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AN OVERVIEW OF THE COPPER SITUATION AND USAGE IN VITICULTURE

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Abstract

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Viticulture is among those agricultural branches, which, along with the intensification of heavy industry and natural processes such as the weathering of bedrock, contribute to an increased environmental intake of copper compounds, so, in many places, levels of this metal in soil are elevated or maximum soil copper limits are exceeded. Organic fruit and grape production is still the major consumer of copper-based plant protection products per unit area. Long-term use of copper fungicides can elevate copper levels even in the lower soil layers. Copper accumulation in soils along with its ecotoxicological impacts necessitates alternatives in the form of new, low copper content formulations or in the form of other means. The adverse effects of copper have also been observed in winemaking, where excessive copper residues in grapes must adversely affect wine quality. Therefore, restrictions on the use of copper fungicides in relation to soil contamination with copper compounds are necessary.

Key words: grapevine, soil, copper compounds, formulations, environment

Introduction

Copper is a heavy metal that acts as a catalyst for many chemical reactions. In plant cells, it participates in the photosynthetic electron transport and is an integral part of enzymes such as polyphenol oxidase, monoamine oxidase, and other phenolases. Organisms are usually unable to fully control copper entering their cellular structures. They also lack the ability to completely neutralize its harmful effects when excessive accumulation in cells and tissues occurs. Organisms developing in an environment with elevated levels of copper compounds are at a higher risk of suffering from a number of developmental and reproductive disorders. The adverse effects of copper have also been noted in winemaking, where excessive copper residues in grapes must impair wine quality.

Copper-based preparations have been used for over 200 years to control fungi and bacterial diseases in cultivated plants. Their use became widespread after the accidental discovery of a Bordeaux mixture in 1880. At the time, winegrowers in the Bordeaux wine region were using a mixture of copper sulphate and lime to deter passersbys from eating the grapes. The French scientist Millardet noted these grapes did not show any signs of downy mildew (Copper Development Association, 2003). By 1885, Millardet had completed experiments, which confirmed this mixture controlled this disease at relatively low cost. Therefore, the Bordeaux mixture became the first fungicide to be used on a large scale, worldwide level (Schneiderhan, 1933).

Viticulture is amongst agricultural sectors that contribute to an increased environmental intake of copper compounds. Due to the excessive use of copper-con-

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taining fungicides, the maximum soil copper limits are exceeded in many places. The detection limit of ecotoxicity for copper is between 60-90 mg Cu $kg^{-1}$of soils (Official Gazette RS no. 84/05). In the past, the annual environmental copper inputs of up to 30 kg ha^{-1} were observed. Nowadays, integrated production limits this intake by restricting the use of copper-based pesticides to the final sprayings. In organic agriculture, a gradual reduction of copper input levels from 8 kg ha^{-1} to 6 kg ha^{-1} over a transitional period of four years (Commission Regulation EC No. 473/2002) is required.

Copper in the Soil

Copper is present in organic matter, oxides, and minerals that readily release copper ions under acidic conditions (Kabata-Pendias et al., 1992; Mortvedt, 2000). In the soil solution, copper can occur in ionic or in complex form, and as an exchangeable cation, plants can absorb that. Long-term use of copper fungicides increases copper levels even in the lower soil layers (Casali et al., 2008).

The average global concentrations of soil copper are 30 mg kg^{-1}, and these concentrations should never exceed the limits of 50 mg kg^{-1} (Adriano, 1986). In Slovenia, copper is present in surface soil samples at concentrations ranging from 2.2 to 151 mg kg^{-1} and in depths of 5 to 20 cm in concentrations of 27 mg kg^{-1}, indicating a natural source (Zupan et al., 2008). Average levels of copper in the top soil layers from 22 experimental vineyards in the Primorska region, were between 62 and 120 mg kg^{-1} (Rusjan et al., 2007), while it was in old vineyards more than 300 mg kg^{-1} (Veber, 1997) (Table 1). In French vineyard soils, these values exceed 100 mg, sometimes even 1000 mg kg^{-1} (Fernandez-Calvino et al., 2008), in central Taiwan they range from 9.1 to 100 mg Cu kg^{-1}, and in central Italy's agricultural soils they range between 50 and 220 mg Cu kg^{-1} (Mantovi et al., 2003). In central Chile, agricultural soils were divided into two categories according to their copper concentrations. Soils from the first group contained 162 mg Cu kg^{-1}, while those from the second one contained 751 mg Cu kg^{-1}. Most of this copper was present in poor soluble forms and so its availability to plants was limited (Badilla-Ohlbaum et al., 2001). In China, agricultural soils have between 5.8 and 66.1 mg Cu kg^{-1} (Wang et al., 2003a). Higher levels of 26 to 199 mg Cu kg^{-1}, found in calcareous soils, were attributed to irrigation with mine waste water and smelting operations in the 1950s (Nan et al., 2001). An analysis of heavy metal concentrations in the soil collected along roadsides at distances from 0.25 to 50 m in Turkey have shown copper values of between 11.1 to 27.9 mg kg^{-1} for soil and 0.8 to 5.6 mg kg^{-1} for plants (Bakirdere and Yaman, 2008).

Availability of copper in soil

Soil types containing large quantities of copper are Kastanozems, Chernozems, Ferrasols, and Fluvisols, while only minor quantities of this metal are present in Podzols and Histosols (Kabata-Pendias, 1992). The

Table 1
Copper accumulation in different depths of vineyards soils (average± SD; control area was lawn) in different vine-growing regions in Slovenia (Veber, 1997)

Depth, cm	Vine-growing region	Young vineyards Cu mg/kg	Lawn (control areas) Cu mg/kg	Old vineyards Cu mg/kg
0 – 20	Podravje	18.2 ± 10.9	14.3 ± 2.2	77.9 ± 4.5
	Posavje	50.2 ± 26.0	4.3 ± 0.9	204.4 ± 57.7
	Primorska	7.0 ± 0.1	4.6 ± 0.5	325.7 ± 16.3
20 – 40	Podravje	14.4 ± 12.6	10.1 ± 4.1	40.5 ± 9.5
	Posavje	5.0 ± 3.4	1.9 ± 1.0	35.1 ± 33.6
	Primorska	4.0 ± 1.9	1.9 ± 0.5	45.4 ± 9.4
40 – 60	Podravje	11.4 ± 9.8	6.3 ± 3.5	26.4 ± 17.0
	Posavje	4.5 ± 2.3	0.9 ± 0.8	12.6 ± 8.6
	Primorska	4.7 ± 1.7	1.3	27.2 ± 14.6

availability of copper is influenced by the soil's pH. Cu^{2+} is the dominant form of copper in a soil solution with a pH value below 6.9, whereas the $Cu(OH)_2$ predominate at pH values above 6.9 (Mortvedt, 2000). Copper concentrations in soil solution of calcareous soils are low (Mengel et al., 2001). Low pH value increases the solubility, hence the mobility of copper (Evans, 1989). 80% of inorganic copper in soil is present in insoluble form that plants can not use and only 20% is present in soluble, plant-available form. Soil copper is most available to plants at pH values between 5 and 6 (Fregoni and Corrallo, 2001). Copper accumulates in the upper soil layers and is mostly bound to clay particles (Van Der Perk et al., 2004) and to organic matter (Adriano, 1986; Yu et al., 2002; Fernandez-Calvino et al., 2008). Copper positively influences micro-and macronutrients intake (Vercesi et al., 2001). It interacts in various ways with other micro and macronutrients present in the soil. Increased N and P levels negatively affect Cu availability in soils. The resulting Cu deficiency hinders Ca transport to upper plant parts. Excessive Cu amounts negatively affect Fe reception, and Fe deficiency can lead to Cu toxicity in plants (Marschner, 1995). Fertilization with compost and manure increases the number of copper complex compounds and reduces copper phytotoxicity (Bolan et al., 2003) but does not affect the content of copper in grape leaves. However, fungicidal application of copper in doses of 15 kg and 20 Cu kg^{-1} resulted in observable effects on wine must and wine (Pinamonti et al., 1999). Application of fertilizers containing rare earth elements (lanthanum, cerium, praseodymium, and neodymium oxides) increases the amount of unbound copper in the soil (Wang et al., 2003b).

Copper deficiency most often occurs in humus soils, which strongly bind Cu^{2+} ions (Mercer and Richmond, 1970). Over-fertilization with nitrogenous fertilizers as well as long-term use of phosphorus fertilizers can lead to copper deficiency in certain soils (Mengel et al., 2001). Deficiencies are common on sandy soils, soils with high organic matter, and on calcareous soils (Martens and Westerman, 1991). Copper contamination of soil often occurs due to excessive use of fertilisers and copper-based crop-protection preparations, as well as due to copper-containing agricultural and urban wastes and heavy industry emissions (Kabata-Pendias et al., 1992).

In a study conducted in organic vineyard located in southwestern Italy, where the annual input of copper averages 7.4 kg of pure Cu per hectare, analyses of copper residues on vines and soil were made. Copper concentrations at depths of 0-20 cm and 20-40 cm were 103 mg kg^{-1} soil and 47 mg kg^{-1} soil, respectively. The leaves showed visible signs of chlorosis due to phytotoxicity. Copper residues on grapes and in wine were within limits but the levels were higher in white wine (Pessanha et al., 2010).

Copper in Plants

Absorption and metabolism

Copper growth and reproduction requirements of organisms are very small and cannot affect the amounts of soil-copper accumulated due to the use of copper fungicides (Borkow and Gabby, 2007). The highest concentrations of copper are usually found in root tissues (Quartacci et al., 2003; Pradubsuk and Davenport, 2011). Approximately 70% of the total leaf copper content is located in chloroplasts (Marschner, 1995; Mengel et al., 2001). Copper affects the metabolism of carbohydrates and nitrogen. Copper deficiency during vegetative growth stages can result in lowered content of soluble carbohydrates (Brown and Clark, 1977). The application of nitrogen can delay translocation of copper from older to newer leaves (Loneragan et al., 1981). It's content in plants ranges between 2 and 20 mg kg^{-1} dry matter. While some studies have shown no correlation between soil copper and copper extracted from plant tissues (Marschner, 1995; Mclaughlin et al., 1998), Romič et al. (2004) argue that the correlation does exist. In vines treated with copper preparations, the highest copper concentrations were found in leaves, followed by old and one-year old wood, and the lowest concentrations were found in grapes (Rusjan et al., 2007; Lai et al., 2010) (Table 2). Certain plants have shown good potential for copper hyper-accumulation in their tissues (Peralta et al., 2001; Yang et al., 2002; Ali et al., 2003).

Copper deficiency and toxicity in plants

In some crops, micronutrient (including copper) deficiencies have increased due to growing yield expectations, and use of mineral fertilizers (*N*, *P*, *K*), containing only small amounts of trace elements, as well as due to a decrease of manure use (Mortvedt, 2000). Because of copper deficiency, the activity of various plant enzymes is limited (Walker and Webb, 1981). Deficiency symptoms are different for each plant species and are often dependent on the level of deficiency. Most plants stop growing, and develop necrotic spots, curved leaves, and ultimately die (Brennan and Bolland, 2003).

Prior to being used as fungicide and micronutrient, copper was used as a herbicide. Copper ions are toxic to all plant cells and must therefore be used in specific doses or relatively insoluble forms to avoid tissue damage of the plants (Ware and Whitacre, 2004). In high levels, copper is toxic to most plant species, probably because of its ability to displace other metal ions, especially iron, from important physiological centres. Excess copper often causes root growth inhibition and chlorosis or paleness that are, at a first glance, hard to distinguish from copper deficiency (Daniels et al., 1972). Copper toxicity is especially critical in acidic soils where copper is not as tightly bound to soil particles and is thus more available to plants. Where copper is lacking, excessive auxine accumulation, resulting in pollen sterility, is likely to occur (Mengel et al., 2001). Growers have a large number of copper-based fungicides available on the market. Overuse of these has caused many soils to be contaminated with this metal (Semu and Singh, 1995).

Excess amounts of copper alter nitrogen metabolism in grapevines, which is reflected in the root system as a reduction of nitrogen, mainly nitrate and free amino acids (especially glutamine and glutamic acid), and enzyme activity (Llorens et al., 2000). Copper accumulation in soil can have a phytotoxic effect on grapevines. Vines potted in different soil types were fertilized with different rates of copper from 0 to 1000 mg kg^{-1} soil. Changes, observable as a reduction of root growth, appeared after addition of 400 mg Cu kg^{-1} soil. In pots containing sandy soils shoot growth was stunted and signs of chlorosis appeared (Toselli et al., 2009). Depending on plant species, symptoms of phytotoxicity appear when average plant-copper concentrations of 15 to 25 Cu mg g^{-1} dry weight are exceeded (Balsberg-Pahlsson, 1989). A comparison study of soil copper residues from conventional and organic vineyards showed lower soil microbial activity in the latter. Soil and vines from organic vineyard also had higher copper concentrations compared to the ones from conventional vineyard. In both cases the highest concentrations were measured in vine leaves. Copper residues in wine were below the legal limit of 1 mg l^{-1} of wine (Beni and Rossi, 2009).

Copper and Organisms

Because copper cycling in biological systems is very slow, it can accumulate in large concentrations that are measurably harmful for agricultural flora and other organisms (Van Zwieten et al., 2004). Elevated concentrations of copper compounds in soil (Van Rhee, 1967), as well as methods of soil management (Vršič, 2011; Vršič et al., 2011) have an impact on the reduction in number of earthworms. A strong correlation was found between copper concentrations of copper in soil and in earthworm tissues (Ma et al., 1983; Morgan and Morgan, 1988). Copper was found to be sub-lethally toxic for earthworms at concentrations as low as 9 to 16 mg kg^{-1} (Kula et al., 1994; Helling et al., 2000). Enchytraeid (*Cognettia sphagnetorum* Vejd.) have been shown to actively avoid copper-contaminated soils (Salminen and Haimi, 2001). Field trials have shown that the use of copper oxychloride-containing fungicides reduced the field earthworm species population (*Aporrectodea caliginosa* Sav.). This reduction was apparent even six

Table 2
Copper accumulation in the lives and berries of vines according to the number of applications with copper preparations (Rusjan, 2007)

	Leaves (Cu; mg/kg)	Berries (Cu; mg/kg)			
Applications	May	July	August	June	September
4 times	9.52 ± 0.29	17.4 ± 0.36	54.8 ± 5.3	61.2 ± 3.3	10.3 ± 0.2
1 times	7.42 ± 0.33	12.1 ± 0.47	11.3 ± 0.6	14.2 ± 1.6	6.0 ± 0.3
Wthout Cu	7.58 ± 0.39	11.9 ± 0.18	13.0 ± 1.1	11.9 ± 1.5	5.6 ± 1.8

months after the application of the fungicides (Maboeta et al., 2003).

In some cases, copper treatments stimulate population increase of certain species such as mites (Michaut and Grant, 2003), entomopathogenic fungi (McCoy et al., 1996), and nematodes (Jaworska and Gorczyca, 2002), which can cause yield loss. The upper limit for copper concentration in feed is 50 mg Cu kg^{-1} dry matter (Dam Kofoed, 1980) while the recommended dietary concentration for most ruminants is 7-12 mg Cu kg^{-1} dry matter (Blood et al., 1983). Copper is also a micronutrient that is essential for humans due to its involvement in the metabolic reactions of amino and fatty acids, as well as vitamins. Copper deficiency can lead to increased risk of cardiovascular disease. On the other hand, excessive amounts of copper are harmful (Olivers and Uauy, 1996).

Copper as a Plant Protection Agent

Copper-based fungicides have multi-site activity, thus there is less risk of pathogens developing resistance. Copper ions (Cu^{2+}) have multisite activity which means that in fungi cells they block the enzymes involved in the process of respiration, inhibit protein synthesis, reduce the activity of cell membrane and cellular organelles, and affect element exchange (El Bilali, 2005). In target organisms copper acts as enzyme inhibitor, as water-soluble free copper ions (Cu^{2+}) enter harmful cells (spores, fungus spawn, bacteria cells, etc.) where they bind with enzymes, rendering them inactivate. Copper oxides and oxychlorides are highly insoluble compounds that act on fungal and bacterial cell walls and membranes without penetrating deep into the cell interior. In the process, pathogens release secondary metabolites, which react with copper compounds resulting in the poisoning and death of the cells (Lešnik et al., 2009). Some data suggest the possibility of occurrence of copper-tolerant strains. Two bacterial species have developed resistance to copper fungicides, namely bacterial blight (*Pseudomonas syringae* L.) (Wilson et al., 1998; Vanneste and Voyle, 2003) and *Xanthomonas campestris pv. vitians* L. (Wilson et al., 1998; Carisse et al., 2000).

The main objective is to develop a formulation with optimal solubility and persistence. The higher the solubility, the higher the rate of ion release, the lower the fungicidal coating persistence, and the higher the extent of ion penetration in treated plants, resulting in copper binding to carrier compounds (proteins, amino acids, EDTA, fatty acids, etc.). Increased entry of copper compounds leads to phytotoxicity and elevated levels of copper in fruits. Particle size and type of crystals fungicidal coating is built from (net, rods, snowflakes, etc.) plays an important role in the ion release. Reduction of particle size (below 0.1 mm – nano-formulations) significantly increases the solubility and activity, but may increase the toxicological risk (inhalation). If environmental criteria were to become stricter, integrated production winegrowers could completely abandon the use of copper products, as there are many organic fungicides for control of downy mildew available on the market. However, consequences of complete copper ban on the organic winegrowing are difficult to predict. Alternative formulations do not provide a reliable protection and current availability of disease-resistant varieties is too small. Some experiments suggest that the intensive use of alternative products (15-20 sprayings per year) could enable organic growers to manage without copper fungicides. In years of severe downy mildew outbreaks however, yield losses of 30-50% and more are possible (Lešnik et al., 2009).

The actual biological efficiency of conventional copper formulations is less than 5% and sometimes even less than 1%; the rest is released into the environment. The current strategy of developing novel formulations is to develop complex copper-based compounds with low copper levels of 2 to 8% and to improve biological efficiency. Modern formulations contain complexes of copper with amino acids, peptides, fatty acids, pectins, sugars, and other organic compounds (Lešnik et al., 2009). In recent years, due to improved formulations of copper fungicides, possibilities of their use as well as their biological efficiency increased substantially. According to data, the efficiency rates of modern fungicides are up to 85% with much lower doses than in the past (Gomez et al., 2007; Mohr et al., 2007; 2008).

Reduction of fungicide use in conventional viticulture, and especially a reduction of copper-based preparations in organic viticulture, is possible by growing fungal disease resistant interspecific varieties such as Rondo, Prior, Cabernet carol, Regent, Phoenix, Johannniter, etc. (Basler and Pfenninger, 2002). The adverse effects of long-term copper fungicide use can be eliminated or at least diminished by reducing the number of applications and doses of conventional copper fungicides and by combining this strategy with increasing use of biological preparations or by totally abandoning the use of copper fungicides and replacing them with others (Goebel et al., 2004). Potassium phosphonates are highly effective against downy mildew of grapes and are a viable alternative to copper-based preparation, especially in organic viticulture (Speiser et al., 2000). Marine algae extracts improve copper intake and thus increase its concentrations in the grapevine but have no effect on the reception of N, P, K, Ca, Fe, Mg, Mn, and Zn (Turan and Kose, 2004).

Effects of Copper Compounds on the Organoleptic Properties of Wine

Identifying factors contributing to high metal content in wine and other grape products helps to improve their quality and to reduce possible adverse effects on health (Mirlean et al., 2005). Small amounts of copper have a favourable influence on yeast activity, whereas large amounts can be toxic, especially to their growth and reproduction. In organic wines a strain of yeast (*Saccharomyces cerevisiae* L.) resistant to elevated concentrations of copper compounds (Brandolini et al., 2002) was discovered. Copper and ascorbic acid, added to chilled grape, must prior to alcohol fermentation a beneficial effect on protecting aromatic compounds from oxidation (Corona, 2010). Recommended copper concentrations in wine are between 0.3 mg and 0.5 mg l^{-1} of wine (Clark and Scollary, 2000). At elevated copper concentration and during oxidation, Cu^{+} ions are converted to Cu^{2+}, which affects the sensory properties of wine (opacity, refraction and metallic taste). High heavy metal content in wine can be lowered by ion exchange technique, which is not allowed in organic winemaking. Treated wines have a lower content of polyphenols and aromatic compounds, which is reflected in the organoleptic properties of wine (Benitez et al., 2002). Wine equilibrium and the relative volatility of aromatic compounds are largely influenced by the redox potential of wine. At low wine redox potential, it is possible for anomalies, such as formation of sulfur compounds (H_2S) to occur in wine. To remove these anomalies (the smell of H_2S) copper sulfate (max. 1 mg l^{-1}), which reacts with hydrogen sulphide and mercaptans, is added to wine (Jakob, 1995).

Sauvignon is a variety, which contains in its flavour spectrum volatile thiol compounds that react with copper compounds, leading to deterioration of the organoleptic properties of wine. Most grapevine varieties contain terpenes and aromatic components, which do not react with copper compounds. Copper treatments applied to foliage only, results in a decrease of copper residues on grapes and in must. Therefore, there is no reduction in the concentration of volatile thiols and no deterioration of the organoleptic characteristics of wine. Wines from grapes produced by this method were very similar to other reference wines (Darriet et al., 2001). Sauvignon contains volatile thiols 4-mercapto-4-methylpentan-2-one, 3-mercaptohexyl acetate and 3-mercapto hexan-1-ol, characterized by the smell of grapefruit and passion fruit (Tominaga et al., 1998a). Copper treatments at annual rate of 3 kg copper ha^{-1} significantly reduced the amount of thiols (4-mercapto-4-methylpentan-2-one) in grapes and wine of Sauvignon variety (Hatzidimitriou et al., 1996). Similar was found for varieties Merlot and Cabernet Sauvignon because of copper residues reacting with volatile thiol compounds during the early stage of alcoholic fermentation of yeast (*Saccharomyces cerevisiae* L.) (Tominaga et al., 1998b).

In organic vineyards on calcerous soils in the Mediterranean, application of copper fungicides in accordance with the European regulation has no affect on organoleptic properties of wine or on health (Provenzano et al., 2010). The content of aromatic compound 4-mercapto-4-methylpentan-2-non (4MMP) was lower by 25% in beer with hops from copper sulphate-treated fields compared to beer with hops from untreated fields. The latter had higher contents of aromatic compounds 3-mercapto hexan -1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) (Morimoto et al., 2010).

Conclusions

Copper is the cornerstone of plant protection against many diseases in organic agriculture (Van Zwieten et al., 2004; Anonymous, 2007). It is necessary to limit the use of copper preparations depending on copper contamination level of the soil. There are currently no efficient alternative products that could completely replace copper fungicides, available on the market (BMU, 2009). Biological effectiveness of the novel copper-based fungicide groups are poorly studied (in Slovenia they are registered as foliar fertilizers). Since these newer copper fungicide groups are systemic, there is a possibility of residues of active substances appearing in fruits and of enhanced mobility of complexes through soil layers. The prices of alternative copper products are higher compared to traditional ones. The use of copper products within the allowable limits has no effect on wine quality and copper products are still indispensable in winemaking and for removing wine anomalies. Copper preparation residues on fruits affect mainly their visual quality. In the literature review, no studies on effects of copper on taste of wine and other produce were found.

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