Copper Fungicide Residues in Australian Vineyard Soils

ADAM M. WIGHTWICK,*,†,§,# MAHABUBUR R. MOLLAH,†,§
DEBRA L. PARTINGTON,⊥ AND GRAEME ALLINSON#

Cooperative Research Centre for Viticulture, P.O. Box 154, Glen Osmond, South Australia 5064,
Australia; Department of Primary Industries—Mildura, P.O. Box 905, Mildura, Victoria 3502,
Australia; Department of Primary Industries—Queenscliff, P.O. Box 114, Queenscliff, Victoria 3225,
Australia; and Department of Primary Industries—Knoxfield, Private Bag 15, Ferntree Gully Delivery
Centre, Ferntree Gully, Victoria 3156, Australia

Copper (Cu) concentrations were measured in Australian vineyard soils to assess the extent and magnitude of Cu accumulation resulting from the use of Cu-based fungicides and to indicate the likely risks to long-term soil fertility. Soil samples were collected from 98 vineyards across 10 grape-growing regions of Australia and analyzed for total Cu concentrations. Ninety-six percent of vineyards surveyed had elevated Cu concentrations in soil compared to the background Cu concentrations in nearby soil in its native state. Concentrations of total B, Co, Cr, Pb, and Zn were similar to background concentrations and below reported toxicity guideline values. Cu concentrations in Australian vineyard soils were generally much lower (6–150 mg kg^{-1}) than those reported in the soils of vineyards in parts of Europe (i.e., 130−1280 mg kg^{-1}). Concentrations of total Cu were generally below those concentrations reported to cause lethal effects to soil invertebrates; however, Cu exceeded concentrations known to cause sublethal effects (i.e., inhibit growth, affect reproduction, induce avoidance behavior) to those (or related) invertebrates.

KEYWORDS: Copper; vineyards; soils; copper fungicide; accumulation

INTRODUCTION

Copper-based fungicides have been used in vineyards throughout the world, including Australia, for many decades to prevent downy mildew (*Plasmopara viticola*), outbreaks of which can occur under warm humid conditions following rain. Historically, copper fungicides were applied as a mixture of copper sulfate and lime [$CuSO_4 \cdot 3Cu(OH)_2 \cdot 3CaSO_4$; Bordeaux Mixture]; however, more recently copper oxychloride [$CuCl_2 \cdot 3Cu(OH)_2$] and copper hydroxide [$Cu(OH)_2$] have been the main forms applied (*1*). Globally, copper-based fungicides are still widely used in vineyard spray programs, including in Australia. For example, a recent survey in Victoria (Australia) found that 90% of grape growers apply copper-based fungicides with annual rates ranging from 0 to 35 kg ha^{-1} (*1*).

Although copper-based fungicides are effective for disease control in vineyards, their use can result in an accumulation of copper (Cu) in surface soils. For example, copper concentrations ranging from 130 to 1280 mg kg^{-1} have been detected in the surface soil of European vineyards with an established history of copper-based fungicide use (*2–6*), and Cu concentrations as high as 3216 mg kg^{-1} have been found in Brazil (*7*). This is an increase from natural background copper concentrations in soils, which worldwide are typically less than ∼20 mg kg^{-1}, but may be as high as 100 mg kg^{-1} depending on the parent soil material (*8, 9*). A similar increase in copper has occurred in stone fruit, pome fruit, and citrus orchard soils, which now range between 110 and 1500 mg kg^{-1} Cu where copper-based fungicides have been used (*3, 10*).

In many regions of the world there are concerns about the risks that the accumulation of copper in agricultural soils may pose to human health, ecological health, and the long-term sustainability of agricultural land (*11*). The phytotoxic effects of copper to plants have been widely studied (*12*), but copper is not expected to cause phytotoxicity in mature grapevines as they tend to have deep root systems that extend below the surface soils where most of the applied copper tends to remain. Young vines may be affected as their root systems are much shallower while establishing; therefore, copper may have phytotoxicity implications for vine nurseries or when established vineyards are replanted (*13*). The impacts on soil biology and implications for soil fertility are more of a concern in established vineyards. Several studies have shown elevated copper concentrations to adversely affect the fertility of vineyard soils (*14, 15*); for example, field studies conducted found clear linkages between increased Cu concentrations (>80 mg kg^{-1})

* Corresponding author (telephone +61-3-52-58-01-11; fax +61-3-52-58-02-70; e-mail adam.wightwick@dpi.vic.gov.au).
† Cooperative Research Centre for Viticulture.
§ Department of Primary Industries—Mildura.
# Department of Primary Industries—Queenscliff.
⊥ Department of Primary Industries—Knoxfield.

Table 1. Number of Vineyards Surveyed and Estimated Use of Cu-Based Fungicides for Each of the 10 Viticultural Regions Surveyed

region[a]	n	years of Cu use			annual Cu use (kg ha^{-1})			estimated total Cu applied[b] (kg ha^{-1})		
		mean	range	CV%	mean	range	CV%	mean	range	CV%
Murray Valley, Vic/NSW	10	62	30–100	36	5	2–14	57	394	60–960	61
Southern Highlands, Vic	9	31	20–38	17	9	2–24	77	317	58–700	75
Riverland, SA	10	49	25–80	41	9	3–15	50	439	75–1200	72
Barossa, SA	10	66	30–100	48	6	5–8	22	410	150–800	58
southeast, SA	10	51	30–100	54	11	8–20	47	496	247–750	42
Riverina, NSW	10	53	38–84	38	13	5–20	46	696	150–1480	57
Mudgee, NSW	9	30	4–50	35	9	3–14	46	256	50–700	74
Swan Valley, WA	10	14	5–30	64	7	2–15	60	105	28–450	120
Margaret River, WA	13	10	1–28	95	5	2–20	102	63	2–400	181
Tasmania	7	35	30–50	21	6	1–10	58	209	30–450	69

[a] Vic, Victoria; SA, South Australia; NSW, New South Wales; WA, Western Australia. [b] Estimated total Cu applied calculated for each vineyard by multiplying the years of Cu use by the annual Cu use (kg ha^{-1}).

in soil and reduced earthworm abundance (*14, 15*). Although the direct link between increased Cu in vineyard soils and reduced vine performance is unclear, studies in Australia have found a link between increased earthworm density and increased yields (*16*); therefore, any harm to earthworm populations could affect the productivity and efficiency of vineyards.

In response to environmental concerns over the use of copper fungicides such as the accumulation of copper in agricultural soils and the potential impact on soil ecology (*11*), regulators in some European countries have imposed restrictions on the use of copper-based fungicides. For example, copper use has been banned in The Netherlands, and Switzerland has restricted the amount of copper that can be applied per hectare (*11*). In Australia, there are currently no restrictions on copper use in conventional viticulture, although organic standards limit annual copper use to a maximum of 8 kg ha^{-1} (*17*).

The Australian viticultural industries recognized the issues surrounding copper fungicide use and required information on the extent and magnitude of copper accumulation in Australian vineyard soils to aid in assessing the need for modifying the management of copper inputs in the future. This paper presents the findings of an Australia-wide survey of copper concentrations in vineyard soils. The aim of the survey was to assess the extent and magnitude of copper accumulation in Australian vineyard soils to provide an indication of the likely risks that copper accumulation poses to the long-term fertility of Australian vineyard soils.

MATERIALS AND METHODS

Balancing an acceptable level of precision with the resources required to collect and analyze samples, we collected one composite soil sample from each of 98 vineyards across Australia. To ensure adequate representation of Australian grape-growing regions, we surveyed between 7 and 13 vineyards in each of 10 regions (**Table 1**). The regions included in the Australia-wide survey were selected to represent a range of growing conditions (e.g., climate, soils) and copper use regimes.

In each region the objective was to survey vineyards with the greatest potential for having high accumulated copper concentrations. This was achieved by selecting properties that had been operated as a vineyard for at least 30 years, but preferably more than 60 years, and that had an established history of copper-based fungicide use. In addition, preference was given to vineyards with heavy textured soils (e.g., clay) for that region (as assessed by soil map information and verbal soil descriptions given by individual vineyard managers) as the adsorption of copper is expected to be greater in these soils than in lighter textured soils. The vineyards included in the survey were selected by contacting regional grape-growing associations and vineyard managers who identified sites that most closely matched the selection criteria.

Soil Sampling. Soils were collected from the vineyards between April and December 2004. In each vineyard, soil samples were collected

from the surface soil (10 cm depth) from 10 different locations within 30 cm of the vine line. Samples were collected from three rows representing the middle row in the vineyard, and rows one-fourth and three-fourths of the way into the vineyard. Within in each of these rows, soil was collected from locations one-fourth, halfway, and three-fourths of the way down the row. In the middle row, an additional sample was collected midway between three-fourths of the way down the row and the end of the row. This sampling layout was selected to account for heterogeneity of soil within the vineyard and to ensure consistency in sampling across vineyards. Soil samples were collected using a 10 cm diameter soil auger. The 10 subsamples were combined to obtain a composite sample (approximately 4 kg) for each vineyard, which was homogenized and then divided into subsamples using a soil splitter, to obtain representative 200 g (approximately) samples in duplicate for each vineyard/site. The duplicate soil samples were dried at approximately 30 °C for at least 7 days, passed through a 2 mm stainless steel sieve, and then homogenized to provide a uniform sample of <2 mm particles for chemical analysis.

A composite soil sample was obtained in the same manner from two or three different "reference" locations in each region. The reference locations were areas of remnant vegetation that had not received any artificial inputs of copper (native soil), generally located within 10 km of surveyed vineyards. The reference samples were collected to provide an indication of the background copper concentrations in soil for each region and were sieved and homogenized in the same manner as the vineyard soil samples.

Operational information such as the number of years the property has been operated as a vineyard, the history of the property prior to the establishment of the vineyard (e.g., other farming systems), and the history of copper-based fungicide use (e.g., years of copper use, number and rate of applications per year) was obtained for each vineyard (**Table 1**).

Soil Analysis. Copper (Cu) and other metals/elements were dissolved from soil samples using the acid dissolution method (*18*). In short, this method involved digesting 0.5 g of soil in 5 mL of aqua regia (HNO$_3$/HCl, 1:3). After digestion, samples were vortexed and passed through a Whatman No. 42 filter paper (Whatman International, Maidstone, Kent, U.K.); the first few milliliters of filtrate was discarded. The soil extracts were then analyzed using inductively coupled plasma atomic emission spectrometry (ICP-AES) by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Land and Water Soils Laboratory, Adelaide, Australia. For quality control purposes, 5% of the soil samples were processed in duplicate, and four samples for each of two different internal soil standards (reference materials) were processed.

Selected physical properties of vineyard soils, including total organic carbon, calcium carbonate, pH, cation exchange capacity, exchangeable Ca, exchangeable Na, electrical conductivity, and particle size analysis, were predicted using mid-infrared (MIR) with partial least squares (PLS) analysis by CSIRO Land and Water, Adelaide, using methods developed by the laboratory (*19*). For these analyses, soil subsamples (10 g) from each vineyard were ground to <100 μm by processing in a steel vibrating puck mill for 60 s. Soil MIR spectra were scanned directly

Copper in Australian Vineyard Soils

J. Agric. Food Chem., Vol. 56, No. 7, 2008 **2459**

Table 2. Selected Physical Chemical Soil Properties: pH, Particle Size Analysis, and Organic Carbon[a]

		physical–chemical soil property[b]														
		pH (H₂O)			sand (%)			silt (%)			clay (%)			TOC[b] (%)		
region[c]		mean	range	CV%	mean	range	CV%	mean	range	CV%	mean	range	CV%	mean	range	CV%
Murray Valley	V	8.7	6.8–9.8	11	66.7	53.6–81.0	11	11.5	3.10–15.9	36	21.7	7.80–33.5	37	1.1	<0.0–2.6	88
	R	6.4	5.8–6.9	12	67.9	54.7–81.2	28	12.9	9.3–16.4	39	19.2	9.50–28.9	71	1.7	0.2–3.3	124
Southern Highlands	V	5.6	5.1–6.3	8	61.9	40.5–81.8	19	23.5	7.80–39.4	37	14.6	10.4–20.1	20	3.6	2.1–4.8	26
	R	4.6	4.2–4.9	10	67.1	61.5–72.8	12	20.2	16.5–23.9	26	12.6	10.7–14.6	22	6.1	6.1	0
Riverland	V	7.7	7.0–9.2	<1	75.4	52.5–87.6	8	7.80	0.60–16.8	5	16.8	6.00–34.3	8	2.8	0.4–7.0	<1
	R	8.1	7.8–8.4	5	71.4	69.0–73.8	5	6.30	0.00–12.6	141	22.3	13.6–31.0	55	1.0	<0.1–2.0	141
Barossa	V	7.2	6.1–8.5	11	75.4	52.5–87.6	15	7.80	0.60–16.8	66	16.8	6.00–34.3	55	2.8	0.4–7.0	82
	R	6.1	5.9–6.4	5	64.5	58.4–70.6	13	21.1	17.4–24.8	25	14.4	12.0–16.8	23	2.6	2.3–2.9	15
southeast, SA	V	7.8	7.2–8.6	6	55.0	44.4–74.5	16	14.2	7.20–19.5	32	30.8	18.4–36.3	16	2.9	1.1–4.6	32
	R	6.3	4.8–7.8	33	85.7	75.1–96.3	17	5.60	0.00–11.3	141	8.70	3.80–13.7	81	3.1	2.7–3.5	17
Riverina	V	6.6	5.9–7.2	5	58.8	49.2–72.0	12	8.70	4.50–13.0	44	32.5	23.5–38.2	14	1.7	0.5–3.1	37
	R	6.8	5.9–7.2	18	61.2	64.4–57.9	8	9.40	8.80–10.0		29.5	26.8–32.1	13	3.2	2.8–3.7	20
Mudgee	V	6.0	5.6–6.4	5	64.3	37.0–75.7	18	19.2	8.20–34.1	41	16.5	11.2–28.9	30	1.6	0.4–3.7	70
	R	5.8	5.6–6.4	16	78.5	72.7–84.3	11	9.40	7.80–10.9	23	12.1	7.80–16.4	50	1.8	1.2–2.4	50
Swan Valley	V	5.9	5.1–7.0	9	91.1	83.8–95.4	5	1.10	0.00–6.0	181	7.80	4.60–12.0	31	1.4	0.7–2.4	39
	R	6.0	5.1–7.0	2	80.5	74.3–86.7	11	5.20	1.55–8.81	99	14.3	11.8–16.9	25	2.1	2.1–2.2	2
Margaret River	V	5.9	5.0–7.0	9	84.1	73.0–94.5	9	6.10	0.00–12.0	93	9.80	4.70–13.9	36	5.2	3.5–6.4	18
	R	5.5	5.0–7.0	12	86.7	81.2–92.2	9	7.50	4.00–11.0	66	5.90	3.90–7.90	48	5.0	3.6–6.4	40
Tasmania	V	6.4	5.7–7.7	11	67.0	26.7–87.1	36	15.3	0.00–31.3	79	18.0	4.90–43.1	78	4.1	2.4–9.0	55
	R	5.4	4.1–6.8	25	64.0	22.8–96.9	59	14.6	0.00–39.0	145	21.4	4.00–38.3	80	6.3	3.0–9.3	50

[a] For vineyards, $n = 10$ except for Southern Highlands and Mudgee ($n = 9$), Margaret River ($n = 13$), Tasmania ($n = 7$); for reference sites, $n = 2$ except for Tasmania $n = 3$. [b] Physical soil properties reported are predicted values determined from the MIR analysis of the soils. [c] V, vineyard sites; R, reference sites.

by diffuse reflectance on the neat powdered soil samples. Spectra were recorded in the 7800–400 cm^{-1} spectral range using a Perkin-Elmer Spectrum-One Fourier transform infra-red (FTIR) instrument with an autofocusing Perkin-Elmer diffuse reflectance accessory, using a 1 min scan (*19*). A 10% subset of samples was analyzed for total organic carbon, calcium carbonate, pH, cation exchange capacity, exchangeable Ca, exchangeable Na, electrical conductivity, and particle size analysis using conventional analytical techniques (*20*) to validate and calibrate the MIR predictions. The subset of samples was selected on the basis of the MIR results and to cover the variability found across the sample set for each analyte. Results from the validation testing were used in PLS calibration training, in which different calibrations were tested by cross-validation performance. This was used to generate the optimum regression model that was then used for prediction from the unknown sample spectrum. The software used was either PLSplus-IQ (GRAMS, Galatic, NH), or Unscrambler (Camo AS, Oslo, Norway) (*19*).

Data Analysis. Data were analyzed using GenStat release 6.1 (6th ed., 2002, Lawes Agricultural Trust, Rothamsted Experimental Station). Regression analysis was used to determine relationships between vineyard copper concentrations and factors that may have influenced the accumulation of copper, such as the estimated total amount of copper applied (i.e., years of copper use × annual copper use) and the properties of the soil within each of the regions. Outliers were removed from the regression analysis in some instances to improve the fit. Goodness of fits from regression analysis are provided as adjusted R^2 values, which is a measure of the proportion of the variation in the dependent variable accounted for by the exploratory variables.

RESULTS

To indicate the accuracy of the MIR predictions of soil physical properties and using the results from calibration samples, an *F* ratio indicator (F^2) value was determined according to the following formula: $F^2 = (F \text{ ratio}/5 + 0.25)$, where *F* ratio is a spectral measure of how well the unknown matches the calibration set. An $F^2 > 3$ indicates a poor spectral fit with the calibration set. The F^2 values for the soil samples were <3, with the exception of one of the Tasmanian reference sites that had F^2 values >3 for all analytes. In addition, one of the Margaret River vineyards had an F^2 value >3 for EC and exchangeable Ca, and a Margaret River reference site also had an F^2 value >3 for exchangeable Ca. On the basis of a large

set of calibration samples ($n = 290–1225$) CSIRO Land and Water, Adelaide, has correlated results from conventional analysis with those predicted from their MIR analysis, and at the time of analyzing our soil samples the cross-validation R^2 and standard error of cross-validation (SECV) values were within the ranges of 0.72–0.98 and 0.14–3.2, respectively. Physical properties of the vineyard soils predicted using MIR varied both between and within the regions (**Tables 2** and **3**).

The results of duplicate samples for copper were within acceptable limits (variance from the mean ranging from 0.7 to 5.9% for each set of duplicates). Likewise, copper recoveries from the internal soil standards (reference materials) processed were within acceptable limits ranging from 96 to 110% (mean of 102%). In addition to Cu, the ICP analysis also produced results for 19 additional elements (Al, As, B, Ca, Cd, Co, Cr, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Zn). There were no (or insufficient) internal soil standard (reference material) data for As, Mn, Mo, and Se, and so these data were disregarded. Likewise, the mean recoveries from internal soil standards were poor for Cd (479%), Ni (71%), and S (152%), and the repeatability of duplicates for Al was poor (mean discrepancy of 43%), and there data have also been disregarded. The mean recoveries from the reference materials for B, Ca, Co, Cr, Fe, K, Mg, P, Pb, and Zn were within acceptable limits (117, 88, 101, 103, 113, 105, 99, 101, 123, and 113%, respectively), and the results from duplicate analysis were also in acceptable agreement with the mean discrepancies [i.e., variance from the mean of duplicate results; B (11%), Ca (0.9%), Co (3.0%), Cr (3.8%), Fe (8.9%), K (7.2%), Mg (0.8%), P (6.6%), Pb (1.3%), and Zn (2.0%)], and so these data have been presented (**Tables 4** and **5**). The recovery of Na from the internal soil standards was poor (mean recovery of 229%); however, it is recognized that Na is a major ion in soil, so these data have been included with the caveat that at best the Na data should be regarded as semiquantitative. Sample concentrations of metals/elements reported have not been adjusted to reflect variation in their respective recoveries from the reference materials.

The concentrations of copper measured in vineyard soils varied from 6 to 223 mg kg^{-1} (**Table 5**). In each region

Table 3. Selected Physical and Chemical Soil Properties: Exchangeable Cations, Salinity, and Calcium Carbonate[a]

		CEC[d] (cmol_c kg^{-1})			exch Ca (cmol_c kg^{-1})			exch Na (cmol_c kg^{-1})			EC[e] (dS m^{-1})			CaCO_3[f] (%)		
region[c]		mean	range	CV%	mean	range	CV%	mean	range	CV%	mean	range	CV%	mean	range	CV%
Murray Valley	V	16.5	6.00–26.8	49	10.0	2.90–17.3	53	0.6	<0.1–2.5	140	0.1	<0.1–0.3	118	1.5	0.1–5.0	105
	R	12.5	3.80–21.2	99	4.40	2.10–6.60	72	0.8	0.5–1.2	60	0.1	<0.1–0.1	141	0.2	0.1–0.2	58
Southern Highlands	V	14.3	5.10–20.9	32	10.6	3.60–16.7	36	0.4	<0.1–0.9	98	<0.1	<0.1–0.1	144	0.5	<0.1–1.2	80
	R	11.1	10.4–11.9	9	6.60	5.80–7.40	17	0.6	<0.1–1.2	141	<0.1	0.0–0.1	141	0.1	0.1–0.2	47
Riverland	V	23.0	11.5–31.2	6	9.4	6.00–12.8	3	0.4	<0.1–1.6	<1	0.2	<0.1–0.8	<1	0.4	0.1–1.0	0.5
	R	17.5	11.4–23.7	50	9.10	7.50–10.7	25	2.9	1.1–4.8	90	4.3	<0.1–8.6	140	0.6	0.3–0.9	67
Barossa	V	14.2	<0.1–37.0	82	9.4	1.10–23.7	78	0.4	<0.1–1.6	148	0.2	<0.1–0.8	128	0.4	0.1–1.0	70
	R	6.70	2.30–11.2	94	6.60	1.10–12.1	119	<0.1	<0.1		0.3	0.1–0.5	113	0.1	0.0–0.1	141
southeast, SA	V	26.7	11.1–35.9	25	15.8	4.90–26.2	36	0.3	<0.1–1.2	119	0.1	<0.1–0.4	97	0.7	0.1–2.5	102
	R	7.90	<0.1–15.9	141	6.80	<0.4–13.5	141	0.5	<0.1–1.0	141	<0.1	<0.1		0.4	0.4–0.5	16
Riverina	V	21.1	14.9–25.4	18	8.4	4.80–12.1	31	0.2	<0.1–0.7	147	<0.1	<0.1–0.2	180	0.3	<0.1–0.8	93
	R	24.5	20.0–29.0	26	12.1	8.10–16.2	47	<0.1	<0.1		0.1	0.1–0.2	20	0.8	0.7–1.0	30
Mudgee	V	8.50	1.50–22.5	78	5.0	0.70–15.4	98	<0.1	<0.1–0.2	300	<0.1	<0.1–0.1	122	0.4	<0.1–0.8	85
	R	6.80	0.50–13.0	132	2.50	<0.4–5.00	141	<0.1	<0.1		<0.1	<0.1		0.2	<0.1–0.5	136
Swan Valley	V	1.60	0.10–5.00	139	1.0	<0.4–5.70	196	0.3	<0.1–2.0	207	0.1	<0.1–0.4	210	0.4	<0.1–0.6	64
	R	6.00	4.30–7.80	42	0.70	<0.4–1.50	141	0.1	<0.1–0.2	141	<0.1	<0.1		0.2	<0.10 0.3	104
Margaret River	V	9.00	3.60–17.3	44	5.1	1.00–12.4	62	0.5	<0.1–1.8	112	0.2	<0.1–0.9	139	0.4	<0.1–1.4	92
	R	7.60	1.70–13.5	111	2.10	<0.4–4.30	141	1.5	0.8–2.1	61	<0.1	<0.1	141	0.1	<0.1–0.2	141
Tasmania	V	19.5	3.60–52.2	95	14.1	7.20–29.7	63	0.3	<0.1–1.3	168	0.2	<0.1–0.5	103	0.6	<0.1–1.4	92
	R	20.7	4.90–33.5	70	4.90	2.80–7.50	50	<0.1	<0.1		0.8	0.1–1.4	84	2.3	0.1–5.5	125

[a] For vineyards, n = 10 except for Southern Highlands and Mudgee (n = 9), Margaret River (n = 13), Tasmania (n = 7); for reference sites, n = 2 except for Tasmania n = 3. [b] Physical soil properties reported are predicted values determined from the MIR analysis of the soils. [c] V, vineyard sites; R, reference sites. [d] CEC, cation exchange capacity. [e] EC, electrical conductivity. [f] CaCO_3, calcium carbonate.

Table 4. Concentrations of Major Ions and P Measured in Vineyard and Reference Site Soils[a]

		Ca			K			Mg			P			Na[d]		
region[c]		mean	range	CV%	mean	range	CV%	mean	range	CV%	mean	range	CV%	mean	range	CV%
Murray Valley	V	15231	1838–51538	107	5570	2975–8066	38	3883	1945–5541	37	863	246–2291	65	224	134–400	35
	R	1965	1827–2103	10	3435	3175–3695	11	2329	1594–3064	45	224	122–325	64	419	124–714	100
Southern Highlands	V	4036	1238–10645	72	1323	728–2940	59	1491	427–3975	76	347	193–720	44	154	84–474	80
	R	1814	987–2640	64	1089	657–1521	56	853	229–477	62	182	125–238	44	144	110–177	33
Riverland	V	5756	2186–12133	54	7291	4456–11775	25	4329	2827–6074	25	654	205–1013	32	318	216–579	33
	R	5177	4857–5497	9	4359	3820–4897	17	3558	2830–4287	29	236	172–300	38	5661	422–10900	131
Barossa	V	5622	1434–11893	55	4310	1522–9798	64	3059	1014–7980	71	716	222–1454	60	170	107–265	32
	R	1783	811–2756	77	2628	2346–2910	15	2232	1942–2522	18	128	124–132	5	149	129–169	19
southeast, SA	V	7157	3341–15355	57	4684	3805–5640	17	1811	1263–2900	28	516	72–1392	80	256	184–326	18
	R	3919	429–7409	126	1040	170–1909	118	514	135–894	104	110	48–172	80	92	64–120	43
Riverina	V	3103	1811–4677	32	4669	4214–5296	8	2167	1703–2765	14	551	169–927	48	189	149–217	12
	R	4132	2168–6096	67	4899	3753–6045	33	2903	1762–4043	56	233	164–302	42	266	151–381	61
Mudgee	V	2529	1061–4615	44	1970	1191–4006	44	1339	505–4279	88	514	191–1319	78	111	60–235	50
	R	1247	514–1980	82	865	528–1202	55	880	321–1438	90	128	86–171	47	102	69–136	46
Swan Valley	V	1777	794–6149	90	491	127–1829	106	273	93–602	54	447	233–808	41	153	73–488	81
	R	1243	1152–1334	10	443	343–542	32	440	411–469	9	104	100–109	6	138	125–150	13
Margaret River	V	5011	2276–20138	94	324	209–549	28	533	291–1657	67	947	510–1894	39	165	86–370	52
	R	1665	545–2786	95	229	182–277	29	585	243–928	83	80	<LOR–159	141	159	96–223	56
Tasmania	V	5652	2042–10497	62	1261	131–2416	67	2138	236–5856	110	430	130–794	55	309	58–874	107
	R	2751	1433–3953	46	835	285–1571	79	2017	428–3896	87	165	37–318	86	1422	222–3795	145

[a] For vineyards, n = 10 except for Southern Highlands and Mudgee (n = 9), Margaret River (n = 13), Tasmania (n = 7); for reference sites, n = 2 except for Tasmania n = 3. [b] Limits of reporting (mg kg^{-1}): Ca, 1.92; K, 11.92; Mg, 4; P, 0.92; Na, 3.04. [c] V, vineyard sites; R, reference sites; [d] Recoveries from internal soil standards were poor for Na (>200%) but data are reported because Na is such an important ion; at best these data should be regarded as semiquantitative.

surveyed, the total copper concentrations in vineyard soils were greater than the reference locations in each region, with the exception of some vineyards in the Swan Valley and Tasmania (**Table 5**). Concentrations of other metals were generally lower than the concentration of Cu and close to background concentrations (**Table 5**).

DISCUSSION

In the survey design, it was necessary to account for the inherent heterogeneity of vineyard soils and the implications that this may have for accumulated concentrations of copper.

Therefore, the design of the survey was based on the recommendations given in ref *21*, where it was found that the most precise estimate of copper can be obtained by taking a single composite sample from as many vineyards in a region as possible rather than many samples from fewer vineyards when regional surveys are conducted (*21*). Surface soil samples (0–10 cm) were collected from within 30 cm of the vine line to give the greatest chance of detecting the highest concentrations of copper accumulated in the vineyard soil, as it had been previously found that the concentrations of copper in vineyard soils decrease substantially at greater soil depths and distances

Copper in Australian Vineyard Soils

J. Agric. Food Chem., Vol. 56, No. 7, 2008   **2461**

Table 5. Concentration of Total Copper and Other Metals Measured in Vineyard and Reference Site Soils[a]

		total concentration[b] (mg kg^{-1})																				
		Cu			B			Co			Cr			Fe			Pb			Zn		
region[c]		mean	range	CV%	mean	range	CV%	mean	range	CV%	mean	range	CV%	mean	range	CV%	mean	range	CV%	mean	range	CV%
Murray Valley	V	66	34–128	44	21	10–26	23	7	3–12	42	23	14–31	29	16058	9294–23766	33	19	7–35	50	50	34–70	25
	R	13	7–19	63	11	7–14	45	5	2–8	75	19	11–27	60	13668	8111–19224	58	12	7–17	58	34	20–47	58
Southern	V	87	30–223	74	7	1–11	56	3	1–7	65	17	12–25	27	11420	6890–19998	41	25	13–53	55	37	22–68	47
Highlands	R	9	7–11	34	5	5–6	6	3	1–4	82	13	9–18	47	11495	4327–18665	88	20	13–27	51	26	12–40	78
Riverland	V	78	55–119	26	20	14–23	15	9	7–10	17	23	21–26	8	16727	11480–20606	20	13	10–20	23	73	59–91	18
	R	24	18–30	35	22	22	2	7	4–9	50	20	14–26	40	17394	10424–24365	57	15	11–19	41	79	50–108	53
Barossa	V	49	24–87	38	16	9–24	31	6	2–11	46	23	9–41	43	15280	7327–29093	44	12	6–25	45	50	17–100	48
	R	16	14–19	20	9	5–12	56	3	2–3	45	17	10–23	55	13665	10493–16837	33	15	14–15	6	18	18–19	4
southeast, SA	V	81	39–150	50	19	12–25	23	11	5–16	32	33	22–42	21	28384	12020–55603	46	30	9–68	63	34	18–63	54
	R	2.2	2.0–2.4	15	11	9–13	27	2	<1–4	109	10	2–18	116	6806	1253–12358	115	5	2–8	81	6	4–9	52
Riverina	V	94	60–136	37	17	11–27	28	7	6–8	9	27	18–34	21	17929	10011–22844	27	16	11–21	23	44	33–56	16
	R	13	12–15	17	14	10–18	42	7	5–8	26	23	20–27	23	16086	12517–19657	31	12	10–14	25	35	29–40	24
Mudgee	V	57	36–91	32	8	2–17	49	12	8–18	36	25	16–38	30	15361	8734–23530	31	17	12–29	37	41	18–83	49
	R	6	4–9	52	4	1–7	91	7	6–7	8	14	12–15	16	14601	14194–15009	4	16	11–21	45	68	10–127	122
Swan Valley	V	21	6–39	53	11	5–17	32	2	<1–5	70	15	7–26	35	6085	2884–10362	36	11	7–17	30	23	14–55	52
	R	12	12–13	8	11	6–15	58	3	3–4	20	24	18–30	34	12555	10710–14340	21	24	16–31	47	11	10–13	22
Margaret River	V	26	11–52	50	9	7–16	29	3	1–7	67	13	6–19	31	18361	3158–44275	64	12	7–24	36	25	17–36	25
	R	1.1	0.8–1.4	35	7	6–8	24	3	3	5	11	11–12	6	28724	13612–43837	74	12	11–14	16	12	12	0
Tasmania	V	49	19–93	51	8	4–13	38	14	1–42	126	45	3–133	116	22827	3587–64992	101	11	6–15	30	67	24–161	72
	R	26	6–40	68	6	3–9	59	12	4–21	72	23	3–46	95	20773	11007–44571	100	22	5–43	90	71	11–136	88

[a] For vineyards, n = 10 except for Southern Highlands and Mudgee (n = 9), Margaret River (n = 13), Tasmania (n = 7); for reference sites, n = 2 except for Tasmania n = 3. [b] Limits of reporting (mg kg^{-1}): Cu, 0.84; B, 0.48; Co, 1.42; Cr, 0.44; Fe, 0.64; Pb, 1.80; Zn, 0.44. [c] V, vineyard sites; R, reference sites.

from the vine line (*21*). Surface soil were also sampled as this is the area in which the majority of the biological activity in soil occurs (*22*).

Whereas the focus of this study was on copper accumulation in vineyard soils, it is recognized that other metals can also affect soil fertility. The concentrations of B, Co, Cr, Pb, and Zn measured were generally similar to those concentrations found in soils from their respective reference sites (**Table 5**), with the exception of Cr in some vineyards in southeastern South Australia, Mudgee, Swan Valley, and Tasmanian regions. Concentrations of Cr, Pb, and Zn were below estimated sublethal hazardous concentrations (HC$_5$ values) from the literature (*23*) of 5, 164, and 160 mg kg^{-1}, respectively. HC$_5$ values are not available for B and Co; however, their concentrations were well below Australian soil quality guideline values (*8, 24*). This suggests that Cu is the most significant metal present in the vineyard soils, in terms of the potential effects to soil organisms.

The total iron (Fe) (aqua regia) content of soils has been suggested as a suitable geochemical indicator for estimating the background concentrations of metals (including Cu) in Australian soils (*25*). On the basis of this suggestion, we compared the Fe concentration of soils collected from the reference sites with those collected from the vineyards in each region as an assessment of the suitability of the reference sites as indicators of background copper concentrations. In most regions the mean Fe concentration in vineyards varied by <20% compared with the mean Fe concentration in soils from regional reference sites. In four regions the variance was ≤10%. This result indicates that in the majority of regions surveyed the reference sites provided a suitable indication of the background copper concentrations expected in the vineyard soils surveyed. The exceptions where a much greater variance from reference sites was found were southeastern South Australia (317%) and, to a lesser extent, Swan Valley (52%) and Margaret River (36%).

The annual usage of copper fungicides was expected to vary across the different regions due to differences in climatic conditions (predominantly humidity) during the growing season (spring/summer) and the subsequent risk of a downy mildew outbreak. However, the difference in annual copper usage (kg ha^{-1}) between the regions [5–13 kg ha^{-1} copper (CV 33%)] was less that the annual Cu usage within regions [CV 46–102%, except Barossa (CV 22%)] (**Table 1**). This suggests that individual vineyard management practices rather than climate (i.e., downy mildew risk) influence the extent of Cu use in vineyards. This finding is consistent with Pietrzak and McPhail (*1*), who also reported no apparent link between climatic regions (i.e., humidity) and annual copper usage, and is probably due to the fact that a downy mildew outbreak requires a unique combination of humidity, temperature, and rainfall, conditions that could feasibly occur in most viticultural regions of Australia. Copper fungicides are commonly applied as preventative sprays, so their usage is also likely to be influenced by the thought processes of individual vineyard managers and their tolerance to downy mildew outbreaks.

The accumulation of applied copper was expected, as copper is known to bind strongly to clay, organic matter, and minerals in soil (*9, 12*). Our results are thus consistent with findings from studies in vineyards within other countries (**Table 6**). The concentrations of copper in our older Australian vineyards (40–100 years old) ranged from 24 to 159 mg kg^{-1} (mean = 73 mg kg^{-1}). These are lower than copper concentrations reported in the soils of vineyards in France (47–845 mg kg^{-1}), northern Italy (297 mg kg^{-1}), Germany (1280 mg kg^{-1}), northwestern Spain (40–301 mg kg^{-1}), and Brazil (1214–3216 mg kg^{-1}) (concentrations also reported in surface soils) (**Table 6**). The fact that copper fungicide usage in Australian vineyards tends to be lower than in Europe is probably a factor contributing to the lower Cu concentrations in the surface soils of Australian vineyards (within older vineyards 40–100 years old) compared with vineyards in parts of Europe. For example, in some European regions typical annual Cu use ranges from 10 to 50 kg ha^{-1}, whereas the average annual use in Australian vineyards in our study was 5–13 kg ha^{-1}. However, soil factors such as organic carbon content, cation exchange capacity, and pH will also clearly influence Cu retention and, thus, accumulation in surface soils; it is therefore probable that the lower extent of Cu accumulation in the surface soils of Australian vineyard is related to differences in soil factors.

Table 6. Summary of Previously Reported Concentrations of Cu in Vineyard Soils in Comparison to the Findings from This Study (Australia-wide Survey)

country	region	no. of vineyards surveyed	Cu concentration (mg kg^{-1}) mean	range	years of Cu use	ref
France	Bordeaux	20	458	305–845	NR	*26*
	southern	8	121	47–177	50–106	*27*
	Roujan	13	161	75–398	NR	*28*
Italy	northern	NR	297	NR	NR	*2*
	southern	NR	75	NR	NR	
Germany		NR	1280	NR	NR	*3*
Spain	northwestern	20	144	40–301	>100	*29*
Canada	Ontario	16	40	10–77	3–25	*30*
Greece	Nemea	24	89	NR	>100	*31*
Brazil	southern	21	2198	1214–3216	100	*7*
New Zealand	nationwide	43	35	1–259	1–100	*32*
Australia	Victoria	5	59	51–77	>90	*1*
		9	90	9–229	20–30	
	New South Wales	4	22	NR	NR	*33*
	nationwide	35	73	24–159	40–100	this study
		63	53	6–223	1–38	

^{a} NR, not reported.

The wide variation in copper concentrations within regions is attributed to differences in copper use history, soil properties, and topography between the vineyards within each region. Contrary to observations in New Zealand (*32*), we found copper in vineyard surface soils to be poorly correlated with copper application history (adjusted $R^2 = 0.278$). There are, however, two exceptions in our study. For instance, copper application history and copper in vineyards soils was strongly correlated (adjusted $R^2 = 0.846$) in the Southern Highlands, Victoria, and Riverland. Although the form of copper fungicides used over the years has changed, from copper sulfate originally to currently copper oxychloride and copper hydroxide, all forms of copper applied are expected to have a strong soil-binding affinity (*3*). Therefore, the form of copper fungicide applied is likely to have been only a relatively minor influence on the extent of accumulation in the surface soils of vineyards. Losses of applied Cu from surface soils (10 cm depth), such as by runoff or leaching, may account for some of the inconsistencies between copper application history and copper concentrations in the surface soils of vineyards in most of the regions studied; however, an assessment of these such losses was outside the scope of this study.

Copper sorption in soil is generally considered to be largely influenced by organic carbon, cation exchange capacity, and clay content (*9*), but no correlation was found between vineyard copper concentrations and these parameters. This is broadly consistent with surveys of vineyard soils in Greece and New Zealand, although in Greece a correlation was found between total copper and clay content (*31, 32*). There was a reasonable relationship between copper in vineyard surface soils and soil pH ($R^2 = 0.685$ found in the Swan Valley). This was perhaps to be expected, as it is known that Cu sorption is greater with higher pH due to an increase in adsorption sites within the soil (*9*). Reasonable correlations between increasing copper concentration and increasing soil salinity (adjusted $R^2 = 0.697$, Barossa, Margaret River, Tasmania), increasing exchangeable sodium in soil (adjusted $R^2 = 0.695$, southeastern South Australia), and increasing exchangeable calcium (adjusted $R^2 = 0.577$, Murray Valley and Riverina) were also found. The reasons for the reasonable correlation between vineyard copper concentration and soil salinity ($R^2 = 0.697$) are not clear, as it is generally considered that the mobility of metals will increase in saline soil due to the formation of chloride complexes (*34*). It has been suggested that the specific adsorption of Cu in soil can occur in the presence of excess quantities of nonspecifically

bonded cations (such as sodium and calcium) (*35*). It has also been suggested that the concentration of Na and Ca ions in soil solution increases following the addition of Cu to soil due to the displacement of exchangeable Na$^+$ and Ca^{2+} by the Cu^{2+} ions (*36, 37*). This may explain the relationships found between vineyard copper and exchangeable sodium (Na) in southeastern South Australia and exchangeable calcium (Ca) in the Murray Valley and Riverina. However, we recognize that this survey was not designed to specifically assess the influence of soil properties on Cu accumulation; therefore, the correlations found may be a statistical artifact caused by targeting soils with high adsorption capacity rather than randomly selecting vineyards to get a range of soils with different adsorption capacities. Further work needs to be undertaken to explore the influence of soil properties on the adsorption and accumulation of Cu in vineyard soils in different regions. Nonetheless, the information on correlations between accumulated Cu and soil properties presented may aid future studies aimed at understanding Cu bioavailability and binding processes at these or similar sites.

Although it was outside the scope of this study to conduct a comprehensive assessment of the risks that accumulated copper poses to soil fertility in Australian vineyards, the potential risks can be indicated by comparing the measured concentrations of copper to guideline values and ecotoxological data. Australian soil quality guideline values are available for copper (*8, 24*); however, these are designed for assessing phytotoxicity to smaller plants (e.g., vegetable, cereals) and human health concerns in urban settings and are thus not relevant to assessing risks to soil fertility. Using species sensitivity distributions (SSDs) Jansch et al. (*23*) and Frampton et al. (*38*) have estimated hazardous concentrations to 5% (HC$_5$) of beneficial soil organisms. A lethal HC$_5$ to soil invertebrates has been estimated to be 183 mg kg^{-1} total Cu (derived using LC$_{50}$ values) (*38*), and a sublethal HC$_5$ to soil organisms has been estimated to be 55 mg kg^{-1} total Cu (derived using EC$_{50}$ values for animals, microbial processes, and plant species) (*23*). These HC$_5$ concentrations provide an indication of potential toxicity while recognizing that site-specific soil factors such as pH, texture, and cation exchange capacity (CEC) will influence the bioavailability of the copper present and therefore toxicity. Most of the ecotoxological data available relate to the biological response of different earthworm species to copper (lethal concentrations = 218−1000 mg kg^{-1}). Considerable data are also available for nematodes (lethal concentrations = 53−1272 mg kg^{-1}) and collembola (lethal concentrations = 1180–6840

Copper in Australian Vineyard Soils

J. Agric. Food Chem., Vol. 56, No. 7, 2008 **2463**

mg kg^{-1}) (*39*). Microbial activity and litter breakdown have been found to be significantly reduced at copper concentrations >100 mg kg^{-1} (*10, 40*). With the exception of one site in the Southern Highlands, Victoria (222 mg kg^{-1} Cu), all of the vineyards investigated were below the lethal HC$_5$ threshold (183 mg kg^{-1} total Cu), indicating that the risk of lethal effects to invertebrates in Australian vineyards is likely to be low. However, it appears likely that the copper in Australian vineyard soils may cause sublethal effects to soil organisms (i.e., inhibit growth, affect reproduction, induce avoidance behavior), resulting in reduced earthworm abundance and disrupting key soil processes (such as the breakdown of plant litter and the cycling of nutrients).

In summary, almost all of the vineyards surveyed (96%) had elevated surface soil copper concentrations compared to the background soil concentration in their region, and this is attributed to the use of copper-based fungicides. The extent of Cu accumulation and retention in the surface soils of Australian vineyards soils appears to be related to different management and soil factors in different regions, including total amount of copper applied, soil pH, salinity, exchangeable Na, and exchangeable Ca. Although it appears the risk of lethal effects on soil invertebrates is low, copper in the vineyard soils surveyed did exceed concentrations that have been shown to have sublethal effects (i.e., inhibit growth, affect reproduction, induce avoidance behavior) on soil organisms. Total copper concentrations were measured in this survey, but it is recognized that bioavailable metal is a more biologically relevant measure. There is, however, a lack of consensus as to which methods best represent bioavailability, and there are few data available that relate measures of bioavailable copper to effects on soil organism (*12, 41*). To fully characterize the impact and risks, further studies are now needed to determine the bioavailability of the copper present in Australian vineyard soils and to assess the potential effects on beneficial soil organisms and long-term soil fertility under Australian field conditions through both laboratory and field studies.

ABBREVIATIONS USED

CaCO$_3$, calcium carbonate; CEC, cation exchange capacity; CSIRO, Commonwealth Scientific and Industrial Research Organisation; Cu, copper; EC, electrical conductivity; ICP-AES, Inductively coupled plasma atomic emission spectrometry; LOR, limit of reporting; MIR, mid-infrared; PLS, partial least squares; TOC, total organic carbon.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Pietrzak, U.; McPhail, D. C. Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. *Geoderma* **2004**, *122*, 151–166.

(2) Delusia, A.; Giandon, P.; Aichner, M.; Bortolami, P.; Bruna, L.; Lupetti, A.; Nardelli, F.; Stringari, G. Copper pollution in Italian vineyard soils. *Commun. Soil Sci. Plant Anal.* **1996**, *27*, 1537–1548.

(3) Tiller, K. G.; Merry, R. H. Copper pollution of agricultural soils. In *Copper in Soils and Plants*; Proceedings of the Golden Jubilee International Symposium, Murdoch University, Perth, Western Australia; Loneragan, J. F., Robson, A. D., Graham, R. D., Eds.; Academic Press: Sydney, Australia, 1981; pp 119–134.

(4) Besnard, E.; Chenu, C.; Robert, M. Influence of organic amendments on copper distribution among particle size and density fractions in Champagne vineyard soils. *Environ. Pollut.* **2001**, *112*, 329–337.

(5) Flores-Velez, L. M.; Ducarior, J; Jaunet, A. M.; Robert, M. Study of the distribution of copper in an acid sandy vineyard soil by three different methods. *Eur. J. Soil Sci.* **1996**, *47*, 523–532.

(6) Magalhaes, M. J.; Sequeria, E. M.; Lucas, M. D. Copper and zinc in vineyards of central Portugal. *Water, Air Soil Pollut.* **1985**, *26*, 8–24.

(7) Mirlean, N; Roisenberg, A; Chies, J. O. Metal contamination of vineyard soils in wet subtropics (southern Brazil). *Environ. Pollut.* **2007**, *149*, 10–17.

(8) NEPC. *National Environment Protection (Assessment of Site Contamination) Measure*; National Environmental Protection Council Service Corporation: Adelaide, Australia, 1999.

(9) Sauve, S. Speciation of metals in soils. In *Bioavailability of Metals in Terrestrial Ecosystems: Importance of Partitioning for Bioavailability to Invertebrates, Microbes, and Plants*; Allen, H. E., Ed.; Society of Environmental Toxicology and Chemistry (SETAC): Pensacola, FL, 2002; pp 7–37.

(10) Merrington, G.; Rogers, S. L.; Van Zwieten, L. The potential impact of long-term copper fungicide usage on soil microbial biomass and microbial activity in an avocado orchard. *Aust. J. Soil Res.* **2002**, *40*, 749–759.

(11) Gardner, J. Copper usage controversy heats up in Europe [online]; Ministry of Agriculture and Food: Ontario, Canada, 2003;available at http://www.gov.on.ca/OMAFRA/english/crops/hort/news/hortmatt/2003/03hrt03a3.htm,accessed 2004.

(12) McLaughlin, M. J.; Hamon, R. E.; McLaren, R. G.; Speir, T. W.; Rogers, S. L. Review: A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australian and New Zealand. *Aust. J. Soil Res.* **2000**, *38*, 1037–1086.

(13) Conradie, K. Copper levels in South African nursery soils: possible effects on the propagation of grapevines. Wynoboer a technical guide for wine producers; 2004;available at http://www.wynboer-.co.za/recentarticles/0406copper.php3.

(14) Van Rhee, J. A. Effects of soil pollution on earthworms. *Poedobiologia* **1976**, *17*, 201–208.

(15) Paoletti, M. G.; Sommaggioa, D.; Favrettoa, M. R.; Petruzzelli, B.; Pezzarossab, B.; Barbafieri, M. Earthworms as useful bioindicators of agroecosytem sustainability in orchards and vineyards with different inputs. *Appl. Soil Ecol.* **1998**, *10*, 137–150.

(16) Buckerfield, J. C.; Webster, K. A. Earthworms, mulching, soil moisture and grape yields. *Wine Ind. J.* **1996**, *11* (1), 47–53.

(17) Organic Industry Export Consultative Committee. National Standard for Organic and Bio-Dynamic Produce (Draft); Australian Quarantine and Inspection Service, Australia, 2005; available at http://www.ofa.org.au/papers/2005%20Draft%20NATIONAL%20STANDARD.doc.

(18) Zarcinas, B. A.; McLaughlin, M. J.; Smart, M. K. The effect of acid digestion technique on the performance of nebulization systems used in inductively coupled plasma spectrometry. *Commun. Soil Sci. Plant Anal.* **1996**, *27*, 1331–1354.

(19) Forrester, S. T.; Janik, L. J.; Beech, T. A.; McLaughlin, M. J. *Recent Developments in Routine Soil Analysis by Mid-Infrared; Rapid and Cost Effective Laboratory Analysis*; Occasional Report 17; Australasian Soil and Plant Analysis Council (ASPAC): New Zealand, 2004.

(20) Rayment, G. E.; Higgins, F. R. *Australian Laboratory Handbook of Soil and Water Chemical Methods*; Inkata Press: Melbourne, Vic, Australia, 1992.

(21) Wightwick, A.; Mollah, M.; Smith, J.; MacGregor, A. Sampling considerations for surveying copper concentrations in Australian vineyard soils. *Aust. J. Soil Res.* **2006**, *44*, 711–717.

(22) Stirling, M. Soil biology and its importance in soil health. In *Soil Health: The Foundation of Sustainable Agriculture*, Proceedings of a Workshop on the Importance of Soil Health in Agriculture. Wollongbar Agricultural Institute,June 20−21, 2001; Lines-Kelly, R., Ed.; New South Wales Agriculture: Wollongbar, NSW, Australia, 2001; pp 8–13.

(23) Jansch, S.; Rombke, J.; Schallanass, H.-J.; Terytze, K. Derivation of soil values for the path 'soil-soil organisms' for metals and selected organic compounds using species sensitivity distributions. *Environ. Sci. Pollut. Res.* **2007**, *14* (5), 308–318.

(24) ANZECC; NHMRC Australian and New Zealand guidelines for the assessment and management of contaminated sites. *Australian and New Zealand Environment and Conservation Council*; National Health and Medical Research Council: Australia, 1992.

(25) Hamon, R. E.; McLaughlin, M. J.; Gilkes, R. J.; Rate, A. W.; Zarcinas, B.; Robertson, A.; Cozens, G.; Radford, N.; Bettenay, L. Geochemical indices allow estimation of heavy metal background concentrations in soils. *Global Biogeochem. Cycles* **2004**, *18*, GB1014.

(26) Delas, J. Copper toxicity in viticultural soils. In *Copper in Animal Waste and Sewerage Sludge*; Hermite, P. L., Dehandtshutter, J., Eds.; Reidel: Dordecht, The Netherlands, 1981; pp 136–142.

(27) Brun, L. A.; Maillet, J.; Richarte, J.; Herrmann, P.; Remy, J. C. Relationships between extractable copper, soil properties and copper uptake by wild plants in vineyard soils. *Environ. Pollut.* **1998**, *102*, 151–161.

(28) Chaignon, V.; Sanchez-Neira, I.; Herrmann, P.; Jaillard, B.; Hinsinger, P. Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vine-growing area. *Environ. Pollut.* **2003**, *123*, 229–238.

(29) Arias, M.; Lopez, E.; Fernandez, D.; Soto, B. Copper distribution and dynamics in acid vineyard soils treated with copper-based fungicides. *Soil Sci.* **2004**, *169*, 796–805.

(30) Frank, R.; Braun, H. E.; Ishida, K.; Suda, P. Persistent organic and inorganic pesticide residues in orchard soils and vineyards of Southern Ontario. *Can. J. Soil Sci.* **1976**, *56*, 463–484.

(31) Vavoulidou, E.; Avramides, E. J.; Papadopoulos, P.; Dimirkou, A. Copper content in agricultural soils related to cropping systems in different regions of Greece. *Commun. Soil Sci. Plant Anal.* **2005**, *36*, 759–773.

(32) Morgan, R. K.; Taylor, E. Copper accumulation in vineyard soils in New Zealand. *Environ. Sci.* **2003**, *1*, 139–167.

(33) Biswas, T.; Christen, E.; Quayle, W.; Beijert, M. Copper toxicity associated with viticulture in southeastern New South Wales, Australia. In *FUTUREsoils*; Conference Proceedings Australian Society of Soil Science National Conference, Perth, Western Australia, Dec 2−6, 2002; Williamson, D., Tang, C., Rate, A.,

Eds.; Australian Society of Soil Science: Australia, 2002; pp 32–33.

(34) Oliver, D.; Naidu, R. *Uptake of Copper (Cu), Lead (Pb), Cadmium (Cd), Arsenic (As) and Dichlorodiphenyltrichloroethane (DDT) by Vegetables Grown in Urban Environments*; Proceedings of the 5th National Workshop on the Assessment of Site Contamination; Langley, A., Gilbey, M., Kennedy, B., Eds.; National Environment Protection Council: Adelaide, Australia, 2003.

(35) McBride, M. B. Forms and distribution of copper in solid and solution phases of soil. In *Copper in Soils and Plants*; Proceedings of the Golden Jubilee International Symposium, Murdoch University, Perth, Western Australia; Loneragan, J. F., Robson, A. D., Graham, R. D., Eds.; Academic Press: Sydney, Australia, 1981; pp 25–45.

(36) Ponizovsky, A. A.; Thakali, S.; Allen, H. E.; Di Toro, D. M.; Ackerman, A. J. Effect of soil properties on copper release in soil solutions at low moisture content. *Environ. Toxicol. Chem.* **2006**, *25*, 671–682.

(37) Ponizovsky, A. A.; Allen, H. E.; Ackerman, A. J. Copper activity in soil solutions of calcareous soils. *Environ. Pollut.* **2007**, *145*, 1–6.

(38) Frampton, G. K.; Jansch, S.; Scott-Fordsmand, J. J.; Rombke, J.; Van Den Brink, P. J. Effects of pesticides on soil invertebrates in laboratory studies: a review and analysis using species sensitivity distributions. *Environ. Toxicol. Chem.* **2006**, *25*, 2480–2489.

(39) U.S. EPA. ECOTOX Database System; Office of Pesticide Programs, Environmental Fate and Effects Division; U.S. Environmental Protection Agency: Washington, DC, 2001; available at http://www.epa.gov/ecotox/.

(40) Bogomolov, D. M.; Chen, S. K.; Parmelee, R. W.; Subler, S.; Edwards, C. A. An ecosystem approach to soil toxicity testing: a study of copper contamination in laboratory soil microcosms. *Appl. Soil Ecol.* **1996**, *4*, 95–105.

(41) Menzies, N. W.; Donn, M. J.; Kopittke, P. M. Evaluation of extracts for estimation of the phytoavailable trace metals in soils. *Environ. Pollut.* **2007**, *145*, 121–130.