

Article published in Environmental Monitoring and Assessment 2012, 184: 3359-3371.

DOI 10.1007/s10661-011-2194-4

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

Eliseo Herrero-Hernández^a, M. Soledad Andrades^b, M. Sonia Rodriguez-Cruz^a, Michele Arienzo^c, Maria J. Sánchez-Martin^{a*}

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

^bDepartamento de Agricultura y Alimentación, Universidad de la Rioja, Madre de Dios 51, 26006 Logroño, Spain,

^cDipartimento di Scienze del Suolo, Pianta, Ambiente e delle Produzioni Animali, Università Federico II, via Università, 80055 Portici, Napoli, Italy.

*Corresponding author: Tel: +34 923219606; fax: +34 923219609. E-mail address: mjesus.sanchez@irnasa.csic.es (M.J. Sánchez-Martín).

1 **Abstract**

2 Long-term variability of total Cu content from fungicides applied in a certified wine region of
3 Spain (La Rioja) and of other metals (Cd, Cr, Ni, Pb and Zn) was evaluated in three young
4 vineyard soils and subsoils unamended and amended with spent mushroom substrates (SMS)
5 over a three-year period (2006-2008). SMS is a promising agricultural residue as an
6 amendment to increase the soil organic matter content but may modify the behaviour of
7 metals from pesticide utilisation in vineyards. Fresh and composted SMS was applied each
8 year at a rate of 25 t ha⁻¹ (dry-weight). Copper concentrations in the three unamended soils
9 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
10 concentrations of the region and reference sub lethal hazardous concentration for soil
11 organism. The concentrations of Cd, Ni, Pb and Zn, were largely below the sub lethal limits.
12 Thus, although Cu levels were lower than those of established vineyards, vine performance
13 and productivity might be affected. The variation in behaviour between different amendments
14 for each soil was high, so a generic conclusion could not be drawn. The amendment practice
15 seemed to have caused temporarily Cu mobilisation respect to untreated soils. Total zinc
16 concentrations fall within the range of the natural soil of La Rioja and were significantly
17 affected (p<0.05) especially by fresh state SMS addition, with increasing up to 75% respect to
18 untreated specimen. Results indicated a build-up of fresh sites for metal retention at both
19 surface and subsurface level, although no accumulation of metals was observed in the short
20 term period. However, benefit for soils as well the negative effects need to be monitored in
21 the long run.

22

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

24

25

26 **Introduction**

27 Copper-based fungicides such as the Bordeaux mixture, $\text{CuSO}_4 \cdot 3\text{Cu}(\text{OH})_2 \cdot 3\text{CaSO}_4$, have been
28 intensively used in Europe since the end of the 19th century to control vine (*Vitis vinifera* L.)
29 fungal diseases, such as downy mildew caused by *Plasmopara viticola*. Additionally, other
30 Cu compounds have been introduced including Cu-oxychloride, $3\text{Cu}(\text{OH})_2 \cdot \text{CuCl}_2$ and Cu
31 hydroxide, $\text{Cu}(\text{OH})_2$ and their long term application and subsequent wash-off from the treated
32 plants have resulted into extensive Cu accumulation in vineyard soils. A recent review of
33 Komárek et al. (2010) on contamination of vineyard soils reported that the levels of Cu
34 usually exceed the legislative limits valid in the EU.

35 Vineyard soils are usually highly degraded soils in terms of biochemical properties
36 (Miguéns et al. 2007) and are thus more susceptible to contamination. Some studies have
37 shown elevated copper concentrations to adversely affect the fertility of vineyard soils
38 (Paoletti et al. 1998); field studies found clear linkages between increased Cu concentrations
39 ($>80 \text{ mg kg}^{-1}$) in soil and reduced earthworm abundance (Paoletti et al. 1998). In response to
40 environmental concerns over the use of copper fungicides such as the accumulation of copper
41 in agricultural soils and the potential impact on soil ecology, regulators in some European
42 countries have imposed restrictions on the use of copper-based fungicides. For example,
43 copper use has been banned in The Netherlands, and Switzerland has restricted the amount of
44 copper that can be applied per hectare (Wightwick et al. 2008).

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

52 comparatively to a natural origin where the metals are strongly associated with soil
53 components.

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

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

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

75 Spent mushroom substrate (SMS) represents a promising renewable agricultural
76 organic resource that can improve soil properties, e.g., soil water retention capacity, nutrient

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

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

99

100 **Materials and Methods**

101

102 Soil sampling

103

104 Soil samples were collected from vineyards of the region of La Rioja (N-Spain) located in
105 Aldeanueva (AL) (42°14'0"N latitude and 1°53'0"W longitude), Sajazarra (SA) (42°35'0"N
106 latitude and 2°57'0"W longitude), and Viana (V) (42°30'0"N latitude and 2°20'0"W
107 longitude). These locations were selected for their different altitudes, topographies and
108 climates. This region represents the most important wine region of Spain and extends over
109 50,000 ha and gives 273 million of litres of certified wine. Soils were classified as a Fluventic
110 Haplocambids (AL), a Typic Calcixerepts (SA), and a Typic Xerorthents (V) (USDA 2006).
111 The soils have been treated with copper from Cu-oxychloride or Cu-calcium sulphate 3-4
112 times per year at a rate of 4-6 kg ha⁻¹ for 12 (AL), 14 (SA) and 15 (V) years (Table 1). Each
113 vineyard plot, with a size between 2.03 ha to 2.74 ha, was divided into three sub-plots, one
114 was not amended and the other two were amended with F-SMS or C-SMS, respectively. The
115 amendment was added for three years, 2006-2008, between February-April at the
116 recommended agronomic rate of 25 t ha⁻¹ (dry-weight). Soil and subsoil samples were taken
117 each year before the addition of the amendment and after grape harvest from 27 sites (three
118 sites per subplot) over the experimental plot. At each site three soil cores (0-60 cm) were
119 taken to make a soil (0-30 cm) and subsoil (30-60 cm) composite sample representative of
120 each site.

121

122 Soils and spent mushroom substrate

123

124 Soil samples were air dried and sieved (<2 mm) and characteristics were determined by usual
125 analytical methods (MAPA 1986) (Table 2). The pH of the soils was determined in soil/water
126 suspensions (ratio 1/2.5) and their particle size distribution was determined using the pipette

127 method, which measures the weight percent of each particle fraction (clay, silt and sand) in the
128 soil sample based on Stokes' law (Gee and Or, 2002). Organic carbon (OC) contents were
129 determined by the modified method of Walkley-Black and the results were multiplied by 1.72
130 to convert the organic carbon into OM. Inorganic carbon was determined as CaCO₃ with a
131 Bernard calcimeter. The dissolved organic carbon (DOC) content was determined using a
132 Shimadzu 5050 (Shimadzu, Columbia, MD) organic carbon analyzer. Clay minerals were
133 identified qualitatively by the X-ray diffraction (XRD) technique, using a Philips PW 1710
134 diffractometer (Eindhoven, the Netherlands). The XRD patterns showed the presence of illite,
135 kaolinite and chlorite-illite interstratified mineral respectively in all soils. In the case of AL
136 soil, the presence of smectitic clay was also detected.

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

144

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

146

147 A sub-sample of 1 g was weighed directly in a dried, cleaned PTFE digestion vessel and 10
148 mL of aqua regia were added. Afterwards, the digestion vessel was placed in the chamber of a
149 microwave system Ethos Sel model (Milestone, Shelton, CT). Metal analysis was performed
150 on a Varian model 720-ES inductively coupled plasma optical emission spectrometer (Varian
151 Instruments, Palo Alto, CA). All determinations were carried out by triplicate. Analytical

152 precision of replicates varied from 9.3 to 19% relative to standard deviation. Analytical
153 accuracy in the determination of the total content of metals was checked with the BCR
154 reference materials. Five samples of CRM 141 and CRM 320 were analysed and variation
155 coefficients <6.5% or <15%, respectively were obtained. All reagents were Merck analytical
156 grade and Milli-Q ultrapure water was used from a Millipore Milli Q system. The extraction
157 procedures were performed using laboratory glassware and polythene bottles pre-cleaned by
158 HCl and rinsed with double distilled water.

159

160 Data analysis

161

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

167

168 **Results and discussion**

169

170 Characteristics of unamended and amended soils

171

172 Soils had generally a sandy clay loam texture (Table 2) with a clay content of 21.4%-38.3%
173 and a carbonate content of 10.6%-48.9%, with peaks for SA and V soils and for samples
174 taken at the subsoil level. The AL soil displayed the lowest levels of clay, carbonates and OM
175 with an average OM content of the unamended specimen of about 1.04% which increased of
176 ~50% when the SMS was added. Fig. 1 shows the variation of the OM content in unamended

177 and amended surface soil and subsoil over the time. The results obtained here show that the
178 use of F-SMS or C-SMS as an amendment at relatively low agronomic doses elicits an
179 increase in the OM content of soils. At the beginning of the trial, the addition of the SMS
180 significantly ($p<0.05$) increased the content of the OM for both surface and subsurface and
181 especially when the amendment was used at fresh state. However, after repeated amendment
182 application the organic load increased more markedly when it was used at composted state.
183 This was the case for V soil, whose OM content increased up to ~100% at latest sampling
184 (2008). The OC richness of the fresh residue was higher than that composted 34.2 vs. 28.6%
185 and even its dissolved organic pool was threefold higher, 13.3% vs. 4.34% (Marín-Benito et
186 al. 2009). Thus, the highest level of SOM of the fresh residue might have sustained a greater
187 lost by leaching of the OC toward the deepest soil layers. A similar trend, even though with
188 more smoothed differences, was observed for the subsoil. Differences in structure and
189 composition of soils might explain the extent of OM variation by amendment addition. No
190 significant soil pH variation was observed with the addition of the amendment with value
191 remaining stable around an alkaline value of 7.8, at all studied depths and at all the
192 experimental sites.

193

194 Total copper content

195

196 Total Cu content in the unamended soils sampled in 2006 varied in the range 31.6-88.5 mg
197 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).
198 These levels exceeded those for unpolluted soil Cu background concentrations of 30 mg kg^{-1}
199 (Adriano 2001). No copper soil accumulation was observed, since the ranges remained
200 unvaried, with no significant differences ($p>0.05$) at later sampling taken in 2007 and 2008.
201 This was likely due to the ‘aging’ effect acting when Cu enters the soil, which reduces metal

202 loss. When Cu is spread over the soil its speciation rapidly changes and Cu is sorbed and
203 (co)precipitated by humic and fulvic acids (Strawn and Baker 2009). This redistribution of Cu
204 into less available chemical fractions of soils decreases its mobility and bioavailability and is
205 referred to as “aging” (Arias-Estévez et al. 2007; Sayen et al. 2009).

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

210 Copper varied within a range similar to that of 6.0-120 mg kg⁻¹ reported for vineyard soils of
211 the Rioja region (Marín et al. 2000). The mean Cu levels were up to five-fold higher, as in the
212 case of V soil, than those indicated for natural soils of La Rioja (14.1 mg kg⁻¹) (Iñigo et al.
213 2006). On the other hand, Cu peak contents of the unamended soil were much lower than
214 those reported for other vineyard areas of N.W. Spain (up to 603 mg kg⁻¹) with high annual
215 rate of antifungal treatment due to climatic conditions of high humidity (mean annual rainfall
216 of around 825 mm (Nóvoa-Muñoz et al. 2007, Fernández-Calviño et al., 2008). This may be
217 due to the limited history of copper-based fungicide use (Table 1) being the studied vines not
218 older than 15 years (V) as well as to the low usage rate of fungicide. In some European
219 regions typical annual Cu use ranges from 10 to 50 kg ha⁻¹, whereas the average annual use in
220 our studied vineyards was 5 kg ha⁻¹. This rate, although in Spain, there are currently no
221 restrictions on copper use in conventional viticulture, appears to be close to foreign organic
222 standards for limit annual of copper use, ~8 kg ha⁻¹ (Wightwick et al. 2008). Even though Cu
223 total soil levels appeared to be lower than the range (130-1280 mg kg⁻¹) detected in the
224 surface soil of European vineyards with an established history of copper-based fungicide use
225 (Delusia et al. 1996; Flores-Velez et al. 1996), the impact on soil fertility and biology can be
226 of a concern especially for these young vines, which have a shallower root system extending

227 over the surface soil where most of the applied copper tends to remain (Wightwick et al.
228 2008).

229 Although it was outside the scope of this study to conduct a comprehensive
230 assessment of the risks that accumulated copper poses to soil fertility in the three selected
231 vineyards of La Rioja, the potential risks can be indicated by comparing the measured
232 concentrations of copper to thresholds known to cause sub lethal effects to soil organisms.
233 Using species sensitivity distributions Jansch et al. (2007) and Frampton et al. (2006) have
234 estimated hazardous concentrations to 5% (HC5) of beneficial soil organisms. A sub lethal
235 HC5 to soil organisms has been estimated to be 55 mg kg⁻¹ total Cu (derived using EC50
236 values for animals, microbial processes, and plant species). This HC5 concentration provide
237 an indication of potential toxicity while recognizing that site-specific soil factors such as pH,
238 texture, and cation exchange capacity will influence the bioavailability of the copper present
239 and therefore toxicity.

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

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

259 For AL soils, Cu dropped from a mean value of ~60 mg kg⁻¹ of the unamended
260 specimen to ~27 mg kg⁻¹ of that amended with fresh state organic material, as observed in
261 2006. A similar Cu level was also observed in AL in 2008 and likely due to the already
262 observed low content of OM and clay. Similarly van Herwijnen (2007) in a study comparing
263 four composts and a liming product found that spent mushroom compost caused an increase
264 in metal concentration in the leachates. Copper has been shown to become mobile in soils
265 through association with the dissolved organic matter (DOM), especially in sandy soils
266 (Temminghoff et al. 1997, Weigand and Totsche 1998; McBride et al. 1999). SOM can
267 influence the mobility of Cu by two different means: while particulate SOM will act as a
268 sorbent for Cu, soluble SOM will actually complex Cu, increasing thus its solubility,
269 especially at alkaline pH (Fernández-Calviño et al. 2008; Martínez-Villegas and Martínez
270 2008). Its association with SOM occurs through inner-sphere complexation (e.g. bidentate
271 inner-sphere coordination with carboxyl or amine ligands) (Strawn and Baker 2009). Thus, it
272 seems that in such vine site, the amendment practice with SMS has made copper more mobile
273 and available, possibly impacting surface and/or ground water.

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

277 >55 mg kg⁻¹, was observed whatever was the form of the amendment added and at all
278 sampled times. In such a soil having a higher clay content, >30%, it is possible that the metal
279 is immobilized by chelation with the OM and adsorbed onto clay minerals and accumulate.
280 Many studies found that the addition of organic amendments can accelerate the natural soil
281 attenuation process (natural remediation), of metals by sorption, precipitation, and
282 complexation reactions (Kabata-Pendias and Pendias 2001; Bolan et al. 2003; Pérez-de-Mora
283 et al. 2005). There has been evidence that the addition of composted bio-amendment can
284 increase Cu (together with other metals) concentrations in vineyard soils as well (Ramos
285 2006). Some authors report on the adsorption of SOM from the washing of SMS in field
286 setting by soil components in particular clay (Guo et al. 2001). The affinity of Cu to soil
287 fractions was reported to vary in the following order: SOM>silicate clays>ferrihydrite,
288 (Martínez-Villegas and Martínez 2008). It has been observed that amendments can decrease
289 heavy metal bioavailability, shifting it from “plant available” forms to fractions associated
290 with clays, carbonates or metal oxides (Walker et al. 2004),

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

296 In subsurface unamended soil, Cu concentration was significantly lower up to 62 %
297 than that in surface soil and this was more evident in AL soil. Surface soil copper
298 accumulation was expected as copper bind strongly to soil components. Even though this, no
299 significant correlations were found in this work between vineyard copper concentrations and
300 these soil components. This was likely due to the limited number of surveyed vineyard since
301 our survey was not designed to specifically assess the influence of soil properties on metal

302 accumulation. Similarly, no significant correlations were observed by Marin et al. (2000)
303 between Cu and soil chemical properties of 37 vineyard soil samples of La Rioja due to the
304 narrow range of variation of the soil properties of the set of soils. Further work needs to be
305 undertaken to explore the influence of soil properties on the adsorption and accumulation of
306 Cu in the vineyard soils in La Rioja region. At the observed significant decrease of Cu level in
307 the surface of SMS-amended Al and V soils, corresponded a significant ($p < 0.05$)
308 accumulation of the metal in the deepest soil layers, especially if the added SMS was at fresh
309 state (Table 3). A peak concentration of Cu of 81.4 mg kg^{-1} was noted for V+F-SMS soil.
310 This could be of great importance for vineyard soils, especially when C-SMS which is more
311 homogeneous than F-SMS is used. No significant differences were observed for treated and
312 untreated SA soil.

313

314 Total Cd, Cr, Ni, Pb and Zn contents

315

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

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

325 The highest load, $\sim 100 \text{ mg kg}^{-1}$, was observed for AL soil, the lowest for the SA soil,
326 $\sim 40 \text{ mg kg}^{-1}$. Zinc soil loads in untreated soil remained quite constant over the time for all the

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

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

351

352 **Conclusions**

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

370

371 **Acknowledgments**

372 This work was funded by the Spanish Ministry of Science and Innovation (projects
373 CIT-310200-2007-63 and AGL2007-61674/AGR). E. Herrero-Hernández thanks CSIC for his
374 JAE-doc contract and Prof. Arienzo thanks the University of Naples, Federico II, for
375 sponsoring his stay in the IRNASA of Salamanca under the international short term exchange
376 visiting programme. The authors thank L.F. Lorenzo, J.M. Ordax, and A. González for

377 technical assistance and CIDA (Dr. E. García-Escudero and J.M. Martínez Vidaurre),
378 INTRAVAL S.L., and the wineries CVNE, Aldeanueva de Ebro and Viña Ijalba from La
379 Rioja, Spain, for their collaboration.

380

381 **References**

382 Adriano, D.C. (2001). *Trace elements in the terrestrial environments: Biogeochemistry,*
383 *bioavailability, and risks of metals.* New York: Springer-Verlag.

384 Arias-Estévez, M., Nóvoa-Muñoz, J. C., Pateiro, M., & López-Periago, E. J. (2007). Influence
385 of aging on copper fractionation in an acid soil. *Soil Science, 172,* 225–232.

386 Barber, S. A. (1995). *Soil nutrient bioavailability* (2nd ed.). New York: John Wiley & Sons,
387 Inc.

388 Belotti, E. (1998). Assessment of a soil quality criterion by means of a field survey. *Applied*
389 *Soil Ecology, 10,* 51–63.

390 Bolan, N. S., Adriano, D. C., Duraisamy, P., & Mani, A. (2003). Immobilization and
391 phytoavailability of cadmium in variable charge soils. III. Effect of biosolid compost
392 application. *Plant and Soil, 256,* 231–241.

393 Delusia, A., Giandon, P., Aichner, M., Bortolami, P., Bruna, L., Lupetti, A., Nardelli, F., &
394 Stringari, G. (1996). Copper pollution in Italian vineyard soils. *Communications in Soil*
395 *Science and Plant Analysis, 27,* 1537–1548.

396 Díaz-Barrientos, E., Madrid, L., Maqueda, C., Morillo, E., Ruiz-Cort, E., Basallote, E., &
397 Carrillo, M. (2003). Copper and zinc retention by an organically amended soil.
398 *Chemosphere, 50,* 911–917.

399 Fernández-Calviño, D., Rodríguez-Suárez, J. A., López-Periago, E., Arias-Estévez, M., &
400 Simal-Gándara, J. (2008). Copper content of soils and river sediments in a winegrowing
401 area, and its distribution among soil or sediment components. *Geoderma, 145,* 91–97.

402 Flores-Velez, L. M., Ducarior, J., Jaunet, A. M., & Robert, M. (1996). Study of the
403 distribution of copper in an acid sandy vineyard soil by three different methods. *European*
404 *Journal of Soil Science*, 47, 523–532.

405 Frank, R., Braun, H. E., Ishida, K., & Suda, P. (1976). Persistent organic and inorganic
406 pesticide residues in orchard soils and vineyards of Southern Ontario. *Canadian Journal*
407 *of Soil Science*, 56, 463–484.

408 Frampton, G. K., Jansch, S., Scott-Fordsmand, J. J., Rombke, J., & Van Den Brink, P. J.
409 (2006). Effects of pesticides on soil invertebrates in laboratory studies: a review and
410 analysis using species sensitivity distributions. *Environmental Toxicology and Chemistry*
411 25, 2480–2489.

412 Gee, G. W., Or, D. (2002). Particle-size analysis. In J. H. Dane, G. C. Topp (Eds.), *Methods*
413 *of Soil Analysis. Part 4. Physical Methods* (pp. 255-293). Madison, WI: Soil Science
414 Society of America.

415 Guo, M., Chorover, J., & Fox, R. H. (2001). Effects of spent mushroom substrate weathering
416 on the chemistry of underlying soils. *Journal of Environmental Quality*, 30, 2127–2134.

417 Helling, B., Reinecke, S. A., & Reinecke, A. J. (2000). Effects of the fungicide copper
418 oxychloride on the growth and reproduction of *Eisenia fetida* (Oligochaeta).
419 *Ecotoxicology and Environmental Safety*, 46, 108–116.

420 Hsu, J. H., & Lo, S. L. (2000). Characterization and extractability of copper, manganese, and
421 zinc in swine manure composts. *Journal of Environmental Quality*, 29, 447–453.

422 Iñigo, V., Andrades, M., Marín, A., & Alonso, J. I. (2006). Determinación de los niveles de
423 referencia de cinc, cobre y níquel en los suelos naturales de la Rioja. In J. F. Gallardo
424 (Ed.), *Medio Ambiente en Iberoamerica* (pp. 249-255). Badajoz: Diputación de Badajoz.

425 Jansch, S., Rombke, J., Schallanass, H-J., & Terytze, K. (2007). Derivation of soil values for
426 the path 'soil-soil organisms' for metals and selected organic compounds using species
427 sensitivity distributions. *Environmental Science and Pollution Research*, *14*, 308–318.

428 Kabata-Pendias, A., & Pendias, H. (2001). *Trace elements in soils and plants*. Florida: CRC
429 Press, Boca Raton.

430 Kiekens, L. (1990). Zinc. In B. J. Alloway (Ed.), *Heavy metals in soils* (pp. 261-279).
431 Glasgow: Blackie.

432 Komárek, M., Száková, J., Rohošková, M., Javorská, H., Chrastný, V., & Balík, J. (2008).
433 Copper contamination of vineyard soils from small wine producers: a case study from the
434 Czech Republic. *Geoderma*, *147*, 16–22.

435 Komárek, M., Čadková, E., Chrastný, V., Bordas, F., & Bollinger, J. C. (2010).
436 Contamination of vineyard soils with fungicides: A review of environmental and
437 toxicological aspects. *Environment International*, *36*, 138–151.

438 MAPA (Ministerio de Agricultura, Pesca y Alimentación). (1986). *Métodos oficiales de*
439 *análisis*. Madrid: Dirección General de Política Alimentaria.

440 MAPA (Ministerio de Agricultura Pesca y Alimentacion). (1990). Real Decreto 1310/1990 de
441 29 de octubre, sobre utilización de los lodos de depuración en el sector agrario. *Boletín*
442 *Oficial del Estado*, *262*, 32339–32340.

443 McBride, M. B., Richards. B. K., Steenhuis, T., & Spiers, G. (1999). Long-term leaching of
444 trace elements in a heavily sludge-amended silty clay loam soil. *Soil Science*, *164*, 613–
445 623.

446 Marín, A., Alonso-Martirena, J. I., & Andrades, M. (2000). Contenido en metales pesados en
447 suelos de viñedo de la D.O.C. La Rioja. *Edafología*, *7*, 351–357.

448 Marín-Benito, J. M., Sánchez-Martín, M. J., Andrades, M. S., Pérez-Clavijo, M., &
449 Rodríguez-Cruz, M. S. (2009). Effect of spent mushroom substrate amendment of

450 vineyard soils on the behaviour of fungicides: 1. Adsorption-desorption of penconazole
451 and metalaxyl by soils and sub-soils. *Journal of Agricultural and Food Chemistry*, 57,
452 9634–9642.

453 Martínez-Villegas, N., & Martínez, C. E. (2008). Solid- and solution-phase organics dictate
454 copper distribution and speciation in multicomponent systems containing ferrihydrite,
455 organic matter, and montmorillonite. *Environmental Science and Technology* 42, 2833–
456 2838.

457 Miguéns, T., Leirós, C., Gil-Sotres, F., & Trasar-Cepeda, C. (2007). Biochemical properties
458 of vineyard soils in Galicia, Spain. *Science of the Total Environment*, 378, 218–222.

459 Mihaljevič, M., Ettler, V., Šebek, O., Strnad, L., & Chrastný, V. (2006). Lead isotopic
460 signatures of wine and vineyard soils, tracers of lead origin. *Journal of Geochemical*
461 *Exploration*, 88, 130–133.

462 Nóvoa-Muñoz, J. C., Queijeiro, J. M. G., Blanco-Ward, D., Álvarez-Olleros, C., Martínez-
463 Cortizas, A., & García-Rodeja, E. (2007). Total copper content and its distribution in acid
464 vineyards soils developed from granitic rocks. *Science of the Total Environment* 378, 23–
465 27.

466 Paoletti, M. G., Sommaggio, D., Favretto, M. R., Petruzzelli, B., Pezzarossa, B., (1998).
467 Barbafieri, M. (1998). Earthworms as useful bioindicators of agroecosystem sustainability
468 in orchards and vineyards with different inputs. *Applied Soil Ecology*, 10, 137–150.

469 Pavel, J., Vávrová, J., Herzogová, L., & Pilařová, V. (2010). Effects of inorganic and organic
470 amendments on the mobility (leachability) of heavy metals in contaminated soil: A
471 sequential extraction study. *Geoderma*, 159, 335–341.

472 Pérez-de-Mora, A., Ortega-Calvo, J. J., Cabrera, F., & Madejón, E. (2005). Changes in
473 enzyme activities and microbial biomass after “in situ” remediation of a heavy metal-
474 contaminated soil. *Applied Soil Ecology*, 28, 125–137.

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

477 Plan Director de Residuos de La Rioja 2007-2015. Gobierno de La Rioja, Spain,
478 <http://www.larioja.org>. Accessed on April 2010.

479 Ramos, M. C. (2006). Metals in vineyard soils of the Penedès area (NE Spain) after compost
480 application. *Journal of Environmental Management*, 78, 209–215.

481 Sanders, J. R. (1980). The use of adsorption equations to describe copper complexing by
482 humified organic matter. *Journal of Soil Science*, 31, 633–641.

483 Sayen, S., Mallet, J., & Guillon, E. (2009) Aging effect on the copper sorption on a vineyard
484 soil: column studies and SEM-EDS analysis. *Journal of Colloid and Interface Science*,
485 331, 47–54.

486 Sauve, S., Hendershot, W., & Allen, H. E. (2000). Solid-solution partitioning of metals in
487 contaminated soils: dependence on pH, total metal burden, and organic matter.
488 *Environmental Science and Technology*, 34, 1125–1131.

489 Strawn, D. G., & Baker, L.L. (2009). Molecular characterization of copper in soils using X-
490 ray absorption spectroscopy. *Environmental Pollution*, 157, 2813–2821.

491 Temminghoff, E. J. M., Van Der Zee, S. A. E. T. M., & De Haan, F. A. M. (1997). Copper
492 mobility in a copper-contaminated sandy soil as affected by pH and solid and dissolved
493 organic matter. *Environmental Science and Technology*, 31, 1109–1115.

494 USDA-United States Department of Agriculture. Soil Survey Staff. (2006). *Keys to soil*
495 *taxonomy*. Washington: Natural Resources Conservation Service.

496 van Herwijnen, R., Laverye, T., Poole, J., Hodson, M. E., & Hutchings, T. R. (2007). The
497 effect of organic materials on the mobility and toxicity of metals in contaminated soils.
498 *Applied Geochemistry*, 22, 2422–2434.

- 499 Walker, D. J., Clemente, R., & Bernal, M. P. (2004). Contrasting effects of manure and
500 compost on soil pH, heavy metal availability and growth of *Chenopodium album* L. in a
501 soil contaminated by pyritic mine waste. *Chemosphere*, *57*, 215–224.
- 502 Weigand, H., & Totsche, K. U. (1998). Flow and reactivity effects on dissolved organic
503 matter transport in soil columns. *Soil Science Society of America Journal*, *62*, 1268–1274.
- 504 Wightwick, A., Mollah, M. R., Partington, D. L., & Allison, G. (2008). Copper Fungicide
505 Residues in Australian Vineyard Soils. *Journal of Agricultural and Food Chemistry*, *56*,
506 2457–2464.
- 507 Wuest, P. J., Levanon, D., & Hadar, Y. (1995). *Environmental, agricultural and industrial*
508 *uses for spent mushroom substrate from mushroom farms*. Emmaus: J G Press, Inc.
- 509

510

511

512

513 **Figure captions**

514

515 **Fig. 1** Evolution of the organic matter content in surface soil (bar) and subsoil (line) amended

516 with composted C- and fresh F-SMS over 2006-2008

517

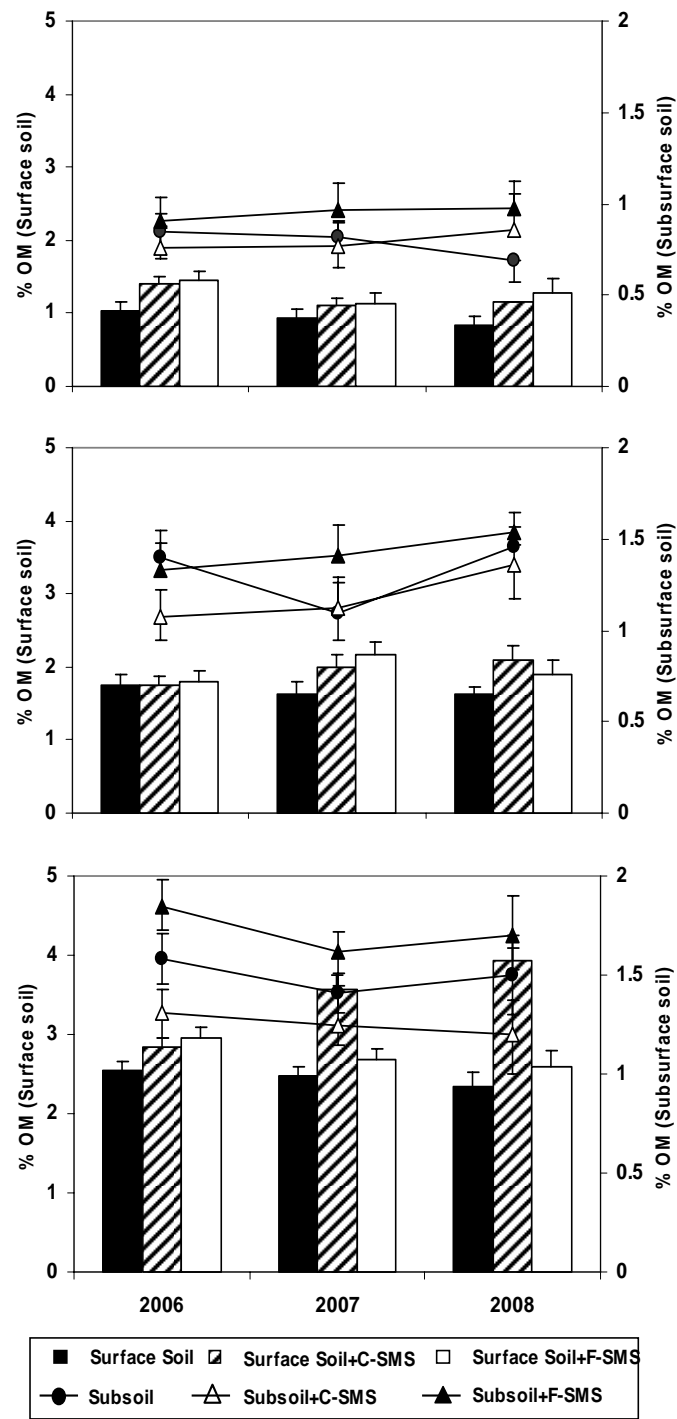


Fig. 1

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 ·3Cu(OH) ₂ ·3CaSO ₄	6	72
Sajazarra (SA)	14	CuSO ₄ ·3Cu(OH) ₂ ·3CaSO ₄ + CuCl ₂ ·3Cu(OH) ₂	4	56
Viana (V)	15	CuSO ₄ ·3Cu(OH) ₂ ·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 ^aUnamended soil, ^bSoil amended with composted SMS, ^cSoil amended with fresh SMS

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

Soils	Cu (mg kg ⁻¹)			Cd (mg kg ⁻¹)		
	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

Soils	Cr (mg kg ⁻¹)			Ni (mg kg ⁻¹)		
	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

Soils	Pb (mg kg ⁻¹)			Zn (mg kg ⁻¹)		
	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