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ORIGINAL PAPER

IMPACT OF SELECTED AGROCHEMICAL TREATMENTS ON THE CONTENT OF MOLYBDENUM IN GRAIN OF WINTER WHEAT AND SPRING BARLEY GROWN ON LESSIVE SOIL DEVELOPED FROM LOESS*

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Abstract

This paper presents results of a study on the molybdenum content in grain of winter wheat and spring barley grown on a permanently fertilized field on loessial soil in the Rzeszow Foothills (Podgórze Rzeszowskie), in Poland. The experiment was set up in randomized sub-blocks, with potato, spring barley, fodder sunflower, and winter wheat plants grown in 4 four-year rotation cycles. Fodder cabbage was grown instead of sunflower in the second crop rotation. The first variable factor was liming (A2) or its lack (A1), while the second one consisted of different doses of mineral fertilization. Mineral NPK nutrition was applied together with constant Mg as well as Mg and Ca fertilization (liming). The general fertilization level ($N_1P_1K_1$) for spring barley was 80 kg N ha^{-1}, 43.6 kg P ha^{-1} and 99.6 kg K ha^{-1}, while for winter wheat it was 90 kg N ha^{-1}, 34.9 kg P ha^{-1} and 83 kg K ha^{-1}. Constant magnesium fertilization was applied at a 24.1 kg Mg ha^{-1} dose. Liming at a dose of 4 t CaO ha^{-1} was applied before starting the experiment and in each year that completed a subsequent crop rotation cycle. There was a significant increase in the molybdenum concentration in grain of winter wheat and spring barley due to the liming applied. A moderate molybdenum content was found in winter wheat grain on non-limed soil 0.278 mg kg^{-1} DM and on limed soil 0.320 mg kg^{-1} DM; while in spring barley grain on non-limed soil, it equalled 0.291 mg kg^{-1} DM and on limed soil 0.455 mg kg^{-1} DM. Mineral fertilization increased the content of this component in wheat grain and usually or most often reduced

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its amount in barley grain. The interaction of liming with mineral fertilization did not have any significant effect on the molybdenum content of winter wheat grain and resulted in an increase in the molybdenum content in spring barley grain.

Keywords: liming, mineral fertilization, molybdenum, cereal grain.

INTRODUCTION

Plants require many elements that perform various physiological and biochemical functions in their growth and development. Among the essential nutrients, there are macroelements, including nutritive and building substances (N, P, K, Ca, Mg, S), and microelements (for example Cu, Mn, Fe, Mo, Zn, B, Co, J, F). Micronutrients have a regulating effect, affect the conversion of building components into the plant mass, and regulate the proper course of biochemical and enzymatic processes (Lošák et al. 2011, Kabata-Pendias, Szteke 2012, Shukla et al. 2014).

Molybdenum is an important microelement, essential for plants, animals and people (Johnsson 2005) because it is included in over 60 enzymes involved in the redox processes (Kabata-Pendias, Szteke 2012). It participates in the metabolism of nitrogen in plants (which is its most important function), also affecting the uptake of nitrogen by winter wheat (Zhong-hua, Sheng-xiu 2005). It is one of the most important trace elements in cereal crops and a component of such enzymes as nitrogenase and nitrate reductase (Modi 2002, Barker, Pilbeam 2015). Other enzymes for the functioning of which the molybdenum is needed are xanthine dehydrogenase, aldehyde oxidase and sulfite oxidase (Mendel 2011). This element is involved in the formation of enzymes needed for protein and chlorophyll synthesis, it increases protein content in seeds and seed efficiency, and it affects the DNA and RNA functions.

Molybdenum often occurs in soil in the form of an MoO_4^{2-} anion and easily interacts with other anions, especially SO_4^{2-}. This element passes from soil to plants, with the largest amounts in swamp and calcareous soils. The same plant species can accumulate different amounts of molybdenum depending on the soil type it comes from. Its interactions with some cations (Fe, Mn, Ca) can be antagonistic or synergistic (Marschner 2011). Availability of molybdenum for plants depends on the grain-size composition of the soil, its organic matter content and pH value. It increases with an increasing soil pH (Kaniuczak et al. 2000, Johnsson 2005, Kirchmann et al. 2005, Gupta et al. 2008). The effect of pH also applies to the uptake of molybdenum by cereals (Hamner, Kirchmann 2015).

The content of molybdenum in plants is directly correlated with its content in soil and bioavailability, but in acidic soils this metal is hardly available to plants (Gembarzewski, Stanisławska 1987, Mengel, Kirkby 2001).

Stanisławska-Glubiak and Korzeniowska (2011) noted a significant increase in the molybdenum content in spring barley grain under the influence of liming of very acidic and acidic light soils. Increased uptake of this element in the presence of liming and phosphate was found by Snowball and Robson (1991).

An increase in the molybdenum concentration in cereals was correlated with the reduction of sulfur deposition and its content in soil because molybdates and sulfates are ions competing for their uptake by plant roots. Reduced molybdenum assimilation in the presence of sulfates was demonstrated by Snowball and Robson (1991) and Kirchmann et al. (2009). Under reducing soil conditions, sulfur compounds (MoS_4^{2-}, $MoO_2S_2^{2-}$), which are easily taken up by plants, are formed (Marschner 2011).

Molybdenum also shows interdependence with copper. If the Cu:Mo ratio is below 1, there is a risk of copper deficiency and molybdenum poisoning. Excessive molybdenum uptake due to copper deficiency may cause an imbalance of these micronutrients in the diet of ruminants, and this reduces the biological value of grain as feed (Jones 2005, Stanisławska-Glubiak, Korzeniowska 2011). Molybdenum content in feed plants above 10 mg kg^{-1} DM is toxic to animals (Barker, Pilbeam 2015).

Manifestations of molybdenum deficiency depend to some extent on the nitrogen content in the soil. With low nitrogen fertilization, cereal leaves are smaller and sloping, and with high levels of molybdenum deficiency, the plants are pale green. Toxic symptoms appear on old leaves, too much nitrogen (Snowball, Robson 1991). Symptoms of molybdenum deficiency during winter wheat cultivation on acidic soils include chlorosis, necrosis and lower yields (Yu et al. 2002). Deficiency of this micronutrient on soils with low pH values can also be shown through the pale, fragile and withered leaves of the plants grown on them and their dwarfing.

Soil fertilization of spring barley with molybdenum increased the content of this component in the grain, depending on a variety. This treatment produces grain that can be used as seed on light and acidic soils. It can also be an alternative to liming (Stanisławska-Glubiak, Korzeniowska 2011) and effectively supplement deficiencies of this microelement in soils (Kaiser et al. 2005). Molybdenum, as an essential trace element for higher plants, also increases the resistance of winter wheat to low temperatures (Sun et al. 2009). Molybdenum and phosphorus fertilization had a particularly beneficial effect on the yield of winter wheat grain by increasing the plant's resistance to cold owing to the accumulation of biological substances (Zhaojun et al. 2015).

Molybdenum is needed in the human diet, even though the requirement for this element is small. It functions as a cofactor of many important enzymes (sulfite, xanthine and aldehyde oxidase), necessary for the absorption of sugars and fats (Vyskočil, Viau 1999, Novotny 2011). It participates in many biochemical processes, also contributing to the proper functioning of the nervous system and kidneys (Novotny 2011). It participates in the synthesis of proteins and is necessary for the proper growth of a young organism (Kulkarni et al. 2014).

The source of this component for humans are fruits and vegetables (especially legumes, green vegetable leaves, red cabbage) and, to a lesser extent, drinking water. Cereal plants as a raw material are the basis for obtaining various products which have a significant share in human nutrition (bread, cereals, cereals, etc.).

The aim of the study was to determine the effect of liming and mineral NPK fertilization against the background of constant Mg nutrition on the content of molybdenum in winter wheat and spring barley grain, cultivated in four 4-year crop rotations on lessive soil developed from loess.

MATERIAL AND METHODS

The research was carried on a long-term, controlled fertilizing field of the University of Rzeszow in Krasne near Rzeszow, situated in the Rzeszow Foothills (50°02′ N; 22°03′ E, 220 m a.s.l.), Poland.

The soil on which the research was carried out was formed from loess of the grey-brown podzolic type (a Haplic luvisol) and had the textural composition of silt-loam. Prior to the experiment (1986), the soil was characterized by very high acidification (with low pH values: pH_{KCl} = 3.92, pH_{H_2O} = 4.93) in the plough humus layer (Ap) and pH_{KCl} = 3.89, pH_{H2O} = 4.90 in the enrichment layer (Bt), with considerable values of hydrolytic acidity: 4.87 cmol (+) kg^{-1} in the Ap layer and 3.6 cmol (+) kg^{-1} in the Bt layer. The soil was low in available P and K, but moderately rich in available magnesium and contained 0.87 g kg^{-1} total N and 7.6 g kg^{-1} organic C. The elements were determined by the following methods: the total nitrogen content by the Kjeldahl method, the organic carbon content by the Tiurin method, the content of available forms of magnesium by the Schachtschabel method and of P and K by the Egner-Riehm method (Kaniuczak 1998). Before starting the trials, the bulk density of the soil amounted 1.405 Mg m^{-3}, porosity equalled 45.33 m^3 (100 m^3)$^{-1}$, water content at $pF_{2.0}$ was 24.17 kg (100 kg)$^{-1}$ and the content of Mo soluble in 1mol HCl dm^{-3} was 0.07 mg kg^{-1}. The experiment was set up in a random sub–block design with four replicates. The first variable factor was liming (A_2) or its lack (A_1), while the second one consisted of different doses of mineral fertilization (14 fertilizer objects) with constant magnesium nutrition. The following crops were cultivated in the rotation system: potato, spring barley, fodder sunflower (fodder cabbage in the 1st crop rotation cycle) and winter wheat. The study covered 4 crop rotation cycles. Winter wheat was grown in 1987, 1991, 1995 and in 1999, whereas spring barley was cultivated in 1989, 1993, 1997 and 2001.

Basic doses of mineral fertilization $N_1P_1K_1$ with constant magnesium nutrition were as follows: spring barley: N_1 – 80 kg N, P_1 – 43.6 kg P, K_1 – 99.6 kg K ha^{-1}, winter wheat: N_1 – 90 kg N, P_1 – 34.9 kg P, K_1 – 83.0 kg K ha^{-1}. Constant magnesium fertilization was applied before sowing in each experimental sub-block at a 24.1 kg Mg ha^{-1} dose for potato, spring barley and winter wheat, and a 72.4 kg Mg ha^{-1} dose for fodder crops. Liming with a dose of 4 t CaO ha^{-1} was used prior to the experiment and after the harvest of the crop last in a rotation. Mineral fertilizers NPK, Mg and Ca were applied in forms of ammonium nitrate, triple superphosphate, potassium salt KCl (58%), synthetic magnesium sulfate and CaO or $CaCO_3$ (dose to according 1 value of the hydrolytic acidity).

Mineral fertilizers used in plant fertilization did not contain molybdenum, except for triple superphosphate (trace amounts to 18 mg kg^{-1} DM) – Kaniuczak (1996).

Plant material samples were collected after wheat and barley harvest. Molybdenum in the dry plant material was determined colorimetrically with the tiocyanate method (Czuba et al. 1970). Thiocyanates create an orange-red complex with molybdenum, which can be quantified by spectrophotometry after its extraction.

The accuracy of the method was estimated by comparing the result of the SRM 1547 - Peach Leaves analysis, obtaining an Mo content not differing by more than 9.6% of the certified value.

The results were statistically processed by a two-factor analysis of variance (liming, mineral NPK fertilization) and calculating the lowest significant difference (LSD) with the Tukey's tests at the significance level of p=0.05.

RESULTS AND DISCUSSION

Molybdenum in winter wheat and spring barley grain varied depending on the applied agrochemical treatments (Tables 1 and 2). The content of molybdenum in winter wheat grain originating from non-limed objects ranged from 0.037 to 0.563 mg kg^{-1}, with an average content of 0.278 mg kg^{-1} DM (Table 1). In winter wheat grain grown on limed soil, the molybdenum content was higher, i.e. from 0.057 to 0.620 mg kg^{-1}, with an average of 0.320 mg kg^{-1} DM. Liming had a statistically significant effect on increasing the content of this element in the grain of this cereal. In seed of various winter wheat cultivars originating from the province of Zielona Góra (currently *lubuskie*), the molybdenum content was found in the range of 0.24-0.26 mg kg^{-1} (Ignatowicz, Żmigrodzka 1972). Kirchmann et al. (2005), when examining the wheat grain from soil with a pH close to 7, found the Mo content of up to 1.18 mg kg^{-1}. The content of molybdenum in consumer

Table 1

Content of molybdenum in grain (mg kg^{-1} DM) of winter wheat cultivated on loessial soil (means from 4 years)

Object no.	Treatments of fertilizers (B)	A_1		A_2		Mean (B)
		mean	range	mean	range	
1.	$N_0 P_0 K_0$	0.037	0.03-0.05	0.057	0.03-0.08	0.047
2.	$N_0 P_1 K_1$	0.160	0.14-0.18	0.193	0.14-0.24	0.177
3.	$N_{0,5} P_1 K_1$	0.097	0.08-0.11	0.147	0.08-0.20	0.122
4.	$N_1 P_1 K_1$	0.277	0.26-0.29	0.313	0.28-0.34	0.296
5.	$N_{1,5} P_1 K_1$	0.083	0.04-0.12	0.100	0.04-0.16	0.092
6.	$N_1 P_0 K_1$	0.157	0.14-0.17	0.190	0.14-0.23	0.173
7.	$N_1 P_{0,5} K_1$	0.283	0.26-0.31	0.327	0.28-0.36	0.305
8.	$N_1 P_{1,5} K_1$	0.447	0.40-0.48	0.460	0.40-0.52	0.453
9.	$N_1 P_1 K_0$	0.360	0.34-0.38	0.417	0.38-0.45	0.388
10.	$N_1 P_1 K_{0,5}$	0.487	0.46-0.52	0.500	0.41-0.57	0.493
11.	$N_1 P_1 K_{1,5}$	0.563	0.51-0.64	0.620	0.56-0.66	0.592
12.	$N_{0,5} P_{0,5} K_{0,5}$	0.433	0.40-0.46	0.493	0.46-0.52	0.463
13.	$N_{1,5} P_{1,5} K_{1,5}$	0.267	0.16-0.38	0.353	0.24-0.48	0.310
14.	$N_2 P_2 K_2$	0.247	0.16-0.34	0.307	0.22-0.42	0.277
Mean (A)		0.278	-	0.320	-	-
LSD at p=0,05		A* = 0.029, B = 0.133 AB = n.s				

A_1 – NPK fertilization + Mg constans, A_2 – NPK fertilization +MgCa constans,
LSD – lowest significant difference for: A – liming, B – mineral fertilization (irrespective from liming), AB – interaction between liming and mineral fertilization, * significant at p=0.001, ns – non-significant differences

plants ranges from 0.07 to 1.75 mg kg^{-1} DM, with an average content in cereal grains at 0.50 mg kg^{-1} DM. Plants are quite resistant to elevated molybdenum concentrations, although cereals are the most sensitive ones, while some vegetables (cauliflower, onion) can tolerate up to 600 mg kg^{-1} DM (Barker, Pilbeam 2015). Papilionaceous plants may contain increased amounts of molybdenum, up to about 5 mg kg^{-1} DM, and in areas covered by industrial pollution – up to 200 mg kg^{-1} DM.

Johansen et al. (2005) did not show significant correlations between the soil pH value and molybdenum content in soil and grass vegetation. Such relationships, i.e. increasing the uptake of this microelement by winter wheat grain as a result of liming, were found by Yu et al. (2002). This may be due to the Mo-Ca interaction, which consists in the formation of a soluble $CaMoO_4$ compound in soil containing free carbonates, which determines an easy uptake of molybdenum. Significant relationships between the content of molybdenum in winter wheat and the content of available forms

Table 2

Content of molybdenum in grain (mg kg^{-1} DM) of spring barley cultivated on loessial soil (means from 4 years)

Object no.	Treatments of fertilizers (B)	A_1		A_2		Mean (B)
		mean	range	mean	range	
1.	$N_0 P_0 K_0$	0.307	0.29-0.32	0.523	0.51-0.54	0.415
2.	$N_0 P_1 K_1$	0.327	0.30-0.34	0.377	0.36-0.39	0.352
3.	$N_{0,5} P_1 K_1$	0.327	0.31-0.34	0.583	0.56-0.60	0.455
4.	$N_1 P_1 K_1$	0.317	0.30-0.34	0.320	0.28-0.35	0.318
5.	$N_{1,5} P_1 K_1$	0.313	0.28-0.33	0.487	0.46-0.51	0.400
6.	$N_1 P_0 K_1$	0.317	0.30-0.33	0.500	0.48-0.52	0.408
7.	$N_1 P_{0,5} K_1$	0.300	0.29-0.33	0.310	0.28-0.32	0.305
8.	$N_1 P_{1,5} K_1$	0.230	0.28-0.34	0.310	0.28-0.34	0.270
9.	$N_1 P_1 K_0$	0.317	0.30-0.33	0.517	0.50-0.54	0.417
10.	$N_1 P_1 K_{0,5}$	0.310	0.29-0.33	0.783	0.76-0.80	0.547
11.	$N_1 P_1 K_{1,5}$	0.317	0.30-0.33	0.390	0.36-0.41	0.363
12.	$N_{0,5} P_{0,5} K_{0,5}$	0.307	0.30-0.32	0.317	0.28-0.33	0.312
13.	$N_{1,5} P_{1,5} K_{1,5}$	0.340	0.32-0.35	0.470	0.46-0.49	0.406
14.	$N_2 P_2 K_2$	0.323	0.30-0.34	0.480	0.46-0.50	0.402
Mean (A)		0.291	-	0.455	-	-
LSD at p=0,05		A* = 0.008, B = 0.036 AB = 0.030				

A_1 – NPK fertilization + Mg constans, A_2 – NPK fertilization +MgCa constans,
LSD – lowest significant difference for: A – liming, B – mineral fertilization (irrespective from liming), AB – interaction between liming and mineral fertilization, * significant at p=0.001

of molybdenum in soil have been demonstrated (Gembarzewski, Stanisławska 1987).

The content of molybdenum in spring barley grain grown on non-limed soil ranged from 0.230 to 0.340 mg kg^{-1}, with an average of 0.291 mg kg^{-1} DM (Table 2). In spring barley grain originating from limed objects, the content of this component ranged from 0.310 to 0.783 mg kg^{-1}, with an average of 0.455 mg kg^{-1} DM. The effect of liming on increasing the amount of molybdenum in this plant was also statistically significant. Molybdenum in acidic soils is difficult to be absorbed by plant (Mengel, Kirkby 2001). There is the Mo-Mn antagonism then, which is caused by increased soil acidity facilitating manganese uptake and limiting the availability of molybdenum to plants. Tyler and Olsson (2001) showed greater solubility of this element and its assimilation by plants growing on limed soils (or having high pH from the beginning). Mercik (1969) showed a distinct increase in the molybdenum content in spring barley harvested during the flowering phase, under the influence of liming used in many years of field experiments conducted in Skierniewice. A significant increase in the molybdenum content in spring barley grain occurred after liming (in the form of hydrated lime) applied to very acid and acidic light soils (Stanisławska-Glubiak, Sienkiewicz 2004, Stanisławska-Glubiak, Korzeniowska 2011). By regulating the soil reaction, liming increases the content of available forms of molybdenum in loess (Kaniuczak et al. 2000) and sandy soil (Rutkowska et al. 2014). Matula and Pechova (2007) showed a decrease in the content of this microelement in spring barley grain as a result of using gypsum in laboratory conditions on soil characterized by sulfur deficiency. At the same time, there were no clear statistical differences in relation to soil pH values.

Mineral fertilization (B), regardless of liming, significantly affected the molybdenum content in winter wheat grain (Table 1), although increasing doses of nitrogen fertilizers did not reveal unequivocal trends of changes for this element. Similarly, in Mercik's study (1969), nitrogen fertilization did not affect the molybdenum content of the dry matter of cereals (wheat and barley) grown on lessive soils. However, in this experiment, the use of increasing doses of phosphorus against the background of constant nitrogen and potassium fertilization was the reason for an increase in the content of the tested component in wheat grain. The smallest content was found in the object without phosphorus fertilization, while the largest appeared in response to the 1.5-fold higher dose of this component (Figure 1). Wheat was grown in the crop rotation after sunflower, a plant significantly accumulating Mo, which resulted in soil depletion of the available forms of Mo. Under such conditions, an increase in the dose of phosphorus fertilizers caused the activation of absorbed molybdate ions and an increase in the uptake of this element by wheat.

Also, potassium fertilization influenced the tendency to increase the molybdenum content in winter wheat grain, excluding the object where fertilizers (NPK) were applied at the same basic dose. NPK mineral fertilization (with constant proportion of components) was also the reason for the increase in molybdenum content in wheat grain compared to non-fertilized soil, but this effect was smaller when using higher doses of fertilizers. In the research of Rutkowska et al. (2009) only nitrogen fertilization reduced the molybdenum content of soil solution, and phosphorus and potassium fertilization did not have any significant effect.

Regardless of liming, mineral fertilization (B) had a statistically significant effect on the molybdenum content in spring barley grain, despite the lack of a clear and unambiguous effect of applying the increasing doses of nitrogen and potassium fertilizers. Increasing the doses of phosphorus fertilizers resulted in changes in the direction of reducing the content of this microelement in barley grain (Fig.1). Potatoes being the preceding crop for barley less depleted the soil of Mo. With the high soil content in Mo, an increase in the dose of phosphorus fertilizers had a competitive impact on the uptake of Mo by barley, unlike wheat previously described. Thus,

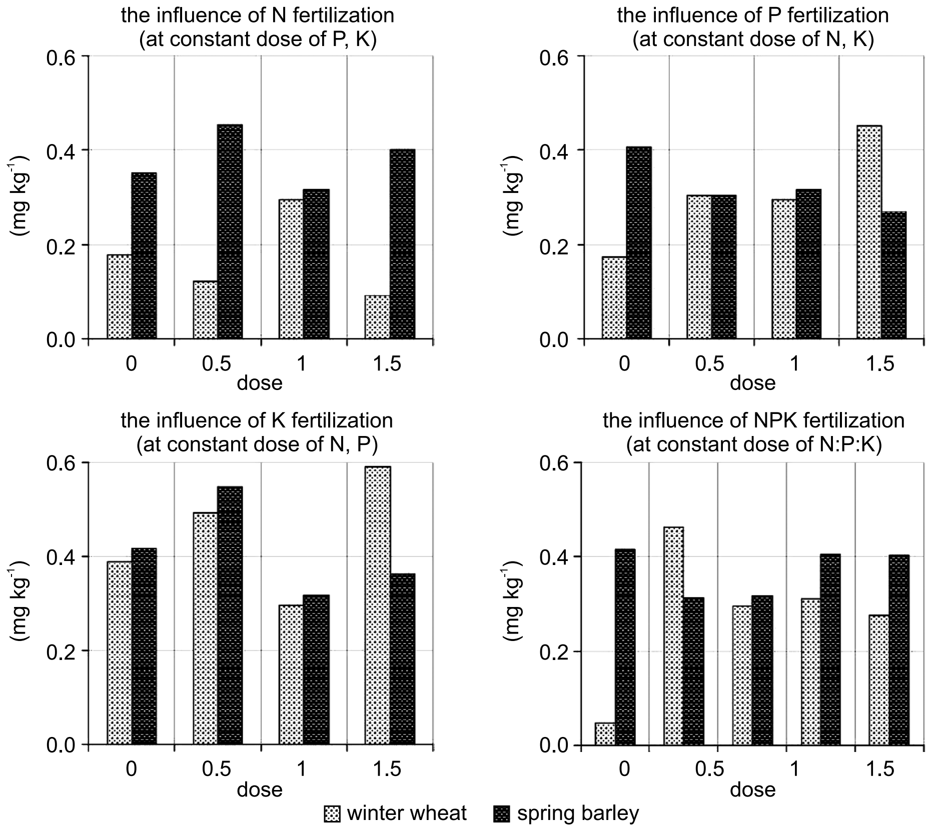

Fig. 1. The influence of different doses of fertilizers N, P, K and NPK on the content of molybdenum in winter wheat and spring barley

Mo-P interactions are variable and highly governed by diverse soil factors, in addition to which they are related to plant metabolic processes. NPK mineral fertilization was also the reason for an increase in the molybdenum content in spring barley cultivated on lessive soils (MERCIK1969). The optimal supply of spring barley with macro- and microelements during cultivation, protected the crop against an excessive uptake of molybdenum, even after its soil application (STANISŁAWSKA-GLUBIAK, KORZENIOWSKA 2011).

The interaction of liming with mineral fertilization (AB) did not cause any significant effect on the molybdenum content in winter wheat grain. However, the content of this element in wheat grain from limed fertilizer objects tended to increase. The synergy of these agrochemical treatments resulted in an increase in the molybdenum content in spring barley grain. Similar relationships were demonstrated by MERCIK (1969) in grain of wheat and barley cultivated on lessive soils in Skierniewice (many years of field experiments). A statistically significant increase in the molybdenum concentration in wheat and barley grain was noted as a result of the interaction of liming and molybdenum fertilization applied to podzolic soils of Eastern Canada, characterized by the lowest pH values (GUPTA, MACLEOD 1978). The simultaneous use of manure and calcium nitrate increased the content of molybdenum in cereal grains grown under long-term experiments on loamy soils in Sweden (HAMNER, KIRCHMANN 2015).

CONCLUSIONS

Liming significantly increased the molybdenum content in grain of winter wheat and spring barley cultivated in four 4-year crop rotations on lessive soil developed from loess and maintained under constant fertilizer conditions.

Mineral fertilization (with or without liming) had a statistically significant effect on the molybdenum content in both winter wheat and spring barley grains. Mineral fertilization increased the content of this component in wheat grain and usually or most often reduced its amount in barley grain.

The use of increasing doses of phosphorus against the background of constant nitrogen and potassium fertilization was the reason for the increase in the content of molybdenum in wheat grain. Increasing the doses of phosphorus fertilizers resulted in a change towards the content of this microelement in barley grain.

Interaction of liming with mineral fertilization resulted in an increase in the molybdenum content in spring barley grain grown on fertilizing objects with liming. The synergy of these agrochemical treatments did not have any significant effect on the molybdenum content of winter wheat grain. However, there was a tendency to increase the content of this element in wheat grain derived from the limed fertilizer objects.

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