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EVALUATION OF GRASS SPECIES FOR ELEMENTS THROUGH ICP-OES TECHNIQUE

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ABSTRACT: The aim of the study is to screen the grasses of South India for mineral nutrition. The results revealed that the grass species accumulate the major and minor elements at different concentrations. The maximum concentration (ppm) of macroelements in the analysed samples were 12270, 31310, 5309 and 3946 for Ca, K, Mg and P respectively. The maximum concentrations (ppm) of microelements were 50.05, 89.68, 2452, 258.3, 64.01 and 76.05 for B, Cu, Fe, Mn, Mo and Zn respectively. 22% of ash content and 4192 cal/g of calorific value were found. The levels of macro and micro elements determined of the grasses were analysed by using ICP – OES. Elemental studies of the grass species revealed that large amounts of major and minor elements were rich in *Aristida hystrix* and *Echinochloa colonum*, which may be suggested for livestock feeding.

INTRODUCTION: A major constraint to livestock production in developing countries is the scarcity and fluctuating quantity and quality of the year-round feed supply. Grasslands provide the primary forage for ruminants particularly cattle, throught much of the year. Many minerals make up the body composition of animals, they play many fundamental roles and many of them are essential to the normal vital function of animals.In the animal body, major minerals constitute more than 100 mg/g (Ca, P, Mg, Na, K, Cl and S), while micro minerals or trace elements (Fe, Zn Cu, Mb, Se, I, Mn, Pb, Cd, Co, Cr, Al, As, Si Ni and Sn) are present in lower amounts [1]. In general fewer mineral deficiencies or toxicities occur in animals when a major portion of the diet consists of concentration levels of production as well as the genotype of the animal influences mineral requirements and tolerance of animal for minerals[2].

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Elements are essential for normal growth of plants, their protection against viruses and completion of their life cycle [3].

There is an increasing awareness of the need to pay greater attention to the role of elements in plant and animal nutrition and welfare. Some elements are essential nutrients for plant growth and often also for food and feed quality because the primary route for their intake by humen and animals is plants. Animals having developed a dependency on these trace elements, which are an important physiological effects when present at concentrations other than those associated with classical toxicity and with extreme deficiency.

The essential elements are involved in many metabolic processes of physiology, especially as enzymes activators, e.g., Fe, Zn and Mn [4]. They can also interact with some organic compounds such as flavonoids, influencing their biological activity [5]. This present study has conducted with the objective of assessing the major and trace elements in ten grass specie, which are used to feed livestock. Major trace elements play a very important role and often toxic in higher doses. The assessment of trace elements is essential which could potentially be useful to animals. The nutritive values of grasses had been studied by [6, 7, 8, 9] but studies on elemental analysis are scarity. Hence the present work includes qualitative and quantitative determination of various elements in ten selected grass species were carried out by using ICP-OES technique. Due to the lack of knowledge on nutritive value of feed and feed ingredients farmers are unable to formulate the balance diet for their animals.

As a result quality status of these feed ingredients and their effect on animal performance is not known properly. Scientific study on the evaluation of the quality of such feeds and feed ingredients thus becomes necessary to satisfy the farmers, scientists and the feed manufacturers for various purposes. So determining the quality of these feed ingredients will help to address this problem.

MATERIAL AND METHODS:

Material

Ten grass species *Alloteropsis cimicina* L., *Aristida hystrix* L.F., *Aristida setacea* Retz., *Brachiaria racemosa* L., *Chloris barbata* Swartz., *Cymbopogon coloratus* (Hook.f) Stapf, *Cynodon dactylon* (L.) Pers., *Dactyloctenium aegyptium* (L.) Willd., *Digitaria sanguinalis* (L.) Scop. and *Echinochloa colonum* (L.) Link. were collected from different places of South India, in 2010, authenticated by BSI Coimbatore (Tamilnadu) and were dried at 1050C for 24h. Dried samples were grinded and homogenized using an agate homogenizer and stored in poly- ethylene bottles until analysis.

Experimental

Apparatus

Perkin Elmer 7000DV model ICP-OES was used for the determination of elements. Its appearance alone sends out a definite signal: The Perkin Elmer optical emission spectrometer (Perkin Elmer 7000DV, USA) is different from conventional ICP-OES. The Perkin Elmer can be out fitted with an interface for either axial or radial plasma observation. The proprietary ICAL system logic automatically monitors operation of the Perkin Elmer guaranteeing continuous optimum operating conditions. The operating parameters of ICP-OES were set as recommended by the manufacturer. The ICP-OES operating conditions and analytical characteristics of elements are listed in **Tables 1** and **2**, respectively. Milestone start D microwave (Sorisole- BgItaly) closed sys- tem (maximum pressure 1500psi, maximum temperature 300C was used.

TABLE.1 THE OPERATING PARAMETERS OF DETERMINATION OF ELEMENTS BY ICP-OES.

Instrument	Perkin Elmer
Viewing height (mm)	12
Wavelength	nm
Replicates	3
RF Power (W)	1300
Spray chamber	cross flow
Nebulizerflow (L/min)	nebulizer
Plasma Torch Quartz, fixed, 3.0	0.8
mm injector tube Replicate read time	30 s per replicate
Plasma Gas Flow (L/min)	15
Auxiliary Gas Flow (L/min)	0.2
Sample aspiration rate (mL/min)	1.0

TABLE 2 ANALYTICAL CHARACTERISTICS OF ANALYTE IONS BY ICP-OES.

Elements	Analyte Wavelength(nm)	Slopes of the calibration curves
Boron	249.677	0.99998
Calcium	317.933	0.99989
Copper	327.393	0.99989
Iron	238.204	0.99987
Potassium	766.490	0.99998
Magnesium	285.213	0.99991
Manganese	257.610	0.99994
Molybdenum	202.031	0.99952
Phosphorus	213.617	0.99954
Zinc	206.200	0.99975

Reagents and solutions

All chemicals used throughout the experiments were of analytical reagent grade (Merck, Darmstadt, Germany). HNO3 (65%), H2O2 (30%) and HCI (37%) were of suprapur quality (E. Merck, Darmstadt, Germany).

All glassware and polyethylene bottles were kept overnight by soaking in 10% HNO3, and cleaned by rinsing five times with distilled de-ionized Ultra High Quality (UHQ, chemical resistivity: 18MXcm1) water (Millipore, Bedford, MA, USA) prior to use. Aliquots of an ICP multi element standard solution (10 mg/L) containing the analyzed elements (B, Ca, Cu, Fe, K, Mg, Mn, Mo, P and Zn) was used in the preparation of calibration solutions. These solutions were prepared by serial dilution with 0.2% (v/v) HNO3 to the required concentrations with UHQ water prior to use. For calibration, commercially available standard solutions were used. The ranges of the calibration curves (6 points) were selected to match the expected concentrations (10–500 mg/L) for all the elements of the sample studied by ICP-OES

Mineral analysis

Grass samples were digested and made up the final solution to volume with UHQ water, the concentrations of each of Ca, K, Mg, P, B, Cu, Fe, Mn, Mo, and Zn were determined by ICP-OES equipped with an auto-sampler. Prior to analysis, the instrument was calibrated according to manufacturer's recommendation. Blank digestion was also carried out by completion of full analytical procedure without plant sample. All determinations were made in triplicate. We used standard addition method for possible matrix effect.

CALORIFIC VALUE

An Digital Bomb Calorimeter (Model – RSTB- 3, Rico, INDIA) was used to determined the calorific value of the dry powders of the by using the following formula sample.

$$\text{Formula: } CV_s = \frac{T \times W - (C_{VT} + C_{VW})}{M}$$

T= Final rise in temperature in Degree Celsius.

M= Mass of sample in grams.

H= Known Calorific Value of Benzoic Acid in cal/gram.

W=Water Equivalent in calories per degrees centigrade.

CV T= Calorific Value of thread.

CV W= Calorific Value of Ignition wire.

CV S=Calorific Value of sample.

ASH CONTENT

Method recommended in pharmacopoeia of India and British Pharmacopoeia.10 were followed for determining Ash value and percentage method.

Preparation of Ash - 3g of dried powder of plant sample was incinerated in a Silica crucible over the burner. The charred material was heated in muffle furnace for six hours at 600-650oC.The ash formed was white and free from carbon. It was cooled and weighed on the ash less filter paper.

RESULTS AND DISCUSSION:

There are various sources contributing to the metal composition of the grass species. The sample pre-treatment procedure must take in to account the analyte interest, the matrix characteristics and the minimal required time period of the analytical technique considered. The major elements like Ca, K, Mg, P, and minor elements like B, Cu, Fe, Mn, Mo, and Zn were analysed by using ICP – OES. The results of the analyses are summarized in **Table 3**.

TABLE 3: ELEMENTAL DETECTION OF TEN GRASS SPECIES ON A DRY WEIGHT BASIS EXPRESSED AS PPM SAMPLE

S. NO	Scientific name of the Grass	Ca	K	Mg	P	B	Cu	Fe	Mn	Mo	Zn
1	*Alloteropsis cimicina* L.	4268 ± 185	12830 ± 123	1194± 68	1138 ± 95	42.18± 1.11	57.91 ±0.14	471.7 ±5.23	72.34 ±1.59	17.98± 1.02	67.42± 2.36
2	*Aristida hystrix* L.F.	4483 ± 223	31310 ± 241	3107± 75	1648 ± 21	50.05± 2.08	39.05 ±0.21	494.9 ±6.01	176.7 ±2.65	18.62± 1.25	72.77± 4.25
3	*Aristida setacea* Retz.	3317 ± 162	11190 ± 230	1155± 23	1085 ±45	31.41± 0.91	24.61 ±0.11	395.5 ±5.20	62.47 ±5.08	10.74± 2.01	55.13± 5.32
4	*Brachiaria racemosa* L.	4743 ± 131	23760 ± 980	2667± 81	1592 ±87	26.16± 0.83	56.85 ±0.41	2325± 7.36	81.50 ±6.36	63.56± 1.23	72.03± 4.98
5	*Chloris barbata* Swartz.	7406 ± 254	22050 ± 845	3484± 59	3946 ±49	29.00± 1.01	86.99 ±0.65	703.7 ±9.12	118.9 ±10.1	24.33± 2.30	76.05± 9.25
6	*Cymbopogon coloratus* (Hook.f) Stapf	4627 ± 160	15460 ± 563	1751± 80	1511 ±94	31.61± 0.76	89.68 ±0.25	444.9 ±5.23	96.45 ±6.35	15.77± 3.20	58.87± 7.58

7	*Cynodon dactylon* (L.) Pers.	12270 ± 194	24440 ± 758	5309± 73	1634 ±20	33.25± 0.86	19.09 ±0.63	260.1 ±4.56	115.9 ±9.36	9.45±1.36	58.21± 1.58
8	*Dactyloctenium aegyptium* (L.) Willd.,	4050 ± 184	19990 ± 652	1994± 69	2225 ±45	37.51± 1.41	60.28 ±0.74	1737± 2.23	246.0 ±12.25	46.58± 4.23	70.41± 4.89
9	*Digitaria sanguinalis* (L.) Scop.	4733± 151	15870 ± 952	2113± 59	1483 ±41	23.38± 1.32	58.96 ±0.52	2452± 5.23	82.53 ±6.87	64.01± 1.25	66.40± 5.69
10	*Echinochloa colonum* (L.) Link.	5468± 156	28320 ± 624	4122± 89	2813 ±29	21.19± 0.94	31.12 ±0.69	453.4 ±2.63	258.3 ±14.20	15.17± 1.65	72.76± 8.36

Results are Mean of triplicate estimation on dry weight basis ± standard error

The calcium concentrations varied from 3317 to 12270 ppm. Aristida setacea had the lowest concentration and Cynodon dactylon had highest. The concentrations of calcium were comparable in sample *Cymbopogon coloratus*, *Digitaria sanguinalis* and *Brachiaria racemosa* with a range of 4627, 4733 and 4733 ppm respectively. Concentration sufficient for growth of plant is 5000 mg/kg Calcium is required by meristematic and differentiating tissues. During cell division it is used in the synthesis of cell wall, particularly as calcium pectate in the middle lamella. It is also needed during the formation of mitotic spindle. It accumulates in older leaves. It is involved in the normal functioning of the cell membranes.

Calcium is the most abundant element in the animal body and it is fundamental for the activity of many enzyme systems, coagulation of blood, transmission of nerve impulses, contraction of muscles, flocculation of casein in the stomach and many other [11]. About 26 to 30% of total ash content of most animals is Ca, NRC lists the maximum tolerable levels of Ca as 2% of diet dry matter. Calcium is used in the development and maintenance of bone structure. It plays functional role in the clotting process, nerve transmission, hormone function and metabolism of vitamin D etc.

The potassium concentrations of selected species varied from 11190 to 30310 ppm. Highest potassium content observed in *Aristida hystrix* followed by *Echinochloa colonum*, *Cynodon dactylon* and *Brachiaria racemosa*. The potassium concentrations were comparable in *Brachiaria racemosa* and *Cynodon dactylon* ranges from 23760 to 24440 ppm. 10,000 mg/kg concentration is sufficient for growth of plant. Potassium together with sodium helps to regulate the water balance within the body and transfer of nutrients to the cell, transmits electrochemical impulses and is necessary for normal growth enzymatic reactions [12]. Potassium is the main intracellular cation and plays a role of primary importance in nerve and muscle excitability [13].

Magnesium concentrations were found 1155 to 5309 ppm. Five samples having the content between 2113 to 4122 ppm, while two samples *Alloteropsis cimicina* and *Dactyloctenium aegyptium* had the equal concentration 1194 ppm. *Cynodon dactylon* had the highest concentration and *Aristida setacea* had the lowest concentration. 2,000 mg/kg concentration is sufficient for growth of plant. Magnesium is a key element in cellular metabolisms. For high metabolic rate, cells require high magnesium. In presence of higher percentage of potassium and phosphorous, absorption of magnesium increases. Loss of magnesium leads to hyper irritability. Adults may suffer muscles tremors, memory loss, inability to concentrate, apathy and depression [12]. About 60-70% of the total magnesium of the organism is localized in the skeleton, where it is closely associated with calcium and phosphorus the remainder is found in the soft tissues and body fluids [1].

The phosphorus concentrations of selected grass species varied from 1085 to 3946 ppm. Chloris barbata had the highest concentration and Aristida setacea had the lowest concentration. The concentration of phosphorus were comparable in Cymbopogon coloratus, Brachiaria racemosa and Cynodon dactylon, Aristida hystrix ranges from 1577 to 1592 and 1634 to 1648 ppm. 2,000 mg/kg is necessary for growth of plant.

Phosphorous is tied to calcium in bone structure and plays a significant role in CNS function. Phosphate is the primary iron in extracellular and intracellular fluid; it aids absorption of dietary constituents, helps to maintain the blood at a slightly alkaline level, regulatory enzyme activity and is involved in the transmission of nerve impulses [12]. The highest amount of Boron recorded in Aristida hystrix 50.05 ppm followed by Alloteropsis cimicina, Dactyloctenium aegyptium and Cynodon dactylon. While least amount was recorded in *Echinochloa colonum* 21.09 ppm. Concentration sufficient for growth of plant is 20 mg/kg. Boron is required for uptake and utilisation of Ca2+, membrane functioning, pollen germination, cell elongation, cell differentiation and carbohydrate translocation. Agricultural practice has well established that adequate B supply is imperative for obtaining high yield and good quality. Knowledge about metabolic functions of B in plants remains incomplete.

Recent research findings have greatly improved our understanding for B uptake and transport processes [14], and roles of B in cell-wall formation [15], cellular membrane functions, and anti-axidative defense systems have been suggested [16]. A beneficial or even essential role of B in animal metabolism is supported by the findings that low B concentrations induce the MAPK pathway in cultured animal cells with a knockout of the B transporter Na B C1, the mammalian homolog of At B or 1, stop to develop and proliferate [17].

The copper concentrations varied from 19.09 ppm to 89.68ppm in ten species. Cynodon dactylon had the lowest concentration and Cymbopogon coloratus had the highest. The concentrations of copper content were comparable in species one and nine ranges from 57.91 to 58.96. Lowest amount of Cu was recorded by 18 in Cynodon dactylon. 6 mg/kg concentration sufficient for growth of plant. Copper is an important mineral in dopamine synthesis. Low level of dopamine results in decrease in activity of central nervous system.

A deficiency of copper may cause hypertension, antibiotic sensitivity, hyperactivity, hyperglycemia, manic disorders insomnia, allergies and osteoporosis [12]. Cu is universally important cofactor and activator of numerous enzymes which are involved in development and maintenance of the cardiovascular system. A Cu deficiency can result in a decrease in the tinsel strength of arterial walls, leading to aneurysm formation and skeletal maldevelopment [19]. Copper is essential for the synthesis of haemoglobin it is involved in the synthesis of coetaneous pigments and crimp tensile strength, elasticity and affinity for dyes of wool. Cu is the main constituent of the bone, connective tissue, brain, heart, and many other body organs [20].

The average Iron content of 973.7 ppm. the highest amount of fe content found in Digitaria sanguinalis 2452 ppm followed by Brachiaria racemosa and Dactyloctenium aegyptium while least amount of fe content found in Cynodon dactylon 260.1. Highest amount were recorded by 18 in Cynodon dactylon. 100 mg/kg concentration is sufficient for growth of plant. Iron plays a significant role in oxygen transport in the body. A deficiency of Iron can impair neuronal development, sweating, rapid pulse, prolonged sleep, cessation of the menses, aversion to eating and heavy feeling of body [21]. Iron is an essential mineral and an important component of proteins involves in oxygen transport and metabolism [22]. Most iron is combined with proteins, it participates in the composition of haemoglobin, myoglobin and cytochromes.

The highest amount of Manganese recorded in *Echinochloa colonum* 258 and the least amount was recorded in Aristida setacea 62.47 ppm. The manganese concentrations were comparable in sample four and seven ranges from 81.50 to 82.53 ppm. Lowest amount were recorded by 18 in *Cynodon dactylon*. 50 mg/kg concentration is sufficient for growth of plant. Mn is a component of several enzymes including manganese-specific glycosyltransferase and phosphoenolpyruvate carboxykinase and essential for normal bone structure.

Mn deficiency can manifest as transient dermatitis, hypocholesterolemia, increased ALP level, skeletal abnormalities, retarded bone growth, change in hair colour to growth, abnormalities in pancreas, and disturbances in lipid and carbohydrate metabolisms [21]. Mn is an important electrolyte also responsible for proper bones, liver, kidneys, pancreas and pituitary gland function. It also works as co-factor for more than 300 metabolic reactions [23]. Manganese was shown to prevent ataxia in lambs. The present results show that the level of 'Mn' is well acceptable. Manganese participates to the non-toxic function of the iron, vitamin C and the potentiation of the hypoglycaemic effect of adrenaline [24].

The molybdenium concertrations varied from 9.451 to 64.01 ppm. Highest molybdenum concentrations observed in Digitaria sanguinalis 64.01 and least concentration in Cynodon dactylon. 0.1 mg/kg is required for growth of plant. The concentrations of molybdenium were comparable in sample Brachiaria racemosa, Digitaria sanguinalis; Echinochloa colonum, Cymbopogon coloratus and Cynodon dactylon, Aristida setacea ranges from 81.50 to 82.53; 15.17 to 15.77 and 9.45 to 10.74 ppm. Molybdenium is a component of coenzyme that is essential for the activity of xanthine oxidase, sulphite oxidase and aldehyde oxidase [25]. It acts as a detoxification agent in the liver as a part of the sulfite oxidase enzyme and it possibly retards degenerative diseases, cancer and ageing. Molybdenum is important essential trace element involved in metabolism through metalloenzymes [26].

The highest amount of Zinc concentration observed in Chloris barbata 76.05 ppm followed by *Aristida hystrix*, *Echinochloa colonum* and *Brachiaria racemosa*, while the lowest amount observed in *Aristida setacea*. While two samples *Echinochola colonum* and *Aristida hystrix* had the equal concentration 72.76 ppm and sample *Cynodon dactylon* and *Cymbopogon coloratus* were comparable concentration ranges from 58.21 to 58.87 ppm. Lowest amount was recorded by 18 in *Cynodon dactylon*. Required content for growth of plant is 20 mg/kg. Zinc deficiency are associated with mental impairments, lethargy, emotional disorder and irritability [27]. Zinc has the tendency to accumulate in bone tissue rather than in the liver, but it is found in every tissue and reaches rather high levels in skin, hair and wool.

It is an activator of several enzyme systems and enteres in the composition of insulin. Zinc is necessary for the functioning of over 300 different enzymes and plays a vital role in an enormous number of biological process [28]. The physiological activities of the plant influence the Zn absorption and the interaction with many elements like Fe and Mn. Cu affects Zn uptake [29] and its deficiency causes many physiological disorders. Besides, it is responsible for stimulating growth of epidermal and epithelial cells [30].

All plant species had the ash content between 14 to 22%. Chloris barbata had the highest content followed Cymbopogon coloratus, Aristida hystrix and Brachiaria racemosa. While sample Aristida hystrix, Brachiaria racemosa and Dactyloctenium aegyptium, Digitaria sanguinalis had the equal content. The results of the analyses are summarized in **Table 4**. The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, treatment etc. The constituents of the ash also vary with time and from organ to organ. Ash usually represents the inorganic part of the plant.

TABLE: 4 ASH AND CALORIFIC VALUES OF TEN GRASS SPECIES ON A DRY WEIGHT BASIS EXPRESSED AS PPM SAMPLE

S.No	Grass name	Calorific value cal/g	Ash %
1	*Alloteropsis cimicina L.*	3240 ± 245	12 ± 0.25
2	*Aristida hystrix* L.F.	3435 ± 320	17± 0.29
3	*Aristida setacea* Retz.	3582 ± 193	11± 0.18
4	*Brachiaria racemosa* L.	3020 ± 112	17± 0.24
5	*Chloris barbata* Swartz.	3338 ± 095	22± 0.45
6	*Cymbopogon coloratus* (Hook.f) Stapf	4192 ± 180	19± 0.12
7	*Cynodon dactylon* (L.) Pers.	3191 ± 224	14± 0.26
8	*Dactyloctenium aegyptium* (L.) Willd.	3606 ± 315	15± 0.36
9	*Digitaria sanguinalis* (L.) Scop.	3118 ± 201	15± 0.16
10	*Echinochloa colonum* (L.) Link.	3411 ± 326	16± 0.33

Results are Mean of triplicate estimation on dry weight basis ± standard error

The calorific value varied from 3020 to 4192 cal/g. *Cymbopogon coloratus* had the highest calorific value and *Brachiaria racemosa* had the lowest concentration. The calorific value were comparable in *Echinochloa colonum*, *Aristida hystrix* and *Digitaria sanguinalis*, *Cynodon dactylon* ranges from 3411 to 3435 and 3118 to 3191 cal/g. Highest calorific values in partly attributed to the slight difference in the moisture content of the material at the time of analysis. The results of the analyses are summarized in **Table 4.** The starch as a binder has been reported to have ability of increasing the calorific values [31]. Calorific concentrations in plants increase with decreasing total available isolation over the span of a growing season.

The differences in concentration of various elements may be due to the differences in botanical structures of the plants and also due to the mineral composition of the soil. Moreover the difference may be due the ability of plants to accumulate the elements from the surrounding aerial or aquatic environment either for their physiological requirement or as a precautionary measure. This in turn enables some plants to be used as bio monitors for environmental pollution[32]. The level of essential trace elements in the plants varies by the geochemical characteristics of the soil and also by the ability of the plants to select and absorb some of these elements. Further, the bioavailability of the elements depends on the nature of their association with the constituents of the soil. Plants are readily assimilate elements through their roots. The additional sources of these elements for plants are rainfall, atmospheric dusts, plant protection agents and fertilizers that can be absorbed though the leaf blendes [33]. Generally it is concluded that the studied products are rich source of essential elements Mg, Ca, Zn, and Cu, hence might play an important role in the maintenance of the nutritional requirements. The results presented in this paper enlarge the knowledge of the elemental composition of these grass species, will be useful and of interest in the toxicological and nutritional fields.

CONCLUSION: The above results indicate that the grass samples are a good source of essential nutrients required for the well being of livestock feed. The presence of potassium, phosphorus, calcium, iron, magnesium, copper etc. in high concentrations in the grass samples suggests its use in therapeutic purposes. Thus the presence of the nutraceutically valued minerals in the plants points toward the possibility of their use to restore the different imbalances caused in the body. It is therefore concluded that the selected grass species of the study are rich in elements may also help in biodiversity function etc.

From the results it indicated that variations in elemental composition and concentration among the species and different ecological areas. There reflecting differences in physiological functioning of the specific plants depending upon the elemental interaction within it. Our preliminary study having baseline information about mineral constituents of grass species of South India, it will be helpful to develop an approach towards direct link between elemental content and its curative probability having coherence with traditional use and livestock feed. The present results may provide an useful information to formers for cultivation of grasses which are consisting high mineral and calorific values.

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