

# Ecotoxicology of Copper in Horticultural Soils: A Review

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**Summary:** Nowadays, the world is facing the problem of environmental pollution because of the increase of man's needs requires development in life activities, progress industrialization, transportation tools, enhancement of agriculture and exploitation of natural resources. Soil and water resources are extremely exposed to pollution from different aspects. Agrochemicals in particular, have created severe problems, since they release thousands of chemicals to the environment. Several studies on the effect of environmental pollutants on agroecosystem have been carried out. On the other hand, the importance of trace elements as environmental pollutants is well known and well documented in literature. Cu contamination to agricultural soils has been accelerated due to its wide and repeated use in agriculture and horticulture as fertilizers or fungicides to protect vines, citrus trees, and other fruit crops against fungus diseases. Applied Cu from different agrochemical sources to agro-environment may be adsorbed and are transported to the groundwater table and pollute it besides polluting the soils. The use of Cu-based fungicides in vineyard soils is widely documented worldwide. It has been found that many countries contain concentrations in excess of 100 mg kg<sup>-1</sup>. Importance of study of transport of Cu arises due to the fact that Cu is absorbed in soils and also reaches the groundwater table, thus polluting both soil and ground water. It is often more important to be able to estimate the mobile fraction, the readily soluble fraction, the exchangeable fraction, or the plant available fraction of Cu content of a soil as a more direct indication of the likelihood of deleterious or toxic effects on soils and groundwater. Therefore, the aim of present work was to highlight the behavior and ecotoxicological effects of copper on horticultural soils.

**Keywords:** Copper, horticultural soils, agrochemical, availability, ecotoxicology

## Introduction

Copper (Cu) is a redox-active transition element with roles in photosynthesis, respiration, C and N metabolism, and protection against oxidative stress. It forms highly stable complexes and participates in electron transfer reactions like Fe. Divalent Cu is reduced readily to monovalent Cu which is unstable (Broadley et al. 2012). It is an essential micronutrient, a constituent of the plastocyanin chloroplast protein, and a component of the electron transport system linking Photosystems I and II in the photosynthetic process. Copper participates in protein and carbohydrate metabolism and N<sub>2</sub> fixation. It is a component of some enzymes that reduce atoms of molecular oxygen (cytochrome oxidase, ascorbic acid oxidase, and polyphenol oxidase) and is involved in the desaturation and hydroxylation of fatty acids (Jones 2003). That means most of the functions of Cu as a plant nutrient are based on enzymatically bound Cu which catalyses redox reactions.

In redox reactions of the terminal oxidases, Cu enzymes react directly with molecular oxygen. Terminal oxidation in living cells is therefore catalysed by Cu and not by Fe (Broadley et al. 2012).

Due to versatile properties, Cu has a wide range of applications in different fields. It can be used for the production of various conductor materials such as fertilizers and pesticides. Due to its bacteriostatic properties, it can be also added to animal fodder (Kabata-Pendias 2011). It is well known that, the use of Cu-based fungicides in vineyard soils is widely documented worldwide. Thus, vineyard soils in Greece, France, Slovenia, Portugal, and the Czech Republic have been found to contain concentrations in excess of 100 mg kg<sup>-1</sup>, above which the biological quality of the soils is substantially degraded through a strong effect on microbial action. Excessive concentrations of Cu can be hindered topsoil rooting in young vines. Also, there is the risk of Cu accumulating in river water or even river sediments by effect of erosion-induced mass movements in

Cu-contaminated soils, resulting in potential environmental problems (Fernández-Calviño et al. 2009a).

Therefore, this review focuses on the occurrence and behavior of Cu in horticultural soils and plants with relevance to the potential risks associated with Cu in the soils of horticultural properties to be developed for residential use.

## Typical Cu concentrations in soils

It is well documented that, Cu has been part of the human civilizations since ancient times up to present days. This element has a history of use that is at least 10,000 years old, and estimates of its discovery place it at 9000 BC in the Middle East; a Cu-pendant was found in northern Iraq that dates to 8700 BC (Rusjan 2012). Cu fabricated in Mesopotamia was quickly introduced to the the Egyptian Empire, where its use flourished thousands of years. These peoples used Cu for the fabrication of different jewels, ornaments, but also in the fields of armament and tools. They soon realized that pure Cu, because of its softness, is not suitable for shaping tools used in agriculture, cultivation. Later, in the era of the Romans other metals, especially iron and bronze as the main metals in weaponry, gained in importance. In that time, Cu was first used also for architectural intentions, what can be witnessed on the roof sheathing of the Pantheon (Rusjan 2012).

The general values for the average total Cu contents in soils of different groups all over the world range between 14 and 109 mg kg<sup>-1</sup> (Kabata-Pendias 2011). The concentration of Cu in a soil can be increased by applying fertilizers or organic wastes containing Cu. Piggery wastes and digested urban sewage may contain relatively large concentrations of Cu. The soils in horticultural areas may also contain elevated concentrations of Cu as a result of using Cu compounds to control plant pests and diseases (Cornforth et al. 2003). The regularity in large-scale Cu occurrence in soils indicates that two main factors including parent material and soil formation processes govern the initial Cu status of soils. Also, the clay fraction contributes significantly to the Cu content of soils. Other soil properties, such as Fe and Mn oxides, and base saturation, explain about 15–25% of all impact factors (Kabata-Pendias 2011).

It is showed that Cu concentrations varied from 7 to 490 mg kg<sup>-1</sup> in a survey of 43 horticultural soils in the Auckland area, New Zealand (Gaw 2002). Sites currently being used for orchards had an average concentration of 209 mg kg<sup>-1</sup> Cu, whereas vineyard soils had an average of 105 mg kg<sup>-1</sup>. About the background Cu concentrations in the soils of forest remnants were 10 mg kg<sup>-1</sup> as reviewed by Cornforth et al. (2003). Generally, Cu is accumulated in the upper few centimeters of soils, however, due to its tendency to be adsorbed by soil clay minerals, carbonates, organic matter, and oxyhydroxides of Mn and Fe, it may be also accumulated in deeper soil layers. It is reported that, humic acids are to reveal a large binding capacity for this metal (Logan et al. 1997). On the other hand, Cu is a rather immobile element in soils

and shows relatively little variation in total contents of soil profiles. Thus, the common characteristic of Cu distribution in soil profiles is its accumulation in the top horizons. This phenomenon is an effect of various factors, but above all, Cu concentration in surface soils reflects its bioaccumulation as well as its anthropogenic sources (Kabata-Pendias 2011). Concentrations of Cu in soil solution range from 0.5 to 135 µg L<sup>-1</sup>, depending on techniques used and on soil types. In soil solution, it may occur as cations: Cu<sup>2+</sup>, Cu<sub>2</sub>(OH)<sub>2</sub><sup>2+</sup>, CuOH<sup>+</sup>, and as anions: Cu(OH)<sup>3-</sup>, Cu(OH)<sub>4</sub><sup>2-</sup>, and Cu(CO<sub>3</sub>)<sub>2</sub><sup>2-</sup> (Kabata-Pendias and Sadurski 2004).

It is reported that, the main variables affecting the Cu mobility in soils include soil organic matter, dissolved organic matter, pH, and Cu soil content. Overall solubility of both cationic and anionic forms of Cu decreases at about pH 7–8 (Ponizovsky et al. 2006). It was estimated also that hydrolysis products of Cu(CuOH)<sup>+</sup> and Cu<sup>2+</sup> + 2(OH)<sub>2</sub> are the most significant species below pH 7, while above pH 8, anionic hydroxy complexes of Cu become important. The precipitation of CuCO<sub>3</sub> in calcareous soils is a main process affecting the Cu activity in soil solution (Ponizovsky et al. 2007) as reviewed by (Kabata-Pendias 2011).

## Effects of Cu-agrochemicals on soils

Soil contamination by Cu compounds has been the subject of detailed studies for several decades and a large database has been already collected and presented in a number of monographs and papers. In this context, several significant sources such as fertilizers, agrochemicals, manures, sewage sludge, industrial by-product wastes and the quality of irrigation waters have contributed to increased Cu levels to agricultural soils (Kabata-Pendias and Mukherjee 2007). Cu contamination to agricultural soils has been accelerated due to its wide and repeated use in horticulture and agriculture as fertilizers or fungicides to protect vines, citrus trees, and other fruit crops against fungus diseases (He et al. 2005). Due to a long history of vineyard or citrus production, Cu has accumulated in the soils. A large proportion of soils under citrus production contains total Cu content above 85 mg kg<sup>-1</sup>, the critical level for ecosystem health (Schuler and Hoang 2008). Soil pollution with Cu causes soil degradation, Cu phytotoxicity, and increased transport of Cu to surface and ground waters (He et al. 2010).

It is well documented that, the most important sources of Cu contamination include old mining area, deactivated Cu ore plant, nonferrous metal mining, metal-processing industry, urban gardens, orchards, and parks, sludged, irrigated, or fertilized farmland, application of fungicides, vineyard soils, and military shooting range (Kabata-Pendias 2011). Dry and wet deposition around mining and smelting sites, wastewater irrigation, compost application, including municipal waste, sewage sludge or their combination, and spraying of heavy metal-containing pesticides or herbicides have been reported to contribute to the input of anthropogenic Cu and other heavy metals into agricultural soils (Yu et al. 2004).

**Table 1:** Effects of soil texture, pH, organic carbon (OC, %), nitrogen content (N, %) and effective cation exchange capacity (eCEC,  $\text{cmol}_c \text{kg}^{-1}$ ) on soil total Cu concentration ( $\text{mg Cu kg}^{-1}$ , as a range) in 6 vineyards locations around the world (from Mackie et al. 2012) comparing with mean of 95 vineyard soils from 6 different wine production areas in the NW Iberian Peninsula (from Soler-Rovira et al. 2013)

Country or location	Total Cu in control soil ( $\text{mg Cu kg}^{-1}$ )	Total Cu in vineyard soil ( $\text{mg Cu kg}^{-1}$ )	Soil texture	Soil pH ( $\text{CaCl}_2$ )	OC (%)	N (%)	eCEC ( $\text{cmol}_c \text{kg}^{-1}$ )
NW Iberian Peninsula	–	11–1120	Sandy loam	5.4	2.5	–	8.6
Germany	74	142–513	Loam	6.6	3.8	0.38	11.2
Germany	105	159–435	Loam	6.7	2.1	0.24	7.3
France	23	201–689	Sandy loam	6.8	2.1	0.17	17.2
Italy	105	194–448	Loam	7.3	9.1	0.22	16.1
Germany	65	276–516	Silty clay loam	7.3	5.9	0.40	29.2
Italy	66	155–455	Loam	7.5	5.6	0.08	15.3

Control soil is the soil adjacent to the vineyard and for the NW Iberian Peninsula, the mean total of Cu is  $176 \text{ mg Cu kg}^{-1}$

It is reported that, Cu levels in plants ranging from 2 to  $50 \mu\text{g g}^{-1}$  DW with  $6 \mu\text{g g}^{-1}$  considered as adequate in the shoots (Epstein and Bloom 2005). The amount of Cu contained in healthy plants varies considerably within this range and depends both on the species and the Cu-feeding status. Typically, symptoms of deficiency start when Cu decreases below  $5 \mu\text{g g}^{-1}$  DW in vegetative tissues, whereas toxicity levels are observed above  $20 \mu\text{g g}^{-1}$  DW or higher in the same tissue (Marschner 2002). However, it may be generally stated that total Cu contents below  $10 \text{ mg kg}^{-1}$  in different soils may indicate deficiency as reviewed by Kabata-Pendias (2011).

## I. Behavior of Cu in soils

It is well known that, Cu-based fungicides (such as the Bordeaux mixture,  $\text{CuSO}_4 + \text{Ca}(\text{OH})_2$ ) have been intensively used in Europe since the end of the 19<sup>th</sup> century to control vine (*Vitis vinifera* L.) fungal diseases, such as downy mildew caused by *Plasmopara viticola*. Besides vineyards, Cu-based fungicides have also been extensively used such as in hop fields (Komárek et al. 2009), coffee (Loland and Singh 2004), apple (Li et al. 2005), avocado orchards (Van Zwieten et al. 2004) and during the cultivation of several vegetables such as tomatoes and potatoes (Adriano 2001) as reviewed by Komárek et al. (2010).

From the background Cu concentrations in unpolluted soils, it is found that this concentration is influenced by the parent material from which the soils are formed and reach an average of  $30 \text{ mg kg}^{-1}$ . As of other metals, its solubility is greatly dependent on soil pH and will be most readily available at pH values below 6 (Table 1; Adriano 2001). For example, in acidic soils, Cu developed on granitic rocks, can migrate throughout soil profiles more easily and thus cause groundwater pollution (Nóvoa-Muñoz et al. 2007). Cu mobility in soils can increase at pH values above  $\sim 7.5$  due to the solubilization of soil organic matter (SOM) and formation of Cu-SOM complexes (Fernández-Calviño et al. 2009a). In general, Cu in soils is mostly associated with SOM, Fe-, Mn-(hydr)oxides and to a lesser extent with clay minerals through specific and non-specific adsorption (Fernández-Calviño et al. 2009b). However, SOM can

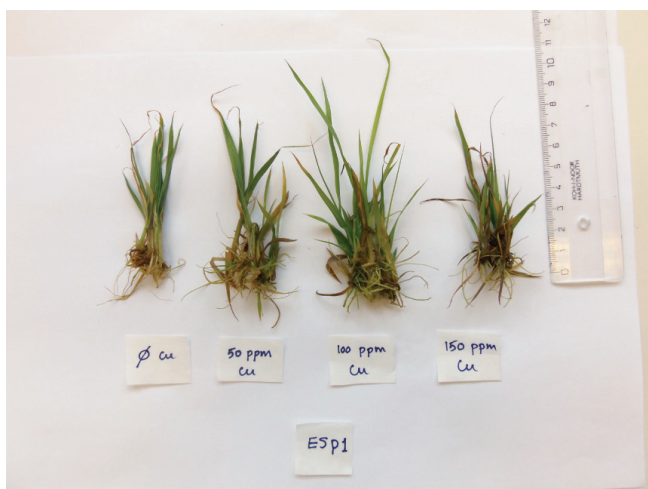
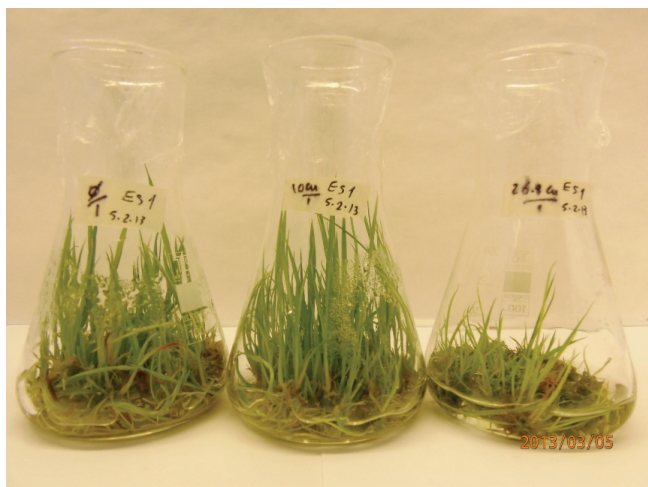
influence the mobility of Cu by two different mechanisms: while particulate SOM will act as a sorbent for Cu, soluble SOM will actually complex Cu, increasing thus its solubility, especially at alkaline pH (above  $\sim 7.5$ ) (Martínez-Villegas and Martínez 2008). When Cu enters the soil, as a result of the wash-off from vine leaves (Paradelo et al. 2008) and accidental spills of the fungicides, its speciation rapidly changes and Cu is sorbed and co-precipitated in the soil (Komárek et al. 2010). This redistribution of Cu into less available chemical fractions of soils which decreases its mobility and bioavailability is referred to as aging (Sayen et al. 2009) as reviewed by Komárek et al. (2010).

## II. Bioavailability and toxicity of Cu in horticultural soils

It is well known that, since about 1850, Cu containing fungicides have been used to protect crops from fungal infections such as downy mildew (*Plasmopara viticola*). Thus, intensive and long term use of these fungicides has increased soil Cu concentrations and this is likely most pronounced in vineyards (Komárek et al. 2010). Nowadays, EC regulation 473/2002 (European Commission 2002) restricts the annual dose of applied Cu to  $6 \text{ kg Cu ha}^{-1}$ , which corresponds to an annual accumulation of about  $5 \text{ mg Cu kg}^{-1}$  soil in the top 10 cm assuming no losses (Ruyters et al. 2013). Such a sustained application since  $> 150$  years would have increased soil Cu concentrations to  $750 \text{ mg Cu kg}^{-1}$  in unplowed vineyard soils, whereas plowing the first 30 cm after removing old vines (every 30–50 years) would yield a topsoil Cu concentrations of about  $250 \text{ mg Cu kg}^{-1}$ . Measured Cu concentrations in vineyard soils range from 77 up to  $3200 \text{ mg Cu kg}^{-1}$  (Komárek et al. 2010) and are above legislative limits affecting the sustainability and potentially the productivity of these agroecosystems as reviewed by Ruyters et al. (2013).

The phytotoxicity of Cu is the highest in acidic soils with a low cation exchange capacity. Cu phytotoxicity to agricultural plants grown on calcareous soils from former vineyards has been observed as well (Michaud et al. 2007). Although Cu concentrations in roots are a good indicator of

Cu bioavailability in soils (Chopin et al. 2008), this time consuming approach is not suitable for routine analyses (Komárek et al. 2010). So, it is needed to point out that Cu uptake by roots is species-dependent and influenced by root type and size (i.e., fine vs. coarse roots) (Fig. 1; Chopin et al. 2008). Total Cu concentrations alone do not provide adequate information about the bioavailability of the metal in soils as well (Wightwick et al. 2008). On the other hand, water-soluble Cu concentrations alone do not give sufficient information either, because a portion of exchangeable Cu (not extractable by water) in soils can be easily taken up by roots (Komárek et al. 2010).



**Fig. 1:** Giant reed (*Arundo donax* L.) tolerant to different concentrations of Cu using liquid media. The Spanish ecotype (ESP 1) was used under different Cu concentrations from 0 to 26.8 mg Cu kg<sup>-1</sup> or from 0 to 150 ppm in two different experiments (photos by T. Alshaal)

### III. Pollution of horticultural soils with Cu

As mentioned before, the mixtures of Cu sulphate and lime (Bordeaux mixture) have been widely used in pome and stone fruit orchards, vineyards and vegetable crops to control fungal diseases for over 100 years (Merry et al. 1983). However, foliar application of these fungicides leads to a significant input of Cu to soil, through direct application, dripping, or drift

of excess sprays from leaf surfaces (Chaignon et al. 2003). Numerous studies indicated that long-term use of Cu-based chemicals resulted in increased soil Cu concentrations, such as 29–131 mg kg<sup>-1</sup> in India (Prasad et al. 1984); 100–1500 mg kg<sup>-1</sup> in France (Besnard et al. 1999) and 11–320 mg kg<sup>-1</sup> in Australia (Wightwick et al. 2008). Consequently, some European countries have introduced restrictions on the use of Cu fungicides to protect the environment, which also satisfy eco-labelling requirements. For example, the Netherlands has banned Cu fungicides, while Switzerland has restricted the amount of Cu that can be applied per hectare (Table 2; Wang et al. 2009).

### Forms and accumulation of soil Cu in plants

It is well known that, the difference between optimum and toxic concentrations of Cu in many economically important crops is very small. Normal and phytotoxic concentrations of Cu in plant leaves are 5–20 and 20–300 mg kg<sup>-1</sup>, respectively although there are some species variations (Alloway 1995). Despite this, examples of Cu toxicity in plants are rare (Cornforth et al. 2003). The proportion of total soil Cu which is available to plants depends on soil composition and pH. Cu is held strongly in soils containing relatively large concentrations of clay, organic material, or oxides of Fe, Al, and Mn. These forms are largely unavailable to plants in the short term but may become slowly available if the more plant-available fractions are depleted (Cornforth et al. 2003). Availability of Cu is also greater in acid conditions although the link between pH and Cu availability is less marked than for other heavy metals. Liming an acid soil increases the amount of Cu adsorbed but does not necessarily decrease the amount of plant available Cu proportionally. This is because lime may increase the amount of Cu released by mineralisation of organic Cu (James and Barrow 1981) as reviewed by Cornforth et al. (2003).

It is well documented that, the bioavailability of soluble forms of Cu depends basically on both the molecular weight of Cu complexes and on the amounts present. Compounds of a low molecular weight liberated during decay of plant and animal residues as well as those applied with sewage sludge may greatly increase the availability of Cu to plants (Kabata-Pendias 2011). It should be also emphasized, however, that concentrations of Cu in soil solutions are principally controlled by both the reactions of Cu with active groups at the surface of the solid phase and by reactions of Cu with specific substances. The behavior, phytobioavailability and toxicity of Cu are influenced by its species, and are not a function of its total concentration (Allen 1993). Several soil variables control the Cu solubility and thus bioavailability; these include soil pH, soil organic matter, oxidation and reduction potential, soil texture, mineral composition, temperature, and water regime. The mobility of Cu is especially reduced at the presence of large mineral colloids with Fe–Al-oxyhydroxide coatings, by oxyhydroxide particles of Al, Mn, Fe, and by organic matter (Kabata-Pendias and Sadurski 2004).

**Table 2:** Total Cu concentration (mg Cu kg<sup>-1</sup> soil) and used methods in upper layers of vineyard soils as published in literature from 2007 to 2013

Country/ location	Depth (cm)	Total Cu (mg Cu kg <sup>-1</sup> )	Method used	Reference
Spain	0–10	125–603	HNO <sub>3</sub> +HCl+HF	Nóvoa-Muñoz et al. (2007)
Spain	0–5	42–583	HNO <sub>3</sub> +HCl+HF	Pateiro-Moure et al. (2007)
France	0–10	17–34	HClO <sub>4</sub> + HF	Dousset et al. (2007)
Croatia	0–20	105–553	HClO <sub>4</sub> + HNO <sub>3</sub> +HCl+HF	Miko et al. (2007)
Slovenia	0–20	87–120	HNO <sub>3</sub> + HCl	Rusjan et al. (2007)
Brazil	0–5	37–3216	HNO <sub>3</sub> + HClO <sub>4</sub> +HF	Mirlean et al. (2007)
Spain	0–20	35–550	HNO <sub>3</sub> +HCl+HF	Díaz-Raviña et al. (2007)
Brazil	0–20	51–665	H <sub>2</sub> O <sub>2</sub> + HClO <sub>4</sub> +HF	Casali et al. (2008)
France	0–10	89–243	HNO <sub>3</sub>	Probst et al. (2008)
France	5–10	232	HNO <sub>3</sub> + HCl +HF	Chopin et al. (2008)
Italy	n.a.	215–372	HNO <sub>3</sub> + HCl	Dell'Amico et al.(2008)
Spain	0–5	42–583	HNO <sub>3</sub> +HCl+HF	Fernández-Calviño et al. (2008a)
Spain	0–20	79–130	HNO <sub>3</sub> +HCl+HF	Fernández-Calviño et al. (2008b)
Spain	0–20	25–272	HNO <sub>3</sub> +HCl+HF	Fernández-Calviño et al. (2008c)
Spain	0–20	61–434	HNO <sub>3</sub> +HCl+HF	Fernández-Calviño et al. (2008d)
Brazil	0–5	433–517	HNO <sub>3</sub> + HClO <sub>4</sub> +HF	Mirlean et al. (2009)
Spain	0–20	55–112	HNO <sub>3</sub> +HCl+HF	Fernández-Calviño et al. (2009a,b)
Slovenia	0–45	364	n.a.	Pociecha and Lestan (2009)
NW Spain	n.a.	100-268	n.a.	Fernández-Calviño et al. (2010)
Italy	n.a.	93	n.a.	Provenzano et al. (2010)
Spain	n.a.	22.5-66.8	n.a.	Herrero-Hernández et al. (2011)
NW Iberian Peninsula	0-20	176	HNO <sub>3</sub> + HCl + HF	Soler-Rovira et al. (2013)
Different *	0-10	435 – 689	Aqua regia extraction	Ruyters et al. (2013)

Compiled from Komárek et al. (2010), Mackie et al. (2012), Soler-Rovira et al. (2013) and Ruyters et al. (2013)

n.a., not available

\* mean of soil samples from France (1 site), Germany (3 sites) and from Italy (2 sites)

The affinity of Cu to separate soil fractions decreases in the following order: Mn-(hydr)oxides > SOM > Fe-(hydr)oxides > clay minerals (Bradl 2004). Dissolved organic matter has a great affinity to fix Cu and thus to inhibit its sorption in soils. These phenomena are attributed to the formation of soluble Cu-organic complexes (Zhou and Wong 2003). In mineral soils, natural attenuation of Cu occurs as an effect of Cu substituting for Ca in calcites present in calcareous soils and as precipitation of Cu(OH)<sub>2</sub> and/or Cu<sub>2</sub>(OH)<sub>2</sub>CO<sub>3</sub> in other soils (Ma et al. 2006). It is observed that the long-term application of Cu fertilizers resulted in a great Cu accumulation in surface soil (0–15 cm) due to its low mobility, since a great proportion of added Cu was bound to mineral soil fractions (Wei et al. 2007). The more readily plant available forms include Cu ions in solution, soluble organic-Cu complexes, and Cu on the soil's exchange complex (Alloway 1995). However, Bolan et al. (2003) distinguish between Cu adsorbed on soluble organic matter and free Cu<sup>2+</sup> ions in the soil solution: the former may increase the mobility of Cu in the soil while the latter represents the plant available fraction as reviewed by Cornforth et al. (2003).

It is reported that, soil carbonates proved to be another important factor controlling Cu mobility (and thus bioavailability) in soils. It is also found that, the activity of Cu in calcareous soils is to a great extent controlled by the surface precipitation of CuCO<sub>3</sub> (Ponizovsky et al. 2007). This is especially important in alkaline soils containing high

concentrations of carbonates, which is the case for many vineyards. The retention of Cu in calcareous soils through co-precipitation with carbonates is associated with the release of Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup> and H<sup>+</sup> into the soil solution at equimolar ratios (Ponizovsky et al. 2007). The precipitation of newly formed Cu phases in the soil presents thus an important retention mechanism of Cu retention in soils. The solubility of Cu minerals in soils decreases in the following order: CuCO<sub>3</sub> > Cu<sub>3</sub>(OH)<sub>2</sub>(CO<sub>3</sub>)<sub>2</sub> (azurite) > Cu(OH)<sub>2</sub> > Cu<sub>2</sub>(OH)<sub>2</sub>CO<sub>3</sub> (malachite) > CuO (tenorite) > CuFe<sub>2</sub>O<sub>4</sub> (cupric ferrite). Copper sulfates, such as CuSO<sub>4</sub> (chalcocyanite) and CuSO<sub>4</sub>·5H<sub>2</sub>O (chalcantite), are highly soluble and require very high Cu concentrations to form in soils (Lindsay 1979) as reviewed by Komárek et al. (2010).

In excessive quantities Cu becomes toxic as it interferes with photosynthetic and respiratory processes, protein synthesis and development of plant organelles (Upadhyay and Panda 2009). Specifically excess Cu can cause chlorosis, inhibition of root growth and damage to plasma membrane permeability, leading to ion leakage (Bouazizi et al. 2010). Reports are also available on induced deficiency of various minerals content under Cu toxicity (Lequeux et al. 2010). Apart from this, the information on plant metabolism is sporadic. Hence, efforts have been made to establish the toxic level of Cu on mung bean plants. It is found that, low Cu concentration (50 mg kg<sup>-1</sup>) had stimulatory effect on growth, dry matter yield and mineral nutrient content of mung bean. Whereas, Cu application beyond these levels (100-250 mg

**Table 3:** Some hyperaccumulators of copper from Democratic Republic of Congo (Zaire)

Plant species	Family of plant	Maximum Cu concentration (mg kg <sup>-1</sup> )	References
<i>Pandiaka metallorum</i>	Amaranthaceae	6,270	Malaisse et al. (1979)
<i>Anisopappus davyi</i>	Compositae or Asteraceae	3,504	Malaisse et al. (1994)
<i>Ascolepis metallorum</i>	Cyperaceae	1,211	Malaisse et al. (1994)
<i>Vigna dolomitica</i>	Fabaceae or Leguminosae	3,000	Malaisse & Gregoire (1978)
<i>Haumaniastrum robertii</i>	Lamiaceae or Labiatae	2,070	Brooks (1977)
<i>Actiniopteris</i> sp.	Pteridaceae	3,535	Malaisse et al. (1994)
<i>Buchnera henriquesii</i>	Scrophulariaceae	3,520	Brooks et al. (1987)
<i>Triumfetta dekindtiana</i>	Liliaceae	1,283	Brooks et al. (1987)

Compiled from Reeves (2006)

kg<sup>-1</sup>) adversely affected the growth, dry matter yield and nutrient content (Manivasagaperumal et al. 2011).

Copper can be presented in several insoluble forms; (1) adsorbed on surface of metal oxides, clay minerals, humic substances and organo-mineral complexes, (2) in structure of secondary minerals or in amorphous iron and manganese oxides; (3) or associated with antigenic sulfides. Gunkel et al. (2003) found that applied Cu was mostly concentrated in the MnO<sub>x</sub>, FeO<sub>x</sub> and organic soil fractions. Although the total copper levels were quite low on Cu-enriched plots, ranging from 68–135 mg kg<sup>-1</sup>, anthropogenic copper in soil influenced the maize crop yield. The recommended method for determine the real bioavailability nowadays is the sequential extraction method and the plant bioassay.

Most of the cases of hyperaccumulation of Cu and Co have been reported from the metalliferous soils of the Democratic Republic of Congo (formerly Zaire), where the two metals occur together at elevated levels in the soils, but in widely varying proportions (Reeves 2006). A few other records of plants with > 1000 mg kg<sup>-1</sup> Cu from Cu-mineralized areas (Dykeman and De Sousa 1966) should be reinvestigated in detail. Normal concentrations of Co and Cu in plants are in the ranges 0.03-2 and 5-25 mg kg<sup>-1</sup>, respectively. The tupelo or black gum of the southeastern United States (*Nyssa sylvatica*) is remarkable in being able to accumulate as much as 845 mg kg<sup>-1</sup> Co from normal soils (Brooks 1977). Plant Cu concentrations are controlled within a remarkably narrow range, and Cu concentrations above 100 mg kg<sup>-1</sup> are rarely found in carefully washed plant leaves, even in the presence of high soil Cu. The Cu hyperaccumulators have been found in more than a dozen plant families; some examples are given in Table 3, and a more complete listing can be found in Reeves and Baker (2000). It should also be noted that most of the Cu accumulators are not restricted to metalliferous soils. Therefore, great care needs to be taken in selecting seed of any of these species for studies connected with metal-accumulation experiments or with studies of their potential for phytoremediation (Reeves 2006).

### Loading limits of Cu in soils

Most studies are limited to the total soil Cu concentration and its distribution among soil components in the orchards receiving long-term application of Cu fungicides

(Fernández-Calviño et al. 2008c). As fungicides are applied to control fungal diseases, they will also affect beneficial soil fungi and other soil organisms. Therefore, in many regions of the world there are increasing concerns that Cu may reach the concentrations in orchard soils toxic to soil organisms, or even phytotoxicity (Viti et al. 2008). Although the total soil Cu concentration is a useful indicator of soil deficiency and/or contamination, it does not provide enough information about its environmental impact (Bending et al. 2004). Since biological properties are sensitive to change, they are being considered to be suitable indicators of environmental impact for properly complementing the soil physicochemical properties as reviewed by Wang et al. (2009).

Increased Cu levels in vineyard soils up to 246 mg kg<sup>-1</sup> affected the Cu accumulation up to 209 mg kg<sup>-1</sup> in river sediments (Fernandez- Calvino et al. 2008b). In the surface soil layer of vineyards, after 50–100 years of applied fungicides, the content of Cu forms dissolved by various extractants were as follows (in mg kg<sup>-1</sup>): aqua regia 220, DTPA 82.5, and CaCl<sub>2</sub> 0.23 (Deluisa et al. 1996). The maximum allowable loading of Cu with wastes to arable soils of the EU countries is estimated at 12 kg ha<sup>-1</sup> year<sup>-1</sup>. Ranges of maximum allowable concentration (MAC) values commonly cited in literature range from 20 to 100 mg kg<sup>-1</sup> (Kabata-Pendias and Sadurski 2004). Precautionary values for Cu in Germany are established for soils of various textures as follows (in mg Cu kg<sup>-1</sup>): clay, 100; loam, 60; and sand, 30 (Eckel et al. 2005) as reviewed by Kabata-Pendias (2011).

It is well documented that, Cu reveals a strong affinity for S, hence its principal minerals are chalcopyrite, CuFeS<sub>2</sub>; bornite, Cu<sub>5</sub>FeS<sub>4</sub>; chalcocite, Cu<sub>2</sub>S; and covellite, CuS. During the weathering of copper sulfides, Cu is incorporated in oxide and carbonate minerals of which cuprite, Cu<sub>2</sub>O; tenarite, CuO; malachite, Cu<sub>2</sub>CO<sub>3</sub>(OH)<sub>2</sub>; and azurite, Cu<sub>2</sub>(CO<sub>3</sub>)<sub>2</sub>(OH)<sub>2</sub> are the most common. Cu is often associated with sphalerite, ZnS; pyrite, FeS; and galena, PbS. Its ores are commonly found in acid igneous rocks and various sedimentary deposits (Table 4; Kabata-Pendias 2011). Cu has a high affinity for peptide and sulphhydryl groups, and thus to cysteine-rich proteins, as well as also for carboxylic and phenolic groups. Therefore, more than 98% of the Cu in plants is present in complexed forms and the concentrations of free Cu<sup>2+</sup> and Cu<sup>+</sup> is extremely low in the cytoplasm (Broadley et al. 2012).

**Table 4:** Selected properties of copper comparing with sulfur as an element-based fungicides for some physical, chemical and biological properties

Properties or items (unit)	Sulphur (S)	Copper (Cu)
Name origin	Latin word <i>sulfur</i> (brimstone)	Latin <i>Cuprum</i> (isle of Cyprus)
Discovery or discoverer of essentiality (year)	von Sachs, Knop (1865)	Sommer (1931)
World mine production in 2012 (metric tons)	70,000 × 10 <sup>3</sup>	17,100 × 10 <sup>3</sup>
Abundance in the Earth's crust	0.06 – 0.1 (%)	55 mg kg <sup>-1</sup>
Abundance or usual soil content	0.01 – 0.1 (%)	20 mg kg <sup>-1</sup>
Ranking in order of abundance in earth crust	14	26
Most important minerals	Gypsum (CaSO <sub>4</sub> ·2H <sub>2</sub> O), Pyrite (FeS <sub>2</sub> ), Chalcopyrite (CuFeS <sub>2</sub> ), Galena (PbS)	Chalcopyrite CuFeS <sub>2</sub> , Malachite Cu <sub>2</sub> (OH) <sub>2</sub> (CO <sub>3</sub> ), Cuprite Cu <sub>2</sub> O
Most important sources	Iron sulfide and sulfate	Copper sulfide, sulfate and carbonate
Most important uses	Matches, gunpowder, medicines	Fertilizers, pesticides, motors and electrical industry
Common valence states	-2, 0, +2, +4, +6	+1 and +2
Ionic Radius (Å)	0.37	1.28
Electronegativity (according to Pauling scale)	2.58	1.90
Atomic Number	16	29
Atomic Mass (atomic mass unit)	32.06	63.54
Atomic Radius (Å)	0.88	1.57
Density at 20°C (g cm <sup>-3</sup> )	2.07	8.94
Boiling point (°C)	444.6	2595
Melting point (°C)	112.8	1083
Crystal Structure	Orthorhombic	Cubic
Functions in Plants	Component of amino acids, glucosides, coenzyme A, vit. B1	constituent of protein and many superoxide dismutases (SOD)
Principal forms for plant uptake	Sulfate SO <sub>4</sub> <sup>-2</sup> anion	Cupric cation (Cu <sup>2+</sup> )
Essentiality for animals and plants	Essential for both	Essential for both
Typical sufficient concentration (plant shoot)	1,000 mg kg <sup>-1</sup> DM	6 mg kg <sup>-1</sup> DM
Critical level in plant leaf (DW, dry weight)	0.10 – 0.50 (%)	1.0 – 5 mg kg <sup>-1</sup>
Toxic level in plant leaf (DW, dry weight)	0.5 – 0.7 (%)	15 – 30 mg kg <sup>-1</sup>
Uptake by plants	Active (SO <sub>4</sub> <sup>-2</sup> )	Active
Major antagonistic elements	As, Fe, Pb, Mo, and Se	Ca, Mg, P, and N
Major synergistic elements	F and Fe	Ca, P, and N
Mobility in plant	Moderately mobile	Immobile
Movement in soil	Mass flow (SO <sub>4</sub> <sup>-2</sup> )	Mass flow
Hydroponic nutrient concentration in solution	70–150 mg S L <sup>-1</sup>	0.01– 0.1 mg Cu L <sup>-1</sup>

Compiled from Jones (2003), Jones (2005), Kabata-Pendias and Mukherjee (2007), White and Brown (2010), Kabata-Pendias (2011), Kirkby (2012), USGS (2013)

## Effects of Cu on soil organisms in horticultural soils

It is found that, there were a number of basic criteria that a microbiological property might be expected to fulfill as an indicator in monitoring soil pollution by metals or other pollutants (Brooks 1995). Soil microbial biomass is considered to be a transformation agent of soil organic materials and a labile pool for plant nutrients. Hence, the change of the soil microbial biomass could lead to a change in the rate of nutrient cycling and the size of the nutrient pool. It is suggested that, soil C mineralization and linked parameters such as CO<sub>2</sub>-C production per unit biomass C and unit time (biomass specific respiration rate) might be useful as indicators for the change of soil function (Brooks 1995). Soil enzyme activities are also useful for detecting changes in soil quality, as they underpin nutrient cycling, and also function as signals of altered microbial community structure caused by environmental impact (Wang et al. 2009).

Because of the human and environmental health hazards that may arise from this pollution, setting soil-Cu limits and adequate management practices are necessary to reach sustainability in this type of crop. High Cu contents can harm soil microbial communities, which are the main agents responsible for long-term sustainability of soil ecosystems (Nannipieri et al. 2003). Some studies have evaluated the effects of Cu on enzymatic activities (Fernández-Calviño et al. 2010a), microbial community structure (Fernández-Calviño et al. 2010b), and bacterial community tolerance (Fernández-Calviño et al. 2011a) in vineyard soils. However, the establishment of limits for Cu accumulation using microbial indicators is difficult because of the confounding effects of soil factors such as texture, pH, and organic matter content (Fernández-Calviño et al. 2011b) as reviewed by (Soler-Rovira et al. 2013).

It is reported that, toxic effects of Cu on the microbial communities in vineyard soils have been observed and the enzyme activities in soil were affected at and above total

concentrations of 150–200 mg Cu kg<sup>-1</sup> soil (**Fernández-Calviño et al. 2010a**), whereas nitrification was impaired in soils contaminated up to 380 mg Cu kg<sup>-1</sup> (**Baroux 1972**). Evidence for increased Cu tolerance of the microbial community in response to the Cu contamination has also been reported in such soils (**Diaz-Ravina et al. 2007**) as reviewed by **Ruyters et al. (2013)**. On the other hand, there is evidence that earthworms (**Martin 1986**) and soil microorganisms (**Aoyama and Nagumo 1997**) are less active in Cu-rich soils than in those with concentrations of Cu in the normal range (2–30 mg kg<sup>-1</sup>) (**Cornforth et al. 2003**). Earthworm populations have been proposed as indicators of the degree of Cu contamination in orchard and vineyard soils (**Paoletti et al. 1998**). This approach assumes that earthworms and higher plants are similar in their sensitivity to Cu or that decreased earthworm activity will damage soil structure to the extent that plant growth suffers (**Cornforth et al. 2003**).

## Environmental risks

The intensive and long-term use of copper salts, promoted, in viticulture over the years, Cu accumulation in soils. Cu is a heavy metal toxic for aquatic and soil organisms, bacteria, fungi and plants 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 et al. 2001**). Despite its environmental and agricultural importance, the concentration, distribution and fractionation of anthropogenic, and naturally occurring, Cu in soils is poorly known. Although the total Cu content in soils is a useful indicator of soil deficiency and/or contamination, it does not provide enough information about its environmental impact (**Pietrzaka and McPhail 2004**). Sorption on SOM by means of complexation especially with humic and fulvic acids presents possibly the most important retention mechanism for Cu in soils (**Strawn and Baker 2009**). Its association with SOM through inner-sphere complexation (e.g., bidentate inner-sphere coordination with carboxyl or amine ligands; **Strawn and Baker 2008**) results in its lower toxicity compared to free Cu<sup>2+</sup> (**Karlsson et al. 2006**). Additionally, Cu-rich SOM is less vulnerable to biodegradation (**Parat et al. 2002**). In most cases, the sorption of Cu in soils follows well either the Langmuir or the Freundlich isotherm as reviewed by **Komárek et al. (2010)**.

Contents of Cu are closely associated with soil texture and usually are the lowest in light sandy soils and the highest in loamy soils. It is found that, Cu toxicity seems to be related to soil texture and a toxicity threshold was established only in light textured soils, for nonbearing potted grapevine and pear plants at a concentration of DTPA-extractable Cu > 141 and 350 mg kg<sup>-1</sup>, 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<sup>-1</sup> with no symptoms on shoot growth (**Toselli et al. 2009**). Cu availability to biota (as a nutrient or toxin) and its mobility are the most important

factors to be considered when assessing its effect on the soil environment. Since Cu bioavailability is influenced not only by soil physical and chemical properties, but also by environmental factors such as climate, biological population, and type and source of contaminants, correlation between total and bioavailable Cu cannot be predicted accurately (**Pietrzaka and McPhail 2004**).

Cu must be absorbed in small amounts on a daily basis to maintain good health. A daily dietary intake of 1–2 mg is required. However, high levels of Cu can be harmful to health. Inhaling high levels can cause irritation to the nasal passages, mouth, eyes and throat, and ingesting high Cu concentrations can lead to nausea, vomiting and diarrhoea. Exposure to very high levels can damage the liver and kidneys and may lead to death. Copper is classified as a hazardous substance. An excess of Cu may result in Wilson's disease, mostly ending in death (**Sharma et al. 2009**).

Fungicides have received little attention compared with other types of pesticides, such as insecticides and herbicides, despite the likely environmental risks (**Wightwick et al. 2010**). Ecological risks of chemicals detected in the environment are often derived by comparisons with environmental quality values and reported ecotoxicological effects values for key sentinel aquatic species (**Wightwick et al. 2012**). The paucity of ecotoxicological data for fungicides is surprising given their frequency of use and the fact that most do not have specific modes of action and thus are likely to be toxic to a wide range of organisms, not just fungi (**Maltby et al. 2009**). There is a widespread presence of residues of many different organic fungicide compounds in the surface waters of a horticultural-production catchment. Whilst, it appears that the fungicides detected are likely to pose a low ecological risk to fish, aquatic invertebrates, and algae, it is important to note that at present there are few ecotoxicological data available describing the effects of fungicides on aquatic fungi and microorganisms despite the fact that these organisms are likely sensitive to fungicides and play a key role in aquatic ecosystems (e.g., decomposition and nutrient cycling) (**Milenkovski et al. 2010**) as reviewed by **Wightwick et al. (2012)**.

## Possible recommendations

It could be recommended that, phosphate application to soils such as fertilizers can reduce the mobility of risk metals through sorption mechanisms on newly formed phases, but probably not through precipitation of Cu-phosphates as observed, for example, for Pb-phosphates (**Pérez-Novo et al. 2009**). It could be also recommended that, some management options (e.g., lime, organic matter,...ect.) are applied to soils in residential areas with a total concentration in excess of Cu of 300 mg kg<sup>-1</sup>, because this concentration represents a threat to humans health (**Cornforth et al. 2003**). On the other hand, lime should be applied to the acidic soils to increase the pH from 6.5 to 7.0 and the organic matter concentration should be maintained or increased by applying organic materials



such as compost or peat. Orchard and vineyard soils should be cultivated to mix the topsoil and thus dilute the large concentrations of Cu found in the surface few centimetres (Cornforth et al. 2003).

It is indicated that, threshold values of total Cu and exchangeable Cu contents indicative of soil Cu pollution cannot be established. However, adequate management practices resulting in soil organic C contents > 2% and pH > 5.5 are recommended for preserving vineyard soil quality (Soler-Rovira et al. 2013).

Significant adverse effects were only found for three bioassays in vineyard samples of one site and for two bioassays in another site. Biological responses in these cases were more importantly explained by other soil properties than soil Cu. Overall, no Cu toxicity to plants, microbial processes and invertebrates was observed in vineyard soil samples at Cu concentrations well above European Union limits protecting the soil ecosystem (Ruyters et al. 2013).

It could be provided valuable information for policy and decision-makers, all over the world, to assess the likely risks the use of fungicide compounds in horticultural production systems poses to aquatic ecosystems. To progress toward a more thorough assessment of the ecological risks posed, future research should focus on gaining a better understanding of the ecotoxicological effects of priority fungicides and fungicide mixtures, particularly to the lower trophic levels of aquatic ecosystems (Wightwick et al. 2012).

## Conclusions

It could be concluded that, Cu compounds applied as pesticides to horticultural soils accumulate in the upper soil layers and some Cu in these soils is attached very strongly to soil particles and organic materials making it unavailable to plants. Despite its environmental and agricultural importance, the concentration, distribution and fractionation of anthropogenic, and naturally occurring, Cu in soils is poorly known. Ecological risks of chemicals detected in the environment are often derived by comparisons with environmental quality values and reported ecotoxicological effects values for key sentinel aquatic species and soil organisms. The paucity of ecotoxicological data for fungicides is surprising given their frequency of use and the fact that most do not have specific modes of action and thus are likely to be toxic to a wide range of organisms, not just fungi. The threshold values of total Cu and exchangeable Cu contents indicative of soil Cu pollution cannot be established. However, adequate management practices resulting in soil organic C contents > 2% and pH > 5.5 are recommended for preserving vineyard soil quality.

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