



Determination and Comparison of Macro, Trace and Toxic Element Content of Some Roughages by ICP OES Technique

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ABSTRACT

It is critical to determine the amount of mineral and toxic elements in roughages, which play an important role in animal nutrition. In this study, macro (Ca, K, Na, Mg, P), trace (Fe, Mn, Zn, Cu, Ni, Co, Cr, Sn, V, Si, Se, Sr, Sb, B), and toxic element (Al, As, Be, Cd, Pb) analysis of 14 roughages were determined by the Inductively Coupled Plasma Optik Emission Spectrometry (ICP OES) method. CRM (NCS DC 73350, poplar leaves) confirmed the accuracy of the method. The samples were solubilized according to the microwave wet digestion method. The macro, trace and toxic element contents of 14 roughages were compared and evaluated according to the results of the analyses. Furthermore, the hierarchical clustering analysis (HCA) displayed the groups as dendrograms. Toxic elements like As, Be, Cd, Co, and Sb were not found in any of the samples. The only one that was found was Pb, which was 0.181±0.12, 0.45±0.07, and 0.40±0.13 mg kg⁻¹ in grain corn leaf, sorghum leaf, and lentil straw, in that order. On the other hand, Sn was found as 0.01±0.005 mg kg⁻¹ only in fodder pea stalk and Si was identified as 1015±140 mg kg⁻¹ only in chickpea straw. In the study, it can be suggested that different roughages contain macro and trace elements at varying rates, and these roughages can be included in the rations according to these element ratios.

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Bazı Kaba Yemlerin Makro, İz ve Toksik Element İçeriklerinin ICP OES Tekniği Kullanılarak Belirlenmesi ve Karşılaştırılması

ÖZET

Ruminant hayvanların beslenmesinde önemli bir yere sahip olan kaba yemlerde mineral ve toksik elementlerin miktarının belirlenmesi oldukça önemlidir. Bu çalışmada, 14 kaba yemin makro (Ca, K, Na, Mg, P), iz (Fe, Mn, Zn, Cu, Ni, Co, Cr, Sn, V, Si, Se, Sr, Sb ve B) ve toksik element (Al, As, Be, Cd, Pb) miktarları İndüktif Eşleşmiş Plazma Optik Emisyon Spektrometresi (ICP OES) yöntemi ile belirlenmiştir. Yöntemin doğruluğu CRM (NCS DC 73350, kavak yaprakları) ile teyit edilmiştir. Örnekler mikrodalga yaş yakma yöntemine göre çözündürülmüştür. Analiz sonuçları çerçevesinde 14 kaba yemin makro, iz ve toksik element içerikleri karşılaştırılmış ve değerlendirilmiştir. Ayrıca, hiyerarşik kümeleme analizi (HCA) ile gruplar dendrogram olarak gösterilmiştir. Çalışma sonucunda, tane mısır yaprağı, sorgum yaprağı ve mercimek samanında sırasıyla 0,181±0,12, 0,45±0,07, 0,40±0,13 mg kg⁻¹ Pb tespit edilirken, toksik elementler (As, Be, Cd, Co ve Sb) hiçbir örnekte tespit edilmemiştir. Öte yandan, Sn sadece yem bezelyesi sapında (0.01±0.005 mg kg⁻¹) ve Si sadece nohut samanında (1015±140 mg kg⁻¹) tespit edilmiştir. Çalışmada farklı kaba yemlerin değişen oranlarda makro ve eser element ihtiva ettiği ve bu kaba yemlerin içerdikleri bu element oranlarına göre rasyonlara dahil edilebileceği önerisi yapılabilir.

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INTRODUCTION

In addition to using high-yield breeds, countries must feed animals with rations that meet their nutritional requirements in an adequate and balanced manner to develop livestock farming. In the feeding of ruminants, various industrial wastes, pulps, and some cereal residues and straws are also used to meet the need for roughage due to insufficient quality roughage resources (Mohamoud & Kılıç, 2018). Ruminants rely heavily on the nutrients in the roughages they add to their rations, their microbial digestion in the rumen, and the conversion of these nutrients into metabolic energy (Selçuk et al., 2023). The contribution of breeding studies to the increase in efficiency in animal production was found to be 30–35%, and the contribution of improvements in the environment, care, nutrition, feed, feed processing, and technologies was found to be 60–65% (Kutlu & Özen, 2009). Sources defined as active substances in animal nutrition are basically examined in two basic groups: minerals and vitamins. The mineral requirements of animals are met by forming feed mixtures in certain ratios. While the needs of farm animals for macrominerals such as sodium, calcium, and phosphorus are met by the direct use of natural resources rich in these minerals, the need for trace minerals and vitamins is met by the addition of premixes prepared based on the relevant standards to the feed. Developments in this field include innovations in production technologies, especially in order to increase the usefulness of minerals and the stability and effectiveness of vitamins, as well as the use of preserved amino acid sources in the rumen for high-yielding dairy cows (Kutlu & Özen, 2009). The most significant advancement in mineral nutrition in recent years has been the discovery of newly defined functions for certain trace minerals and their use as feed supplements. Especially studies on Se are gaining importance with its antioxidant power (Surai, 2002a, 2005; Yu et al., 2008) and it improves reproductive quality in animals. (Surai, 2002b; Juniper et al., 2009). Despite ongoing detailed discussions on the essential properties of Cr, a trace element, experts agree that this element plays a crucial role in energy, fat, and protein metabolism (Pechova & Pavlata, 2007; Wang et al., 2007; Wang & Xu, 2008). This element, which forms the core of the glucose tolerance factor, aids insulin and optimizes glucose metabolism, prevents fat synthesis from carbohydrates, encourages the burning of fat for energy in the cell (mitochondria), and improves performance while reducing blood glucose and cholesterol concentrations. Within the scope of trace minerals and their importance in animal nutrition, a significant number of studies have been conducted on the B element in recent years, showing that boron is related to immune function (Hunt, 2003), reduces cholesterol and triglyceride levels, reduces lipid peroxidation, and increases the durability of antioxidant enzymes (Bakken & Hunt, 2003), improves mineral balance, and affects calcium metabolism and bone development (Qin & Klandorf, 1991; Kurtoğlu et al., 2001; Kurtoğlu et al., 2002; Eren et al., 2004; Bozkurt et al., 2007). It has been stated that it improves egg shell quality (Yeşilbağ & Eren, 2007) and increases the synthesis of some steroid hormones (Small et al., 1997). On the other hand, under field conditions, V phytotoxicity (chlorosis and dwarfing) is extremely rare. Intake of feed with 10 to 300 mg kg⁻¹ feed dry matter animals induced black diarrhea, weakness, spontaneous abortions, decreased milk production, and high mortality in animals.

Minerals are essential because they cannot be synthesized in the organism, that is, they are necessary for the continuation of vital activities (Ergül, 1993; Kutlu et al., 2005); therefore, a significant part of these substances that animals need are met by feed (Gökkuş et al., 2013; Eğritaş & Önal Aşçı, 2015). Plants, on the other hand, can obtain the nutrients they need from the soil as long as they are in an available form (Özyazıcı, 2019). Animals need to receive minerals with their rations, as their bodies cannot synthesize them biologically. These inorganic substances are essential for their healthy survival and optimal genetic structure. In addition to vitamins, the effective utilization of both vitamins and other nutrients is crucial. Macro elements, especially phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), which are among the mineral substances, are as important as other nutrients in the nutrition of animals (Ergül, 1993; Gülümser et al., 2017). The skeletal system of animals, the structure of teeth and bones, the development of the muscle and nervous system, and certain vital elements in animal physiology all depend on these minerals. It is well known that these minerals play a crucial role in various activities; Minerals with more than 50 milligrams per kilogram of body weight are called macrominerals (Kutlu et al., 2005). Calcium is essential for milk yield as well as for the skeleton, eggshell, and teeth. It enters the structure and takes part in the transmission of nerve impulses; its deficiency resulted in rickets, osteomalacia, and hypocalcaemia (Gürsoy & Macit, 2017). A deficiency in Ca can lead to bone softening, particularly in young animals, and deformation of bones in older animals. Acid-base balance involves K, and a deficiency leads to tetany (Gürsoy & Macit, 2017). Moreover, Mg functions as an enzyme activator, causing meadow tetany in cases of deficiency (Aksoy et al., 2000; Türkmen et al., 2011; Gürsoy & Macit, 2017). Magnesium deficiency is associated in decreased functions of some elements such as Ca, Mg, vitamin D, methionine, and arginine (Aksoy et al., 2000; Demir, 2005; Türkmen et al., 2011; Çimrin & Demirel, 2012). In addition, in Mg deficiency, various diseases may occur in

animals, and even death may occur if necessary precautions are not taken. (Ensminger et al., 1990; Ergül, 1993; Sabah & Çelik, 2001; Tekeli et al., 2003). In general, phosphorus (P) is essential for fertility, bone, and it functions in the formation of teeth, in case of deficiency, especially pica (abnormal labour) syndrome is observed. Additionally, P deficiency causes bone development to stop and teeth to become damaged. It causes fractures, loss of appetite, and weakness. On the other hand, minerals that contain less than 50 milligrams per kilogram of body weight, known as microminerals, include Fe, Cu, Zn, Mn, B, I, F, Se, Mo, Cr, V, Sn, Si, and Ni (Kutlu et al., 2005). Boron is involved in sugar transport in plants, cell wall formation, lignin formation, carbohydrate, respiration, and phenol metabolism, as well as the structural and functional properties of biological membranes, the endocrine system, the immune system, and the brain. Boron is involved in Ca and Mg metabolism. Iron is involved in the structure of hemoglobin in milk-fed animals, pregnant animals, and ovulation. Animals during the period have a high need for Fe, and a deficiency can lead to anemia (Gürsoy & Macit, 2017). In this regard, adding it to rations can effectively address anemia problems in animals. Copper forms the coenzyme moiety, or essential moiety, of many enzymes, participates in the structure of feather pigments, and contributes to hemoglobin synthesis in cases of deficiency, nervous ailments, anemia, and discoloration of hair and fleece (Gürsoy & Macit, 2017). Manganese is involved in bone development and cholesterol synthesis; deficiency, perosis (deformities of foot and leg skeletons in chickens), eggshell formation, deterioration, and reproductive impairment are observed (Gürsoy & Macit, 2017). Zinc has an antioxidant effect and takes part in the structure of enzymes in deficiency, decreased food intake, decreased feed utilization, impaired reproductive functions, growth retardation, and abnormalities in feathers, skin, hair, and wool (Gürsoy & Macit, 2017). On the other hand toxic elements; Cadmium (Cd) is a mineral element highly toxic to animals and humans, and not essential to physiological and biochemical functions (Reis et al., 2010). Cadmium, when more than 15 µg is taken daily, it accumulates in the kidneys, disrupts renal arterioles and glomeruli. In excess, it can be fatal. For cattle, diet containing 5 to 30 mg of Cd kg⁻¹ and, for sheep, diet containing > 40 mg of Cd kg⁻¹ of DM (dry matter) it would be toxic effect. In animal diets, the maximum concentration of Cd tolerated is 0.5 mg kg⁻¹ (McDowell, 1992; NRC, 2001). When Cd is ingested, it is absorbed by intestinal cells and transported by blood flow to the liver. In the liver, this mineral element induces metallothionein synthesis, a protein that is involved in detoxification of heavy metals. Lead (Pb) is also a ubiquitous heavy metal. Lead has biological functions in the animal body but is highly toxic to animals and humans (NRC, 2001). Being one of the most dangerous minerals to animal health, has worldwide distribution and is accumulated in the environment by industrial pollution (Patra et al., 2006; Patra et al., 2007; Swarup et al., 2007). Lead poisoning in animals can be considered a risk to public health, since there is an accumulation of this mineral in meat and milk (when animals have blood concentration ≥ 0,20 µg of Pb mL⁻¹), for human consumption, it may intoxicate them (Swarup et al., 2005). For cattle, 400 to 600 mg of Pb kg⁻¹ of BW (body weight), for sheep 4,5 mg of Pb kg⁻¹ of BW, for goats 400 mg of Pb kg⁻¹ is considered toxic. Arsenic (As) causes poisoning and even deaths in animals and humans. Efforts are being made to combat arsenic, which is widely found in nature, but is frequently brought to the agenda due to its use in the production of various industrial, medical and agricultural chemicals (Kaya et al., 1990). Conversely, elemental As is not toxic, although some arsenical compounds are highly toxic. There are no reports of animal toxicity attributed to naturally occurring Al in the environment. Bentonite, kaolin, Al₂(SO₄)₃, Al₂O₃, and Al(OH)₃ have been used as growth stimulants for rabbits, chickens, sheep, cattle, and hogs without adverse effects as long as they composed no more than 1-2 percent of the diet (Sorenson et al., 1974). Under natural dietary conditions because of the low concentrations of Sb believed generally to occur in plants, toxicity to animals from plant sources is unlikely. Sittig (1976) reported, however, that marine animals concentrate Sb in their muscle tissue, and that studies had indicated certain levels in this tissue that were toxic to some fish. Moreover, many reports do not specify the chemical form of an element when discussing its toxicity. It can be assumed for most of these reports that, under the specified natural or experimental conditions, the element existed in an undetermined compound and that only the total concentration of the element was known. For most reports in which the basis for expressing concentrations of an element in plant or animal tissues is not specified, a dry-weight basis can be assumed. Some reports define the minimum lethal dose (MLD); that is, the amount of a substance that kills the organism in one dose. In reports that relate toxicity to body weight of the experimental animals, "live" or "wet" weight of the animal is commonly used. Selenium (Se) is an essential micromineral to animals (Kommisrud et al., 2005; Haddad and Alves, 2006; Carroll and Forsberg, 2007) and humans. This mineral element is part of several selenoproteins, including glutathione peroxidase (GSH-Px). On the other hand, when Se is ingested on high concentrations above the maximum tolerable dose of Se in diet (2 ppm) (McDowell, 1992) may cause poisoning in animals and humans, and is also known as selenosis (Papp et al., 2007). Furthermore, foods containing ≥ 200 mg Se kg⁻¹ for cattle and more than 0.8 mg Se kg⁻¹ BW for sheep are considered toxic. The toxic doses have not been known yet because there is disagreement between results of published studies, and still the physiological mechanisms involved in mineral poisoning are also not yet understood. For some mineral elements, the published papers that have evaluated these mechanisms are lacking probably due to the difficulty of describing the

physiological mechanisms, changes involved in the process of poisoning and also difficulty of establishing toxic dose of a mineral element due to the dependence of each mineral element, its form, specie and age of animals and the interaction between minerals in metabolism (Reis et al., 2010)

Graphite furnace atomic absorption spectrometry (GFAAS) (Rusin et al., 2021), flame atomic absorption spectrometry (FAAS) (Ahmadi & Ziraati, 2015), inductively coupled plasma optic emission spectrometry (ICP OES) (Tokay & Bağdat, 2022), mass spectrometry (ICP MS) (Kowalska et al., 2020; Bağdat et al., 2019), and polarography and voltammetry (Palisoc et al., 2018) are some of the methods used to find elements in substances. Among these techniques, ICP OES offers a wide dynamic linear range.

The purpose of this study was to compare the macro, trace, and potential toxic elements of 14 roughages, which play a significant role in animal nutrition, and to make a valuable contribution to the literature and research on this topic.

MATERIAL and METHOD

After harvesting the crops, 14 roughages were collected from the field in sufficient quantities and placed in polythene bags. Coarse impurities were removed from the samples, and then the samples were kept in an oven at 65 °C for 24 hours.

Reagents and Digestion Procedure

The samples were thoroughly crumbled in a blender, and 0.3 g of each sample was weighed into TFM containers for microwave (Milestone Start D) wet digestion and 2 ml of 30% H₂O₂, then 8 ml of 65% HNO₃ was added to the samples, and solubilization was carried out according to the digestion program in Table 1.

Table 1. Microwave digestion program for roughages samples
Çizelge 1. Kaba yemler için mikrodalga yakma programı

Step (plant samples)	Time (min)	T	Power (W) (°C)
1	20:00	180	1200
2	15:00	180	1200

Laboratory Analyses

After solubilization, the samples were made up to 50 ml with ultrapure water (Millipore Corporation, USA) and analyzed in ICP OES (Thermo ICAP 6300, England). The operating conditions of the ICP OES device are shown in Table 2. In addition, the element content of certified reference materials (NCS DC 73350, poplar leaves) and recovery of elements by method are shown in Table 3, and the amount of elements contained in the roughage is shown in Table 4.

Table 2. Instrumental operating conditions for Thermo ICAP 6300 ICP-OES
Çizelge 2. Thermo ICAP 6300 ICP-OES için enstrümantal çalışma koşulları

Parameter	Normal	Hydride System
RF Power	1100 W	1300 W
Flush Pump rate	100 rpm	-
Pump speed	50 rpm	30 rpm
Purge gas	Argon	Argon
Pump tubing type	Tygon Orange/white	-
Coolant Gas		
Flow	12 L min. ⁻¹	16 L min. ⁻¹
Auxiliary gas		
Nebulizer flow	0.6 L min. ⁻¹	0.3 L min. ⁻¹
Auxiliary gas flow	0.5 L min. ⁻¹	0.5 L min. ⁻¹
View Mode	Axial, Radial	Axial
Auto sampler	Cetac ASX-260	-

Table 3. Element content of certified reference materials (NCS DC 73350, poplar leaves) and recovery of elements by method

Çizelge 3. Sertifikalı referans maddenin (NCS DC 73350, kavak yaprakları) element içeriği ve elementlerin metotla geri kazanımı

Elements	Found value (mg kg ⁻¹)	Certified value (mg kg ⁻¹)	Recovery (%)
Al (Aluminium)	842.4±11	1040±60	81
As (Arsenic)	0.344±0.2	0.37±0.09	93
B (Boron)	51.4±0.6	53±5	97
Ca (Calcium)	11615±78	13506±161	86
Cd (Cadmium)	0.316±0.006	0.32±0.07	99
Co (Cobalt)	0.428±0.006	0.42±0.03	102
Cr (Chromium)	0.555±0.46	0.55±0.007	101
Cu (Copper)	7.22±0.07	9.3±1	77.6
Fe (Iron)	275±2.4	274±17	100.4
K (Potassium)	102.9±93	13800±70	74.6
Mg (Magnesium)	4855±15	6500±50	74.7
Mn (Manganese)	36.9±0.25	45±4	82
Ni (Nickel)	2.01±0.2	1.9±0.3	106
P (Phosphorus)	2569±6.8	2435±15	105.5
Pb (Lead)	1.635±0.76	1.5±0.3	109
Sr (Strontium)	113.9±1	154±9	74
Zn (Zinc)	32.5±0.25	37±3	88

Statistical Analyses

Hierarchical clustering analysis (HCA) was performed according to the between-group linkage method in the SPSS-22 statistical program, and results are shown as a dendrogram in Figure 4.

RESULTS and DISCUSSION

Macro elements: In terms of Ca, lentil straw had the highest value (11711±33 mg kg⁻¹) followed by camelina stalk (7343±5.8 mg kg⁻¹), while the lowest value was in the grain corn cob (309±4.1 mg kg⁻¹). The study found lentil straw to be a very good source of calcium for animals. Furthermore, in terms of K, sweet corn stalk (30685±120 mg kg⁻¹) and fodder pea stalk (27251±48 mg kg⁻¹) had the highest value, while sorghum straw (4054±33 mg kg⁻¹) had the lowest value. Mg was highest in sweet corn leaf (3437±91 mg kg⁻¹) and lowest in grain corn cob (351±2 mg kg⁻¹). On the other hand, wheat straw had the highest Na value (369±6.7 mg kg⁻¹), while grain corn leaf (102±9.4 mg kg⁻¹) had the lowest value. In terms of P, sorghum stalk had the highest value (497±3.5 mg kg⁻¹), followed by sweet corn leaf (435±14 mg kg⁻¹), while grain corn cob (107±0.07 mg kg⁻¹) and soybean stalk (107±0.4 mg kg⁻¹) had the lowest value. Table 4 and Figure 1 display the results. When compared with the study conducted by Gürsoy & Macit, (2017), P, K, Ca, and Mg ratios of macronutrients of legume forage crops samples were changed 1.16-1.28%, 0.70-2.69%, 0.56-1.61%, and 0.11-0.51%, respectively. While the macronutrients of wheat forage crops, P ranged between 1.10-1.19%, K content 1.99-3.25%, Ca content 0.09-1.15%, and Mg content 0.07-0.26%. On the other hand, the P values obtained in this study were found to be considerably lower than those of both wheat forage crops and legume forage crops. While Ca values were in the same range in Camelina stalk, rice straw, and wheat straw with wheat forage crops, but lower than legume forage crops. Mg values of rice straw were in the same range of both wheat forage crops and legume forage crops, but Mg values of wheat straw and camelina stalk were lower than the others. On the other hand, K values were in the same range as legume forage crops but lower than wheat forage crops. Emmanuel et al. (2022) used AAS method in the analysis of sweet sorghum stalk samples and reported that the elemental concentrations were 25.02-22.87 for Ca, 27.14-45.40 for K, 25.02-22.52 for Na, 8.60-8.50 for Mg, 12.81-14.98 for Fe, 3.71-2.19 for Mn, 0.96-0.52 for Cu, and 18.15-17.90 mg/kg for P.

Figure 1 presents the radar graph of macro elements in roughages. This graph displays the prominent elements in each roughage.

Table 4. The amount of elements contained in some roughages (mg kg⁻¹, n=3, dry weight)
Çizelge 4. Kaba yemlerin içerdiği element miktarı (mg kg⁻¹, n=3, kuru ağırlık)

Elements	Al	As	B	Be	Ca	Cd	Co	Cr	Cu	Fe
Grain corn stalk	14.6±1	nd	6.9±0.2	nd	4503±0.2	nd	nd	0.52±0.5	4.1±0.35	39.5±1.6
Grain corn leaf	10±2.5	nd	10±0.2	nd	5281±270	nd	nd	0.8±0.8	3.9±1.3	38.5±0.5
Grain corn cob	32.9±0.9	nd	4.4±0.04	nd	309±4.1	nd	nd	nd	2±0.5	20.1±0.7
Sweet corn stalk	13.4±0.6	nd	8.6±0.14	nd	2774±21	nd	nd	0.38±0.16	1.3±0.5	31±1.5
Sweet corn leaf	29±2	nd	11.4±0.5	nd	6963±181	nd	nd	0.75±0.6	3.48±0.5	26±0.3
Sorghum straw	nd	nd	5.7±0.13	nd	1473±27	nd	nd	1.1±0.25	4.14±0.26	9.25±0.7
Sorghum leaf	41±1.3	nd	4.1±0.02	nd	4952±150	nd	nd	1.69±0.4	7.6±0.44	90±2.7
Wheat straw	21±2	nd	5.8±	nd	2679±53	nd	nd	1.7±1.2	4.2±0.9	51±0.9
Lentil straw	278±3	nd	20±0.02	nd	11711±33	nd	nd	1.4±0.4	7±0.5	334±5
Fodder pea stalk	22.8±0.12	nd	9.2±0.13	nd	5626±103	nd	nd	0.48±0.34	3.9±0.54	56±0.8
Soybean stalk	7±1.7	nd	14.6±0.36	nd	6381±310	nd	nd	0.85±0.07	4.8±1.4	28.8±0.4
Camelina stalk	64.9±3	nd	15±0.01	nd	7343±5.8	nd	nd	0.56±0.07	4.5±0.45	96±1.1
Rice straw	nd	nd	4.5±0.1	nd	2019±1.9	nd	nd	0.61±0.19	3.9±0.2	19.8±0.6
Chickpea straw	4.4±1.2	nd	9.7±0.35	nd	4874±168	nd	nd	0.50±1	2.7±0.13	10.5±0.34
Elements	K	Mg	Mn	Na	Ni	P	Pb	Sb		
Grain corn stalk	10319±23.1	2378±6.3	89±0.4	122±0.1	0.03±0.06	227±1.4	-	nd		
Grain corn leaf	11780±467	2614±127	109±4.7	102±9.4	nd	181±1.5	0.18±0.12	nd		
Grain corn cob	5154±26	351±2	6.6±0.01	132±3.2	0.60±0.05	107±0.07	nd	nd		
Sweet corn stalk	30685±120	1301±1.2	24.9±0.2	171±2.8	0.55±0.05	223±2.5	nd	nd		
Sweet corn leaf	18866±532	3437±91	90±2.5	138±5	1±0.2	435±14	nd	nd		
Sorghum straw	4054±33	490±3.6	16±0.08	229±6	0.18±0.04	215±5.8	nd	nd		
Sorghum leaf	12238±269	1548±35	42±1	117±4.5	0.46±0.004	497±3.5	0.45±0.07	nd		
Wheat straw	19182±241	680±10	14±0.2	369±6.7	1.6±0.008	162±0.8	-	nd		
Lentil straw	18933±127	1936±5	43±0.3	157±0.5	2.8±0.1	225±2	0.40±0.13	nd		
Fodder pea stalk	27251±48	989±2	7.3±0.13	180±0.1	0.30±0.005	216±3	nd	nd		
Soybean stalk	9043±200	2507±51	17±0.1	133±3.5	1±0.005	107±0.4	nd	nd		
Camelina stalk	11934±101	698±1.6	10±0.18	197±0.9	0.97±0.03	309±3	nd	nd		
Rice straw	14213±23	1962±12	60.3±0.5	320±3.9	0.80±0.1	118±0.3	nd	nd		
Chickpea straw	19244±565	1001±38	4.9±0.06	115±10	nd	144±3.75	nd	nd		
Elements	Se	Si	Sn	Sr	V	Zn				
Grain corn stalk	0.8±0.3	nd	nd	41.2±0.09	3.8±0.9	20.1±0.05				
Grain corn leaf	0.52±0.5	nd	nd	39.3±1.7	3.2±0.6	15.9±0.06				
Grain corn cob	1.2±0.2	nd	nd	6.2±0.03	0.20±0.04	74±0.05				
Sweet corn stalk	nd	nd	nd	79.4±0.35	1.8±0.2	25±0.2				
Sweet corn leaf	0.73±0.3	nd	nd	53.6±1.7	4.7±0.7	22.6±0.7				
Sorghum straw	0.51±0.02	nd	nd	9±0.1	0.53±0.14	5.8±0.2				
Sorghum leaf	0.62±0.08	nd	nd	35±0.8	1.4±0.2	14.6±0.1				
Wheat straw	0.92±0.06	nd	nd	43±0.6	0.34±0.48	14.6±0.07				
Lentil straw	0.8±0.9	nd	nd	33.5±0.09	nd	16±0.12				
Fodder pea stalk	1.6±0.4	nd	0.01±0.005	71.3±0.25	1.2±0.04	3.9±0.07				
Soybean stalk	1.4±0.002	nd	nd	64±1.1	3.5±0.3	5.9±0.1				
Camelina stalk	0.96±0.79	nd	nd	65±0.5	nd	8.6±0.08				
Rice straw	1.75±0.2	nd	nd	30±0.1	2.5±0.01	15±0.14				
Chickpea straw	0.55±0.1	1015±140	nd	46.5±1.3	1.25±0.5	8±0.19				

nd: not detected

Trace Elements

Lentil straw had the highest B content (20±0.02 mg kg⁻¹), Fe (334±5 mg kg⁻¹) and Ni (2.8±0.1 mg kg⁻¹) (Table 4, Figure 2). Furthermore, when it came to Fe content, lentil straw outperformed other roughages by a significant margin. Wheat straw had the highest value in Cr (1.7±1.2 mg kg⁻¹), followed by sorghum leaf (1.69±0.4 mg kg⁻¹). On the other hand, Cu was highest in sorghum leaf (7.6±0.44 mg kg⁻¹) and lowest in sweet corn stalk (1.3±0.5 mg kg⁻¹). Grain corn leaf (109±4.7 mg kg⁻¹) and sweet corn leaf (90±2.5 mg kg⁻¹) were found to be high in Mn, but, chickpea straw (4.9±0.06 mg kg⁻¹) had the lowest Mn content. In terms of Ni, in the grain corn leaf and the chickpea straw was below the LOD. The highest value for Se was found in rice straw (1.75±0.2 mg kg⁻¹). The stalk of sweet

corn was also below the LOD. In terms of Sr, sweet corn stalk ($79.4 \pm 0.35 \text{ mg kg}^{-1}$) had the highest value, followed by fodder pea stalk ($71.3 \pm 0.25 \text{ mg kg}^{-1}$) and the lowest value was found in grain corn cob ($6.2 \pm 0.03 \text{ mg kg}^{-1}$). The highest V value was observed in sweet corn leaf ($4.7 \pm 0.7 \text{ mg kg}^{-1}$), while lentil straw and camelina stalk were below the LOD, and Zn was highest in grain corn cob ($74 \pm 0.05 \text{ mg kg}^{-1}$), and lowest in fodder pea stalk ($3.9 \pm 0.07 \text{ mg kg}^{-1}$).

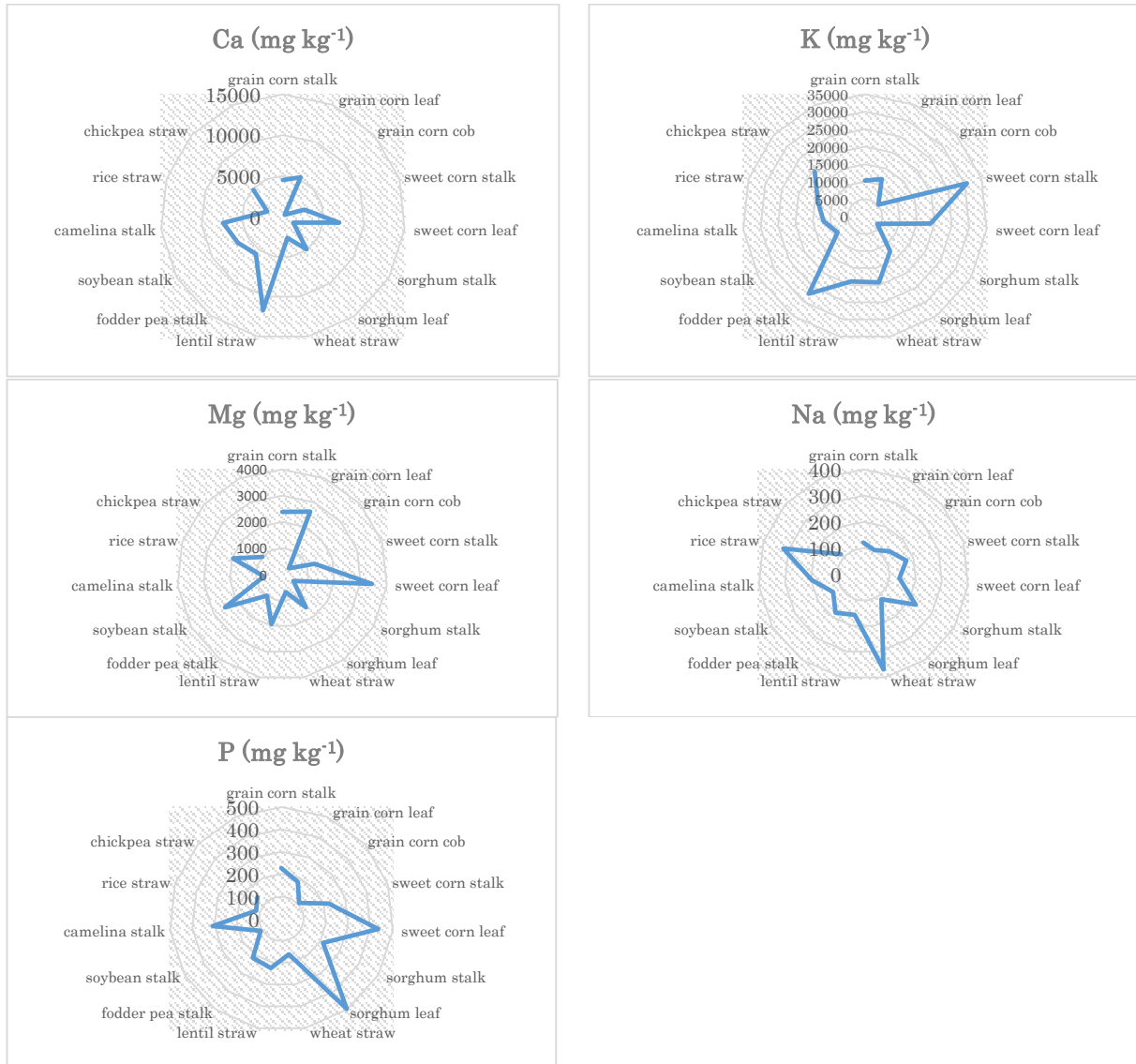
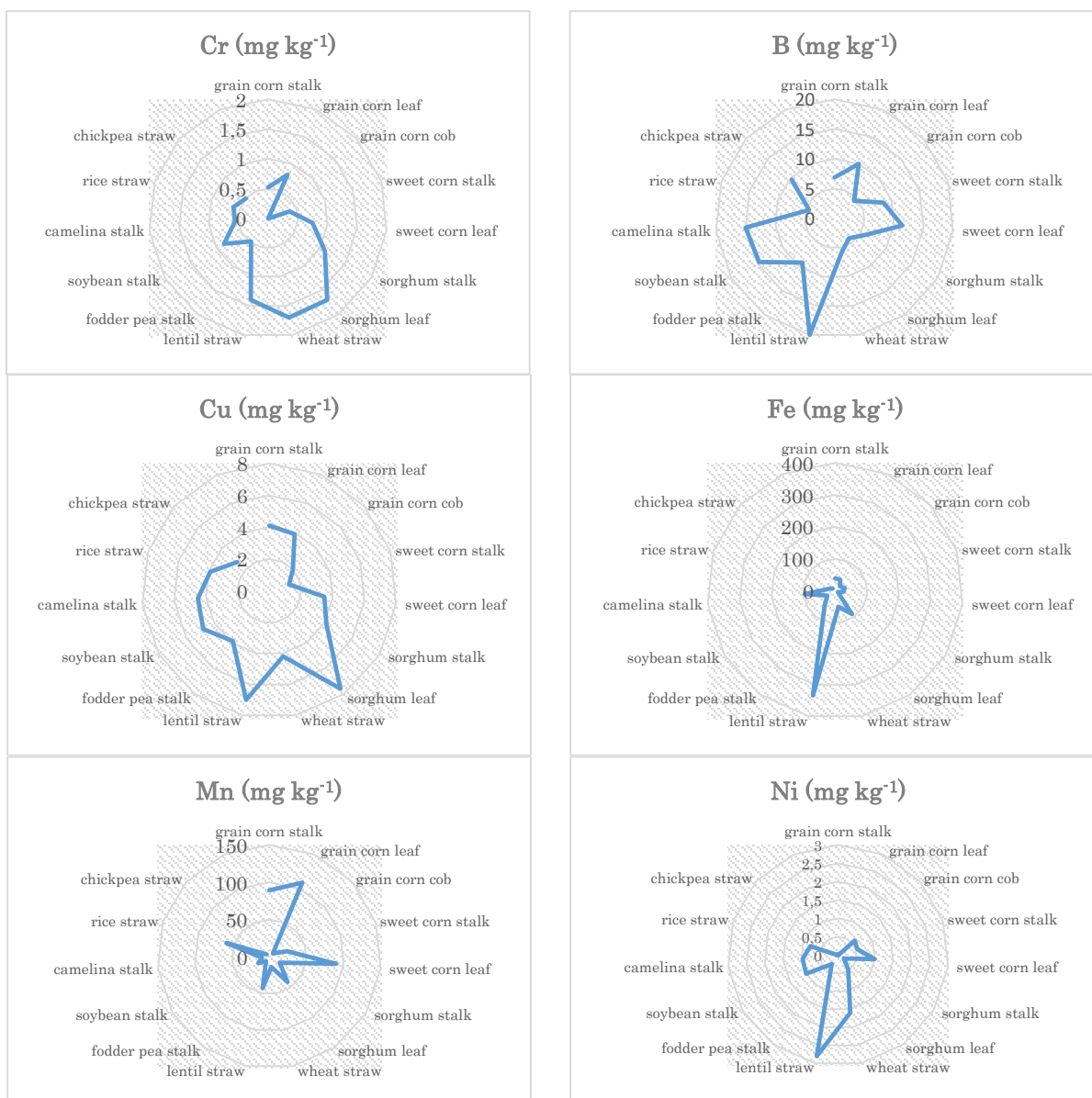


Figure 1. Graphical representation of macro element concentrations in roughages
 Şekil 1. Kaba yemlerdeki makro element konsantrasyonlarının grafiksel gösterimi

Sn was found only in fodder pea stalk ($0.01 \pm 0.005 \text{ mg kg}^{-1}$), while Si was found only in chickpea straw ($1015 \pm 140 \text{ mg kg}^{-1}$). All samples contained Co and Sb below the limit of detection. According to Gürsoy & Macit, (2017), micronutrient amounts of legume forage crops were changed 105.9-893.7 ppm, 2.22-12.36 ppm, 14.11-195 ppm, 18.18-66.58 ppm and 5.91-40.39 ppm for Fe, Cu, Zn, Mn and B, respectively. While micronutrient amounts determined for Fe, Cu, Zn, Mn and B in wheat forage crops varied between 74.90-630.6 ppm, 4-9.84 ppm, 31.49-335.6 ppm, 24.63-94.51 ppm and 0.35-26.64 ppm, respectively. In this study, the trace elements of lentil straw, chickpea straw, and soybean stalk, which were used as legume forage, were compared with those of legume and wheat forage crops in Gürsoy and Macit (2017) study. The current study found that lentil straw had about the same Fe content as wheat and legume forage crops. On the other hand, the amount of Fe in chickpea straw and soybean stalk was less than that in wheat and legume forage crops. On the other hand, Cu was in the same range with both wheat and legume forage crops. In lentil straw, Zn was only in the same range as legume forage crops, but in chickpea straw and soybean stalk, it was considerably lower than the others. On the other hand, Mn in lentil straw was in the same range as both wheat and legume forage crops. However, chickpea straw and soybean stalk

had lower values. B was in the same range across all crops. On the other hand, according to Baloda et al. (2018),, in a study conducted in India, elemental contents in green and dry roughages was reported to be average 0.49% for Ca in sorghum as green roughage with a range of 0.46-0.55% and ranged 0.28-0.52% for P with an average of 0.42%, and the critical levels for Ca and P were reported to be 0.30 and 0.25%. It was reported that the range for Zn was 19.78-29.55 ppm, the average value for Zn was 27.16 ppm and the critical level was 30 ppm. It was reported that the Cu range was around 27.3-50.2 ppm. It was reported that the range of Fe was between 147.3-203.6 ppm and the average was 179 ppm. It was stated that the highest Fe value was above 500 ppm in feeds. Mn concentration in sorghum was 37.9-64.3 ppm and the average value was 50.98. It was reported that the critical value was around 40 ppm. In dry roughage, Ca in wheat straw averaged 0.37% and the range was 0.28-0.45 ppm. Some samples were 0.30 % below the critical level. The range of P concentration was 0.14-0.25 % with an average of 0.20 % and below the limit of 0.25 %. Zn content averaged 19.6 ppm and the range was found to be between 12.6-27.7 ppm with values below the critical level (30 ppm). Cu content in wheat straw was 7.70 ppm on average and the range was 5.8-9.5 ppm. The average Fe content was 227.2 ppm and the range was 207.3-247.2 ppm and the values were 50 ppm above the critical level. Mn content in wheat straw averaged 43.8 ppm with a range of 36.9-54.04 ppm.

Figure 2 presents the radar graph of trace elements in roughages. This graph displays the prominent element in each roughage.



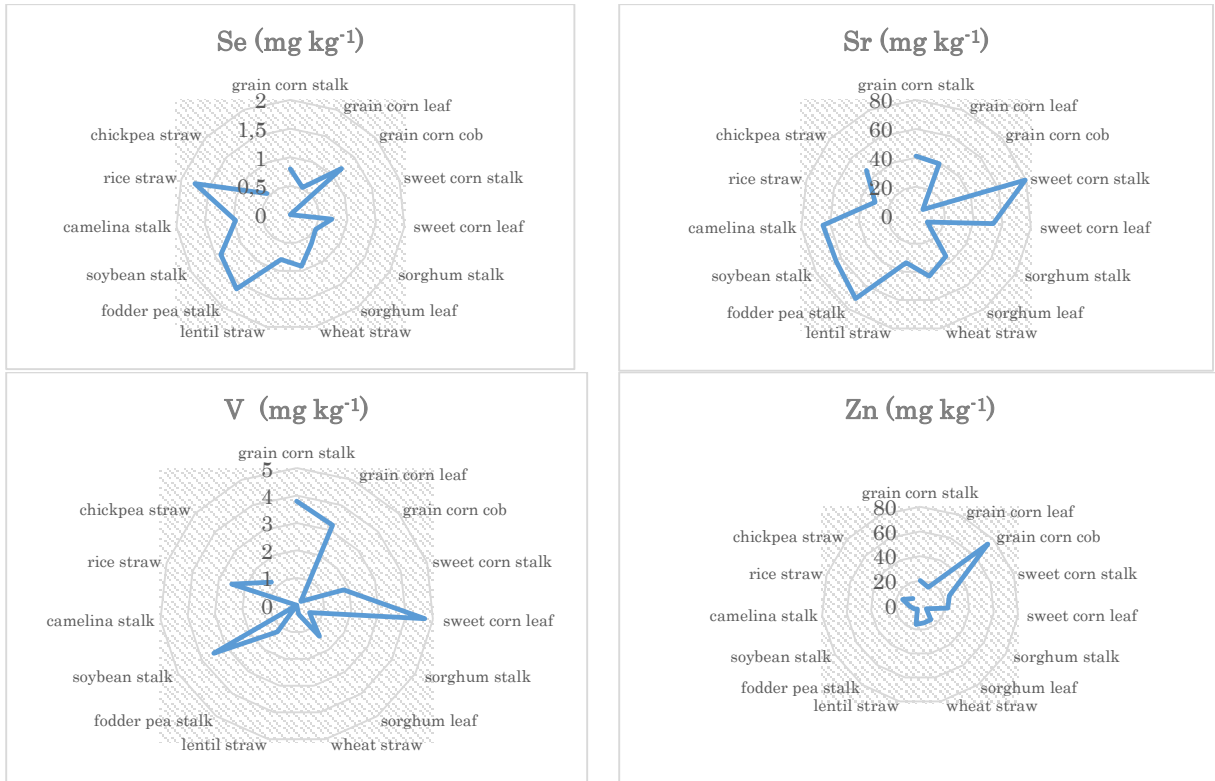


Figure 2. Graphical representation of trace element concentrations in roughages
 Şekil 2. Kaba yemlerdeki eser element konsantrasyonlarının grafiksel gösterimi

Toxic Elements

Al concentration was highest in lentil straw ($278\pm3\text{ mg kg}^{-1}$) and below the LOD in rise straw and sorghum straw. Pb was detected in grain corn leaf ($0.18\pm0.12\text{ mg kg}^{-1}$), sorghum leaf ($0.45\pm0.07\text{ mg kg}^{-1}$), and lentil straw ($0.40\pm0.13\text{ mg kg}^{-1}$), whereas it was below LOD in other roughages (Table 4, Figure 3). Other toxic elements, all samples (Table 4) showed that Be, As, and Cd were below the limit of detection (LOD).

Figure 3, the radar graph of toxic elements in roughages, is given below. This graph displays the dominant element in each roughage. Al, As, Cd, Pb, Hg, and Be are toxic to all animals.

The SPSS-22 program evaluated the hierarchical cluster analysis (HCA) of the samples, dividing the roughages into two groups and five clusters. The first cluster includes grain corn leaves, sorghum leaves, grain corn stalks, soybean stalks, camelina stalks, and rice straw. The second cluster includes grain corn cob and sorghum straw. The third cluster includes wheat straw, chickpea straw, and sweet corn leaves. The fourth cluster includes lentil straw. The fifth cluster includes sweet corn stalks and fodder pea stalks. Clusters that are close to each other in the dendrogram can be characterised as close to each other in terms of elemental composition.

Figure 4 shows the dendrogram of 14 roughages as a result of HCA analysis.



Figure 3. Graphical representation of toxic element concentrations in roughages
 Şekil 3. Kaba yemlerdeki toksik element konsantrasyonlarının grafiksel gösterimi

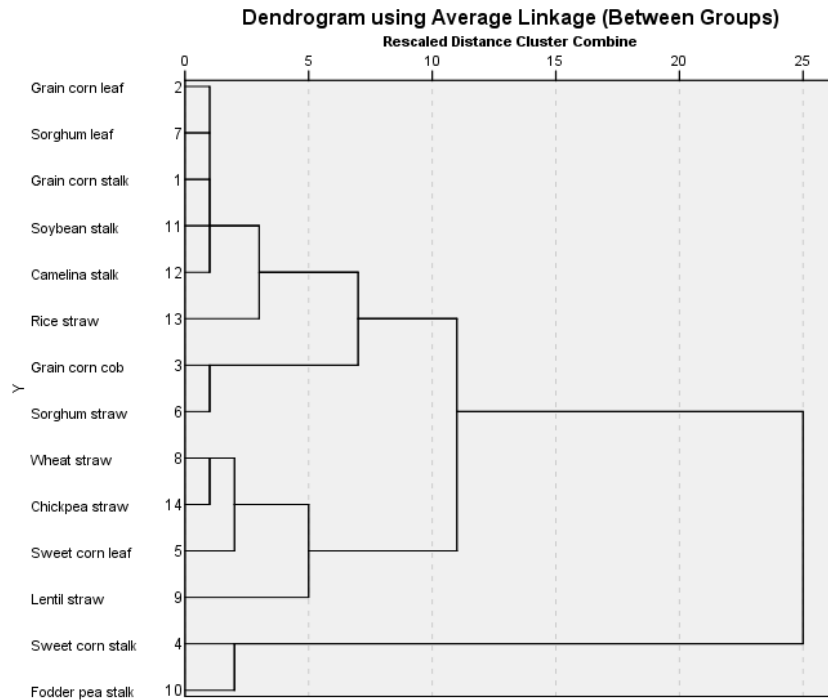


Figure 4. Dendrogram consisting of cluster analysis of roughages
Şekil 4. Kaba yemlerin küme analizinden oluşan dendrogram

CONCLUSION

Macro, trace, and potentially toxic element analyses of 14 roughages used in ruminant rations were carried out, and comparisons were made in terms of these elements. Each element has certain functions in the organism. Animals that consume these elements in high doses will undoubtedly experience health disturbances. Some elements will help in the treatment of various disorders, among which Zn, Fe, Ca, and Se are the most important ones. The analyses revealed that each roughage possesses prominent elements; lentil straw, for instance, exhibited high concentrations of Ca, Fe, and B, while Al and Cr exceeded acceptable levels. Sweet corn stalks were rich in K, while maize cobs were rich in zinc. Only chickpea straw exhibited a high Si content. None of the samples contained the toxic elements (As, Be, Cd, Co, and Sb), but samples of grain corn leaves, sorghum leaves, and lentil straw showed acceptable levels of Pb. However, the experiments were conducted only in the laboratory; these results need to be verified in the field. It is clear that such studies will benefit researchers and the feed industry.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors declare that there is no conflict of interest between them.

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