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Copper in surface layer of Croatian vineyard soils

Elda Vitanović [1]*, Željko Vidaček [2], Miro Katalinić [1], Sonja Kačić [1] and Boško Miloš [1]
*[1] Institute for Adriatic Crops and Karst Reclamation, Put Duilova 11, 21000 Split, Croatia. [2] Faculty of Agriculture, Department of Pedology, Svetošimunska 25, 10000 Zagreb, Croatia. *e-mail: elda@krs.hr*

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Abstract

The frequent use of copper fungicides for the control of vine diseases results in increased accumulation of total copper in the surface layer of vineyard soils. The objective of this research was to survey copper concentration in surface soils of vineyards in coastal Croatia, which is a part of the Adriatic Coast, Europe. Sampling was undertaken on anthropogenic colluvial soils, flysch, terra rossa and terrace soils on cretaceous limestone. Each sample was physically and chemically analyzed. The survey was also carried out among local wine-growers. A questionnaire was provided to growers to determine total copper loading on an annual basis. Concentrations of total copper in the vineyard soils under research range from 70.50 to 625.79 mg/kg, compared to 21.85 to 290.11 mg/kg total copper found on the nearby control sites. Copper concentrations were significantly ($p<0.05$) higher in vineyard terrace soils than on other soils sites tested. This was shown to be a result of the treatment of the vines with copper fungicides, with the subsequent introduction of 2.90 kg/ha (Kaštela-Trogir vineyard area) to 4.20 kg/ha (Primošten vineyard area) of copper into vineyard soils each vegetative year. Seventeen out of twenty vineyard soils under research were contaminated with copper, according to the specifications provided within the "Regulations on Protection of Cultivated Land from Contamination by Hazardous Substances". Considering the average concentrations of the metal under research, quaternary colluvial anthropogenic soils (So 1.03) and those of terra rossa (So 1.94) were contaminated, while those on flysch (So 2.13) and terraced on cretaceous limestones (So 2.88) were polluted with copper. The results of this research provide further data relevant to the inventory of heavy metals in vineyard soils of this part of the Adriatic Coast.

Key words: Heavy metals, copper, copper fungicides, anthropogenic soils, polluted soils.

Introduction

The problem of soil contamination with heavy metals is a central and current issue in modern ecology, especially agroecology. Agricultural soils are particularly exposed to excessive contamination by heavy metals, the reasons being traffic, households and anthropogenic impact. Anthropogenic impact is especially conspicuous in vineyard soils, orchards and gardens. Copper sulphate has been in use with wine-growing as a plant-protection product against fungal diseases *(Plasmopara viticola* (B. and C.) Berl. and De Toni and *Phomopsis viticola* (Sacc.) Sacc) since the 18th century [3]. In Croatia, in addition to copper sulphate there are other copper-based fungicides in use against fungal diseases in wine-growing, such as copper (I) oxide, copper oxichloride, copper hydroxide, copper-hydroxide-potassium sulphate complex, copper-hydroxide-Ca-chloride complex, combinations of copper and organic fungicides, and combinations of copper and mineral oils [4].

According to information gathered to date, long-term use of copper fungicides in wine-growing results in ingression of significant quantities of copper, which remain in the surface soil layer at 0-0.2 m, as confirmed by a number of researchers. Arable soils usually contain Cu 5-30 mg/kg, while concentrations of total copper in treated vineyards can range between 100 and 1500 mg/kg [5-10]. Besnard *et al.*[10] determined that vineyard soils of the French Mediterranean contain from 31 to 250 mg/kg of total copper. Pietrzak *et al.*[11] indicate that the use of copper fungicides led to an increase of total copper (250 mg/kg) in some vineyard soils in Victoria, Australia, while Wightwick *et al.*[12] determined that copper concentrations in Australian vineyard soils were generally much lower (6-150 mg/kg) than those reported in vineyards soils in parts of Europe (130-1280 mg/kg). High concentrations of fungicide-derived copper in orchard and vineyard soils have been reported from India (29-131 mg/kg) [13] and Australia (11-320 mg/kg) [14, 15]. The bulk of copper accumulated in the leaves and soil after treatment of the vine with copper fungicides returns to the surface layer of soil through tillage or the biological cycle [8, 9, 16]. Copper can be a micronutrient and toxic element simultaneously, depending on its concentration in soil. Previous studies indicated that copper in soil is bound to soil organic matter, adsorbed to clay surface, bound to Fe and Mn oxides, in the matrix of primary silicate minerals, in secondary minerals or within amorphous matter[17]. The sum of all above can be defined as total copper in soil. Determination of the total content of metals in soils is an important step in estimating the hazards to the vital roles of soil in the ecosystem, and also in comparison with the quality standards in terms of the effects of pollution and sustainability of the system. From the ecotoxicological aspect, however, it is equally important to determine the bioavailability of Cu accumulated in vineyards. The total copper content in soils is a useful indicator of soil deficiency and/or contamination. Copper availability to biota and its mobility are the most important factors to be considered when assessing its effect on the soil environment[11]. Copper bioavailability is influenced not only by soil physical and chemical properties [18-20] but also by environmental factors such as climate, biological population and type and source of contaminants [21, 22]. Toxicity of copper for soil organisms and plants is essentially observed in acid soils, but not in calcareous soils, and for copper contents as high as those reported in vineyard soils [23]. Van Zwieten *et al.* [24] indicate that earthworms occurred at lower density in orchard soils with a history of copper fungicide use. According to Merrington *et al.*[25], data suggest that the copper residues are responsible for significant reductions in biomass carbon (C_{moic}) even though the orchard soils had similar or elevated levels of total organic carbon (C_{org}). Many authors have found a positive correlation between copper retention and pH [26, 27] and the sum of bases or exchangeable calcium [28]. When the cation exchange capacity or the level of organic matter increases, the bioavailability of copper has also been reported to decrease [27, 29].

There is no published evidence of elevated copper concentrations in vineyard soils of coastal Croatia. It is well understood [30] that copper is used to control plant fungal diseases in this region, so it is expected that elevated concentrations of Cu are present, and 2-5 kg/ha is introduced into vineyard soils each vegetative year. According to Parat *et al.* [31], the amount of Cu introduced into the soil is 0-7.5 kg/ha. Delas and Juste [32] present information that up to 15 kg/ha was introduced some twenty years ago.

All similar research carried out in Croatia to date has been carried out with the vineyard soils of continental Croatia [1, 23, 30, 33-36]. The vineyard area of continental Croatia comprises vineyards with deep and fertile soils, the area of which exceeds 1 ha. Namely, in researched vineyard areas, the number of treatments is conditioned by a micro-climate favorable for development of vine diseases, as well as traditional methods used in cultivation and protection. In this study, we undertook a survey of copper concentrations in coastal Croatian vineyards and determined which soil types accumulated the most copper. In order to determine possible deviations, the research also included various types of forest soils (control areas), which had not been exposed to the use of copper-based plant protection products. Regarding the existing condition and according to the obtained results, the soils under research are defined as either contaminated or polluted [1, 2].

Materials and Methods

Selection of locations, vineyards and control areas: For this research we selected vineyard soils within the region of coastal Croatia. This region consists of a number of sub-regions, while each sub-region comprises a number of vineyard areas. Following an extensive analysis of the region, the vineyards of Kaštela-Trogir and Primošten vineyard areas, which differ in geology and grape varieties, were selected for the research. In Kaštela-Trogir vineyard area, sampling was made at three different locations, which had been selected by taking into account differences between respective types of soil typical in the area under research (Table 1).

Areas with anthropogenic colluvial soils were selected as location A (N 43°53', E 16°43'). Location B was comprised of areas with anthropogenic soils on flysch (N 43°56', E 16°33'), while areas with anthropogenic soils on terra rossa were selected for location C (N 43°55', E 16°28'). In Primošten vineyard area, sampling was made on one location only, D (N 43°56', E 16°94'), since anthropogenic terrace soils on cretaceous limestones are the prevalent and typical type of soil in the area. These sites were sampled because tourism is important to the region, which was nominated for the UNESCO's World Heritage list.

Table 1. Schematic presentation of vineyard areas, locations, vineyards and control areas.

Vineyard area	Location	Anthropogenic soil	Vineyard	Control area
Kaštela–Trogir	A	colluvial soils	1 – 5	6 – 8
	B	soils on flysch	1 – 5	6 – 8
	C	soils on terra rossa	1 – 5	6 – 8
Primošten	D	terrace soils	1 – 5	6 – 8

Five vineyards were selected from each of the locations. The age of the vineyards had an important role in the selection, owing to long-term use of copper fungicides. Each of the selected vineyards is 40 to 70 years old, although the vine has been grown on the same locations for over 100 years. In each of said locations, sampling was also made in three control areas. Forest soils were selected in order to identify the so-called "background" concentration, for no copper-based plant protection products had ever been used on them.

Soil sampling: Soil sampling was carried out in all locations in each of the five vineyards and on each control sites. Samples were taken by a 30-mm diameter cylindrical probe from 0 - 0.2 m [37, 38]. The ten samples in each of vineyards and on each control site were taken and homogenized to obtain one average sample. Each of ten samples was taken at random from various lines (in between vines) in each vineyard. An average sample from each control area comprises ten randomly taken subsamples of forest soil, adjacent to each location (500-1000 m), characteristic for the same type of soil as the pertinent location. Samples were air-dried, passed through 2-mm sieve and stored in plastic boxes at room temperature. After that, samples were used for current physical and chemical analyses.

Soil analysis: In the prepared samples the following values were determined: pH, active lime, total carbonates, humus, exchangeable potassium and exchangeable phosphorus. Soil reaction (pH) was determined potentiometrically with a glass electrode in water and 1 M KCl, active lime by the Druines-Galet method, total carbonates by the Scheibler calcimeter and humus content by the permanganate method according to Kotzmann. Exchangeable potassium was extracted with 1 M ammonium acetate and phosphorus according to Truog. All methods of soil chemical analyses were in line with standard methods [39].

Total copper in the surface layer (0 - 0.2 m) was evaluated after digestion with aqua regia [40]. Three g of soil (previously prepared and crushed to dust) was measured into a 250 ml reaction flask and 0.5-1 ml of double-distilled water, drop by drop, 21 ml of 12.0 M hydrochloric acid and 7 ml of 15.8 M nitric acid were added. The prepared mixture was left at room temperature for 16 h to allow oxidation of organic components within it. After settling, the content above the sediment was filtered through filter paper (8 µm pore width, 150 mm diameter) into a 100-ml volumetric flask. The sediment from the filter paper was carefully washed with diluted nitric acid. The flask was filled with double-distilled water up to the mark, and total copper was measured in such prepared sample. The choice of this method was dictated by Croatian Government regulations, which set norm values for this procedure[2]. The extraction process was done by the microwave technique on a Perkin Elmer Multiwave 6MF 100 (1000W). Total copper content in soil was determined by flame atomic absorption spectrometry (Varian 220).

Soil skeleton (rock fragments greater than 2 mm) [30] and mechanical composition of soil by means of Na-pyrophosphate were determined in all samples. Classification of soils by texture was according to the U.S. system [61].

Survey method: A survey was used in order to collect information concerning vineyard (soil, size, age), agrotechnical measures applied and plant protection products in use (particularly the copper-based ones). Twenty wine-growers were interviewed. The core element comprised all wine-growers in whose vineyards the research was carried out. For this purpose, a questionnaire with ten open questions was prepared. It was a written survey, and the examinees completed the questionnaire on their own.

Data processing: All indicators were processed by analysis of variance (ANOVA). The software used for this purpose was Statview (SAS, Version 5.0).

Results and Discussion

Soil chemical and physical properties: Characteristics of anthropogenic colluvial soils (A) vary greatly depending on the origin of quaternary deposit, depth, properties of substrate onto which colluvium is deposited, as well as hydrological and geomorphological-lithological conditions of the location in which colluvium is accumulated [41]. The results of chemical analyses of these soils are presented in Table 2. Loam texture dominates in these soils. The soils are skeletal, their water capacity is low, while the air capacity is high.

The significant variations of the properties of anthropogenic soils on flysch (B) (Table 3) are a result of the lithological complexity of flysch [41]. There was a great variability in the mechanical composition. These soils are equally skeletal to anthropogenic colluvial soils. Generally, chemical and hydrophysical properties of this type of soil are totally converse in comparison to other researched soils.

Anthropogenic soils on terra rossa (C) (Table 4) are deep, with a specific red color [41]. The textures are clay loam and silty clay loam and these soils are very skeletal. Generally, this type of soil, in addition to a heavy texture composition, has favorable hydrophysical properties.

A characteristic of anthropogenic terrace soils (D) (Table 5) is a shallow A-horizon of small-grained soil, located above the parent material[42]. An analysis of the mechanical composition indicates that soils are comprised of loam and silt loam. The soils are skeletal, mainly medium gravelly to cobbly, the water capacity is low, while their air capacity and water permeability are high. The structure of soils in these vineyards is mainly stable and well-defined.

Copper introduction in plant protection products: The survey method helped collect and subsequently describe the basic technological properties of vineyards in which the research was carried out. Considering the use of various copper fungicides and different numbers of treatments, it was necessary to calculate the amount of copper introduced by means of copper fungicides into vineyard soils during one vegetative year, for the research vineyard area.

Table 6 shows two examples of copper fungicide application in research areas. The survey revealed that Bordeaux mixture and Cuprablau Z are most frequently used in Kaštela–Trogir vineyard area. Each comprises different quantities of active ingredients, applied in different concentrations. In four treatments with copper fungicides in this vineyard area, an average of 2700 L of water is consumed per hectare during each vegetative year. Aware of this information, and taking into consideration the quantity of the active ingredient, as well as concentrations of each applied preparation, the total quantity of copper introduced into one hectare of vineyards during one vegetative year has been calculated. The result shows that Cu is introduced 2.90 kg/ha each vegetative year.

Copper fungicides used in Primošten vineyard area are Bordeaux mixture, in use for a number of years, and Nordox, used during the last few years. Four treatments with copper fungicides are carried out each year in this vineyard area as well, so that the average consumption of water during one vegetative year amounts up to 3250 L/ha. With regard to the quantity of consumed water, as well as the concentration of preparations used and quantities of the active ingredient, it has been calculated that each vegetative year Cu is introduced some 4.20 kg/ha into these vineyards. The results of this research match those of Romić *et al.* [23], who noted that 2 to 5 kg/ha of copper is introduced annually into vineyard soils of continental Croatia through use of copper fungicides. Gračanin *et al.* [30, 34, 36] point out that number of treatments in one year in the past was substantially higher (8-14), particularly in the areas which were exposed to more severe disease attacks. Besnard *et al.* [43] reveal that until the 1970's vineyards in France were treated 3 to 10 times per year with Bordeaux mixture. They also claim that in recent years the number of treatments was reduced to a mere 1 to 3 times a year, and 3 to 5 kg/ha of copper is introduced by one treatment with Bordeaux mixture. In Burgundy (France), in an average year, approximately 5.5 kg/ha (11 kg/ha max) of Bordeaux mixture is applied to grapevines to control diseases [44, 45]. In a similar study, Parat *et al.*[31] found out that 0 to 7.5 kg/ha Cu was introduced annually into vineyard soils of Burgundy, while twenty years ago 15 kg/ha have been introduced per year [32].

Total copper in experimental soils: The results show that the total copper concentrations in vineyard soils, in each location, was higher than in the control soils (Table 7).

The total copper concentrations in anthropogenic colluvial soils ranged between 70.50 and 181.62 mg/kg, while concentrations from 21.85 to 49.05 mg/kg were recorded in control areas. The highest concentration of total copper was found in vineyard soil (181.62 mg/kg).

Anthropogenic soils on flysch contained Cu 163.68-302.05 mg/kg, while the concentrations of this metal in control areas ranged from 44.42 to 124.77 mg/kg. The highest concentration of total copper was found in vineyard soil (302.05 mg/kg). It is also apparent that the total copper concentrations in anthropogenic soils on flysch are also somewhat higher than those in anthropogenic colluvial soils.

The total copper concentrations in vineyard and control areas

Table 2. Chemical properties of anthropogenic colluvial soils.

Vineyard/ control area	Depth m	pH H_2O	pH M KCl	Total carbonate %	Active lime %	Humus %	N ‰	K_2O mg/100 g	P_2O_5 mg/100 g
A1	0-0.2	8.36	7.52	56.2	17.45	1.94	0.97	36.0	13.0
A2	0-0.2	8.10	7.41	37.2	11.49	2.10	1.05	84.0	23.5
A3	0-0.2	8.35	7.34	26.4	10.00	3.10	1.55	40.0	1.0
A4	0-0.2	8.22	7.44	49.6	22.07	2.46	1.23	64.0	10.2
A5	0-0.2	8.61	7.57	52.8	15.75	1.51	0.75	60.8	12.1
A6	0-0.2	8.09	7.35	34.3	7.19	9.30	4.65	32.8	1.7
A7	0-0.2	8.38	7.61	70.2	5.79	2.17	1.08	27.9	3.5
A8	0-0.2	8.00	7.30	39.6	16.30	11.12	5.56	42.8	5.4

Table 3. Chemical properties of anthropogenic soils on flysch.

Vineyard/ control area	Depth m	pH H_2O	pH M KCl	Total carbonates %	Active lime %	Humus %	N ‰	K_2O mg/100 g	P_2O_5 mg/100 g
B1	0-0.2	8.42	7.39	31.4	10.85	2.48	1.24	60.0	11.7
B2	0-0.2	8.16	7.19	23.2	7.44	3.62	1.81	54.9	9.7
B3	0-0.2	8.33	7.35	44.8	11.28	2.69	1.34	45.7	4.1
B4	0-0.2	8.25	7.17	8.6	4.02	3.07	1.53	81.5	17.5
B5	0-0.2	7.95	7.38	51.2	18.29	3.08	1.54	63.9	17.2
B6	0-0.2	8.12	7.29	52.8	13.00	5.61	2.80	42.2	5.3
B7	0-0.2	8.17	7.27	29.3	4.35	5.71	2.85	58.9	4.8
B8	0-0.2	8.04	7.22	48.8	19.82	4.11	2.05	30.8	3.7

Table 4. Chemical properties of anthropogenic soils on terra rossa.

Vineyard/ control area	Depth m	pH H_2O	pH M KCl	Total carbonates %	Active lime %	Humus %	N ‰	K_2O mg/100 g	P_2O_5 mg/100 g
C1	0-0.2	8.08	6.99	1.2	0.00	4.04	2.02	93.0	126.0
C2	0-0.2	7.55	6.76	0.4	0.00	4.54	2.27	164.8	164.1
C3	0-0.2	8.26	7.25	12.6	3.57	3.46	1.73	98.3	38.2
C4	0-0.2	7.84	7.05	2.4	0.00	2.96	1.48	108.0	144.3
C5	0-0.2	8.35	7.19	25.2	8.66	3.13	1.56	41.2	2.4
C6	0-0.2	7.91	6.97	0.8	0.00	5.89	2.94	45.6	8.0
C7	0-0.2	7.66	6.81	0.4	0.00	4.62	2.31	34.3	5.4
C8	0-0.2	8.32	7.12	9.9	2.22	3.01	1.50	40.1	23.1

Table 5. Chemical properties of anthropogenic soils on terraces.

Vineyard/ control area	Depth m	pH H_2O	pH M KCl	Total carbonates %	Active lime %	Humus %	N ‰	K_2O mg/100 g	P_2O_5 mg/100 g
D1	0-0.2	7.82	7.06	1.2	0.00	3.50	1.75	101.2	80.9
D2	0-0.2	7.51	6.86	1.2	0.00	8.88	4.44	363.8	254.2
D3	0-0.2	7.79	7.08	4.1	0.00	8.03	4.01	84.8	107.8
D4	0-0.2	8.00	7.18	9.9	0.69	10.07	5.03	57.1	22.4
D5	0-0.2	8.18	7.18	2.9	0.00	5.30	2.65	62.1	29.2
D6	0-0.2	7.80	7.03	40.9	9.11	9.66	4.83	48.8	9.1
D7	0-0.2	7.97	7.22	35.9	16.25	11.24	5.62	62.7	6.9
D8	0-0.2	7.90	7.11	47.0	22.72	7.68	3.84	27.9	2.1

Table 6. Use of copper fungicides during one vegetative year in each location.

Preparation	Active ingredient	Concentration of respective preparations (%)	Quantity of active ingredient (Cu g/L)	Total quantity in a vegetation year (Cu kg/ha)
		Location A, B and C		
Bordeaux mixture	Copper-hydroxide-potassium sulphate complex	1.00	200	1.00
Cuprablau Z	Copper-hydroxide-Ca-chloride complex	0.25	350	1.90
				2.9
		Location D		
Bordeaux mixture	Copper-hydroxide-potassium sulphate complex	1.00	200	1.40
Nordox	Copper (I) oxide	0.15	750	2.80
				4.2

Table 7. Concentration of total copper in surface layer of researched soils (0-0.2 m).

	Location*			
	A	B	C	D
	Cu (mg/kg)			
Vineyards				
1	70.50	204.86	252.89	166.46
2	71.84	211.46	248.37	138.79
3	106.19	183.33	162.23	625.79
4	85.40	302.05	196.47	297.81
5	181.62	163.68	113.46	213.74
Control areas				
6	45.53	57.42	52.03	87.80
7	21.85	124.77	53.32	45.94
8	49.05	44.42	290.11	140.01

*A anthropogenic colluvial soils; B anthropogenic soils on flysch; C anthropogenic soils on terra rossa; D anthropogenic soils on terraces.

on terra rossa ranged from 113.46 to 252.89 mg/kg in vineyard soils, whereas in control area they ranged from 52.03 to 290.11 mg/kg. The highest concentration of total copper was recorded in the soil from the control area C8. The other two control area soils contained a very low concentration of this metal (52.03, 53.32 mg/kg). This high background total copper concentration can be linked with the vicinity of wine-growing areas. We didn't expect this. Namely, this control area is adjacent to vineyards, so it can be concluded that the higher concentrations of the metal are conditioned by erosion (by water and wind). Leaching of wine-growing areas resulted in accumulation of copper in forest soil, which is located at a somewhat lower altitude than the vineyard under research. Similar conclusion was also reached by other authors [43, 46-50].

Increased total copper concentrations in anthropogenic soils on terra rossa had also been confirmed by Gračanin [30]. According to Romić *et al.* [33, 17], there is a stronger bond of copper with soil organic matter, and thus soils with higher humus content are rich in copper. Equivalent results have been obtained through this research. It can be concluded that the total copper concentrations in anthropogenic soils on terra rossa are significantly higher than in control area soils.

Total copper concentrations in anthropogenic terrace soils on cretaceous limestones ranged from 138.79 to 625.79 mg/kg. Concentrations of this metal in control area soils varied from 45.94 to 140.01 mg/kg. The highest concentration of total copper was found in vineyard soil (625.79 mg/kg). It is also the soil with the overall highest concentration of the metal in all soils covered by the research. Their concentrations are also higher in comparison with other types of anthropogenic soils under research. Vineyard soils of coastal Croatia contained from 70.50 to 625.79 mg/kg of total copper, while the concentrations in control areas were lower (21.85 - 290.11 mg/kg). These results match those obtained by other local and foreign authors. Romić *et al.* [33, 23] confirmed that the average concentration of copper in vineyard soils in continental Croatia were higher than their background concentrations, which can be associated with long-term intensive protection of grapevines. The same authors declared that vineyard soils of continental Croatia contained from 30 to 700 mg/kg of total copper. Similar results had also been confirmed in research of vineyard soils in some other world regions. Some results of maximum recorded concentrations of total copper (mg/kg) in surface layers of vineyard soils in the world are as follows: South Italy 75 and North Italy 297[8]; Greece 100 [51]; Moldova 230[52]; Australia 250 [11]; South France 250 [53]; Bordeaux (France) 1500 [9]; South Brazil 3200 [54]; India 131 [13] and New Zealand 304 [55].

Romić *et al.* [23] indicated in their research that the background concentrations of total copper in continental Croatia ranged from 14 to 71 mg/kg. Mirlean *et al.* [54] determined total copper background concentrations ranging from 7.2 to 49.8 mg/kg. The background concentrations of total copper in their research amounted to approx. 10 mg/kg [11]. Chaignon *et al.*[17] reported that the concentration of total copper in control areas was 22 mg/kg. Brun *et al.* [53] described typical background copper concentrations of 14-29 mg/kg in uncontaminated woodland plots. Arable land usually contains quantities of copper between 5 and 30 mg/kg [5-7, 9]. Concentrations of copper in uncontaminated soils vary between 2 and 40 mg/kg [56-58]. The total copper concentrations exceeding 60 mg/kg in soils normally require environmental risk assessments[59].

Impact of plant protection products on soil copper: Based on the analysis of variance ($F_{exp} = 12.895^* > F_{tab\,0.05} = 9.28$) of total copper concentrations in all researched soils, it follows that there is a significant difference in concentrations of total copper between the vineyard and control areas. Significantly higher concentrations of this metal were found in vineyard soils. Based on the obtained results, it can be concluded that total copper accumulates in the surface layer of vineyard soils due to long-term use of copper fungicides.

As already mentioned, many authors have researched this issue of pollution of vineyard soils with copper, and their results confirm those obtained by our research. Seventeen out of twenty vineyard soils under research deviate from the maximum allowed concentrations, i.e. their soils were contaminated with copper [2]. In Croatia, total copper concentrations of up to 60 mg/kg are allowed in agricultural soils. The maximum allowed concentrations of total copper throughout the world vary from one country to another. The limit of total copper concentrations in soil in the Netherlands is 36 mg/kg [60]. In Australia and New Zealand, total copper concentrations exceeding 60 mg/kg in soil require environmental investigation [61]. Considering the average concentrations of the metal under research, quaternary colluvium anthropogenic soils (So 1.03) and anthropogenic soils of terra rossa (So 1.94) were contaminated with copper [1]. Anthropogenic soils on flysch (So 2.13) and anthropogenic terrace soils on cretaceous limestones (So 2.88) were polluted with copper. This classification is determined by comparison of actual pollution with cutoff value of pollution [2] or by the degree of pollution So (content in soil - mg/kg/cutoff value). Based on this, soils are grouped into five classes: pure soil (So<0.25), increased soil pollution (So 0.25-0.50), high soil pollution (So 0.50-1.00), polluted soil (So 1.00-2.00) and contaminated soil (So>2.00). Any matter that enters the human environment (food, drink, air) is either directly or indirectly connected with soil, i.e. its quality is directly dependent on soil properties. Therefore, the results of this research are very important, since they are the basis for defining soils as polluted, which will remain as such irreversible, and contaminated soils, in which the contamination can still be reduced. It is very important to determine bioavailability of accumulated copper in vineyard soils, because total copper content in soils is a useful indicator of soil contamination. Copper availability to biota and its mobility are the most important factors to be considered when assessing its effect on the soil environment [11].

The analysis of variance of total copper concentrations ($LSD_{exp} = 2.687^* > LSD_{tab\ 0.05} = 2.120$) indicates a considerable difference in total copper concentrations (with 95% certainty) between colluvial anthropogenic soils and anthropogenic terrace soils on cretaceous limestones. Significantly higher concentrations of this metal were identified in anthropogenic terrace soils on cretaceous limestones. These soils are of loam and silt loam, and are located in Primošten vineyard area. They are somewhat heavier than the anthropogenic colluvial soils, which are of loam and located in Kaštela–Trogir vineyard area. The reason for higher concentrations of total copper in heavier soils can lie in a stronger bond of copper with particles of heavier soils. Copper is known to leach in lesser quantities and slower into lower layers in such soils, while it's leaching is more excessive and faster in lighter soils. There are no significant differences in concentrations of the metal under research between anthropogenic soils on flysch and terra rossa and other researched anthropogenic soils.

Conclusions

This research showed that application of copper fungicides to vineyards of coastal Croatia has resulted in the accumulation of copper residues in surface soils. Concentrations up to 625.79 mg/kg were found in anthropogenic terrace soil on cretaceous limestones. Concentrations of total copper are higher in all researched vineyard soils than in control area soils. An average of 2.90 to 4.20 kg/ha of copper is introduced into vineyards each vegetative year through treatment of the vine with copper fungicides. Loam and silt loam soils which belong to vineyard terrace soils on cretaceous limestones contain significantly higher concentrations of total copper compared to other soils under research. Seventeen out of twenty vineyard soils under research were contaminated with copper [2]. Considering the average concentrations of the metal under research, quaternary colluvium anthropogenic soils and those of terra rossa were contaminated, while those on flysch and terraced on cretaceous limestones were polluted with copper. The results of this research provide further input towards the inventory of heavy metals in vineyard soils of coastal Croatia. Future research should be extended to include distribution of total copper within the profiles of soils in this area, and also perhaps to the impact of total copper on soil ecology.

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