Agronomy Research **4**(2), 493–498, 2006

Determination of manganese with Methylene Blue in various vegetable crops

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Abstract. A study was made of the manganese content in the crop production of 28 vegetable species, using the most common cultivars within the species. The manganese content was determined by a new extraction – spectrophotometric method using Methylene Blue (MB). The ion – associate of Mn(VII) was completely removed in a single extraction of only 5 s, and the 1,2-dichloroethane layer remained constant for 2 days under the usual laboratory conditions. To compare results, manganese was determined by the atomic – absorption method. The results obtained showed that the manganese content varies significantly in the different vegetable species and cultivars. The manganese content in more widely used varieties of tomatoes, peppers, head cabbage, melons, and radishes was studied in order to establish differences with respect to the level of this element in their production.

Key words: manganese determination, extraction – spectrophotometry, plants, *Solanaceae, Cucurbitaceae, Fabaceae, Brassicaceae, Alliaceae, Apiacea, Lamiacea*

INTRODUCTION

In the conditions of the intensive chemization in agriculture, the effect of fertilization on the soil-plant system becomes an object of a careful study. The studies of the setting in changes in the content of the microelements are of a particular interest.

Manganese is one of the microelements which are actively absorbed by plants (Jiang, 1999; Mutaftchiev, 2001; Jiang, 2002; Wei, 2002; Mutaftchiev, 2003a, b; Noroozifar & Khorasani-Motlagh, 2004) and have a significant effect on the formation of plant mass. Manganese is involved in a number of important physiological and biological processes (Vlasjuk, 1970; Udintseva et al., 1981; Sidorovich, et al., 1987) and is important for the synthesis of the organic substance in plants and the metabolism of a number of nutrient elements in a plant organism. Manganese insufficiency causes a decrease in Ca(II) and Mg(II) levels in plants and disease in some (Epstein, 1961; Shkolnik, 1974).

The optimal content of manganese, its critical level and toxic concentration, at which the growth is depressed and the yield decreased, have been established for a great number of crops (Potatueva & Targimanyan, 1990).

The objective of this study is to determine the manganese content using a new extraction – spectrophotometric method with Methylene Blue (Beck, Kostova, Zhang, *no published*) in a greater number of vegetable crops. It should be noted how the content of manganese changes in different species and cultivars.

MATERIALS AND METHODS

The manganese content was studied in 28 vegetable species of the different families (Table 1).The crops were grown by the technology adopted for the region and systemof planting, the number of plants, optimal mineral and organic 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, a humus content of 2.2% (by Tjurin) and a slightly acid to neutral soil reaction (pH 6.8 – 7.0).

The experiments were set by the block method in four replications. Nitrogen was twice introduced with ammonium nitrate as feeding during crop growth, 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.

Methylene Blue (MB) as a cation forms an ion – association complex of manganese (VII) (Beck, Kostova, Zhang, *no published*). The ion – associate of Mn(VII) was completely removed in a single extraction of only 5 s, and the 1,2-dichloroethane layer remained constant for 2 days under the usual laboratory conditions. The absorption maximum of the associate was approximately 245 nm.

Apparatus. Atomic absorption spectrometer "Perkin – Elmer" - 3030, Germany. A Carl Zeiss VSU 2 – P spectrophotometer (Jena, Germany) was used with 1-cm light path quartz cells measured at 245 nm.

Reagents. Stock manganese (VII) solution. A $2x10^{-2}$ M aqueous solution was prepared by dissolving 0.79 g of $KMnO_4$ in 250 ml of distilled water. After 8 days the exact concentration of manganese(VII) was checked by oxalate titration. A working solution $1.8x10^{-3}$ M Mn(VII) was prepared by dilution.

A $1x10^{-2}$ M stock solution of Methylene Blue (MB) Fluka was prepared by dissolving 0.373 g of MB in distilled water and diluting it to 100 ml. Other MB concentrations were prepared by appropriate dilution.

Hydrochloric acid at concentration of 1.2 M and 1.2-Dichloroethane were used.

Procedure. A wet burning of the plant sample was carried out and a mixture of sulphuric and nitric acids was 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 4ml distilled water; 5ml of conc. sulphuric 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. If the oxidation of the organic substance wasn't complete, additional HNO_3 was added and the solution was heated again. When all the organic material was oxidized, the solution was heated at a higher temperature for 10min. (Vazhenin, 1974). After cooling, the solution was diluted with water and filtered. Portions of 3 ml conc. sulfuric acid, 2 ml conc. phosphoric acid and 0.02 g potassium periodate were added. It was heated to a boiling point and the temperature was maintained for 10 min, then cooled. It was transferred into a volumetric flask of 50 ml and diluted up to the mark with distilled water. Aliquot parts of this solution were taken for analysis.

The following solutions were introduced in a separate 100ml funnel: 4 ml of 1.2 M HCl, 1 ml of $1x10^{-3}$ M Methylene Blue, and an aliquot part of the plant sample solution, 2 ml each of saturated solutions of ascorbic acid and tartaric acid (to mask the

interfering ions). It was diluted up to a volume of the aqueous phase of 12 ml with distilled water and extracted with 3 ml dichloroethane for 5 s. The organic layer was then transferred through paper filter into a 1cm cuvette and photometered on spectrophotometer VSU 2-P at 245 nm against the pure solvent. In the absence of a plant sample, a blank was run in parallel. A calibration graph was constructed with similarly treated standards (Beck, Kostova, Zhang, *not published*).

RESULTS AND DISCUSSION

The method using Methylene Blue was applied to the determination of manganese in various plant samples. Manganese content was studied in 28 vegetable species of the following families: *Solanaceae, Cucurbitaceae, Fabaceae, Brassicaceae, Alliaceae, Apiacea, Lamiacea.* The manganese content in more widely used varieties of tomatoes, peppers, radish, melons and head cabbage was studied in order to establish differences in respect to the level of this element in their production.

The results obtained showed that manganese content in the production of the various vegetable crops grown under the same soil-climatic conditions varied considerably (Table 1). The highest concentrations of manganese were found in the leaf parsley (family *Apiaceae)*, viz. 239.50 Mn $mgkg^{-1}$ dry mass, in eggplants (family *Solanaceae)*, viz. 171.00 Mn $mgkg^{-1}$ dry mass, in lentils (family *Fabaceae)*, viz. 119.10 Mn $mgkg^{-1}$ dry mass, in dill (family *Apiaceae)*, viz. 115.50 Mn $mgkg^{-1}$ dry mass, and in savory (family *Lamiaceae)*, viz. 97.00 Mn $mgkg^{-1}$ dry mass.

Some species also had high manganese content: parsley 86.60 $mgkg^{-1}$, broccoli 75.50 $mgkg^{-1}$, onions 75.80 $mgkg^{-1}$, vegetable marrows 66.00 $mgkg^{-1}$, parsley (rhizome) 52.50 $mgkg^{-1}$, okra 52.20 $mgkg^{-1}$ dry mass. Legumes of the family *Fabaceae* were found to have manganese content from 30.00 (broad beans) to 52.20 (okra) $mgkg^{-1}$ Mn dry mass except for lentils, which were estimated to have a higher content: 119.10 $mgkg^{-1}$ dry mass. With the exception of eggplant, where manganese content was high – 171.00 $mgkg^{-1}$ dry mass, it was relatively lower – 7.30 (potatoes) to 17.50 (peppers) $mgkg^{-1}$ dry mass in other species in the family *Solanaceae.* The *Cucurbitaceae* family species also exhibited different manganese content: 9.00 (pumpkins) to 66.00 (vegetable marrows) $mgkg^{-1}$ Mn dry mass.

It could be pointed out that in leaf and root vegetable crops (family *Apiaceae*) the content of manganese was much higher than in fruit (families *Solanacea, Cucurbitaceae, Fabaceae, Brassicaceae, Alliaceae, Lamiaceae*).

The content of manganese also varied in the different varieties widely grown in the country (Table 2). In various sorts of tomatoes it was 8.00 (variety Topaz) to 21.00 (variety Jana) $mgkg^{-1}$ Mn dry mass; in a variety of peppers it was 5.60 (variety Sivria 66) to 22.00 (variety Zlaten medal) $mgkg^{-1}$ Mn dry mass; in a variety of onions 9.60 (variety Ispanski 482) to 408.00 (variety Assenovgradski 5) $mgkg^{-1}$ Mn dry mass; in a variety of white cabbage 15.70 (variety Atleta F_1) to 25.20 (variety Monter F_1) $mgkg^{-1}$ Mn dry mass; in a variety of radishes 41.10 (variety Red with white tails) to 664.20 (variety Saxa, red rhizomes) $mgkg^{-1}$ Mn dry mass.

These results showed that the manganese content depended not only on the biologic features of the species, but on the particular variety as well. The least manganese content variation was found in white cabbage, viz. 15.70 to 25.20 $mgkg^{-1}$ Mn dry mass; the highest, in radishes, viz. 41.10 to 664.20 $mgkg^{-1}$ Mn dry mass.

Table 1. Manganese content in the production of different vegetable crops.

Species	Mn $mgkg^{-1}$ in dry matter		RSD, %* (n = 3)
	Methylene Blue method	Atomic absorption	
SOLANACEAE			
Tomatoes *(Lycopersicom esculentium Mill)*	12.50	12.90	0.9
Peppers *(Capsicum annuum L.)*	17.50	17.70	1.3
Eggplant *(Solanum melongena L.)*	171.00	171.25	1.1
Potatoes *(Solanum tuberosum L.)*	7.30	7.00	0.7
CUCURBITACEAE			
Vegetable marrow *(Cuc. peposer. var. giromontia L.)*	66.00	66.30	1.2
Water melons *(Citrulus edulis Pang)*	27.50	28.00	1.4
Melons *(Cucumis melo L.)*	25.50	25.10	0.8
Pumpkins *(Cucurbita ssp.)*	9.00	8.70	0.8
FABACEAE			
Green beans *(Phaseolus vulgaris L.)*	38.50	38.65	1.1
Broad beans *(Vicia faba L.)*	30.00	29.70	1.4
Okra *(Hibiscus esculentus L.)*	52.20	52.45	0.8
Lentils (*Lens esculenta Moench)*	119.10	119.40	1.2
BRASSICACEAE			
White cabbage *(Brassica oleracea L. var. capitata)*	21.50	21.10	1.2
Cauliflower *(Br. oleracea var. botrylis L.)*	45.15	45.50	1.4
Savoy *(Br. oleracea var. sabauda L.)*	4.80	5.00	0.6
Brussels sprouts *(Br. oleracea var. gemmifera D.C.)*	6.30	6.50	0.9
French turnip *(Br. oleracea var. gongilodes L.)*	15.60	15.80	1.2
Radishes *(Raphanus sativus var. minor D.C.)*	28.40	28.15	1.0
Kohlrabi	27.00	26.60	0.8
Broccoli	75.50	75.25	0.9
ALLIACEAE			
Onions *(Allium cepa L.)*	75.80	75.40	0.7
Green onions – stems	5.20	4.85	1.1
Green onions – leaves	15.30	15.00	1.3
APIACEA			
Parsley (leafy) *(Petroseliumhortence Hoffm.)*	86.60	86.90	0.8
Parsley - rhizomes	52.50	53.00	0.9
Parsley - leaves	239.50	239.90	1.3
Parsnip *(Pastinaca sativa L.)*	37.75	38.00	1.2
Dill *(Anethum graveolens L.)*	115.50	115.15	1.0
LAMIACEA			
Savory *(Satureia hortensis L.)*	97.00	96.80	1.3
Coriander	40.00	39.60	0.7

*Relative Standard Deviation for Methylene Blue method.

The established differences could hardly be explained through differing vegetative and reproductive behavior of the various species (varieties) of the vegetable crops. Different plant species, grown under the same conditions, accumulated different quantities of manganese from the nutrient medium, depending upon the biological features of the species. An analysis of the manganese content by plant sample was carried out using a new extraction-spectrophotometric method with Methylene Blue. Pre-isolation of manganese from most other ions was not necessary.

Table 2. Varietal differences in the manganese content in crops.

Species/varieties	Mn $mgkg^{-1}$ in dry matter		RSD, %* (n=3)
	Methylene Blue method	Atomic absorption	
TOMATOES			
Topaz	8.00	8.20	1.1
Miliana	11.50	11.25	0.8
Slava	14.00	14.15	1.3
Jana	21.00	21.35	1.2
PEPPERS			
Sivria 66	5.60	5.25	0.7
Kalinkov 805	17.70	17.40	1.4
Zlaten medal	22.00	21.70	1.0
ONIONS			
Ispanski 482	9.60	10.00	0.9
Jubilee 50	14.25	14.50	1.1
Trimontium	14.00	14.15	1.5
Konkurent	16.20	16.10	1.2
Plovdivski 10	25.00	25.20	1.3
Liaskovski 58	48.00	47.70	0.8
Assenovgradski 5	408.00	407.85	1.3
WHITE CABBAGE			
Atleta F_1	15.70	15.95	1.3
Ditmarsko	22.00	22.25	0.9
Prospera F_1	22.80	22.40	1.4
Monter F_1	25.20	25.30	1.2
RADISHES			
Red with white tails	41.10	41.00	0.8
Bisser (with white rhizomes)	312.00	312.40	0.9
Saxa (red rhizomes)	664.20	664.70	1.2

*Relative Standard Deviation for Methylene Blue method.

The accuracy of the method was checked by an atomic absorption method. The results obtained by the two methods were largely in agreement. The method using Methylene Blue could successfully be used for the determination of manganese traces in plant material. The reproducibility of the method (tested with 3 samples and with a 95 % confidence interval using Student's test) was evaluated. The relative standard deviation of the method was $S_r = 1.2\%$.

CONCLUSIONS

The experiment was conducted using Methylene Blue, a new extraction – spectrophotometric method for the determination of the concentration of manganese in various vegetable crops.

The manganese content in the production of various vegetable crops grown under the same soil-climatic conditions varied considerably. Significant differences in the content of manganese were observed in the production of the different varieties, especially in the species with a high concentration of manganese, namely onions and radishes. The smallest differences were found in white cabbage varieties.

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