Inter-Regional Variability in Environmental Availability of Fungicide Derived Copper in Vineyard Soils: An Australian Case Study

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This study determined the environmental availability of copper (Cu) in Australian vineyard soils contaminated with fungicide derived Cu residues, and investigated the soil characteristics correlated with differences in Cu availability between regions. Concentrations of 0.01 M calcium chloride extractable Cu, measured in surface soils collected from 98 vineyards in 10 different grape-growing regions of Australia, ranged from <0.1 to 0.94 mg/kg and accounted for 0.10−1.03% of the total Cu concentrations in the soils. Differences in the calcium chloride extractable Cu concentrations were related to the total Cu concentration and soil properties, including pH, clay, exchangeable K, silt, and calcium carbonate. The information generated from this study may prove useful in devising strategies to reduce the availability and toxicity of Cu in agricultural soils.

KEYWORDS: Copper; vineyard; soil; fungicide; availability; calcium chloride

INTRODUCTION

The regular use of copper-based fungicides (e.g., copper sulfate, copper oxychloride) to prevent downy mildew (*Plasmopara viticola*) on grapevines results in an accumulation of copper (Cu) in the surface soils of vineyards, which can pose a risk to the biological health of the soil. For instance, Cu concentrations in the surface soils of vineyards with histories of Cu-fungicide use have been reported to range from 130 to 1280 mg/kg in European vineyards (*1−5*), and to be generally in the range of 24 to 159 mg/kg, but as high 249 mg/kg, in Australian vineyards (*6, 7*). This is an increase from the natural background Cu concentrations in soils, which were typically less than ∼20 mg/kg. The accumulated Cu in vineyard soils is not expected to cause phytotoxicity in mature grapevines because they tend to have deep root systems that extend below the surface soils and, thus, below most of the accumulated Cu (*8*), although Cu may be toxic to shallow rooted plants used for cover crops, or in the case of changed land use (*9*). The potential impacts on soil organisms (e.g., earthworms, microorganisms), and implications for long-term soil fertility, may be of concern in established vineyards. For example, field studies have found correlations between increased Cu concentrations (> 80 mg/kg total Cu) in vineyard soils and reduced earthworm abundance (*10*). Likewise, Diaz-Ravina et al. (*11*) reported adverse effects to the microbial community of vineyard soils with > 100 mg/kg total Cu. The Cu accumulated in vineyard soils can also pose a risk to nearby waterways if it is transported off-site via runoff or leaching (*12*).

A major challenge in assessing the risks of Cu in agricultural soils is that much of the Cu present is not considered available to organisms or for off-site transport because it binds strongly to minerals and organic matter in soil (*13, 14*). In addition to the total Cu concentration of the soil, the chemical availability or "environmental availability" of Cu in the soil is controlled by soil factors such as pH, CEC, clay content, and organic matter content which influence the extent and strength of Cu adsorption in soil (*13, 15, 16*). While many different soil extraction and analysis techniques have been developed to estimate the environmental availability of Cu and other trace elements in soils, neutral salt extractants have often been reported to provide the most useful indication of metal phytoavailability (*17*). The calcium chloride soil extraction technique, a neutral salt extraction, is a widely used and internationally recognized technique (*13, 15*). Calcium chloride ($CaCl_2$) extractable Cu concentrations in vineyard soils have been reported in several viticultural regions of the world, including Southern France (0.10−9.24 mg/kg, $n = 22$) (*3*); Roujan region of France (1.00−39.0 mg/kg, $n = 6$) (*4*); Slovenia (0.04−0.07 mg/kg, $n = 22$) (*18*); Czech Republic (0.53−0.70 mg/kg, $n = 5$) (*19*); and Brazil (0.20−5.00 mg/kg, $n = 21$) (*20*). Currently, information on the availability of Cu in Australian vineyard soils is limited to a study of 14 vineyards within four

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regions of Victoria by Pietrzak and McPhail (*7*). While CaCl₂ extractable Cu concentrations were not determined, the magnesium chloride (MgCl₂) extractable Cu concentrations (a similar measurement of Cu availability) were reported to be in the range of 0.40−2.90 mg/kg (*7*).

To inform future policy decisions on the management of Cu-based fungicide use, there is a need to assess the risks to long-term soil fertility posed by the use of Cu-based fungicides in different viticultural regions. However, as the availability of Cu varies greatly across soils with different physical-chemical properties, it is difficult to make generic conclusions regarding the likely risks posed. This presents a particular challenge from an Australian perspective, as there are approximately 60 different viticultural production regions with a wide variety of soil types (e.g., in terms of pH, texture, organic matter content). The aims of the current study were to determine the environmental availability of fungicide derived Cu in the surface soils of vineyards in 10 different viticultural regions of Australia, and to investigate the factors (e.g., soil characteristics) correlated with differences in Cu availability between vineyards from different regions. For the purposes of this study, the environmental availability of the Cu was characterized as the 0.01 M CaCl₂ extractable soil concentration (*21, 22*). This technique was selected over other approaches, such as EDTA soil extraction, other weak salt extractants (e.g., 0.1 M NaNO₃, 1 M NH₄NO₃), and free Cu^{2+} measurements, as it represents an acceptable intermediate for determining freely available Cu in terms of ease of use, suitability for a range of different soils, and applicability to biological response (*13*).

MATERIALS AND METHODS

Soils. The soil samples used in this study had previously been collected in a survey of total Cu accumulation in 98 Australian vineyards (between April and December 2004), as described by Wightwick et al. (*6*). The samples were collected from between 7 and 13 vineyards in each of 10 different viticultural regions across Australia, namely, Murray Valley, Victoria (Vic)/New South Wales (NSW); Southern Highlands, Vic; Riverland, South Australia (SA); Barossa, SA; Riverina (NSW); Mudgee (NSW); Swan Valley, Western Australia (WA); Margaret River (WA); and Tasmania. The vineyards surveyed were cultivating grapes for wine production, with the exception of some vineyards in the Murray Valley region which were cultivating for dried grape production; information on the varieties of grapes was not collected. The regions included in the survey were selected to represent a range of growing conditions (e.g., climate, soils) and Cu-fungicide use regimes, while the vineyards surveyed in each region were chosen on the basis that they had the greatest potential for having high accumulated Cu concentrations (i.e., operated as a vineyard for at least 30 years with an established history of Cu-fungicide use, with heavy textured soils likely to have high Cu sorption). In each vineyard, a composite surface soil sample (0−10 cm), comprising 10 subsamples, was collected from the under vine area within 30 cm of the vine trunk. Moreover, to act as "reference" sites, two or three composite soil samples were collected from areas of remnant vegetation in each region that had not received any artificial Cu inputs. Sample preparation, total Cu and other elemental concentrations (determined in aqua regia digests), along with physical-chemical soil properties (predicted using mid-infrared with partial least-squares analysis) had been previously reported as detailed in Wightwick et al. (*6*) (see also the Supporting Information). Information on the history of Cu-based fungicide use (i.e., years of Cu use and typical rate and number of applications per year) had previously been obtained by surveying the individual vineyard managers, as reported in Wightwick et al. (*6*) (see also the Supporting Information).

0.01 M CaCl₂ Soil Extraction Procedure. Soluble Cu, and other elements, were extracted from the soil samples based on the 0.01 M CaCl₂ extraction method of Houba et al. (*21*). This involved weighing 4 g of air-dry soil into a 50 mL polythene centrifuge tube to which 40 mL of

Analar 0.01 M CaCl₂ dihydrate (BDH, West Chester, PA) extraction reagent was added to achieve a soil to extraction solution ratio of 1:10. The tubes containing the soil and extraction solution were then mechanically shaken in an end-over-end rotating shaker at 30 rpm for 2 h at room temperature (∼20 °C). The tubes were centrifuged at a relative centrifugal force (RCF) of 2100*g* for 10 min, after which 25 mL of the clear supernatant was transferred to a separate polythene tube and 0.25 mL of 1 M HCl was added to each tube to acidify the sample (0.1% HCl). As recommended by Houba et al. (*21*), the supernatant was not filtered, as the filter could absorb extracted metals or introduce contaminants into the filtrate; however, as a consequence, it is possible that some of the metals/elements in the soil extracts were bound to colloidal material. The soils were extracted in three separate batches with each extraction batch including a 0.01 M CaCl₂ reagent blank sample and samples of four reference soils (ISE 953, ISE 967, ISE 968, ISE 990) from the Wageningen Evaluating Programs for Analytical Laboratories (WEPAL) (Wageningen University, Netherlands). There were a total of four replicates for the reagent blank and each of the four reference soils. In addition, 7% of the samples (randomly selected) were processed in duplicate, with at least two duplicate samples being included in each extraction batch.

Analysis of 0.01 M CaCl₂ Soil Extracts. Copper and other elements in the 0.01 M CaCl₂ soil extracts were analyzed by inductively coupled plasma emission spectrometry (ICP-ES) using a SPECTROFLAME-EOP instrument (Spectro Analytical Instruments GMBH, Kleve, Germany). Each analytical batch included standard calibration solutions (every 20−25 injections) to check for instrumental drift and continuing check verification standards (CCV1A and/or CCV1B) (High Purity Standards, Charleston, SC) to assess the accuracy of the instrument. Instrument limits of determination (LOD) for ICP-ES were as follows: Al, 0.014; B, 0.012; Cd, 0.0001; Cr, 0.011; Cu, 0.012; Fe, 0.015; K, 0.691; Mg, 0.004; Mn, 0.0002; Na, 0.037; Ni, 0.002; P, 0.002; Pb, 0.002; S, 0.008; and Zn, 0.005 mg/L. The method detection limits (MDL) were based on instrumental limits of determination (LOD), which in turn were based on mean + 3 standard deviations for the eight extraction blanks (*23*), and were 10 × LOD values. Concentrations of Cu in the CaCl₂ extracts were confirmed by inductively coupled plasma mass spectrometry (ICP-MS) using an Ultramass ICP-MS instrument (Varian, Australia) (MDL of 0.10 mg/kg). The concentrations of Cu determined from ICP-MS analysis have only been reported where concentrations were found to be between 0.10 and 0.12 mg/kg.

Where higher reliability consensus values (based on at least 16 results, CV < 25%) were available for the WEPAL reference soils (typically only for K, Mg, and Na), the recoveries of the elements were generally within 90−110% of the stated value. This indicates that the 0.01 M CaCl₂ soil extractions provided acceptable extraction efficiency for K, Mg, and Na. Recoveries for trace elements for which only low reliability indicative (based on 8−16 results, CV 25−50%) or informative values (based on < 8 results, CV > 50%) have been stated (Al, B, Cd, Cr, Cu, Fe, Ni, Pb, S, and Zn) were generally poorer (e.g., outside the range of 90−110%). However, recoveries for these elements were still within the same order of magnitude of the stated value. For example, in relation to Cu, the median and median of absolute deviation (MAD) values stated by WEPAL for the standard reference were as follows: ISE 953 (median 0.19 mg/kg, MAD 0.013 mg/kg, *n* = 6), ISE 967 (median 0.30 mg/kg, MAD 0.089 mg/kg, *n* = 10), ISE 968 (median 0.12 mg/kg, MAD 0.021 mg/kg, *n* = 7), and ISE 990 (median 0.35 mg/kg, MAD 0.094 mg/kg, *n* = 4). The Cu concentrations we determined in the four replicates for each of these reference soils were as follows: ISE 953 (< 0.12 mg/kg), ISE 967 (mean 0.85 mg/kg, CV 19%), ISE 968 (mean 0.26 mg/kg, CV 29%), and ISE 990 (1.09 mg/kg, CV 20%). While the recoveries of Cu (and other trace metals) from the WEPAL reference soils, based on the low reliability informative values stated, were less than ideal, the fact that the recoveries of K, Mg, and Na were within acceptable limits indicates that the 0.01 M CaCl₂ soil extraction step was of an acceptable standard (i.e., provided acceptable extraction efficiency). Other analytical quality control checks (i.e., CCV standards, drift standards) were within acceptable limits, demonstrating that the analytical step of the 0.01 M CaCl₂ determinations was conducted to an acceptable standard.

Consequently, the 0.01 M CaCl₂ trace metals data generated can be considered valid, while recognizing the limitations to the quality control

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Table 1. Total and CaCl$_2$ Extractable Cu Concentrations in Australian Vineyard and Reference Site Soils[a]

| | | | total Cu (mg/kg)[b] | | | | CaCl$_2$ extractable Cu | | | | | | | |
| | | | | | | | mg/kg | | | | percentage of total Cu[c] | | | |
		n	mean	min	max	CV	mean	min	max	CV	mean	min	max	CV
Murray Valley	V	10	66	34	128	44	0.22	0.12	0.34	38	0.37	0.17	0.84	53
	R	2	13	7.0	19.0	63	<0.10	<0.10	<0.10	ND	ND	ND	ND	ND
Southern Highlands	V	9	87	30	223	74	0.42	0.06	0.94	71	0.61	0.40	1.03	35
	R	2	9.0	7.0	11.0	34	<0.10	<0.10	<0.10	ND	ND	ND	ND	ND
Riverland	V	10	78	55	119	26	0.24	0.10	0.42	43	0.30	0.19	0.46	28
	R	2	24	18	30.0	35	0.13	<0.10	0.16	39	ND	ND	ND	ND
Barossa	V	10	49	24	87.0	38	0.20	0.10	0.40	49	0.41	0.26	0.56	26
	R	2	16	14	19.0	20	<0.10	<0.10	<0.10	ND	ND	ND	ND	ND
southeast, SA	V	10	81	39	150	50	0.13	<0.10	0.23	45	0.17	0.12	0.21	16
	R	2	2.0	2.0	2.00	15	<0.10	<0.10	<0.10	ND	ND	ND	ND	ND
Riverina	V	10	94	60	136	37	0.29	0.12	0.80	65	0.30	0.20	0.50	31
	R	2	13	12	15.0	17	<0.10	<0.10	<0.10	ND	ND	ND	ND	ND
Mudgee	V	9	57	36	81.0	32	0.18	<0.10	0.41	66	0.37	0.25	0.48	53
	R	2	6.0	4.0	9.00	52	<0.10	<0.10	<0.10	ND	ND	ND	ND	ND
Swan Valley	V	10	21	6.0	39.0	53	<0.10	0.01	0.19	51	0.58	0.26	1.00	42
	R	2	12	12	13.0	8.0	<0.10	<0.10	<0.10	ND	ND	ND	ND	ND
Margaret River	V	13	26	11	52.0	50	<0.10	<0.10	0.12	70	0.23	0.23	0.23	0
	R	2	1.0	1.0	1.00	35	<0.10	<0.10	<0.10	ND	ND	ND	ND	ND
Tasmania	V	7	49	19	93.0	51	0.13	<0.10	0.25	68	0.38	0.10	0.53	45
	R	3	26	6.0	40.0	68	<0.10	<0.10	<0.10	ND	ND	ND	ND	ND

[a] V, Vineyard sites; R, reference sites; CV, coefficient of variation (%); ND, not determined/calculated. [b] Data from Wightwick et al. (6). [c] For vineyards $n = 10$ except for Southern Highlands, southeast, SA ($n = 8$); Mudgee, Swan Valley, Tasmania ($n = 6$); Margaret River ($n = 1$).

applied as dictated by the low reliability of the stated values for the standard reference soils used. Data for Mn and P have not been reported, as recoveries were consistently much greater than the stated WEPAL values, including where consensus values were available. The results for the duplicate samples for the most part were within acceptable limits (variation <10 to 15%), noting that trace elements (e.g., Al, B, Cd, Cr, Cu, Ni, Pb, Zn) were not always above detectable limits in the samples randomly selected as duplicates. Concentrations of Cu could be detected in four of the seven duplicate samples analyzed, with the CVs ranging from 17 to 36%. Other than low concentrations of K (~3 mg/kg), no elements were detected in the 0.01 M CaCl$_2$ extraction blanks. Sample concentrations of elements have not been adjusted to reflect variation in their respective recoveries from the reference soils.

Data Analysis. Data were analyzed using SPSS version 17.0 (XL Statistics, Chicago, IL) and XLStatistics 08.05.12, XLent Works, Australia. The data did not conform to the assumptions associated with parametric statistical methodologies, and therefore, the Kruskal−Wallis one-way ANOVA and Mann−Whitney U tests were used to determine significant differences in CaCl$_2$ Cu availability between regions ($\alpha = 0.05$). Where necessary, the Bonferroni correction was used for multiple post hoc comparisons. The backward stepwise approach to multiple regression was used to determine factors that were significantly contributing to the CaCl$_2$ extractable Cu concentrations, including Cu-fungicide use history (typical Cu use per year, years of Cu use), physical-chemical soil properties (pH, particle size analysis, CEC, exchangeable cations, total organic C, CaCO$_3$, EC), and total Cu concentration. The final model containing only those predictors that were found to have a significant effect on modeled outcomes ($\alpha = 0.05$).

RESULTS

Concentrations of CaCl$_2$ extractable Cu in the vineyard soils varied from <0.1 to 0.94 mg/kg (**Table 1**). Calcium chloride extractable Cu was not detected in the soil from the reference sites, with the exception of one of the reference sites in the Riverland (0.16 mg/kg). The percentage of CaCl$_2$ extractable Cu relative to the total Cu concentration in the vineyard soils varied from 0.10 to 1.03% (**Table 1**), excluding sites in which the extractable Cu concentrations were below the limit of detection (<0.1 mg/kg). Concentrations of CaCl$_2$ extractable Al, B, Cd, Fe, Ni, and Zn in vineyard site soils were generally found to be similar to, or less

than, those of their respective reference sites in each region (data not shown). Chromium and Pb concentrations in the CaCl$_2$ soil extracts were all below the LOD of 0.1 mg/kg for Cr and 0.02 mg/kg for Pb.

There were statistically significant differences ($P < 0.001$) between the regions for both CaCl$_2$ extractable Cu concentration and the percentage of CaCl$_2$ extractable Cu (relative to total Cu) (**Table 1**). In relation to the concentration of CaCl$_2$ extractable Cu, the Southern Highlands region was significantly ($P < 0.01$) higher than all regions other than Mudgee, Riverina, and Murray Valley. The Swan Valley region had significantly ($P < 0.01$) lower CaCl$_2$ extractable Cu concentrations than the Southern Highlands, Mudgee, Riverina, and Riverland regions. The Swan Valley and Southern Highlands regions were found to have significantly ($P < 0.01$) higher percentages of CaCl$_2$ extractable Cu than the southeast, SA, Riverina, and Riverland regions. The southeast, SA region had significantly ($P < 0.05$) lower percentage of CaCl$_2$ extractable Cu than all other regions.

The CaCl$_2$ extractable Cu concentration of the vineyard soils was found to increase ($R^2 = 0.48$, $P < 0.01$, $n = 75$) with increasing total Cu concentration (**Figure 1**). When data from the Murray Valley and Tasmania (where no significant relationships were found) and southeast, SA ($R^2 = 0.75$, $P < 0.01$, $n = 8$) (for which the slope of the regression differed) were excluded from the regression, total Cu concentration could explain 67% of the variation ($P < 0.001$, $n = 51$) in the CaCl$_2$ extractable Cu concentrations (**Figure 1**). When all samples were considered, no significant relationships ($R^2 < 0.20$, $P > 0.05$) were found between CaCl$_2$ extractable Cu concentration and years of Cu use, typical annual rate of application (kg/ha), and total estimated Cu use (years of Cu use × annual Cu application). In relation to total estimated Cu use, analysis using Student's t test, found that the slopes of the linear regressions for the Southern Highlands and Mudgee regions (group 1) ($R^2 = 0.47$) were significantly ($P = 0.007$) steeper than those of the Tasmania, Riverland, and Swan Valley regions (group 2) ($R^2 = 0.64$) (**Figure 2**). No significant relationships
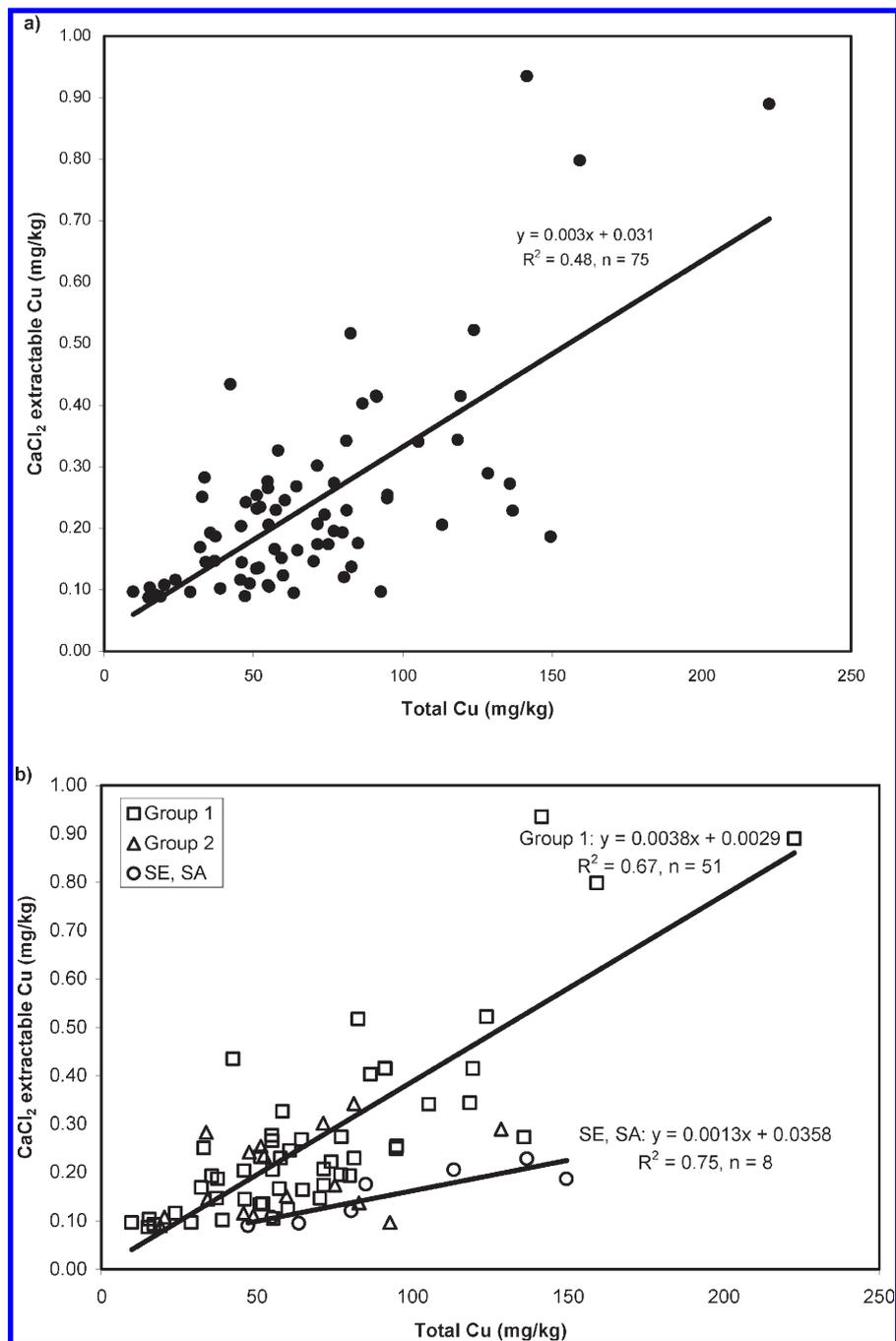
Figure 1. Relationship between $CaCl_2$ extractable Cu concentration and total Cu concentration in Australian vineyard soils. (**a**) Data pooled from all regions. (**b**) Data for vineyards in different regions. Group 1: Barossa, Mudgee, Riverina, Riverland, Yarra Valley, and Swan Valley. Group 2: Murray Valley, Tasmania. SE, SA: southeast, SA.

between $CaCl_2$ extractable Cu concentration and total estimated Cu use were found for the remaining regions (Riverina, Barossa, southeast, SA, and Murray Valley).

To determine the influence of soil properties on Cu availability, we ran a multiple stepwise (backward) regression using available data on the main physical-chemical soil properties ($CaCO_3$, CEC (and exchangeable cations), EC, pH, total organic C, clay, silt, and sand, along with total Cu (data previously presented in ref 6). It was found that total Cu, pH, clay, silt, exchangeable K, and $CaCO_3$ could explain 70% of the variation in the $CaCl_2$ extractable Cu concentrations (eq **1**). Based on the sums of squares, total Cu (0.99), pH (0.15), clay (0.14), and exchangeable K (0.12) were the greatest contributors to the regression. The data on the physical-chemical soil properties used in the regression analysis is provided as Supporting Information.

$$CaCl_2 \; Cu \; (mg/kg) = 0.382 + 0.003 \; total \; Cu + 0.034 \; CaCO_3$$
$$+ 0.107 \; exchangeable \; K - 0.062 \; pH - 0.10 \; clay$$
$$+ 0.004 \; silt \; R^2 = 0.70, \quad P < 0.001, \quad n = 75 \quad (1)$$

Analysis by simple linear regression found only a weak relationship ($R^2 = 0.06$, $P = 0.02$) between pH and the concentration of $CaCl_2$ extractable Cu. A significant relationship ($R^2 = 0.39$, $P < 0.001$, $n = 29$) was found between pH and $CaCl_2$ extractable Cu concentration when data for neutral to acidic soils (pH < 7) were analyzed separately (excluding data from the Swan Valley and Tasmania regions). No relationship was found between pH and
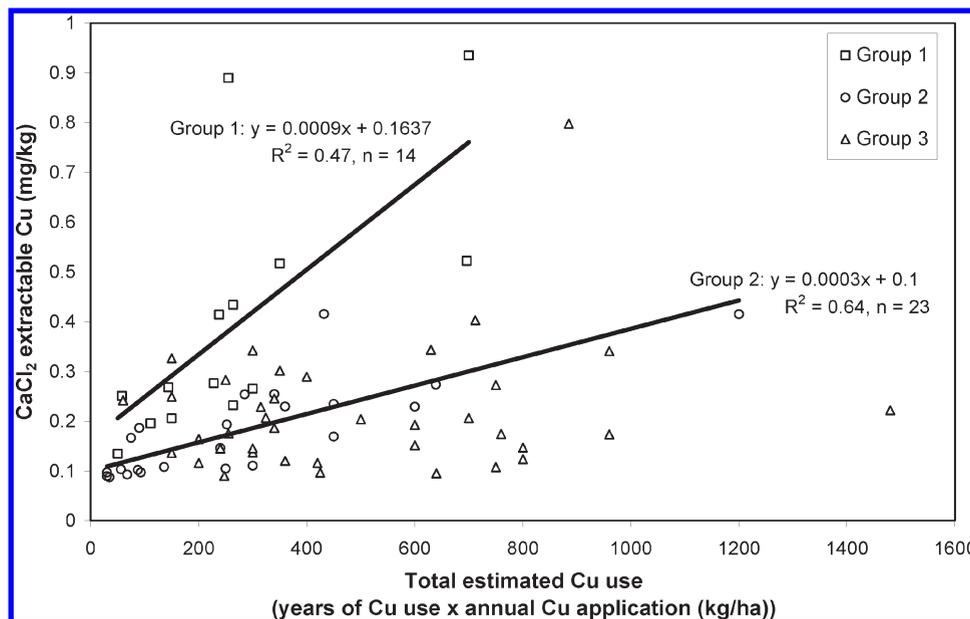
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Figure 2. Relationship between $CaCl_2$ extractable Cu concentration and total Cu-fungicide use (years of Cu use $\times$ annual Cu application kg/ha) for vineyards in different regions of Australia. Group 1: Mudgee, Southern Highlands (n = 14). Group 2: Tasmania, Riverland, Swan Valley (n = 23). Group 3: Riverina, Barossa, southeast, SA, Murray Valley (n = 38).
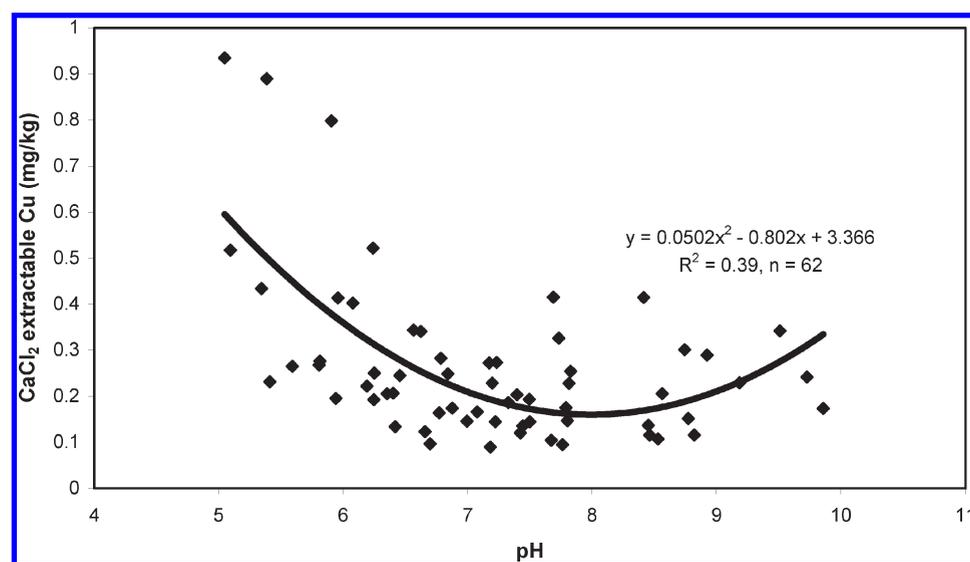

Figure 3. Relationship between $CaCl_2$ extractable Cu concentration and the pH of Australian vineyard soils.

$CaCl_2$ extractable Cu concentration for the neutral to alkaline soil (pH > 7) (n = 33). Using a polynomial regression model, a significant relationship ($R^2 = 0.39$, $P < 0.01$, $n = 62$) (**Figure 3**) was found between soil pH and the concentration of $CaCl_2$ extractable Cu (excluding data from the Swan Valley and Tasmania regions). Analysis of the neutral to alkaline (pH > 7) soils from the Barossa, southeast, SA, and Murray Valley regions (n = 28) found a significant relationship between $CaCO_3$ and the concentration of $CaCl_2$ extractable Cu ($R^2 = 0.37$, $P < 0.01$).

DISCUSSION

Currently, there is no published information on the concentrations of $CaCl_2$ extractable Cu in Australian vineyard soils with which to directly compare the results from the present study. However, the $CaCl_2$ extractable Cu concentrations (< 0.1−0.94 mg/kg, 0.10 and 1.03% of the total Cu concentration) found in this study are within a similar range to the $MgCl_2$ extractable Cu

concentrations (a similar measurement of Cu availability) (0.4−2.9 mg/kg, 0.11−1.16% of the total Cu concentration) reported from 14 vineyards within four different regions of Victoria (Australia) (*7*). Calcium chloride extractable Cu concentrations in vineyard soils have been reported in other regions of the world, where like in this study concentrations have, for the most part, been reported to be < 1 mg/kg, and to represent < 1%, and often < 0.5%, of the total Cu concentration in the soil. Even in Brazil, where very high concentrations of accumulated Cu (1214−3216 mg/kg total Cu) have been reported in vineyard soils, the $CaCl_2$ extractable Cu still only represented 0.1−0.9% of the total Cu (*20*). This supports the theory that Cu has low availability due to its high affinity to clay and organic matter in soil (*24*). Considerably higher $CaCl_2$ extractable Cu concentrations (up to 30 mg/kg) have been reported in acidic vineyard soils. For example, Brun et al. (*3*) found acidic vineyard soils (pH 4.5−5.5) to have much higher and greater variability in $CaCl_2$ Cu

availabilities (0.4−9.24 mg/kg, 1.05−7.24% of total Cu) than neutral to alkaline soils (pH 6.5−8.6) (0.10−0.56 mg/kg, 0.13−0.54% of total Cu). Similarly, relatively high concentrations of ammonium acetate (NH_4OAc) extractable Cu concentrations (which provides a similar indication of availability as $CaCl_2$ extractable Cu) (0.1−30.3 mg/kg, mean 4.9 mg/kg) have been reported in a recent study of 170 acidic vineyard soils (pH 4.0−7.9, mean 5.6). These studies highlight the strong influence pH has on controlling the availability of Cu in soil.

While not covering all of the potential Cu contamination scenarios in Australia, the 10 viticultural regions included in this study have provided a relatively good representation of the environmental availability of Cu in vineyard soils for the major grape growing areas of Australia. This is in terms of both the geographical diversity of the regions included, which incorporated five different states of Australia, and the diversity of soil types surveyed, for example, the pH of the vineyard soils varied from 5−9.8. It is noted, however, that the vineyards selected for the study in each region where chosen on the basis that they had long histories of Cu-based fungicide use (at least 30 years) and soils with higher Cu adsorption potential (e.g., clayey soils) for the region.

Significant differences in the environmental availability of Cu between regions (**Table 1**) were expected, as vineyards in (and within) different regions have different Cu-fungicide use histories (e.g., years of use, rates of application) and thus different total Cu concentrations in the soils. Likewise, the vineyards in different regions have different physical-chemical soil properties which are known to influence the adsorption and thus the exchangeability of Cu in soil (e.g., pH, CEC, organic matter, clay) (*5*, *16*). That fact that the highest concentrations (0.06−0.94 mg/kg) and percentages of $CaCl_2$ extractable Cu (0.40−1.03%) were found in the Southern Highlands region is most likely because of the combination of relatively high total Cu concentrations in the soil (30−223 mg/kg) (**Table 1**) and the acidic nature of these soils (pH 5.1−6.3). This because it is known that the availability of Cu increases with increasing total Cu concentration and decreasing soil pH (particularly < pH 6) (*16*). The vineyard soils from the Swan Valley region were also relatively acidic (pH 5.1−7.0), which could explain why these soils had high percentages of $CaCl_2$ extractable Cu (0.26−1.00%) despite having the lowest $CaCl_2$ extractable (< 0.10−0.19 mg/kg) and total Cu concentrations (6−39 mg/kg). Despite having relatively high total Cu concentrations (39−150 mg/kg), the vineyard soils from the southeast, SA region had significantly ($P < 0.05$) lower percentages $CaCl_2$ extractable Cu (0.12−0.21%) than all other regions. This is most likely because of the combination of the neutral to slightly alkaline nature (pH 7.2−8.8) and relatively high clay content (18.4−36.3%) and CEC (11.1−35.9 cmol$_c$/kg) contributing to the strong adsorption of Cu in these soils.

As expected, regression analysis found that the total Cu concentration of soil could explain much of the variation (48%) in the $CaCl_2$ extractable Cu concentration across all the soil samples (**Figure 1**). This relationship was even stronger when data from the Murray Valley, Tasmania, and southeast, SA regions were excluded ($R^2 = 0.67$). While based on only a small sample size ($n = 8$), the relationship between $CaCl_2$ extractable Cu and total Cu was the strongest in the southeast, SA region, explaining 75% of the variation (**Figure 1**). Interestingly, the slope of the relationship for the southeast, SA is considerably flatter than the relationship based on data for all the other regions (except Murray Valley and Tasmania) (**Figure 1**). Once again, this indicates that Cu has adsorbed more strongly to the soils from this region due to the soils being neutral to slightly alkaline and having high clay content and CEC. The fact that a significant

relationship with total Cu was not found in the Murray Valley and Tasmania regions is unclear. In the case of Tasmania, it may be due to insufficient spread in the data because of the low total Cu concentrations (i.e., only one of the six soils had total Cu > 52 mg/kg) and therefore low $CaCl_2$ extractable Cu concentrations (**Table 1**). In relation to the Murray Valley region, perhaps the poor relationship with total Cu is an artifact of the $CaCl_2$ extraction being unsuitable for the alkaline nature of these soils. It is recognized that the $CaCl_2$ extraction method may be less suited to alkaline soils, as the $CaCl_2$ solution may misrepresent the natural ionic composition of the soil solution, which tends to be more Na dominated in alkaline soils, resulting in an underestimate of Cu availability (*25*).

The strong relationships found between $CaCl_2$ extractable Cu and total Cu were not unexpected, as several other studies have found that the total Cu concentration of the soil has a strong influence on the available Cu concentration (*3*, *5*, *16*, *26*, *27*). For example, in assessing 170 vineyard soils, Fernandez-Calvino et al. (*5*) found that the total Cu concentration of the soil could explain 52% of the variation in the NH_4OAc extractable Cu concentrations (which is similar to $CaCl_2$ extractable Cu). The findings from Fernandez-Calvino et al. (*5*) compare well with this study; however, the relationships from both studies indicate that a considerable proportion (around 30−50%) of the variation is still unexplained by differences in total Cu concentrations. This indicates that other factors, most likely physical-chemical soil properties such a pH and CEC, which influence the adsorption and exchangeability of Cu in soil are likely to be having a significant influence on the availability of Cu.

As the total Cu concentration in the vineyard soils is influenced by the use of Cu-fungicides (*6*), it is reasonable to consider that the concentration of $CaCl_2$ extractable Cu would also be related to the history of Cu-fungicide use in the vineyards. However, no relationships were found between $CaCl_2$ extractable Cu concentration and years of Cu use, typical annual rate of application (kg/ha), and total estimated Cu use (years of Cu use × annual Cu application). This may well be due to inaccuracies in the Cu-fungicide usage data on which the regression analysis was based, as this data was self-reported by the individual vineyard managers, and the types, rates, and annual applications of Cu-fungicides used in individual vineyards are likely to have varied considerably between different years (e.g., due to disease pressure, different vineyard management regimes). Thus, it is difficult to obtain a robust estimate of the total amount of Cu that had been applied to each of the vineyard soils studied. Nonetheless, when the data from the Tasmania, Riverland, and Swan Valley regions were pooled together, total estimated Cu usage could explain 64% of the variation in $CaCl_2$ extractable Cu concentrations. Similarly, a relationship using data from the Southern Highlands and Mudgee regions could explain 47% of the variation (**Figure 2**). Interestingly, the slope of the Southern Highlands/ Mudgee regression was found to be steeper than that of the Tasmania/Riverland/Swan Valley grouping (**Figure 2**). The differences in the rate at which the available Cu concentration increased with increasing Cu usage may be indicative of differences in the extent of Cu aging, where added Cu can become increasingly strongly bound within the mineral component of the soil over time (*28*). The steeper increases in $CaCl_2$ extractable Cu with increasing Cu use seen in the Southern Highlands and Mudgee regions may have been due to these aging effects occurring to a lesser extent, due to the acidic nature of the soils (pH 5.1−6.4), contributing to the weaker sorption of Cu to soil solid phases (*24*). That no significant relationships with total estimated Cu use could be found for the Riverina, Barossa, southeast, SA, and Murray Valley regions is most likely an

artifact of inherent inaccuracies in Cu-fungicide usage data reported by the vineyard managers.

The CaCl$_2$ extractable Cu data were analyzed by multiple stepwise regression to determine the physical-chemical soil properties which best explained the variation between vineyards, in order to gain a further understanding of the key factors influencing the environmental availability of Cu in the soils. The multiple stepwise regression analysis found that total Cu, pH, clay, silt, exchangeable K, and CaCO$_3$ could explain 70% of the variation in the CaCl$_2$ extractable Cu concentrations (eq **1**). Total Cu, pH, clay, and exchangeable K were identified as the greatest contributors to the regression. It is known that Cu adsorbs strongly to clay minerals in soil, with Cu bound in this manner considered to be largely unavailable (*24*). The inclusion of clay content in the regression was therefore expected, as it represents the capacity for the soil to adsorb Cu. Much of the Cu in soil (around 20−50%) is known to be less strongly bound to organic matter and thus considered reactive or labile. The desorption of organically bound Cu is known to be closely related to soil pH, as this controls the magnitude of the negative charge on the surface of organic matter and thus cation exchange processes (*5, 7, 24*). This explains why pH and exchangeable K (as a component of total CEC) were identified as important factors in the regression. Similarly, CaCO$_3$ plays a role in Cu desoprtion and cation exchange processes, as in alkaline soils Cu is known to form complexes with carbonates in the soil solution, thus reducing the adsorption of Cu to the solid phase of the soil (*24*). The finding from this study agrees well with other similar relationships reported in the literature (*3, 5, 16, 26, 27*). For example, Brun et al. (*3*) found that, in 29 vineyard soils, total Cu and pH could explain 67% of the variation in the CaCl$_2$ extractable Cu concentrations. Similarly, based on a large data set of 353 soils (nonvineyard soils), Sauve et al. (*16*) reported a significant regression relationship between soluble Cu concentration and pH, total Cu, and organic matter content ($R^2 = 0.61$, $P < 0.001$). More recently, Fernandez-Calvino et al. (*5*) found that 66% of the variation in NH$_4$OAc extractable Cu concentrations could be explained by total Cu, total C, silt, Fe bound to organic matter, CEC, and pH based on data from 170 vineyard soils.

While considered significant contributors in their own right, it is recognized that there are some interactions between the physical-chemical soil properties included in the regression model. For instance, clay content and exchangeable K ($R^2 = 0.58$) were closely related. Likewise, pH was associated with exchangeable K ($R^2 = 0.37$) and to a lesser extent CaCO$_3$ ($R^2 = 0.27$), further highlighting the importance of pH in controlling Cu availability. While pH was found to be the most significant physical-chemical soil property influencing Cu availability, a simple linear relationship could not be found between pH and the concentration of CaCl$_2$ extractable Cu. However, when the data for neutral to acidic soils (pH < 7) were analyzed separately, 39% of the variation was able to be explained by differences in soil pH. No such relationship could be found for the neutral to alkaline (pH > 7) soils. Interestingly, when a polynomial regression model was fitted to the whole data set (excluding data from the Swan Valley and Tasmania regions, as the CaCl$_2$ Cu concentrations were mostly close to the LOD of 0.1 mg/kg), 39% of the variation in the percentage of CaCl$_2$ extractable Cu concentration could be explained by pH (**Figure 3**). The fitted model suggesting a trend where the percentage of CaCl$_2$ extractable Cu is lowest between ∼pH 7.5 and 8.5 and then increases sharply as pH drops below pH 7.5. As pH rises above pH 8.5, the CaCl$_2$ extractable Cu concentration appears to rise slightly or plateau. It is noted however that there is uncertainty around the increase in Cu availability with increasing alkalinity, as only 13 out of the

62 data points were above pH 8. While fitting this polynomial model does not improve the regression for the neutral to acidic soils, it does enable the neural to alkaline soils to be considered in a single model.

The relationships with pH are consistent with other studies that have also reported that Cu availability is generally lowest in neutral soils (pH ∼ 7) and increases with increasing soil acidity and alkalinity (pH > 8) (*24, 29, 30*). For example, Burton et al. (*30*) also demonstrated a strong influence of pH, with the soil−liquid partitioning coefficient (K_D) for Cu reported to increase from 11.4 L/kg at pH 2.7 to 196 L/kg at pH 5.0. The curve below pH 7 (**Figure 3**) can be explained by the extent to which Cu forms strong inner-sphere complexes (specific adsorption) with organic matter and the surface of clay minerals. Adsorption decreases as the soil becomes more acidic (*24, 29, 30*). The magnitude of the negative charge on the surface of organic matter and oxides also decreases with more acidic pH, resulting in a decrease in the extent to which Cu is nonspecifically sorbed to solid phases in the soil (*24, 29, 30*). The increase in Cu availability above pH > 8 (**Figure 3**) can be explained by the formation of Cu complexes with carbonate and hydroxy groups and dissolved organic matter which in turn reduces Cu sorption to the soil solid phase (*24, 29, 30*). The results from this study also highlighted the influence of carbonates on Cu availability. When data related to the neutral to alkaline (pH > 7) soils from the Barossa, Murray Valley, and southeast, SA regions were considered, the concentration of CaCl$_2$ extractable Cu was found to increase with increasing CaCO$_3$ content of the soil ($R^2 = 0.37$). Increases in CaCl$_2$ extractable Cu with decreasing pH will most likely correspond to an increase in toxicity to soil organisms as the available Cu is expected to be present as free Cu^{2+}, the most biologically available form (*13*). However, the same may not hold true where Cu availability increases with increasing soil alkalinity, as most of the Cu is expected to be present as complexes with dissolved organic matter and inorganic ligands (e.g., carbonates) (*24, 29, 30*). As the analysis of CaCl$_2$ soil extracts determines both free Cu^{2+} and complexed Cu, the CaCl$_2$ extractable Cu concentration may not prove to be a good predictor of toxicity in alkaline soils.

Total Cu concentration, as dictated by Cu-fungicide usage, is clearly a strong determinant of Cu availability in vineyard soils. Thus, from a risk management perspective, the obvious way of reducing the concentrations of available Cu in vineyard soils would be to stop or restrict the use of Cu-fungicides. However, this may not be practical because potentially environmentally safer alternatives to Cu-fungicides (e.g., phosphorous acid, plant extracts, and biological controls) require further evaluation before they can be widely adopted (*31*). In the meantime, Cu-fungicide use is considered economically important for disease control, as downy mildew outbreaks can cause considerable crop losses (*32*). The relationship between pH and Cu availability may well be important in considering strategies to reduce Cu availability and toxicity in vineyard soils, as it appears that Cu availability can be maintained at a minimum by keeping soil pH in the range of ∼7−8. This is also the soil pH range that is generally considered within an optimum range for the health of grapevines in Australia (*33*). If deemed necessary, soil pH management may prove to be a cost-effective in situ remediation strategy, as the pH of most agricultural soils can be relatively easily raised through the use of lime. Alternatively, the organic matter content of the soil could be increased, such as through the application of composts, to increase the cation exchange capacity of the soil and thus reduce the availability of Cu (*34*).

Few studies have published ecotoxicological data for soil organisms based on CaCl$_2$ extractable Cu concentration; hence,

there is little reference information with which to compare the extractable Cu concentrations determined in this study. In relation to microbial activity, Broos et al. (*35*) reported the CaCl$_2$ extractable Cu EC$_{50}$ values for substrate induced nitrification to be generally in the range of ~5−32 mg/kg and those for substrate induced respiration to be in the range of ~10−100 mg/kg. In comparison, soil enzyme activities have been found to be more sensitive. For example, data presented by Kim et al. (*36*) showed that adverse effects on urease activity began at CaCl$_2$ extractable Cu concentrations less than 5 mg/kg. In relation to invertebrates, data reported by Lock and Janssen (*37*) and Arnold et al. (*38*) suggest that the LC$_{50}$ values for the pot worm, *E. albidus*, and the composting worm, *E. fetida*, and the EC$_{50}$ reproduction value for the springtail, *F. candida*, lay somewhere between a soil solution Cu concentration of 0.1 and 1 mg/L (*37, 38*). These data suggest that the CaCl$_2$ extractable Cu concentrations found in Australian vineyards soils (**Table 1**) are in the range that could potentially cause adverse effects to soil enzymatic activities and invertebrates. It is noted, however, that the environmental availability of Cu may not necessary relate to the toxicological bioavailability, as some forms of Cu considered to be environmentally available (e.g., Cu complexed with organic matter) may not necessarily be taken up by the organism. Also, it is known that soil organisms can influence Cu availability by modifying the chemistry of the surrounding soil and/or soil solution, for example, changing pH, ionic strength, and macronutrient cation concentrations or through the excretion of organic ligands (*13*).

In summary, the CaCl$_2$ extractable Cu concentrations in the vineyard soils from 10 different viticultural regions of Australia ranged from < 0.1 to 0.94 mg/kg, accounting for 0.10−1.03% of the total Cu concentrations in the soils. The concentration of CaCl$_2$ extractable Cu was primarily influenced by the total Cu concentration of the soil. The CaCl$_2$ extractable Cu concentration was also related to soil properties which influence cation exchange processes and the capacity for soil to adsorb Cu, including pH, clay, exchangeable K, silt, and CaCO$_3$. This information may prove useful in devising strategies to reduce the availability of Cu in agricultural soils. The concentrations of CaCl$_2$ extractable Cu determined in this study are in the range of those concentrations reported to have adverse effects on soil invertebrates and the enzyme activity of soils. Further studies are now needed to determine the extent of adverse biological effects in Australian vineyard soils, and to determine how well the effects relate to CaCl$_2$ extractable Cu concentrations.

ABBREVIATIONS USED

CaCl$_2$, calcium chloride; CaCO$_3$, calcium carbonate; CEC, cation exchange capacity; EC, electrical conductivity; EC$_{50}$, effect concentration to 50% of organisms (or causing a 50% reduction); EDTA, ethylenediaminetetraacetic acid; ICP-ES, inductively coupled plasma emission spectroscopy; LOD, limit of determination; SIN, substrate induced nitrification; SIR, substrate induced respiration; WEPAL, Wageningen Evaluating Programs for Analytical Laboratories.

ACKNOWLEDGMENT

Supporting Information Available: Copper fungicide usage history and physical-chemical soil property data used in the regression analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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