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DETERMINATION OF MOLYBDENUM WITH TRIPHENYLTETRAZOLIUM CHLORIDE IN PLANT SAMPLES

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ABSTRACT

An analysis for molybdenum content by variants of lettuce samples was carried out using an extraction-spectrophotometric method with Triphenyl-tetrazolium chloride.

The influence of fertilization has been studied upon the content of molybdenum in black lettuce. Nitrogen sources – $(NH_4)_2SO_4$, NH_4NO_3 and $CO(NH_2)_2$ have been used. The soil treating on plants is carried out with 150 mg N кg^{-1} soil and 300 mg N кg^{-1} soil.

It can be seen a decrease in concentration of molybdenum in black lettuce in mineral fertilization with $(NH_4)_2SO_4$ and $CO(NH_2)_2$ with 1 mg kg^{-1} Mo introduced in soil, with the increase of the fertilization norm of nitrogen in soil.

Key words: molybdenum(VI), triphenyltetrazolium chloride, spectrophotometry, plants

INTRODUCTION

It has been established that molybdenum takes part in fixing and assimilation of the atmospheric nitrogen. Molybdenum also affects the biochemical processes and chemical composition of plants. It is known that micro-amounts of molybdenum favorably affect plant development. Low levels cause some functional diseases in the plants. The interest in molybdenum has increased recently. Molybdenum toxicity can rarely be observed in plants but the excess of this element causes illness in humans and animals. For this reason the molybdenum content in the crop production should be controlled [1-3].

In literature there are lots of data about the influence of molybdenum content in plants. With betterment of molybdenum feeding-up in plants sets in positive changes in the content of chlorophyll and carotene. The intensity of photosynthesis reinforces. On condition of molybdenum scarcity he synthesization of amino acids upsets, which reflects on quality and quantity of yield [4].

The objective of this study is to determine the molybdenum content using a new extraction – spectrophotometric method with Triphenyltetrazolium chloride in plant samples. To explore the influence of the mineral fertilization with molybdenum upon the assimilation of molybdenum on plants of black lettuce.

MATERIAL AND METHODS

Soil samples from a layer of 0 – 20 cm deep were taken for agronomical analysis and analyzed for: mineral nitrogen (NH_4-N + NO_3-N) 1.2 mg kg^{-1} soil ; mobile P_2O_5 and K_2O respectively 16.8 mg and 18.8 mg kg^{-1} soil by the method of Egner-Rhiem [9].

The crops were grown on a highly leached meadow-cinnamonic soil with comparatively light mechanical composition, a humus content of 1.6 % (by Tjurin) and neutral soil reaction pH 7-7.1 (potentiometrically) in aqueous extract.

A soil treating on plants of sort black lettuce has been carried out. Nitrogen sources – $(NH_4)_2SO_4$, NH_4NO_3 and $CO(NH_2)_2$ have been used. The soil treating on plants is carried out with 150 mg N kg^{-1} soil (N_1) and 300 mg N kg^{-1} soil (N_2). Molybdenum at dose of 1 mg kg^{-1} soil was applied like ammonium molybdate.

An analysis for molybdenum content in lettuce samples was carried out by using a new extraction-spectrophotometric method with Triphenyltetrazolium chloride [5]. All measurements were carried out in 1-cm quartz cells with measurement at 250 nm on UV-VIS spectrophotometer.

A wet burning of the plant samples was carried out and a mixture of sulfuric and nitric acids used for the oxidation of the organic substance. A portion of 2 g of air dry plant material was placed into a Kjeldahl flask and moistened with 4 mL distilled water. 5 mL conc. sulfuric acid and 10 mL conc. nitric acid were added. The flask was slightly heated to avoid splashing of the solution, decomposition and fuming away of nitric acid. When all the organic material was oxidized, the solution was heated at a higher temperature for 10 min [8]. After cooling, the solution was diluted with water and filtered. It was transferred into a volumetric flask of 50 mL and diluted up to the mark with distilled water. Aliquote parts of this solution were taken for analysis.

In separatory funnel of 100 mL the following solutions were introduced: 0.5 mL phosphoric acid $2x10^{-2}$ mol L^{-1}, 0.5 mL triphenyltetrazolium chloride 1.10^{-3} mol.L^{-1}, aliquote of the prepared solution of plant sample. The mixture was diluted up to a volume of the aqueous phase of 10 mL with distilled water and extracted with 3 mL of 1,2-dichloroethane for 30 sec. The organic phase was filtered through a dry paper into a 1 cm cuvette and the absorbance was measured at 250 nm. A blank was run in parallel in the absence of plant sample. A calibration graph was constructed with standards similarly treated.

RESULTS AND DISCUSSION

Molybdenum(VI) forms an ion-pair with Triphenyltetrazolium chloride [5]. The studies showed that the ion associate is better dissolved in 1,2-dichloroethane. Beer's law is obeyed in the range of 0.5 – 10 μg ml^{-1} molybdenum(VI). The molar absorptivity of the ion-pair is $1x10^{6}$ L mol^{-1} cm^{-1}. This method is applied in the present research for determination of microquantities of molybdenum in plant material.

The microelement molybdenum exerts influence on the normal development and nutritious qualities of the black lettuce. In conjunction with the mineral fertilization with the three nitrogen fertilizers $(NH_4)_2SO_4$, NH_4NO_3 and $CO(NH_2)_2$ it has been done a treating with molybdenum soil feeding-up with 1 mg Mo introduced like ammonium molybdate in kg soil. Complete information about the molybdenum content in soils and plants is needed to study its importance and to establish the need for molybdenum in fertilizers.

The fertilization brought considerable changes in the molybdenum content of lettuce plant material. The experimental data for content of molybdenum in a black lettuce cultivar in mineral fertilization and treating with molybdenum soil feeding-up are stated on Table 1.

The experimental data Table 1 show that the content of molybdenum in the plant mass stays the same 16.8 mg kg^{-1} in mineral fertilization with $(NH_4)_2SO_4$ in different levels of nitrogen in soil N_1 – 150 mg N kg^{-1} soil and N_2 – 300 mg N kg^{-1} soil (variants 3 and 5). In fertilization with $CO(NH_2)_2$ (variants 11 and 13) in different levels of nitrogen in soil the content of molybdenum is almost equal 16.00 mg kg^{-1} and respectively 15.8 mg kg^{-1}. The experimental data show that the content of molybdenum in black lettuce increases at about three times in fertilization with NH_4NO_3 (N_2 – 300 mg N kg^{-1} soil).

Consequently the mineral fertilization with NH_4NO_3 contributes to the better extraction of molybdenum in the plant mass. In control ($N_0P_0K_0$) the content of molybdenum is low 14.7 mg kg^{-1}.

Table 1. *Content of molybdenum in black lettuce (fertilization with 1 mg Mo kg^{-1} soil)*

N	Variants	Fertilizer	Mo, mg kg^{-1} TTC method	Reliab. P=99%	RSD* %	Mo, mg kg^{-1} TV** method [7]
1	$N_0P_0K_0$ (control)		14.7	a	1.4	15.2
2	$N_0P_1K_1$ (background)		14.0	d	2.0	14.6
3	N_1 + background	$(NH_4)_2SO_4$	16.8	d	1.8	16.2
4	N_1+background + 1 mg Mo	$(NH_4)_2SO_4$	31.5	d	1.6	31.0
5	N_2 + background	$(NH_4)_2SO_4$	16.8	a	1.5	16.0
6	N_2 +background +1 mg Mo	$(NH_4)_2SO_4$	19.5	f	1.9	19.9
7	N_1 + background	NH_4NO_3	18.0	c	1.5	18.7
8	N_1+ background +1 mg Mo	NH_4NO_3	8.7	d	2.3	9.3
9	N_2 + background	NH_4NO_3	55.5	c	1.4	55.0
10	N_2+ background +1mg Mo	NH_4NO_3	24.3	b	1.9	25.0
11	N_1 + background	$CO(NH_2)_2$	16.0	b	1.9	16.4
12	N_1+ background +1 mg Mo	$CO(NH_2)_2$	44.8	c	1.2	44.2
13	N_2 + background	$CO(NH_2)_2$	15.8	c	1.6	16.5
14	N_2 + background +1mg Mo	$CO(NH_2)_2$	14.7	c	1.5	15.0

*Relative Standard Deviation for TTC method (n = 6)
**Tetrazolium violet (TV)
a, b, c, A degree of reliability

Indices:
N_1 – 150 mg N kg^{-1} soil
N_2 – 300 mg N kg^{-1} soil
P_1 – 150 mg P_2O_5 kg^{-1} soil introduced like double superphosphate
K_1 – 150 mg K_2O kg^{-1} soil introduced like potassium sulphate

The obtained results from the study in connection with the developed method by us for determination of molybdenum with Triphenyltetrazolium chloride are worked with a computer programme for multispecified comparative analysis in Duncan's method [6].

It can be seen a decrease in concentration of molybdenum in black lettuce in mineral fertilization with $(NH_4)_2SO_4$ and $CO(NH_2)_2$ with 1 mg Mo kg^{-1} introduced in soil, with the increase of the fertilization norm of nitrogen in soil. In N_1 + background + 1 mg Mo (variant 4) fertilized with $(NH_4)_2SO_4$ molybdenum is 31.5 mg kg^{-1}, where as in N_2 – 300 mg N kg^{-1} (variant 6) the concentration of molybdenum decreases to 19.5 mg kg^{-1} (Fig.1). Hence the high content of nitrogen in soil decreases the quantity of the assimilated molybdenum by plants.

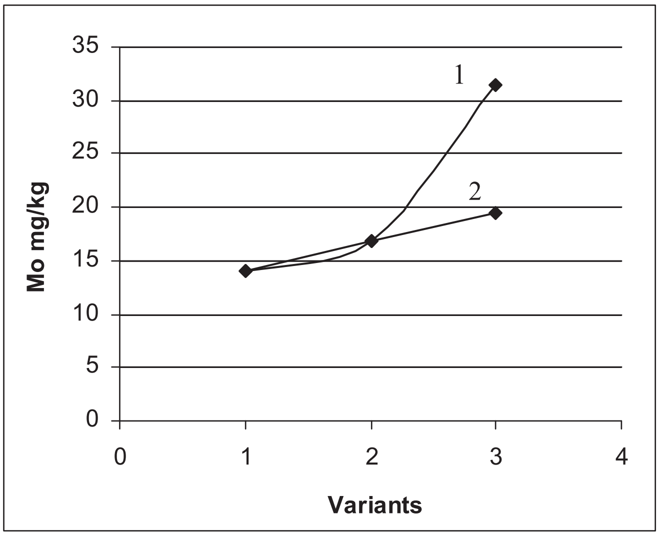

Figure 1. *Content of Mo in black lettuce in different levels of fertilization with* $(NH_4)_2SO_4$ *+ 1 mg Mo: 1 –* N_1 *150 mg N* kg^{-1} *soil; 2 –* N_2 *300 mg N* kg^{-1} *soil. Variants: 1 –* $N_0P_1K_1$ *(background); 2 – N + background; 3 – N + background + 1 mg Mo*

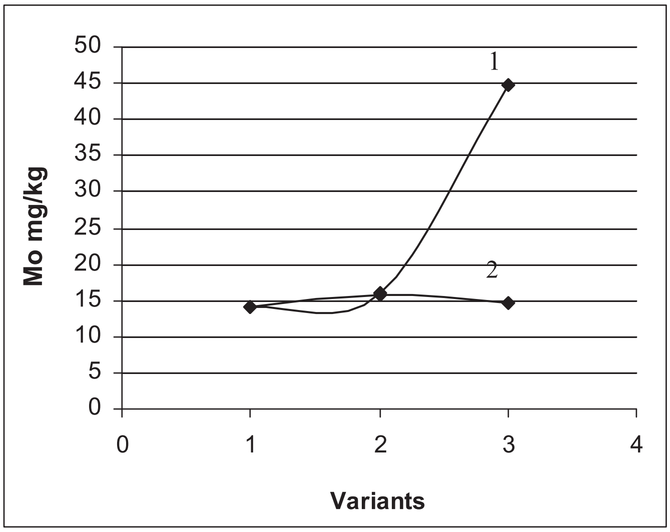

Figure 2. *Content of Mo in black lettuce in different levels of fertilization with* $CO(NH_2)_2$ *+ 1 mg Mo: 1 –* N_1 *150 mg N* kg^{-1} *soil; 2 –* N_2 *300 mg N* kg^{-1} *soil. Variants: 1 –* $N_0P_1K_1$ *(background); 2 – N + background; 3 – N + background + 1 mg Mo*

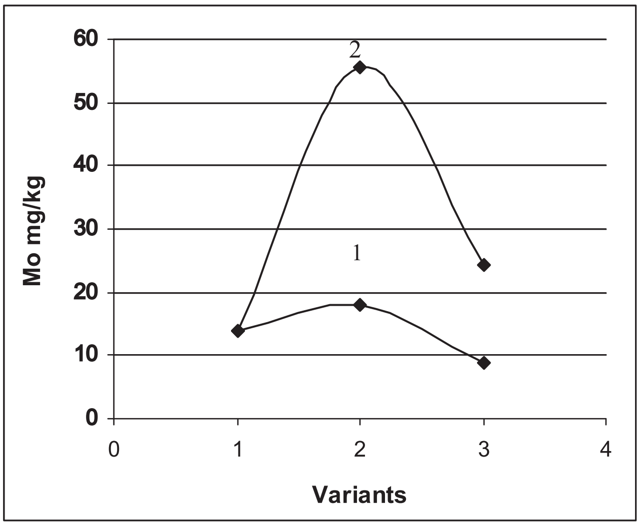

Figure 3. *Content of Mo in black lettuce in different levels of fertilization with NH_4NO_3 + 1 mg Mo: 1 – N_1 150 mg N kg^{-1} soil; 2 – N_2 300 mg N kg^{-1} soil. Variants: 1 – $N_0P_1K_1$ (background); 2 – N + background; 3 – N + background + 1 mg Mo*

A similar subordination can be seen in variants 12 and 14 fertilized with $CO(NH_2)_2$. In N_1 + background + 1 mg Mo the content of molybdenum is 44.8 mg kg^{-1}. With increase of the fertilization norm of nitrogen in soil in N_2 – 300 mg N kg^{-1}, the content of molybdenum decreases to 14.7 mg kg^{-1} (Fig. 2). Hence nitrogen introduced in soil in the form of $CO(NH_2)_2$ and $(NH_4)_2SO_4$ exerts the same influence on the accumulation of molybdenum by plants.

There is one exception and this is the fertilization with NH_4NO_3, that has a reverse influence upon the accumulation of molybdenum in black lettuce (Fig. 3). In fertilization with N_1 + background + 1 mg Mo(variant 8) the content of molybdenum in the plant mass is 8.7 mg kg^{-1} dry mass. The concentration of molybdenum increases three times to 24.3 mg kg^{-1} (variant 10), if we increase the fertilization norm of nitrogen to N_2 – 300 mg N kg^{-1} soil. Data from the minerel fertilization and treating with 1 mg Mo kg^{-1} soil show a positive influence of the added molybdenum in soil, if we only fertilize with $(NH_4)_2SO_4$.

To check the method that we propose, a parallel determination of molybdenum content was carried out by the method with Tetrazolium violet(TV) [7].

The experimental data (Table 1) by both methods show that the proposed method with Triphenyltetrazolium chloride can be successfully used for determination of microquantities of molybdenum in plant material.

CONCLUSIONS

Plants accumulate more molybdenum in additional bringing in soil of this microelement in the form of ammonium salt together with the base fertilization with $(NH_4)_2SO_4$ and $CO(NH_2)_2$.

An exception can be discerned in fertilization with NH_4NO_3. The additional addition of molybdenum in soil causes its decrease in the content in plant mass. Maybe this is due to the high nitrogen content in these variants of fertilization.

In mixed soil fertilization with $(NH_4)_2SO_4$ and $CO(NH_2)_2$ with 1 mg Mo, the less content of nitrogen in nutrient medium helps the better extraction of molybdenum in the plant mass.

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