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1 **Copper accumulation and fractionation in vineyard soils from temperate humid**
2 **zone (NW Iberian Peninsula)**

3

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19

20 **Abstract**

21 The occurrence of total Cu and Cu fractions (exchangeable Cu, Cu bound to organic
22 matter, Cu bound to amorphous inorganic materials, Cu bound to crystalline Fe and
23 Al oxides and residual Cu) was studied in 170 surface layers of soils from seven
24 vineyard regions located in the NW Iberian Peninsula (Rías Baixas, Ribeira Sacra,
25 Ribeiro, Monterrei, Valdeorras, O Bierzo and Vinhos Verdes). The data showed that
26 long-term application of Cu based fungicides has led to increased concentrations,
27 mainly in superficial vineyard soil layers. Thus, 64% of the samples showed total Cu
28 levels higher than 100 mg kg⁻¹, threshold for soil contamination. Total Cu content in
29 Ribeiro (248±130 mg kg⁻¹) and Ribeira Sacra (259±118 mg kg⁻¹) soils were
30 significantly higher than those observed for the rest of the vineyard regions (169±90,
31 139±122, 115±42, 103±42 and 100±48 mg kg⁻¹ in Valdeorras, Rías Baixas, O Bierzo,
32 Vinhos Verdes and Monterrei, respectively). Cu distribution among various soil
33 fractions also differed between winegrowing regions; however, in general, Cu in these
34 vineyard soils is present mainly in less mobile fractions (average values were 48±12%
35 for Cu bound to soil organic matter; 15±9% for Cu associated to amorphous inorganic
36 materials; 12±9% for Cu bound to crystalline Fe and Al oxides and 23±12% for
37 residual Cu), whereas exchangeable Cu constituted approximately 3±3% of total Cu.
38 All soil samples were separated in three well differentiated groups according their
39 physical, chemical and Cu fractionation data (Ribeiro, Ribeira Sacra and Monterrei
40 samples; O Bierzo, Valdeorras and Monterrei samples; Rías Baixas and Vinhos
41 Verdes samples), being soil type, climate and fungicide application the most
42 determinant factors for soil quality.

43

44 **Keywords:** vineyards, Cu fractionation, soil properties, multivariate analysis

45 **Introduction**

46

47 The fungicidal properties of copper have been recognized for more than two centuries.
48 Thus, most of soils devoted to vineyard are characterized by a Cu contamination due
49 to successive applications of Cu-based fungicides such as Bordeaux mixture
50 ($\text{Ca(OH)}_2 + \text{CuSO}_4$) and, more recently, Cu oxychloride ($\text{CuCl}_2 \cdot 3\text{Cu(OH)}_2$) in order
51 to fight fungal diseases, especially downy mildew (*Plasmopara viticola*). Because of
52 its worldwide and prolonged use, particularly in European vineyards, average values
53 above of 100 mg kg^{-1} of total Cu have been reported in traditional wine-producing
54 countries (Magalhaes *et al.*, 1985 in Portugal; Deluisa *et al.*, 1996 in Italy; Parat *et al.*,
55 2002 in France; and Arias *et al.*, 2004 in Spain). Although Cu concentrations below
56 100 mg kg^{-1} can negatively influence plant growth and soil functioning, this has been
57 accepted as the threshold mean value for plant toxicity (Kabata-Pendias & Pendias,
58 2001); therefore Cu presence above this value constitutes a serious threat to soil
59 quality of many European vineyard soils.

60 The spread of high Cu concentrations in vineyard soils lead to a serious
61 environmental issue due to their non-biodegradable nature and long-term biological
62 half-lives. To evaluate the ecotoxicological risks that Cu can generate in such soils, it
63 is first of all necessary to predict the bioavailability of Cu for the plants. Copper
64 added to vineyard soils is mainly retained in upper soil layers due to their low
65 mobility in soils (Komárek *et al.*, 2008), and its accumulation can affect adversely soil
66 microorganisms in terms of numbers, activity and diversity (Díaz-Raviña *et al.*, 2007;
67 Miguéns *et al.*, 2007) but also the detritivores (Paoletti *et al.*, 1998; Daoust *et al.*,
68 2006) and hence to cause serious agricultural and environmental problems. The
69 copper contained in topsoils is susceptible both to lateral water erosion of soil

70 phenomena and leaching down to deeper horizons (Ribolzi *et al.*, 2002) and hence it
71 can affect negatively aquatic organisms (Zyadah & Abdel-Baky, 2000). In order to
72 assess those potential damages related to Cu accumulation in vineyard soils, total Cu
73 content was widely studied. However total Cu content is not a good predictor of the
74 metal concentration in plants and its measurement only can be taken into account as a
75 reference for Cu-contaminated soils. Copper can be associated with various soil
76 components that differ in their ability to retain or release Cu. Thus, the distribution of
77 Cu among soil components (fractionation of Cu) will strongly influence the mobility
78 and hence the bioavailability of Cu. The assessment of soil metal bioavailability using
79 chemical extractions is a conventional approach used in soil testing (Tessier *et al.*,
80 1979; Ribolzi *et al.*, 2002). Although these methods are subject to analytical
81 limitations and too much criticism, they are still being used.

82 Most studies concerning metal contamination in vineyards are based on the
83 analysis of total and available Cu content or on Cu fractionation of a small amount of
84 samples, obtaining only a scatter view of this issue. However, studies of Cu
85 accumulation, distribution and fractionation in wide range of vineyard soils are scarce.
86 In this sense, this kind of studies in France, Italy and Spain, which are the most wine-
87 producing countries in the world, are absent. Thereby, there is a need to investigate
88 the role of relevant chemical soil properties (carbon, nutrient elements, heavy metals)
89 in vineyards in order to evaluate soil quality and risk assessment associated with the
90 presence of Cu.

91 North-west of Iberian Peninsula, which can be included in the European
92 temperate humid region, has enjoyed a long tradition of intensive grapevine growing,
93 with frequent use of copper containing fungicides dating back to the late 19th century.
94 Today, vineyards still cover a large area of land in Galicia (NW Spain, 33,273 ha),

95 which definitely contributes to environmental contamination since high humidity and
96 rains demands more often application of copper-based fungicide. In addition, there is
97 a high potential risk for soil and water contamination with Cu due to climatic
98 conditions (abundant precipitation), topography and soil properties (sandy texture,
99 acid pH, high organic matter content). Physical and chemical characterization of
100 vineyard soils located in Galicia, including Cu accumulation and fractionation, has
101 recently been undertaken. These preliminary results, performed with a reduced set of
102 samples, have showed that high contents of Cu were accumulated in the topsoil and
103 river sediments in winegrowing areas of Galicia (Arias *et al.*, 2004; Fernández-
104 Calviño *et al.*, 2008a). However, although viticulture is considered as one of the land
105 uses that causes the most intense deterioration of soil, there is no detailed information
106 concerning the impact of vine cropping on soils of this environmentally sensitive area
107 located in the NW of Iberian Peninsula. The objectives of this study were: a) to
108 characterize a wide range of soil samples collected in seven selected winegrowing
109 regions of the North-west of Iberian Peninsula (Galicia, NW Castilla-León and N
110 Portugal) and determine the accumulation and fate of Cu in vineyard soils; b) to
111 compare the soil data obtained in different selected winegrowing regions of Galicia,
112 NW Castilla-León and N Portugal in terms of Cu fractionation.

113

114 **Material and methods**

115

116 *Study area*

117

118 Vineyard soils selected for this study were located in an area corresponding to the
119 NW of the Iberian Peninsula which cover a surface of 35,600 km² approximately

120 (Figure 1). These vineyards are distributed in 7 regions of quality wines, five in
121 Galicia (Monterrei, Rías Baixas, Ribeira Sacra, Ribeiro and Valdeorras), one in
122 North-west of Castilla-León (O Bierzo) and one in North of Portugal (Vinhos
123 Verdes), representing all together roughly 48,000 ha. The selection of the vineyards
124 was based on climatic, topographical, socio-economical and ecological reasons. From
125 the climatic point of view, the study area is characterized by a marked Atlantic
126 influence giving smooth annual mean temperatures of 10-15°C, together with low to
127 moderate annual rainfalls of 700-1,500 mm. The Atlantic Ocean influence goes
128 progressively down from the coastal regions to the inner lands, where there is a clear
129 Mediterranean influence. As a result of this climatic gradient, temperatures in the
130 coastal regions (Rías Biexas and the Western part of Vinhos Verdes area) are
131 generally lower than in inner lands (Monterrei, Ribeiro, Ribeira Sacra, Valdeorras, O
132 Bierzo and the Easter part of Viños Verdes region) and very variable through the year,
133 giving rise to warm summers. Likewise, mean annual rainfall also shows a clear
134 gradient from coastal (>1400 mm year) to the most inner regions (Valdeorras and O
135 Bierzo, 650 mm). Lithologically, the studied area is more homogeneous, being
136 granites together with granodiorites the main parent material from vineyard-devoted
137 soils in the area (40% of the total samples). However, there are also other soil parent
138 materials such as schists and gneiss (24% of the samples, in Ribeiro and Ribeira
139 Sacra), slates (5% of the samples, in Ribeira Sacra and Valdeorras) and tertiary
140 sediments, limestone and quaternary terraces (31% of the samples in Ribeiro,
141 Monterrei, Valdeorras and O Bierzo). Typical mineralogy of clay size fraction in soils
142 developed from granite and granodiorites is represented by 1:1 dioctaedric
143 phyllosilicates (mainly halloysite), vermiculites (usually interlayered Al hydroxide),
144 Fe oxyhydroxides and gibbsite (Macias *et al.*, 1982). Phyllosilicates 1:1, illite-

145 vermiculite interstratifieds and Fe oxyhydroxides are most common clay minerals in
146 soils developed from gneiss, slate and schist (Macias and Calvo, 1992), whereas Fe
147 oxyhydroxides dominated in soils developed from tertiary sediments and terraces. In
148 all winegrowing regions, the high degree of humidity makes necessary an intensive
149 application of Cu-based fungicides in the fight against fungus. Copper-based
150 fungicides were added to soils mainly in the form of Bordeaux mixture [$\text{Ca}(\text{OH})_2 +$
151 CuSO_4] and Cu oxychloride. Moreover, vines have been grown mainly on shallow
152 soils located at steep slopes and the erosion events can mobilize a part of
153 accumulated Cu, therefore there is a potential risk of contamination of other soils and
154 water ecosystems with this metal.

155

156 *Sampling*

157

158 Soil samples were collected from a total of 170 vineyards distributed in seven
159 winegrowing regions in NW of Iberian Peninsula (Monterrei, M, $n= 36$ samples; Rías
160 Baixas, RB, $n= 24$ samples; Ribeira Sacra, RS, $n= 18$ samples; Ribeiro, R, $n= 25$
161 samples; Valdeorras, V, $n= 24$ samples; O Bierzo, OB, $n=25$; Vinhos Verdes, VV,
162 $n=18$ samples). In order to ensure consistency in sampling across vineyards, several
163 soil sub-samples (0-20 cm depth) were collected with a soil auger to an overall
164 amount of 2 kg (8-10 subsamples) along each vineyard. Soil sub-samples of each
165 vineyard were subsequently mixed into a single composite soil sample. This sampling
166 strategy tries to overcome the well known variability in soil characteristics of vineyards
167 (Fernández-Calviño *et al.*, 2008b; Wightwick *et al.*, 2008). Once in the laboratory,
168 composite soil samples were air-dried, sieved through 2 mm and stored in
169 polyethylene bottles until analysis.

170

171 *Physicochemical and chemical properties of soils*

172

173 The proportions of sand (2–0.05 mm), silt (0.05–0.002 mm) and clay (<0.002 mm) of
174 the soils were determined by wet sieving for the size fractions greater than 0.05 mm
175 and using the international pipette method for all others. The pH in water (pH_w) and
176 0.1 M KCl (pH_k) were measured at a soil/water ratio of 1:2.5 after 10 and 120 minutes
177 respectively. Total carbon and nitrogen contents were determined on a
178 ThermoFinnigan 1112 Series NC elemental analyser. The cation exchange capacity at
179 soil pH (eCEC) was estimated as the combination of exchangeable base cations (K_e,
180 Na_e, Ca_e, Mg_e) extracted with 0.2 M NH₄Cl (Sumner & Miller, 1996) and
181 exchangeable Al (Al_e) extracted with 1 M KCl (Bertsch & Bloom, 1996). The
182 distribution of Al and Fe in the soils was studied by the selective extraction with 0.1
183 M Na-pyrophosphate at pH 10 (Fe and Al bound to organic matter, Al_p and Fe_p) and
184 0.2 M oxalic acid-ammonium oxalate at pH 3 (Fe and Al bound to organic matter and
185 non-crystalline inorganic forms, Al_o and Fe_o). The contents in Ca, Mg, Al and Fe were
186 determined by flame atomic absorption spectrometry, and those in Na and K by flame
187 atomic emission spectrometry.

188

189 *Total copper in soils*

190

191 Total copper concentration (Cu_T) was determined as follows: 0.5 g of soil was
192 digested in a microwave oven at 100 psi with 5 ml of HNO₃, 4 ml of HF, and 1 ml of
193 HCl. Then we measured copper in the extracts by atomic absorption
194 spectrophotometry using a Thermo Solar M series spectrometer. For quality assurance

195 and control (QA/QC) purposes, two certified reference materials endorsed by the
196 Community Bureau of Reference, Commission of the European Communities (*viz.*
197 CRM 141 calcareous loam soil and CRM 143 sewage sludge amended soil) was used.
198 After measurement of Cu in triplicate samples of reference material digested by the
199 above-described method, the copper recoveries ($32.7 \pm 1.1 \text{ mg kg}^{-1}$ for CRM 141 and
200 $229.7 \pm 3.2 \text{ mg kg}^{-1}$ for CRM 143) were in the range of certified values (32.6 ± 1.4
201 and $236.5 \pm 8.2 \text{ mg kg}^{-1}$, respectively).

202

203 *Fractionation of Cu in soils*

204

205 It should be taken into account that there is not a selective dissolution scheme that can
206 be recognized to be accurate for metal partitioning, and they usually have several
207 shortcomings. Despite of this, methods for selective dissolution provide useful
208 information on metal binding, mobility and bioavailability. In the present study, the
209 distribution of Cu in the soil solid phase was determined in triplicate following the
210 methods applied by Fernández-Calviño *et al.* (2008c). A briefly description of these
211 methods is given as follows:

212

213 1) Cu_a (ammonium acetate extractable Cu): 10 g of soil was shaken for 1 hour in 50
214 ml of 1 M ammonium acetate solution (pH 7).

215 2) Cu_p (pyrophosphate-extractable Cu): 1 g of soil was shaken for 16 h in 100 ml of
216 0.1 M sodium pyrophosphate (pH 10.1) solution.

217 3) Cu_o (oxalic/oxalate-extractable Cu): 1 g of soil was shaken in darkness for 4 h in 50
218 ml of 0.2 M oxalic acid - ammonium oxalate (pH 3) solution.

219 4) Cu_{ao} (ascorbic/oxalic/oxalate-extractable Cu): 1 g of soil was shaken for 30 min at
220 96 °C in 50 ml of 0.2 M oxalic acid - ammonium oxalate + 0.1 M ascorbic acid (pH
221 3.25) solution.

222

223 In all soil extracts, copper was measured by atomic absorption
224 spectrophotometry using a Thermo Solar M series spectrometer. Based on the
225 amounts of Cu extracted, the following operationally-defined fractions were
226 determined:

227

228 - Exchangeable copper (Cu_{EX}): is considered the copper measured in the NH_4OAc
229 extracts (Cu_a).

230 - Copper bound to soil organic matter (Cu_{OM}): estimated as the difference between Cu
231 extracted with Na pyrophosphate and exchangeable Cu ($Cu_p - Cu_a$).

232 - Copper bound to amorphous inorganic materials (Cu_{IA}): the difference between Cu
233 extracted with oxalic acid–ammonium oxalate and Na pyrophosphate ($Cu_o - Cu_p$).

234 - Copper bound to crystalline Fe and Al hydrous oxides (Cu_C): the difference between
235 Cu extracted with oxalic acid-ammonium oxalate plus ascorbic acid and Cu extracted
236 with oxalic acid-ammonium oxalate ($Cu_{oa} - Cu_o$).

237 - Residual copper (Cu_R): the difference between total Cu and Cu extracted with oxalic
238 acid–ammonium oxalate plus ascorbic acid ($Cu_T - Cu_{oa}$).

239

240 *Statistical analyses*

241

242 To facilitate comparison of data between soils collected from different winegrowing
243 regions, the average values and standard deviation (mean \pm SD) were calculated using

244 all soil samples from same region. Calculations of basic descriptive statistics and
245 lineal regressions, analyses of variance (ANOVA), least significant difference tests (at
246 the 95% probability level), multiple linear regressions, principal component analysis
247 and two step cluster analysis were all performed using SPSS v.17.0 for Windows.

248

249 **Results and discussion**

250

251 *Physicochemical and chemical soil properties*

252

253 The overall mean values and standard deviation (mean±SD) as well as the range
254 (minimum and maximum values) of physicochemical and chemical soil properties
255 analyzed in seven different vineyard regions are summarized in Tables 1 and 2. The
256 texture in most of the vineyard-devoted soils studied is characterized by the
257 predominance of the sand fraction (>53%) versus silt and clay fractions, which is
258 expected due to the fact that granite is the dominant soil parent material in most areas.
259 The lowest mean values of sand fraction were founded in V region (46±10%) and
260 especially in OB region (35±9%), where the dominance of sedimentary materials
261 gives to the soils a more fine texture. Mean silt percentage ranged between 21±6 in R
262 region and 41±8% in OB region. This increase in silt content could be due to the
263 influence of parent materials such as slates, sediments, schists. Clay is in general the
264 lowest fraction in all regions and ranged from 25%, in regions where soils developed
265 from sediments, to 13-18% where soils are mainly derived from granite.

266 Mean total C content in soils ranged from 12±4 g kg⁻¹ (in OB soils) to 35±9 g
267 kg⁻¹ (in RB soils), suggesting the existence of a C gradient with a trend to decrease
268 from the coastal regions (RB and VV) to the inner regions (OB and V). Mean total N

269 content in soils followed a similar trend than total C, ranging from $1.2\pm 0.3 \text{ kg}^{-1}$ (in V
270 and OB soils) to $2.7\pm 0.7 \text{ mg kg}^{-1}$ (in RB soils). In the Atlantic coastal areas, the
271 higher soil humidity and lower temperatures can reduce organic matter decomposition
272 increasing its accumulation as compared with vineyards further from the coast.
273 Anyway, vineyard-devoted soils are not characterized by large C contents and
274 therefore, differences in soil management techniques can also be responsible for
275 variations in total C content. The relatively high C content in RS soils is due to extra
276 addition of organic matter through mulching which contributes to reduce soil erosion
277 in steep slope vineyards soils and to preserve soil humidity during summer. The C:N
278 ratio values were relatively low ranging from 9 ± 2 (in V and OB soils) to 13 ± 2 (in rest
279 of winegrowing regions).

280 Most of studied soils are acid, being moderately acid (mean $\text{pH}_w < 5.0$) for M,
281 R and RS soils and slightly acid (mean $\text{pH}_w < 5.7$) in soils from V and VV regions.
282 However, pH_w values close to neutrality (mean pH_w 6.5-6.6) were observed for OB
283 and RB vineyard soils. In RB region is traditional the addition of calcium carbonate
284 (as milled bivalve shells) to granite vineyard soils in order to increase soil pH,
285 whereas in soils of OB region pH_w values can be due to the presence of traces of
286 calcium carbonate in sediments. Mean soil pH_k are 0.7 to 1.1 units lower than pH_w but
287 showed a similar trend; the lowest pH_k (≈ 4.1) in soils from R, RS and M regions are
288 likely due to the presence of exchangeable Al. Mean values of effective cationic
289 exchange capacity (eCEC) in studied vineyard soils is also low ($< 11 \text{ cmol}_c \text{ kg}^{-1}$; Table
290 2), which is the result of the combination of a low content of minerals with high
291 specific surfaces in terms of weatherable minerals and a low content of soil organic
292 matter. However, in soils from RB region, eCEC mean value increased notably due to
293 Ca addition from bivalve shells ($21 \text{ cmol}_c \text{ kg}^{-1}$). Aluminium in the cation exchange

294 complex ranged from 0.09 cmol_c kg⁻¹ in RB soils to 0.83 cmol_c kg⁻¹ in R soils,
295 representing between 0.1 and 15% of effective cation exchange capacity. As it was
296 expected, Ca was the dominant cation in the cation exchange complex ranging from
297 1.09 cmol_c kg⁻¹ (in RS soils) to 9.39 cmol_c kg⁻¹ (in RB soils), contributing to 31 to
298 77% of effective cation exchange capacity in soils from RS and OB regions,
299 respectively.

300 The distribution of Al in the soil solid phase was dominated by Al bound to
301 soil organic matter (Table 2), since the ratio Al_p/Al_o showed mean values from 0.6 in
302 OB soils to 1.0 in soils from M and RS regions. In the case of Fe, non-crystalline
303 inorganic Fe was the dominant fraction (Fe_p/Fe_o<0.3) in soils from M, RS, OB and
304 VV regions, whereas Fe bound to soil organic matter was dominant with in soils from
305 RB, R and V regions (Fe_p/Fe_o>0.6).

306 When results of physicochemical soil properties are compared to those from
307 undisturbed soil samples located at the same area (González-Prieto *et al.*, 1996;
308 Miguéns *et al.*, 2007), a clear vision of severe soil degradation can be maintain for
309 these vineyard soils from N.W. Iberian Peninsula, which show a very low organic
310 matter content and high pH and exchangeable cations due to the intense addition of
311 liming agents and inorganic fertilizers.

312

313 *Total copper in vineyard soils*

314

315 Mean value of total copper content (Cu_T) in all studied samples ($n = 170$) was 157 mg
316 kg⁻¹, with a range that varies from 25 to 666 mg kg⁻¹ (Table 3). These Cu_T levels are
317 much greater than those reference values obtained for uncultivated soils of the same
318 area that ranged from 12 to 39 mg kg⁻¹ (Macías *et al.*, 1993), which is a clear evidence

319 of Cu accumulation in vineyard soils due to application of Cu-based fungicides.
320 According to the 86/278/CEE European Directive, Cu permissible threshold range
321 from 50 to 150 mg kg⁻¹, being 50 mg kg⁻¹ the maximum content allowed in acid soils.
322 Detrimental effects on organic matter turnover and soil organisms were also observed
323 in soils with more than 100 mg kg⁻¹ (Paoletti *et al.*, 1998; Merrington *et al.*, 2002;
324 Díaz-Raviña *et al.*, 2007). Therefore most of the soils studied can be considered
325 copper polluted since in 94% and 43% of them, the value of Cu_T exceeded 50 and 140
326 mg kg⁻¹, respectively (Figure 2), which can have important implications in both soil
327 quality and wine production.

328 Despite of relatively high Cu accumulation in soils, a discrepancy between the
329 amount of added Cu and their content in the soils devoted to vine crop was observed.
330 Taking into account that dose of Cu added as fungicide (3-5 kg per ha) and the
331 number of applications (3-6 treatments), the expected content of Cu_T in old vineyard
332 soils (50 years) should be around three times higher than those observed. This
333 suggests that a relevant proportion of added Cu may have been lost from vineyard
334 soils by runoff, leaching or soil erosion as was shown in several studies (Besnard *et*
335 *al.*, 2001; Ribolzi *et al.*, 2002). A previous study carried out in NW Spain also
336 supported this hypothesis since sediments originated by erosion in vineyard soils
337 showed a significant Cu enrichment compared to soil (Fernández-Calviño *et al.*,
338 2008c). Likewise, the Cu_T levels found in the present study are consistent with those
339 in French vineyards with values of 111-138 mg kg⁻¹ (Brun *et al.*, 1998; Chaignon *et*
340 *al.*, 2003) or Greece vineyards where the average of Cu_T was approximately 156 mg
341 kg⁻¹ (Vavoulidou *et al.*, 2005). However, Italian vineyards showed a slightly higher
342 mean content of Cu_T reaching up to 196 mg kg⁻¹ (Deluisa *et al.*, 1996). Total copper
343 values slightly lower than those found in our study are present in soil vineyards from

344 other countries such as Slovenia (87 mg kg⁻¹, Rusjan *et al.*, 2007), Australia (about 90
345 mg kg⁻¹, Pietrzak & McPhail, 2004; Wightwick *et al.*, 2008), New Zealand (range 12-
346 59 mg kg⁻¹, Morgan and Taylor, 2003) or Czech Republic (55 mg kg⁻¹, Komárek *et*
347 *al.*, 2008). In contrast, Brazilian vineyards showed significantly higher mean Cu_T
348 values than those in our study (353 to 2197 mg kg⁻¹, Mirlean *et al.*, 2007), which can
349 be partly explained by a more intensive application of Cu-base fungicides as
350 consequence of severe climatic conditions (precipitation, temperature).

351 A step-wise multiple regression analysis was carried out to investigate the
352 relationships between total Cu content as a function of soil physico-chemical
353 parameters, resulting in the following significant relationships:

354

$$355 \text{Cu}_T (\text{mg kg}^{-1}) = 312.4 \pm 49.1 - 27.9 \pm 8.7 \text{pH}_w \quad (1)$$

$$356 R^2=0.052 \quad F=10.3 \quad P=0.002$$

357

$$358 \text{Cu}_T (\text{mg kg}^{-1}) = 320.3 \pm 53.0 - 26.2 \pm 8.5 \text{pH}_w + 11.7 \pm 5.5 \text{C} - 1.5 \pm 0.8 \text{Silt} \quad (2)$$

$$359 R^2=0.093 \quad F=6.8 \quad P=0.000$$

360 The result showed that total Cu is related to soil pH, carbon content and silt
361 content (Equation 1 and 2). However, taking into account the low percentage of
362 variance explained in both equations by these variables (5-9%), the results clearly
363 supported that viticultural practices related to the addition of antifungal products
364 (doses and number of applications of Cu-based fungicides) rather than soil properties
365 analyzed are determinant for Cu accumulation in these vineyard soils from NW
366 Iberian Peninsula.

367 When comparing mean contents of Cu_T in soils from the different
368 winegrowing regions it is observed that RS (260±120 mg kg⁻¹) and R (248±130 mg

369 kg^{-1}) soils exhibited the highest Cu_T levels, followed by V ($174\pm 88 \text{ mg kg}^{-1}$) and RB
370 ($139\pm 122 \text{ mg kg}^{-1}$) soils, and finally OB, VV and M soils showed the lowest values
371 (115 ± 39 , 103 ± 42 and $100\pm 48 \text{ mg kg}^{-1}$, respectively). Differences in soil
372 characteristics, vineyard age and management practices can explain the magnitude of
373 Cu_T range observed in the different regions. Thus, soil characteristics such as pH,
374 organic matter content or cation exchange capacity are known to have a strong
375 influence on Cu retention and their potential accumulation in vineyard soils (Arias *et al.*,
376 2004; Wightwick *et al.*, 2008). On the other hand, vineyard age has been
377 demonstrated that also behaves as a significant factor in Cu accumulation, increasing
378 Cu content in soil with vineyard age (Morgan & Taylor, 2003; Rusjan *et al.*, 2007),
379 although in other studies a significant relationship between both terms was not found
380 (Wightwick *et al.*, 2008). Differences in management practices such as number and
381 doses of applied Cu-based fungicides, organic matter addition, ploughing, etc., also
382 can contribute to the disparity in total Cu values. Thus, higher Cu_T values observed in
383 RS and R soils can be explained by climatic conditions with higher levels of humidity
384 and temperature (they are located in the valley of Miño and Sil rivers) that make
385 necessary to use high fungicides doses as well as the repeated application of organic
386 residues that can contribute to Cu accumulation in soil. In fact, the percentage of
387 samples that exceed the phytotoxic level of 100 mg kg^{-1} ranged from 88% to 100% for
388 RS and R soils, 50-70% for V, OB and VV soils and 42-46% for M and RB soils.
389 This agrees with studies of several authors who also attributed differences in Cu_T
390 among vine-growing regions in Italy, France and New Zealand to climatic conditions
391 (Deluisa *et al.*, 1996; Brun *et al.*, 1998; Morgan & Taylor, 2003) as well as to soil
392 factors and management practices (Wightwick *et al.*, 2008). Within same wine
393 producing area, higher variability was found in samples collected in RB region

394 (139±122 mg kg⁻¹, Table 3), which can be due to a significant establishment of new
395 vineyard plantations during the last decades resulting in a mixture of young (with low
396 Cu inputs) and old vineyards (with high Cu inputs).

397

398 *Copper distribution in soils*

399

400 Values of Cu extracted using different reagents described in material and methods
401 section is showed in Table 3, whereas values of Cu fractions (meaning metal
402 associations to various soil components) are present in Table 4. Values of Cu_{EX} (Cu
403 extracted with ammonium acetate, Cu_a) ranged between 0.1 and 30.3 mg kg⁻¹ for the
404 whole set of soil samples studied (Table 3), which represent as average only 3% of
405 Cu_T (Figure 3). When wine-producing regions are compared, the highest Cu_{EX}
406 absolute values were exhibited by R soils followed by soils of the RB, RS and V
407 regions, whereas OB, VV and M soils showed the lowest values (Table 4). The
408 highest relative values of Cu_{EX} (as percentage of Cu_T) were observed in RB and R
409 soils (Figure 3). These exchangeable Cu values are similar to those obtained in other
410 studies focused on Cu fractionation in vineyard soils, which reported absolute values
411 usually below 10 mg kg⁻¹ and a percentage of about 5 % of Cu_T (Parat *et al.*, 2002;
412 Arias *et al.*, 2004; Fernández-Calviño *et al.*, 2008d). Since Cu_{EX} is considered the
413 most mobile fraction in the soil, our results suggest that only a small amount of Cu
414 could be mobilized to soil solution. Despite these relatively low values of Cu_{EX} with
415 regard to Cu_T, 85 % of the samples analyzed showed values higher than those
416 observed in natural soils (McLaren & Crawford, 1973). Thus, it is probable that these
417 additional levels of Cu_{EX} can alter the diversity of soil microorganisms and the
418 biochemical properties of these vineyard soils as was suggested recently by Lejón *et*

419 *al.* (2008). A step-wise multiple regression analysis was carried out to investigate the
420 relationship between Cu_{EX} as a function of several soil parameters, resulting in the
421 following regression:

422

$$423 \quad Cu_{EX} \text{ (mg kg}^{-1}\text{)} = 7.8 \pm 2.0 + 0.03 \pm 0.00 Cu_T - 1.2 \pm 0.2 C - 0.09 \pm 0.02 \text{ Silt} + \\ 424 \quad 0.9 \pm 0.3 Fe_p + 0.17 \pm 0.05 eCEC - 0.9 \pm 0.4 pH_w \quad (3)$$

$$425 \quad R^2 = 0.659, \quad F = 55.5 \quad P = 0.000$$

426

427 Equation 3 reveals that Cu_{EX} was mainly related to Cu_T (52% of variance), whereas
428 variables such as total C (4%), silt (4%), Fe_p (4%), eCEC (1%) and pH_w (1%) only
429 explained a low percentage of variance of Cu_{EX} .

430 The distribution of Cu is mainly dominated by non-exchangeable fractions
431 ($Cu_{OM} + Cu_{IA} + Cu_C + Cu_R$) which, in average, are 97% of Cu_T (Tables 3 and 4, Figure 3).
432 Other studies also have reported the predominance of these Cu fractions in soils
433 devoted to vine crop (Morgan & Taylor, 2003; Arias *et al.*, 2004; Komárek *et al.*,
434 2008). In most of the analyzed samples (85%), the main Cu fraction is that bound to
435 soil organic matter (Cu_{OM}) which showed values that ranged between 3.6 and 367 mg
436 kg^{-1} representing, as average, 49% of Cu_T (Figure 3). The highest absolute mean
437 values of Cu_{OM} were found in RS and R soils, followed by V and RB soils and finally,
438 soils from the regions OB, VV and M showed the lowest values (Table 4). Vineyard
439 soils from regions OB and VV exhibited a relative slightly lower mean values of
440 Cu_{OM} (38-39% of Cu_T) than those observed in the other analyzed winegrowing
441 regions (47-56% of Cu_T) as can be seen in Figure 3. These values contrast with those
442 from natural soils in which Cu_{OM} only achieve 5% (Graña *et al.*, 1991). Since organic
443 matter showed a high affinity for Cu reducing considerably its mobility (McBride *et*

444 *al.*, 1998, Hernández *et al.*, 2007), the predominance of this copper fraction in the
445 surface layer of soils is of great interest to reduce adverse effects of Cu on soil
446 microorganisms and plants (Bolan *et al.*, 2003). However, since Cu_{OM} fraction
447 constitutes a latent source of Cu contamination due to potential liberation of this Cu
448 pool when mineralization rather than humification processes are dominant, situation
449 that will be even worst take into account the high levels of Cu_{OM} fraction were
450 detected in the soils studied. In this sense, it will be necessary to control soil
451 conditions that can alter organic matter dynamics in order to avoid environmental
452 problems. An appropriate management of the natural (endogenous) and/or added
453 (exogenous) organic matter to soil is, therefore, an essential strategy for controlling
454 Cu toxicity and hence the sustainability of these Cu-contaminated vineyard soils.

455 The fraction of Cu bound to inorganic amorphous colloids (Cu_{IA}) showed
456 absolute values ranging from 0.4 to 267 mg kg⁻¹ (calculated as Cu_o-Cu_p from Table 3),
457 with a relative mean value for all soils of 15% of Cu_T (Figure 3), which is similar to
458 those reported in a previous study performed with other vineyard soils (Fernández-
459 Calviño *et al.*, 2008c), but much lower than the 33% founded in natural soils (Graña
460 *et al.*, 1991). In contrast, Parat *et al.* (2002) in soils from vineyards located in France
461 found that Cu_{IA} represented around 57% of total Cu. Differences in particle size
462 distribution as well as in content of Fe and Al inorganic colloids can partly explain the
463 results obtained for Cu distribution among Spanish and French vineyard soils. The
464 highest absolute mean value of Cu_{IA} was found in RS soils (Table 4), whereas relative
465 mean Cu_{IA} values were lower in soils from OB, R and V regions (9-12% of Cu_T) than
466 in the rest of regions (15-21% of Cu_T) (Figure 3).

467 The relative mean value of Cu bound to crystalline colloids (Cu_C) is 12% of Cu_T
468 (Figure 3), with a range for absolute values from 0.6 to 80.4 mg kg⁻¹ (calculated as

469 $Cu_{oa}-Cu_o$ from Table 3). This relative mean value is clearly lower than the 21%
470 reported by Graña *et al.* (1991) for natural soils in NW Iberian Peninsula. Values of
471 this fraction in other studies ranged from 10-12% of Cu_T (Parat *et al.*, 2002; Arias *et*
472 *al.*, 2004). The minor relevance of Cu_C than Cu_{IA} in Cu distribution in these soils can
473 be explained by the lower affinity of crystalline colloids than inorganic amorphous
474 colloids for Cu (Arias *et al.*, 2002). The absolute mean Cu_C values were significantly
475 higher in soils from RS, R and OB regions than in soils from V, RB, VV and M
476 regions (Table 4). Soils from OB region exhibited the highest relative mean Cu_C
477 values (24% of Cu_T versus 9-11% in the rest of regions) (Fig. 3).

478 Residual Cu (Cu_R), which corresponded to the most recalcitrant Cu in the soils,
479 showed a range from 1 to 138 mg kg⁻¹ (calculated as Cu_T-Cu_{ao} from Table 3) and a
480 relative mean value of 22% of Cu_T (Figure 3). This is the second fraction in
481 quantitative importance in analyzed soils whereas in natural soils this percentage is
482 almost double, 41% (Graña *et al.*, 1991). It is noticeable that in 13 % of analyzed
483 soils, Cu_R is the main Cu fraction which occurs mainly in soils with lowest Cu_T levels.
484 Absolute Cu_R values obtained in this study are higher than total Cu content in non-
485 contaminated soils developed from the same parent materials (Macías *et al.*, 1993). This
486 accumulation of residual Cu could take place through occlusion processes and/or co-
487 precipitation especially when high concentrations of Cu were accumulated in soils
488 with high pH. Regarding to this, in experiments with incubated Cu, the proportions of
489 the most labile Cu fractions (exchangeable Cu and organically bound Cu) decreased
490 with increasing incubation time, whereas those of Cu bound to crystalline and
491 amorphous materials and residual Cu exhibited the opposite trend (Arias-Estévez *et*
492 *al.*, 2007). This processes can justified the dominance of Cu distribution by Cu_R in
493 some soil samples from OB region characterized by a low organic matter content and

494 pH higher than 6.5. In this sense, recent studies have shown that in a time span lower
495 than two years it is possible to observe significant changes in Cu distribution towards
496 the dominance of recalcitrant fractions (Arias *et al.*, 2007). The highest absolute mean
497 value for Cu_R were found in RS and R vineyard soils and the lowest in RB soils
498 (Table 4), which also exhibited the lowest relative mean value for Cu_R (10% of Cu_T
499 versus 20-23% in the soils from the rest of regions) (Figure 3).

500 When the whole set of soil samples was considered, most soil Cu fractions
501 analyzed were strongly correlated between them (data not shown) and strong
502 associations were also established with Cu_T (Figure 4). The results clearly showed
503 positive and significant correlations between total Cu and all the different Cu fractions
504 ($r = 0.724, 0.948, 0.613, 0.550$ and 0.690 for Cu_{EX} , Cu_{OM} , Cu_{IA} , Cu_C and Cu_R ,
505 respectively). It would be pointed out that similar results were observed when
506 relationships between Cu total and Cu in different fractions were considered
507 independently for each vineyard region (data not shown). The relationship is closer
508 for Cu_T and Cu_{OM} , showing the important role that organic matter plays in the soil Cu
509 accumulation due to repeated application of Cu-based fungicides (whole set of
510 samples, $n = 169$, $y = 0.61x - 14.92$, $R^2 = 0.938$; M region, $n = 36$, $y = 0.58x - 9.32$, R^2
511 $= 0.948$; RB region, $n = 24$, $y = 0.62x - 6.85$, $R^2 = 0.978$; RS region, $n = 23$, $y = 0.66x$
512 $- 36.03$, $R^2 = 0.932$; R region, $n = 25$, $y = 0.62x - 16.18$, $R^2 = 0.905$; V region, $n = 24$,
513 $y = 0.55x - 9.31$, $R^2 = 0.953$; OB region, $n = 25$, $y = 0.36x - 1.65$, $R^2 = 0.762$; VV
514 region, $n = 18$, $y = 0.48x - 8.56$, $R^2 = 0.889$). Likewise the Cu_T content was
515 significantly correlated with Cu_{EX} and Cu_R but in a less extent, and weakly related
516 with Cu_C and Cu_{IA} . The intercorrelation among all soil variables related with
517 accumulation and fractionation of Cu indicated that all Cu fractions responded
518 similarly to the Cu-based fungicide application increasing their concentrations; the

519 data also emphasized the interdependence of Cu_T with Cu associated with various soil
520 components that differ in their ability to retain or release Cu, especially Cu complexed
521 with organic matter. This is coincident with results published by Flores-Velez *et al.*
522 (1996), who found that organic matter is responsible for a substantial retention of Cu
523 in acid sandy vineyard soils.

524

525 *Combined interpretation of all soil parameters analyzed*

526

527 To compare soil quality in different wine producing regions, all physico-chemical and
528 chemical soil properties, including parameters related with Cu accumulation and
529 fractionation, should be used simultaneously. One method of examining combinations
530 of variables or parameters as well as to determine if there is differentiation between
531 sites based on these variables is by principal component analysis (PCA). Thus, the
532 PCA of SPSS was used to analyze 24 physico-chemical and chemical soil variables of
533 the whole set vineyard samples analyzed. The four main factors identified together
534 account for 66% of the variance; the loading of the 24 soil characteristics considered
535 on each factor are listed in Table 5. Factor I, which accounts for 19% of the total
536 variance, is defined at its positive pole by pH_w , pH_k , $eCEC$, Ca_e , Mg_e and K_e and at its
537 negative pole by Al_e , and may thus be regarded as related to the saturation of cation
538 exchange complex. Factor II, which accounts for 18% of the total variance, exhibits
539 close positive correlations with total C, total N, Al_o , Al_p , Fe_o , Fe_p , suggesting that it is
540 related to the accumulation of soil organic matter. Factor III, which accounts for 16%
541 of the total variance, may be regarded as Cu accumulation and fractionation, being
542 defined by Cu_T , Cu_{OM} , Cu_{EX} , Cu_C , Cu_{IA} . Factor IV accounts for 13% of the total
543 variance. As its negative pole is defined by silt and clay content and at its positive pole

544 by sand content, these associations suggest that it is related to soil texture. The data
545 clearly showed the usefulness of PCA in order to quantify the importance of Cu
546 contamination as source of variation in soil environment for vineyard regions from
547 NW Iberian Peninsula. The samples were homogeneously distributed in planes
548 defined by these four factors; however, the high number of samples make impossible
549 to discriminate between soil samples from different wine producing regions.

550 To investigate similarities between soil samples collected from seven different
551 vineyard regions, two step clustering of the 170 samples on basis all physico-chemical
552 and chemical soil properties analyzed ($n=24$), were performed and significant
553 differences between clusters were determined. This cluster analysis grouped all soil
554 samples into three different groups: a first group formed by 65 samples, a second
555 group with 60 samples and a third group composed of 45 samples. The main
556 characteristics that define these three groups are indicated in Table 6. These three
557 clusters differed significantly in most analyzed variables with the following
558 exceptions: Na_e , K_e , Fe_p , Al_p and Cu_c for Cluster 1 and 2; C/N ratio, sand and silt
559 content for cluster 1 and 3, and Cu accumulation and fractionation (Cu_t , Cu_{ex} , Cu_{om} ,
560 Cu_{ia} , Cu_r), Al_e and Fe_o for clusters 2 and 3. The detailed observation of samples
561 distribution showed that 89% and 80% of soils from R and RS regions respectively,
562 and 50% of soils from M region were included in cluster 1. All vineyards from OB
563 region, 67% of soils from V region and 42% of samples from M region were included
564 in cluster 2. Cluster 3 was compounded by 92% and 89% of soils from RB and VV
565 regions, respectively. Therefore, the data clearly indicated that this analysis allowed
566 us to discriminate vineyard soil samples according their geographical location and
567 proximity (see Figure 1).

568 As was indicated above, the whole set of vineyard soils analyzed in this study
569 are considered highly degraded when they are compared with uncultivated reference
570 soils of same regions, that is, low organic matter content, high pH and nutrient
571 availability, high Cu content. However, differences among these soils could be
572 established on the basis of a cluster analysis (Table 6). Thus, three main groups could
573 be distinguished on the basis of physico-chemical and chemical soil properties
574 analyzed. Group 1 is formed by R and RS vineyard samples characterized by coarse
575 texture, moderately acid pH, the lowest eCEC, intermediate levels of organic matter
576 and the highest Cu levels. Group 2 is made up by soils from OB and V regions which
577 showed finer texture, slightly acid pH, moderate content of exchangeable cations and
578 Cu levels and the lowest organic matter content. Group 3 is formed by RB and VV
579 vineyard soils that exhibited coarse texture, the highest values for organic matter
580 content, pH and eCEC and the lowest Cu content. Soils from M region were included
581 in groups 1 and 2. These samples were grouped clearly and the result obtained shows
582 that soil type, climatic conditions and repeated applications of high doses of
583 fungicides are a key factor for soil quality of these degraded soils devoted to vineyard.
584 Therefore, since Cu is a serious threat to soil quality for these soils, Cu-based
585 pesticide might include a higher environmental risk in soils from Ribeiro and Ribeira
586 Sacra than in the other regions due to the excessive Cu accumulation and the acid pH.

587

588 **Conclusions**

589

590 The paper is the first detailed report on copper soil contamination of vineyards in the
591 temperate humid winegrowing region in the Iberian Peninsula. The amounts of total
592 Cu and Cu associated to different soil components in vineyards were many times

593 higher than the background levels of forest soils in same region. Since total Cu
594 concentrations higher than 100 mg kg⁻¹ in most of analyzed soils, Cu is therefore a
595 serious threat to soil quality of these soils. Principal component analysis of the
596 combined physical, chemical and Cu fractions data for these soils indicated that Cu
597 accumulation and fractionation accounted for 16% of variation in soil environment,
598 whereas pH and cation exchange complex, organic matter and related properties, and
599 soil texture contributed about 19%, 18% and 13%, respectively, of soil variations.
600 Cluster analyses performed with the same matrix of data allow us to separate soil
601 samples in three well differentiated groups, a first group formed by soils from Ribeiro
602 and Ribeira Sacra regions, a second group made up by soils from O Bierzo and
603 Valdeorras regions, a third group constituted by soils from Rías Baixas and Vinhos
604 Verdes regions and finally, soils from Monterrei region which are included in first and
605 second groups. Obtained results should promote the care for environment in these
606 vineyards from NW Iberian Peninsula and it should be reduced the use of copper-
607 based sprays as much as possible in the future, especially in soils from Ribeiro and
608 Ribeira Sacra regions that exhibited very high Cu concentrations and acid pH.

609

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611

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744

745 **Table 1.** Mean, standard deviation, maximum and minimum values of selected physico-
 746 chemical and chemical properties of studied vineyard soils in the NW Iberian Peninsula.

Region		Sand	Silt	Clay	pH _w	pH _k	C	N	C/N
		----- (%) -----			- (g kg ⁻¹) -				
Monterrei (M)	Mean	53	32	16	4.9	4.1	22	1.6	13
	SD	14	11	4	0.6	0.6	12	0.8	2
	Max	74	56	24	6.7	6.3	55	4.0	16
	Min	23	16	9	4.0	3.3	3	0.4	8
Rías Baixas (RB)	Mean	63	21	16	6.6	5.8	35	2.7	13
	SD	7	6	2	0.7	0.8	9	0.7	1
	Max	74	35	21	7.9	7.2	66	4.4	15
	Min	46	13	12	5.3	4.6	22	1.9	11
Ribeira Sacra (RS)	Mean	60	27	13	5.0	4.1	34	2.6	13
	SD	11	9	3	0.6	0.7	20	1.3	2
	Max	77	46	20	6.3	5.9	86	5.9	16
	Min	36	14	8	4.3	3.4	15	1.2	11
Ribeiro (R)	Mean	60	22	18	4.9	4.1	21	1.8	12
	SD	8	6	4	0.6	0.7	15	1.2	2
	Max	72	35	28	6.9	6.6	77	7.0	17
	Min	44	14	12	4.1	3.5	11	0.8	8
Valdeorras (V)	Mean	46	31	24	5.6	4.5	14	1.6	9
	SD	10	6	8	0.4	0.5	7	0.6	2
	Max	70	42	40	6.5	5.6	34	3.0	13
	Min	26	18	7	4.4	3.8	5	0.6	6
O Bierzo (OB)	Mean	35	41	25	6.5	5.8	12	1.2	10
	SD	9	8	4	0.9	1.1	4	0.3	3
	Max	52	56	37	7.9	7.3	28	2.1	23
	Min	13	23	19	4.8	3.8	7	0.8	8
Vinhos Verdes (VV)	Mean	57	25	18	5.7	4.8	32	2.7	12
	SD	13	11	3	0.4	0.4	12	0.9	1
	Max	70	52	25	6.5	5.7	55	4.5	14
	Min	27	16	13	4.8	4.1	17	1.5	9

747 **Table 2.** Mean, standard deviation, maximum and minimum values of characteristics of cation
748 exchange complex and Al and Fe extractions of studied vineyard soils in the NW Iberian
749 Peninsula.

Region		Na _e	K _e	Ca _e	Mg _e	Al _e	eCEC	Al _p	Al _o	Fe _p	Fe _o
		----- (cmolc kg ⁻¹) -----						----- (g kg ⁻¹) -----			
Monterrei (M)	Mean	0.09	0.64	2.38	0.49	0.36	3.9	1.2	1.0	0.3	2.0
	SD	0.04	0.34	1.75	0.44	0.28	1.9	0.4	0.4	0.2	1.0
	Max	0.26	1.65	8.88	1.87	1.00	11.5	2.2	1.9	0.8	5.0
	Min	0.05	0.16	1.11	0.16	<0.06	2.3	0.4	0.1	0.1	0.5
Rías Baixas (RB)	Mean	2.43	7.68	9.39	1.41	0.09	21.0	2.6	3.2	2.1	3.0
	SD	3.36	3.62	6.72	0.60	0.08	8.4	1.4	1.4	1.0	1.1
	Max	18.0	13.82	29.78	3.70	0.41	42.4	5.4	6.5	4.4	5.7
	Min	0.94	2.98	3.49	0.66	<0.06	8.6	0.7	1.5	0.3	1.3
Ribeira Sacra (RS)	Mean	0.68	0.36	1.09	0.85	0.48	3.5	1.6	1.6	0.4	2.0
	SD	0.09	0.09	0.46	0.54	0.66	0.8	0.7	0.7	0.2	0.9
	Max	0.90	0.50	2.25	2.56	2.63	5.7	3.1	3.2	0.7	4.1
	Min	0.56	0.20	0.40	0.30	<0.06	2.4	0.4	0.6	0.1	0.7
Ribeiro (R)	Mean	0.07	0.85	2.99	0.63	0.83	5.4	1.2	1.5	1.1	1.8
	SD	0.06	0.99	2.14	0.54	0.70	2.9	1.0	1.1	0.8	0.8
	Max	0.20	5.36	7.71	2.17	2.19	15.2	4.3	5.0	3.9	3.4
	Min	0.00	0.08	0.56	0.12	<0.06	2.4	0.2	0.4	0.3	0.5
Valdeorras (V)	Mean	1.54	0.50	2.70	0.37	0.40	5.6	0.9	1.1	1.3	2.2
	SD	0.35	0.35	1.31	0.24	0.46	1.4	0.5	0.6	0.5	1.1
	Max	1.91	1.55	4.99	0.78	1.48	8.2	2.6	2.8	2.1	4.2
	Min	0.07	0.15	0.64	0.09	<0.06	3.6	0.4	0.5	0.5	0.7
O Bierzo (OB)	Mean	0.26	0.91	8.32	1.19	0.13	10.7	0.4	0.7	0.4	1.9
	SD	0.05	0.36	5.54	0.71	0.20	5.8	0.2	0.2	0.2	0.7
	Max	0.44	1.90	20.48	2.75	0.81	22.7	0.6	1.2	0.9	3.6
	Min	0.20	0.51	1.10	0.28	<0.06	2.7	<0.1	0.4	0.1	0.5
Vinhos Verdes (VV)	Mean	0.67	0.76	3.75	1.02	0.12	6.4	3.1	3.5	0.6	1.9
	SD	0.16	0.36	1.54	0.40	0.08	2.0	1.4	1.4	0.6	1.6
	Max	1.14	1.72	7.17	1.91	0.33	10.0	7.4	6.9	2.1	6.3
	Min	0.47	0.28	1.34	0.44	<0.06	2.9	1.2	1.4	0.2	0.5

751 **Table 3:** Mean, standard deviation, maximum and minimum values of total copper
752 and copper extractions in vineyard soils from NW Iberian Peninsula. Cu_a: ammonium
753 acetate extractable Cu; Cu_p: pyrophosphate-extractable Cu; Cu_o: oxalic/oxalate-
754 extractable Cu; Cu_{ao}: ascorbic/oxalic/oxalate-extractable Cu; Cu_T: total Cu.
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Fraction		Monterrei	Rías Baixas	Ribeira Sacra	Ribeiro	Valdeorras	Bierzo	Vinhos Verdes
		----- (mg kg ⁻¹) -----						
Cu _a	Mean	1.1	6.0	4.7	12.2	5.1	3.1	2.2
	SD	0.6	1.7	4.3	7.0	4.1	1.1	1.0
	Max	2.5	9.0	15.9	30.3	14.9	6.0	4.0
	Min	0.1	2.5	0.9	0.6	0.6	1.1	0.4
Cu _p	Mean	50	85	131	151	102	46	43
	SD	29	77	73	91	53	17	22
	Max	163	348	302	398	223	77	86
	Min	4	16	44	10	29	14	9
Cu _o	Mean	69	112	187	174	122	58	59
	SD	43	97	111	98	63	22	31
	Max	240	429	404	448	260	100	126
	Min	6	30	73	12	35	16	13
Cu _{ao}	Mean	78	125	212	198	137	84	71
	SD	44	111	114	108	67	29	35
	Max	243	503	437	528	275	134	144
	Min	7	32	77	27	45	29	17
Cu _T	Mean	100	139	260	248	174	115	103
	SD	48	122	120	130	88	39	42
	Max	272	560	511	666	387	184	190
	Min	25	33	122	47	55	43	34

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760 **Table 4.** Mean and standard deviation values of total and copper fractions obtained
 761 for different winegrowing regions from NW Iberian Peninsula. Cu_{EX}: Exchangeable
 762 Cu; Cu_{OM}: Cu bound to organic matter; Cu_{IA}: Cu bound to amorphous inorganic
 763 materials; Cu_C: Cu bound to crystalline Fe and Al hydrous oxides; Cu_R: Residual Cu;
 764 Cu_T: Total Cu. Different letters denote significant differences at P<0.05 level between
 765 winegrowing regions.

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	Ribeira Sacra (RS)	Ribeiro (R)	Valdeorras (V)	Rías Baixas (RB)	O Bierzo (OB)	Vinhos Verdes (VV)	Monterrei (M)
	----- (mg kg ⁻¹) -----						
Cu_T	260±120 ^a	248±130 ^a	174±88 ^b	139±122 ^{bc}	115±39 ^c	103±42 ^c	100±48 ^c
Cu_{EX}	5±4 ^{bc}	12±7 ^a	5±4 ^b	6±2 ^b	3±1 ^{cd}	2±1 ^{de}	1±1 ^e
Cu_{OM}	126±70 ^{ab}	138±85 ^a	97±49 ^{bc}	79±76 ^c	43±16 ^d	41±21 ^d	49±29 ^d
Cu_{IA}	56±66 ^a	23±18 ^{bc}	20±18 ^{bc}	27±24 ^b	11±8 ^c	16±10 ^{bc}	19±15 ^{bc}
Cu_C	25±16 ^a	24±20 ^a	15±10 ^b	13±17 ^b	27±10 ^a	11±6 ^b	9±7 ^b
Cu_R	48±29 ^{ab}	50±31 ^a	37±26 ^{bc}	14±15 ^e	31±16 ^{cd}	32±11 ^{cd}	22±11 ^{de}

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771 **Table 5.** Loadings of physico-chemical and chemical soil properties on the axes
 772 identified by principal component analysis of their values in the studied vineyard soils
 773 samples from NW Iberian Peninsula.

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Variable	Factor I	Factor II	Factor III	Factor IV
pH _k	0.92	-0.01	-0.08	-0.16
pH _w	0.91	-0.02	-0.10	-0.14
eCEC	0.87	0.32	-0.05	0.06
Ca _e	0.87	0.05	-0.06	-0.08
Mg _e	0.66	0.20	-0.01	0.04
K _e	0.56	0.51	-0.08	0.21
Al _e	-0.52	-0.02	0.28	0.18
Na _e	0.34	0.33	0.02	0.04
N	0.05	0.85	0.07	0.08
C	0.02	0.83	0.04	0.22
Al _o	0.15	0.81	-0.04	0.20
Al _p	0.15	0.77	-0.07	0.11
Fe _p	0.19	0.65	0.09	-0.20
Fe _o	0.04	0.61	-0.06	-0.49
Cu _T	-0.15	0.11	0.96	0.06
Cu _{OM}	-0.18	0.15	0.89	0.09
Cu _{EX}	-0.03	-0.06	0.82	0.18
Cu _R	-0.17	-0.12	0.71	-0.14
Cu _C	0.11	-0.20	0.64	-0.05
Cu _{IA}	-0.08	0.26	0.50	0.12
Sand	-0.06	0.05	0.12	0.94
Clay	0.19	-0.05	0.03	-0.81
Silt	-0.03	-0.04	-0.18	-0.80
C/N	-0.04	0.32	-0.06	0.49

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779 **Table 6.** Two step cluster analysis of the studied vineyard soil samples from NW

780 Iberian Peninsula on the basis of their physico-chemical and chemical soil properties.

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Variable	Cluster 1		Cluster 2		Cluster 3	
	Mean	SD	Mean	SD	Mean	SD
Sand / %	61	9	39	9	59	11
Silt / %	24	7	38	9	24	9
Clay / %	15	4	23	6	17	3
C / %	2.3	1.5	1.5	0.6	3.6	1.3
N / %	0.19	0.1	0.14	0.04	0.28	0.20
C/N	12	2	10	3	13	1
pH _w	4.9	0.5	5.8	0.9	6.2	0.8
pH _k	4.0	0.5	5.0	1.0	5.4	0.8
Na _e / cmol _c kg ⁻¹	0.46	0.55	0.59	0.65	1.49	2.63
K _e / cmol _c kg ⁻¹	0.62	0.83	0.77	0.37	4.38	4.39
Ca _e / cmol _c kg ⁻¹	2.19	1.84	4.95	4.64	6.81	5.72
Mg _e / cmol _c kg ⁻¹	0.54	0.47	0.78	0.63	1.28	0.56
Al _e / cmol _c kg ⁻¹	0.64	0.60	0.20	0.27	0.13	0.20
eCEC / cmol _c kg ⁻¹	4.43	2.62	7.23	4.90	14.05	9.63
Fe _p / g kg ⁻¹	0.65	0.56	0.74	0.63	1.40	1.12
Fe _o / g kg ⁻¹	1.72	0.85	2.30	0.97	2.44	1.38
Al _p / g kg ⁻¹)	1.29	0.68	1.01	0.71	2.67	1.50
Al _o / g kg ⁻¹)	1.37	0.70	0.91	0.43	3.20	1.58
Cu _T / mg kg ⁻¹	229	131	116	50	108	58
Cu _{EX} / mg kg ⁻¹)	7.4	6.7	2.5	1.8	3.9	2.5
Cu _{OM} / mg kg ⁻¹	125	78	52	28	51	39
Cu _{IA} / mg kg ⁻¹	33	41	15	13	21	14
Cu _C / mg kg ⁻¹	21	19	18	11	10	6
Cu _R / mg kg ⁻¹	43	30	28	15	22	15

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784 **Figure captions**

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786 Figure 1. Location map of the study area showing winegrowing regions.

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788 Figure 2. Soil sample distribution according to total copper concentrations.

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790 Figure 3. Mean percentages of Cu fractions with respect to total Cu obtained for
791 different winegrowing regions from NW Iberian Peninsula. Cu_{EX} : Exchangeable
792 copper; Cu_{OM} : Cu bound to organic matter; Cu_{IA} : Cu bound to amorphous inorganic
793 materials; Cu_C : Cu bound to crystalline Fe and Al hydrous oxides; Cu_R : Residual Cu.

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795 Figure 4. Relationship between total copper (Cu_T) and copper fractions in vineyard
796 soil samples from NW Iberian Peninsula. Cu_{EX} : Exchangeable copper; Cu_{OM} : Cu
797 bound to organic matter; Cu_{IA} : Cu bound to amorphous inorganic materials; Cu_C : Cu
798 bound to crystalline Fe and Al hydrous oxides; Cu_R : Residual Cu.

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