Bulgarian Journal of Agricultural Science, 22 (No 5) 2016, 733–739
Agricultural Academy

TRACE ELEMENTS CONTENT OF PLANT MATERIAL GROWING ON ALKALINE ORGANIC SOILS AND ITS SUITABILITY FOR SMALL RUMINANT EXTENSIVE FARMING

A. Z. PAPAZAFEIRIOU[1], Ch. LAKIS[2], S. STEFANOU[3*], M. YIAKOULAKI[4], P. MPOKOS[4] and K. PAPANIKOLAOU[4]
[1] *Halkidikis 101, 54248, Thessaloniki, Greece*
[2] *Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, Laboratory of Applied Soil Science, 541 24 Thessaloniki, Greece*
[3] *Alexander Technological Educational Institute of Thessaloniki, Department of Agricultural Technology, Laboratory of Soil Science, 57 400 Sindos Thessaloniki, Greece*
[4] *Aristotle University of Thessaloniki, Faculty of Agriculture, Department of Range Science, Forestry & Natural Environment, 541 24 Thessaloniki, Greece*

Abstract

PAPAZAFEIRIOU, A. Z., Ch. LAKIS, S. STEFANOU, M. YIAKOULAKI, P. MPOKOS and K. PAPANIKOLAOU, 2016. Trace elements content of plant material growing on alkaline organic soils and its suitability for small ruminant extensive farming. *Bulg. J. Agric. Sci.*, 22: 733–739

The soils in the basin of Philippoi, Greece are organic with an alkaline pH, due to topographic, hydraulic and climatic conditions. The main agricultural activity is extensive small ruminant farming, based on the grazing of natural forage species and silage coming from corn cultivated locally. Fe, Zn, Cu, Mn and Se concentrations in forage species and corn were investigated in order to evaluate their suitability to meet the nutrient requirements for small ruminants. Twenty sites were selected and plant samples were collected in the peak of the growth period. Results showed that Zn and Mn concentrations in the plant material followed the distribution of organic matter in the topsoil, while the opposite is true for Cu. The distribution of Fe and Se was independent of the organic matter content. Zn, Mn and Se concentrations met the animals needs with no danger for either deficiency or toxicity. Fe and Cu concentrations exceeded those of maximum tolerable level. Consumption of high level of Fe may present hazardous conditions for small ruminants. Therefore, the use of plant material growing on these soils in the animals' feeding schedule must be considered with caution, in order to avoid problems to their health.

Key words: mineral concentration, peat soils, forage species, nutrient requirements

Introduction

Small ruminant farming plays an important role in the economic and social life of many agricultural regions in Greece. Information about the dietary habits and nutrient requirements of these animals is crucial in managing their welfare and in contributing to the livelihoods of people who depend on them.

The basin of Philippoi represents the most south and lower part of the greater plain of Drama (Eastern Macedonia – Greece), which is surrounded by mountains and hills composed mainly from limestone and marble. Before 1930 the area was an extensive marsh. The draining started in 1930 by deepening the gorge of Aggitis River and the construction of the central draining trench (Philippoi trench) and the perpendicular secondary trenches every 1000 m approximately. After the construction of the land reclamation works during the years 1930–1940, the marsh was drained and approximately 11 500 ha were given to agriculture, out of which 5000 ha are in the center of the old marsh. Whether artificially drained

**E-mail:* azafipap@gmail.com, chlakis@geo.auth.gr, *stefst2@cp.teithe.gr (corresponding author), yiak@for.auth.gr

for cultivation or left in their natural water–saturated state, peat soils (Histosols) possess unique properties resulting from their high organic matter contents. These include dark brown to black colours, very low bulk densities (0.2 to 0.4 Mg m^{-3}), very high water-holding capacities and high cation exchange capacities (typically 150 to 300 $cmol_c$ kg^{-1}) that increase with increasing soil pH (Brady and Weil, 2008). In general, most peat soils are acidic. In the basin of Philippoi, though, the soil is alkaline due to the presence of minute layers of calcium carbonate as a product of weathering of the surrounding rocks (NAGREF, 2001).

The micronutrient contents of organic soils depend on the extent of the washing or leaching of these elements into the bog area as the soils were formed. In most cases, this rate of movement is too slow to produce deposits as high in micronutrients as are the surrounding mineral soils. The ability of organic soils to bind certain elements, notably copper, also accentuates micronutrient deficiencies (Brady and Weil, 2008) although it has been reported that lower molecular weight organic compounds in the soil (such as humic and fulvic acids) may increase the mobility of metals, therefore affecting their solubility and the form in which they are present. Metal toxicity depends on their bioavailability, i.e. the ability to be uptaken by a living organism. Bioavailability is not a function of the total concentration in the soil only, but mainly depends on its physical, chemical and biological properties (Leyval et al., 1997). Soil properties such as cation exchange capacity, pH and microflora greatly affect the uptake of metals by plants (Clijsters and Van Assche, 1985). The uptake of mobile ions from the soil solution depends mainly on the total quantity of the ions in the soil and on the sorption capacity of the root system (Alloway, 1995; Reichman, 2002), as well as on the permeability and sensitivity of the cell membranes of each organism (Purkayastha et al.,

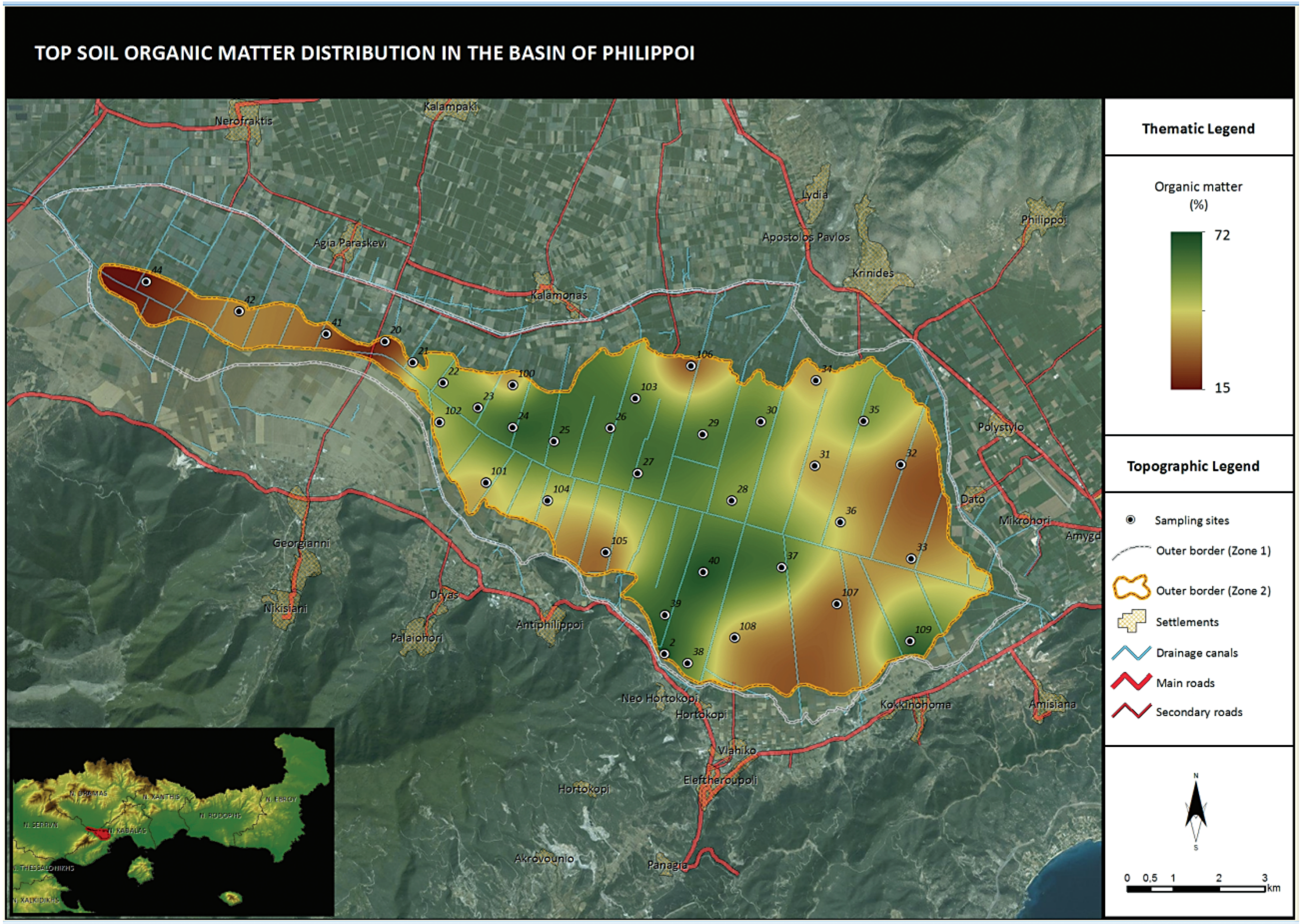

Fig. 1. Top soil organic matter distribution in the basin of Philippoi (adapted from Papazafeiriou, 2011)

1994). The presence of mycorrhizae may increase the absorbing surface of the roots and contribute to the uptake of nutrients (Alloway, 1995), or may lead to reduction of metal uptake and the increased plant resistance (Leyval et al., 1997; Reichman, 2002). Even high temperatures may cause a higher availability of metals to plants (Antoniadis and Alloway, 2001). The uptake mechanism is also related to the metal ion, while it is possible that antagonism occurs between ions that are absorbed by the root using the same mechanism (Alloway, 1995). Moreover, the presence of a specific metal in the plant cells may reduce the uptake or toxicity of other metals due to antagonism (Sawidis et al., 2001).

Besides root uptake, plants may acquire considerable quantities of metals through leaf uptake (Chamberlain, 1983; Koricheva et al., 1997; Seregin and Ivanov, 2001; Assunçao et al., 2003), with the foliage playing an important part in metal retention (Öztürk and Türkan, 1993). So, the concentration of metals in plants is a complex procedure affected by a multitude of parameters, only one of which is the concentration of the ion in the soil.

The aim of this study was i) to determine the concentrations of trace elements – such as Fe, Zn, Cu, Mn, and Se – in two plant groups (corn and forage species) growing on the alkaline organic soils of the basin of Philippoi, ii) to investigate their distribution in the study area and, iii) to evaluate if these concentrations are prohibitive for small ruminant extensive farming systems.

Materials and Methods

The basin of Philippoi is located in the NW of the city of Kavala and the SE of Drama, in Northern Greece. The soils in the area are categorized as Histosols Medisaprists with a high percentage of organic matter (greater than 20 to 30% organic matter by weight) as presented in Figure 1 (Papazafeiriou, 2011).

The low altitude, flat terrain and bad drainage conditions combined with the climate and the inorganic matter transferred via the surface runoff, were the main parameters that influenced the formation and accumulation of peat, which goes deeper than 298 m in the center of the basin (NAGREF, 2001).

The dominant forage species growing in the study area are *Phragmites communis, Aster tripolium, Lythrum virgatum, Bolboschoenus maritiumus, Apium graveolens, Trifolium fragiferum, Agropyron elongatum, Crepis foetida, Hordeum murinum, Phalaris canariensis, Bromus erectus, Avena barbata* και *Chamomila recutita*. These species were identified using the Treatises Mountain Flora of Greece (Strid, 1986; Strid and Tan, 1991) and Flora Europaea (Tutin et al., 1964-1980). The area is grazed by sheep and goat flocks during the spring and summer months. Corn is also cultivated, exclusively for silage.

Twenty sample sites were selected in the organic soils of the study area and plant material was collected from each site. Plant material includes forage species and corn. Forage species were collected by hand plucking, similarly to those grazed by small ruminants in the peak of the growth period (summer) of 2009. Corn samples were collected from the adjacent fields in the same sites. These samples included leaves, shoots and seeds. Three samples, both of forage species and corn, were collected from each site. The samples were oven-dried at 60°C for 24 h, burned at 480°C for 2 h and the concentrations of Fe, Zn, Cu, Mn and Se were determined by atomic absorption spectrophotometry (AOAC, 1999). Afterwards the measured values of Fe, Zn, Cu, Mn and Se for both forage and corn were compared to the nutrient requirements for small ruminants of 30 kg weight and moderate activity according to NRC (1975, 1980, 1981, 1985, 2001, 2005). The animals' nutrient requirements refer to maintenance unless otherwise stated in the text.

Mean values of Fe, Zn, Cu, Mn and Se concentrations and their standard deviation were calculated using JMP-8 statistical software of SAS (Lehman et al., 2005; Sall et al., 2007). Statistical analysis was carried using analysis of variance, level of significance a=0.05. Mean differences were compared using the least significance difference criterion.

Results and Discussion

The average values and standard deviations of Fe, Zn, Cu, Mn and Se concentrations for both forage and corn are shown in Table 1.

Table 1

Mean values and standard deviations of Fe, Zn, Cu, Mn and Se concentrations in forage species and corn growing on the alkaline organic soils of the basin of Philippoi - Greece

Plant group	Fe	Zn	Cu	Mn	Se
	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
Forage species	3766.2*±1500.9	95.3±25.5	23.1±7.9	105.4±56.8	0.32±0.1
Corn	4023.5±1589.5	95.5±32.7	23.6±7.6	127.0±81.8	0.30±0.2

* Statistically significant differences were not found for any of the five elements between forage species and corn ($P < 0.05$)

Table 2
Results of plant material analysis for each sampling site

Sampling site*	Plant material**	Fe	Zn	Cu	Mn	Se
		mg kg^{-1}				μg kg^{-1}
100	1	3287.50	77.50	17.25	87.50	210.00
101	1	4625.00	84.00	23.75	145.00	345.00
101	2	6262.50	78.50	12.40	92.50	260.00
102	1	4937.50	162.00	31.20	116.00	280.00
102	2	5020.00	126.00	32.85	143.50	330.00
103	1	5887.50	113.50	22.00	313.50	445.00
103	2	4680.00	96.00	19.30	275.00	365.00
104	1	4550.00	66.00	14.10	77.00	325.00
104	2	6600.00	82.00	30.10	113.50	200.00
105	1	4200.00	115.50	26.45	89.50	55.00
105	2	5725.00	88.50	22.05	97.50	175.00
107	1	2500.00	70.00	20.55	32.00	250.00
107	2	5025.00	85.00	26.20	106.00	430.00
108	1	1612.50	110.50	32.15	37.50	195.00
108	2	1875.50	72.00	22.60	37.50	285.00
109	1	2675.00	17.00	40.10	353.50	115.00
2	1	1800.00	53.50	13.85	55.50	108.00
2	2	5087.50	76.00	26.35	77.50	310.00
20	1	2348.00	79.00	27.20	85.50	205.00
20	2	2262.50	74.50	26.85	90.00	360.00
21	1	2487.50	108.00	18.05	60.50	260.00
21	2	2012.50	71.00	14.55	72.50	605.00
24	1	1887.50	112.00	19.85	69.00	80.00
24	2	4750.00	127.00	33.35	160.50	170.00
27	1	5250.00	135.00	28.75	105.50	385.00
28	1	2895.00	92.50	13.50	70.50	210.00
28	2	2512.50	155.50	12.70	54.50	190.00
30	1	6358.00	85.00	28.75	220.00	375.00
30	2	2550.00	107.00	20.85	192.00	240.00
37	2	3287.50	117.00	22.55	103.00	310.00
39	1	6580.00	98.50	21.45	96.50	255.00
39	2	3750.00	140.00	28.00	156.00	170.00
41	1	2782.50	122.50	42.85	92.00	390.00
41	2	4225.00	84.50	15.85	80.50	345.00
44	1	3580.50	68.00	13.75	135.00	495.00
44	2	4860.00	82.50	18.25	180.50	400.00

* The identification number of the sampling sites refers to Fig.1
** 1: Forage species, 2: Corn

There is no significant difference between the two plant groups for any of the micronutrients measured. In order to view the fluctuation of the concentrations by site, analytical results are shown in Table 2.

The evaluation of suitability of the forage material for small ruminant extensive farming is presented separately for each of the five trace elements taken into account in this study.

Iron

Iron concentration ranged from 1612.5 mg kg^{-1} to 6600 mg kg^{-1} (Table 2). The maximum concentrations of Fe in plants were found in sites 104, 39 and 30 situated in the central and south part of the basin as shown in Figure 1. The minimum values occurred in the NW part of the basin (sites 20, 21 and 24).

The plant ability to absorb Fe is variable and is affected by changing conditions of soil and climate and by phenological stages of plant and specific genotype properties. Some plants, like e.g., *Alyssum bertoloni*, exhibit a special capability to absorb Fe and can accumulate this metal to about 4.000 mg kg^{-1} in roots and 1.300 mg kg^{-1} in leaves (Brooks, 1998). The variation among plants in their ability to absorb Fe is not always consistent and is affected by changing conditions of soil and climate and by stages of plant growth. Generally, legumes are known to accumulate more Fe than other plants. However, where Fe is easily soluble, plants may take up a very large amount of Fe. This is clearly shown by vegetation grown in soils derived from serpentine, where grass contained Fe within the range of 2.127–3.580 mg kg^{-1} (Johnston and Proctor, 1977).

Concentrations in the study area highly exceed the maximum tolerable level for small ruminants (500 mg kg^{-1} DM) (NRC, 2005) both for forage species and corn by three to more than ten times, which should be taken into account when determining the animals' feed. According to NRC (2001), the daily dietary iron requirement for sheep is 30 mg kg^{-1} DM. For goats higher values (30-100 mg kg^{-1} DM) may be justified depending on the age and iron status of the animal (Haenlein, 1987). According to Ivan et al. (1990) high levels of iron in forages (549–990 mg kg^{-1} DM) may cause Cu deficiency in lambs and goats.

Zinc

Zinc concentrations ranged from 17 mg kg^{-1} to 162 mg kg^{-1} (Table 2). The maximum concentration of Zn in the samples occurred in sites 102, 28 and 24 on the upper north part of the basin following the distribution of organic matter in the topsoil (Figure 1). The lower concentrations occurred in the perimeter of the basin, again following the distribution of organic matter (sites 2, 104 and 44) (Figure 1).

Generally, Zn concentrations in plants vary considerably, reflecting impact of different factors of the various ecosystems and of genotypes. It has been referred by Kabata-Pendias (2011) that mean contents of zinc in grasses worldwide vary from 25 to 47 mg kg^{-1} and is fairly similar to those in clover. However there are local deviations from the average (15 to 80 mg kg^{-1}) for plants growing on the acid organic soils of Germany. The high measured values of Zn in the study area can be attributed to the high content of organic matter and to bioavailability (Papazafeiriou, 2011).

Zinc concentrations in plant tissues in the basin of Philippoi are higher than the dietary requirements for sheep and goats, but below toxicity levels. There is no toxicity danger and the Zn requirements of the animals are met. The dietary requirements for sheep were set as 20 mg kg^{-1} DM for growing animals (NRC, 1985). Zn requirement for growth in goats was set at 10 mg kg^{-1} DM (NRC, 1981). These requirements were differentiated by NRC (1985) at 20 mg kg^{-1} DM for growing animals, while AFRC (1997) set the requirements at 50 mg kg^{-1} DM in the presence of antagonists. Ruminants have less tolerance to high zinc intake than non-ruminant animals. The maximum tolerable level for sheep is 300 mg Zn kg^{-1} DM (NRC, 2005).

Copper

Copper concentrations in the selected samples ranged from 12.40 mg kg^{-1} to 42.30 mg kg^{-1} (Table 2). The maximum values of Cu occurred in sites 41, 109, 24 and 102 in the NW to SW part of the basin (Figure 1) with low organic matter content. On the other hand, minimum levels of Cu in plants were observed in sites 44, 28 and 101, which coincide with the central part of the basin where a high organic matter percentage is also present (Figure 1).

Copper contents in plants vary greatly and are controlled by several factors of which the pool of mobile Cu in soils and plant properties play significant roles. Higher contents of Cu appear in grasses, in the range of 2–10 mg kg^{-1} and in clovers, from 7 to 15 mg kg^{-1} (Kabata-Pendias and Pendias, 2001).

The copper requirement for sheep was set at 5 mg kg^{-1} DM (NRC, 1975) and later increased to 7–11 mg kg^{-1} DM (NRC, 1985). Underwood and Suttle (1999) estimate Cu requirements for small ruminants to 4.3–28.4 mg kg^{-1} DM. Specifically, copper requirements for goats were established by Kessler (1991) and adopted by Meschy (2000) to be 8–10 mg kg^{-1} DM. The great variation in absorption coefficients of copper combined with variable levels of absorption antagonists, such as Fe, Zn, Cd, and metabolic reactions result in an approximate measure of dietary need (Suttle et al., 2002). In the study area, Cu concentrations in the forage material are two times higher than those limits.

Manganese

Manganese concentrations in the forage material varied from 32 mg kg^{-1} to 353 mg kg^{-1} in the study area (Table 2). The maximum values of Mn occurred in the center and eastern part of the basin (sites 109, 103 and 106) with high concentrations of organic matter (Figure 1). The minimum concentrations of Mn in plants were observed in the lower SE part of the basin with low concentrations of organic matter, as shown in Figure 1 (sites 2, 28, 108 and 107).

Concentrations of Mn fluctuate greatly within plant species, plant parts and during the vegetative period (Kabata-Pendias, 2011). Usually, Mn concentrations increase in older parts of plants. Apart from the plants' characteristics, the content of Mn in plants is also influenced by the pool of available Mn in the soil, which, in turn, is highly controlled by soil properties. Although Mn uptake is metabolically controlled, its passive absorption, especially at high and/or toxic ranges, is also observed. According to Kabata-Pendias and Mukherjee (2007) grasses and clovers from various countries contain relatively high and fairly similar amounts of Mn (71–127 mg kg^{-1} for grasses and 25–89 mg kg^{-1} for clover).

There are several different views about the dietary Mn requirements for small ruminants. NRC (1985) suggests a value of 20 mg kg^{-1} DM for sheep, while Masters et al. (1988) found that 13 mg kg^{-1} DM were adequate for growth. Requirements suggested for goats include 20–25 mg kg^{-1} DM (ARC, 1980), 20–40 mg kg^{-1} DM (Haenlein, 1992), 40–50 mg kg^{-1} DM (Meschy, 2000), 60–120 mg kg^{-1} DM (Lamand, 1981) and 60 mg kg^{-1} DM (AFRC, 1997). Again whichever approach is used, Mn requirements are covered for both sheep and goats in the area of study. The maximum tolerable level of Mn, given by NRC (2005) is 2000 mg kg^{-1} DM well above the concentration of Mn in study area. Therefore, there is no toxicity danger for either grazing sheep or goats.

Selenium

As indicated by the results in Table 2, selenium concentrations in the plant material vary from 55 μg kg^{-1} to 605 μg kg^{-1}. Maximum values occurred in sites 21, 44 and 103, while the minimum values were observed in the lower SW part of the basin (sites 105, 24, 2 and 39) regardless of the percentage of organic matter.

Most plants contain rather low foliar Se, in general, 25 μg kg^{-1}, and rarely exceed 100 μg kg^{-1}. However, some plants exhibit a great capability to accumulate Se (Kabata-Pendias and Mukherjee, 2007) and they may concentrate Se to extremely high levels (over 1000 μg kg^{-1}). Leguminous plants (clover and alfalfa) usually contain more Se than grasses. Ge and Yang (1993) analyzed a number of feedstuffs and forage samples from China and found that samples contain Se at the levels (in μg kg^{-1}) of: <20, from severely Se-deficient areas; 30–50, from deficient areas; 60–90, from moderate areas; and >100, from adequate areas.

The maximum tolerable level of Se for small ruminants was increased from 2.0 mg kg^{-1} DM (NRC, 1980) to 5.0 mg kg^{-1} DM (NRC, 2005). The latter value was set considering animal health. However, it was stated that lower values are necessary to avoid excessive accumulation in edible tissues (NRC, 2005). In general, the proposed value for Se requirements is 0.1 mg kg^{-1} DM for all small ruminants during maintenance. This value rises to 1.0 mg kg^{-1} DM during pregnancy (NRC, 1985).

Conclusions

The concentrations of Zn and Mn in the plant material growing on the alkaline organic soils of the basin of Philippoi follow the distribution of organic matter in the topsoil. On the other hand, the minimum levels of Cu coincide with high organic matter content and vice versa. Fe and Se concentrations were independent of the organic matter content.

Regarding the concentrations of minerals meeting the nutrient requirements of small ruminants, Fe concentrations exceeded toxicity levels. However, the forage species and corn can be combined with feedstuffs of lower Fe concentration in order to eliminate this problem. The same precautions can also be applied to Cu. Zn concentrations were higher than the nutritional requirements of small ruminants, but there is no toxicity danger. Mn concentrations were within the range of acceptable nutritional requirements of small ruminants with no toxicity danger. Finally, Se concentrations covered the maintenance requirements of the animals, but Se needs to be supplemented during pregnancy.

The plant material coming from the alkaline organic soils of the basin of Philippoi can be used in the extensive farming system of small ruminants in the area with caution and regular monitoring of Fe and Cu concentrations.

References

AFRC, 1997. Technical Committee on Responses to Nutrients, Report 10. The nutrition of goats. *Nutrition Abstracts and Reviews (Series B),* **67**: 806–815.

Alloway, B. J., 1995. Heavy Metals in Soils. *Blackie Press*, London.

Antoniadis, V. and B. J. Alloway., 2001. Availability of Cd, Ni and Zn to ryegrass in sewage sludge-treated soils at different temperatures. *Water Air Soil Pollution,* **132**: 201–214.

AOAC, 1999. Official Methods of Analysis, Method 968.08, 16th ed. *Association of Official Analytical Chemists*, Gaithersburg, MD, USA (5th rev.).

ARC, 1980. The nutrient requirements of Ruminant Livestock. Slough, *Commonwealth Agricultural Bureaux,* UK.

Assunção, A. G. L., H. Schat and M. G. M. Aarts, 2003. *Thlaspi caerulescens*, an attractive model species to study heavy metal hyperaccumulation in plants. *New Phytologist,* **159**: 351–360.

Brady, N. C. and R. R. Weil, 2008. The Nature and Properties of Soils. 14th ed., *Prentice Hall*, Inc., Upper Saddly River, NJ. 980 pp.

Brooks, R. R., 1998. Plants That Hyperaccumulate Heavy Metals: Their Role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining. *CAB*, Wallingford.

Chamberlain, A. C., 1983. Fallout of lead and uptake by crops. *Atmospheric Environment,* **17**: 693–706.

Clijsters, H. and F. Van Assche, 1985. Inhibition of photosynthesis by heavy metals. *Photosynthesis Research,* **7**: 31–40.

Ge, K. and G. Q. Yang, 1993. The epidemiology of selenium deficiency of endemic disease in China. *American Journal of Clinical Nutrition Supplement,* **57**: 259–263.

Haenlein, G. F. W., 1987. Mineral and vitamin requirements and deficiencies, In: O. P. Santana, A. G. da Silva and W. C. Foote (Eds.) Proc. IV International Conference on Goats, vol. 2, Brazil, pp. 1249–1266.

Haenlein, G. F. W., 1992. Advances in the nutrition of macro and micro elements in goats. In: Proc. V. International Conference on Goats, New Delhi, India, pp. 933–950.

Ivan, M., M. Hidiroglou, S. L. Al-Ismaily, H. S. Al-Sumry and R. B. Harper, 1990. Copper deficiency and posterior paralysis (Shalal) in small ruminants in the Sultanate of Oman. *Tropical Animal Health and Production,* **22**: 217–225.

Johnston, W. R. and J. Proctor, 1977. Metal concentrations in plants and soils from two British serpentine sites. *Plant and Soil,* **46**: 275.

Kabata-Pendias, A., 2011. Trace Elements in Soils and Plants. 4th ed., *CRC Press LLC*, USA.

Kabata-Pendias, A. and A. B. Mukherjee, 2007. Trace Elements from Soil to Human. *Springer-Verlag*, Berlin Heidelberg.

Kabata-Pendias, A. and H. Pendias, 2001. Trace Elements in Soils and Plants. 3rd ed., *CRC Press LLC*, USA, pp. 331.

Kessler, J., 1991. Mineral nutrition of goats. *Goat Nutrition,* **46**: 104–119.

Koricheva, J., S. Roy, J. A. Vranjic, E. Haukioja, P. R. Hughes and O. Hãnninen, 1997. Antioxidant responses to simulated acid rain and heavy metal deposition in birch seedlings. *Environmental Pollution,* **95**: 249–258.

Lamand, M., 1981. Metabolism and requirements of microelements by goats. Proceedings ITOVIC-INRA International Symposium Nutrition Systems Goat Feeding, May 12–15, 1981, Tours, France, I: 210.

Lehman, A., N. O'Rourke, L. Hatcher and E. J. Stepanski, 2005. JMP® for Basic Univariate and Multivariate Statistics: A Step-by-Step Guide. Cary, NC: *SAS Institute Inc*.

Leyval, C., K. Turnau and K. Haselwandter, 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza,* **7**: 139–153.

Masters, D. G., D. I. Paynter, J. Briegel, S. K. Baker and D. B. Purser, 1988. Influence of manganese intake on body, wool and testicular growth of young rams and on the concentration of manganese and activity of manganese enzymes in tissues. *Australian Journal of Agricultural Research,* **39**: 517–524.

Meschy, F., 2000. Recent progress in the assessment of mineral requirements of goats. *Livestock Production Science,* **64**: 9–14.

N.AG.RE.F (National Agricultural Research Foundation), 2001. Soil Survey of the basin of Philippoi. Athens (Gr).

NRC, 1975. Nutrient Requirements of Sheep. *National Academy Press,* Washington DC.

NRC, 1980. Mineral Tolerance of Domestic Animals. *National Academy Press,* Washington DC.

NRC, 1981. Nutrient Requirements of Goats: Angora, Dairy and Meat Goats in Temperate and Tropical Countries. *National Academy Press,* Washington DC.

NRC, 1985. Nutrients Requirements of Sheep, 6th rev. ed. *National Academy Press*, Washington DC.

NRC, 2001. Nutrient requirements of Dairy Cattle, 7th rev. ed. *National Academy Press*, Washington DC.

NRC, 2005. Mineral Tolerance of Animals, 2nd rev. ed. *National Academy Press*, Washington DC.

Öztürk, M. and I. Türkan, 1993. Heavy metal accumulation by plants growing alongside the motorroads: A case study from Turkey. In: B. Markert (Ed.) Plants as Biomonitors: Indicators for Heavy Metals in the Terrestrial Environment, *VCH Publishers*, Germany, pp. 515–522.

Papazafeiriou, A., 2011. Impact of soil parameters on the transfer of heavy metals in the food chain through forage material in areas of Greece. Ph.D. dissertation, *Aristotle University of Thessaloniki*, Greece, pp. 235.

Purkayastha, R. P., A. K. Mitra and B. Bhattacharyya, 1994. Uptake and toxicological effects of some heavy metals on Pleurotus sajor-caju. Singer. *Ecotoxicology and Environmental Safety*, **27**: 7–13.

Reichman, S. M., 2002. The responses of plants to metal toxicity. A review focusing on copper, manganese and zinc. Occasional Paper No.14, *Australian Minerals and Energy Environment Foundation*, Melbourne.

Sall, J., L. Creighton and A. Lehman, 2007. JMP® Start Statistics: A Guide to Statistics and Data Analysis Using JMP®, 4th Edition. *SAS Institute Inc.,* Cary, NC.

Sawidis, T., M. K. Chetrri, A. Papaioannou, G. Zachariadis and J. Stratis, 2001. A study of metal distribution from lignite fuels using trees as biological monitors. *Ecotoxicology and Environmental Safety,* **48**: 27–35.

Seregin, I. V. and V. B. Ivanov, 2001. Physiological aspects of cadmium and lead toxic effects on higher plants. *Russian Journal of Plant Physiology,* **48**: 523–544.

Strid, A., 1986. Mountain Flora of Greece, Vol.1. *Cambridge University Press*, Cambridge, p. 819.

Strid, A. and K. Tan, 1991. Mountain Flora of Greece, Vol. 2. *Edinburgh University Press*, Edinburgh, p. 974.

Suttle, N. F., R. M. Lewis and J. N. W. Small, 2002. Effects of breed and family on rate of copper accretion in the liver of purebred Charolais, Suffolk and Texel lambs. *Animal Science,* **75**: 295–302.

Tutin, T., V. Heywood, N. Burges, D. Valentine, S. Walters and D. Webb (eds.), 1964–1980. Flora Europaea, Vol. 1–5. Cambridge.

Underwood, E. J. and N. Suttle, 1999. The Mineral Nutrition of Livestock, 3rd ed. *CAB International*, New York.

Received November, 14, 2015; accepted for printing August, 29, 2016