

Copper content and distribution in vineyard soils of central Serbia

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Abstract

This research studied the copper content of vineyard soils as affected by the long-term use of copper-based fungicides. The soil samples were taken from individual vineyards located in the central region of Serbia, from two depths: 0-30 and 30-60 cm. At the same time, at each site, control samples were collected from a nearby forest in order to determine the background concentrations. The pseudototal (Cu_T) and available (Cu_{EDTA}) copper content were analysed in 60 soil samples in total, 46 of which represented vineyard soils and 14 control samples. The maximum value of copper was 200.1 mg/kg of pseudototal and 82.1 mg/kg of available copper. Comparison of the copper content in vineyards to the background concentrations of control samples clearly confirmed anthropogenic influence. Out of 46 vineyard soil samples, about one half (22 of them) had the Cu_T concentration above the critical level of 60 mg/kg. Eleven samples had the pseudototal content over the MAC of 100 mg/kg. Anthropogenic influence was also confirmed on the basis of copper bioavailability and copper distribution along the soil profile. Available content of over 50 mg/kg was found in 8 out of 46 analysed samples of vineyard soils. According to the percentage contribution of available Cu_{EDTA} to pseudototal Cu_T , half of the samples were above 36%, which is potentially phytotoxic. The concentration of copper was the highest in the surface layer in the vineyard soil samples. A check of the background Cu levels has shown that the distribution of Cu_T and Cu_{EDTA} is uniform throughout the soil profile. Data from some of the analysed plots indicate that the process of erosion is under way at the site. The soil on lower-lying terrain has been found to be more exposed to copper pollution than the soil of higher terrain. Since copper at the surveyed sites is very persistent and accumulates in a short period of time, focus should be placed on the preventive measures of reducing the use of copper-based fungicides to an optimal level.

Article Info

Received : 04.07.2014

Accepted : 14.10.2014

Keywords: copper, soil, vineyards

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Introduction

Soils where grapevines are grown are especially threatened by copper accumulation due to long-term and intensive application of copper-based pesticides. Bordeaux mixture ($Ca(OH)_2 + CuSO_4$) has a traditional use as a protective agent against downy mildew (*Plasmopara viticola*), one of the most dangerous pathogens of grapevine. Bordeaux mixture originated in vineyards of France in 1885 and it has been used for over a century. Copper-based pesticides, most commonly in the form of copper oxychloride $CuCl_2 \cdot 3Cu(OH)_2$ and copper hydroxide $Cu(OH)_2$ are used in vineyards worldwide, as well as on coffee and hops plantations, orchards and vegetable crops. Numerous recent studies revealed that intensive and long-term application of these agents has negative effect on the environment because it leads to soil contamination with copper

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ISSN: 2147-4249

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(Besnard et al., 2001; Komarek et al., 2010). Copper accumulation in the soil negatively affects soil biota primarily (Paoletti et al. 1998; Merrington et al. 2002). In addition, it can be phytotoxic (Kabata-Pendias and Pendias, 2001) and it can negatively affect vine quality and yield (Mirlean et al., 2005; Garcia-Esparza et al., 2006). Elevated copper concentration in the soil can lead to pollution of river sediment in broader area (Fernandez-Calvino et al., 2008b). The most recent studies indicated paradox that soils in organic production contain more copper than soils under conventional production, which is a consequence of exclusive application of copper-based protective agents (Vavoulidou et al., 2005; Bjorn et al., 2008; Coll et al., 2011).

Copper content studies of the vineyard soils worldwide indicate a serious risk of using copper compounds, which paradoxically until recently were considered safe in comparison to other pesticides (Dixon, 2004). In agreement with previous studies, this problem was also present in soils of Serbia (Ninkov et al., 2010; 2012). Copper content in vineyard soils depends on soil type, i.e. specific physical and chemical properties (pH value, organic matter content, mechanical composition, CEC, C/N ratio, etc.), vineyard age, quantity of applied copper and climate conditions. Researchers in this field (Schramel et al., 2000; Pietrzak and McPhail, 2004) indicate that determination of total content in the soil is insufficient for assessment, but it is necessary to determine its bioavailability, mobility and toxicity. The most recent studies are directed towards development of different techniques to remediate soils loaded with copper. However, since the techniques of remediation are relatively expensive, time-consuming and insufficiently effective, the optimal solution to this problem is preventive measure that will restrict excessive intake of copper in the soil.

The aim of this study was general assessment of soil copper contamination in vineyards of central Serbia.

Material and Methods

Study area

Conforming to regional viticultural classification, Republic of Serbia is divided into 3 regions and 22 production areas. Research presented in this study took place in central part of Serbia, in Šumadija region (Figure 1).

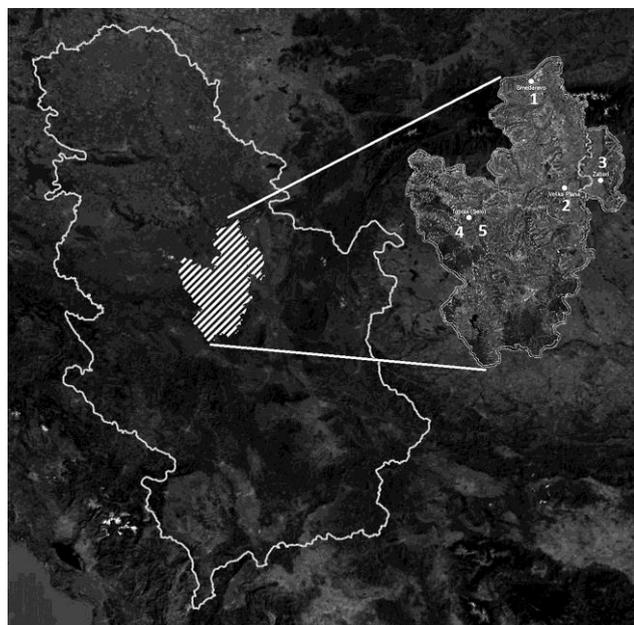


Figure 1. Location map of the study area

Šumadija extends between 4 major rivers (Danube, Sava, Velika and Zapadna Morava) and has hilly macro-landscape geomorphological forms. As reported in World Reference Base (WRB) for soil resources, these are the following reference soil groups: cambisol (eutric) and vertisol (FAO, 2006). According to climate data for last 30 years, average annual temperature was 11.6°C, average amount of precipitation was 618.5 mm. Average amount of potential evapotranspiration was 785.6 mm annually, and 612.6 mm in vegetation period. Number of frost days was 76 annually, the largest number of sunshine hours was recorded during June (8.8 h/day) and average annual value was 5.5 h/day. By virtue of favourable climate, terrain configuration and fertile soil, this region has long tradition in growing grapevine. King's vine cellar (former property of the royal family Karadjordjevic) and Vinča village, which is well known by growing grapevine since the Roman era, are settled in this production region. Studies included five large and well-known complexes of vineyards at sites: 1. Smederevo, 2. Velika Plana, 3. Žabari, 4. and 5. Topola village (Figure 1).

Collecting samples

Samples were collected using agrochemical probes at two depths (0-30 and 30-60 cm). One composite sample represented 20-25 subsamples. Some of the observed plots were divided into more subsamples, according to macro-landscape and previously applied cultivation practices. Terrain altitude of the surveyed

plots was between 103 and 355 m. Size of sub-plot ranged from 0.3 to 3 ha. Total sampled area was 23 ha. At least one more sample from two depths was collected as control sample from every surveyed site. In order to determine background concentration of Cu, control samples were collected from nearby forests and areas that previously had not been under vineyards. Total of 60 samples were collected, where 46 represented vineyard soil and 14 samples were control samples.

Laboratory analyses

The soil samples were air-dried and sieved to a particle size of <2 mm, in accordance with ISO 11464:2006. Particle size distribution was determined in the <2mm fraction by the pipette method. Soil form was determined according to the ISSS soil texture classification. The pH value in 1:2.5 (v/v) suspension of soil in 1 M KCl was determined using a glass electrode upon the ISO method 10390:2005. Organic matter content was measured by sulfochromic oxidation method ISO 14235:1998. The free CaCO₃ content was determined by volumetric method ISO 10693:1995. The samples were analysed for pseudototal contents of Cu after digesting the soil in concentrated HNO₃ and H₂O₂ (5 HNO₃ : 1 H₂O₂, and 1 : 12 solid : solution ratio) by stepwise heating up to 180°C using a Milestone Vario EL III for 55 min. Available Cu concentrations e.g. EDTA-extractable Cu were determined by the EDTA extraction protocols for IRMM BCR reference materials CRM-484: 5 g soil/50 ml EDTA concentration 0.05 mol/L pH=7.00. The concentrations of Cu were determined by ICP-OES (Vista Pro-Axial, Varian).

Results and Discussion

Basic soil properties

Relating to pH reaction of soil, 4 surveyed sites were characterized by dominantly neutral reaction, i.e. pH was between 6.5 and 7.2. Site 3 stands out from the rest, since all of the surveyed plots were in the class of strongly acidic soil (pH < 4.5). Free CaCO₃ content is in accordance with pH reaction, so that site 3 had non-calcareous soil, while other surveyed sites had slightly calcareous soil. Site 5 had slightly calcareous soil in one half, and strongly calcareous soil in another half of surveyed soil. High-carbonate soil in site 5 stands out by the organic matter content, which was 3.5%, while all other plots had organic matter content from 1 to 2%. Tested soil predominantly belongs to textural class of loamy clay (57 samples out of 60 tested). Clay content was in range from 27 to 47% with simultaneous presence of fraction silt and fine sand. Soils of relevant controls, since it is forest soil, have slightly lower pH reaction and more organic matter compared to vineyard plots.

Pseudototal copper content Cu_T

Copper concentration in vineyard soil fully depends on quantity of its intake in agroecosystem, which is related to vineyard age and number of treatments during a year. Number of treatments depends on climate conditions. As stated in the previous studies, vineyard soils in more humid climate areas are loaded with higher copper concentration (Delusia et al., 1996; Brun et al., 1998; Mirlean et al., 2007). Beside quantity of applied copper fungicides, Cu content in the soil depends on soil type, i.e. its specific properties. Pseudototal copper concentration in the whole study of vineyard soil ranges from 26 to 200 mg/kg. There is a high standard deviation in pseudototal Cu_T concentration, which is present among sites, as well as with each site (Table 1). Result heterogeneity indicates different application of cultivation practices and vineyard age, and, besides that, many plots were established on the soil that was under vineyards in the past. The highest copper content was confirmed at site 5, which was the oldest vineyard, and the lowest copper content at site 3, which was the youngest vineyard (established in 2006 on the plot that was under vineyards 40 years ago).

Out of 46 surveyed vineyard soil samples, half of them (22 samples) had elevated Cu_T content, over 60 mg/kg, which represents critical concentration according to literature (Schramel et al., 2000; Pietrzak and McPhail, 2004; Wightwick et al., 2006; Rusjan et al., 2007). Relating to the size of total observed area of vineyards (23 ha), 17 ha is threatened by copper accumulation, i.e. monitoring and reduction of copper-based fungicide application should be introduced on ³/₄ of observed areas. Out of 22 samples over 60 mg/kg, 11 of them exceeded the maximum allowable concentration (MAC), which is area of 4.8 ha that is seriously threatened by copper contamination. In the whole study, only one plot at site 5, which is historically the oldest vineyard, had Cu_T content at the level of 200 mg/kg where further remediation of the soil is needed. Based on literature data for copper content in vineyard soils worldwide (Komarek et al., 2010; Ninkov et al., 2012; Toselli et al., 2009), results differ upon every study. Except specified comparison by climate conditions of individual world regions, it is hard to establish other generalizations. Even in the same region, some

authors report different concentrations depending on research year, since copper can accumulate in soil in a very short time. Different manners of laboratory sample digestion are used for total copper content, and, besides that, some authors determine different depths as topsoil, which also hinders result comparison. In every previous study (Komarek et al., 2010), Cu content above critical concentration of 60 mg/kg was found, and larger number of studies indicate Cu_T content above 200 mg/kg, necessitating remediation. Additionally, in accordance with specified data copper content elevates as vineyard ages.

Table 1. Pseudototal Cu_T and available Cu_{EDTA} copper content at surveyed sites in vineyard soils and relevant controls

Site	1	2	3	4	5
Min-Max Cu _T 0-30 cm [mg/kg]	27.8–167.0	50.4–143.5	38.1–50.8	35.1–106.6	62.9–200.1
Average Cu _T ± SD 0-30 cm [mg/kg]	69.4 ± 57.0	105.0±35.4	45.7±5.8	62.9±31.3	132.4±64.1
Control average Cu _T ± SD 0-30 cm [mg/kg]	26.6±2.6	37.7	43.3	38.5±5.3	31.3
Min-Max Cu _T 30-60 cm [mg/kg]	26.1–128.9	47.2–130.1	32.0–43.1	36.2–68.5	50.2–132.4
Average Cu _T ± SD 30-60 cm [mg/kg]	50.2±44.1	80.3±30.7	39.3±5.1	50.6±13.7	100.7±36.5
Control average Cu _T ± SD 30-60 cm [mg/kg]	24.8±0.9	37.5	42.8	34.1±2.5	28.2
Min-Max Cu _{EDTA} 0-30 cm [mg/kg]	6.3–82.1	14.9–68.9	12.8–20.8	6.9–36.2	24.5–74.0
Average Cu _{EDTA} ± SD 0-30 cm [mg/kg]	26.6±31.9	41.9±19.3	16.6±3.3	17.6±12.9	50.5±23.9
Control average Cu _{EDTA} ± SD 0-30 cm [mg/kg]	5.9±0.8	12.8	12.5	7.6±0.8	6.0
Min-Max Cu _{EDTA} 30-60 cm [mg/kg]	5.3–59.2	11.7–52.7	9.8–15.8	3.5–19.2	19.5–49.0
Average Cu _{EDTA} ± SD 30-60 cm [mg/kg]	17.4±23.5	29.0±15.3	14.0±2.8	10.9±7.9	38.5±13.7
Control average Cu _{EDTA} ± SD 30-60 cm [mg/kg]	5.1±0.3	10.3	10.8	4.1±2.3	4.6

Apart from substantial heterogeneity of results in this study, by comparing average concentration of pseudototal copper to relevant control sample for every site, it can be concluded that Cu_T content is elevated in vineyard soil, in relation to background concentration (Table 1), which confirms its anthropogenic origin. Average copper content in the vineyard soil is higher than control samples in topsoil (0-30 cm) for every surveyed site. In deeper layer of the soil (30-60 cm), average Cu_T is higher than control sample at four surveyed sites, while site 3 stands out from this rule (Table 1). Site 3 represents historically youngest vineyard and has the lowest copper content in relation to other sites, so that it has not relocated from topsoil to deeper layer yet.

Available copper content

Available copper content Cu_{EDTA} in this study was determined by extraction with EDTA and contains water-soluble fraction, exchangeable and associated with carbonates (Ramos, 2006). Available copper concentration is highly correlated with pseudototal copper content, which is in accordance with literature data (Brun et al., 1998; Fernandez-Calvino et al., 2008a). Like Cu_T, Cu_{EDTA} content is highest at site 5, which represents historically oldest vineyard, and lowest at site 3, which is the youngest vineyard. Cu_{EDTA} content in the whole study ranged from 6.3 to 82.1 mg/kg in topsoil (Table 1). In comparison to relevant control samples, Cu_{EDTA} content was also higher in vineyard soil than in background concentration in topsoil, as well as in deeper layer, except at site 3, which stands out from this rule in soil layer 30-60 cm. In a study conducted in Spain (Fernandez-Calvino et al., 2008a), average Cu_{EDTA} value was 45±13 mg/kg in vineyards that are 30-50 years old. Cu_{EDTA} content over 50 mg/kg is considered potentially phytotoxic (Wasterhoff et al., 1995). In this study, 8 samples exceeded this limit (6 from topsoil). All these samples simultaneously have Cu_T content over the maximum allowable concentrations (MAC) of 100 mg/kg.

Percentage contribution of available Cu_{EDTA} to pseudototal Cu_T

Percentage contribution of $\text{Cu}_{\text{EDTA}/T}$ represents indicator of copper bioavailability. According to literature data, percentage contribution of over 36% $\text{Cu}_{\text{EDTA}/T}$ pose a threat of copper phytotoxicity for some plant species that are intolerant to elevated copper concentrations, especially in acidic soils (Novoa-Mundoz et al., 2007). A half of surveyed plots had $\text{Cu}_{\text{EDTA}/T}$ over this limit of 36%. In vineyard soil at site 3, which had the lowest copper content, percentage contribution of $\text{Cu}_{\text{EDTA}/T}$ ranged from 33 to 41%. Increased Cu bioavailability at site 3 can be explained by acidic soil reaction, which was determined here. Percentage contribution of $\text{Cu}_{\text{EDTA}/T}$ in control soil ranged from 19 to 32%. High contribution of $\text{Cu}_{\text{EDTA}/T}$ in this study is in accordance with literature data, where copper bioavailability was confirmed to be higher in vineyard soils than in unpolluted soils. According to Pietrzak and McPhail (2004), 60% of total copper content in topsoil was potentially bioavailable and this bioavailability decreased by profile depth. Fernandez-Calvino et al. (2008a) report 45 to 51% in old and abandoned vineyards, while $\text{Cu}_{\text{EDTA}/T}$ was 27% in young vineyards. Lower values obtained in this study can also be explained by clay fractions presence in surveyed samples, since clay fraction largely affects reduced copper bioavailability in the soil (Ninkov et al., 2013).

Copper along soil profile

Copper distribution by soil depth primarily depends on soil type, i.e. series of its specific physical and chemical properties. In largest number of previous studies, copper concentration was highest in topsoil and rapidly decreased by soil depth under vineyards (Pietrzak and McPhail, 2004; Li et al., 2005; Rusjan et al., 2006; 2007; Mirlean et al., 2007), which was also confirmed in this study. Average Cu_T content was higher in topsoil than in deeper layer (Figure 2), while copper in control soil was uniform by profile depth. Each of 23 vineyard soil subsamples confirms this rule, as well as by Cu_T and Cu_{EDTA} content. Copper accumulates in the first several centimetres of topsoil, it is poorly mobile by soil depth, since it firmly binds to soil organic components, carbonates, clay minerals and manganese and iron oxides (Kabata-Pendias and Mukherjee, 2007). Process of copper relocation from topsoil into deeper layers was found to be very slow (Pietrzak and McPhail, 2004). In our present study, 22 samples that exceeded critical concentration of 60 mg/kg originated from 12 individual surveyed plots. Out of these 12 plots, 4 have elevated Cu content just in topsoil, while 9 plots have elevated Cu content along the whole profile (both in 0-30 cm and 30-60 cm layer). Such distribution indicates that process of copper relocation is under way and represents risk for agroecosystem.

Based on determined Cu distribution by soil depth, anthropogenic influence on Cu accumulation in vineyard soils was confirmed.

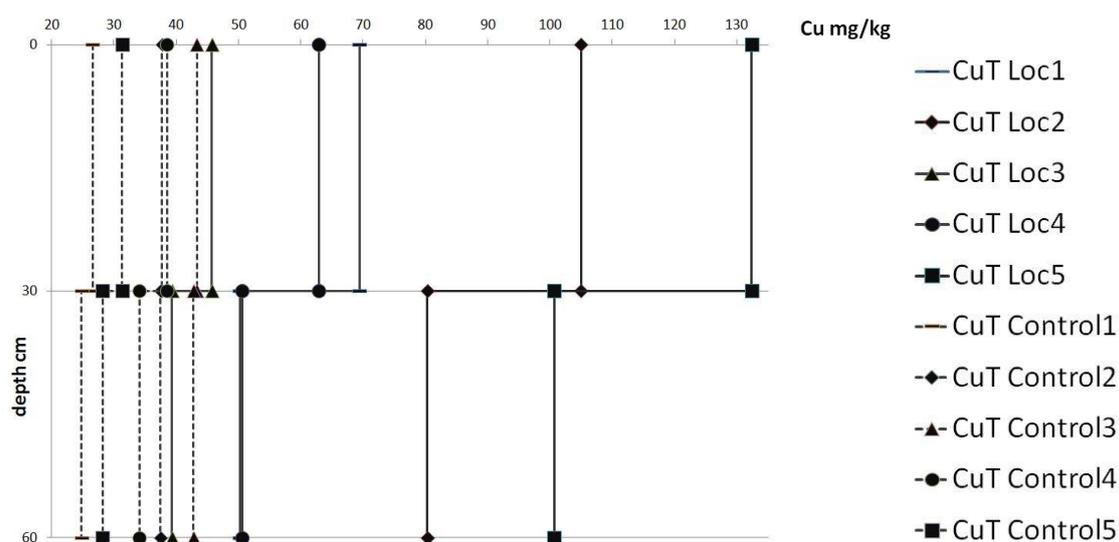


Figure 2. Distribution of pseudototal copper content Cu_T in vineyard soils (solid line) and in control soil (dashed line) by soil profile depth

Copper content and soil erosion

Vineyard soils are prone to erosion to the greatest extent, compared to other soil uses. Cu content in topsoil (0-10 cm) does not indicate quantity of Cu intake in soil and vineyard age, due to leaching or process of water and wind erosion (Wightwick et al., 2006). In this study, erosion was analysed from obtained data

from 6 plots with same history and applied cultivation practices, which were divided in two during sampling, due to present altitude difference. Altitude difference between plot halves ranged from 6 to 14 m (Table 2). As for topsoil, surveyed plots of lower altitude had higher organic matter content and lower carbonate content than higher altitudes, which indicate erosion. According to pseudototal content and bioavailable copper, every observed area had higher copper content at lower altitudes in comparison to higher altitudes (Table 2). Accumulated copper quantity from topsoil of higher altitudes can lead to contamination of lower altitude soils through water and wind erosion (Fernandez-Calvino et al., 2008b). Copper that enters open watercourses is highly toxic and poses a risk for the environment, which is another reason for vineyard soils protection from copper contamination.

Table 2: Pseudototal Cu_T and available Cu_{EDTA} copper content in relation to altitude on plots with same history and cultivation practices

Site	ALT Plot [m]	Cu_T [mg/kg] lower plot	Cu_T [mg/kg] higher plot	Cu_{EDTA} [mg/kg] lower plot	Cu_{EDTA} [mg/kg] higher plot
1	176-193	167.0	57.5	82.1	12.4
2	164-178	127.3	91.2	50.5	31.4
2	168-174	143.5	132.2	69.0	53.2
2	150-159	85.3	50.4	32.5	14.9
3	246-259	49.6	44.1	16.7	16.0
5	321-328	200.1	171.4	74.0	67.2

Conclusion

Pseudototal copper content over the critical concentration of 60 mg/kg was found at $\frac{3}{4}$ of surveyed vineyard areas. Cu_T content that exceeds MAC of 100 mg/kg was found on 4.8 ha, so that this area is seriously threatened by copper contamination. Available Cu_{EDTA} content was also very high. Available content above phytotoxic concentration of 50 mg/kg was confirmed in 8 out of 46 surveyed samples of vineyard soil.

Anthropogenic influence on vineyard soil contamination was attested based on comparison to control samples, since Cu_T and Cu_{EDTA} contents were higher than background concentration. It was also validated by profile depth copper distribution, because Cu content was higher in topsoil and significantly decreased with soil depth, while Cu content in sample soils was uniform. Based on percentage contribution of Cu_{EDTA} in Cu_T , copper in vineyard soils was available at higher extent, compared to control samples.

According to general assessment in this study, soil monitoring and reduction of copper-based fungicide application should be implemented on $\frac{3}{4}$ of viticultural areas of central Serbia. Since copper accumulates in the soil very fast and stays in it for a long time, special attention should be directed toward preventive measures of copper-based fungicide application reduction. A broader education of producers about copper fungicides application harmfulness is also a significant precautionary approach.

Acknowledgements

This study was conducted as part of the Project No. TR 31072: "Status, trends and possibilities to increase the fertility of agricultural land in the Vojvodina Province", which is supported by the Ministry of Education, Science and Technological Development and Project „Preservation and Development of Vineyard Soils in Republic of Serbia“ which is supported by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia.

References

- Besnard, E., Chenu, C., Robert, M., 2001. Influence of organic amendments on copper distribution among particle-size and density fractions in Champagne vineyard soils. *Environmental Pollution* 112: 329-337.
- Bjorn, P., Shuler, C., Joergensen, R.G., 2008. Vineyard soils under organic and conventional management - microbial biomass and activity indices and their relation to chemical properties. *Biology and Fertility Soils* 44: 443-450.
- Brun, L.A., Maillet, J., Richarte, J., Herrmann, P., Remy, J.C., 1998. Relationships between extractable copper, soil properties and copper uptake by wild plants in vineyard soils. *Environmental Pollution* 102: 151-161.
- Coll, P., le Cadre, E., Blanchart, E., Hinsinger, P., Villenave, C., 2011. Organic viticulture and soil quality: A long-term study in Southern France. *Applied Soil Ecology* 50: 37-44.
- Delusia, A., Giandon, P., Aichner, M., Bortolami, P., Bruna, L., Lupetti, A., Nardelli, F., Stringari, G., 1996. Copper pollution in Italian vineyard soils. *Communication in Soil Science and Plant Analyses* 27: 1537-1548.
- Dixon, B., 2004. Pushing Bordeaux mixture. *The Lancet Infect. Dis.* 4, 594.

- Fernandez-Calvino, D., Novoa-Munoz, J.C., Lopez-Periago, E., Arias-Estevez, M., 2008a. Changes in copper content and distribution in young, old and abandoned vineyard acid soils due to land use changes. *Land Degradation and Development* 19: 165-177.
- Fernandez-Calvino, D., Rodriguez-Suarez, J.A., Lopez-Periago, E., Arias-Estevez, M., Simal-Gandara, J., 2008b. Copper content of soils and river sediments in a winegrowing area, and its distribution among soil or sediment components. *Geoderma* 145: 91-97.
- Food and Agriculture Organization (FAO), International Soil Reference and Information Centre (ISRIC) and International Union of Soil Science (IUSS), 2006. World Reference Base for Soil Resources WRB, 2nd edition, World Soil Resources Reports No. 103., Rome
- Garcia-Esparza, M. A., Capri, E., Pirzadeh, P., Trevisan, M., 2006. Copper content of grape and wine from Italian farms. *Food Additives and Contaminants* 23: 274-280.
- Kabata-Pendias, A., Pendias, H., 2001. Trace Elements in Soils and Plants. Third Edition. CRC Press, USA.
- Kabata-Pendias, A., Mukherjee, A.B., 2007. Trace Elements from Soil to Human. Springer, New York. 2007.
- Komarek, M., Čadkova, E., Chrastny, V., Bordas, F., Bollinger J.C., 2010. Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects. *Environment International* 36: 138-151.
- Li, W.Q., Zhang, M., Shu, H.R., 2005. Distribution and fractionation of copper in soils of apple orchards. *Environmental Science and Pollution* 12: 168-172.
- Merrington, G., Rogers, S.L., van Zwieten, L., 2002. The potential impact of long-term copper fungicide usage on soil microbial biomass and microbial activity in an avocado orchard. *Australian Journal of Soil Research* 40: 749-759.
- Mirlean, N., Roisenberg, A., Chies, J.O., 2005. Copper-based fungicides contamination and metal distribution in Brazilian grape products. *The Bulletin of Environmental Contamination and Toxicology* 75: 968-974.
- Mirlean, N., Roisenberg, A., Chies, J.O., 2007. Metal contamination of vineyard soils in wet subtropics (southern Brazil). *Environmental Pollution* 149: 10-17.
- Ninkov, J., Zeremski-Škorić, T., Sekulić, P., Vasin, J., Milić, S., Paprić, Đ., Kurjački, I., 2010. Teški metali u zemljištima vinograda Vojvodine. *Field and Vegetable Crops Research* 47: 273-279.
- Ninkov, J., Paprić, Đ., Sekulić, P., Zeremski-Škorić, T., Milić, S., Vasin, J., Kurjački, I., 2012. Copper content of vineyard soils at Sremski Karlovci (Vojvodina Province, Serbia) as affected by the use of copper-based fungicides. *International Journal of Environmental Analytical Chemistry* 92: 592-600.
- Ninkov, J., Milić, S., Sekulić, P., Vasin, J., Zeremski, T., 2013. Influence of soil particle size on copper availability in vineyard soils. Conference Proceedings of the 13th SGEM GeoConference on Water Resources. Forest, Marine and Ocean Ecosystems. 563 – 568.
- Novoa-Munoz, J.C., Queijeiro, J.M., Blanco-Ward, D., Alvarez-Olleros, C., Martinez-Cortizas, A., Gracia-Rodeja, E., 2007. Total copper content and its distribution in acid vineyards soils developed from granitic rocks. *Science of the Total Environment* 378: 23-27.
- Paoletti, M.G., Sommaggio, D., Favretto, M.R., Petruzeelli, G., Pezzarossa, B., Barbaferri, M., 1998. Earthworms as useful bioindicators of agroecosystem sustainability in orchards and vineyards with different inputs. *Applied Soil Ecology* 10: 137-150.
- Pietrzak, U., McPhail, D.C., 2004. Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. *Geoderma* 122: 151-166.
- Ramos, M.C., 2006. Metals in vineyard soils of the Penedes area (NE Spain) after compost application. *Journal of Environmental Management* 78: 209-215.
- Rusjan, D., Strlič, M., Pucko, D., Šelih, V. S., Korošec-Koruza, Z., 2006. Vineyard soil characteristics related to content of transition metals in a sub-Mediterranean winegrowing region of Slovenia. *Geoderma* 136: 930-936.
- Rusjan, D., Strlič, M., Pucko, D., Korošec-Koruza, Z., 2007. Copper accumulation regarding the soil characteristics in Sub-Mediterranean vineyards of Slovenian. *Geoderma* 141, 111-118.
- Schramel, O., Michalke, B., Kettrup, A., 2000. Study of the copper distribution in contaminated soils of hop fields by single and sequential extraction procedures. *Science of the Total Environment* 263: 11-22.
- Toselli, M., Schiatti, P., Ara, D., Bertacchini, A., Quartieri, M., 2009. The accumulation of copper in soils of the Italian region Emilia-Romagna. *Plant Soil and Environment* 55: 74-79.
- Ubavić, M., Bogdanović, D., 1995. Agrohemija. Univerzitet u Novom Sadu, Poljoprivredni fakultet, Institut za ratarstvo i povrtarstvo, Novi Sad.
- Vavoulidou, E., Avramides, E.J., Papadopoulos, P., Dimirkou, A., Charoulis, A., Konstantinidou-Doltsinis, S., 2005. Copper content in agricultural soils related to cropping systems in different regions of Greece. *Communication in Soil Science and Plant Analyses* 36: 759-773.
- Wightwick, A., Mollah, M., Smith, J., MacGregor, A., 2006. Sampling considerations for surveying copper concentrations in Australian vineyard soils. *Australian Journal of Soil Research* 44: 711-717.