ECOTOX, AQUATIC AND TERRESTRIAL ECOTOXICOLOGY CONSIDERING THE SOIL: WATER CONTINUUM IN THE ANTHROPOCENE CONTEXT

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

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Abstract

Accumulation of copper (Cu) in soils due to the application of fungicides may be toxic for organisms and hence affect winegrowing sustainability. Soil parameters such as pH and dissolved organic matter (DOM) are known to affect the availability of Cu. In this study, we investigated the contribution of chromophoric and fluorescent DOM properties to the prediction of Cu availability in 18 organic vineyard soils in the Bordeaux winegrowing area (France). The DOM parameters, assessed through absorbance and fluorescence analyses, and proxies for Cu availability (total soluble Cu and free ionic Cu^{2+}) were measured in 0.01 M KCl extracts. Total soluble Cu (Cu_{KCl}) varied 23-fold while free ionic Cu^{2+} varied by a factor of 4600 among the soils. DOC concentrations were similar among the soils, but the samples differed in the quality of DOM as assessed by optical spectroscopy. Multilinear regression models with and without DOM quality parameters were investigated to predict Cu availability. The best model for Cu_{KCl} successfully explained 83% of variance and included pH, Cu_T, and two DOM fluorescence quality indices, the FI fluorescence index, which distinguishes between microbial and higher plant origins, and the HIX humification index. For the prediction of Cu^{2+}, pH alone explained 88% of variance and adding DOM parameters did not improve modelling. The two Cu availability proxies were related to pH. This study confirms the prominent role of pH in Cu availability and underlines the importance of DOM quality to better predict Cu solubility.

Keywords Copper · Organic matter · Fluorescence · Absorbance · Vineyard soils · Availability · Modelling

Introduction

Copper-based fungicides like Bordeaux mixture have been repeatedly applied in vineyards since the end of the nineteenth century to control downy mildew of grapevine, sometimes causing Cu to accumulate in the soils (Brun et al. 2001; Ballabio et al. 2018). In European soils, the median of total Cu concentrations is 13 mg kg^{-1}. In vineyards soils, this value reaches 49 and 91 mg kg^{-1} in Europe and France, respectively

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(Ballabio et al. 2018). In old vineyards, Cu concentrations in the topsoil can reach several hundred mg Cu kg^{-1}, as widely reported in several European countries including France, Italy, Spain, Greece, Portugal, and Czech Republic (Fernández-Calviño et al. 2009; Komárek et al. 2010; Fernández-Calviño et al. 2010; Flores-Álvarez et al. 2011; Kelepertzis et al. 2017). Despite the reduction of the authorized application rate to 4 kg Cu ha^{-1} $year^{-1}$ (EFSA et al. 2018), Cu-based fungicides continue to be used, particularly in organic viticulture for which there is currently no alternative that offers equally effective protection (Andrivon et al. 2018).

Cu is an essential micronutrient for all living organisms. Microorganisms need trace levels of Cu for their growth because it is a cofactor in enzymatic reactions (Trevors and Cotter 1990). In plants, copper plays key roles in cell wall metabolism and in photosynthetic electron transport (Yruela 2009). A Cu deficiency can affect vital functions in plant metabolism. However, excess copper can cause phytotoxicity through the formation of oxygen radicals which damage cells

and inactivate protein structure, or by disturbing photosynthetic activity due to the photoinhibition induced by excessive Cu (Yruela 2005). In calcareous soils, excess Cu can also lead to Fe deficiency in graminaceous species such as durum wheat (Michaud et al. 2007) since Cu(II) has sufficient affinity for phytosiderophores to divert them from their initial objective of mobilizing Fe(III). All these problems may affect plant growth and reduce agricultural productivity. Cu phytotoxicity has been reported in durum wheat grown in former vineyard soils with total Cu concentrations beginning at 114 mg kg^{-1} (Michaud et al. 2007). Exposure to Cu at soil concentrations above 16 mg Cu kg^{-1} in the soil has also been shown to have a serious impact on earthworm *Eisenia fetida* reproduction by reducing the production of cocoons (Helling et al. 2000). Fernández-Calviño et al. (2010) reported that microbial enzymatic activity (phosphatase) in Portuguese vineyard soils decreased drastically above 250 mg Cu kg^{-1}. A recent meta-analysis of Cu in vineyard soils showed that microbial respiration was reduced by half above a stock of 200 kg Cu ha^{-1} (Karimi et al. 2021). Excess copper may thus cause ecotoxicity in vineyard soils, although the ecotoxic thresholds vary widely depending on certain soil parameters like organic matter (OM) content or pH. Total Cu inducing 50% of biomass decrease (EC$_{50}$) is a widely used indicator in ecotoxicity studies. It has been shown that EC$_{50}$ of lettuce and tomatoes increases with increasing OM content or decreasing pH (Recatalá et al. 2012; Sacristán et al. 2016). Cu toxicity may therefore be linked not only to total Cu amounts but also to certain bioavailable forms of Cu in the soils.

The bioavailability of metals is defined as the fraction of its environmentally available compound an organism takes up through physiologically driven processes (ISO 2006). Environmental availability is defined as the availability of metals in the soil measured using chemical methods (Harmsen 2007). Sauvé et al. (1996) showed that Cu concentrations in the tissues of several plants were closely linked to Cu in solution, more specifically to free Cu^{2+} activity. The free ion Cu^{2+} is thought to be the bioavailable form, and this hypothesis is supported by other authors who reported that Cu toxicity for different organisms (algae, earthworms, zooplankton) in soils and aquatic systems depended strongly on free Cu^{2+} activity (Verweij et al. 1992; Qiu et al. 2013; Tait et al. 2016).

The concentration of Cu in soil solutions and Cu^{2+} activity is controlled by soil properties such as total Cu content, pH, and soil organic matter (SOM) content (Sauvé et al. 1997; Sauvé et al. 2000). pH is known to affect Cu solubility (Temminghoff et al. 1994), but also copper speciation in solution (Bravin et al. 2009). Cu biogeochemistry is controlled to a large extent by its binding to organic matter, since SOM contains acidic functional groups (mainly phenolic and carboxylic groups) which have a particular and strong affinity for Cu (Manceau and Matynia 2010). Organic matter supplied to soils in different forms (fresh materials, compost, manure, sludge) has been shown to significantly reduce Cu availability in its soluble form (Navel and Martins 2014; Ferreira et al. 2018; Gonzaga et al. 2020) and its ionic form (Oustriere et al. 2016). Dissolved organic matter (DOM) plays a key role in the fate of metals in the soil. While SOM immobilizes Cu through sorption onto solid phases (Sauvé et al. 2000), DOM increases Cu solubility by forming Cu complexes in soil solution. However, the free Cu fraction may also decrease due to complexation with DOM. Cu mobilization from the solid phase into solution has been reported to be exacerbated with increasing aromatic DOM (Amery et al. 2007; Amery et al. 2008; Araújo et al. 2019). What is more, prediction of Cu in soil leachates was improved when the DOM aromaticity was taken into account in geochemical modelling (Amery et al. 2008) rather than only considering a percentage of reactive DOM (Djae et al. 2017). Phenolic and O-functional groups of aromatic molecules have high affinity for Cu binding and are highly stable, which may facilitate its solubilization by DOM (Fuentes et al. 2013). These findings clearly underline the importance of DOM characteristics in the control of Cu dynamics in soil solutions.

The capacity of DOM to complex Cu and to promote its mobilization from the solid phase not only depends on DOM quantity, but also on DOM quality (Baken et al. 2011; He et al. 2016; Kikuchi et al. 2017). DOM quality can be described through its optical properties assessed by ultraviolet visible (UV-Vis) absorption and excitation-emission matrix (EEM) fluorescence spectroscopy (Jaffé et al. 2014). In the soil literature, DOM is largely characterized by its specific UV-absorbance index at 254 nm (SUVA$_{254}$), which is related to its aromaticity (Weishaar et al. 2003). But the measurements of SUVA$_{254}$ may be biased due to the presence of colloids or iron at high concentrations (Weishaar et al. 2003). Other spectroscopic indices are widely used in studies on aquatic environments to characterize DOM (Yang and Hur 2014; Derrien et al. 2017) and its interactions with Cu (He et al. 2016). Another UV-Vis absorption index is the spectral slope ratio S$_R$ which is inversely proportional to molecular size (Helms et al. 2008). Fluorescence indices include the humification index HIX, which expresses the degree of DOM condensation (Zsolnay et al. 1999), the biological index BIX, used to assess the recent production of autochthonous components (Huguet et al. 2009), and the fluorescence index FI, which makes it possible to distinguish microbial versus higher plant origin of DOM (McKnight et al. 2001). In aquatic media, concentrations of metals (Cu, Zn, Cd) in sediment solutions were shown to be related to DOM aromaticity (HIX) and to the autochthonous (BIX) or microbial (FI) origins of molecules and not to DOM molecular size (S$_R$) (He et al. 2016; Zhang et al. 2020).

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

Material and methods

Soil sampling and analyses

Eighteen vineyard topsoils were sampled in the vine inter-rows at a depth of between 0 and 15 cm in March 2019 in the region of Bordeaux (France), located in the AOC (i.e., controlled designation of origin) zones of "*Bordeaux supérieur*," "*Blaye Côtes de Bordeaux*," and "*Graves*." All the vineyards sampled are organic and the only fungicide treatment they receive is Cu. The fungicide applied was mainly Bordeaux mixture (mixture of copper sulfate and lime).

The 18 soils were contrasted in terms of organic matter and pH based on the pedological bedrock and agronomic practices. After sampling, the fresh soils were sieved to 4 mm and kept at 4 °C before analysis to minimize the effects of sampling on dissolved organic carbon (Jones and Willett 2006).

Soil pH (pH_{water}), total copper (Cu_T), organic carbon (C_{org}), and total nitrogen contents were determined on separate air-dried soils. pH_{water} was determined by potentiometry in distilled water in a 1:2.5 (w/v) soil-to-solution ratio (NF ISO 10390). Cu_T was determined after *aqua regia* microwave-assisted digestion (Anton Paar Multiwave PRO Microwave Reaction System®). Aliquots (0.5 g) of crushed dried sample were placed in a microwave Teflon flask with an acid mix HNO_3:HCl (3.75:1.25, v:v) (Aristar® for trace analysis, VWR Chemicals). Extracts were obtained after centrifugation at 1300 W at 180 °C for 20 min and filtration on a 2-μm Whatman cellulose acetate filter. The concentration of Cu in the extracts was measured by ICP-OES (Agilent®). A certified reference material (BCR-143R, 98% Cu recovery rate) was used to validate extraction and quantification.

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

Extraction of soil solutions

Before the soil solution was extracted, the wet soils were all adjusted to 80% water holding capacity (WHC) and incubated at 20 °C for 1 week to avoid a possible microbial flush and its effects on Cu solubility and speciation (Cornu et al. 2007).

Soil solutions were extracted as follows: 15 g of wet soil was shaken with 30 mL of 0.01 M KCl (purity 99.5%, Merck®) with 1:2 w/v (soil-to-solution ratio) at 35 rev. mn^{-1} for 2 h. The soil suspensions were centrifuged at 4600 g for 10 min. The supernatants were collected and filtered through 0.2-μm cellulose acetate filters. Extractions were performed in triplicate. The extracted soil solutions were stored at 4 °C in the dark before spectroscopic analysis, which was performed less than 7 days after extraction.

The 0.01 M KCl extraction was chosen for two reasons: (i) to be in the range of common ionic strength for soil pore water and (ii) to avoid DOM coagulation which may occur in the case of $CaCl_2$ extraction (Römkens and Dolfing 1998). The 1:2 w/v ratio was used because lower extraction ratios tend to overestimate the concentrations of DOC recovered (Jones and Willett 2006).

Analyses of soil extracts

Total dissolved metals and Cu^{2+} concentrations

The pH of the extracts (pH_{KCl}) was measured immediately after extraction by potentiometry using a microelectrode (E16M331, Radiometer Analytical®). The total concentrations of dissolved Ca, Cu, Fe, Mn, and Zn were determined by ICP-OES (Agilent®) in a 5-mL aliquot acidified with 2% HNO_3 (Aristar® for trace analysis purity 69%, VWR Chemicals®). Free Cu activity was measured in non-acidified 5-mL aliquots using a cupric ion-selective electrode (ISE, 9629BNWP, Thermo Scientific Orion®). The ISE was calibrated in Cu solutions buffered with iminodiacetic acid and potassium phthalate with pCu ($= -\log_{10} aCu^{2+}$) ranging from 5.5 to 13 as described in Bravin et al. (2009). The calibration curve ($R^2 = 0.98$, $n = 67$) showed 85% slope efficiency compared to the theoretical Nernstian slope (Fig. S1). The concentrations of free Cu^{2+} were determined from pCu values and from the Cu^{2+} activity coefficient, calculated from the extended Debye-Hückel equation (Ritsema 1993).

Dissolved organic matter parameters

The concentrations of dissolved organic carbon (DOC) in the soil extracts were determined using a TOC analyser (TOC-VCPH, Shimadzu®, Japan). The DOC was calculated as the difference between total carbon (TC) and inorganic carbon (IC) (DOC = TC − IC). The analyser was calibrated with a solution prepared from sodium carbonate, sodium

bicarbonate, potassium hydrogen phthalate, and the 0.01 M KCl was used as the zero point in the calibration.

Absorbance indices

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

$$SUVA254 = \frac{A_{254}.\ 1000}{b.\ DOC}$$

where A_{254} is the measured absorbance at 254 nm (dimensionless) (Fig. S2), b is the path length (cm), and DOC the organic carbon concentration (mg L^{-1}).

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

Fluorescence indices

The excitation-emission matrix (EEM) fluorescence spectra were recorded in a 0.5-cm quartz cuvette, thermostatted at 20 °C in the cell holder, using an Aqualog spectrofluorometer (Horiba Scientific®). Excitation scans were performed using a double-grating monochromator, a 150-Watt Xenon lamp with a 5-nm bandpass, and 2-s integration time at wavelengths of 240–800 nm at 5-nm intervals. Emission spectra were collected with high CCD detector gain at approximately 0.58 nm (1 pixel) intervals at wavelengths of 245–830 nm. A blank EEM was acquired daily from the 0.01 M KCl solution used for soil extraction and subtracted from EEM spectra of all the samples to eliminate scattering peaks. EEM spectra were corrected for instrumental biases. When the maximum intensity of the absorbance spectrum was higher than 0.1, the samples were diluted with 0.01 M KCl to avoid inner filter effects. Fluorescence data are expressed in Raman units (R.U) after normalization to the area under the Raman peak of an ultra-pure water blank (Milli-Q, Millipore®) at an excitation wavelength of 350 nm. TreatEEm software (https://sites.google.com/site/daromasoft/home/treateem) was used to remove remaining scatter peaks in EEMs and to calculate the fluorescence indices.

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

Statistical analyses

All statistical analyses were performed with R software version 4.0.2 (R core Team 2020). First, principal component analysis (PCA) was used to visualize correlations between variables and possibly groups of soils. PCA was performed using the "FactomineR" package (Husson et al. 2008). For the PCA, all the variables were log-transformed except pH_{water} and pH_{KCl}. Second, a correlation matrix was performed to investigate the relationships between the same variables using the R "corrplot" package (Wei and Simko 2021). The Pearson correlation coefficients were considered as statistically significant at $p < 0.01$.

Multilinear log–log regressions were used to identify and rank the contributions of soil variables to the prediction of Cu availability proxies (Cu_{KCl} and Cu^{2+}). Multilinear regressions were performed using the "car" package (Fox and Weisberg 2019). Each replicate was considered as one sample. For Cu_{KCl} prediction, soil and solution variables were entered as predictors (Cu_T, pH_{water}, C:N, C_{org}, $SUVA_{254}$, S_R, DOC, HIX, BIX, FI). Only solution variables were used for Cu^{2+} prediction (Cu_{KCl}, pH_{KCl}, $SUVA_{254}$, S_R, DOC, HIX, BIX, FI). The variables (except pH_{water} and pH_{KCl}) were log-transformed before the computation. A total of 2047 and 255 models with one to maximum four predictors (to avoid overparameterization) were exhaustively tested for Cu_{KCl} and Cu^{2+} predictions, respectively. The best models had the maximum R^2_{adj} (goodness of fit) with a minimum number of predictors (parsimony). The selected models also had a variation inflation factor (VIF) below 2.3 to avoid multicollinearity. The root mean square error (RMSE) was calculated to compare the accuracy of the models including or not DOM parameters.

Modelling Cu^{2+} activity

Cu speciation in solution was predicted with ORCHESTRA software (Meeussen 2003) using the DOM ion-binding model NICA-Donnan (Kinniburgh et al. 1996; Koopal et al. 2005).

The objective was to compare simulations of amounts of free Cu^{2+} with the corresponding experimental measurements. The input parameters used for the calculations were pH_{KCl}, total DOM, Cu^{2+}, Ca^{2+}, Zn^{2+}, Fe^{3+}, and Mn^{3+} concentrations. Cl^- and K^+ concentrations were set to 0.01 M, i.e., their concentration in the extraction solution. DOM concentrations were calculated assuming that DOM contained 50% of C. Activity coefficients were calculated using the Davies equation. Fe activity was calculated by assuming its equilibrium with $Fe(OH)_3$ ($logK_S=3.19$). Equilibrium reactions were described taking the equilibrium with atmospheric CO_2 ($pCO_2 = 3.9\times10^{-4}$ atm) into account.

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

Results and discussion

Soil properties

The main soil parameters are listed in Table 1.

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

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

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

Elemental composition of the soil extracts and Cu availability

Mean Fe, Mn, Ca, and Zn concentrations are listed in Table S1 in supplementary information. The pH of the soil extracts (pH_{KCl}) fluctuated between 5.9 and 7.6 (Table S1).

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

The mean total concentration of Cu in the extracts (Cu_{KCl}) ranged from 0.11 to 2.64 μM, i.e., a factor of 23 (Table S1), with a median of 0.3 μM. Many studies in the literature used salt solutions—generally $CaCl_2$—to extract Cu and considered it to be a correct surrogate for soil pore water Cu (Sauvé et al. 1997; Chaignon et al. 2003). Our Cu_{KCl} values were similar to those found by Djae et al. (2017) (3×10^{-2}– 3.7 μM Cu) with 2 mM $KNO_3/Ca(NO_3)_2$ extractions. Similar ranges of total Cu assessed in $CaCl_2$ extracts were found in the literature: 0.11–2 μM (Sauvé et al. 1996), 0.01–5 μM (Sauvé et al. 1995).

The mean Cu^{2+} concentrations varied between 2.1×10^{-4} and 0.95 μM, i.e., by a factor of 4600 (Table S1). This variability shows that our samples were more contrasted in terms of Cu^{2+} than Cu_{KCl}. The highest Cu^{2+} values were found in the most acidic soils. The percentage of free Cu, defined as the ratio of concentrations in Cu^{2+} and Cu_{KCl} (fCu^{2+}), ranged from 0.07 to 50% (Table S1). Our results are in agreement with Djae et al. (2017) who found Cu^{2+} concentrations between 3.10^{-7} and 2 μM and fCu^{2+} ranged from 0.01 to 44% for Cu-contaminated soils. But in moderately contaminated vineyard soils, Cornu et al. (2019) found Cu^{2+} of between 6×10^{-5}–0.3 μM and fCu^{2+} of between 0.002 and 10%. These results reveal dramatic differences based on free Cu^{2+}, even within a collection of soils with the same source of Cu contamination.

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

Table 1 Chemical properties of the 18 vineyard soils analyzed 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 mg kg^{-1}	pH$_{water}$	CaCO$_3$ g kg^{-1}	C_{org} g kg^{-1}	C:N
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 Camisol	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

Beyond total soluble Cu, the bioavailable form of Cu is generally considered to be its ionic species Cu^{2+} (Sauvé et al. 1998), so Cu^{2+} concentrations would be a far more reliable tool to assess Cu toxicity for organisms. In the present study, the Cu^{2+} measured in some KCl extracts was the same as that which caused a 50% reduction ($EC_{50} = 6.3 \times 10^{-2}$ μM) in root growth in durum wheat (Bravin et al. 2010) (Table 2). This EC_{50} value was found at 2.2 μM Cu^{2+} for cucumber (Kader et al. 2016). To our knowledge, no such toxicity indicator assessed via ionic Cu^{2+} has been established for grapevine. Four soils of our panel (Table S1) may be problematic if converted from grapevine to widely consumed crops like wheat. Qiu et al. (2013) reported that Cu^{2+} concentration

was also an appropriate proxy to assess Cu toxicity for earthworms and found an EC_{50} of 1 μM Cu^{2+} for *Eisenia fetida* mortality (Table 2). In our panel of soils, this suggests that one soil represents a potential toxic risk for this earthworm species (Table 2, Table S1).

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

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

|	Toxicity thresholds	Values (μM)	References
Total Cu in solution (Cu_{KCl} in this study)	**Max value in this study**	**2.64**	
	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)
Free ionic Cu^{2+}	**Max value in this study**	**0.94**	
	EC_{50} durum wheat	0.06	Bravin et al. (2010)
	EC_{50} cucumber	2.20	Kader et al. (2016)
	EC_{50} *Eisenia fetida*	1.00	Qiu et al. (2013)

Diversity of soils in terms of DOM properties

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

Fluorescence EEM spectra (Fig. S4) were acquired to describe heterogeneities in DOM in the samples more precisely by calculating indices such as FI, HIX, and BIX. The FI values ranged from 1.10 to 1.34 (median 1.22) (Fig. 1, Table S1). FI values are usually used to distinguish sources of DOM. McKnight et al. (2001) found that low FI values (~1.3)

correspond to higher plant-derived molecules, while high values (~1.9) indicate microbial or microbially processed DOM. This suggests that the majority of DOM collected from our samples originated from vascular plants, and that microbial contributions were limited. The BIX index is used to assess the relative production of recent DOM and usually ranges from 0.5 to 1. In the present study, about 75% of the samples had BIX between 0.6 and 0.7, indicating a relatively low contribution of fresh autochthonous DOM substances (Table S1), while the other 25% had BIX between 0.7 and 0.9, suggesting fresh DOM production by organisms (Huguet et al. 2009; He et al. 2016). In our samples, the HIX values were contrasted, with almost 10-fold variation from 1.31 to 10.33 (median 5.75) (Fig. 1, Table S1). Two of the soils (D and L) were characterized by very low HIX values (Table S1) due to the presence of tyrosine-like fluorescence at an excitation wavelength of 240–260 nm and emission of 300–340 nm (Fig. S4a). This tyrosine-like fluorescence band was absent from all other EEM spectra that were similar to that of soil B (Fig. S4b). HIX stands for degree of humification or condensation of DOM (Zsolnay et al. 1999) but has also been used to characterize DOM aromaticity (Ohno et al. 2007). Our HIX results are in the same range as those reported by Daouk et al. (2015) for Swiss vineyard soils (0.1–7.0) and as those reported by Tang et al. (2019) (2 to 10). However, HIX values varying

Fig. 1 Distribution of DOM optical parameters (mean of replicates), the red points represent the mean values of all the soils
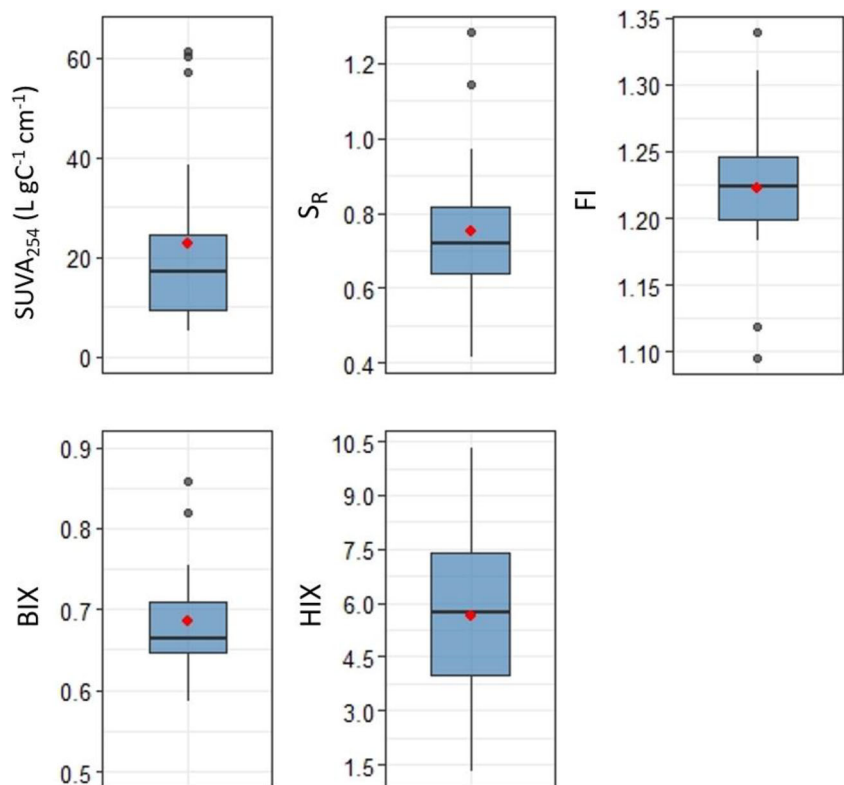
from 10 to 30 have been reported in several arable soils (Kalbitz et al. 2003). Compared to the latter range, our soils had low HIX values and can be characterized as weakly humified.

Relationships between the variables

We performed a principal component analysis (PCA) of all 18 soil extracts to describe the relationships between the solution variables and to identify homogeneous groups: 64% of total variance is explained by the first two principal components (Fig. 2a). The first principal component revealed 44.5% variance of data and was positively associated with Cu_{KCl}, Cu^{2+}, Fe concentrations, and $SUVA_{254}$ and was negatively associated with pH_{water}, pH_{KCl}, and HIX. The second principal component explained 19.2% of variance and was positively associated with C_{org}, the C:N ratio, and negatively associated with FI and BIX. Two groups can be distinguished on the principal plan plot (Fig. 2b): group 1 is composed of the eight most alkaline soils ($7.8 < pH_{water} < 8.3$), while group 2 is composed of the 10 most acidic soils ($6.3 < pH_{water} < 7.5$). The differences between the groups are mainly explained by the pH_{water} values (PCA correlation coefficient with Component 1: -0.92; with component 2: 0.03). On the first axis, the soils in group 1 share high values of pH and HIX, and low values of Cu_{KCl}, Fe_{KCl}, and Cu^{2+}. The opposite trend was observed in group 2. On the second axis, soils in group 2 have more contrasted FI and BIX than those in group 1. Indeed, in group 1, FI values ranged from 1.20 to 1.31; and BIX values from 0.67 to 0.75. Comparatively, these indices show a larger range in group 2, with 1.10–1.34 for FI and 0.59–0.86 for BIX. Group 1 is composed of alkaline soils with low Cu concentrations in

solution and relatively highly humified DOM, while group 2 is composed of more acidic soils with high concentrations of Cu_{KCl} and Cu^{2+}, and contrasted contributions of microbial DOM.

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

Cu^{2+} concentrations and fCu^{2+} were strongly linked to the pH of the extracts, with correlation coefficients of -0.94 and -0.91, respectively (Fig. 3, Fig. 4d), suggesting that Cu^{2+} decreased with increasing pH. The strong relationship between pH and Cu^{2+} has already been reported (McBride and Blasiak 1979; Sauvé et al. 1995; Laurent et al. 2020), confirming that pH is the main factor controlling Cu
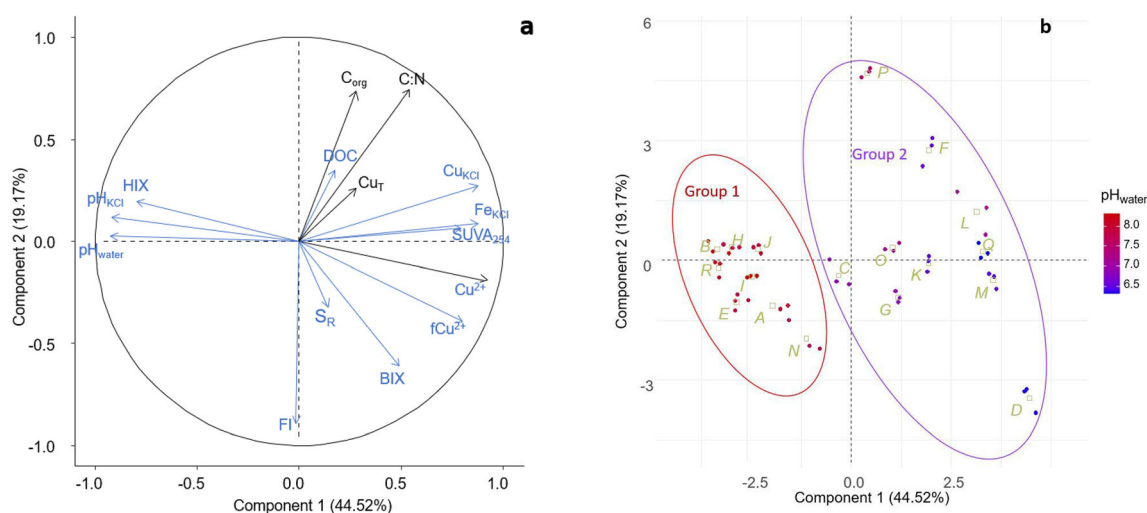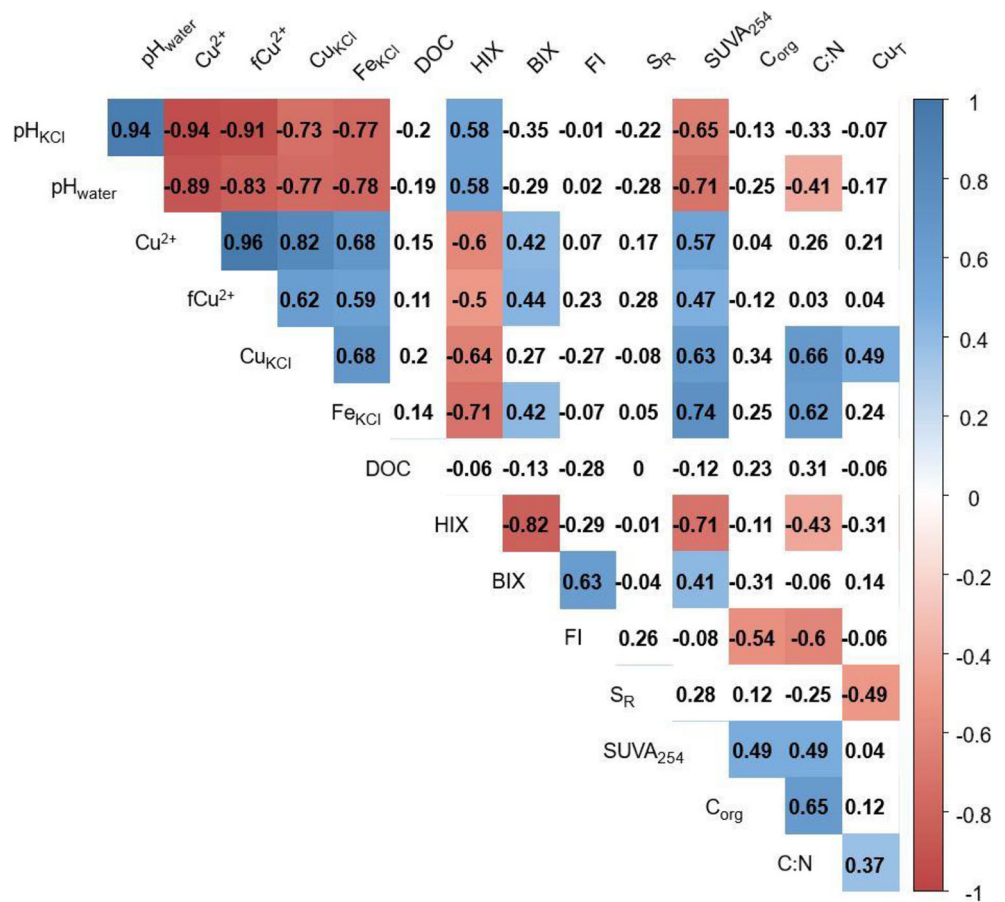

Fig. 2 Principal component analysis (PCA) of soil solution chemistry and Cu availability proxies in the dataset. All the variables except pH_{water} and pH_{KCl} were log-transformed. The correlation plot (**a**) shows the contribution of each variable to the two first principal components. The soil

variables are shown in black, the extract variables in blue. Soil samples (grouped in triplicate) are colored 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 (fCu^{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 ($p < 0.01$)

	pH$_{water}$	Cu^{2+}	fCu^{2+}	Cu$_{KCl}$	Fe$_{KCl}$	DOC	HIX	BIX	FI	S$_R$	SUVA$_{254}$	C$_{org}$	C:N	Cu$_T$
pH$_{KCl}$	0.94	-0.94	-0.91	-0.73	-0.77	-0.2	0.58	-0.35	-0.01	-0.22	-0.65	-0.13	-0.33	-0.07
pH$_{water}$		-0.89	-0.83	-0.77	-0.78	-0.19	0.58	-0.29	0.02	-0.28	-0.71	-0.25	-0.41	-0.17
Cu^{2+}			0.96	0.82	0.68	0.15	-0.6	0.42	0.07	0.17	0.57	0.04	0.26	0.21
fCu^{2+}				0.62	0.59	0.11	-0.5	0.44	0.23	0.28	0.47	-0.12	0.03	0.04
Cu$_{KCl}$					0.68	0.2	-0.64	0.27	-0.27	-0.08	0.63	0.34	0.66	0.49
Fe$_{KCl}$						0.14	-0.71	0.42	-0.07	0.05	0.74	0.25	0.62	0.24
DOC							-0.06	-0.13	-0.28	0	-0.12	0.23	0.31	-0.06
HIX								-0.82	-0.29	-0.01	-0.71	-0.11	-0.43	-0.31
BIX									0.63	-0.04	0.41	-0.31	-0.06	0.14
FI										0.26	-0.08	-0.54	-0.6	-0.06
S$_R$											0.28	0.12	-0.25	-0.49
SUVA$_{254}$												0.49	0.49	0.04
C$_{org}$													0.65	0.12
C:N														0.37

speciation in solution. When pH increases, DOM is more negatively charged due to the deprotonation of its functional groups. This promotes its complexation with Cu, leading to a drastic decrease in Cu^{2+} activity in solution (Bravin et al. 2009; Ren et al. 2015). Conversely, at low pH, competition for DOM binding sites is high between Cu^{2+} and H$^+$, which promotes increasing Cu^{2+} activity. A pH above 6.5 seems to be the safety threshold for the soils in our collection in terms of Cu toxicity when Cu^{2+} is used as a proxy (Fig. 4d).

No tangible relationship was found between C$_{org}$ values and DOC concentrations in the extracts (Fig. 3), meaning that the solubilization rates of C$_{org}$ may differ with the soil conditions or properties. None of DOM optical indices was significantly linked to DOC concentrations in the KCl extracts, indicating that DOM quality was not related to the quantity of DOM in solution. No significant relationship was found between the S$_R$ and the SUVA$_{254}$ indices. SUVA$_{254}$ and HIX indices were negatively linked in this study ($r=-0.71$, $p < 0.01$) (Fig. 3). This negative relationship is all the more surprising as these indices are both related to DOM aromaticity. In the literature, SUVA$_{254}$ and HIX are generally positively correlated (He et al. 2016; Tang et al. 2019). However, such unexpected differences between these two indices have already been observed (Inamdar et al. 2011; Broder et al.

2017). Considering the differences in pH between soils, and given that fluorescence is pH dependent, one may wonder if the variations observed in these two indices are not affected by the effect of pH on spectroscopic analyses. However, an impact on fluorescence analyses is unlikely in our range of soil pH and would, in any case, be less than differences due to the sources and composition of DOM (Hudson et al. 2007). The presence of protein-like fluorescence was observed in EEM spectra of only two soils (D and L) and cannot explain the negative correlation between HIX and SUVA$_{254}$. Moreover, it has been reported that SUVA$_{254}$ measurements may be subject to some bias since Fe, colloids, and other constituents also absorb at 254 nm (Weishaar et al. 2003; Yang and Hur 2014). Since there were huge differences in Fe concentrations among our samples (i.e., varying by a factor of 12, Table S1), the SUVA$_{254}$ results in this study should be interpreted with caution. In a collection of samples with such contrasted concentrations of dissolved Fe as those measured in the present study, it would therefore not be appropriate to use the SUVA$_{254}$ index to assess DOM aromaticity, and HIX appears to be a more reliable tool for that purpose. No significant relationship was found between HIX and S$_R$, suggesting that, in our soil extracts, DOM condensation may not be directly related to its molecular size. Moreover, the
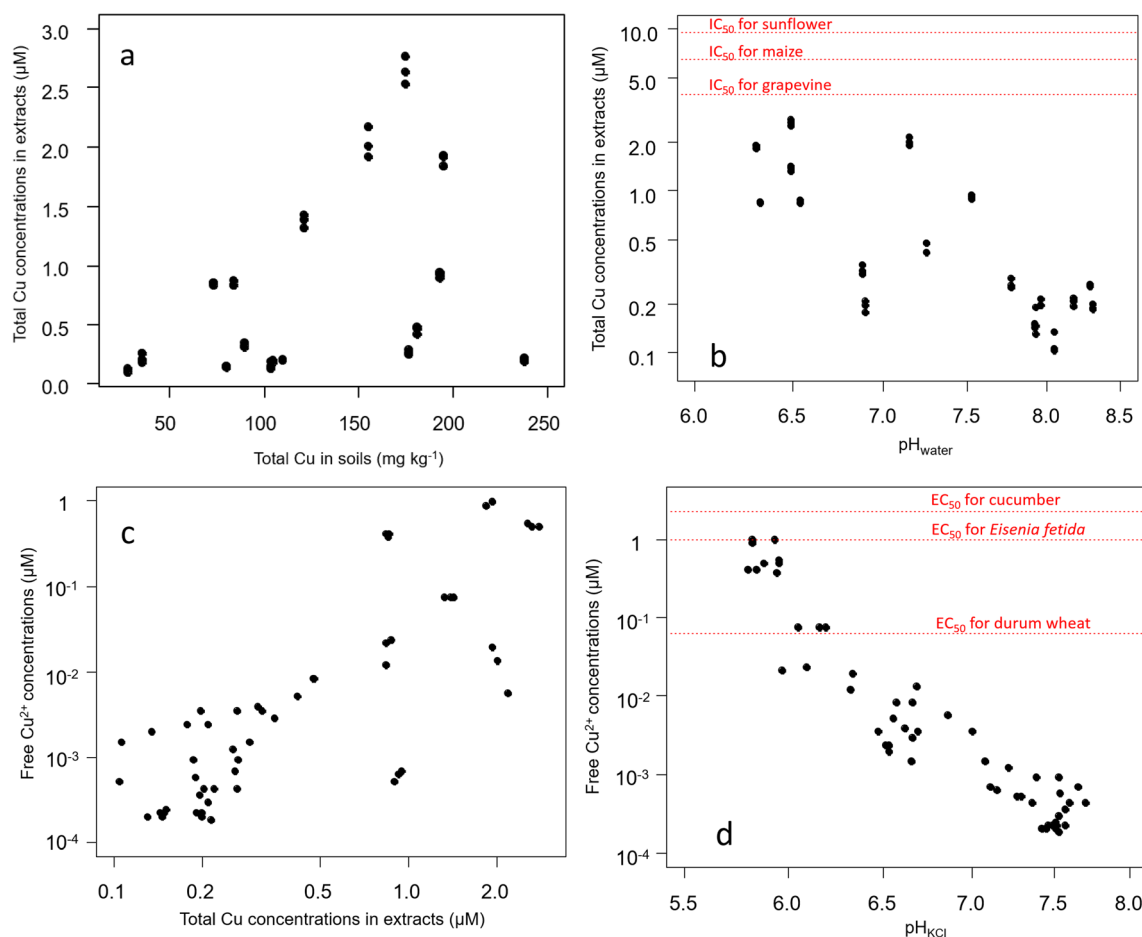
Fig. 4 Total Cu concentrations in extracts (Cu$_{KCl}$) as a function of total Cu in soils Cu$_T$ (**a**) and pH of the soils (pH$_{water}$) (**b**); free ionic Cu concentrations (Cu^{2+}) as a function of total Cu concentrations in extracts (**c**) and pH of the extracts (pH$_{KCl}$) (d**d**). The log–log plot is shown in (**c**) and only the *y*-axis is log-transformed in (**b**) and (**d**)

negative correlation between HIX and BIX (Fig. 4b) ($r = -0.82$, $p < 0.01$) indicates that the most aromatic DOM is associated with older molecules derived from higher plants (Tang et al. 2019), which is quite consistent. No significant link was found between HIX and FI. Nevertheless, the positive relationship between BIX and FI ($r = 0.63$, $p < 0.01$) was not surprising and suggested that the soils with DOM originating from a microbial source were related to the highest autochthonous contributions, as reported in other works (Tang et al. 2019; Musadji et al. 2020).

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

Prediction of Cu availability using the properties of the soils and the extracts

The risk of Cu environmental toxicity could be better assessed if its availability could be predicted using the variables measured in the soil and in the soil solution. However, organisms often use mechanisms to resist the stress caused by Cu toxicity. For instance, some plants take up and sequestrate Cu in cells where it cannot cause harm (Küpper et al. 2009). Nevertheless, as Cu environmental availability is linked to its total (Cu$_{KCl}$) and ionic (Cu^{2+}) concentrations in soil solutions, predicting these concentrations could enable more accurate evaluation of potential Cu toxicity to a certain extent (see "Elemental composition of the soil extracts and Cu availability"). Table 3 shows the best models calculated to predict Cu$_{KCl}$ and Cu^{2+} among the 18 vineyard soils sampled in this study.

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

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

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

Prediction of total Cu in extracts Cu_{KCl}

Among the one-variable models tested to predict Cu_{KCl}, the model with pH_{water} (a) showed the best goodness of fit ($R^2_{adj} = 0.575$, Table 3). This suggests that pH is the main factor driving Cu solubilization in the soils sampled in this study. However, this model showed marked dispersion of points around the 1:1 line (Fig. 5a), more visible in soils in group 1 than in soils in group 2. The prediction was better when Cu_T was added to pH (model (b)), the R^2_{adj} value raised to 0.71 and the RMSE decreased from 0.28 to 0.23 (Table 3). The points were then less dispersed than with the preceding model,

except in soils in group 2 (Fig. 5b). The respective coefficients of pH and Cu_T are suitable for the presumed mechanisms. Indeed, it has been reported that metal desorption increases with a decrease in pH, which is why metals tend to be more soluble in acidic environments (McBride and Blasiak 1979; Degryse et al. 2009). Moreover, at higher pH, Cu oxyhydroxides or carbonates may form and precipitate thereby reducing the Cu in solution (McBride 1989). As expected, the model showed that the higher the Cu_T, the higher the concentration of soluble Cu in solution (Cu_{KCl}). Sauvé et al. (1997) underlined the importance of pH and Cu_T in Cu solubilization, but in the reverse hierarchy. These authors found
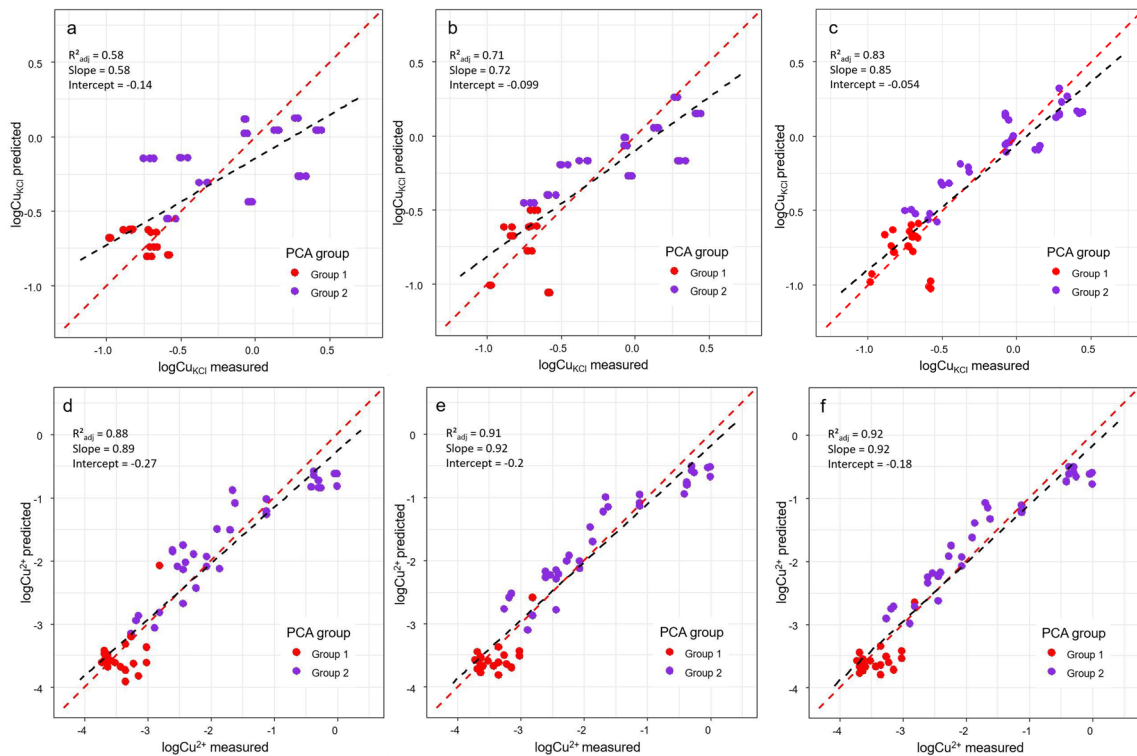

Fig. 5 Comparison of the multilinear models predicting Cu_{KCl} (**a, b, c**) and Cu^{2+} concentrations (**d, e, f**) in the 0.01 M KCl extracts. The dashed red line represents the 1:1 line, the dashed black line represents the regression line

that in 66 soils with diverse sources of Cu contamination, 90% of variation in soluble Cu was explained by total Cu in the soils and the importance of pH was minimal. This could be explained by the wider range of Cu_T in their study (14–3000 mg kg^{-1}) which reinforced the contribution of Cu_T in predicting soluble Cu.

The concentration of DOC did not appear in the best models, presumably because of its limited variability in our initial dataset. However, Cu_{KCl} predictability was dramatically improved when DOM quality parameters were added as predictors in addition to pH and Cu_T. This suggests that in the present study, Cu availability depended more on DOM characteristics than on its concentration. Among all the DOM indices tested in the regressions, only HIX and FI emerged significantly, and the best prediction was obtained by equation (c) (Table 3, Fig. 5c), which explained 83.2% of variance, showing good accuracy of this model for the prediction of Cu_{KCl}. Compared to model (b), model (c) produced lower RMSE (0.17) and a slope closer to 1 (0.85) (Fig. 5c). The contributions of pH_{water}, Cu_T, FI, and HIX were all significant in the model ($p < 0.001$) (Table 3). The variance inflation factor (VIF) did not identify correlations between the predictors (VIF < 2.3). HIX and FI were both expressed with negative coefficients in the model, suggesting that the solubilization of Cu is enhanced with lowly condensed plant-derived DOM molecules. This suggests that, in the vineyard soils studied here, molecules with low aromaticity have the most affinity for Cu for its solubilization. This finding contradicts previous studies suggesting that the most aromatic DOM had higher affinity for Cu and promoted its solubilization through complexation (Amery et al. 2007; Amery et al. 2008; Araújo et al. 2019). In the present study, DOM composed of more hydrophilic molecules may have higher Cu-binding capacity because of its higher proportion of O-containing functional groups (hydroxyl, carboxylic acid, sulfonic acid, and amine) than aromatic and hydrophobic substances (Baham et al. 1978; Leenheer 1981). Moreover, the negative coefficient of FI in the model suggests a higher plant origin for the DOM components that bind Cu. Our results are consistent with those of He et al. (2016), who found higher concentrations of heavy metals in sediment solutions with lower aromatic DOM molecules, which the authors linked to amino acids. Moreover, it has previously been reported that the ability of DOM hydrophilic and low aromatic components to transport Cu is greater than that of hydrophobic molecules (Han and Thompson 1999, 2003). These authors explained that low aromatic and hydrophilic molecules were more mobile, while aromatic and complex components tended to be adsorbed on soil particles.

Prediction of free ionic Cu^{2+}

Among the models with just one predictor, the one with pH_{KCl} (model (d)) appeared to be the most effective and explained 88.3% of Cu^{2+} variance (Table 3). The RMSE value was low (0.4), implying good accuracy of the prediction (Fig. 5d). The high R^2_{adj} (0.88) of model (d) highlights the importance of pH in predicting Cu^{2+} concentrations.

We hypothesized that free Cu^{2+} may be linked to the solubility of Cu, as we found a positive relationship between Cu^{2+} and Cu_{KCl} (Fig. 4c). When adding Cu_{KCl} to pH_{KCl} as predictors of Cu^{2+} (model (e)), the R^2_{adj} increased from 0.88 to 0.91. This slight improvement can be observed in Fig. 5e. The corresponding VIF was the maximum receivable (2.3), certainly due to the relative effect of pH on Cu solubilization. The contributions of pH_{KCl} and Cu_{KCl} were all significant ($p < 0.001$) in explaining Cu^{2+} variability. The coefficients in the model suggest that Cu^{2+} increases with Cu_{KCl}, and decreasing pH. Compared to model (d), model (e) was more accurate with a lower RMSE (0.34 instead of 0.4) (Table 3).

BIX was the DOM index that appeared in the best three-variable models (model (f), Table 3). Models with more than three variables did not present any statistical improvement (data not shown). In model (f), adding BIX to pH_{KCl} and Cu_{KCl} did not enhance the effectiveness of the prediction compared to model (e) (Fig. 5f), with similar R^2_{adj} and RMSE (0.92 and 0.32, respectively) (Table 3). Even though the contribution of BIX to the model was significant ($p < 0.05$), the addition of BIX was not needed to explain the variability of our collection of samples. Moreover, the max VIF of model (f) (2.44) was higher than the fixed threshold of 2.3, illustrating multicollinearity between the predictors that probably weakens the stability of the prediction. Taken together, these results suggest that including DOM indices does not improve the modelling of Cu^{2+} and that only pH_{KCl} and Cu_{KCl} were needed.

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

Influence of DOC composition on Cu speciation

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

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

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

Conclusions

The main goal of this work was to assess the contribution of DOM spectroscopic indices to predicting two Cu availability proxies in 18 organic vineyard soils. Our results showed that in the KCl extracts, the total concentration of Cu varied 23-fold while that of free ionic Cu^{2+} varied 4600-fold among soils, pointing to wide diversity of soils in terms of Cu availability. Four soils had concentrations of Cu^{2+} higher than the toxicity levels for organisms reported in the literature. These samples were considered as potentially harmful for vineyard soil organisms.

Our results also showed that Cu environmental availability was weakly related to the level of Cu contamination in the soils. pH was the main factor controlling Cu environmental availability, as it was closely and positively correlated with the concentrations of total Cu and free ionic Cu^{2+} measured in KCl extracts. Thus, in theory, toxicity problems can occur even in moderately Cu-contaminated soils when the soils are particularly acidic. It would thus be safer to maintain the pH above 6.5. To this end, liming is possible but should be applied with caution, so as not to jeopardize plant uptake of other nutrients like Fe, whose availability is low at alkaline pH.

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

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

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Amery F, Degryse F, Cheyns K, De Troyer I, Mertens J, Merckx R, Smolders E (2008) The UV-absorbance of dissolved organic matter predicts the fivefold variation in its affinity for mobilizing Cu in an agricultural soil horizon. Eur J Soil Sci 59(6):1087–1095. https://doi.org/10.1111/j.1365-2389.2008.01078.x

Amery F, Degryse F, Degeling W, Smolders E, Merckx R (2007) The copper-mobilizing-potential of dissolved organic matter in soils varies 10-fold depending on soil incubation and extraction procedures. Environ Sci Technol 41(7):2277–2281. https://doi.org/10.1021/es062166r

Andrivon D, Bardin M, Bertrand C, Brun L, Daire X, Fabre F, Gary C, Montarry J, Nicot P, Reignault P, Tamm L, Savini I (2018) Can organic agriculture give up copper as a crop protection product? Synthesis of the scientific assessment report. Institut national de la recherche agronomique (INRA) :68. hal-02944872

Araújo E, Strawn DG, Morra M, Moore A, Ferracciú Alleoni LR (2019) Association between extracted copper and dissolved organic matter in dairy-manure amended soils. Environ Pollut 246:1020–1026. https://doi.org/10.1016/j.envpol.2018.12.070

Baham J, Ball NB, Sposito G (1978) Gel filtration studies of trace metal-fulvic acid solutions extracted from sewage sludges. J Environ Qual 7(2):181–188. https://doi.org/10.2134/jeq1978.00472425000700020006x

Baken S, Degryse F, Verheyen L, Merckx R, Smolders E (2011) Metal complexation properties of freshwater dissolved organic matter are explained by its aromaticity and by anthropogenic ligands. Environ Sci Technol 45(7):2584–2590. https://doi.org/10.1021/es103532a

Ballabio C, Panagos P, Lugato E, Huang J-H, Orgiazzi A, Jones A, Fernández-Ugalde O, Borrelli P, Montanarella L (2018) Copper distribution in European topsoils: an assessment based on LUCAS soil survey. Sci Total Environ 636:282–298. https://doi.org/10.1016/j.scitotenv.2018.04.268

Bravin MN, Le Merrer B, Denaix L, Schneider A, Hinsinger P (2010) Copper uptake kinetics in hydroponically-grown durum wheat (Triticum turgidum durum L.) as compared with soil's ability to supply copper. Plant Soil 331(1–2):91–104. https://doi.org/10.1007/s11104-009-0235-3

Bravin MN, Martí AL, Clairotte M, Hinsinger P (2009) Rhizosphere alkalisation — a major driver of copper bioavailability over a broad pH range in an acidic, copper-contaminated soil. Plant Soil 318(1):257–268. https://doi.org/10.1007/s11104-008-9835-6

Broder T, Knorr K-H, Biester H (2017) Changes in dissolved organic matter quality in a peatland and forest headwater stream as a function of seasonality and hydrologic conditions. Hydrol Earth Syst Sci 21(4):2035–2051. https://doi.org/10.5194/hess-21-2035-2017

Brun LA, Maillet J, Hinsinger P, Pépin M (2001) Evaluation of copper availability to plants in copper-contaminated vineyard soils. Environ Pollut 111(2):293–302. https://doi.org/10.1016/S0269-7491(00)00067-1

Chaignon V, Sanchez-Neira I, Herrmann P, Jaillard B, Hinsinger P (2003) Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vine-growing area. Environ Pollut 123(2):229–238. https://doi.org/10.1016/S0269-7491(02)00374-3

Coll P, Le Cadre E, Blanchart E, Hinsinger P, Villenave C (2011) Organic viticulture and soil quality: a long-term study in Southern France. Applied Soil Ecology :S0929139311001570. https://doi.org/10.1016/j.apsoil.2011.07.013

Cornu JY, Denaix L, Schneider A, Pellerin S (2007) Temporal evolution of redox processes and free Cd dynamics in a metal-contaminated soil after rewetting. Chemosphere 70(2):306–314. https://doi.org/10.1016/j.chemosphere.2007.06.003

Cornu JY, Randriamamonjy S, Gutierrez M, Rocco K, Gaudin P, Ouerdane L, Lebeau T (2019) Copper phytoavailability in vineyard topsoils as affected by pyoverdine supply. Chemosphere 236:124347. https://doi.org/10.1016/j.chemosphere.2019.124347

Cory R, Mcknight D (2005) Fluorescence spectroscopy reveals ubiquitous presence of oxidized and reduced quinones in dissolved organic matter. Environ Sci Technol 39:8142–8149. https://doi.org/10.1021/es0506962

Daouk S, Frege C, Blanc N, Mounier S, Redon R, Merdy P, Lucas Y, Pfeifer H-R (2015) Fluorescence spectroscopy to study dissolved organic matter interactions with agrochemicals applied in Swiss vineyards. Environ Sci Pollut Res 22(12):9284–9292. https://doi.org/10.1007/s11356-015-4086-6

Degryse F, Smolders E, Parker DR (2009) Partitioning of metals (Cd, Co, Cu, Ni, Pb, Zn) in soils: concepts, methodologies, prediction and applications – a review. Eur J Soil Sci 60(4):590–612. https://doi.org/10.1111/j.1365-2389.2009.01142.x

Derrien M, Yang L, Hur J (2017) Lipid biomarkers and spectroscopic indices for identifying organic matter sources in aquatic environments: a review. Water Res 112:58–71. https://doi.org/10.1016/j.watres.2017.01.023

Djae T, Bravin MN, Garnier C, Doelsch E (2017) Parameterizing the binding properties of dissolved organic matter with default values skews the prediction of copper solution speciation and ecotoxicity in soil: heterogeneity in reactive DOM impacts soil Cu ecotoxicity. Environ Toxicol Chem 36(4):898–905. https://doi.org/10.1002/etc.3622

El Hadri H, Chéry P, Jalabert S, Lee A, Potin-Gautier M, Lespes G (2012) Assessment of diffuse contamination of agricultural soil by copper in Aquitaine region by using French national databases. Sci Total Environ 441:239–247. https://doi.org/10.1016/j.scitotenv.2012.09.070

European Food Safety Authority (EFSA), Arena M, Auteri D, Barmaz S, Bellisai G, Brancato A, Brocca D, Bura L, Byers H, Chiusolo A, Court Marques D, Crivellente F, De Lentdecker C, Egsmose M, Erdos Z, Fait G, Ferreira L, Goumenou M, Greco L et al (2018) Peer review of the pesticide risk assessment of the active substance copper compounds copper(I), copper(II) variants namely copper hydroxide, copper oxychloride, tribasic copper sulfate, copper(I) oxide, Bordeaux mixture. EFSA J 16(1). https://doi.org/10.2903/j.efsa.2018.5152

Fernández-Calviño D, Nóvoa-Muñoz JC, Díaz-Raviña M, Arias-Estévez M (2009) Copper accumulation and fractionation in vineyard soils from temperate humid zone (NW Iberian Peninsula). Geoderma 153(1–2):119–129. https://doi.org/10.1016/j.geoderma.2009.07.024

Fernández-Calviño D, Soler-Rovira P, Polo A, Díaz-Raviña M, Arias-Estévez M, Plaza C (2010) Enzyme activities in vineyard soils long-term treated with copper-based fungicides. Soil Biol Biochem 42(12):2119–2127. https://doi.org/10.1016/j.soilbio.2010.08.007

Ferreira PAA, Marchezan C, Ceretta CA, Tarouco CP, Lourenzi CR, Silva LS, Soriani HH, Nicoloso FT, Cesco S, Mimmo T, Brunetto G (2018) Soil amendment as a strategy for the growth of young vines when replanting vineyards in soils with high copper content. Plant Physiol Biochem 126:152–162. https://doi.org/10.1016/j.plaphy.2018.03.003

Flores-Álvarez JM, González I, García-de la Rosa LA, Mancilla-Peña F, Reyes-Gómez J (2011) Carbon paste electrodes modified with biosolids, soils and biocomposites utilized to study the interaction

between organic matter and copper. J Environ Manag 92(3):448–456. https://doi.org/10.1016/j.jenvman.2010.08.033

Fox J, Weisberg S (2019) An R companion to applied regression, Third Edition. Sage Publications

Fuentes M, Olaetxea M, Baigorri R, Zamarreño AM, Etienne P, Laîné P, Ourry A, Yvin J-C, Garcia-Mina JM (2013) Main binding sites involved in Fe(III) and Cu(II) complexation in humic-based structures. J Geochem Explor 129:14–17. https://doi.org/10.1016/j.gexplo.2012.12.015

Gonzaga MIS, de Matias MIAS, Andrade KR, de Jesus AN, da Cunha GC, de Andrade RS, de Santos JCJ (2020) Aged biochar changed copper availability and distribution among soil fractions and influenced corn seed germination in a copper-contaminated soil. Chemosphere 240:124828. https://doi.org/10.1016/j.chemosphere.2019.124828

Guo X, Zhu N, Chen L, Yuan D, He L (2015) Characterizing the fluorescent properties and copper complexation of dissolved organic matter in saline-alkali soils using fluorescence excitation-emission matrix and parallel factor analysis. J Soils Sediments 15(7):1473–1482. https://doi.org/10.1007/s11368-015-1113-7

Han N, Thompson ML (1999) Copper-binding ability of dissolved organic matter derived from anaerobically digested biosolids. J Environ Qual 28(3):939–944. https://doi.org/10.2134/jeq1999.00472425002800030026x

Han N, Thompson ML (2003) Impact of dissolved organic matter on copper mobility in aquifer material. J Environ Qual 32(5):1829–1836. https://doi.org/10.2134/jeq2003.1829

Harmsen J (2007) Measuring bioavailability: from a scientific approach to standard methods. J Environ Qual 36:1420–1428. https://doi.org/10.2134/jeq2006.0492

He W, Lee J-H, Hur J (2016) Anthropogenic signature of sediment organic matter probed by UV–Visible and fluorescence spectroscopy and the association with heavy metal enrichment. Chemosphere 150:184–193. https://doi.org/10.1016/j.chemosphere.2016.01.116

Helling B, Reinecke SA, Reinecke AJ (2000) Effects of the fungicide copper oxychloride on the growth and reproduction of Eisenia fetida (Oligochaeta). Ecotoxicology and Environmental Safety 46(1):108–116. https://doi.org/10.1006/eesa.1999.1880

Helms JR, Stubbins A, Ritchie JD, Minor EC, Kieber DJ, Mopper K (2008) Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnol Oceanogr 53(3):955–969. https://doi.org/10.4319/lo.2008.53.3.0955

Hudson N, Baker A, Reynolds D (2007) Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters—a review. River Res Appl 23(6):631–649. https://doi.org/10.1002/rra.1005

Huguet A, Vacher L, Relexans S, Saubusse S, Froidefond JM, Parlanti E (2009) Properties of fluorescent dissolved organic matter in the Gironde Estuary. Organic Geochem 40(6):706–719. https://doi.org/10.1016/j.orggeochem.2009.03.002

Husson F, Josse J, Lê S (2008) FactoMineR: an R package for multivariate analysis. Journal of Statistical Software 25. 10.18637/jss.v025.i01

Inamdar S, Singh S, Dutta S, Levia D, Mitchell M, Scott D, Bais H, McHale P (2011) Fluorescence characteristics and sources of dissolved organic matter for stream water during storm events in a forested mid-Atlantic watershed. J Geophys Res Biogeosci 116(G3). https://doi.org/10.1029/2011JG001735

ISO 2006 Soil quality — requirements and guidance for the selection and application of methods for the assessment of bioavailability of contaminants in soil and soil materials

Jaffé R, Cawley KM, Yamashita Y (2014) Applications of excitation emission matrix fluorescence with parallel factor analysis (EEM-PARAFAC) in assessing environmental dynamics of natural dissolved organic matter (DOM) in aquatic environments: a review.

In: Rosario-Ortiz F (ed) ACS Symposium Series. American Chemical Society, Washington, DC, pp 27–73

Jones D, Willett V (2006) Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol Biochem 38(5):991–999. https://doi.org/10.1016/j.soilbio.2005.08.012

Juang K-W, Lee Y-I, Lai H-Y, Wang C-H, Chen B-C (2012) Copper accumulation, translocation, and toxic effects in grapevine cuttings. Environ Sci Pollut Res Int 19(4):1315–1322. https://doi.org/10.1007/s11356-011-0657-3

Kader M, Lamb DT, Wang L, Megharaj M, Naidu R (2016) Predicting copper phytotoxicity based on pore-water pCu. Ecotoxicology 25(3):481–490. https://doi.org/10.1007/s10646-015-1605-7

Kalbitz K, Schmerwitz J, Schwesig D, Matzner E (2003) Biodegradation of soil-derived dissolved organic matter as related to its properties. Geoderma 113(3):273–291. https://doi.org/10.1016/S0016-7061(02)00365-8

Karimi B, Masson V, Guilland C, Leroy E, Pellegrinelli S, Giboulot E, Maron P-A, Ranjard L (2021) Ecotoxicity of copper input and accumulation for soil biodiversity in vineyards. Environ Chem Lett. https://doi.org/10.1007/s10311-020-01155-x

Kelepertzis E, Massas I, Fligos G, Panagiotou M, Argyraki A (2017) Copper accumulation in vineyard soils from Nemea, Greece. Bulletin of the Geological Society of Greece 50:2192. 10.12681/bgsg.14275

Kikuchi T, Fujii M, Terao K, Jiwei R, Lee YP, Yoshimura C (2017) Correlations between aromaticity of dissolved organic matter and trace metal concentrations in natural and effluent waters: a case study in the Sagami River Basin, Japan. Sci Total Environ 576:36–45. https://doi.org/10.1016/j.scitotenv.2016.10.068

Kinniburgh DG, Milne CJ, Benedetti MF, Pinheiro JP, Filius J, Koopal LK, Van Riemsdijk WH (1996) metal ion binding by humic acid: application of the NICA-Donnan model. Environ Sci Technol 30(5):1687–1698. https://doi.org/10.1021/es950695h

Kolbas A, Marchand L, Herzig R, Nehnevajova E, Mench M (2014) Phenotypic seedling responses of a metal-tolerant mutant line of sunflower growing on a Cu-contaminated soil series: potential uses for biomonitoring of Cu exposure and phytoremediation. Plant Soil 376(1):377–397. https://doi.org/10.1007/s11104-013-1974-8

Komárek M, Čadková E, Chrastný V, Bordas F, Bollinger J-C (2010) Contamination of vineyard soils with fungicides: a review of environmental and toxicological aspects. Environ Intl 36(1):138–151. https://doi.org/10.1016/j.envint.2009.10.005

Koopal LK, Saito T, Pinheiro JP, van Riemsdijk WH (2005) Ion binding to natural organic matter: general considerations and the NICA–Donnan model. Coll Surf A Physicochem Eng Asp 265(1):40–54. https://doi.org/10.1016/j.colsurfa.2004.11.050

Küpper H, Götz B, Mijovilovich A, Küpper FC, Meyer-Klaucke W (2009) Complexation and toxicity of copper in higher plants. I. Characterization of Copper Accumulation, Speciation, and Toxicity in Crassula helmsii as a New Copper Accumulator. Plant Physiol 151(2):702–714. https://doi.org/10.1104/pp.109.139717

Laurent C, Bravin MN, Crouzet O, Pelosi C, Tillard E, Lecomte P, Lamy I (2020) Increased soil pH and dissolved organic matter after a decade of organic fertilizer application mitigates copper and zinc availability despite contamination. Sci Total Environ 709:135927. https://doi.org/10.1016/j.scitotenv.2019.135927

Leenheer JA (1981) Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. Environ Sci Technol 15(5):578–587. https://doi.org/10.1021/es00087a010

Li S, Li M, Wang G, Sun X, Xi B, Hu Z (2019) Compositional and chemical characteristics of dissolved organic matter in various types of cropped and natural Chinese soils. Chem Biol Technol Agric 6(1):20. https://doi.org/10.1186/s40538-019-0158-z

Manceau A, Matynia A (2010) The nature of Cu bonding to natural organic matter. Geochimica et Cosmochimica Acta 74(9):2556–2580. https://doi.org/10.1016/j.gca.2010.01.027

Meeussen JCL (2003) ORCHESTRA: an object-oriented framework for implementing chemical equilibrium models. Environ Sci Technol 37(6):1175–1182. https://doi.org/10.1021/es025597s

McBride M, Sauve S, Hendershot W (1997) Solubility control of Cu, Zn, Cd and Pb in contaminated soils. Eur J Soil Sci 48(2):337–346. https://doi.org/10.1111/j.1365-2389.1997.tb00554.x

McBride MB (1989) Reactions controlling heavy metal solubility in soils. In: Stewart BA (ed) Advances in Soil Science: Volume 10. Springer, New York, NY, pp 1–56

McBride MB, Blasiak JJ (1979) Zinc and copper solubility as a function of pH in an acid soil1. Soil Sci Soc Am J 43(5):866. https://doi.org/10.2136/sssaj1979.03615995004300050009x

McKnight DM, Boyer EW, Westerhoff PK, Doran PT, Kulbe T, Andersen DT (2001) Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnol Oceanogr 46(1):38–48. https://doi.org/10.4319/lo.2001.46.1.0038

Michaud AM, Bravin MN, Galleguillos M, Hinsinger P (2007) Copper uptake and phytotoxicity as assessed in situ for durum wheat (Triticum turgidum durum L.) cultivated in Cu-contaminated, former vineyard soils. Plant Soil 298(1):99–111. https://doi.org/10.1007/s11104-007-9343-0

Michaud AM, Chappellaz C, Hinsinger P (2008) Copper phytotoxicity affects root elongation and iron nutrition in durum wheat (Triticum turgidum durum L.). Plant Soil 310(1):151–165. https://doi.org/10.1007/s11104-008-9642-0

Milne CJ, Kinniburgh DG, Tipping E (2001) Generic NICA-Donnan model parameters for proton binding by humic substances. Environ Sci Technol 35(10):2049–2059. https://doi.org/10.1021/es000123j

Milne CJ, Kinniburgh DG, van Riemsdijk WH, Tipping E (2003) Generic NICA−Donnan model parameters for metal-ion binding by humic substances. Environ Sci Technol 37(5):958–971. https://doi.org/10.1021/es0258879

Musadji NY, Lemée L, Caner L, Porel G, Poinot P, Geffroy-Rodier C (2020) Spectral characteristics of soil dissolved organic matter: long-term effects of exogenous organic matter on soil organic matter and spatial-temporal changes. Chemosphere 240:124808. https://doi.org/10.1016/j.chemosphere.2019.124808

Navel A, Martins JMF (2014) Effect of long term organic amendments and vegetation of vineyard soils on the microscale distribution and biogeochemistry of copper. Sci Total Environ 466–467:681–689. https://doi.org/10.1016/j.scitotenv.2013.07.064

Ohno T, Fernandez IJ, Hiradate S, Sherman JF (2007) Effects of soil acidification and forest type on water soluble soil organic matter properties. Geoderma 140(1):176–187. https://doi.org/10.1016/j.geoderma.2007.04.004

Oustriere N, Marchand L, Galland W, Gabbon L, Lottier N, Motelica M, Mench M (2016) Influence of biochars, compost and iron grit, alone and in combination, on copper solubility and phytotoxicity in a Cu-contaminated soil from a wood preservation site. Sci Total Environ 566–567:816–825. https://doi.org/10.1016/j.scitotenv.2016.05.091

Ouzounidou G (1995) Responses of maize (Zea mays L.) plants to copper stress—I. Growth, mineral content and ultrastructure of roots. Environ Exp Bot 35(2):167–176. https://doi.org/10.1016/0098-8472(94)00049-B

Parat C, Chaussod R, Leveque J, Dousset S, Andreux F (2002) The relationship between copper accumulated in vineyard calcareous soils and soil organic matter and iron. Eur J Soil Sci 53(4):663–670. https://doi.org/10.1046/j.1365-2389.2002.00478.x

Qiu H, Vijver MG, He E, Peijnenburg WJGM (2013) Predicting copper toxicity to different earthworm species using a multicomponent Freundlich model. Environ Sci Technol 47(9):4796–4803. https://doi.org/10.1021/es305240n

R core Team (2020) R A Lang. Environ. Stat. Comput. R Found. Stat. Comput, Vienna, Austria URL. https://www.r-project.org/

Recatalá L, Sacristán D, Arbelo C, Sánchez J (2012) Can a single and unique cu soil quality standard be valid for different Mediterranean agricultural soils under an accumulator crop? Water Air Soil Pollut 223. https://doi.org/10.1007/s11270-011-0960-0

Ren Z-L, Tella M, Bravin MN, Comans RNJ, Dai J, Garnier J-M, Sivry Y, Doelsch E, Straathof A, Benedetti MF (2015) Effect of dissolved organic matter composition on metal speciation in soil solutions. Chem Geol 398:61–69. https://doi.org/10.1016/j.chemgeo.2015.01.020

Ritsema CJ (1993) Estimation of activity coefficients of individual ions in solutions with ionic strengths up to 0.3 mol dm−3. J Soil Sci 44(2):307–315. https://doi.org/10.1111/j.1365-2389.1993.tb00454.x

Romero CM, Engel RE, D'Andrilli J, Chen C, Zabinski C, Miller PR, Wallander R (2017) Bulk optical characterization of dissolved organic matter from semiarid wheat-based cropping systems. Geoderma 306:40–49. https://doi.org/10.1016/j.geoderma.2017.06.029

Römkens PFAM, Dolfing J (1998) Effect of Ca on the solubility and molecular size distribution of DOC and Cu binding in soil solution samples. Environ Sci Technol 32(3):363–369. https://doi.org/10.1021/es970437f

Sacristán D, Viscarra Rossel RA, Recatalá L (2016) Proximal sensing of Cu in soil and lettuce using portable X-ray fluorescence spectrometry. Geoderma 265:6–11. https://doi.org/10.1016/j.geoderma.2015.11.008

Sauvé S, Cook N, Hendershot WH, McBride MB (1996) Linking plant tissue concentrations and soil copper pools in urban contaminated soils. Environ Pollut 94(2):153–157. https://doi.org/10.1016/S0269-7491(96)00081-4

Sauvé S, Dumestre A, McBride M, Hendershot W (1998) Derivation of soil quality criteria using predicted chemical speciation of Pb2+ and Cu2+. Environ Toxicol Chem 17(8):1481–1489. https://doi.org/10.1002/etc.5620170808

Sauvé S, Hendershot W, Allen HE (2000) Solid-solution partitioning of metals in contaminated soils: dependence on pH, total metal burden, and organic matter. Environ Sci Technol 34(7):1125–1131. https://doi.org/10.1021/es9907764

Sauvé S, McBride MB, Hendershot WH (1995) Ion-selective electrode measurements of copper(II) activity in contaminated soils. Arch Environ Contam Toxicol 29(3):373–379. https://doi.org/10.1007/BF00212503

Sauvé S, McBride MB, Norvell WA, Hendershot WH (1997) Copper solubility and speciation of in situ contaminated soils: effects of copper level, pH and Organic matter. Water Air Soil Pollut 100(1):133–149. https://doi.org/10.1023/A:1018312109677

Sun R, Song C, Sun Y, Wang Q, Liu H, Wang J (2020) Agricultural soil organic matters and microbiome are shaped by management and hedgerows. Agronomy 10(9):1316. https://doi.org/10.3390/agronomy10091316

Supriatin S, Terrones CA, Bussink W, Weng L (2015) Drying effects on selenium and copper in 0.01M calcium chloride soil extractions. Geoderma 255–256:104–114. https://doi.org/10.1016/j.geoderma.2015.04.021

Swift MJ, Heal OW, Anderson JM (1979) Decomposition in terrestrial ecosystems. Studies in Ecology (USA)

Tait TN, Cooper CA, McGeer JC, Wood CM, Smith DS (2016) Influence of dissolved organic matter (DOM) source on copper speciation and toxicity to Brachionus plicatilis. Environ Chem 13(3):496. https://doi.org/10.1071/EN15123

Tang J, Wang W, Yang L, Cao C, Li X (2019) Variation in quantity and chemical composition of soil dissolved organic matter in a peri-urban critical zone observatory watershed in Eastern China. Sci

Total Environ 688:622–631. https://doi.org/10.1016/j.scitotenv.2019.06.270

Temminghoff EJM, Van Der Zee SE, TM A, Keizer MG (1994) The influence of pH on the desorption and speciation of copper in a sandy soil. Soil Sci 158(6):398–408

Trevors JT, Cotter CM (1990) Copper toxicity and uptake in microorganisms. J Ind Microbiol 6(2):77–84. https://doi.org/10.1007/BF01576426

Verweij W, Glazewski R, Haan HD (1992) Speciation of copper in relation to its bioavailability. Chem Spec Bioavail 4(2):43–51. https://doi.org/10.1080/09542299.1992.11083177

Wei T, Simko V (2021) R package "corrplot": visualization of a correlation matrix. https://github.com/taiyun/corrplot. Accessed 5 Jan 2021

Weishaar JL, Aiken GR, Bergamaschi BA, Fram MS, Fujii R, Mopper K (2003) Evaluation of Specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environ Sci Technol 37(20):4702–4708. https://doi.org/10.1021/es030360x

Weng L, Temminghoff EJM, Lofts S, Tipping E, Van Riemsdijk WH (2002) Complexation with dissolved organic matter and solubility control of heavy metals in a sandy soil. Environmental Science & Technology 36(22):4804–4810.10.1021/es0200084

Xu H, Zou L, Guan D, Li W, Jiang H (2019) Molecular weight-dependent spectral and metal binding properties of sediment dissolved organic matter from different origins. Sci Total Environ 665:828–835. https://doi.org/10.1016/j.scitotenv.2019.02.186

Yang L, Hur J (2014) Critical evaluation of spectroscopic indices for organic matter source tracing via end member mixing analysis based on two contrasting sources. Water Research 59:80–89. https://doi.org/10.1016/j.watres.2014.04.018

Yeh Y-L, Yeh K-J, Hsu L-F, Yu W-C, Lee M-H, Chen T-C (2014) Use of fluorescence quenching method to measure sorption constants of phenolic xenoestrogens onto humic fractions from sediment. J Hazard Mater 277:27–33. https://doi.org/10.1016/j.jhazmat.2014.03.057

Yruela I (2009) Copper in plants: acquisition, transport and interactions. Func Plant Biol 36(5):409. https://doi.org/10.1071/FP08288

Yruela I (2005) Copper in plants. Brazilian J Plant Physiol 17(1):145–156. https://doi.org/10.1590/S1677-04202005000100012

Zhang S, Chen X-W, Ye Q, Zhang Z-T, Kong S-F, Cao C, Wang J-J (2020) Dissolved Metal(loid) concentrations and their relations with chromophoric and fluorescent dissolved organic matter in an urban river in Shenzhen, South China. Water 12(1):281. https://doi.org/10.3390/w12010281

Zsolnay A (1996) Dissolved humus in soil waters. In: Humic Substances in Terrestrial Ecosystems. Elsevier, pp 171–223

Zsolnay A, Baigar E, Jimenez M, Steinweg B, Saccomandi F (1999) Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. Chemosphere 38(1):45–50. https://doi.org/10.1016/S0045-6535(98)00166-0