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SCREENING OF FIVE PLANT SPECIES FOR MACRO/MICRO NUTRIENTS AND HEAVY METALS AT VARIOUS PHENOLOGICAL STAGES

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Abstract

This study presents the results of chemical analysis of five plant species. A total of 15 minerals were analyzed in these species at pre-reproductive and post-reproductive stages. These included 5 macronutrients i.e. P, K, Mg, Ca and S; 7 micronutrients i.e. Fe, Co, Ni, Cu, Zn, Mo, Mn and 3 trace elements i.e. Na, Si and Ba. Highest values were recorded for Ca while Co and Mo were below detection range in all samples. Based on mean values of all minerals the trend for all minerals in descending order for *Aristida cyanantha* was Ca > K > S > Mg > P > Fe > Si > Na > Zn > Cu: Ba > Mn, for *Andrachne cordifolia* Ca > K > Mg > P > S > Fe > Na > Mn > Zn >Ba > Cu > Si > Ni, for *Quercus baloot* it was found to be Ca > K > Mg > S > P > Fe > Mn > Ba > Si > Na > Zn > Cu > Ni, for *Indigofera heterentha* var. *heterantha* it stood as Ca > K > Mg > S > P > Fe > Si > Na > Ba > Mn > Zn > Cu > Ni and for *Cotoneaster nummularia* it was recorded as Ca > K > Mg > S > P > Fe > Si > Ba > Na > Mn > Zn > Cu > Ni. Factorial analysis confirmed that these minerals varied significantly in both phenological stages.

Key words: Macronutrients, Micronutrients, Trace elements, Chemical screening, Phenological stages.

Introduction

This study was designed to analyze 15 elements in five selected plant species at two phenological stages i.e., pre-reproductive and post-reproductive stages. During growth and development of plant species they not only undergo morpho-anatomical changes but they also show variations in secondary metabolites as well as differences in elemental composition (Gull *et al*., 2015: Dastagir *et al*., 2014). These changes in mineral composition determine the palatability preferences of grazing and browsing animals in a habitat. A total of 15 elements were analyzed which included 5 macro-nutrients, 7 micro-nutrients and 3 trace elements. These plant species were collected from Hindukush range of Swat, Pakistan, between 72^{o} 32' 1" to 72^{o}43' 3" longitude and 35^{o}3' 40" to 35^{o} 11' 40" latitude.

Arsitida cyanantha Nees ex Steud. belongs to family Poaceae and is a common grass in the locality. It is a highly palatable species in post-reproductive stage (Hussain *et al*., 2007). It is usually fed to cattle in dry condition. *Andrachne cordifolia* (Wall. ex Dcne.) Muell. Arg. belongs to family Euphorbiaceae and is a common shrub along the Northern slopes in Chail, Bishigram, Shinku and Dabargai area. It is a poisonous species and is not palatable, but is often used by locals as vermifuge for cattle (Khan *et al*., 2012; Sher *et al*., 2012). *Quercus baloot* Griffth belongs to family Fagaceae and is a common tree species in the locality. Its leaves are used as fodder and is less palatable in post-reproductive stage but palatable in pre-reproductive stage. Its wood is used as fuel while its fruit is reported to have medicinal uses (Hassan *et al*., 2015). *Indigofera heterantha* Wall. ex Brandis var. *heterantha* belongs to family Papilionaceae. It is a common shrub on Northern and Southern slopes in Chail, Dabargai and Shinku area. It is a palatable species and its leaves are used to cure scabies. In addition to this it is also used to treat digestive disorders (Akhtar *et al*., 2013). *Cotoneaster nummularia* Fisch. & Mey. belongs to family Rosaceae, shrubby in nature and a palatable species. Its fruit is used as astringent and expectorant. Decoction of its fruits is an effective therapy for treating cough (Youssef, 2013; Ahmad *et al*., 2011).

Materials and Methods

Sampling: Plant specimens were collected at pre-reproductive and post reproductive stage from Chail valley of Hindukush ranges. Each collected sample weighed 2.5 kg. Contaminants were removed and each sample was labeled and taken in perforated polythene bags. Samples were dried in laboratory and later grounded to 0.5-1mm particle size.

Microwave digestion of plant samples: Grounded plant material was digested by taking 200 mg of each sample. Each sample was digested with H_2O_2 and HNO_3. Both H_2O_2 and HNO_3 were taken in a 2:1 ratio. Closed vessel microwave method of digestion was applied.

Mineral analyses: After digesting the material, each sample was diluted by deionized H_2O. Macronutrients, micronutrients and trace elements were estimated by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). ICP-OES spectrometer iCAP 6500 (Thermo Fisher, UK) was used for analysis.

Results

1. *Aristida cyanantha* Nees ex Steud.

Macronutrients: In *Aristida cyanantha*, phosphorus was estimated as 262 μg/g at pre-reproductive stage while at post-reproductive stage it slightly decreased to 215μg/g. Considerable drop in potassium levels was observed with maturity of the plant species. Potassium content was 6151 μg/g in pre-reproductive stage which came down to 3591 μg/g in post-reproductive stage. There was no marked variation in magnesium levels, which remained 244 µg/g and 264 µg/g in pre-reproductive and post reproductive stages respectively. Calcium was low in pre-reproductive stage i.e. 1460 µg/g and it increased considerably in post reproductive stage (1789 µg/g). A decrease in sulphur content was observed with increasing maturity of the plant. Its levels were recorded as 440 µg/g at pre-reproductive stage and 379 µg/g at post-reproductive stage.

Micronutrients: Iron content increased with maturity, it was 94 µg/g in pre-reproductive stage while 109 µg/g in post-reproductive stage. Though cobalt and molybdenum were below detection range at both phenological stages, traces of nickel were recorded at post-reproductive stage i.e. 1 µg/g. Nickel was not detected in pre-reproductive stage. Only 16 µg/g of copper was found in pre-reproductive stage which further decreases to 3 µg/g at post-reproductive stage. Zinc levels remained almost the same at both phenological stages i.e., 14 µg/g and 13 µg/g. Similarly, manganese levels showed no considerable variation at both phenological stages i.e., 5 µg/g and 7 µg/g.

Trace elements: An increase in sodium content was recorded at post-reproductive stage (76 µg/g) while at pre-reproductive stage it was 21 µg/g. Similar trend was seen in case of silicon, at pre-reproductive stage it was found to be 66 µg/g which slightly increased to 75 µg/g at post-reproductive stage. Lowest values were recorded for barium at both phenological stages i.e. 2 µg/g and 17 µg/g at pre-reproductive and post-reproductive stage respectively (Table 1, Fig. 1).

***Andrachne cordifolia* (Wall. ex Dcne.) Muell. Arg.**

Macronutrients: Except phosphorous, a decrease was observed in all macronutrients with increasing maturity. This may be one of the reasons that this species in non-palatable (Sher *et al*., 2012). Phosphorous was estimated as 381 µg/g in pre-reproductive stage which increased to 574 µg/g in post-reproductive stage. Potassium levels were just 6187 µg/g in post-reproductive stage while they were 12659 µg/g at pre-reproductive stage. Considerable decrease in magnesium content was recorded i.e. from 4146 µg/g to 1612 µg/g in pre-reproductive and post-reproductive stages respectively. Calcium clicked just 11914 µg/g in post-reproductive stage while it was 17723 µg/g in pre-reproductive stage. A decrease in sulphur content was recorded with increasing maturity of the plant, which was 822 µg/g in post-reproductive stage and 1198 µg/g in pre-reproductive stage.

Micronutrients: With maturity, the micronutrients decreased as well. Iron contents decreased from 349 µg/g to 284 µg/g in pre-reproductive and post-reproductive stage. Cobalt levels were below detection range in both stages while nickel remained 1 µg/g at both phenological stages. Marked decreased was noticed in copper content, its levels were 23 µg/g in pre-reproductive stage while just 4 µg/g in post-reproductive stage. Zinc was estimated as 32 µg/g and 20 µg/g in successive stages. Molybdenum was not detected in either stage. Manganese remained almost same in both phenological stages i.e., 30 µg/g and 31 µg/g.

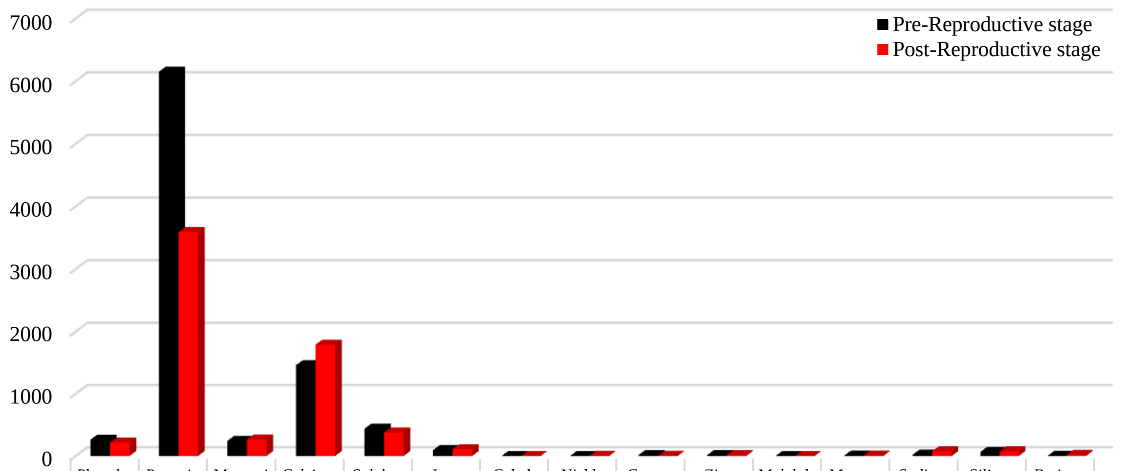
Trace elements: Sodium contents decreased from 276 µg/g to 66 µg/g in post-reproductive stage. Silicon levels remained almost unchanged at both phenological stages (8 µg/g and 9 µg/g). A slight decrease in barium content was recorded with increasing maturity, in pre-reproductive stage it was 37 µg/g while just 12 µg/g in post-reproductive stage. The results of this study indicate that high levels of Cu, Fe, Na and Ca are responsible for non-palatability of *A. cordifolia.* Moreover, it has a very bitter taste for which grazing animals don't touch it in the wild. Findings of this study are also backed by work of Gillani *et al*., (2010) who have reported toxic secondary metabolites in this species (Table 1, Fig. 2).

Table 1. Macro-Micro mineral and Trace elements profile.

S. No.	Parameter	Symbol	*Aristida cyanantha*		*Andrachne cordifolia*		*Quercus baloot*		*Indigogera heterantha* var. *heterantha*		*Cotoneaster nummularia*	
			I	II	I	II	I	II	I	II	I	II
	Macronutrients											
1.	Phosphorous	P	262	215	381	574	377	965	344	1019	632	551
2.	Potassium	K	6151	3591	12659	6187	5696	4664	3654	8274	7173	8393
3.	Magnesium	Mg	244	264	4146	1612	1597	1968	3729	4344	3756	2101
4.	Calcium	Ca	1460	1789	17723	11914	10905	7205	29802	35269	18460	14127
5.	Sulphur	S	440	379	1198	822	719	654	696	3970	659	916
	Micronutrients											
6.	Iron	Fe	94	109	349	284	147	364	136	179	188	281
7.	Cobalt	Co	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8.	Nickel	Ni	ND	1	1	1	1	3	2	2	3	1
9.	Copper	Cu	16	3	23	4	8	6	5	8	13	5
10.	Zinc	Zn	14	13	32	20	14	18	13	20	29	18
11.	Molybdenum	Mo	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12.	Manganese	Mn	5	7	30	31	65	139	18	53	24	30
	Trace elements											
13.	Sodium	Na	21	76	276	66	4	33	146	60	95	41
14.	Silicon	Si	66	75	8	9	11	39	205	94	171	16
15.	Barium	Ba	2	17	37	12	37	17	47	32	52	32

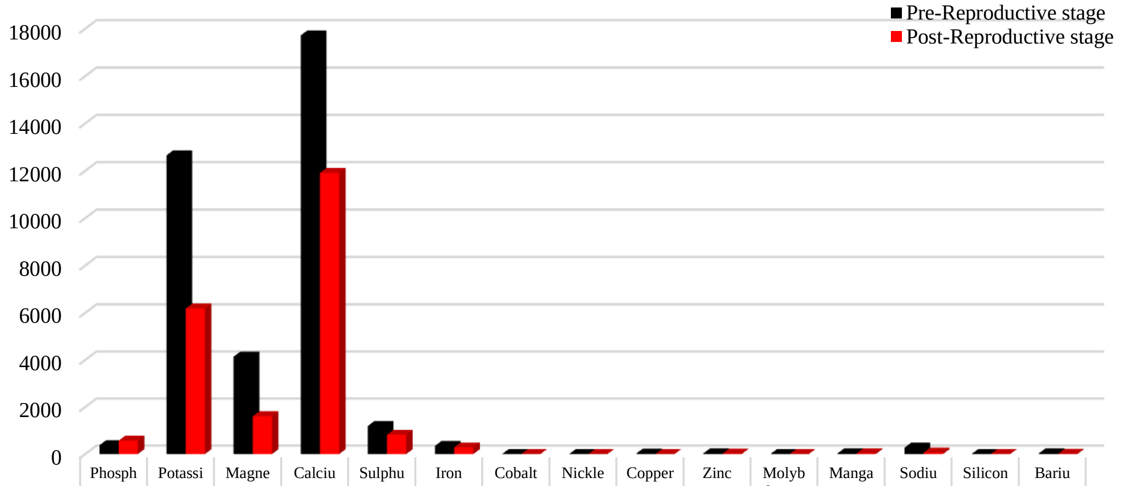
I = Pre-Reproductive stage, II = Post-Reproductive stage; ND = value below detection range

*All values in µg/g



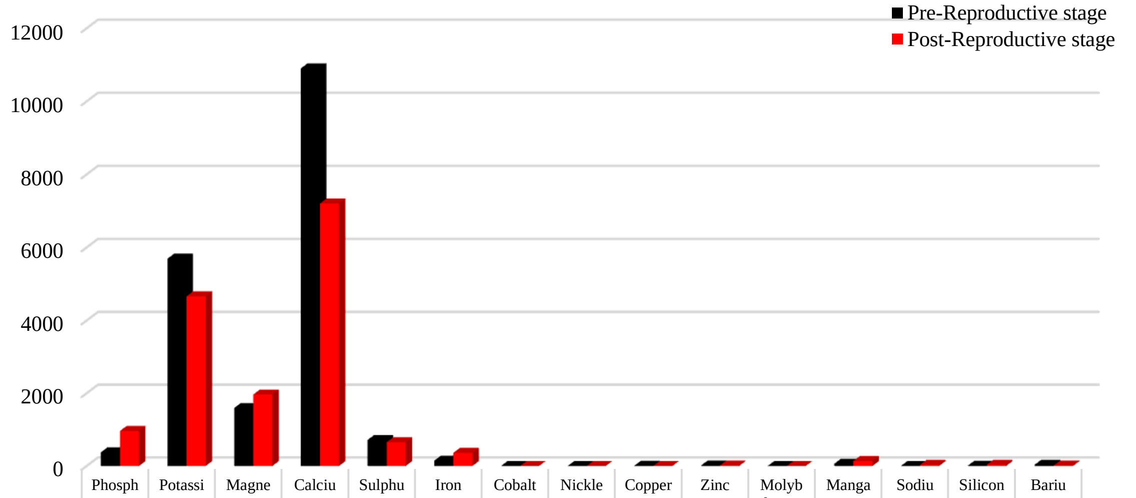
	Phosphorous	Potassium	Magnesium	Calcium	Sulphur	Iron	Cobalt	Nickle	Copper	Zinc	Molybdenum	Manganese	Sodium	Silicon	Barium
Pre-Reproductive stage	262	6151	244	1460	440	94	0	0	16	14	0	5	21	66	2
Post-Reproductive stage	215	3591	264	1789	379	109	0	1	3	13	0	7	76	75	17

Fig. 1. Macro- micronutrients and trace element analysis of *Aristida cyanantha*.



	Phosphorous	Potassium	Magnesium	Calcium	Sulphur	Iron	Cobalt	Nickle	Copper	Zinc	Molybdenum	Manganese	Sodium	Silicon	Barium
Pre-Reproductive stage	381	12659	4146	17723	1198	349	0	1	23	32	0	30	276	8	37
Post-Reproductive stage	574	6187	1612	11914	822	284	0	1	4	20	0	31	66	9	12

Fig. 2. Macro-micronutrients and trace element analysis *Andrachne cordifolia*.



	Phosphorous	Potassium	Magnesium	Calcium	Sulphur	Iron	Cobalt	Nickle	Copper	Zinc	Molybdenum	Manganese	Sodium	Silicon	Barium
Pre-Reproductive stage	377	5696	1597	10905	719	147	0	1	8	14	0	65	4	11	37
Post-Reproductive stage	965	4664	1968	7205	654	364	0	3	6	18	0	139	33	39	17

Fig. 3. Macro-micronutrients and trace element analysis *Quercus baloot*.

Quercus baloot **Griffth**

Macronutrients: In *Quercus baloot* a significant increase in phosphorous levels was noticed with increasing age of the plant. Phosphorous content was 377 µg/g in pre-reproductive stage which increased to 965 µg/g in post-reproductive stage. Potassium was recorded as 5696 µg/g in pre-reproductive stage which decreased to 4664 µg/g in post-reproductive stage. Magnesium content showed an increase with maturity of the plant, it was 1597 µg/g in pre-reproductive stage and 1968 µg/g in post-reproductive stage. Calcium was found to be 10905 µg/g in pre-reproductive stage which decreased to 7205 µg/g in post-reproductive stage. Usually the livestock consume its foliage, especially the young leaves, or its inflorescence.

Micronutrients: With growing age of the plant, increase in micronutrients was observed. Iron content was recorded as 147 µg/g in pre-reproductive stage which increased to 364 µg/g in post-reproductive stage. Nickel levels showed no marked variation at both phenological stages i.e. 1 µg/g and 3 µg/g. Cobalt and molybdenum were not detected in either of the phenological stages. Copper content was low i.e. 8 µg/g and 6 µg/g in pre-reproductive and post-reproductive stages respectively. Zinc content was 14 µg/g and 18 µg/g in successive phenological stages. Significant increase in manganese levels was noticed which showed an increase from 65 µg/g in pre-reproductive stage to 139 µg/g in post-reproductive stage.

Trace elements: An increase in sodium levels was noticed, it was recorded as 4 µg/g in pre-reproductive stage and 33 µg/g in post-reproductive stage. Silicon levels were also raised in post-reproductive stage (39 µg/g) which were only 11 µg/g in pre-reproductive stage. Barium showed a contrasting trend, it was found to be 37 µg/g in pre-reproductive stage and decreased to 17 µg/g in post-reproductive stage (Table 1 Fig. 3).

Indigofera heterantha **Wall. ex Brandis var. *heterantha***

Macronutrients: An increase in all macronutrients was recorded with maturity of the plant. Phosphorous in pre-reproductive stage (344 µg/g) increased to 1019 µg/g in post-reproductive stage. There was a marked increase in potassium content in post-reproductive stage i.e. 8274 µg/g while in pre-reproductive stage it was just 3654 µg/g. In pre-reproductive stage magnesium levels were 3729 µg/g which became 4344 µg/g in post-reproductive stage. Calcium levels were 29802 µg/g in pre-reproductive stage and 35269 µg/g in post-reproductive stage. Considerable increase in sulphur content was recorded, it was found to be 696 µg/g in pre-reproductive stage and 3970 µg/g in post-reproductive stage.

Micronutrients: There was no substantial change in iron levels, which ranged between 136 µg/g and 179 µg/g during both the stages. Nickel content was low while molybdenum and cobalt were not detected in both phenological stages. Copper content was also low during both stages (5µg/g and 8µg/g). Zinc levels were estimated as 13 µg/g and 20 µg/g in pre-reproductive and post-reproductive stages respectively. Manganese content showed an increase with maturity and it was estimated as 53 µg/g in post-reproductive stage while it was 18 µg/g in pre-reproductive stage.

Trace elements: Trace elements showed a decrease with maturity. Sodium levels decreased in post-reproductive stage (60 µg/g) which were 146 µg/g in pre-reproductive stage. Similar trend was recorded for silicon, it was 205 µg/g in pre-reproductive stage and just 94 µg/g in post-reproductive stage. Barium content also decreased from 47 µg/g to 32 µg/g (Table 1, Fig. 4).

Cotoneaster nummularia **Fisch. & Mey.**

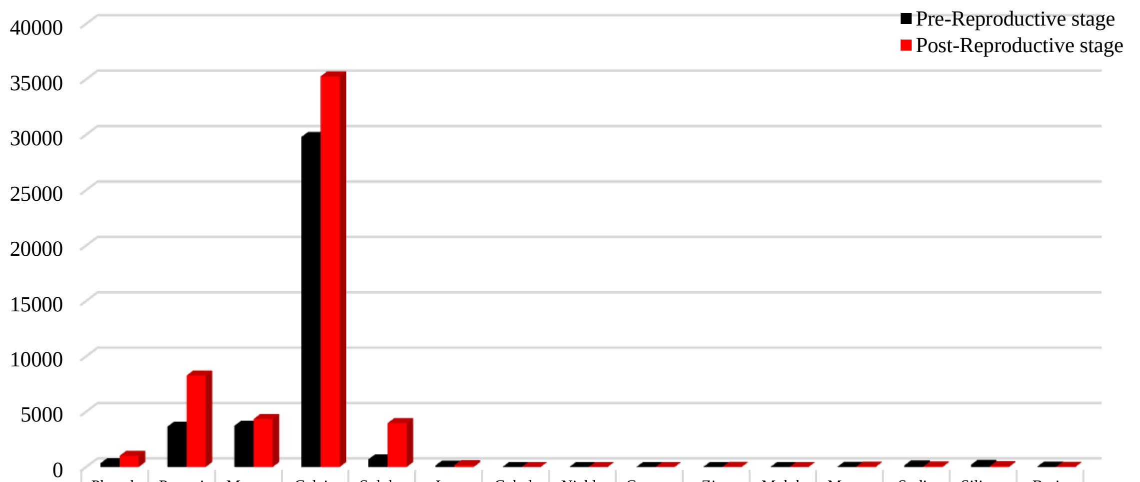
Macronutrients: Phosphorous levels showed a decrease with age of the plant, they were estimated as 632 µg/g and 551 µg/g in pre-reproductive and post-reproductive stages respectively. On the contrary, potassium content was 7173 µg/g in pre-reproductive stage which increased to 8390 µg/g in post-reproductive stage. Magnesium levels decreased from 3756 µg/g in pre-reproductive stage to 2101 µg/g in post-reproductive stage. Significant variation in calcium levels was recorded in both phenological stage, in pre-reproductive stage they were found to be 18460 µg/g which were reduced to 14127 µg/g in post-reproductive stage. Slight increase in sulphur content was recorded, in pre-reproductive stage they were 659 µg/g while 916 µg/g in post-reproductive stage.

Micronutrients: Iron levels showed an increase with maturity, 188 µg/g in pre-reproductive stage and 281 µg/g in post-reproductive stage. Copper and zinc showed a decrease in post-reproductive stage, while molybdenum and cobalt were below the detection range in either of the phenological stages. Manganese levels increased from 24 µg/g in pre-reproductive stage to 30 µg/g in post-reproductive stage.

Trace elements: Sodium levels were 95 µg/g in pre-reproductive stage which dropped to 41 µg/g in post-reproductive stage. Marked decrease was recorded for silicon levels, they were estimated as 171 µg/g in pre-reproductive stage which decreased to just 16 µg/g in post-reproductive stage. No drastic change was seen in case of barium, they stayed at 52 µg/g and 32 µg/g in successive stages (Table 1, Fig. 5).

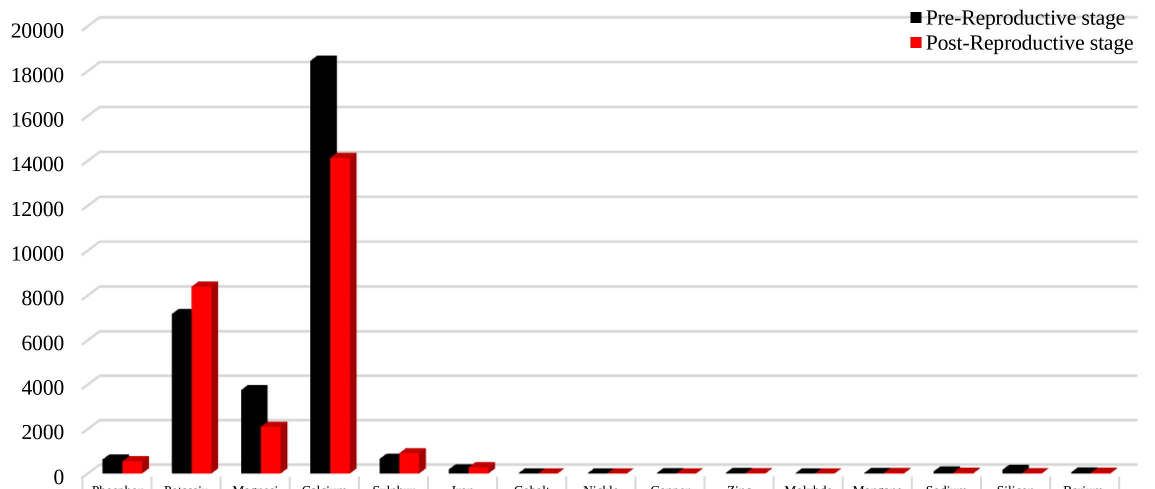
Figure 6 shows the mean values for all the minerals enumerated. The highest mean value for P was recorded in *Indigofera heterentha* (68.51 µg/g), followed by *Quercus baloot* (671 µg/g), *Cotoneaster nummularia* (591.5 µg/g) and *Andrachne cordifolia* (477.5 µg/g) while the lowest P levels were estimated in *Aristida cyanantha* (238.5 µg/g). Similarly, highest K levels were recorded in *A. cordifolia* (9423 µg/g) followed by *C. nummularia* (7783 µg/g), *I. heterentha* (5964 µg/g), and *Q. baloot* (5180 µg/g) whereas *A. cyanantha* (4871 µg/g) showed lowest concentration. Mg levels were highest in *I. heterantha* (4036.5 µg/g), followed by *C. nummularia* (2928.5 µg/g), *A. cordifolia* (2879 µg/g) and *Q. baloot* (1782 µg/g) and lowest in and *A. cyanantha* (254 µg/g).

Ca was abundant in *I. heterantha* (32535 µg/g), *C. nummularia* (16293.6 µg/g), *A. cordifolia* (14818.5 µg/g), and *Q. baloot* (9055 µg/g) while lowest in *A. cyanantha* (1624.5 µg/g). *I. heterantha* (2333 µg/g) had highest mean value for S, followed by *A. cordifolia* (1010 µg/g), *C. nummularia* (787.5 µg/g) and *Q. baloot* (686.5 µg/g) while the least S was recorded in *A. cyanantha* (409.5 µg/g). Fe levels were low in all selected plants with highest mean value observed in *A. cordifolia* (316.5 µg/g) and lowest in *A. cyanantha* (101.5 µg/g). Ni was not detected in *A. cyanantha* while *Q. baloot*, *I. heterantha* and *C. nummularia* had 2 µg/g mean value each while *A. cordifolia* had a mean value of 1 µg/g. Cu content was high in *A. cordifolia* (13.5 µg/g) and low in *I. heterantha* (6.5 µg/g). However highest mean value for Zn was observed in *A. cordifolia* (26 µg/g) followed by *C. nummularia* (23.5 µg/g), *I. heterantha* (16.5 µg/g) and *Q. baloot* (16 µg/g) and lowest in *A. cyanantha* (13.5 µg/g). Highest mean value for Mn was recorded in *Q. baloot* (102 µg/g), *I. heterantha* (35.5 µg/g) followed by *A. cordifolia* (30.5 µg/g) and *C. nummularia* (27 µg/g) and lowest in *A. cyanantha* (6 µg/g). Na exhibited highest value in *A. cordifolia* (171 µg/g) followed by *I. heterantha* (103 µg/g), *C. nummularia* (68 µg/g) and *A. cyanantha* (48.5 µg/g) while lowest in *Q. baloot* (18.5 µg/g). Similarly, Si levels were high in *I. heterantha* (149.5 µg/g) and low in *A. cordifolia* (8.5 µg/g). Ba exhibited high concentration among the plant viz. *C. nummularia* (84 µg/g) followed by *I. heterantha* (39.5 µg/g), *Q. baloot* (27 µg/g) and *A. cordifolia* (24.5 µg/g). Based on mean values the trend for *A. cyanantha* remained as Ca > K > S > Mg > P > Fe > Si > Na > Zn > Cu: Ba > Mn. For *A. cordifolia* it was Ca > K > Mg > P > S > Fe > Na > Mn > Zn >Ba > Cu > Si > Ni, for *Q. baloot* it stood as Ca > K > Mg > S > P > Fe > Mn > Ba > Si > Na > Zn > Cu > Ni, for *I. heterentha* var. *heterantha* it remained as Ca > K > Mg > S > P > Fe > Si > Na > Ba > Mn > Zn > Cu > Ni and for *C. nummularia* it was Ca > K > Mg > S > P > Fe > Si > Ba > Na > Mn > Zn > Cu > Ni.



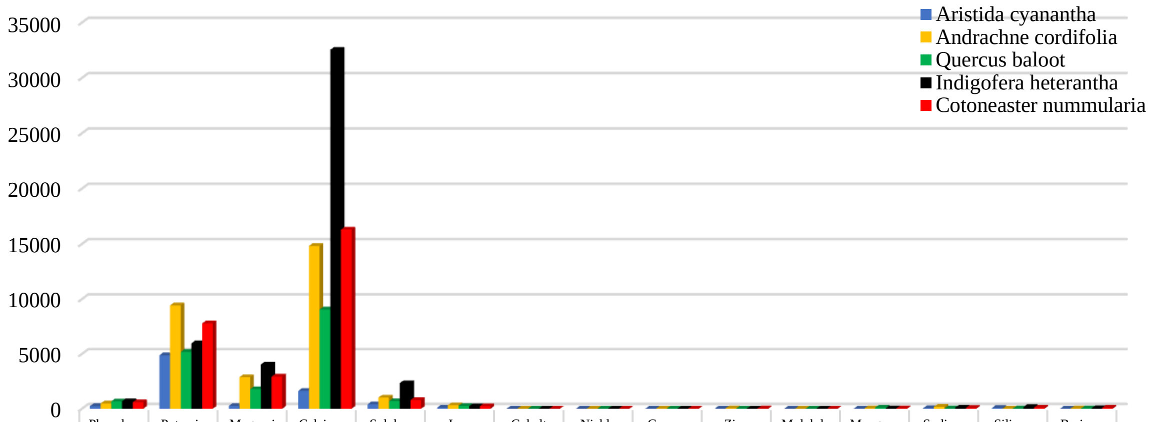
	Phosphorous	Potassium	Magnesium	Calcium	Sulphur	Iron	Cobalt	Nickle	Copper	Zinc	Molybdenum	Manganese	Sodium	Silicon	Barium
Pre-Reproductive stage	344	3654	3729	29802	696	136	0	2	5	13	0	18	146	205	47
Post-Reproductive stage	1019	8274	4344	35269	3970	179	0	2	8	20	0	53	60	94	32

Fig. 4. Macro-micronutrients and trace element analysis *Indigofera heterantha* var. *heterantha*.



	Phosphorous	Potassium	Magnesium	Calcium	Sulphur	Iron	Cobalt	Nickle	Copper	Zinc	Molybdenum	Manganese	Sodium	Silicon	Barium
Pre-Reproductive stage	632	7173	3756	18460	659	188	0	3	13	29	0	24	95	171	52
Post-Reproductive stage	551	8393	2101	14127	916	281	0	1	5	18	0	30	41	16	32

Fig. 5. Macro-micronutrients and trace element analysis *Cotoneaster nummularia*.



	Phosphorous	Potassium	Magnesium	Calcium	Sulphur	Iron	Cobalt	Nickle	Copper	Zinc	Molybdenum	Manganese	Sodium	Silicon	Barium
Aristida cyanantha	238.5	4871	254	1624.5	409.5	101.5	0	0	9.5	13.5	0	6	48.5	70.5	9.5
Andrachne cordifolia	477.5	9423	2879	14818.5	1010	316.5	0	1	13.5	26	0	30.5	171	8.5	24.5
Quercus baloot	671	5180	1782	9055	686.5	255.5	0	2	7	16	0	102	18.5	25	27
Indigofera heterantha	681.5	5964	4036.5	32535	2333	225.5	0	2	6.5	16.5	0	35.5	103	149.5	39.5
Cotoneaster nummularia	591.5	7783	2928.5	16293.6	787.5	234.5	0	2	9	23.5	0	27	68	93.5	84

Fig. 6. Mean values for minerals in all plants analyzed.

Discussion

It is the chemical profile of a plant species which determines its palatability status and its relative preference by grazing and browsing animals (Sultan *et al*., 2009 & 2007). Chemical changes during growth and development of plant species either attract or repel grazing animals. Some plants become more palatable after maturity while some become less or even non-palatable with growing age. Potential intake of a plant by grazing animals and their relative preference is largely dependent on its chemical constituents (Khan & Hussain, 2012; Hussain & Durrani, 2009). Kothmann (1980) reported that some plants become less palatable as they age, mainly due to raised mineral content. Results reported here are supported by previous works of similar nature which reported an increase in K, P, Ca, Mg, Co and Cu levels in post-reproductive stage (Sher *et al*., 2012; Bano *et al*., 2009; Sultan *et al*., 2009 and Rahim *et al*., 2008). Phosphorous is an important mineral required by plants for vegetative growth and its deficiency leads to shorter internodes in plants. Phosphorous levels were not low at both phenological stages in all five plants, though seasonal variation was recorded in its values. These findings are backed by reports of Gull *et al*., (2015) and Dastagir *et al*., (2014). Potassium affects plant growth as it is an important activator of several plant enzymes (Sultan *et al*., 2008; Hussain & Durrani, 2007; Khan *et al*., 2007). Previous studies suggest that potassium content is higher in pre-reproductive stages in free grazing lands (Sultan *et al*., 2008), this was found true for *A. cyanantha, A. cordifolia* and *Q. baloot.* But in *I. heterantha* var. *heterantha* and *C. nummularia,* potassium content increased with maturity. Similarly, Minson (1990) reported low levels of potassium in grasses and forbs while present study contradicts his findings as in *A. cyanantha* potassium content was high in both phenological stages (6151 µg/g and 3591 µg/g). Magnesium is an important component of chlorophyll molecule and it is a must for ribosomal sub-unit association. Though magnesium levels were slightly low in *A. cyanantha* in both phenological stages, in rest of the plant species analyzed, higher values were recorded which suggest them to be good for lactating livestock. Findings presented here are strongly supported by Rahim *et al*., (2008); Khan *et al*., (2006); Islam *et al*., (2003); Skerman & Riveros (1990) and Georgievskii (1982). Calcium levels were considerably high in all plant species tested. Variable calcium levels in different plant species are reported by Hameed & Hussain (2015); Zafar *et al*., (2010); Bano *et al*., (2009); Hanif *et al*., (2006); White & Broadly (2003). but our results contradict their findings. Role of sulphur is well established as a macronutrient which governs the proper growth of plants and enables them to tolerate stress (Matraszek *et al*., 2016). Sulphur levels varied with age in all plant samples. Highest levels of iron were found in post-reproductive stage of *Q. baloot* i.e., 364 µg/g followed by 349 µg/g in pre-reproductive stage of *A. cordifolia.* Iron is an important component required for chlorophyll synthesis hence having an impact on overall photosynthetic yield. Our results are favored by work of Hameed & Hussain (2015); Adnan *et al*., (2010) and Khan *et al*., (2006) who reported high levels of iron in plant of moist regions and from grazing pastures. Nickel content was low in all plant species analyzed and below detection range in pre-reproductive stage of *A. cyanantha*. This is in line with the work of Musharaf *et al*., (2017). In pre-reproductive stages of *A. cyanantha*, *A. cordifolia* and *C. nummularia* copper levels were 16 µg/g, 23 µg/g and 13 µg/g respectively which are above the permissible limit (10 µg/g) reported by Khuda *et al*., (2012); Demirezen & Aksoy (2006). Our findings are backed by reports of Hameed *et al*., (2008); Hussain & Durrani (2008); Garg *et al*., (2007) and Said *et al*., (1996) who have reported raised levels of copper in various plant species. Zinc is required by 200 different enzymes for their activity hence it is present in all living organisms in varying concentrations. It is also required for nucleic acid metabolism. Khuda *et al*., (2012) reported the permissible limit of 50 µg/g in medicinal plants. In our findings, the values for Zinc were found well below this limit and these results are supported by work of Demirezen & Aksoy (2006). Manganese is an important micronutrient but if its levels are raised it can prove to be injurious (Hameed &

Hussain, 2015). Our results are in line with this, apart from post-reproductive stage of *Q. baloot* which exhibited 139 µg/g of manganese. This may be a reason that in post-reproductive stage plant leaves are seldom browsed by the livestock. Low sodium content was detected in all plant samples. Our results are in line with work of Adnan *et al.*, (2010) but contradictory to the reports of James *et al.*, (2010) and Hanif *et al.*, (2006) who reported high levels of sodium in other plant species. In most of the elemental studies, silicon is usually neglected but recent studies suggest its important role in influencing the plant nutrition as well as nutrient cycling (Schaller *et al.*, 2016). Lowest silicon levels were recorded in *A. cordifolia* i.e., 8 and 9 µg/g in both phenological stages respectively while highest values were found in *I. heterantha* var. *heterantha* in pre-reproductive stage (205 µg/g). Former is a non-palatable species while later is a palatable one hence it clearly indicates that silicon is an important factor in deciding the palatability status of a plant. Some of the problems related to animal growth and reproduction are linked to low mineral content in soils and forage plants in the locality (Tiffany *et al.*, 2000). Variation in concentrations of macro and micronutrients are reported by Gull *et al.*, (2015) and Dastagir *et al.*, (2014). This supports findings of this study which reports a variation in concentration of macro and micronutrients in forage plants at different phenological stages. Studies of similar kind were conducted for Ba, Ca, Co, Cu, Fe, Mg, Mn, Mo, Ni, P, K, Si, Na, S and Zn (Gull *et al.*, 2015; Abreu 2012; Sher *et al.*, 2012; Cheema *et al.*, 2011; Sultan *et al.*, 2010, 2008; Bano *et al.*, 2009; Ahmad *et al.*, 2008 and Rahim *et al.*, 2008).

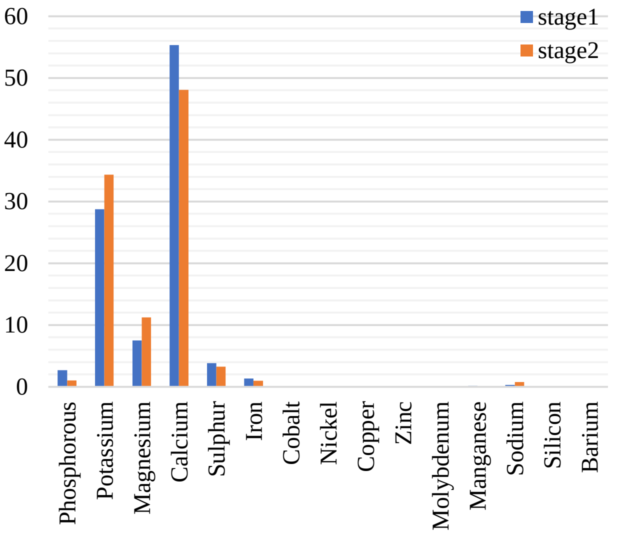


Fig. 7. Comparison of all nutrients at two phonological stages.

Statistical analysis

Anova was applied to analyze the results of this study. Detail of statistical analysis are given below (Fig. 7).

ANOVA. Overall analysis of Macronutrients, Micronutrients and Trace elements at Pre-reproductive stage.

	Sum of squares	Df	Mean square	F	Sig.
Between 5 plants	42782033.387	4	10695508.347	0.434	0.784
Within 5 plants	1724817138.400	70	24640244.834		
Total	1767599171.787	74			

Micronutrients - (Phosphorous, Potassium, Magnesium, Calcium, Sulphur),
Micronutrients - (Iron, Cobalt, Nickel, Copper, Zinc, Molybdenum, Manganese) & Trace elements (The amount of Sodium, Silicon, Barium) are non-significant at stage – I

ANOVA. Macronutrients, micronutrients and trace elements at post-reproductive stage.

	Sum of squares	Df	Mean square	F	Sig.
Between 5 plants	82452861.520	4	20613215.380	0.893	0.473
Within 5 plants	1615658333.867	70	23080833.341		
Total	1698111195.387	74			

Micronutrients - (Phosphorous, Potassium, Magnesium, Calcium, Sulphur),
Micronutrients - (Iron, Cobalt, Nickel, Copper, Zinc, Molybdenum, Manganese) & Trace elements (The amount of Sodium, Silicon, Barium) are non-significant at stage – II.
Post – Hock test
The LSD value = 2456.10
Sample mean – 1= 435.9333
Sample mean – 2 = 1435.733
Sample mean – 3 = 1071.667
Sample mean – 4 = 3554.933
Sample mean – 5= 1767.467

Comparison of means	LSD	Remarks
1 vs. 2	<	Non-significant
1 vs. 3	<	Non-significant
1 vs 4	>	Significant
1 vs. 5	<	Non-significant
2 vs. 3	<	Non-significant
2 vs. 4	<	Non-significant
2 vs. 5	<	Non-significant
3 vs. 4	>	Significant
3 vs 5.	<	Non-significant
4 vs. 5	<	Non-significant

In further analysis sample means 1, sample mean 3 and sample mean 4 are significant

ANOVA. For stage – I Macronutrients.

	Sum of squares	Df	Mean square	F	Sig.
Between micronutrients – 1	124218761.040	4	31054690.260	0.526	0.718
Within Groups	1180847863.200	20	59042393.160		
Total	1305066624.240	24			

Phosphorous, Potassium, Magnesium, Calcium, Sulphur
From above ANOVA, the amount of Phosphorous, Potassium, Magnesium, Calcium, Sulphur are non-significant at stage –1

ANOVA. For stage – II Macronutrients.

	Sum of squares	Df	Mean square	F	Sig.
Between groups	246881024.640	4	61720256.160	1.162	0.357
Within groups	1062711672.800	20	53135583.640		
Total	1309592697.440	24			

From above ANOVA, we conclude that the amount of Phosphorous, Potassium, Magnesium, Calcium, Sulphur are significant at stage-2

ANOVA. For stage – I Macronutrients.

	Sum of squares	Df	Mean square	F	Sig.
Between groups	7833.714	4	1958.429	0.357	0.837
Within groups	164560.571	30	5485.352		
Total	172394.286	34			

Iron, Cobalt, Nickel, Copper, Zinc, Molybdenum, Manganese. Non-significant

ANOVA. For stage – II Macronutrients.

	Sum of squares	Df	Mean square	F	Sig.
Between groups	11865.429	4	2966.357	0.321	0.861
Within groups	276849.714	30	9228.324		
Total	288715.143	34			

Non-significant

ANOVA. For stage – I Trace elements.

	Sum of squares	Df	Mean square	F	Sig.
Between groups	31885.733	4	7971.433	1.207	0.367
Within groups	66038.000	10	6603.800		
Total	97923.733	14			

The amount of Sodium, Silicon, Barium is significant at stage -1

ANOVA. For stage – II Trace elements.

	Sum of squares	Df	Mean square	F	Sig.
Between groups	3199.600	4	799.900	1.168	0.381
Within groups	6847.333	10	684.733		
Total	10046.933	14			

The amount of Sodium, Silicon, Barium is significant at stage -II

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