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LONG-TERM VARIABILITY OF METALS FROM FUNGICIDES APPLIED IN AMENDED YOUNG VINEYARD FIELDS OF LA RIOJA (SPAIN)

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Abstract

Long-term variability of total Cu content from fungicides applied in a certified wine region of Spain (La Rioja) and of other metals (Cd, Cr, Ni, Pb and Zn) was evaluated in three young vineyard soils and subsoils unamended and amended with spent mushroom substrates (SMS) over a three-year period (2006-2008). SMS is a promising agricultural residue as an amendment to increase the soil organic matter content but may modify the behaviour of metals from pesticide utilisation in vineyards. Fresh and composted SMS was applied each year at a rate of 25 t ha^{-1} (dry-weight). Copper concentrations in the three unamended soils were 21.2-88.5 mg kg^{-1}, 25.5-77.1 mg kg^{-1} and 29.4-78.4 mg kg^{-1}. They exceeded natural Cu concentrations of the region and reference sub lethal hazardous concentration for soil organism. The concentrations of Cd, Ni, Pb and Zn, were largely below the sub lethal limits. Thus, although Cu levels were lower than those of established vineyards, vine performance and productivity might be affected. The variation in behaviour between different amendments for each soil was high, so a generic conclusion could not be drawn. The amendment practice seemed to have caused temporarily Cu mobilisation respect to untreated soils. Total zinc concentrations fall within the range of the natural soil of La Rioja and were significantly affected ($p<0.05$) especially by fresh state SMS addition, with increasing up to 75% respect to untreated specimen. Results indicated a build-up of fresh sites for metal retention at both surface and subsurface level, although no accumulation of metals was observed in the short term period. However, benefit for soils as well the negative effects need to be monitored in the long run.

Keywords Fungicide; soil; subsoil; vineyard; spent mushroom substrate; heavy metal

Introduction

Copper-based fungicides such as the Bordeaux mixture, $CuSO_4 \cdot 3Cu(OH)_2 \cdot 3CaSO_4$, have been intensively used in Europe since the end of the 19th century to control vine (*Vitis vinifera L.*) fungal diseases, such as downy mildew caused by *Plasmopara viticola*. Additionally, other Cu compounds have been introduced including Cu-oxychloride, $3Cu(OH)_2 \cdot CuCl_2$ and Cu hydroxide, $Cu(OH)_2$ and their long term application and subsequent wash-off from the treated plants have resulted into extensive Cu accumulation in vineyard soils. A recent review of Komárek et al. (2010) on contamination of vineyard soils reported that the levels of Cu usually exceed the legislative limits valid in the EU.

Vineyard soils are usually highly degraded soils in terms of biochemical properties (Miguéns et al. 2007) and are thus more susceptible to contamination. Some studies have shown elevated copper concentrations to adversely affect the fertility of vineyard soils (Paoletti et al. 1998); field studies found clear linkages between increased Cu concentrations (>80 mg kg^{-1}) in soil and reduced earthworm abundance (Paoletti et al. 1998). 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, 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 (Wightwick et al. 2008).

Copper in soils is mostly associated with organic matter (OM), Fe-, Mn-(hydr)oxides and to a lesser extent with clay minerals through specific and non-specific adsorption (Arias-Estévez et al. 2007; Nóvoa-Muñoz et al. 2007). Sorption on OM by means of complexation especially with humic and fulvic acids presents possibly the most important retention mechanism for Cu in soils (if not the most important one) (Strawn and Baker 2009). Such strong sorption/complexation properties make it one of the least mobile metals in soils. However, metals of anthropogenic origin present in general a greater mobility in soil comparatively to a natural origin where the metals are strongly associated with soil components.

During the last few decades, some European vineyards have been abandoned, which has led to intensive soil erosion and subsequent dispersion of the pollutants into the environment. Copper applied to eroded vineyard soils can easily reach ground and surface waters either as water-soluble species or associated to colloidal soil particles and concentrate in surface water sediments (Fernández-Calviño et al. 2008). Stabilization strategies that lower the intensity of erosion (such as the application of organic amendments and vegetation cover) are needed for decreasing Cu runoff from vineyard soils via wind and water erosion. The application of organic wastes to improve soil physical characteristics in mechanized vineyards planted is becoming a common practice in Mediterranean areas. It may be useful as an additional source of organic matter and nutrients.

Many studies have focused on the sorption of metals by solid phase soil organic matter (SOM) (Sanders 1980; Sauve et al. 2000). Generally, SOM in environmental systems is implicated in retention, decreased mobility, and reduced bioavailability of trace metals. On the other hand other studies have found significant relationships between increasing dissolved organic matter (DOM) and metal mobilization in environmental systems. (Hsu and Lo 2000).

Besides Cu from fungicide, increased concentrations of other risk elements were found in vineyard soils often in bioavailable forms (Adriano 2001). These include Zn (e.g., from insecticides, manure and compost applications) (Ramos 2006), Pb (e.g., from atmospheric depositions, but also from the use of lead arsenate as insecticide) (Frank et al. 1976; Mihaljevič et al. 2006; Komárek et al. 2008) and Cd (from phosphate fertilizers) (Komárek et al. 2008). Like Cu, these metals can also affect soil fertility (Wightwick et al. 2008).

Spent mushroom substrate (SMS) represents a promising renewable agricultural organic resource that can improve soil properties, e.g., soil water retention capacity, nutrient content, pH. This spent substrate is the pasteurized organic material remaining after mushroom harvesting. Its richness in nutrients and OM can advantage other agricultural sectors (Wuest et al. 1995). However, the application of SMS involves an addition of solid and liquid OM from these residues to soil, which could affect the fate of metals in soils. Thus, a more effective use and exploitation of this residual material represents an issue of paramount importance. Recently, the production of mushrooms in the region of La Rioja (northern Spain), increased from 51 372 tons in 1999 to 83 500 tons in 2005. For many years, the SMS produced in this region (>183 000 tons in 2005; Plan Director de Residuos de La Rioja 2007-2015) were disposed in landfills. This method has generated an environmental problem and the mushroom industry is seeking the reuse of such material as soil amendment.

So far, no studies addressing the extent to which SMS contributes to increase the copper total content in soils and subsoils from fungicides application and other metals have been published. The Spanish viticulture industries recognized the issues surrounding copper fungicide and SMS use and required information on the extent and magnitude of copper accumulation in amended vineyard soils to aid in assessing the need for modifying the management of copper inputs in the future. The objective of the present work was to study the temporal variation of Cu and metals (Cd, Cr, Ni, Pb,Zn) concentrations in surface and subsoil of a certified wine region of Spain, La Rioja, after long term addition (2006-2008) of fresh and composted SMS (F-SMS and C-SMS) from mushroom *A. bisporus* processing. The aim of the study was also to provide an indication of the likely risks that metal accumulation poses to soil fertility. Soils were from experimental plots in vineyard areas, unamended and amended with SMS in the field.

Materials and Methods

Soil sampling

Soil samples were collected from vineyards of the region of La Rioja (N-Spain) located in Aldeanueva (AL) (42º14'0"N latitude and 1º53'0"W longitude), Sajazarra (SA) (42º35'0"N latitude and 2º57'0"W longitude), and Viana (V) (42º30'0"N latitude and 2º20'0"W longitude). These locations were selected for their different altitudes, topographies and climates. This region represents the most important wine region of Spain and extends over 50,000 ha and gives 273 million of litres of certified wine. Soils were classified as a Fluventic Haplocambids (AL), a Typic Calcixerepts (SA), and a Typic Xerorthents (V) (USDA 2006). The soils have been treated with copper from Cu-oxychloride or Cu-calcium sulphate 3-4 times per year at a rate of 4-6 kg ha^{-1} for 12 (AL), 14 (SA) and 15 (V) years (Table 1). Each vineyard plot, with a size between 2.03 ha to 2.74 ha, was divided into three sub-plots, one was not amended and the other two were amended with F-SMS or C-SMS, respectively. The amendment was added for three years, 2006-2008, between February-April at the recommended agronomic rate of 25 t ha^{-1} (dry-weight). Soil and subsoil samples were taken each year before the addition of the amendment and after grape harvest from 27 sites (three sites per subplot) over the experimental plot. At each site three soil cores (0-60 cm) were taken to make a soil (0-30 cm) and subsoil (30-60 cm) composite sample representative of each site.

Soils and spent mushroom substrate

Soil samples were air dried and sieved (<2 mm) and characteristics were determined by usual analytical methods (MAPA 1986) (Table 2). The pH of the soils was determined in soil/water suspensions (ratio 1/2.5) and their particle size distribution was determined using the pipette method, which measures the weight percent of each particle fraction (clay, silt and sand) in the soil sample based on Stokes' law (Gee and Or, 2002). Organic carbon (OC) contents were determined by the modified method of Walkley-Black and the results were multiplied by 1.72 to convert the organic carbon into OM. Inorganic carbon was determined as $CaCO_3$ with a Bernard calcimeter. The dissolved organic carbon (DOC) content was determined using a Shimadzu 5050 (Shimadzu, Columbia, MD) organic carbon analyzer. Clay minerals were identified qualitatively by the X-ray diffraction (XRD) technique, using a Philips PW 1710 diffractometer (Eindhoven, the Netherlands). The XRD patterns showed the presence of illite, kaolinite and chlorite-illite interstratified mineral respectively in all soils. In the case of AL soil, the presence of smectitic clay was also detected.

The spent substrate was collected after the cultivation of *Agaricus bisporus* mushroom and was kindly supplied by INTRAVAL Environmental Group TRADEBE, S.L. (La Rioja, Spain). F-SMS was obtained immediately after it was removed from mushroom houses and may have a heterogeneous composition, depending on its origin. Alternatively, SMS can be further composted under aerobic conditions to obtain C-SMS to increase the uniformity and stability level of the SMS. The characteristics of the SMS are described elsewhere (Marín-Benito et al. 2009).

Determination of total Cu, Cd, Cr, Ni, Pb and Zn

A sub-sample of 1 g was weighed directly in a dried, cleaned PTFE digestion vessel and 10 mL of aqua regia were added. Afterwards, the digestion vessel was placed in the chamber of a microwave system Ethos Sel model (Milestone, Shelton, CT). Metal analysis was performed on a Varian model 720-ES inductively coupled plasma optical emission spectrometer (Varian Instruments, Palo Alto, CA). All determinations were carried out by triplicate. Analytical precision of replicates varied from 9.3 to 19% relative to standard deviation. Analytical accuracy in the determination of the total content of metals was checked with the BCR reference materials. Five samples of CRM 141 and CRM 320 were analysed and variation coefficients <6.5% or <15%, respectively were obtained. All reagents were Merck analytical grade and Milli-Q ultrapure water was used from a Millipore Milli Q system. The extraction procedures were performed using laboratory glassware and polythene bottles pre-cleaned by HCl and rinsed with double distilled water.

Data analysis

Analysis of variance (ANOVA) was used to evaluate the effects of the different treatments and the least significant difference (LSD), at a confidence level of 95%, was used to separate means. Partial correlations between metal total contents and different soil parameters were made in order to analyse possible relations. The Statgraphics Plus ver. 5.1 statistical software was used.

Results and discussion

Characteristics of unamended and amended soils

Soils had generally a sandy clay loam texture (Table 2) with a clay content of 21.4%-38.3% and a carbonate content of 10.6%-48.9%, with peaks for SA and V soils and for samples taken at the subsoil level. The AL soil displayed the lowest levels of clay, carbonates and OM with an average OM content of the unamended specimen of about 1.04% which increased of ~50% when the SMS was added. Fig. 1 shows the variation of the OM content in unamended and amended surface soil and subsoil over the time. The results obtained here show that the use of F-SMS or C-SMS as an amendment at relatively low agronomic doses elicits an increase in the OM content of soils. At the beginning of the trial, the addition of the SMS significantly ($p<0.05$) increased the content of the OM for both surface and subsurface and especially when the amendment was used at fresh state. However, after repeated amendment application the organic load increased more markedly when it was used at composted state. This was the case for V soil, whose OM content increased up to ~100% at latest sampling (2008). The OC richness of the fresh residue was higher than that composted 34.2 vs. 28.6% and even its dissolved organic pool was threefold higher, 13.3% vs. 4.34% (Marín-Benito et al. 2009). Thus, the highest level of SOM of the fresh residue might have sustained a greater lost by leaching of the OC toward the deepest soil layers. A similar trend, even though with more smoothed differences, was observed for the subsoil. Differences in structure and composition of soils might explain the extent of OM variation by amendment addition. No significant soil pH variation was observed with the addition of the amendment with value remaining stable around an alkaline value of 7.8, at all studied depths and at all the experimental sites.

Total copper content

Total Cu content in the unamended soils sampled in 2006 varied in the range 31.6-88.5 mg kg^{-1}, 35.5-56.7 mg kg^{-1} and 55.2-78.4 mg kg^{-1} for AL, SA and V, respectively (Table 3). These levels exceeded those for unpolluted soil Cu background concentrations of 30 mg kg^{-1} (Adriano 2001). No copper soil accumulation was observed, since the ranges remained unvaried, with no significant differences ($p>0.05$) at later sampling taken in 2007 and 2008. This was likely due to the ‘aging’ effect acting when Cu enters the soil, which reduces metal loss. When Cu is spread over the soil its speciation rapidly changes and Cu is sorbed and (co)precipitated by humic and fulvic acids (Strawn and Baker 2009). This redistribution of Cu into less available chemical fractions of soils decreases its mobility and bioavailability and is referred to as "aging" (Arias-Estévez et al. 2007; Sayen et al. 2009).

On the overall, the levels of Cu in the subsoil, Table 3, were significantly lower ($p<0.05$), up to three-fold (AL soil taken in 2006, 59.3 vs. 22.5). This agrees with previous finding that report decreases of copper concentration in vineyard soils by depth and distances from the vine line (Pietrzak and McPhail 2004; Wightwick et al. 2008).

Copper varied within a range similar to that of 6.0-120 mg kg^{-1} reported for vineyard soils of the Rioja region (Marín et al. 2000). The mean Cu levels were up to five-fold higher, as in the case of V soil, than those indicated for natural soils of La Rioja (14.1 mg kg^{-1}) (Iñigo et al. 2006). On the other hand, Cu peak contents of the unamended soil were much lower than those reported for other vineyard areas of N.W. Spain (up to 603 mg kg^{-1}) with high annual rate of antifungal treatment due to climatic conditions of high humidity (mean annual rainfall of around 825 mm (Nóvoa-Muñoz et al. 2007, Fernández-Calviño et al., 2008). This may be due to the limited history of copper-based fungicide use (Table 1) being the studied vines not older than 15 years (V) as well as to the low usage rate of fungicide. In some European regions typical annual Cu use ranges from 10 to 50 kg ha^{-1}, whereas the average annual use in our studied vineyards was 5 kg ha^{-1}. This rate, although in Spain, there are currently no restrictions on copper use in conventional viticulture, appears to be close to foreign organic standards for limit annual of copper use, ~8 kg ha^{-1} (Wightwick et al. 2008). Even though Cu total soil levels appeared to be lower than the range (130-1280 mg kg^{-1}) detected in the surface soil of European vineyards with an established history of copper-based fungicide use (Delusia et al. 1996; Flores-Velez et al. 1996), the impact on soil fertility and biology can be of a concern especially for these young vines, which have a shallower root system extending over the surface soil where most of the applied copper tends to remain (Wightwick et al. 2008).

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 the three selected vineyards of La Rioja, the potential risks can be indicated by comparing the measured concentrations of copper to thresholds known to cause sub lethal effects to soil organisms. Using species sensitivity distributions Jansch et al. (2007) and Frampton et al. (2006) have estimated hazardous concentrations to 5% (HC5) of beneficial soil organisms. A sub lethal HC5 to soil organisms has been estimated to be 55 mg kg^{-1} total Cu (derived using EC50 values for animals, microbial processes, and plant species). This HC5 concentration provide an indication of potential toxicity while recognizing that site-specific soil factors such as pH, texture, and cation exchange capacity will influence the bioavailability of the copper present and therefore toxicity.

Copper levels in the unamended soils were close to the HC5 limits of 55 mg kg^{-1} (Jansch et al. 2007). The concentrations of Cd, Ni, Pb and Zn (Tables 3-5) were largely below the HC5 limits of 7, 64, 164, and 160 mg kg^{-1}, respectively. This suggests that Cu was the most significant metal present in the studied vineyard soils, in terms of the potential effects to soil organisms. Field studies found clear linkages between increased Cu concentrations (>80 mg kg^{-1}) in soil and reduced earthworm abundance (Paoletti et al. 1998). Belotti (1998) set the critical Cu concentration in soils (above which populations of several earthworm species are negatively affected) to 33 mg kg^{-1}, a value easily met in most of our studied vineyards (Table 3). Helling et al. (2000) published even a lower concentration of Cu originating from Cu-oxychloride (16 mg kg^{-1}). This means that although in the studied regions, copper levels remained below those of established vineyards, vine performance and productivity might be affected.

Metal concentrations in SMS (Marín-Benito et al. 2009) were much lower than those legally permitted in Spain for use of urban sludge in agriculture (MAPA 1990). The concentrations of Cu and Zn were 30-15 times lower than the rules for disposal onto alkaline soil, 1750 and 4000 mg kg^{-1}, respectively. On the overall and with the exception of the SA soil, the amendment of soil with SMS caused a decrease of copper concentration and with significant differences ($p<0.05$) respect to the unamended soil more evident at the beginning of the experimentation.

For AL soils, Cu dropped from a mean value of ~60 mg kg^{-1} of the unamended specimen to ~27 mg kg^{-1} of that amended with fresh state organic material, as observed in 2006. A similar Cu level was also observed in AL in 2008 and likely due to the already observed low content of OM and clay. Similarly van Herwijnen (2007) in a study comparing four composts and a liming product found that spent mushroom compost caused an increase in metal concentration in the leachates. Copper has been shown to become mobile in soils through association with the dissolved organic matter (DOM), especially in sandy soils (Temminghoff et al. 1997, Weigand and Totsche 1998; McBride et al. 1999). SOM can influence the mobility of Cu by two different means: while particulate SOM will act as a sorbent for Cu, soluble SOM will actually complex Cu, increasing thus its solubility, especially at alkaline pH (Fernández-Calviño et al. 2008; Martínez-Villegas and Martínez 2008). Its association with SOM occurs through inner-sphere complexation (e.g. bidentate inner-sphere coordination with carboxyl or amine ligands) (Strawn and Baker 2009). Thus, it seems that in such vine site, the amendment practice with SMS has made copper more mobile and available, possibly impacting surface and/or ground water.

Decreasing the total Cu level below the phototoxic threshold limit of 60 mg kg^{-1} (Kabata-Pendias and Pendias 2001) represented also an advantage. By contrast for the SA soil a more significant ($p<0.05$) level of the metal up to 75.5 mg kg^{-1}, exceeding the HC5 limit of

>55 mg kg^{-1}, was observed whatever was the form of the amendment added and at all sampled times. In such a soil having a higher clay content, >30%, it is possible that the metal is immobilized by chelation with the OM and adsorbed onto clay minerals and accumulate. Many studies found that the addition of organic amendments can accelerate the natural soil attenuation process (natural remediation), of metals by sorption, precipitation, and complexation reactions (Kabata-Pendias and Pendias 2001; Bolan et al. 2003; Pérez-de-Mora et al. 2005). There has been evidence that the addition of composted bio-amendment can increase Cu (together with other metals) concentrations in vineyard soils as well (Ramos 2006). Some authors report on the adsorption of SOM from the washing of SMS in field setting by soil components in particular clay (Guo et al. 2001). The affinity of Cu to soil fractions was reported to vary in the following order: SOM>silicate clays>ferrihydrite, (Martínez-Villegas and Martínez 2008). It has been observed that amendments can decrease heavy metal bioavailability, shifting it from ‘‘plant available’’ forms to fractions associated with clays, carbonates or metal oxides (Walker et al. 2004),

V soil behaved similarly to AL soil with significant loss of copper by amendment addition only at the beginning of the trial. These contrasting effects seem to agree with other studies using SMS and/or other amendments (van Herwijnen et al. 2007), which demonstrated that organic wastes have different effects on metal immobilisation, depending on the type of amendment used and the soil used.

In subsurface unamended soil, Cu concentration was significantly lower up to 62 % than that in surface soil and this was more evident in AL soil. Surface soil copper accumulation was expected as copper bind strongly to soil components. Even though this, no significant correlations were found in this work between vineyard copper concentrations and these soil components. This was likely due to the limited number of surveyed vineyard since our survey was not designed to specifically assess the influence of soil properties on metal accumulation. Similarly, no significant correlations were observed by Marin et al. (2000) between Cu and soil chemical properties of 37 vineyard soil samples of La Rioja due to the narrow range of variation of the soil properties of the set of soils. Further work needs to be undertaken to explore the influence of soil properties on the adsorption and accumulation of Cu in the vineyard soils in La Rioja region. At the observed significant decrease of Cu level in the surface of SMS-amended Al and V soils, corresponded a significant ($p<0.05$) accumulation of the metal in the deepest soil layers, especially if the added SMS was at fresh state (Table 3). A peak concentration of Cu of 81.4 mg kg^{-1} was noted for V+F-SMS soil. This could be of great importance for vineyard soils, especially when C-SMS which is more homogeneous than F-SMS is used. No significant differences were observed for treated and untreated SA soil.

Total Cd, Cr, Ni, Pb and Zn contents

The levels of Cd, Cr, Ni and Pb (Tables 3-5) were within the range reported by the literature for vineyard and natural soils of La Rioja (Marin et al. 2000; Iñigo et al. 2006) and were not significantly affected by the amendment practice.

Total zinc concentrations (Table 5) in unamended soils and at the beginning of the trial ranged between 36.3 and 102 mg kg^{-1}, falling within the range of the natural soil of La Rioja (Iñigo et al. 2006). Kiekens (1990) reported a typical range of zinc in soils of 10-300 mg kg^{-1} with a mean of 50 mg kg^{-1}. The values were higher than those observed in other animal manure amended soils but lower than those recorded in vineyards soil in non-industrailly polluted areas (Ramos 2006).

The highest load, ~100 mg kg^{-1}, was observed for AL soil, the lowest for the SA soil, ~40 mg kg^{-1}. Zinc soil loads in untreated soil remained quite constant over the time for all the surveyed soils. Initially, the amendment addition markedly increased total contents of Zn up to 75%, especially when SMS was used at fresh state. Respect to the untreated specimen, the addition of fresh SMS to SA soil shifted Zn content from 41.3 to 71.8 mg kg^{-1}. The same amendment significantly increased Zn content from 73 to 127 mg kg^{-1} in V soil. Lower increases, even though still significant and also more time stable, of Zn in soil were observed when SMS was used in its composted form as observed for Al soil in 2006 and V soil in 2007 and 2008. In unamended subsoil, Zn contents were fairly similar, up to 15% lower, than those of surface soil. In subsoil as for surface soils a significant and marked increase of the metal load was noted for all soils when F-SMS was used. This behaviour, respect to Cu, where at a surface impoverishment corresponded a subsoil enrichment, indicated a higher soil retention of Zn after the amendment practice. The greater sorption in F-SMS amended soils indicates a build-up of fresh sites for metal retention. Similarly, Pavel et al. (2010) reported increased amounts of Zn retained in a sandy soil that had been amended in the field with different organic wastes. Díaz-Barrientos et al (2003) also report that the amounts of Zn retained by a sandy soil increased largely after two years of amendment treatment.

The solid components of the organic wastes added to soils will provide new exchange sites for Zn as well as other binding sites. Barber (1995) stated that more than 90% of Zn in soils is in mineral insoluble form, although it may also be present in organically complexed forms and in the soil solution. Kiekens (1990) reported for Zn the formation of stable organic complexes with the solid-state OM in amended soils. It is likely that in our alkaline soils Zn would be more likely adsorbed by chemisorption on calcium carbonate and complexation by organic ligands. Kiekens (1990) also studied the adsorption of zinc on a calcareous soil and found that the reaction was not reversible due to some of the zinc being irreversibly fixed by the soil.

Conclusions

In summary, Cu and Zn were the only metals affected by the amendment practice. Metals total concentrations in the surveyed areas were lower compared to the levels reported for well established vineyards. This was due to the short history of fungicide and insecticide application in the young surveyed vineyard and the low rate of pesticide application. However, Cu levels appeared to be close to the thresholds sub lethal effects to soil organisms. The application of SMS amendment if for some vineyard site decreased such a risk, decreasing metal concentrations, for other vine area a build up of the metal load was observed. This could impact surface and/or ground water contamination. The variation in behaviour between different amendments for each soil was high, so a generic conclusion could not be drawn. In most cases, the moderate doses application of SMS did not cause any significant accumulation above regulatory limits or impoverishment of metals in the three year period. However, the benefit for the soils as well the negative effects need to be monitored. Future studies are needed to understand the correlations between metal loads and soil properties and binding processes at these or similar sites. This will optimise the use of SMS as suitable materials for vineyard amendment practice for vitalising the soil and immobilise metals, thereby breaking contaminant-receptor pathways and reducing the ecotoxicity.

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Figure captions

Fig. 1 Evolution of the organic matter content in surface soil (bar) and subsoil (line) amended with composted C- and fresh F-SMS over 2006-2008

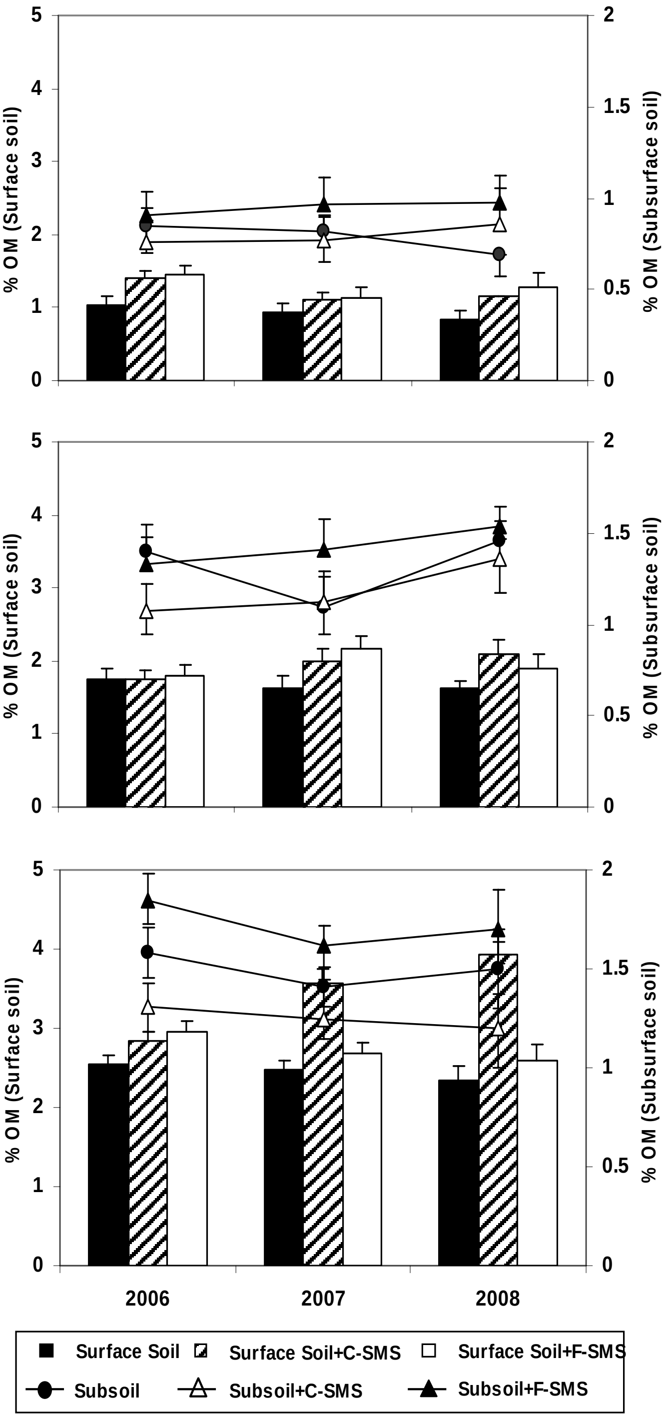
% OM (Surface soil)
% OM (Subsurface soil)
0
1
2
3
4
5
0
0.5
1
1.5
2
2006
2007
2008
Surface Soil
Surface Soil+C-SMS
Surface Soil+F-SMS
Subsoil
Subsoil+C-SMS
Subsoil+F-SMS

Fig. 1

Table 1 Estimated use of Cu for each of the three vineyards studied

Soil	Years of Cu use	Form of fungicide	Annual Cu use (kg ha^{-1})	Estimated total Cu applied (kg ha^{-1})
Aldeanueva (AL)	12	$CuSO_4 \cdot 3Cu(OH)_2 \cdot 3CaSO_4$	6	72
Sajazarra (SA)	14	$CuSO_4 \cdot 3Cu(OH)_2 \cdot 3CaSO_4$ + $CuCl_2 \cdot 3Cu(OH)_2$	4	56
Viana (V)	15	$CuSO_4 \cdot 3Cu(OH)_2 \cdot 3CaSO_4$	4	60

Table 2 Characteristics of soil samples taken in 2006

Soils	Soil texture	pH	OM %	$CaCO_3$ %	Sand %	Silt %	Clay %	Clay mineralogy[d]
Surface soil								
AL[a]	Sandy clay loam	7.8	1.04	11.3	64.4	14.2	21.4	I, K, M, Chl
AL+C-SMS[b]	Sandy clay loam	7.8	1.41	11.3	59.1	15.2	25.7	I, K, M, Chl
AL+F-SMS[c]	Sandy clay loam	7.8	1.46	11.2	56.3	18.3	25.4	I, K, M, Chl
SA	Sandy clay loam	7.7	1.74	27.9	57.9	10.5	31.6	I, K, Chl-I
SA+C-SMS	Sandy clay loam	7.8	1.74	32.0	51.3	23.3	25.4	I, K, Chl-I
SA+F-SMS	Sandy clay loam	7.8	1.81	29.9	54.7	10.4	34.9	I, K, Chl-I
V	Sandy clay loam	7.8	2.54	34.6	51.8	13.5	34.7	I, K, Chl-I
V+C-SMS	Sandy clay	7.7	2.83	30.7	50.5	14.3	35.2	I, K, Chl-I
V+F-SMS	Sandy clay	7.6	2.96	30.3	48.8	13.9	37.3	I, K, Chl-I
Subsoil								
AL	Clayed loam	7.9	0.85	10.6	43.6	19.5	36.9	I, K, M, Chl
AL+C-SMS	Sandy clay loam	7.7	0.76	12.2	60.9	14.2	24.9	I, K, M, Chl
AL+F-SMS	Sandy clay loam	7.8	0.91	11.6	45.8	22.4	31.8	I, K, M, Chl
SA	Sandy clay loam	7.9	1.41	39.2	59.7	10.0	30.3	I, K, Chl-I
SA+C-SMS	Clayed loam	8.0	1.07	48.9	41.8	19.9	38.3	I, K, Chl-I
SA+F-SMS	Sandy clay loam	7.9	1.33	42.6	60.3	9.7	30.0	I, K, Chl-I
V	Sandy clay loam	7.7	1.58	40.0	51.3	15.1	33.6	I, K, Chl-I
V+C-SMS	Sandy clay loam	7.7	1.31	36.1	52.0	16.1	31.9	I, K, Chl-I
V+F-SMS	Sandy clay	7.6	1.85	32.6	47.3	16.1	36.6	I, K, Chl-I

I, Illite; K, Kaolinite; M, Montmorillonite, Chl-I, Chlorite-Illite interstratified [a]Unamended soil, [b]Soil amended with composted SMS, [c]Soil amended with fresh SMS

Table 3 Total content of Cu and Cd in unamended and amended vineyard surface soils and subsoils over 2006-2008

	Cu (mg kg $^{-1}$)			Cd (mg kg $^{-1}$)		
Soils	2006	2007	2008	2006	2007	2008
Surface						
AL	59.3[a] 31.6-88.5[b]	45.2 21.2-65.3	27.0 22.1-38.7	0.94 0.91-0.99	1.52 1.40-1.58	1.14 0.82-1.44
AL+C-SMS	50.5 30.2-75.3	30.0 21.3-47.5	34.2 21.8-65.7	1.23 0.99-1.51	1.44 1.13-1.73	1.13 0.94-1.47
AL+F-SMS	27.8 23.8-34.0	33.7 24.4-48.6	35.1 23.3-60.7	1.28 1.23-1.36	1.58 1.39-2.0	1.44 1.06-1.90
SA	46.7 35.5-56.7	42.5 29.8-58.6	49.8 25.5-77.1	1.55 0.65-3.06	1.22 0.77-1.68	0.96 0.71-1.18
SA+C-SMS	59.3 45.2-86.7	73.0 40.2-102	66.5 22.6-110	0.95 0.92-1.00	1.21 0.99-1.48	1.10 0.91-1.25
SA+F-SMS	68.2 42.9-115	75.5 50.2-114	71.4 28.5-107	0.91 0.85-0.98	1.29 1.18-1.54	1.04 0.91-1.19
V	66.8 55.2-78.4	46.0 29.4-65.8	49.9 32.1-74.1	1.05 0.82-1.67	1.01 0.63-1.21	1.14 0.92-1.50
V+C-SMS	38.4 35.7-41.1	52.4 34.5-81.1	46.9 35.8-54.0	1.02 0.89-1.11	1.40 1.04-1.74	1.04 0.70-1.34
V+F-SMS	45.3 42.7-48.4	57.0 21.9-112	44.3 25.7-64.7	1.18 1.14-1.23	1.25 0.99-1.56	0.97 0.50-1.31
LSD ($p<0.05$)	20.1	10.5	34.0	0.80	0.56	0.48
Subsoil						
AL	22.5 17.3-28.5	34.4 17.7-49.7	26.1 17.2-51.1	1.14 0.85-1.44	1.48 0.77-1.93	1.40 0.91-1.49
AL+C-SMS	42.5 21.3-54.5	36.9 20.2-70.8	43.6 22.1-73.9	1.14 1.03-1.20	1.51 1.23-1.94	0.96 0.92-1.17
AL+F-SMS	31.4 27.1-39.6	37.0 23.8-53.2	22.2 18.7-24.6	1.67 1.56-1.77	1.71 1.22-2.48	1.16 1.00-1.38
SA	40.6 20.6-60.9	23.1 8.26-55.8	32.1 12.7-48.3	0.79 0.52-1.01	1.05 0.75-1.58	0.88 0.42-1.14
SA+C-SMS	15.2 10.2-23.2	29.3 12.4-68.5	49.4 6.40-130	0.83 0.62-1.16	1.06 0.81-1.29	0.75 0.62-0.92
SA+F-SMS	23.7 13.1-30.9	28.5 7.21-91.2	35.4 8.5-58.1	1.17 1.16-1.17	1.10 0.73-1.45	0.83 0.74-0.97
V	36.6 16.3-71.2	36.9 10.6-70.8		1.05 0.96-1.22	1.10 0.63-1.24	
V+C-SMS	38.6 25.9-62.4	26.9 21.1-33.7		1.15 0.88-1.36	1.07 0.85-1.19	
V+F-SMS	81.4 71.9-94.7	38.1 18.2-57.8		1.30 1.05-1.42	1.21 0.90-1.49	
LSD ($p<0.05$)	12.4	10.6	14.6	0.85	0.74	0.45

LSD least significant difference [a] Mean of total metal contents corresponding to all samples taken per year in three sampling sites of each experimental plot [b] Range of total metal contents corresponding to all samples taken per year in three sampling sites of each experimental plot

Table 4 Total content of Cr and Ni in unamended and amended vineyard surface soils and subsoils over 2006-2008

	Cr (mg kg $^{-1}$)			Ni (mg kg $^{-1}$)		
Soils	2006	2007	2008	2006	2007	2008
Surface						
AL	27.8[a] 26.7-29.0[b]	31.4 27.5-36.4	26.1 21.7-38.6	26.2 18.2-34.0	40.1 20.2-41.4	25.0 19.7-31.8
AL+C-SMS	37.6 31.7-42.8	31.0 20.2-39.6	31.5 25.6-45.3	25.9 25.3-26.0	37.0 20.2-96.6	25.1 21.1-34.0
AL+F-SMS	37.4 35.1-40.8	37.4 28.6-60.1	39.9 30.1-54.5	23.4 22.1-25.0	30.7 27.1-41.3	27.3 23.8-36.2
SA	27.8 22.5-31.7	32.3 23.7-55.6	35.4 25.3-45.6	13.1 10.9-15.2	23.5 12.3-31.2	22.2 16.9-29.9
SA+C-SMS	29.3 24.1-34.1	34.9 25.5-45.5	38.9 33.5-45.0	18.2 16.6-19.6	19.8 15.9-22.5	18.4 14.9-21.0
SA+F-SMS	29.7 24.2-32.1	35.9 27.1-43.8	33.1 23.5-45.7	15.0 14.7-15.0	22.3 13.7-45.5	25.9 12.3-44.4
V	40.2 28.2-50.7	38.5 31.0-45.8	38.9 35.0-43.8	16.0 12.5-21.8	25.8 17.4-29.8	31.1 18.8-42.6
V+C-SMS	36.1 34.5-39.0	40.5 30.9-48.8	38.2 29.6-50.5	24.2 19.5-33.1	25.8 20.6-30.8	25.3 19.3-33.6
V+F-SMS	40.7 28.7-45.1	44.4 32.8-63.6	38.3 30.9-43.1	22.6 21.5-24.0	23.7 19.5-26.2	21.4 18.2-27.2
LSD ($p<0.05$)	14.7	8.5	14.8	7.1	9.3	10.0
Subsoil						
AL	29.3 23.4-37.1	33.2 21.3-40.2	28.2 17.1-38.6	24.6 12.8-32.5	36.0 17.8-58.1	23.1 18.3-28.0
AL+C-SMS	27.8 24.2-30.8	37.8 27.4-55.8	32.8 27.6-43.0	24.3 20.2-27.6	26.1 20.0-43.3	42.0 24.7-51.4
AL+F-SMS	41.6 35.1-48.1	47.4 33.8-58.7	34.9 25.4-47.5	40.8 30.0-61.0	36.7 30.1-45.4	25.0 18.1-33.8
SA	26.1 19.7-30.1	27.6 17.9-50.4	31.6 20.7-38.1	24.0 20.2-28.7	24.9 9.2-52.5	21.1 10.1-45.1
SA+C-SMS	30.2 26.0-36.7	34.7 30.9-44.8	29.5 16.2-39.3	14.8 13.6-16.3	17.6 15.8-23.7	18.8 7.50-30.1
SA+F-SMS	21.5 18.3-25.9	24.4 18.3-36.5	27.8 13.6-39.1	18.7 11.5-24.4	16.5 12.7-36.8	16.9 8.7-23.5
V	35.5 28.1-41.1	34.9 17.2-51.5		16.9 14.4-19.5	17.3 11.0-19.5	
V+C-SMS	36.7 32.6-39.5	21.9 17.8-24.8		21.6 20.3-22.8	18.7 17.5-19.9	
V+F-SMS	45.5 42.0-48.9	38.2 22.7-57.2		24.5 21.9-27.1	27.7 23.2-37.0	
LSD ($p<0.05$)	18.6	21.5	7.9	16.9	18.6	19.6

LSD least significant difference [a] Mean of total metal contents corresponding to all samples taken per year in three sampling sites of each experimental plot [b] Range of total metal contents corresponding to all samples taken per year in three sampling sites of each experimental plot

Table 5 Total content of Pb and Zn in unamended and amended vineyard surface soils and subsoils over 2006-2008

	Pb (mg kg $^{-1}$)			Zn (mg kg $^{-1}$)		
Soils	2006	2007	2008	2006	2007	2008
Surface						
AL	33.2[a] 32.1-34.0[b]	47.1 36.5-62.0	37.0 28.3-52.0	91.3 84.2-102	105 79.6-132	88.4 76.1-120
AL+C-SMS	42.5 39.0-45.1	44.3 35.8-52.1	38.9 31.4-50.1	130 84.4-200	102 81.6-116	96.0 86.0-113
AL+F-SMS	37.4 34.9-40.5	49.1 37.7-67.6	45.1 36.0-60.3	98.8 92.0-103	113 85-150	114 84.9-142
SA	23.2 18.8-26.1	33.9 22.2-54.9	31.4 24.5-43.4	41.3 36.3-45.6	56.2 42.2-90.8	55.2 42.0-67.0
SA+C-SMS	25.3 23.1-28.6	38.8 30.4-47.5	35.5 28.9-49.1	45.4 40.2-48.4	64.8 48.7-77.4	62.3 52.3-88.0
SA+F-SMS	29.3 24.5-34.6	36.8 24.7-51.4	31.1 25.9-42.3	71.8 44.6-124	63.5 54.3-81.3	58.3 48.6-70.8
V	41.6 22.6-62.0	36.6 26.1-42.3	37.3 34.5-43.6	73.0 63.1-85.8	84.0 74.0-98.2	79.2 66.0-96.0
V+C-SMS	40.1 32.6-45.0	46.1 36.9-59.3	36.1 20.1-52.4	80.7 77.1-84.0	109 77.7-138	100 78.0-120
V+F-SMS	38.6 37.9-39.3	38.9 30.6-46.9	39.7 26.7-82.3	127 106-138	107 67.2-169	86.5 59.6-130
LSD ($p<0.05$)	18.2	13.2	22.5	20.5	26.8	10.1
Subsoil						
AL	36.2 27.8-43.7	45.2 30.6-56.0	37.6 34.4-48.1	80.8 46.2-117	108 69.4-134	89.7 75.4-115
AL+C-SMS	38.5 35.4-41.5	48.1 32.8-64.7	36.6 29.8-42.4	93 82.4-103	108 84.3-146	85.4 72.4-101
AL+F-SMS	57.0 52.0-60.0	50.8 46.7-77.4	37.9 33.0-48.1	120 112-128	127 90.6-174	87.7 75.4-98.2
SA	29.6 17.0-44.9	27.0 18.4-39.9	25.7 17.2-31.9	55.9 32.0-90.4	42.8 27.4-71.5	45.2 26.2-61.0
SA+C-SMS	24.0 17,0-29.9	27.3 22.4-33.2	25.1 19.7-32.2	66.7 36.3-114	51.4 43.5-61.9	43.8 25.0-53.2
SA+F-SMS	24.2 17.2-31.1	24.0 13.1-34.0	23.3 15.5-32.0	71.0 33.7-134	43.5 31.0-68.6	45.2 27.1-55.4
V	30.5 27.6-32.6	35.1 17.2-52.9		61.0 49.5-74.0	68.7 33.1-106	
V+C-SMS	42.1 27.1-53.3	38.4 33.3-46.4		67.3 49.2-77.9	61.3 54.1-69.1	
V+F-SMS	41.2 40.1-42.4	36.4 20.0-54.2		133 102-178	93.1 47.3-163	
LSD ($p<0.05$)	12.5	10.8	4.5	13.1	15.4	10.6

LSD least significant difference [a] Mean of total metal contents corresponding to all samples taken per year in three sampling sites of each experimental plot [b] Range of total metal contents corresponding to all samples taken per year in three sampling sites of each experimental plot