



Effect of spent mushroom substrate applied to vineyard soil on the behaviour of copper-based fungicide residues

E. Herrero-Hernández^a, M.S. Andrades^b, M.S. Rodríguez-Cruz^a, M.J. Sánchez-Martín^{a,*}

^a Instituto de Recursos Naturales y Agrobiología (IRNASA-CSIC), Cordel de Merinas 40-52, 37008 Salamanca, Spain

^b Departamento de Agricultura y Alimentación, Universidad de La Rioja, Madre de Dios 51, 26006 Logroño, Spain

ARTICLE INFO

Article history:

Received 29 July 2010

Received in revised form

1 February 2011

Accepted 8 March 2011

Available online 1 April 2011

Keywords:

Copper distribution

Vineyard soil

Spent mushroom substrate

Chemical forms

ABSTRACT

The effect of the addition of spent mushroom substrate (SMS) to the soil as an amendment on the distribution and/or fate of copper from a copper-based fungicide applied to a vineyard soil in La Rioja (N. Spain) was studied. The study was carried out on experimental plots amended or not with SMS at rates of 40 and 100 t ha⁻¹. The variation in total Cu content in the topsoil (0–10 cm) and in the soil profile (0–50 cm), and the distribution of Cu in different fractions of the topsoil were studied as a function of the dose of Cu added (5 and 10 kg ha⁻¹) and of the time elapsed since application (0–12 months). In addition, the changes in the chemical properties (solid organic carbon (OC), dissolved organic carbon (DOC) and pH) of the soils were studied. A greater capacity for Cu retention by the amended soils than by the unamended one was observed only when the fungicide was applied at the high dose. No effect of the amendment rate was noted on this retention capacity. The metal content in the topsoil decreased over time in step with the disappearance of the OC in the amended soil due to its oxidation, mineralization and/or leaching. This decrease in total Cu content was possibly due to the formation of soluble Cu complexes with the DOC, which facilitated its transport through the soil. A re-distribution of Cu in the different soil fractions was also observed over time, mainly from the organic to the residual fraction. The results obtained indicate that the increase in OC due to the application of SMS at the rates used does not lead to any significant increase in the persistence of Cu in the soil over time. Of greater interest would be the assessment of the risk for groundwater quality, owing to possible leaching of the fungicide enhanced by the SMS when SMS and Cu-based fungicides are jointly applied to vineyard soils.

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1. Introduction

The use of organic and inorganic fungicides in vineyards is a common practice in many wine-producing countries (Komárek et al., 2010). Copper-based fungicides have been intensively used to control fungal vine diseases such as downy mildew, caused by *Plasmopara viticola*. They are even used for organic vine cultivation, where the application of organic fungicides is prohibited (EC regulation 473/2002). Although copper-based fungicides are effective for the control of fungal diseases, their long-term use can result in increased copper levels in surface soils with respect to natural background Cu concentrations (<30 mg kg⁻¹) (Adriano, 2001). Cu concentrations varying over a broad range have been detected in the surface horizons of vineyard soils in Europe (14–1280 mg kg⁻¹), Australia (6–249 mg kg⁻¹), and Brazil

(37–3216 mg kg⁻¹) (Komárek et al., 2010; Wightwick et al., 2008). They depend on factors such as the parent soil material (Adriano, 2001), the age of the vineyard (Fernández-Calviño et al., 2008; Pietrzak and McPhail, 2004), the landform of the soil (terrace, plateau or plain) (Rusjan et al., 2007), and the influence of climate (Fernández-Calviño et al., 2009). Most published values exceed the warning and critical legislative limits (50 and 140 mg kg⁻¹, respectively) established in the EU for Cu concentrations in agricultural soils (Komárek et al., 2010). These data indicate that potential soil contamination could occur through the use of copper-based fungicides, although data on Cu mobility in soils and its availability to biota are also necessary for the assessment of its environmental impact (Ahumada et al., 2009; Brun et al., 1998; Flores-Vélez et al., 1996).

Increases in Cu content in soils occur as a result of its retention through mechanisms of specific adsorption, cation exchange, and the precipitation of new solid phases. Factors such as pH, clay mineral content, carbonates and organic matter (OM) content are important in this process (Arias et al., 2004; Komárek et al., 2009;

* Corresponding author. Tel.: +34 923 219 606; fax: +34 923 219 609.

E-mail address: mjesus.sanchez@irnasa.csic.es (M.J. Sánchez-Martín).

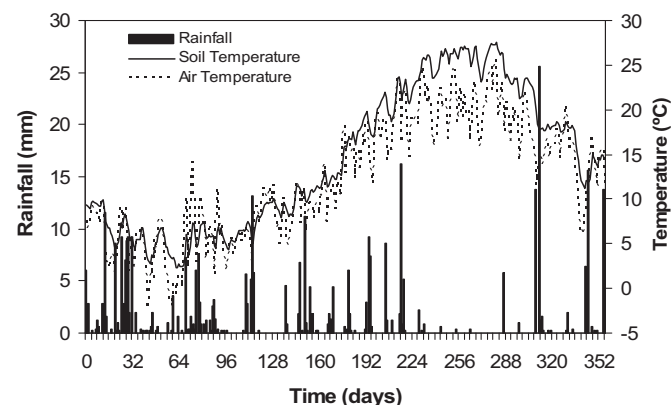


Fig. 1. Weather conditions at the site throughout the experiment.

Rodriguez-Rubio et al., 2003). The adsorption of Cu by solid OM through complexation, especially with humic and fulvic acids (inner-sphere complexes), is one of the most important mechanisms involved in the retention of this element by the soil (Boudesocque et al., 2007). However, dissolved OM (DOM) may also form soluble complexes with Cu and thus increase its mobility, especially at alkaline pH (Doelsch et al., 2010; Stevenson, 1994; Temminghoff et al., 1997), and its possible leaching/transport to groundwater and surface waters.

Vine cultivation is the main agricultural activity in the region of La Rioja (N. Spain). Its importance lies in the considerable economic activity it generates (D.O. Ca. Rioja, 2009). Some of these soils have low OM contents, and attempts are currently being made to improve their fertility through the application of organic amendments. Spent mushroom substrate (SMS) is the pasteurized organic material remaining after a mushroom crop has been harvested. In the La Rioja region, this spent substrate is produced in large amounts (>183,000 tons in 2005) and for many years SMS was disposed of in landfills. However, this method has recently become an environmental issue and industries are now proposing to reuse these materials as soil amendments. The addition of such residues to vineyard soils may be beneficial because they are rich in nutrients and OM (Paredes et al., 2009). However, the application of SMS involves the addition of the solid and liquid OM from these residues to the soil, which could affect Cu dynamics when it is applied as a fungicide in vineyard soils.

The high affinity of Cu for OM has led many authors to study the influence of organic amendments such as sewage sludge, urban compost, biochar, greenwaste compost or agro-industrial by-products in the fate of Cu in the soil, since both the solid OM from these organic residues (Ramos, 2006; Vega et al., 2009; Xiao and Huang, 2009) and their dissolved fractions, DOM (Ashworth and Alloway, 2004; Gondar and Bernal, 2009; Beesley et al., 2010) may modify the sorption and/or mobility of Cu in soils. However, only a few authors have included SMS among the organic residues studied (van Herwijnen et al., 2007). Moreover, these studies were

generally conducted in the laboratory, and there are far fewer studies that have explored the situation under field conditions (Besnard et al., 2001). Accordingly, additional research is needed because results in laboratory systems may differ from those obtained under field conditions.

The objective of this work has been to study the effect of adding SMS used as an amendment on the distribution and/or fate of Cu from a Cu-based fungicide applied to a vineyard soil. The study was carried out at experimental field plots, determining over one year: 1) the variation in the parameters of the unamended and amended soils (pH, OC, and DOC) that can modify the behaviour of Cu; 2) the changes in the total Cu content in the topsoil and in the soil profile to assess its possible accumulation and/or transfer to groundwater, and 3) the re-distribution of Cu in the different fractions of the topsoil as estimated by a sequential extraction procedure to determine the evolution of its potential (bio)-availability for plants and other organisms. Two rates of SMS (40 and 100 t ha⁻¹) and two doses of a Cu-based fungicide (5 and 10 kg ha⁻¹) were used to approximate the results of the experiments conducted to the agricultural conditions usually employed.

2. Materials and methods

2.1. Site description and experimental design

A field experiment was conducted in a vineyard soil located in Sajazarra (SA), La Rioja, Spain, (42°35'0"N latitude and 2°57'0"W longitude). Sajazarra lies at an altitude of 500 m above sea level, with a temperate climate and gentle topography. Experimentation was conducted in a sandy clay loam soil developed on calcareous sandstones and classified as Typic Calcixerept according to the Soil Survey Staff (2006).

An experimental layout of randomized complete blocks was designed with six treatments (unamended and amended soils at two rates of SMS treated with two doses of Cu) and three replicates per treatment (18 plots of 1.50 × 3.90 m). Prior to soil amendment, the soil was tilled using a field cultivator. Unamended vineyard soil and the same soil amended with SMS at rates of 40 t ha⁻¹ (SA-40) or 100 t ha⁻¹ (SA-100) (dry weight) were prepared on 10 November 2008. SMS was manually mixed with the topsoil (0–10 cm) in each plot. Three more plots (unamended, and amended with low and high rates of SMS) did not receive Cu application and were used as untreated control SA soils. Solutions of Cu at two doses (5 and 10 kg ha⁻¹) corresponding to the recommended dose and twice this dose were prepared from the commercial formulation Cuprosan 500 (50% w/w of Cu as copper oxychloride). They were applied to the plots by surface soil spraying the day after soil was amended using a manual backpack sprayer. Thus, nine of the plots were treated with the low dose and the other nine plots were treated with the high dose of the Cu-based fungicide. The plots did not receive any other treatment until the experiment was completed (November 2009).

Weather conditions (precipitation and soil and air temperature) were monitored at the site throughout the experiment (Fig. 1). The data recorded gave an annual mean temperature for the air of 12 °C

Table 1
Selected properties of soil used.

Depth (cm)	Soil texture	pH	OC g kg ⁻¹	N g kg ⁻¹	CaCO ₃ g kg ⁻¹	Sand g kg ⁻¹	Silt g kg ⁻¹	Clay g kg ⁻¹	Total Cu mg kg ⁻¹
0–10	Sandy loam	7.7	13.1	1.3	295	607	202	171	25.8 ± 2.29
10–20	Sandy loam	7.8	12.9	1.3	292	626	183	191	16.3 ± 0.43
20–30	Sandy loam	7.8	12.1	1.3	296	641	214	145	18.4 ± 1.15
30–40	Sandy loam	7.8	10.9	1.2	305	636	230	134	10.9 ± 2.24
40–50	Sandy clay loam	7.9	8.4	0.9	436	633	157	210	6.60 ± 3.16

Table 2
Characteristics of SMS.

Parameter	SMS
pH	7.5
Ash%	44.4
Moisture%	53.0
OC g kg ⁻¹	271
DOC g kg ⁻¹	12.2
OM g kg ⁻¹	556
N g kg ⁻¹	22
C/N	14.8
Cd mg kg ⁻¹	0.32 ± 0.05
Cr mg kg ⁻¹	89.4 ± 13.8
Cu mg kg ⁻¹	38.7 ± 6.69
Ni mg kg ⁻¹	28.3 ± 14.1
Pb mg kg ⁻¹	29.6 ± 3.96
Zn mg kg ⁻¹	179 ± 7.31

and 14 °C for the soil (at 5 cm depth), with a cumulative rainfall of 377 mm.

Five soil cores (3 cm diameter, 50 cm depth) from the untreated and unamended soil (control SA plot) were collected at the beginning of the experiment, and composite soil samples representative of this plot were obtained from depths of 0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm and 40–50 cm in five soil cores. Additionally, from the 18 treated plots we collected: 1) five soil cores (9 cm diameter, 10 cm depth) at 1 (T0) and 36 (T36) days after Cu application and 2) five soil cores (3 cm diameter, 50 cm depth) at 121 (T121), 212 (T212) and 359 (T359) days after Cu application. All soil samples were taken on a 1.05 × 2.50 m grid over the experimental area and were transferred to polypropylene bottles, transported to the laboratory in portable refrigerators and stored in a freezer until analysis.

2.2. Soil and spent mushroom substrate analysis

The characteristics of the unamended and amended soils were determined with the usual methods of analysis (MAPA, 1986) (Table 1). The soil samples were air-dried and sieved (<2 mm). The pH of the soils was determined in suspensions with a soil:water ratio of 1:2.5 and soil particle size distribution was determined using the pipette method. Organic carbon (OC) contents were determined using a modified version of the Walkley–Black method (Prat Pérez and Sánchez, 1973). Dissolved organic carbon (DOC) was determined in a suspension of soil in deionized water (1:2) after shaking (24 h), centrifugation (20 min at 12,800 g), and filtering. The DOC content was determined using a Shimadzu 5050 (Shimadzu, Columbia, MD, USA) carbon analyzer. Determinations were carried out in triplicate. Inorganic carbon was determined as CaCO₃ with a Bernard calcimeter. Primary minerals (quartz and calcite) and clay minerals (illite and kaolinite) were qualitatively identified in all soil samples by X-ray diffraction using CuK α radiation on a Philips PW1710 diffractometer (Eindhoven, The Netherlands).

Table 3
Chemical extraction scheme used in this work for copper fractionation.

Fraction	Reagent conditions ^a
Exchangeable/acid extractable (F1)	40 mL of 0.11 M acetic acid. Shaking 16 h at 22 °C
Associated with reducible phases (metal bound to Fe and Mn oxo-hydroxides) (F2)	40 mL of 0.5 M hydroxylammonium chloride. Shaking 16 h at 22 °C
Associated with oxidisable phases (metal bound to organic matter) (F3)	20 mL of 8.8 M hydrogen peroxide. Digestion 2 h at 85 °C 50 mL of 1 M ammonium acetate adjusted to pH 2 with HNO ₃ . Shaking 16 h at 22 °C
Residual (F4)	Digestion with 10 mL of aqua regia

^a Separation between steps was by decantation of the supernatant after centrifugation at 2500 g for 15–30 min. Sample weight was 1 g.

SMS (commercial name: Intracompost SPCH-SPC) from *Agaricus bisporus* (75%) and *Pleurotus* sp. (25%) cultivation was supplied by INTRAVAL (La Rioja, Spain). The SMS obtained immediately after it had been removed from the mushroom houses was placed in compost piles (2.5 m high) and further composted for several weeks under aerobic conditions to obtain commercial composted SMS. The characteristics of the SMS used are shown in Table 2. pH was determined in suspensions with a SMS:water ratio of 1:5, and ash was determined by loss on ignition at 540 °C. OM was calculated as 100 – % ash. OC and DOC were determined as indicated previously. Total N was determined with the Kjeldahl method. Metals (Cd, Cr, Cu, Ni, Pb and Zn) were determined as indicated for sewage sludge in García-Delgado et al. (2007). It should be noted that the metal concentrations obtained were well below the maximum allowed in Spain for land-applicable wastes (MAPA, 1990).

2.3. Copper determination

Total Cu content was determined in 1 g samples of unamended soil and SMS-amended soil (ground to less than 1 mm) weighed directly in a dried, clean PTFE digestion vessel, and 10 mL of aqua regia (HCl + HNO₃ 3:1 ratio) was added. The digestion vessel was then placed in the chamber of a microwave system Ethos Sel model (Milestone, Shelton, CT, USA). Cu analysis was performed on a Varian model 720-ES inductively coupled plasma-optical emission spectrometer (Varian Instruments, Palo Alto, CA, USA). The detection limit was 0.005 mg L⁻¹. All determinations were carried out in triplicate. The analytical precision of the replicates varied from 8.4 to 22% relative to the standard deviation. Analytical accuracy in the determination of total Cu content was checked with certified reference materials (BCR) from Community Bureau of Reference. Five samples of CRM 141 and CRM 320 were analysed, and recoveries of 92 ± 8.8% and 115 ± 12.6%, respectively, were obtained.

The chemical forms of Cu were determined by a sequential extraction method of four-step chemical fractionation based on the work of Tessier et al. (1979), proposed by the BCR (Ure et al., 1993) and applied by Rauret et al. (2000) to characterize extractable trace metals in sewage sludge-amended soils. Triplicate samples of SMS and unamended soil (untreated with Cu) and samples of unamended and amended soils after the application of Cu were treated with the appropriate reagents and shaken at room temperature for the specified times to carry out the extractions (F1 to F4). Table 3 shows the experimental procedure employed. After the first three extractions, the remaining sample was transferred to a dry, clean PTFE digestion vessel, and 10 mL of aqua regia was added for the determination of the residual content of metal. The differences between the total metal content estimated by the sum of the fractions and the total metal content obtained by acid digestion of the samples were in all cases less than 25%. All reagents were of Merck analytical grade, and Milli-Q ultrapure water from a Millipore Milli Q system was used. All extraction procedures were performed using laboratory glassware and polyethylene bottles pre-cleaned with HCl and rinsed with double-distilled water.

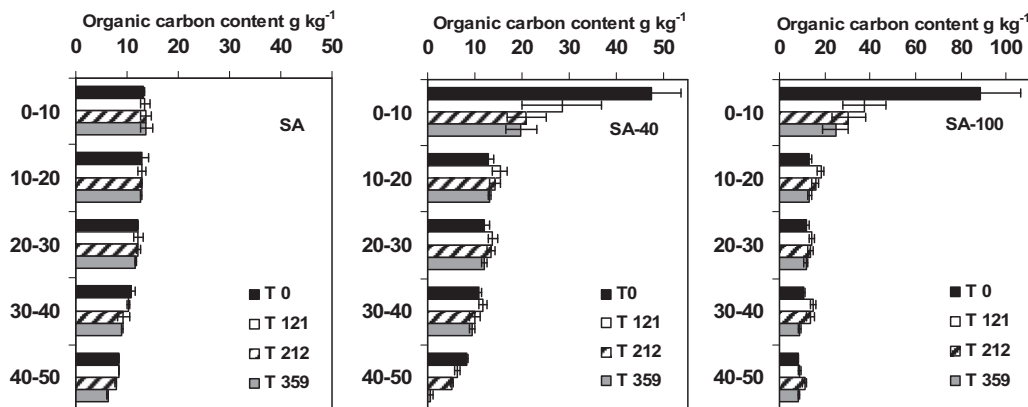


Fig. 2. Organic carbon contents of unamended soil SA (a) and amended soils SA-40 (b) and SA-100 (c) along the soil profile at different times after SMS addition. Error bars indicate standard deviation of replicates.

2.4. Sorption experiments

Sorption isotherms of Cu (from $\text{CuSO}_4 \cdot 6\text{H}_2\text{O}$) by unamended and amended soils were obtained using the batch equilibrium technique. Duplicate 1 g soil samples were equilibrated with 20 mL of a Milli-Q ultrapure water solution of Cu at concentrations of 0.1, 0.25, 0.5, 0.75, 1, 2 and 5 mg L^{-1} . The suspensions were shaken at $20 \pm 2^\circ\text{C}$ for 24 h in a temperature-controlled chamber, with intermittent shaking for 2 h at 3 h intervals. Preliminary experiments revealed that contact for 24 h was long enough to reach equilibrium. The suspensions were subsequently centrifuged at 2500 g for 15–30 min and the equilibrium concentrations of Cu were determined as previously indicated. pH values and the DOC of solutions were measured before and after sorption equilibrium was reached.

The amount of Cu sorbed was considered to be the difference between that initially present in solution and that remaining after equilibration with the soil. Sorption coefficients (K_f and n_f) were obtained from the sorption data fitted to the linear form of the Freundlich equation ($\log C_s = \log K_f + n_f \log C_e$) with r values ≥ 0.92 ($p < 0.01$).

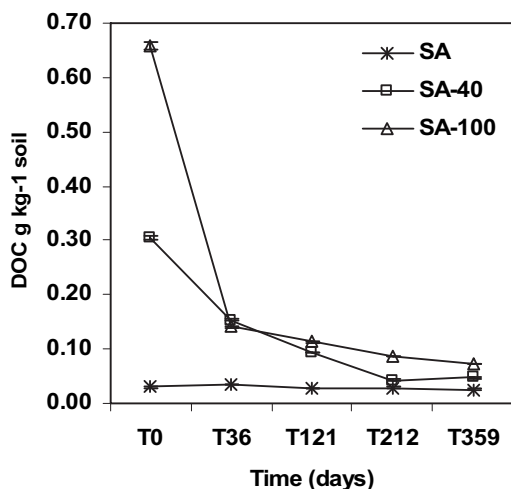


Fig. 3. Changes in dissolved organic carbon content in unamended and amended soils (0–10 cm depth) at different times after SMS addition. Error bars indicate standard deviation of replicates.

2.5. Data analysis

Analysis of variance (ANOVA) was used to evaluate the effects of the different treatments. Standard deviation (SD) was used to indicate variability among replicates, and the least significant difference (LSD), at a confidence level of 95%, was used to separate means. Statgraphics Plus ver. 5.1 statistical software was used.

3. Results and discussion

3.1. Changes in pH and organic carbon content of soils

The temporal and spatial variations in the pH, OC and DOC soil parameters that may considerably modify the behaviour of Cu in the soil were determined. The pH values of the amended soils were 7.7 (SA-40) and 7.6 (SA-100), with a difference of 0.1–0.2 units to the unamended soil (7.8). These values remained stable throughout the time the soil was treated with SMS. Statistical analysis revealed that the pH values of the unamended and amended soils along the soil profile over time were not significantly different ($p < 0.05$).

The OC content of the unamended soil varied between 13.1 and 8.4 g kg^{-1} along the soil profile (Fig. 2) and remained almost constant throughout the experiment. The addition of SMS to the soil increased the OC content 3.5 times (SA-40) and 6.5 times (SA-100) in the upper horizon (0–10 cm) of the soils in the amended plots. After 121 days of contact between the residues and the soil, the OC content decreased by 40–58%, possibly due to the oxidation and/or mobility of the most labile forms of carbon (Fig. 2). Despite this decrease, a significant increase ($p < 0.05$) in the OC content of the 10–20 cm horizon up to 212 days and in the 0–10 cm horizon up to 359 days was recorded in the amended soils with respect to

Table 4

Total Cu content (mg kg^{-1}) in soils of experimental plots (0–10 cm depth) unamended and amended with SMS at rates of 40 and 100 t ha^{-1} and treated with copper at two different doses.

	T 0	T 36	T 121	T 212	T 359
Dose 5 kg ha^{-1}					
SA	67.2 \pm 7.13	42.8 \pm 3.11	34.0 \pm 8.85	34.2 \pm 4.39	34.3 \pm 5.68
SA-40	67.5 \pm 3.72	47.1 \pm 8.07	35.7 \pm 10.7	35.9 \pm 6.71	37.8 \pm 8.69
SA-100	80.9 \pm 13.6	50.7 \pm 9.66	40.6 \pm 8.78	33.8 \pm 8.43	34.5 \pm 6.35
Dose 10 kg ha^{-1}					
SA	87.9 \pm 7.57	51.8 \pm 7.77	46.5 \pm 1.79	40.5 \pm 8.44	38.7 \pm 4.23
SA-40	109 \pm 8.40	45.6 \pm 4.22	40.4 \pm 6.36	41.8 \pm 6.01	42.6 \pm 5.04
SA-100	100 \pm 5.08	74.9 \pm 11.3	49.0 \pm 8.19	46.8 \pm 4.33	42.5 \pm 11.6

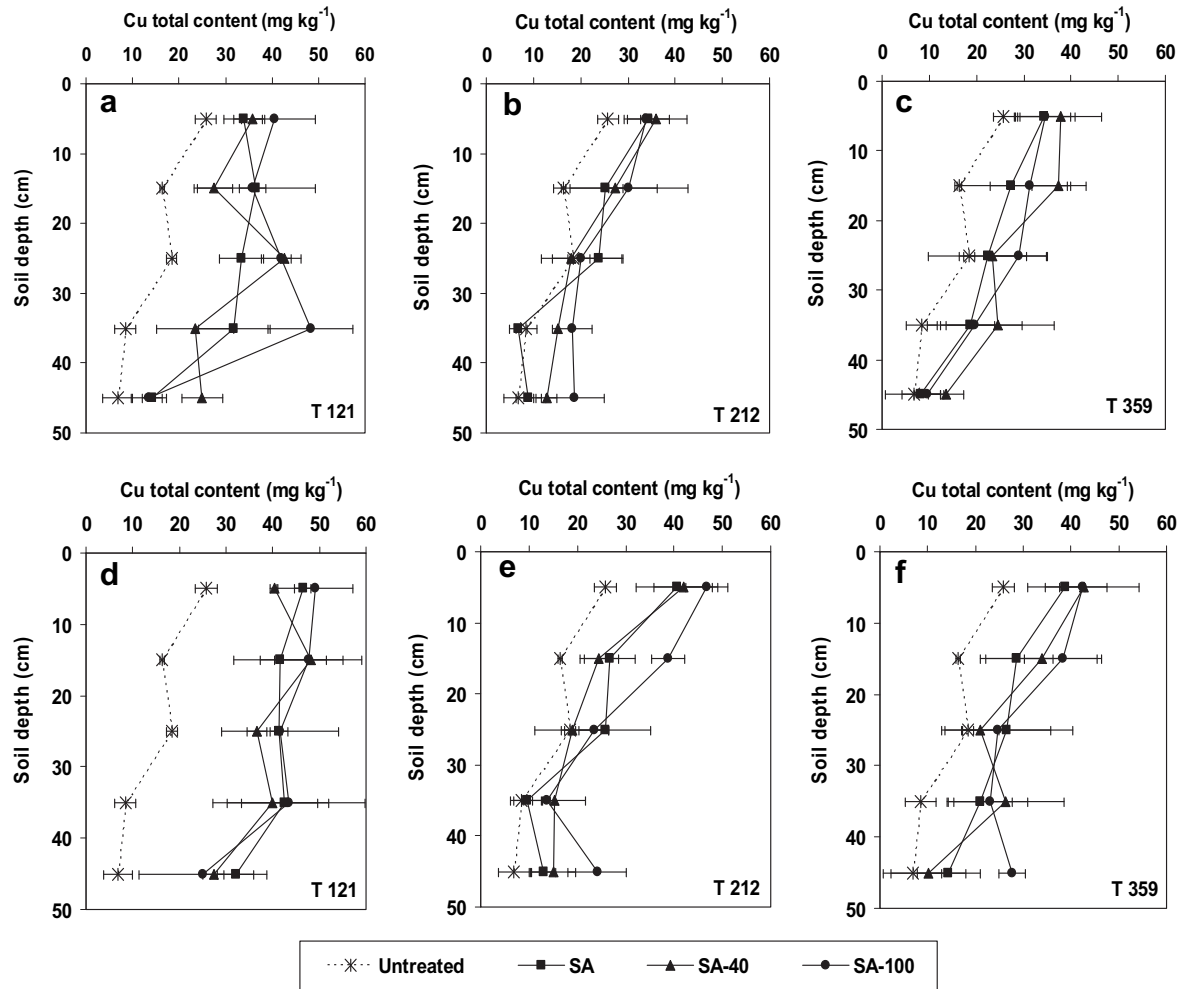


Fig. 4. Distribution patterns of Cu in the unamended and amended soils treated with low (a–c) and high (d–f) doses of Cu. Error bars indicate standard deviation of replicates.

the unamended soils, indicating that the application of the SMS to the soil could improve its fertility.

In addition, the DOC content was determined in the unamended topsoil (0–10 cm). It was 0.03 g kg^{-1} and this remained almost constant until the end of the experiment. After the addition of the SMS to the soil (T0), the DOC increased more than 10-fold and more than 20-fold in the SA-40 and SA-100 soils, respectively (Fig. 3). This DOC content decreased dramatically 36 days after treatment (T36) and continued to decrease, although much more slowly, until the end of the experiment, in parallel to the decrease in soil OC ($r = 0.98$, $p < 0.01$). After one year (T359), the DOC content in the amended soils decreased by more than 80%, although the content in soils SA-100 and SA-40 was higher than as determined in SA ($p < 0.05$). The DOC may have been leached out of the soil profile, especially under conditions of $\text{pH} > 7$ for the soil studied, as reported by some authors (Temminghoff et al., 1997). This process might have affected the fate of Cu to a certain extent immediately after the addition of the fungicide to the soil when the highest DOC decrease occurred.

3.2. Changes in total Cu content

The total Cu content in the unamended soil prior to the addition of the metal was 25.8 mg kg^{-1} . This content was within the $6.35\text{--}121.5 \text{ mg kg}^{-1}$ range given for vineyard soils in La Rioja (Spain) (Marin et al., 2000). It was also close to the mean standard

values reported for natural soils in La Rioja (14.1 mg kg^{-1}) (Iñigo et al., 2006) and the rest of the world ($<30 \text{ mg kg}^{-1}$) (Adriano, 2001). As expected, application of the Cu-based fungicide increased the levels of this element in the soils in all the plots

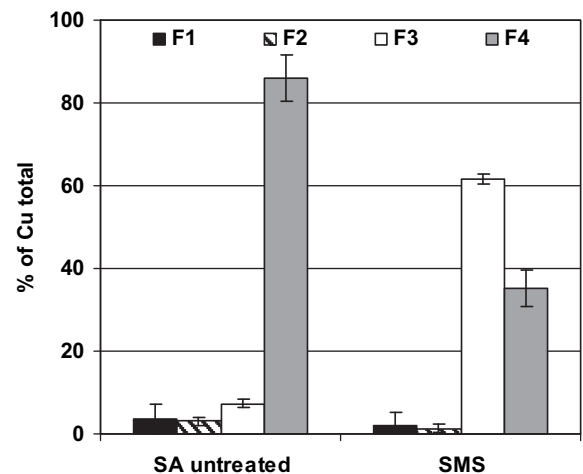


Fig. 5. Fractionation of Cu expressed as percentages of total content (F1: exchangeable/acid extractable, F2: bound to Fe and Mn oxyhydroxides, F3: bound to organic matter and F4: residual) for SA untreated soil and SMS. Error bars indicate standard deviation of replicates.

(Table 4). There were no significant differences ($p < 0.05$) between the levels found in the unamended and amended soils when the fungicide was applied at a dose of 5 kg ha^{-1} . When the application of Cu was 10 kg ha^{-1} , the increase in the Cu content was greater in the amended soils than in the unamended ones ($\text{LSD} = 14.3$, $p < 0.05$). These observations suggest a greater initial retention capacity of Cu by the amended soils than by the unamended ones, although no effect of the amendment rate was observed on this retention capacity. The fungicide may have been sorbed to a greater

extent by the amended soils owing to their higher OC content, and hence it was less affected by the rainfall immediately after its application to the soil (Fig. 1). This effect, or other effects related to the physical factors of the unamended soil (porosity, permeability, etc), could have elicited greater transport (surface and/or at depth) of the compound in the unamended soils than in the amended ones (Besnard et al., 2001).

With time, the Cu content decreased in all the soils, although values obtained from four months (T121) up to one year (T359)

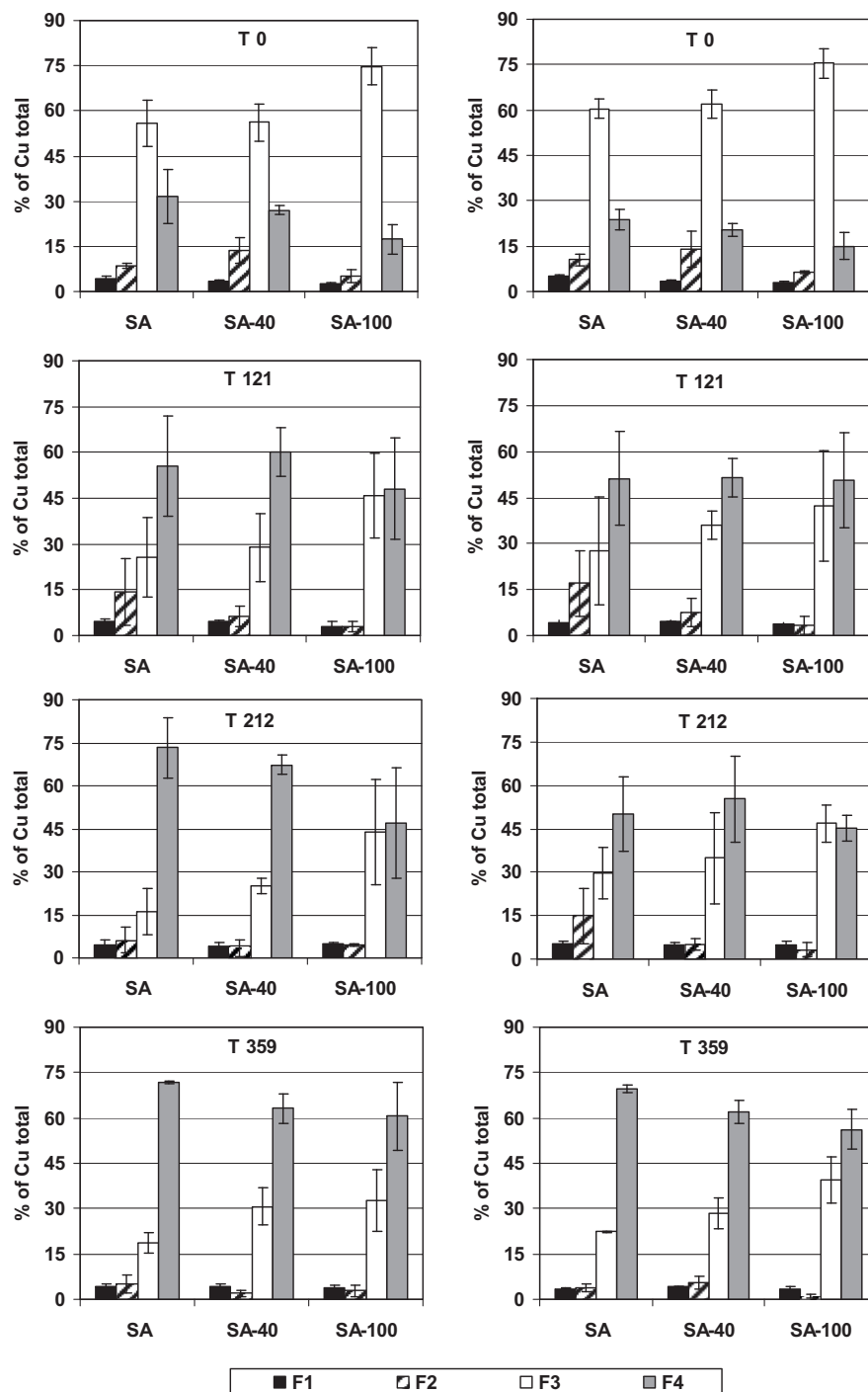


Fig. 6. Fractionation of Cu expressed as percentages of total content (F1: exchangeable/acid extractable, F2: bound to Fe and Mn oxyhydroxides, F3: bound to organic matter and F4: residual) for unamended and amended soils treated with low (left side) and high (right side) dose of Cu after 0, 121, 212 and 359 days. Error bars indicate standard deviation of replicates.

after application of the fungicide were statistically similar ($LSD = 8.33$, $p < 0.05$). At the end of the experiment, the mean Cu contents obtained for unamended soil and amended soils were not significantly different and revealed that the organic amendment did not increase the persistence of Cu in the surface horizon of the soil after 359 days. However, these Cu contents were slightly higher than the native Cu content in the soil before application of the fungicide ($LSD = 6.11$, $p < 0.05$) (Table 4).

The Cu applied may be sorbed and/or moved in the soil. Sorption data were obtained from the sorption isotherms of Cu by soils from the control plots (data not shown). The values of the Freundlich sorption constants (Kf) indicated a far greater extent of sorption by the unamended soil (12.4) than by the amended ones (0.42 for SA-40 and 0.12 for SA-100). The high carbonate content or the clay minerals, illite and kaolinite, in the soils must have affected the sorption of Cu by the unamended soil (Rodríguez-Rubio et al., 2003). The lower sorption of Cu by the amended soils compared to the unamended one obtained by the batch equilibrium system could be due to the presence of DOC in solution, as the pH values of the soil solutions (7.5–7.9 range) did not change when sorption experiments were performed. DOC mean concentrations measured in initial suspensions of SA-40 and SA-100 (16.5 and 21.9 mg L⁻¹) were higher than those in SA soil (1.17 mg L⁻¹) and increased after shaking of suspensions until 21.6 and 31.4 mg L⁻¹, while remaining similar in SA soil (1.01 mg L⁻¹). The DOC generated could have enhanced the interaction of Cu in solution with soluble organic compounds. The formation of soluble Cu complexes could decrease their sorption by the solid OM in the soil, as reported by other authors (Gondar and Bernal, 2009; Plaza et al., 2005).

The DOC from the soils could also govern the behaviour of Cu in the experimental plots over time, especially in the amended soils, where it is produced in greater amounts (Fig. 3). DOC may be adsorbed or leached, depending on the pH of the soil. At pH > 7, determined for the soil in this study, DOC adsorption would be low and/or weak at the soil surface owing to the increase in negative charges of the DOC, and thereby leading to a high degree of leaching (Temminghoff et al., 1997). The interaction of Cu with the DOC derived from the SMS could have contributed to increasing the mobility of the element in the soil studied.

With a view to studying the effect of adding SMS to the soil on the possible mobility of Cu, the content of the element in the soil profile was studied from concentrations determined in soil columns (0–50 cm) collected from the plots at 121, 212 and 359 days after the addition of the fungicide to the soil (Fig. 4). For comparative purposes, the Cu concentration in the soil profile was previously determined in the control plots (unamended and untreated SA).

A decrease in Cu concentration was observed in the untreated soil with increasing depth, and the distribution pattern of Cu was similar to the soil OC content (Figs. 2 and 4). At 121 days after application in the fungicide-treated soils, an enrichment in Cu was observed in the soil profile with respect to the untreated soil, with this being more significant in the soils with the high dose of Cu. The distribution patterns of Cu and OC in the amended and unamended soils were also generally similar, but they exhibited effects of increases or decreases in the concentration of Cu along the profile of amended soils. In amended soils, Cu concentrations higher than those observed at the surface were found (42 mg kg⁻¹ and 48.3 mg kg⁻¹, in soils SA-40 and SA-100, respectively, with the low dose of Cu). These effects were not related to the solid OC contents (which decreased in the soil profile) and could be due to the leaching of Cu in soluble form with the DOC from the SMS, as indicated above. These effects were also reported at 212 and 359 days after application of the fungicide, although they were less significant. This would be consistent with the observed decrease in DOC (Fig. 3) over time.

Additionally, at 212 and 359 days after treatment the distribution pattern of the fungicide indicated a sharper decrease in total Cu concentration, and similar values of total Cu content were found at 40–50 cm in the treated and untreated soils. A sharp decrease in total Cu concentration with increasing depth was reported by Pietrzak and McPhail (2004) in young vineyards as opposed to older ones, and ascribed to an increase in Cu mobility. Furthermore, Xiaorong et al. (2007) reported the potential for Cu leaching when used as a fertilizer after long-term applications.

3.3. Changes over time in the chemical forms of Cu in topsoils

An initial Cu fractionation was conducted in untreated SA and in SMS. Fig. 5 shows the amounts of Cu in each fraction, expressed as percentages with respect to the total amount. Cu was found mainly in the residual fraction (F4) (>80%) of the soil and in the fraction associated with oxidisable phases (F3) (>60%) in SMS. This behaviour was similar to that reported for this element in other soils (Pietrzak and McPhail, 2004) and in organic residues such as sewage sludge (Sánchez-Martín et al., 2007).

The fractionation of Cu expressed as a percentage of the total content in SA, SA-40 and SA-100 treated with the two doses of Cu was obtained after different treatment times (T0, T121, T212 and T359) (Fig. 6). The distribution of the metal in the different fractions of the soils at T0 appeared to be similar to that of the SMS, with most of the metal applied being concentrated in the fraction associated with oxidisable phases, even in the unamended soil with an OM content of ~2%. The amounts in this fraction were >50% of the total Cu content in SA and SA-40 and >70% in SA-100. However, the chemical forms of Cu bound to OM could in time evolve or be re-distributed when the OM in all the soils decomposes and/or solubilises.

The results shown in Table 5 reveal significant differences between the average distribution fractions for each treatment time for soils SA, SA-40 and SA-100, considering jointly soils with Cu applied at low and high doses, as indicated by the LSD value at a confidence level of 95%. Thus, the percentages of Cu bound to OM (F3) at T0 were reduced to >50% (SA), >45% (SA-40) and >40% (SA-100) after 121 days of treatment, and the values obtained remained statistically similar until the end of the experiment (T359). Changes were also recorded in the Cu forms bound to oxides (F2). Thus, the percentages of Cu increased in this fraction in SA and decreased in

Table 5

Average values of distribution fractions of Cu (% of the total content) (F1: exchangeable/acid extractable, F2: bound to Fe and Mn oxy-hydroxides, F3: bound to organic matter and F4: residual) for unamended and amended soils considering jointly soils treated with low and high dose of Cu over time.

Soil/fractions	T0	T121	T212	T359	LSD
SA					
F1	4.666 ^a	4.303 ^a	4.685 ^a	3.888 ^a	1.23
F2	9.523 ^{ab}	15.64 ^b	12.28 ^{ab}	5.601 ^a	8.04
F3	58.12 ^a	26.22 ^b	23.06 ^b	20.59 ^b	14.1
F4	27.69 ^a	53.33 ^b	61.67 ^{bc}	70.76 ^c	14.8
SA-40					
F1	3.278 ^a	4.685 ^b	4.267 ^b	4.219 ^{ab}	0.97
F2	13.82 ^a	7.002 ^b	4.343 ^b	3.764 ^b	4.11
F3	59.14 ^a	32.35 ^b	30.07 ^b	29.54 ^b	9.77
F4	23.74 ^b	55.96 ^b	61.34 ^b	62.47 ^b	9.12
SA-100					
F1	2.890 ^a	3.342 ^a	4.800 ^b	3.679 ^a	0.95
F2	5.812 ^a	3.123 ^b	3.781 ^{ab}	1.932 ^b	2.16
F3	75.12 ^a	44.11 ^b	45.35 ^b	36.02 ^b	15.4
F4	16.17 ^a	49.44 ^b	46.06 ^b	58.36 ^b	15.4

Numbers within a file followed by the same letter are not significantly different according to LSD value at a confidence level of 95%.

SA-40 and SA-100 after 121 days of treatment. The changes in the exchangeable forms of Cu were less significant, and the slight increases in this fraction over time were only statistically significant in SA-40 and in SA-100 after 121 days. Additionally, a remarkable increase in Cu in the residual fraction (F4) was observed from T0 to T121, as much as ~2–3 times in the unamended and amended soils. Similar effects were reported and referred to as ageing by Arias-Estévez et al. (2007) in the distribution fractions of Cu added to an acid agricultural soil with increasing incubation time (>500 days) in a laboratory study.

After one year of treatment, the highest concentration of Cu was found in the residual fraction for all soils (~70% in SA, ~60% in SA-40 and SA-100). The distribution of Cu followed a pattern similar to that found for native Cu in the soil before the addition of the fungicide (Fig. 5), although the percentage of Cu associated with oxidisable phases was higher for the amended soils than for the unamended one.

4. Conclusions

The results obtained show the influence of the OC from the SMS organic residue in the retention of Cu from a Cu-based fungicide immediately after its application to the soil. The decrease in the concentration of the metal in the topsoil of the amended soils with time occurred parallel to the decrease and/or loss of the OC in the soil. This effect, together with the ability of Cu to form soluble complexes with the DOC derived from SMS, facilitates the leaching of Cu and the decrease in total Cu content in the topsoil. In the case of the unamended soil, the decrease in the total content of Cu in the topsoil cannot be linked to the decrease in the OC content, since the degree of mineralization of the OC was low. In this soil, Cu redistributed over time from the fraction associated with oxidisable phases towards the bound to oxides (initially) and residual fractions, and it is possible that physical factors related to the structure of the soil could have been involved in the mobilization of the metal. The sorption of Cu by other soil fractions (clay minerals, oxides and carbonates) has previously been reported in the literature, but these effects did not appear to be significant in the unamended soil for maintaining the concentration of Cu applied to the topsoil. Additional studies addressing the leaching of Cu in SMS-amended soils could be of interest to assess the risk for groundwater quality when SMS and Cu-based fungicides are applied together in vineyard soils.

Acknowledgements

This work was funded by the Spanish Ministry of Science and Innovation (project AGL2007-61674/AGR). E. Herrero-Hernández thanks CSIC for his JAE-doc contract. The authors thank J.M. Ordax, L.F. Lorenzo and A. González for their technical assistance, and INTRAVAL Environmental Group TRADEBE S.L., and the CVNE winery in La Rioja, Spain, for their collaboration.

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