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Professional paper

THE INFLUENCE OF THE DEVELOPMENT PHASE AND THE AGROCHEMICAL COMPOSITION OF THE SOIL ON THE MOLYBDENUM (MO) CONTENT IN ALFALFA (BANAT ZMS II)

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Abstract

Alfalfa cultivation (Banat ZMS II) was carried out in the polish conditions of four localities: Saraj, Radusha (outskirt of Skopje), Jegunovce (Tetovo), and Kodzilari (Veles) with known content of nitrogen, phosphorus, potassium, iron and pH value.
The developmental phase showed a significant impact on the molybdenum (Mo) content in the dry matter (DM) of the experimental alfalfa (leaves and stalks), whereas in the stage of flowering the average Mo values were lower by 20.45% (1.44mg/kg-1 DM), while in the two stages of development the average content of Mo in the mass of leaves (1.90 mg/kg-1DM) along with that of stalks (1.28 mg/kg-1 DM) was higher by 65.89%. Also, the quantities of Mo uptake or absorption in cultivated alfalfa variants were correlated with the different nitrogen, phosphorus, and iron contents found in the experimental plots, a ratio especially expressed in the alfalfa of the Radusha and Kodzilari localities.

Keywords: alfalfa, dry matter (DM), molybdenum, stage of development, localities

1. Introduction

Molybdenum is an essential micronutrient required for plants and animals [16]. The optimal Mo content according to [20] in alfalfa ranges from 0.5 - 5.0 mg/kg DM. Contents up to 0.2 mg/kg are considered low, i.e. toxic when higher than 5 mg/kg DM [27].

Alfalfa as a fodder crop for ruminants is a basic source of protein and other nutrients [17]. Numerous studies to date have shown that the quality of alfalfa plants decreases with plant age, primarily due to the increased percentage of the total mass of stalks at the expense of leaves expressed through total yield [3;25]. The nutritional value of the leaves changes insignificantly during the development of the plant, unlike the stem where the qualitative composition changes dramatically with age, primarily the content of cellulose and lignin increases while the protein, sugars, vitamins, and other nutrients decrease [9;25;22]. It has also been observed in peas that the bioaccumulation of Mo and Fe was generally higher in the early stages of growth while decreasing during generative development [1].

In alfalfa, molybdenum compared to other trace elements shows the greatest activity on nitrogen fixation. In soil solution, Mo occurs as molybdate oxyanion (MoO4). Once molybdenum is captured from plant roots, it remains biologically inactive until it forms a complex with a unique paternal compound called molybdenum cofactor (MoCo). Moco binds to various apoproteins used in both reductive and oxidative reductions [15], including nitrate reductase (NR), aldehyde oxidase (AO), xanthine oxidase/dehydrogenase [14], and sulfate oxidase [23].

In conditions of larger amounts of molybdenum in the soil of alfalfa, there is an increased presence of absorbed molybdenum in the dry matter which is followed by a decrease in the content of nitrates and iron in it [18].

In plants, it has been suggested that molybdenum cotransport can be accomplished through a variety of mechanisms, including through a phosphate absorption system [8].

This study was conducted to investigate the changes that occur in terms of molybdenum content in alfalfa leaves and stalks in two stages of development as well as the impact of soil mineral composition, ie the supply of experimental soils with nitrogen, phosphorus, and iron important in the molybdenum uptake process. Also, according to the obtained results, the level of Mo content in leaves and stalks important in the diet of ruminants is considered, as well as the existing ratio of the adopted amounts of iron and molybdenum in the dry mass of leaves and stalks.

2. Material and methods

Alfalfa variety banat ZMS II is grown in polish conditions in the localities Saraj, Jegunovce, Radusa, and Kodzilari (Veles) - Republic of North Macedonia.
Experimental area in:

1. Saraj - is taken as a control variant
2. Jegunovce, near the area of the factory Jugohrom Jegunovce, Tetovo.
3. Radusa, near an abandoned mineral mine
4. Kodzilari, a locality near the factory for the production of artificial fertilizers "HIV" in Veles.

The soil of the sites with experimental alfalfa has a known agrochemical composition [4], ie the content of total nitrogen, phosphorus (P2O5), and iron (Fe) is given in Table 1.

Table 1. Agrochemical composition of soil from experimental areas

Location	Available amounts in the soil			pH KCl
	mg/100g		mg/kg	
	NO3-N + NH4-N	P2O5	Fe	
Saraj	7.52	36.02	10.70	7.68 ± 0.05
Jegunovce	7.62	23.17	23.03	7.80 ± 0.05
Radusha	9.75	28.90	94.04	7.90 ± 0.11
Kodzilari	9.38	50.75	8.26	7.48 ± 0.06

The dry matter of leaves and stalks of the experimental alfalfa has a known content of total nitrogen (%) and iron (mg/kg DM) (Tab.3;4), [4;5].

- The content of molybdenum (Mo) in the dry mass of alfalfa was determined by the HPLC method.

3. Examination results and discussion

The tests of the molybdenum content in the dry mass of the experimental alfalfa in the individual localities during the two phases of development ranged within the allowed quantities, i.e., from 0.5 to 5.0 mg/kg dry mass (DM), [20], except the variant of the village Radusha where the amount of measured Mo was lower than 0.5 mg/kg DM.
In the before-flowering stage (Tab.2) the content of Mo (mg/kg) in the dry mass of leaves and stalks of the experimental variants: Jegunovce (2.42; 1.55mg/kg) Radusha (> 0.75;> 0.51mg /kg) and Kodzilari (2.09; 1.46 mg/kg) which compared to the measured quantities in the variant of Saraj (3.50; 2.20 mg/kg) are lower in the variant of Jegunovce by 30.09% and 29.05%, of Radusha by 78.6% and 76.8%, and of Kodzilari by 40.03% in the leaves, ie by 33.6% in the stalks. The average content of Mo in the dry mass of the stalks of 1.43 mg/kg DM about the leaves (2.19 mg/kg) is lower by 34.7%.
In the flowering phase in the experimental variants significantly lower Mo content was measured compared to those in the before-flowering phase, ie in the leaves, the average Mo content (1.73 mg/kg) was lower by 21%. The ratio of the measured quantities of Mo in the dry mass in the flowering phase is generally similar to that found in the pre-flowering phase. In the dry mass of leaves, the largest decrease of 33.3% is in the variant of Radusha, followed by the variant of Kodzilari, lower by 25.4%, and Jegunovce and Saraj lower by 17.4% and 17.3%, respectively.

Table 2. Molybdenum content in dry alfalfa matters (Banat ZMS II)

Location	Mo mg/kg DM						
	Leaves			Stalks			Mo stalks/leaves
	Before flowering						
		%			%		%
Saraj	3.50 ± 0.40	100.0	100.0	2.20 ± 0.51	100.0	100.0	62.85
Jegunovce	2.42 ± 0.72	69.1	100.0	1.55 ± 0.34	70.5	100.0	64.05
Radusha	0.75 ± 0.37	21.4	100.0	0.51± 0.00	23.2	100.0	68.00
Kodzilari	2.09 ± 0.55	59.7	100.0	1.46 ± 0.18	66.4	100.0	69.85
X1	2.19 ± 0.51	-	100.0	1.43 ± 0.36	-	100.0	66.19
	Flowering						
Saraj	2.88 ± 0.59	100.0	82.3	1.56 ± 0.57	100.0	70.9	54.16
Jegunovce	2.00 ± 0.85	69.4	82.6	1.36 ± 0.34	87.2	87.7	68.00
Radusha	>0.50± 0.0	17.7	66.7	>0.50± 0.0	32.1	-	-
Kodzilari	1.56 ± 0.29	54.2	74.6	1.14 ± 0.12	73.1	78.1	73.07
X2	1.73 ± 0.57	-		1.35 ± 0.34	-		65.30
Xmean	1.96 ± 0.54		76.55	1.39 ± 0.35		78.0	65.74

In the flowering phase, the amount of Mo in the stalks shows a significant decrease compared to the same in the pre-flowering phase. Higher average content was measured in the

Saraj variant (1.56 mg/kg) which compared to the same in the before-flowering phase is lower by 30.01%, while in the Radusha variant the contents were lower than 0.50 mg/kg. Kodzilari variant showed lower Mo content in the stalks by 26.9% (1.14 mg/kg), ie Jegunovce 1.36 mg/kg, ie lower by 13.8% compared to the measured quantities Mo in the stalks of the Saraj variant. The average amounts of Mo in the dry mass of the stalks in the flowering stage compared to those in the before-flowering stage were lower by 5.6 % (1.35 mg/kg DM).

Higher contents of measured Mo in the dry mass in the before-flowering stage indicate the influence of the developmental stage on the degree of Mo adoption and translocation [1; 21; 7; 6; 25]. The higher amounts of adopted Mo in the dry mass of the Saraj variant are primarily correlated with the higher presence of P2O5 in the soil (36.02 mg/100g) compared to other sites as well as the lower Fe content (10.71 mg kg DM). [12;15;2;19;26].

The lowest contents of Mo in the dry mass of the variant of Radusha are primarily conditioned by higher Fe content in the soil (94.04 mg/kg) which is competitive with Mo uptake [7] as well as higher NH4-N content (7.90 mg/kg) which causes a decrease in Mo uptake due to conditioned pH lowering [10;7;14].

The lower quantities of measured Mo in the variant of Jegunovce versus the variant Saraj and Kodzilari are correlated with the lower soil availability in the village. Jegunovce with available amounts of phosphorus (23.17 mg/100 g) and significantly higher iron content (23.03 mg/kg) in the soil compared to the measured Fe contents in the soils of Saraj and Kodzilari (10.71; 8.26 mg/kg) [24;2;15]. In the Kodzilari variant, in addition to the higher soil availability with P2O5 (50.75 mg/100g) which intensifies the adoption, the significantly lower content of adopted Mo in dry mass compared to the Saraj variant is due to the significantly higher potassium content in the soil, [5]. The negative influence of K on the adoption of Mo is also noted by other authors [28;14;11].

According to the presented values for the amount of molybdenum in the dry mass of the experimental alfalfa (tab.2) and the previously known quantities of total nitrogen in the dry mass of the simultaneously taken samples of alfalfa at the same experimental sites [4] in Tab.3. is shown their ratio where a positive correlation is found, ie in the phase before flowering and flowering the higher content of Mo measured in the leaves and stalks of alfalfa of the Saraj variant (3.50; 2.88 mg/kg and 2.20; 1.56 mg/kg) is followed by the higher content of total nitrogen in them (5.62; 2.64 and 4.93; 2.10%), i.e. significantly lower Mo content in the Radusha variant (0.75;> 0.50 mg/kg and> 0.51;> 0.50 mg/kg DM) are followed by significant lower content of total nitrogen in them (4.81; 2.05% and 4.44; 1.72%). A similar regularity between the content of Mo and total nitrogen (N) is observed in the dry mass of experimental alfalfa in Jegunovce and Kodzilari. This correlation and dynamics of Mo and total N in plants have been observed and determined by other authors. [14;5;13;9;6].

Table 3. Content of total nitrogen (N %) and molybdenum (Mo mg/kg) in DM of alfalfa

Location	Stage. of develop.	Leaves		Stalks	
		Total N	Mo	Total N	Mo
Saraj	Before flower	5.62 ± 0.35	3.50 ± 0.40	2.64 ± 0.02	2.20 ± 0.51
Jegunovce		5.01 ± 0.04	2.42 ± 0.72	2.26 ± 0.03	1.55 ± 0.34
Raduscha		4.81 ± 0.27	0.75 ± 0.37	2.05 ± 0.23	0.51± 0.00
Kodzilari		5.47 ± 0.07	2.09 ± 0.55	2.63 ± 0.29	1.46 ± 0.18
Saraj	Flowering	4.93 ± 0.04	2.88 ± 0.59	2.26 ± 0.03	1.56 ± 0.57
Jegunovce		4.68 ± 0.21	2.00 ± 0.85	1.85 ± 0.02	1.36 ± 0.34
Raduscha		4.44 ± 0.22	>0.50 ± 0.0	1.81 ± 0.02	>0.50 ± 0.0
Kodzilari		4.82 ± 0.19	1.56 ± 0.29	2.01 ± 0.04	1.14 ± 0.12

Table 4. Shows the ratio of the measured quantities of Mo in the dry mass of alfalfa in two stages of development and the known quantities of Fe present in the same samples [4] and it

Location	Stage. of develop.	Leaves		Stalks	
		Fe	Mo	Fe	Mo
Saraj	Before flower	137.0 ± 11.5	3.50 ± 0.40	65.3 ± 6.5	2.20 ± 0.51
Jegunovce		117.3 ± 10.6	2.42 ± 0.72	50.8 ±11.6	1.55 ± 0.34
Raduscha		131.3 ± 8.1	0.75 ± 0.37	66.2 ± 4.3	0.51± 0.0
Kodzilari		126.7 ± 12.7	2.09 ± 0.55	63.0 ± 6.7	1.46 ± 0.18
Saraj	Flowering	117.1 ± 16.3	2.88 ± 0.59	49.2 ± 6.90	1.56 ± 0.57
Jegunovce		102.8 ± 6.1	2.00 ± 0.85	39.5 ± 10.7	1.36 ± 0.34
Raduscha		111.5 ± 3.9	>0.50 ± 0.0	53.7 ± 4.51	> 0.50 ± 0.0
Kodzilari		105.5 ± 3.5	1.56 ± 0.29	50.1 ± 2.53	1.14 ± 0.12

Table 4. Content of iron (Fe mg/kg) and molybdenum (Mo mg/kg) in DM of alfalfa is concluded that there is not always an antagonism between these two elements, in fact, in both phenophases in the Saraj variant the higher Fe contents in leaves (137.0 and 117.0 mg/kg) are followed by higher Mo contents (3.50; 2.88 mg/kg), ie lower Mo contents in the dry mass of leaves of the Radusha variety (3.50 2.88 mg/kg) are not followed by a lower Fe content (131.3; 111.5 mg/kg DM). Also, in both phenophases, the low content of Mo in the dry mass of leaves of the Kodzilari variant (2.09 and 1.56 mg/kg DM) and Fe in the leaves (126.7mg and 105.5 mg/kg DM) indicates that these two elements in their adoption are in a competitive relationship where the Fe content is very important for molybdenum absorption, while Mo in this respect is of significantly less important for the uptake of Fe from the soil [1; 21].

4. Conclusions

The content of molybdenum (Mo) in the DM of the experimental alfalfa was within the optimal values except the Radusha variant where the measured quantities are less than 0.50 mg/kg;

The influence of the development phase on the content of Mo proved to be very significant, with the significant reduction of Mo in the DM of leaves and stems being observed in the flowering phase of the samples;

Mo content showed dependence on the agrochemical composition of the soil as follows: the present amounts of available forms of nitrogen (N), and phosphorus (P2O5), showed a stimulating effect on the uptake and translocation of Mo. The amount the iron (Fe) in the soil proved to be an inhibitor and competitive trace element in terms of Mo uptake of alfalfa.

The DM of leaves showed a significantly better Mo supply compared to the stalks;

The measured amounts of Mo in DM showed a positive correlation with the present amounts of total N in DM.

The ratio between the present amounts of Fe and Mo in DM showed the significant role of iron in the process of Mo absorption.

References

[1]. Andrzej Wysokinski * , Izabela Lozak and Beata Kuziemska :The Dynamics of Molybdenum, Boron, and Iron Uptake,Translocation and Accumulation by Pea(Pisum sativum L.) Journal Agronomy 2022, 12, 935.

[2]. Barrow, N.J., P. Cartes and M.L. Mora, 2005. Modifications to the Freundlich equation to describe anion sorption over a large range and to describe the competition between pairs of ions. Eur. J. Soil Sci., 56: 601-6.

[3]. BUXTON, D.R.; HORNSTEIN, J.S.; WEDIN, W.F.; MARTEN, G.C.(1985): Forage quality in strat field canopies of alfalfa, birdsfoot trefoil and ol. 28, 342-347.

[4]. Elmi Jusufi & Mentor Zekiri (2012): Content of the adopted amounts of iron and manganese in dry mass of alfalfa (BANAT ZMS II) cultivated in different localities in R. Macedonia. Intern. Conference on Towards future sustainable development”. Proceedings book, pp.487-490.University of Shkodra, Albania.

[5]. Elmi Jusufi, Mentor Zekiri, Natasha Gjorgovska, Vesna Levkov (2012): Contents of some macrobiogen elements in soil and their uptake at alfalfa. Macedonian Journal of Animal Science, Vol. 2, No. 1, pp: 37-40.

[6]. Fredeen A. H. 1989. Effect of maturity of alfalfa (Medicago sativa) at harvest on calcium absorption in goats. Can. J. Anim. Sci' 69:365-371.

[7]. Gupta, U.C., 1997. Soil and Plant Factors Affecting Molybdenum Uptake by Plants. In: Molybdenum in Agriculture, Gupta, U.C. (Ed.). Chapter 5, Cambridge University Press, Cambridge, UK., ISBN-13: 9780521571210, pp: 71-91.

[8]. H. Heuwinkel, E.A.Kirkby, J.Le Bot and H.Marschner: Phosphorus deficiency enhances molybdenum uptake by tomato plants.J.of Plant Nutr. 15(5) ,549-568 (1992).

[9]. IGNJATOVIĆ, S., DINIĆ, B., KOLARSKI, D., UROŠEVIĆ, B. (1998): Chemical composition of first and second cut of lucerne (Medicago sativa L.) at different stages of maturity. Proc.17th General Meeting EGF, may 18- 21, Debrecen, Hungary.

[10]. Kaiser,B.N.,Gridley,K.L.,NgaireBrady,J.,Phillips,T.,andTyerman,S.D.(2005). The role of molybdenum in agricultural plant production. Ann.Bot. 96, 745–754.

[11]. Kastori, R. : Uloga elemenata u ishrani biljaka.Matica Srpska,N.Sad (1983).

[12]. Liu, H., C. Hu, X. Hu, Z. Nie, X. Sun, Q. Tan, and H. Hu, 2010. Interaction of molybdenum and phosphorus supply on uptake and translocation of phosphorus and molybdenum by brassica napus. J. Plant Nutr., 33: 1751-1760.

[13]. Львов, Н.П.,(1989). Молибден в ассимилЎации азота у растениЎ и микроорганизмов. М., Наука.

[14]. Marschner,H.:Mineral nutrition of Higher Plants,U.S.Sec.ed.Pub.Ac. Press, 1995.

[15]. Mendel, R.R. and R. Hansch, 2002. Molybdoenzymes and molybdenum cofactor in plants. J. Exp. Bot., 53: 1689-1698.

[16]. Mengel, K., E.A. Kirkby, H. Kosegarten and T. Appel, 2001. Principles of Plant Nutrition. 5th Edn., Kluwer Academic Publishers, Dordrecht

[17]. MINSON, D.J. (1990): Forage in ruminant nutrition. Academic Press, Inc., San Diego, CA.

[18]. L. M. Romero Monreal and A. Aguilar Villalvilla (1982).: Relationship between Mo and Fe in lucerne plants.Journal of Plant Nutrition, 5(4-7), 743-746.

[19]. Mortvedt, J.J. and H.G. Cunningham, 1971. Production, Marketing and use of other Secondary and Micronutrient Fertilizer. In: Fertilizer Technology and Use, Olson, R.A. (Ed.). 2nd Edn., SSSA, Madison, WI., USA., pp: 413-454.

[20]. Neubert, P.,Warzidlo, W., Vielemeyer, H.P., Hundt, I., Gollmick, E., Bergmann, W.: Tabellen zur Pflanzenanalyse.

Erste orientierende Ubersicht. Inst. fur Pflanzenernahrung der Akademie der Landwirtschaftswiss, der DDR, Jena, 1970.
[21]. Ociepa, E.; Pachura, P.; Ociepa-Kubicka, A. Effect of fertilization unconventional migration of heavy metals in the soil-plant system. Eng. Prot. Environ. 2014, 17, 325–338.
[22]. Ocokoljic, S. (1975): Leptiraste biljke u ishrani stoke (lucerka, crvena detelina i žuti zvezdan). Nolit, Beograd, 251.
[23]. Russ Hille, Takeshi Nishino and Florian Bittner: Molybdenum enzymes in higher organisms Coord Chem Rev. Volume 255, Issues 9–10, May 2011, Pages 1179-1205.
[24]. Rebafka,F.P.(1993); Deficiency of phosphorus and molybdenum as major growth limiting factors of pearl milet and ground nuton an acid sandy soil in Niger ,West Africa.Ph.D.Thesis University Hohenheim.
[25]. Sanderson, A., Hornstein, S., Wedin, F. (1989): Alfalfa morphological stage and its relation to in situ digestibility of detergent fiber fraction of stems. Crop Sci., Vol. 29, p.1315-1319 red clover. Crop Sci. Vol. 25, p. 273-279.
[26]. Sanderson ,M.A.,and R.M.Jones 1993.Stand dynamics and yield compoundof alfalfa as affected by phosphorus fertility .Agron.J.85: 241-246.
[27]. Umesh C.Gupta.: Boron and molybdenum – Critical Plant Levels in Forage Legumes (Canada).Better Crops with plant food.Vol.LXXV(75), N.0 4, p.8-9; Fall 1991 Atlanta.
[28]. Villora, G., D.A. Moreno and L. Romero, 2003. Potassium supply influences molybdenum, nitrate and nitrate reductase activity in eggplant. J. Plant Nutr., 26: 659-669.