Casanova et al. / Agro Sur 51(1): 43-52, 2023
DOI:10.4206/agrosur.2023.v51n1-04

AGROSUR
Universidad Austral de Chile
Facultad de Ciencias Agrarias y Alimentarias

Cover crop's potential to remediate vineyard soils affected by copper. Mediterranean Chile

Potencial de cultivos cubierta para remediar suelos de viñas afectados por cobre. Chile mediterráneo

Casanova, M.[a*], Salazar, O.[a], Govan, J.[a], Tapia, Y.[a], Silva, G[a].

[a] Departamento de Ingeniería y Suelos. Facultad de Ciencias Agronómicas. Universidad de Chile.

ARTICLE INFO

Article history:
Received 05-04-2023
Accepted 31-08-2023

Keywords:
Vineyards
Soil copper
Cover crops
Bioaccumulation
Bioconcentration

Original Article,
Plant Science

**Corresponding author:*
Casanova, M.
E-mail address:
mcasanov@uchile.cl

ABSTRACT

Our main goal was to evaluate the potential of cover crops (CC: ryegrass and/or white clover) to remediate a Mollisol under table grape production in central Chile that was affected by copper (Cu), with the crop displaying fruit yields directly related to soil Cu concentrations caused by regular and long-term application of fungicides. Surface soils from sites with high (LY: low fruit yields), intermediate (MY: medium fruit yields) and low (HY: high fruit yields) available Cu (Cu_{DTPA}) were used in a glasshouse essay, where CC was sowed. After 9 months they were harvested to obtain the dry matter and Cu contents from their tissues (shoots and roots), also measuring Cu forms in the soil. A reduction of Cu_{DTPA} (67%) and exchangeable Cu (90%) was obtained. Among sites, the shoot biomass of clover tends to decrease as Cu increases, whilst ryegrass alone or associated decreases its shoot biomass and increases its root biomass. At HY and MY no statistical differences in Cu uptake between ryegrass and mixed crops were detected, but the highest Cu uptake for ryegrass was observed at LY. Among the sites, ryegrass exhibited similar Cu uptake while in clover (alone or associated) roots the Cu uptake was higher in contaminated soils. In conclusion, CC growing in more contaminated soils exhibited highest root-to-shoot ratio, being lower in clover than ryegrass in all soils. The highest tolerance index was found in more contaminated soils for the roots of ryegrass (alone or associated) and clover was less affected by Cu stress than ryegrass and mixed CC.

RESUMEN

El principal objetivo de este trabajo fue evaluar el potencial de cultivos de cobertura (CC: ballica y/o trébol blanco) para remediar un Mollisol bajo producción de uva de mesa en la zona central de Chile, afectado por cobre (Cu), con rendimientos de fruta directamente relacionados con las concentraciones de Cu en el suelo, debido a la aplicación regular y prolongada de fungicidas. Los horizontes superficiales de los sitios con un alto (LY: rendimiento bajo de fruta), intermedio (MY: rendimiento medio de fruta) y bajo (HY: rendimiento alto de fruta) disponible de Cu (Cu_{DTPA}) se dispusieron en un ensayo de invernadero, donde se sembraron los CC. Luego de 9 meses, se cosecharon para obtener contenidos de materia seca y Cu en sus tejidos (brotes y raíces), midiendo también las formas de Cu en los suelos. Se alcanzó una reducción de Cu_{DTPA} (67%) y Cu intercambiable (90%). Entre los sitios, la biomasa de brotes del trébol tiende a disminuir a medida que aumenta el Cu, mientras que la ballica sola o asociada disminuye su biomasa de brotes y aumenta su biomasa de raíces. En HY y MY no se detectaron diferencias estadísticas en la absorción de Cu entre ballica y la mezcla de ambas especies, pero se observó la mayor absorción de Cu para ballica en LY. Además, entre sitios, ballica exhibió una absorción de Cu similar y las raíces del trébol (solo o asociado) absorbieron más Cu en suelos más contaminados. En conclusión, los CC que crece en suelos más contaminados exhibieron la mayor relación de raíces: brotes, siendo esta menor en trébol que en ballica en todos los suelos. Se registraron los índices de tolerancia más altos en raíces de ballica (sola o asociada) en suelos más contaminados y el trébol resultó menos afectado por el estrés de Cu que ballica y que ambas especies mezcladas.

Palabras clave: Viñedos; Cobre del suelo; Cultivos cubierta; Bioacumulación; Bioconcentración.

Research highlights

- Sites with previous regular application of fungicides and far from mining areas, show copper levels that seriously affect fresh table grape yields in Mediterranean areas.
- Mixed cover crops (ryegrass and white clover) decreased both available and exchangeable soil copper.
- Mixed cover crops are a low-cost and environmentally friendly strategy to remediate long-term soils contaminated by copper-containing fungicides.

INTRODUCTION

Chile is a primary exporter of table grapes (*Vitis vinifera* L.) in the southern hemisphere and globally, being among the ten leading exporters of this fruit for the USA, the European Union and the Far East during winter months (Seccia et al., 2015). Table grapes are mainly grown in alluvial valleys that are located between the Atacama Desert in the northern (27° SL) zone and the Mediterranean central zone of the country (35° SL), with the Aconcagua valley basin (33° SL) being one of the most important areas to produce and export fresh fruit.

Copper (Cu) is accumulated in the top surface layer of most soils, mainly through bioaccumulation and anthropogenic sources. Despite this, is considered to be among the most labile metals available in surface environments, with evidence for migration through soil profiles in vineyards (Komárek et al., 2010) and being a worldwide concern in terms of soil–grapevine–human transfer (Lai et al., 2010; Juang et al., 2012). Depending on some basic soil properties (pH, clay and organic matter content), Cu represents a significant risk to groundwater quality. Strategies such as cover crops (CC) or phytoremediation are proposed for decreasing Cu concentration in the upper soil profile or the runoff from vineyard soils, although the use of individual plants in vineyards has not resulted in efficient outcomes (Pietrzak and Uren 2011; Mackie et al., 2012).

Considering the capacity of perennial grasses to uptake heavy metals and generate high biomass, Santibáñez et al. (2008) used ryegrass (*Lolium perenne*) in the remediation of Cu tailings in a glasshouse and Chen et al. (2006) used chelators to enhance Cu uptake by white clover (*Trifolium repens*) in contaminated soils. However, the interactions of mixed ryegrass/white clover in contaminated soil have been studied up to now only by Dong et al. (2008) and Bidar et al*.* (2009).

Grapevines (*Vitis vinifera*) are reported to be a Cu-tolerant species and could stand much higher concentrations of anthropogenic Cu than background levels, without showing symptoms of Cu toxicity when grown in clay-rich soils (Toselli et al., 2009). Several authors have reported that agricultural soils surrounding areas of mining activity in the Aconcagua River basin of Chile may have a high Cu concentration (700-4000 mg kg^{-1}), but in areas where these activities are absent the soil background Cu concentration ranges between 70 and 155 mg kg^{-1} (Aguilar et al., 2011; Verdejo et al., 2015; Mondaca et al., 2017). Indeed, Cu values about 100-fold greater than the geochemical background level in soils have been observed by Fernandez-Calviño et al. (2010). Although total soil Cu concentration rarely correlates to plant availability and critical total Cu level depends on soil properties, thresholds have been defined between >36 mg kg^{-1} and >140 mg kg^{-1} (de Gregori et al., 2003; Pietrzak and McPhail, 2004; Mackie et al., 2012).

Although Cu toxicity is extremely rare in established vineyards due to older vine's deeper root systems, it can result in stunted growth for newly planted vines, replanted in old vineyard sites or nurseries (superficial roots), limiting potential success for renewal vineyards (Miotto et al., 2014; Baldi et al., 2018). Moreover, accompanied by slower plant growth, changes in root morphology and anatomical structure, reduced root and shoot biomass and decreased rate of photosynthesis were observed by Brunetto et al. (2016) and Ambrosini et al. (2018).

The main objective of this study was to evaluate, in a glasshouse experiment, the potential of cover crops (ryegrass and/or white clover) to remediate soils from fields being utilized for table grapes production in central Chile (Aconcagua river basin) and possessing copper excess.

MATERIAL AND METHODS

Study area and sampling

The study was carried out (Figure 1) in vineyards of central Chile (32º 44' SL - 70º 39' WL; 155 m a.s.l.), southern Aconcagua river basin (Valparaíso Region, Santa María borough), where fresh table grape production accounts for over 23% (11200 ha) of the total national area cultivated with vines. Three adjoining fields occupied with Superior Seedless cultivar (growing on its own roots and with 3 m × 3 m plant distance) were selected, under plant vigor and registered fruit yields criteria. Fresh fruit yields reported for field owners were low (LY: 8 Mg ha^{-1}), medium (MY: 12 Mg ha^{-1}) and high (HY: 24 Mg ha^{-1}).

At all fields, pedons belong to the same cartographic unit within an alluvial terrace (Fluventic Haploxerolls), corresponding to loamy soils resting over an alluvial substrate with abundant rounded gravels and a sandy clay loam matrix, where a plough pan is observed (Casanova et al., 2013). A steppe climate with an arid moisture regime (BS, Köppen classification) is characteristic in almost all river basins, exhibiting a dry summer-spring period and a wet autumn-winter.

Soil characterization and plant tissue sampling

Similar genetic horizons at soil pits in each field were observed, being sampled (n = 3) for general chemical and physical characterization, according to the Chilean standard analysis procedures (Sadzawka et al., 2006; Sadzawka et al., 2007; Sandoval et al., 2012). Soil electrical conductivity in saturation extracts, soil organic matter content by wet digestion method (Walkley-Black), soil pH in a 1:2.5 ratio (soil:water) and cation exchange capacity with NH_4OAc (1 N) percolation at pH 8.2 were determined. Soil bulk density was measured

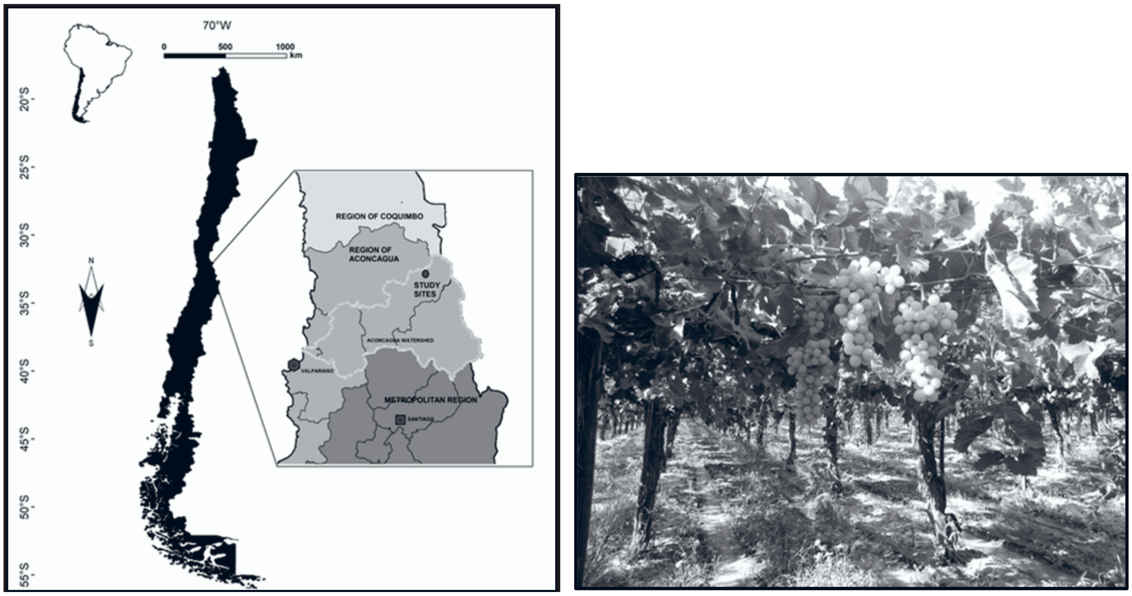


Figure 1. Location of study sites in central Chile (left) and one of three sampled sites at the berry ripening stage (right).

Figura 1. Localización de los lugares de estudio en Chile central (izquierda) y uno de los tres lugares muestreados en la fase de maduración de las bayas (derecha).

by the core method, particle density with pycnometers, soil texture by the Bouyoucos hydrometer method, and water holding capacity at 33 and 1500 kPa with pressure devices. A composed sample of fine roots (25 g) and leaf blades (n = 50) of *V. vinifera* from each site was collected during a preliminary characterisation of Cu contents (by atomic absorption spectrometry after acid digestion with HF and $HClO_4$) in tissues.

Glasshouse experiment

In each site, soils were sampled (0-20 cm) and transported to a glasshouse (Figure 2) belonging to the Universidad de Chile in Santiago, filling 2 L plastic pots with it (Figure 2). Ryegrass (*L. perenne*) and/or white clover (*T. repens*) were seeded with 5 replicates, defining 9 treatments (Table 1). Both species were chosen because they were representative grasses and legumes, commonly used in revegetation practices which have different soil rooting characteristics. The experiment was carried out between April and December, with soil water content at field capacity applying distilled water.

In addition to soil chemical analyses performed following standard methods, available soil Cu was extracted with DTPA, total soil Cu determined by acid digestion (Reed and Martens, 1996) and exchangeable soil Cu extracted with NH_4OAc (1 mol L^{-1}) at pH 7.0 (Tan, 2005), with all of them being were measured at the beginning and the end of the experiment. Harvested plant material was separated into roots and shoots (stems + leaves) and oven-dried at 65°C for 96 h to determine dry matter (DM) content. Total Cu uptake (mass unit/pot) in plant tissues (roots and shoots) was calculated using DM and Cu concentration in tissues. Buscarolli (2017) reviewed various factors, coefficients and indexes that evaluated terrestrial plant performance regarding metal dynamics in soil remediation experiments, observing diverse ways to calculate them. Hence, Figure 3 provides evaluated metrics used by us here for soils and both plant (tissues) species.

Statistical analyses

The study was carried out in a complete randomised factorial experimental design with two factors: plants (3 levels: *L. perenne*, *T. repens* and *L. perenne* + *T. repens*) and vine yields responding to soil Cu values (3 levels: HY, MY and LY) with five replicates. Data were analysed using a two-way ANOVA and when significant F values were observed, statistically significant ($p< 0.05$) differences between means were separated by Student Newman Keuls or SNK test. The software InfoStat (di Rienzo et al., 2014) was used for these purposes.

RESULTS AND DISCUSSION

Initial soil characteristics

Despite certain variability in some soil properties (Tables 2 and 3) of the evaluated fields included in the same cartographic unit, all horizons were slightly acid to neutral and non-salty, with a narrow distribution of particle size (loamy), soil density and water holding capacities. Soil organic matter contents were moderate to low in upper horizons and very low in deeper soil, with a moderate cation exchange capacity, which is mainly due to soil texture. However, vineyard fields varied significantly (p ≤ 0.05) in terms of soil Cu forms (Table 3) and previous land use reported by farmers, which can be linked to reported fruit yields. Indeed, all fields are far from mining areas but in the case of HY field, previously a walnut orchard, Cu values could correspond to the geological setting, while in the other two fields, different horticultural rotations were used

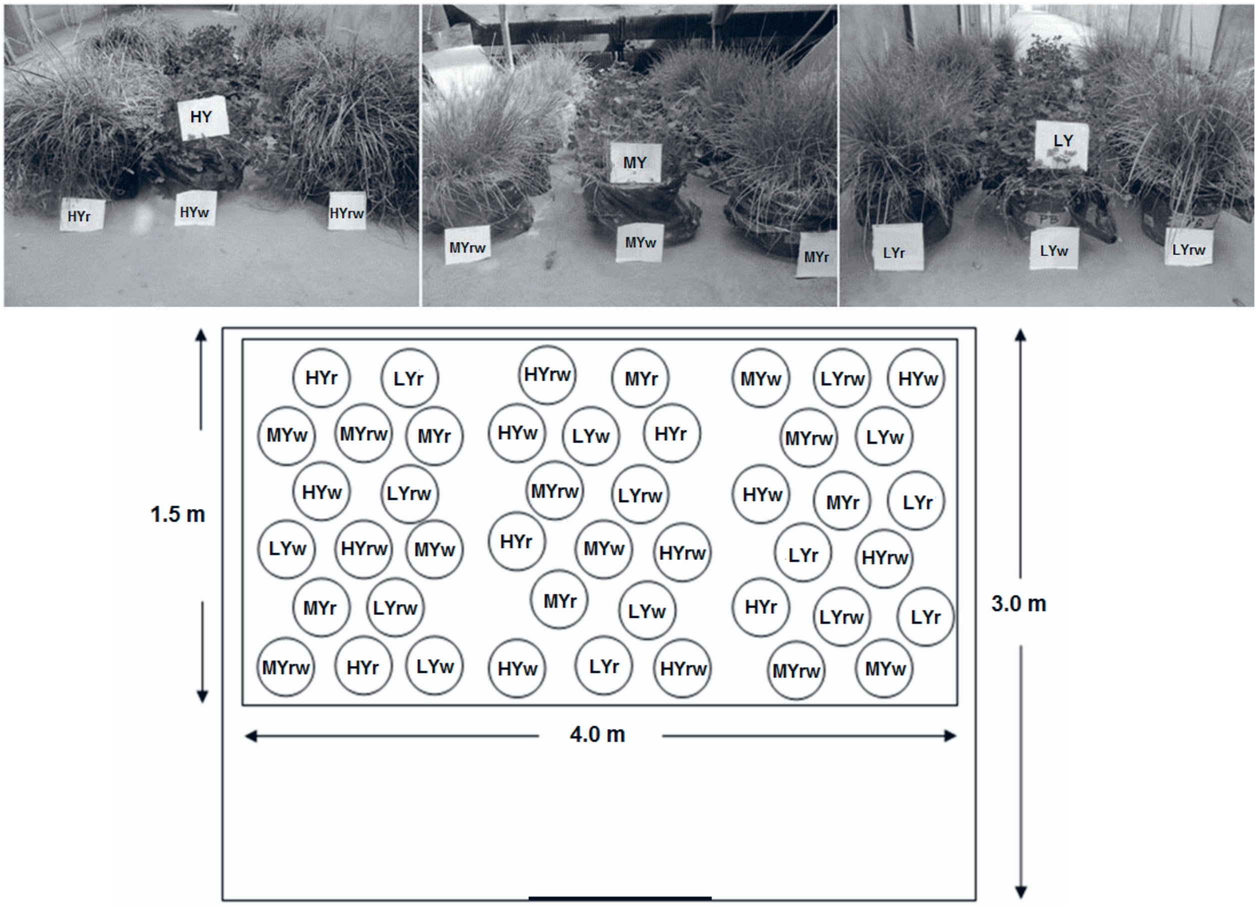


Figure 2. Experimental units with *Lolium perenne* (r) and/or *Trifolium repens* (w) and its spatial distribution within the glasshouse (see treatment details in Table 1).

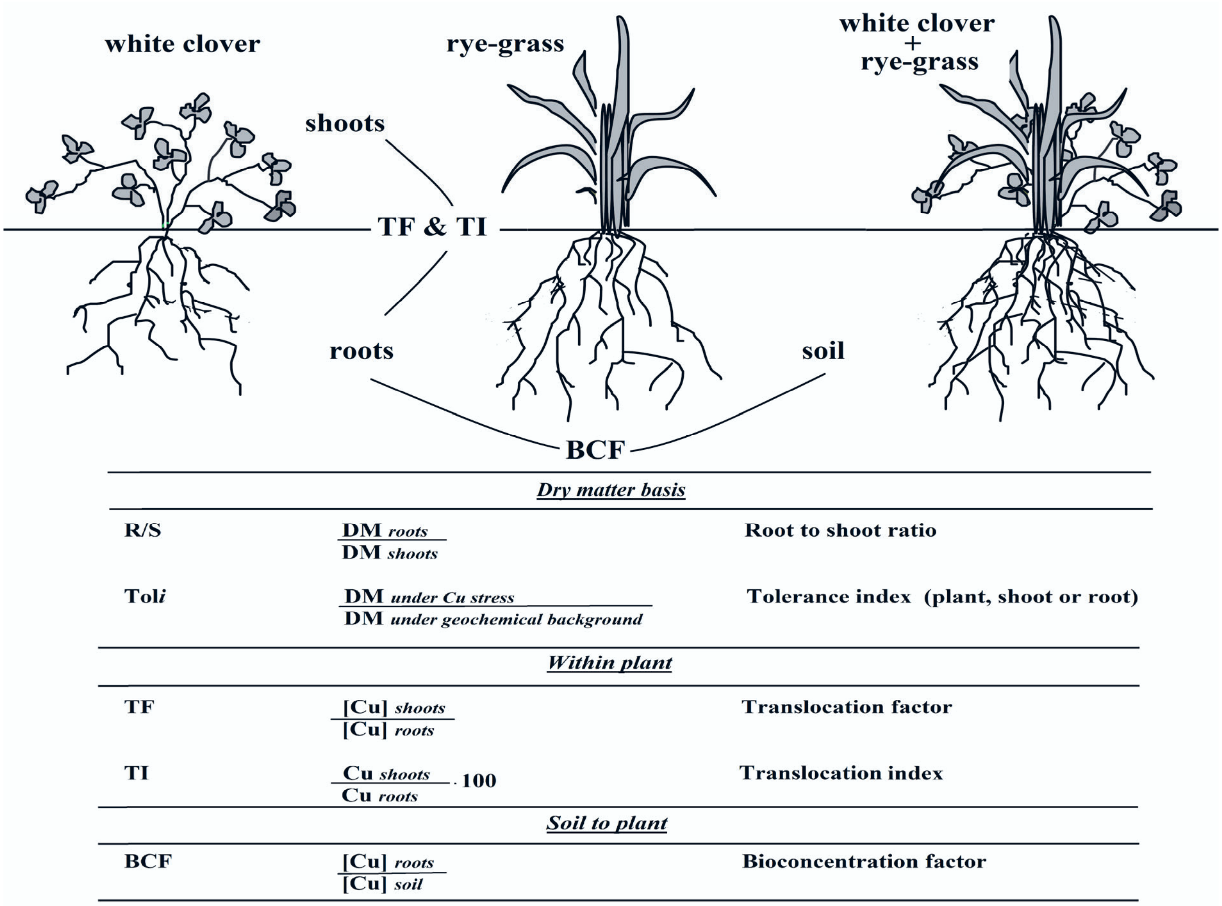
Figura 2. Unidades experimentales con *Lolium perenne* (r) y/o *Trifolium repens* (w) y su distribución espacial dentro del invernadero (ver detalles de tratamientos en Cuadro 1).

Table 1. Treatments (n = 5) in pots with soil from three fields with high (HY), medium (MY) and low (LY) fruit yields of *Vitis vinifera* (Superior seedless cv.).

Cuadro 1. Tratamientos (n = 5) en macetas con suelo procedente de tres parcelas con rendimientos de fruta alto (HY), medio (MY) y bajo (LY) de *Vitis vinifera* (cv. Superior seedless).

Treatments	Plant species
HYr or MYr or LYr	Ryegrass (*Lolium perenne* , var. Nui)
HYw or MYw or LYw	White clover *(Trifolium repens*, var. Blanco Hui)
HYrw or MYrw or LYrw	Ryegrass associated to white clover

Seed doses (g/pot): 5.1 at ryegrass, 3.8 at white clover and 3.1/2.4 at ryegrass+white clover.



	Dry matter basis		
R/S	$\frac{\text{DM}_{roots}}{\text{DM}_{shoots}}$	**Root to shoot ratio**	
Tol*i*	$\frac{\text{DM}_{under\ Cu\ stress}}{\text{DM}_{under\ geochemical\ background}}$	**Tolerance index (plant, shoot or root)**	
	Within plant		
TF	$\frac{[\text{Cu}]_{shoots}}{[\text{Cu}]_{roots}}$	**Translocation factor**	
TI	$\frac{\text{Cu}_{shoots}}{\text{Cu}_{roots}} \cdot 100$	**Translocation index**	
	Soil to plant		
BCF	$\frac{[\text{Cu}]_{roots}}{[\text{Cu}]_{soil}}$	**Bioconcentration factor**	

Figure 3. Quantitative analyses to assess copper behaviour in *Lolium perenne* and/or *Trifolium repens* at Cu-contaminated soils. DM_i, dry matter of tissue *i* (shoots or roots, in mass units); Cu, copper content in mass units; [Cu], copper concentration (in mass/mass units).

Figura 3. Análisis cuantitativos para evaluar el comportamiento del cobre en *Lolium perenne* y/o *Trifolium repens* en suelos contaminados con Cu. DMi, materia seca del tejido i (brotes o raíces, en unidades de masa); Cu, contenido de cobre en unidades de masa; [Cu], concentración de cobre (en unidades de masa/masa).

Table 2. General soil physical characterisation at three fields with different table grape yields.

Cuadro 2. Caracterización física general del suelo en tres parcelas con diferentes rendimientos de uva de mesa.

Sites	Soil depth	Sand (2-0.05 mm)	Silt (<0.05-0.002 mm)	Clay (< 0.002 mm)	Bd	Pd	W_{33}	W_{1500}
	cm	---------------------%---------------------			----- Mg m^{-3} -----		--------------%-------------	
HY	0-50	33.21±0.40	42.60±0.50	24.20±1.45	1.40±0.08	2.70±0.06	24.98±1.09	13.38±0.12
	50-100	39.13±0.23	37.30±0.59	23.60±1.17	1.49±0.21	2.60±0.09	26.92±0.07	13.45±1.90
MY	0-50	44.52±0.86	38.19±2.48	17.29±1.62	1.54±0.19	2.50±0.04	23.69±1.99	10.56±1.14
	50-100	46.20±3.31	35.90±2.50	17.90±0.81	1.70±0.38	2.53±0.10	22.31±1.25	12.00±1.47
LY	0-50	29.28±0.72	38.52±1.11	32.20±0.95	1.59±0.11	2.63±0.03	26.65±0.16	14.87±0.34
	50-100	42.26±1.13	32.97±1.26	24.78±0.77	1.58±0.35	2.64±0.60	27.88±0.24	14.13±0.34

Bd, soil bulk density; Dp, soil particle density; W_{33} and W_{1500}, soil gravimetric water contents at 33 and 1500 kPa, respectively. Vineyard fields with high (HY), medium (MY) and low (LY) fresh fruit yields. Values are means (n = 3) ± SD, where SD represents sampling variability.

Table 3. General soil chemical characterisation at three fields with different table grape yields.

Cuadro 3. Caracterización química general del suelo en tres parcelas con diferentes rendimientos de uva de mesa.

Sites	Soil depth	SOM	pH_w	ECe	CEC	Cu_{DTPA}	Exchange. Cu	Total Cu
	cm	%	-	dS m^{-1}	cmol$_c$ kg^{-1}	--------------- mg kg^{-1} ---------------		
HY	0-50	1.96±0.02	7.28±0.09	0.59±0.01	12.54±0.19	40.0±0.6**c**	27.0±1.4**c**	328.5±50.2**c**
	50-100	0.60±0.02	6.83±0.03	0.41±0.03	13.87±0.09	nd	nd	nd
MY	0-50	1.59±0.03	6.82±0.08	0.39±0.03	11.62±0.79	91.0±5.9**b**	50.5±0.7**b**	492.5±51.6**b**
	50-100	0.47±0.06	6.76±0.04	0.51±0.20	12.57±0.55	nd	nd	nd
LY	0-50	1.67±0.03	6.84±0.04	0.46±0.02	13.13±0.14	146.4±14.1**a**	126.0±2.8**a**	771.5±41.7**a**
	50-100	0.70±0.02	6.34±0.11	0.44±0.04	13.85±0.10	nd	nd	nd

SOM, soil organic matter content; pH_w, soil pH in water (1:2.5); ECe, electrical conductivity at saturated extract; CEC, cation exchange capacity. Vineyard fields with high (HY), medium (MY) and low (LY) fresh fruit yields. nd: non determined.

Values are means (n = 3) ± SD, where SD represents sampling variability. Only values followed by different lowercase letters in the same column are significantly different ($p < 0.05$).

with regular application of Cu-containing fungicides. There is abundant evidence that Cu-based fungicides can have a long-term impact on soil chemistry in vineyards and orchards (El Azzi et al., 2013; Yruela, 2015). Aguilar *et al*. (2011) reported that Cu sprayed on crops in the Aconcagua Valley would contribute to soil concentrations of up to 100 mg kg^{-1}. In this regard, initially, a total Cu (mg kg^{-1} DM) content of 34, 37 and 54 in *V. vinifera* leaves was found, whilst values of 172, 218 and 372 were registered in roots by us in HY, MY and LY fields, respectively.

In soils cultivated with grapevines and investigated as part of our study, Cu potentially available to plants (Cu_{DTPA}) and exchangeable Cu represented ~12% to ~18% and ~8% to ~16% of total Cu, respectively. Since soil Cu reference values are rather site-specific for each field studied, the total soil Cu value from vineyards with high yield (HY, ~328 mg kg^{-1}) was assumed as the background level. Moreover, the contamination Factor (CF, ratio between total soil Cu concentration and their background values in uncontaminated soils) was employed and, in accordance with different authors (Shaheen et al., 2017; Shen et al., 2019), a moderate contamination degree ($1 \leq CF < 3$) was estimated for soils from MY and LY fields (Table 3). Lillo-Robles et al*.* (2020), studied the Aconcagua Valley and proposed exchangeable Cu as a better indicator of soil phytotoxicity than either total Cu or free Cu^{2+} in solution, given an estimated moderate and high CF value for soils from MY and LY fields, respectively.

Glasshouse experiment

At the end of the glasshouse essay, practically no changes (< 16%) in total soil Cu contents were observed from initial values, while mean decreases in Cu_{DTPA} and exchangeable Cu of 67% and 90% were observed, respectively (Tables 3 and 4). Differences in growth patterns may be responsible for changes in DM and Cu concentration in both plants after the experimental period (Figure 4).

Ren *et al*. (2017) reported that *T. repens* competes poorly with *L. perenne* because of inter and intraspecific root interaction, indicating that interspecific root interaction regulates the competitive ability of grass and legume in mixtures and further makes a contribution to yielding. In this context and within each site, *T. repens* showed significantly lower ($p < 0.05$) shoots and roots biomass than *L. perenne* (alone or associated), whilst no significant ($p > 0.05$) difference in shoot biomass between *L. perenne* and mixed cover crops was observed. Root growth in *T. repens* alone did not express differences among sites, but their shoot biomass tended to decrease as contamination increased. However, *L. perenne* alone and associated cover crops decrease their shoot biomass and increase their root biomass in more contaminated sites (MY and LY).

Certain root-mediated phenomena are reported as a plant attempt to stabilise metals outside the root, limiting their availability in soil and plant uptake, particularly at rhizosphere of intercropped plant species (tolerant and intolerant), where both species can benefit from it regardless of relative contribution to metal stabilisation (Wan et al., 2017; De Conti et al., 2018). With regard to Cu uptake, it was the only plant factor that turned out to have a significant effect ($p \leq 0.05$). Therefore, within each evaluated site and those less contaminated sites (HY and MY), no statistical differences in Cu uptake between *L. perenne* and mixed cover crops were detected, but the highest values were obtained for *L. perenne* at more contaminated sites (LY). These results agree with those of Arienzo et al. (2004) who demonstrated

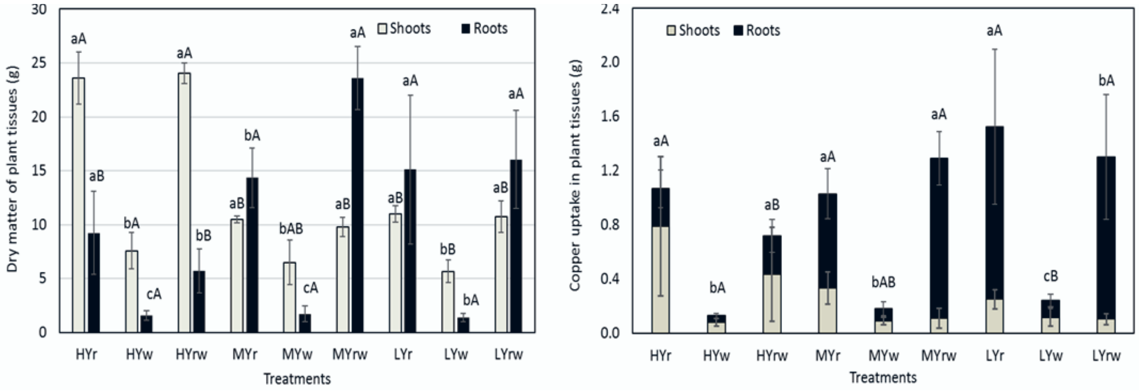


Figure 4. Plant tissue biomass (left) and copper uptake (right) for ryegrass and/or white clover in experimental pots after nine months of growth. Means in the same soil (from HY, MY or LY vineyards) followed by the same minor letter and in the same plant species or mixture (r, w or rw) followed by the same capital letter are not significantly different (p> 0.05). See Table 1 for treatment explanation.

Figura 4. Biomasa de tejidos vegetales (izquierda) y absorción de cobre (derecha) de ballica y/o trébol blanco en macetas tras nueve meses de crecimiento. Las medias en el mismo suelo (de viñedos HY, MY o LY) seguidas de igual letra minúscula y en la misma especie vegetal o mezcla (r, w o rw) seguidas de igual letra mayúscula no son significativamente diferentes (p> 0,05). Ver Cuadro 1 para la explicación de los tratamientos.

an important phytoaccumulation potential of *L. perenne* in soils contaminated with metals. Consequently, *T. repens* alone showed significantly lower ($p \leq 0.05$) Cu uptake than *L. perenne* (alone or associated). Likewise, when analysing the three sites, *L. perenne* showed the same Cu uptake at all sites, with *T. repens* (alone or associated) uptaking more Cu in contaminated soils (MY and LY), particularly at the root level (Figure 4).

Metrics

Total plant/tissue Cu contents ($Cu_{plant/tissue}$) and Cu concentrations ($[Cu_{plant/tissue}]$) were used to measure the actual plant Cu uptake, besides dry biomass (DM) to assess growth responses for the plants used (Figure 3). The root-to-shoot ratio (R/S) does not consider Cu concentrations and is a highly representative indicator of the environmental stress that affects terrestrial plants. Therefore, plants growing in soils with high Cu concentrations exhibited the highest R/S, a possible mechanism by which some plants can tolerate high Cu concentrations (Chiu et al., 2006; Rouphael et al., 2008). After 90 days, white clover showed lower R/S than ryegrass (Table 5) in all soils and a trend towards increased partitioning of DM to shoots under Cu stress, whereas in ryegrass alone and associated with clover a reverse trend was apparent.

In our essay, none plant species showed metal concentrations >1000 mg kg^{-1} in shoots (Table 4), i.e. none was considered hyperaccumulator, although their ability to tolerate and accumulate Cu was assessed (Figure 3) to estimate the plant's potential for remediation purposes. The tolerance index (Tol*i*) representing a ratio of total biomass for plants grown in soils with elevated levels of Cu compared to plants grown in control soils with baseline elemental contents (Figure 3), showed a negative trend with Cu concentration in shoots of ryegrass + clover and in ryegrass cropped alone, which decreased when increasing soil contamination (Table 5).

Given the absence of mining activities near the fields used in this study, total Cu concentrations in the range of 70-155 mg kg^{-1} were considered a background concentration for the alluvial soil at the Aconcagua Valley (Aguilar et al., 2011; Mondaca et al., 2017). However, since these reference values are rather site-specific for each studied area, soil Cu value from a vineyard with high yield (HY, ~272 mg kg^{-1}) was assumed as the background concentration level.

According to Antoniadis et al. (2017), a Tol*i* <1 indicates a net decrease in biomass suggesting that plants are stressed, Tol*i* = 1 indicates no difference relative to control soils, whereas Tol*i* > 1 indicates positive effects, such as those expected in fertility tests with added nutrients and suggesting that plants express a growth dilution effect. Here, the highest tolerance index (> 1) for roots of ryegrass (alone or associated) in contaminated soils was recorded (Table 5).

A translocation factor (TF) above 1, as previously defined (Figure 3), indicates higher Cu translocation from roots to above-ground parts (shoots) and was only observed for *L. perenne* in less contaminated soils (HYr). It is also highlighted that *T. repens* was less affected by Cu increase, decreasing their TF by only 26% in more contaminated sites, compared with ryegrass and mixed crops where this factor decreased by around 74

Table 5. Experimental metrics for cover crops in three soil types after 90 day growth period.

Cuadro 5. Métricas experimentales de los cultivos cubierta en tres tipos de suelo tras un periodo de crecimiento de 90 días (n= 5).

Treatment	R/S	Tol_{plant}	Tol_{shoots}	Tol_{roots}	TF	TI	BCF
	-	-	-	-	-	(%)	-
HY*r*	0.38±0.14**a**	-	-	-	1.09±0.82**a**	70±13**c**	1.12±0.30**a**
MY*r*	1.37±0.52**b**	0.78	0.45	1.64	0.65±0.16**a**	32±3**b**	1.01±0.14**a**
LY*r*	1.36±0.23**b**	0.74	0.43	1.55	0.28±0.11**a**	18±10**a**	1.04±0.15**a**
HY*w*	0.21±0.05**a**	-	-	-	0.34±0.12**a**	61±12**a**	1.16±0.28**a**
MY*w*	0.24±0.03**a**	0.77	0.75	0.88	0.26±0.06**a**	51±9**a**	1.19±0.43**a**
LY*w*	0.26±0.05**a**	0.90	0.86	1.11	0.25±0.18**a**	47±15**a**	1.17±0.43**a**
HY*rw*	0.24±0.09**a**	-	-	-	0.44±0.48**a**	56±19**b**	1.84±0.34**b**
MY*rw*	1.49±0.30**b**	0.90	0.45	2.81	0.22±0.11**a**	8±4**a**	1.08±0.17**a**
LY*rw*	2.44±0.44**c**	1.12	0.41	4.14	0.13±0.05**a**	8±4**a**	0.97±0.13**a**

R/S, root-to-shoot ratio; Tol*i*, tolerance index; TF, translocation factor; TI, translocation index; BCF, bioconcentration factor.
Values are means (n = 5) ± SD, where SD represents sampling variability. Only values followed by different lowercase letters in the same plant species or mixture (r, w or rw) are significantly different ($p < 0.05$).

and 70%, respectively (Table 5). The ability of both species regarding Cu translocation to shoots was estimated also by translocation index (TI), which again expressed the best *T. repens* proficiency in more contaminated soils (47%).

Greater bioconcentration factor (BCF) values indicate relatively high efficiency of plant shoots and roots to uptake metals. Therefore, for assessing bioaccumulation, a common threshold value adopted (Alagić et al., 2013, Pachura et al., 2015) was used, where BCF > 1 indicate that metal is accumulated in roots from soil, being observed for *L. perenne* and/or *T. repens* in all soil conditions (Table 5).

CONCLUSION

The Contamination Factor confirms the medium and high levels of soil contamination by copper estimated in *Vitis vinifera* orchards with moderate (MY) and low (LY) fruit yields, respectively.

Ryegrass (*L. perenne*) associated with white clover (*T. repens*) as cover crops (CC) showed potential to remediate vineyard soils affected by Cu, considering:

a) Total soil Cu contents were not statistically different from initial values after the harvesting of a glasshouse essay, but available and exchangeable Cu decreased by 67% and 90%, respectively.

b) Among sites, *T. repens* express no differences in root growth and its shoot biomass tends to decrease as Cu contamination increases, whilst *L. perenne* alone or associated with *T. repens* decreases its shoot biomass and increases its root biomass in contaminated sites (MY and LY).

c) Cu plant uptake, within HY and MY (less contaminated sites), was not different between *L. perenne* and mixed cover crops, yet the highest Cu uptake for *L. perenne* at more contaminated sites (LY) was observed. Besides, among the three sites, *L. perenne* exhibited the same Cu uptake and *T. repens* (alone or associated) uptake more Cu in contaminated soils (MY and LY), particularly at roots.

d) Both species growing in more contaminated soils exhibited the highest root-to-shoot ratio (R/S) as a mechanism against high Cu concentrations. White clover showed lower R/S than ryegrass in all soils, showing a trend to increase its shoot´s dry matter under Cu stress, whereas ryegrass (alone and associated) showed a reverse trend.

e) The highest tolerance index (> 1) for roots of ryegrass (alone or associated) in more contaminated soils was observed. Besides, the translocation factor (TF) above 1 was only found in the case of *L. perenne* at less contaminated soil (HYr). *T. repens* was less affected by Cu stress than *L. perenne* and mixed CC, a fact that was ratified by the translocation index (TI).

Acknowledgements This work was funded by the Soil and Engineering Department at the Faculty of Agricultural Sciences, Universidad de Chile.

REFERENCES

Aguilar, R., Hormazábal, C., Gaete, H., Neaman, A. 2011. Spatial distribution of copper, organic matter and pH in agricultural soils affected by mining activities. Journal of

Soil Science and Plant Nutrition 11 (3), 125-145. http://dx.doi.org/10.4067/S0718-95162011000300010

Alagić, S.Č., Šerbula, S,S., Tošic, S.B., Pavlović, A.N., Petrović, J.V. 2013. Bioaccumulation of Arsenic and Cadmium in Birch and Lime from the Bor Region. Archives of Environmental Contamination and Toxicology 65 (4), 671-682. http://dx.doi.org/10.1007/s00244-013-9948-7

Ambrosini, V.G., Rosa, D.J., Bastos de Melo, G.W., Zalamena, J., Cella, C., Simao, D., Souza da Silva, L., Pessoa dos Santos, H., Toselli, M., Tiecher, T.L., Brunetto, G. 2018. High copper content in vineyard soils promotes modifications in photosynthetic parameters and morphological changes in the root system of 'Red Niagara' plantlets. Plant Physiology and Biochemistry 128, 89-98. http://dx.doi.org/10.1016/j.plaphy.2018.05.011

Antoniadis, V., Levizou, E., Shaheen, S.M., Ok, Y.S., Sebastian, A., Baum, C., Prasad, M.N.V., Wensel, W.W., Rinklebe, J. 2017. Trace elements in the soil-plant interface: Phytoavailability, translocation, and phytoremediation – A review. Earth-Science Reviews 171, 621-645. http://dx.doi.org/10.1016/j.earscirev.2017.06.005

Arienzo, M., Adamo, P., Cozzolino, V. 2004. The potential of *Lolium perenne* for revegetation of contaminated soil from a metallurgical site. Science of the Total Environment 319 (1-3),13-25. http://dx.doi.org/10.1016/S0048-9697(03)00435-2

Baldi, E., Miotto, A., Ceretta, C.A., Quartieri, M., Sorrenti, G., Brunetto, G., Toselli, M. 2018. Soil-applied phosphorous is an effective tool to mitigate the toxicity of copper excess on grapevine grown in rhizobox. Science Horticulturae 227, 102-111. http://dx.doi.org/10.1016/j.scienta.2017.09.010

Bidar, G., Pruvot, C., Garcon, C., Verdin, A., Shirali, P., Douay, F. 2009. Seasonal and annual variations of metal uptake, bioaccumulation, and toxicity in *Trifolium repens* and *Lolium perenne* growing in a heavy metal-contaminated field. Environmental Science and Pollution Research 16, 42–53. https://doi.org/10.1007/s11356-008-0021-4

Brunetto, G., de Melo, G.W.B., Terzano, R., Del Buono, D., Astolfi, S., Tomasi, N., Pii, Y., Mimmo, T., Cesco, S. 2016. Copper accumulation in vineyard soils: Rhizosphere processes and agronomic practices to limit its toxicity. Chemosphere 162, 293-307. http://dx.doi.org/ 10.1016/j.chemosphere.2016.07.104

Buscarolli, A. 2017. An overview of indexes to evaluate terrestrial plants for phytoremediation purposes. Ecological Indicators 82, 367-380. http://dx.doi.org/10.1016/j.ecolind.2017.07.003

Casanova, M., Salazar, O., Seguel, O., Luzio, W. 2013. The Soils of Chile. World Soils Book Series, Springer International Publishing. Dordrecht, The Netherlands.

Chen, Y., Wang, Y., Wu, W. 2006. Impacts of chelate-assisted phytoremediation on microbial community composition in the rhizosphere of a copper accumulator and non-accumulator. Science of The Total Environment 356 (1-3), 247-255. https://doi.org/10.1016/j.scitotenv.2005.04.028

Chiu, K.K., Ye, Z.H., Wong, M.H. 2006. Growth of *Vetiveria zizanioides* and *Phragmities australis* on Pb/Zn and Cu mine tailings amended with manure compost and sewage sludge: A greenhouse study. Bioresource Technology 97 (1), 158-70. https://doi.org/10.1016/j.biortech.2005.01.038

De Conti, L., Ceretta, C.A., Tiecher T., da Silva, L.O.S., Tassinari, A., Mosavilla, L.M., Mimmo, S, Cesco, S. Brunetto, G. 2018. Growth and chemical changes in the rhizosphere of black oat (*Avena strigosa*) grown in soils contaminated with copper. Ecotoxicology and Environmental Safety 163, 19-27. https://doi.org/10.1016/j.ecoenv.2018.07.045

De Gregori. I., Fuentes, E., Rojas, M., Pinochet, H., Potin-Gautier, M. 2003. Monitoring of copper, arsenic and antimony levels in agricultural soils impacted and non-impacted by mining activities, from three regions in Chile. Journal of Environmental Monitororing 5 (2), 287-295. https://doi.org/10.1039/B211469K

Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W. 2014. InfoStat, FCA, Universidad Nacional de Córdoba, Argentina (http://www.infostat.com.ar).

Dong, Y., Zhu, Y.G., Smith, F.A., Wang, Y., Chen, B. 2008. Arbuscular mycorrhiza enhanced arsenic resistance of both white clover (*Trifolium repens* L.) and ryegrass (*Lolium perenne* L.) plants in an arsenic-contaminated soil. Environmental Pollution 155, 174-181. https://doi.org/10.1016/j.envpol.2007.10.023

El Azzi, D., Viers, J., Guiresse, M., Probst, A., Aubert, D., Caparros, J., Charles, F., Guizien, K., Probst, J. 2013. Origin and fate of copper in a small Mediterranean vineyard catchment: New insights from combined chemical extraction and δ^{65} Cu isotopic composition. Science of The Total Environment 463-464, 91-101. https://doi.org/10.1016/j.scitotenv.2013.05.058

Fernandez-Calviño, D., Martín, A., Arias-Estévez, M., Bååth, E., Díaz-Raviña, M. 2010. Microbial community structure of vineyard soils with different pH and copper content. Applied Soil Ecology 46 (2), 276-282. https://doi.org/10.1016/j.apsoil.2010.08.001

Juang, K., Lee, Y., Lai, H., Wang, C., Chen, B. 2012. Copper accumulation, translocation, and toxic effects in grapevine cuttings. Environmental Science and Pollution Research 19, 1315-1322. https://doi.org/10.1007/s11356-011-0657-3

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

Lai, H.Y., Juang, K.W., Chen B.C. 2010. Copper concentrations in grapevines and vineyard soils in central Taiwan. Soil Science and Plant Nutrition 56 (4), 601-606. https://doi.org/10.1111/j.1747-0765.2010.00494.x

Lillo-Robles, F., Tapia-Gatica, J., Díaz-Siefer, P., Moya, H., Youlton, C., Celis-Diez, J., Santa-Cruz, J., Ginocchio, R., Sauvé, S., Brykov, V.A., Neaman, A. 2020. Which soil Cu pool governs phytotoxicity in field-collected soils contaminated by copper smelting activities in central Chile?. Chemosphere 242, 125176. https://doi.org/10.1016/j.chemosphere.2019.125176

Mackie, K.A., Müller, T., Kandeler, E. 2012. Remediation of copper in vineyards - A mini review. Environmental Pollution 167, 16-26. https://doi.org/10.1016/j.envpol.2012.03.023

Miotto, A., Ceretta, C.A., Brunetto, G., Nicoloso, F.T., Girotto, E., Farías, J.G., Tiecher, T.L., de Conti, L., Trentin, G. 2014. Copper uptake, accumulation and physiological changes in adult grapevines in response to excess copper in soil. Plant and Soil 374, 593-616. https://doi.org/10.1007/s11104-013-1886-7

Mondaca, P., Catrin, J., Verdejo, J., Sauvé, S., Neaman, A. 2017. Advances on the determination of thresholds of Cu phytotoxicity in field-contaminated soils in central Chile. Environmental Pollution 223, 146-152. https://doi.org/10.1016/j.envpol.2016.12.076

Pachura, P., Ociepa-Kubicka, A., Skowron-Grabowska, B. 2015. Assessment of the availability of heavy metals to plants based on the translocation index and the bioaccumulation factor. Desalinisation and Water Treatment 57 (3), 1469-1477. https://doi.org/10.1080/19443994.2015.1017330

Pietrzak, U., McPhail, D.C. 2004. Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. Geoderma 122 (2-4), 151-166. https://doi.org/10.1016/j.geoderma.2004.01.005

Pietrzak, U., Uren, N.C. 2011. Remedial options for copper-contaminated vineyard soils. Soil Research 49 (1), 44-55. https://doi.org/10.1071/SR09200

Reed, S.T., Martens D.C. 1996. Copper and Zinc. In: Sparks D.L. (ed). Methods of Soil Analysis. Part 3: Chemical Methods. SSSA Book Series Nr.5. Madison, Wis. USA. pp. 703-722. https://doi.org/10.2136/sssabookser5.c26

Ren. H., Gao, T., Hu, J., Yang, G. 2017. The effects of arbuscular mycorrhizal fungi and root interaction on the competition between *Trifolium repens* and *Lolium perenne*. PeerJ 5, e4183. https://doi.org/10.7717/peerj.4183

Rouphael, Y., Cardarelli, M., Rea, E., Colla, G. 2008. Grafting of cucumber as a means to minimize copper toxicity. Environmental and Experimental Botany 63 (1), 49-58. https://doi.org/10.1016/j.envexpbot.2007.10.015

Sadzawka, A., Carrasco, M., Demanet, R., Flores, H., Grez, R., Mora, M.L., Neaman, A. 2007. Methods of Vegetal Tissue Analyses. Instituto de Investigaciones Agropecuarias, Serie Actas INIA Nº 40, Santiago, Chile. (In Spanish).

Sadzawka, M.A., Carrasco, M.A., Grez, R., Mora, M.L., Flores, H., Neaman, A. 2006. Recommended analysis methods for Chilean soils. Instituto de Investigaciones Agropecuarias, Serie Actas INIA N° 34, Santiago, Chile. (In Spanish).

Sandoval, M., Dörner, J., Seguel, O., Cuevas, J., Rivera, D. 2012. Soil physical analyses methods. Departamento de Suelos y Recursos Naturales. Universidad de Concepción. Chillán, Chile. (In Spanish).

Santibañez. C., Verdugo, C., Ginocchio, R. 2008. Phytostabilization of copper mine tailings with biosolids: Implications for metal uptake and productivity of *Lolium perenne*. Science of The Total Environment 35, 1-10. https://doi.org/10.1016/j.scitotenv.2007.12.033

Shaheen, S.M., Shams, M.S., Khalifa, M.R., El-Dali, M.A., Rinklebe, J. 2017. Various soil amendments and wastes affect the (im)mobilization and phytoavailability of potentially toxic elements in a sewage effluent irrigated sandy soil. Ecotoxicology and Environmental Safety 142, 375-387. https://doi.org/10.1016/j.ecoenv.2017.04.026

Seccia, A., Santeramo, F.G., Nardone, G. 2015. Trade competitiveness in table grapes: a global view. Outlook on Agriculture 44, 127-134. https://doi.org/10.5367/oa.2015.0205

Shen, F., Mao, L., Sun, R., Du, J., Tan, Z., Ding, M. 2019. Contamination Evaluation and Source Identification of Heavy Metals in the Sediments from the Lishui River Watershed, Southern China. International Journal of Environmental Research and Public Health 16 (3), 336. https://doi.org/10.3390/ijerph16030336

Tan, K.H. 2005. Soil Sampling, Preparation and Analysis. 2nd Ed., CRC Press, Taylor & Francis group, Boca raton, FL. USA. https://doi.org/10.1201/9781482274769

Toselli, M., Baldi, E., Marcolini, G., Malaguti D., Quartieri M.,Sorrenti G., Marangoni, B. 2009. Response of potted grapevines to increasing soil copper concentration. Australian Journal of Grape and Wine Research 15 (1), 85-92. https://doi.org/10.1111/j.1755-0238.2008.00040.x

Verdejo, J., Ginocchio, R., Sauvé, S., Salgado, E., Neaman, A. 2015. Thresholds of copper phytotoxicity in field-collected agricultural soils exposed to copper mining activities in Chile. Ecotoxicology and Environmental Safety 122, 171-177. https://doi.org/10.1016/j.ecoenv.2015.07.026

Wan, X., Lei, M., Chen, T., Yang, J. 2017. Intercropped *Pteris vittata* L. and *Morus alba* L. presents a safe utilization mode for arsenic-contaminated soil. Science of The Total Environment 579, 1467-1475. https://doi.org/10.1016/j.scitotenv.2016.11.148

Yruela, I. 2015. Copper, in: Barker, A., Pilbeam, D. (Eds.) Handbook of Plant Nutrition. Taylor and Francis Group, Boca Raton. pp. 367-398.