

Investigation on Some Trace Elements (Cu, Mo, Zn, Co, Mn) and sulphate in Soil, Grass and Sheep's Blood*

Hayati ÇAMAŞ¹ Ayşegül BİLDİK² Füsün GÜLSER³

¹ Department of Biology, Faculty of Science and Letters, Kafkas University - KARS

² Department of Biochemistry, Faculty of Veterinary Medicine, Yüzüncü Yıl University - VAN

³ Department of Soil Science, Faculty of Agriculture, Yüzüncü Yıl University - VAN

SUMMARY

In this study, the quantitative determination of copper, molybdenum, zinc, cobalt, manganese and sulphate in soil, grass and sheep's blood from eighty villages and eight towns of Van, Turkey, is reported. There are direct correlations between copper in soil and in sheep's blood ($r=0.281$, $p<0.01$); between sulphate and manganese in grass ($r=0.212$, $p<0.05$); between sulphate and cobalt in grass ($r=0.230$, $p<0.05$); for copper and manganese and for cobalt and zinc in sheep's blood ($r=0.257$, $p<0.05$ and $r=0.271$, $p<0.05$, respectively) and between the Mo: $[SO_4]^{2-}$ ratio in grass and copper in sheep's blood ($r=0.252$, $p<0.05$), negative correlations between copper in grass and cobalt in sheep's blood ($r=-0.238$, $p<0.05$) and between the Cu:Mo ratio in grass and the amount of copper in sheep's blood ($r=-0.609$, $p<0.01$). The results are presented and discussed stressing the importance that the deficiency of these trace elements has for animal and human health.

Key Words: Soil, Grass, Sheep blood, Trace elements, Copper, Molybdenum, Manganese, Zinc, Cobalt, Sulphate ion.

Toprakta, Bitkide ve Koyun Kanında Elementler (Cu, Mo, Zn, Co, Mn) ve Sülfat Miktarlarının Araştırılması

ÖZET

Bu çalışmada Türkiye'nin Van yöresinin sekiz ilçesine bağlı seksen köyden elde edilen toprak, ot ve koyun kanındaki; bakır, molibden, çinko, kobalt, manganez ve sülfat miktarları tayin edilmiştir. Topraktaki ve koyun kanındaki bakır değerleri ($r=0.281$, $p<0.01$); ottaki sülfat ve manganez düzeyleri ($r=0.121$, $p<0.05$); ottaki sülfat ve kobalt miktarları ($r=0.230$, $p<0.05$); koyun kanındaki bakır ve manganez; kobalt ve çinko düzeyleri ($r=0.257$, $p<0.05$ ve $r=0.271$, $p<0.05$) olmak üzere pozitif bir korelasyon bulunmuştur. Yine ottaki Mo: $[SO_4]^{2-}$ oranı ve koyun kanındaki bakır oranı arasında pozitif korelasyon ($r=0.252$, $p<0.05$) tespit edilmiştir. Ayrıca ottaki bakır ve koyun kanındaki kobalt ($r=-0.238$, $p<0.05$); ottaki Cu:Mo oranı ve koyun kanındaki bakır miktarı negatif bir korelasyon ($r=-0.609$, $p<0.01$) göstermiştir. Bu veriler üzerinde tartışılarak bu iz elementlerinin eksikliğinin hayvan ve insan sağlığı üzerindeki önemi vurgulanmıştır.

Anahtar Kelimeler: Toprak, Ot, Koyun kanı, İz elementler, Bakır, Molibden, Manganez, Çinko, Sülfat iyonu.

INTRODUCTION

After intense research during the last two decades, it has been established that some elements in very small or trace quantities, play an important role in vegetal, animal and human life the chemical composition of soils affect the mineral content of foodstuffs thus influencing the amount of trace elements available for animals and humans (19,25,30).

The concentrations of trace elements in grass depends on the concentration of these elements in soil (20,25) and may be the cause for the deficiency of several elements in commercially valuable animals (9,22,29,31,34).

It has been found that molybdenum (Mo) plays an important role in the deficiency of other elements, such as copper, by limiting the storage of Cu in sheep's liver (11,12).

Several investigators have suggested that Cu deficiency may result from Cu, Mg and $[SO_4]^{2-}$ imbalances (29), or because of an improper Cu:Mg: $[SO_4]^{2-}$ ratio in foodstuffs (13,16, 20,22,32).

Interactions between various elements in living organisms has also been reported to play a role in a particular element deficiency (14,25).

In Turkey, previous studies (1,6) have reported copper deficiency in some areas. The present work was undertaken to determine the amount of Cu, Mo, Zn, Co, Mn and inorganic $[SO_4]^{2-}$ and their correlation in soils, grass and sheep's blood in towns of the Van area of Turkey. The corresponding element profiles were obtained and recommendations for improving animal breeding in the area given.

MATERIALS AND METHODS

For this study, eight sampling sites were selected in the area of Van. Soil samples were collected from 80 villages, taking four samples per village at depths of 0-20 cm. The samples were mixed, dried and sifted through a 2 mm sieve (15).

Grass samples were collected from at least 10 pasture points, representing the available pastures during the May-

* This work has been supported by Turkish Technical Research Institute (TUBİTAK) (The project number VHAG-966)

June period. The grass samples were prepared for analysis by washing with doubly-distilled water and dried in an oven at 140°C for 24 hours (18).

The blood samples were taken from at least four sheep living in the same areas where soil and grass samples were collected. The blood was frozen and kept at -20°C until the analysis was performed (5,8,33,36).

For determination of trace elements in soil samples 10g of soil were treated with 20 ml DTPA solution (see below) in a beaker and stirred for hours.

The grass samples were first weighed (0.5 g) dried and ground prior to digestion with 1 ml 5% sulphuric acid in ethanol in a porcelain ash pot. The excess alcohol was burned in air and the ash pot placed in a cold furnace. The temperature of the furnace was gradually increased to 500 °C (± 50°C) and maintained for 2-6 hours. After the ash became grey, the ash pot was taken out of the furnace and cooled to room temperature, wetted with a small amount of water and added with 4.0 ml 3.0 N HCl, heated for 10-15 minutes until all minerals (except silicon) are dissolved, and diluted to 100 ml with d.d.i. water (15,18,26).

The DTPA solution consists of 14.920 g triethanolamine +1.967 g diethylenetriamine-pentaacetic acid +1.47 g anhydrous calcium chloride. The reagents are suspended in deionized water and carried to a volume of about 0.91, adjusting the pH to 7.3 ± 0.05 with 6 N HCl. After the pH is regulated, the final volume is adjusted to 1.01. The suspension is filtered and then used for trace element analysis.

For the determination of [SO₄]²⁻ in grass, 100 g dry samples were placed in a Kjeldahl flask and treated with 2.0 ml concentrated nitric acid, heating it in a water bath for 30 min. After that, 1ml 60% perchloric acid was added and heated with a small gas flame, increasing the flame gradually. Heating was continued for at least 1 hour after perchloric acid flames appear.

After cooling, the samples were added with 3.0 ml of 6.0 N HCl and heated again until all residues of perchloric acid were eliminated. The samples were cooled again and diluted to 50.0 ml, 10.0 ml aliquots were obtained and added with 1.0 g BaCl₂ and 0.25% arabic gum solution.

With this treatment the samples were ready for turbidimetry analysis using standart curves prepared with known [SO₄]²⁻ values in the 50-100 ppm range (4).

The same procedure is used for the preparation of the blood samples for [SO₄]²⁻ analysis.

The preparation of soil samples for [SO₄]²⁻ analysis requires that 10.0 g soil be placed in a 100 ml flask with 50.0 ml of acetate buffer (100 g AcONa, 30 ml 99.5% AcOH in 500 ml water).

The flasks were shaken every 5 minutes and then set aside for 30 minutes. After that, the solution was filtered and treated for [SO₄]²⁻ determination by turbidimetry.

The metallic elements were determined by means of a Shimadzu 680 atomic absorption spectrometer. Air-acetylene flame was used for the determined of Cu, Zn, Mn and Co in soil and grass.

Molybdenum was determined by means of an acetylene-nitrogen peroxyde flame (3,18, 26).

Sulphate in soils and in blood was determined by turbidimetry using a blue filter and precipitating the ion with barium chloride (4,10,28).

The statistical analysis was performed by using the SYSTAT application program.

RESULTS

The results obtained are presented in Tables 1-3. Table 4 summarizes the correlations obtained for the results obtained in sampling sites shown in tables 1-3, the average values of trace elements in soil were found to be: Cu, 2.10 ± 0.16 µg/g; Mo, 6.20 ± 0.71 µg/g; Co, 0.26 ± 0.03 µg/g; Zn, 0.67 ± 0.16 µg/g and Mn, 12.34 ± 2.21 µg/g. For inorganic sulphate the value obtained for soil is 9.01 ± 0.91 µg/g.

The values reported here for Cu, Zn and Mn in soil show some differences with those reported in a previous study carried out in the same region by Gulser (15).

This may be explained by the fact that in that case samples were obtained at different depths and in different types of soil.

For grass, the values are Cu, 2.10±0.16 µg/g; Mo, 6.20 ± 0.71 µg/g; Co, 0.26±0.03 µg/g; Zn, 0.67±0.16 µg/g; Mn, 12.34±2.21 µg/g and [SO₄]²⁻, 1074.61± 56.29 µg/g. In the blood samples we found the following values: Cu, 285.45±6.64 µg/100ml; Mo, 20.58±1.37 µg/100 ml; Co, 2.69±0.30 µg/100ml; Zn, 25.48±4.44 µg/100ml; Mn, 0.13±0.02 µg/100 ml and [SO₄]²⁻, 84.38±2.75 µg/100ml

Table 1.-Trace Elements¹, Inorganic Sulphate¹ and ratios Between Cu:Mo and Mo: [SO₄]²⁻ in Soils from Eight sampling Sites in Van, Turkey and its Towns

Site	(Town Name)	n	Cu	Mo	Co	Zn	Mn	[SO ₄] ²⁻	Cu:Mo	Mo: [SO ₄] ²⁻
1	Edremit	10	2.14±0.23	8.97 ± 1.47	0.18 ± 0.02	0.71 ± 0.21	11.30 ± 3.61	10.68±2.79	0.28 ± 0.13	2.17 ± 0.64
2	Ercis	10	1.79 ± 0.16	7.90 ± 0.57	0.39 ± 0.09	1.35 ± 0.62	19.86 ± 19.86	7.20 ± 1.40	0.38 ± 0.07	1.10 ± 0.29
3	Caldıran	10	1.65 ± 0.17	4.41 ± 0.76	0.21 ± 0.03	15.05±3.15	15.05 ± 3.15	10.20 ± 2.99	0.42 ± 0.04	1.15 ± 0.52
4	Gürpınar	10	1.72 ± 0.12	4.80 ± 0.83	0.26 ± 0.06	0.66 ± 0.19	8.91 ± 1.15	5.80 ± 1.45	0.55 ± 0.17	2.04 ± 0.72
5	Muradiye	10	2.41 ± 0.39	8.74 ± 1.25	0.23 ± 0.03	0.34 ± 0.06	11.07 ± 2.07	12.05 ± 4.09	0.32 ± 0.07	3.05 ± 1.25
6	Gevaş	10	1.68 ± 0.41	5.53 ± 0.85	0.37 ± 0.11	0.24 ± 0.09	22.07 ± 7.00	12.17 ± 4.36	0.67 ± 0.29	2.76 ± 1.62
7	Özalp	10	2.64 ± 0.33	5.08 ± 1.16	0.25 ± 0.04	0.62 ± 0.17	5.27 ± 0.48	6.30 ± 1.99	0.95 ± 0.26	1.48 ± 0.48
8	Van	10	2.75 ± 0.26	4.18 ± 0.73	0.18 ± 0.03	0.19 ± 0.05	5.21 ± 1.06	7.70 ± 2.74	1.03 ± 0.31	1.53 ± 0.55
Averages			2.10	6.20	0.26	0.67	12.34	9.01	0.58	1.91
S.D.			0.16	0.71	0.03	0.16	2.21	0.91	0.10	0.26

¹ All concentrations in µg/g

² n = 8

Table 2.-Trace Elements¹, Inorganic Sulphate¹ and Ratios Between Cu:Mo and Mo: [SO₄]² in Grass Samples from Eight Sampling Sites in Van, Turkey and its Towns

Site Number	n	Cu	Mo	Co	Zn	Mn	[SO ₄] ²	Cu:Mo	Mo: [SO ₄] ²
1	10	2.10±0.20	3.29 ± 1.49	0.07 ± 0.02	1.06 ± 0.26	5.07 ± 1.58	884.6±101.1	6.58 ± 3.26	4.0 ± 2
2	10	1.91 ± 0.12	7.62 ± 2.42	0.12 ± 0.03	0.93 ± 0.30	18.48 ± 4.79	1302.2 ± 215.8	1.08 ± 0.59	6.5 ± 2
3	10	2.05 ± 0.06	3.43 ± 1.34	0.09 ± 0.03	1.97 ± 0.88	10.76 ± 2.66	972.0 ± 120.7	5.03 ± 2.35	4.4 ± 2
4	10	2.06 ± 0.07	6.80 ± 3.96	0.05 ± 0.01	1.55 ± 0.60	5.95 ± 2.07	1187.1±127.1	8.67 ± 4.25	5.0 ± 3
5	10	2.07 ± 0.07	16.70 ± 5.9	0.05 ± 0.01	2.11 ± 0.92	5.93 ± 1.77	1165.5 ± 162.4	2.39 ± 1.86	2.0 ± 8
6	10	2.10 ± 0.06	6.34 ± 1.58	0.09 ± 0.02	1.82 ± 0.32	11.31 ± 3.04	918.0 ± 187.7	5.93 ± 2.93	1.0 ± 3
7	10	2.10 ± 0.05	7.53 ± 3.85	0.08 ± 0.03	1.11 ± 0.27	10.60 ± 3.6	1202.5 ± 204.0	4.17 ± 2.27	9.0 ± 6
8	10	1.94 ± 0.07	2.41 ± 0.75	0.04 ± 0.04	2.54 ± 0.95	5.77 ± 1.52	963.0±100.0	3.00 ± 1.72	2.0 ± 0.6
Averages ²		2.10	6.20	0.26	0.67	12.34	1074.61	4.64	8.0
S.D.		0.16	0.71	0.03	0.16	2.21	55.50	0.88	2.0

¹ All concentrations in µg/g
² n = 8

Table 3.-Trace Elements¹, Inorganic Sulphate¹ in Sheep's Blood from Van, Turkey and its Towns

Site Number	n	Cu	Mo	Co	Zn	Mn	[SO ₄] ²
1	10	84.10±7.76	18.11 ± 2.53	2.20 ± 0.33	18.82 ± 4.60	0.02±0.003	86.75±8.30
2	10	81.20 ± 6.16	17.24 ± 2.50	2.68 ± 0.43	18.77 ± 2.02	0.18± 0.02	92.80±8.49
3	10	86.20 ± 4.18	19.80 ± 3.14	3.30 ± 0.60	23.40± 5.30	0.13 ± 0.03	82.20 ± 8.40
4	10	54.50 ± 4.60	25.40 ± 3.90	1.51 ± 0.30	7.14 ± 1.80	0.15 ± 0.03	76.20±5.60
5	10	99.50 ± 9.00	15.90 ± 3.70	2.20 ± 0.40	28.70 ± 1.70	0.18 ± 0.02	97.90 ± 11.30
6	10	67.02 ± 6.50	21.20 ± 2.80	3.00 ± 0.60	29.40 ± 3.80	0.03 ± 0.03	82.90± 10.60
7	10	114.20 ± 7.10	27.00 ± 2.30	2.31 ± 0.41	26.70 ± 3.10	0.22 ± 0.11	80.70 ± 7.42
8	10	96.90 ± 7.00	20.00 ± 4.60	4.30 ± 0.90	50.90 ± 19.1	0.14 ± 0.03	75.60±8.80
Averages ²		285.45	20.58	2.69	25.48	0.13	84.38
S.D.		6.64	1.37	0.30	4.44	0.02	2.75

¹ All concentrations in µg/g
² n = 8

Table 4.- (a) Element Correlations (r and p values) in Soil and Sheep's blood from Van, Turkey (Eight Sampling Sites)

	Cu/Zn	Cu/Co	Cu/Mn	Cu/Mo	Mo/Mn	Zn/Mn
Blood	0.271 (<0.05)	0.238 (<0.05)	0.257 (<0.05)			
Soil					0.374 (<0.05)	0.227 (<0.05)

(b) Element, Element Ratios and Sulphate Correlations

C (Soil)/Cu(Blood)	0.281 (<0.01)
Cu (Grass)/Co (Blood)	-0.238 (<0.05)
Co (Grass)/[SO ₄] ² (Grass)	0.230 (<0.05)
Cu:Mo(Grass)/Cu(Blood)	-0.690 (<0.01)
Mo: [SO ₄] ² (Grass) / Cu (Blood)	0.252 (<0.05)
Mn (Grass)/[SO ₄] ² (Grass)	0.212 (<0.05)

DISCUSSION

Molybdenum has an important antagonistic effect in the metabolism of copper in ruminants. So for feeds that are low in copper, high levels of molybdenum are not desirable (37). Thus it has been estimated that a Mo:Cu ratio of 1:4 is optimal to avoid the risk of nutritional Cu deficiency in ruminants (16). Others have suggested a Mo:Cu ratio not lower than 1:10 to avoid copper deficiency in animals (17). We have found a 1:0.34 Mo:Cu ratio in the Van area soils, indicating a high risk of copper deficiency. Previous studies report that grasses that contain more than 3 µg/g Mo and less than 5 µg/g Cu are associated to illnesses related to Cu-deficiency in animals (27). Molybdenum and inorganic

sulphate play an important role in the Cu availability to animals. In the grasses growing in areas where animals suffer from Cu deficiency, the ratio of Cu:Mo: [SO₄]² is reported to be about 1:0.6:330-890, whereas in regions where Cu deficiency is not a problem the corresponding ratio is 1:0.2:180 (22). In the region studied here, it has found a Cu:Mo: [SO₄]² ratio of 1:3.3:525 also indicating a high risk of copper deficiency in animals grazing in the area. Climatic conditions are a factor that determine if Mo is stored in greater amounts by grass. In cases of increased rainfall the Mo concentration in grass is decreased (23,24), thus making the Mo:Cu ratio more favorable. Large variations of the Cu concentration in grass have been reported (16), failing

between 5 and 15 µg/g and depending upon the concentrations of Mo and $[SO_4]^{2-}$ as previously stated. Copper levels below 5 µg/g are considered unfavorable (7,27). Copper levels in the blood of sheep grazing in sites 1-3 and 5 are within normal levels, but are lower in sites 4 and 6, Table 3. If the value of 60 µg/100ml in sheep's blood is accepted as the lower limit to avoid copper deficiency (34), only animals from site 4 would be classified at risk, although pathological conditions associated to copper deficiency such as enzootic ataxia has been reported in site 6, where Cu levels are slightly above the 60 µg/100ml limit.

Considering the Mo:Cu ratio in soil and the Cu:Mo: $[SO_4]^{2-}$ ratio in grass given above, the risk of copper deficiency in the Van area of Turkey can not be ruled out, in particular if climatic conditions change and the Mo content in grass increases, such as in a drought.

The amounts Cu and Mo in soil and Cu, Mo and sulphate in grass and of trace elements in sheep's blood were correlated. We found a negative correlation between Cu and Mo, with $r = -0.609$, $p < 0.01$, helping to explain the relatively high levels of copper in blood. We can not find, however, an explanation for the positive correlations between Mo and sulphate in grass and in sheep's blood, $r = 0.252$, $p < 0.05$. While 6 µg/100ml molybdenum in ruminant blood is considered normal, a high Mo content in the blood of sheep from Van and surrounding towns (20.58 ± 1.37 µg/100ml) is observed along with normal blood copper levels.

This topic is still to be explored by looking at the roles of xantine oxidase and molybdenum the purine metabolism. In the case of zinc, its concentration grass depends on the type and age of the grass itself and on the geographical structure, pH and chemical composition of the soil where it grows. Nitrogen-rich soils will have larger zinc concentrations, whereas lime-containing soils will show a decreased zinc content. In sheep, optimal zinc uptakes have been set at 15-18 µg/day (16,34) and at 35-50 µg/day (21).

It has been reported that the amount of zinc in grass is in the range 14-138 µg/g and that the daily Zn requirement for sheep is 30-40 µg/g (16). In lambs and calves, serum zinc has been reported at 80-120 µg/100ml. Levels of ≤ 40 µg/100ml are considered characteristic of a zinc-deficient animal (16,34).

In the Van area, we have found that the average zinc concentration in grass is 1.64 ± 0.20 µg/g. This level seems too low to adequately sustain the grazing animals in the region. It should be remembered that high calcium, cadmium and pyrate concentrations in grass will impose a higher zinc demand.

All these factors point to a possible Zn-deficiency in the region. For cobalt, the low levels found in soil, grass and blood, 0.26 ± 0.03 µg/g, 0.07 ± 0.01 and 2.69 ± 3 µg/100ml, respectively, make it difficult to reach a conclusion or to suggest cobalt supplementation to cattle in the region. Deficiency in ruminants has been suggested if grass where they feed contains 0.05-0.06 µg/g Co or less (2). Signs of Co deficiency have been reported for feeds containing ≤ 0.07 µg/g (16,34).

The cobalt requirement for ruminants has been set at 0.07-0.10 µg/g dry grass. By looking at the concentrations of

cobalt in sheep's liver it is possible to arrive at more reliable conclusions (34).

In this study we did not have an opportunity to measure liver Co values, but focused on its concentration in grass. We have found average Co values in grass of 0.07 µg/g, which may be considered at the critical threshold for this element. Sites 4, 5 and 8 are definitely below the lower acceptable limit, although this is not reflected in relatively normal values in blood nor the cobalt content of the soil.

This may be explained if the negative correlation between Zn and Co is taken into consideration. Further investigations are in progress to establish if there is risk of cobalt deficiency in this area. Manganese concentrations in grass depend on several factors (2,35).

Plants get their Mn as Mn^{2+} ions. Formation of Mn^{2+} ions depends on the redox potential in soil. Mn^{2+} ions are continuously formed in soil through Mn (III) and Mn (IV) species. This process is affected by various factors that include the pH of grass, drought conditions, sunlight exposure levels and rainfall. Thus, Mn concentrations in plants vary accordingly. For instance, Mn deficiency appears more often in drought years, as compared to rainy years because Mn^{2+} ions are oxidized and consequently are unavailable for plants (36,37). Optimal Mn content in cattle feed is set at 40 µg/g, the minimal content is set at 20 µg/g (36).

Others have suggested a 35-50 µg/g range as acceptable for sustaining the needs of ruminants (35). Our results show values for grass that are well below the minimal manganese content. It is concluded that grass in the Van area does not meet the minimal Mn requirement for ruminants (33). Accordingly, this element is negatively correlated to Cu, Zn and Co. The great potential that the Van area of Turkey has for animal breeding, together with its geographical situation, geological structure, climate, social, cultural and economical characteristics, merit attention to the risks of essential trace elements deficiencies found in this study. Steps to prevent animal diseases associated to lack of adequate intake of Cu, Zn, Co and other elements should be taken by supplementing either the soils or the cattle itself. This in turn will be beneficial for humans who are at latter stages of the food chain.

Acknowledgements

The authors wish to thank the Turkish Scientific and Technical Research Institute for their generous support of this work.

REFERENCES

- 1-Ağaoğlu Z., Akgül, Y., Bildik A., (1992): A Study on the Distribution of Enzootic Ataxia in Van and its environment. Journal of the Veterinary Medicine of Yüzüncü Yıl University, 3 (1-2), 71-90.
- 2-Anke M., (1961): Der Spurenelementgehalt von Grünland und Ackerpflanzen Verschiedener Böden in Thüringen. Acker Z., Pflanzenbau 112, 113-140.
- 3-Anonym, (1982): Atomic Absorption Spectrometry; John Edward, Cattle elsevier, Scientific Publishing Company, Amsterdam-Oxford-New York.
- 4-Aydeniz A., (1969): Radiational Analysis in Feed Plants for the Fertility of Land. Ankara University press.

- 5-Baruthio F., Francis P., (1993):** Cobalt Determination in Serum and urine by Electrothermal Atomic Absorption Spectrometry. *Biological Trace Elements Research*, 39, 21-31.
- 6-Baysu N.,(1968):** Studies about the Copper Level in the Blood of Healthy and Enzootic Ataxia Suspected Lambs in Senirkent Town. *Journal of the Association of Turkish Veterinary Medicine*, 38(9), 8-12 .
- 7-Bennets H.W., Beck A.B. (1941):** Falling Disease of Cattle in the South West of Australia. *Austral.Vet.J.*17,85-88 (1941).
- 8-Çamaş H.(1979):** Research on The Copper Values of The Blood Serum of Normal Akkaraman Sheep Raised by The People of Some Villages of Ankara. A thesis of PhD, Publications of Ankara University, Faculty of Veterinary Medicine,351,Institute of Ialahan Zootechny Research, Ankara.
- 9-Curthbertson D.(1970):** Introduction to the Symposium Trace Element Metabolism in Animals. *Proc.Intern. Symposium,C.F.Mills, Ed.Livingstone, Edinburgh and London* 1-2.
- 10-Denis W. (1921):** Sulfates in Blood. *J. Biol. Chem.* 49, 311-317.
- 11-Dick A.T.,(1969):** The Copper-Molibdenum Complex in Ruminant Nutrition. *Outlook On Agriculture* 6, 14-18.
- 12-Dick A.T.,(1963):** The Control of Copper Storage in the Liver of Sheep by Inorganic Sulphate and Molibdenum. *Aust. Vet. J.* 29, 233-239 .
- 13-Dowdy R.P., Matrone G.(1968):**A Copper-Molibdenum Interaction in Sheep and Chicks. *J. Nutr.* 95, 191-196.
- 14-Dynna O., Havre G.N.,(1963):** Interrelationship of Zinc and Copper in the Nutrition of Cattle. *Acta Vet. Scand.* 4,197-208
- 15-Gülser F.,(1992):** Productivity of the Land in The Catchment Area of Van Lake. A thesis of master, Van .
- 16-Gürtler H.,(1971):**Die Bedeutung der Spurenelemente und Erkrankungen Infolge Einer Unzureichenden Oder Übermaessigen Aufnahme an Spurenelementen. In *Ernaehrung- Physiologie Der Lantwirtschaftlichen Nutztiere*, E. Kolb H. Gürtler, Ed., Gustav Fischer Verlag, Jena 695-814.
- 17-Hasler A., Besson J.M., (1972):** Über den Gehalt an Kupfer und Molibdaen Sowie Deren Verhaeltnis in Wiesenfutter. *Schweizerische Landwirtschaftliche Forschung*, 11, 313-332.
- 18-Kaçar B.,(1984):** Guide of plant Raising. Publication of Ankara University; Faculty of Agriculture .
- 19-Kay H.D., (1965):** Copper In Biology. *Cuprum Pro Vita, Copper's Role In Plant and Animal Life*. Transactions of a Symposium, Vienna.
- 20-Kirchgessner M.,MullerH.L. and Voigtlaender G.,(1971):**Spurenelementgehalte (Fe,mn,Cu,Zn,Co,Mo) des Weidegrases in abhaengigkeit von Wachstumsdauer und Vegetationsperiode. *Das Wirts Chaftseigene Fülter*17(3),179-189
- 21-Kirchgessner M., Voigtlaender G., Maier D.A., Pahl E., (1968):**Zum Einfluss des Vegetationsstadiums auf den Spurenelementgehalt von Rotklee (*Trifolium pratense* L.) und Luzerne (*Medicago varia* Mart.). *Wirtschaftseigenes Futter* 14, 112-122.
- 22-Kovaisky V.V.,(1970):** The Geochemical Ecology of Organisms Under Condition of Varying contents of Trace Elements in the Environment. *Proc. Intern. Symposium. Trace Element Metabolism In Animals*, C.F. Mills Ed., Livingstone, Edinburgh and London 452-456.
- 23-Lang V., Kirchgessner M., Voigtlaender G.,(1972):** Spurenelementgehalte des Wiesenschwingsels (*Festuca pratensis* Huds.) in Abhaengigkeit von Wuchshöhe, Entwicklung und Witterung. *Z. Acker- U. Pflanzenbau* 135, 216-225.
- 24-Mengel K.,(1968):** Ernaehrung und Stoffwechsel der Pflanze. 3.Aufl., Verlag Güstav Fischer.
- 25-Mitchel R.L.,(1963):**Soil Aspects of Trace Element Problems in Plants and Animals. *J.Royal, Agric. Soc. England* 124, 75-86.
- 26-Page A.L.,Miller R.H., Keeney D.R., (1982):** Methods of Soil Analyses Chemical and Microbiological Properties, 2nd Ed., Agronomy No: 9, Science of Soil Society of America.
- 27-Perigaud S.,(1970):** Les Carences en Oligo-Elements Chez Les Ruminants en France. Leur Diagnostic. Les Problemes Souleves par l'intensification fourragere. *Ann. Agron.* 21, 635-669.
- 28-Reed L. (1926):** Methods for the Determination of Some of the Nonprotein J. *Biol. Chem.* 71, 191-204.
- 29-Rish M.A. (1970):** The Geochemical Ecology of Organism in Deficiency and Excess of Copper.. *Proc. Intern. Symposium. Trace Element Metabolism In Animals*, C.F. Mills, Ed., Livingstone, Edinburgh and London 452-456.
- 30-Schwarz K. (1970):** Control of Environmental Conditions In Research of Trace Elements, In *Proc. Intern. Symposium, Trace Element Metabolism In Animals*, C.F. Mills, Ed., Livingstone, Edinburgh and London 497-504.
- 31-Spaıs A.G.(1959):** Le Cuivre En Pathologie Ovine und Bovine. *Rec. Med. Veter.* 135, 161-194.
- 32-Suttle N.F., Field A.C., (1968):** Effect of Intake of Copper, Molibdenum and Sulphate on Copper Metabolism in Sheep. II. Copper Status of the Newbornlambs. *J. Comp. Path.* 78, 363-370 .
- 33-Taylor A. and Walker A.W., (1991):**TraceElement Analyses-Obtaining Good Results-TRACE'89 Trace Elements in Health and Disease.G.T.Yüregir, O.Donna, L.Kayrı, Ed., Çukurova University Medical Faculty, Adana, Turkey 1-9 .
- 34-Underwood E.J., (1971):** Trace Element Metabolism in Human and Animal Nutrition, Third Ed., Academic Press Inc., New York and London .
- 35-Voigtlaender G., Lang V., Kirchgessner M.,(1972):** Spurenelementgehalte der Luzerne (*Medicago varia* Mart.)in Abhaengigkeit von Wachstum, Entwicklung undWitterung in drei Versuchsjahren. *Z.Aacker-u.Pflanzenbau*135,204-215 (1972).
- 36-Wasowiz W., Wolkani P., Bednarski M., Gromadzinska J., Sklodowska M. Grzybowska K.,(1993):** Plasma Trace Element (Se, Zn, Cu) Concentration in Maternal and Umbilical Cord Blood in Poland. *Biological Trace Element Research* 38 (2), 205-215.
- 37-Zuber R.,(1956):** Spurenelementgehalt des Futters Einerer Düngungsversuche. *Die Grüne* 84, 627-634.