

# Copper and zinc accumulation, fractionation and migration in vineyard soils from Santa Catarina State, Brazil

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**ABSTRACT:** The purpose of this study was to evaluate Cu and Zn migration and fractions in sandy soil of vineyards. In Urussanga (SC), Brazil, soil samples were collected from a 4-year-old and 15-year-old vineyard, and from a forested area. In the soils, the chemical characteristics of Cu and Zn were analyzed by the EDTA method, which determines the fraction available in soil; 3050B method of the USEPA for total concentrations, which represents the pseudo-soil contents in the soil; and chemical fractionation, which estimates soluble fraction, exchangeable fraction, fraction associated with clay minerals, fraction associated with organic matter and residual fraction. The results show that there is accumulation of Cu and Zn in sandy soils cultivated with grapevines and with frequent fungicide applications.

These higher levels were found in soils with longer cultivation time (15 years old), but were restricted to the superficial layers of the soil. Most of the Cu was extracted by EDTA method, and it may be considered as available to plants. The EDTA also extracted a small part of Zn. Most of the Cu in the vineyard soils can be characterized by low geochemical mobility, but in the uppermost soil layers of the oldest vineyard, there was an increase in Cu content associated with soil organic matter. Most of the Zn in the vineyard soil was associated with minerals, which indicates low mobility and also low potential for toxicity to plants and microorganisms.

**Key words:** availability, chemical fractionation, toxicity, environmental contamination, *Vitisvinifera*.

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## INTRODUCTION

In the southern region of Santa Catarina (SC), Brazil, vineyards are planted on soils located on lands near sea level with sandy texture, predominance of 1:1 clay, low organic matter content, acidic soil and low natural fertility (Schmitt et al. 2013). The grapevines are subjected to the application of Cu- and Zn-bearing fungicides for control of fungal leaf diseases (Mackie et al. 2012; Tiecher et al. 2016).

Because of washing of trace-elements by rainwater or deposition of senescent leaves or pruned branches containing Cu and Zn derived from the applications, the concentration of the two elements in the soil may increase through the years. Therefore, there may be an increase in Cu concentration in vineyard soils of traditional winegrowing regions of the world (Fernández-Calviño et al. 2008; Toselli et al. 2009; Fernández-Calviño et al. 2010), including those in Brazil, as reported in some exploratory studies on copper (Casali et al. 2008; Nogueirol et al. 2010; Brunetto et al. 2016) and zinc (Fernández-Calviño et al. 2012).

Copper and Zn in the soil are retained by physical-chemical bonds and their lability depends on the ligand, especially on the content of minerals, oxides and hydroxides of iron (Fe), aluminum (Al) and manganese (Mn), carbonates and organic matter (Bradl 2004; Couto et al. 2015), but also on the soil pH value (Chaignon et al. 2003; Shaheen et al. 2015), cation exchange capacity (CEC) and the composition of the organic matter (Fernández-Calviño et al. 2010). Normally, adsorption of Cu and Zn in the soil first occurs in the most avid binding sites and then the remaining trace elements are redistributed in fractions that are retained with less energy and, consequently, with greater availability and mobility (Miotto et al. 2013). However, frequent applications of fungicides can increase the Cu and Zn content in the soil, modifying distribution of the fractions, increasing toxicity to plants (Nagajyoti et al. 2010) and transfer by the runoff solution on the soil surface and percolated in the soil profile, increasing water contamination (Fernández-Calviño et al. 2012).

Methods for chemical analysis are used to estimate the Cu and Zn content in the soil. The total content of the two trace elements, like that estimated by the 3050B method of the USEPA (USEPA 1996), has been a useful indicator, but it may, for example, present low correlation with bioavailability of the element to plants (Miotto et al. 2013). For that reason, extractions with chemical products in an

isolated manner have been used, such as chelating agents, like Ethylenediamine tetra acetic acid (EDTA) (Schramel et al. 2000). However, for some soils, EDTA may present limited capacity for predicting the contents of the trace elements that may be available to plants or potentially transferred to the environment (Chaignon and Hinsinger 2003). Thus, the results of Cu and Zn in the soil obtained in by an isolated method of analysis may be improved by joining them with chemical fractionation. Through the sequential use of chemical extractors, it removes the Cu and the Zn of the most labile fractions up to the most stable (Tessier et al. 1979).

Thus, it is possible to separate the total quantity of the trace element in the soils or sediments into bioavailable fractions (soluble in water or exchangeable), potentially bioavailable (associated with clay minerals, oxides, carbonates and organic matter) and residual (mineral structure) (Tessier et al. 1979). With the use of this technique, an increase of Cu has been verified in vineyard soils, especially in the mineral and organic fraction (Casali et al. 2008; Nogueirol et al. 2010) and of Zn in the residual or mineral fraction (Fernández-Calviño et al. 2012). But studies in traditional winegrowing regions of the world reporting Cu and Zn distribution in different soil fractions are scarce, and in Brazil are non-existent. This study aims to evaluate Cu and Zn migration and fractions in sandy soils of vineyards with a history of fungicide application.

## MATERIALS AND METHODS

The vineyard and forest soils used in the present study were collected in the municipality of Urussanga (lat 28°31'22'S, long 49°19'3'W), in the southern region of SC, Brazil. The soil of the vineyards and the forest was sandy Typic Hapludalf (Soil Survey Staff 2006) (Table 1). The soil had a sandy surface horizon, which was derived from a granite substrate, exhibited predominance of 1:1 clay and was located on flat land (Santos et al. 2015). Climate in the region is humid subtropical (Cfa), with annual mean temperature of 19.4 °C and mean annual rainfall of 1,619mm (Embrapa 2003).

The vineyards were of different ages and, consequently, had different histories of application of Cu-bearing fungicides, such as Bordeaux mixture ( $\text{Ca}(\text{OH})_2 + \text{CuSO}_4$ ) and Cu-oxychloride ( $\text{CuCl}_2 \cdot 3\text{Cu}(\text{OH})_2$ ), and Zn-bearing

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**Table 1.** Main physical and chemical characteristics of the forest, vineyard 1 (4 years) and vineyard 2 (15 years) soils, at 0.0-0.05, 0.05-0.10 and 0.1-0.20m depth.

Characteristics	Forest			Vineyard 1			Vineyard 2		
Clay(g·kg <sup>-1</sup> ) <sup>(1)</sup>	36 <sup>(7)</sup>	135 <sup>(8)</sup>	81 <sup>(9)</sup>	97 <sup>(7)</sup>	112 <sup>(8)</sup>	104 <sup>(9)</sup>	88 <sup>(7)</sup>	135 <sup>(8)</sup>	202 <sup>(9)</sup>
Silt(g·kg <sup>-1</sup> ) <sup>(1)</sup>	825	826	804	623	614	604	586	561	523
Sand (g·kg <sup>-1</sup> ) <sup>(1)</sup>	139	39	116	280	274	292	323	304	275
OM(g·kg <sup>-1</sup> ) <sup>(2)</sup>	6.0	4.0	1.5	18.1	14.9	15.9	26.7	19.4	16.6
pH in water <sup>(2)</sup>	4.5	4.0	4.0	5.3	5.3	5.4	6.3	6.4	6.2
P <sub>a</sub> (mg·kg <sup>-1</sup> ) <sup>(3)</sup>	10.0	7.0	4.0	82.3	48.3	22.7	483.0	424.6	196.0
K <sub>e</sub> (mg·kg <sup>-1</sup> ) <sup>(3)</sup>	30.0	20.0	18.0	249.9	157.0	129.8	275.6	237.6	257.2
Ca <sub>e</sub> (cmol <sub>c</sub> ·kg <sup>-1</sup> ) <sup>(4)</sup>	1.0	0.5	0.5	3.7	4.1	3.8	5.5	4.9	4.1
Mg <sub>e</sub> (cmol <sub>c</sub> ·kg <sup>-1</sup> ) <sup>(4)</sup>	0.4	0.3	0.2	3.4	3.1	2.9	3.3	3.2	3.2
Al <sub>e</sub> (cmol <sub>c</sub> ·kg <sup>-1</sup> ) <sup>(4)</sup>	0.2	0.4	0.4	0.5	0.6	0.7	0.0	0.0	0.0
CEC <sub>e</sub> (cmol <sub>c</sub> ·kg <sup>-1</sup> ) <sup>(5)</sup>	1.67	1.25	1.14	12.9	12.6	12.1	12.0	11.3	11.1
CEC <sub>pH7.0</sub> (cmol <sub>c</sub> ·kg <sup>-1</sup> ) <sup>(5)</sup>	3.77	2.91	2.74	8.2	8.2	7.7	9.5	8.7	8.0
Fe <sub>d</sub> (g·kg <sup>-1</sup> ) <sup>(6)</sup>	4.6	4.9	5.4	27.1	28	29.6	17.5	21.5	27.7
Fe <sub>o</sub> (g·kg <sup>-1</sup> ) <sup>(6)</sup>	0.4	0.5	0.6	6.9	6.9	7.0	2.8	3.1	3.3

<sup>(1)</sup>Pipette method (Embrapa 1997); <sup>(2)</sup>Determined according to Tedesco et al. (1995); <sup>(3)</sup>Extracted Mehlich 1 extractor (Tedesco et al. 1995); <sup>(4)</sup>Extracted by KCl 1 mol·L<sup>-1</sup> extractor (Tedesco et al. 1995); <sup>(5)</sup>Determined according to CQFS-RS/SC,2016; <sup>(6)</sup>Extracted by (NH<sub>4</sub>)<sub>2</sub>C<sub>2</sub>O<sub>4</sub>·H<sub>2</sub>O 0.2 mol·L<sup>-1</sup> (Tedesco et al. 1995); <sup>(7)</sup>Depht 0.00-0.05; <sup>(8)</sup>Depht 0.05-0.10; <sup>(9)</sup>Depht 0.10-0.20.

fungicides, such as Mancozeb. Vineyard 1 was established in 2006 at a density of 2.525 plants per hectare and vineyard 2 was established in 1995 at a density of 1.429 plants per hectare. Vineyard 1 (lat 28°34'34,2"S, long 49°16'07"W and altitude of 29m) and vineyard 2 (lat 28°33'49,5"S, long 49°15'50,2"W and altitude of 24m) were formed of the *Niágara Rosada* cultivar, grafted onto the rootstock Paulsen 1103. The plants of the two vineyards were trained in the pergola trellis training system and each one of the vineyards occupied an area of approximately one hectare. Before setting up the vineyards, limestone was applied on the soil surface, with later incorporation, to raise the pH in water to 6.0. At planting, 17.5kg P·ha<sup>-1</sup> and 63.3kg K·ha<sup>-1</sup> were applied on each one of the vineyards for fertilization. Throughout the annual grapevine cycle on the soil surface of each vineyard, approximately 50kg N·ha<sup>-1</sup>, 20kg K·ha<sup>-1</sup> and 22kg P·ha<sup>-1</sup> were applied. The forest soil, located near the vineyards, had never been cultivated and did not have a history of fertilizer application.

Soil collection was made in December 2010, 4 and 15 years after establishment of vineyard 1 and vineyard 2, respectively. Sampling was performed in a representative area of the vineyards through the opening of six trenches with dimensions of 0.3 × 0.5 × 0.5m at random points near the plant row. The soil samples from the forest area,

used as a reference, were collected at random in trenches with the same dimensions as those of the vineyards. In the vineyards and in the forest, soil was collected at the 0.00-0.05, 0.05-0.10, 0.10-0.15, 0.15-0.20, 0.20-0.25, 0.25-0.30, 0.30-0.40, 0.40-0.50 and 0.10-0.20m layers. The soil samples were dried in open air, passed through a sieve with a 2mm mesh and reserved for analyses. The physical and chemical attributes were determined in the 0.0-0.05, 0.05-0.10 and 0.10-0.20m layers. Analysis of granulometric distribution of the soil constituents was performed by the pipette method and of organic matter (OM) by the Walkley-Black method (Embrapa 1997). Analysis of pH in water, the available phosphorus content (P<sub>a</sub>), exchangeable contents of K<sup>+</sup> (K<sub>e</sub>), Ca<sup>2+</sup> (Ca<sub>e</sub>), Mg<sup>2+</sup> (Mg<sub>e</sub>) and Al<sup>3+</sup> (Al<sub>e</sub>) were determined according to Tedesco et al. (1995). The crystalline iron (Fe<sub>d</sub>) contents were extracted by sodium citrate-bicarbonate-dithionite, and amorphous Fe (Fe<sub>o</sub>) by ammonium oxalate (Tedesco et al. 1995).

Total Cu contents (Cu<sub>EPA</sub>) and Zn contents (Zn<sub>EPA</sub>) were determined in all the soil samples by the 3050B method of the USEPA (USEPA, 1996). Available Cu contents (Cu<sub>EDTA</sub>) and Zn contents (Zn<sub>EDTA</sub>) were analyzed with the use of the EDTA extractor (Chaignon et al., 2003). Chemical fractionation of Cu and Zn was carried out in the soil of

the 0.0-0.05, 0.05-0.10 and 0.10-0.20m layers (Tessier et al. 1979). There was thus determination of the following:

- i. Soluble fraction ( $\text{Cu}_{\text{Sol}}$  and  $\text{Zn}_{\text{Sol}}$ );
- ii. Exchangeable fraction ( $\text{Cu}_{\text{E}}$  and  $\text{Zn}_{\text{E}}$ );
- iii. Fraction associated with clay minerals ( $\text{Cu}_{\text{Min}}$  and  $\text{Zn}_{\text{Min}}$ );
- iv. Fraction associated with organic matter ( $\text{Cu}_{\text{OM}}$  and  $\text{Zn}_{\text{OM}}$ ); and
- v. Residual fraction ( $\text{Cu}_{\text{R}}$  and  $\text{Zn}_{\text{R}}$ ).

Total Cu and Zn ( $\text{Cu}_{\text{T}}$  and  $\text{Zn}_{\text{T}}$ ) were analyzed in unfractionated soil (Miotto et al. 2013). Determination of  $\text{Cu}_{\text{Sol}}$  and  $\text{Zn}_{\text{Sol}}$  contents was carried out by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (Perkin Elmer, Optima 2100 DV) and the content of the other fractions was by Atomic Absorption Spectrometry (AAS). The Cu and Zn contents were subjected to analysis of variance and when the effects were significant, the mean values were compared by the Tukey test ( $p < 0.05$ ). The data on  $\text{Cu}_{\text{EPA}}$  and  $\text{Cu}_{\text{EDTA}}$  and  $\text{Zn}_{\text{EPA}}$  and  $\text{Zn}_{\text{EDTA}}$  were subjected to correlation analysis.

## RESULTS AND DISCUSSION

### Accumulation and migration of Cu and Zn in the soil profile

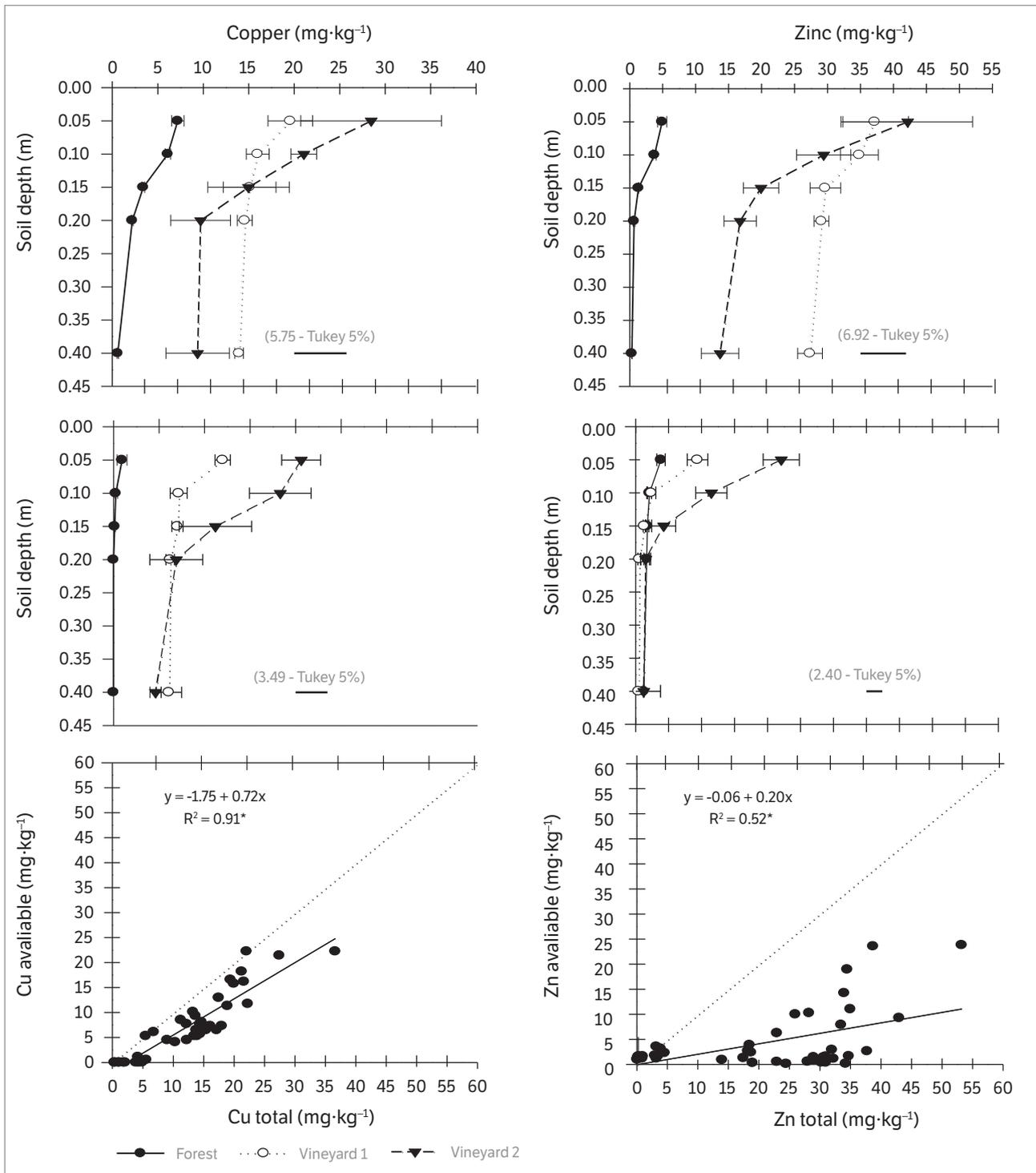
Total Cu contents ( $\text{Cu}_{\text{EPA}}$ ) were higher at the 0.0-0.05m layer (Figure 1a) in the 15-year-old vineyard soil, while in the 4-year-old vineyard,  $\text{Cu}_{\text{EPA}}$  contents were the same at all the soil depths sampled in this study. The lowest levels of  $\text{Cu}_{\text{EPA}}$  were found in the forest soil.

The highest  $\text{Cu}_{\text{EDTA}}$  contents were observed up to the depth of 0.15m in the vineyard 2 soil (Figure 1b). The  $\text{Cu}_{\text{EDTA}}$  levels in the soil of vineyards 1 and 2 were significantly higher than the forest soil. On the other hand, the  $\text{Zn}_{\text{EPA}}$  contents at all depths in the vineyard 1 and 2 soils were higher than the content observed in the forest soil (Figure 1d). The highest  $\text{Zn}_{\text{EPA}}$  contents in the vineyard soils were observed in the 0.10 m to 0.40 m layers of the vineyard 1 soil. At the depth of 0.20 and 0.40m, higher  $\text{Zn}_{\text{EPA}}$  contents were observed in comparison to the vineyard 2 soil. The highest  $\text{Zn}_{\text{EDTA}}$  contents were observed up to the depth of 0.10m in the vineyard 2 soil (Figure 1e). The higher contents of  $\text{Cu}_{\text{EPA}}$  and  $\text{Zn}_{\text{EPA}}$ , and  $\text{Cu}_{\text{EDTA}}$  and  $\text{Zn}_{\text{EDTA}}$ , frequently used to assess the mobility of Cu and Zn in the soil profile and, in the case of EDTA, the availability of the element to the

plants, may be explained by the frequent leaf applications of fungicides over the years for control of fungal leaf diseases (Mackie et al., 2012; Santiago-Martin et al. 2015). In vineyard 1 (4-year-old),  $\text{Zn}_{\text{EPA}}$  levels at the 0.10 to 0.40 m layers were higher than those observed in vineyard 2 (15-year-old). This can be attributed to the longer history of Zn fungicide application on grapevine leaves and also from poultry waste application used as source of N, P and K in fertilization and grapevine production (Fernández-Calviño et al. 2012).

The highest contents of  $\text{Cu}_{\text{EPA}}$  and  $\text{Zn}_{\text{EPA}}$ ,  $\text{Cu}_{\text{EDTA}}$  and  $\text{Zn}_{\text{EDTA}}$  in the uppermost layer of the soil (0.00-0.10 m) (Figure 1 a,b,d,e) can be mainly explained by the fact that the soil was not tilled (Miotto et al. 2013). Copper has high binding affinity to the organic matter functional groups, like the groups containing S, N, carboxylic and phenolic groups (Croué et al. 2003), increasing its complexation and, consequently, reducing its availability. Zinc normally has higher binding affinity to functional groups of the mineral solid phase of the soil, but a part may also be associated with functional groups of the organic phase (Giroto et al. 2010; Fernández-Calviño et al. 2012). However, part of the Cu and the Zn migrated in depth, and this may have occurred through water flow in the soil mass, through the turbulent water flow in macropores and through cracks in the soil or even by bioturbation (Giroto et al. 2010). For Zn, solute flow in the soil mass appears to be the most important mechanism since a fraction ( $\text{Zn}_{\text{EPA}}$ ) of the element tends to remain in the soil solution in free form or in soluble ion pairs (Citeau et al. 2003). On the other hand, the free Cu content in the soil solution is very low, due to its high reactivity with the functional groups of organic particles (Croué et al. 2003). Thus, Cu migration in the soil profile occurs especially in the colloidal form (Giroto et al. 2010). In addition, part of the Cu and the Zn may bind to low molecular weight organic acids, increasing their mobility in the soil profile. In addition, the mobility of Cu and Zn may have been facilitated because the soil of the present study has a sandy silty texture, low organic matter contents in the soil profile (Table 1) and the presence of 1:1 clay mineral (kaolinite). In addition, reduction of pH in water values in the deeper soil layers (Table 1) may also have contributed to impede the formation of stable complexes (inner-sphere) between Cu and Zn with functional groups of the organic and mineral fractions of the soil, increasing mobility in the soil (Giroto et al. 2010).

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**Figure 1.** Total copper content (CuEPA) (a); copper available to plants (CuEDTA) (b); correlation between CuEPA and CuEDTA at all depths (n=45) (c); total zinc (ZnEPA) (d); zinc available to plants (ZnEDTA) (e); and correlation between ZnEPA and ZnEDTA at all depths (n=72), in the forest, vineyard 1 (4 years) and vineyard 2 (15 years) soils; (\*= means  $\pm$  standard deviation do not differ from each other according to Tukey's test  $p < 0.05$ ). Bars represent standart deviation of the mean. Dashed line represents reference line.

The increase of total Cu and Zn, but especially of bioavailable Cu and Zn extracted by EDTA, particularly in the uppermost soil layers, may raise toxicity to plant roots

(Nagajyoti et al. 2010), as well as to spontaneous or sown cover crops that cohabitate the vineyards, and lead to the transfer of the two trace elements on the soil surface either

through the runoff solution or adsorbed in the sediment (Fernández-Calviño et al. 2012), increasing contamination of surface waters adjacent to the vineyards.

Vineyard 1 and vineyard 2 soils exhibited a linear relationship with the  $Cu_{EPA}$  contents at all depths of the forest (Figure 1c). The slope of the straight line (0.72) indicates that 72% of the  $Cu_{EPA}$  was extracted by EDTA, which is available to plants. The  $Zn_{EDTA}$  contents at all depths exhibited a linear relationship with the  $Zn_{EPA}$  contents, but the slope of the straight line (0.20) suggests that only 20% of the  $Zn_{EPA}$  was extracted by EDTA (Figure 1f). Some studies, for example, those undertaken with Cu in acidic or calcareous soils, report that EDTA may extract from less than 10% up to

95% of total Cu (Chaignon et al. 2003; Toselli et al. 2009). Normally, the quantity of Cu and Zn extracted with EDTA shows a strong correlation with the total quantity, but it is not always correlated with the quantity taken up by the plants (Chaignon and Hinsinger 2003).

## Cu and Zn fractions in the soil

In the vineyard 1 soil, the highest contents of  $Cu_{Sol}$ ,  $Cu_E$ ,  $Cu_{Mf}$ ,  $Cu_{OM}$ ,  $Cu_R$  and the sum of the fractions in general were observed in the uppermost soil layers, 0.00-0.05 and 0.10-0.20m (Table 2). The highest  $Cu_{Min}$  and  $Cu_{OM}$  contents were observed in the 0.00-0.10m layer in the vineyard 2

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**Table 2.** Forms of copper and zinc in three layers of forest, vineyard 1 (4 years) and vineyard 2 (15 years) soils.

Copper (mg·kg <sup>-1</sup> )							
Depth (m)	Soluble	Exchangeable	Mineral fraction	Organic fraction	Residue	Sum fractions	Total
<b>Forest</b>							
0.00-0.05	0.05 aC <sup>1</sup>	0.10 aC	0.50 aC	0.78 aC	3.00 aB	4.43 aC	5.86 aB
0.05-0.10	0.05 aC	0.10 aC	0.60 aC	0.20 aC	1.50 aB	2.45 aB	3.71 aB
0.10-0.20	0.05 aC	0.06 aC	1.10 aB	0.04 aB	0.70 aC	2.95 aB	3.92 aB
<b>Vineyard 1 (4 years)</b>							
0.00-0.05	0.23 aB	0.20 aB	4.75 aB	7.94 aB	32.64 aA	45.76 aB	55.17 aA
0.05-0.10	0.16 bB	0.20 aB	3.93 bB	5.21 bB	29.10 aA	38.61 aA	48.57 aA
0.10-0.20	0.13 bB	0.15 aAB	3.26 bA	6.41 abA	26.11 aA	36.05 aA	45.57 aA
<b>Vineyard 2 (15 years)</b>							
0.00-0.05	0.42 aA	0.45 aA	7.63 aA	17.40 aA	31.57 aA	57.48 aA	66.24 aA
0.05-0.10	0.45 aA	0.34 aA	8.18 aA	11.47 bA	25.83 bA	45.73 bA	45.42 aA
0.10-0.20	0.29 bA	0.24 bA	3.4 bA	4.69 cA	19.71 bB	28.33 bA	49.37 aA
<b>Zinc (mg.kg<sup>-1</sup>)</b>							
Depth (m)	Soluble	Exchangeable	Mineral fraction	Organic fraction	Residue	Sum fractions	Total
<b>Forest</b>							
0.00-0.05	3.00aA	1.30 aC	3.00 aC	0.70 aC	0.06 aC	8.06 aB	10.23 aC
0.05-0.10	2.80 aA	0.70 aC	1.30 bC	0.40 aC	1.10 aC	6.30 aB	7.65 aC
0.10-0.20	1.82 aA	0.30 bC	1.20 bB	0.30 aC	0.05 aC	3.67 aC	5.03 aC
<b>Vineyard 1 (4 years)</b>							
0.00-0.05	0.17 bB	7.21 aA	13.10 aB	6.05 aA	54.41 aA	80.94 aA	143.63 aA
0.05-0.10	0.31 aB	5.28 bA	9.58 bB	5.8 abA	57.06 aA	78.03 aA	119.00 bA
0.10-0.20	0.33 aB	2.59 cAB	7.67 cA	5.63 bA	62.93 aA	79.16 aA	113.25 bA
<b>Vineyard 2 (15 years)</b>							
0.00-0.05	0.19 aB	6.49 aA	29.00 aA	4.13 aB	35.25 aB	75.06 aA	83.33 aB
0.05-0.10	0.09 aB	4.28 bAB	18.81 bA	3.47 bC	41.50 aB	68.15 aA	58.33 bB
0.10-0.20	0.08 aB	3.13 bA	7.34 cA	2.89 cB	46.22 aB	59.67 bB	59.82 bB

<sup>1</sup>Means ± standard deviation followed by the same uppercase letter in the column and by the same lowercase letter on the line do not differ from each other according to Tukey's test ( $p < 0.05$ ); nsi: non-significant interaction.

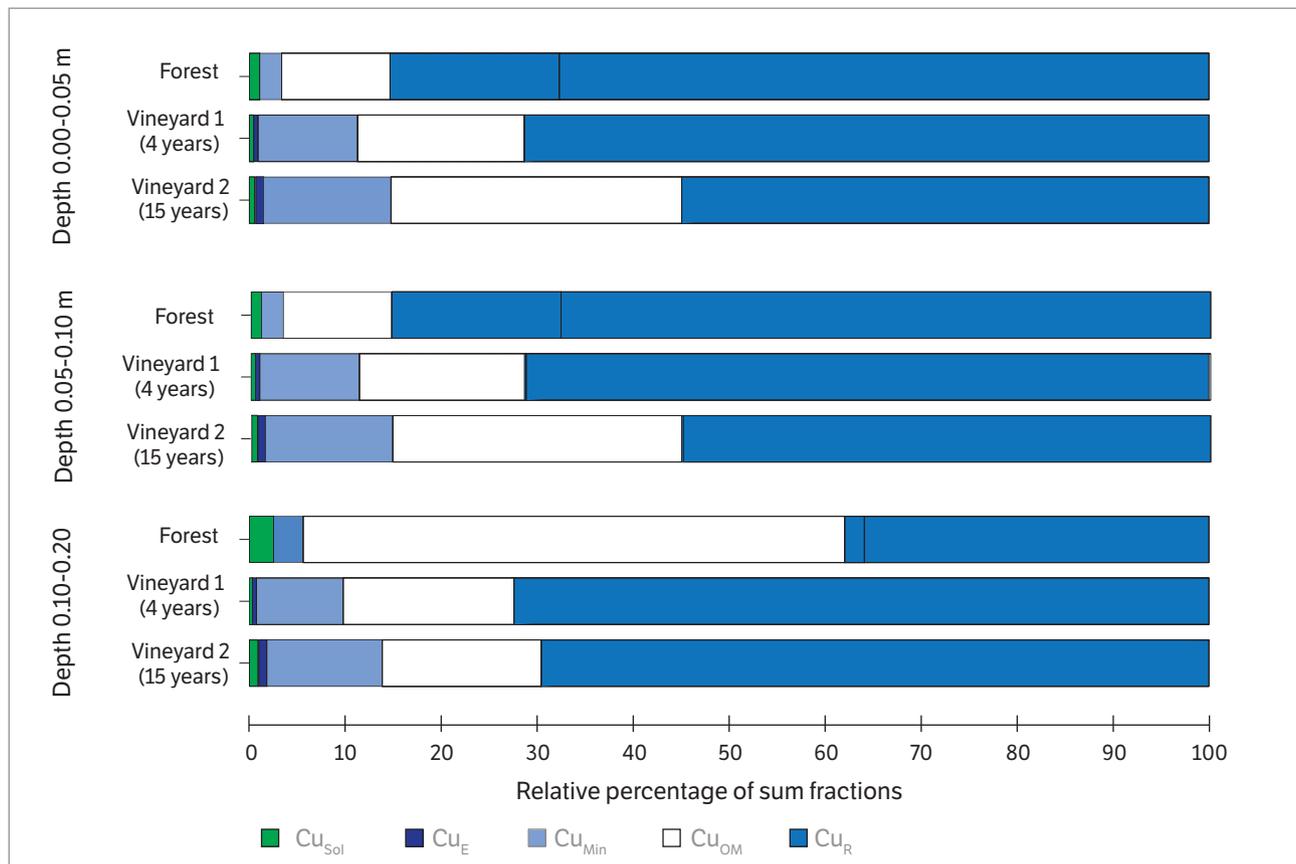
soil, and decreased with depth (0.10-0.20m) (Table 2), while  $Cu_{SOL}$  contents,  $Cu_R$  contents, sum of the fractions and  $Cu_E$  contents were equal among the 0.00-0.05, 0.05-0.10 and lower at the 0.10-0.20m layers.

The  $Cu_T$  content was equal in the three layers assessed. In the three soil layers of vineyard 2, higher contents of  $Cu_{SOL}$  and  $Cu_E$  were observed in comparison with the vineyard 1 and forest soil. In the vineyard 2 soil, but in the 0.00-0.05 and 0.10-0.20m layers, the highest contents of  $Cu_{Min}$  and  $Cu_{OM}$  were observed, compared to the vineyard 1 and forest soils. But the  $Cu_R$ , sum of the fractions and  $Cu_T$  contents in the three layers were similar between the soil of vineyard 2 and vineyard 1. Nevertheless, in all the layers, the contents of the Cu fractions observed in vineyard 1 and, especially, in vineyard 2, were higher than those seen in the forest soil. In the 0.00-0.05, 0.05-0.10 and 0.10-0.20m layers of the forest, vineyard 1 and vineyard 2 soil, in general, the highest percentages of Cu were observed in the residual fraction ( $Cu_R$ ), followed by the fraction associated with organic matter ( $Cu_{OM}$ ) and  $Cu_E$  (Figure 2). But in the vineyard 2 soil where,

in general, the highest Cu contents were seen (Table 2), a decrease in the  $Cu_R$  percentage and an increase especially in the  $Cu_{OM}$  fraction, followed by the  $Cu_{Min}$  fraction, were observed, especially in the 0.00-0.05 and 0.05-0.10m layer. It should be emphasized that in the 0.10-0.20m layer of the forest soil, a higher percentage of Cu was observed in the exchangeable fraction ( $Cu_E$ ), which may be attributed to the lower pH in water value in this layer (Table 1) in comparison with the soils of the two vineyards.

The higher contents of most of the Cu fractions in the uppermost soil layers of the vineyard 1 and 2 soils agree with the results obtained by  $Cu_{EPA}$  (Figure 1a) and  $Cu_{EDTA}$  (Figure 1b). The higher contents of the Cu fractions in the soil of vineyard 2 may be attributed to the higher age of the vineyard and, consequently, the history of application of copper-bearing leaf fungicides (Casali et al., 2008). The accumulation of Cu in the residual fraction ( $Cu_R$ ) in all the layers of the forest, vineyard 1 and vineyard 2 soils, indicates that the trace element is in a more stable or unavailable fraction (Tessier et al. 1979).

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**Figure 2.** Percent average distribution of Cu fractions ( $Cu_E$  exchangeable;  $Cu_{SOL}$  soluble;  $Cu_{MIN}$  bonded to oxides;  $Cu_{OM}$  bonded to organic matter and  $Cu_R$  residual Cu) in soil layers of the forest, vineyard 1 (4 years) and vineyard 2 (15 years).

The capacity for inactivating the Cu in the residual fraction when in soil surface layers may be attributed to the higher presence of recalcitrant organic carbon and, in the deeper layers, as in the 0.10-0.20m layer, to the clay and silt content (Table 1), but also to amorphous inorganic matter and clay minerals, as well as reaction time (Tessier et al. 1979). Nevertheless, the results showing an increase in Cu in the fraction associated with organic matter ( $Cu_{OM}$ ), especially in the uppermost layers (0.00-0.05 and 0.05-0.10 m) of the oldest vineyard, agree with those obtained by Casali et al. (2008). This occurs because Cu has the electron configuration  $[Ar]3d^{10}4s^1$ , and has high reactivity with the functional groups containing S and N, in addition to the carboxylics and phenolics of the soil organic matter (Croué et al. 2003). However, the  $Cu_{OM}$  contents, for example, in the two uppermost layers (0.00-0.05 and 0.05-0.10 m) of the vineyard 2 soil were less than the contents reported in studies already undertaken in soils of other vineyards of traditional winegrowing regions, which report contents oscillating from 40 to 400 mg kg<sup>-1</sup> (Fernández-Calviño et al. 2009). This may be explained, not only, but most probably, by the low organic matter contents (Table 1), in comparison to the studies already undertaken, but may also be associated with the composition of the organic matter (Fernández-Calviño et al. 2010).

The increase in the percentage of  $Cu_{Min}$ , especially in the uppermost layers of vineyard 2, may happen because of the saturation of the organic matter functional groups (Croué et al. 2003). In the mineral fraction, Cu is associated with the functional groups of Fe and even Mn oxides (Bradl 2004), but also as the soil of the present study has low oxide contents (Table 1), it is possible that part of it is adsorbed with less binding energy to the silanol and aluminol groups of the phyllosilicates.

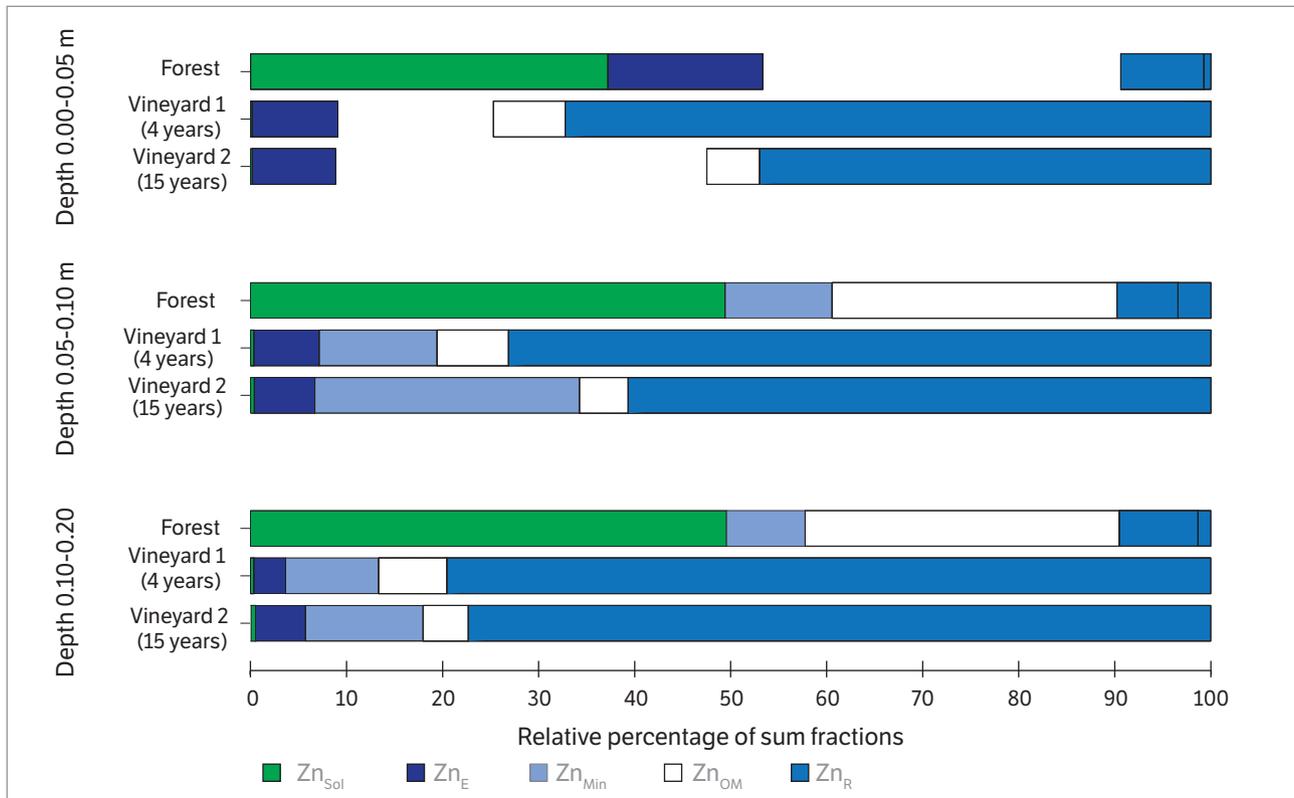
The highest  $Zn_E$ ,  $Zn_{Min}$ ,  $Zn_{OM}$  and  $Zn_T$  contents in the vineyard 1 and vineyard 2 soils were observed in the uppermost soil layers, especially in the 0.00-0.05m layer (Table 2). But the  $Zn_R$  contents among the vineyard 1 soil layers were equal, and in the vineyard 2 soils, the highest  $Zn_R$  contents were observed in the 0.00-0.05 and 0.05-0.10 m layers. The highest contents of the  $Zn_E$ ,  $Zn_{Min}$ ,  $Zn_{OM}$ ,  $Zn_R$  fractions, sum of the fractions and  $Zn_T$  fraction in the three layers were observed in the vineyard soils

(Table 2). But the highest  $Zn_{OM}$ ,  $Zn_R$  and  $Zn_T$  contents were observed in the vineyard 1 soil layers, and in the vineyard 2 soil layers, the highest  $Zn_{Min}$  contents were observed, with the  $Zn_E$  contents being equal for the soils of the two vineyards. In the 0.00-0.05, 0.05-0.10 and 0.10-0.20 m layers, the highest percentages of Zn in the forest, vineyard 1 and vineyard 2 soils were observed in the residual fraction ( $Zn_R$ ) (Figure 3).

In the 0.00-0.05 and 0.05-0.10m layer of the vineyard 1 soil, where the highest  $Zn_T$  contents were observed (Table 2), the highest percentages observed were of  $Zn_R$ . But in the same soil layers of vineyard 2, which exhibited lower  $Zn_T$  contents (Table 2), higher percentages of Zn were observed in the mineral fraction ( $Zn_{Min}$ ) in comparison to the vineyard 1 soil. It should be highlighted that especially in the deepest layers (0.05-0.10 and 0.10-0.20 m) in the forest soil, the highest percentages of Zn were observed in the solution ( $Zn_{sol}$ ), which may be explained by the low pH values (Table 1), which decreases Zn adsorption with the soil and, consequently, increases its availability to plants.

The higher contents of all the Zn fractions in the soils of the two vineyards in comparison to the forest soil may be attributed to the application of Zn-based leaf fungicides, and in the vineyard 1 soil, also the application of poultry manure. In addition, the higher contents of Zn fractions, such as  $Zn_E$ ,  $Zn_{Min}$ ,  $Zn_{OM}$  and even  $Zn_T$  in the uppermost layer of the vineyard 1 and vineyard 2 soils agree with the  $Zn_{EPA}$  and  $Zn_{EDTA}$  contents (Figure 1d, 1e) and with the data of the Cu fractions (Table 2),  $Cu_{EPA}$  and  $Cu_{EDTA}$  (Figure 1a, 1b). The higher  $Cu_R$  contents that were reflected in the percentages in the vineyard soils, especially in the uppermost layers of vineyard 1, but also the  $Zn_{Min}$  in the 0.00-0.05 and 0.05-0.10 m layers of vineyard 2, represent two fractions of the trace element that are more stable, characterized by low availability and mobility (Fernández-Calviño et al., 2012), explaining in part the low quantity of Zn extracted by EDTA, in relation to the  $Zn_{EPA}$  (Figure 1f). Fernández-Calviño et al. (2012) report that in vineyards of three important winegrowing regions of Spain, 93% of the Zn was observed in these two fractions. One thus expects low toxicity potential of the Zn for the roots of cover plant species that cohabitate the vineyards, the grapevines, the soil microorganisms and small quantities transferred by the runoff or percolated solution (Fernández-Calviño et al. 2012).

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**Figure 3.** Percent average distribution of Zn fractions ( $Zn_E$  exchangeable;  $Zn_{Sol}$  soluble;  $Zn_{Min}$  bonded to oxides;  $Zn_{OM}$  bonded to organic matter and  $Zn_R$  residual Zn) in soil layers of the forest, vineyard 1 (4 years) and vineyard 2 (15 years).

## CONCLUSION

Cu and Zn were accumulated in the uppermost layers of the vineyard soils and especially in higher quantity in the older vineyard. Approximately 72% of the total Cu was extracted by the EDTA method and, thus, may be considered as available to plants; however, only 20% of the total Zn was extracted by EDTA.

Most of the Cu in the vineyard soils was distributed in the residual fraction, characterized by low geochemical mobility, but in the uppermost soil layers of the oldest vineyard, there was an increase in the Cu content associated

with soil organic matter. Most of the Zn in the vineyard soil was distributed in the residual fraction and associated with minerals, which indicates low mobility and also low potential for toxicity to plants and microorganisms.

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