Status of Fe, Mn and Zn in red beet due to fertilization and environment

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Abstract

Soil is a non-renewable resource, so it should be taken care of it. High quality food with sufficient yield for a growing human population can be produced only if plant nutrients are added to the soil by fertilizers to increase soil fertility, but the fertilizers have to be used economically, with the aim of raising the nutrient content available in the soil in order to meet plants' needs. The goal of the present investigation was to determine the effect of application of organic and mineral fertilizers as well as the effect of research environment on micronutrient content in red beet root. A field trial (2003–2005) was set up in a hilly part of Croatia according to the Latin square method, with four types of fertilization (control, 50 t stable manure*ha^{-1}, 500 and 1,000 kg NPK 5-20-30*ha^{-1}). After digestion of plant material with concentrated HNO_3, iron, manganese, and zinc were determined by an atomic absorption spectrophotometer (AAS). The highest average red beet root micronutrient contents (270 mg Fe*kg^{-1} in dry matter, 96 mg Zn*kg^{-1} in dry matter, and 53 mg Mn*kg^{-1} in dry matter) were determined in the first research environment (Brašljevica in 2003) with low potassium soil content. There was a general decreasing trend in contents of the studied micronutrients (Fe, Mn and Zn) with NPK 5-20-30 fertilization (and by application of high doses of potassium), certainly due to antagonistic activity with potassium. It is suggested to fertilize with both 500 kg NPK 5-20-30*ha^{-1} and microelement fertilizers.

Keywords: *Beta vulgaris* var. *conditiva* Alef., iron, manganese, microelement, vegetable, zinc

Introduction

The understanding that soil is actually a non-renewable resource is more and more highlighted lately (Abawi and Widmer, 2000), and it has become clear that the economics of agricultural production depend heavily on the strength of concern for both soil fertility and productivity. The biggest serious threat for the survival of humanity is the constantly increasing difference between population growth and food supply (Wallace et al., 1998). The high yields that are essential for overcoming hunger and securing sufficient quantities of high quality food for a growing human population can be achieved only if plant nutrients are added to the soil by organic or mineral fertilizers that affect soil fertility (Bergmann, 1992; Petek et al., 2008; Herak Ćustić et al., 2009). In 1960's and 1970's, increased use of various types of fertilizers led to an impressive increase in yields in developing countries (Sillanpää, 1982). However, it should be kept in mind that fertilizers have to be used economically, with the aim of raising the nutrient content available in the soil in order to meet plants' needs for optimal growth and development (Bergmann, 1992; Madhava Rao et al., 2006). Also, along with combining different forms and formulations of fertilizers, balanced fertilization is greatly important for agriculture production and sustainable investing (Jordão et al., 2003). At the end of the 20^{th} century, concern about the environmental impact of fertilization increased (Davenport et al., 2005). Environmental problems can occur when excessive and unprofessional fertilization is applied and fertilizers remain unused in the soil after the harvest. Application of fertilizer without considering the amount of crop residues may cause excessive nutrients in the soil that can be leached into groundwater (Jackson et al., 2004; Hartz, 2006; Schenk, 2006). This is one more reason why it is so important to perform soil analysis before fertilization.

Many authors investigated effect of different mineral and organic fertilization on different cultures as *Cynodon dactylon* (Imoro et al., 2012), potato (Mitova et al., 2014), stevia (Xiangyang et al., 2011) and amaranth (Onyango et al., 2012).

Micronutrients

Micronutrients are essential for the growth and development of plants, animals, and humans (He et al., 2005), because they are an integral part of metalloenzymes (Horvatić et al., 1999). Mobility and availability of these elements control the number of chemical and biochemical processes such as precipitation-dissolution, adsorption-desorption, complexation-dissociation, and oxidation-reduction. Not all processes are equally important for each element, but soil pH and biological activity affect all these processes (Sillanpää, 1982; Borkert and Cox, 1999; He et al., 2005). Application of organic matter in agricultural soils shows no significant impact on the total soil micronutrient content, but increases the availability of micronutrients compared with the soil fertilized with mineral fertilizers (Moustaoui and Verloo, 1995; Tamoutsidis et al., 2002; Herencia et al., 2008). Perez-Murcia et al. (2005) mentioned that the contents of iron, copper, manganese, and zinc in plant tissue were increased by increasing application of sewage sludge. Herencia et al. (2008) stated that sequence analysis showed that the addition of organic matter to the soil favoured the oxidation fraction, except in the case of zinc, which showed the opposite trend, and Zhang et al. (2003) emphasized that copper, zinc, and manganese were mainly found in the carbonate or oxide fraction, which indicated that they had a high mobility potential.

Iron

Iron is an essential micronutrient for the growth and development of plants (López-Millán et al., 2009). Plants uptake iron as ions (Fe^{2+} and Fe^{3+}) and in the chelated form. Translocation in the plant is in the form of chelates. Mobility from old to young leaves is poor, so plants must uptake iron constantly (Bergmann, 1992). Iron plays an important role in metabolism, due to the possibility of changing its oxidation state ($Fe^{2+} \leftrightarrow Fe^{3+}$). The metabolically active form is Fe^{2+}, which is built into biomolecular compounds. It is an integral part of many active groups of enzymes (catalase, peroxidase, dehydrogenase, and hydrogenase), heme protein, cytochrome, leghaemoglobin, and non-heme proteins such as feredoxin and lipoxygenase. It is also located in the mitochondria, where it participates in electron transfer in the process of respiration (Bergmann, 1992; Madhava Rao et al., 2006). As mentioned above, iron is part of feredoxin, which is essential in the first part of photosynthesis (the conversion of light energy into chemical), where feredoxin is an electron acceptor and transporter (Belkhodja et al., 1998). Iron is essential for the synthesis of chlorophyll and reduction of nitrate and sulphate, as well as assimilation of molecular nitrogen (N_2) (Bergmann, 1992; Marschner, 1995; Madhava Rao et al., 2006). Iron deficiency reduces pigment content in the plant (chlorophyll, carotene, xanthine, and lutein) and chlorosis appears on young leaves (Madhava Rao et al., 2006), especially on alkaline soils (Rashid and Ryan, 2004; Herak Ćustić et al. 2008; López-Millán et al., 2009).

Manganese

Plants uptake manganese from the soil in the form of Mn^{2+}, which competes with Mg^{2+} and can reduce its uptake (Mukhopadhyay and Sharma, 1991). A high soil pH (Borkert and Cox, 1999) and high contents of iron, zinc, and copper in the soil (Madhava Rao et al., 2006) can also reduce Mn uptake. Although mobility and remobilisation of Mn^{2+} in plants are low, they are greater than those of calcium, boron, copper, and iron. Manganese affects photosynthesis by chloroplast and chlorophyll synthesis, as well as water dissociation in Hill's reaction in Photosystem I. The role of manganese is similar to magnesium, which can perform a non-specific replacement in many phosphorylation reactions (Bergmann, 1992; Marschner, 1995; Madhava Rao et al., 2006). Lime application with manganese positively affects the normal growth of red beet, which is sensitive to the toxicity of nickel and manganese deficiency (Siebielec and Chaney, 2006; Ronaghi and Ghasemi-Fasaei, 2008). Manganese toxicity occurs in plants that have a low silicon content or are deficient in calcium, iron, magnesium, and phosphorus (El-Jaoual and Cox, 1998).

Zinc

As with the other micronutrients, zinc is an essential trace element for all living beings (He et al., 2004). Plants uptake zinc as the Zn^{2+} ion, which can be inhibited by high contents of iron and copper in the plant. Mobility of zinc in the plant is low, but better than that of iron, boron, and molybdenum. Zinc is important in metabolism as a specific and non-specific activator of more than 300 enzymes in all six groups (Parisi and Vallee, 1969; Bergmann, 1992; McCall et al., 2000; He et al., 2004). Chizzola and Mitteregger (2005) have reported that zinc antagonisms cadmium uptake. He et al. (2004) have reported similar results, and have also highlighted that zinc has a synergistic effect on calcium and magnesium, although calcium and magnesium have an antagonistic effect on zinc.

The goal of the present investigation was to determine the effect of application of organic and mineral fertilizers as well as research environment on micronutrient content in red beet root.

Materials and methods

Field work

A field fertilization trial with beetroot (*Beta vulgaris* var. *conditiva* Alef.), cultivar 'Bikor', was carried out in Brašljevica and Hrvatsko Polje (Croatia) (Figure 1) from 2003 to 2005 (Brašljevica in 2003, B-2003; Hrvatsko Polje in 2004, HP-2004 and Hrvatsko Polje in 2005, HP-2005) using the Latin square method with four treatments (unfertilized control, 50 t stable manure*ha^{-1}, 500 and 1,000 kg NPK 5-20-30*ha^{-1}). Untreated beetroot seed was sown (22nd May 2003, 21st May 2004 and 29th June 2005) directly into soil with a plant spacing of 0.07 m x 0.4 m and a main plot area of 12 m^2. Beetroot were harvested (21st Aug 2003, 24th Aug 2004 and 28th Sep 2005) after ~90 days.

Figure 1. Map of Croatia with highlighted locations of investigation (Brašljevica and Hrvatsko Polje)

Chemical plant analysis

The edible parts of six plants from each plot at harvest were randomly selected for analyses. Samples of plant material (dried at 105 °C) were analysed in triplicate and the results presented as mean values. After digestion of plant material with concentrated HNO_3 (MILESTONE 1200 Mega Microwave Digester), iron, manganese and zinc were analysed by an atomic absorption spectrophotometer (AAS) (Association of Official Analytical Chemists [AOAC], 1995).

Chemical soil analysis

Field investigations were carried out on silty loam soil with a soil reaction (pH_{H2O}) of 6.1-6.6, with low to moderate humus and nitrogen content, poor in phosphorus and low to rich in potassium (Table 1). Air-dried, ground and homogenized soil was analysed according to following methods: soil pH was determined electrometrically using a combined electrode (pH-meter MA5730) for a soil:water suspension (1:2.5, w/v) (active acidity) (Škorić, 1982); humus by the Tjurin method (JDPZ, 1966); potassium and phosphorus by the Egner-Riehm-Domingo method (Egner et al., 1960) and nitrogen by Kjeldahl method (AOAC, 1995).

Table 1. Chemical properties of the soils collected

		%	%	Al – mg*100 g^{-1}	
Environment[x]	pH_{H2O}	Humus	N	P_2O_5	K_2O
B-2003	6.5	2.17	0.12	0.1	6
HP-2004	6.1	2.65	0.13	1.5	15.3
HP-2005	6.6	3.1	0.16	6.2	32.8

[x]B-2003 – Brašljevica, year 2003; HP-2004 – Hrvatsko Polje, year 2004; HP-2005 – Hrvatsko Polje, year 2005

Statistical data analysis

Statistical data analyses were performed using the SAS 8.2 System (2002-2003). Where the ANOVA was significant, Tukey's multiple comparison test (Tukey's HSD - Tukey's Honestly Significant Difference test) was applied for the effects of fertilization, the environment and their interactions. The statistical level of significance for all analyses was defined as an error of 10% (with tags: P<1% = **; P<5% = *; P<10% = $^+$).

Climate conditions

The closest meteorological station for Brašljevica is Jastrebarsko and for Hrvatsko Polje is Otočac.

Brašljevica, year 2003

The total precipitation throughout the year 2003 (Figure 2a) was 766 mm, which is less than a multi annual average (935 mm, Table 3). Precipitation during the vegetation months of red beet growing was 247 mm. Mean daily air temperature during the period of red beet growing were 19-23 °C and were higher for 2 to 4 °C in comparison to multi annual average (Table 2). There was an arid period since the beginning of February, so plants were not able to use the water reserves from the soil, as well as above average daily temperature during that period. Generally, year 2003 was relatively unfavourable for red beet growing because of low precipitation and poor ratio of temperature and precipitation.

Hrvatsko Polje, year 2004

In year 2004 (Figure 2b) weather conditions during the growing season were favourable for red beet growing thanks to the reserves of soil water before the growing, as well as to the rain during the first half of the growing period. Total precipitation during the year was 1,238 mm, which is 133 mm higher than the multi annual average (1,105 mm, Table 2). Temperatures were lower, the ratio between temperature and precipitation was good and had favourable influence on the growth and development of red beet. Generally, temperatures were lower so there was not so much difference to the temperature and precipitation ratio what resulted with a favourable impact on the growth and development of red beet.

Hrvatsko Polje, year 2005

The total precipitation in year 2005 was 1,339 mm and was higher for 234 mm than a multi annual average (1,105 mm, Table 2). Also, during the vegetation period (July-September) the total precipitation was 423 mm and was higher for 198 mm than a multi annual average (225 mm, Table 3). Temperatures were favourable for the growth of red beet. During the whole growing period precipitation and temperature ratio was favourable, except in August, when fell 231 mm of precipitation, significantly more than required which could adversely affect the soil water-air ratio, and thus the red beet growth and minerals accumulation.

Comparing climatological conditions in all three investigation years during the growing period, it is evident that the most favourable conditions for normal growth and development of red beet prevailed in year 2004. Precipitation in 2003 was 247 mm and was poorly distributed. Temperatures were between 19 °C and 23 °C. In year 2004 was less precipitation (176 mm) during the red beet growth, air temperatures ranged from 17 °C to 21 °C, so this year was favourable for red beet growing because of favourable temperatures, and better distribution of precipitation. Air temperatures in year 2005 (15-20 °C) were also within the biological optimum for the growth and development of red beet, but the precipitation was excessive (423 mm) so the weather conditions were unfavourable.

Table 2. Multi annual (1961-1991) climate data from the Jastrebarsko and Otočac meteorological stations

Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Jastrebarsko												
TMP*	54	51	60	70	74	100	78	87	105	92	88	76
AMT**	-0.4	1.1	5.9	10.6	15.6	18.7	20.7	20.2	15.6	10.8	4.9	0.9
Otočac												
TMP*	79	68	75	89	86	77	47	81	127	113	137	127
AMT**	-1	0.1	3.9	8.9	14.1	17.8	19.7	19	13.9	10.5	5	0.1

* TMP – total month precipitation (mm); ** AMT – average month temperature (°C).

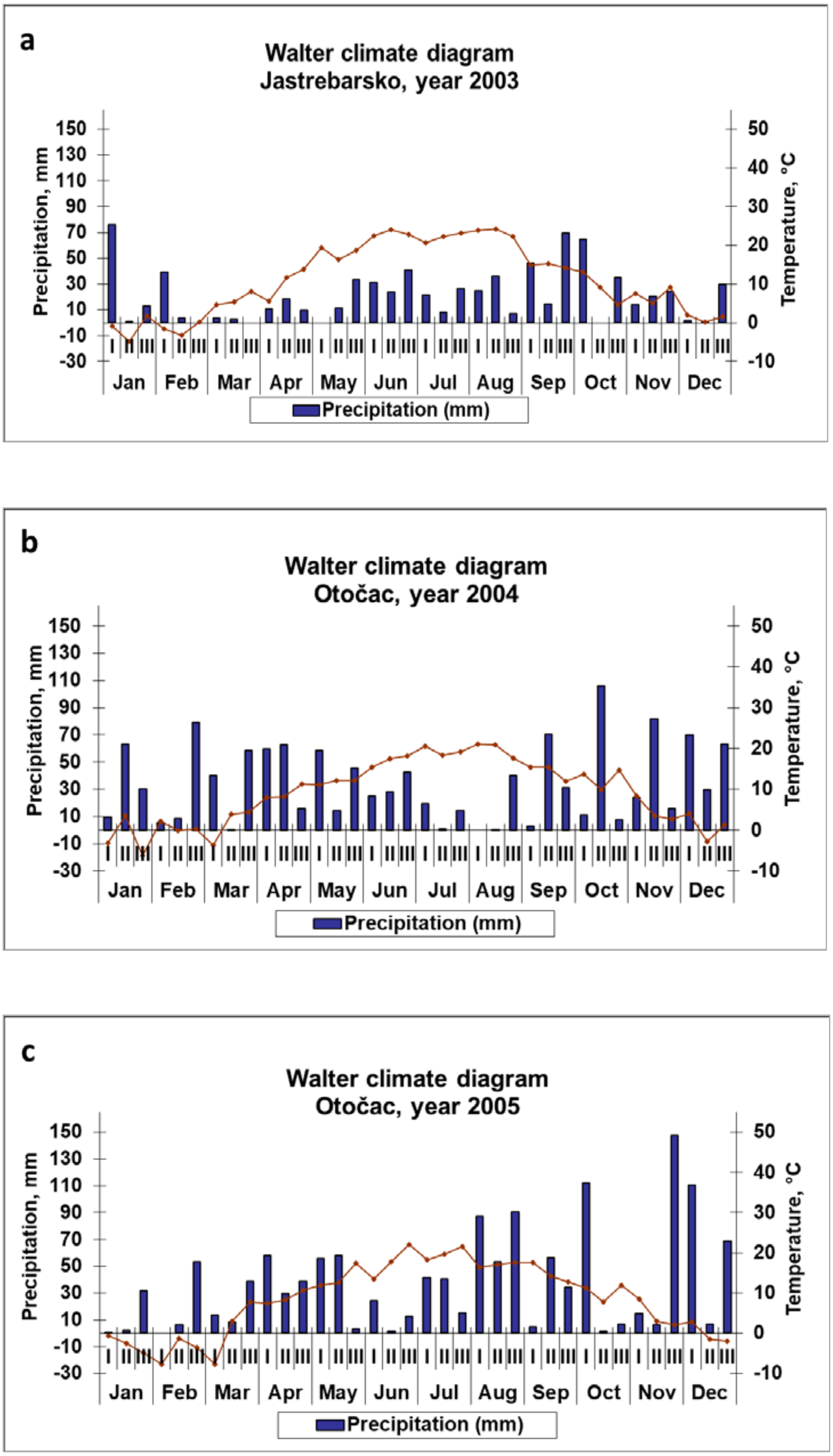

Figure 2 (a, b and c). Walter climate diagrams for Jastrebarsko and Otočac meteorological stations

Results

Table 3 shows the results of variance analysis for fertilization across the environments. Table 4 shows the results of the combined variance analysis in the three environments. From this, it is evident that the weather conditions (environment) significantly affected the value of all studied micronutrients. Statistical analysis showed a slight interaction between environment and fertilization treatments for red beet zinc and a slightly stronger interaction for red beet manganese content in dry matter.

Table 3. Results of variance analysis for fertilization in the three environments for red beet root micronutrient content and significance levels[1]

Micronutrient ($mg*kg^{-1}$ in dry matter)		Environment[2]				
		B-2003		HP-2004		HP-2005
Fe	F_{exp3}	1.21		0.57		0.32
	Pr > F[4]	0.384		0.6559		0.8081
Mn	F_{exp}	5.97	*	3.59	+	2.99
	Pr > F	0.0311		0.0854		0.1178
Zn	F_{exp}	3.58	+	3.7	+	2.57
	Pr > F	0.0859		0.0811		0.1499

[1] Statistical significance level: ** P=1%; * P=5%; + P=10%; [2] B-2003 – Brašljevica, year 2003; HP-2004 – Hrvatsko Polje, year 2004; HP-2005 – Hrvatsko Polje, year 2005; [3] F_{exp} – F experimental factor; [4] Pr > F – error probability F factor.

Table 4. Results of combined variance analysis in the three environments for red beet root macronutrient content and significance levels[1]

Micronutrient ($mg*kg^{-1}$ in dry matter)		Fertilization	Environment		Environment x Fertilization	
Fe	F_{exp2}	1.31	47.8	**	1.07	
	Pr > F[3]	0.3562	0.0036		0.4156	
Mn	F_{exp}	2.38	50.48	**	3.85	*
	Pr > F	0.1686	<0.0001		0.0123	
Zn	F_{exp}	2.52	17.61	**	2.35	+
	Pr > F	0.1548	0.0001		0.0745	

[1] Statistical significance level: ** P=1%; * P=5%; + P=10%; [2] F_{exp} – F experimental factor;
[3] Pr > F – error probability F factor.

Iron

The average red beet iron content in dry matter is shown in Table 5. In terms of annual average values, the highest red beet iron content in dry matter was determined in Brašljevica in 2003 (270 mg Fe*kg^{-1} in dry matter), while the iron content was not significantly different between Hrvatsko Polje in 2004 and 2005 (102 and 96 mg Fe*kg^{-1} in dry matter, respectively). In the first research environment (Brašljevica in 2003), the highest iron content was determined in the control treatment (310 mg Fe*kg^{-1} in dry matter) and the lowest in fertilization with 1,000 kg NPK*ha^{-1} (223 mg Fe*kg^{-1} in dry matter). In Hrvatsko Polje in 2004. A slightly higher value of iron was determined in red beet that was not fertilized (109 mg Fe*kg^{-1} in dry matter) with respect to the fertilizer treatments. Considering the average values for the fertilization treatments, the highest red beet iron content in the three studied environments was determined in the control treatment (172 mg Fe*kg^{-1} in dry matter), while the lowest iron content was determined in treatment with 1,000 kg NPK*ha^{-1} (140 mg Fe*kg^{-1} in dry matter).

Table 5. Red beet iron content (mg Fe*kg^{-1} in dry matter) for different fertilization treatments and environments

	mg Fe*kg^{-1} in dry matter			
Fertilization treatments	B-2003^{x}	HP-2004	HP-2005	Average
Control	310	109	97	172
50 t stable manure*ha^{-1}	267	103	97	156
500 kg NPK*ha^{-1}	278	98	93	156
1,000 kg NPK*ha^{-1}	223	99	97	140
Average	270 A^{y}	102 B	96 B	

^{x} B-2003 – Brašljevica, year 2003, HP-2004 – Hrvatsko Polje, year 2004; HP-2005 – Hrvatsko Polje, year 2005; ^{y} Factor level means accompanied by different letters are significantly different, with error P≤0.05 according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Manganese

Table 6 shows red beet manganese content in dry matter. The highest manganese content was found in Brašljevica in 2003 (96 mg Mn*kg^{-1} in dry matter) and the lowest in Hrvatsko Polje in 2004 (44 mg Mn*kg^{-1} in dry matter). In the first research environment (Brašljevica in 2003), manganese content ranged from 83 to 106 mg Mn*kg^{-1} in dry matter. The highest manganese content was found in red beet that was not fertilized (106 mg Mn*kg^{-1} in dry matter). Manganese values in Hrvatsko Polje in 2004 ranged between 42 and 46 mg Mn*kg^{-1} in dry matter. The highest value was determined in red beet that was not fertilized. Neither in Hrvatsko Polje in 2005 were no statistical differences in red beet manganese content due to different fertilization. The highest manganese content was again determined in the red beet that was not fertilized (62 mg Mn*kg^{-1} in dry matter). The highest manganese content among the three studied environments was determined in the control treatment (71 mg Mn*kg^{-1} in dry matter).

Table 6. Red beet manganese content (mg Mn*kg^{-1} in dry matter) for different fertilization treatments and environments

	mg Mn*kg^{-1} in dry matter			
Fertilization treatments	B-2003[x]	HP-2004	HP-2005	Average
Control	106 a[y]	46	62	71
50 t stable manure*ha^{-1}	83 b	42	57	61
500 kg NPK*ha^{-1}	100 ab	44	58	67
1,000 kg NPK*ha^{-1}	94 ab	42	60	65
Average	96 A	44 C	59 B	

[x] B-2003 – Brašljevica, year 2003, HP-2004 – Hrvatsko Polje, year 2004; HP-2005 – Hrvatsko Polje, year 2005

[y] Factor level means accompanied by different letters are significantly different, with error P≤0.05 according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Zinc

A statistically significant difference was found in red beet zinc content in dry matter between the environments (Table 7). The zinc content determined in Brašljevica in 2003 (53 mg Zn*kg^{-1} in dry matter) was significantly higher than that for Hrvatsko Polje in 2005 and 2004 (40 and 30 mg Zn*kg^{-1} in dry matter, respectively). In Brašljevica in 2003, the zinc values ranged from 47 mg Zn*kg^{-1} in dry matter in treatment with 1,000 kg NPK*ha^{-1} to 60 mg Zn*kg^{-1} in dry matter in the control treatment. Zinc values in Hrvatsko Polje in 2004 ranged from 28 to 31 mg Zn*kg^{-1} in dry matter, and in Hrvatsko Polje in 2004 from 39 to 41 mg Zn*kg^{-1} in dry matter. Looking at the fertilizer treatments in the three research environments, the highest average zinc content was determined in the control treatment (44 mg Zn*kg^{-1} in dry matter), while the lowest was determined in fertilization with 1,000 kg NPK*ha^{-1} (38 mg Zn*kg^{-1} in dry matter).

Table 7. Red beet zinc content (mg $Zn*kg^{-1}$ in dry matter) for different fertilization treatments and environments

	mg $Zn*kg^{-1}$ in dry matter			
Fertilization treatments	B-2003[x]	HP-2004	HP-2005	Average
Control	60	30	41	44
50 t stable $manure*ha^{-1}$	50	29	40	40
500 kg $NPK*ha^{-1}$	55	31	41	42
1,000 kg $NPK*ha^{-1}$	47	28	39	38
Average	53 A[y]	30 C	40 B	

[x] B-2003 – Brašljevica, year 2003, HP-2004 – Hrvatsko Polje, year 2004; HP-2005 – Hrvatsko Polje, year 2005

[y] Factor level means accompanied by different letters are significantly different, with error P≤0.05 according to Tukey's HSD test. Small letters refer to fertilization treatments. Capital letters refer to average values of environments.

Discussion

Statistical analysis of the studied environments has shown variations in red beet iron, manganese, and zinc contents, which were greatly affected by the initial soil nutrient level (especially potassium) and weather conditions (precipitation and mean daily temperature) during the study period. Despite the finding that fertilization did not significantly increase the red beet iron content, there were very interesting and important findings, namely, that the red beet iron content obtained in this study (annual average values ranged from 96 to 270 mg $Fe*kg^{-1}$ in dry matter) is significantly higher than that reported in the literature (Silanpää ,1982; Maynard and Hochmuth, 1997; Kołota and Adamczewska-Sowinska, 2006) (71, 69 and 69 mg $Fe*kg^{-1}$, respectively), and the values in Brašljevica in 2003 (223-310 mg $Fe*kg^{-1}$ in dry matter) are even up to 10 times higher than those reported by some authors (Lisiewska et al., 2006; Ekholm et al., 2007) (26 and 29 mg $Fe*kg^{-1}$, respectively).

The red beet manganese content was highest in Brašljevica in 2003 (83-106 mg $Mn*kg^{-1}$ in dry weight), and among the treatments, the highest value was obtained in the control treatment. This value is also higher than those reported by many authors (Lindow and Peterson, 1927; Mikula and Indeka, 1997; Lisiewska et al., 2006; Ekholm et al. ,2007; and Nizioł-Łukaszewska and Gawęda, 2016) (35-76, 26, 27, 30 and 26 mg $Mn*kg^{-1}$, respectively). In terms of red beet zinc content, variation among studied environments was found. Again, the highest values were recorded in Brašljevica in 2003 (50-60 mg $Zn*kg^{-1}$ in dry matter). It is worth highlighting that the obtained red beet zinc content is higher or similar to the data in the literature Silanpää (1982), Mikula and Indeka (1997), Sękara et al. (2005), Lisiewska et al. (2006), Ekholm et al. (2007) and Nizioł-Łukaszewska and Gawęda (2016) (52, 44, 33, 41, 16 and 45 mg $Zn*kg^{-1}$ respectively).

Looking at the effect of fertilization in general for all three studied micronutrients (iron, manganese, and zinc) there was a noticeable general decreasing trend of the content of these microelements with fertilization treatments. This was certainly the result of antagonistic activity of the investigated nutrients, especially potassium (Bergmann, 1992; Madhava Rao et al., 2006), which was confirmed in this study (the highest values of micronutrients were determined in the control treatment). The antagonism theory was also confirmed by the finding that the highest average red beet micronutrient content (270 mg $Fe*kg^{-1}$ in dry matter, 96 mg $Zn*kg^{-1}$ in dry matter, and 53 mg $Mn*kg^{-1}$ in dry matter) was determined in Brašljevica in 2003, where the starting soil potassium content was lower (6 mg $K_2O*100\ g^{-1}$ soil), compared with Hrvatsko Polje in 2004 and 2005 (15.3 mg $K_2O*100\ g^{-1}$ soil). But on the other hand, there is possibility that reducing content of micronutrients can be a result of dilution as the effect of increased mass of plants, especially in Hrvatsko Polje in 2004 when the obtained yield was 43,4 $t*ha^{-1}$ (data not shown).

Despite the finding that the red beet micronutrient content was influenced more by environment than by NPK fertilization, fertilization with micronutrients would also increase the content and thus increase the nutritional value of red beet as a functional food. Red beet is a very valuable foodstuff that should be included very often on the daily menu. In addition, as already mentioned, the obtained micronutrient values are mostly considerably higher or at the upper level of the data in the literature. Therefore, to increase the micromineral content in plants, it is necessary to fertilize with microelements also, not just with NPK only, as is common practice.

Conclusions

The environment has a great effect on red beet micronutrient content. The highest average red beet root micronutrient content (270 mg $Fe*kg^{-1}$ in dry matter, 96 mg $Zn*kg^{-1}$ in dry matter, and 53 mg $Mn*kg^{-1}$ in dry matter) was determined in Brašljevica in 2003 with low potassium soil content. In general, according to fertilization treatments, there was a general decreasing trend in contents of the studied micronutrients (iron, manganese and zinc) with NPK 5-20-30 fertilization (and by application of high doses of potassium). This was certainly the result of antagonistic activity of the investigated elements with potassium. Therefore, it is suggested to fertilize with both 500 kg NPK 5-20-30*ha^{-1} and microelement fertilizers in order to increase the content of all nutrients to produce highly valuable food.

References

Abawi, G.S., Widmer, T.L. (2000) Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. Applied Soil Ecology, 15, 37. https://doi.org/10.1016/S0929-1393(00)00070-6

Association of Official Analytical Chemists [AOAC] (1995) Official method of analysis of AOAC International. 16th edition. Arlington, USA: Association of Official Analytical Chemists.

Belkhodja, R., Morales, F., Quílez, R., López-Millán, A.F., Abadía, A., Abadía, J. (1998) Iron deficiency causes changes in chlorophyll fluorescence due to the reduction in the dark of the Photosystem II acceptor side. Photosynthesis Research, 56, 265-276. Available at: https://link.springer.com/content/pdf/10.1023%2FA%3A1006039917599.pdf

Bergmann, W. (1992) Nutritional disorders of plants. Stuttgart, New York: Gustav Fischer Verlag Jena.

Borkert, C.M., Cox, F.R. (1999) Effects of acidity at high soil zinc, copper, and manganese on peanut, rice, and soybean. Communications in Soil Science and Plant Analysis, 30 (9-10), 1371-1384. http://dx.doi.org/10.1080/00103629909370293

Chizzola, R., Mitteregger, U.S. (2005) Cadmium and zinc interactions in trace element accumulation in chamomile. Journal of Plant Nutrition, 28 (8), 1383-1396. http://dx.doi.org/10.1081/PLN-200067470

Christiansen, J.S., Thorup-Kristensen, K., Kristensen, H.L. (2006) Root development of beetroot, sweet corn and celeriac, and soil N content after incorporation of green manure. Journal of Horticultural Science and Biotechnology, 81 (5), 831-838. http://dx.doi.org/10.1080/14620316.2006.11512146

Davenport, J.R., Milburn, P.H., Rosen, C.J., Thornton, R.E. (2005) Environmental impacts of potato nutrient management. American Journal of Potato Research: an official publication of the Potato Association of America. 82 (4), 321-328. Available at: https://link.springer.com/content/pdf/10.1007%2FBF02871962.pdf

Egner, H., Riehm, H., Domingo, W.R. (1960) Untersuchung über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nahrstoffzustanden der Boden. II, Chemische Extraktionsmethoden zur Phosphor und Kaliumbestimmung–K. Lantbr. Hogsk. Annir. W.R., 26, 199-215.

Ekholm, P., Reinivuo, H., Mattila, P., Pakkala, H., Koponen, J., Happonen, A., Hellström, J., Ovaskainen, M.J. (2007) Changes in the mineral and trace element contents of cereals, fruits and vegetables in Finland. Journal of Food Composition and Analysis, 20, 487–495. https://doi.org/10.1016/j.jfca.2007.02.007

El-Jaoual, T., Cox, D.A. (1998) Manganese toxicity in plants. Journal of Plant Nutrition, 21 (2), 353-386. http://dx.doi.org/10.1080/01904169809365409

Hartz, T.K. (2006) Vegetable production best management practices to minimize nutrient loss: July-September 2006. Horttechnology, 16 (3), 398-403. Available at: http://www.ucanr.org/sites/nm/files/76754.pdf

He, P.P., Lv, X.Z., Wang, G.Y. (2004) Effects of Se and Zn supplementation on the antagonism against Pb and Cd in vegetables. Environment International, 30, 167-172. http://dx.doi.org/10.1016/S0160-4120(03)00167-3

He, Z.L., Yang, X.E., Stoffella, P.J. (2005) Trace elements in agroecosystems and impacts on the environment. Journal of Trace Elements in Medicine and Biology, 19, 125–140. https://doi.org/10.1016/j.jtemb.2005.02.010

Herak Ćustić, M., Gluhić, D., Čoga, L., Petek, M., Gošćak, I. (2008) Vine plant chlorosis on unstructured calcareous soils and leaf Ca, Mg and K content. Cereal Research Communications, 36, 1S, 439-442.

Herak Ćustić, M., Horvatić, M., Pecina, M. (2009) Nitrogen fertilization influences protein nutritional quality in red head chicory. Journal of Plant Nutrition, 32 (4), 598-609. Available at: http://www.tandfonline.com/doi/abs/10.1080/01904160802714987

Herencia, J.F., Ruiz, J.C., Morillo, E., Melero, S., Villaverde, J., Maqueda, C. (2008) The effect of organic and mineral fertilization on micronutrient availability in soil. Soil Science, 173 (1), 69-80. http://dx.doi.org/10.1097/ss.0b013e31815a6676

Horvatić, M., Gačić, M., Vedrina-Dragojević, I. (1999) Accumulation of iron, copper, manganese and nickel during maize grain maturation. Journal of Agronomy and Crop Science, 182, 99-103. http://dx.doi.org/10.1046/j.1439-037x.1999.00278.x

Imoro, Z.A., Khan, A.T., Lawer, E.A. (2012) Effects of organic and inorganic fertilizers on mineral composition of *Cynodon dactylon*. Greener Journal of Agricultural Sciences, 2 (7), 323-328.

Jackson, L.E., Ramirez, I., Yokota, R., Fennimore, S.A., Koike, S.T., Henderson, D.M., Chaney, W.E., Calderon, F.J., Klonsky, K. (2004) On-farm assessment of organic matter and tillage management on vegetable yield, soil, weeds, pests, and economics in California. Agriculture, Ecosystems and Environment, 103 (3), 443-463. https://doi.org/10.1016/j.agee.2003.11.013

JDPZ (1966) Priručnik za ispitivanje zemljišta. Knjiga I. Kemijske metode ispitivanja zemljišta. Beograd.

Jordão, C., Cecon, P., Pereira, J. (2003) Evaluation of metal concentrations in edible vegetables grown in compost amended soil. International Journal of Environmental Studies, 60 (6), 547-562.

Kołota, E., Adamczevska-Sowińska, K. (2006) Burak ćwikłowy i liściowy. Warszawa: Hortpress.

Kristensen, H.L., Thorup-Kristensen, K. (2007) Effects of vertical distribution of soil inorganic nitrogen on root growth and subsequent nitrogen uptake by field vegetable crops. Soil Use Manage, 23 (4), 338-347. https://doi.org/10.1111/j.1475-2743.2007.00105.x

Lindow, C.W., Peterson, W.H. (1927) The manganese content of plant and animal materials. Journal of Biological Chemistry, 169-176. http://www.jbc.org/content/75/1/169.full.pdf

Lisiewska, Z., Kmiecik, W., Gebczynnski, P. (2006) Effects on mineral content of different methods of preparing frozen root vegetables. Food Science and Technology International, 12, 497-503. http://dx.doi.org/10.1177/1082013206073273

López-Millán, A.F., Morales, F., Gogorcena, Y., Abadía, A., Abadía, J. (2009) Metabolic responses in iron deficient tomato plants. Journal of Plant Physiology, 166, 375-84. https://doi.org/10.1016/j.jplph.2008.06.011

Madhava Rao, K.V., Raghavendra, A.S., Janardhan Reddy, K. (2006) Physiology and molecular biology of stress tolerance in plants. Netherlands: Springer.

Marschner, H. (1995) Mineral nutrition of higher plants. San Diego: Academic Press Limited.

Maynard, D.N., Hochmuth, G.J. (1997) Knott's handbook for vegetable growers. New York: John Wiley & sons, Inc.

McCall, K.A., Huang, C., Fierke, C.A. (2000) Function and mechanism of zinc metalloenzymes. Journal of Nutrition, 130, 5S, 1437-1446. Available at: http://jn.nutrition.org/content/130/5/1437S.full.pdf+html

Mikuła, W., Indeka, L. (1997) Heavy metals in allotment gardens close to an oil refinery in Płock. Water, Air, & Soil Pollution, 96, 61-71. Available at: https://link.springer.com/content/pdf/10.1007%2FBF02407196.pdf

Mitova, I., Dinev, N., Vassileva, V. (2014) Effects of mineral and organic fertilization on early potato production. Bulgarian Journal of Agricultural Science, 20 (5), 1182-1188. Available at: http://www.agrojournal.org/20/05-25.pdf

Moustaoui, D., Verloo, M. (1995) Effect of soil dunging on absorption of iron, copper, zinc and manganese. Agrochimica, 39 (5-6), 316-325.

Mukhopadhyay, M.J., Sharma, A. (1991) Manganese in cell metabolism of higher plants. Botanical Review, 57 (2), 117-149. Available at: https://link.springer.com/article/10.1007/BF02858767

Nizioł-Łukaszewska, Z., Gawęda, M. (2016) Influence of cultivar on the content of selected minerals in red beet roots (*Beta vulgaris* L.). Folia Horticulturae, 28 (2), 143-150. https://doi.org/10.1515/fhort-2016-0017

Onyango, C.M., Harbinson, J., Imungi, J.K., Shibairo, S.S., Van Kooten, O. (2012) Influence of organic and mineral fertilization on germination, leaf nitrogen, nitrate accumulation and yield of vegetable amaranth. Journal of Plant Nutrition, 35, 342-365. http://dx.doi.org/10.1080/01904167.2012.639917

Parisi, A.F., Vallee, B.L. (1969) Zinc metalloenzymes: characteristics and significance in biology and medicine. American Journal of Clinical Nutrition, 22, 1222-1239. Available at: http://ajcn.nutrition.org/content/22/9/1222.abstract

Perez-Murcia, M.D., Moreno-Caselles, J., Moral, R., Perez-Espinosa, A., Paredes, C., Rufete, B. (2005) Use of composted sewage sludge as horticultural growth media: effects on germination and trace element extraction. Communications in Soil Science and Plant Analysis, 36 (4-6), 571-582. http://dx.doi.org/10.1081/CSS-200043286

Petek, M., Herak Ćustić, M., Čoga, L., Pecina, M. (2008) Phosphorus content in soil and in fresh and cooked red beet in dependence on different fertilization. Cereal Research Communication, 36, 435-438.

Rashid, A., Ryan, J. (2004) Micronutrient constraints to crop production in soils with Mediterranean-type characteristics: a review. Journal of Plant Nutrition, 27 (6), 959-975. http://dx.doi.org/10.1081/PLN-120037530

Ronaghi, A., Ghasemi-Fasaei, R. (2008) Field evaluations of yield, iron-manganese relationship, and chlorophyll meter readings in soybean genotypes as affected by iron-ethylenediamine di-o-hydroxyphenylacetic acid in a calcareous soil. Journal of Plant Nutrition, 31 (1-3), 81-89. http://dx.doi.org/10.1080/01904160701741925

Schenk, M.K. (2006) Nutrient efficiency of vegetable crops. Acta Horticulturae, 700, 21-33. http://dx.doi.org/10.17660/ActaHortic.2006.700.1

Sękara, A., Poniedziałek, M., Ciura, J., Jędrszczyk, E. (2005) Zinc and copper accumulation and distribution in the tissues of nine crops: implications for phytoremediation. Polish Journal of Environmental Studies, 14 (6), 829-835. Available at: http://www.pjoes.com/pdf/14.6/829-835.pdf

Siebielec, G., Chaney, R.L. (2006) Manganese fertilizer requirement to prevent manganese deficiency when liming to remediate Ni-phytotoxic soils. Commun. Journal of Soil Science and Plant Nutrition, 37 (1-2), 163-179. http://dx.doi.org/10.1080/00103620500408548

Sillanpää, M. (1982) Micronutrients and the nutrient status of soils. FAO Soils Bulletin.

Škorić, A. (1982) Priručnik za pedološka istraživanja. Zagreb: Fakultet poljoprivrednih znanosti.

Tamoutsidis, E., Papadopoulos, I., Tokatlidis, I., Zotis, S., Mavropoulos, T. (2002) Wet sewage sludge application effect on soil properties and element content of leaf and root vegetables. Journal of Plant Nutrition, 25 (9), 1941-1955. http://dx.doi.org/10.1081/PLN-120013286

Wallace, P.A., Marfo, E.K., Plahar, W.A. (1998) Nutritional quality and antinutritional composition of four non-conventional leafy vegetables. Food Chemistry, 61 (3), 287-291. https://doi.org/10.1016/S0308-8146(97)00062-9

Xiangyang, L., Guangxi, R., Yan, S. (2011) The effect of organic manure and chemical fertilizer on growth and development of *Stevia rebaudiana* Bertoni. Energy Procedia, 5, 1200-1204. https://doi.org/10.1016/j.egypro.2011.03.210

Zhang, M.K., He, Z.L., Calvert, D.V., Stoffella, P.J., Yang, X.E., Lamb, E.M. (2003) Accumulation and partitioning of phosphorus and heavy metals in a sandy soil under long-term vegetable crop production. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, 38 (9), 1981-1995. http://dx.doi.org/10.1081/ESE-120022894