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A comparative analysis of different vegetable crops for content of manganese and molybdenum

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Abstract. The microelements manganese and molybdenum are actively absorbed by plants. The manganese and molybdenum content in 12 widely used vegetable species of the families *Fabaceae, Malvaceae, Brassicaceae, Cruciferae* were studied to establish the different levels of these elements in their production.

Under the influence of nitrogen fertilization (400 mg N / 1000 g soil), different plant species, grown under the same soil-climatic conditions, accumulate different quantities of manganese and molybdenum from the nutrient medium. The resulting content varies considerably.

Lentils are described with the highest content of manganese, 120.83 mg kg^{-1} dry matter (family Fabaceae). The concentration of molybdenum is highest in broccoli, 29.53 mg kg^{-1} dry matter (family Brassicaceae). Brussels sprouts and okra accumulate the least manganese and molybdenum - brussels sprouts, 5.50 mg kg^{-1} Mn dry matter, okra, 5.23 mg kg^{-1} Mo dry matter.

The manganese and molybdenum content in the vegetable species were determined by a new extraction-photometric method with Toluidine Blue and Tetrazolium Violet.

Manganese and molybdenum has also been determined by atomic-absorption method, in order to compare the results.

Key words: manganese, molybdenum, plants, toluidine blue, tetrazolium violet *Fabaceae, Malvaceae, Brassicaceae, Cruciferae*

INTRODUCTION

The determination of micro-quantities of manganese and molybdenum in plants and other naturally occurring materials is of particular importance in connection with the growing interest in environmental concerns. Manganese and molybdenum are actively absorbed by plants. The formation of plant mass depends on the manganese and molybdenum supply. Manganese and molybdenum take part in a number of important physiological and biological processes – in the nitrogen metabolism, photosynthesis, breathing and maintaining the needed oxidation-reduction conditions in the cell (Panayotov, 2004; Panayotov, 2005; Panayotov et al., 2005).

Manganese is important for the synthesis of the organic substance in plants and for the metabolism of a number of nutrient elements in a plant organism. Plants need small amounts of manganese - from 0.0001% to 0.02% but it is indispensable for their normal growth and development. For most cultivated plants the critical concentration of manganese in leaves is 25 mg kg^{-1} dry matter (Epstein, 1961; Dobrolubskii, 1982; Bergmann, 1976; Jiang, 2002; Mutaftchiev, 2001; Mutaftchiev, 2003).

Interest in molybdenum has increased recently. It is known that micro-amounts of molybdenum favorably affect plant development, biochemical processes and chemical composition of plants. The normal content of molybdenum for most of the crops is 2-4 mg kg^{-1} dry matter; symptoms of scarcity are discernible when the content of Mo in plants is less than 0.1 mg kg^{-1} dry matter. Although molybdenum toxicity can rarely be observed in plants, an excess of this element causes illness in humans and animals. Symptoms of molybdenum toxicity are manifested with content above 20 mg kg^{-1} dry matter. Therefore the molybdenum content in crop production should be controlled (Kania, 2000; Maslowska, 1993; ZaiJun, 2001; Fontes, 2000; ZaiJun, 2005).

The aim of the present research is to make a comparative analysis of content of the essential trace elements manganese and molybdenum in the main vegetable crops that are cultivated on the same soil-climatic conditions. Results are based on a comparative evaluation of the manganese and molybdenum absorbed by the plants and the ratio between the two microelements, using a new method for determination of manganese with Toluidine Blue (Kostova, no published) and for determination of molybdenum with Tetrazolium Violet (Kamburova, 2008).

MATERIALS AND METHODS

The crops were grown according to customary technology adopted for the region and manner of planting, the number of plants, optimal mineral fertilization as well as for the appropriate agricultural practices during vegetation, such as irrigation and insect pest control.

The crops were grown on a highly leached meadow-cinnamonic soil with comparatively light mechanical composition; humus content of 2.2% (by Tjurin); neutral soil reaction $pH_{(H2O)}$ = 6.9; (mineral nitrogen in 1N KCl – by way of destillation) = 5.4 mg/100g; P_2O_5 and K_2O (by Ergner-Rim) = 214 mg/100g, respectivly 47 mg/100g and salt concentration (by electrical conductivity) 0.212 mS cm^{-1}.

The experiments were set according to the block method in four replications. Nitrogen was twice introduced with ammonium nitrate during the vegetation of the crops, with the first earthing-up and an equal dose a month later. For radish only, the nitrogen fertiliser was introduced once before the sowing of the seeds.

An analysis for manganese content of samples was carried out using a new extraction-photometric method with Toluidine Blue (Kostova, not published). Manganese(VII) forms an ion-pair with triphenylmethane dye Toluidine Blue (TB). The studies showed that the associate is best dissolved in 1,2-dichloroethane. The absorption spectra of triphenylmethane dye and ion-association complex in perchloric acid show that the absorption maxima are around 290 nm. The molar absorptivity at 290 nm is (0.8 ± 0.09) x 10^4 l mol cm^{-1}. The relative standard deviation (8 determinations with 2 μg ml^{-1} of manganese (VII), 95% confidence level) of the method is ± 1.5%. The Sandell sensitivity index is 7.14 x 10^{-3} μg cm^{-2} (Sandell, 1959).

An analysis for molybdenum content of samples was carried out using a new extraction-photometric method with Tetrazolium Violet (TV) (Kamburova, 2008). The absorption spectra of solutions of TV and the ion-association complex of molybdenum(VI) show that the absorption maximum is around 230 nm. The molar absorptivity of the associate, calculated by the method of Komar-Tolmatchov, was $(1.30 \pm 0.08) \times 10^5$ L mol^{-1} cm^{-1}. This indicates the high sensitivity of the given reaction. The sensitivity of the method according to Sandell is 7.4×10^{-4} μg cm^{-2}. The relative standard deviation (6 determinations with 5 μg of Mo(VI), 95% confidence level) is ± 1.4 % .

Apparatus

Spectrophotometer UV-VIS with 1 cm quarts cuvette, Germany.

Atomic absorption spectrometer, Germany.

Procedure

A wet burning of the plant samples was carried out and a mixture of sulfuric and nitric acids was used for 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. sulphuric acid, 2 ml conc. perchloric acid and 10 ml conc. nitric acid were added. The flask was heated only slightly 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 (Vazhenin, 1974). After cooling, the solution was diluted with water and filtered, transferred into a volumetric flask of 50 ml and diluted up to the mark with distilled water.

For determination of manganese in a separatory funnel of 100 ml, the following solutions were introduced: 1.8 ml of Toluidine Blue 0.5×10^{-3} mol l^{-1}, 0.5 ml of perchloric acid 9 mol l^{-1}, aliquote of the prepared solution of plant sample. It 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 1 min. The organic phase was filtered through a dry paper into a 1 cm cuvette and the absorbance measured at 290 nm. A blank was run in parallel in the absence of a plant sample. A calibration graph was constructed with standards similarly treated.

For determination of molybdenum in a separatory funnel of 100 ml, the following solutions were introduced: 1.2 ml of 1×10^{-4} M Tetrazolium Violet, 0.2 ml of 1×10^{-2} M 3,4,5-trihydroxybenzoic acid (R), aliquote of the prepared solution of plant sample. Dilute to a volume of 10 ml with distilled water and extract with 3 ml of 1,2-dichloroethane for 30 s. The organic layer was then transferred through filter paper into a 1-cm cell and measured at 230 nm. A blank solution containing all reagents except molybdenum was prepared and treated in the same way.

RESULTS AND DISCUSSION

This is the first time a research has been carried out including 12 vegetable species of the families Fabaceae, Malvaceae, Brassicaceae, and Cruciferae regarding content of manganese and molybdenum, two significant microelements affecting the biological activity of plants. All of the plants were fertilized and cultivated in the same soil-climatic conditions. The data was collected using one cultivar per species. The studies of changes occurring in the content of the microelements manganese and molybdenum are of particular interest.

Content of manganese and molybdenum in the plants of families *Fabaceae* and *Malvaceae*

The results, from the comparative analysis (Mokreva, 1988; Mokreva, 1997) for appraising the reliability of the differences between the middle values of concentrations of manganese and molybdenum obtained for a family FABACEAE, are stated in Table 1. The content of manganese and molybdenum is different for every member of this family; the differences between the middle values are statistically reliable.

Table 1. A comparative appraisal between the middle values of concentrations of Mn and Mo in a family FABACEAE.

Species	Mn mg kg^{-1} TB method	Reliab. P = 99%	Mn mg kg^{-1} AAS	Mo mg kg^{-1} TV method	Reliab. P= 99%	Mo mg kg^{-1} AAS
FABACEAE						
Green beans (*Phaseolus vulgaris L.*)	38.40	a	38.00	8.50	b	8.23
Broad beans (*Vicia faba L.*)	28.75	b	28.93	22.25	d	22.00
Lentils (*Lens esculenta Moench)*	120.83	c	120.75	15.40	a	15.67

a, b, c, …. - a degree of reliability

The highest variance in the manganese content was found in the crop products of the *Fabaceae* species from 28.93 mg kg^{-1} Mn dry matter in Broad beans to 120.83 mg kg^{-1} Mn dry matter in lentils, i.e. the difference is four times. The difference is similar between the highest and lowest value of molybdenum which is 8.23 mg kg^{-1} dry matter in Green beans and respectively 22.00 mg kg^{-1} dry matter in Broad beans. The data shows that the tested crops of this family need different amounts of these microelements.

The data for the content of tested microelements in every crop in the vegetable production shows that the content of manganese in lentils is 4 times higher than that in broad beans, and, compared to green beans, is 3 times higher.

The comparative analysis shows that the high content of one of the elements does not correlate to high content of the other element. It creates the impression that in the species of this family the content of molybdenum is considerably lower than that of manganese. In lentils, the molybdenum content is 8 times lower than that of manganese, and in Green beans it is 4.5 times (Fig. 1). The ratio between the two microelements is in good proximity in Broad beans - Mn : Mo = 1: 0.75.

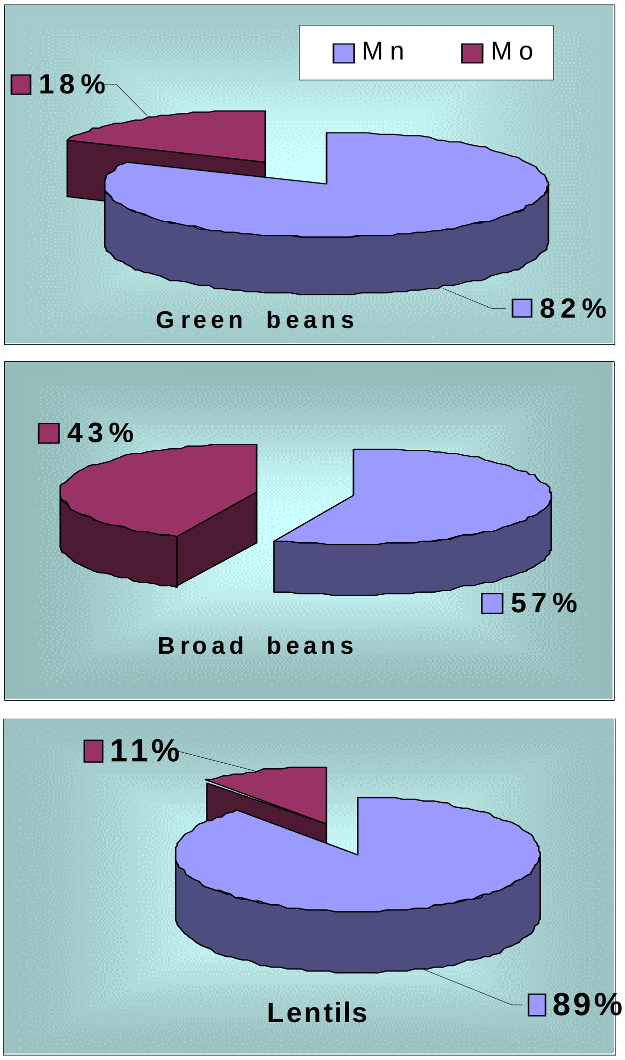

Fig. 1. Ratio between the content of manganese and molybdenum in crops of family *Fabaceae.*

The results show not only that manganese and molybdenum are indispensable to vegetable plants, but that they also have a practical purpose. Lentils have a markedly different requirement for manganese;, a correction of pH- value to more acid reaction can be recommended. It has been determined that in soil acidity in the limits of pH – value 5.8 –7.0, the non-assimilated Mn(IV) by plants reduces to the assimilated Mn(II). The correction of the pH-value can be achieved with a physiological acid nitrogen fertilizer (ammonium sulphate). In this way, the requirement for nitrogen, a basic element for plant nutrition, can be satisfied.A relatively strong correlation (-0.71) is obtained between the concentrations of manganese and molybdenum in the FABACEAE family and Okra MALVACEAE family. It can be seen from Fig. 2 that as one of the elements increases, the concentration of the other decreases.

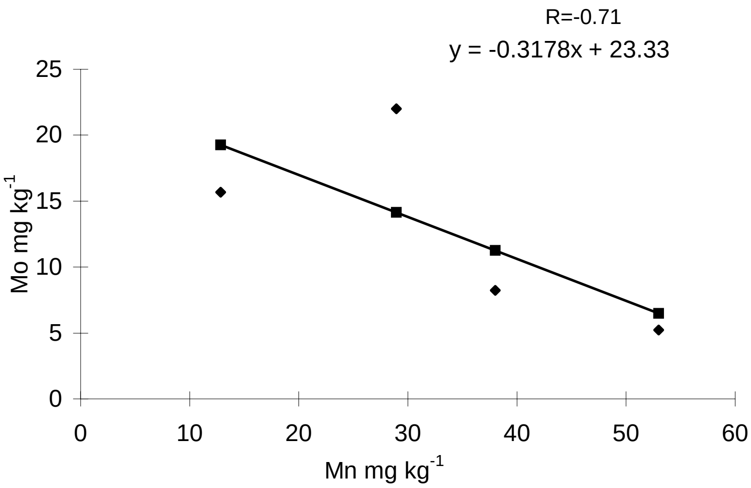

Fig. 2. A correlation between the concentrations of Mn and Mo in a family FABACEAE and Okra family MALVACEAE; correlation coefficient.
R = -0.71.

Okra, from family *Malvaceae,* was included in the experiment. The analysis shows that this crop absorbs a great amount of manganese (Table 2). There is a significant difference between the two microelements: molybdenum content is 10 times lower than that of manganese.

Table 2. A comparative appraisal between the middle values of concentrations of Mn and Mo in Okra family MALVACEAE.

Species	Mn mg kg^{-1} TB method	Reliab. P = 99%	Mn mg kg^{-1} AAS	Mo mg kg^{-1} TV method	Reliab. P = 99%	Mo mg kg^{-1} AAS
MALVACEAE						
Okra (*Hibiscus esculentus L.*)	52.70	c	53.00	5.23	d	5.20

a, b, c,… - a degree of reliability

Content of manganese and molybdenum in the plants of family *Brassicaceae*

Table 3 shows the results for manganese and molybdenum content in species of the *Brassicaceae* family, indicating that broccoli has the highest concentrations of manganese and molybdenum. A similar subordination was found in vegetable marrow.

The content of manganese in the broccoli is 75.97 mg kg^{-1} dry matter and 29.53 mg kg^{-1} dry matter molybdenum. The lowest content 5.50 mg kg^{-1} Mn dry matter was found in Brussels sprouts, and for Mo in White cabbage 7.00 mg kg^{-1} dry matter.

The cabbage crops exhibit a strong need for the nutrient medium, especially nitrogen. Accordingly, the use of physiological acid fertilizers such as such as $(NH_4)_2SO_4$ is recommended to avoid an increase of pH and to decrease the risk of oxidation of Mn(II) to Mn (IV) that plants cannot absorb.

Table 3. A comparative appraisal between the middle values of concentrations of Mn and Mo in family BRASSICACEAE.

Species	Mn mg kg^{-1} TB method	Reliab. P = 99 %	Mn mg kg^{-1} AAS	Mo mg kg^{-1} TV method	Reliab. P = 99%	Mo mg kg^{-1} AAS
BRASSICACEAE						
White cabbage (*Brassica oleracea L.var.capitata*)	22.20	a	22.07	7.15	c	7.00
Cauliflower (*Br. oleracea var. botrylis L.*)	46.70	d	46.50	9.35	b	9.53
Savoy (*Br. oleracea var. sabauda L.*)	5.75	c	5.97	14.25	f	14.07
Brussels sprouts (*Br. olerasea var. gemmifera)*	5.50	d	5.60	17.35	d	17.60
French turnip (*Br. olerasea var. gongilodes)*	14.55	g	14.77	13.25	f	13.00
Kohlrabi	27.15	a	27.00	23.30	d	23.47
Broccoli	75.80	c	75.97	29.53	b	29.50

a, b, c,…- a degree of reliability

The comparative analysis for evaluation of the reliability of the differences between the mean values of the concentrations of manganese and molybdenum obtained for the *Brassicaceae* family is stated in Table 3. The content of manganese and molybdenum in every species of this family is different; the differences between the mean values are statistically reliable.

Compared with White cabbage, a basic crop in this family, Brussels sprouts absorbs 4 times less maanganese, Savoy - 3 times less, and Cauliflower - 2 times more than White cabbage.

The ratio of the absorbed molybdenum differs: White cabbage has the lowest content of this microelement. Compared with White cabbage, the molybdenum content in the other crops of this family is 4.2 times higher in Broccoli; 3.4 times in Kohlrabi; 2.5 times in Brussels sprouts; 2 times in Savoy and 1.4 times in Cauliflower.

Thus, the caried-out research for the content of microelements manganese and molybdenum in the vegetable crops of family *Brassicaceae* shows the great differences in their absoption, and in the ratio of the absorbed microelements manganese and molybdenum (Fig. 3).

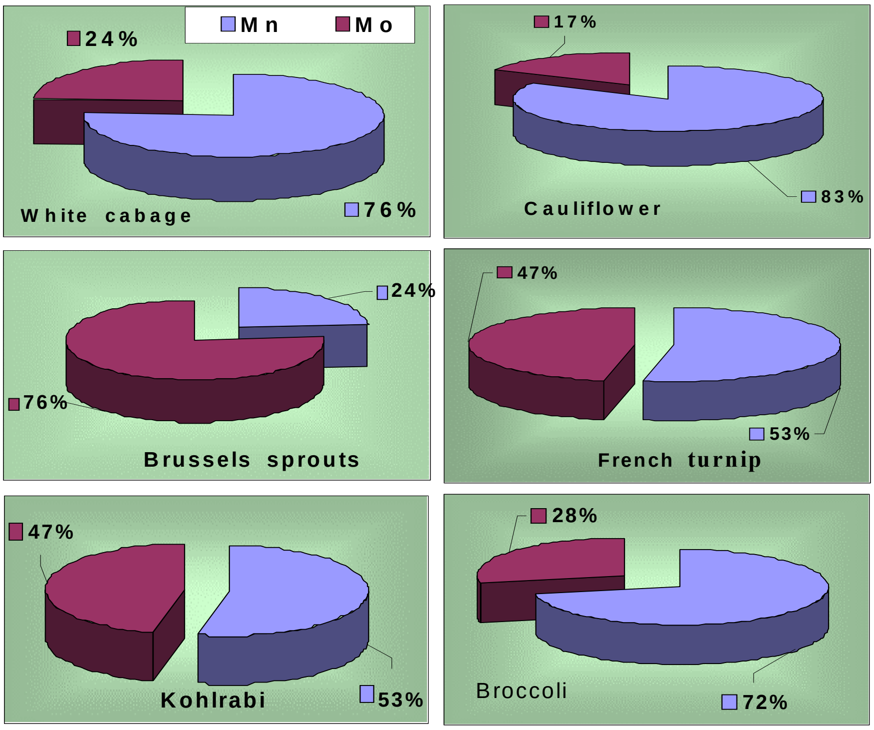

Fig. 3. Ratio between the content of manganese and molybdenum in crops of family *Brassicaceae.*

Cauliflower absorbs manganese and molybdenum in a ratio of Mn : Mo = 1: 0.2; White cabbage – 1: 0.32; Broccoli – 1: 0.39; French turnip and Kohlrabi – 1: 0.89; Savoy – 1: 2.33 and Brussels sprouts – 1: 3.17.

The obtained ratios between the microelements manganese and molybdenum have to be taken into consideration in feeding the crops with micromanures, for avoidance of symptoms of scarcity or excess, that will increase the risk of obtaining less yield and production with poor quality.

The correlation between the concentrations of manganese and molybdenum in the BRASSICACEAE family are stated in Fig. 4. A weak positive correlation (0.46) determines the concentration of the elements. With increasing concentration of one of the elements, the concentration of the other also weakly increases.

The differences found in the microelements manganese and molybdenum content of different species are probably due to the biological peculiarities in its assimilation and accumulation in products.

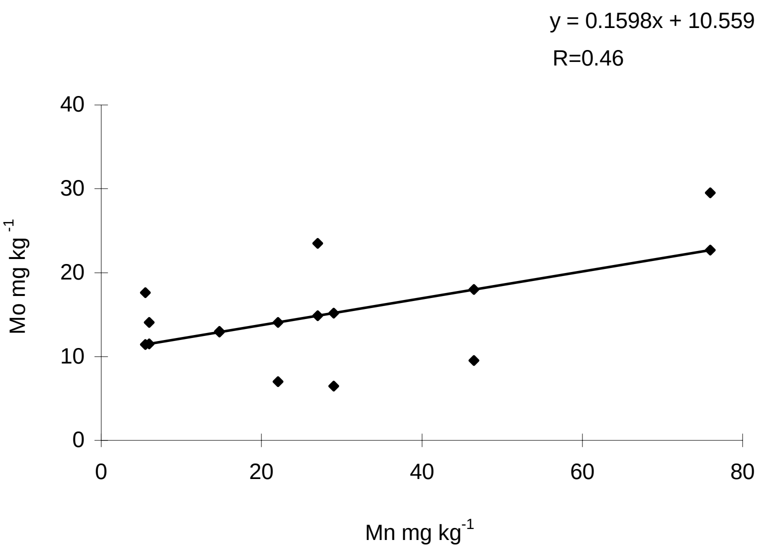

Fig. 4. A correlation between the concentrations of Mn and Mo in family BRASSICACEAE; correlation coefficient. R = 0.46.

Content of manganese and molybdenum in the plants of family *Cruciferae*

Radishes have been incorporated into this experiment with crops of family *Cruciferae*. The analysis shows that they need a significant amount of manganese (Table 4).

Table 4. A comparative appraisal between the middle values of concentrations of Mn and Mo in Radishes family CRUCIFERAE.

Species	Mn mg kg^{-1} TB method	Reliab. P = 99%	Mn mg kg^{-1} AAS	Mo mg kg^{-1} TV method	Reliab. P = 99%	Mo mg kg^{-1} AAS
CRUCIFERAE						
Radishes (*Raphanus sativus var. minor D.C.*)	29.30	b	29.00	6.40	d	6.50

a, b, c,…- a degree of reliability

The ratio between the two microelements in the pulp is Mn : Mo = 1: 0.22 (Fig. 5). If we take into account the fact that radishes differ in their fast rate of growing and poorly developed root system, this data can be used for developing of the programme of fertilization.

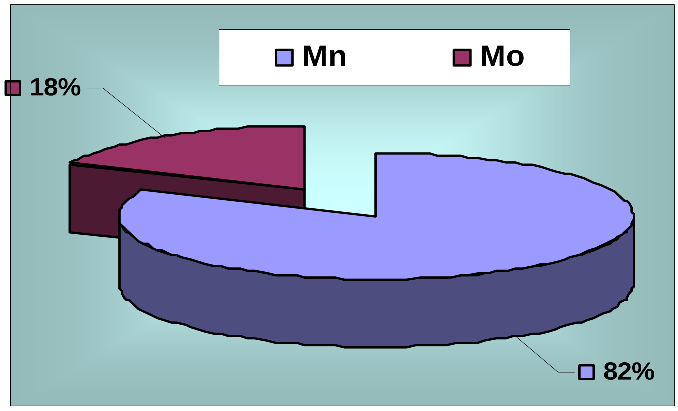

Fig. 5. Ratio between the content of manganese and molybdenum absorbed by Radishes.

To check the methods that we propose, a parallel determination of manganese and molybdenum content was carried out by the atomic-absorption method (AAS). The results obtained show a satisfactory accuracy of the method for determination of manganese in plant material with Toluidine Blue and for molybdenum with Tetrazolium Violet. The experimental data from both methods show that the proposed extraction-photometric methods with Toluidine Blue and Tetrazolium Violet can be successfully used for determination of microquantities of manganese and molybdenum in plant material.

The manganese and molybdenum content in the more widely used 12 vegetable species were studied in order to establish the kinds of differences in respect to the level of these elements in their production.

Under the influence of nitrogen fertilization (400 mg N / 1000 g soil), different plant species, grown under the same soil-climatic conditions, accumulate considerably different quantities of manganese and molybdenum from the nutrient medium. The formation of plant mass depends on the manganese and molybdenum supply.

The carried-out research for the content of microelements manganese and molybdenum in the basic vegetable crops shows the great differences in their absoption. The content of manganese is higher in Lentils, and lowest in Savoy and Brussels sprouts; the difference between the highest and lowest value of manganese is 24 times.

In comparison with manganese, the difference in the content of molybdenum in the tested vegetable crops is less. The content of molybdenum is highest in Broccoli and lowest in Okra. The difference between them is 6 times.

The differences in the ratio between manganese and molybdenum between the tested crops are great, from 1:3.17 in Brussels sprouts to 1: 0.75 in Broad beans.

CONCLUSIONS

A new method for determination of manganese with Toluidine Blue and for molybdenum with Tetrazolium Violet in plant material was used. The method is characterized by high selectivity and sensitivity.

Under the influence of nitrogen fertilization (400 mg N / 1000 g soil), different plant species, grown under the same soil-climatic conditions, accumulate different quantities of manganese and molybdenum from the nutrient medium, resulting in considerably varying content. The content of manganese is highest in lentils, and lowest in Savoy and Brussels sprouts. The difference between the highest and lowest value of manganese is 24 times.

In comparison with manganese, the difference in molybdenum content in the tested vegetable crops is less, highest in Broccoli and lowest in Okra. The differece between them is 6 times.

The differeces in the ratio between manganese and molybdenum between the tested crops are great. The ratio is from 1: 3.17 in Brussels sprouts to 1: 0.75 in Broad beans.

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