Effect of Mo, Se, Zn, and Cr Treatments on the Yield, Element Concentration, and Carotenoid Content of Carrot

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A study was undertaken to evaluate the uptake of Mo, Se, Zn, and Cr in soil–plant systems and to investigate the effect on the yield and carotenoid composition of carrot. Despite the beneficial effects of Se and Cr to plant growth, at low concentration they were extremely phytotoxic when presented at relatively high concentration in the soils. In the case of Mo and Zn treatments, carrot plants showed good adaptation to high levels of these elements. Although Mo treatments had no phytotoxicity on the plants, the highest dose resulted in a significant decrease in the carotenoid content of the roots. Amending the soil with Se and Zn stimulated an increase of α-carotene and lutein synthesis and a decrease of β-carotene with slight increase in the total carotenoid content.

Keywords: *Metal elements; micronutrients; carrot; carotenoids*

INTRODUCTION

Trace metals are that group of elements that are of environmental importance, usually occurring in minute quantities, and whose presence in the soil–plant system is primarily via human activities. The content of available harmful elements in soil is increased by some orders of magnitude in urban–industrial areas or through addition to the soils of sewage sludges, swine manure, and some pesticides with high metal content (Adriano, 1986).

Composition and quality of the soil may effect qualitative changes in the plants growing on these soils and in the humans and animals eating the plants. According to literature data (Purves, 1985; Fergusson, 1991), the concentration of some heavy metals increased by orders of magnitude in the blood, urine, hair, and other tissues of urban populations. Moreover, soil contamination by toxic/harmful metals represents one form of chemical burden on the environment, and it exerts numerous sanitary, economical, and ecological aftereffects.

A spinoff area of toxicity research is plant adaptation to high levels of metals in soils. Depending on types and genotypes, plants show different responses to heavy and toxic metals (Brown et al., 1961; Adriano, 1986). Growth, yield, and sometimes chemical composition of the edible part can be taken as indices of plant adaptation or response to metal contamination in the soils.

It is well established that micronutrients interact with organic compounds in metabolism as metal–ligand complexes. Interference of micronutrients with metabolic pathways of a living plant can reduce the biosynthesis of important dietary nutrients in plants. Of the vital nutrients in human diet, carotenoids have aroused greater interest lately most likely due to their provitamin A activity and role in prevention of cancer (Moon and Micozzi, 1988).

In accordance with an earlier investigations (Kádár, 1992), mainly tuber and root corps, leafy vegetables, and fodder plants are threatened by the contaminant originating from traffic, industry, and heavily polluted urban areas.

The objective of this work is to investigate the response of the carrot to high levels of Mo, Se, and Zn as well as to study the changes in carotenoid composition of the roots as a function of high element load in the soils.

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Table 1. Treatments in the Field Experiment, Calcareous Chernozem, Nagyhörcsök Research Station, 1991

	amt applied in spring 1991				
element	0 kg/ha	1 kg/ha	2 kg/ha	3 kg/ha	chemicals used
Mo	0	90	270	810	$(NH_4)_6Mo_7O_{24}$
Se	0	90	270	810	Na_2SeO_3
Zn	0	90	270	810	$ZnSO_4$
Cr	0	90	270	810	K_2CrO_4

MATERIALS AND METHODS

The soil of the experimential station (Nagyhörcsök, Hungary) is a calcareous loamy chernozem with about 25% clay, developed on loess. In its plowed layer it contains humus and $CaCO_3$ in about 3% and 5%, respectively. To ensure a sufficient macronutrient supply in the whole experiment, 100 kg/ha N, P_2O_5, and K_2O were given yearly.

In this work we focused on four of the applied elements (Mo, Se, Zn, and Cr) because of their high accumulation in plant roots as reported by Kádár (1992). The four selected microelements were applied to the soil in 1991 at four levels (0, 90, 270, and 810 kg/ha) and the $4 \times 4 = 16$ treatments arranged in a split-plot design with 2 replications, 21 m/plot.

Carrots (cv. vörös óriás, Hermes Co., Budapest, Hungary) were planted in April 1992 at 2–3 cm deep, 36 cm between rows, and 10 cm between plants. Treatments and chemicals used in this work are summarized in Table 1. The plants were harvested between October 6 and 8, 1992, and roots from each plot were combined.

Microelement Analysis. Soil samples were taken after harvest to make composite samples consisting of 20 subsamples per plot. Twenty carrot roots were used to prepare a composite sample per plot. Plant samples were digested in Teflon bombs using concentrated HNO_3, and their total element content was determined, while soil samples were extracted by NH_4-Ac + EDTA (Lakanen and Erviö, 1971) and their available element content was measured by means of ICP

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Table 2. Effect of Treatments on the Available Element Content of Soil after Harvesting the Carrot (Milligrams per Kilogram in Soil, 1992)

	amt applied in spring 1991					
element	0 kg/ha	90 kg/ha	270 kg/ha	810 kg/ha	$LSD_{5\%}$	CV%
Mo	0	12	22	43	4.5	7.3
Se	0	7	66	81	14.0	11.0
Zn	3	29	68	213	13.5	5.5
Cr	0	2	6	30	7.0	6.0

technique using a Model JY-24 (Joben Yvon, France) ICP instrument with the following parameters: gas, argon; flow, auxiliary gas 0.4 L/min, nebulizator gas 0.4 L/min, carrier gas 12 L/min; wavelength, Cr 205.552 nm, Zn 213.856 nm, Se 196.030 nm, Mo 202.030 nm.

For quantitation of microelements in soil and plant samples standard stock solutions (Spex Co., Metuchen, NJ) were used.

Carotenoid Analysis. A 1-cm-length slice was taken from the middle of each root, cut into small pieces by a plastic knife, and mixed thoroughly to make a homogeneous sample. Five grams in triplicate were groud in a crucible mortar with quartz sand. The pigments were extracted by shaking the sample with 50 mL of acetone for 15 min. The mixture was then filtered through Rundfilter Mn 640d filter paper, and the procedure was repeated twice until pale yellow color remained on the residues. The acetone fractions were collected, and the solvent was evaporated under vacuum by using rotatory evaporator. To the remaining liquor were added 10 mL of NaCl-saturated bidistilled water and 80 mL of 1:1 diethyl ether−benzene. The mixture was mixed thoroughly in a separatory funnel. The organic layer was separated, washed twice with water, and dried on anhydrous Na_2SO_4. The solvent was then evaporated and the residues were redissolved in 2 mL of chloroform. The volume was brought to 10 mL with the HPLC eluent.

High-performance liquid chromatographic (HPLC) separation of carotenoids was performed on a Lichrosorb ODS column of 6 μm particles using 39:52:5:4 acetonitrile−2-propanol−methanol−water as the mobile phase (Biacs and Daood, 1994). Standard α-carotene, β-carotene, and lutein (Sigma, St. Louis, MO) were used for the qualitative and qantitative analysis. Identification of carotenoids in the samples was based on comparison of spectral characteristics and retention times of the unknowns with those of the standard materials. A combination of a Beckman 116 solvent delivery pump, a Beckman 165 variable-wavelength UV−visible detector, and a Shimadzu integrator was used for chromatographic analysis, while spectral characteristics of the individual peaks were studied by using a photodiode array detector (Waters 990) under the control of chromatographic software.

RESULTS AND DISCUSSION

Element Uptake and Phytotoxicity. As shown in Table 2, availablity of different elements increased as the dose of treatment was raised to 810 kg/ha. When calculated on a molar basis, availability of elements in soils appeared to be Zn > Se > Mo > Cr. Table 3 shows the content of Mo, Se, and Zn in the roots as influenced by element treatments. As a consequence of element availability in soil, the concentration of these elements increased significantly in carrot roots. Maximum levels of 99.0, 63, and 34 mg/kg were estimated for Mo, Se, and Zn, respectively, in the roots of plants given the highest dose (810 kg/ha). Except for Zn (34.4 mg/kg), which poses no problem for human and animal nutrition, the highest concentrations of the elements in the roots are high enough to make the product unsafe for human consumption.

Phytotoxicity of the elements, as evaluated on the basis of plant growth and amount of harvestable yield, is shown in Table 3. Of the applied elements Se and Cr were found to be phytotoxic for carrot plants. Response of carrot to the high level of Se and Cr was similar to that of corn and potato when cultivated under the same conditions (Kádár, 1992).

Table 3. Effect of Treatments on the Yield and Element Concentration of Carrot Root at Harvest (Oct 7, 1992)

	amt applied in spring 1991					
element	0 kg/ha	90 kg/ha	270 kg/ha	810 kg/ha	$LSD_{5\%}$	CV%
	Fresh Root Yield, Tons/ha					
Mo	11.4	15.9	14.2	13.1	4.7	11
Se	12.8	13.9	14.4	7.2	4.0	10
Zn	11.9	14.3	16.0	15.6	10	22
Cr	12.9	7.1	*a*	*a*	3.7	2.9
	Element Concentration, mg/kg of Dry Wt					
Mo	0	21	54	99	5.4	3.8
Se	0	16	33	63	7.0	4.0
Zn	18	19	23	34	7.5	10
Cr	0	0.2	*a*	*a*	0.5	47

[a] No harvestable yield.

Table 4. Effect of Element Treatments on the Carotenoid Content of Carrot Roots at Harvest

	amt applied in spring 1991					
element	0 kg/ha	90 kg/ha	270 kg/ha	810 kg/ha	$LSD_{5\%}$	CV%
	α-Carotene, mg/kg of Fresh Wt					
Mo	34.9	43.1	38.3	27.7	5.2	4.6
Se	34.7	39.4	50.6	64.5	10.8	8.4
Zn	34.3	37.8	44.8	54.5	9.5	7.0
	β-Carotene, mg/kg of Fresh Wt					
Mo	55.9	60.7	56.1	46.9	8.8	5.1
Se	54.9	75.1	66.1	60.5	10.5	5.0
Zn	54.0	56.8	79.0	71.3	4.3	2.1
	Lutein, mg/kg of Fresh Wt					
Mo	5.4	7.0	4.1	3.4	0.9	5.5
Se	5.2	6.0	6.3	10.1	1.5	8.0
Zn	5.0	5.6	6.4	9.2	1.1	5.4
	Total Carotenoids, mg/kg of Fresh Wt					
Mo	97	112	98	79	13.7	4.5
Se	95	120	123	136	12.0	3.4
Zn	94	102	132	135	10.4	2.8

Changes in the Root Yield. Different elements had different effects on the yield of carrot. Data in Table 3 indicate that no significant change occurred in the yield as a function of Mo treatments, revealing high adaption of carrot to the high concentration of this element in the soil. In the case of Zn treatment a slight increase in the yield with no phytotoxicity was recorded even at the highest dose. The detrimental effect of Cr and Se in soil was observed. A decrease of 40% in the yield was recorded when the concentration of available Cr and Se reached 2 and 81 mg/kg in the soil respectively. These results agree with those of Pais (1991), who concluded that the elements are either essential or beneficial when they exist at very low concentration in the biosphere of plants and animals but toxic when their concentration exceeds a level over which a physiological disorder is initiated in the living organisms.

Changes in the Carotenoid Content. Because of their increasing importance in human nutrition and disease prevention, carotenoids have aroused much interest. Table 4 shows the results obtained from the HPLC analysis of carotenoids in carrot samples. Percent coefficient of variation (CV%) was 2.3 for total carotenoids when five measurements were performed using a well-homogenized carrot sample. This indicates that the extraction method and HPLC determination were more than precise.

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As a result of Mo treatment at 90 kg/ha a slightly significant increase was recorded in the total carotenoid content of carrot, but the highest dose caused a marked decrease. In contrast, Se and Zn treatments significantly increased the total carotenoid content of the roots even at the highest levels. The unexpected consequence of a high concentration of Se and Zn in the soil was the conversion of carotene from β to α structure, perhaps due to the effect on activity of some isomerase or on electron transport that has been reported to be influenced by toxic concentrations of some elements (Van Assche and Clijsters, 1986). The only difference between Se and Zn in their effect on carotenoid formation was that the former promoted β to α conversion at lower level of element load in the soil (270 kg/ha). This conversion was not observed in Mo-treated plants in which both β- and α-carotenes were decreased by the highest doses.

Lutein (dihydroxy-α-carotene) tended to change, as a function of increasing element dose, in a similar pattern to that of α-carotene in all of the samples tested.

Because α-carotene has lower vitamin A activity (50%), the β to α conversion of carotene as a result of high element concentration in the soil can reduce the nutritive value of carrot.

In conclusion, high levels of Mo, Zn, and Se in soil can unfavorably affect the production and chemical composition of plant-derived foods. Harmful accumulation of microelements could be observed in carrot roots as well as in the vegetative parts. It is interesting and perhaps significant that with tolerant plants soil pollution by some microelements may increase not only carotenoid content but also carotenoid production per hectare since both yield and carotenoid content are increased. It would be interesting to perform comparative studies on tolerant and nontolerant plants to see to what degree metal tolerance is related not only to plant growth but also to differential vital micronutrient composition of human foods.

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