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EFFECTS OF Mo, Zn, Sr AND Ba LOADS ON THESE ELEMENTS' UPTAKE AND OIL CONTENT AND FATTY ACID COMPOSITION OF RAPESEED

ABSTRACT: Studied in the present paper were the long-term effects of the application of high Mo, Zn, Sr and Ba rates (0, 90, 270, and 810 kg ha^{-1}) on rapeseed oil content and oil fatty acid composition. The trace elements were applied in the spring of 1991, while the rapeseed was sown on a calcareous chernozem soil in 2001. The trace elements differed significantly in their rates of accumulation in rapeseed plants. Relative to the control, the Mo content of the stem increased up to 1,000 times, that of the chaff over 100 times, and that of the seed around 60 times. The levels of the other trace elements increased considerably less relative to the control. The increases were typically twofold to threefold, depending on the plant part involved. The trace elements accumulated the most in the vegetative plant parts, except for Zn, a major quantity of which was found in the seed as well. The application of the high rates of Sr, Zn and, to an extent, Mo reduced the seed oil content of rapeseed. However, the differences were not statistically significant. The application of the trace elements had no significant effect on the fatty acid composition of the rapeseed oil, either. The increased levels of the trace elements found in the rapeseed plants indicate that 11 years after application significant amounts of the applied elements are still present in the soil in a form available to plants. However, the rates were not high enough to affect the synthesis of oil and its fatty acid composition.

KEY WORDS: Mo, Zn, Sr, Ba — loads, rapeseed, oil content, fatty acids

INTRODUCTION

Rapeseed (*Brassica napus* L. ssp. *oleifera*) is a highly productive oil crop. Brassica species constitute the world's third most important source of vegetable oil at present. The nutritive value, oxidative stability and melting point of vegetable oils depend on their levels of certain fatty acids (Beare-Rogers, 1988; Galliard, 1980). Oil content and fatty acid composition are quantitative traits of a plant. They are most often inherited intermediately and they are controlled by minor genes. Plants' quantitative traits can be affected by numerous external factors. The environmental factor with the largest effect on fatty acid content is temperature during seed development (Beringer, 1971; Pleines and Friedt, 1988). Other factors found to have an effect on this trait include light (Trémoliéres et al., 1982), oxygen concentration in the atmosphere (Dompert, 1976), frost damage (Daun et al., 1985), and agronomic practices (May et al., 1994).

The objective of this study was to determine how high rates of Mo, Zn, Sr and Ba — trace elements having different physical-chemical properties and physiological and environmental importance — affect the oil content and fatty acid composition of rapeseed. Zn and Mo are biogenic, transition elements, and, like all heavy metals, they are toxic at higher concentrations, which means they are potential environmental pollutants (Kiekens, 1990; Jones et al., 1990). Sr and Ba are not biogenic elements, they belong in the group of alkaline earth metals and have no major environmental impact. In addition, these elements differ significantly when it comes to the intensity of their accumulation and distribution in plants (Marschner, 1995).

Knowing how excess concentrations of heavy metals affect the chemical composition of vegetable oils is particularly important not only from the academic standpoint but also from the point of view of actual agronomic practice and the production of biologically valuable food. Knowledge of the effects of environmental factors on the levels and composition of vegetable oils is also necessary for the design of informative genetic experiments as well as for the correct interpretation of the results of such experiments.

MATERIALS AND METHODS

Plant Material and Treatments

A small-plot field experiment was set up in the spring of 1991 on the loamy-textured calcareous chernozem soil formed on loess at the Nagyhőrcsők Experimental Station of the Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, Budapest, to investigate the effect of high trace element rates. The plowed layer of the growing site contained approximately 5% $CaCO_3$ and 3% humus, was satisfactorily supplied with available Ca, Mg, Md and Cu, moderately supplied with N and K, and poorly supplied with P and Zn. The groundwater was at a depth of 15 m and the area had a negative water balance tending toward drought. Salts of the 13 trace elements examined were each applied at four levels in the spring of 1991 prior to maize sowing. In 2001, the winter oilseed rape variety Doublal was sown. The trace element levels were 0, 90, 270 and 810 kg ha^{-1}. Studied in the present paper were only the effects of Mo, Zn, Sr and Ba. These elements were applied as $(NH_4)_6MO_70_{24}$, $ZnSO_4$, $SrSO_4$, and $BaCl_2$, respectively. All the plots received basic fertilization with 100 kg ha^{-1} each of N, P_2O_5 and K_2O in the form of ammonium nitrate, superphosphate and potassium chloride, respectively.

Plant Analysis

Mo, Zn, Sr and Ba levels were determined separately in the seed, stem (stem plus leaves) and chaff. After breaking down the plant materials with cc HNO_3 + cc H_2O_2, the levels were determined using the ICP technique. The total seed oil content was determined by extraction using petroleum ether according to Soxhlet. In order to analyze the fatty acid composition, the oil was extracted by a hydraulic press, after which the preparation of fatty acid methyl esters of the oil was carried out using trimethyl sulfonium hydroxide according to Butte (1983). The qualitative and quantitative compositions of the mixture of fatty acid methyl esters were determined by gas chromatography with a flame-ionizing detector (HP 5890 with FID) and a capillary column (HP--INNOWax cat. No 19091N-133). The results were statistically processed by calculating the least significant difference.

RESULTS AND DISCUSSION

Mo, Zn, Sr and Ba Contents, Accumulation and Distribution

Plants differ in their capacity for the uptake, accumulation, translocation and use of different mineral elements. Among the trace elements studied, Mo in particular has an especially high accumulation rate. The results shown in Table 1 support this. The Mo content in the stem increased over 1,000 times relative to the check treatment, in the chaff over 100 times, and in the seed about 60 times. A significant characteristic of plant nutrition with Mo is a wide variation between the critical deficiency and toxicity levels. These levels may differ by a factor of up to 10^4 (Marschner, 1995). Molybdenum contents above 5—10 mg kg^{-1} dry wt are considered critical for humans and herbivorous animals. Values obtained in the present study significantly exceeded this threshold value. The levels of the other trace elements — Zn, Sr and Ba — increased to a considerably smaller extent as a result of their application, in most cases as little as two to three times, depending on the plant part involved. The zinc content of the vegetative plant parts was low, which may have been due to the low level of available zinc in the soil. Despite the fact that the mobility of Zn in plants is not large (Mengel and Kirkby, 1987), the seed Zn content was considerably higher than the Zn content of the vegetative plant parts. Strontium is not an essential element for plants, and it is not considered to be particularly toxic, either. Still, the uptake, distribution and accumulation of Sr in plants is extremely important in the soil-plant-human system. This problem has gained weight since the Chernobyl accident in 1986. In this connection, it is important to know the effects of single factors on Sr accumulation in plants. Agroecological factors have relatively little effect on the Sr content of rapeseed and wheat (Haneklaus, 1989). This is partly supported by the results of the present study too. Sr levels increased to about the same extent relative to the control in all rapeseed plant parts under study. The measured levels were quite below the toxicity threshold for plants (500 mg kg^{-1})

and were also lower than the toxic concentration for food, especially in the seed (150 mg kg^{-1}) (Pais, 1980). Similar Sr levels in rapeseed are reported by Haneklaus (1989). Similarly to Sr, the Ba content in our study increased to aproximately the same extent relative to the check treatment in all the rapeseed plant parts under investigation. Plant Ba levels vary across a wide range and this element is considered to be more toxic to plants than Sr (Scharrer, 1955).

Table 1. Effect of Mo, Zn, Sr and Ba loads on their levels in rapeseed

Plant part	Rate (kg · ha^{-1})*				LSD 5%
	0	90	270	810	
Mo content (mg Mo · kg^{-1} DM)					
Stem	0.1	73	144	137	42
Chaff	0.5	78	164	266	48
Seed	0.5	6	13	29	3
Zn content (mg Zn · kg^{-1} DM)					
Stem	3.5	8.0	9.6	13.0	1.2
Chaff	5.0	8.2	7.3	10.5	1.4
Seed	32.0	41.0	45.0	49.0	6.0
Sr content (mg Sr · kg^{-1} DM)					
Stem	58	56	72	94	6
Chaff	88	99	124	211	10
Seed	16	22	19	32	4
Ba content (mg Ba · kg^{-1} DM)					
Stem	6.0	7.0	11.8	20.1	2.7
Chaff	6.1	7.0	9.3	19.4	2.4
Seed	1.8	2.7	3.0	3.9	1.3

* The trace elements were incorporated into the soil 11 years before.

In some studies involving other crop species that had been carried out in previous years in the same trial as our experiment, the trace elements concerned were found to have similar effects on their levels in plants. In those studies, too, there was a particularly large increase of Mo levels resulting from this element's application in the trial (Kádár and Prokisch, 2000b; Kádár et al., 2000a; Kádár et al., 2000c, Kádár 2001).

The accumulation of the trace elements involved in our study varied (Figure 1). In relation to the check, Mo accumulated the most as a result of the treatment, followed by Ba, Zn, and, lastly, Sr. In the check treatment, it was Sr that accumulated the most, followed by Zn, Ba, and Mo. Rapeseed's great capacity for Sr accumulation was also confirmed by Haneklaus (1989), who reported that the Sr content of rapeseed leaves was six times larger than that of wheat leaves.

The large accumulation of the trace elements indicates that 10 years after application the soil still contained a significant amount of them in the form available to plants. Results of the 2000 soil analysis showed that the topsoil

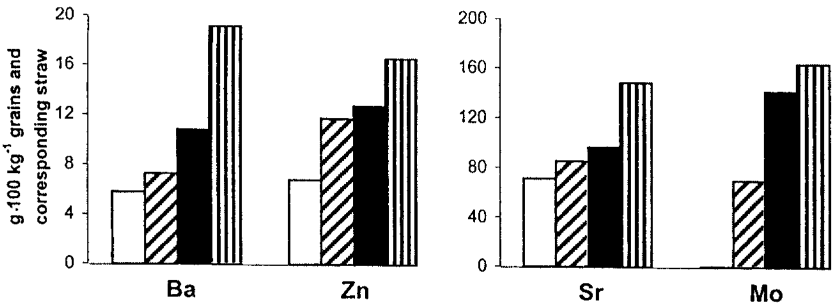

Figure 1. Accumulation of Mo, Zn, Sr and Ba in harvest unit of 100 kg grains and corresponding quantities of straw in rape. Analyses were done on plants grown on the soil loaded eleven years before with 0 (□, control), 90 (▨), 270 (■) and 810 (▥) kg of Mo, Zn, Sr and Ba per ha, respectively.

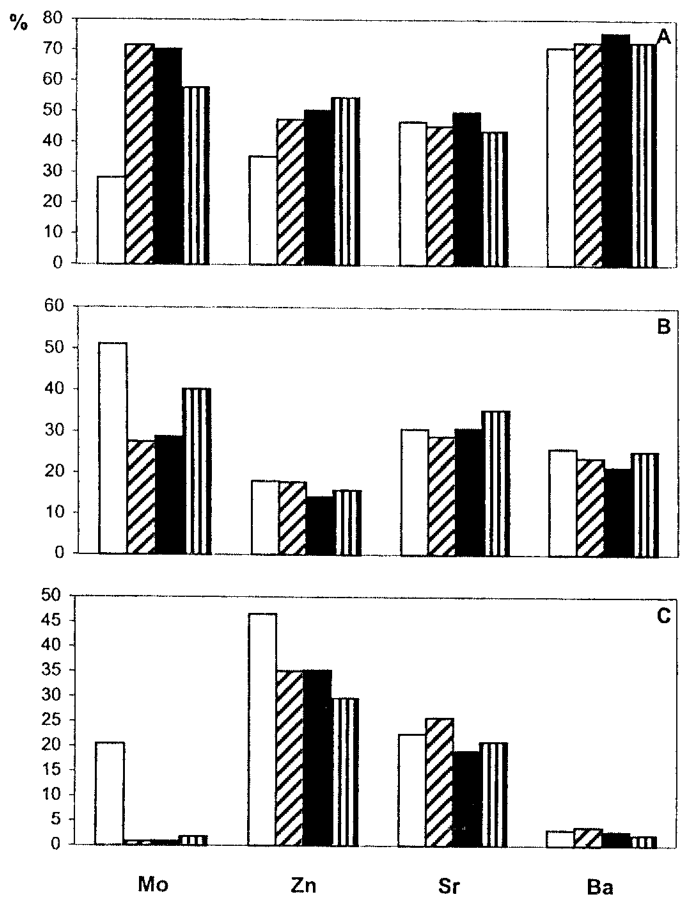

Figure 2. Distribution of Mo, Zn, Sr and Ba in stem (A), chaff (B) and seed (C) of rape (in % of total content). Analyses were done on plants grown on the soil loaded eleven years before with 0 (□, control), 90 (▨), 270 (■) and 810 (▥) kg of Mo, Zn, Sr and Ba per ha, respectively.

had 4—5% of Mo, 42—50% of Zn, 28—30% of Sr and 15—20% of Ba in a readily soluble form (extracted by NH_4-acetate + EDTA).

In rapeseed, storage lipids are synthesized in the seed tissue during sedd development. The accumulation of the trace elements and their overall distribution across the plant could therefore be of importance from the point of view of oil content and the oil's fatty acid composition (Figure 2).

The distribution of individual elements in plants is specific and dependant on numerous biotic and abiotic factors. The seed can be very rich in Mo (Rebafka, 1993). In the control treatment, around 20% of the total accumulated Mo were located in the seeds. The application of Mo led primarily to its accumulation in the stem. Of all four elements, it was zinc that accumulated the most in the seeds. Similar to what happened with Mo, the application of zinc increased the zinc level in the stem. Strontium accumulated the most in the stem, followed by chaff and, finally, the seeds. Similar distribution has been reported in wheat as well (Kastori et al., 1992; Lásztity, 1996). The application of Sr and Ba had no effect on their distribution, since their presence increased uniformly in the rapeseed parts concerned. Of the four elements, Ba accumulated the most in the stem, while only a small amount of it (only 3% of the total amount accumulated) was found in the seeds.

The uneven accumulation of the trace elements in the seed suggests that these elements potentially pose varying degrees of danger when it comes to their entry into the food chain. In this connection, a question also arises of the extent to which these trace elements actually end up in the products of rapeseed processing.

Oil content and its fatty acid composition

The oil content and its fatty acid composition both change in the course of plant growth and development and plant aging. Genetic, biotic and environmental factors play an important role in these changes. Environmental factors can affect plant lipid metabolism in several ways. They can cause changes of adaptive nature (nonhereditary variability) and stress-induced degenerative changes and affect biochemical processes that are not directly related to lipid metabolism (Nyitri, 1998). High concentrations of trace elements, especially heavy metals, may induce stress in the plant and affect plant metabolism and plant physiological processes (Kabata-Pendias and Pendias, 1984). It is therefore reasonable to expect that their excessive accumulation in the plant, especially the seeds, will cause changes in lipid metabolism and hence affect the seed oil content and its fatty acid composition.

Among the environmental factors, there has been relatively little study of the effects of mineral nutrition, particularly trace elements, on the levels and chemical composition of vegetable oils (Yermanos et al., 1964).

Most of the studies concern the effects of nitrogen, whose application most often reduces the oil content (Dybing, 1964; Yermanos et al., 1964). In Beringer (1966), the application of nitrogen reduced the oil content of oat grains to a negligible extent and practically had no effect on the oil's fatty acid composition. According to Kádár (2001), increasing nitrogen rates decreased the oil content of poppyseed, while increased phosphorus supply had no effect on this trait, although it reduced the oleic and linolenic acid contents and increased the linoleic acid level. Szirtes and Lukács (1980) reported that the foliar application of Mn, Cu and Zn had no major impact on the oil content of sunflower seeds. As for the effect of these elements on the oil's fatty acid composition, there was an increase in the linoleic acid content and a drop in the oleic acid one. In the absence of nutrient deficiencies, foliar applications of iron chelates did not affect the safflower and flax oil contents of seed and the iodine value of oil (Yermanos et al., 1964).

Table 2. Effect of Mo, Zn, Sr and Ba loads on oil content of rapeseed (%)

Element	Rate (kg · ha^{-1})*				LSD 5%
	Ø	90	270	810	
Mo	43.15	42.15	41.65	42.53	4.07
Zn	43.11	43.16	43.72	41.91	4.81
Sr	43.75	43.16	43.76	42.13	2.34
Ba	43.75	44.58	44.29	45.00	4.89

* The trace elements were incorporated into the soil 11 years before.

Table 3. Effect of Mo, Zn, Sr and Ba loads on the fatty acid composition of rapeseed oil (%)

Treatment* (kg ha^{-1})	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenic acid
	16:0	18:0	18:1	18:2	18:3	20:1
Mo Ø	3.89	2.04	65.17	17.25	10.02	1.50
Mo 90	3.81	1.99	65.99	16.76	10.12	1.44
Mo 270	3.87	2.01	66.20	17.06	9.85	1.42
Mo 810	3.86	2.01	66.05	16.92	9.80	1.46
LSD 5%	0.09	0.55	1.95	1.74	0.41	0.13
Zn Ø	3.85	2.06	66.89	16.80	9.65	1.46
Zn 90	3.93	2.03	66.25	16.79	6.46	1.43
Zn 270	3.80	2.07	67.08	16.57	9.36	1.45
Zn 810	3.89	2.07	66.87	16.56	9.17	1.45
LSD 5%	0.14	0.09	1.20	0.38	0.50	0.13
Sr Ø	3.86	2.10	66.92	17.02	9.84	1.44
Sr 90	3.96	2.14	67.35	16.37	9.40	1.42
Sr 270	4.07	2.07	66.78	16.35	9.27	1.40
Sr 810	3.93	2.06	66.15	17.04	9.31	1.42
LSD 5%	0.22	0.12	1.53	0.70	0.63	0.06
Ba Ø	3.86	2.06	66.32	17.02	9.82	1.45
Ba 90	3.90	2.06	65.29	17.35	9.53	1.42
Ba 270	4.01	2.07	66.83	16.45	9.35	1.40
Ba 810	4.04	2.06	66.65	16.42	9.41	1.41
LSD 5%	0.19	0.11	2.27	0.93	0.89	0.13

* The trace elements were incorporated into the soil 11 years before.

The application of high rates of Sr, Zn and, to an extent, Mo reduced the seed oil content of rapeseed (Table 2). However, the differences were not statistically significant. The application of these trace elements had no significant effect on the fatty acid composition of the rapeseed oil, either (Table 3). The accumulation of the trace elements in plants indicates that 10 years after application significant amounts of them are still present in the soil in the form available to plants. However, the rates were not high enough to affect plant metabolism and hence plant growth and development itself. This is supported by the fact that there were no significant differences between the yields in the trial treatments and those in the check treatment. The application of high Mo, Zn, Sr and Ba rates had no significant effect on the oil content of rapeseed and its fatty acid composition, in spite of the significant accumulation of these elements in the plant.

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УТИЦАЈ ПРИМЕНЕ ВИСОКИХ ДОЗА Mo, Zn, Sr и Ba НА ЊИХОВО УСВАЈАЊЕ, САДРЖАЈ УЉА И САСТАВ МАСНИХ КИСЕЛИНА У СЕМЕНУ УЉАНЕ РЕПИЦЕ

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Резиме

Уљана репица је високо продуктивна уљана биљка и по значају је трећа у свету. У раду је испитан утицај продуженог дејства примене високих доза Mo, Zn, Sr и Ba (0, 90, 270 и 810 kg/ha) на садржај уља и састав масних киселина у семену уљане репице. Микроелементи су примењени 1991. године. Оглед је изведен на земљишту типа чернозема, на огледном пољу Института за земљиште и агрохемију Мађарске академије наука. Примењени микроелементи су се у различитој мери накупљали у уљаној репици. Највеће је било накупљање Mo. Високе дозе Sr, Zn и донекле Mo смањиле су садржај уља у семену уљане репице. Настале разлике у односу на контролу у садржају уља и саставу масних киселина уља нису биле сигнификантне. Добијени резултати указују да 11 година после примене високих доза Mo, Zn, Sr и Ba на земљишту типа чернозема још увек значајна количина остане у приступачном облику за биљке. На то указује њихово велико накупљање у биљкама. Поред тога, није дошло до значајније промене у садржају уља и саставу масних киселина и семену уљане репице.