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# STUDIES ON VARYING NUTRIENT STATUS AT PRE-REPRODUCTIVE, REPRODUCTIVE AND POST-REPRODUCTIVE STAGES OF FIVE PLANT SPECIES FROM KOH-E-SUFAID RANGE, KURRAM VALLEY, PAKISTAN

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**Abstract**

This study was designed to investigate the mineral composition at three phenological stages of some selected fodder forage. Therefore, five taxa viz., *Indigofera gerardiana*, *Tagetes minutia*, *Rubus fruticosus*, *Medicago sativa*, and *Quercus baloot* were tested for different minerals such as Mg, K, Na, Ca, Zn, Co, Fe, Cr, Mn and Cu. The highest concentration of macronutrients i.e. Ca (96700 µg/g), Mn (99800 µg/g) were found at post-reproductive phase in *Indigofera gerardiana* and K (90200 µg/g) was recorded during pre-reproductive phase in *Tagetes minutia* while the *Rubus fruticosus* exhibited (17800 µg/g) and (59900µg/g) Co and Cu respectively. Similarly, the highest level of Chromium (Cr) recorded (76600µg/g) at reproductive phase in *Tagetes minuta,* Nickle concentration was recorded maximum i.e. 46400 µg/g at pre-reproductive phase in *Rubus fruticosus*. Furthermore, Zn contents were (88800 µg/g) at pre-reproductive both in *Medicago sativa* and *Tagetes minuta* and subsequently Fe (59300 µg/g) at pre-reproductive phase in *Indigofera gerardiana*. This study helped in correlating the mineral status of these plant species to their palatability status. The mineral profile of the plants analyzed indicates that mineral levels were as per the required standards and their concentrations were not drastically different from other parts of Pakistan.

**Key words:** Palatability, Grazing, Preferences, Minerals composition, Environment.

## Introduction

Grazing is an important biotic stress in land ecosystems (Nawaz *et al.,* 2008; Yin *et al.,* 2017; Ali *et al.,* 2018) and some plants are eaten by herbivores in their fresh state while some animals prefer to eat dry plants. This is due to the fact that at different phenological stages, concentrations of various mineral components vary (Hussaein *et al.,* 2016; Ibrahim *et al.,* 2016; Sultan *et al.,* 2009). Sensory impulses of grazing animals determine which plant and which plant part they will consume (Badshah & Hussain, 2011; Hussain & Durrani, 2008). Notably, work of Palkova and Leps, (2008) proposed a range of principles for classifying desirable palatable species to animals.

The palatability involves two practices, it is done either by direct observation in the field or it is determined by progressive loss of plants in a locality (Amjad *et al.,* 2014; Anderson, 1994; Anderson & Robert, 1987). A positive correlation is observed between palatability and $C/N_2$ ratio, $N_2$ content and moisture content in the above ground plant parts (Miller & Thompson, 2005). Additionally, minerals like nitrogen, magnesium, sodium, iron, calcium are essential for the normal functioning of the body process of animals. On the other hand, arborescent plants which influence the biochemical activities in the rhizosphere by altering the soil mineral composition, also effect the forb's palatability as it enhances dry matter digestibility and N2 content of their leaves (Sagilicco & Bruzzese, 2004; Ganskopp & Bohnert, 2003; Jackson, 1962) have been reported similar findings about the forage quality of forbs (Wilkinson & Gross, 1967). Minerals and elements play a vital role in the various metabolic processes of animals and are also important for the formation of active components (Ali *et al.,* 2018; Hussain *et al.,* 2009). Different plants known to accumulate and concentrate different minerals at different phenological stages which in turn affects their palatability status (Gunassekran *et al.,* 2014). Previous studies corroborated that minerals contents increase or decrease at different life phases of plant species (Khan & Hussain, 2012; Smith, 1970; Wilkinson & Gross, 1967; Fleming, 1963). This study was designed to assess the mineral content of selected plant species at three phonological stages viz. pre-reproductive, reproductive and post-reproductive stages. The varying mineral status of plants in turn determines the palatability preferences of herbivores in a locality; hence this study will help in enumerating the palatability statuses of these plants in future studies.

## Materials and Methods

**Study area:** Kurram is a newly established Tribal District of Khyber Pakhtunkhwa, Pakistan. It is a beautiful, green valley located in the northwest of the country. The newly formed Kurram district is located between 33 ° 20 "and 34 ° 10" N latitude and 69 ° 50 "and 70 ° 50" E longitude (Abbas *et al.,* 2020). The Kurram Valley is 115 km long and covers a total area of 3380 square kilometers. Phytosociologically, the vegetation and forests of Koh-e-Sufaid slopes are unique with representatives from Sino-Japanese vegetation type (Hussain *et al*., 2019). Kurram's natural forests cover about 8% of the area. The land being cultivated is 35% while the rest of 47% is barren. The main forest types of the Kurram are dry tropical forest and subalpine scrub (Hussain *et al.,* 2020). The area is very rich in plant resources, but little ecological work has been done in the region. The dry tropical vegetation occupies the southern parts, while dry temperate and alpine vegetation types occur in the northern parts of the area. In the area studied, the climate is of the highland type and varies at different altitudes.

**Sampling:** Five plant species viz: *Indigofera heterantha* voucher number (B. Huss.615.UOP), *Medicago sativa* (B. Huss.622.UOP), *Quercus baloot* (B. Huss.495.UOP), *Rubus fruticosus* (B. Huss.733.UOP) *and Tagates minuta* (B. Huss. 362.UOP) were selected for elemental analysis at pre-reproductive, reproductive and post-reproductive phases. These plant species exhibited a uniform dispersion across the study area and they had high density and cover values (Ali *et al.,* 2018; Hussain *et al.,* 2019). Of these plant species, *Tagatus minuta* is rarely palatable species, which was chosen to make a comparison with those of the palatable plant species. It is evident from a number of previous studies that (Anderson & Roberts, 1984; Dastagir *et al.,* 2014; Del *et al*., 2016; Ali *et al.,* 2018) mineral status of plants differ at different phonological stages and that is why some of the plant species are less palatable at pre-reproductive stage while others are at post-reproductive stage. Plant samples were collected randomly from 15 monitoring sites in Koh-e-Sufaid range, District Kurram. These plants specimens were preserved, identified and deposited in Herbarium Department of Botany, University of Peshawar. Before analysis, plants were oven dried at $65^{o}$ C for a time period of 72 hours. Clean and clear polythene bags were used to store the powdered plant material.

**Digestion of plant samples:** One gram dried sample of selected samples were taken in the flask and then digested by 12 ml of concentrated Nitric acid and left over night. The 5ml of per chloric acid was added to the solution, heated on hot plates for 20 minutes till the solution appeared transparent. The sample was cooled and then filtered using Watmann filter paper No.42. The filtrate was then transferred to 100 ml volumetric flask. Each filtrate was stored in glass bottles and duly labeled (Dastagir *et al.,* 2014).

**Mineral analyses:** Five samples solution were investigated for different elements by Atomics Absorptions Spectrometers (Shimadzu AA-670) with suitable hollows cathodes lamps. The values of various minerals were recorded by CSC curves got by applying standards AR grades solution of minerals potassium, magnesium, calcium, sodium, iron, cobalt, manganese, copper, chromium and zinc.

## Results and Discussion

All 5 plants species selected for minerals composition were investigated to estimate the proportion of 11 elements at three phonological phases (pre reproductive, reproductive and post-reproductive stages). These mineral comprised of three macro, seven micronutrients and one trace element. The *I. gerardiana, R. fruticosus and T. minuta* are found throughout the valley while M. sativa is restricted to plains and clay loamy and moisture rich soil while the distribution of Q. baloot was confined to the northern slopes.

### 1. *Indigofera gerardiana* Baker

*I. gerardiana* is a common shrub in study area. Its palatable plant species eat by herbivores. Higher values of K, Fe while lower concentrations of Cu, Cr, Zn, Mg, Na, Fe, Co, Mn and Ca make this plant mostly consumed in pre-reproductive phase. More concentrations of Cr, Cu, Zn, Na, Mg, Co, Mn and Mg at post reproductive stage made it less palatable for the herbivores.

**Macronutrients:** In *I. gerardiana*, Mg levels were low at reproductive phase (54600 µg/g) and highestat pre-reproductive phase (99800 µg/g). On the other hand, Ca levels were maximum at post-reproductive phase (96700µg/g). K contents were maximum during early phase of life (32300 µg/g) and lowest at mature stage (19900 µg/g). K levels were noted as 23500 µg/g at reproductive phase.

**Micronutrients:** A gradual decrease in the micronutrient content of *I. gerardiana* was noted with increasing age of the plant. Cu concentration was 61800 µg/g at reproductive phase, 42100 µg at pre reproductive phase while 53600 µgg at post reproductive phase. Hence, there was a substantial decrease of 8200 µg/g from reproductive to post- reproductive phases. Similar trend was observed in Mg content; it was recorded 99800 µg/g at post-reproductive phase while just 54600 µg/g during floral period. Zn concentration was 38600 µg/g at mature stage while they were 18800 µg/g and 20800 µg/g at pre-reproductive and reproductive phases. Values for Fe were 11300 µg/g at pre-reproductive stage which drastically decreased to 9000 µg/g at flowering period and further reduced to 2000 µg/g at mature phase. Cobalt content was recorded as 16600 µg/g in post-reproductive phase and just 800 µg/g at pre-reproductive stage. Nickle content reduce to 1200 µg/g at post-reproductive stage while it was 13400 µg/g at pre-reproductive stage. Nickle levels again increased during the reproductive phase 16000 µg/g. Chromium concentrations was maximum at reproductive phase (8800 µg/g) and it reduced during post reproductive stage (5600 µg/g).

**Trace elements:** In this plant species highest Na levels were recorded during pre-reproductive phase (87500 µg/g), which is slightly decreased to (10350 µg/g) at reproductive stage. During mature phase Na concentrations was 99500 µg/g (Table 1, Fig. 1). Our results are strongly supported by similar studies carried out by Ali, 2017; Del *et al*., 2016; Hussain *et al*., 2016; Tariq *et al.,* 2015 and Ahmad *et al.,* 2014.

**Table 1. Mineral profile of five selected palatable plants growing wild in Koh-e-Safaid Range, Upper Kurram.**

| S. No | Parameters | *Indigofera gerardiana* | | | *Medicago sativa* | | | *Quercus baloot* | | | *Rubus fruticosus* | | | *Tagetes minuta* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PR stage (µg/g) | R stage (µg/g) | POR stage (µg/g) | PR stage (µg/g) | R stage (µg/g) | POR stage (µg/g) | PR stage (µg/g) | R stage (µg/g) | POR stage (µg/g) | PR stage (µg/g) | R stage (µg/g) | POR stage (µg/g) | PR stage (µg/g) | R stage (µg/g) | POR stage (µg/g) |
| 1. | K | 32300 | 23500 | 19900 | 69600 | 67000 | 59000 | 6301 | 5321 | 4993 | 89300 | 53500 | 33500 | 90200 | 22400 | 21500 |
| 2. | Ca | 89000 | 89100 | 96700 | 8000 | 8700 | 8900 | 11615 | 10350 | 9352 | 69900 | 44400 | 8800 | 39600 | 49900 | 69900 |
| 3. | Mg | 19900 | 54600 | 99800 | 9900 | 10800 | 11800 | 1612 | 1832 | 2129 | 5900 | 8100 | 96700 | 8800 | 9300 | 10800 |
| 4. | Co | 1800 | 800 | 16600 | 8100 | 6000 | 6100 | 8800 | 9721 | 8973 | 17800 | 8800 | 8800 | 8800 | 1800 | 8100 |
| 5. | Cu | 42100 | 61600 | 53600 | 29900 | 21700 | 12700 | 9.9 | 5.9 | 9.9 | 8700 | 59900 | 18900 | 9900 | 18800 | 9200 |
| 6. | Cr | 4500 | 8800 | 5600 | 8800 | 8800 | 8800 | 56.0 | 23.5 | 31.5 | 35600 | 3500 | 8800 | 16600 | 76600 | 3700 |
| 7. | Fe | 59300 | 25300 | 20900 | 2900 | 800 | 8800 | 260 | 269 | 62 | 2900 | 9100 | 18800 | 18300 | 9100 | 20900 |
| 8. | Ni | 13400 | 16000 | 1200 | 1700 | 600 | 11900 | 2.0 | 104.2 | 122.9 | 46400 | 16600 | 4200 | 11300 | 11300 | 1100 |
| 9. | Mn | 45500 | 54600 | 99800 | 88000 | 88000 | 88000 | 54.0 | 244.0 | 89.0 | 19900 | 8000 | 93300 | 9700 | 9700 | 9700 |
| 10. | Zn | 18800 | 20800 | 38600 | 88000 | 69600 | 59800 | 7.9 | 39.9 | 6.9 | 17800 | 69800 | 8800 | 88000 | 18800 | 17000 |
| 11. | Na | 87500 | 103500 | 99500 | 20200 | 20460 | 29900 | 12 | 49 | 51 | 87500 | 19900 | 29900 | 28700 | 30400 | 18900 |

**Key to abbreviations:** PR = Pre-Reproductive stage; R = Reproductive stage; POR = Post-Reproductive stage

**2. *Medicago sativa* L.**

Its most common persistent herbs found mainly in the plains of study area. It is a compactly tufted growing forage plant. The whole plant is very much palatable as they are readily consumed by cows, goats, sheep, donkeys and horses. Our findings revealed that Co, K, Cu, Mn. Fe and Zn levels were greater in pre-reproductive phase while the concentrations levels of Cr, Cu, Na and Ni were recorded lower during early stage of their life.

**Macronutrients:** In *M. sativa*, Mg levels showed an increase from pre- reproductive phase to reproductive Phase (9900 µg/g and 10800 µg/g respectively). But K levels decreased from 6900 µg/g at pre-reproductive to 6700 µg/g at reproductive phase. At post-reproductive stage K levels stood at their highest i.e. 59900 µg/g. Calcium content was recorded as 8000 µg/g reproductive, 8700 µg/g pre reproductive and 8900 µg/g during post-reproductive stages respectively. Though increase in Ca content progressively was not significant but still a gradual increase in Ca content with age of the plant noted.

**Micronutrients:** A decrease in Fe content was noted in *M. sativa* from pre reproductive to reproductive phases. Iron levels were 2900 µg/g at pre reproductive stage that markedly decrease to 800 µg/g at reproductive stage. Quite a significant increase in Fe levels was noted at post reproductive phase (8100 µg/g). A gradual decrease in Co levels was noted with increasing age of the plant, which were 8100 µg/g and 6000 µg/g at pre reproductive and reproductive phases respectively. At post-reproductive stage an increase of 100 µg/g in Co levels was noted (6100 µg/g). Substantial decrease in Cu content was noted as the plant aged. Copper content was 29900 µg/g at pre reproductive, 21700 µg/g during reproductive and 12700 µg/g at post-reproductive stages. For Ni, highest values were recorded at post-reproductive phase (11900 µg/g) while at pre reproductive phase Ni content remained at 1700 µg/g, which dropped to just 600 µg/g at reproductive phase. Manganese levels showed no decrease or increase at any phenological stage and remained more or less the same. Zinc was found to be 8800 µg/g in pre-reproductive phase that rose (69900 µg) decrease to 59800 µg/g at post-reproductive phase, which indicates that this plant increased its Zn uptake during flower set.

**Traces elements:** Sodium levels were almost same at pre-reproductive i.e 2020o µg/g and post-reproductive stages (2046 µg/g). Notable increase of Na content was observed 2990 µg at postreproductive phase (Table 1, Fig. 2). Our results are similar with work of (Dastagir *et al.*, 2014; Tiffany *et al.*, 2000; Rominger *et al.*, 1975; Smith, 1970; Wilkinson & Gross, 1967 and Fleming, 1963).

**3. *Quercus baloot* Griffth**

*Quercus baloot* is an evergreen shrub or tree that may reach to 5-12 meters height. The leaves are green, elliptical and inverted to elongated and highly palatable. The sheep and goats are eating the buds and young leaves of *Quercus baloot.*

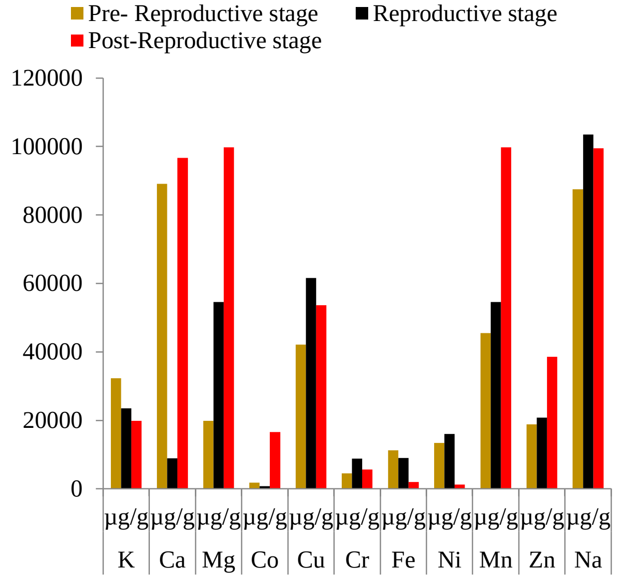


Fig. 1. Mineral composition of *Indigofera gerardiana* Baker.

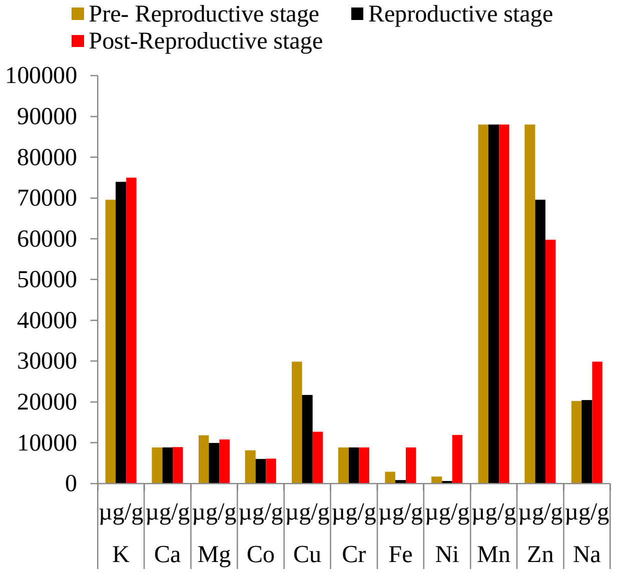


Fig. 2. Mineral composition of *Medicago sativa* L.

**Macronutrients:** The shoots and softer plant parts of *Q. baloot* are preferred by the browsing animals of the locality. A gradual increase in Mg content was noted. Magnesium concentrations were 1612 µg/g during Pre-reproductive phase that rose up to (1832 µg/g) reproductive phase. As Mg uptake increases with age in *Q. baloot*, at post-reproductive stage the Mg content clicked at 2129 µg/g. Potassium remained at 6302 µg/g in pre- reproductive phase but decreased to 5321 µg/g during reproductive phase and post-reproductive phase in that order. Calcium concentrations were recorded (11615 µg/g) at pre-reproductive, (10350 µg/g) reproductive and (9352 µg/g) at post-reproductive phases.

**Micronutrients:** Iron contents were as 260 µg/g during pre-reproductive and 269 µg/g at reproductive phase. Significant decrease was seen in Fe content at post-reproductive phase (62 µg/g). Cobalt concentration remained at 8800 µg/g at pre-reproductive phase that rose up to 9721 µg at reproductive phase. At post reproductive phase, Co levels fell to 8973 µg/g. Copper levels were low at all phenological stages. At pre-reproductive phase Cu contents were 9.9 µg/g, 5.9 µg/g at reproductive phase & 6.9 µg/g at post-reproductive phase. Zinc levels stood at 7.90 µg at pre-reproductive phase that showed substantial increase i.e 39 µg/g at reproductive phase. Mn was recorded as 54 µg/g at pre reproductive, 244 µg/g during reproductive and 89 µg post-reproductive phase. Ni content increased slightly with the age of the plant as it was just 2 µg/g, 4 µg/g, and 8 µg/g at pre-reproductive and post-reproductive phases in order. Similar pattern was seen in case of chromium. Chromium levels were 5 µg/g, 23 µg/g and 31 µg/g at reproductive, pre and post-reproductive phases in order.

**Trace elements:** At pre-reproductive stage Na content was recorded as 12 µg/g which showed four times increase at reproductive stage (49 µg/g), it further rose up 51.0 µg/g at post-reproductive stage. Our results are backed by the results of Ali *et al*., 2018; Cheema *et al*., 2011; Zafar *et al*., 2010 and Adnan *et al*., 2010) (Fig. 3).

### 4. *Rubus fruticosus* L.

*R. fruiticosus* is among the preferred species for grazers and browsers. It is highly palatable at young stage as it has higher levels of potassium, magnesium, cobalt, sodium, nickel and chromium during pre-reproductive phase. During reproductive phase that one had higher levels of Cu and Zn. During post reproductive stage it had greater levels of Fe and Mn (Sagliocco & Bruzzese, 2004).

**Macronutrients:** A decrease in K levels was noticed from pre-reproductive phase (53500 µg/g) to post reproductive phase (33500 µg/g). Highest K levels observed at reproductive phase i.e., 89300 µg/g. Mg levels were noted 5900 µg/g at pre-reproductive phase which rose to 8100 µg/g and 9670 µg/g at reproductive and post- reproductive phases. Ca concentrations reduced with the age of plant. At pre-reproductive phase Ca concentration was noted 69900 µg/g at pre-reproductive while 44400 µg/g at reproductive and 8800 µg/g at post-reproductive phases.

**Micronutrients:** As the plant aged, iron content increased. Iron levels were noted as 2900 µg/g at pre- reproductive phase that substantially rose to 9100 µg/g at reproductive and 18800 µg at post-reproductive phase. The Cobalt concentration was an exception as its values remained 17800 µg/g at pre reproductive phase while it decreased to 8800.0 µg/g equally at post-reproductive & reproductive phases. Similarly, Cu, contents were greater at reproductive phase (59900 µg/g) which reduced to (18900 µg/g) Postreproductive phase. Zinc concentrations were lowest at post-reproductive phase (8.8 µg/g). Manganese levels were 19900 µg/g at pre-reproductive phase that drastically reduced (8000 µg/g) at reproductive phase. At post reproductive phase manganese content again increased markedly to 93300 µg/g. Nickle content was lowest at post-reproductive phase (42 µgg.) At pre reproductive phase, Ni levels remained 46400 µg/g, and then dropped to (16600 µg/g) at reproductive phase. Similar pattern was noticed for Cr, which remained (35600 µg/g), at pre-reproductive phase while (3500 µgg) at reproductive and post-reproductive phases respectively (Fig. 4).

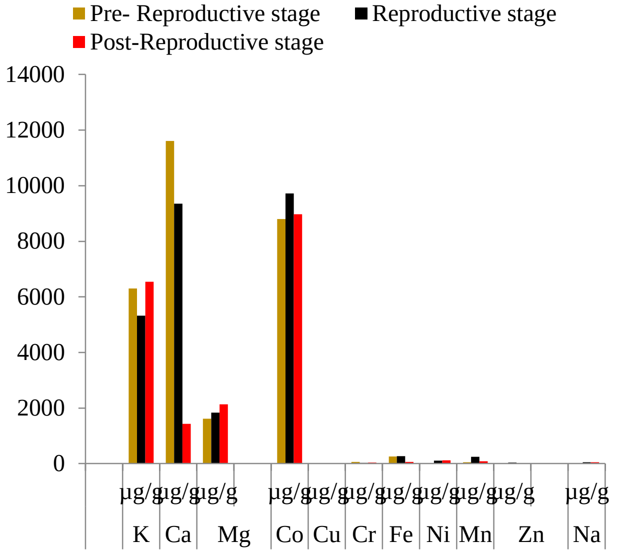


Fig. 3. Mineral composition of *Quercus baloot* Griffth.

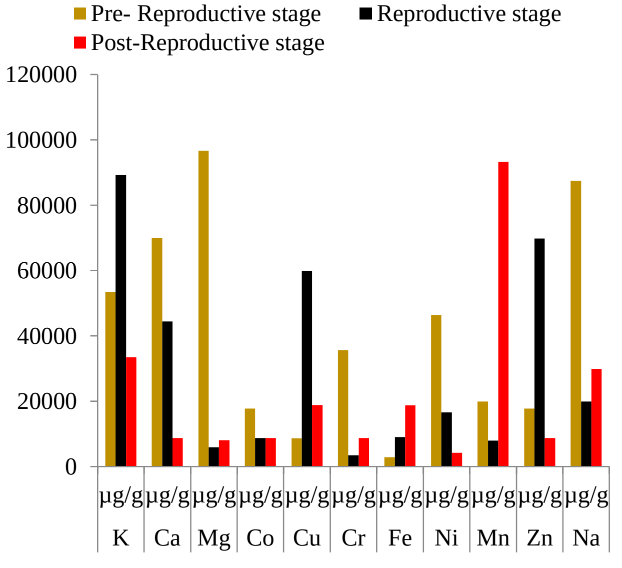


Fig. 4. Mineral composition of *Rubus fruitcosus* L.

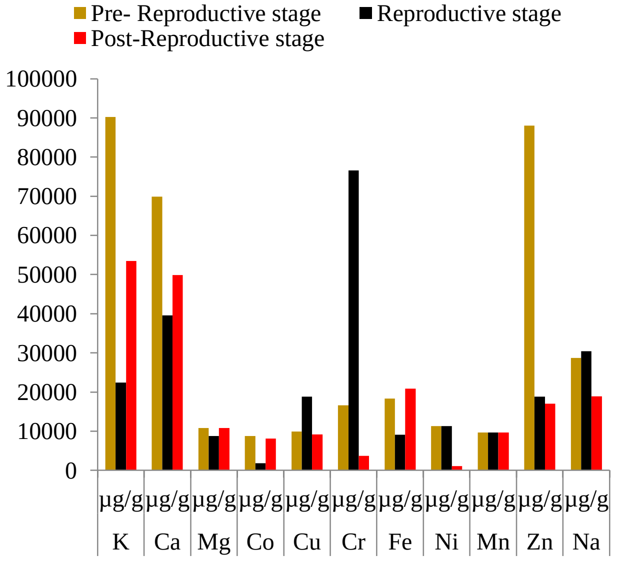


Fig. 5. Mineral composition of *Tagates minuta* L.

**Trace elements:** Highest Na levels were recorded at pre-reproductive phase (87500 µg/g). Sodium levels dropped to19900 µg/g at reproductive phase but once again improved at post-reproductive phase (29900 µg/g). These results are supported by studies carried out by Cheema *et al*., 201; Zafar *et al*., 2010, James *et al*., 2010 and Hanif *et al*., (2006. Ali *et al*., (2018) have documented lesser levels of sodium in rest of the plants.

**5. *Tagetes minuta* L.**

*T. minuta* is palatable annual herb, commonly found along the road sides and in plains of Upper Kurram valley. Phytochemical analysis revealed that this plant had higher levels of Ca, Fe, Mn and Mg at post-reproductive stage. This might be a cause of its palatability at this stage (Deel *et al*., 2016).

**Macronutrients:** Highest K concentration was recorded during pre-reproductive phase i.e., 90200 µg/g that decreased with time and at reproductive stage, the K levels were 22400 µg/g. Potassium was recorded (53500 µg/g) at Post-reproductive phase. Magnesium level was greater than before with maturity of the plant. Magnesium contents were (88000 µg/g) during reproductive phase which gradually decreased to 9300 µg/g at reproductive phase. Highest Mg concentrations were observed at Post-reproductive phase 10800µgg. Calcium concentrations were recorded (39600 µg/g) Pre-reproductive phase, 49900 µg/g at reproductive phase and 69900 µg/g during Post-reproductive phase. This clearly indicates that Ca uptake in *T. minuta* increases with the age of the plant.

**Micronutrients:** Among micronutrients, Fe content was noticed 18300 µg/g during pre-reproductive phase that dropped 9100 µg/g during reproductive phase. A notable increase in Fe levels was seen at Post-reproductive phase (20900µg/g). Co contents were greater during Pre-reproductive and Post-reproductive phases. At pre-reproductive phase Co content remained 8800 µg/g while 8100 µg/g at post-reproductive phase. Lowest Co levels were observed during the reproductive phase (1800 µg/g). Copper content was found to be 9900 µg/g at pre-reproductive phase which raise to 18800 µg/g at reproductive phase. Lowest Cu values were found at post reproductive phase (9200µg/g). Zinc content also decreased as the plant matured. Zn concentration was 88000µg/g at Pre-reproductive, 18800µg/g at reproductive and 1700µg/g during Post-reproductive phases. Ni concentrations remained the same at equally pre and reproductive phases 11300 µg/g. Nickle content was at its lowest at post reproductive phase (1100 µg/g). No substantial change was observed in Mn level at all phenological stages. Highest levels of Cr were note down during reproductive phase (76600 µg/g). Cr content was lowest at the Post-reproductive phase that is3700 µg/g (Fig. 5).

**Trace elements:** The Na levels were 28700 µg/g during reproductive, 30400µg/g at pre-reproductive, and 30400 µg/g at post reproductive phase (Fig. 5). Lowest Na levels were noticed during post-reproductive stage (18900 µg/g). These findings are line with the Del *et al*., 2016;

Cheema *et al*., 2011, Zafar *et al*., 2010; James *et al*., 2010 and Hanif *et al*., (2006). It is important to mention in some other plant species low Na concentration was documented (Ali *et al*., 2018).

## Conclusions

This research was designed to understand that how palatability preferences of herbivores change with changing mineral content of plants during three phenological stages. A total of 11 minerals were studied in 5 different plant species belonging to different palatability classes. Results clearly indicate that the concentration of these minerals was different in all selected plant species as well as different at various phenological stages. It was noticed that Ca, Mg and K levels showed an increase as the passage of plants aged. In *Q. baloot* Ca levels decreased while other micronutrients varied at three phenological stages. At pre-reproductive and post-reproductive phases, Fe, Mn, Mg and Ca levels increased. This may be the reason behind palatable status of selected palatable plants at different phenological stages while rest of the micro minerals varied at different phonological stages due to edaphic and environmental variables. The mineral profile of the plants analyzed indicated that mineral levels were as per the required standards and their concentrations were not drastically different from other parts of Pakistan.

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