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Copper content and distribution in vineyard soils from Betanzos (A Coruña, Spain)

Contenido y distribución de cobre en suelos de viñedo de Betanzos (A Coruña, España)
Teor em cobre e sua distribuição em solos de vinha de Betanzos (Corunha, Espanha)

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ABSTRACT

The sustained use of cupric compounds in grape production has contributed to a pronounced Cu accumulation in vineyard soils. We studied 32 surface (0-20 cm) vineyard soil samples from the Atlantic-influenced NW Iberian Peninsula, in order to assess the Cu levels in these soils. The total Cu (Cu_T) contents were high and variable (between 63-730 mg kg^{-1}), similar to those previously found in the Ribeira Sacra and O Ribeiro denominations of origin, which were until now the areas with the highest Cu_T concentrations detected in vineyard soils of the NW Iberian Peninsula. Most Cu in the solid phase of the soil was bound to organic matter (18-373 mg kg^{-1}), contrary to what happens in natural soils or with elements from natural sources, such as Zn, for which the residual fraction is quantitatively the most important. The exchangeable Cu fraction (Cu_E) was the fraction that showed the highest geochemical mobility and its variance in the studied soils was mainly related to Cu_T and, secondly, to exchangeable Ca levels. The results of fractionation were clearly different from those of Zn, an element with a mainly natural origin, which was characterized by a dominant residual fraction. Finally, agricultural practices aimed at maintaining or increasing the amount of organic matter in vineyard soils should be encouraged, due to its role in decreasing the eventual toxic effects of high Cu levels both on plants and on soil microorganisms.

RESUMEN

El uso sostenido de compuestos de cobre para la producción de uva ha contribuido a la acumulación pronunciada de Cu en sueños de viñedo. Se estudiaron 32 muestras superficiales (0-20 cm) de suelos dedicados al cultivo de la vid en una zona de influencia atlántica en el NW de la Península Ibérica. Los contenidos de cobre total (Cu_T) fueron, en general, altos y muy variables (entre 63-730 mg kg^{-1}). Los valores son similares a los encontrados en las denominaciones de origen Ribeira Sacra y O Ribeiro, que eran las zonas vitivinícolas cuyos suelos de viñedo presentaban el mayor contenido conocido de Cu_T del NW peninsular hasta el momento. La distribución del Cu en la fase sólida del suelo indica que la fracción mayoritaria es la unida a la materia orgánica (18-373 mg kg^{-1}), a diferencia de lo que ocurre en otros suelos naturales o con elementos procedentes de fuentes naturales como el Zn, donde la fracción residual es la más importante. La fracción intercambiable de Cu (Cu_E) fue la que mostró una mayor movilidad geoquímica y su varianza estaba principalmente relacionada con el Cu_T y, en menor medida, con el Ca intercambiable. Cuando se compara con un elemento predominantemente de origen natural, como es el Zn, la distribución también es muy diferente, ya que la fracción residual es la mayoritaria para el Zn (mientras que era la unida a la materia orgánica en el caso del Cu). Finalmente, las prácticas agrícolas encaminadas a mantener o mejorar la presencia de materia orgánica en los suelos de viñedo son de especial relevancia, puesto que contribuyen a disminuir los posibles efectos tóxicos de los altos contenidos de Cu, tanto en la vid como en la actividad de los microorganismos del suelo.

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RESUMO

O uso continuado de produtos cúpricos na produção vitivinícola tem conduzido a uma intensa acumulação de cobre nos solos de vinha. Neste estudo foram analisadas 32 amostras superficiais (0-20 cm) de solos vitícolas numa área de influência atlântica do NW da Península Ibérica. O teor de cobre total (Cu_T) foi, em geral alto e muito variável (entre 63-730 mg kg^{-1}). Os valores obtidos são semelhantes aos encontrados nas denominações de origem de Ribeira Sacra e O Ribeiro, que são as áreas vitivinícolas cujos solos até hoje apresentaram o maior teor de Cu_T conhecido do NW Peninsular. A distribuição de Cu na fase sólida do solo indica que a fracção principal está ligada à matéria orgânica (18-373 mg kg^{-1}), ao contrário do que acontece nos outros solos naturais ou com elementos derivados de fontes naturais, como o Zn, onde a fracção residual é quantitativamente a mais importante. A fração correspondente ao cobre de troca (Cu_E) foi a que apresentou maior mobilidade geoquimica e a sua variação nos solos estudados está essencialmente relacionada com o Cu_T e, em menor grau, com o Ca de troca. Quando comparado com um elemento principalmente de origem natural, como é o Zn, a distribuição é também muito diferente na medida em que a fracção residual é a maioritária para o Zn (enquanto que no caso de Cu é maioritária a fração ligada à matéria orgânica). Finalmente, as práticas agrícolas com o objetivo de manter ou melhorar a presença de matéria orgânica em solos de vinha são particularmente importantes e devem ser incentivadas, uma vez que ajudam a diminuir os possíveis efeitos tóxicos de altos teores de Cu, nas videira e na atividade dos microrganismos do solo.

KEY WORDS
Fractionation, organic matter, contamination, heavy metals, Iberian Peninsula

PALABRAS CLAVE
Fraccionamiento, materia orgánica, contaminación, metales pesados, Península Ibérica

PALAVRAS-CHAVE
Fracionamento, matéria orgânica, contaminação, metais pesados, Península Ibérica

1. Introduction

Copper compounds, such as the Bordeaux mixture [$Ca(OH)_2$ + $CuSO_4$], or copper oxychloride [$CuCl_2$*$3Cu(OH)_2$] are broadly used in conventional agriculture where they are used to fight fungi diseases affecting commercial crops. As a consequence, high Cu accumulation of anthropic-origin has been found in soils devoted to cultivation of hops (Schramel et al. 2000), avocado (Van Zwieten et al. 2004), orange trees (Zhou et al. 2011), coffee plants (Loland and Sing 2004) and cacao (Josanidia 1994). In vineyard soils, the longevity of plantations and the sustained use of cupric compounds (from the end of the XIX century in Europe, Gessler et al. 2011) have contributed to a more pronounced Cu accumulation. Furthermore, the need for increasing grape production for a growing and more competitive market has favored a higher frequency and intensity of Cu-based fungicide applications. Thus, some vineyard soils clearly exceed the average toxicity threshold level for plants (around 100 mg kg^{-1}, Kabata-Pendias and Pendias 2001), mainly in countries with a long tradition in this practice such as Portugal (Magalhaes et al. 1985), Italy (Deluisa et al. 1996), France (Brun et al. 1998; Chaignon et al. 2003) and Spain (Arias et al. 2004; Fernández-Calviño et al. 2009). However, the rapid development of the sector during recent decades has led to Cu accumulation in vineyard soils of other countries, such as the Czech Republic (Komarec et al. 2008), Slovenia (Rusjan et al. 2007), Greece (Vavoulidou et al. 2005), Australia (Pietrzak and McPhail 2004; Wightwick et al. 2008), New Zealand (Morgan and Taylor 2004) and Brazil (Mirlean et al. 2007). In addition to fungicide management practices, weather conditions also affect Cu accumulation in vineyard soils. Some studies indicate that total Cu levels are higher in vineyard soils from humid areas (Brun et al. 1998; Deluisa et al. 1996). However, moderate precipitation and temperatures with a high air humidity are optimal for the development of fungal diseases in wine-grape plants, requiring reiterative use of Cu-based fungicides and so promoting Cu accumulation in vineyard soils of the Atlantic facade of Europe (Fernández-Calviño et al. 2009). A similar situation affects vineyard soils in South Brazil (Mirlean et al. 2007), where annual rainfall is up 2000 mm.

Although Cu is an essential nutrient for plants, its accumulation in vineyard soils can cause environmental problems. From the eco-toxicological point of view, high Cu concentrations may alter cellular division in some plants (Eleftheriou and Karataglis 1989; Arduini et al. 1994), affect the activity and diversity of soil microorganisms (Díaz-Raviña et al. 2007; Fernández-Calviño et al. 2010a, 2010b), and cause damage in the detritivorous populations (Paoletti et al. 1998; Daoust et al. 2006). Moreover, copper's preferential association with organic matter favors Cu accumulation in the top-most surface layers of soils (Arias-Estévez et al. 2007; Fernández-Calviño et al. 2008a), resulting in an easier transfer to sedimentation zones by means of erosion processes (Ribolzi et al. 2002; Fernández-Calviño et al. 2008b, 2008c), reaching groundwater and then potentially affecting aquatic fauna (Zyadah and Abdel-Baky 2000). Cu accumulated in the top-most surface layers of soils can migrate downwards and laterally affecting wider areas, which is more probable in acid soils where Cu solubility and incorporation to the soil solution is enhanced (Strobel et al. 2005).

The potential hazard of Cu accumulation in vineyard soils is mainly related to its mobility and bio-availability for soil microorganisms, related to Cu transfer from the solid phase to the soil solution (Kabata-Pendias 2004). Existing regulations on heavy metals in soils are usually based on their total contents, although these are a poor marker of metal mobility, bio-availability or impact on the natural environment (Rao et al. 2008). Total metal content is not appropriate to evaluate heavy metal toxicity due to the association of metals with soil organic matter, silt, clay and Fe and Al oxy-hydroxides, all of which have different potentials for metal retention and release. Thus, it is necessary to determine the way in which metals are distributed in solid media, with different fractions characterized by various binding energies. In this way, it is usual to carry out selective extractions in order to estimate the fractions of metal that are bound or associated with different soil compounds, thus yielding information on the amounts of metals that are mobile or potentially mobile (Rao et al. 2008).

The main objectives of this work are: firstly, to determine total Cu levels in order to provide information on Cu accumulation in the vineyard soils in the area of the Protected Geographical Indication (PGI) "Betanzos" (A Coruña, Spain), an area that is characterized by a strong Atlantic influence; and, secondly, to elucidate the Cu distribution in the solid phase of these soils. This is relevant considering the risks of Cu mobilization in this geographic zone that has registered huge increases in wine-production activities in the last years.

2. Materials and Methods

2.1. Study area

The study area corresponds to the Protected Geographical Indication (PGI) "Betanzos", which extends for several municipalities of the province of A Coruña (Spain), in the northwest corner of the Iberian Peninsula. Although the total surface of the territory included in the PGI "Betanzos" is approximately 340 km^2, vineyards occupy only about 600 ha. The climatology of the area is characterized by a marked Atlantic influence, showing a mean annual temperature of 14 ºC, with a narrow temperature range (< 10 Cº). Mean annual precipitation is 1100 mm, and it is evenly distributed throughout the year. The territory is dominated by biotite-rich schist in the western half, whereas granitic rocks are abundant in the eastern part of the PGI "Betanzos". Natural soils in this PGI are mostly Leptosols, Umbrisols, Cambisols and Regosols, with the occasional presence of Gleysols, Andosols, Fluvisols and Arenosols (IUSS Working Group WRB 2006).

2.2. Soil sampling and physico-chemical characterization

Thirty-two vineyard plots placed along the PGI Betanzos were selected for this study. At each plot, 10 soil subsamples (0-20 cm depth) were collected with a soil auger in the vine line, and then pooled into a single composite soil sample. The parent material was biotite schist for all soil samples. Prior to their physico-chemical characterization, samples were air-dried and sieved (mesh size 2 mm), and the fine earth fraction was stored in polyethylene bottles until analysis.

The particle-size distribution, distinguishing between sand (2-0.05 mm), silt (0.05-0.002 mm) and clay (< 0.002 mm) fractions, was determined by the internationally-recognized pipette method. The soil pH in water (pHw) and 0.1 M KCl (pHk) solutions were measured at a soil/solution ratio of 1:2.5. The total contents of carbon and nitrogen were determined in finely-milled (agate mortar) samples, using a soil analyser. The effective cation exchange capacity (eCEC) was estimated as the sum of the basic cations (K, Na, Ca, Mg) in 1 M NH_4Cl (Peech et al. 1947) and the Al extracted with 1 M KCl (Bertsch and Bloom 1996).

2.3. Copper in soils: total content and fractionation

To determine the total Cu content (Cu_T), an aliquot of 0.5 g of finely-milled soil was digested in a microwave oven (at 100 psi) using a mixture of concentrated strong acids (5 mL HNO_3 + 4 mL HF + 1 mL HCl). In the resulting extract, Cu was measured by flame atomic absorption spectrometry. For quality assurance and control (QA/QC) purpose, certified reference materials (CRM 141 and CRM 143) were also digested in triplicate, with the results showing that the Cu concentration in their extracts deviated from certified values by less than 5%.

Regarding Cu fractionation, a non-sequential extraction procedure was carried out, as indicated in Fernández-Calviño et al. (2008b, 2009). A brief description of these methods is given as follows:

- *Exchangeable Cu (Cu_E):* 50 mL of 1 M NH_4Ac (pH 7) solution was added to 10 g of soil and shaken for 1 hour.
- *Pyrophosphate-extracted Cu (Cu_p):* 100 mL of 0.1 M Na-pyrophosphate solution was added to 1 g of soil and the suspension was shaken for 16 hours. Sodium pyrophosphate is assumed to recover organically bound Cu.
- *Oxalic-oxalate-extracted Cu (Cu_o):* 50 mL of 0.2 M oxalic acid-ammonium oxalate (pH 3) solution was added to 1 g of soil and shaken in the dark for 4 hours. This solution dissolves non-crystalline hydrous oxides of Al and Fe, releasing the Cu bound to them and associated to soil organic matter.
- *Ascorbic-oxalic-oxalate-extracted Cu (Cu_{ao}):* 50 mL of 0.2 M oxalic acid-ammonium oxalate + 0.1 M ascorbic acid (pH 3.25) solution was added to 1 g of soil and shaken for 30 minutes in a water bath at 96 ºC. This reagent can mobilize Cu associated with crystalline Al and Fe oxy-hydroxides as well as Cu bound to soil organic matter and non-crystalline Al and Fe oxy-hydroxides.

After the period of shaking, the different extracts obtained were centrifuged (700 × *g* for 15 minutes) and then filtered through acid-washed filter paper (2-5 µm pore size). In the filtered soil extracts, Cu was measured by flame atomic absorption spectrometry.

The application of the procedure provides useful information on Cu mobility and bioavailability in soils. Thus, the following operationally-defined fractions were determined:

- Exchangeable copper (Cu_{EX}) is the Cu measured in the NH_4OAc extracts (Cu_E) and provide an estimate of the amount of Cu adsorbed to exchangeable sites.
- Copper bound to soil organic matter (Cu_{OM}) is estimated as the difference between Cu extracted with Na-pyrophosphate and exchangeable Cu (Cu_p-Cu_E).
- Copper bound to non-crystalline Al and Fe oxy-hydroxides (Cu_{IA}) is calculated as the difference between Cu extracted with oxalic acid-ammonium oxalate and Na-pyrophosphate (Cu_o-Cu_p).
- Copper bound to crystalline Al and Fe oxy-hydroxides (Cu_C): Cu extracted with oxalic acid-ammonium oxalate plus ascorbic acid minus Cu extracted with oxalic acid-ammonium oxalate (Cu_{ao}-Cu_o).
- Residual copper (Cu_R): estimated as the difference between total Cu and Cu extracted with oxalic acid-ammonium oxalate plus ascorbic acid (Cu_T-Cu_{ao}).

Moreover, the potentially bioavailable Cu (Cu_{ED}) was extracted using as reagent 0.02 M Na_2-EDTA + 0.5 M NH_4Ac (pH 4.65) following the procedure described by Lakanen and Ervio (1971).

2.4. Statistical analysis

Calculations of basic descriptive statistics and lineal regressions were performed using SPSS v. 22 for Windows.

3. Results and Discussion

3.1. General characteristics of the studied soils

The studied soils are acidic, with pH in water ranging from 3.8 to 6.0 (mean pH 5.0 ± 0.4) and pH in KCl somewhat lower (between 3.3-5.5), indicating predominance of negatively charged soil colloids. Organic carbon content ranged between 1.1-2.6%, and cation exchange capacity was relatively low, ranging between 4.8-16.1 $\text{cmol}_{(+)}\ \text{kg}^{-1}$ (**Table 1**). The predominant soil textures were loamy (samples 4-15, 18-22, 25 and 26), with some of them being silty loam (samples 1-3, 16-17, 23 and 27-31) and one clay loam (sample 32). Selective extractions revealed higher Al than Fe contents (**Table 1**). In general, a significant part (more than 50%) of the Al and Fe contents was associated with organic matter, in view of the Al_p/Al_o and Fe_p/Fe_o relationships.

Table 1. General characteristics of the soils

Sample	pH (H_2O)	pH (KCl)	O.C. (%)	eCEC $\text{cmol}_{(+)}\text{kg}^{-1}$	Sand (%)	Silt (%)	Clay (%)	Al_p (g kg^{-1})	Al_o (g kg^{-1})	Fe_p (g kg^{-1})	Fe_o (g kg^{-1})
1	4.9	3.6	1.77	6.7	24.5	52.3	23.2	1.7	2.8	0.6	1.8
2	5.0	3.7	2.43	5.2	31.3	50.6	18.1	2.1	2.9	0.4	0.9
3	4.8	3.7	2.23	8.0	33.4	49.3	17.3	1.9	3.0	1.1	0.8
4	5.0	3.7	1.12	6.6	38.3	45.0	16.7	1.1	1.8	0.2	0.4
5	4.9	3.8	2.25	6.8	36.3	44.0	19.7	1.6	2.2	0.4	0.8
6	4.9	4.1	2.31	9.8	41.5	40.3	18.2	2.1	3.2	0.5	1.2
7	4.9	3.6	1.88	5.0	37.1	46.2	16.6	1.3	2.1	0.2	0.6
8	4.7	3.8	1.96	6.2	41.1	41.0	17.9	1.4	2.6	0.4	0.6
9	4.6	3.6	2.05	5.0	39.0	42.1	18.9	1.4	2.3	0.5	1.3
10	4.9	4.0	2.57	8.9	37.6	42.5	19.9	1.1	2.0	0.5	0.9
11	5.0	3.9	1.76	7.4	41.4	35.1	23.5	1.3	2.0	0.6	1.6
12	4.9	4.6	1.69	10.4	32.4	44.3	23.3	1.1	2.0	0.5	0.9
13	4.7	4.0	1.73	7.7	40.7	41.3	18.0	1.0	1.7	0.4	0.8
14	3.8	3.3	1.89	6.3	34.2	44.5	21.4	1.6	2.1	0.9	1.6
15	4.9	4.2	2.05	9.9	27.6	50.6	21.8	1.0	1.8	0.5	1.5
16	5.3	4.4	1.33	8.9	34.5	50.0	15.5	1.0	1.8	0.4	0.8
17	5.7	5.5	1.79	14.9	37.3	42.0	20.7	1.1	2.0	0.4	0.7
18	5.9	4.3	2.03	9.3	35.5	42.8	22.1	1.3	2.3	0.4	1.0
19	4.7	3.3	2.44	9.9	41.2	41.8	17.0	1.3	1.9	0.6	1.6
20	4.9	3.6	2.11	6.4	37.1	44.4	18.5	1.9	2.7	0.6	1.7
21	5.0	3.9	1.91	10.1	33.4	46.7	19.8	1.2	1.9	0.3	0.7
22	5.0	3.7	2.27	7.3	30.0	50.9	19.1	1.3	1.9	0.4	0.9
23	5.6	5.3	1.83	16.1	28.1	46.8	25.0	1.0	2.4	0.7	0.8
24	5.4	3.7	2.09	6.9	32.8	45.8	21.4	1.7	3.1	0.6	1.5
25	5.0	3.6	2.05	4.8	32.6	48.2	19.2	1.5	2.8	0.5	1.2
26	6.0	5.2	1.95	13.3	23.7	53.8	22.5	1.3	3.0	0.9	1.4
27	5.9	4.7	1.96	11.7	23.6	56.8	19.6	1.4	2.3	0.4	1.4
28	5.0	3.7	1.39	7.7	20.7	59.8	19.6	1.2	1.9	0.6	1.5
29	4.8	3.8	2.23	8.0	27.4	52.8	19.8	1.4	2.3	0.6	1.4
30	5.1	4.0	2.55	8.4	23.3	60.1	16.6	1.4	2.0	0.5	1.5
31	4.9	3.5	1.49	6.8	23.4	47.0	29.6	1.0	1.6	0.6	1.6
32	4.5	3.6	2.12	6.1	25.5	52.1	22.4	1.9	2.9	0.6	1.6

O.C.: organic carbon; eCEC: Effective cation exchange capacity; Al_p and Fe_p: Al and Fe extracted with sodium pyrophosphate; Al_o and Fe_o: Al and Fe extracted with ammonium oxalate-oxalic acid.

3.2. Total and bio-available copper

The average Cu$_T$ value for the 32 samples examined was 224 ± 151 mg kg^{-1} (range 63-730 mg kg^{-1}). These levels are clearly higher than those corresponding to surface horizons of non-cultivated soils in Galicia (Macías and Calvo 2008), ranging between 12-39 mg kg^{-1}, and are also higher than the threshold levels for Cu indicated by the European Union (50-100 mg kg^{-1}), which is further limited to 50 mg kg^{-1} in the case of acid soils (Council Directive 86/278/EEC). Moreover, Díaz-Raviña et al. (2007) signal that Cu levels above 100 mg kg^{-1} have negative effect on the turnover of soil organic matter and microbiological activity.

In view of our Cu$_T$ levels, most of the soils studied here can be considered as polluted, with all of them presenting > 50 mg kg^{-1}, 72% of them having > 100 mg kg^{-1}, and 56% of them containing > 150 mg kg^{-1} Cu. **Figure 1** shows that these Cu$_T$ levels are similar to those found in other vineyard areas in the NW of the Iberian Peninsula, as Ribeira Sacra (260 ± 120 mg kg^{-1}), Ribeiro (248 ± 130 mg kg^{-1}), Valdeorras (174 88 mg kg^{-1}), Rías Baixas (139 ± 122 mg kg^{-1}), Bierzo (115 ± 38 mg kg^{-1}), Vinhos Verdes (Portugal) (103 ± 42 mg kg^{-1}), and Monterrei (100 ± 48 mg kg^{-1}). As shown in **Figure 1**, Betanzos is characterized by high Cu concentrations, in the order of that found in Ribeira Sacra and Ribeiro, which can be justified by the high rainfall and relative humidity during summer in these areas, causing higher applications of Cu-based fungicides to decrease risks of fungal diseases. The same explication could justify high Cu$_T$ levels (> 600 mg kg^{-1}) in some vineyard soils situated in the Galician Ulla valley (Rodríguez-Lovelle et al. 1991).

Cu$_T$ concentrations around or above 100 mg kg^{-1} have been also found in other countries with tradition in vineyard production (**Table 2**), showing that high Cu levels affect vineyards on all continents. In Europe, Cu$_T$ contents > 100 mg kg^{-1} have been reported in countries other than Spain, such as France, Italy, Croatia and the Czech Republic. In Australia Cu$_T$ concentrations as high as 223 mg kg^{-1} have been detected in vineyard soils (**Table 2**). In America, Brazil is the country where the highest Cu concentrations have been found in vineyard soils (> 3000 mg kg^{-1}, **Table 2**).

Cu$_T$ variability depends on various factors, including edaphic variables. However, in the present study no significant correlation was found between Cu$_T$ and any of the edaphic variables shown in **Table 1**. In agreement with this, a previous study with 170 vineyard soil samples from the NW Iberian Peninsula indicated that only 5-10% of the variation in Cu$_T$ levels could be explained by edaphic properties (Fernández-Calviño et al. 2009). In the present study, climate and geomorphology would not justify Cu$_T$ variation, given that all samples were taken at sites characterized by similar

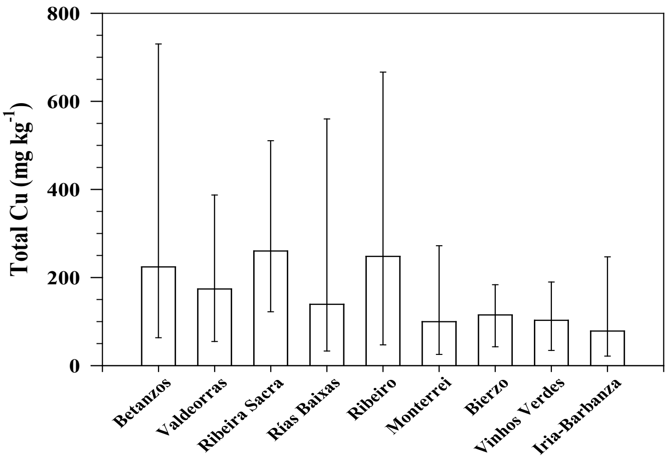

Figure 1. Comparison of the values of total Cu in vineyard soils from Betanzos with those from other wine regions in the NW of the Iberian Peninsula.

Table 2. Total Cu (Cu_T) ranges in surface samples of vineyard soils in different countries

Country	Cu_T (mg kg^{-1})	Depth (cm)	Reference
Australia	6-223	0-10	Wightwick et al. 2008
Brazil	51-665	0-20	Casali et al. 2008
Brazil	37-3216	0-5	Mirlean et al. 2007
Brazil	433-517	0-5	Mirlean et al. 2009
Croatia	105-553	0-20	Miko et al. 2007
Czech Republic	20-168	0-20	Komárek et al. 2008
Slovenia	87-120	0-20	Rusjan et al. 2007
Spain	38-63	0-20	Ramos 2006
France	14-251	0-15	Brun et al. 1998, 2001
France	22-398	0-30	Chaignon et al. 2003
Greece	<157	0-30	Vavoulidou et al. 2005
Italy	220	0-10	Deluisa et al. 1996
New Zealand	4-259	0-10	Morgan and Taylor 2004
Portugal	58-130	0-30	Magalhaes et al. 1985
Switzerland	58-489	--	Celardin et al. 2004
Thailand	115-238	0-10	Joannon et al. 2001

climate conditions and low slope gradient, thus minimizing Cu erosion and transport downslope. In view of that, differences in Cu_T levels found in this study would be related to the age of the vineyard and variations in cultivation practices.

Regarding Cu extracted with EDTA salts (Cu_{ED}), it provides an estimate of potentially bioavailable Cu (Brun et al. 2001; Pietrzak and McPhail 2004). The values of Cu_{ED} in the studied soils ranged from 11 to 232 mg kg^{-1}, with a mean value of 66 mg kg^{-1}, which is somewhat higher than that obtained in previous studies (Rodríguez-Lovelle et al. 1991; Fernández-Calviño et al. 2009). The Cu_{ED} values were also greater than those reported for vineyard soils in other countries, being above the range of 38-53 mg kg^{-1} in soils of France (Brun et al. 1998; Chaignon et al. 2003), 38 mg kg^{-1} in Czech Republic vineyards (Komárec et al. 2008) and 48 mg kg^{-1} in Australian vineyard soils (Pietrzak and McPhail 2004). However, our Cu_{ED} values were considerably below the score of 390 mg kg^{-1}, reported by Mirlean et al. (2009) for subtropical vineyard soils.

Potentially bioavailable Cu (i.e. Cu_{ED}) accounted for 28% of total Cu, which suggests that a significant proportion of Cu accumulated into soil was ready to contribute to potential toxicity, especially for plants and microorganisms.

3.3. Copper distribution in soils

Table 3 shows Cu results corresponding to selective extractions, whereas **Table 4** shows mean, maximum and minimum values corresponding to each of the different Cu fractions in the soils.

Cu_E values (Cu extracted with ammonium acetate) ranged between 0.6 and 21.8 mg kg^{-1} (mean 3.9 mg kg^{-1}) for the whole set of samples, representing between 0.5-3.0% of Cu_T (Cu_{EX} in **Table 4**), which is similar to that found in other samples from the NW Iberian Peninsula (Cu_E mean 1-12 mg kg^{-1}).

Multiple regression statistical analysis indicated that Cu_E is related to Cu_T and exchangeable Ca (**Eq. 1**): higher Cu_T levels correspond with higher Cu_E levels while higher exchangeable Ca levels are associated with lower Cu_E levels (inverse relationship). Cu_T and exchangeable Ca explain 88% of the variation in Cu_E, with Cu_T justifying 85%, and exchangeable Ca just 3%.

$$Cu_E = -2.42 \pm 0.52 + 0.03 \pm 0.00 Cu_T - 0.27 \pm 0.11 Ca \quad \textbf{Eq. 1}$$

R^2 adjusted: 0.878

where Cu_T is total Cu (mg kg^{-1}) and Ca is exchangeable Ca ($cmol_{(+)}kg^{-1}$).

Table 3. Copper content in the selective extractions of Cu (mg kg^{-1})

Sample	Cu_{ED}	Cu_E	Cu_P	Cu_o	Cu_{ao}	Cu_T
1	29.9	1.4	49.8	53.5	80.6	103.1
2	112.6	3.5	161.5	161.9	191.9	249.4
3	86.9	5.0	171.2	177.3	212.5	280.0
4	22.7	1.0	38.1	38.8	59.8	91.5
5	104.4	6.4	185.0	194.2	277.1	381.5
6	121.0	6.7	225.0	269.8	336.0	383.6
7	88.9	6.1	158.2	162.0	240.6	293.1
8	232.2	21.8	372.8	375.2	503.8	730.3
9	25.2	1.0	42.4	43.5	62.5	90.0
10	64.4	1.7	87.8	106.3	138.3	177.7
11	37.5	1.1	64.1	73.3	89.0	145.0
12	35.6	0.8	60.0	72.6	116.3	168.2
13	126.5	8.9	209.1	242.1	296.7	396.2
14	94.8	6.4	172.8	174.2	240.1	321.1
15	36.8	1.0	54.0	62.7	91.7	140.8
16	14.7	<0.5	23.2	26.1	40.3	73.4
17	107.1	2.6	155.3	189.7	253.1	374.9
18	51.7	1.7	75.8	104.9	124.3	211.9
19	78.8	4.6	149.1	165.8	211.4	270.8
20	21.3	1.1	38.2	45.6	59.5	96.1
21	83.2	2.2	119.3	133.8	180.2	280.0
22	22.6	0.8	31.4	32.7	48.3	84.4
23	16.4	<0.5	17.8	25.3	35.5	63.3
24	85.4	4.9	163.2	186.6	216.1	312.5
25	17.4	0.6	30.7	33.0	51.7	93.8
26	11.2	<0.5	17.8	24.9	32.2	63.4
27	27.4	<0.5	42.6	46.1	55.9	91.4
28	22.8	1.2	39.9	40.8	35.4	101.6
29	150.1	9.5	239.0	273.9	343.0	489.8
30	112.0	4.0	179.9	206.2	237.3	323.8
31	50.6	2.8	81.3	92.2	123.4	180.0
32	33.8	1.2	54.0	59.1	80.3	110.4

Cu_{ED}: extractable Cu in a solution of NH_4OAc and EDTA; Cu_E: Cu extractable in ammonium acetate; Cu_p: Cu extractable in sodium pyrophosphate; Cu_o: Cu extractable in oxalic acid-ammonium oxalate; Cu_{ao}: Cu extractable in oxalic acid-ammonium oxalate-ascorbic acid; Cu_T: Total Cu.

Table 4. Percentages of the different Cu fractions with respect to total Cu

Fraction Cu	Cu_{ED}	Cu_{EX}	Cu_{OM}	Cu_{IA}	Cu_C	Cu_R
Mean	28.0	1.3	44.4	5.4	17.3	31.7
Maximum	45.2	3.0	63.4	13.8	26.8	49.1
Minimum	17.7	0.5	28.1	0.1	9.1	12.4
SD	5.4	0.6	8.8	3.9	5.0	8.7

Cu_{ED}: Cu potentially available to plants; Cu_{EX}: exchangeable Cu; Cu_{OM}: Cu associated with organic matter; Cu_{IA}: Cu associated with amorphous inorganic components; Cu_C: Cu associated with crystalline oxides; Cu_R: Residual Cu.

Cu distribution is dominated by the non-exchangeable fractions (Cu_{OM}, Cu_{IA}, Cu_C and Cu_R), representing in average 99% of Cu_T (**Table 4**). Previous studies also signaled the dominance of non-exchangeable Cu fractions in vineyard soils (Morgan and Taylor 2003; Arias et al. 2004).

In the present study, the most abundant Cu fraction is that bound to soil organic matter (Cu_{OM}), ranging between 28-63% of Cu_T (mean 44 ± 9%, **Table 4**). These values are similar to those found in vineyard soils from other areas in the NW Iberian Peninsula (Fernández-Calviño et al. 2009), but are much higher than those detected in Galician natural soils that showed Cu_{OM} levels around 5% (Graña et al. 1991). Given the high affinity existing between Cu and organic matter (McBride et al. 1998), the dominance of this Cu fraction in the surface layers of the studied soils is very relevant, reducing the potential adverse effects that Cu could cause plants and soil microorganisms. In view of that, agricultural practices favoring organic matter mineralization could increase Cu in solution thus enhancing risks of toxicity. Conservation of optimal organic matter contents should therefore be promoted in order to preserve the quality of soils in the present and in the future, especially when focusing on lowering risks of toxicity by Cu.

Cu bound to inorganic amorphous compounds (Cu_{IA}) ranged between 0.1-14% of Cu_T (mean 5 ± 4%, **Table 4**), similar to that found by Fernández-Calviño et al. (2009) in other vineyard soils, but clearly below the average value of 57% detected for this fraction in vineyards from France (Parat et al. 2002). Moreover, in Galician natural soils this fraction represents 33% of Cu_T. Differences between French and Galician soils regarding this fraction may be attributed to the lower Fe and Al oxides content characterizing the French soils.

Cu bound to crystalline colloids (Cu_C fraction) ranged between 9-27% of Cu_T (mean 17 ± 5%, **Table 4**), similar to that found by Graña et al. (1991) in Galician natural soils (21%), meaning that an important amount of Cu included in this fraction may be considered to be from a natural origin, the final content thus being conditioned by the parent material in which the soil was developed.

Finally, the residual Cu (Cu_R), which can be considered the most recalcitrant Cu fraction, ranged between 12-49% (mean 32 ± 9%, **Table 4**). This is quantitatively the second in order of importance, usually presenting lower values than those found by Graña et al. (1991) in Galician natural soils, reaching up to 41% of the total Cu. It is also important to note that a relevant proportion of the Cu entering these soils can become recalcitrant in a short lapse of time, incorporated into the residual fraction as was shown by laboratory incubation trials (Arias-Estévez et al. 2007).

The comparison between Cu and Zn distribution in these soils, knowing that Zn in soils derives mainly from natural origin, allow us to state that Cu has a predominant anthropic origin in the soils studied. **Figure 2** shows Cu and Zn distribution in these soils, showing that Zn is incorporated into the residual fraction and that most of the Cu in the fraction is bound to organic matter. Another substantial difference, confirming the diverse origin of both elements, is that some Cu remains as exchangeable, while this fraction is almost non-existent in the case of Zn (**Figure 2**).

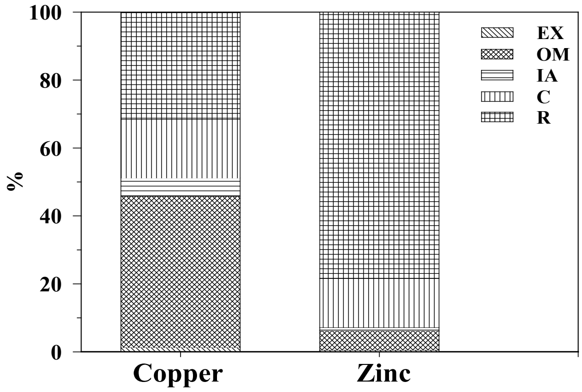

Figure 2. Average distribution of Cu and Zn fractions in the studied soils, expressed as percentage of the corresponding total metal content. EX: exchangeable; OM: organically-bound; IA: bound to inorganic non-crystalline compounds; C: bound to crystalline compounds; R: residual metal.

4. Conclusions

In the present study, 72% of the vineyard soil samples had total Cu (Cu_T) concentration > 100 mg kg^{-1}. Considering the whole set of samples, the variability of Cu_T levels was high, similar to other areas in the NW of the Iberian Peninsula and to other countries. Regarding Cu distribution, non-exchangeable fractions clearly dominated, averaging 99% of Cu_T, whereas the most mobile fraction (exchangeable Cu -Cu_E-) ranged between 0.6 and 21.8 mg kg^{-1} (mean 3.9 mg kg^{-1}), representing 0.5-3.0% of Cu_T. Cu_E variability was dependent on Cu_T and exchangeable Ca, the former justifying 85% of the variability, and the latter explaining 3% of it. The most abundant Cu fraction was that bound to organic matter (Cu_{OM}), ranging between 28-63% of Cu_T (mean 44 ± 9%), which means that cultural practices favoring mineralization of soil organic matter could result in increasing Cu concentrations in the soil solution, thus enhancing the risks of toxicity affecting vine-grape plants and soil microorganisms. In view of that, organic matter conservation is fundamental to preserving soil quality.

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