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Microelements Changes in Leaves and Fruits of Raspberry (*Rubus idaeus* L.) Under the Influence of Ameliorative Measures

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ABSTRACT

The study evaluated the effects of lime and dolomite application (2 and 4 t ha^{-1}) in combination with NPK fertilizer (1 t ha^{-1}) or borax (50 kg ha^{-1}) on micronutrient concentrations in the raspberry leaves and fruits grown in the soil with a strongly acid reaction. Changes in the content of micronutrients (Fe, Mn, Cu, Zn, and B) and Al in the soil and raspberry leaves and fruits were monitored during a two-year period. Calcification reduced the content of micronutrients and Al in the soil, as well as their content in the plant. Applied doses of lime or dolomite of 4 t ha^{-1} almost completely neutralized the high to toxic content of mobile Al in the soil, but the values in the leaves although reduced were still high. In all applied treatments, high and very high concentrations of available forms of Fe and Mn in the soil and/or leaves and fruits were significantly reduced. However, the Mn concentrations in the soil, leaves, and raspberry fruits were very high. Calcification did not reduce the available Cu in the soil, while this effect was observed in raspberry leaves and fruits where the Cu concentrations were within optimal values. After calcification, the Zn and B concentrations decreased in the soil and plant, but they mostly remained within the optimal values. The results indicate that due to the very high concentrations of Al and Mn in the soil and plant after lime application, additional doses of lime materials in more than one season are required.

INTRODUCTION

In the last few decades, the areas under intensive raspberry plantations have been rapidly expanding in the world, which is primarily due to tasteful and healthy raspberry fruit. Raspberry production in Serbia has experienced a sharp rise in previous years (Mišić and Nikolić, 2003). Owing to the favorable climatic and geographical conditions, such as the widespread hilly and mountainous areas with an altitude of 400–800 m, this fruit culture has become of strategic importance for Serbia. The areas under raspberries have rapidly increased, as well as the production of first-class raspberry fruits. In the period from 2008 to 2017 among the largest raspberry producers in the world, Serbia was ranked fourth, with an average annual production of 86,530.4 tonnes during the observed decade (Šapić et al., 2020). Only fertile soil with a regulated pH value and an optimal nutrient content ensures adequate plant yields with desirable quality parameters (Buskiene and Uselis, 2008), and accurate assessment of macronutrients and micronutrients is required to obtain the expected yield (Kowalenko, 2005).

In Serbia, the raspberry plantations were raised on large areas on very acidic soils (Stevanović et al., 2003), which are characterized by unfavorable chemical and physical properties. In acid soils, the deficiency of many biogenic elements can be observed (NH_4^+, NO_3^-, P, K, Ca, Mg, B, and Zn), and also very high to toxic concentrations of some elements can be present, primarily mobile Al and accessible forms of Fe and Mn (Voight and Staley, 2004; Von Uexkull and Mutert, 1995). Previous studies found positive effects of calcification on the repair of soil acidity and an increase in the deficient content of some macrobiogenic elements (Fageria and Nascente, 2014). Lime application increases soil pH and reduces mobile Al toxicity (Grewal and Williams, 2003). The lime or other lime materials provide Ca and/or Mg for the plant, increase available P content, and decrease heavy metals content (Fe and Mn) in the soil (Dugalić et al., 2006). The increase in soil pH, after liming, intensifies the microbiological processes in soil, resulting in increased decomposition of soil organic matter, which could lead to increased mineral N (Mkhonza et al., 2020). Physical properties of soil were also improved by lime application. Previously, in the raspberry plantation established on acid soil, the positive effect of lime materials on soil basic properties (pH) and macronutrients (Ca, Mg, P, and K) in soil and plant was noted (Sikirić et al., 2015). Adequate ratio of micronutrients and their favorable content in the soil, whose uptake can be affected by low soil pH, ensures plants optimal supply (Karaklajić-Stajić et al., 2012; Lanauskas et al., 2006). Plant requirements for micronutrients are very low, as opposed to their need for macronutrients, but they are evenly significant for growth and reproduction of plants (Kirkby and Romheld, 2004).

Therefore, the aim of this study was to determine the effect of different types and dose of lime materials in combination with boron fertilizer that will lead to the greatest reduction of potentially toxic concentrations of mobile Al, as well as available Fe and Mn in raspberry plantation: soil, plant leaves, and fruits. In addition, the concentrations of other micronutrients, Cu, Zn, and B were measured.

Material and Methods

The trial was conducted in the 4-year-old raspberry plantation (Wilamet variety), established on the soil with extremely acid reaction (Eutric Cambisol, WRB, 2014), in the village Prijanovici (43° 51′2.00″N, 20°4′9.39″E), near Pozega city (West Serbia). The initial soil characteristics, before the experiment was set up, showed that the soil is extremely acidic (pH_{KCl} 3.60; pH_{H20} 4.4) (Supplementary Table 1). Values of hummus (3.64), organic carbon (C 2.11%), and total nitrogen (N 0.23%), as well as C/N values (9), P, and K were at the satisfactory level, and their changes under calcification were given in Sikirić et al. (2015). The value of mobile Al in the soil examined was very high 9.84 mg 100 g^{-1}, which is considered as a boundary of toxicity for raspberry cultivation. Concentrations of available Fe and Mn were very high, and the concentration of available Cu was high and above optimal values. The concentrations of available Zn and B were sufficient (Supplementary Table 1). The micronutrient values in the soil were evaluated according to the limits given by Ankerman and Large (1974) and Alloway (2008).

The experiment was arranged in a randomized block design with three replications. The experiment with 7 treatments with different fertilization was conducted. Each treatment was set up in three replications where the experimental plot of each repetition was 10 m x 2.5 m (25 m^2 surface). The treatments were: 2 and 4 t ha^{-1} lime; 2 and 4 t ha^{-1} dolomite, 2 t ha^{-1} lime + 50 kg ha^{-1} borax and 2 t ha^{-1} dolomite + 50 kg ha^{-1} borax. Lime (with 98.5% of $CaCO_3$), dolomite (with 97% of $CaCO_3 \times MgCO_3$ in 1:1 ratio), and borax ($Na_2B_4O_2 \times 10 H_2O$), were applied once in autumn of 2002 and their effect was observed in the two following growing seasons (2003 and 2004). The lime materials were applied on the soil surface of the whole treatment, avoiding the raspberry bush directly. To provide raspberries with the necessary nutrients to achieve high yields (> 10 t ha^{-1}) according to Ubavić et al. (2001), as well as to complement nutrients leached from the soil and nutrients taken up by raspberry

plants, in early spring every year, 1 t ha^{-1} of NPK fertilizer (15% N, 6.54% P, and 12.45% K) was added to each treatment of the experiment. In the control treatment only 1 t ha^{-1} of NPK fertilizer was added. The fertilization was done throughout the whole plantation.

The experiment was followed for two years. At the beginning of June in two subsequent years, soil sampling and leaf sampling were conducted. The fruits were collected at the beginning of July. Soil samples were collected from the rows near raspberry bushes (about 20 cm from the plant), from a depth of 0–30 cm, at each treatment. Leaf samples were taken from the middle of the one-year-old fruiting branches, several different shrubs, fruit samples from several native shoots, and different raspberry bushes. Soil samples were dried at room temperature and ground in a grinding mill, and available forms of Mn, Fe, Zn, and Cu in soils were extracted by DTPA (Soltanpour et al., 1996) and determined using a SensAA dual atomic absorption spectrophotometer (Dandenong, Australia). Mobile Al (exchangeable) in the soil was determined by the aluminon acetat method by Barnhisel and Bertsch (1982) as well as in the leaf samples. The B contents in soil and plant, after hot-water extraction, were determined by colorimetry (curcumin method) (Dible et al., 1954). For determination of plant Mn, Fe, Zn, and Cu, leaves and fruits were dried at 105°C and ground and burned to ash at 550°C and acid digestion with HCl was performed according to Chapman and Pratt (1961) and determined using a SensAA dual atomic absorption spectrophotometer (Dandenong, Australia).

Statistical analysis

The effect of the treatments was evaluated using the analysis of variance (SPSS 16.0, Chicago, USA), and significant differences between means were tested by Duncan's multiple range test. Dependencies between individual soil characteristics and between some soil and plant characteristics are determined by the Pearson correlation coefficient.

RESULTS

Agrochemical Properties of Soil in the Field Trial

In the control treatment, the mobile Al concentration was 10.35 mg 100 g^{-1}, average for 2 years (Table 1), which is considered potentially toxic for most of the cultivated plants (Sikirić et al., 2009). The slight increase in Al of control treatment compared to soil before the start of the experiment (9.84 mg 100 g^{-1}) could be the result of terrain heterogeneity and/or further NPK fertilization. In all treatments with lime materials, Al concentrations were significantly decreased. Application of 2 t ha^{-1} of lime reduced Al concentration by 43% and 4 t ha^{-1} of lime for 96% (average decrease for two years). In the first year, lime application completely neutralized mobile Al (Table 1). Maximum reduction of Al by dolomite application was obtained in the second year. The mobile Al values were significantly reduced by 40% in the treatment with 2 t ha^{-1} and for 65% in the treatment with 4 t ha^{-1} of dolomite (average decrease for two years). Treatments with combination of lime, dolomite, and borax also significantly decreased the mobile Al concentrations (in average by 38 and 46%).

The concentration of Fe in soil in the control treatment in both years of investigation was extremely high (98 mg kg^{-1}) according to Ankerman and Large (1974). Terrain heterogeneity and high variability of available Fe concentrations in soil compared to the other microelements probably caused Fe decrease compared with soil before the start of the experiment. A significant decrease compared to the control in the first year was obtained in all treatments (except with 2 t ha^{-1} of dolomite), and in the second year, only in the treatment with 4 t ha^{-1} of lime. On average, for two years, the highest decrease of Fe concentration was in the treatment with 4 t ha^{-1} of lime by 18% (Table 1). In the treatments with combined application of lime and dolomite with borax, in the first year, the content of this element was significantly lower by 27%, and in the second year, the changes were not significant.

Concentrations of available Mn in soil of all treatments in both evaluated years were very high, from 164 up to 256 mg kg^{-1} (Table 1), according to Ankerman and Large (1974). Increased Mn concentrations in soil of almost all treatments compared with values before the start of the experiment can be the result of continuous NPK fertilization. Compared to the control, a significant reduction in

Table 1. Influence of lime treatments on content of Al, Fe, Mn, Cu, Zn, and B in soil.

Treatments	Al	Fe	Mn	Cu	Zn	B
	mg 100 g^{-1}			mg kg^{-1}		
First year						
Control (1 t ha^{-1} NPK)	10.84 a	96 a	252 a	2.3 a	1.6 a	0.8 b
CaCO$_3$ (2 t ha^{-1})	5.50 d	81 b	213 e	2.6 a	1.4 bc	0.7 c
CaCO$_3$ (4 t ha^{-1})	0.00 f	77 bc	164 f	2.7 a	1.1 d	0.6 c
MgCaCO$_3$ (2 t ha^{-1})	6.54 b	86 ab	246 b	2.4 a	1.6 a	0.7 c
MgCaCO$_3$ (4 t ha^{-1})	5.90 cd	84 b	238 c	1.9 a	1.4 c	0.7 c
CaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	4.58 e	70 c	230 d	2.6 a	1.5 ab	1.4 a
MgCaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	6.20 bc	71 c	248 ab	2.5 a	1.5 a	1.3 a
Second year						
Control (1 t ha^{-1} NPK)	9.86 a	99 ab	232 bc	2.5 a	1.6 a	0.6 cd
CaCO$_3$ (2 t ha^{-1})	6.30 c	89 ab	240 ab	2.7 a	1.5 ab	0.6 c
CaCO$_3$ (4 t ha^{-1})	0.80 f	80 c	215 cd	2.9 a	1.0 c	0.5 d
MgCaCO$_3$ (2 t ha^{-1})	5.97 c	92 ab	230 bc	2.6 a	1.5 ab	0.5 d
MgCaCO$_3$ (4 t ha^{-1})	1.50 e	89 ab	204 d	2.0 a	1.1 c	0.5 d
CaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	8.19 b	110 a	256 a	2.4 a	1.4 b	1.4 a
MgCaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	5.05 d	106 a	252 a	2.4 a	1.4 b	1.1 b
Average for two years						
Control (1 t ha^{-1} NPK)	10.35 a	98.0 a	242.0 a	2.40 d	1.60 a	0.70 c
CaCO$_3$ (2 t ha^{-1})	5.90 cd	85.0 b	226.5 b	2.65 b	1.45 b	0.65 cd
CaCO$_3$ (4 t ha^{-1})	0.40 f	78.5 b	189.5 c	2.80 a	1.05 d	0.55 e
MgCaCO$_3$ (2 t ha^{-1})	6.25 bc	89.0 ab	238.0 a	2.50 c	1.55 a	0.60 de
MgCaCO$_3$ (4 t ha^{-1})	3.70 e	86.5 ab	221.0 b	1.95 e	1.25 c	0.60 de
CaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	6.38 b	90.0 ab	243.0 a	2.50 c	1.45 b	1.40 a
MgCaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	5.62 d	88.5 ab	250.0 a	2.45 c	1.45 b	1.20 b
ANOVA						
Treatment	***	ns	***	***	***	***
Year	*	***	ns	ns	**	***
Interaction	***	*	***	ns	**	**

a-e: Means in a column followed by the same subscript letters are not significantly different according to the Duncan multiple range test ($p < 0.05$). * – significant at P ≤ 0.05, ** – significant at P ≤ 0.01, *** – significant at P ≤ 0.01, ns – not significant according to two-way ANOVA.

available Mn in the first year was found in almost all treatments (except for the treatment: dolomite + borax). In the second year, the effect of calcification on the reduction of available Mn in the soil was generally weaker in all treatments, with a significant reduction measured only in the treatment with 4 t ha^{-1} of dolomite. In this year, in the treatments with borax, a certain increase in Mn was noticed.

Concentrations of available Cu in the soil were not influenced by calcification and in all treatments, were high or very high.

The content of available Zn in all examined treatments was from 1.0–1.6 mg kg^{-1}, that is at sufficient to medium content according to Ankerman and Large (1974) and Alloway (2008). The application of lime fertilizers in accordance with dose increase showed small significant reductions of available Zn in the soil. In the first year, a significant reduction of Zn compared to the control was in the treatments with both doses of lime and treatment with a higher dose of dolomite, and in the second year, not only in treatments with 4 t ha^{-1} of lime or dolomite but also in combined treatments of lime or dolomite with borax. The greatest effect on Zn reduction was measured on treatments with 4 t ha of lime (two-year average 34%) and 4 t ha^{-1} of dolomite (two-year average 22%).

Available B content in the soil of the control treatment, depending on the year, varied from 0.8–0.6 mg kg^{-1} (middle level of supply). The optimal values are in the range of 0.5–2.0 mg kg^{-1}, and toxic higher than 3 mg kg^{-1} (Brdar-Jokanović, 2020). Less than 0.5 mg kg^{-1} is rated as marginal to deficient. Application of doses of both lime and dolomite (without borax), in the first year, significantly decreased the B concentration compared to the control, but not below the optimal level. In the second year, there were no differences between these treatments and control. The application of lime with 50 kg ha^{-1} of borax caused an increase in the B concentrations in the soil by 100%, and the application of borax with dolomite by 71% (average for two years) compared to the control.

Table 2. Influence of lime treatments on content of Al, Fe, Mn, Cu, Zn, and B in a raspberry leaf.

Treatments	Al	Fe	Mn	Cu	Zn	B
			mg kg^{-1}			
First year						
Control (1 t ha^{-1} NPK)	896 a	153 a	1676 a	22 a	40 a	14 b
CaCO$_3$ (2 t ha^{-1})	763 b	148 ab	1492 b	18 b	36 bc	9 c
CaCO$_3$ (4 t ha^{-1})	710 b	130 c	1320 cd	17 cd	35 cd	10 c
MgCaCO$_3$ (2 t ha^{-1})	745 b	135 bc	1510 b	18 bc	35 cd	9 c
MgCaCO$_3$ (4 t ha^{-1})	764 b	128 c	1400 bc	15 e	34 d	9 c
CaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	523 c	164 a	1189 e	16 de	37 b	76 a
MgCaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	540 c	156 a	1235 de	17 bc	35 bcd	77 a
Second year						
Control (1 t ha^{-1} NPK)	962 a	139 a	2453 a	35 f	39 a	14 c
CaCO$_3$ (2 t ha^{-1})	810 bc	122 bc	1834 b	106 d	35 b	8 de
CaCO$_3$ (4 t ha^{-1})	783 bc	104 d	1353 e	114 c	29 e	7 e
MgCaCO$_3$ (2 t ha^{-1})	770 bc	132 ab	1686 c	121 b	31 d	9 d
MgCaCO$_3$ (4 t ha^{-1})	715 c	109 cd	1317 e	79 e	30 de	7 e
CaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	852 ab	133 ab	1650 cd	80 e	36 b	46 b
MgCaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	886 ab	127 ab	1520 d	126 a	34 c	49 a
Average for two years						
Control (1 t ha^{-1} NPK)	929.0 a	146.0 ab	2064 a	28.5 f	39.5 a	14.0 c
CaCO$_3$ (2 t ha^{-1})	786.5 b	135.0 bc	1663 b	62.0 d	35.5 b	8.5 de
CaCO$_3$ (4 t ha^{-1})	746.5 bcd	117.0 d	1336 c	65.5 c	32.0 e	8.5 de
MgCaCO$_3$ (2 t ha^{-1})	757.5 bc	133.5 c	1598 b	69.5 b	33.0 d	9.0 d
MgCaCO$_3$ (4 t ha^{-1})	739.5 bcd	118.5 d	1358 c	47.0 e	32.0 e	8.0 e
CaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	687.5 c	148.5 ab	1419 c	48.0 e	36.5 b	61.0 b
MgCaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	713.0 cd	141.5 abc	1377 c	71.5 a	34.5 c	63.0 a
ANOVA						
Treatment	***	***	***	***	***	***
Year	***	***	***	***	***	***
Interaction	***	ns	***	***	***	***

a-e: Means in a column followed by the same subscript letters are not significantly different according to Duncan multiple range test ($p < 0.05$). * – significant at P ≤ 0.05, ** – significant at P ≤ 0.01, *** – significant at P ≤ 0.01, ns – not significant according to two-way ANOVA.

The Al and Microelements Concentrations in Raspberry Leaves and Fruits

The concentration of Al in raspberry leaves, in the control treatment, was high, with an average of 930 mg kg^{-1} of dry matter (Table 2). The application of all lime and dolomite rates significantly reduced the Al concentrations in the leaves in both experimental years. However, the Al values were still high in all treatments. The decrease ranged between 26% and 19% in treatment with a higher rate of dolomite or lime (average for two years). No significant differences between the applied rates were detected. Fertilization in combination with lime/dolomite and borax also significantly reduced the Al in the leaves. In the first year, these treatments recorded the largest decrease in Al in the leaf (by 35%), compared to the control, but in the second year, its content increased significantly and was higher than that in lime treatments without borax. There was a significant correlation between Al concentrations in soil and leaves in the second year ($r = 0.766$) (Supplementary Table 2).

The concentrations of Fe in raspberry leaves were in the range from 128 up to 164 mg kg^{-1} in the first year and 109–139 mg kg^{-1} in the second year (Table 2). The application of lime and dolomite without borax, in almost all treatments, showed a significant reduction of Fe concentration in raspberry leaves (the exception was the treatment with 2 t ha^{-1} of lime in the first year). In the raspberry fruits, lime and dolomite application decreased the Fe concentration in both years. The decrease was more pronounced in the treatments with higher doses of lime materials. In the treatments with the combined application of lime or dolomite with borax, Fe was not significantly decreased in the leaves compared to the control (4–9% in the second year), while in the fruits, a significant reduction of this element was found (on average by 26%). No significant correlation was found between the content of Fe in the soil and plant organs, as well as between Fe in leaves and fruits (Supplementary Table 2).

In all treatments, during both years, extremely high concentrations of Mn were measured in the leaves and raspberry fruits. Mn in the leaves was up to 2453 mg kg^{-1} of dry matter and in the fruits, was up to 251 mg kg^{-1} of dry matter in the controls. All treatments reduced these Mn values compared to control, but still they remained high. Differences in the Mn content between the same treatments with different doses of fertilization were significant. No significant correlation was found between the Mn content in soil and plant (Supplementary Table 2), while there were significant positive correlations between the Mn content in leaves and fruits in both years.

In the first year, the Cu concentrations in the leaves in all treatments were satisfactory. The application of lime and dolomite in the first year showed a significant reduction of Cu in all lime treatments. A higher dose of lime and dolomite affected the reduction of Cu in the leaves by 23% and 32%, respectively. In the second year, Cu concentrations in the leaves are much higher than those in the first year, up to 126 mg kg^{-1}. The Cu content in the fruits in the control treatments was from 10–11 mg kg^{-1}, which is in accordance with the previous results (Kowalenko, 2005; Tešović and Dulić, 1989). The application of lime materials reduced the Cu concentrations in fruits in both years by 20–30% (up to 7 mg kg^{-1})(Table 3).

The concentrations of Zn in raspberry leaves (29–40 mg kg^{-1}) and fruits (10–16 mg kg^{-1}) during both examined years were within the optimal values or values commonly detected in previous researches (Hanson, 1996; Kowalenko, 2005; Milinković et al., 2021). In relation to the control, a significant reduction of Zn was found in all lime treatments: in leaves by 7–26% (in the second year) and in fruits by 18–37% (in both years). A significant effect of the applied dose of lime materials on the Zn content in the leaves was also detected (average for two years), so treatments with a higher dose of lime materials had a greater decrease in the

Table 3. Influence of lime treatments on content of Fe, Mn, Cu, Zn, and B (mg kg^{-1}) in the fruit of the raspberry.

Treatments	Fe	Mn	Cu	Zn	B
			mg kg^{-1}		
First year					
Control (1 t ha^{-1} NPK)	72 a	251 a	10 a	16 a	8 d
CaCO$_3$ (2 t ha^{-1})	60 b	216 b	8 bc	13 b	8 d
CaCO$_3$ (4 t ha^{-1})	53 c	192 c	8 bc	13 bc	9 cd
MgCaCO$_3$ (2 t ha^{-1})	61 b	195 c	8 bc	12 c	9 cd
MgCaCO$_3$ (4 t ha^{-1})	52 c	179 d	7 c	13 bc	10 c
CaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	60 b	176 d	8 b	11 d	19 a
MgCaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	62 b	150 e	8 bc	10 e	17 b
Second year					
Control (1 t ha^{-1} NPK)	50 a	203 a	11 a	16 a	7 bc
CaCO$_3$ (2 t ha^{-1})	38 c	133 c	8 d	13 bc	6 c
CaCO$_3$ (4 t ha^{-1})	32 d	117 de	9 bc	12 c	6 c
MgCaCO$_3$ (2 t ha^{-1})	46 ab	120 de	10 b	13 b	6 bc
MgCaCO$_3$ (4 t ha^{-1})	37 c	110 e	9 c	12 bc	7 bc
CaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	30 d	148 b	9 cd	12 c	10 a
MgCaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	44 b	123 cd	9 cd	11 b	9 a
Average for two years					
Control (1 t ha^{-1} NPK)	61.0 a	227.0 a	10.5 a	16.0 a	7.5 de
CaCO$_3$ (2 t ha^{-1})	49.0 c	174.5 b	8.0 d	13.0 b	7.0 c
CaCO$_3$ (4 t ha^{-1})	42.5 d	154.5 d	8.5 bc	12.5 b	7.5 de
MgCaCO$_3$ (2 t ha^{-1})	53.5 b	157.5 cd	9.0 b	12.5 b	7.5 de
MgCaCO$_3$ (4 t ha^{-1})	44.5 d	144.5 e	8.0 d	12.5 b	8.5 c
CaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	45.0 d	162.0 c	8.5 bc	11.5 c	14.5 a
MgCaCO$_3$ (2 t ha^{-1}) and Borax (50 kg ha^{-1})	53.0 bc	136.5 f	8.5 bc	10.5 d	13.0 b
ANOVA					
Treatment	***	***	***	***	***
Year	***	***	***	**	***
Interaction	***	***	*	***	***

a-e: Means in a column followed by the same subscript letters are not significantly different according to Duncan multiple range test ($p < 0.05$). * – significant at P ≤ 0.05, ** – significant at P ≤ 0.01, *** – significant at P ≤ 0.01, ns – not significant according to two-way ANOVA.

concentration of Zn in the leaves, which was not the case with fruits. In addition, the decrease in Zn in the leaves was more influenced by 4 t ha^{-1} of dolomite than lime. The applied quantities of lime materials, although they reduced the Zn content in plant organs, did not cause its deficiency.

The B concentration in raspberry leaves in all treatments without borax was below the optimal values (25–50 mg kg^{-1}, Hanson, 1996) although the soil is well supplied with this element. The application of lime fertilizers, without borax, significantly reduced the B concentration in the leaves, during both years (from 28 up to 50%). In the treatments with borax (in combination with lime and dolomite), the B content in the leaves increased by 550% (in the first year) and 350% (in the second year).

The concentration of B in the fruit, in all treatments was within the values given by Kowalenko (2005): 2–16 mg kg^{-1}. In treatments with lime but without borax, during both years, only a slight variation of B concentration in fruits was found, without significance, while in the treatments with borax, a significant increase in this element was found. A significant correlation was found between the content of available B in the soil and its content in leaves and fruits during both years (Supplementary Table 2).

Discussion

In our research, the application of lime materials caused the decrease of Al and Mn toxic concentrations and decrease of extremely high Fe concentrations in the soil, which is in agreement with the previous findings (Caires et al., 2008; Dugalić et al., 2006; Sikirić et al., 2009). An increase in soil pH (up to 0.65 units) after calcification in the same raspberry plantation, as observed by Sikirić et al. (2015), might have caused the decrease of these elements. Similarly, Sikirić et al. (2009) reported that the application of 3 t ha^{-1} of lime or dolomite induced a small increase in pH of 0.4 units, but influenced a significant decrease up to 90% in soil mobile Al and up to 54% in available Fe concentrations. It was noted that the decrease in pH for only 0.1 units can double the mobile Al concentration in the root system zone (Foy et al., 1974). In addition, the positive effect of lime application in acidic soils in terms of the pH increase and mobile soil Al concentrations decrease was confirmed in several studies (Edmeades and Ridley, 2003; Grewal and Williams, 2003).

Dolomite had a weaker effect on the Fe reduction compared to lime, which can be not only the results of the lower solubility of dolomite (CaMgCO$_3$) but also the results of the antagonistic action of Ca and Mg in soil solution as was observed in the previous studies (Järvan and Poldma, 2004; Michalk and Huang, 1992; Sikirić et al., 2015). Besides lime and dolomite, their combination with borax also influenced the reduction of Fe in the soil in the first year; however, in the second year these treatments with borax did not differ compared to the control. Barman et al. (2014) reported a significant reduction of soil Fe by lime application, while lime and borax combination did not affect the available Fe status in acidic soil. On the other hand, introduction of a larger amount of borax (Na$_2$B$_4$O$_2$) into the soil in the first year of our experiment could have increased the Na concentration in the soil solution, causing a stronger binding of this element in the soil adsorption complex, whereby other cations, primarily Ca and Mg, were pushed into the soil solution influencing the reduction of the Fe concentration (Džamić and Stevanović, 2000). This is supported by the research of Sikirić et al. (2015) where a significant increase in Ca and Mg in the soil solution due to the application of borax was found. None of the treatments in the examined soil reduced the concentration of available Fe to the optimal level.

Similar to our results, the decrease of the Mn concentration in the soil after calcification was previously detected (Grewal and Williams, 2003). Very high concentrations of Mn in our soil can be the results of low soil pH and soil reducing properties. Sharma et al. (2003) reported strong negative

correlation between pH and the content of available Mn. It is well known that soil Mn available concentrations are mainly influenced by soil redox potential and the other factors that affect its value (pH, organic matter content, microbiological activity, soil moisture, etc.)(Kastori, 1990).

Calcification has a generally positive effect on reducing high concentrations of available Cu (Barman et al., 2014). In our study, weak changes in soil Cu under the influence of calcification can be the result of a low pH increase in the soil and/or its strong connection with organic matter, as was also noted in previous works (Fageria et al., 2002; Moreira et al., 2017). The soil pH influences the Cu^{2+} solubility, and each pH unit increase decreases its solubility 100-fold (Fageria et al., 2002). In our experimental field, the highest pH increase was noted for 0.65 units, as was published by Sikirić et al. (2015). The application of higher doses of lime materials and the establishment of higher pH values would probably lead to a significant reduction of Cu content in the soil.

The presence of $CaCO_3$ is crucial in reducing the solubility of Zn (Moreira et al., 2017). In our study, the changes of Zn were about the mean content, and the applied doses of lime materials did not lead to a Zn-deficient content in the soil (Alloway, 2008). The lower values of Zn in the second year are the result of an increased precipitation and the tendency of this element to be easily rinsed in acidic soils.

Liming generally decreases the B content in the soil and a significant negative correlation exists between soluble B and $CaCO_3$ content (Prodromu, 2004; Rosolem and Bíscaro, 2007). In our work, application of lime materials without borax slightly decreased the available content of B in the soil only in the first year, probably due to the low pH increase as was previously found (Sikirić et al., 2015). This reduction of the available content of B did not lead to its deficiency in the soil, i.e. to the critical pH level at which boron deficiency occurs (Bročić, 1994). The values of B in the second year were generally lower, compared to the first year. Heavy rainfall in the second year of testing, especially in the first half of the year, could cause intensive leaching of B from the soil (similar to Zn). Very acidic soil influences increased B solubility, as well as other elements, where B is easily rinsed, and its deficiency is often seen in such soils (Broadley et al., 2012).

Although being reduced, the Al values in leaves in our study were still high in all treatments compared to the most common values for different plants (100–200 mg kg^{-1})(Negreanu-Pirjol et al., 2019). The Al reduction in the soil was not followed by its proportional reduction in the leaves, which was also previously seen (Sikirić et al., 2009). It was shown that soil mobile Al and its concentration in the root are the most accurate parameters for determination of detrimental effects on plants (Ramaškevičiene et al., 2002).

The applied doses of lime materials slightly reduced the concentrations of Fe in leaves and fruits. The highest measured Fe concentration in leaves was 161 mg kg^{-1}, which is close to the critical concentration for cultivated plants (Kastori, 1990), but below the potentially toxic concentrations. According to some authors, the Fe concentrations from 207 to 535 mg kg^{-1} recorded in raspberry leaves late in the season should be considered toxic (threshold of 250 mg Fe kg^{-1})(Kowalenko, 2005). Furthermore, Hanson (1996) reports that the optimal Fe values in raspberries are 50–300 mg kg^{-1}. In previous studies, high Fe concentrations in leaves were also found (mean Fe concentration 191 mg kg^{-1})(Dresler et al., 2015). The concentrations of Fe in fruits in the first year were somewhat higher, and in the second year mainly within to the previously detected values of 38 mg kg^{-1}(Tešović and Dulić, 1989) and 27–55 mg kg^{-1} (Kowalenko, 2005). Therefore, the values from our study in leaves and fruits are among the commonly found values, despite the high Fe concentrations in the soil. According to Hanson (1996), an increase in the Fe content in the soil affected its slight increase in the aboveground part of the plant, and in some cases, a decrease. At the same time, the Fe content in the root increased up to 5 times. In many cases, the correlation between the total Fe content in plants and its level in the soil cannot be determined (De Kock, 1981).

In our research, high concentrations of Mn in the leaves and raspberry fruits were detected even after the liming. The optimal values of Mn in raspberry leaves are 35–150 mg kg^{-1} (Hanson, 1996) while Mn concentrations found in fruits were 23 mg kg^{-1} (Tešović and Dulić, 1989) and 13–27 mg kg^{-1}

(Kowalenko, 2005). Previously, very high concentrations of Mn in raspberry leaves (the mean concentration of 702 mg kg^{-1}) were also found (Dresler et al., 2015), hypothesizing that the low pH of the soil, may be one of the causes. High to toxic concentrations of Mn in plants occur in soils with unfavorable physical properties with a strong reducing ability and in the soils with an acidic reaction (Karaklajić-Stajić et al., 2012). Especially in rainy years (the second year of our experiment), due to the lower permeability, this soil becomes very unfavorable, moisty, and heavy, with pronounced reducing properties, which greatly affects the increase in toxic concentrations of Mn (Millaleo et al., 2010). The application of all lime materials significantly reduced the Mn concentration in leaves and fruits. The decrease was consistent with the applied dose and the increase in soil pH (Fageria and Nascente, 2014). It is considered that, when the pH increases, due to the pronounced antagonism with Ca, the content of any other element does not decrease to the same extent as the content of Mn (Kastori, 1990). Generally, the applied doses of lime materials during the calcification in our research, as well as the calcification measure itself, were insufficient to reduce such high values of Mn in the soil and plant organs.

After liming, the Cu concentrations in leaves and fruits decreased, but not below the optimal values. Optimal concentrations of Cu in raspberry leaves are 10–30 mg kg^{-1} (Hanson, 1996). Symptoms of Cu deficiency in the leaves of higher plants occur at concentrations lower than Cu 4 mg kg^{-1} of dry matter, and symptoms of excess at concentrations higher than 30 mg kg^{-1} of dry matter (Ubavić et al., 2001). Previously, the concentration of Cu in the fruits of the Wilamet variety was 8 mg kg^{-1} of dry matter (Tešović and Dulić, 1989) and in the range from 4 to 6 mg kg^{-1} (Kowalenko, 2005; Milinković et al., 2021), which is similar to our study. In our research, no correlation was found between the available Cu content in the soil and in the raspberry leaf or fruit, while a significant correlation was found between the Cu content in leaves and fruits in the first year ($r = 0.951$). Although the effect of calcification on the Cu content was not shown in the soil, due to the influence of other factors, the reduction of the Cu content became noticeable in leaves and fruits (Ginocchio et al., 2002). Generally, the application of lime materials, due to the effect of increasing the pH value, affected the reduction of solubility and absorption of Cu in leaves and fruits (Lutz et al., 1972). The application of NPK fertilizer with the Ca component influenced the variation of Cu in raspberry fruits (Lenartowicz, 1986). A significant increase of Cu concentrations in the leaves in the second year of our experiment cannot be related to the influence of lime materials and is considered a result of copper-based products application for disease protection as was also observed in previous studies (Milinković et al., 2021).

The applied quantities of lime materials reduced the Zn content in plant organs, but did not cause its deficiency. Similarly, there were no changes in Zn in soybean leaves after liming (Moreira et al., 2017). The Zn concentration in the raspberry leaves of our experiment was between 35 and 40 mg kg^{-1}, indicating no risk of its deficiency since the optimal content of Zn in leaves is 20–50 mg kg^{-1} (Hanson, 1996). Moreover, lower Zn values from the ones in our study were previously often measured in raspberry leaves (Dresler et al., 2015; Kowalenko, 2005; Tešović and Dulić, 1989). The Zn values detected in fruits in our study were similar to the values detected by Milinković et al. (2021) (12.5–14.3 mg kg^{-1}) or somewhat lower than Zn values published by Kowalenko (2005) (19–24 mg kg^{-1}). It is recommended to further monitor the Zn content in the soil and plants, due to the various factors that can affect it. First, if the soil reaction is very acidic, the possibility of faster leaching of Zn occurs. Second, the application of higher doses of lime and dolomite can significantly inhibit Zn uptake. Finally, during the uptake of elements by plants, various antagonisms can occur at high concentrations, e.g. between Cu and Zn (Džamić and Stevanović, 2000). Since the uptake of Zn in soil solution is inhibited by alkaline earth metals, the dolomite containing Ca and Mg would strongly inhibit Zn uptake compared to lime, which contains only Ca (Kastori, 1990).

In our study, the application of lime and dolomite reduced B in the soil (not below the optimal values for soil), which was reflected in its decreased content in the leaves being below the optimal values given by Hanson (1996). In the previous findings, the B decrease in soil and plant due to the lime effect on the soil pH increase was also noted (Krug et al., 2011; Lehto and Malkonen, 1994; Steiner and Lana, 2013). In addition, there is an interaction between the adoption of B and Ca which can

further decrease the B content in plants (Gupta, 1993). Krug et al. (2011) reported that the lime ingested through calcification (depending on the dose) increased Ca in plants while at the same time B in plants decreased. On the other hand, the introduction of B into the soil through fertilization increased its content in plants regardless of the lime application (Lehto and Malkonen, 1994). Similarly, in our treatments with lime or dolomite in combination with borax fertilization, the B concentrations increased in the soil and plant.

Conclusion

Application of lime materials significantly reduced the Al, Fe, Mn, Zn, and B in the soil, while Cu remained unaffected. However, very high concentrations of Al in the leaves remained, as well as Mn concentrations in leaves and fruits, while Fe concentrations could be considered acceptable. The lime materials did not reduce Zn in leaves and fruits below the common values. In the treatments without borax, the B concentrations in leaves were below the optimal values, indicating a need for boron product application. Higher doses or periodical application of lime materials should be evaluated in raspberry cultivation in extremely acidic soils, with the aim of further decreasing Al and Mn in leaves and fruits.

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B.S. designed experiments, performed analytical measurements, and wrote the manuscript; O.S.S. performed statistical analysis and wrote the manuscript; E.S. and A.L. wrote and edited the manuscript; M.J. performed statistical analysis; V. M. supervised the work.

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