.004 mg/Kg of DM) and Mn (29.69±1.61 mg/Kg of DM) concentrations were lower than dietary requirements for sheep. In different administrative divisions (tehsils) like Chakwal, Talagang, Choa Saidan Shah, and Kalar Kahar of Chakwal district, the trace minerals Zn, Mn, and Co, showed non-significant variation, whereas Se, Mo, and Cu showed substantial variation. In conclusion, rangeland forages contained a sufficient concentration of most trace minerals (Co, Cu, Se, Zn) to meet the requirements of grazing sheep in the study district. However, Mn and Mo supplementation seems to be essential to maintain the optimal production capabilities of sheep in this area.

Key Words: dietary requirements, rangeland forages, sheep flocks, trace minerals
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Introduction

In livestock production systems, natural vegetation and agro-grazing are the most important components. Regional situation and climate regulate the kind, nature, nutritive value, composition, and distribution of forage plants in different grazing sites and cropping belts (Khan *et al*., 2007). Experiments, conducted in the Punjab province of Pakistan, identified trace mineral contents of many plant species (*Cichorium intybus, Coronopus didymus, Mazus reptans, Cynodon dactylon, Malva neglecta, Trifolium alexandrinum, Parthenium hysterophorus,* and *Medicago polymorpha*) grazed by sheep and goats (Khan *et al*., 2007; Rizwan *et al*., 2019; Ahmad *et al*., 2020). Identification of feed, soil, and pastures deficient in minerals is important for the rationalization of nutrient supplies, which may result in enhanced production performance and immune status of animals (Rizwan *et al*., 2021a,b). In reference to livestock, forages, pasture, and supplements (concentrates) are the main sources of trace minerals like Cu, Mn, Co, and Zn. Trace minerals are considered essential for the proper functioning of various physiological systems of the body and are important for normal immune function and protection against oxidative damage to tissues. In livestock, the amount of these trace minerals depends upon environmental conditions, nature, type of soil, plant types, species of plants, the magnitude of dry matter, and the maturity status (Rizwan *et al*., 2021a,c).

Rangelands, covering more than 60% of the area, are a major part of the agricultural system in Pakistan and offer grazing facilities for livestock (Khan *et al*., 2007). It is important to explore the triangle of soil–plant–animal to calculate the concentration of trace minerals because of its effects on animal production capabilities (Qudoos *et al*., 2017). Information regarding the available pastures along with their seasonal nutritive values is a prerequisite to obtaining maximum output from livestock production (Khan *et al*., 2007; Qudoos *et al*., 2017; Rizwan *et al*., 2019). It is well known that the mineral profiles of forages may fluctuate seasonally (Khan *et al*., 2007). Consequently, it is beneficial to know the mineral profiles of forages and pastures before deciding on a particular mineral(s) supplementation for livestock in a particular season. Limited efforts by Ahmad *et al*. (2020) and Rizwan *et al*. (2021b) have been made for this purpose and incomplete data is available regarding the status of trace minerals of forages utilized by rangeland sheep of Chakwal district, Pakistan. Hence, the study was planned to estimate the trace mineral profiles of rangeland forages utilized by sheep in this district. Under natural grazing conditions, forages are the main source of minerals. Determination of trace elements in grazing forages is important to regulate the requirement of animals. Grazing on trace element-rich forages may increase the resilience against infections, particularly in resource-poor countries like Pakistan. This study may elucidate the status of the mineral profiles of available forages to recommend specific mineral supplementation in instances of deficiency.

Materials and Methods

The present study was completed in autumn (October to December, 2020) in Chakwal district, Punjab, Pakistan, located in the southern part of Rawalpindi district between 72° 54' east and 32° 56' north. The district of Chakwal, which covers an area of 6,524 km^2, is subdivided into four tehsils i.e., Tehsil Kalar Kahar, Talagang, Chakwal, and Choa Saidan Shah. Chakwal is in the Potohar plateau and the Salt Range. The southern portion runs up into the Salt Range and includes the Chail peak, 3,701 feet (1,128 m) above sea level, the highest point in the district. The southeast and southern area is hilly and scrub forest covers the rocky surfaces. The climate of Chakwal is sub-humid; rainfall is 350–500 mm and occurs during the monsoon.

Forages that are commonly grazed by sheep were collected in triplicate (n = 3) from each grazing site located in the Kalar Kahar, Talagang, Chakwal, and Choa Saidan Shah tehsils. All stages (seedling, vegetating, budding, flowering, and ripening) of selected forages were collected in equal amounts. The fresh green samples were collected in polythene bags and labelled for shipment to the University of Agriculture, Faisalabad, Pakistan. The forage samples were shipped on the same day of collection. The identification of collected forages was done by a professional of the Botany Department, University of Agriculture, Faisalabad. Hydrochloric acid (1%) followed by distilled water was used to wash the leaves of forages. Forage samples were dried in the open air under shade and then placed in a hot air oven (65 ± 5 °C) for 24 h. An electrical grinding machine (Secura®, Netherlands: Type: Blade Coffee Grinders, Power (W): 3000w, Housing Material: Stainless steel, Model Number: 800Y) was used to grind dried leaves, which were then subjected to wet digestion. In short, 1 g dried sample of the plant was digested with nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 3:1 ratio on a hot plate. The final volume was adjusted to 50 mL with de-ionized water when the material was cleared. Filtration was then done using a Whatman filter (No. 42) and stored in air-tight bottles for determination of the mineral profile (Miller, 1998).

Evaluation of Cu, Co, Zu, and Mn was done at the Central Hi-Tech Laboratory, University of Agriculture, Faisalabad, using an atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200) using the standard protocols as described by Anan *et al*. (2001). Standard solutions for Zn, Cu, Mn, and Co were prepared using the available reagent-grade salts. Each standard solution was run one by one for these minerals and their absorbance was recorded. Standard curves for each mineral were constructed by plotting the absorbance of standards against their concentrations. Concentrations of respective minerals in the samples were calculated from their respective standard curves. Values of the atomic absorption spectrophotometer, used for the determination of Cu, Co, Zn, and Mn, are given in Table 1. An inductively coupled plasma-optical emission spectrophotometer (Optima 2100-DV, Perkin Elmer) was used to calculate the ultra-trace minerals, Se and Mo. Zero emission was set by using a blank solution. The emissions of prepared standards were noted and plotted to obtain a calibration curve. The emissions of the samples were determined and concentrations were calculated from the respective calibration curve. Values from the inductively coupled plasma-optical emission spectrophotometer used for the determination of Se and Mo, are given in Table 2.

Table 1 Values of atomic absorption spectrophotometer used for the determination of Cu, Co, Zn, and Mn

Parameters	Fixed values			
	Co	**Cu**	**Mn**	**Zn**
Wavelength (nm)	241.5	325.6	278.5	212.8
Slit width (nm)	0.3	1.4	0.5	1.2
Lamp current (mA)	11.9	6.8	7.3	9.8
Flame	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2
Pressure (Flow rate) (kpa)	165	165	165	165
Burner height (mm)	7.3	7.3	7.3	7.3
Burner head	Standard type	Standard type	Standard type	Standard type
Fuel gas pressure (kpa)	6	6	6	6

Table 2 Values from the inductively coupled plasma-optical emission spectrophotometer used for the determination of Se and Mo

Parameters	Fixed values
Sample flow rate	1.50 mL/Min
Gas	Argon (99.99%)
Plasma flow rate	15.0 L/min (Ar + liquid sample)
Auxiliary flow rate	0.20 L/min
Nebulizer flow rate	0.80 L/min
R.F. power	1300 watts

The mean concentration of each mineral was measured, together with the standard errors. Differences in mineral concentration of forages were evaluated using one-way analysis of variance (ANOVA). Tukey's *t*-test was used in case of significant (P <0.05) differences between treatment means (Schork and Remington, 2010).

Results

The dominant forage species consumed by sheep (Kajli and Fat tail) in the study area were: *Buxus pappillosa, Prosopis juliflora, Ficus palmata, Acacia modesta, Trichodesma indica, Olea permiginea, Lantana camara, Justicia adhatoda, Morus nigra,* and *Ipomoea carnea*. The highest concentration of Zn was detected in *Buxus pappillosa* (39.28 ± 1.34 mg kg^{-1} of DM), whereas the lowest was in *Acacia* *modesta* (31.12 ± 0.93 mg kg^{-1} of DM). *Ipomoea carnea* (32.88 ± 0.56 mg kg^{-1} of DM) had the highest concentration of Cu, while *Lantana camara* (22.68 ± 0.53 mg kg^{-1} of DM) had the lowest concentration. The concentration of Mn was highest in *Prosopis juliflora* (35.23 ± 0.73 mg kg^{-1} of DM) and lowest in *Justicia adhatoda* (27.35 ± 0.59 mg kg^{-1} of DM). The mean concentration of trace minerals (mg kg^{-1} of DM) in forages sampled collected from Chakwal district, Pakistan is given in Table 3. The overall concentration of Co, Zn, and Mn in forages collected from the lands of different tehsils was similar (P >0.05). However, the overall concentration of Cu, Mo, and Se in forages reflected a marked fluctuation (P <0.05) in different tehsils. The overall mean trace mineral concentration in forages collected from different tehsils of Chakwal district are shown in Table 4. The overall mean concentrations of Zn (35.56 mg kg^{-1} of DM), Cu (28.06 mg kg^{-1} of DM), and Co (1.27 mg kg^{-1} of DM) in the sampled forages of different tehsils were higher than the recommended dietary requirements for lactating ewes. The overall mean concentrations of Mn (29.69 mg kg^{-1} of DM) and Mo (0.030 mg kg^{-1} of DM) in forages were lower than the suggested critical dietary concentration for lactating ewes, however; that of Se (0.194 mg kg^{-1} of DM) remained within the recommended range. The critical concentration of minerals required by lactating ewes was described by NRC (2007). A compression of overall mean concentration of trace mineral with the critical concentration of minerals required for sheep is given in Figure 1.

Table 3 Concentration of trace minerals (mg kg^{-1} of DM) in forages sampled collected from Chakwal district, Pakistan (mean ± SE)

Plants	Zn	Cu	Mn	Co	Mo	Se
Buxus pappillosa	39.28±1.34^{a}	32.51±1.32^{a}	32.77±0.27^{b}	1.37±0.42^{b}	0.037±0.005^{b}	0.268±0.024^{a}
Prosopis juliflora	38.67±0.98^{a}	30.07±0.76^{b}	35.23±0.73^{a}	1.19±0.26^{c}	0.027±0.003^{d}	0.183±0.031^{ab}
Trichodesma indica	32.49±1.16^{cd}	29.31±1.48^{b}	29.44±0.65^{c}	1.09±0.37^{c}	0.042±0.004^{a}	0.226±0.016^{ab}
Ipomoea carnea	33.75±0.74^{cd}	32.88±0.56^{a}	31.35±1.04^{b}	0.95±0.57^{d}	0.032±0.005^{c}	0.210±0.031^{ab}
Olea permiginea	32.83±c0.83^{d}	27.57±1.12^{c}	27.85±0.82^{d}	1.14±0.19^{c}	0.036±0.003^{b}	0.163±0.026^{b}
Ficus palmata	35.02±1.28^{bc}	23.97±0.78^{e}	30.99±1.05^{c}	1.35±0.24^{b}	0.022±0.002^{d}	0.165±0.025^{b}
Justicia adhatoda	34.60±1.53^{bc}	25.89±1.42^{d}	27.35±0.59^{d}	1.30±0.42^{b}	0.033±0.005^{c}	0.175±0.014^{ab}
Acacia modesta	31.12±0.93^{d}	29.43±0.68^{b}	28.65±0.72^{d}	1.15±0.33^{c}	0.021±0.004^{d}	0.156±0.043^{b}
Lantana camara	37.41±1.25^{ab}	22.68±0.53^{e}	31.54±1.21^{b}	1.75±0.18^{a}	0.025±0.003^{d}	0.197±0.032^{ab}
Morus nigra	34.25±1.42^{bc}	25.35±1.42^{d}	28.32±0.93^{d}	1.64±0.47^{a}	0.015±0.004^{e}	0.178±0.025^{ab}

Different letters mean there is a significant difference between tehsils (P < 0.05)

Table 4 Concentration of trace minerals (mg kg^{-1} of DM) in forages sampled from 4 tehsils of Chakwal district, Pakistan (mean ± SE).

Tehsils	Zn	Cu	Mn	Co	Mo	Se
Chakwal	37.88±1.13^{a}	33.96±1.64^{a}	30.04±1.85^{a}	1.09±0.29^{a}	0.042±0.004^{a}	0.253±0.058^{a}
Talagang	34.78±0.79^{a}	26.15±0.50^{bc}	27.06±1.72^{a}	1.17±0.32^{a}	0.035±0.006^{b}	0.174±0.037^{b}
Choa Saidan Shah	32.65±1.45^{a}	28.43±1.54^{b}	29.62±1.54^{a}	1.21±0.27^{a}	0.026±0.003^{c}	0.168±0.014^{c}
Kalar Kahar	35.26±1.73^{a}	23.70±1.49^{c}	32.05±1.31^{a}	1.62±0.06^{a}	0.018±0.004^{d}	0.179±0.005^{b}

Different letters mean there is a significant difference between tehsils (P <0.05)

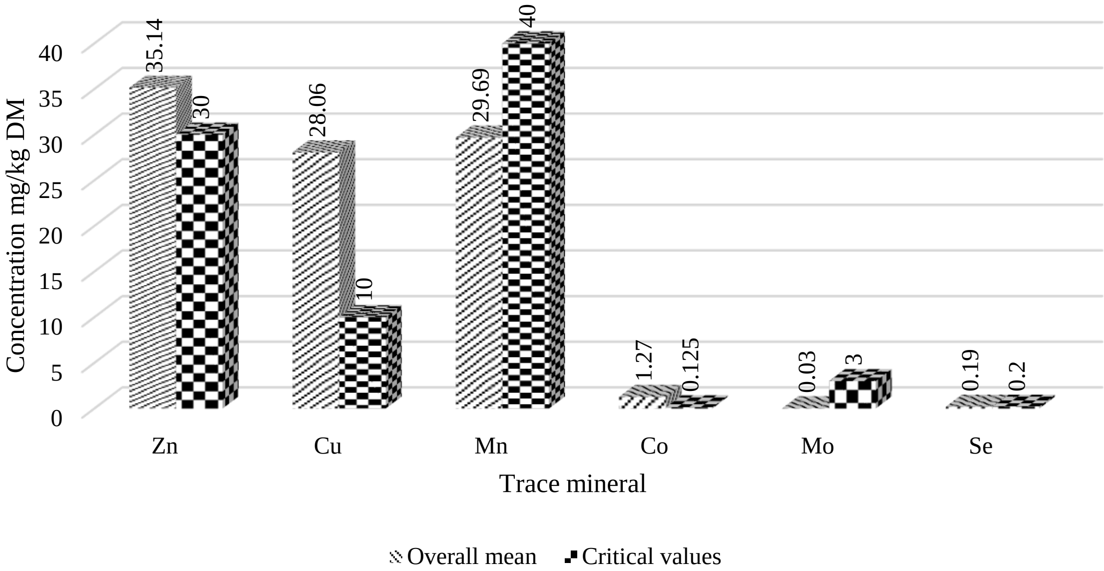

Figure 1. The overall mean concentration of trace minerals determined in the forages of Chakwal district compared to the critical concentration of minerals required for sheep (NRC, 2007)

Discussion

Rangelands represent an important source of livestock feed in Pakistan. In the Potohar plateau, small ruminants depend mainly on grazing for feed. In the salt range of Pakistan, `the forage species (*Acacia modesta, Butea monosperma, Olea feruginea, Tamarix aphylla, Ziziphus maritiana, Astragalus psilocentros, Buxus papillosa*), preferred for grazing by sheep, have been listed by Nawaz *et al*. (2012). Various other authors (Qudoos *et al*., 2017; Rizwan *et al*., 2019; Ahmad *et al*., 2020) have also reported the forages found in the present study along with other species which comprise the vegetation potential of this plateau. A diverse flora is consumed by small ruminants in other parts of the world, including Botswana, Kenya, and Nigeria (Odo *et al*., 2001; Aganga and Mesho, 2008; Lengarite *et al*., 2013), which vary from forages identified in our study. The difference in forage variety is related to differences in soil composition and structure, the topography of the area, and climatic conditions (Odo *et al*., 2001). Additionally, patterns of grazing may also affect the diversity of floral species. Forages offer many nutrients including trace minerals to animals in a natural grazing system.

Analyses of forages were done by various scientists for the determination of trace minerals, as they generally reflect the mineral intake profile of grazing livestock (Ogeba and Ayoade, 1995; Khan *et al*., 2007; Qudoos *et al*., 2017). The higher-than-recommended critical dietary values of Zn (35.14 mg kg^{-1} of DM) for lactating ewes in our analysis are in close agreement with reports published in Pakistan and Kenya (Khan *et al*., 2007; Lengarite *et al*., 2013). However, some reports from Pakistan also report a lower concentration of forage Zn than the recommended critical dietary concentration (Khan *et al*., 2015). Or *et al*. (2005) and Johannesson *et al*. (2007) reported much higher values of Zn in forage grazed by sheep than we found. Suggested requirements of Zn for lactating ewes are 20–40 mg kg^{-1} DM, which is compatible with our observations (NRC, 2007). Deficiency of Zn has been described in animals grazing on forages with low concentrations of Zn or high in compounds like S, Mn, Mg, Fe, and Cd, which interfere with the utilization of Zn by cattle (Ndebele *et al*., 2005). The composition of forage minerals differs depending on grazing pressure, type, variety of soil, and fertilizer use. The Zn concentration in forages is also influenced by these factors. Moreover, profiles of trace minerals in plants may vary amongst different species and seasons (Ben-Shahar and Coe, 1992; Khan *et al*., 2003).

Mean concentration of Cu (28.06 mg kg^{-1} of DM) in sampled forages was higher than the suggested concentration for lactating ewes (NRC, 2007). Our findings are supported by those of Ahmad *et al*. (2020), Rizwan *et al*. (2021b), Or *et al*. (2005), and Khan *et al*. (2003), who reported similar Cu concentrations in forages consumed by small ruminants. Forage species, naturally grown in different geographical localities, may contain lower (Johannesson *et al*., 2007; Khan *et al*., 2015) or higher (Khan *et al*., 2003) quantities than the suggested requirements. The main factor accountable for changes in Cu concentration of forage is low soil pH which raises Fe solubility and thus results in decreased absorption of Cu by plants. A lower concentration of Cu in forages may also be linked to the presence of antagonistic elements such as S, Fe, and Mo (Beeson and Matrone, 1976). The formation of thiomolybdates by the interaction of S, Mo, and Fe with Cu is another cause of Cu deficiency in animals, as this complex is not absorbed by the animals (Spears, 2003).

The manganese concentration (29.69 mg kg^{-1} of DM) in forage species in our study was lower than the critical concentration of 40 mg kg^{-1} DM recommended by the NRC (2007) for lactating ewes, which concurs with earlier reports (Khan *et al*., 2015; Rizwan *et al*., 2019; Ahmad *et al*., 2020). However, higher Mn concentrations in forages have been reported by Johannesson *et al*. (2007). Forage Mn concentration is generally linked to soil Mn abundance. However, forages, grown even in Mn-deficient soils, have been found to have a sufficient concentration (Beeson and Matrone, 1976).

Cobalt deficiency is often considered the most prevalent deficient mineral of grazing livestock in many parts of the world (NRC, 2007). The concentration we found (1.27 mg kg^{-1} of DM) in the collected forage species was considerably higher than the critical range (0.1–0.125 mg kg^{-1}) established by NRC (2007) and reported elsewhere (Johannesson *et al*., 2007; Ahmad *et al*., 2020). Deficiencies of Co in forages have also been observed in different geographical areas (Spears, 2003; Lengarite *et al*., 2013; Nordlokken *et al*., 2015). However, Rizwan *et al*. (2019; 2021b) observed a normal concentration of Co in forages. Differences in Co concentration in forage are primarily linked to its uptake by plants, ultimately depending on Mn and Co concentration in soils. Higher concentrations of Mn in soil reduces the uptake of Co in forages (Beeson and Matrone, 1976). Variation in forage Co concentration may also be related to the plant maturity stage because Co concentration tends to decrease as the plant matures (Beeson and Matrone, 1976; Spears, 2003).

The concentration of Mo in forages (0.030 mg kg^{-1} DM) reported here was not different from that noted by Johannesson *et al*. (2007). Plants, grown in soils having an insufficient concentration of certain trace minerals, may contain a reduced concentration of these minerals (Beeson and Matrone, 1976; Spears, 2003). The concentration of Se in the study forages was within the recommended dietary requirements for lactating ewes (NRC, 2007), which supports the findings of Nordlokken *et al*. (2015). However, lower forage Se concentrations were observed by Johannesson *et al*. (2007) and Khan *et al*. (2003). Variations in forage Se concentration might be attributable to variations in S concentration in forages. Consequently, owing to the chemical similarity between Se and S, the availability of S plays a key role in Se accumulation. Moreover, Se uptake by plants increases with increases in soil pH (Pope *et al*., 1979).

The present study was conducted in a specific period of a specific zone of Pakistan to determine the trace mineral profiles of forages. However, further studies are recommended to compare the variation in forage species in different seasons and their trace mineral profiles. Such kind of studies will be helpful to understand the status of mineral profiles of available forages and to recommend specific mineral supplementation in instances of deficiency.

Conclusion

This study on profiles of trace minerals in rangeland forage consumed by sheep suggested that Co, Cu, Se, and Zn concentrations in forages were sufficient to meet sheep requirements in the rangeland of Chakwal district, Punjab, Pakistan. However, forage concentrations of Mo and Mn were below the dietary requirements, and optimal productivity of sheep in this area may require supplementation with these minerals. Forages like *Buxus pappillosa* (Zn: 39.28±1.34 mg kg^{-1} DM; Se: 0.268±0.024 mg kg^{-1} DM)*, Ipomoea carnea* (Cu: 32.88±0.56 mg kg^{-1} DM)*, Prosopis juliflora* (Mn: 35.23±0.73 mg kg^{-1} DM)*, Lantana camara* (Co: 1.75±0.18 mg kg^{-1} DM)*,* and *Trichodesma indica* (Mo: 0.042±0.004 mg kg^{-1} DM) showed the highest concentration of trace minerals. It is recommended to use these forages to feed the sheep to fulfil the requirement of trace minerals naturally.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Authors' contributions

MSS, AQ, and HMR conceived and planned the experiments. MI, NL, RHAN, and IJ contributed to sample preparation and carried out the experiments. NL and ZI contributed to the interpretation of the results. MR, HMR, and AQ took the lead in writing the manuscript. MSS supervised the project. MSS and HMR contributed to the final version of the manuscript. All authors have read and approved the finalized manuscript.

Conflict of interest declaration

The authors declare there is no conflict of interest.

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