***Pak. J. Bot.*, 56(3): 1041-1048, 2024.** **DOI: http://dx.doi.org/10.30848/PJB2024-3(31)**

PROXIMATE AND ELEMENTAL ASSESSMENT OF SIX SELECTED PLANT SPECIES AT VARIOUS PHENOLOGICAL STAGES FROM DISTRICT BAJAUR, PAKISTAN

AMINUL HAQ[1,2*] AND LAL BADSHAH[1]

[1]Phytoecology Lab. Department of Botany, University of Peshawar, Pakistan
[2]Department of Botany, Govt. Post Graduate College Khar, Bajaur, Pakistan
**Corresponding author's email: aminulhaq_bot@yahoo.com, aminulhaq.bot@gmail.com*

Abstract

Proximate and elemental analysis can be used to assess the nutritional value of plants. The health and feed of cattle are influenced by the phytochemical contents. This study is designed to investigate the proximate and elemental composition of selected plant species at three different phenological stages i.e. pre-reproductive, reproductive and post-reproductive. The proximate analysis revealed that *Conyza canadensis* had the highest content of carbohydrates (59.2%) in all six species. *Olea ferruginea* was found with the highest concentration of fiber (37%) and protein (18.38%), while *Rubus fruticosus* had the highest content of fibers (33%), protein (13.13%) and moisture (10.6%) next to carbohydrates. The proximate parameter's correlation matrix showed a strong negative correlation. The highest concentrations of the macro-nutrients N (120950 µg/g), P (10900 µg/g), K (82575 µg/g), Ca (62550 µg/g) and Mg (3675 µg/g) were found in *Conyza canadensis* in the pre-reproductive and reproductive stages. Elevated concentrations of micro-nutrients like Cu, Fe and Zn were detected in *Rubus fruticosus, Daphne mucronata* and *Alnus nitida.* Potassium had a significant correlation with Ca (0.768***) and Mg (0.761***) while nitrogen has a negative correlation with all macro-nutrients. Fe has a significant correlation with Zn (0.649**) and Cu displayed a negative correlation with all macro-nutrients. The proximate and minerals profiles of the selected plants were found within permissible limits and the concentrations were similar to those reported in other regions of Pakistan.

Key words: Palatability, Grazing, Preferences, Macro-nutrients, Micro-nutrients.

Introduction

The nutritive and medicinal potential of plants can be learned from their chemical profiling (Hameed & Hussain, 2015). The nutritional value of a plant is assessed by its proximate and elemental analysis (Hussain *et al.*, 2021; Batool *et al.*, 2022). The physiological function and metabolism of both humans and cattle are significantly impacted by the nutritional and elemental composition of the diet (Gull *et al.*, 2015). Cattle health and performance are impacted by the availability and concentration of nutrients (Khan *et al.*, 2013). The proximate composition and elemental content of feed plants may be affected by various factors, including plant species, their phenological stages, climate and harvesting season (Minson, 1990; Jumba *et al.*, 1996). Plant nutrient levels vary according to their phenological stage, weather, grazing pressure, soil fertility and geographic distribution (Khan *et al.*, 2018). Nutrient deficiency in livestock is associated with many physiological abnormalities, reproductive problems and illnesses. Low milk production and other physiological disorders are directly related to the mineral deficiency of major and trace elements (Khan *et al.*, 2013). The overwhelming majority of compounds with medicinal and dietary benefits are found in plants (Edeoga & Gomina, 2001).

The livestock industry is one of the agricultural subsectors with the fastest growth. The livelihoods and nutritional security of nearly a billion people depend on livestock (Robinson *et al.*, 2014; Geng *et al.*, 2020). The production of crops and cattle is competing more ferociously for agricultural land and manpower as a result of an unexpected increase in consumer demand for animal goods. The cost of commercial livestock feed is increasing while at the same time environmental stress, such as heat stress, is lowering milk production (Geng *et al.*, 2020). The sustainability of traditional smallholder livestock farmers in the mountains is imperiled by factors such as the scarcity of grazing pasture for livestock, the high cost of commercial feed and increasingly rising environmental stresses (Toth *et al.*, 2017).

The relevance of any fodder species depends on its flavour and nutritional content because fodder is provided to animals for a specified purpose (Khan *et al.*, 2018). Nutritious feed security is crucial for ensuring optimum agricultural output (FAO, 2006; White *et al.*, 2012). Plants are rich in essential elements and there is a strong link between their nutritional value and growth (Newall *et al.*, 1996). The nutrition of grazing animals is a dynamic interaction of soils, plants and animals (Khan *et al.*, 2007). Seasonal variation can significantly alter the composition of minerals consumed through feeding, as well as have an impact on plant growth, pasture supply and soil moisture content (Smith & Loneragon, 1997). The objectives of this research were to investigate the proximate composition and elemental analysis of macro-nutrients (N, P, K, Ca and Mg) and micro-nutrients (Cu, Fe, Zn, Mn and Na) in the pre-reproductive, reproductive and post-reproductive stages of the plants. These plants are grown well in the valley, are easily accessible throughout the year, and may have nutritional benefits to be utilized as feed. The palatability preferences of herbivores in a given area are determined by the varying mineral and proximate composition of plants, therefore, this analysis will contribute to enumerating the palatability status of these plants in future studies.

Material and Methods

Plants samples collection, drying and grinding: Six plant species i.e., *Alnus nitida*, *Conyza canadensis*, *Daphne mucronata*, *Heteropogon contortus*, *Olea ferruginea* and *Rubus fruticosus* were collected at three phenological stages (pre-reproductive, reproductive and post-reproductive). These species were identified with the flora of Pakistan (Ali & Qaiser, 1995-2018). The voucher specimens were deposited in the herbarium of the Department of Botany, University of Peshawar. The collected parts of plants were cleaned thoroughly with deionized water and spread over paper for air drying. Following air drying, oven-dried for 72 hours at 65°C. The samples were then ground into powder. The powdered dry samples were put in plastic bags for further analysis.

Proximate analysis: The moisture content, total ash, crude fiber, crude oil and crude protein of the powdered samples were determined using standard methods (Anon., 2016; Hussain *et al*., 2021).

$$\text{Moisture content (\%)} = \frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

$$\text{Total ash (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \qquad \text{Crude fiber (\%)} = \frac{W2 - W3}{W1} \times 100$$

where "W1" is the weight of the sample that has been dried out completely and extracted with ether, "W2" is the weight of the sample that has been cooled for 30 minutes and "W3" is the weight of the sample that has been cooled for an hour.

$$\text{Crude fat (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

$$\text{Crude protein (\%)} = \frac{\text{ml. of 0.1N sulphuric acid used} \times 0.1\ \text{(normality)} \times 1.4007 \times \text{dilution factor} \times F}{\text{Weight of sample}} \times 100$$

To calculate the amount of non-fiber carbohydrates (NFC), the following formula was employed (Unuofin *et al*., 2017).

NFC (%) = 100 – (% Ash + % Crude fat + % Crude protein + % Crude fiber

The correlation matrix between the six parameters was calculated using SPSS software, version 16.0 (Beshaw *et al*., 2022).

Heatmaply and ggplot2 analysis: A cluster heat map was evaluated for plant proximate analysis using the Heatmaply and ggplot2 packages in R programming to understand more about the correlation and coherence among the proximate variables (Galili *et al*., 2018).

Elemental analysis: The solutions for various elements were examined using an atomic absorption spectrometer, a Shimadzu AA-670 and the appropriate hollow cathode lamps. The required standard calibration curves were generated by calculating the measurements of various elements using standard AR-grade solutions.

Jaffrey amazing statistic program (JASP): A correlation-based heat map for the analysis of plant macro-nutrients and micro-nutrients was evaluated using JASP statistical software version 0.9 (Love *et al*., 2019).

Results

Proximate and elemental analysis: The nutritional value and palatability status of plants are determined by their proximate composition and elemental contents (Akinleye *et al*., 1996). The palatability of forage plants is determined by their proximate and elemental composition. At various phenological stages, different plant species have distinctive proximate and elemental concentrations. The current study analyzed the proximate and elemental compositions of six plants (Table 1).

***Alnus nitida* (Spach) Endl. Gen.:** Proximate analysis revealed that the reproductive stage of *Alnus nitida* had the maximum moisture content (9.6%), while the pre and post-reproductive stages had the lowest ash content (5.5%) respectively. The reproductive stage had higher protein (18.38%), fiber (16.0%) and fat (4.1%) contents than the post-reproductive stage which had a higher content of carbohydrates (57.3%) (Table 2). The current study shows that at the pre-reproductive stage, the concentrations of nitrogen, potassium, calcium and magnesium in the macro-elements were 98850 µg/g, 17850 µg/g, 36175 µg/g and 3350 µg/g respectively. Compared to the reproductive and post-reproductive stages, these concentrations were higher, except for the reproductive stage having the greatest phosphorus level (32350 µg/g). Similarly, sodium and manganese concentrations in micro-elements varied somewhat throughout the three phenological periods. At the post-reproductive stage, the maximum Fe concentration (3300 g/g) was observed (Table 3).

***Conyza canadensis* (L.) Cronquist:** *Conyza canadensis* proximate composition shows that moisture content (10.2%) was nearly the same at all three phenological stages, while carbohydrate content (59.2%) was higher at the pre-reproductive stage, protein (14%), fiber (16.5%), ash (15.5%) and lipid (1.1%) contents were higher in the reproductive stages (Table 2). Macro-elements like nitrogen concentration were found to be at their highest level (67925 µg/g) during the pre-reproductive stage. The highest concentrations of calcium (62559 µg/g), potassium (82575 µg/g), magnesium (3675 µg/g) and phosphorus (10900 µg/g) were detected in the reproductive stage. The values of P content in the current study exhibit variation at various phenological stages. While manganese (3560 µg/g), zinc (2220 µg/g) and iron (1150 µg/g) were estimated more at the reproductive stage, sodium content (6120 µg/g) was assessed more at the post-reproductive stage (Table 3).

***Daphne mucronata* Royle:** The proximate analysis of *Daphne mucronata* shows that the highest concentrations of carbohydrate (53.55%) and lipid (3.1%) were found at the pre-reproductive stage, fiber (28.5%) and protein (7.28%) were found at the reproductive stage and moisture (10.2%) and ash (5.5%) were found at the post-reproductive stage (Table 2). At both the reproductive and pre-reproductive stages, the nitrogen content in micro-elements was 120000 µg/g. The highest concentration of calcium (20475 µg/g) and manganese (2300 µg/g) was found in the post-reproductive stage, respectively. In the pre-reproductive stage, there was a higher concentration of potassium (12300 µg/g). The levels of micro-elements varied between the three phenological stages, with the post-reproductive stage having the greatest copper and sodium concentrations at 2120 µg/g and 1960µg/g, respectively (Table 3).

***Heteropogon contortus* (L.) P. Beauv. ex. Roem & Schult.:** The pre-reproductive stage of *Heteropogon contortus* had the highest concentrations of carbohydrates (58.1%) and protein (10.5%), while the reproductive stage had higher concentrations of fiber (34.5%), moisture (9.3%), ash (5.5%) and fat (1.3%) (Table 2). The elemental composition shows that at the reproductive stage, nitrogen concentration was 105125 µg/g, potassium was 18200 µg/g, magnesium was 900 µg/g and phosphorus was 560 µg/g. Similarly, at the pre-reproductive stage, the calcium content was 7425 µg/g. The highest concentrations of iron (3440 µg/g) and zinc (3320 µg/g) in the micro-elements were found at the post-reproductive stage, as shown in Table 3.

***Olea ferruginea* Wall. ex Aitch.:** The pre-reproductive stage of *Olea ferruginea* had a high protein content (7.88%), moisture content (7.77%) and ash content (6.6%), whereas the reproductive stage had a greater fiber content (37.0%) and the post-reproductive stage had the highest carbohydrate (42.6%) and lipid (16.4%) contents (Table 2). When it came to macro-elements, the pre-reproductive stage had a magnesium content of 3025 µg/g and calcium content of 43625 µg/g, a phosphorus content of 2125 µg/g and a nitrogen content of 111400 µg/g. The stage after reproduction had the maximum potassium levels, 22500 g/g (Table 3). The post-reproductive stage has the maximum copper concentration in micro-elements, at 3600 µg/g. With high concentrations of 2140 µg/g and 3380 µg/g, respectively, of iron and manganese, the reproductive stage had high iron and manganese levels. In the same way, during the post-reproductive phase, zinc concentration was 3600 µg/g, while sodium concentration was 2410 µg/g (Table 3).

***Rubus fruticosus* L.:** The approximate composition of *Rubus fruticosus* reveals that the reproductive stage had the highest levels of fiber (33%) and protein (13.13%), the post-reproductive stage had the highest levels of carbohydrate (53.1%), moisture (10.6%) and lipid (1.0%), while the pre-reproductive stage had the highest levels of ash (6%) as shown in table 2. At the reproductive stage, the concentration of macro-elements such as nitrogen (111375 µg/g), magnesium (3050 µg/g), calcium (21525 µg/g) and phosphorus (1225 µg/g) was highest, whereas potassium level (118700 µg/g) was highest at the post-reproductive stage (Table 3). In micro-nutrients, the reproductive stage was found to have high concentrations of copper (3650 µg/g), iron (2530 µg/g) and sodium (1850 µg/g), whereas the pre-reproductive stage had the highest zinc content (1680 µg/g) and the post-reproductive stage had the highest level (1850 µg/g) of manganese (Table 3).

Table 1. Forage plants selected for proximate and elemental analysis.

Species / Family	Habit	Distribution	Palatability classes
Alnus nitida (Spach) Endl. Gen., **Betulaceae**	Tree	Occasional	Non-palatable
Conyza canadensis (L.) Cronquist, **Asteraceae**	Herb	Common	Moderately palatable
Daphne mucronata Royle, **Thymelaeaceae**	Shrub	Common	Non-palatable
Heteropogon contortus (L.) P. Beauv. ex. Roem & Schult., **Poaceae**	Herb	Common	Less palatable
Olea ferruginea Wall. ex Aitch., **Oleaceae**	Tree	Common	Moderately palatable
Rubus fruticosus L., **Rosaceae**	Shrub	Common	Highly palatable

Table 2. Proximate analysis at different phenological stages.

Parameters and mean value	**Species name and phenological stages**																	
	Alnus nitida			***Conyza canadensis***			***Daphne mucronata***			***Heteropogon contortus***			***Olea ferruginea***			***Rubus fruticosus***		
	PR (%)	**R (%)**	**POR (%)**	**PR (%)**	**R (%)**	**POR (%)**	**PR (%)**	**R (%)**	**POR (%)**	**PR (%)**	**R (%)**	**POR (%)**	**PR (%)**	**R (%)**	**POR (%)**	**PR (%)**	**R (%)**	**POR (%)**
Moisture	9.5	9.6	9.0	10.1	10.2	9.5	9.2	9.8	10.2	9.1	9.3	9.4	7.7	7.2	7.3	10.0	10.4	10.6
Mean	**9.36 ± 0.05**			**9.93 ± 0.22**			**9.73 ± 0.29**			**9.26 ± 0.08**			**7.40 ± 0.15**			**10.33 ± 0.17**		
Ash	5.5	5.5	5.2	10.5	15.5	14	4.5	4.0	5.2	4.0	5.5	3.7	6.6	4.0	6.0	6.0	6.0	5.3
Mean	**5.4 ± 0.1**			**13 ± 1.48**			**4.56 ± 0.34**			**4.40 ± 0.55**			**5.53 ± 0.78**			**5.8 ± 0.23**		
Fiber	10.5	16	12.5	9.5	16.5	10.2	21.0	28.5	22.4	17.5	34.5	30.5	34.0	37.0	32.0	33.0	31.0	30.0
Mean	**13.0 ± 1.61**			**12.07 ± 2.23**			**23.97 ± 2.30**			**27.50 ± 5.13**			**34.33 ± 1.45**			**31.33 ± 0.88**		
Fat	3.5	4.1	2.5	0.2	1.1	0.2	3.0	2.3	2.5	0.8	1.3	1.2	1.8	8.0	5.4	0.5	0.5	1.0
Mean	**3.36 ± 0.46**			**0.5 ± 0.30**			**2.6 ± 0.21**			**1.10 ± 0.15**			**5.06 ± 1.79**			**0.66 ± 0.16**		
Protein	18.38	11.38	13.5	10.5	14.0	12.5	8.75	7.88	6.7	10.5	4.38	4.5	7.88	7.0	6.7	12.25	13.13	11.5
Mean	**14.42 ± 2.01**			**12.33 ± 1.02**			**7.77 ± 0.59**			**6.46 ± 2.02**			**7.19 ± 0.35**			**12.69 ± 0.47**		
NFC	52.62	53.42	57.3	59.2	42.7	54.1	53.55	47.52	53.0	58.1	45.02	50.7	42.02	36.8	53.1	38.25	38.97	53.1
Mean	**54.45 ± 1.44**			**52.0 ± 4.87**			**51.36 ± 1.93**			**51.27 ± 3.78**			**40.47 ± 1.84**			**43.44 ± 4.84**		

Key: PR= Pre-reproductive, R= Reproductive, POR= Post-reproductive

Table 3. Elemental analysis at different phenological stages.

Plant species, phenological stages	Macro-elements (µg/g)					Micro-elements (µg/g)				
	N	K	P	Ca	Mg	Cu	Fe	Mn	Zn	Na
Alnus nitida										
PRP	98850	17850	2425	36175	3350	1210	3075	4110	2660	4660
R	80325	11725	1400	28975	2725	1280	1425	4330	2710	4700
PR	55650	20360	32350	33525	2250	1310	3300	4360	2740	4720
Mean	**78275**	**16645**	**12058**	**32892**	**2775**	**1267**	**2600**	**4267**	**2703**	**4693**
Conyza canadensis										
PRP	120950	51900	4525	36500	2775	760	1550	1210	1140	1440
R	67925	82575	10900	62550	3675	1110	1225	3560	2220	1480
PR	89000	69350	12875	59300	2950	1140	1250	1590	1290	6120
Mean	**92625**	**67942**	**9433**	**52783**	**3133**	**1003**	**1342**	**2120**	**1550**	**3013**
Daphne mucronata										
PRP	120000	12300	2350	15275	1925	1120	3350	2220	3540	1870
R	120000	11175	2850	20475	1225	2010	3411	2520	3610	1910
PR	96500	8475	2035	17500	2300	2120	3550	3660	3630	1960
Mean	**112167**	**10650**	**2412**	**17750**	**1817**	**1750**	**3437**	**2800**	**3593**	**1913**
Heteropogon contortus										
PRP	72575	14900	125	7425	875	1310	3330	1980	2140	1650
R	105125	5275	87.5	5625	575	1970	3370	2011	2260	1660
PR	88350	18200	560	4575	900	2010	3440	2120	3220	1720
Mean	**88683**	**12792**	**257.5**	**5875**	**783**	**1763**	**3380**	**2037**	**2540**	**1677**
Olea ferruginea										
PRP	103100	14375	2125	43625	3025	3310	2025	2320	2110	2120
R	111400	15475	350	35200	1900	3530	2140	3380	1690	2230
PR	91750	22500	1150	41000	2850	3600	1850	2400	2212	2410
Mean	**102083**	**17450**	**1208**	**39942**	**2592**	**3480**	**2005**	**2700**	**2004**	**2253**
Rubus fruticosus										
PRP	79325	13450	430	16850	2825	2520	2200	1740	1680	1786
R	111375	13400	1225	21525	3050	3650	2530	1700	596	1850
PR	87900	18700	975	19300	2450	2050	1270	1850	1050	1585
Mean	**92867**	**15183**	**876.7**	**19225**	**2775**	**2740**	**2000**	**1763**	**1109**	**1740**

Table 4. Proximate parameters correlation matrix.

Parameters	Correlation coefficient	P-value	Status
Ash vs Carbohydrate	-0.98	0.354 (>0.05)	NS
Ash vs Fat	-0.038	0.062 (>0.05)	NS
Ash vs Fiber	-0.475	0.027 (<0.05)	SNC
Ash vs Protein	0.4	0.056 (>0.05)	NS
Fat vs Carbohydrate	0.106	0.106 (> 0.05)	NS
Fat vs Fiber	0.176	0.176 (>0.05)	NS
Fiber vs Carbohydrate	-0.761	0.00 (<0.05)	SNC
Moisture vs Ash	0.252	0.165 (>0.05)	NS
Moisture vs Carbohydrate	0.337	0.093 (>0.05)	NS
Moisture vs Fat	-0.709	0.001 (<0.05)	SNC
Moisture vs Fiber	-0.353	0.083 (>0.05)	NS
Moisture vs Protein	0.407	0.053 (>0.0.5)	NS
Protein vs Carbohydrate	0.168	0.260 (>0.05)	NS
Protein vs Fat	-0.289	0.234 (>0.05)	NS
Protein vs Fiber	-0.621	0.004(<0.05)	SNC

Key: NS= Non-significant, SNC= Significant negative correlated

Correlation matrix of proximate parameters: According to the statistical analysis of the data for the proximate content, there is a significant inverse relationship between moisture and fat (-0.709), moisture and fibers (-0.352), ash and fat (-0.0388), ash and carbohydrates (-0.98), protein and fat (0.189), protein and fibers (-0.621), fibers and carbohydrates (-0.761) as shown in (Table 4). The Heat map tree diagram illustrated the distribution of various proximate concentrations in different phenological stages of six chosen plant species, including non-fibrous carbohydrates (NFC), fiber, moisture, ash, fat and protein. Dark colour has been used to indicate the highest concentration, while lighter colour has been used to represent the lowest concentration. To determine the stage at which these proximate concentrations differ from one another within a species, we have assessed them. *Conyza candensis* showed the highest concentration of NFC throughout the reproductive and post-reproductive stages, whereas *Heteropogon contortous* showed the highest concentration of NFC during the pre-reproductive stage. In *Olea feruginea*, the pre-reproductive and post-reproductive stages showed a high content of fibers (Fig. 1).

Correlation matrix of nutrient contents

Correlation among the macro-nutrients: The plant species' concentrations of various macro-nutrients at various phenological stages were examined using a correlation-based heat map. Various macro-nutrients were evaluated, including N, K, P, Ca and Mg. In a correlation-based heat map diagram (Fig. 2a), the concentration and p* values for each macro-nutrient have been graphed. The K has a substantial link with both Ca and Mg (p* value 0.768*** and 0.497*). Nitrogen has a negative correlation with all macro-nutrients, as shown by the heat map. While Ca has a significant correlation with K (p* value 0.768***), P (p*0.423*) and Mg (p*0.761***). Similarly, P has a positive correlation with Ca (p* value 0.423*) and Ca correlates with K (p* 0.768***) and Mg (p* 0.761***). Mg has a negative correlation with N (-0.141), K (0.497*) and Ca (0.761***).

Correlation among the micro-nutrients: The correlation-based Heat map also explained the concentration of important micro-nutrients at distinct phenological stages in six plant species. Cu, Fe, Mn, Zn and Na were the micro-nutrients that were assessed. The correlation-based heat map diagram has been used to graph the concentration and p* values for each micro-nutrient. (Fig. 2b) illustrates that Fe has a significant correlation with Zn (p* value 0.649**), Mn with Zn (p* value 0.496*) and Na (p* value 0.437*), whereas all macro-nutrients showed a negative correlation with Cu.

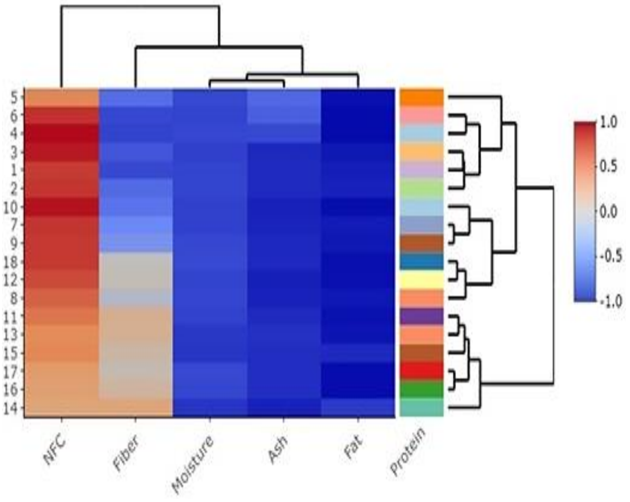

Fig. 1. Clustered heat map showing correlation and coherence among the proximate parameters.

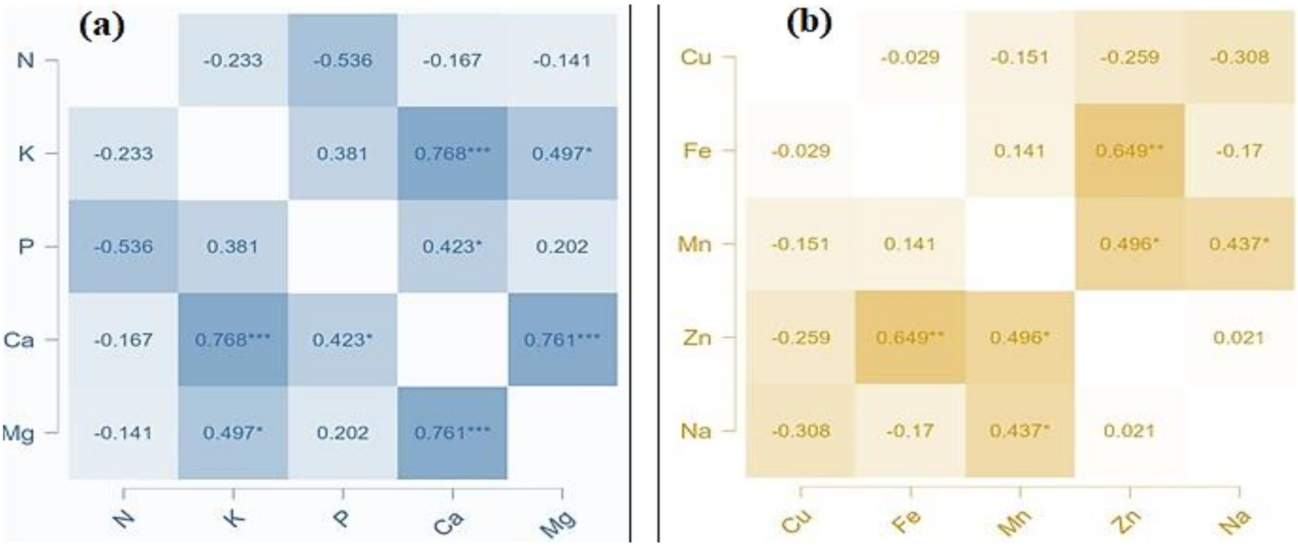

Fig. 2. Heat map showing the correlation among the macro-nutrients (a) and micro-nutrients (b)

Discussion

Proximate analysis has a serious influence on the suitability of plant species for specific livestock requirements in various phases and subsidiary communities. The inhabitants frequently graze livestock in the area to build their livelihoods. The main issue in the study area was the low productivity of cattle due to their poor health. Six fodder plant species from Pashat Valley, Bajaur, were assessed for their ash, carbohydrate, protein, fiber, fat, moisture and elemental content in three phenological stages. The nutritional requirements of livestock vary with the age and physiological functions of grazing animals such as maintenance of growth, fattening and lactation. The efficiency of range animals will depend on the availability of plant species with high nutritional value to primary consumers.

The proximate and elemental composition of forage plants is used to quantify their palatability. Different plant species have unique proximate and elemental concentrations at various phenological stages. Differences in moisture content among the species result from the physiological nature of the plant and the climate condition (Begum *et al*., 2018; Hussain *et al*., 2021; Anwar *et al*., 2022; Batool *et al*., 2022; Shahid *et al*., 2023). Additionally, the proximity of plants changes according to their phenological stage, climate, grazing pressure, soil texture and geographic distribution (Khan *et al*., 2018). In the present investigation, high moisture content for *Rubus fruticosus* and low moisture content for *Olea ferruginea* were found. Plants with green leaves have more moisture than dry ones (Sheikh *et al*., 2019). The moisture level of a feed ingredient is important because it affects only the weight of the feed and not its nutritional value for the animals. The normal moisture content permits the water-soluble enzyme and coenzyme activity required for the plant's metabolic processes (Adamu *et al*., 2017).

The pre-reproductive stages of plants consistently contain more crude protein than the reproductive and post-reproductive stages (Ganskopp & Bohnert, 2003; Hussain & Durrani, 2008). The protein content of the examined species ranged from 6.46±2.02 % (*Heteropogon contortus*) to 14.42±2.01 % (*Alnus nitida*). Due to the high concentration of crude protein, grazing animals often prefer the pre-reproductive stage of *Heteropogon contortus* over the reproductive and post-reproductive stages (Sultan *et al*., 2008). The availability of energy is directly correlated with the uptake and digestion of protein (McCarthy *et al*., 1989). The health of animals is connected to the range of plants' protein content (Ahmed *et al*., 2013). Protein-rich plants help in tissue growth and repair, the regulation of biological processes and the production of hormones, enzymes and other substances that assist the body in fighting infection (Ogidi *et al*., 2019). Usually, cattle graze more during the spring and summer because of the abundance availability of proteins that support animal growth and development (James *et al*., 2010). Along with protein content, dietary fat enhances feed palatability and also retains its flavor (Antia *et al*., 2006). The concentration of fat was found highest for *Olea ferruginea* (5.06±1.79%) and lowest for *Conyza canadensis* (0.5±030%). A species' usefulness as a feed source, as well as its level of palatability and vital function in energy production, are indicated by its highest fat content (Dangoggo *et al*., 2012). The high protein content of plants suggests that they can significantly help meet daily protein needs (FAO, 2006).

Crude fiber is made up of plant cell structural elements such as cellulose, hemicellulose, lignin and pectin. As the plant becomes mature, the content of crude fiber increases (Ahmed *et al*., 2013). The content of crude fiber ranges from 34.33±1.45% for *Olea ferruginea* to 12.07±2.23% for *Conyza canadensi*s. It is essential for the digestion of food materials in animals' digestive tracts (Deka *et al*., 2013). Because of its well-balanced nutritional composition, *Conyza canadensis* was found to be more palatable and preferred by animals in the pre-reproductive stage than in the reproductive and post-reproductive stages (Haq & Badshah). This supports previous research by Hussain & Durrani (2008) and Ahmad *et al*. (2014) that found that in early developmental stages, herbaceous plants and grasses are rich in nutritional content. The findings also revealed that the plant fiber content had a beneficial nutritional effect because dietary fiber is known to improve the body's assimilation of trace minerals and decrease the absorption of cholesterol (Adamu *et al*., 2017). Crude fiber is crucial for preserving the bacterial balance and overall health of the hindgut (Cherian, 2020). The digestibility of food is increased by a larger fiber intake, although a high intake sometimes can irritate the intestines (Hussain *et al*., 2010). The content of carbohydrates was found in the range from 40.47±1.84 to 54.45±1.44 in the various phenological stages of the selected species. The current findings are consistent with (Satter *et al*., 2016) whose reported range for the carbohydrate content of wild plants was 52.78-76.34%).

The proximate parameters showed both significant and non-significant correlation coefficients. We analyzed the statistically significant and non-significant data to check for any link between these variables. It has been found that species with a high moisture content also contain a low amount of fat and fiber. A high ash concentration also reduces the quantities of fat and carbohydrates. Protein and fat content, as well as protein and fibers, act as opponents. Additionally, the fiber and carbohydrate contents interact negatively.

Cattle receive health benefits from fodder plants that are directly related to their nutritional value. Mineral components are essential for animal metabolism and active metabolite development. Mineral deficiency causes poor growth, delayed development, and abnormalities. Forage plants' elemental content directly correlates with nutritional value (Khan *et al*., 2018). According to Onwuka (2005), the mineral composition is often evaluated based on ash content and this ash content indicates the mineral composition that may be the source of any therapeutic benefit (Tomescu *et al*., 2015). *Conyza canadensis'*s pre-reproductive and reproductive stages have the highest concentrations of N and K, whereas the reproductive stage has higher concentrations of Ca and Mg. Hussain *et al*., (2021) evaluated the high concentration of K, Mg and Ca in the pre-reproductive stage of the same plant species in a related study. This is consistent with the current study's conclusion. Magnesium is required for the proper functioning of the enzymes involved in the metabolism of carbohydrates, while calcium insufficiency has the consequences of poor development, delayed growth and several malformations (Naqbi *et al*., 2022). Results for macro elements indicated that potassium content was at the peak during the post-reproductive stage, while nitrogen, magnesium, phosphorus and calcium content was highest during the reproductive stage. According to Ahmad *et al*., (2008), there are constant variations in the potassium concentration in various plant parts and phenological stages. My findings are supported by Khan *et al*., (2013) who investigated that post-reproductive stages had the greatest potassium contents. Similarly, the plants had a high concentration of phosphorus in the early stages of growth, but as they mature, this concentration diminishes (Ganskopp & Bohnert, 2003). Samreen *et al*. (2016) found that all pre-reproductive forage species had high phosphorus concentrations, which is consistent with the current findings. The proximate and mineral contents of plants fluctuated during the phenological stage as a result of the soil's differing mineral composition, which is consistent with the findings of Hussain & Durani (2009) and Ahmad *et al* (2014). In micro-nutrients, the post-reproductive stage of *Daphne mucronata* had the highest concentration of Fe and Zn while *Olea ferruginea*'s had the highest concentration of Cu. Overall, the post-reproductive stages of the selected plants showed higher concentrations of Na, Zn, Mn, and Cu. In this investigation, the levels of Cu, Fe and Zn are within acceptable limits. Cu is a key component of an enzyme that incorporates Fe into red blood cells and prevents anemia. Cattle with a copper deficiency often have anemia and deformed bones. Manganese is recognized as an antioxidant nutrient and is essential for the metabolism of lipids and cholesterol, while zinc, is a component of several metalloenzymes and promotes wound healing (Naqbi *et al*., 2022). The variations in mineral content might be attributed to climatic and edaphic factors. This is consistent with the findings of Khan *et al*., (2018), who reported fluctuations in mineral content caused by environmental factors. The mineral element deficiency affects digestibility and intake, which lowers animal productivity (Khan *et al*., 2013). Changes in the composition of macro- and micro-nutrients have a great impact on a plant's nutritional value. Hussain & Durrani (2008) reported a similar concentration in plants from the Harboi rangeland, which is consistent with this finding. The research is also strongly supported by the results of Khan *et al*., (2007), Hussain & Durani (2008), Khan *et al*., (2013), Ahmad *et al*., (2014), Tariq *et al*., (2015), Ali *et al*., (2018), Khan *et al*., (2018), Shaheen *et al*., (2020) and Hussain *et al*., (2021).

Literature review shows that a wide range of variables, including soil type, chemical composition, growing conditions and seasonal variations, can affect a plant's nutritional value (Abule, 2003). Usually, palatable species of leafy plants with a short stem, low leaf table and weak leaves are preferred (Meissner, 1997; de Lucena *et al*., 2012). They claim that animals allowed to forage freely can choose the best species, avoiding dangerous or less nutritious ones (Shaheen *et al*., 2020). They back up this claim by comparing the milk yield of cattle fed on fodder to that of animals that are allowed to graze freely, as well as by stating that when free grazing is unavailable during the dry season, animal health and milk yield drastically decline (Meissner, 1997; Provenza *et al*., 2003).

Conclusion

The goal of this study was to determine the proximate and elemental composition of the plant during different phenological stages that affect herbivores' preferences for palatability. The quantitative analysis of the proximate parameters such as moisture, ash, crude protein, fats and non-fibrous carbohydrates with elemental contestants of the six plants were examined in the different phenological stages. *Olea ferruginea* was found to have a significant amount of fat and fiber, *Alnus nitida* had more protein. Ca, Mg and K levels were observed to increase as the plants became mature. Ca levels were declining in the case of *Heteropogon contortus,* but other nutrients showed more variability across all phenological stages. Almost all plants had an increase in Fe, Mn, Mg and Ca levels in pre-reproductive and post-reproductive periods. In the various phenological stages, high content of Cu, Fe, Mn, Na, and Zn lead to non-palatability, whereas low concentrations of these elements combined with high proportions of moisture, protein, and carbohydrates promote plant palatability. It was concluded that proximate and elemental analysis provides accurate feed assessments by comparing feed nutrients that are essential for animal health, milk production and defence against microbial infections. The proximate and mineral profiles of the selected plants were found within permissible limits and did not differ from those examined in other regions of Pakistan.

Acknowledgments

This work is part of the first author's Ph.D. dissertation. For proximate analysis, the authors acknowledge the laboratory staff from the Feed and Water Testing Laboratory of the Poultry Research Institute, Murree Road, Shamsabad Rawalpindi, Pakistan, and the Centralized Research Laboratory (CRL) of the University of Peshawar for elemental analysis of plant samples. The authors also acknowledge the help of Mr. Abdullah, Ph.D. Scholar, Quaid-e-Azam University, Islamabad, Pakistan for the statistical analysis of the research data.

References

Abule E. 2003. Rangeland evaluation in relation to pastoralists' perceptions in the Middle Awash valley of Ethiopia. PhD thesis submitted to the University of the Free State, Bloemfontein, South Africa, pp 297.

Adamu, A.U., E.D. Paul, C.E. Gimba and I.G. Ndukwe. 2017. Phytochemical and proximate analysis of *Aspillia kotschyi* (Sch. bipex, hochst) Oliv. *Niger. J. Technol.,* 36(4): 1135-1137.

Ahmad, K., M. Ashraf, Z.I. Khan and E.E. Valeem. 2008. Evaluation of macromineral concentrations of forages in relation to ruminants' requirements: a case study in Soone Valley, Punjab, Pakistan. *Pak. J. Bot*., 40(1): 295-299.

Ahmad, K., Z.I. Khan, A. Ashfaq, M. Ashraf and S. Yasmin. 2014. Assessment of heavy metal and metalloid levels in Spinach (*Spinacia oleracea* L.) growth in waste water irrigated agricultural soil of Sargodha, Pakistan. *Pak. J. Bot.*, 46(5): 1805-1810.

Ahmed, K., M. Shaheen, F. Mirzaei, Z. I. Khan, S. Gondal, A. Fardous, A. Hussain, F. Arshad and Mehmood. 2013. Proximate analysis: Relative feed values of various forage plants for ruminants investigated in a semi-arid region of Punjab, Pakistan. *Agric. Sci.*, 4(6): 302 308.

Akinleye, O.B., O.E. Adu and I.A. Ayeni. 1996. Studies of some biological and chemical characteristics of Mexican sunflower (*Tithonia diversifolia*). *Consult. Res. J.*, 1(1): 35-38.

Ali, A., L. Badshah and F. Hussain. 2018. Screening of five plant species for macro/micro-nutrients and heavy metals at various phenological stages. *Pak. J. Bot*., 50(5): 1941-1949.

Ali, S.I. and M. Qaiser. 1995-2018. Flora of Pakistan. Department of Botany, University of Karachi.

Anonymous. 2016. Official Methods of Analysis of AOAC International. In: Latimer J GW (20th ed. AOAC International, Maryland, USA.

Antia, B.S., E.J. Akpan, P.A. Okon and P.A. Umoren. 2006. Nutritive and antinutritive evaluation of sweet potatoes (*Ipomoea batatas*) leaves. *Pak. J. Nutr.*, 5: 166-168.

Anwar, S., Z. Mohammad, W. Hussain, N. Ali, A. Ali, J. Hussain and D. Hussain. 2022. Evaluation of mineral, proximate compositions and anti-oxidant activities of some wild edible vegetables of District Kurram Khyber Pakhtunkhwa, Pakistan. *Plant Sci. Today*, 9(2): 1-11.

Batool, W., W. Hussain, R. Hassan, Tasleem, S. Ullah and A. Ali. 2023. A study of wild vegetables: Proximate and mineral analysis of selected wild edible vegetables of Parachinar, District Kurram KP, Pakistan. *Pak. J. Bot*., 55(1): 291-298.

Begum, H.A., M. Hamayun, N. Shad, V. Yaseen and F. Asad. 2018. Nutritional analysis of some selected medicinal plants of Khyber Pakhtunkhwa, Pakistan. *Pure Appl. Boil.,* 7(3): 955-964.

Beshaw, T., K. Demssie, M. Tefera and A. Guadie. 2022. Determination of proximate composition, selected essential and heavy metals in sesame seeds (*Sesamum indicum* L.) from the Ethiopian markets and assessment of the associated health risks. *Toxicol. Rep.*, 9: 1806-1812.

Cherian, G. 2020. A guide to the principles of animal nutrition. Oregon State University.

Dangoggo, S., M. Bunu, A. Uba and Y. Saidu. 2012. Study of proximate, mineral and anti-nutrient composition of *Punica granatum* seeds from North-Western Nigeria and Saudi Arabia. *Researcher*, 4: 4-9.

de Lucena, R.F.P., P.M. de Medeiros, E. de Lima Arau´jo, A.G.C. Alves and U.P. de Albuquerque. 2012. The ecological apparency hypothesis and the importance of useful plants in rural communities from Northeastern Brazil: An assessment based on use value. *J. Environ. Manag.*, 96(1): 106-115.

Deka, M., A.K. Medhi and J.C. Kalita. 2013. Biochemical estimation of primary metabolites and mineral composition of *Clitoria ternatea* (L.) Roots. *Int. J. Life Sci. Res.*, 8(2): 713-716.

Edeoga, H.O. and A. Gomina. 2001. Nutritional values of some non-conventional leafy vegetables of Nigeria. *J. Econ. Taxon. Bot.,* 25(2): 439-447.

FAO. 2006. Plant Nutrition for Food Security. A Guide for Integrated Nutrient Management; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy.

Galili, T., A. O'Callaghan, J. Sidi and C. Sievert. 2018. Heatmaply: an R package for creating interactive cluster heatmaps for online publishing. *Bioinformatics*, 34(9): 1600-1602.

Ganskopp, D. and D. Bohnert. 2003. Mineral concentration dynamics among 7 northern great Basin grasses. *J. Range Manag.*, 5(6): 174-184.

Geng, Y., S. Ranjitkar, Q. Yan, Z. He, B. Su, S. Gao, J. Niu, D. Bu and J. Xu. 2020. Nutrient value of wild fodder species and the implications for improving the diet of Mithun (*Bos frontalis*) in Dulongjiang area, Yunnan Province, China. *Plant Diver.*, 42(6): 454-463.

Gull, T., Z. Mahmood, F. Anwar, B. Sultana, W. Nouman, S.A. Shahid and M.Z. Iqbal. 2015. Variation of proximate composition and minerals within different parts of *Capparis decidua* (Forssk*.) Edgew. as a function of harvesting seasons. Pak. J. Bot.*, 47(5): 1743-1748.

Hameed, I. and F. Hussain. 2015. Proximate and elemental analysis of five selected medicinal plants of family Solanaceae. *Pak. J. Pharm. Sci.*, 28(4): 1-13.

Haq, A. and L. Badshah. 2023. Palatability status and animals' preferences of forage plants in Pashat Valley, Pak-Afghan border, District Bajaur, Pakistan. *Ethnobot. Res. Appl.* 26: 1-22.

Hussain, A., M.A. Farooq, M. Ahmed, M. Akbar and M.U. Zafar. 2010. Phytosociology and structure of Central Karakorm National Park (CKNP) of Northern areas of Pakistan. *World Appl. Sci.*, 9(12): 1443-1449.

Hussain, F. and M.J. Durrani. 2009. Nutritional evaluation of some forage plants from Harboi rangeland, Kalat, Pakistan. *Pak. J. Bot.*, 41(3): 1137-1154.

Hussain, F., and M.J. Durrani. 2008. Mineral composition of some range grasses and shrubs from Harboi rangeland Kalat, Pakistan. *Pak. J. Bot*., 40: 2513-2523.

Hussain, W., L. Badshah, A. Ali, H. Hussain and F. Hussain. 2021. Studies on varying nutrient status at pre-reproductive, reproductive and post-reproductive stages of five plant species from Koh-e-Sufaid range, Kurram valley, Kakistan. *Pak. J. Bot.*, *53*(5): 1771-1777.

James, O., A.A. Rotimi and B.O.J. Bamaiyi. 2010. Phytoconstituents, proximate and nutrient investigations of *Saba florida* (Benth.) from Ibaji forest. *Int. J. Nutr. Metab.,* 2(5): 88-92.

Jumba, I.O., N.F. Suttle and S.O. Wandiga. 1996. Mineral composition of tropical forages in the mount Elgan region of Kenya. 1. Macro minerals. *Trop. Agric.*, 73: 108-112.

Khan, A., I. Ullah, S.U. Khan, K. Zhang, Z. Muhammad, S.M. Wazir, F. Ullah, S. Shah, L. Kang and M. Ullah. 2018. Elemental and nutritional values of wild fodder plants of Poaceae in district Bannu Pakistan. *Int. J. Agron. Agric. Res.*, 13: 53-61.

Khan, M., F. Hussain and Musharaf. 2013. Macro-mineral contents in ten species at three phenological stages in Tehsil Takhte-Nasrati, district Karak, Pakistan. *Afr. J. Agric. Res.*, 8: 65475-5484.

Khan, R., M.N. Khan, U. Ullah, A. Basit, A. Razzaq, M. Ahmad and F.A. Ozdemir. 2018. A comparative assessment of proximate and elemental composition six weedy grasses for their potential use as fodder. *Prog. Nutr.*, 20(1-S): 182-190.

Khan, Z.I., M. Ashraf and A. Hussian. 2007. Evaluation of macro mineral contents of forages: Influence of pasture and seasonal variation. *Asian-australas. J. Anim. Sci.*, 20(6): 908-913.

Love, J., R. Selker, M. Marsman, T. Jamil, D. Dropmann, J. Verhagen and E.J. Wagenmakers. 2019. JASP: Graphical statistical software for common statistical designs. *J. Stat. Softw.*, 88: 1-17.

McCarthy, R.D., T.H. Klusmeyer, J.L. Vicini, J.H. Clark and D.R. Nelson. 1989. Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. *J. Dairy Sci.*, 72: 2002-2016.

Meissner, H. 1997. Recent research on forage utilization by ruminant livestock in South Africa. *Anim. Feed Sci. Technol.*, 69(1-3): 103-119.

Minson, D.J. 1990. Forage in ruminant nutrition. Academic Press, Sun Diego, USA, pp. 483.

Naqbi, K.M.A.A., K. Karthishwaran, S.S. Kurup, M. Abdul Muhsen Alyafei and A. Jaleel. 2022. Phytochemicals, proximate composition, mineral analysis and in vitro antioxidant activity of *Calligonum crinitum* Boiss. *Hort.*, 8(2): 1-14.

Newall, C.A., L.A. Anderson and J.D. Phillipson. 1996. Herbal medicines: A Guide for health care professionals. London, the Pharmaceuticals Press.

Ogidi, O.I., N.G. Esie and O.G. Dike. 2019. Phytochemical, Proximate and Mineral compositions of *Bryophyllum pinnatum* (Never die) Medicinal plant. *J. Pharm. Phytochem.,* 8(1): 629-635.

Onwuka, G.I. 2005. Food analysis and instrumentation; Theory and Practice. Naphthalic prints, Surulere, Lagos, Nigeria, 219-230.

Provenza, F.D., J.J. Villalba, L. Dziba, S.B. Atwood and R.E. Banner. 2003. Linking herbivore experience, varied diets and plant biochemical diversity. *Small Rumin. Res.*, 49(3): 257-274.

Robinson, T.P., G.R.W. Wint, G. Conchedda, T.P. Van Boeckel, V. Ercoli, E. Palamara, G. Cinardi, L. Aietti, S. Hay and M. Gilbert. 2014. Mapping the global distribution of livestock. *PloS One,* 9(5): 1-13.

Samreen, U., M. Ibrar and L. Badshah. 2016. Floristic composition, ecological characters and biological characters of Darazinda, F.R.D.I. Khan, Pakistan. *Int. Invent. J. Agric. Soil Sci.*, 4(1): 9-21.

Satter, M.M.A., M.M.R.L. Khan, S.A. Jabin, N. Abedin, M.F. Islam and B. Shaha. 2016. Nutritional quality and safety aspects of wild vegetables consume in Bangladesh. *Asian Pac. J. Trop. Biomed.*, 6(2): 125-131.

Shaheen, H., R. Qureshi, M.F. Qaseem and P. Bruschi. 2020. The fodder grass resources for ruminants: A indigenous treasure of local communities of Thal desert Punjab, Pakistan. *PloS One,* 15(3): 1-25.

Shahid, M., R.K. Singh and S. Thushar. 2023. Proximate Composition and Nutritional Values of Selected Wild Plants of the United Arab Emirates. *Molecules*, 28(3): 1-15.

Sheikh, S.A., B.N. Mirani, S. M. Nizamani, A. A. Panhwar and M. Memon. 2019. Nutritional composition of wild-non-traditional vegetables of Sindh, under different postharvest processing methods. *Pak. J. Bot.*, 51(1): 259-267.

Smith, F.W. and J.F. Loneragon. 1997. Interpretation of plant analysis: concepts and principles. Plant Analysis: An Interpretation Manual. 2nd Ed CSIRO Publishing: Melbourne, 3-33.

Sultan, J.I., I.U. Rahim, M. Yaqoob, H. Nawaz and M. Hameed. 2008. Nutritive value of free rangeland grasses of northern grasslands of Pakistan. *Pak. J. Bot.*, 40(1): 249-258.

Tariq, S.A., T. Bashharat, N. Khan, B.S. Hamid and Y. Rehman. 2015. Elemental analysis of *Indigofera gerardiana* Wall by Atomic Absorption Spectrophotometer (AAS). *Adv. Basic Med. Sci.*, 1(1): 85-90.

Tomescu, Rus, C., G. Pop, E. Alexa, I. Radulov, I. M. Imbrea and M. Negrea. 2015. Researches regarding proximate and selected elements composition of some medicinal plants belonging to the Lamiaceae family. *Lucrari Stiintifce Seria Agronomie,* 58(2): 175-180.

Toth, G.G., P.R. Nair, C.P. Duffy and S.C. Franzel. 2017. Constraints to the adoption of fodder tree technology in Malawi. *Sustain. Sci.*, 12(5): 641-656.

Unuofin, J.O., G.A. Otunola and A.J. Afolayan. 2017. Nutritional evaluation of *Kedrostis africana* (L.) Cogn: An edible wild plant of South Africa. *Asian Pac. J. Trop. Biomed.*, 7: 443-449.

White, P.J., M.R. Broadley and P.J. Gregory. 2012. Managing the nutrition of plants and people. *Appl. Environ. Soil Sci.*, 1-14.

(Received for publication 21 December 2022)