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ABSTRACT

The use of copper (Cu)-based fungicides to control diseases in vineyards can cause excessive Cu accumulation in soils. Greater Cu availability is expected in acid sandy soils with low organic matter (OM), where the risk of toxicity to plants and environment contamination is high. This study aimed to study the evolution of Cu in acid, sandy soils planted with grapevines for a period of time between 2 and 32 years. Two studies were carried out in South Brazil: in the first study, soil samples, from 19 vineyards were collected and analyzed in the first 0.2 m depth-layer for the total and available Cu. In the second study, 3 soils were selected among the previous 19 according to the age of vineyard (5, 11, and 30 year-old), and their 0.60 m-depth profile was analyzed for 5 fractions of Cu (soluble, exchangeable, mineral, organic, and residual). All the soils were classified as Ultisols. The vineyards with more than 25 years under cultivation had a mean total Cu concentration of 90 mg kg^{-1} in the 0–0.10 m layer. Approximately 80% of it was potentially available to plants. The greatest total and available Cu were found in the soil surface layers (0.2 m depth), where up to 75% of the total Cu is adsorbed in clay minerals and only 20% is complexed in the OM.

Introduction

Copper (Cu)-based fungicides are frequently applied during grapevine cultivation to control fungal and bacterial diseases. This practice leads to Cu accumulation in the soil and raises the potential toxicity to plants and the risk of environmental contamination. The oldest primary winegrowing Brazilian region is the Serra Gaúcha (mountainous region of Rio Grande do Sul), where some soils may contain up to 665 mg kg^{-1} total Cu (Casali et al. 2008). Nevertheless, the soil Cu concentration varies widely in vineyard soils, with 20 to 500 mg kg^{-1} in France (Brun et al. 1998; Flores-Veles et al. 1996), 35 to 600 mg kg^{-1} in Spain (Arias et al. 2004; Nóvoa-Muñoz et al. 2007), 50 to 300 mg kg^{-1} in Italy (Toselli et al. 2009; Viti et al. 2008), 100 to 210 mg kg^{-1} in Greece (Vavoulidou et al. 2005), and 40 to 250 mg kg^{-1} in Australia (Pietrzak and McPhail 2004; Wightwick et al. 2008). This variation may be caused by physicochemical soil characteristics and by climatic conditions, such as the average rainfall, which determines the incidence of fungal diseases and, consequently, the quantity of Cu needed to protect the plants (Komárek et al. 2010; Vavoulidou et al. 2005).

The total soil Cu availability also depends on the functional groups of the soil reactive particles and their sorption capacity, which is a result of diverse factors. The sorption capacity increases in accordance with the clay mineral content, iron (Fe) oxides, hydroxides of Fe, aluminum (Al) and manganese (Mn), carbonates, and organic matter (OM) (Bradl 2004; McBride 1994). Soil pH (Bradl

2004; Chaignon et al. 2003), cation exchange capacity (CEC), and OM quality are important factors that regulate metal sorption and, therefore, Cu availability (Arias et al. 2005; Brun et al. 2001). Soils with neutral to alkaline pH values may generally have lower Cu availability relative to acid soils, which is explained by the presence of carbonates that favor Cu precipitation and adsorption (Bradl 2004). Copper availability is generally greater in acid soils (Brun et al. 2001; Chaignon et al. 2003; Pietrzak and McPhail 2004), with an increase of the potential toxicity to plants and water contamination. The total soil Cu concentration is widely used as a reference for comparing different situations. Nevertheless, the total soil Cu concentration is weakly correlated with the fraction available for plants (Brun et al. 2001, 1998). The available Cu is controlled by complex physicochemical reactions between Cu and diverse soil components (Bradl 2004), which prevents more exact diagnoses. So that the best manner for evaluating Cu availability in soils is by growing plants (Brun et al. 1998; Chaignon, Quesnoit, and Hinsinger 2009). However, because of time constraints and the difficulty of establishing routine, single, or sequential chemical extraction methods are used instead. As a result, single extraction methods are normally used to determine Cu availability, with diluted acids, saline solutions (calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), ammonium acetate (NH_4OAc), etc.), chelating agents (diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA)) or a mixture of these compounds (Schramel, Michalke, and Kettrup 2000). One of the most common extraction methods for evaluating plant Cu availability employs EDTA.

Sequential extraction methods are common when it is desirable to identify the Cu forms in the soil. Nevertheless, although it can cause interpretation problems as a consequence of its analytical limitations (Schramel, Michalke, and Kettrup 2000), chemical fractionation is highly important for research on metals in soils (Kennedy et al. 1997). By using extraction methods with different extraction forces until complete soil dissolution, the total accumulated metals can be divided in several fractions, depending on the method (Kennedy et al. 1997; Tessier, Campbell, and Bisson 1979). The method indicates the chemical species and the bond strength of the metal with the soil, which allows knowing the metal availability and potential dangers from plant toxicity to plants or environmental contamination.

The purpose of the present study was to evaluate the evolution of fractions of Cu in sandy, acid soil profiles, subjected to Cu applications for different periods of time during grapevine cultivation. Two studied were carried on, the first one involved 19 vineyard soils analyzed in their superficial (0.2 m) layer. The second one involved three vineyard soils selected from those used in the first study, according to their age: 5, 11, and 30 year old that were analyzed in the 0.6 m-layer for 5 fraction of Cu: soluble, extractable, organic, mineral and residual.

Materials and methods

Study # 1

This study was conducted in commercial vineyard soils in the Campanha Gaúcha region, in Santana do Livramento, which is located in the state of Rio Grande do Sul, in the extreme south of Brazil (approx 30 S, 30 W). Ultisol soil is predominant in the region, since derives from sedimentary rocks (arenite with high silicon content), has a sandy texture and a lightly sloped to rolling topography (Streck et al. 2002). The climate in the region is subtropical and classified as Cfa (Köppen classification) with an average rainfall of 1388 mm year^{-1} (IPA 1989). Although dry summer with high evapotranspiration daily rate, frequent rainfalls occur during grapevine planting and productive cycles, requiring frequent copper-based fungicides, to prevent fungal leaf diseases. The quantities of Cu applied via fungicides prior to 1993 ranged from 10 to 15 kg Cu ha^{-1} year^{-1}, then from 1993 to the present, the quantities of applied Cu are shown in Table 1.

The espalier trellis training system was used in all study vineyards. Until the late 1980s, the soil between the grapevine rows was frequently harrowed for weed control, and the weeds located in the plant row were desiccated with an herbicide. From 1990, between-row weeds were cut periodically,

Table 1. Cultivars, time under cultivation, and geographic location of the 19 vineyards used in Study # 1.

Variety	Planting year	Vineyard age	Assigned class	Location	
		year		Latitude	Longitude
Tannat	2007	2	1–5	30° 47.872′ S	55° 21.933′ W
Tannat	2004	5	1–5	30° 46.807′ S	55° 23.108′ W
Cabernet Sauvignon	2004	5	1–5	30° 46.689′ S	55° 22.576′ W
Cabernet Sauvignon	2004	5	1–5	30° 46.853′ S	55° 22.471′ W
Cabernet Sauvignon	2003	6	6–10	30° 46.831′ S	55° 21.618′ W
Cabernet Sauvignon	2003	6	6–10	30° 47.524′ S	55° 21.980′ W
Cabernet Sauvignon	2000	9	6–10	30° 47.849′ S	55° 21.793′ W
Cabernet Sauvignon	1999	10	6–10	30° 47.722′ S	55° 21.933′ W
Tannat	1996	13	11–15	30° 46,957′ S	55° 22.571′ W
Tannat	1996	13	11–15	30° 46,863′ S	55° 23.080′ W
Cabernet Sauvignon	1981	28	>25	30° 46.890′ S	55° 21.348′ W
Tannat	1980	29	>25	30° 47.122′ S	55° 21.648′ W
Tannat	1980	29	>25	30° 47.134′ S	55° 21.659′ W
Cabernet Sauvignon	1980	29	>25	30° 47.170′ S	55° 22.566′ W
Cabernet Sauvignon	1978	31	>25	30° 47.616′ S	55° 22.060′ W
Tannat	1977	32	>25	30° 47.594′ S	55° 22.659′ W
Cabernet Sauvignon	1977	32	>25R[a]	30° 47.470′ S	55° 23.455′ W
Cabernet Sauvignon	1977	32	>25R[a]	30° 47.701′ S	55° 23.396′ W
Cabernet Sauvignon	1977	32	>25R[a]	30° 47.746′ S	55° 23.398′ W

[a]Vineyard whose soil was turned over by disking for replanting after 30 years under cultivation. This management procedure was performed in 2007, two years before soil sampling.

and their residues were deposited on the soil surface and the weeds in the plant row continued to be desiccated. In June 2009, 19 commercial vineyards of different ages were selected, for their histories of copper-based fungicide application (Table 1). The vineyards were placed in five classes according to their age, namely five year-old or younger, from six to 10 year-old, from 11 to 15 year-old, older than 25 years, and older than 25 years under cultivation after plowing (Table 1). The last group was composed of three soils that were ploughed after 30 years under cultivation in order to plant new grapevines. In addition, a natural field (NF) without a history of agricultural use adjacent to the vineyards was selected, and the Cu soil concentrations were used as a reference. Soil was collected from the vineyards and NF area at 0–0.10 and 0.10–0.20 m layers, with a spade and each sample was composed of 12 subsamples collected at random within and between the rows of each vineyard. The soil was dried, passed through a 2-mm sieve screen and analyzed for the total Cu (CuEPA) and available Cu (CuEDTA), as described below.

Part of the samples from the 19 vineyards and NF was used for granulometric analysis according to the pipette method (Embrapa 1997). Soil from the 0–0.20 m layer of the NF area was subjected to X-ray diffractometry analysis to identify the main clay minerals in the soil. For that purpose, a clay fraction sample was placed on glass slides and analyzed in a Phillips X-ray diffractometer (PW 3710) equipped with a Cu tube. Angular variations from 4 to 35° 2θ were used with step recording (step = 0.02° 2θ/s) and times of 0.5 seconds per step. The diffractogram showed high intensity peaks corresponding to the 1:1 kaolinite clay minerals. Peaks related to 2:1 clay minerals revealed very low intensities.

On another part of the soil samples was determined pH, by using a soil: water suspension in a 1:1 v/v proportion after 30 min of equilibration (Tedesco et al. 1995). The OM was determined by the Walkley–Black method (Empresa 1997). The CEC at pH 7.0 (CEC $_{pH}$7.0) was calculated by the sum of the exchangeable calcium (Ca^{2+}) + magnesium (Mg^{2+}) + potassium (K^+) cations plus estimated hydrogen plus aluminum (H+Al) (Tedesco et al. 1995). The exchangeable Ca^{2+} and Mg^{2+} concentrations were extracted using potassium chloride (KCl) 1 mol L^{-1} solution and determined in an atomic absorption spectrophotometer (AAS) GBC (932 AA). The exchangeable K^+ was extracted with a Mehlich 1 solution and determined in a flame photometer (Tedesco et al. 1995). The H+Al value was estimated from the equilibrium pH from the soil, water and SMP buffer solution (Shoemaker, McLean, and Pratt 1961) at a proportion of 2:2:1, m/v/v (Tedesco et al. 1995). The estimate value was made by using the equation H+Al = ($e^{10.665-1.1488pHSMP}$)/10 (CQFS-RS/SC 2004).

The remaining sample portion was subjected to an analysis of the plant-available Cu concentrations (CuEDTA) using EDTA solution (Chaignon, Quesnoit, and Hinsinger 2009). A sample of 0.25 g dry soil was added to a 15 mL capacity centrifuge tube and agitated for 2 h with 10 mL of solution containing disodium EDTA 0.01 mol L^{-1} + ammonium acetate (NH_4CH_3COO) 1.0 mol L^{-1}, with the pH corrected to 7.0. After agitation, the suspension was centrifuged, filtered and the Cu was determined in the atomic absorption spectrophotometry (AAS). Soil total Cu (CuEPA) was determined by using the 3050B USEPA method (United States Environmental Protection Agency) (USEPA 1996). One gram of the soil sample was transferred to a 250 mL beaker covered with a watch glass for reflux and placed on a heating plate, oxidized with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2), and treated with concentrated hydrochloric acid (HCl). At the end of the extraction, the remaining particles were separated by filtration, and the Cu concentration was determined in an EAA.

Study # 2

In July 2009, three vineyards were selected among the 19 vineyards and designated, according to their age as vineyard 1 (VN1), 2 (VN2), and 3 (VN3). VN1 was 5, VN2 was 11, and VN3 was 31 years of age. These three vineyards exhibited average conditions in term of clay, OM, CEC, and soil depth values, as well as soil topography. The history of Cu application in VN1, VN2, and VN3 from 1993 to 2009 is described in Table 2. The history of Cu application before 1993 could not be recovered. In October 2009, three trenches were dug in each of the three vineyards and in NF adjacent area with dimensions of $1.0 \times 1.5 \times 0.6$ m in a transversal direction to the grapevine row, which was sufficient for sampling the plant row and inter-row. Soil samples were then collected from the 0–0.05, 0.05–0.10, 0.10–0.15, 0.15–0.20, 0.20–0.25, 0.25–0.30, 0.30–0.40, and 0.40–0.60 m layers. Mixtures were made from equal masses of soils collected from the same layers in order to make composite samples from the 0–0.05, 0.05–0.15, and 0.15–0.30 m layers for the vineyards and NF. The soil was air-dried in a forced air laboratory oven at 45ºC until reaching a constant mass, passed through a sieve with a 2 mm screen and set aside for analyses. Undeformed soil samples were collected from the same layers to determine the apparent density (mass/volume) by using metallic cylinders of 5.72 cm diameter and 4.0 cm height. The soil contained in the metallic rings was dried in a laboratory oven at 105ºC for 24 h and, soon after, its mass was determined with an analytical balance.

Table 2. Quantity of copper applied each year and total quantity applied in three Cabernet Sauvignon vineyards (Study # 2).

Crop	Vineyard 1	Vineyard 2	Vineyard 3
	Cu, kg ha^{-1}		
93/94			9.62
94/95			19.16
95/96			12.17
96/97			6.82
97/98			8.80
98/99			12.36
99/00			9.25
00/01			17.24
01/02			13.14
02/03		7.43	13.68
03/04		12.33	21.05
04/05		4.16	8.63
05/06		4.31	3.36
06/07	3.17	9.54	2.18
07/08	2.01	5.66	0.25
08/09	1.16	1.60	-
Average	**2.11**	**6.41**	**10.51**
Total	6.34	45.05	157.72

Soil from the 0–0.05, 0.05–0.15, and 0.15–0.30 m layers of VN1, VN2, VN3 and NF were subjected to Cu chemical fractionation (Tessier, Campbell, and Bisson [1979]). Triplicate 1.0 g soil samples were transferred to 50 mL Falcon polypropylene centrifuge tubes, to which the following extraction solutions were applied in sequence: 1) Soluble fraction (CuS), 8 mL of Milli-Q water; 2) Exchangeable fraction (CuE), 8 mL of $MgCl_2$ solution 1.0 mol L^{-1} at pH 7.0; 3); 3) Fraction bound to clay minerals (CuMin), 20 mL of NH_2OHHCl 0.04 mol L^{-1} in CH_3COOH 25% (v/v) at pH 2.0; 4) Fraction bound to organic matter (CuOM), 3 mL of HNO_3 solution 0.02 mol L^{-1} + 8 mL of H_2O_2 (30%) corrected to pH 2.0 with HNO_3; and 5) Residual fraction (CuR), total digestion with HF and $HClO_4$. The total Cu (CuT) was analyzed in 1.0 g samples of non-fractionated soil according to their CuR. After each extraction, the samples were centrifuged at 3500 RPM for 30 min and a supernatant aliquot was filtered and set aside to determine the Cu concentration. The CuS determination was performed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (Perkin Elmer, Optima 2100 DV) and all the other parameters were analyzed by EAA. With the CuEPA concentrations, the quantities of Cu accumulated in the 0–0.60 m layer of VN1, VN2, VN3 and NF were calculated per hectare. For this calculation, the mean CuEPA from the eight evaluated layers were multiplied by the total mass of the soil layer (volume × apparent density). The values were expressed in kg ha^{-1}.

The results were presented as mean values with standard deviations. The differences among soil layers and vineyards were subjected to analysis of variance and when significant differences ($p < 0.05$) were observed, a Tukey test was used to separate means.

Results

Study # 1

The 19 vineyard 0–0.2 m-depth had similar clay, silt, sand and OM (Table 3). The mean clay was 61 g kg^{-1}, sand 800–900 g kg^{-1} and the mean OM were 10.5 g kg^{-1} in the 0–0.10 m layer and 7.1 g kg^{-1} in the 0.10–0.20 m layer, which was a little greater than the original soil contents, which were 9.0 and 4.8 g kg^{-1}, respectively (Table 3). There was no significant correlation between the cultivation period and the OM of the soil (data not shown). The pH values for the 0–0.10 m layer were close to 6.0; in the 0.10–0.20 m layer the values were close to 5.5; they were greater than the pH from the NF area. The $CEC_{pH\ 7.0}$ values of the vineyard soils ranged from 3.0 to more than 6.0 cmol$_c$ kg^{-1} (Table 3). There was a positive correlation between $CEC_{pH7.0}$ and OM values ($r = 0.93$, $p < 0.01$, in

Table 3. Chemical and physical characteristics of the surface layer (0.00–0.10 and 0.10–0.20 m) of 19 soils collected from vineyards with different histories of copper-based fungicide application and from a natural field area (Study # 1).

Vineyard Age Years	N	Depth M	Clay	Silt	Sand	OM[b]	pH	$CEC_{pH\ 7.0}$[c] cmol$_c$ kg^{-1}
					g kg^{-1}			
0	1	0.00–0.10	54	47	899	9.0	5.2	3.2
		0.10–0.20	63	51	886	4.8	4.9	2.9
1–5	4	0.00–0.10	66 ± 13	73 ± 22	861 ± 23	10.3 ± 0.9	5.7 ± 0.2	4.4 ± 0.7
		0.10–0.20	70 ± 36	79 ± 18	850 ± 18	7.0 ± 2.0	5.3 ± 0.3	3.9 ± 0.9
6–10	4	0.00–0.10	45 ± 23	72 ± 7	883 ± 21	7.9 ± 0.9	6.2 ± 0.5	3.8 ± 0.5
		0.10–0.20	48 ± 20	68 ± 6	885 ± 14	4.9 ± 0.3	5.7 ± 0.2	3.0 ± 0.3
11–15	2	0.00–0.10	66 ± 21	85 ± 1	848 ± 21	12.1 ± 6.1	5.6 ± 0.3	5.3 ± 1.6
		0.10–0.20	75 ± 19	71 ± 9	854 ± 11	7.2 ± 2.4	5.5 ± 0.3	4.4 ± 1.0
>25	6	0.00–0.10	55 ± 27	66 ± 18	879 ± 14	9.7 ± 4.0	5.9 ± 0.3	4.1 ± 1.4
		0.10–0.20	63 ± 51	73 ± 22	863 ± 30	6.6 ± 2.1	5.4 ± 0.3	3.4 ± 0.8
>25[a]	3	0.00–0.10	59 ± 29	95 ± 15	846 ± 33	12.5 ± 2.6	6.2 ± 0.1	5.2 ± 0.8
		0.10–0.20	72 ± 38	80 ± 18	848 ± 23	9.9 ± 2.0	5.9 ± 0.1	4.9 ± 1.0

[a]Group of soils that were turned over with a disk to replant vineyards after 30 years of cultivation. This management procedure was performed two years before soil sampling.
[b]OM. organic matter = Organic carbon × 1.72.
[c]$CEC_{pH\ 7.0}$. cation exchange capacity at pH 7.0 = sum of exchangeable Ca^{2+}, Mg^{2+}, K^+ and titratable H + Al.

the 0–0.10 layer and $r = 0.92$, $p < 0.01$, in the 0.10–0.20 m layer). The pH, clay, silt and sand of the soils were not significantly correlated with the $CEC_{pH7.0}$ values (data not presented).

The natural CuEPA concentration of the soil was only 3.0 mg kg^{-1}, as observed in the NF (Figure 1a). In the vineyard soils, the 0–0.10 m layer showed, on average, a CuEPA of 13.8; 21.7 and 31.2 mg kg^{-1} in vineyards with less than 5, from 6–10 and 11–15 years of age, respectively. In the oldest vineyards with more than 25 years of cultivation, the mean CuEPA concentration was 90.3 mg kg^{-1}, approximately 30 times greater than that observed in the NF area. In the 0.10–0.20 m layer, the CuEPA concentration ranged from 6.0 to 10.0 mg kg^{-1} for vineyards ≥15 years under cultivation, increasing to 32.0 mg kg^{-1} in the soils with more than 25 years under cultivation (Figure 1a).

The CuEDTA concentration showed behavior similar to that observed for the CuEPA. The natural CuEDTA concentration in the soil from the NF area was 0.65 mg kg^{-1} for both studied layers (Figure 1b). In vineyards ≥15 years under cultivation, the mean CuEPA concentrations in the 0–0.10 m layer ranged from 8.9 to 22.0 mg kg^{-1}, reaching 73.8 mg kg^{-1} in vineyards older than 25 years (Figure 1b). In the 0.10–0.20 m layer, the mean CuEPA concentration was 4.7 mg kg^{-1} in vineyards with up to 15 years under cultivation, increasing to 22.0 mg kg^{-1} in vineyards with older than 25 years. Wide variation was observed in the CuEDTA and CuEPA from vineyard soils of similar ages, especially those with more than 15 years of age.

Tilling the soil reduced the Cu concentrations in the uppermost soil layer. In the 0–0.10 and 0.10–0.20 m layers, the mean CuEPA and CuEDTA concentrations were 90.4 and 73.3 for the vineyard soils >25 years of age, and 40.0 and 28.9 mg kg^{-1} in soils with the same time under cultivation but turned over by tillage, respectively (Figure 1a and 1b).
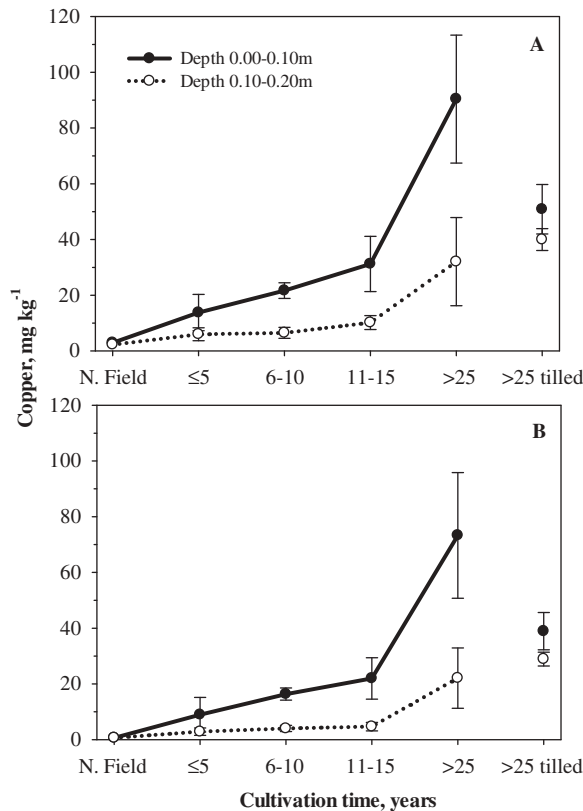

Figure 1. Total copper "CuEPA" (A) and available copper "CuEDTA" (B) concentrations in two layers (0.00–0.10 and 0.10–0.20 m) of 19 soils collected in vineyards with different histories of copper-based fungicide application and of a natural area. Bars represent standard error (n = 2–6). (Study # 1).

The CuEDTA concentrations of the 0–0.10 and 0.10–0.20 m layers had a positive linear correlation with the CuEPA concentrations (Figure 2), indicating that nearly 80% of the CuEPA in the soil is extractable by EDTA.

Study # 2

The analysis of soil layers of 0.60 m of depth showed that Cu accumulation in the soil profile occurred mainly in the surface layers (Figure 3). The natural Cu concentration was constant along the soil profile, less than 3.0 mg kg^{-1} for CuEPA and approximately 1.0 mg kg^{-1} for CuEDTA. In VN1, VN2, and VN3, the Cu mostly accumulated in the 0.05, 0.20, and 0.30 m layers, respectively. The highest values were found in the 0–0.05 m layer of VN3 and accounted for 113 and 98 mg kg^{-1} concentrations of CuEPA and CuEDTA, respectively, which decreased with depth (Figure 3). A large Cu concentration gradient was observed among the study layers; in the first three layers (0–0.05, 0.05–0.10, 0.10–0.15 m) of VN2 and four layers (0–0.05, 0.05–0.10, 0.10–0.15, 0.15–0.20 m) of VN3, the CuEPA concentrations decreased by approximately 50% for each 0.05 m of depth.

The total CuEPA accumulated in the 0–0.60 m layer of vineyard soils for 5, 11 and 31 year-old resulted of 15, 66, and 175 kg of Cu ha^{-1}, respectively (Table 3). VN3 showed the greatest concentration of CuEPA, which was significantly greater in all the layers above 0.20 m. In VN2, the accumulated quantity of CuEPA was greater than in VN1 and NF, but only in the 0–0.10 m layer. No difference of CuEPA concentration between NF and VN1 was observed in all the studied layers, with the exception of the 0–0.05 m layer (Table 3 and Figure 3a) that exhibited greater quantities of this Cu fraction. The Cu applied to VN1, VN2, and VN3 was less than the quantity found in the 0–0.60 m layer for the three vineyards (Table 4). The difference was almost 9 kg for VN1 and near 20 kg for VN2 and VN3.

The natural CuT concentration of the 3 studied soils was a little greater than 4 mg kg^{-1} in the 0.30 m layer, as observed in the NF area soil (Table 5). In VN1, VN2, and VN3 the CuT concentration was 10.5, 51.1, and 92.1 in the 0–0.05 m layers, respectively, and decreased with depth (Table 5). With the exception of the 0.15–0.30 m VN1 layer, the CuT concentration was always greater in the vineyard soils in comparison to the NF soil. The CuEPA and CuT concentrations were similar among the soils and the layers evaluated (Table 5).
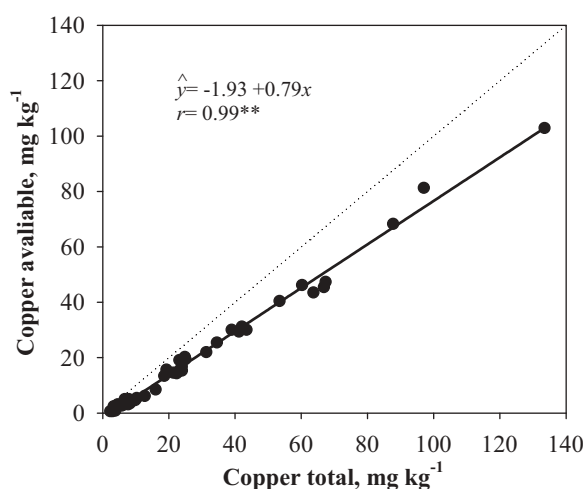

Figure 2. Correlation between total "CuEPA" and available "CuEDTA" Cu in the layers (0.00–0.10 and 0.10–0.20 m) for 19 soils collected from vineyards with different copper-based fungicide application histories and in a natural field ($n = 40$). ** Significant at $p < 0.01$ (Study # 1).
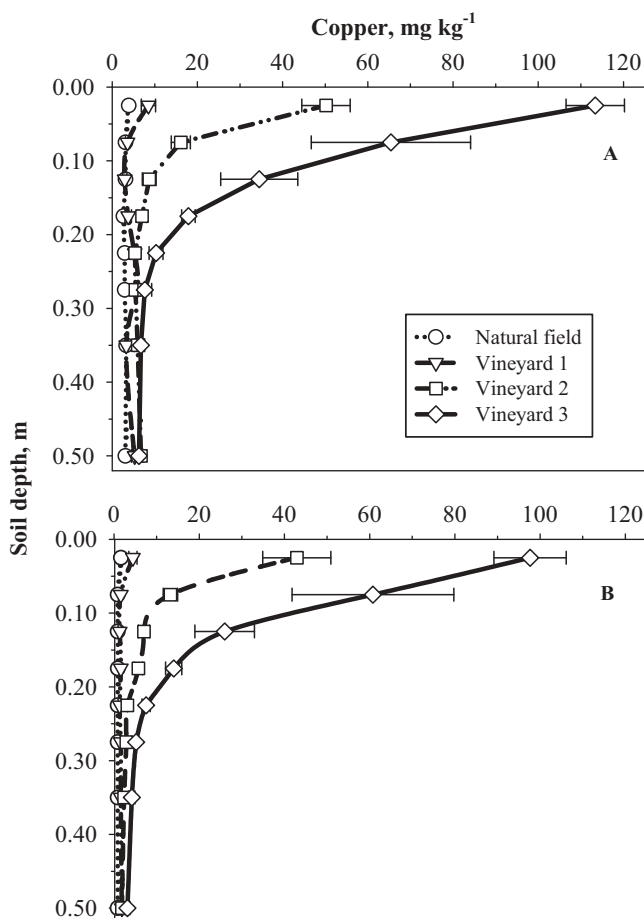
Figure 3. Soil concentration of CuEPA (A) and plant-available CuEDTA (B) of 8 soil layers collected from three vineyards with different histories of copper-based fungicide application and in a natural field area (Study # 2).

In comparison to the NF area, the vineyard soils exhibited greater CuSol, CuE, Cu-Min, and Cu-OM fractions (Table 5). The concentrations obtained from all the Cu fractions were greater in VN3 in all layers. In the VN2 soil, Cu concentrations were proportional to the time under cultivation (11 years) and the quantity of accumulated Cu, which were greater in the layers above 0.15 m. In VN1 soil, Cu concentration was lower than in VN2 and the Cu accumulation interested preferentially the 0.00–0.05 m layer.

The Cu-Min was the most abundant fraction in VN2 and VN3 while the CuR concentration was highest in NF and VN1 (Table 4 and Figure 4). In the VN3 soil, the clay minerals adsorbed 74% of the total Cu found in the 0–0.15 m layer and 52% in the 0.15–0.30 m layer (Figure 4). Similar behavior was observed in relation to the quantity adsorbed by clay minerals in the 0–0.05 m VN1 layer and the 0–0.15 m VN2 layer.

The greatest concentration of Cu-OM fraction was 22.1 mg kg^{-1}, observed in the 0–0.05 m layer of the VN3 soil (Table 5), which already exhibited 92.1 mg kg^{-1} of CuT in this same layer. The percentage of Cu complexed by the OM in vineyard soils was barely changed in relation to the NF area soil, where it represented 17% to 33% of the total. In the 0–0.30 m layer of vineyard soil, the Cu-OM represented 14% to 30% of the sum of the total Cu (Figure 4).

The greatest CuE concentrations were observed in VN3 from the 0–0.05 and 0.05–0.15 m layers and in the VN2 0–0.05 m layer (Table 4), where it increased from 4.5 to 8.8 times. At the same time,

Table 4. Quantity of total copper CuEPA per hectare in different layers of soil collected from three vineyards with different histories of copper-based fungicide application and in a natural field area in Study # 2.

Depth M	Natural field		Vineyard 1		Vineyard 2		Vineyard 2	
	Copper. kg ha^{-1}							
0.00–0.05	2.59	[NS]C[a]	5.80	[NS]C	32.90	aB	76.11	aA
0.05–0.10	2.30	C	2.73	C	11.59	bB	46.72	bA
0.10–0.15	2.37	B	2.28	B	6.38	bB	25.84	cA
0.15–0.20	2.04	B	2.95	B	5.45	bB	13.54	dA
0.20–0.25	2.23		4.04		4.15	b [ns]	7.70	de
0.25–0.30	2.22		4.44		4.16	b	5.75	e
0.30–0.35	2.44		2.51		4.49	b	5.02	e
0.35–0.40	2.44		2.51		4.49	b	5.02	e
0.40–0.45	2.29		3.92		5.01	b	4.64	e
0.45–0.50	2.29		3.92		5.01	b	4.64	e
0.50–0.55	2.29		3.92		5.01	b	4.64	e
0.55–0.60	2.29		3.92		5.01	b	4.64	e
Sum	27.79		42.94		93.65		204.26	
Net increase[b]	-		15.15		65.86		176.47	
Applied amount[c]	-		6.34		45.05		157.72	
Difference			−8.81		−20.81		−18.75	

** Significant at $p < 0.01$. NS Not significant.
[a]Mean values followed by the same small letter in the column and capital letters in the row are not statistically different by Tukey test at $p < 0.05$.
[b]The existing quantity of Cu in the natural field was subtracted (27.79 kg).
[c]Values obtained by summing the quantity applied from 1993 to 2009.
CV for layers = 34.17; CV for vineyards = 37.48.

CuT concentrations greater than 50 mg kg^{-1} was observed. In contrast, CuT concentrations lower than 15 mg kg^{-1} had little influence on the CuE fraction (Table 4). Data also showed that the percentage of CuE in relation to the total extract decreased as the CuT increased (Figure 4).

In the 0–0.05 m layer, there was the greatest accumulation of CuSol that increased from undetectable in uncultivated NF (<40 μg kg^{-1}) to 42.5, 292 and 617 μg kg^{-1} for the VN1, VN2 and VN3 soils, respectively. The CuSol concentrations in the vineyards decreased with depth, but in VN3, the 0.15–0.30 m layer still exhibited 189 μg kg^{-1}. In fact, the CuSol fraction represented 0.5 to 1.4% of the fractional sum in vineyard soil layers under cultivation (Figure 4).

Discussion

The Ultisols used in this study had low OM and clay and a predominance of 1:1 clay minerals (kaolinite), which reduce sorption capacity for metals such as Cu. Kaolinite has low isomorphic substitution, which results in a low density of negative charges (CEC), that are important for metal sorption (McBride 1994) and are instead abundant in 2:1 clay minerals. In addition, the soils used in this study originated from acid rocks and do not contain carbonates. In soils of limestone origin and in the presence of carbonates with a neutral to alkaline pH, Cu may be adsorbed to the carbonates or precipitate as hydroxides or carbonates (Bradl 2004). These chemical reactions allow greater Cu concentrations without causing harm to plants (Brun et al. 1998; Chaignon and Hinsinger 2003).

During vineyard plantation, soil liming is recommended to raise the pH up to 6.0 (CQFS RS/SC 2004), which explains the greater pH of the vineyard soils in comparison to the soil from the NF area. Nevertheless, the acid nature of the soils maintain available forms of Cu (Bradl 2004; McBride 1994). Earlier studies showed that Cu is more available in acid soils, which could lead to plant toxicity (Brun et al. 2001; Chaignon et al. 2003). Nevertheless, the main objective of liming is not to decrease Cu availability through the increase in pH values of the soil, but by Al^{+3} neutralization, which is considered sufficient for good plant growth when the pH is 5.5 (CQFS RS/SC 2004). Liming

Table 5. Soil concentration of Cu fractions collected from three vineyards with different histories of copper-based fungicide application and in a natural field area (Study # 2).

Soil depth	Natural field		Vineyard 1		Vineyard 2		Vineyard 3		CV%
(m)	Soluble (H$_2$O), µg kg^{-1}								
0.00–0.05	ND	[a]	42.51		292.37		616.88		–
0.05–0.15	ND		ND		113.04		430.91		
0.15–0.30	ND		ND		54.73		188.64		
	Exchangeable (MgCl$_2$), mg kg^{-1}								
0.00–0.05	0.27	bC[b]**	0.45	bC	1.51	aB	2.39	aA	11.46[c]
0.05–0.15	0.55	aB	0.70	aB	0.67	bB	2.50	aA	6.67[d]
0.15–0.30	0.60	aBC	0.70	aAB	0.41	cC	0.85	bA	
	Mineral fraction (NH$_2$OHHCl), mg kg^{-1}								
0.00–0.05	0.21	bD**	2.05	aC	37.84	aB	85.78	aA	4.62
0.05–0.15	0.67	abC	0.67	bC	7.86	bB	37.38	bA	3.92
0.15–0.30	1.48	aBC	0.32	cB	2.40	cB	7.08	cA	
	Organic fraction (HNO$_3$ + H$_2$O$_2$), mg kg^{-1}								
0.00–0.05	1.61	NSC**	2.70	aC	10.83	aB	22.11	aA	12.73
0.05–0.15	0.95	C	1.50	abC	3.36	bB	6.89	bA	13.15
0.15–0.30	1.06	A	1.28	bA	1.61	cA	2.05	cA	
	Residue (HF), mg kg^{-1}								
0.00–0.05	2.79	bC**	3.63	bA	3.21	aB	3.91	aA	5.35[V]
0.05–0.15	2.79	bB	3.48	bA	3.07	aB	3.07	cB	3.34[b]
0.15–0.30	3.07	aBC	5.87	aA	2.79	bC	3.35	bB	
	Sum fractions, mg kg^{-1}								
0.00–0.05	4.91	NSD**	8.87	aC	53.69	aB	114.8	aA	5.74
0.05–0.15	5.00	C	6.39	bC	15.07	bB	50.28	bA	4.27
0.15–0.30	6.24	B	8.21	abB	7.26	cB	13.51	cA	
	Total (H$_2$O$_2$ + HF), mg kg^{-1}								
0.00–0.05	4.86	aC**	10.53	aC	51.09	aB	92.07	aA	10.82
0.05–0.15	4.18	aC	7.01	aC	14.9	bB	46.23	bA	15.61
0.15–0.30	4.65	aB	6.85	aAB	7.64	cAB	12.85	cA	
	Total EPA (H$_2$O$_2$ + HNO$_3$ + HCl), mg kg^{-}								
0.00–0.05	3.10	NSD**	7.92	aC	49.76	aB	117.20	aA	2.20
0.05–0.15	2.38	D	4.49	bC	12.08	bB	46.94	bA	4.63
0.15–0.30	2.86	C	4.25	bC	7.67	cB	12.33	cA	

**Significant at $p < 0.01$. NS Not significant.
[a]ND. Not detected (<40 µg kg^{-1}).
[b]Mean values followed by the same small letter in column and capital letters in row are not statistically different by the Tukey test at $p < 0.05$.
[c]CV for vineyards.
[d]CV for soil layers.

to a pH of 6.5 to 7.0 could decrease Cu availability, but the magnitude of this effect and its results on grapevine nutrition still requires further studies.

In contrast, the maintenance of OM in vineyard soils, reflected in this study, are the result of management techniques adopted in the 1990s. During that decade, cover crops such as oats (*Avena strigosa*) and clovers (*Trifolium* spp.) were introduced to the vineyards, which increased the input of organic matter into the soil together with the senescent leaves and residues from grapevine trimming (Brunetto et al. 2011). At the same time, between-row grapevine soils were no longer turned over, which resulted in cover crop maintenance, slower organic residue decomposition and, consequently, in reduced erosion. The maintenance of OM is of great importance for the fertility of soils, especially those with a sandy texture with variable charges. In these soils, a large part of the CEC is derived from negative charges on the OM (Syers, Campbell, and Walker 1970), such as those observed in this study. An increased CEC results in a greater capacity to retain nutrients such as Ca^{2+}, Mg^{2+}, and K$^+$, which improves plant nutrition and may help to decrease the phytotoxic effects of Cu (Marschner 1995). In addition, OM controls Cu availability because its high affinity sites and a binding energy capable of making strong complex with this metal (Besnard, Chenu, and Robert 2001; Bradl 2004; Croué et al. 2003).

In the studied vineyards, it was observed that the use of Cu-based fungicides for approximately 30 years resulted in relatively high metal contents in the soils. Nevertheless, the total concentrations observed in our study are similar and in many cases less than those reported in the literature. Diverse
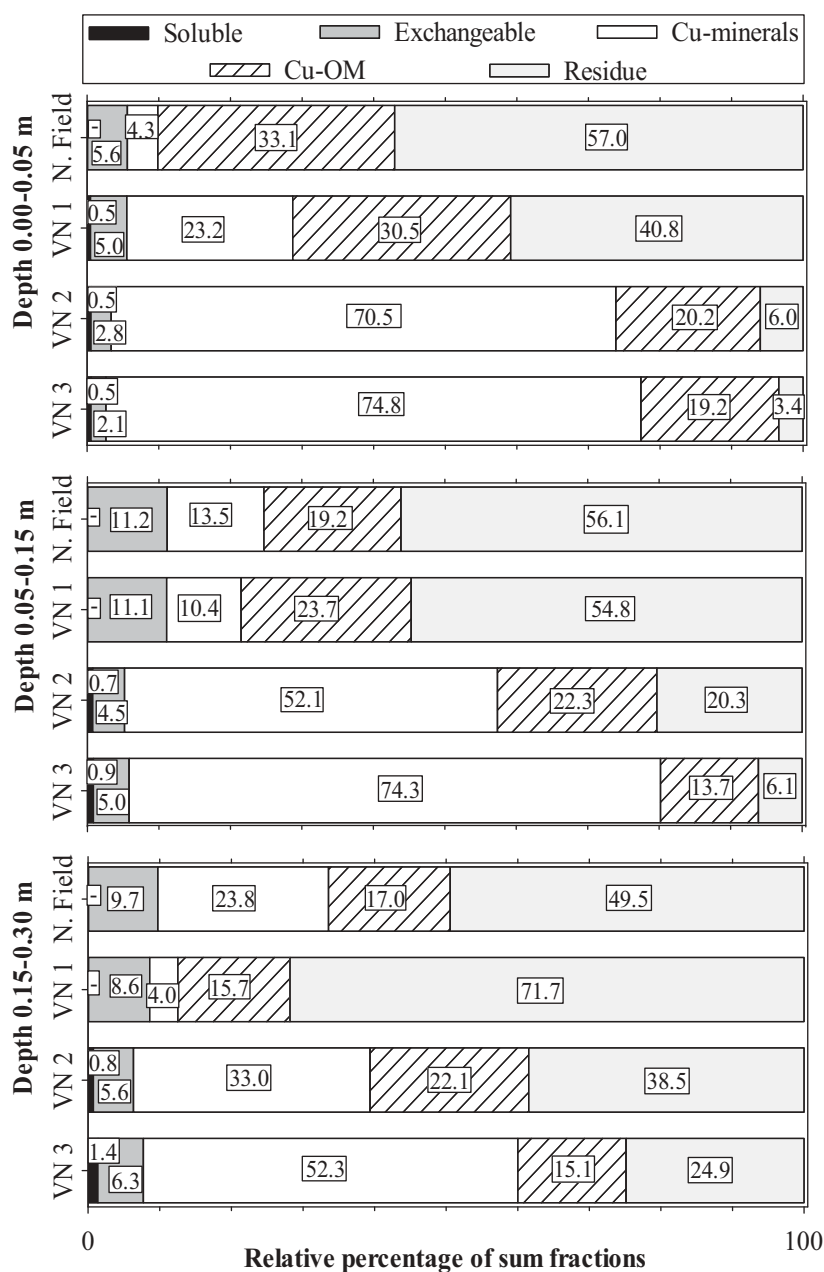

Figure 4. Relative percentage of each fraction of Cu obtained by sequential extraction of soil collected in three selected vineyards (Study # 2). The numbers in boxes placed over the bars represent the percentage of the respective fraction. N. F. Natural field. VN. Vineyard.

studies showed that the total Cu concentration ranges from 40 to 665 mg kg^{-1} in vineyard soils (Brun et al. 1998; Casali et al. 2008; Fernández-Calviño et al. 2009; Flores-Veles et al. 1996). In Australia, in the 0–0.10 m layer of acid, sandy soils of vineyards of 20 to 30 years, a Cu total mean

concentrations ranging from 30 to 113 mg kg^{-1} Cu was found. The values ranged between 34 and 52 mg kg^{-1} in vineyards older than 90 years (Pietrzak and McPhail 2004). In Spain, acid soils with less than 230 g kg^{-1} of clay and with a history of more than 100 years of fungicide applications exhibited 40 to 300 mg kg^{-1} of the total Cu in the 0–0.20 m layer (Arias et al. 2004).

The reference values established by government regulatory bodies are not specific for fungicide use and, in general, they are intended to regulate the use of sewage sludge and waste products. In Brazil, the total Cu concentrations of 60 and 200 mg kg^{-1} are the prevention and intervention (maximum permitted) concentrations, respectively, for Cu in agricultural soils (CETESB 2005; CONAMA 2009). The USEPA established a maximum limit of 1500 mg kg^{-1} (USEPA 1993), and the European Community allows 50 to 140 mg kg^{-1} of Cu for agricultural soils with pH 6.0 to 7.0 (Council Directive 86/278/EEC 1986). In Australia and New Zealand, 60 mg kg^{-1} of Cu indicates the need for an environmental evaluation (ANZECC/NHMRC 1992). Although they are still within the limits established by regulatory bodies and those reported in the literature, the accumulation of Cu in the vineyard soils here investigated was already equal to that of older vineyards in other Countries. In addition, some of the vineyards exhibited the total Cu greater than 60 mg kg^{-1}, which indicates the need for preventive practices to avoid polluting these soils (CETESB 2005; CONAMA 2009).

The accumulation of Cu in soils is directly connected to the rate of Cu application, the sorption capacity of the soils and losses through erosion or leaching. In Regions with greater mean rainfall, the accumulation of Cu is greater in vineyard soils (Komárek et al. 2010; Vavoulidou et al. 2005). This trend occurs because the greater the rainfall, the greater the incidence of fungal diseases and, consequently, the greater the number of fungicide applications. In our investigation, we observed that the quantity of applied Cu ranges from less than 5 to more than 20 kg ha^{-1} year^{-1}. This variation is explained by rainfall variability, especially during the spring and summer period (la niña and el niño phenomena), which influences the need for fungicides. Nevertheless, the application rate is less than that reported in Serra Gaúcha another important wine growing region in the south of Brazil, where up to 30 kg Cu ha^{-1} year^{-1} is applied (Casali et al. 2008). In Australia, the annual application rate is 2–14 kg Cu ha^{-1} (Pietrzak and McPhail 2004; Wightwick et al. 2008). In Europe, rates of 0.5 to 8.7 kg Cu ha^{-1} year^{-1} are reported in Italy (García-Esparza et al. 2006; Toselli et al. 2009), and from 5 to 8 applications of 0.5 to 2.5 kg of Cu per year are used in Greece (Vavoulidou et al. 2005). Nevertheless, in some regions, such as Bordeaux, France, the application rate was much greater, i.e. 15 to 30 kg Cu ha^{-1} year^{-1} in past decades (Brun et al. 1998). In the current organic production system, the European Community permits the application of up to 30 kg Cu ha^{-1} every 8 years (mean value of 3.75 kg Cu ha^{-1} year^{-1}) (Council Regulation 2092/91/EEC 1991).

Our data show that Cu accumulation occurred mainly in the surface layers of the soil, because of the high affinity of Cu for soil constituents (McBride 1994). Once in contact with the soil, Cu binds to the functional groups of diverse crystalline phase constituents and the OM (Schramel, Michalke, and Kettrup 2000). In the presence of a large quantity of high affinity functional groups, for example groups containing sulfur, nitrogen, carboxyl, and phenols (Croué et al. 2003) present in OM, Cu is complexed with a high binding energy in forms that are not easily soluble. The same pattern occurs in limestone soils in which the Cu binds to carbonates or precipitates at alkaline pH (Bradl 2004).

Our data showed that the increased Cu concentration in the 0.10–0.20 m layer has begun after 11 to 15 years of cultivation, and became more intense after this period. In fact, the Cu concentration was highest in the 0.60-m layer in a vineyard with only 31 years under cultivation, but this did not represent a potential risk to groundwater because the concentration in the deeper layers was low. This Cu movement in the soil profile was not the result of periodic soil turnover operations, as occurs in some tillage systems in the world, rather was a consequence of the sandy texture and the predominance of 1:1 clay minerals such as kaolinite resulting in a limited Cu sorption capacity. In addition, the organic acids released by root exudation and OM decomposition might have chelated Cu and facilitated its percolation. In addition, particles move in the soil profile in response to the galleries formed by biota and by decomposing plant roots. A similar Cu distribution in the profile of sandy vineyard soils with an acid soil was reported in France (Brun et al. 1998) and Australia

(Pietrzak and McPhail 2004). However, in the soils of the present study, percolation occurred with lower total Cu concentrations than those reported by these authors.

The movement of Cu in the soil profile may be accelerated by soil turnover. The vineyards in the southern region of Brazil, like those of the present study, have a commercial life time of about 30 years. At the time of vineyard renewal, the plowing and harrowing operations undertaken to level the soil result in Cu movement in the soil profile. An inversion of the soil layers reduced the CuT and CuEDTA concentrations in the 0–0.10 m layers and raised it in the 0.10–0.20 m layer, but not in the same proportion, indicating that the concentration was also distributed along the layers below 0.20 m. Nóvoa-Muñoz et al. (2007) reported similar total Cu concentrations (250 to 270 mg kg^{-1}) up to 0.30 m depth and they attributed this finding to frequent soil turnover in the vineyards. Turning over the soil to dilute the Cu in the lower profile has been presented as a remedial technique for soils with excessive metal accumulation (Pietrzak and Uren 2011). Nevertheless, it increased the Cu concentrations in the layers below 0.10 m, leading to greater plant root exposure to Cu, and possible greater potential for toxicity.

The total quantities of Cu accumulated in soil were a little greater than the quantities applied via Cu-based fungicides. The fungicides are not the only inputs that contain Cu. Frequent use of organic or chemical fertilizers, often enriched with micronutrients such as Cu, may also transfer this metal to the soil. In addition, other heavy metals are also found in chemical fertilizers (Sharpley and Menzel 1987), frequently used in vineyards. In contrast, the quantity of Cu applied during the first 15 years in VN3 cultivation (1978 to 1992) was not found in the 0.00–0.60 m layer, indicating Cu leaching over the years and also adsorbed to particles transported by erosion. The reason of this response is related to the sandy texture of the studied soils that makes them susceptible to water erosion, even though the topography is only lightly rolling (Streck et al. 2002). However, disking between the grapevine rows was a common form of weed control in the 1980s. This management technique exposed soil to raindrop impact, which led to soil degradation and surface runoff. In fact, sediments coming from vineyard generally contain high Cu concentrations (Fernández-Calviño et al. 2008; Pietrzak and McPhail 2004). Under other conditions, the total accumulated quantity of Cu in soil planted to vineyards may be much greater than that observed in the present study. Brun et al. (1998) reported accumulations of up to 215 and 469 kg Cu ha^{-1} in acid and limestone soils, respectively, in the 0–0.15 m layer alone.

EDTA is frequently used to evaluate soil Cu availability for plants. In this study, EDTA extracted nearly 80% of the total Cu. This finding indicates that the Cu accumulated in the vineyard soils was available and was 50 or more times greater than plant requirement, which is 1.0 mg kg^{-1} (CQFS RS/SC 2004). Interestingly, the total concentration of CuEPA and EDTA-extractable exhibited proportional values in all the soils and layers in this study. This finding shows a proportional distribution of high and low binding energy forms for Cu, regardless of the layer and total Cu concentration. In addition, differences in soil texture, OM, pH and CEC$_{pH7.0}$ caused little interference with EDTA extraction of Cu. The literature reports that EDTA has different extraction capacities depending on the different soils and total metal concentrations, ranging from less than 10% from both acid and limestone soils (Brun et al. 1998), to 20, 50% (Brun et al. 1998; Chaignon et al. 2003; Toselli et al. 2009) and up to 95% of the total Cu (Schramel, Michalke, and Kettrup 2000). The quantity extracted with EDTA generally presents a strong correlation with the total quantity, but it is not always correlated with availability to plants (Brun et al. 1998; Chaignon and Hinsinger 2003). Because of its activity and time of contact with the soil, this extractor is not sufficiently strong to extract the most stable forms of Cu. Nevertheless, EDTA is a strong extractor, and it is even capable of extracting Cu bound to OM (Arias et al. 2004), which is considered a quite stable form because of its high binding energy (McBride, Martínez, and Sauvé 1998).

Regardless of the soil layer, the greatest quantity of Cu accumulation in this study was connected with clay minerals (Table 3 and Figure 4). This fraction is reported as the most important in terms of Cu sorption in acid and with low OM soils (Casali et al. 2008; Yu et al. 2004). Cu has a high affinity for the functional groups present in Fe and Mn oxides (Bradl 2004), but may also be adsorbed with

less binding energy in the silanol and aluminol groups of phyllosilicates (McBride 1994; Sposito 1989). However, soil pH alterations greatly affect the sorption capacity of clay minerals, especially iron oxides (Sposito 1989). In acid soils, the sorption is lower and desorption is more likely (Arias et al. 2005; Bradl 2004; Casali et al. 2008). For these reasons, the Cu fraction bound to minerals is considered available (Tessier, Campbell, and Bisson 1979).

The quantity of Cu complexed in the OM was many times lower than the quantity adsorbed in the clay minerals (<22 mg kg^{-1}) being among the lowest reported in the literature. Previous studies showed that the OM of the soil may retain the majority of total Cu in contaminated soils, reaching concentration of 40 to 400 mg kg^{-1} (Fernández-Calviño et al. 2009; Nóvoa-Muñoz et al. 2007; Pietrzak and McPhail 2004). In relative terms, these concentrations represent 20% (like those observed in the present study), 40% to even 80% of the total accumulated quantity. This amplitude is explained by differences in the OM and Cu concentrations, but is also explained by methodology differences (Kennedy et al. 1997). Nevertheless, our data showed that soil OM exhibited a low Cu sorption capacity and may be saturated in the surface layers of the oldest vineyards. This hypothesis is based on the fact that even if there is greater affinity for Cu in the OM groups (Besnard, Chenu, and Robert 2001; Croué et al. 2003; McBride 1994), the greatest quantity of Cu was found accumulated as CuMin fraction.

The soluble and exchangeable fractions are considered to be mobile in the soil and easily available (Tessier, Campbell, and Bisson 1979). The exchangeable phase is in rapid equilibrium with the soil solution (Sposito 1989). In solution, the Cu moves in accordance with and in the direction of water flow, and may be taken up by roots (Marschner 1995) or percolate into the soil profile. As expected the soluble Cu observed in the present study represented a little fraction (<1.4%) of the total Cu accumulated, as a consequence of the high Cu affinity for the soil, which results in its low solubility (McBride 1994; Sposito 1989). Although the soluble concentration has increased from non-detectable at 616.9 µg kg^{-1} or 0.6 mg kg^{-1}, it is within the range normally observed in soils of an acid and sandy nature with accumulated Cu. Pietrzak and McPhail (2004) reported a soluble Cu concentrations from <1.0 mg kg^{-1} to 1.4 mg kg^{-1} when the total Cu was <150 and 250 mg kg^{-1}, respectively. Nóvoa-Muñoz et al. (2007) showed that soluble Cu may vary from 0.5 to 3.8 mg kg^{-1} with a total concentrations range of 104–632 mg kg^{-1}.

The same behavior may be observed with the exchangeable Cu concentrations (<2.5 mg kg^{-1}). This response occurs when certain maximum sorption capacity limits have been reached, which are different for each soil, leading to a more pronounced increase of soluble and exchangeable forms. This happens in the surface layers of vineyards with a greater Cu accumulation (Table 5). In the literature, exchangeable Cu concentrations extracted with different salts have been reported (for example, CaCl$_2$, NH$_4$CH$_3$COO, ammonium nitrate (NH$_4$NO$_3$) or magnesium chloride (MgCl$_2$), as used in the present study) which generally are less than 10 mg kg^{-1} (Arias et al. 2004; Casali et al. 2008; Fernández-Calviño et al. 2009; Pietrzak and McPhail 2004). However, when the total Cu concentrations are higher, the exchangeable Cu may even range from 20 to 36 mg kg^{-1} (Fernández-Calviño et al. 2009; Nóvoa-Muñoz et al. 2007). The increase in the exchangeable and soluble Cu is connected with the equilibrium between the diverse forms of Cu and the partial saturation of sites with greatest binding energy for the Fe, Mn, and OM oxides (Bradl 2004; McBride 1994; Sposito 1989).

Regardless of the fractionation scheme used, the residual fraction represented the most stable or unavailable forms of Cu. Under natural conditions, Cu was predominantly found in the residual fraction, which is frequently reported in fractionations. In contrast, when Cu was accumulated, the behaviour of the residual fraction differed among the soils. In the soils evaluated in the present study, the residual fraction was small (<4 mg kg^{-1}) with differences of ~1 mg kg^{-1} among the layers and vineyards, showing the scarcity of high binding energy groups. The low residual Cu concentration shown in this study may be attributed to low OM and clay, which are predominated by kaolinite. These characteristics determine the maintenance of nearly all the Cu in available or potentially available forms. These data are among the lowest already reported and they differ from most previous studies, which in general show that Cu accumulation is followed by an increase in the residual fraction. In soils with clay

less than 20%, the Cu residual fraction may consist of less than 30 mg kg^{-1} (often <10 mg kg^{-1}) (Arias et al. 2004; Pietrzak and McPhail 2004), but in some soils and textures, it may reach values from 40 to 200 mg kg^{-1} (Casali et al. 2008; Fernández-Calviño et al. 2009; Nóvoa-Muñoz et al. 2007). These concentrations represent an average of 15 to 25% of the total Cu, but in some soils, this percentage is greater than 35%. This capacity to inactivate the Cu in the residual fraction is directly related to the presence of recalcitrant organic carbon, amorphous inorganic materials and high crystallinity Fe and Al oxyhydroxides (Fernández-Calviño et al. 2009; McBride 1994; Tessier, Campbell, and Bisson 1979).

Conclusions

In sandy acid soils used to cultivate grapevines, there should be greater caution with regards to Cu accumulation, because approximately 80% of the total Cu accumulated in these soils is potentially available to plants (and extractable with EDTA), and may cause phytotoxicity. Fungicide application rates increased Cu concentration in the top 0.30 m soil layer over 30 years of cultivation. The low amount of Cu in the residual fraction indicates the percolation of this metal in the soil profile and links lower energy binding clay minerals with increased soluble and exchangeable Cu.

Acknowledgments

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