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1 **Do DOM optical parameters improve the prediction of copper availability**
2 **in vineyard soils?**

3

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21 **Abstract**

22 Accumulation of copper (Cu) in soils due to the application of fungicides may be toxic for
23 organisms and hence affect winegrowing sustainability. Soil parameters such as pH and
24 dissolved organic matter (DOM) are known to affect the availability of Cu. In this study, we
25 investigated the contribution of chromophoric and fluorescent DOM properties to the prediction
26 of Cu availability in 18 organic vineyard soils in the Bordeaux winegrowing area (France). The
27 DOM parameters, assessed through absorbance and fluorescence analyses, and proxies for Cu
28 availability (total soluble Cu and free ionic Cu^{2+}) were measured in 0.01 M KCl extracts. Total
29 soluble Cu (Cu_{KCl}) varied 23-fold while free ionic Cu^{2+} varied by a factor of 4,600 among the
30 soils. DOC concentrations were similar among the soils, but the samples differed in the quality
31 of DOM as assessed by optical spectroscopy. Multilinear regression models with and without
32 DOM quality parameters were investigated to predict Cu availability. The best model for Cu_{KCl}
33 successfully explained 83 % of variance and included pH, Cu_{T} and two DOM fluorescence
34 quality indices, the FI fluorescence index, which distinguishes between microbial and higher
35 plant origins, and the HIX humification index. For the prediction of Cu^{2+} , pH alone explained
36 88 % of variance and adding DOM parameters did not improve modelling. The two Cu
37 availability proxies were related to pH. This study confirms the prominent role of pH in Cu
38 availability and underlines the importance of DOM quality to better predict Cu solubility.

39

40 **Keywords**

41 Copper, organic matter, fluorescence, absorbance, vineyard soils, availability, modelling.

42 **1. Introduction**

43 Copper-based fungicides like Bordeaux mixture have been repeatedly applied in vineyards
44 since the end of the 19th century to control downy mildew of grapevine, sometimes causing Cu
45 to accumulate in the soils (Brun et al. 2001; Ballabio et al. 2018). In European soils, the median
46 of total Cu concentrations is 13 mg kg⁻¹. In vineyards soils, this value reaches 49 and 91 mg kg⁻¹
47 ¹ in Europe and France, respectively (Ballabio et al. 2018). In old vineyards, Cu concentrations
48 in the topsoil can reach several hundred mg Cu kg⁻¹, as widely reported in several European
49 countries including France, Italy, Spain, Greece, Portugal, Czech Republic (Fernández-Calviño
50 et al. 2009; Komárek et al. 2010; Fernández-Calviño et al. 2010; Flores-Álvarez et al. 2011;
51 Kelepertzis et al. 2017). Despite the reduction of the authorized application rate to 4 kg Cu ha⁻¹
52 ¹ year⁻¹ (EFSA et al. 2018), Cu-based fungicides continue to be used, particularly in organic
53 viticulture for which there is currently no alternative that offers equally effective protection
54 (Andrivon et al. 2018).

55 Cu is an essential micronutrient for all living organisms. Microorganisms need trace levels of
56 Cu for their growth because it is a cofactor in enzymatic reactions (Trevors and Cotter 1990). In
57 plants, copper plays key roles in cell wall metabolism and in photosynthetic electron transport
58 (Yruela 2009). A Cu deficiency can affect vital functions in plant metabolism. However, excess
59 copper can cause phytotoxicity through the formation of oxygen radicals which damage cells
60 and inactivate protein structure, or by disturbing photosynthetic activity due to the
61 photoinhibition induced by excessive Cu (Yruela 2005). In calcareous soils, excess Cu can also
62 lead to Fe deficiency in graminaceous species such as durum wheat (Michaud et al. 2007) since
63 Cu(II) has sufficient affinity for phytosiderophores to divert them from their initial objective of
64 mobilising Fe(III). All these problems may affect plant growth and reduce agricultural
65 productivity. Cu phytotoxicity has been reported in durum wheat grown in former vineyard
66 soils with total Cu concentrations beginning at 114 mg kg⁻¹ (Michaud et al. 2007). Exposure to

67 Cu at soil concentrations above 16 mg Cu kg⁻¹ in the soil has also been shown to have a serious
68 impact on earthworm *Eisenia fetida* reproduction by reducing the production of cocoons
69 (Helling et al. 2000). Fernández-Calviño et al. (2010) reported that microbial enzymatic activity
70 (phosphatase) in Portuguese vineyard soils decreased drastically above 250 mg Cu kg⁻¹. A
71 recent meta-analysis of Cu in vineyard soils showed that microbial respiration was reduced by
72 half above a stock of 200 kg Cu ha⁻¹ (Karimi et al. 2021). Excess copper may thus cause
73 ecotoxicity in vineyard soils, although the ecotoxic thresholds vary widely depending on certain
74 soil parameters like organic matter (OM) content or pH. Total Cu inducing 50 % of biomass
75 decrease (EC₅₀) is a widely used indicator in ecotoxicity studies. It has been shown that EC₅₀
76 of lettuce and tomatoes increases with increasing OM content or decreasing pH (Recatalá et al.
77 2012; Sacristán et al. 2016). Cu toxicity may therefore be linked not only to total Cu amounts
78 but also to certain bioavailable forms of Cu in the soils.

79 The bioavailability of metals is defined as the fraction of its environmentally available
80 compound an organism takes up through physiologically driven processes (ISO 2006).
81 Environmental availability is defined as the availability of metals in the soil measured using
82 chemical methods (Harmsen 2007). Sauvé et al. (1996) showed that Cu concentrations in the
83 tissues of several plants were closely linked to Cu in solution, more specifically to free Cu²⁺
84 activity. The free ion Cu²⁺ is thought to be the bioavailable form, and this hypothesis is
85 supported by other authors who reported that Cu toxicity for different organisms (algae,
86 earthworms, zooplankton) in soils and aquatic systems depended strongly on free Cu²⁺ activity
87 (Verweij et al. 1992; Qiu et al. 2013; Tait et al. 2016).

88 The concentration of Cu in soil solutions and Cu²⁺ activity are controlled by soil properties such
89 as total Cu content, pH and soil organic matter (SOM) content (Sauvé et al. 1997; Sauvé et al.
90 2000). pH is known to affect Cu solubility (Temminghoff et al. 1994), but also copper
91 speciation in solution (Bravin et al. 2009). Cu biogeochemistry is controlled to a large extent

92 by its binding to organic matter, since SOM contains acidic functional groups (mainly phenolic
93 and carboxylic groups) which have a particular and strong affinity for Cu (Manceau and
94 Matynia 2010). Organic matter supplied to soils in different forms (fresh materials, compost,
95 manure, sludge) has been shown to significantly reduce Cu availability in its soluble form
96 (Navel and Martins 2014; Ferreira et al. 2018; Gonzaga et al. 2020) and its ionic form (Oustriere
97 et al. 2016). Dissolved organic matter (DOM) plays a key role in the fate of metals in the soil.
98 While SOM immobilises Cu through sorption onto solid phases (Sauvé et al. 2000), DOM
99 increases Cu solubility by forming Cu complexes in soil solution. However, the free Cu fraction
100 may also decrease due to complexation with DOM. Cu mobilisation from the solid phase into
101 solution has been reported to be exacerbated with increasing aromatic DOM (Amery et al. 2007;
102 Amery et al. 2008; Araújo et al. 2019). What is more, prediction of Cu in soil leachates was
103 improved when the DOM aromaticity was taken into account in geochemical modelling (Amery
104 et al. 2008) rather than only considering a percentage of reactive DOM (Djae et al. 2017).
105 Phenolic and O-functional groups of aromatic molecules have high affinity for Cu binding and
106 are highly stable, which may facilitate its solubilisation by DOM (Fuentes et al. 2013). These
107 findings clearly underline the importance of DOM characteristics in the control of Cu dynamics
108 in soil solutions.

109 The capacity of DOM to complex Cu and to promote its mobilisation from the solid phase not
110 only depends on DOM quantity, but also on DOM quality (Baken et al. 2011; He et al. 2016;
111 Kikuchi et al. 2017). DOM quality can be described through its optical properties assessed by
112 Ultraviolet visible (UV-Vis) absorption and excitation-emission matrix (EEM) fluorescence
113 spectroscopy (Jaffé et al. 2014). In the soil literature, DOM is largely characterised by its
114 specific UV-absorbance index at 254 nm ($SUVA_{254}$), which is related to its aromaticity
115 (Weishaar et al. 2003). But the measurements of $SUVA_{254}$ may be biased due to the presence
116 of colloids or iron at high concentrations (Weishaar et al. 2003). Other spectroscopic indices

117 are widely used in studies on aquatic environments to characterise DOM (Yang and Hur 2014;
118 Derrien et al. 2017) and its interactions with Cu (He et al. 2016). Another UV-Vis absorption
119 index is the spectral slope ratio S_R which is inversely proportional to molecular size (Helms et
120 al. 2008). Fluorescence indices include the humification index HIX, which expresses the degree
121 of DOM condensation (Zsolnay et al. 1999), the biological index BIX, used to assess the recent
122 production of autochthonous components (Huguet et al. 2009), and the fluorescence index FI
123 which makes it possible to distinguish microbial versus higher plant origin of DOM (McKnight
124 et al. 2001). In aquatic media, concentrations of metals (Cu, Zn, Cd) in sediment solutions were
125 shown to be related to DOM aromaticity (HIX) and to the autochthonous (BIX) or microbial
126 (FI) origins of molecules and not to DOM molecular size (S_R) (He et al. 2016; Zhang et al.
127 2020).

128 Up to now, only a few studies combined all these tools to describe DOM in contrasted
129 agricultural soils (Romero et al. 2017; Li et al. 2019; Sun et al. 2020) and their effects on metal
130 speciation in soil solution (Guo et al. 2015) or sediments (Xu et al. 2019). To our knowledge,
131 only one paper reported the use of these spectroscopic indices for vineyard soils exposed to
132 herbicide glyphosate and Cu-based fungicides (Daouk et al. 2015). The main goal of the present
133 study was thus to examine the contribution of quality of DOM in predicting Cu availability in
134 organic vineyard soils. We hypothesized that taking the DOM optical indices into account
135 would improve the prediction of Cu environmental availability, assessed with total soluble Cu
136 or free Cu^{2+} .

137 **2. Material and methods**

138 **2.1 Soil sampling and analyses**

139 Eighteen vineyard topsoils were sampled in the vine inter-rows at a depth of between 0 and 15
140 cm in March 2019 in the region of Bordeaux (France), located in the AOC (i.e., controlled

141 designation of origin) zones of ‘*Bordeaux supérieur*’, ‘*Blaye Côtes de Bordeaux*’ and ‘*Graves*’.
142 All the vineyards sampled are organic and the only fungicide treatment they receive is Cu. The
143 fungicide applied was mainly Bordeaux mixture (mixture of copper sulphate and lime).
144 The 18 soils were contrasted in terms of organic matter and pH based on the pedological
145 bedrock and agronomic practices. After sampling, the fresh soils were sieved to 4 mm and kept
146 at 4 °C before analysis to minimise the effects of sampling on dissolved organic carbon (Jones
147 and Willett 2006).

148 Soil pH (pH_{water}), total copper (Cu_{T}), organic carbon (C_{org}) and total nitrogen contents were
149 determined on separate air-dried soils. pH_{water} was determined by potentiometry in distilled
150 water in a 1:2.5 (w/v) soil-to-solution ratio (NF ISO 10390). Cu_{T} was determined after *aqua*
151 *regia* microwave-assisted digestion (Anton Paar Multiwave PRO Microwave Reaction
152 System®). Aliquots (0.5 g) of crushed dried sample were placed in a microwave Teflon flask
153 with an acid mix $\text{HNO}_3\text{:HCl}$ (3.75:1.25, v:v) (Aristar® for trace analysis, VWR Chemicals).
154 Extracts were obtained after centrifugation at 1,300 W at 180 °C for 20 minutes and filtration
155 on a 2- μm Whatman cellulose acetate filter. The concentration of Cu in the extracts was
156 measured by ICP-OES (Agilent®). A certified reference material (BCR-143R, 98 % Cu
157 recovery rate) was used to validate extraction and quantification.

158 C_{org} and N contents in soils were assessed by dry combustion (950 °C) with a CN analyser
159 (Flashea 1112 series®) after carbonates were removed with HCl (NF ISO 10694). An organic
160 soil (ISE 973) was used as control.

161 **2.2 Extraction of soil solutions**

162 Before the soil solution was extracted, the wet soils were all adjusted to 80 % water holding
163 capacity (WHC) and incubated at 20 °C for one week to avoid a possible microbial flush and
164 its effects on Cu solubility and speciation (Cornu et al. 2007).

165 Soil solutions were extracted as follows: 15 g of wet soil were shaken with 30 mL of 0.01 M
166 KCl (purity 99.5 %, Merck®) with 1:2 w/v (soil-to-solution ratio) at 35 rev. mn⁻¹ for 2 h. The
167 soil suspensions were centrifuged at 4,600 g for 10 min. The supernatants were collected and
168 filtered through 0.2 µm cellulose acetate filters. Extractions were performed in triplicate. The
169 extracted soil solutions were stored at 4 °C in the dark before spectroscopic analysis, which was
170 performed less than seven days after extraction.

171 The 0.01 M KCl extraction was chosen for two reasons: (i) to be in the range of common ionic
172 strength for soil pore water and (ii) to avoid DOM coagulation which may occur in the case of
173 CaCl₂ extraction (Römken and Dolfing 1998). The 1:2 w/v ratio was used because lower
174 extraction ratios tend to overestimate the concentrations of DOC recovered (Jones and Willett
175 2006).

176 **2.3 Analyses of soil extracts**

177 **2.3.1 Total dissolved metals and Cu²⁺ concentrations**

178 The pH of the extracts (pH_{KCl}) was measured immediately after extraction by potentiometry
179 using a microelectrode (E16M331, Radiometer Analytical®). The total concentrations of
180 dissolved Ca, Cu, Fe, Mn and Zn were determined by ICP-OES (Agilent ®) in a 5-mL aliquot
181 acidified with 2 % HNO₃ (Aristar® for trace analysis purity 69 %, VWR Chemicals®). Free
182 Cu activity was measured in non-acidified 5-mL aliquots using a cupric ion-selective electrode
183 (ISE, 9629BNWP, Thermo Scientific Orion®). The ISE was calibrated in Cu solutions buffered
184 with iminodiacetic acid and potassium phthalate with pCu (= - log₁₀ aCu²⁺) ranging from 5.5 to
185 13 as described in (Bravin et al. 2009). The calibration curve (R²=0.98, n=67) showed 85 %
186 slope efficiency compared to the theoretical Nernstian slope (**Fig. S1**). The concentrations of
187 free Cu²⁺ were determined from pCu values and from the Cu²⁺ activity coefficient, calculated
188 from the extended Debye-Hückel equation (Ritsema 1993).

189 **2.3.2 Dissolved organic matter parameters**

190 The concentrations of dissolved organic carbon (DOC) in the soil extracts were determined
191 using a TOC analyser (TOC-VCPH, Shimadzu®, Japan). The DOC was calculated as the
192 difference between total carbon (TC) and inorganic carbon (IC) (DOC=TC - IC). The analyser
193 was calibrated with a solution prepared from sodium carbonate, sodium bicarbonate, potassium
194 hydrogen phthalate, and the 0.01 M KCl was used as the zero point in the calibration.

195 **Absorbance indices**

196 Soil solution UV-visible absorbance was measured using a Jasco® V-560 spectrophotometer,
197 scanning from 210 to 700 nm with a 0.5 cm path length quartz cuvette. A 0.01 M KCl solution
198 was used as a blank. Several indices were determined from absorbance spectra to characterise
199 the DOM in the extracts. The specific UV absorbance at 254 nm (SUVA₂₅₄, L gC⁻¹ cm⁻¹) was
200 calculated as follows:

201
$$\text{SUVA}_{254} = \frac{A_{254} \cdot 1000}{b \cdot \text{DOC}}$$

202 where A₂₅₄ is the measured absorbance at 254 nm (dimensionless) (**Fig. S2**), b is the path length
203 (cm) and DOC the organic carbon concentration (mg L⁻¹).

204 The S_R index was calculated as the spectral slope ratio over 275-295 nm to 350-400 nm (Helms
205 et al. 2008).

206 **Fluorescence indices**

207 The excitation-emission matrix (EEM) fluorescence spectra were recorded in a 0.5 cm quartz
208 cuvette, thermostatted at 20 °C in the cell holder, using an Aqualog spectrofluorometer (Horiba
209 Scientific®). Excitation scans were performed using a double-grating monochromator, a 150-
210 Watt Xenon lamp with a 5 nm bandpass, and 2-s integration time at wavelengths of 240–800 nm
211 at 5-nm intervals. Emission spectra were collected with high CCD detector gain at

212 approximately 0.58 nm (1 pixel) intervals at wavelengths of 245-830 nm. A blank EEM was
213 acquired daily from the 0.01 M KCl solution used for soil extraction and subtracted from EEM
214 spectra of all the samples to eliminate scattering peaks. EEM spectra were corrected for
215 instrumental biases. When the maximum intensity of the absorbance spectrum was higher than
216 0.1, the samples were diluted with 0.01 M KCl to avoid inner filter effects. Fluorescence data
217 are expressed in Raman units (R.U) after normalization to the area under the Raman peak of an
218 ultrapure water blank (Milli-Q, Millipore®) at an excitation wavelength of 350 nm. TreatEEM
219 software (<https://sites.google.com/site/daromasoft/home/treateem>) was used to remove
220 remaining scatter peaks in EEMs and to calculate the fluorescence indices.

221 Several indices were obtained from fluorescence analysis. As defined by Zsolnay et al. (1999),
222 the humification index HIX was calculated as the ratio H/L of the area under the curve for two
223 spectral regions (between emission wavelengths 435 nm and 480 nm for H and between 300
224 nm and 345 nm for L) from the emission spectrum scanned for excitation at 254 nm (**Fig. S3**).
225 The higher the HIX, the higher the degree of condensation and aromaticity. The biological index
226 BIX was determined as the ratio of fluorescence intensity at an emission wavelength of 380 nm
227 to that at 430 nm, at an excitation wavelength of 310 nm (Huguet et al. 2009). High BIX values
228 are related to predominantly recent production of DOM linked to biological activity (Huguet et
229 al. 2009). The fluorescence index FI was calculated as the ratio of the fluorescence intensity at
230 emission wavelength of 450 nm to that at 500 nm while excitation was set at 370 nm (McKnight
231 et al. 2001). FI is an indicator of the source of DOM, low values are associated with vascular-
232 plant-derived compounds, while high values indicate microbial origin or microbially processed
233 terrestrial DOM (Cory and McKnight 2005; Broder et al. 2017).

234 **2.4 Statistical analyses**

235 All statistical analyses were performed with R software version 4.0.2 (R core Team 2020). First,
236 principal component analysis (PCA) was used to visualise correlations between variables and

237 possibly groups of soils. PCA was performed using the ‘FactomineR’ package (Husson et al.
238 2008). For the PCA, all the variables were log-transformed except pH_{water} and pH_{KCl} . Second,
239 a correlation matrix was performed to investigate the relationships between the same variables
240 using the R ‘corrplot’ package (Wei and Simko 2021). The Pearson correlation coefficients
241 were considered as statistically significant at $p < 0.01$.

242 Multi-linear log-log regressions were used to identify and rank the contributions of soil
243 variables to the prediction of Cu availability proxies (Cu_{KCl} and Cu^{2+}). Multi-linear regressions
244 were performed using the ‘car’ package (Fox and Weisberg 2019). Each replicate was
245 considered as one sample. For Cu_{KCl} prediction, soil and solution variables were entered as
246 predictors (Cu_{T} , pH_{water} , C:N, C_{org} , SUVA_{254} , S_{R} , DOC, HIX, BIX, FI). Only solution variables
247 were used for Cu^{2+} prediction (Cu_{KCl} , pH_{KCl} , SUVA_{254} , S_{R} , DOC, HIX, BIX, FI). The variables
248 (except pH_{water} and pH_{KCl}) were log-transformed before the computation. A total of 2,047 and
249 255 models with one to maximum four predictors (to avoid overparameterization) were
250 exhaustively tested for Cu_{KCl} and Cu^{2+} predictions, respectively. The best models had the
251 maximum R^2_{adj} (goodness of fit) with a minimum number of predictors (parsimony). The
252 selected models also had a variation inflation factor (VIF) below 2.3 to avoid multicollinearity.
253 The root mean square error (RMSE) was calculated to compare the accuracy of the models
254 including or not DOM parameters.

255 **2.5 Modelling Cu^{2+} activity**

256 Cu speciation in solution was predicted with ORCHESTRA software (Meeussen 2003) using
257 the DOM ion-binding model NICA-Donnan (Kinniburgh et al. 1996; Koopal et al. 2005). The
258 objective was to compare simulations of amounts of free Cu^{2+} with the corresponding
259 experimental measurements. The input parameters used for the calculations were pH_{KCl} , total
260 DOM, Cu^{2+} , Ca^{2+} , Zn^{2+} , Fe^{3+} and Mn^{3+} concentrations. Cl^- and K^+ concentrations were set to
261 0.01 M, i.e., their concentration in the extraction solution. DOM concentrations were calculated

262 assuming that DOM contained 50 % of C. Activity coefficients were calculated using the
263 Davies equation. Fe activity was calculated by assuming its equilibrium with
264 Fe(OH)₃ (logK_S=3.19). Equilibrium reactions were described taking the equilibrium with
265 atmospheric CO₂ (pCO₂ = 3.9x10⁻⁴ atm) into account.

266 Several hypotheses regarding DOM composition were examined: (i) 100 % humic acids (HA),
267 (ii) 100 % fulvic acids (FA), or an inert DOM pool and an active DOM pool comprised of (iii)
268 65 % FA, or (iv) 30 % HA and 30 % FA. In each case, proton and metal binding parameters
269 were set at their generic value (Milne et al. 2001, 2003). Sixty-five percent is the approximation
270 generally used to determine the amount of free metal cations in soil solutions (Weng et al. 2002,
271 Djae et al. 2017). In the second step, free Cu was optimised by adjusting the reactive DOM pool
272 (noted rDOM) in each sample to match the experimental measurements.

273

274 **3. Results and discussion**

275 **3.1 Soil properties**

276 The main soil parameters are listed in **Table 1**.

277 Total concentration of Cu in the soil (Cu_T) ranged from 28 to 238 mg kg⁻¹ with a median of 107
278 mg kg⁻¹. In the Aquitaine region of France, large quantities of Cu-based fungicides are used to
279 prevent downy mildew caused by the wet climate. Accordingly, El Hadri et al. (2012) found
280 that Cu_T in soils in the Aquitaine region ranged from 0.5 to 491 mg kg⁻¹, with maximum values
281 found in vineyard soils. All the vineyard soils in our study fell within this range. It is worth
282 noting that Cu_T concentrations may be very different in the same location, mainly depending
283 on the age of the vineyard (Fernández-Calviño et al. 2009). This may explain the wide range of
284 Cu_T (i.e., 8-fold) observed in the present study.

285 The pH_{water} of the soils ranged from 6.3 to 8.3 (**Table 1**). The most alkaline soils were associated
286 with the highest carbonate contents (**Table 1**). The organic carbon content (C_{org}) ranged from
287 5 to 31 g kg^{-1} . Vineyard soils are generally poor in organic carbon. Coll et al. (2011) reported
288 C_{org} values ranging from 10 to 13 g kg^{-1} for vineyard soils in the Languedoc-Roussillon region
289 (South of France). Our results are also similar to those obtained by Parat et al. (2002) ($11 < C_{\text{org}}$
290 $< 45 \text{ g kg}^{-1}$) in a study conducted in Burgundy viticultural region (east-central France).

291 The C:N ratio varied between 10.3 and 17.4 (**Table 1**). The C:N ratio varied between 10.3 and
292 17.4 (**Table 1**). This ratio provides information on the rate of SOM mineralisation (high for
293 values below 12) and also on the carbon storage capacity of the soil (Swift et al. 1979).

294

295 **3.2 Elemental composition of the soil extracts and Cu availability**

296 Mean Fe, Mn, Ca and Zn concentrations are listed in **Table S1** in supplementary information.

297 The pH of the soil extracts (pH_{KCl}) fluctuated between 5.9 and 7.6 (**Table S1**).

298 Mean concentrations of DOC in KCl extracts ranged only from 15 to 28 mg L^{-1} (median 18)
299 (**Table S1**). This corresponds to 0.08 and 0.33 % of total C_{org} , illustrating the low solubility of
300 SOM. Furthermore, Zsolnay (1996) reported that DOC is the most mobile and reactive fraction
301 of C_{org} .

302 The mean total concentration of Cu in the extracts (Cu_{KCl}) ranged from 0.11 to 2.64 μM , i.e., a
303 factor of 23 (**Table S1**), with a median of 0.3 μM . Many studies in the literature used salt
304 solutions - generally CaCl_2 - to extract Cu and considered it to be a correct surrogate for soil
305 pore water Cu (Sauvé et al. 1997; Chaignon et al. 2003). Our Cu_{KCl} values were similar to those
306 found by Djae et al. (2017) (3×10^{-2} - 3.7 μM Cu) with 2 mM KNO_3 / $\text{Ca}(\text{NO}_3)_2$ extractions.
307 Similar ranges of total Cu assessed in CaCl_2 extracts were found in the literature: 0.11 – 2 μM
308 (Sauvé et al. 1996), 0.01 – 5 μM (Sauvé et al. 1995).

309 The mean Cu^{2+} concentrations varied between 2.1×10^{-4} and $0.95 \mu\text{M}$, i.e., by a factor of 4,600
310 (**Table S1**). This variability shows that our samples were more contrasted in terms of Cu^{2+} than
311 Cu_{KCl} . The highest Cu^{2+} values were found in the most acidic soils. The percentage of free Cu,
312 defined as the ratio of concentrations in Cu^{2+} and Cu_{KCl} ($f\text{Cu}^{2+}$), ranged from 0.07 % to 50 %
313 (**Table S1**). Our results are in agreement with Djae et al. (2017) who found Cu^{2+} concentrations
314 between 3.10^{-7} and $2 \mu\text{M}$ and $f\text{Cu}^{2+}$ ranged from 0.01 % to 44 % for Cu-contaminated soils.
315 But in moderately contaminated vineyard soils, Cornu et al. (2019) found Cu^{2+} of between 6×10^{-5} – $0.3 \mu\text{M}$
316 and $f\text{Cu}^{2+}$ of between 0.002 % - 10 %. These results reveal dramatic differences
317 based on free Cu^{2+} , even within a collection of soils with the same source of Cu contamination.

318 Soil pore water Cu has been used as a proxy for Cu phytotoxicity (Michaud et al. 2008),
319 assuming that few complex forms of Cu present in the solution play a role in Cu uptake by plant
320 roots. The toxicity threshold found in the literature for various plants (3.94 - $9.55 \mu\text{M}$) are all
321 higher than the maximum Cu_{KCl} ($2.64 \mu\text{M}$) found in the present study (**Table 2**), suggesting
322 that our 18 vineyard soils do not *a priori* represent a risk for grapevine and also possibly maize,
323 grapevine and sunflower production (Ouzounidou 1995, Juang et al. 2012, Kolbas et al. 2014).

324 Beyond total soluble Cu, the bioavailable form of Cu is generally considered to be its ionic
325 species Cu^{2+} (Sauvé et al. 1998), so Cu^{2+} concentrations would be a far more reliable tool to
326 assess Cu toxicity for organisms. In the present study, the Cu^{2+} measured in some KCl extracts
327 was the same as that which caused a 50 % reduction ($\text{EC}_{50} = 6.3 \times 10^{-2} \mu\text{M}$) in root growth in
328 durum wheat (Bravin et al. 2010) (**Table 2**). This EC_{50} value was found at $2.2 \mu\text{M}$ Cu^{2+} for
329 cucumber (Kader et al. 2016). To our knowledge, no such toxicity indicator assessed via ionic
330 Cu^{2+} has been established for grapevine. Four soils of our panel (**Table S1**) may be problematic
331 if converted from grapevine to widely consumed crops like wheat. Qiu et al. (2013) reported
332 that Cu^{2+} concentration was also an appropriate proxy to assess Cu toxicity for earthworms and
333 found a EC_{50} of $1 \mu\text{M}$ Cu^{2+} for *Eisenia fetida* mortality (**Table 2**). In our panel of soils, this

334 suggests that one soil represents a potential toxic risk for this earthworm species (**Table 2,**
335 **Table S1**).

336 Overall, the Cu^{2+} and Cu_{KCl} levels in the soils sampled in this study cannot be considered
337 completely risk-free for various organisms, and may therefore affect the sustainability of the
338 vineyards. The Cu^{2+} concentrations in about one-quarter of the soils were above the toxicity
339 thresholds reported in the literature and may be toxic for organisms. It is thus important to
340 examine and understand the factors that control Cu availability and potential toxicity.

341

342 **3.3 Diversity of soils in terms of DOM properties**

343 We calculated several spectroscopic indices to assess DOM quality (**Fig. S4**). SUVA_{254} values
344 varied between 5 and 61 $\text{L g C}^{-1} \text{cm}^{-1}$ (**Fig. 1, Table S1**). This range is similar to that reported
345 in other studies on agricultural soils: 6–65 $\text{L g C}^{-1} \text{cm}^{-1}$ (Amery et al. 2007); 27–46 $\text{L g C}^{-1} \text{cm}^{-1}$
346 (Amery et al. 2008); 8–58 $\text{L g C}^{-1} \text{cm}^{-1}$ (Supriatin et al. 2015). DOM is considered to be more
347 aromatic when the SUVA_{254} index is higher (Weishaar et al. 2003). It has been reported that
348 SUVA_{254} values below 30 $\text{L g C}^{-1} \text{cm}^{-1}$ suggest that DOM is composed mainly of hydrophilic
349 substances (Yeh et al. 2014), which is the case of about 80 % of our samples. S_R values varied
350 three-fold between 0.42 and 1.28 (**Fig. 1, Table S1**), which is the same range as those reported
351 by Tang et al. (2019) for Chinese agricultural soils (0.36–1.7). The S_R index is inversely related
352 to molecular size, high S_R values indicate low molecular weight DOM (Helms et al. 2008) while
353 low S_R values indicate high molecular weight and highly condensed organic molecules (Gao et
354 al. 2017).

355 Fluorescence EEM spectra (**Fig. S4**) were acquired to describe heterogeneities in DOM in the
356 samples more precisely by calculating indices such as FI, HIX and BIX. The FI values ranged
357 from 1.10 to 1.34 (median 1.22) (**Fig. 1, Table S1**). FI values are usually used to distinguish

358 sources of DOM. McKnight et al. (2001) found that low FI values (~1.3) correspond to higher
359 plant-derived molecules, while high values (~1.9) indicate microbial or microbially-processed
360 DOM. This suggests that the majority of DOM collected from our samples originated from
361 vascular plants, and that microbial contributions were limited. The BIX index is used to assess
362 the relative production of recent DOM and usually ranges from 0.5 to 1. In the present study,
363 about 75 % of the samples had BIX between 0.6 and 0.7, indicating a relatively low contribution
364 of fresh autochthonous DOM substances (**Table S1**), while the other 25 % had BIX between
365 0.7 and 0.9, suggesting fresh DOM production by organisms (Huguet et al. 2009; He et al.
366 2016). In our samples the HIX values were contrasted, with almost 10-fold variation from 1.31
367 to 10.33 (median 5.75) (**Fig. 1, Table S1**). Two of the soils (D and L) were characterised by
368 very low HIX values (**Table S1**) due to the presence of tyrosine-like fluorescence at an
369 excitation wavelength of 240-260 nm and emission of 300-340 nm (**Fig. S4a**). This tyrosine-
370 like fluorescence band was absent from all other EEM spectra that were similar to that of soil
371 B (**Fig. S4b**). HIX stands for degree of humification or condensation of DOM (Zsolnay et al.
372 1999), but has also been used to characterise DOM aromaticity (Ohno et al. 2007). Our HIX
373 results are in the same range as those reported by Daouk et al. (2015) for Swiss vineyard soils
374 (0.1-7.0) and as those reported by Tang et al. (2019) (2 to 10). However, HIX values varying
375 from 10 to 30 have been reported in several arable soils (Kalbitz et al. 2003). Compared to the
376 latter range, our soils had low HIX values and can be characterized as weakly-humified.

377 **3.4 Relationships between the variables**

378 We performed a principal component analysis (PCA) of all 18 soil extracts to describe the
379 relationships between the solution variables and to identify homogeneous groups. 64% of total
380 variance is explained by the first two principal components (**Fig. 2a**). The first principal
381 component revealed 44.5 % variance of data and was positively associated with Cu_{KCl} , Cu^{2+} ,
382 Fe concentrations, and SUVA_{254} , and was negatively associated with pH_{water} , pH_{KCl} and HIX.

383 The second principal component explained 19.2 % of variance and was positively associated
384 with C_{org} , the C:N ratio, and negatively associated with FI and BIX. Two groups can be
385 distinguished on the principal plan plot (**Fig. 2b**): group 1 is composed of the eight most alkaline
386 soils ($7.8 < pH_{water} < 8.3$), while group 2 is composed of the 10 most acidic soils ($6.3 < pH_{water}$
387 < 7.5). The differences between the groups are mainly explained by the pH_{water} values (PCA
388 correlation coefficient with Component 1: -0.92; with Component 2: 0.03). On the first axis,
389 the soils in group 1 share high values of pH and HIX, and low values of Cu_{KCl} , Fe, and Cu^{2+} .
390 The opposite trend was observed in group 2. On the second axis, soils in group 2 have more
391 contrasted FI and BIX than those in group 1. Indeed, in group 1, FI values ranged from 1.20 to
392 1.31; and BIX values from 0.67 to 0.75. Comparatively, these indices show a larger range in
393 group 2, with 1.10 - 1.34 for FI and 0.59 - 0.86 for BIX. Group 1 is composed of alkaline soils
394 with low Cu concentrations in solution and relatively highly-humified DOM, while group 2 is
395 composed of more acidic soils with high concentrations of Cu_{KCl} and Cu^{2+} , and contrasted
396 contributions of microbial DOM.

397 Pearson's correlation coefficients were analysed to describe the links between the variables
398 measured in the soils and in the extracts (**Fig. 3**). pH_{water} and pH_{KCl} were strongly and positively
399 correlated ($r=0.94$, $p < 0.01$). Total dissolved Fe and Cu concentrations were negatively
400 correlated with pH (**Fig. 3**), with $r=0.77$, 0.73 ($p < 0.01$), respectively. **Fig. 4a** reports total Cu
401 in extracts as a function of total Cu in the soils. We found no immediate relationship between
402 Cu availability and the level of contamination. Cu_{KCl} concentrations globally increased with
403 decreasing soil pH (**Fig. 4b**). This result confirms the fact that metals are more soluble in acidic
404 soils, as reported by McBride et al. (1997). None of the Fe and Cu concentrations were
405 significantly correlated with DOC (**Fig. 3**). Cu^{2+} was strongly linked to Cu_{KCl} ($r=0.82$, $p < 0.01$)
406 (**Fig. 3, Fig. 4c**), showing that Cu solubilisation affects its concentration of free ions. A slightly
407 positive relationship was found between pH_{KCl} and HIX (**Fig. 3**), suggesting that the most

408 humified DOM was present in the most alkaline samples, as confirmed by the results of the
409 PCA.

410 Cu^{2+} concentrations and $f\text{Cu}^{2+}$ were strongly linked to the pH of the extracts, with correlation
411 coefficients of -0.94 and -0.91 respectively (**Fig. 3, Fig. 4d**), suggesting that Cu^{2+} decreased
412 with increasing pH. The strong relationship between pH and Cu^{2+} has already been reported
413 (McBride and Blasiak 1979, Sauvé et al. 1995, Laurent et al. 2020), confirming that pH is the
414 main factor controlling Cu speciation in solution. When pH increases, DOM is more negatively
415 charged due to the deprotonation of its functional groups. This promotes its complexation with
416 Cu, leading to a drastic decrease in Cu^{2+} activity in solution (Bravin et al. 2009; Ren et al. 2015).
417 Conversely, at low pH, competition for DOM binding sites is high between Cu^{2+} and H^+ , which
418 promotes increasing Cu^{2+} activity. A pH above 6.5 seems to be the safety threshold for the soils
419 in our collection in terms of Cu toxicity when Cu^{2+} is used as a proxy (**Fig. 4d**).

420 No tangible relationship was found between C_{org} values and DOC concentrations in the extracts
421 (**Fig. 3**), meaning that the solubilisation rates of C_{org} may differ with the soil conditions or
422 properties. None of DOM optical indices was significantly linked to DOC concentrations in the
423 KCl extracts, indicating that DOM quality was not related to the quantity of DOM in solution.
424 No significant relationship was found between the S_R and the SUVA_{254} indices. SUVA_{254} and
425 HIX indices were negatively linked in this study ($r=-0.71$, $p < 0.01$) (**Fig. 3**). This negative
426 relationship is all the more surprising as these indices are both related to DOM aromaticity. In
427 the literature, SUVA_{254} and HIX are generally positively correlated (He et al. 2016; Tang et al.
428 2019). However, such unexpected differences between these two indices have already been
429 observed (Inamdar et al. 2011; Broder et al. 2017). Considering the differences in pH between
430 soils, and given that fluorescence is pH-dependent, one may wonder if the variations observed
431 in these two indices are not affected by the effect of pH on spectroscopic analyses. However,
432 an impact on fluorescence analyses is unlikely in our range of soil pH, and would, in any case,

433 be less than differences due to the sources and composition of DOM (Hudson et al. 2007). The
434 presence of protein-like fluorescence was observed in EEM spectra of only two soils (D and L)
435 and cannot explain the negative correlation between HIX and SUVA₂₅₄. Moreover, it has been
436 reported that SUVA₂₅₄ measurements may be subject to some bias since Fe, colloids, and other
437 constituents also absorb at 254 nm (Weishaar et al. 2003; Yang and Hur 2014). Since there were
438 huge differences in Fe concentrations among our samples (i.e., varying by a factor of 12, **Table**
439 **S1**), the SUVA₂₅₄ results in this study should be interpreted with caution. In a collection of
440 samples with such contrasted concentrations of dissolved Fe as those measured in the present
441 study, it would therefore not be appropriate to use the SUVA₂₅₄ index to assess DOM
442 aromaticity, and HIX appears to be a more reliable tool for that purpose. No significant
443 relationship was found between HIX and S_R, suggesting that, in our soil extracts, DOM
444 condensation may not be directly related to its molecular size. Moreover, the negative
445 correlation between HIX and BIX (**Fig. 4b**) ($r = -0.82$, $p < 0.01$) indicates that the most aromatic
446 DOM is associated with older molecules derived from higher plants (Tang et al. 2019), which
447 is quite consistent. No significant link was found between HIX and FI. Nevertheless, the
448 positive relationship between BIX and FI ($r = 0.63$, $p < 0.01$) was not surprising and suggested
449 that the soils with DOM originating from a microbial source were related to the highest
450 autochthonous contributions, as reported in other works (Tang et al. 2019; Musadji et al. 2020).

451 Overall, these results indicate that the 18 soils sampled in our study differed in DOM quality
452 even though they had similar concentrations of DOC. Given the significant affinity of Cu for
453 DOM, these differences in DOM quality are expected to affect the geochemistry of Cu in
454 solution.

455 **3.5 Prediction of Cu availability using the properties of the soils and the extracts**

456 The risk of Cu environmental toxicity could be better assessed if its availability could be
457 predicted using the variables measured in the soil and in the soil solution. However, organisms

458 often use mechanisms to resist the stress caused by Cu toxicity. For instance, some plants take
459 up and sequester Cu in cells where it cannot cause harm (Küpper et al. 2009). Nevertheless,
460 as Cu environmental availability is linked to its total (Cu_{KCl}) and ionic (Cu^{2+}) concentrations in
461 soil solutions, predicting these concentrations could enable more accurate evaluation of
462 potential Cu toxicity to a certain extent (see **Section 3.2**). **Table 3** shows the best models
463 calculated to predict Cu_{KCl} and Cu^{2+} among the 18 vineyard soils sampled in this study.

464

465 **3.5.1. Prediction of total Cu in extracts Cu_{KCl}**

466 Among the one-variable models tested to predict Cu_{KCl} , the model with pH_{water} (a) showed the
467 best goodness of fit ($R^2_{adj} = 0.575$, **Table 3**). This suggests that pH is the main factor driving
468 Cu solubilisation in the soils sampled in this study. However, this model showed marked
469 dispersion of points around the 1:1 line (**Fig. 5a**), more visible in soils in group 1 than in soils
470 in group 2. The prediction was better when Cu_T was added to pH (model (b)), the R^2_{adj} value
471 raised to 0.71 and the RMSE decreased from 0.28 to 0.23 (**Table 3**). The points were then less
472 dispersed than with the preceding model, except in soils in group 2 (**Fig. 5b**). The respective
473 coefficients of pH and Cu_T are suitable for the presumed mechanisms. Indeed, it has been
474 reported that metal desorption increases with a decrease in pH, which is why metals tend to be
475 more soluble in acidic environments (McBride and Blasiak 1979; Degryse et al. 2009).
476 Moreover, at higher pH, Cu oxyhydroxides or carbonates may form and precipitate thereby
477 reducing the Cu in solution (McBride 1989). As expected, the model showed that the higher the
478 Cu_T , the higher the concentration of soluble Cu in solution (Cu_{KCl}). Sauv e et al. (1997)
479 underlined the importance of pH and Cu_T in Cu solubilisation, but in the reverse hierarchy.
480 These authors found that in 66 soils with diverse sources of Cu contamination, 90 % of variation
481 in soluble Cu was explained by total Cu in the soils and the importance of pH was minimal.

482 This could be explained by the wider range of Cu_T in their study (14–3000 mg kg⁻¹) which
483 reinforced the contribution of Cu_T in predicting soluble Cu.

484 The concentration of DOC did not appear in the best models, presumably because of its limited
485 variability in our initial dataset. However, Cu_{KCl} predictability was dramatically improved when
486 DOM quality parameters were added as predictors in addition to pH and Cu_T . This suggests that
487 in the present study, Cu availability depended more on DOM characteristics than on its
488 concentration. Among all the DOM indices tested in the regressions, only HIX and FI emerged
489 significantly, and the best prediction was obtained by equation (c) (**Table 3, Fig. 5c**), which
490 explained 83.2 % of variance, showing good accuracy of this model for the prediction of Cu_{KCl} .
491 Compared to model (b), model (c) produced lower RMSE (0.17) and a slope closer to 1 (0.85)
492 (**Fig. 5c**). The contributions of pH_{water} , Cu_T , FI and HIX were all significant in the model ($p <$
493 0.001) (**Table 3**). The variance inflation factor (VIF) did not identify correlations between the
494 predictors ($VIF < 2.3$). HIX and FI were both expressed with negative coefficients in the model,
495 suggesting that the solubilisation of Cu is enhanced with lowly-condensed plant-derived DOM
496 molecules. This suggests that, in the vineyard soils studied here, molecules with low aromaticity
497 have the most affinity for Cu for its solubilisation. This finding contradicts previous studies
498 suggesting that the most aromatic DOM had higher affinity for Cu and promoted its
499 solubilisation through complexation (Amery et al. 2007; Amery et al. 2008; Araújo et al. 2019).
500 In the present study, DOM composed of more hydrophilic molecules may have higher Cu-
501 binding capacity because of its higher proportion of O-containing functional groups (hydroxyl,
502 carboxylic acid, sulfonic acid and amine) than aromatic and hydrophobic substances (Baham et
503 al. 1978; Leenheer 1981). Moreover, the negative coefficient of FI in the model suggests a
504 higher plant origin for the DOM components that bind Cu. Our results are consistent with those
505 of He et al. (2016), who found higher concentrations of heavy metals in sediment solutions with
506 lower aromatic DOM molecules, which the authors linked to amino acids. Moreover, it has

507 previously been reported that the ability of DOM hydrophilic and low aromatic components to
508 transport Cu is greater than that of hydrophobic molecules (Han and Thompson 1999; 2003).
509 These authors explained that low aromatic and hydrophilic molecules were more mobile, while
510 aromatic and complex components tended to be adsorbed on soil particles.

511 **3.5.2. Prediction of free ionic Cu²⁺**

512 Among the models with just one predictor, the one with pH_{KCl} (model (d)) appeared to be the
513 most effective and explained 88.3 % of Cu²⁺ variance (**Table 3**). The RMSE value was low
514 (0.4), implying good accuracy of the prediction (**Fig. 5d**). The high R²_{adj} (0.88) of model (d)
515 highlights the importance of pH in predicting Cu²⁺ concentrations.

516 We hypothesised that free Cu²⁺ may be linked to the solubility of Cu, as we found a positive
517 relationship between Cu²⁺ and Cu_{KCl} (**Fig. 4c**). When adding Cu_{KCl} to pH_{KCl} as predictors of
518 Cu²⁺ (model (e)), the R²_{adj} increased from 0.88 to 0.91. This slight improvement can be observed
519 in **Fig. 5e**. The corresponding VIF was the maximum receivable (2.3), certainly due to the
520 relative effect of pH on Cu solubilisation. The contributions of pH_{KCl} and Cu_{KCl} were all
521 significant ($p < 0.001$) in explaining Cu²⁺ variability. The coefficients in the model suggest that
522 Cu²⁺ increases with Cu_{KCl}, and decreasing pH. Compared to model (d), model (e) was more
523 accurate with a lower RMSE (0.34 instead of 0.4) (**Table 3**).

524 BIX was the DOM index that appeared in the best three-variables model (model (f), **Table 3**).
525 Models with more than three variables did not present any statistical improvement (data not
526 shown). In model (f), adding BIX to pH_{KCl} and Cu_{KCl} did not enhance the effectiveness of the
527 prediction compared to model (e) (**Fig. 5f**), with similar R²_{adj} and RMSE (0.92 and 0.32
528 respectively) (**Table 3**). Even though the contribution of BIX to the model was significant ($p <$
529 0.05), the addition of BIX was not needed to explain the variability of our collection of samples.
530 Moreover, the max VIF of model (f) (2.44) was higher than the fixed threshold of 2.3,

531 illustrating multicollinearity between the predictors that probably weakens the stability of the
532 prediction. Taken together, these results suggest that including DOM indices does not improve
533 the modelling of Cu^{2+} and that only pH_{KCl} and Cu_{KCl} were needed.

534 In the literature, pH is widely reported to play an important role explaining free ionic Cu^{2+}
535 activities and the log-log relationship between these two variables is usually linear (McBride
536 and Blasiak 1979; Sauvé et al. 1995; McBride et al. 1997; Sauvé et al. 1997; Bravin et al. 2009).
537 Sauvé et al. (1997) proposed the following equation to predict Cu^{2+} activity in CaCl_2 extracts
538 of a collection of wide Cu-contaminated soils: $\text{pCu} = -1.7 \log_{10}\text{Cu}_T + 1.4 \text{pH} + 3.4$. In contrast
539 to our study, the OM parameters (concentrations and quality) were not included in the model
540 calculations and the authors underlined the importance of the degree of Cu contamination in
541 predicting Cu^{2+} . However, interestingly, the pH coefficient in our study (model (e)) is consistent
542 with the results of McBride (1989) who reported that slopes for $\log\text{Cu}^{2+}$ - pH plots are generally
543 lower than 2. This suggests that pH plays a constant role in Cu^{2+} prediction. But the optical
544 parameters describing the quality of DOM in soil solutions were of no use in explaining the
545 concentrations of Cu^{2+} .

546 **3.6. Influence of DOC composition on Cu speciation**

547 We calculated Cu speciation in solution based on thermodynamic models and on several
548 hypotheses concerning DOM composition. We found that considering DOM as 100 % HA led
549 to underprediction of free Cu^{2+} compared to experimental observations in all samples (**Fig. S5**).
550 Considering all DOM as FA produced better results, but even using only 65 % of active DOM
551 as FA (a widespread assumption in similar studies) resulted in marked discrepancies between
552 model and experimental results. This observation - made here using the NICA-Donnan model
553 to describe ion binding to DOM - is in agreement with previous estimations made using the
554 Windermere humic aqueous model (WHAM) on a large set of soil solutions (Djae et al. 2017).

555 For the model results to fit the experimental observations, the percentage of active DOM,
556 rDOM, was adjusted separately for each sample. The values of rDOM obtained ranged between
557 0.10 and 1.68, reflecting the wide variability of the active pool within DOM. This is consistent
558 with the physically meaningful range of 35-215 % estimated by Djae et al. (2017). The
559 variations in rDOM were compared to the variations in the chemical and optical parameters,
560 but no multilinear regression model could be established (data not shown).

561 The amount of Cu bound to carboxylic or phenolic groups of DOM can be estimated using
562 thermodynamic modelling. Results show that binding to phenolic groups becomes predominant
563 above pH 7.4 (**Fig. S6**). This suggests that these DOM binding groups, deprotonated at alkaline
564 pH, have a very high affinity for Cu. Thus, in alkaline soils, the nature of DOM binding sites
565 will be important in controlling Cu speciation.

566

567 **4. Conclusions**

568 The main goal of this work was to assess the contribution of DOM spectroscopic indices to
569 predicting two Cu availability proxies in 18 organic vineyard soils. Our results showed that in
570 the KCl extracts, the total concentration of Cu varied 23-fold while that of free ionic Cu^{2+} varied
571 4,600-fold among soils, pointing to wide diversity of soils in terms of Cu availability. Four soils
572 had concentrations of Cu^{2+} higher than the toxicity levels for organisms reported in the
573 literature. These samples were considered as potentially harmful for vineyard soil organisms.

574 Our results also showed that Cu environmental availability was weakly related to the level of
575 Cu contamination in the soils. pH was the main factor controlling Cu environmental
576 availability, as it was closely and positively correlated with the concentrations of total Cu and
577 free ionic Cu^{2+} measured in KCl extracts. Thus, in theory, toxicity problems can occur even in
578 moderately Cu-contaminated soils when the soils are particularly acidic. It would thus be safer
579 to maintain the pH above 6.5. To this end, liming is possible but should be applied with caution,

580 so as not to jeopardise plant uptake of other nutrients like Fe, whose availability is low at
581 alkaline pH.

582 This work highlighted the fact that even though the DOC concentrations were similar among
583 the soils, noticeable differences were found in DOM quality, as assessed by optical
584 spectroscopy. Absorbance and fluorescence analyses are thus appropriate to identify these
585 differences in chromophoric and fluorescent DOM in soils.

586 The prediction of total Cu in KCl extracts was clearly improved when DOM spectral indices
587 (HIX and FI) were taken into account, in addition to pH and Cu_T . This study thus emphasizes
588 that the quality of DOM affects the availability of Cu in the soil more than its quantity. Although
589 this finding now needs to be tested on a wider dataset (with different sources of contamination),
590 it suggests that organic management in Cu-contaminated vineyards needs to be redesigned, to
591 better control quality of dissolved organic matter production as a function of the nature of
592 organic matter amendments.

593

594 **Declarations**

595 **Ethics approval and consent to participate**

596 Not applicable

597 **Consent for publication**

598 Not applicable

599 **Availability of data and materials**

600 All data generated or analysed during this study are included in this published article and its
601 supplementary information files.

602 **Competing interests**

603 The authors declare that they have no competing interests.

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609 **Authors' contributions**

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612 Noémie Janot: Formal analysis, Supervision, Writing-review & editing

613 Christophe Nguyen: Formal analysis, Writing-review & editing

614 Mahaut Sourzac: Investigation

615 Edith Parlanti: Conceptualization, Funding acquisition, Supervision, Writing-original draft

616 Laurence Denaix: Conceptualization, Funding acquisition, Supervision, Writing-original draft

617 All authors read and approved the final manuscript.

618

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Figure 1

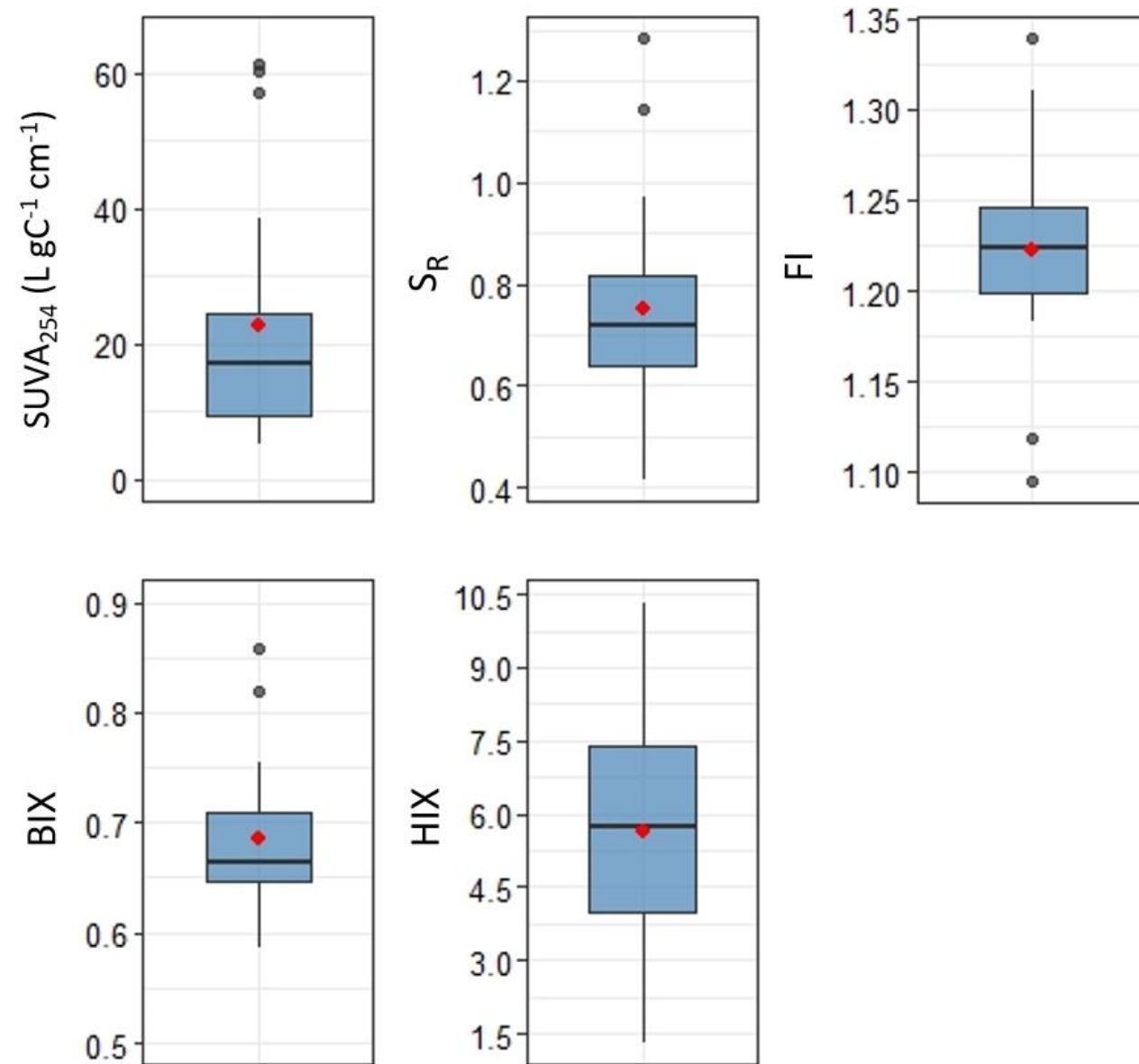


Figure 2

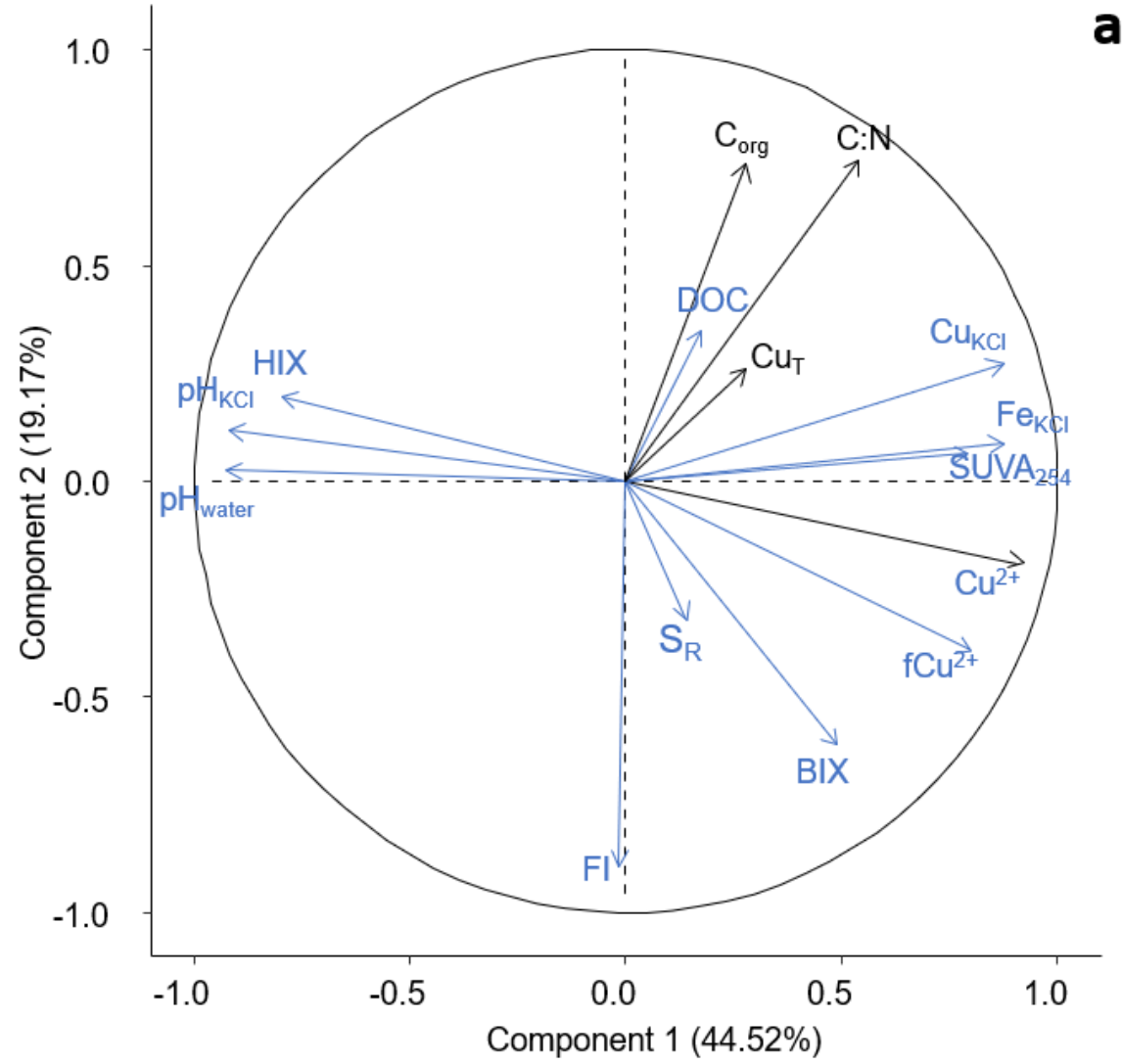


Figure 2

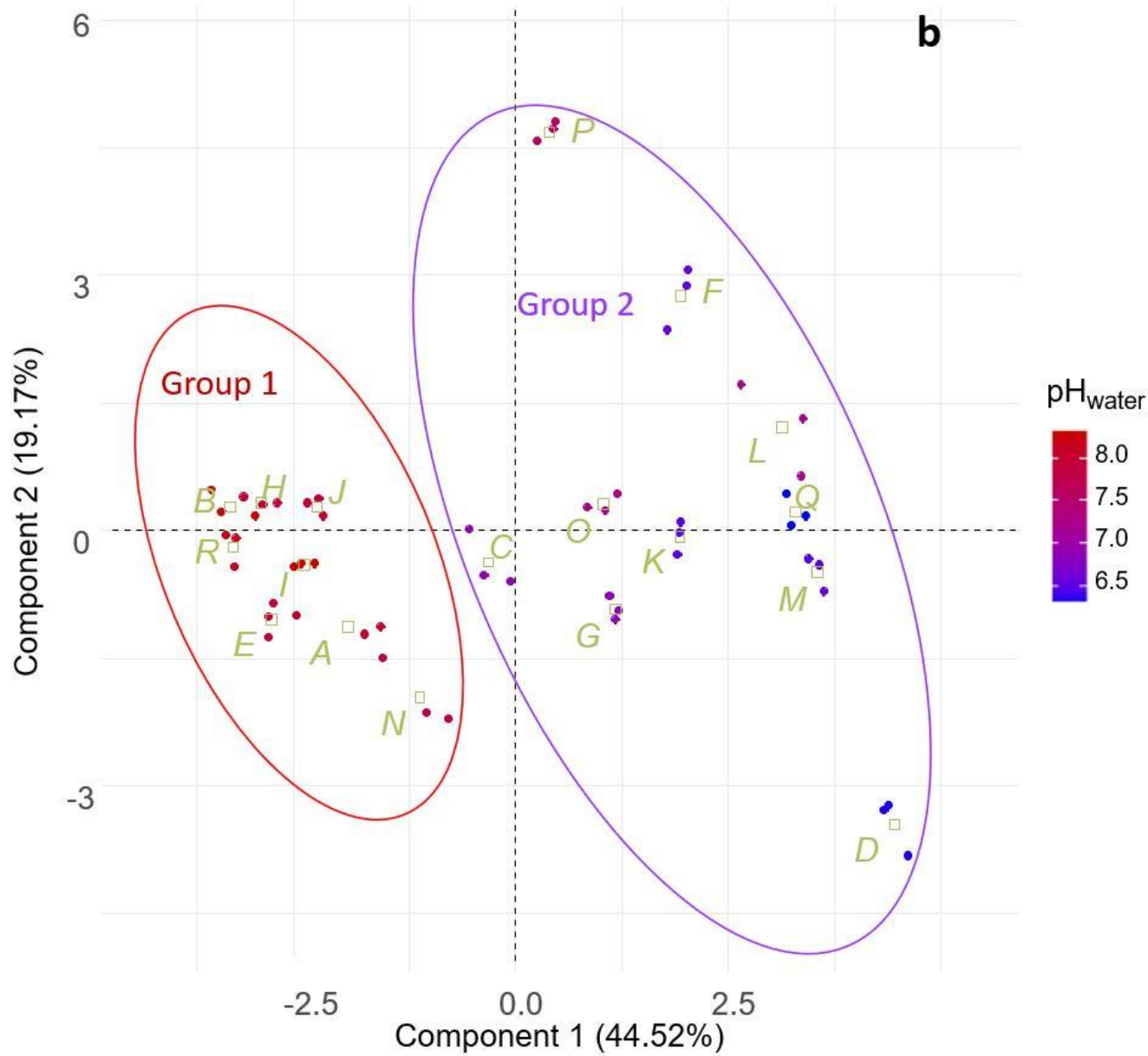


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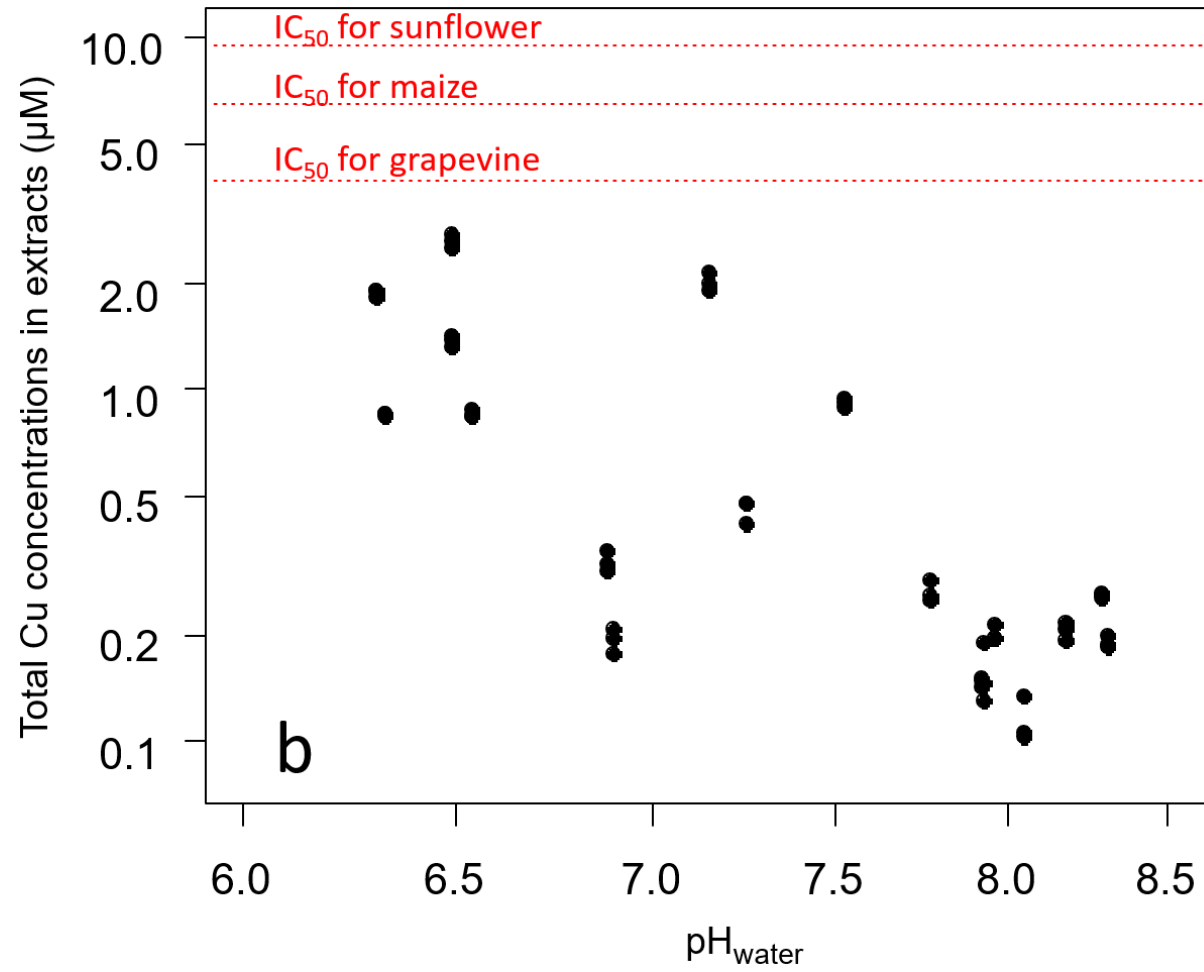
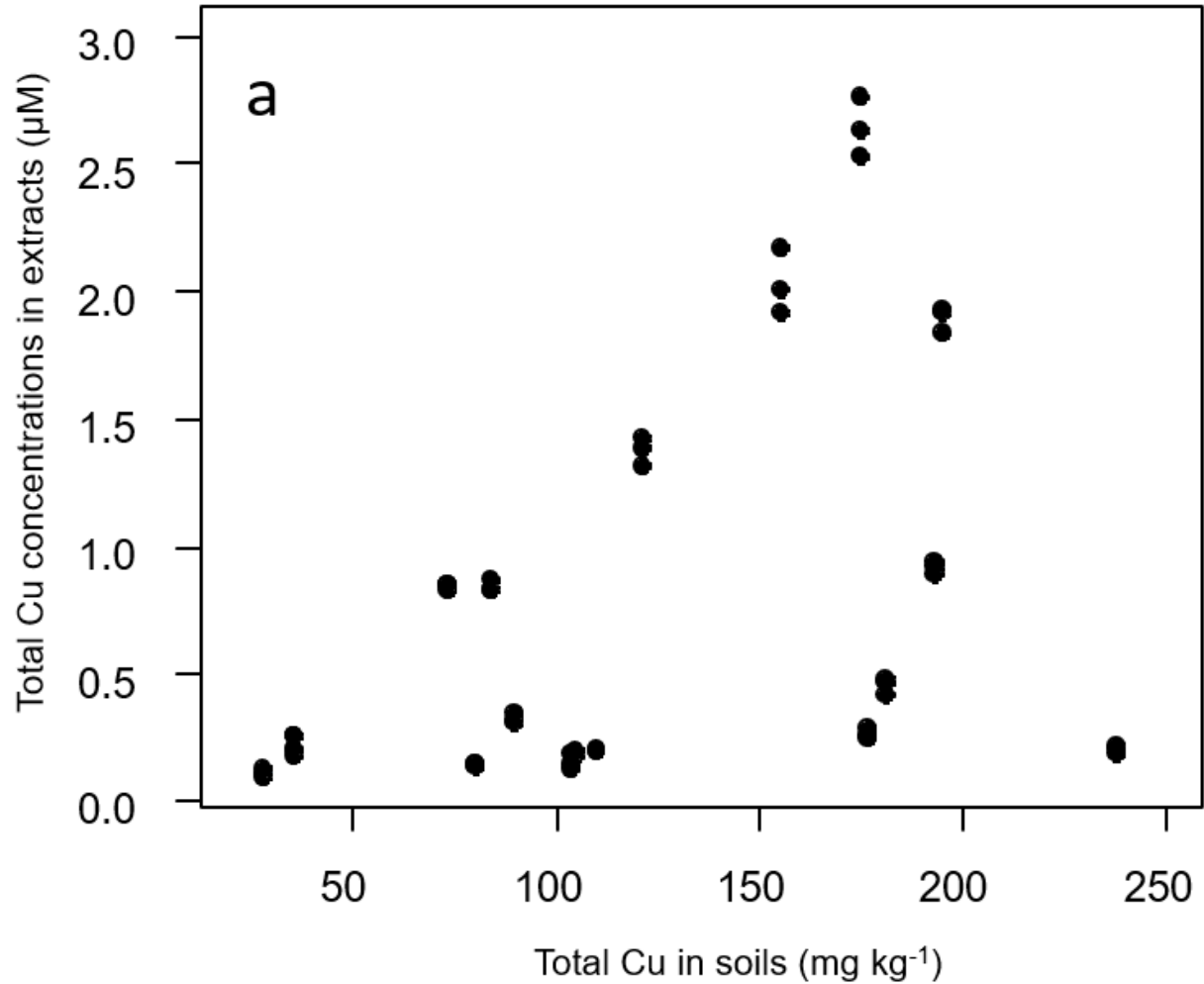


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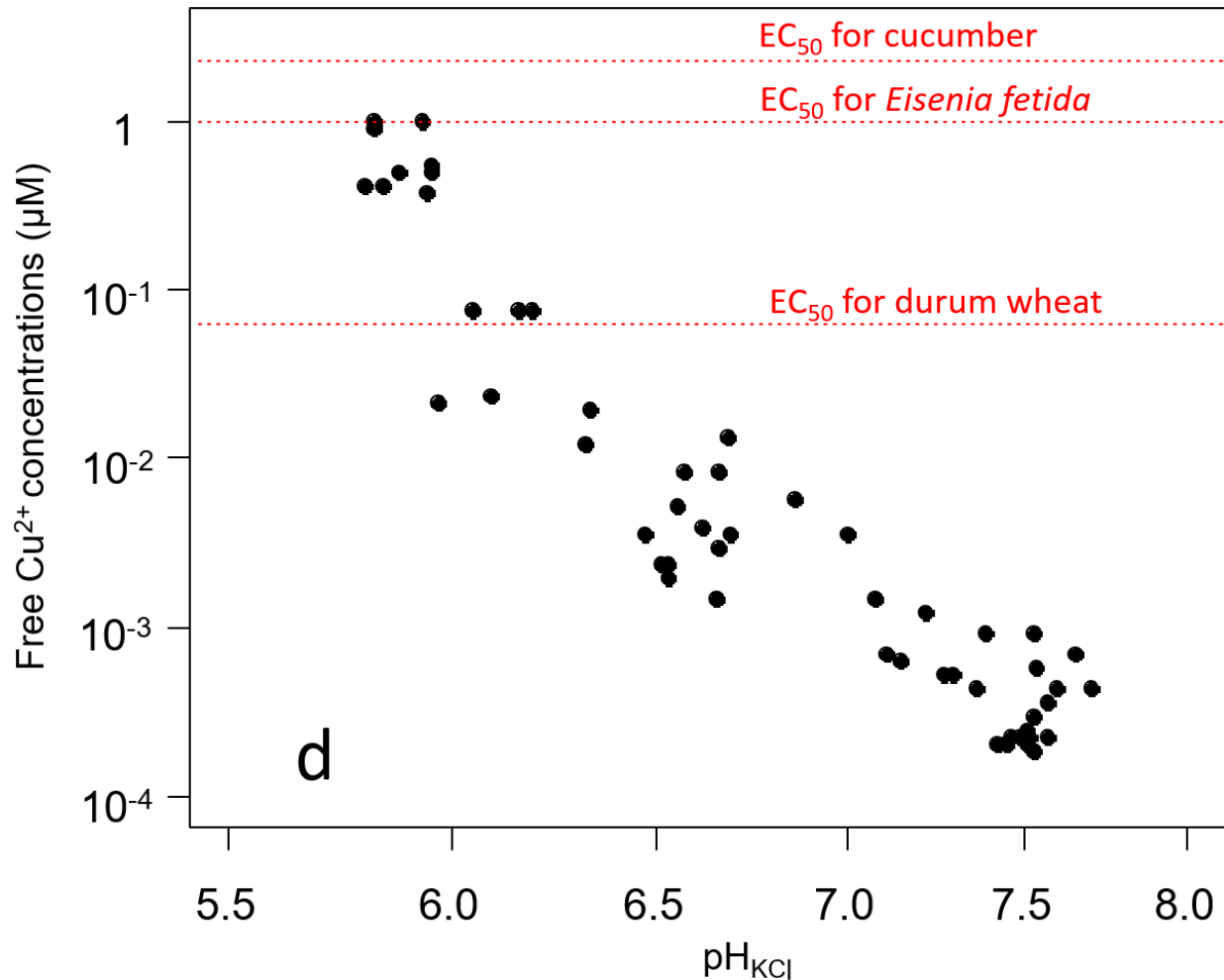
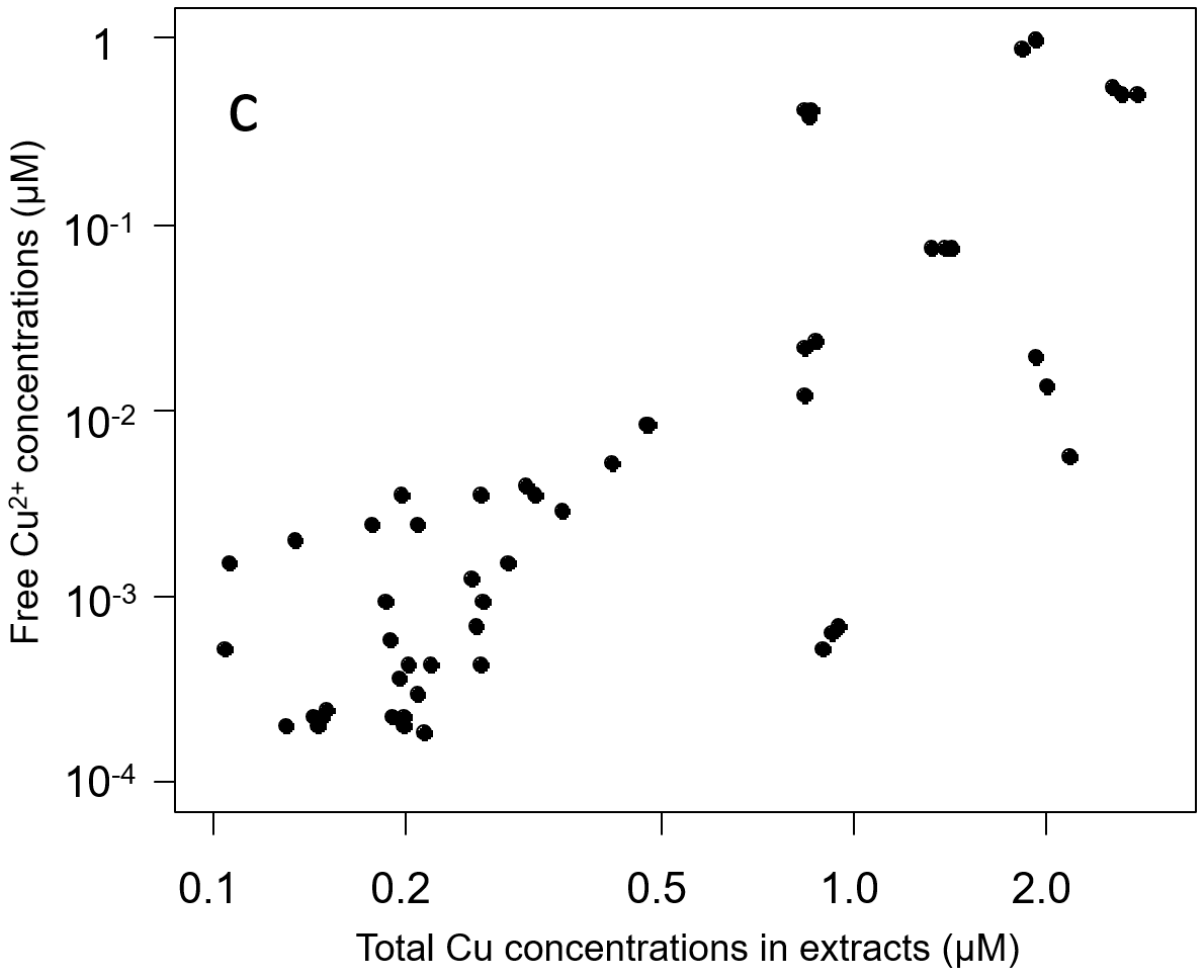


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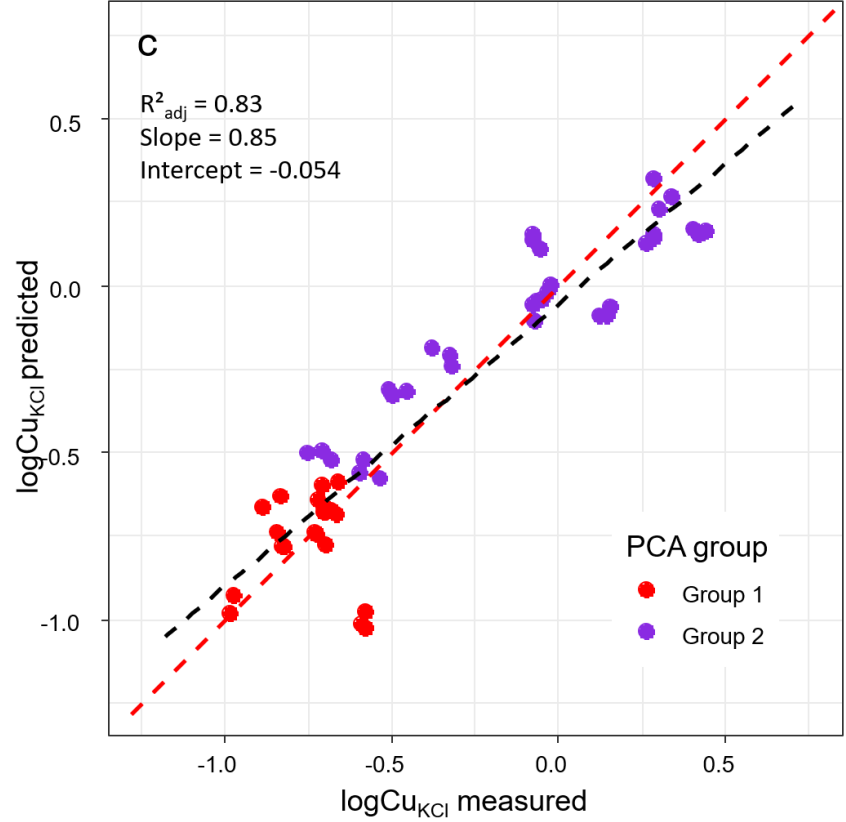
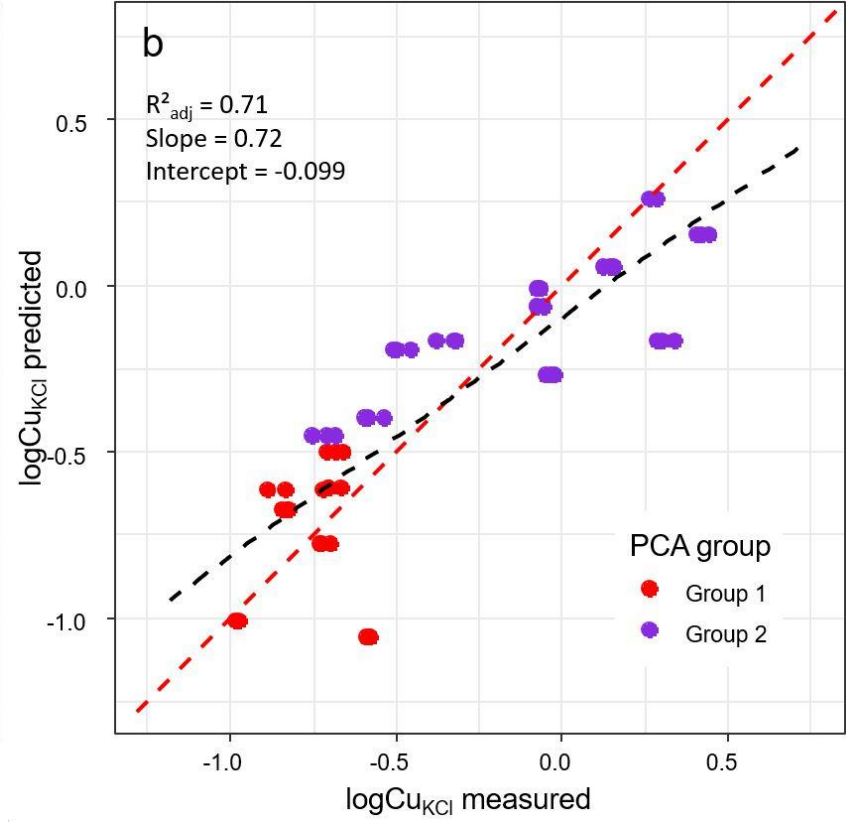
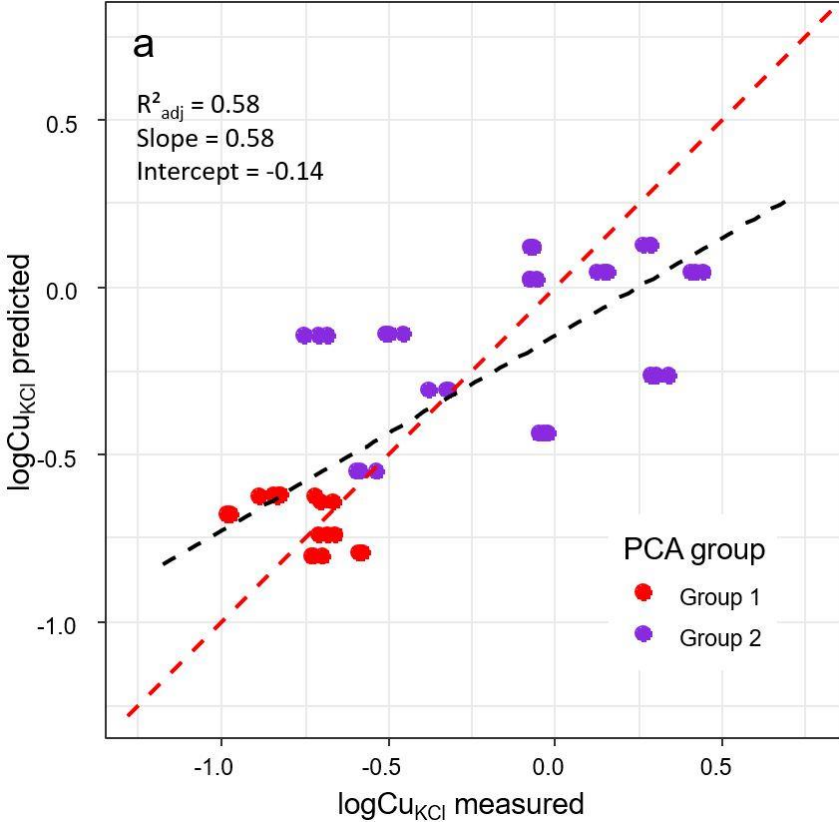


Figure 5

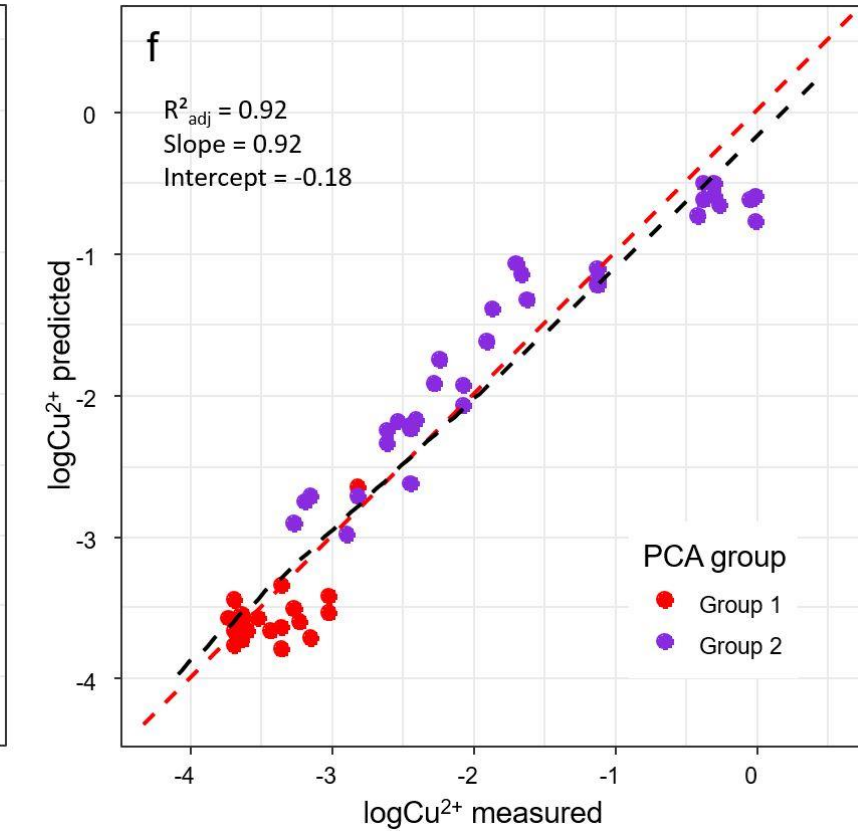
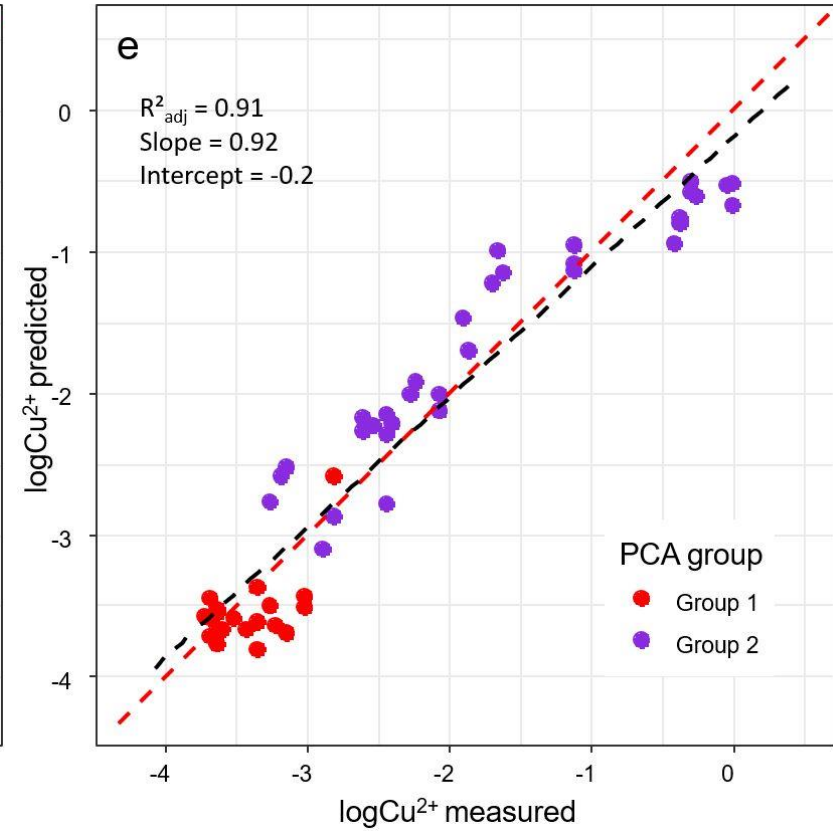
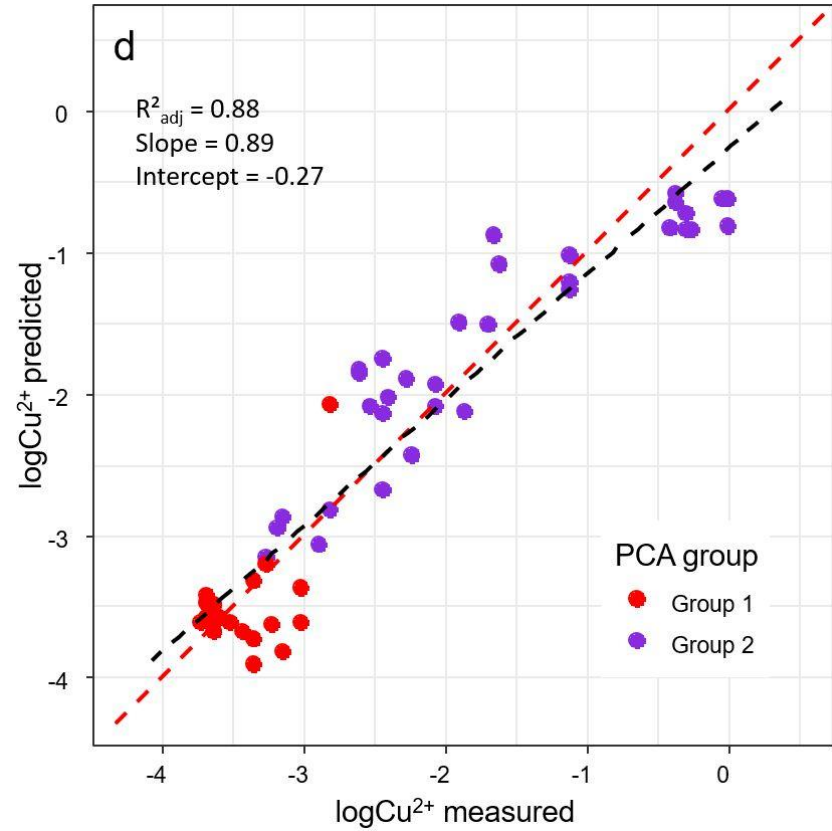


Figure captions

Fig. 1: Distribution of DOM optical parameters (mean of replicates), the red points represent the mean values of all the soils

Fig. 2: Principal component analysis (PCA) of soil solution chemistry and Cu availability proxies in the dataset. All the variables except pH_{water} and pH_{KCl} were log-transformed. The correlation plot (a) shows the contribution of each variable to the two first principal components. The soil variables are shown in black, the extract variables in blue. Soil samples (grouped in triplicate) are coloured according to their pH_{water} values (b)

Fig. 3: Correlation matrix between soil properties (pH_{water} , C_{org} , C:N, Cu_{T}), solution parameters: pH of the extracts (pH_{KCl}), free ionic concentrations (Cu^{2+}), free ionic fractions ($f\text{Cu}^{2+}$), total dissolved metals (Cu_{KCl} , Fe_{KCl}) and DOM properties (DOC, HIX, BIX, FI, S_{R} , SUVA_{254}). The colored boxes represent significant correlations ($p < 0.01$)

Fig. 4: Total Cu concentrations in extracts (Cu_{KCl}) as a function of total Cu in soils Cu_{T} (a) and pH of the soils (pH_{water}) (b); free ionic Cu concentrations (Cu^{2+}) as a function of total Cu concentrations in extracts (c) and pH of the extracts (pH_{KCl}) (d). The log-log plot is shown in (c) and only the y-axis is log-transformed in (b) and (d)

Fig. 5: Comparison of the multilinear models predicting Cu_{KCl} (a, b, c) and Cu^{2+} concentrations (d, e, f) in the 0.01 M KCl extracts. The dashed red line represents the 1:1 line, the dashed black line represents the regression line

Tables

Table 1: Chemical properties of the 18 vineyard soils analysed in this study. C_{org} stands for organic carbon, Cu_T for total Cu. Carbonate values <1 were not quantifiable

Soil id	AOC Zone	Soil classification (WRB)	Cu _T	pH _{water}	CaCO ₃	C _{org}	C:N
			mg kg ⁻¹		g kg ⁻¹	g kg ⁻¹	
A	Blaye Côtes de Bordeaux	Cambisol	28	8.1	< 1	9.9	10.6
B	Blaye Côtes de Bordeaux	Cambisol	35	8.3	27.6	13.7	10.9
C	Blaye Côtes de Bordeaux	Luvic Cambisol	35	6.9	< 1	10.2	11.4
D	Bordeaux supérieur	Luvisol	73	6.3	< 1	10.5	11.5
E	Blaye Côtes de Bordeaux	Cambisol	80	7.9	< 1	8.3	10.7
F	Graves	Podzosol	84	6.5	< 1	16.9	15.3
G	Bordeaux supérieur	Luvisol	89	6.9	< 1	19.2	11.7
H	Bordeaux supérieur	Luvisol	103	7.9	3.2	12.6	10.6
I	Graves	Cambisol	104	8.3	19.5	7.5	11.5
J	Blaye Côtes de Bordeaux	Luvisol	110	8	1.1	11.3	11.6
K	Blaye Côtes de Bordeaux	Podzosol	121	6.5	< 1	11.8	13.0
L	Graves	Leptosol	155	7.1	< 1	14.6	15.5
M	Blaye Côtes de Bordeaux	Luvisol	175	6.5	< 1	9.3	11.9
N	Blaye Côtes de Bordeaux	Luvisol	176	7.8	< 1	5.4	11.7
O	Blaye Côtes de Bordeaux	Luvisol	181	7.3	< 1	15.6	12.6
P	Graves	Leptosol	193	7.5	< 1	30.8	17.4

Q	Blaye Côtes de Bordeaux	Luvisol	195	6.3	< 1	13.3	12.6
R	Blaye Côtes de Bordeaux	Cambisol	238	8.2	30.4	10.6	10.3

Table 2: Values of total Cu in solution and Cu²⁺ reported as toxicity thresholds in the literature, compared with the maximum values found in the present study

	Toxicity thresholds	Values (μM)	References
	Max value in this study	2.64	
Total Cu in solution (Cu _{KCl} in this study)	IC ₅₀ grapevine	3.94	Juang et al. 2012
	IC ₅₀ maize	6.50	Ouzounidou 1995
	IC ₅₀ sunflower	9.55	Kolbas et al. 2014
	Max value in this study	0.94	
Free ionic Cu ²⁺	EC ₅₀ durum wheat	0.06	Bravin et al. 2010
	EC ₅₀ cucumber	2.20	Kader et al. 2016
	EC ₅₀ <i>Eisenia fetida</i>	1.00	Qiu et al. 2013

Table 3: Best models for predicting Cu_{KCl} and Cu^{2+} in KCl extracts and associated statistics.

R^2_{adj} : adjusted r-squared, RMSE: root mean square error, max VIF: maximum value of the variance inflation factor, N: number of observations

Statistical significance: *** for p-value < 0.001, ** for p-value < 0.01, * for p-value < 0.05

	Models	R^2_{adj}	max VIF	RMSE (μM)	N
$\log_{10}\text{Cu}_{\text{KCl}} =$	$-0.465^{***} \text{pH}_{\text{water}} + 3.066$ (a)	0.575	-	0.28	53
	$-0.434^{***} \text{pH}_{\text{water}} + 0.615^{***} \log_{10}\text{Cu}_{\text{T}} + 1.598$ (b)	0.707	1.02	0.23	53
	$-0.312^{***} \text{pH}_{\text{water}} + 0.443^{***} \log_{10}\text{Cu}_{\text{T}} - 7.362^{***} \log_{10}\text{FI}$	0.832	1.91	0.17	53
	$-0.664^{***} \log_{10}\text{HIX} + 2.148$ (c)				
$\log_{10}\text{Cu}^{2+} =$	$-1.751^{***} \text{pH}_{\text{KCl}} + 9.589$ (d)	0.883	-	0.40	53
	$-1.380^{***} \text{pH}_{\text{KCl}} + 0.731^{***} \log_{10}\text{Cu}_{\text{KCl}} - 7.307$ (e)	0.912	2.30	0.34	53
	$-1.316^{***} \text{pH}_{\text{KCl}} + 0.73^{***} \log_{10}\text{Cu}_{\text{KCl}} + 2.818 \log_{10}\text{BIX} + 7.338$ (f)	0.919	2.44	0.32	53