

## LONG-TERM EFFECTS OF ORGANIC FERTILIZERS ON MICROELEMENTS STATUS IN GRAPEVINE LEAF ON CALCAREOUS SOIL

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### Abstract

*The aim of this study was to determine the effect of different doses of organic fertilizers on the content and dynamics of microelements in vine leaves on carbonate soil during three vegetation. The trial was performed according to randomized complete block design with 6 treatments (unfertilized, farmyard manure 20 t ha<sup>-1</sup> and 40 t ha<sup>-1</sup>, peat 20 000 L ha<sup>-1</sup> and 40 000 L ha<sup>-1</sup>, NPK (5-20-30) 500 kg ha<sup>-1</sup>+2x100 kg UREA kg ha<sup>-1</sup>) in 4 repetitions. Samples of vine leaves were taken three times during the growing period: at the flowering, 2 weeks after flowering and veraison stage. Statistically significant difference in iron leaf content was determined in the first vegetation year (verasion). The highest amount was determined in the treatment with 40 t ha<sup>-1</sup> of farmyard manure (94 mg kg<sup>-1</sup> Fe) and the lowest in the treatment with mineral fertilizers (79 mg kg<sup>-1</sup> Fe). Statistically significant difference in the content of manganese was recorded in the second year of research (flowering). The highest amount was determined in control treatment (30.50 mg Mn kg<sup>-1</sup>), and the lowest amount in the treatment with NPK fertilizer 500 kg ha<sup>-1</sup>+2x100 kg UREA (18.50 mg Mn kg<sup>-1</sup>). In three years of research there were no significant differences between average values of zinc and copper.*

**Key words:** farmyard manure, peat, microelements, grapevine, alkaline soil.

### INTRODUCTION

Viticulture in Croatia is an important part of agriculture and economy. Growing on inadequate rootstock on carbonate soils often reduces the height and quality of yield. On soils with high carbonates content, uptake of iron, zinc, manganese, and copper is significantly lower by the poor solubility of compounds containing the trace elements (Ksouri et al., 2005). Therefore, on such soils, chlorosis often occurs on grapevine, which is a physiological disorder often caused by insufficient or imbalance of essential cations for physiological processes in the plant. This may be due to the high amount of calcium in the soil (carbonate soil) which is usually bound in calcite (CaCO<sub>3</sub>), a relatively poorly soluble calcium mineral, but in the presence of CO<sub>2</sub> and H<sub>2</sub>O turns into a soluble form of calcium bicarbonate (Ca(HCO<sub>3</sub>)<sub>2</sub>), whose dissociated forms of HCO<sub>3</sub><sup>-</sup> and Ca<sup>2+</sup> affect the increase of soil pH (Imas, 2000; Ksouri et al., 2005). The occurrence of

grapevine chlorosis in the Plešivica vineyards is most common in June, during flowering or immediately after flowering, and thus has the strongest impact on reducing the yield of grapes due to poor flowering and fertilization. The most common visible symptoms in grapevine are the appearance of pale green to yellow color on the leaves. If the symptoms appear on younger leaves, we talk about the lack of microelements: iron, zinc, manganese, while on older leaves there are symptoms due to magnesium deficiency (Herak Ćustić et al., 2007). Fertilization of vineyards has a great impact on yield and quality of must and wine. Fertilizer application results in increased yields, however, excessive or unbalanced fertilization can have a negative impact on quality (Delgado, 2004).

The application of organic matter to agricultural soils does not show a significant effect on the total amount of trace elements in the soil, but increases their availability compared to soil fertilized with mineral

fertilizers (Herencia et al., 2008; Moustouli and Verloo, 1995; Tamoutsidis et al., 2002, cit. according to Petek, 2009).

Furthermore, one-side application of mineral or organic fertilizers cannot achieve sustainable agricultural production. Even with a balanced application of mineral fertilizers, high and quality yields are unsustainable over many years due to the negative effect on the physical and biological properties of the soil (Khan et al., 2008). In addition, one-side application of mineral fertilizers and other chemicals often used in agriculture, in addition to adverse environmental effects, may lead to changes in the composition of fruits, vegetables and reduce the amount of vitamins, minerals and other nutrients (Masoud, 2012). The main role of microelements in the plant organism refers to their participation in the activity of enzymes (as a component, cofactor or activator). Because of their ability to change valence, they participate in oxido-reduction processes by transporting electrons (Marschner, 1995). Availability of micronutrients is highly dependent on soil characteristics such as pH, CaCO<sub>3</sub>, organic matter, and available phosphorus (Christensen et al., 1951; Jenne, 1968; Lutz et al., 1997; Olomu et al., 1973; Yuan, 1983; Shuman, 1988, cit. according to Wei, 2005). Interactions with macronutrients also significantly affect micronutrient uptake (Aulakh and Malhi, 2005). It should be noted that temperature and humidity are important factors influencing the availability of micronutrients in the soil. The availability of most micronutrients in the soil decreases at low temperatures and low moisture content due to reduced root activity and reduced dissolution and diffusion (Frageria et al., 2002). Gao et al. (2000) state that organic fertilizer is a better source of available iron, manganese and zinc compared to mineral fertilizers. This is particularly pronounced on carbonate soils because the decomposition of organic fertilizers releases organic acids and CO<sub>2</sub> which leads to lower pH and better nutrient availability (Wei et al., 2005), while Herencia et al. (2008) state that the application of compost does not lead to a significant increase in the total content of micronutrients in the soil, but the available forms increase compared to mineral fertilization. Mann et al. (2006) point out that the highest availability of

micronutrients was found in the combination of manure and mineral fertilizer.

Iron is necessary for chlorophyll synthesis, nitrite and sulfate reduction, nitrogen assimilation and electron transport in the process of photosynthesis (Marschner, 1995; Bergman, 1992). Interactions between iron and calcium in soil and plant are very complex. Calcium reducing the availability of iron and lead to chlorosis on plants on carbonate soils (Kabata-Pendias, 2011). In carbonate soils, most iron is found in the form of oxides and other insoluble forms that are not available to plants (Miller et al., 1984). Iron in the form of Fe (III) oxide is hardly soluble in water, but in the presence of various organic compounds it forms chelates, thus becoming available to plants (Kabata-Pendias, 2011). Zinc is part and activator of many enzymes. The great importance of zinc is in the biosynthesis of RNA and DNA, proteins, auxins, and in the uptake and transport of phosphorus. It is the basic catalytic component of over 300 enzymes (Marschner, 1995). According to Vukadinović and Lončarić (1998) zinc increases plant resistance to disease (through its effect on protein synthesis), drought (reduces transpiration) and low temperatures. Zinc deficiency is most common in soils with a pH of 6.5-8.0, and in the case of carbonate soils, zinc deficiency is often associated with iron deficiency (Herak Čustić et al., 2011). Manganese has great importance in water photolysis (Salisbury and Ross, 1992) and in activation of many enzymes (Marschner, 1995; Bergmann, 1992). Factors affecting the availability of Mn<sup>2+</sup> ions and its deficiency or excess in the plant are: concentration of Mn<sup>2+</sup> ions and other readily reducing manganese compounds in soil, concentration of other cations in soil, cation exchange capacity (CEC), temperature, organic matter, microbiological activity and redox soil potential. However, the deciding factor is the pH value of the soil and the balance between Mn<sup>2+</sup> – Mn<sup>3+</sup> – Mn<sup>4+</sup> ions (Masoud, 2012). The aim of this study was to determine the effect of different doses of organic fertilizers on the content and dynamics of microelements in vine leaves on carbonate soil during three vegetation.

## MATERIALS AND METHODS

Three years fertilization trial was set up on Plešivica wine-growing region, Borička location (northwestern Croatia), in a 10-year old vineyard, cv. 'Sauvignon White' grafted on Kobber 5BB rootstock, planted on soil with quite high pH for grapevine growing (pH<sub>H2O</sub> 8.02), containing 2 mg P<sub>2</sub>O<sub>5</sub> 100 g<sup>-1</sup> soil, 14 mg K<sub>2</sub>O 100 g<sup>-1</sup> soil and 13.5% CaO. The trial was set up according to randomize complete block design with 6 treatments: unfertilized (C), farmyard manure 20 t ha<sup>-1</sup> (FM 1) and 40 t ha<sup>-1</sup> (FM 2), peat 20 000 L ha<sup>-1</sup> (P 1) and 40 000 L ha<sup>-1</sup> (P 2), NPK (5-20-30 500 kg ha<sup>-1</sup>+2x100 kg UREA kg ha<sup>-1</sup>) in 4 repetitions. Samples of grapevine leaves were taken three times during the growing period: at the flowering, two weeks after flowering and at the veraison. Average leaf samples were formed from 80 healthy, fully developed and undamaged leaves, taken opposite to clusters from 40 vinestocks (4 replicates x 10 vinestocks). Dried (105°C) homogenized grapevine leaf samples were analyzed in triplicate and the results are presented as mean values.

After digestion of dry plant material with aqua regia microelements (iron, manganese, zinc and copper) were determined by atomic absorption spectrometry (HRN ISO 11466:2004).

Statistical data analyses were performed using the SAS 8.2 System (2002-2003).

## RESULTS AND DISCUSSIONS

Statistically significant effect of fertilization on iron grapevine leaves content (Table 1) was determined in the first year of the study in third sampling (veraison).

Significantly the highest amount of iron was determined with fertilization of 40 t ha<sup>-1</sup> of farmyard manure (94 mg Fe kg<sup>-1</sup>), while the lowest amount was recorded with fertilization of 500 kg ha<sup>-1</sup> NPK 5-20-30 + 200 kg ha<sup>-1</sup> UREE (79 mg Fe kg<sup>-1</sup>).

In all three years (Figure 1), the average values decreased from the first to the third sampling. Statistically significantly the highest values were found in the first sampling - flowering (136.60, 180.88 and 93.27 mg Fe kg<sup>-1</sup>), and the lowest values in the third sampling - veraison (86.42, 101.21 and 54.56 mg Fe kg<sup>-1</sup>)

Table 1. Iron grapevine leaves content (mg Fe kg<sup>-1</sup> in DW) in grapevine leaves under different fertilization treatments in three year experiment

mg Fe kg <sup>-1</sup> DW				
2008				
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	125,25	83,50	84,75 ab	<b>97,83</b>
FM 1	131,35	94,75	93,50 ab	<b>106,53</b>
FM 2	157,25	87,50	94,00 a	<b>112,92</b>
P 1	143,75	101,25	81,00 ab	<b>108,67</b>
P 2	135,50	91,25	86,25 ab	<b>104,33</b>
NPK	126,50	92,50	79,00 b	<b>99,33</b>
Average	<b>136,60 a</b>	<b>91,79 b</b>	<b>86,42 b</b>	
2009				
C	180,00	103,50	106,00	<b>129,83</b>
FM 1	181,25	119,50	96,00	<b>132,25</b>
FM 2	178,75	113,00	115,00	<b>135,58</b>
P 1	174,00	105,75	96,00	<b>125,25</b>
P 2	187,00	109,25	95,75	<b>130,67</b>
NPK	184,25	114,25	98,50	<b>132,33</b>
Average	<b>180,88 a</b>	<b>110,88 b</b>	<b>101,21 c</b>	
2010				
C	83,73	70,45	50,90	<b>68,36 b</b>
FM 1	102,68	67,63	54,13	<b>74,81 ab</b>
FM 2	104,18	80,28	56,68	<b>80,38 a</b>
P 1	88,55	70,48	58,23	<b>72,42 ab</b>
P 2	92,68	67,60	56,30	<b>72,19 ab</b>
NPK	87,83	67,63	51,15	<b>68,87 b</b>
Average	<b>93,27 a</b>	<b>70,68 b</b>	<b>54,56 c</b>	

Factor level means accompanied by different letters are significantly different, with error p<0.05 according to Tukey's HSD test. Means without any letter indicate no significant differences.

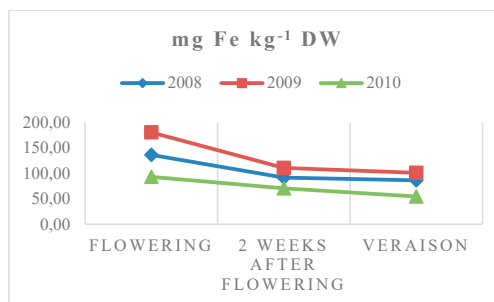


Figure 1. Average iron content (mg Fe kg<sup>-1</sup> in DW) in grapevine leaves by sampling

Determined values are in accordance with Petek et al. (2008), who state that iron grapevine leaves content of Sauvignom blanc variety ranged between 60.17-161.90 mg kg<sup>-1</sup> Fe per dry matter, as well as Fregoni (1998) who determined values 65-300 mg kg<sup>-1</sup> Fe. Furthermore, according to the average annual values of treatments in all three years of research, the highest values of iron in leaves were determined on the treatment with 40 t ha<sup>-1</sup> manure with 40 t ha<sup>-1</sup> (112.92, 135.58 and 80.38 mg Fe kg<sup>-1</sup>) while in the third year the stated value was statistically significantly the highest.

These results are consistent with research by many authors (McCaslin et al., 1987; Parsa and Wallace, 1979; Prasad, 1981, cit. according to Chen et al., 1998) who conclude that iron deficiency in carbonate soils can be corrected by applying organic fertilizers, and Chen et al. (1988) who state that the application of carbon-rich organic fertilizers (manure, compost) on carbonate soils increases the number of microorganisms that produce Fe-chelators.

Furthermore, authors state that the beneficial effect of organic matter in preventing Fe-chlorosis is not only the result of chelation of iron with humic and fulvo components, but also a stimulating effect on soil microorganisms.

Fertilization had no a significant effect on average zinc values (Table 2) in any year of research by individual sampling (flowering, two weeks after flowering, veraison). However, according to the annual average values, in the first and the second year of the study, relatively the highest values was determined on treatment with 40 t ha<sup>-1</sup> farmyard manure (22.80 mg Zn kg<sup>-1</sup>; 11.25 mg Zn kg<sup>-1</sup>).

Table 2. Zinc grapevine leaves content (mg ZN kg<sup>-1</sup> in DW) in grapevine leaves under different fertilization treatments in three year experiment

mg Zn kg <sup>-1</sup> DW				
2008				
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	16,08	17,15	16,25	<b>16,49</b>
FM 1	19,19	16,35	18,25	<b>17,93</b>
FM 2	16,88	15,53	36,00	<b>22,80</b>
P 1	18,04	16,13	17,75	<b>17,30</b>
P 2	17,70	16,30	14,68	<b>16,22</b>
NPK	16,74	16,00	14,00	<b>15,58</b>
Average	<b>17,44</b>	<b>16,24</b>	<b>19,49</b>	
2009				
C	7,20	5,10	19,73	<b>10,68</b>
FM 1	9,15	4,33	12,40	<b>8,63</b>
FM 2	8,18	6,05	19,53	<b>11,25</b>
P 1	7,00	4,88	14,88	<b>8,92</b>
P 2	7,30	8,23	11,70	<b>9,08</b>
NPK	7,98	7,20	15,78	<b>10,32</b>
Average	<b>7,80 b</b>	<b>5,96 b</b>	<b>15,67 a</b>	
2010				
C	20,00	8,85	14,68	<b>14,51</b>
FM 1	13,88	5,40	12,70	<b>10,66</b>
FM 2	18,15	3,68	10,73	<b>10,85</b>
P 1	18,40	6,15	12,94	<b>12,50</b>
P 2	17,38	10,35	11,53	<b>13,08</b>
NPK	21,50	6,13	14,75	<b>14,13</b>
Average	<b>18,22 a</b>	<b>6,76 c</b>	<b>12,89 b</b>	

Factor level means accompanied by different letters are significantly different, with error p≤0.05 according to Tukey's HSD test. Means without any letter indicate no significant differences.

According to average values by individual sampling, the same trend was recorded in all three years (Figure 2). From flowering stage (17.44, 7.8 and 18.22 mg Zn kg<sup>-1</sup>) to two weeks after flowering (16.24, 5.96 and 6.76 mg Zn kg<sup>-1</sup>) values were decreased, and than to veraison stage again were raised (19.49, 15.67 and 12.89 mg Zn kg<sup>-1</sup>).

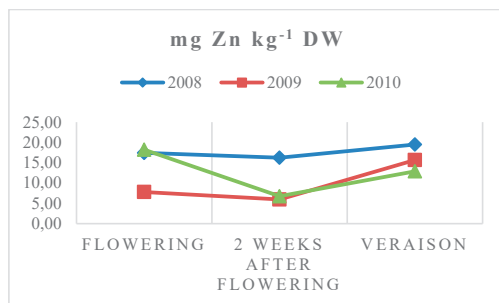


Figure 2. Average zinc content (mg Zn kg<sup>-1</sup> in DW) in grapevine leaves by sampling

Results are consistent with Čoga et al. (2011) who obtained significantly higher values of zinc in grapevine leaves on pseudogley (18.4 mg Zn kg<sup>-1</sup>) in flowering stage compared to rendzina (carbonate soil) (13.4 mg Zn kg<sup>-1</sup>). However, these values are generally low according to Jackson (2000) who stated optimal supply of grapes with zinc is in the range of 25-150 mg Zn kg<sup>-1</sup> while Fregoni (1998) states values of 20-250 mg kg<sup>-1</sup> Zn. Furthermore, relatively low values are in agreement with Herak Čustić et al. (2011) who found a reduced possibility of zinc uptake on soils with a high percentage of physiologically active lime.

The effect of fertilization on the amount of manganese in grapevine leaves (Table 3) was recorded in the second year of research in the first sampling (flowering). The highest amount of manganese was determined on control treatment (30.5 mg Mn kg<sup>-1</sup>) and treatment with 20 t ha<sup>-1</sup> manure (27.75 mg kg<sup>-1</sup> Mn), while the lowest amount was determined on treatment with 500 kg ha<sup>-1</sup> NPK 5-20-30 + 200 kg ha<sup>-1</sup> UREE (18.50 mg Mn kg<sup>-1</sup>).

Table 3. Manganese grapevine leaves content (mg Mn kg<sup>-1</sup> in DW) in grapevine leaves under different fertilization treatments in three year experiment

mg Mn kg <sup>-1</sup> DW				
2008				
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	29,75	37,50	37,75	<b>35,00 a</b>
FM 1	29,40	33,75	36,25	<b>33,13 ab</b>
FM 2	23,98	31,75	31,00	<b>28,91 b</b>
P 1	27,92	28,75	34,25	<b>30,31 ab</b>
P 2	25,06	32,50	34,00	<b>30,52 ab</b>
NPK	26,91	26,75	32,00	<b>28,55 b</b>
Average	<b>27,17 b</b>	<b>31,83 a</b>	<b>34,21 a</b>	
2009				
C	30,50 a	29,75	66,50	<b>42,3</b>
FM 1	27,75 a	32,00	65,0	<b>41,6</b>
FM 2	23,75 ab	33,50	72,75	<b>43,3</b>
P 1	25,50 ab	35,75	73,5	<b>45,0</b>
P 2	25,25 ab	30,50	58,3	<b>38,0</b>
NPK	18,50 b	35,00	69,0	<b>41,0</b>
Average	<b>25,21 c</b>	<b>32,75 b</b>	<b>67,5 a</b>	
2010				
C	18,13	14,88	24,80	<b>19,27 a</b>
FM 1	14,70	12,15	24,43	<b>17,09 ab</b>
FM 2	13,90	11,05	20,60	<b>15,18 b</b>
P 1	18,08	12,68	24,35	<b>18,37 ab</b>
P 2	18,45	13,68	21,40	<b>17,84 ab</b>
NPK	18,75	14,00	27,30	<b>20,02 a</b>
Average	<b>17,00 b</b>	<b>13,07 c</b>	<b>23,81 a</b>	

Factor level means accompanied by different letters are significantly different, with error  $p \leq 0.05$  according to Tukey's HSD test. Means without any letter indicate no significant differences.

According to trend, in the first two years of the study the amount of manganese in grapevine leaves was increase from flowering to veraison, and in the third year it was decrease from the first to the second sampling, and again increase to the third sampling (Figure 3).

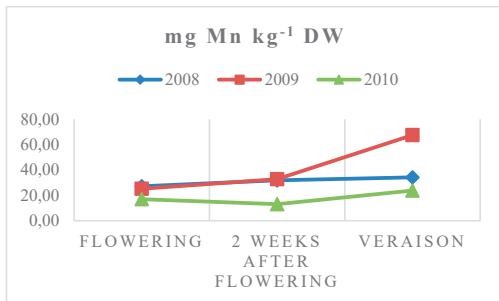


Figure 3. Average manganese content (mg Mn kg<sup>-1</sup> in DW) in grapevine leaves by sampling

However, these values are lower than optimal (30-100 mg Mn kg<sup>-1</sup>) as reported by Bergman (1992). Furthermore, in the first two years of the study, the amount of manganese increased from flowering to veraison, what is consistent

with Čoga et al. (2008) who states the lowest values in flowering (33 mg Mn kg<sup>-1</sup>) and the highest in harvest (100 mg Mn kg<sup>-1</sup>).

Fertilization had no a significant effect on average copper values in any year of research by individual sampling (flowering, two weeks after flowering, veraison), and between average annual values of individual treatments, as well (Table 4).

Table 4. Copper grapevine leaves content (mg Mn kg<sup>-1</sup> in DW) in grapevine leaves under different fertilization treatments in three year experiment

mg Cu kg <sup>-1</sup> DW				
2008				
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	11,63	715,00	501,25	<b>409,29</b>
FM 1	10,87	717,50	274,48	<b>334,28</b>
FM 2	11,22	712,50	266,00	<b>329,91</b>
P 1	11,09	715,00	432,50	<b>386,20</b>
P 2	11,56	747,50	388,25	<b>382,44</b>
NPK	9,79	667,50	452,50	<b>376,60</b>
Average	<b>11,02 c</b>	<b>712,50 a</b>	<b>385,83 b</b>	
2009				
C	7,70	2,78	123,08	<b>44,5</b>
FM 1	8,48	3,50	107,23	<b>39,7</b>
FM 2	8,80	2,13	105,78	<b>38,9</b>
P 1	7,98	2,85	102,35	<b>37,7</b>
P 2	9,33	4,20	95,20	<b>36,2</b>
NPK	8,73	3,80	100,65	<b>37,7</b>
Average	<b>8,50 b</b>	<b>3,21 b</b>	<b>105,7 a</b>	
2010				
C	397,25	505,25	276,25	<b>392,92</b>
FM 1	383,75	526,50	263,50	<b>391,25</b>
FM 2	390,25	508,25	252,00	<b>383,50</b>
P 1	353,75	566,75	273,25	<b>397,92</b>
P 2	394,00	487,50	261,50	<b>381,00</b>
NPK	372,50	468,25	269,00	<b>369,92</b>
Average	<b>381,92 b</b>	<b>510,42 a</b>	<b>265,92 c</b>	

Determined values in individual samples varied in very wide ranges (2.13 to 747 mg Cu kg<sup>-1</sup>). According to the average values of individual sampling, the highest values in the first (712.5 mg Cu kg<sup>-1</sup>) and third (510.42 mg kg<sup>-1</sup> Cu) year were recorded two weeks after flowering, and in the second year of the study in veraison stage (105.70 mg Cu kg<sup>-1</sup>). Determined lowest values differed significantly depending on the year of the study. Thus, in the first year of the study, the lowest value was recorded in flowering (11.02 mg Cu kg<sup>-1</sup>), in the second two weeks after flowering (3.21 mg Cu kg<sup>-1</sup>), and in the third in veraison (265.92 mg kg<sup>-1</sup> Cu) (Figure 4). These differences can be attributed with use of copper plant protection products.



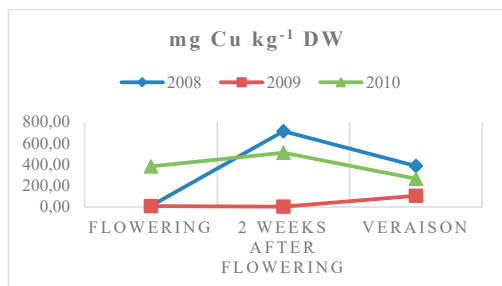


Figure 4. Average copper content (mg Cu kg<sup>-1</sup> in DW) in grapevine leaves by sampling

## CONCLUSIONS

Fertilization with organic fertilizers affected the amount of iron and manganese in grapevine leaves, while the effect of fertilization on the amount of zinc and copper was not determined.

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