# The accumulation of copper in soils of the Italian region Emilia-Romagna

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## ABSTRACT

The investigation was carried out in 2005, on 30 plots chosen in the Central-Eastern part of the Emilia Romagna region, and cultivated with pear, grapevine and vegetable crops under the organic management system. For each crop, 5 plots with a level of calcium carbonate > 10% and 5 plots with a level of calcium carbonate < 3% were selected. For pear and vine, soil analyses were performed at the depths of 0–20 cm and 20–50 cm, for vegetable at the depth of 0–50 cm. Organic matter content was higher in pear-cultivated plots, followed by grapevine and vegetable crops. Copper application rate, from 1998 to 2004, was higher in pear and grapevine than in vegetable plots. Soil total and DTPA-extractable Cu were higher in pear and grapevine than in vegetable-cultivated plots. Soil DTPA-extractable Cu concentration was higher in the upper horizon than at 20–50 cm soil depth. The increase of total Cu in pear and vine-cultivated plots was combined with the increase of soil inactive Cu.

**Keywords:** calcium carbonate; EDTA-extractable Cu; grapevine; pear

Inorganic copper (Cu) is used as a broad-spectrum fungicide (Baligar et al. 1998) and bactericide in horticultural crops because it combines effectiveness and low cost. Copper sprays are suitable for frequent use in controlling such pear (*Pyrus communis* L.) diseases as brown spot (*Stemphylium vesicarium*), European canker (*Nectria galligena*), fire-blight (*Erwinia amylovora*), and pear scab (*Venturia pirina*). In viticulture, Cu is used to control downy mildew (*Plasmopara viticola*), oidium (*Uncinula necator*), botrytis (*Botrytis cinerea*), etc. The intensive and long-term use of copper salts (Garcia-Esperanza et al. 2006), promoted, over the years, Cu accumulation in soil (Moolenaar and Beltrami 1998). Copper is a heavy metal toxic for aquatic and soil organisms, bacteria, fungi (Giller et al. 1998) and plants (Woolhouse and Walker 1981) it also has a negative effect on human health (Turnlund et al. 2004). In soil, Cu is restricted mainly in the top layer because of its ability to tightly bind with carbonates, clay minerals, hydrous oxides of Al, Fe and Mn and organic matter (Mengel and Kirkby 2001). Copper mobility along the soil profile, bioavailability for root uptake and consequently phytotoxicity threshold for crops depend on soil pH (Chaignon et al. 2003), cation exchange capacity (CEC), quality of organic matter, texture etc. (Brun et al. 2001, Parat et al. 2002). In Italy, soil total Cu concentration was reported to range between 2 and 375 mg/kg (Fregoni and Corallo 2001, Mantovi et al. 2003). The potential risk of soil contamination with Cu prompted the European Union (EU) to restrict the rate of Cu application to fruit trees (Commission Regulation [EC] No 473/2002 amending Annexes I, II and VI to Council Regulation [EEC] No. 2092/91). The objective of this investigation was to evaluate the accumulation of Cu in calcareous and non-calcareous soils of the Emilia Romagna region, cultivated with pear, grapevine (*Vitis vinifera* L.) and vegetable crops, under organic farming management.

## MATERIAL AND METHODS

The investigation was carried out in 2005, on 30 plots chosen in the Central-Eastern part of the Emilia Romagna region (between 44°15' and

44°30' North and between 10°45' and 12°15' East), and cultivated with pear, grapevine and vegetable crops (10 plots per crop). For each crop, 5 plots with a high level of carbonates (calcium carbonate > 10%) and 5 plots with a low level of carbonates (calcium carbonate < 3%) were selected. For pear and vine, soil analyses were performed at the depths of 0–20 cm and 20–50 cm, for vegetable only at the depth of 0–50 cm. Soil analyses included soil texture, total and active calcium carbonate, organic matter (O.M.), pH, total and DTPA-extractable Cu. Total soil Cu was extracted by wet mineralisation after US EPA Method 3052 (Kingston 1988) by treating 0.5 g of dry soil with 8 ml of nitric acid (65%) and 2 ml of hydrogen peroxide (30%) at 180°C in an Ethos TC microwave lab station (Milestone, Bergamo, Italy). This method was used because it allows the same results of extraction with $HF/HClO_4/HNO_3$ and aqua regia (Pietrzack and McPhail 2004). DTPA-extractable Cu was extracted according to Lindsay and Norvell (1978) and modified by shaking 5 g of dry soil for 2 h at 60 cycles per min with 25 ml of a solution made with diethylentriaminepentaacetic acid (DTPA) 1.97 g/l, triethanolamine 14.9 g/l and $CaCl_2$ 1.46 g/l buffered to pH 7.3 with HCl, and centrifuging. All Cu fractions were determined by atomic absorption spectrophotometry (Varian AA200, Mulgrave, Victoria, Australia). We assumed that the amount of Cu measured by DTPA-extraction was the active Cu that includes the water soluble, exchangeable and sorbed Cu fraction (Pietrzak and McPhail 2004). Consequently the difference between total and DTPA-extractable Cu was considered as inactive soil Cu fraction. Soil texture, calcium carbonate, active calcium carbonate, organic matter and C.E.C. were determined according to the procedure of the Italian Ministry of Agriculture, Food and Forestry and the International Union of Soil Sciences (Violante 2000), by an external laboratory (ARPA, Ravenna, Italy): calcium carbonate by volumetric determination of carbon dioxide after soil treatment with HCl; active calcium carbonate by titration of the excess ammonium oxalate; organic matter by elemental C analysis; and CEC by barium chloride method.

At the same time, the amount of Cu supplied, from 1998 to 2004, was investigated consulting the field book registers of 30 selected farms. Data were statistically analyzed as in a factorial experimental design (5 farms as replications) with 2 factors: crop (3 levels: grapevine, pear and vegetable) × soil (2 levels: calcareous and non calcareous). In

Table 1. Soil taxonomy, number of observations, texture, total and active carbonate, organic matter (O.M.) and pH of the investigated soils (In parenthesis are the standard errors)

| Soil taxonomy (FAO, 1990) | *n* | Sand (%) | Loam (%) | Clay (%) | $CaCO_3$ (%) | Active $CaCO_3$ (%) | O.M. (%) | pH |
|---|---|---|---|---|---|---|---|---|
| Calcaric cambisols | 12 | 26 (± 8.8) | 53 (± 6.8) | 21 (± 8.2) | 16.2 (± 3.5) | 5.92 (± 2.7) | 1.94 (± 0.47) | 7.7 (±0.18) |
| Eutri vertic cambisols | 6 | 21 (± 5.1) | 53 (± 3.8) | 26 (± 8.0) | 0.80 (± 0.44) | 0 | 2.44 (± 1.2) | 7.2 (±0.26) |
| Vertic calcisols | 4 | 19 (± 5.2) | 53 (± 4.0) | 28 (± 8.1) | 0.75 (± 0.5) | 0 | 2.48 (± 1.38) | 7.3 (±0.26) |
| Bathicalci eutric cambisols | 3 | 19 (± 3.6) | 60 (± 3.2) | 21 (± 6.8) | 0.67 (± 0.58) | 0 | 2.23 (± 0.74) | 7.1 (±0.15) |
| Calcari fluvic cambisols | 2 | 30 (± 0) | 49 (± 1.4) | 21 (± 1.4) | 13.5 (± 3.5) | 3.0 (± 1.4) | 1.55 (± 0.07) | 7.9 (±0.14) |
| Ferri stagnic luvisols, thapto vertic | 2 | 12 (± 2.1) | 62 (± 5.7) | 26 (± 7.1) | 0.5 (± 0.71) | 0 | 1.85 (± 0.92) | 7.4 (±0.07) |
| Eutric cambisols | 1 | 50 | 38 | 12 | 1 | 0 | 1 | 7.1 |
| Bathicalcic cambisols | 1 | 15 | 56 | 29 | 1 | 0 | 2.5 | 7.2 |
| Calcari hypovertic calcisols | 1 | 12 | 36 | 52 | 13 | 9 | 2.4 | 7.8 |
| Haplic calcisols | 1 | 25 | 57 | 18 | 1 | 0 | 3.1 | 7.4 |
| Hyposkeletic luvisols | 1 | 32 | 43 | 25 | 1 | 0 | 1.7 | 7.5 |
| Chromic cutanic luvisols | 1 | 37 | 41 | 22 | 1 | 0 | 3.1 | 6.9 |

Table 2. Chemical and physical characteristics of calcareous and not calcareous soils cultivated with grapevine, pear and vegetable crops

| | Sand (%) | Loam (%) | Clay (%) | $CaCO_3$ (%) | Active $CaCO_3$ (%) | O.M. (%) | pH |
|---|---|---|---|---|---|---|---|
| **Crop** | | | | | | | |
| Grapevine | 26.4 | 53.0 | 26.5 | 8.20 | 3.40 | $2.07^b$ | 7.49 |
| Pear | 27.2 | 51.3 | 21.5 | 8.30 | 3.30 | $2.73^a$ | 7.38 |
| Vegetable | 20.6 | 52.9 | 20.7 | 8.70 | 2.50 | $1.53^c$ | 7.49 |
| Significance | n.s. | n.s. | n.s. | n.s. | n.s. | *** | n.s. |
| **Soil** | | | | | | | |
| Calcareous | 25.0 | 52.3 | 22.7 | 16.1 | 6.13 | 1.97 | 7.68 |
| Not calcareous | 24.5 | 52.5 | 23.1 | 0.73 | 0 | 2.25 | 7.23 |
| Significance | n.s. | n.s. | n.s. | *** | *** | n.s. | *** |
| Interaction | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |

Values followed by the same letter are not statistically different ($P \leq 0.05$); n.s., ***: effect of treatment not significant or significant at 0.001, respectively

one case, a third factor, soil depth (with 2 levels, 0–20 and 20–50 cm), was also considered. When analysis of variance showed a statistical ($P \leq 0.05$) effect of treatment, means were separated by the Student-Neuman-Kuel test.

## RESULTS

Among the calcareous soils investigated there were, according to the Food and Agriculture Organization (FAO 1990) Calcaric Cambisols (12 locations), Calcari-Fulvic Cambisols (2) and Calcari-Hypovertic Calcisols (1). These soils, the most common in the investigated area, are characterized by silt loam, loam and clay texture, respectively; they have a relatively high concentration of total (13–16%) and active calcium carbonate (3–9%), O.M. concentration between 1.5 and 2.4% and average pH between 7.6 and 7.9 (Table 1). The other analyzed soils were Eutri-Vertic Cambisols (6 locations), Vertic Calcisols (4), Bathicalci-Eutric Cambisols (3), Ferri-Stagni Luvisols, Thapto Vertic (2), Eutric Cambisols (1), Bathicalcic Cambisols (1), Haplic Calcisols (1), Hyposkeletic Luvisols (1), Chromi-Cutanic Luvisols (1). They had a texture ranging from loam, silt loam, to silty-clay loam (Table 1), roughly 1% of calcium carbonate, 1 to 3.1% of O.M. and pH between 6.9 and 7.3 (Table 1). The number of soils reported in Table 1 is higher than 30 because in few fields two soils were present (i. e. a combination of Eutri-Vertic Cambisols and Vertic Calcisols and between Bathicalci-Eutric Cambisols and Bathicalcic Cambisols). The percentage of sand, loam and clay was similar in the 3 types of cultivation and in calcareous and non-calcareous soils (Table 2). Organic matter was found higher in soils of pear orchards, followed by vineyards, and vegetable crop. On average, calcareous soils contained 16.1% of total and 6.1% of active $CaCO_3$, significantly higher than those in non-calcareous soils (Table 2), where active $CaCO_3$ was not found.

Copper application rate was higher in pear and grapevine plots than in vegetable crops (Table 3). When only orchards and vineyards were considered, the highest amount of Cu yearly sprayed was observed during 1999, 2000 and 2001, before the introduction of the EU limits, with 13.3, 14.3 and 14.4 kg Cu/ha, respectively (data not reported); then it decreased below 8 kg Cu/ha.

Total and DTPA-extractable Cu were higher in orchards and vineyards than in vegetable-cultivated plots (Table 4). Total Cu concentration was > 100 mg/kg in 14 out of 50 soil samples and always in pear or grapevine-cultivated plots (data not showed). While total Cu did not change through soil profile, DTPA-extractable Cu was higher in the upper horizon than at 20–50 cm depth. Soil carbonates did not affect the concentration of both the fractions of Cu (Table 4).

Table 3. Copper application rate to calcareous and non calcareous soils cultivated with vine, pear and vegetable crops

| | Cu (kg/ha) |
|---|---|
| **Crop** | |
| Grapevine | 8.71$^{a}$ |
| Pear | 9.04$^{a}$ |
| Vegetable | 0.27$^{b}$ |
| Significance | *** |
| **Soil** | |
| Calcareous | 5.10 |
| Not calcareous | 4.82 |
| Significance | n.s. |
| Interaction | n.s. |

Values followed by the same letter are not statistically different ($P \leq 0.05$); n.s., ***: effect of treatment not significant or significant at 0.001, respectively

Table 4. DTPA-extractable and total Cu fractions as observed at 2 depths of calcareous and non calcareous soils cultivated with vine, pear and vegetable crops

| | DTPA-Cu (mg/kg) | Total Cu (mg/kg) |
|---|---|---|
| **Crop** | | |
| Grapevine | 21.3$^{a}$ | 86.7$^{a}$ |
| Pear | 18.5$^{a}$ | 87.8$^{a}$ |
| Vegetable$^{z}$ | 8.98$^{b}$ | 48.9$^{b}$ |
| Significance | * | ** |
| **Soil** | | |
| Calcareous | 20.1 | 91.4 |
| Not calcareous | 19.7 | 83.1 |
| Significance | n.s. | n.s. |
| **Soil depth (cm)$^{y}$** | | |
| 0–20 | 25.2 | 90.9 |
| 20–50 | 14.5 | 83.6 |
| Significance | ** | n.s. |
| Interactions | n.s. | n.s. |

Values followed by the same letter are not statistically different ($P \leq 0.05$); n.s., *, **: effect of treatment not significant or significant at 0.05 and 0.01, respectively; $^{z}$samples from 0–50 cm depth; $^{y}$data of grapevine and pear crop only

## DISCUSSION

Disease control in pear and vine requires more Cu than in vegetable crops and consequently a higher Cu accumulation in pear orchard and vineyard soils was expected. Although the history of the investigated farms refers to a 7-year long period of time, we believe that the same use of Cu was going on for longer. Orchards have an economical life of 25–30 years, and often they are replanted on the same soil several times. So we can speculate that the amount of Cu found in the investigated plots, is the result of several years of the same crop management.

The Cu active fraction (water soluble, exchangeable and adsorbed Cu), available for root uptake (Brun et al. 2001), was increased by 105% and 137% in pear orchards and vineyards, respectively compared to vegetable-cultivated soil. Although, the higher concentration of DTPA-extractable Cu in the upper horizon indicates the low mobility of Cu through the soil profile, however a migration of Cu to the deeper layers was observed in vineyard and pear orchard as demonstrated by the higher concentration of both DTPA-extractable and total Cu the depth of 20–50 cm of grapevine and pear cultivated soil compared to vegetable crop. This is probably the result of the soil tillage that, over the years, promoted a deeper distribution of Cu applied with pesticide sprays. Pietrzak and McPhail (2004) showed that both active and total Cu concentration decreased with increasing soil depth, and this trend was more rapid in young than in old vineyards. In orchards and vineyards of our investigation, total Cu, which includes besides the active fraction also the Cu bound to carbonates, organic matter and Fe and Al oxides, was not affected by soil depth. In addition in the deeper soil profile, compared to the upper layer, there was a decrease of the DTPA-extractable but not of the total Cu, meaning that, moving down along the soil profile, there was a conversion of active Cu to inactive forms (total Cu-DTPA-extractable Cu). Since this process is estimated in decades (Pietrzak and McPhail 2004), this response confirms the hypothesis that, in the investigated area, the same cultivations (pear orchard/vineyard) was adopted for a long time. In fact as much as 72% (at 0–20 cm-depth) and 83% (at 20–50 cm-depth) of the total Cu was inactive, indicating that most of the Cu added to the soil was retained almost irreversibly (Arias et al. 2004). Nóvoa-Muñoz et al. (2007) in acid soils of the Spanish region of Galicia reported a similar Cu distribution (251–271 mg/kg) along the soil profile as a consequence of the traditional intense management carried out by the wine growers. The same authors also reported fractions of active ($Na_2$-EDTA-extracted) Cu of about 36% higher than that found in this investigation. The difference might be related to the lower soil pH of the Spanish soils compared to our conditions, which might have increased the amount of Cu bound to organic matter extracted with $Na_2EDTA$, with the result of an over-estimation of the amount of bioavailable Cu. Soil pH is considered an important factor in determining Cu adsorption by the soil and in particular, near neutrality (condition similar to those studied here) the O.M. was found to be the soil component that most influenced the Cu adsorption (Vega et al. 2007). In our investigation (pH between 7.2 and 7.7) the highest percentage of inactive Cu (ratio between Cu not extracted by DTPA and total Cu) corresponded to the higher O.M. (2.7% in pear plots).

The level of carbonates in the soil did not affect the amounts of Cu sprayed, and consequently the fractions of Cu in the soil, and the percentage of inactive Cu, indicating that $CaCO_3$ does not affect disease susceptibility of pear and vine investigated and does not contribute significantly to sequestrate Cu. In the pedological conditions of the Italian Po valley, including the investigated Region, rather than $CaCO_3$, Cu toxicity seems to be related to soil texture. A toxicity threshold was established only in light textured soils, for non-bearing potted grapevine (Toselli et al. 2009) and pear (Toselli et al. 2008) plants at a concentration of DTPA-extractable Cu > 141 and 350 mg/kg respectively, while in clay-loam soils, both the fruit species showed the possibility to tolerate levels of DTPA-extractable Cu as high as 1000 mg/kg with no symptoms on shoot growth. Total Cu concentration was similar to that found in a Haplic Cambisol near Prague (Komárek et al. 2008), and in several vineyard soils of Victoria, Australia (Pietrzak and McPhail 2004), but lower than the concentrations found in Northern Spain by Nóvoa-Muñoz et al. (2007). Most of the investigated soils presented a total Cu concentration below the Italian legislative (DL 99/92) limit of 100 mg/kg (value above which the application of sewage sludge is not allowed), but above the warning limit valid in the EU, i.e. 50 mg/kg (Council directive 86/278/EC, 1986).

## CONCLUSIONS

In the investigated area, soil Cu contamination seems to be the result of many horticultural cycles which interested grape and pear cultivations. The concentration of Cu was found to be close to the critical level of attention (100 mg/kg) set by the Italian legislation, most of it was however found as inactive in a soil profile as deep as 50 cm.

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