Original scientific paper

Copper and Zinc Accumulation in Vineyard Soils Treated with Cu and Zn Containing Phytopharmaceuticals

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ABSTRACT

Long term use of copper and zinc containing phytopharmaceuticals in vineyard areas causes increased accumulation of total Cu and Zn in the surface layer of soil. Copper and zinc accumulation and distribution in vineyard soils is discussed as a step towards understanding Cu and Zn existence in soils and their availability to plants. Several single extractions were performed to the determination of total Cu and Zn content after aqua regia digestion. The use of Cu containing phytopharmaceuticals has increased the total Cu concentration in analyzed vineyard soils to 97 mg/kg compared to background levels of approximately 30 mg/kg.

The amounts of plant available Cu and Zn extracted by the EDTA ranged as follows, when expressed relative to the total Cu or Zn content in the soils: 13.6 % – 40.4 % for Cu and 3.5 % – 11.6 % for Zn.

The study shows that copper stays active in soils longer than ten years, and may result in leaching and can transfer to deeper soil layers and consequently to ground or the surface waters.

KEYWORDS:

Vineyard, Soil, Copper, Zinc, Phytopharmaceuticals.

Received: 4. 12. 2009
Accepted: 10. 7. 2010

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INTRODUCTION

In order to control vine (*Vitis vinifera L.*) diseases, such as downy mildew caused by *Plasmopara viticola*, different phytopharmaceuticals, especially Cu-based ones, have been applied extensively to vineyards. The application of these phytopharmaceuticals ($CuSO_4$ + $Ca(OH)_2$); Cu_2O), including the Bordeaux mixture $CuSO_4 \cdot 3Cu(OH)_2 \cdot 3CaSO_4$ and more recently copper oxychloride $CuCl_2 \cdot 3Cu(OH)_2$ and hydroxide ($Cu(OH)_2$), resulted into increased Cu concentrations in vineyard soils. The content of Cu significantly increases with a vineyard's age.

Heavy metals are the main pollutants which accumulate in vineyards´ ecosystems. Because of viticulture intensive practice, the phytopharmaceuticals and fertilizers are the main source of metal pollution. While the soil purifies slowly and partially, metal pollutants tend to accumulate [1].

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In many regions of the world there is a concern about the risks that the accumulation of copper in agricultural soils may pose to human health, ecological health, and the long-term sustainability of agricultural lands. The phytotoxic effects of copper to plants have been widely studied [2,3], but it is not expected that copper causes phytotoxicity in mature grapevines as they tend to have deep root systems that extend below the surface soils where most of the applied copper tends to remain.

The impacts on soil biology and implications for soil fertility are more of the concern in established vineyards. Several studies have shown the elevated copper concentrations to adversely affect the fertility of vineyard soils. Increased Cu concentrations in soil (more than 80 mg/kg) reduce earthworm abundance; therefore, any harm to earthworm populations could affect the productivity and efficiency of vineyards [4].

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In response to environmental concerns over the use of copper fungicides such as the accumulation of copper in agricultural soils and the potential impact on soil ecology, regulators in some European countries have imposed restrictions on the use of copper-based fungicides. For example, copper use has been banned in The Netherlands, and Switzerland has restricted the amount of copper that can be applied per hectare [2].

Micronutrient elements like Cu and Zn form insoluble compounds and also bond strongly to mineral particles and organic matter. Considering availability to vines, only a nutrient in the soil solution is immediately available to the vines. Deficiency occurs when a nutrients supply is unsufficient to meet a vines demand; conversely, when supply exceeds demand or a nonnutrient element accumulates in the root zone, toxicity may occur.

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In previous research main focus has been pointed out to Zn determination in vineyard soils. Zinc additions in the Orlica vineyard soil in Meranovo were much larger than uptake; the consequence was accumulation of zinc in the soil. The analysis of the Meranovo stream sediment which springs in the Pohorje territory and runs below the vineyard to the river Drava showed much lower zinc concentrations in the stream sediment before the stream reaches the vineyard and than after it leaves the vineyard. The increasing zinc content in the sediment is caused by the use of zinc containing phytopharmaceutical substances and mineral fertilizers [5].

Zinc is hard to wash away, therefore the contents of zinc are the highest in the surface layer.

Mobility of copper and zinc in soil is closely connected to soil reaction. With increased acidity of the soil there is also the increase of the quantity of element available to plants. The content of zinc is considerably lower in acid than in alkaline soil. Zinc is hard to wash away, therefore the contents of zinc are the highest in the surface layer [6,7].

Contents of copper, zinc and other heavy metals in Slovene soil on the country area were researched by Andjelov and Šajn [8,9]. Increased contents of copper and zinc were established in the Carinthia area (the Mežica mine), in Celje area (the Celje zinc processing plant) and in the areas of intensive plantation production of fruit and grape (organometallic phytopharmaceutical pesticides and fertilizers).

High levels of phosphorous, zinc, iron, manganese and aluminum may also restrict copper absorption by roots.

Micronutrients present the limitation of growth, development and consequently production of vine. Considerable deficiency as well as larger surplus of specific nutrients can be detected on vine by visual inspection. Zinc deficiency in vine occurs in the situation where soil is rich with phosphorus, phosphate ions surrounded the roots and cause the change of zinc ions to insoluble zinc phosphate when contacting the roots. The surplus of phosphate ions deactivates zinc ions into insoluble zinc phosphate as well. High phosphorus content in vineyard soil samples is caused by use of mineral fertilizers containing phosphorus (superphosphate, Thomas phosphate, complex NPK fertilisers). Reports of copper deficiency in vineyards are rare. More common are reports of copper toxicity. Copper is an essential element for living organisms, as a constituent of several enzymes and as a redox catalyst in a variety of metabolic pathways. At high concentrations, however, copper inhibits growth and interferes with several cellular processes, including photosynthesis, respiration, enzyme activity, pigment and protein synthesis and cell division [22]. Copper is strongly bound to organic matter. Peat soils and mineral soils with high levels of organic matter (6-10%) are most likely to be deficient in plant available copper. Copper availability is reduced as pH increases to 7 and above. High levels of phosphorous, zinc, iron, manganese and aluminum may also restrict copper absorption by roots. Copper is an important constituent of many proteins like ascorbic acid oxidase, cytochrome oxidase, diamine oxidase, and polyphenol oxidase. Copper is an important nutrient for many microbes. It controls molds and often alleviates perceived zinc deficiencies. Copper interacts with iron and manganese [23].

Predicting the mobility of heavy metals in soils requires the mathematical models that accurately describe metal adsorption in the presence of competing cations. A reaction-based sorption model was developed using a combination of nonspecific cation exchange reactions and competitive sorption reactions to sites with high affinity to heavy metals. The proposed model describes competitive sorption and transport of Cd, Zn, and Ni in pH range between pH 4.6 and 6.5 [8].

Heavy metal extraction efficiency depends on the combination of many factors, such as the liability of heavy metals in soil, the concentration and nature of the chelant, pH and the properties of the soil matrix. Raising soil pH is a prevention mechanism for elution of heavy metals. Chelant assisted soil washing has been widely studied for remediation of contaminated soil. A number of chelants have been tested. For soils contaminated with Pb, Zn, Cu and Cd, ethylenediamine tetraacetate (EDTA) has often been found to be the most effective [13].

The study of transition metals (Cd, Co, Cu, Mn, Mo, Pb and Zn) in the soils of the sub-Mediterranean winegrowing areas in Slovenia confirmed that the main source of transition metals pollutant in the region is intensive viticulture practice, especially the use of copper substances [10,11].

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The object of this research was to investigate the influence of organometallic phytopharmaceutical fertilizers use on copper and zinc dynamic and distribution in vineyard soil. With the use of the control soil samples, differentiation of natural and anthropogenic soil enrichment in heavy metals in wineyard areas, was possible. Content of Cu and Zn in a control sample gives natural soil profile of these two elements in soil, including the atmospheric influence. Total element contents, EDTA- and AL- (ammonium lactate) extractable (plant available) Cu and Zn with flame atomic absorption spectrometry (FAAS) method was determined. Contents of Cu and Zn were determined in wine, produced on vineyard areas, included in research. Weight balance was calculated from the data on copper content in wine (Cu uptake) and content of copper in used phytopharmaceutical substances (Cu addition). In addition, content of Cu in the sediment of the stream at a foothill of the vineyard Orlica was measured. The sediment of the stream was sampled on several sites from the spring to the river Drava. Increased copper concentration in the stream sediment was expected as the evidence of the long term use of the phytopharmaceutical substances containing copper.

METHODS

Soils of two vineyards from both riverbanks of the river Drava near Maribor, Slovenia, were sampled. Surface of the Orlica vineyard in Meranovo is 50,000 m^2 with 17,800 vines. Surface of the Kalvarija vineyard is 45,000 m^2 with 15,000 vines. Upper part of the vineyard Kalvarija (soil sample named Kalvarija 2) and lower part of the vineyard Kalvarija (soil sample named Kalvarija 3) were sampled and analysed separately. The particular vineyards were chosen because of their diversity in acidity of soil – vineyard Orlica possesses acid soil whereas Kalvarija possesses alkaline soil. Quite different content of free carbonates in vineyardsoil Kalvarija was the reason to separate this vineyard in two parts regarding to the altitude. Control soil sample was sampled above the two vineyards on uncultivated surfaces. The distance between sampling sites was approximately 10 m. In all vineyards, soil samples were collected as close to the vine lines as possible and in the middle of the row, between two sampled lines. The samples were sampled with metal probe (2 cm diameter) at depth of 0-20 cm and 20-40 cm. Two lay-

The distance between sampling sites was approximately 10 m.

ers were sampled to assess the distribution of Cu and Zn with depth. We sampled according to ÖNORM L1057 which regulates soil samples taken in vineyards and orchards [14].

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Soil samples were first dried in the air to the constant weight. Roots and larger stones were removed from the dried samples. Samples were ground in the mill and passed through 1 mm sieve. Humidity was determined by weighing 10 g of air-dried sample into paper bag and dryed at 60 °C in a drying machine to the constant weight. Contents of Cu and Zn were calculated according to the dry weight of the sample.

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Copper and zinc were determined by flame atomic absorption spectrometer, SpectrAA-10 (Varian, Mulgrave, Australia) in air/acetylene flame. Zinc absorption was measured with the use of deuterium lamp for background correction at 213.9 nm and copper absorption at 324.7 nm wavelength. Soil samples were digested in PFTE (polytetrafluoroethylene) containers in MDS 2000 microwave oven produced by CEM (CEM, Matthews's n. c., USA).

Phosphorus concentration was determined by spectrophotometric ammonium molybdate method on Varian Cary 1E spectrophotometer (Varian, Mulgrave, Australia) at 659 nm wavelength. For measuring pH in solution, Iskra MA 5750 pH meter was used (Iskra, Horjul, Slovenia) with HEC 0101 combined glass electrode.

Microwave-assisted digestion of soil samples with aqua regia: 0.5000 g ± 0.0001 g of air-dried soil sample were weighted in PTFE container and 16 mL of aqua regia (HNO_3 and HCl, 1+3) were added. The containers were covered and puted in the microwave oven. Heating programme for 12 containers was following: 1 min at 180 W, 4 min at 480 W and 60 min at 650 W. Containers were cooled to room temperature. Contents were poured through wrinkled filter paper (MN 6191/4) into 50 mL volumetric flask, deionized water were added to the mark. Contents of Cu and Zn in filtrate were determined by the FAAS method in air/acetylene flame. Calibration curve was prepared with standard solutions, added the same amount of aqua regia as the samples. A blank sample was prepared for controlling acid purity.

Total microwave-assisted digestion of soil samples: in PTFE container for digestion 1.0000 g ± 0.0001 g of air-dried soil sample were weighted, 10 mL of deionized water, 5 mL of HNO_3, 2 mL of HCl and 3 mL of HF were added. The containers were covered and put in the microwave oven.

The pressure control was programmed on $6.9 \cdot 10^5$ Pa. Twelve containers were heated for 60 min at 650 W. The containers were cooled to room temperature and the content was poured through wrinkled filter paper into 50 mL PE volumetric flask. Deionized water was added to the mark. Cu and Zn contents in the filtrate were determined by FAAS method in air/acetylene flame.

The calibration curve was prepared with standard solutions added the same amount of acids as to the samples. Blank sample was prepared for acid purity control.

Extraction of soil samples in EDTA-solution: 10.000 g ± 0.001 g of fine air-dried soil sample were weighed to 250 mL plastic container and poured over by 100 mL concentration of EDTA-solution (0.05 mol/L). Preparation of EDTA-extraction solution is described in literature [5]. The plastic container was sealed and shaken on horizontal shaker for two hours with 180 min^{-1}. After the shaking process the suspension was filtered through wrinkled filter paper. At first a few mL of the filtrate were discharged. Contents of Cu and Zn in the filtrate were determined by the FAAS method.

Soil sample extraction with AL-solution: into plastic container with cover 5.000 g ± 0.001 g of soil sample were weighted and 100 mL of AL-extraction solution was added. The container was covered and shooked for two hours on rotary shaker at 35 min^{-1}. Suspension was filtered through dry wrinkled filter paper of medium porosity (white strip). The first few mL of the filtrate were discarded. Concentration of phosphorus in the filtrate was determined by measuring molecular absorption at 659 nm wavelength in air/acetylene flame. AL–extraction solution was used as a blank sample. Preparation of AL-solution is described in literature [5]

Soil acidity was determined according to ÖNORM (16), free carbonates by Scheibler [15].

RESULTS AND DISCUSSION

In our research the main focus was pointed to the copper and zinc determination in vineyard soils. Copper addition in the Orlica vineyard soil in Meranovo is much larger than uptake, the consequence being accumulation of Cu in soil.

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Vineyard soils polluted with heavy metals present a serious environmental concern. Copper and zinc originating from the intensive application of Cu- and Zn-based phytopharmaceuticals belongs to the most important contaminants of the vineyard soils. Both elements can migrate through the soil layers in vineyard and pose thus an important risk for groundwater quality.

Detection and quantification limits for total and extractable copper determination methods in soil samples are shown in the Table 1.

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To verify the accuracy of total Cu, standard reference material 2709 (San Joaquin Soil, National Institute of Standards and Technology) was analysed. Comparison of our results with certified values in SRM is given in the Table 2.

It is evident from the Table 1 and Table 2 that sensitivity, precision and accuracy of analytical values were satisfactory.

Considering pH soil value, the particular vineyards were chosen for their diversity in acidity of soil. Vineyard Orlica possesses acid soil whereas vineyard Kalvarija possesses alkaline soil. Control soil samples were sampled above the two vineyards on uncultivated surfaces. Acid soil reaction is caused by the lack of exchanging metal ions. Cu and Zn con-

Table 1:
Linear ranges, detection and quantisation limits for determination of Cu by FAAS and P_2O_5 by MAS.

	Linear range (mg/L)	Detection limit (mg/L)	Quantisation limit (mg/kg)
Cu – total	0.2 – 1.0	0.1060	10.6
Cu – EDTA	0.5 – 5.0	0.0350	3.5
Cu – AL	0.5 – 5.0	0.0300	2.8
P_2O_5	0.1 – 4.0	0.0002	30.0

Table 2:
Precision of total Cu content determination in SRM 2709.

	Mean conc.* (mg/kg)	RSD (%)	Certified value (mg/kg)	Measured value (mg/kg)
Cu	34.6	1.9	34.6 ± 0.7	34.6 ± 1.1

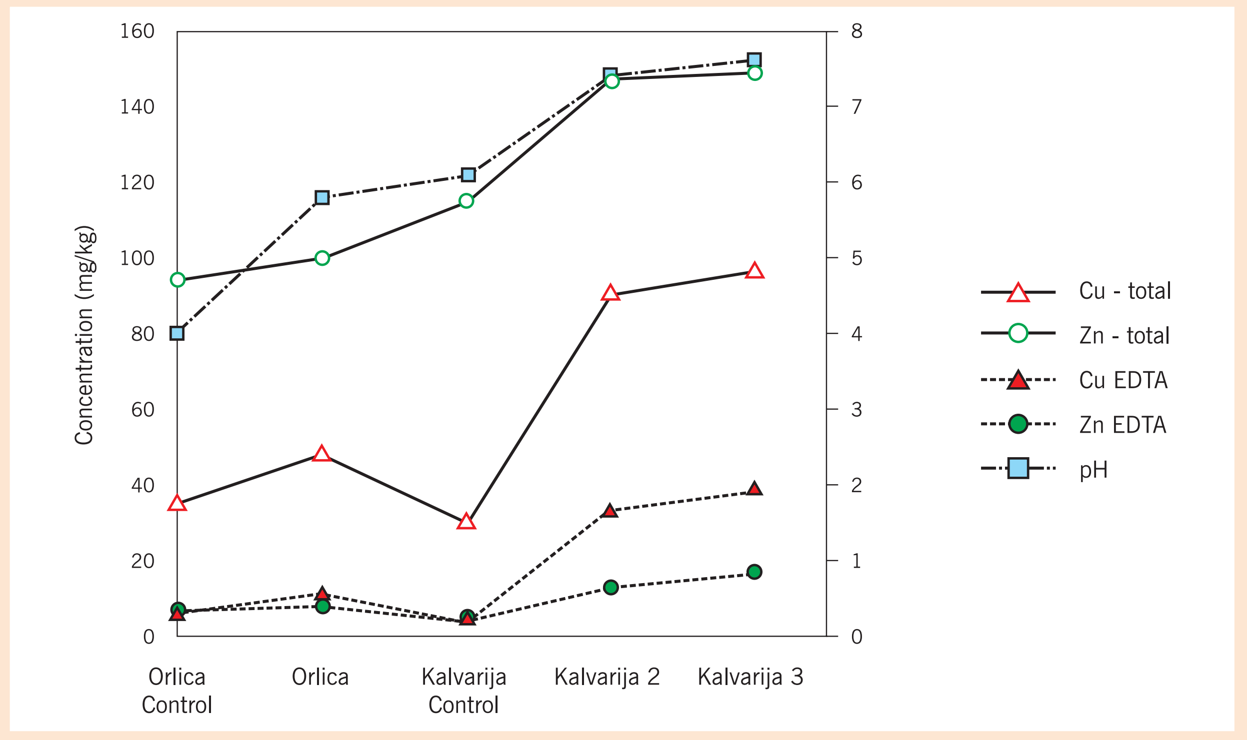
*Mean concentration, result of ten determinations of the same sample.

tent therefore much lower in acid that in alkaline soil. Results of comparison between total copper and zinc content in the vineyard soil samples and control samples to pH value of the samples are shown in the Figure 1.

The use of Cu containing phytopharmaceuticals has increased to total Cu concentration in analysed vineyard soil, 97 mg/kg, compared to background levels of approximately 30 mg/kg.

Content of the Zn increases with soil pH value in all analysed vineyard soil samples and control samples. Content of the Cu trended increasing

Figure 1.
Correlation between total and EDTA extractable Cu and total and EDTA extractable Zn content (amounts in mg/kg on primary axis) and pH soil value (amounts on secondary axis).



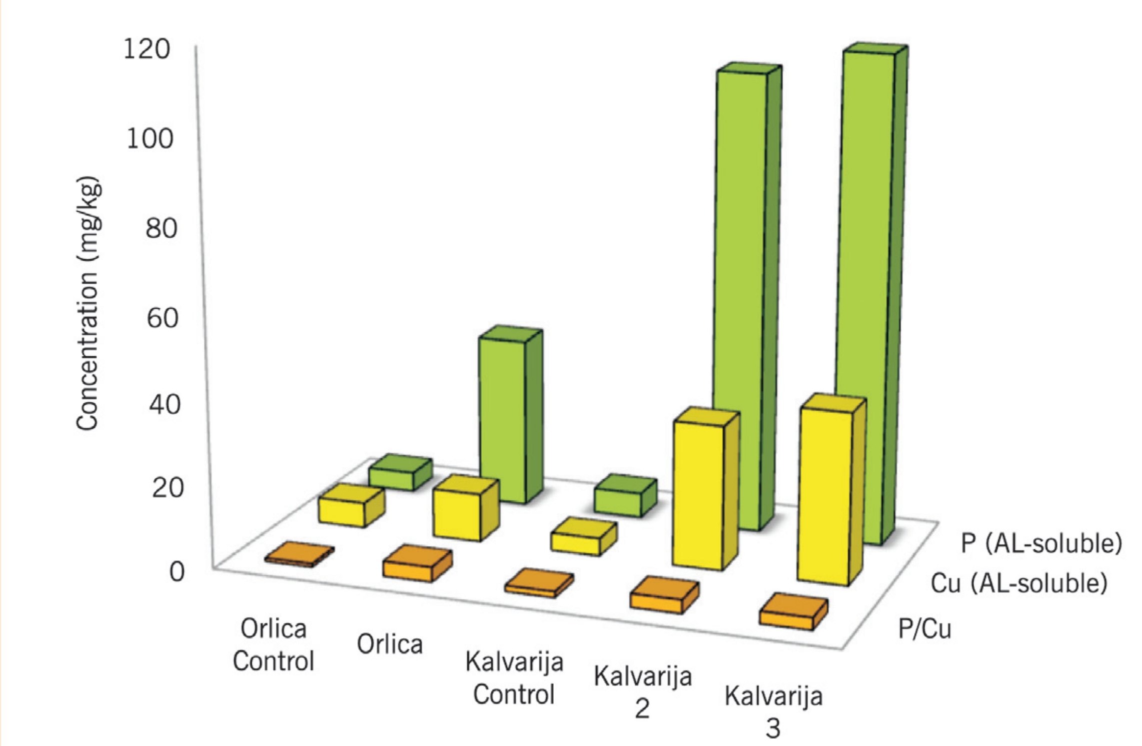


Figure 2.
Content of AL-soluble copper and phosphorus, and P/Cu proportion in soil samples.

with pH soil value with one exception – the control soil sample from Kalvarija vineyard area. Mobility of copper and zinc in soil is closely connected to soil reaction. With increased pH value of soil there is also an increase in the quantity of element available to the plants. Contents of copper and zinc are considerably lower in acid than in alkaline soil.

Amounts of plant available Cu and Zn extracted by the EDTA ranged as follows, when expressed relative to the total Cu or Zn content in the soils: between 13.6 % and 40.4 % for copper and between 3.5 % and 11.6 % for zinc.

Proportion between AL-extractable phosphorus and copper content in vineyard soil samples and control soil samples is shown in the Table 3 and graphically in the Figure 2.

It is evident from the Figure 2 that there is obvious difference between amounts of EDTA extractable copper and phosphorus in vineyard soil samples (Orlica, Kalvarija 2, Kalvarija 3) and amounts of the same elements in control soil samples (Orlica control, Kalvarija control). Concentrations of AL-soluble phosphorus are up to nineteen times higher in vineyard soils than in control soil samples (uncultivated surfaces). Phosphorus and copper proportion is 2 to 4 times lower in control soil samples than in intensively cultivated soil samples.

Addition and uptake of copper in the Orlica vineyard was also calculated. Annual copper addition was calculated from copper content data in phytopharmaceutical substances used for vineyard protection in one-year period. The data are shown in the Table 4.

Annual copper addition with phitopharmaceuticals amounts to 8.5 kg per vineyard surface which is 1.7 kg of Cu per ha. Since 1996 the vine-

Table 3:
Phosphorus and copper content in AL- extracts from soil samples and P/Cu proportion.

Soil sample	P (AL-soluble) (mg/kg)	Cu (AL-soluble) (mg/kg)	P/Cu Mass proportion
Orlica control	4.6	6.0	0.8
Orlica	40.6	11.5	3.5
Kalvarija control	5.9	4.2	1.4
Kalvarija 2	109.1	34.2	3.2
Kalvarija 3	115.4	40.4	2.9

Table 4:
Content of Cu in phytopharmaceutical substances used for Orlica vineyard protection.

	Dosage (kg)	Cu (%)	Cu (kg)
Cu – Antracol	40	17.5	7
Cu – Euparen	10	15	1.5

yard Orlica is not treated with the mineral fertilizers. Annual Cu uptake was calculated from contents data in the Sauvignon wine and than the amount of wine produced in the Orlica vineyard. Content of Cu in wine was determined by flame atomic absorption method. The wine contained 0.05 mg Cu per liter.

Estimated quantity of wine produced in the vineyard Orlica is 24,000 L meaning that our copper uptake with wine from this vineyard is 1.2 g per year. It is important to highlight that with wine analysis the total uptake of copper from soil could not be included. Determination of Cu in pomace and the rest of the vine (leaves, shoots …) may count significantly for total uptake of Cu from vineyard soil.

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Another consideration is transfer of copper caused by rain to the nearest surface waters. Sediment of the Meranovo stream which springs in the Pohorje territory above the vineyard Orlica, and runs below the vineyard to the river Drava, was analysed. Cooper and zinc contents in the sediment are shown in the Table 5. Copper content in the Orlica vineyard soil was 48 mg/kg, zinc content was 99 mg/kg.

Due to the fact that Cu is strongly immobilized by soil organic matter and Fe, Mn, Al-(hydr)oxides, elevated Cu concentrations originating from phytopharmaceutical applications were observed in superficial ho-

Table 5:
Copper and zinc content in the Meranovo stream sediment.

Sampling site	Zn (mg/kg)	Cu (mg/kg)
Spring	96	37
Below the vineyard	95	51
Behind the vineyard	120,5	89
Mouth of the Drava	130	107

Table 6:
Limit, warning and critical imission values for Cu and Zn in soil.

	Limit imission value (mg/kg)	Warning imission value (mg/kg)	Critical imission value (mg/kg)
Cu	60	100	300
Zn	200	300	720

rizons of vineyard soils. The highest Cu concentrations were found in the surface layer (0 cm – 20 cm).

Concentrations of Cu and Zn in the stream sediment are much lower before the stream reaches the vineyard than after it leaves it. Increased concentrations of both elements are a consequence of spraying the vineyard with zinc and copper containing phytopharmaceutical substances.

With analysis of the stream sediment which springs in the Pohorje territory and runs below the vineyard Orlica to river Drava can be inferred that the increasing Cu and Zn contents in the sediment were caused by the use of phytopharmaceuticals containing those two elements. Rain washes away the vineyard soil and the silt gathers in streams. Amounts of copper in stream sediment behind the vineyard Orlica and in the mouth of the Drava River exceed the limit imission value for Cu in soil. Contents of zinc in soil and stream sediment do not exceed limit emissions.

Regulation about limit, warning and critical imission values of hazardous substances in soil deals with heavy metals in soil in Slovenia (Official gazette of the Republic of Slovenia, Vol. 68/96). Limit, warning and critical imission values for copper and zinc in soil are set out in the Table 6.

The copper amounts in vineyard Kalvarija exceed the limit imission value for Cu in soil for both parts of the vineyard. Zinc concentrations were not exceeded in discussed vineyards soils. Amounts of copper in stream sediment behind the vineyard Orlica with 89 mg/kg and in the mouth of the Drava River with 107 mg/kg exceed the limit imission value for Cu in soil.

There is a much higher portion of available copper in the vineyard soils relative to the control soil samples; there is no overall relationship between total and available copper in soils. Concentrations of plant available copper in soils cannot be predicted on the basis of total copper concentration, which is important for developing environmental guidelines.

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