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MINERAL EVALUATION OF FODDER TREE LEAVES AND SHRUBS CONSUMED BY LIVESTOCK IN THE MOUNTAIN REGION OF PAKISTAN

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Abstract

The current study aimed to investigate the macro-micro mineral profile of four fodder trees (*Salix nigra, Salix alba, Populus nigra,* and *Elaeagnus angustifolia*) and two shrubs (*Hippophae rhamnoides* and *Rosa webbiana*) commonly utilized as livestock feed. A study was undertaken in three villages (Holshal, Broshal, and Hakalshal) of Hoper Valley, Gilgit-Baltistan, to determine the species and seasonal changes in macro and micro-mineral concentrations. Six composite samples from each village were collected across three seasons and measured via wet digestion. Except for sulfur (0.82%) in *Salix alba*, substantial amounts of macro-micro minerals of phosphorus (0.41), potassium (2.14), calcium (2.64%), zinc (217.5), and copper (11.63mg/kg DM) were identified in *Populusnigra*, whereas magnesium (0.45%), manganese (90.4), and iron (728.3 mg/kg DM) were found in *Rosa webbiana*. Phosphorus (0.54), potassium (1.87%), and copper (12.4 mg/kg DM) were found in high concentrations in the spring, while calcium (3.04), magnesium (0.34), sulfur (0.53%), zinc (102.0), manganese (69.6), and iron (514.1 mg/kg DM) were found in high concentrations in the autumn. The study suggests that the leaves from all fodder species should be mixed before being fed to ruminant animals to ensure the supply of all minerals in the ration. Mineral concentrations vary by species and season, necessitating a proper feed plan to meet the nutritional needs of ruminant animals, and local farmers should use diverse feeding strategies throughout the year.

Key words: Macro-minerals, Micro-minerals, Concentration, Fodder, Ruminant.

Introduction

In Gilgit-Baltistan, which covers 72,971 square kilometers, livestock is one of the most important income sources. The area's climatic change is influenced significantly by altitudinal differences. Precipitation is scarce below 3000 meters, rarely exceeding 200 millimeters per year. At 6000 meters, however, the annual snowfall is 2000 mm (Khan, 2003). Despite the abundance of natural resources in the area, the local inhabitants are constantly and heavily reliant on them. Animals are set aside to assist persons with financial difficulties, long-term severe illness, burial bills, loan repayment, and wedding costs. Sheep and goats are commonly served as gifts (batikushi in Burushaski) instead of money at weddings, reserved for religious occasions such as Eid-ulAzha, and slaughtered in late December to be consumed as a winter meal to mitigate feed shortages.

Due to a lack of cultivable land for feed, the majority of ruminants graze on summer pastures and winter rangelands. Droughts in Misgar, Chipurson, and portions of the Central Karakoram National Park, such as the Bagrote Valley, have wreaked havoc on pastures in the last decade (Beg, 2010). Rangelands, which are an important source of food, are routinely grazed beyond their carrying capacity (Anon., 1987; Alvi & Sharif, 1995; Beg, 2010). Agricultural residues, hay, dried fodder tree leaves, household leftovers, homegrown grains of mainly poor quality, and mineral mixture in rare cases are stall-fed during severe and extended winters. The main feed resources in the highlands are natural pastures and agricultural leftovers, which have low digestibility, protein, and mineral concentrations (Seyoum & Zinash, 1998). The availability of incorrect and insufficient nutrients in feed has mostly impacted low milk production, poor growth, high death rates, and poor reproduction performance (Qureshi *et al*., 2002; Tiwari *et al*., 2007; Sarwar *et al*., 2009; Pasha & Khan, 2010). During the winter months, a shortage of quality and quantity of feed is the most common cause of cattle underperformance. Because traditional breeds are less productive and require less maintenance, they are preferred by the local inhabitants.

Fodder tree leaves and shrubs contribute to ruminant animals' feed at various periods throughout the year. These browsing species are increasingly being recognized as valuable animal feed ingredients, notably for sheep and goats. In comparison to grass fodder, tree fodder has more crude protein, minerals, and digestible components (Devendra, 1990; Topps, 1992). There is very little information on mineral concentrations in native fodder tree leaves and shrubs across seasons in this region. As a result, the goal of this study was to investigate the species and seasonal variation in macro and micro mineral concentrations in tree leaves and shrubs often used as animal feed in this area.

Material and Methods

Study area: To evaluate the seasonal change of macro and micro-mineral concentrations in regularly used fodder tree leaves and shrubs, a study was conducted in three villages (Holshal, Broshal, and Hakalshal) in Hoper Valley, GilgitBaltistan. The Hoper Valley is about 10 kilometers from Nagar Khas and is situated at (36°N74°E) at an elevation of 2900 meters above sea level. The valley is in a single cropping zone and is snowbound throughout the winters (Fig. 1).

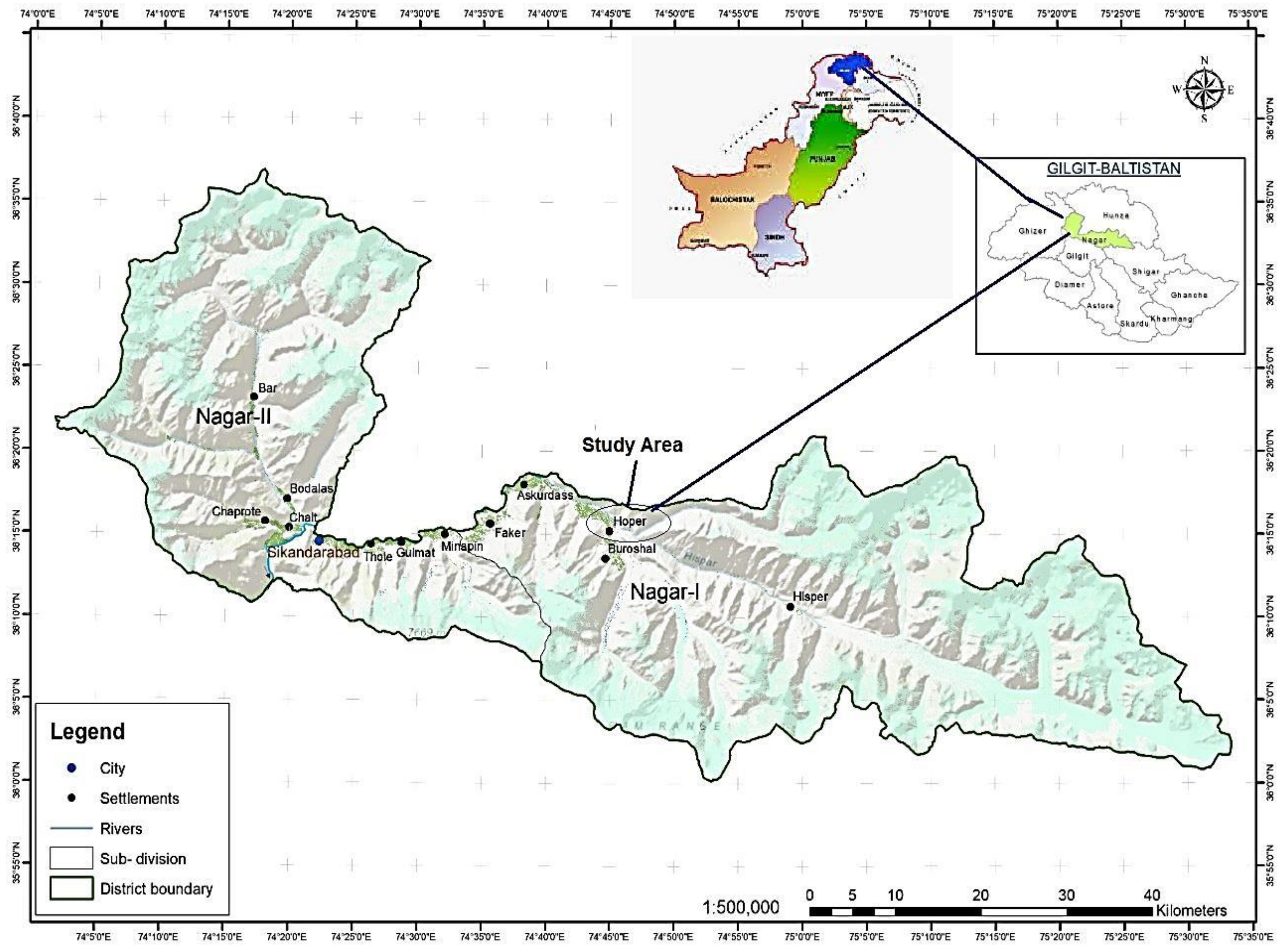

Fig. 1. Map of the study area.

Sample collection: Samples of four commonly used fodder tree leaves (*Salix nigra, Salix alba, Populus nigra,* and *Elaeagnus angustifolia*) and two commonly used shrub leaves (*Hippophae rhamnoides* and *Rosa webbiana*) were handpicked from randomly selected 15–20 plants of each fodder type and combined into one composite sample, with six composite samples collected from each village. On all three sides (top, middle, and bottom), a minimum of 10–15 leaves per plant were collected. Spring, summer, and autumn samples were obtained at various times during the three seasons.

Processing of samples: The samples were air-dried for four to seven days. These samples were placed in empty paper bags that had been pre-weighed and labeled with their identification and collection date. The air-dried samples were then dried for another 24 hours in a hot air oven at 60°C. The samples were then ground in a Wiley mill through a 1mm screen and stored in clean and dry labeled bottles with sample identification numbers and dates for the determination of macro and micro-minerals at the USDA Dairy Forage Research Center in the United States of America.

The mineral concentrations were calculated using wet digestion. In duplicate, a half gram of each plant sample was placed in a folin tube. The tubes were then filled with 5mL concentrated nitric acid, covered with a glass funnel, and stored for predigestion overnight. Samples were placed in a digestion block at 120°C for four hours. After removing the samples from the oven, they were allowed to cool. 1mL of 30% peroxide (H_2O_2) was added to each tube and heated in the digesting block for 20 minutes. The samples were taken out of the oven and allowed to cool. In each tube, 1mL of 30% peroxide was added and the operation was repeated. Each folin tube was filled with 50mL of distilled water and analyzed with Perkin Elmer Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) Optima 8000.

Data analyses: The data were analyzed using the analysis of variance process in the Statistical Analysis System with the GLM procedure (SAS). The results were reported as means and standard errors of means across species and seasons, with three villages serving as replicates. The significance level was set to 0.05.

Results

Mineral (macro-micro) profile of selected tree species and shrubs: The results regarding macro-micro mineral profile in fodder tree leaves and shrub species are shown in (Table 1). Species wise high concentrations of macro-minerals like phosphorus (0.41±0.24), potassium

(2.14±0.37), and calcium (2.64±1.3) were found in *Populus nigra* whereas, magnesium (0.45±0.03) and sulfur (0.82±0.31% DM) were found *in Rosa webbiana* and *Salix alba,* respectively. High concentrations of micro-minerals like zinc (217.5±71.63) and copper (11.63±6.15) were recorded in *Populus nigra* while manganese (90.4±37.18) and iron (728.3±335.6mg/kg DM) were found in *Rosa webbiana.* Season wise high concentrations of phosphorus (0.54±0.099), potassium (1.87±0.38% DM) and copper (12.4±5.24mg/kg DM) were recorded in spring whereas; calcium (3.04±0.7), magnesium (0.34±0.08), sulfur (0.53±0.35% DM), zinc (102.0±111.5), manganese (69.6±41.93) and iron (514.1±281.7mg/kg DM) were recorded in autumn (Table 2).

Principal component analysis: Species-wise data were analyzed by using PCA to establish a relationship between species-wise mineral profiles. The species-wise score plots generated from PCA are presented in (Fig. 2a) and the concentration of mineral in space defined by the first and second PCA dimensions is shown in (Fig. 2b). The sum of principal components (PC1 and PC2) accounted for 74.0% of variations among mineral profiles. PC1, the first component contributed for 46.6% of the total variation and the second component accounted for 27.4% of the total variation. All species and minerals were positively correlated except for *Rosa webbiana*, iron, and manganese. Season-wise data were analyzed by using PCA to establish a relationship between season-wise mineral profiles. The season-wise score plots generated from PCA are presented in (Fig. 4a) and the concentration of mineral in space defined by the first and second PCA dimensions is shown in (Fig. 4b). The sum of principal components (PC1 and PC2) accounted for 100.0% of variations among mineral profiles. PC1, the first component contributed for 79.5% of the total variation and the second component accounted for 20.5% of the total variation. Season-wise mineral profile was positively correlated except for spring, potassium, phosphorus, and copper.

Cluster analysis: Species-wise cluster analysis of the mineral concentration of four tree leaves and two shrubs divided into two major linkage groups (A and B). Linkage A contained 56% of minerals whereas linkage B contained 44% minerals. These linkages were further divided into nine clusters, contained phosphorus, zinc, potassium, copper, and sulfur of nine clusters and B contained calcium, magnesium, manganese, and iron as shown in (Fig. 3).

Season-wise cluster analysis of the mineral concentration of four tree leaves and two shrubs divided into two major linkage groups (A and B). Linkage A contained 33% of minerals whereas linkage B contained 67% minerals. These linkages were further divided into nine clusters, A contained phosphorus, copper, and potassium of nine clusters and B contained calcium, sulfur, manganese, magnesium, iron, and zinc as shown in (Fig. 5).

Table 1. Mineral profile of selected fodder tree leaves and shrubs.

	Species	Macro minerals(% of DM)					Micro minerals(mg/kg DM)			
		Phosphorus	Potassium	Calcium	Magnesium	Sulfur	Zinc	Manganese	Iron	Copper
Shrubs tree leaves	*Salix nigra*	0.37 ± 0.13^{ab}	1.60 ± 0.14^{cd}	2.40 ± 0.79^{ab}	0.35 ± 0.05^{b}	0.51 ± 0.17^{b}	183.4 ± 52.6^{a}	55.7 ± 23.98^{cd}	285.6 ± 5038^{cd}	7.94 ± 5.16^{bc}
	Salix alba	0.34 ± 0.18^{bc}	2.10 ± 0.49^{ab}	2.28 ± 0.7^{b}	0.33 ± 0.06^{b}	0.82 ± 0.31^{a}	122.9 ± 31.8^{b}	45.4 ± 19.95^{cd}	277.7 ± 55.2^{d}	6.29 ± 5.48^{c}
	Populusnigra	0.41 ± 0.24^{a}	2.14 ± 0.37^{a}	2.64 ± 1.3^{a}	0.36 ± 0.04^{b}	0.32 ± 0.04^{cd}	217.5 ± 71.63^{a}	64.4 ± 33.8^{bc}	340.3 ± 41.78^{cd}	11.63 ± 6.15^{a}
	Elaeagnusangustifolia	0.30 ± 0.16^{c}	1.85 ± 0.28^{bc}	1.81 ± 0.76^{c}	0.28 ± 0.02^{c}	0.59 ± 0.13^{b}	23.9 ± 13.44^{c}	37.1 ± 11.79^{d}	506.9 ± 69.57^{b}	11.33 ± 5.07^{ab}
	Hippophaerhamnoides	0.31 ± 0.12^{c}	1.43 ± 0.20^{d}	1.42 ± 0.58^{d}	0.23 ± 0.05^{d}	0.35 ± 0.02^{c}	28.0 ± 2.30^{c}	89.4 ± 33.5^{ab}	414.9 ± 99.62^{bc}	7.60 ± 2.93^{c}
	Rosa webbiana	0.31 ± 0.10^{c}	1.46 ± 0.25^{d}	2.30 ± 0.74^{ab}	0.45 ± 0.03^{a}	0.24 ± 0.04^{d}	20.7 ± 9.37^{c}	90.4 ± 37.18^{a}	728.3 ± 335.6^{a}	5.53 ± 4.0^{c}

The values represent the means of three replications and ± SD. The means with different letter/letters are statistically significant at $p<0.05$

Table 2. Seasonal variation of the mineral profile of selected fodder tree leaves and shrubs.

	Macro minerals(% of DM)					Micro minerals(mg/kg DM)			
Season	Phosphorus	Potassium	Calcium	Magnesium	Sulfur	Zinc	Manganese	Iron	Copper
Spring	0.54 ± 0.099^{a}	1.87 ± 0.38^{a}	1.31 ± 0.35^{c}	0.33 ± 0.07^{a}	0.42 ± 0.12^{b}	101.0 ± 70.0^{a}	55.5 ± 19.28^{a}	376.7 ± 99.0^{b}	12.4 ± 5.24^{a}
Summer	0.28 ± 0.06^{b}	1.82 ± 0.45^{a}	2.08 ± 0.58^{b}	0.33 ± 0.09^{a}	0.46 ± 0.18^{b}	95.22 ± 86.11^{a}	66.1 ± 36.1^{a}	386.1 ± 199.0^{b}	6.7 ± 2.99^{b}
Autumn	0.20 ± 0.05^{c}	1.60 ± 0.37^{b}	3.04 ± 0.7^{a}	0.34 ± 0.08^{a}	0.53 ± 0.35^{a}	102.0 ± 111.5^{a}	69.6 ± 41.93^{a}	514.1 ± 281.7^{a}	6.0 ± 4.76^{b}

The values represent means of three replications and ±SD; the means with different letters/letters are statistically significant at $p<0.05$

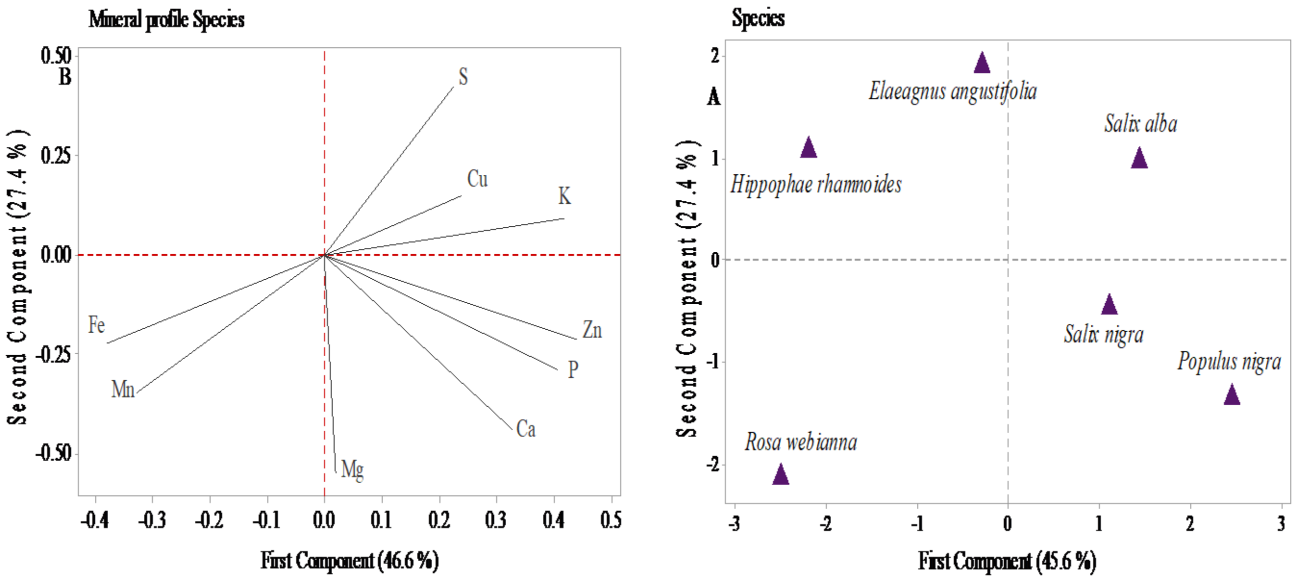

Fig. 2. Principle component analysis of fodder tree leaves and shrubs subject to species wise (a) and mineral profile wise (b).

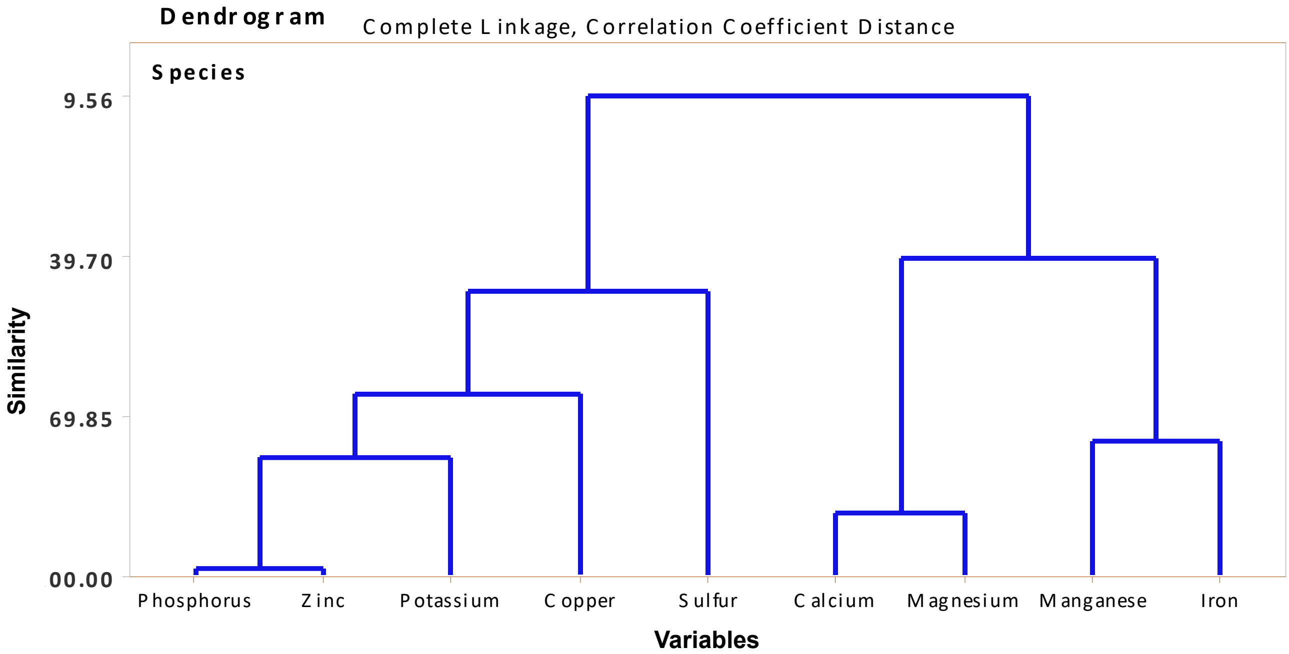

Fig. 3. Dendrogram of the mineral profile of tree leaves and shrubs based on species-wise concentration.

Discussion

Macro-minerals: Minerals are necessary for ruminant livestock to function at their best. Minerals are mostly obtained from plant sources by grazing animals. They can withstand vitamin insufficiency for longer than mineral shortage (Grunes & Welch, 1989). Minerals are chemical components that the body uses in a variety of ways. They play an important role in a diversity of human, domestic animal, and plant activities (Soetan *et al*., 2010). The macro and micro-mineral content of leaves from various temperate tree and shrub species are unknown, although they are required for animal growth, health, and reproduction (Smart *et al*., 1981; Prados *et al*., 2015). Mineral shortages result in a variety of unsatisfactory outcomes, including decreased output and reproductive capacity (Mohebbi-Fani *et al*., 2010). Ewes require 0.15–0.20 and 0.25–0.30% phosphorus for maintenance and lactation, respectively, according to Holechek *et al*., (1998). The concentration of phosphorus in fodder species decreases with maturity, which is consistent with Minson (1990), who reported a mean P concentration of 0.31% in the wet season and 0.08% with maturity, and Wilson (1969), who reported 0.8% in spring and 0.2% in fall. Sampson & Jesperson (1963) also found that deciduous trees and shrubs had acceptable phosphorus levels, with 0.8 percent in the spring and 0.2 percent in the fall, whereas non-deciduous shrubs showed low levels, with 0.22% in the spring and 0.11% in the fall. The P values found in this investigation were consistent with Anon., (2001) recommendations for dairy cow dietary needs (0.31–0.40% DM. Phosphorus needs for cattle range from

0.63–0.88% live weight increase to 0.65% live weight gain (Anon., 1997). Phosphorus concentrations were adequate for specific physiological stages of ewes in this investigation, but not for cattle. As a result, phosphorus should be supplemented all year, especially from the start of summer until the end of winter. According to a recent study, reducing dietary P from 0.57 percent to 0.37 percent had no negative impact on milk production but dramatically reduced P excretion into the environment (Wang *et al.,* 2014).

The potassium values of the current study were higher than the NRC's 1985 recommendation of 0.50% of diet DM for growing sheep and 0.80% for lactating ewes. NRC found that the K values of the evaluated species were higher than the dietary requirement (0.80% DM) for dairy cattle (2001). According to the findings of this study, phosphorus and potassium in diet dry matter are almost within the range of the maximum tolerated values (Anon., 2005) of phosphorus (0.6%) and potassium (2.0%). The most abundant mineral element is calcium. Bones and teeth comprise 99% of the calcium in an animal's body (Thompson, 1978). Calcium insufficiency is uncommon in grazing animals, according to Underwood (1981), except in high milk-yielding cows. The current Ca levels were higher than the crucial threshold suggested by Reid & Harvath (1980), who said that calcium insufficiency in animals is uncommon unless the grass contains less than 0.2% calcium. The current study indicated that calcium levels increased with the advent of maturity, agreeing with Minson (1990), who discovered that Ca levels are often low during active growth and high during slow growth. In other studies, no change in Ca occurred with the progress of maturity. According to Georgievskii (1981), the ideal level of calcium in plants is 0.4–0.6%, whereas levels beyond 1% are considered high. All the fodder species studied in this study had a Ca concentration of more than 1%, comparable to Gaikwad *et al*., (2021), an estimate of 2.18%, indicating their potential as a good supply of Ca for ruminants. In the present study, Ca content (2.64% DM) was higher than the NRC's recommendations (2001) for dairy cow dietary requirements (0.43–0.60% DM). Ca concentrations of more than 1% have been linked to reduced DM intake, as well as a reduction in micro-mineral absorption (particularly zinc) and dairy cattle performance (Anon., 2001). The maximum level of dietary Ca, according to Anon., (2005), is 1.5% DM, which is lower than the previous estimate of 2% DM (Anon., 1980). Hypercalcemia and soft tissue calcification are caused by calcium toxicity (Mc Dowell, 1989).

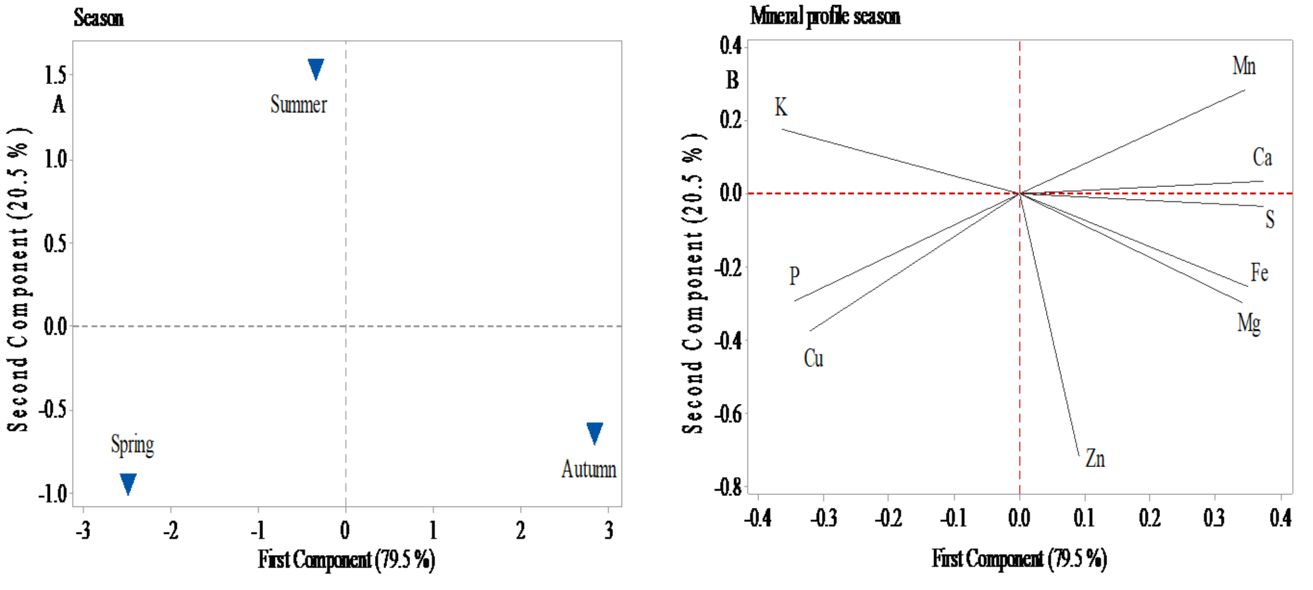

Fig. 4.Principle component analysis of fodder tree leaves and shrubs subject to season-wise (a) and mineral profile wise (b).

Ibeawuchi *et al*., (2002) reported Ca: P ratios of 1.52:0.35, 1.22:0.22 and 1.15:0.32 percent for heavily, moderately, and occasionally browsed species, respectively and Ahamefule (2006) found Ca: P ratios of 1.57:0.37, 1.38:0.39 and 1.45:0.37 percent for heavily, moderately, and occasionally browsed species, respectively. The current calcium and magnesium findings do not correspond with those of Fadel *et al*., (2002), who reported that changes in Ca and Mg content among fodder tree species throughout the dry season were inconsistent.

The current magnesium levels were above the required levels of 0.12, 0.15, and 0.18% of DM for growing lambs, ewes in late gestation, and ewes in early lactation, respectively (Anon., 1985), but below the maximum tolerable levels of 0.6% in the diet DM for growing lambs, ewes in late gestation and ewes in early lactation, respectively (Anon., 2005). While the current study's sulfur mean value (0.47% DM) was almost equal to the maximum acceptable concentration of 0.5% of DM for animals fed a high forage diet (Anon., 2005). Higher values were found in *Salix alba* and *Elaeagnus angustifolia* than what was considered acceptable. The absorption of selenium and copper is hampered by a high sulfur level in the diet. Therefore, feeding these fodder species as a sole source of nutrition for an extended period should be avoided. Mineral nutrition can significantly impact both female and male animal reproduction. It has been demonstrated that either a deficiency or an increase in quantity can affect reproduction (Balamurugan *et al.,* 2017).

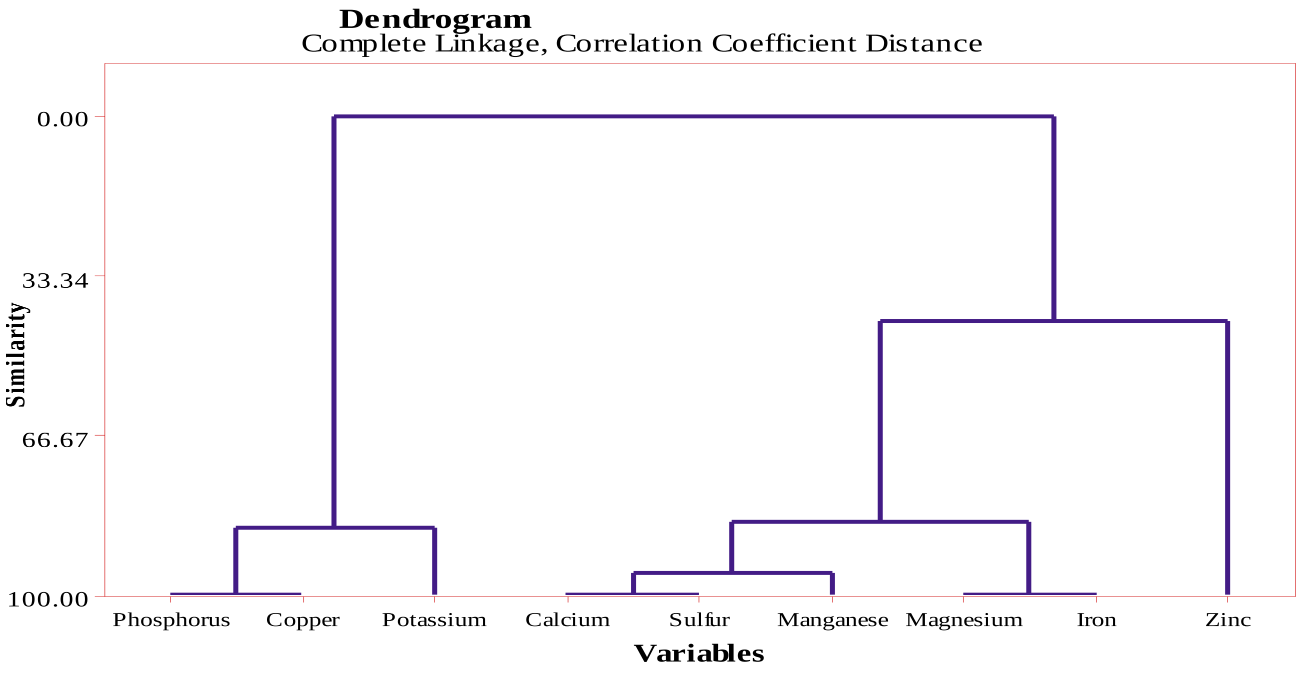

Fig. 5. Dendrogram of the mineral profile of tree leaves and shrubs based on season-wise concentration.

Micro-minerals: The findings of the present study for zinc were significantly different among fodder species and were in line with recommended levels of Anon., (1985) as for sheep, the requirements of zinc were set as 20mg Zn/kg DM for growing animals and 33mg Zn/kg DM for lactating ewes and maximum reproduction performance of males and females. Goats required only 10mg Zn/kg DM for growth, production, and reproduction. The NRC recommends 300mg Zn/kg DM for sheep as the optimal sustainable dose (2005). The current findings corroborated those of Negri *et al*., (1996), who found that poplars and willows collect higher levels of Zn than grassland plants. The dietary requirement of Zn varied from 20–33 mg/kg DM for sheep (Anon., 1985). The Zn contents of *Elaeagnus angustifolia* (23.9), *Hippophae rhamnoides* (28.0), and *Rosa webbiana* (20.7mg/kg DM) were within the recommended range, whereas the Zn contents of *Salix nigra* (183.4), *Salix alba* (122.9), and *Populus nigra* (217.5mg/kg DM) were above the recommended range; however, this could be influenced by high Ca levels (Mills *et al.,* 1967).

Manganese is necessary for a healthy progeny, as well as regular estrus, conception, pregnancy, and gestation (Doisey, 1973).The mean value of fodder species was higher than the dietary needs for growth and skeletal development of sheep, which were previously determined at 10 and 20–25 mg Mn/kg DM (Anon., 1990) and 20mgMn/kg DM, respectively by Anon., (1985). Manganese requirements for goats were set at 20–25 mgMn/kg DM of the diet by Anon., (1980), 40–50 mgMn/kg DM of the diet by Meschy (2000), 60–120 mgMn/kg DM of the diet by Lamand (1981) and 60 mgMn/kg DM of the diet by Anon., (1997). The current findings except for *Populus nigra,* were below the Anon., (2005) recommended maximum acceptable levels of 200 mgMn/kg DM of diet.

One of the most significant micro-minerals is iron. It is the most important component of hemoglobin and serves as the body's oxygen carrier. Iron levels in fodder species are below the NRC's recommended sustainable threshold of 500mgFe/kg DM (2005). *Rosa webbiana* and *Elaeagnus angustifolia* had greater values than the required levels in this study. Ivan *et al*., (1990) also found high levels of iron in forages, ranging from 549–990 mgFe/kg DM, which could lead to copper insufficiency in goats and lambs.

Copper levels in fodder species were above the recommended range of 5mg Cu/kg DM for sheep (Anon., 1975), later increased to 7–11 mgCu/kg DM (Anon., 1985). Anon., (1980) recommended a Cu/kg DM diet range of 1–8.6mg for sheep, depending on their physiological state. Underwood & Shuttle (1999) assumed a Cu/Kg DM of diet range of 4.3–28.4mg. Tolerable values of 15 mgCu/kg DM for sheep were supplied by Anon. (2005), while bearable levels for goats were not discovered. Except for Cu, the mean values of Zn and Fe in this study were higher than those discovered by Shekhar*et al*., (2017), who found mean values of Zn (29.07±0.373), Fe (145.34±8.0980), and Cu (12.07±0.251µg/g) in fodder. The present mean value of Cu was 8.39mg/kg as compared to the critical limit of 8ppm for dairy cattle (Anon., 2001). Mineral deficiencies and surpluses in plants vary according to agro-climatic conditions (Singh *et al*., 2011). The ability of plants to accumulate mineral substances within their bodies is influenced by the plant's development period, nutrient content, root structure, soil structure and mineral matter content in the growing area, and the amount and distribution of precipitation during the vegetation period (Chetri *et al*., 1999; Muhammad *et al*., 2013; Temel *et al*., 2016; Temel & Surmen, 2018; Temel, 2019). Trace mineral nutrition is critical for cattle's optimum development, performance, health, and reproduction, irrespective of the supplementing technique used. The total productivity of the cattle grazing system is dependent on efforts to meet the trace mineral requirements of grazing animals (Arthington & Ranches, 2021).

Conclusion

The macro and micro-minerals of the investigated species were in a moderate range. Mineral concentrations differ by species and season, requiring an appropriate feed plan for different seasons of the year to meet ruminant animals' nutritional demands. This knowledge may lead to opportunities to graze or gather leaves from specific species at different periods of the year to supplement the nutrition of animals that require more nutrients (lactating or young growing animals). Furthermore, it may allow animals with lower requirements to consume lower quality leaves (non-lactating mature animals).

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